Psyche

Ecological Interactions of Bark Beetles with Host Trees

Guest Editors: John A. Byers, Steven J. Seybold, Brian T. Sullivan, and Qing-He Zhang

Ecological Interactions of Bark Beetles with Host Trees

Psyche

Ecological Interactions of Bark Beetles with Host Trees

Guest Editors: John A. Byers, Steven J. Seybold,
Brian T. Sullivan, and Qing-He Zhang

Editorial Board

Contents

Ecological Interactions of Bark Beetles with Host Trees, John A. Byers
Volume 2012, Article ID 252961, 3 pages

Semiochemical Diversity in Practice: Antiattractant Semiochemicals Reduce Bark Beetle Attacks on Standing Trees—A First Meta-Analysis, Fredrik Schlyter
Volume 2012, Article ID 268621, 10 pages

Bark Beetles, *Pityogenes bidentatus*, Orienting to Aggregation Pheromone Avoid Conifer Monoterpene Odors When Flying but Not When Walking, John A. Byers
Volume 2012, Article ID 940962, 10 pages

Pheromone Production, Attraction, and Interspecific Inhibition among Four Species of *Ips* Bark Beetles in the Southeastern USA, Göran Birgersson, Mark J. Dalusky, Karl E. Espelie, and C. Wayne Berisford
Volume 2012, Article ID 532652, 14 pages

Host-Tree Monoterpenes and Biosynthesis of Aggregation Pheromones in the Bark Beetle *Ips paraconfusus*, John A. Byers and Göran Birgersson
Volume 2012, Article ID 539624, 10 pages

A Population Genetic Model of Evolution of Host-Mate Attraction and Nonhost Repulsion in a Bark Beetle *Pityogenes bidentatus*, John A. Byers
Volume 2012, Article ID 529573, 9 pages

2-methyl-3-buten-2-ol: A Pheromone Component of Conifer Bark Beetles Found in the Bark of Nonhost Deciduous Trees, Qing-He Zhang, Fredrik Schlyter, and Göran Birgersson
Volume 2012, Article ID 414508, 7 pages

Declining Bark Beetle Densities (*Ips typographus*, Coleoptera: Scolytinae) from Infested Norway Spruce Stands and Possible Implications for Management, Alexander Angst, Regula Rüegg, and Beat Forster
Volume 2012, Article ID 321084, 7 pages

Performance of *Tomicus yunnanensis* and *Tomicus minor* (Col., Scolytinae) on *Pinus yunnanensis* and *Pinus armandii* in Yunnan, Southwestern China, Tao Zhao and Bo Långström
Volume 2012, Article ID 363767, 6 pages

Mechanisms of Odor Coding in Coniferous Bark Beetles: From Neuron to Behavior and Application, Martin N. Andersson
Volume 2012, Article ID 149572, 14 pages

Attraction of *Tomicus yunnanensis* (Coleoptera: Scolytidae) to Yunnan Pine Logs with and without Periderm or Phloem: An Effective Monitoring Bait, Rong Chun Lu, Hong Bin Wang, Zhen Zhang, John A. Byers, You Ju Jin, Hai Feng Wen, and Wen Jian Shi
Volume 2012, Article ID 794683, 5 pages

***Hylastes ater* (Curculionidae: Scolytinae) Affecting *Pinus radiata* Seedling Establishment in New Zealand**, Stephen D. Reay, Travis R. Glare, and Michael Brownbridge
Volume 2012, Article ID 590619, 9 pages

Coexistence and Competition between *Tomicus yunnanensis* and *T. minor* (Coleoptera: Scolytinae) in Yunnan Pine, Rong Chun Lu, Hong Bin Wang, Zhen Zhang, John A. Byers, You Ju Jin, Hai Feng Wen, and Wen Jian Shi
Volume 2012, Article ID 185312, 6 pages

Hindawi Publishing Corporation
Psyche
Volume 2012, Article ID 252961, 3 pages
doi:10.1155/2012/252961

Editorial

Ecological Interactions of Bark Beetles with Host Trees

John A. Byers

US Arid-Land Agricultural Research Center, ARS USDA, 21881 North Cardon Lane, Maricopa, AZ 85138, USA

Correspondence should be addressed to John A. Byers, john.byers@ars.usda.gov

Received 9 October 2012; Accepted 9 October 2012

Certain species of bark beetles in the insect order Coleoptera, family Curculionidae (formerly Scolytidae), are keystone species in forest ecosystems. However, the tree-killing and wood-boring bark and ambrosia beetles are also among the most damaging insects of forest products including lumber, paper, and ornamental/recreational trees. The pest status of these beetles has been elevated with the advance of global warming and moderate to severe area-wide droughts, exacerbated by mismanagement and prevention of fires over decades. The ecology and chemical ecology of bark beetles has been and still is an exciting area of research, particularly since bark beetles utilize a wide array of semiochemicals in communication and in interactions with plants. Bark beetle chemical ecology is intimately connected and intertwined with behavioral and physiological processes that are still largely unknown in many species. Development of more efficient pest management practices will require a much deeper understanding of the ecology of bark beetles facilitated by interdisciplinary observations and experiments on many levels. Potential topics for this special issue include host-tree finding and selection, resistance by the tree, avoidance of tree defenses, insect/tree microbial associations, regulation of colonization density, ecology of predators and parasitoids, communication, biosynthesis of semiochemicals, behavioral assays and antennal responses, population management, models of dispersal and trapping, and reviews. Many of these topics and others are covered in part in the 12 articles in the special issue on "*Bark beetle ecology and interactions with host trees*."

F. Schlyter in his article "*Semiochemical diversity in practise: Antiattractant semiochemicals reduce bark beetle attacks on standing trees—a first meta-analysis*" brings together the results of 33 recent studies on attractive traps and repellent chemicals (verbenone and/or nonhost tree volatiles) designed to reduce bark beetle colonization of host trees. He found using Cohen's effect size that the use of repellent chemicals caused a significant reduction in attacked and killed trees in most studies. The results were not affected by publication year and the plots indicated there were little bias in reporting of only those studies showing effects of repellents. The use of natural repellents at higher dosages can allow stressed trees to survive by protecting themselves with their innate defenses. He recommends more precise reporting of results, more unified experimental designs, and further meta-analyses that include "grey literature" and more beetle species.

S. D. Reay et al. in their article "*Hylastes ater (Curculionidae: Scolytinae) affecting Pinus radiata seedling establishment in New Zealand*" report on the pine bark beetle *Hylastes ater* that was introduced into New Zealand about 100 years ago. In the 1950–1970s, biological control was attempted with limited success, and now there is renewed interest in developing a better understanding of the pest status on seedlings and to evaluate the role of the beetle in vectoring sapstain fungi in order to develop options for management. A number of findings relevant to the New Zealand exotic forest industry are presented that reveal the role of secondary bark beetles.

A. Angst et al., Rüegg, and Forster report in their article "*Declining bark beetle densities (Ips typographus, Coleoptera: Scolytinae) from infested Norway spruce stands and possible implications for management*" that eight-toothed spruce bark beetles (*Ips typographus*) during the last 20 years have killed millions of cubic meters of standing spruce in central Europe. Beetle densities were monitored using pheromone traps along transects from infested stands to spruce-free buffer zones. The beetle densities decreased rapidly with increasing distance from the infested spruce stands, falling below high risk thresholds within a few hundred meters from the infested stands. The decrease in catches was more pronounced in open land and in an urban area than in a broadleaf stand. Buffer zones without spruce of 500 m width can probably reduce densities of spreading beetles.

Several articles deal with pine shoot beetles of the genus *Tomicus*; for example, T. Zhao and B. Långström in their article "*Performance of Tomicus yunnanensis and Tomicus minor (Col., Scolytinae) on Pinus yunnanensis and Pinus armandii in Yunnan, southwestern China*" report that *T. yunnanensis* and *Tomicus minor* have caused substantial tree mortality to Yunnan pine (*Pinus yunnanensis*) in southwestern China, whereas the beetles rarely attack Armand pine (*Pinus armandii*). The suitability of *P. armandii* as host material for the two *Tomicus* species was tested by introduction of the beetles to branches and logs of the two pines during shoot feeding and trunk attack phases. Successful shoot feeding was observed by both species on both host trees, but the performance of both *Tomicus* species was much better on *P. yunnanensis* than on *P. armandii*. In the laboratory, *T. yunnanensis* and *T. minor* produced similar progeny in both pine species' logs, but the brood beetles emerging from Armand pine weighed less than those from Yunnan pine. Thus, *P. armandii* may be a potential host for *T. yunnanensis* and *T. minor*,but more experiments should assess the risk of these insects to stands of *P. armandii*.

In the first of two articles by R. C. Lu et al. on the "*Attraction of Tomicus yunnanensis (Coleoptera: Scolytinae) to Yunnan pine logs with and without periderm or phloem: an effective monitoring bait,*" the authors report experiments with host log baits to develop a pest monitoring system using host tree kairomone. Yunnan pine logs with peeled-off periderm (outer bark) with sticky adhesive areas captured significantly more beetles than on untreated control logs with adhesive. *T. yunnanensis* fly mostly during the afternoon according to trap catches. Attraction to the periderm-peeled logs decreased considerably when they were peeled further to remove the phloem, indicating that phloem volatiles play a role in selection of the host by the beetle. The readily available log baits appear useful for monitoring pine shoot beetle populations in integrated pest management programs. In a second paper by these authors, "*Coexistence and competition between Tomicus yunnanensis and T. minor (Coleoptera: Scolytinae) in Yunnan pine*," they found that *T. yunnanensis* initiated dispersal from pine shoots to trunks in November, while the majority of *T. minor* begin to transfer in December. The patterns of attack densities of these two species were similar, but with *T. yunnanensis* colonizing the upper section of the trunk and *T. minor* the lower trunk. The highest attack density of *T. Yunnanensis* was 297 egg galleries/m^2, and the highest attack density of *T. minor* was 305 egg galleries/m^2. Although there was significant overlap for the same bark areas, the two species generally colonize different areas of the tree, which reduces the intensity of competition for the relatively thin layer of phloem-cambium tissues where the beetles feed and reside.

Q.-H. Zhang et al. in their article "*2-Methyl-3-buten-2-ol, a pheromone component of conifer bark beetles found in the bark of nonhost deciduous trees*" report that volatiles from the bark of aspen, *Populus tremula* and two species of birch (*Betula pendula* and *B. pubescens*), were collected from both newly cut bark chips and undamaged stems and subjected to combined gas chromatography and mass spectrometry (GC-MS) analysis. The results showed the presence of 2-methyl-3-buten-2-ol (MB), one of the two principal aggregation pheromone components of the spruce bark beetle, *Ips typographus*, in samples of all three tree species. In addition, an oxygenated hemiterpene, 3-methyl-3-buten-2-one, and (*E*)-3-penten-2-ol were collected. Only trace amounts of MB were detected in some aeration samples of the fresh bark chips, and no MB was found from the aeration samples of undamaged stems. The occurrence of MB was also confirmed in the bark of four exotic birch species: *B. albo-sinensis, B. ermanii, B. jacquemontii,* and *B. maximowicziana*, but not in the European pines/spruces and the common yeasts. The results raise major questions regarding the evolution, the biosynthesis, the tropospheric chemistry, and the ecological role of MB.

G. Birgersson et al. report on the "*Pheromone production, attraction, and interspecific inhibition among four species of Ips bark beetles in the southeastern USA.*" Hindgut volatiles from attacking, unmated males of *Ips avulsus*, *I. calligraphus*, *I. grandicollis*, and *I. pini* were analyzed by GC-MS and these results were used to formulate baits for the four bark beetle species. The bioassays were subtractive for the compounds identified in the hindgut analysis of each species, and volatiles identified in sympatric species were added as potential inhibitors alone and in combination. The subtractive assays showed that *I. grandicollis* and *I. calligraphus* share (−)-(4*S*)-*cis*-verbenol as one pheromone component. The second synergistic pheromone component of *I. grandicollis*, (−)-(*S*)-ipsenol, acts as an interspecific inhibitor of *I. calligraphus*, while the second synergistic pheromone component of *I. calligraphus*, (±)-ipsdienol, acts as an interspecific inhibitor to *I. grandicollis*. *I. avulsus* and *I. pini* were found to be very similar in hindgut volatiles, and both use ipsdienol and lanierone as synergistic pheromone components. Lanierone was found to be an interspecific inhibitor for both *I. calligraphus* and *I. grandicollis*.

J. A. Byers reports that "*Bark beetles, Pityogenes bidentatus, orienting to aggregation pheromone avoid conifer monoterpene odors when flying but not when walking*." Previous studies and data in this paper suggest that odors from healthy host Scotch pine (*Pinus sylvestris*) and nonhost Norway spruce (*Picea abies*), as well as major monoterpenes of these trees at natural release rates, significantly reduce the attraction of flying bark beetles, *Pityogenes bidentatus*, of both sexes to their aggregation pheromone components grandisol and *cis*-verbenol in the field. In contrast, *P. bidentatus* males and females walking in an open-arena olfactometer in the laboratory did not avoid monoterpene vapors at release rates spanning several orders of magnitude in combination with aggregation pheromone. This apparent contradiction can be explained if the bark beetle avoids monoterpenes when flying as a mechanism for avoiding nonhost species, vigorous, and thus unsuitable host trees, as well as harmful resinous areas of hosts. After landing, inhibition of this flight avoidance response in beetles would allow them to initiate, or to find and enter, gallery holes with high monoterpene vapor concentrations in order to feed and reproduce.

J. A. Byers and G. Birgersson write about "*Host-tree monoterpenes and biosynthesis of aggregation pheromones in the bark beetle Ips paraconfusus.*" In the 1970–80s, a paradigm

developed that *Ips* bark beetles utilize the host tree's preformed monoterpene myrcene as a precursor that is simply hydroxylated in order to minimize pheromone (ipsenol and ipsdienol) biosynthetic costs. In 1990, however, amounts of ipsenol and ipsdienol produced by male *I. paraconfusus* feeding in five host pine species were found to be very similar, even though GC-MS showed that there was no detectable myrcene precursor in one of the species of pine (*Pinus sabiniana*). Subsequent research showed that ipsenol and ipsdienol are biosynthesized from smaller precursors such as acetate and mevalonate, and this *de novo* pathway is the major one, while host tree myrcene conversion by the beetle is the minor one. Concentrations of myrcene, α-pinene, and other major monoterpenes in five pine hosts (*Pinus ponderosa*, *P. lambertiana*, *P. jeffreyi*, *P. sabiniana*, and *P. contorta*) of *I. paraconfusus* are reported and a scheme for biosynthesis of ipsdienol and ipsenol from myrcene and possible metabolites such as ipsenone is presented. Coevolution of bark beetles and host trees is discussed in relation to pheromone biosynthesis, host plant selection/suitability, and plant resistance.

The issue deals with theoretical topics regarding evolutionary selection for host tolerance and choice in the paper by J. A. Byers "*A population genetic model of evolution of host-mate attraction and nonhost repulsion in a bark beetle Pityogenes bidentatus*." Studies have shown that the bark beetle *Pityogenes bidentatus* avoids volatiles of nonhost trees (Norway spruce, birch, and oak) and healthy host Scotch pine when orienting to aggregation pheromone. A population genetic model of two behavioral genes was hypothesized where AA, Aa, and aa were allele combinations regulating orientation to host tree and pheromone odors, and BB, Bb, and bb were combinations allowing avoidance of nonhost and unsuitable host odors. The nine possible genotypes were assigned different survival factors that remained constant during simulation. The initial proportion of aabb genotype (little aggregation/host response and little avoidance of nonhosts) was $\sim$1.0 when a mutation was hypothesized that caused better orientation to host/beetle odors (Aabb) and another mutation causing more efficient avoidance of nonhosts (aaBb). After these initial mutations, the model used indiscriminate mating of genotypic proportions and subsequent survival as input for each successive generation. The results indicate that AABB eventually fixates in the populations in some scenarios, while AABB and other genotypes reach stable equilibriums in other models depending on genotypic survival values supported by ecologically sound assumptions. The models indicate how the development of insecticide resistance in pest insects as well as host selection and tolerance may proceed.

M. N. Andersson in a comprehensive review "*Mechanisms of odor coding in coniferous bark beetles: from neuron to behavior and application*" discusses how coniferous bark beetles locate their hosts by means of olfactory signals, such as pheromones, and host- and non-host compounds. Behavioral responses to these volatiles are well known, but apart from the olfactory receptor neurons (ORNs) detecting pheromones, information on the peripheral olfactory physiology has been limited until recently. Several new classes of ORNs for the spruce bark beetle, *Ips typographus*, have been described and the odor encoding mechanisms and links between behavioral responses and ORNs responses were investigated, allowing for a more profound understanding of bark beetle olfaction. This paper reviews the physiology of bark beetle ORNs and their relevance in a behavioral context focusing on *I. typographus*.

While the articles are limited in number they do address a wide variety of topics regarding bark beetle interactions directly with the host tree as well as with other colonizing bark beetles in the host tree or during the process of finding hosts and avoiding nonhost plants and bark areas with severe intra- and interspecific competition and thus maximize fitness.

John A. Byers

Hindawi Publishing Corporation
Psyche
Volume 2012, Article ID 268621, 10 pages
doi:10.1155/2012/268621

Review Article

Semiochemical Diversity in Practice: Antiattractant Semiochemicals Reduce Bark Beetle Attacks on Standing Trees—A First Meta-Analysis

Fredrik Schlyter

Division of Chemical Ecology, Department of Plant Protection Biology, Swedish University of Agricultural Sciences (SLU), 230 53 Alnarp, Sweden

Correspondence should be addressed to Fredrik Schlyter, fredrik.schlyter@slu.se

Received 15 January 2012; Revised 16 April 2012; Accepted 17 April 2012

Academic Editor: John A. Byers

Reduction of tree mortality caused by bark beetle attacks is not only important for forestry, but also essential for the preservation of biodiversity and forest carbon sinks in the face of climate change. While bark beetle mass trapping (a "pull" approach) is implemented in practice, few studies exist to estimate its effect. The more complex "push-pull" tactic has, in contrast, been repeatedly tested during the last decade. I analysed published data from 32 experiments in 9 papers published during 2000–2011 on *Ips typographus* and *Dendroctonus ponderosae*, to test if there was an overall effect of antiattractant semiochemicals, that is, if treatments reduced the number of attacks on standing trees at the habitat or stand scale. This meta-analysis showed a substantial overall effect size (treatment-control means divided by their SD) of −0.96, with some heterogeneity but little evidence of publication bias. There was no effect of beetle species or publication year. Heterogeneity resulted from different designs and beetle population levels (as year of study). The conventional "% Reduction" measure correlated well with effect size ($r^2 = 0.7$). Recommendations include more precise reporting of responses (avoiding dichotomous data), more unified experimental designs, and further meta-analyses that include "grey literature" and more beetle species.

1. Introduction

Strategies to reduce tree mortality caused by bark beetle attacks [1–5] are becoming urgent, not only for forest industry, but also for preservation of forest cover and forest carbon sinks in the face of climate change [6–9]. There is a need for quantitative reviews of management alternatives such as mass-trapping and push-pull [10]. While bark beetle mass trapping (a "pull" tactic) is partly implemented in practise, few replicated studies exist to estimate the efficacy of this approach [1–4]. The more complex "push-pull" (as defined by Cook et al. [11]) and "push" (as defined by Gillette and Munson [12]) tactics have, in contrast, been experimentally tested and reported in >10 papers in the last decade, so a review effort on the subject seems to be timely.

I chose the quantitative method of meta-analysis that uses the descriptive data obtained (means, standard deviations, sample sizes), rather than relying on the *P* values and other analytical statistics which are heavily dependent on sample size when declaring an experiment "significant" or not. Meta-analysis is the method of choice for estimating interventions in complex systems such a clinical medicine, social work, and education [13–16], but also in resolving complex ecological issues [17–20], though less often in applied ecology [10, 21]. In principle, a meta-analysis describes the effect of a treatment among controlled experimental studies by analysing the distribution of effect sizes of the relevant studies [10, 22, 23]. The effect size measure in simple terms is the difference between control and treatment, gauged by the size of their standard deviations, which provides a common scale for the magnitude of effects for the experiments performed [24, 25]. A common "currency" such as the effect size is mandatory for any comparisons between experiments of different designs, sample sizes, and so forth, as measures dependent on sample size like *P* values cannot

be used. Effect sizes can be calculated in several ways; I use the simplest possible often referred to as Cohen's d [24]:

$$d = \frac{\text{Mean}_{\text{Control}} - \text{Mean}_{\text{Treatment}}}{\text{SD}_{\text{Pooled}}}, \quad (1)$$

where d above is (1) in [24] and $\text{SD}_{\text{Pooled}}$ is (2) in [24] which takes in account different sample sizes for control and treatment samples.

This meta-analytical review is limited to studies of standing trees at the plot or stand scale, with just two of the recently best studied bark beetle species, *Ips typographus* and *Dendroctonus ponderosae*, and published from 2000 and onwards in readily available international journals (thus excluding government reports, trade journals, and other so called "grey literature"). I keep to (1) only newer studies in order to concentrate on the latest development of semiochemicals representing "state of the art" and (2) stand or plot level tests, because they are the only type likely to give information for future practical applications. Tree scale experiments are nowadays essentially a thing of the past and do not provide essential information for forest management. The intended coverage of the literature is not the most comprehensive possible, but is clearly stated and thus open to future challenges.

In this first meta-analysis I hope to address the key question of whether there is any overall effect in reducing or stopping bark beetle attacks on standing trees by antiattractants. Distribution of effect sizes will be scrutinised for evidence of publication bias and heterogeneity. The latter will be studied for meaningful variation among results that may shed light on factors giving low or high effects of semiochemical interventions against beetle attacks.

The intended audience for this paper is not primarily those familiar with the workings of meta-analysis and its application to yet another field, but the colleagues involved in designing and performing field experiments with antiattractants against bark beetle attack on the stand or habitat scale and the forest managers interested in application of such tactics in forestry.

2. Materials and Methods

I have tried to adhere to the standards suggested by PRISMA and Cochrane collaborations [13, 15, 25–28], while acknowledging that these standards are primarily oriented to well-controlled clinical or laboratory studies. The somewhat less rigorous standards of ecological meta-analysis [10, 18, 19] must be used here, in particular to arrive at a sufficient number of experiments to allowing meaningful number for plots and tests for publication bias and sensitivity analysis [29, 30].

Studies were searched using references in published papers, including a review-like paper in the "grey literature" by Gillette and Munson 2009 [12], and "back-tracking" from these by consulting papers citing these first known papers in Google Scholar. This "centrifugal" multidimensional strategy was iterated by expanding to citing papers, and so forth, until no new relevant papers were discovered.

In some cases reports in noninternational sources were difficult to locate and in addition were sometimes partly overlapping with papers later published in international journals. Therefore, I choose to eliminate any studies that were not published in international journals.

A vast majority of recent papers located concerned either the mountain pine beetle of North America, *Dendroctonus ponderosae*, mostly on ponderosa and lodgepole pine (*Pinus ponderosae, P. contorta*) or the European spruce engraver, *Ips typographus*, on Norway spruce (*Picea abies*). Thus, I decided to concentrate only on these two species, while ignoring the earlier published works on the Southern pine beetle (*D. frontalis*) and the Western pine beetle (*D. frontalis*) and a few other spp with one paper each, in order to limit the overall size and heterogeneity of the material.

Numerical data sets were extracted from papers of various designs, by means and SD or frequencies when provided but sometimes from raw or nearly raw data depending on the presentation and were further summarised in MS Excel to avoid pseudoreplication [31–33]. Care was taken to ascertain the true number of replicates but to allow the different data sets to represent cases depending on variation in design, treatment, or time periods (season, year). In bark beetle population dynamics the variation over time and space is overwhelming and I could conclude after reading the experimental papers that multiple experimental outcomes (data points) are in most studies not autocorrelated. I did my best to avoid autocorrelated data, like from time series within a year as in Jakuš et al. [34] by using it as only one data point, or avoided pseudoreplications within experiments by pooling of data [35, 36].

Further analysis and plotting were done by SPSS 19 software package using command syntax (command files available on request) following suggested algorithms [23, 37, 38]. For a meta-analysis, an effect size for each study or experiment must be extracted from the central and secondary moments (means and a measure of variance, respectively) of the distributions of the treatment and control data (details on the data available are given in Table 1). Depending on the reporting and study design, data were often continuous (trees killed or attacked per plot or area, attack density etc.) allowing simple extraction of means and SDs [24, 25], but are sometime dichotomous (data from outcomes that can be divided into two categories), given as total trees killed/attacked versus alive/unattacked per treatment and experiment [39, 40]. In the latter case, a simple transformation of the ln (odds ratio) to Cohen's d is possible by $d = \ln(\text{OR})/(\pi/\sqrt{3})$ [41] which then allows a comparison of studies with both types of data. This transformation made it possible to use the Cohen's d also for the dichotomous data.

A random effect model was used, as there was no reason to assume that all studies were functionally identical, as in a fixed model where only a single effect plus error is estimated [38]. Instead the random model includes also variation due to heterogeneity, like different species and phases of the population dynamics were likely to influence results [37, 38] which lowers the magnitude of the estimated overall effect size (θ) and widens its associated confidence interval. Bootstrap re-sampling was not used.

Table 1: Input data for meta-analysis with basic effect size and standard error estimates and weights for individual studies.

Expt nr	Author	Year Publ	Year Expt	Location[1]	Beetle species	Treatment[2]	Measure	Dicho-tomous	*n* Ctrl	Effect size (Cohen's *d*)	Standard error of *d*	Inverse SE	*Z* score of *d*	Standardised residual of *d*	Weighted *d* (by Inv. SE)	% Reduction
1	Borden	2006	03	BC	*D. ponderosae*	Vn 100 u/ha	% mass att.		10	**−3.30**	0.73	1.38	**−2.80**	**−3.17**	**−4.54**	85%
2	Borden	2006	03	BC	*D. ponderosae*	Vn + NHV 100 u/ha	% mass att.		10	**−2.90**	0.68	1.48	**−2.30**	**−2.80**	**−4.28**	83%
3	Jakuš	2011	08	SK	*I. typographus*	Vn + NHV	Freq tree kill		5	**−1.81**	0.85	1.17	**−0.99**	**−0.96**	**−2.13**	73%
4	Borden	2006	03	BC	*D. ponderosae*	NHV 100 u/ha	% mass att.		10	**−1.79**	0.56	1.79	**−0.96**	**−1.41**	**−3.20**	59%
5	Progar	2005	02	ID	*D. ponderosae*	Vn	% kill/Suit.		7	**−1.68**	0.68	1.48	**−0.83**	**−1.01**	**−2.49**	71%
6	Borden	2007	05	BC	*D. ponderosae*	Vn + tree rem.	Sum trees	Yes	4	**−1.59**	0.11	9.33	**−0.72**	**−5.50**	**−14.82**	85%
7	Progar	2005	00	ID	*D. ponderosae*	Vn	% kill/Suit.		7	**−1.54**	0.66	1.51	**−0.66**	**−0.82**	**−2.33**	83%
8	Jakus	2003	00	PL	*I. typographus*	Šumava01	Sum trees	Yes		**−1.45**	0.81	1.23	**−0.55**	**−0.55**	**−1.78**	92%
9	Bentz	2005	01–03	ID, MT	*D. ponderosae*	Vn "High"	Att. trees/plo		38	**−1.35**	0.26	3.88	**−0.43**	**−1.36**	**−5.24**	78%
10	Progar	2005	01	ID	*D. ponderosae*	Vn	% kill/Suit.		7	**−1.35**	0.64	1.55	**−0.42**	**−0.54**	**−2.09**	68%
11	Gillette	2009	05	CA	*D. ponderosae*	Vn arial appl.	Att. trees/ha		5	**−1.27**	0.76	1.27	**−0.33**	**−0.34**	**−1.61**	69%
12	Jakus	2003	01	CZ	*I. typographus*	Šumava03	Sum trees	Yes		**−1.16**	0.28	3.63	**−0.19**	**−0.58**	**−4.20**	84%
13	Jakus	2003	00	CZ	*I. typographus*	Šumava02	Sum trees	Yes		**−1.02**	0.60	1.66	**−0.02**	**−0.03**	**−1.69**	83%
14	Borden	2003	01	BC	*D. ponderosae*	High Vn	Att. trees/plot		10	**−0.94**	0.50	2.01	0.07	0.12	**−1.89**	57%
15	Gillette	2009	05	ID	*D. ponderosae*	Vn arial appl.	Att. trees/ha		5	**−0.94**	0.76	1.32	0.07	0.08	**−1.24**	65%
16	Borden	2006	03	BC	*D. ponderosae*	Vn + NHV 400 u/ha	Freq tree kill		10	**−0.93**	0.50	2.01	0.09	0.15	**−1.86**	59%
17	Schiebe	2011	06	SK	*I. typographus*	Vn + NHV × 4	Prop tree kill		9	**−0.88**	0.67	1.50	0.14	0.18	**−1.32**	56%
18	Borden	2006	03	BC	*D. ponderosae*	Vn + NHV 100 u/ha	Freq tree kill		10	**−0.87**	0.49	2.02	0.15	0.25	**−1.77**	51%
19	Borden	2006	03	BC	*D. ponderosae*	Vn + NHV 178 u/ha	Freq tree kill		10	**−0.85**	0.49	2.03	0.18	0.30	**−1.73**	55%
20	Schiebe	2011	07	SK	*I. typographus*	Vn + NHV × 2	Prop tree kill		9	**−0.82**	0.66	1.51	0.22	0.28	**−1.23**	49%
21	Schiebe	2011	07	SK	*I. typographus*	Vn + NHV × 4	Prop tree kill		9	**−0.76**	0.66	1.51	0.29	0.36	**−1.15**	63%
22	Borden	2007	05	BC	*D. ponderosae*	Vn	Sum trees	Yes	3	**−0.66**	0.13	7.52	0.41	2.54	**−4.96**	55%
23	Jakuš	2003	00	CZ	*I. typographus*	Šumava01	Sum trees	Yes		**−0.65**	0.17	5.94	0.42	2.07	**−3.86**	52%
24	Schiebe	2011	06	SK	*I. typographus*	Vn + NHV × 1	Prop tree kill		9	**−0.63**	0.65	1.53	0.45	0.56	**−0.97**	34%
25	Borden	2003	01	BC	*D. ponderosae*	High Vn + NHV	Att. trees/plot		10	**−0.44**	0.48	2.09	0.68	1.16	**−0.92**	23%
26	Borden	2003	01	BC	*D. ponderosae*	Low Vn	Att. trees/plot		10	**−0.40**	0.48	2.09	0.72	1.25	**−0.84**	23%
27	Borden	2006	03	BC	*D. ponderosae*	Vn + NHV 114 u/ha	Freq mass att.		10	**−0.39**	0.48	2.10	0.74	1.27	**−0.82**	38%
28	Schiebe	2011	06	SK	*I. typographus*	Vn + NHV × 2	Prop tree kill		9	**−0.32**	0.64	1.56	0.82	1.05	**−0.50**	40%
29	Schiebe	2011	07	SK	*I. typographus*	Vn + NHV × 1	Prop tree kill		9	**−0.27**	0.64	1.56	0.88	1.13	**−0.43**	47%
30	Borden	2003	01	BC	*D. ponderosae*	Low Vn + NHV	Att. trees/plot		10	**−0.27**	0.47	2.11	0.89	1.53	**−0.57**	18%
31	Progar	2005	04	ID	*D. ponderosae*	Vn	% kill/Suit.		7	0.02	0.58	1.72	1.23	1.74	0.03	**−2**%
32	Progar	2005	03	ID	*D. ponderosae*	Vn	% kill/Suit.		7	1.26	0.64	1.57	2.74	3.55	1.97	**−396**%

[1] BC: British Columbia, Canada, SK: Slovak Republic, ID: Idaho, USA, MT: Montana, USA, CA: California, USA, CZ: Czech Republic.
[2] Vn: verbenone, NHV: nonhost volatiles (combination of green leaf volatiles and bark compounds), Šumava01–Šumava03: dispensers with Vn + NHV.

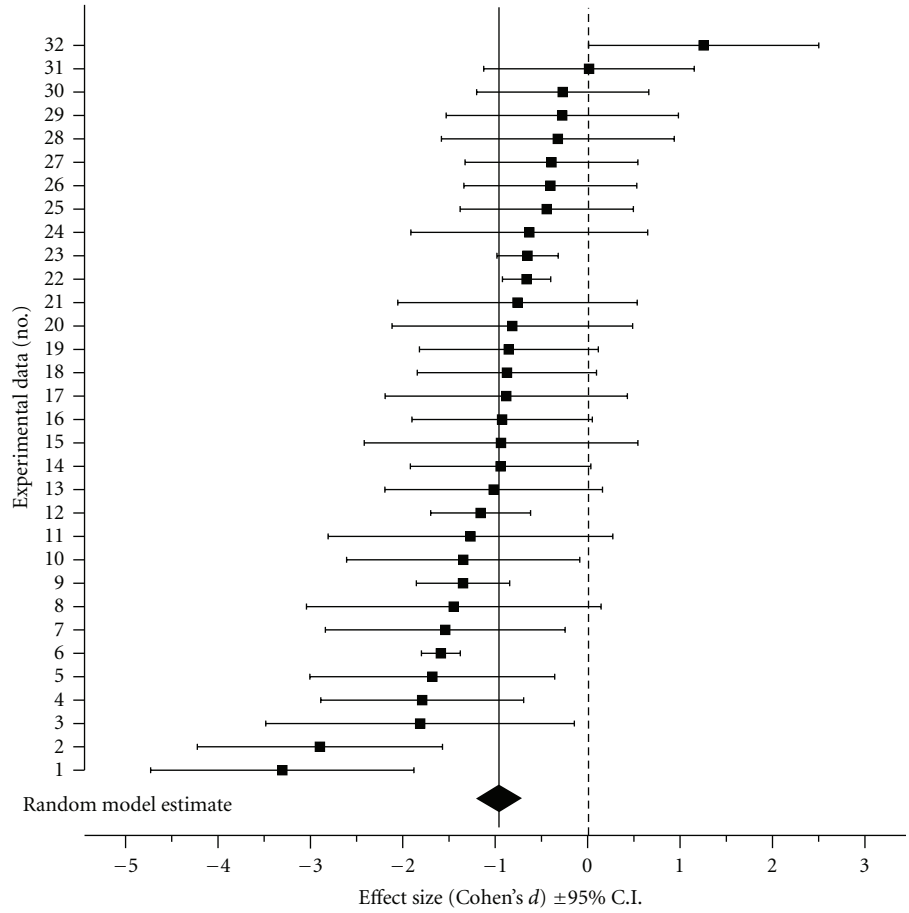


Figure 1: Ordered plot of individual study effect sizes and their ±95% confidence intervals; known as a "forest plot". Vertical lines: — estimated overall effect size (θ), - - - effect size = 0 or no effect. The "diamond" on last row shows the midpoint and width of the 95% CI of grand mean effect size by its location and width, based on a random model estimate [37, 38]. Numbers on the dependent axis correspond to the "Expt nr" in Table 1.

Heterogeneity in recorded effect size distributions may stem from several sources, including true biological variation as well as variation in study designs. Overall for the meta-analysis I followed the results and recommendations of Bax et al. [29] for use of more readily understandable plots for the applied ecologist in the assessment of reporting bias and heterogeneity, rather than the more arcane statistics of Q_T, I^2, τ^2, and so forth, [13, 22, 30, 42, 43]. Sensitivity plots and subgroup analysis as well as attempts of metaregression, based on data of different quality, origin, and treatment levels, respectively, were included to explore heterogeneity and deviations from normality [13].

An important consideration for any review is the "file drawer problem" where a publication bias usually exists against the publication of negative outcomes with low effects. A notable exception is the combination of failures and successes by Jakuš et al. [40]. The subject of this paper, in particular may suffer from this problem, as applied experiments with unclear or negative results may often either not be submitted for publication (stays in the file drawer) or get hidden in the "grey literature" of various internal reports, trade journals, and so forth. Fortunately, there exists graphical tools such as "funnel plots" to aid in detection of such publication biases [29].

3. Results

3.1. Effect Sizes. The analysis included 32 data points from 9 papers [35, 36, 39, 40, 44–48], where several papers reported experiments from different years or seasons and/or different semiochemical blends [36, 40, 47, 48] which were considered independent studies (Table 1). Overall, the effect sizes fell below zero, meaning that there was a lower density or number of attacks on trees in the treated plots, with only one exception (Figure 1). The effect size confidence intervals for individual studies, however, included zero for all but 12 cases (Figure 1). The overall estimate of effect was $\theta = -0.96 \pm 0.25$ (the midpoint and ± the 95% CI of grand mean effect size based on a random model, $\mathrm{SE}\{\theta\} = 0.126$). There was a narrow confidence interval for the grand mean compared to those of the individual studies (Figure 1, lowest point, "diamond"). The effect sizes followed in general a smooth

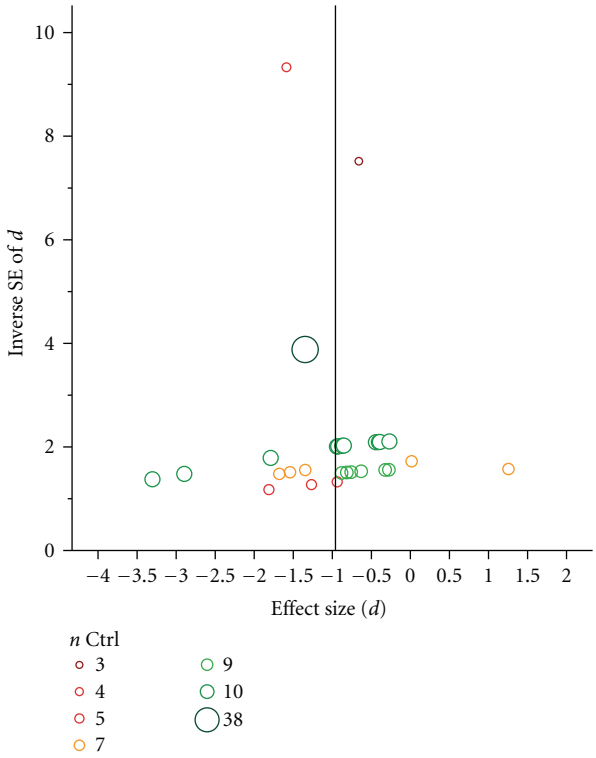


FIGURE 2: Funnel plot for dissemination or publication bias detection. — estimated overall mean effect size. Points distributed symmetrically around the mean effect size indicate little evidence of dissemination bias [29].

progression when ordered after size from low to high, while one study stood out with a reversed ($d > 0$) effect and two with large values ($|d| > 2$) (Figure 1).

3.2. Sensitivity Analysis

3.2.1. Publication Bias. This kind of field research, with highly variable forest conditions and bark beetle populations, and many factors outside the control of authors, might result in "failed studies" (negative or unclear results) that tend to remain unreported. An example to the contrary is the study of Jakuš et al. [40], reporting a string of "failures" before successes. Unfortunately, several of these "failures" could not be included here because they incorporated felled trees or logs, or did not provide sufficient details for estimation of d values. Still, the "funnel plot" (Figure 2) is reasonably symmetrical. If a strong bias existed against publication of unsuccessful studies, this plot would give very few points to the right of the mean d estimate of ≈ -1 and no points at or above $d = 0$. Interestingly, "good" studies with high n values or high inverse SE_d values do not have a clear bias to the left of $d \approx -1$, but have a rather symmetrical distribution (Figure 2).

There was no effect whatsoever of publication year on effect size ($R^2 = 0.002$).

3.2.2. Heterogeneity. The plots in Figure 3 indicate an overall moderate heterogeneity [29]. In Figure 3(a), the box plot of d values weighted by their inverse SE, there is one extreme value (from [39]) and one outlier (from [47]). The median d (middle line of box) falls lower than the mean d. The histogram of standardized residuals, Figure 3(b), shows no clear deviations from the normal distribution overlay (indicating a normal sample distribution) or any clumping of values indicating possible subpopulations (indicating little heterogeneity in the sample). Similarly, the normal quantile plots indicate a normal distribution (points close to a straight line) and little heterogeneity (no clustering of points).

Similarly, there was no variation between the effect sizes based on studies providing continuous data ($n = 26$) or dichotomous data ($n = 6$) in effect of antiattractants (continuous data d, mean ± SE [median] = -0.98 ± 0.18 [-0.88], dichotomous ditto = -1.09 ± 0.16 [-1.09]; $F_{1,31} = 0.08$, $P \gg 10\%$).

3.3. Subgroup Analysis.

There was little variation between the two species in overall effect of antiattractants (*Ips* mean ± SE [median] = -0.89 ± 0.14 (-0.82), *Dendroctonus* ditto = -1.06 ± 0.21 [-0.94]; $F_{1,31} = 0.3$, $P \gg 10\%$).

From a practical point it would be of considerable interest to know if verbenone alone is as efficient as a blend of verbenone combined with sometimes more expensive nonhost volatiles (NHV); however, the studies are not balanced in this respect. All *Ips* studies included an NHV blend plus verbenone, as the combination is known to be clearly needed [5, 40], but for *Dendroctonus* only one study included an NHV blend [36].

3.4. Meta-Regression

3.4.1. Year of Study. There was a relatively strong effect of year since the start of study (ANOVA, full corrected model: $F_{6,9} = 11.0$, $P = 3.8\%$, $\eta^2 = 0.96$) which most likely corresponds to an effect of beetle population variation between years (factor year-of-experiment: $F_{4,9} = 16.0$, $P = 2.3\%$, $\eta^2 = 0.96$). However, there was also some variation due to factor species ($F_{1,9} = 5.4$, $P = 10.2\%$, $\eta^2 = 0.64$) but there was no interaction due to year-of- experiment × species ($F_{1,9} = 0.1$, $P \gg 10\%$, $\eta^2 = 0.24$).

3.4.2. Comparison to the Conventional "% Reduction" Measure. The conventional measure of inhibitory effect, "% Reduction":

$$\%\ \text{Reduction} = \frac{\text{Attacks (kills) in control group} - \text{Attacks (kills) in treatment group}}{\text{Attacks (kills) in control group} \cdot 100} \quad (2)$$

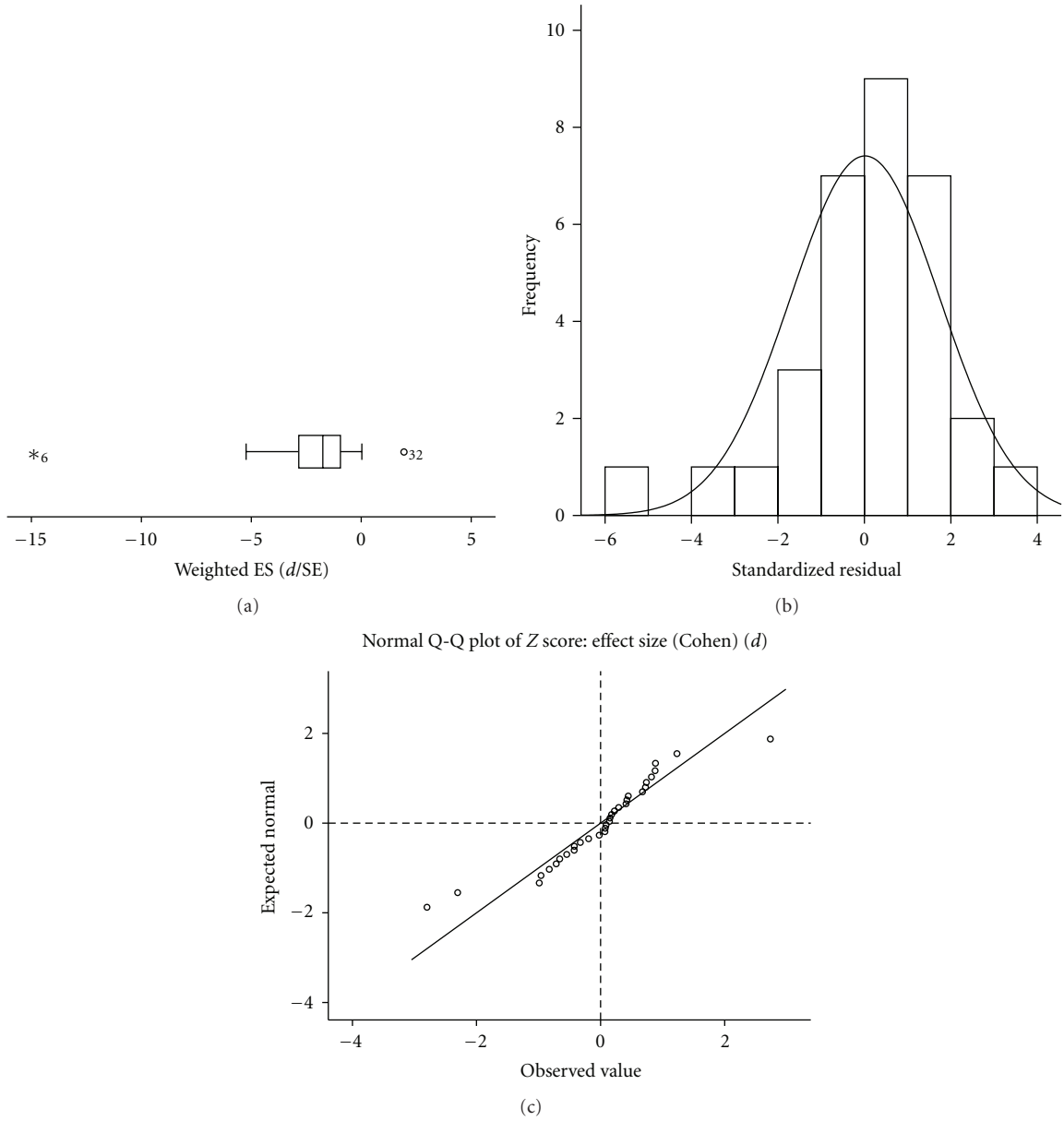


Figure 3: Plots to check normality and heterogeneity of effect size in the whole data set ($n = 32$) [29]. (a) Weighted box plot (data points used weighted by division of SE, vertical line is median, box enclose 50% of observations, whiskers 2 SD or 95% of observations, "o" are outliers not enclosed by whiskers, and "$*$" extremes), where asymmetry indicates heterogeneity and deviations from normality. Numbers near points correspond to the "Expt nr" in Table 1. (b) Histogram of residuals (data-grand mean)/SD allowing check of normality. (c) Normal Quantile Plot of Z-scores (normalised data by subtraction of mean and division by SD), allowing check of normality and of outliers contributing to heterogeneity.

is used in papers on reduction of trap catches, attack densities, or tree kills and is common in applied entomology. However, the stated "% reduction" probably has a wide SE (as it includes the subtraction of two variables both estimated with uncertainty, and then divided by one of them), but I have seen no attempts to quantify this uncertainty. Thus, there is a strong incentive to get a relation to the effect size and its well-defined uncertainty. "% Reduction" was regressed upon effect size and corresponded, as expected, overall rather well ($R^2 = 0.68$) with effect size (Figure 4(a)), but only when omitting the three points that were >2 SDs away from the mean (the three deviates from line in Figure 3(c)). There was little variation between the slopes or the strength of correlation (R^2) based on studies providing continuous data or dichotomous data (Figure 4(a)). The *Ips* data points, which had a smaller spread along the independent axis (x-axis), had a weaker correspondence ($R^2 = 0.58$) than those for *Dendroctonus* ($R^2 = 0.79$) (Figure 4(b)).

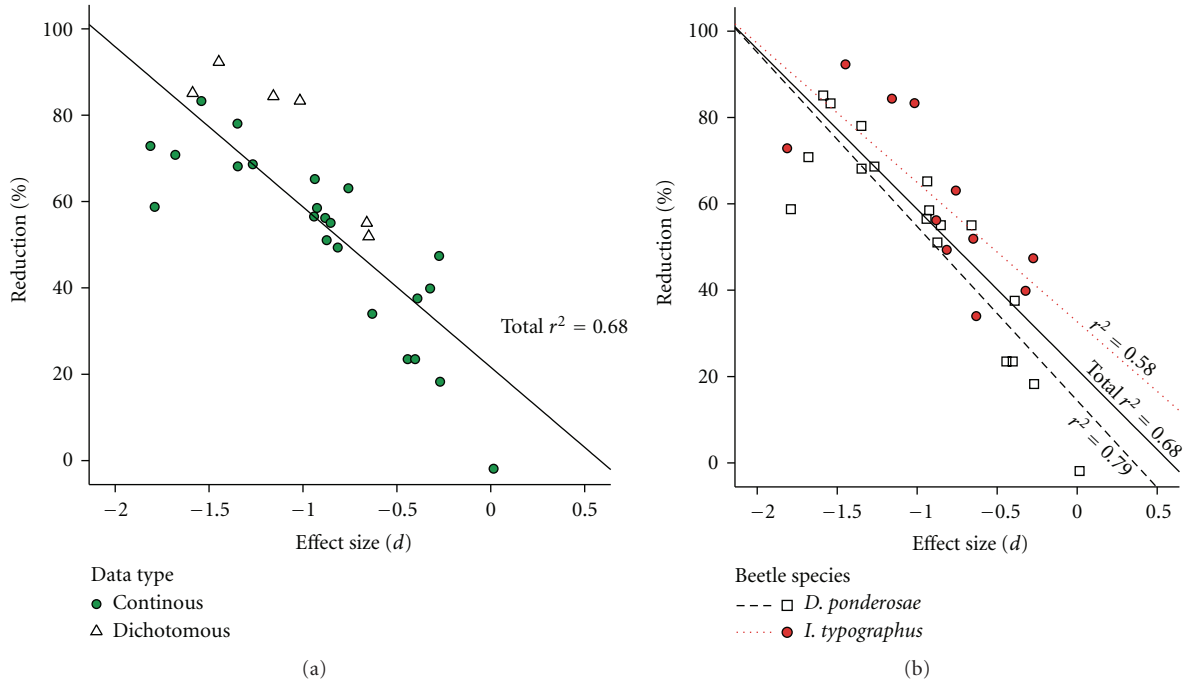


Figure 4: Correspondence between effect size and a standard measure of effect magnitude in applied entomology "% Reduction" ([attacked in control − attacked in treatment/attacked in control] ∗ 100) for (a) different data types and (b) the two different species (*Ips*/*Dendroctonus*).

4. Discussion

In applied ecological applications such as forest entomology, critical reviews in the form of meta-analysis are not widespread [10] but see [7, 20, 21]. Sometimes titles of reviews may imply similarity in scope but, due to criteria set for included studies, have little overlap, as in [42], which covers semiochemical manipulation of pest insects but not insect pest management *per se*. Interestingly, the paper by Szendrei and Rodriguez-Saona [42] does not show a single experimental paper in common with the present review.

In principle, a meta-analysis describes the effect of a treatment among controlled experimental studies by analysing the distribution of effect sizes of the relevant studies [10, 22, 23]. By using results of many studies, it may allow detection of effects not easy to confirm by single studies [49].

The literature was screened by a "centrifugal" multidimensional strategy using citations to some core papers and then iterated by expanding to citing papers, and so forth, until no new papers turned up. This manual approach, based on my knowledge of the field, and the omission of a traditional one-dimensional database search step was justified by the finding that a single data base search may locate only 1/3 of relevant studies [50]. Back-tracking might have created an "inbreeding effect" of focus on papers by a possible citing bias, but I found no clear such indication and would leave this problem for a future discussion based on more studies.

Heterogeneity in effect size may come from several sources, including both true biological variation such as beetle population size and tree vigour in the field, as well as variation in study design and treatment levels. Some papers present details of "different" experiments that are more of replications of the same design rather than different experiments, while others join or would allow joining of data of similar experiments conducted in different years under different conditions [47, 48]. A clear example is that of Progar [47] who clearly showed a reasonable variation in effect over the years of an outbreak, where in the beginning treated plots were protected from attacks by an escalating beetle population, but later became the victims of being the only remnants of mature trees left in the landscape, suitable to be attacked by the beetles at the peak of the outbreak [51].

An important consideration for any metastudy is the "file drawer problem" where a publication bias usually exists against the publication of negative outcomes with low effects. A notable exception is Jakuš et al. [40]. For the current analysis in particular, this aspect is an important consideration as the "grey literature" is not covered, which may cover some less successful experiments. The analysis of effect size distribution in this meta-analysis did, however, not detect any clear pattern of publication or dissemination bias.

The development of complete anti-attractant (verbenone) technology for the Southern Pine Beetle *Dendroctonus frontalis* was successfully finished in 1990s. The evaluation of treatments was based on growth rates of infestations. Clarke et al. [52] has shown that verbenone-only tactic completely suppressed 69% of infestation rate and verbenone-plus-felling tactic suppressed 86%, corresponding to an effect size of $|d| > 1$ and >1.5, respectively, based on the regression in this paper (Figure 4). If one may rely on the regression of % Reduction on effect size in this paper and on these

higher numbers for *D. frontalis* as representative, it is likely that the development of "push and pull" technology for *I. typographus* and *D. ponderosae* has some promise for future development. There is in my view a need for more complex anti-attractant mixture for both *I. typographus* and *D. ponderosae* (push) and for combinations with pheromone traps (pull) [34, 36, 40, 53–55]. It could be interesting to note that an effect size grand mean estimate in this paper $\theta \approx -1$ corresponds to a reduction of attacks of about 60%, a value found already in 2003 by Jakuš et al. [40] on data from 2000 and 2001 for *Ips*.

Patterns and mechanisms of the response by the tree to beetle attack are well researched in *Dendroctonus* [51, 56] and information is now becoming available for *Ips* [57, 58]. Contrarily, beetle response to signals from tree or beetle is in recent years better covered in *Ips*, especially at the antennal and single sensillum level of peripheral detection [59–62].

A deeper understanding of the olfactory interface with the environment for *D. ponderosae* will hopefully emerge from on-going study of the recently published transcriptomes [63, 64] and genome (Keeling et al., unpublished) and the transcriptome of antenna compared for the two species in progress (Andersson et al., unpublished). The present stage of analysis indicates a number of closely related pairs of antennal olfactory receptor genes (*OR*) that are not found in the other genomic coleopteran *Tribolium* [60]. Such understanding will in the future help in the study and manipulation of beetle olfaction and resulting behaviours.

This meta-analysis sets the basis that will further the development of antiattractants by quantitatively establishing a substantial overall effect of such interventions in the two species recently most studied. Such confirmed efficacy of antiattractants against attacks by these two "aggressive" bark beetles might encourage better designed and larger-scale applied studies on these and other economically important conifer bark beetle species in the future. The semiochemical-based "push-pull" tactic has played and will continue to play an important role in the pest management practices in agricultural, forestry, medical and urban settings [11]. Future meta-analysis and experimental studies on the subject would include more new study data points (more species) and "grey literature", but also better reporting (clear provision of means and SDs [65], avoiding dichotomous data) of experimental responses and more of novel designs. Would it, for instance, be possible to use lower densities of dispensers, but with higher release rates as indicated by the distance effects noted in one study [48]?

When anti-attractant technology and treatment designs once comes of age, we will still have to keep in mind that we are not "protecting trees" in reality, but rather helping them to protect themselves. If we design forest landscapes for biodiversity and semiochemical diversity [11, 20, 66], we will in the long run help trees even better.

Acknowledgments

For a constructive critique on language and statistics, respectively, the author thank two anonymous referees. For critical comments on earlier drafts he thank C. Schiebe, Q.-H. Zhang (Sterling International, Inc., Spokane, USA), and R. Jakuš (Inst. Forest Ecology, SAV, Zvolen, Slovak Republic). For analysis, support was provided by Department of Plant Protection Biology, SLU and by Linnaeus-program "Insect Chemical Ecology, Ethology, and Evolution" (ICE^3).

References

[1] L. Dimitri, U. Gebauer, R. Lösekrug, and O. Vaupel, "Influence of mass trapping on the population dynamic and damage-effect of bark beetles," *Journal of Applied Entomology*, vol. 114, no. 1–5, pp. 103–109, 1992.

[2] M. Faccoli and F. Stergulc, "Damage reduction and performance of mass trapping devices for forest protection against the spruce bark beetle, *Ips typographus* (Coleoptera Curculionidae Scolytinae)," *Annals of Forest Science*, vol. 65, no. 309, pp. 1–9, 2008.

[3] J.-C. Grégoire and H. F. Evans, "Damage and control of Bawbilt organisms—an overview," in *Bark and Wood Boring Insects in Living Trees in Europe, A Synthesis*, F. Lieutier, K. R. Day, A. Battisti, J.-C. Grégoire, and H. F. Evans, Eds., pp. 19–37, Springer, Amsterdam, The Netherlands, 2004.

[4] B. Wermelinger, "Ecology and management of the spruce bark beetle *Ips typographus*—a review of recent research," *Forest Ecology and Management*, vol. 202, no. 1–3, pp. 67–82, 2004.

[5] Q. H. Zhang and F. Schlyter, "Olfactory recognition and behavioural avoidance of angiosperm nonhost volatiles by conifer-inhabiting bark beetles," *Agricultural and Forest Entomology*, vol. 6, no. 1, pp. 1–19, 2004.

[6] B. J. Bentz, J. Rgnire, C. J. Fettig et al., "Climate change and bark beetles of the western United States and Canada: direct and indirect effects," *BioScience*, vol. 60, no. 8, pp. 602–613, 2010.

[7] H. Jactel, J. Petit, M.-L. Desprez-Loustau et al., "Drought effects on damage by forest insects and pathogens: a meta-analysis," *Global Change Biology*, vol. 18, no. 1, pp. 267–276, 2012.

[8] P. Schlyter, I. Stjernquist, L. Bärring, A. M. Jönsson, and C. Nilsson, "Assessment of the impacts of climate change and weather extremes on boreal forests in northern Europe, focusing on Norway spruce," *Climate Research*, vol. 31, no. 1, pp. 75–84, 2006.

[9] F. Schlyter and J. Witzell, "Forests: carbon storages in peril," *Public Service Review*, vol. 3, pp. 136–137, 2009.

[10] G. Stewart, "Meta-analysis in applied ecology," *Biology Letters*, vol. 6, no. 1, pp. 78–81, 2010.

[11] S. M. Cook, Z. R. Khan, and J. A. Pickett, "The use of push-pull strategies in integrated pest management," *Annual Review of Entomology*, vol. 52, pp. 375–400, 2007.

[12] N. E. Gillette and A. S. Munson, "Semiochemical sabotage: behavioral chemicals for protection of western conifers from bark beetles," in *The Western Bark Beetle Research Group: A Unique Collaboration With Forest Health Protection. General Technical Report PNW-GTR-784*, J. L. Hayes and J. E. Lundquist, Eds., pp. 85–127, United States Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, Ore, USA, 2009.

[13] M. J. Bown and A. J. Sutton, "Quality control in systematic reviews and meta-analyses," *European Journal of Vascular and Endovascular Surgery*, vol. 40, no. 5, pp. 669–677, 2010.

[14] G. V. Glass, B. McGaw, and M. L. Smith, *Meta-Analysis in Social Research*, vol. 56, Sage, Beverly Hills, Calif, USA, 1981.

[15] H. J. Schünemann, A. D. Oxman, G. E. Vist et al., "Interpreting results and drawing conclusions," in *Cochrane Handbook for Systematic Reviews of Interventions*, pp. 359–387, John Wiley and Sons, Chichester, UK, 2008.

[16] A. J. Sutton and J. P. T. Higgins, "Recent developments in meta-analysis," *Statistics in Medicine*, vol. 27, no. 5, pp. 625–650, 2008.

[17] R. Chaplin-Kramer, M. E. O'Rourke, E. J. Blitzer, and C. Kremen, "A meta-analysis of crop pest and natural enemy response to landscape complexity," *Ecology Letters*, vol. 14, no. 9, pp. 922–932, 2011.

[18] J. Gurevitch and L. V. Hedges, "Statistical issues in ecological meta-analyses," *Ecology*, vol. 80, no. 4, pp. 1142–1149, 1999.

[19] M. J. Lajeunesse, "Achieving synthesis with meta-analysis by combining and comparing all available studies," *Ecology*, vol. 91, no. 9, pp. 2561–2564, 2010.

[20] H. Jactel and E. G. Brockerhoff, "Tree diversity reduces herbivory by forest insects," *Ecology Letters*, vol. 10, no. 9, pp. 835–848, 2007.

[21] G. Shrivastava, M. Rogers, A. Wszelaki, D. R. Panthee, and F. Chen, "Plant volatiles-based insect pest management in organic farming," *Critical Reviews in Plant Sciences*, vol. 29, no. 2, pp. 123–133, 2010.

[22] T. D. Pigott, *Advances in Meta-Analysis*, Springer, New York, NY, USA, 2012.

[23] M. Borenstein, L. V. Hedges, J. P. T. Higgins, and H. R. Rothstein, *Introduction to Meta-Analysis*, John Wiley and Sons, Chichester, UK, 2009.

[24] S. Nakagawa and I. C. Cuthill, "Effect size, confidence interval and statistical significance: a practical guide for biologists," *Biological Reviews*, vol. 82, no. 4, pp. 591–605, 2007.

[25] J. J. Deeks, J. Higgins, D. G. Altman et al., "Analysing data and undertaking meta-analyses," in *Cochrane Handbook for Systematic Reviews of Interventions*, pp. 243–296, John Wiley and Sons, Chichester, UK, 2008.

[26] K. Thorlund, S. D. Walter, B. C. Johnston, T. A. Furukawa, and G. H. Guyatt, "Pooling health-related quality of life outcomes in meta-analysis—a tutorial and review of methods for enhancing interpretability," *Research Synthesis Methods*, vol. 2, no. 3, pp. 188–203, 2011.

[27] A. Liberati, D. G. Altman, J. Tetzlaff et al., "The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration," *Italian Journal of Public Health*, vol. 62, no. 10, pp. e1–e34, 2009.

[28] D. Moher, J. Tetzlaff, A. Liberati, and D. G. Altman, "The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration," *PLoS Medicine*, vol. 6, no. 7, Article ID e1000100, 2009.

[29] L. Bax, N. Ikeda, N. Fukui, Y. Yaju, H. Tsuruta, and K. G. M. Moons, "More than numbers: the power of graphs in meta-analysis," *American Journal of Epidemiology*, vol. 169, no. 2, pp. 249–255, 2009.

[30] W. Huf, K. Kalcher, G. Pail, M. E. Friedrich, P. Filzmoser, and S. Kasper, "Meta-analysis: fact or fiction? How to interpret meta-analyses," *World Journal of Biological Psychiatry*, vol. 12, no. 3, pp. 188–200, 2011.

[31] M. J. Ryan, "Replication in field biology: the case of the frog-eating bat," *Science*, vol. 334, no. 6060, pp. 1229–1230, 2011.

[32] S. H. Hurlbert, "The ancient black art and transdisciplinary extent of pseudoreplication," *Journal of Comparative Psychology*, vol. 123, no. 4, pp. 434–443, 2009.

[33] S. H. Hurlbert, "Pseudoreplication and the design of ecological field experiments," *Ecological Monographs*, vol. 54, no. 2, pp. 187–211, 1984.

[34] R. Jakuš, M. Blaženec, and O. Vojtěch, "Use of anti-attractants in specific conditions of protected areas," *Folia Oecologica*, vol. 38, no. 1, pp. 46–51, 2011.

[35] B. J. Bentz, S. Kegley, K. Gibson, and R. Thier, "A test of high-dose verbenone for stand-level protection of lodgepole and whitebark pine from mountain pine beetle (Coleoptera: Curculionidae: Scolytinae) attacks," *Journal of Economic Entomology*, vol. 98, no. 5, pp. 1614–1621, 2005.

[36] J. H. Borden, A. L. Birmingham, and J. S. Burleigh, "Evaluation of the push-pull tactic against the mountain pine beetle using verbenone and non-host volatiles in combination with pheromone-baited trees," *Forestry Chronicle*, vol. 82, no. 4, pp. 579–590, 2006.

[37] J. J. Deeks and J. P. T. Higgins, (2010, 2012-01-15). Statistical algorithms in Review Manager 5, http://ims.cochrane.org/revman/documentation/Statistical-methods-in-RevMan-5.pdf.

[38] M. Borenstein, L. V. Hedges, J. Higgins, and H. R. Rothstein, "A basic introduction to fixed-effect and random-effects models for meta-analysis," *Research Synthesis Methods*, vol. 1, no. 2, pp. 97–111, 2010.

[39] J. H. Borden, G. R. Sparrow, and N. L. Gervan, "Operational success of verbenone against the mountain pine beetle in a rural community," *Arboriculture and Urban Forestry*, vol. 33, no. 5, pp. 318–324, 2007.

[40] R. Jakuš, F. Schlyter, Q. H. Zhang et al., "Overview of development of an anti-attractant based technology for spruce protection against *Ips typographus*: from past failures to future success," *Anzeiger für Schädlingskunde*, vol. 76, no. 4, pp. 89–99, 2003.

[41] S. Chinn, "A simple method for converting an odds ratio to effect size for use in meta-analysis," *Statistics in Medicine*, vol. 19, no. 22, pp. 3127–3131, 2000.

[42] Z. Szendrei and C. Rodriguez-Saona, "A meta-analysis of insect pest behavioral manipulation with plant volatiles," *Entomologia Experimentalis et Applicata*, vol. 134, no. 3, pp. 201–210, 2010.

[43] G. Rücker, G. Schwarzer, J. R. Carpenter, and M. Schumacher, "Undue reliance on I^2 in assessing heterogeneity may mislead," *BMC Medical Research Methodology*, vol. 8, no. 1, article 79, 2008.

[44] J. H. Borden, L. J. Chong, T. J. Earle, and D. P. W. Huber, "Protection of lodgepole pine from attack by the mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Scolytidae) using high doses of verbenone in combination with nonhost bark volatiles," *Forestry Chronicle*, vol. 79, no. 3, pp. 685–691, 2003.

[45] N. E. Gillette, N. Erbilgin, J. N. Webster et al., "Aerially applied verbenone-releasing laminated flakes protect *Pinus contorta* stands from attack by *Dendroctonus ponderosae* in California and Idaho," *Forest Ecology and Management*, vol. 257, no. 5, pp. 1405–1412, 2009.

[46] R. Jakus, L. Zajíčkova, P. Cudlín et al., "Landscape-scale *Ips typographus* attack dynamics: from monitoring plots to GIS-based disturbance models," *IForest*, vol. 4, pp. 256–261, 2011.

[47] R. A. Progar, "Five-year operational trial of verbenone to deter mountain pine beetle (*Dendroctonus ponderosae*; Coleoptera: Scolytidae) attack of lodgepole pine (*Pinus contorta*)," *Environmental Entomology*, vol. 34, no. 6, pp. 1402–1407, 2005.

[48] C. Schiebe, M. Blaženec, R. Jakuš, C. R. Unelius, and F. Schlyter, "Semiochemical diversity diverts bark beetle attacks from Norway spruce edges," *Journal of Applied Entomology*, vol. 135, no. 10, pp. 715–786, 2011.

[49] M. Marvier, "Using meta-analysis to inform risk assessment and risk management," *Journal für Verbraucherschutz und Lebensmittelsicherheit*, vol. 6, supplement 1, pp. s113–s118, 2011.

[50] R. J. McManus, S. Wilson, B. C. Delaney et al., "Review of the usefulness of contacting other experts when conducting a literature search for systematic reviews," *British Medical Journal*, vol. 317, no. 7172, pp. 1562–1563, 1998.

[51] C. K. Boone, B. H. Aukema, J. Bohlmann, A. L. Carroll, and K. F. Raffa, "Efficacy of tree defense physiology varies with bark beetle population density: a basis for positive feedback in eruptive species," *Canadian Journal of Forest Research*, vol. 41, no. 6, pp. 1174–1188, 2011.

[52] S. R. Clarke, S. M. Salom, R. F. Billings et al., "A scentsible approach to controlling southern pine beetles. Two new tactics using verbenone," *Journal of Forestry*, vol. 97, no. 7, pp. 26–31, 1999.

[53] M. N. Andersson, M. Binyameen, and F. Schlyter, "Attraction modulated by spacing of pheromone components and anti-attractants in a bark beetle and a moth: a smaller scale of action in the moth," *Journal of Chemical Ecology*, vol. 37, no. 8, pp. 899–911, 2011.

[54] C. J. Fettig, S. R. McKelvey, C. P. Dabney, R. R. Borys, and D. P. W. Huber, "Response of *Dendroctonus brevicomis* to different release rates of nonhost angiosperm volatiles and verbenone in trapping and tree protection studies," *Journal of Applied Entomology*, vol. 133, no. 2, pp. 143–154, 2009.

[55] D. P. W. Huber and J. H. Borden, "Protection of lodgepole pines from mass attack by mountain pine beetle, *Dendroctonus ponderosae*, with nonhost angiosperm volatiles and verbenone," *Entomologia Experimentalis et Applicata*, vol. 99, no. 2, pp. 131–141, 2001.

[56] K. F. Raffa and A. Berryman, "Physiological differences between lodgepole pines resistant and susceptible to the mountain pine beetle and associated microorganisms," *Environmental Entomology*, vol. 11, pp. 486–492, 1982.

[57] C. Schiebe, A. Hammerbacher, G. Birgersson et al., "Inducibility of chemical defences in Norway spruce bark is correlated with unsuccessful mass attacks by the spruce bark beetle," *Oecologia*, vol. 170, no. 1, pp. 183–198, 2012.

[58] T. Zhao, P. Krokene, J. Hu et al., "Induced terpene accumulation in Norway spruce inhibits bark beetle colonization in a dose-dependent manner," *PLoS ONE*, vol. 6, no. 10, Article ID e26649, 2011.

[59] M. N. Andersson, M. C. Larsson, M. Blaženec, R. Jakuš, Q. H. Zhang, and F. Schlyter, "Peripheral modulation of pheromone response by inhibitory host compound in a beetle," *Journal of Experimental Biology*, vol. 213, no. 19, pp. 3332–3339, 2010.

[60] M. N. Andersson, J. M. Bengtsson, E. Grosse-Wilde et al., "Olfactory receptors in *Ips typographus*: transcriptome from antenna analysed and preliminary compared to *Dendroctonus ponderosae* and *Tribolium castaneum* (Coleoptera: Curculionidae & Tenebrionidae)," in *Proceedings of the Genetics of Bark Beetles and Associated Microorganisms*, Sopron, Hungary, 2011.

[61] M. N. Andersson, M. C. Larsson, and F. Schlyter, "Specificity and redundancy in the olfactory system of the bark beetle *Ips typographus*: single-cell responses to ecologically relevant odors," *Journal of Insect Physiology*, vol. 55, no. 6, pp. 556–567, 2009.

[62] M. N. Andersson, "Mechanisms of odor coding in coniferous bark beetles: from neuron to behavior and application," *Psyche*, vol. 2012, Article ID 149572, 14 pages, 2012.

[63] T. Aw, K. Schlauch, C. I. Keeling et al., "Functional genomics of mountain pine beetle (*Dendroctonus ponderosae*) midguts and fat bodies," *BMC Genomics*, vol. 11, no. 1, article e215, 2010.

[64] C. I. Keeling, H. Henderson, M. Li et al., "Transcriptome and full-length cDNA resources for the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, a major insect pest of pine forests," *Insect Biochemistry and Molecular Biology*, vol. 42, no. 8, pp. 525–536, 2012.

[65] S. Gripenberg, P. J. Mayhew, M. Parnell, and T. Roslin, "A meta-analysis of preference-performance relationships in phytophagous insects," *Ecology Letters*, vol. 13, no. 3, pp. 383–393, 2010.

[66] Q. H. Zhang and F. Schlyter, "Redundancy, synergism, and active inhibitory range of non-host volatiles in reducing pheromone attraction in European spruce bark beetle *Ips typographus*," *Oikos*, vol. 101, no. 2, pp. 299–310, 2003.

Hindawi Publishing Corporation
Psyche
Volume 2012, Article ID 940962, 10 pages
doi:10.1155/2012/940962

Research Article

Bark Beetles, *Pityogenes bidentatus*, Orienting to Aggregation Pheromone Avoid Conifer Monoterpene Odors When Flying but Not When Walking

John A. Byers

US Arid-Land Agricultural Research Center, ARS, USDA, 21881 North Cardon Lane, Maricopa, AZ 85138, USA

Correspondence should be addressed to John A. Byers, john.byers@ars.usda.gov

Received 17 December 2011; Revised 14 March 2012; Accepted 25 April 2012

Academic Editor: Brian T. Sullivan

Previous studies and data presented here suggest that odors from healthy host Scotch pine (*Pinus sylvestris*) and nonhost Norway spruce (*Picea abies*), as well as major monoterpenes of these trees at natural release rates, significantly reduce the attraction of flying bark beetles, *Pityogenes bidentatus*, of both sexes to their aggregation pheromone components grandisol and *cis*-verbenol in the field, as tested by slow rotation of trap pairs. In contrast, *P. bidentatus* males and females walking in an open-arena olfactometer in the laboratory did not avoid monoterpene vapors at release rates spanning several orders of magnitude in combination with aggregation pheromone. The bark beetle may avoid monoterpenes when flying as a mechanism for avoiding nonhost species, vigorous and thus unsuitable host trees, as well as harmful resinous areas of hosts. Inhibition of this flight avoidance response in beetles after landing would allow them to initiate, or to find and enter, gallery holes with high monoterpene vapor concentrations in order to feed and reproduce.

1. Introduction

The bark beetle *Pityogenes bidentatus* (Herbst) (Coleoptera: Curculionidae: Scolytinae) is a relatively small (2-3 mm long) insect that attacks only Scotch pine, *Pinus sylvestris* L. [1]. The beetle is common in Scotch pine and mixed conifer forests of Europe where it begins a seasonal flight in spring and colonizes smaller diameter trunks and limbs of weakened hosts [1–3]. The species builds up in slash and logging residue and can harm adjacent young stands of Scotch pine [3]. *P. bidentatus* have been caught more frequently in traps baited with pine logs left for several weeks compared to freshly-cut logs, indicating that the beetle is attracted to odors from aging tissue [4]. The aggregation pheromone of *P. bidentatus* consists of two components, (*S*)-*cis*-verbenol (cV) and grandisol (G1) as determined in previous studies [5–9]. Grandisol is well known as a pheromone component of the boll weevil, *Anthonomus grandis* Boheman [10].

Injured conifers such as pines and spruce produce resin, commonly consisting of about 80% mildly toxic monoterpenes, to defend against the penetrations of attacking insects [11]. Some species of bark beetles may be attracted to these tree-specific blends of monoterpenes [12, 13] since they indicate both the appropriate host and the likely susceptibility to colonization [14–17]. Bark beetles in the genus *Tomicus* exhibit relatively strong attraction to host Scotch pine and its major monoterpenes, especially α-pinene (both enantiomers), (+)-3-carene, and terpinolene [14–17]. Camphene, (+)- and (−)-α-pinene, and (−)-β-pinene are major monoterpenes of Norway spruce, *Picea abies* L., the host of *P. chalcographus* L. These monoterpenes enhance the attraction of flying *P. chalcographus* to traps baited with its aggregation pheromone, and increase entry rates of the beetles through 2.5 mm diameter holes into the traps [18, 19]. Furthermore, several studies have found that certain monoterpenes enhance attraction to pheromone components in some of the more aggressive bark beetles that kill standing trees [18–25].

On the other hand, a sufficient flow of resin can expel or kill attacking bark beetles. Hence less aggressive species of bark beetles that specialize on hosts with compromised resin defenses may have evolved olfactory mechanisms and

behaviors for the avoidance of specific volatile monoterpenes in tree resins indicative of a vigorous and resistant tree. Likewise beetles apparently avoid certain monoterpenes or other volatile chemicals associated specifically with nonhost tree species [26–37]. This appears to be the case for flying *P. bidentatus* when orienting to their aggregation pheromone components. Odors from freshly-cut host Scotch pine or from non-host Norway spruce, as well as non-host deciduous trees (birch, *Betula pendula* Roth.; mountain ash, *Sorbus aucuparia* L.; oak, *Quercus robur* L. and alder buckthorn, *Frangula alnus* P. Mill.) reduced attraction to the aggregation pheromone components [7, 8]. Many individual monoterpenes and blends released at rates comparable to that released from physical wounds of trees also inhibited flight attraction to their aggregation pheromone [7–9].

Three previous studies [7–9] tested effects of monoterpenes on flying *P. bidentatus* by using a pair of traps separated 6 m apart that were mechanically rotated slowly at 2 rph to even out any trap position effects [7, 8, 38]. Both traps contained aggregation pheromone (G1 and cV), while one trap also released host or nonhost odors (specific monoterpenes, cut bark, or twigs) that reduced attraction of flying *P. bidentatus* (Figure 1). The objective of my study was to assess the response of both flying and walking *P. bidentatus* of both sexes to monoterpenes released in association with the aggregation pheromone (assayed with either rotating traps in the field or a laboratory olfactometer, resp.). The hypothesis was that aggregation pheromone responses by beetles walking in the olfactometer would exhibit the same inhibition to monoterpenes as when flying in the field. This hypothesis was based on earlier studies with bark beetle semiochemicals in which the same behavioral responses (attraction or repulsion) were found both when beetles were walking in a laboratory olfactometer and when flying in the field to baited traps [7, 8, 14, 15, 18, 19, 39–42].

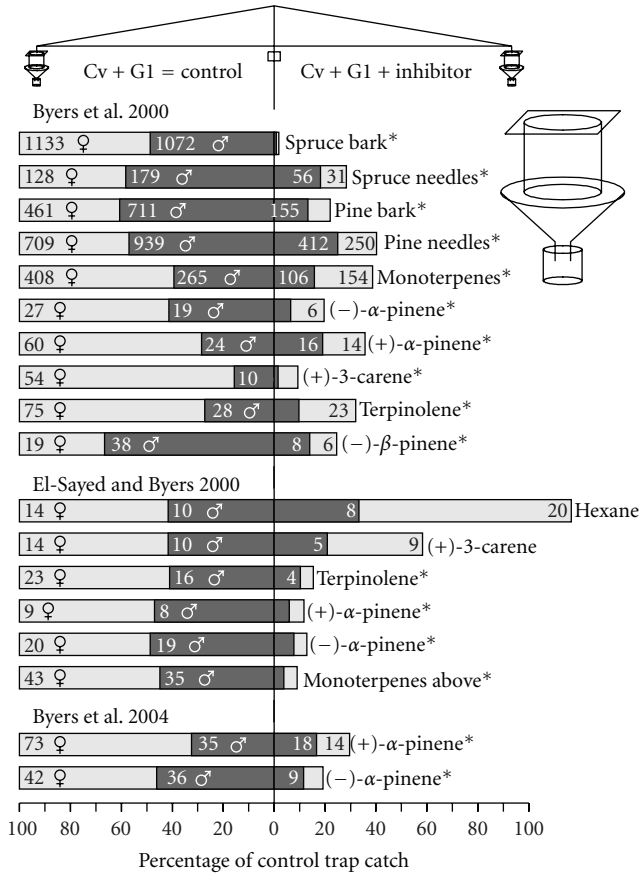


Figure 1: Selected comparisons from three studies (Byers et al. [7], El-Sayed and Byers [9], and Byers et al. [8]) showing reduced catches of flying *Pityogenes bidentatus* on traps releasing pheromone plus inhibitor volatiles (monoterpenes released at 1 mg/day, 100–200 g bark or needles of spruce and pine) compared to control traps with pheromone alone (Sjöbo/Veberöd, Sweden, April-May 1998–2000, see Methods for details). Total catches of both sexes with asterisks were significantly lower than the pheromone controls in the same test at $P < 0.01$ (chi-square goodness of fit).

2. Materials and Methods

2.1. Inhibition of Attractive Response of Flying P. bidentatus to Aggregation Pheromone Components by Monoterpenes. Field tests similar to those mentioned above [7–9] were conducted in mixed forests/plantations (primarily Scotch pine) near Sjöbo/Veberöd, Sweden in May 2001 with three sets of rotating trap pairs. Traps in each pair were 6 m apart, suspended at 1.2 m height, and rotated around a central axis at 2 rph. Synthetic aggregation components G1 (racemic, *cis*-grandisol, (1*R*, 2*S*)-1-(2-hydroxyethyl)-1-methyl-2-(1-methylethenyl) cyclobutane, >98%, Frank Enterprises, Inc., Columbus, Ohio) and cV ((1*S*,4*S*,5*S*)-*cis*-verbenol, 96%, Borregaard) were placed inside each trap in a pair. Each pheromone bait had 25 μL G1 at the bottom of a small glass tube (34 mm × 4.45 mm i.d. opening) and ~25 mg of crystalline cV at the bottom of a polyethylene tube (31 mm × 6.15 mm i.d. opening) so that releases were nearly constant (0.05 mg G1/day and 0.5 mg cV/day at 22°C).

Each trap consisted of an 18 cm diameter × 28 cm high transparent polycarbonate cylinder covered at the top but open at the bottom and suspended over a large white plastic funnel (31 cm diameter) that collected beetles striking the cylinder (Figure 1). Experimental runs of each rotating trap pair were conducted for at least 1 hour during 11 : 00–18 : 00 when temperature was above 18°C. After each run the inhibitory source, but not the attractants, was switched to the other trap of the pair such that from two to five runs were conducted for each monoterpene test. The monoterpenes tested as inhibitors included (+)-α-pinene ($[\alpha]_D^{20} = +57°$, 99%, Fluka, Stockholm, Sweden), (−)-α-pinene ($[\alpha]_D^{20} = -50°$, >99.5%, Fluka), (+)-3-carene ($[\alpha]_D^{20} = +15°$, 95%, Aldrich, Stockholm, Sweden), terpinolene (97% Carl Roth Gmbh, Karlsruhe, Germany), and (−)-β-pinene ($[\alpha]_D^{20} = -21°$, 99%, Aldrich), myrcene (95%, Aldrich), (−)-limonene ($[\alpha]_D^{20} = -94°$, 96%, Aldrich), and (+)-limonene ($[\alpha]_D^{20} = +123°$, 97%, Aldrich). The release rates (μg/h) were about 1400 for α-pinene enantiomers, 800 for (−)-β-pinene, 740 for myrcene, 385 for each enantiomer of limonene, and 240 for terpinolene released individually from glass tubes as described above (weight loss at 22°C in laboratory).

The catches on inhibitor and control were pooled for each treatment comparison and tested for significant differences by comparing the two catches to the average catch with a chi-square test ($df = 1$). If the tests were statistically significant ($P < 0.05$), then catches of each sex were compared within a treatment comparison for significant differences using a chi-square test. Previous results (Figure 1) [7, 8] were also analyzed for gender differences in regard to inhibition of attraction.

Previously reported dose-response data for monoterpene reduction of *P. bidentatus* attraction to G1 and cV (Figure 4 in [9]) was subjected to user-defined logarithmic and logistic dose-response regressions (Statistica 5.1, StatsSoft Inc., Tulsa OK) to find a better relationship than originally presented. In this previously reported test, responses of both sexes to the pheromone-baited, slow-rotating trap pairs were compared after one trap of the pair was amended with an exact release of four host monoterpenes ranging from 0.01 to 10 Scotch pine log equivalents (0.1 to 100 μg/min each monoterpene) provided by a piezoelectric sprayer and syringe pump [9].

2.2. Tests for Inhibition of the Attractive Response of Walking P. bidentatus to Aggregation Pheromone Components in a Laboratory Open-Arena Olfactometer. Adults of *P. bidentatus* that had been caught live in traps baited with aggregation pheromone components, G1 and cV at the same field sites described above during May 1999 and 2000 were separated by sex in the laboratory. They were then stored at 4°C on moistened tissue paper in Petri dishes for up to four days until use in bioassays. The responses of *P. bidentatus* to semiochemicals were tested in a modified open-arena olfactometer [14, 15, 19, 41, 43]. This consisted of a suction fan that drew air out of the room through a fine metal screen (0.4 mm mesh) on one side of an opaque plastic manifold (60 cm wide × 25 cm × 25 cm) placed on the "downwind" side of a glass tabletop covered with white construction/poster paper (0.84 × 1.1 m). At the "upwind" end of the table (0.7 m from the manifold), laboratory air was forced through a clear acrylic manifold (46 cm wide × 5 cm high × 8.5 cm deep) with three rows of 13, 12, and 13 holes (1.5 mm diameter) starting 7.5 mm above the surface (with spacing between holes 3 cm horizontally and 1 cm vertically; middle row of 12 holes centered). The two manifolds maintained an approximately laminar airflow with a speed of 0.9 m/s at the semiochemical source (5 cm from the center of upwind manifold) and 0.6 m/s where the beetles were released (21 ± 2 cm "downwind" from the source). Ten beetles of a single sex were released together initially. A positive response was recorded when a beetle arrived within 2 cm of the odor source. Beetles that walked outside a 25 cm radius circle centered on the release point, or that had not reached the odor source in the time required for various mixtures of the semiochemicals in diethyl ether to finish eluting (126 ± 10 sec) from a 5 μL glass capillary (Drummond Scientific, Broomall, PA), were placed temporarily in a plastic Petri dish until the first trial was completed. These nonresponding beetles were released a second time to a newly filled capillary tube and the numbers reaching the odor source in the two trials were summed (e.g., two of ten may have responded in the first trial and two of eight in the second trial giving a 40% response in total).

The aggregation pheromone components cV and G1 were released together in the laboratory in two ways: (1) diluted in diethyl ether and placed in a 5-μL capillary tube open at both ends, or (2) in the field dispensers described above. The release rates for the second method were estimated by weight loss at 22°C to be 350 ng/min for cV and 35 ng/min for G1, the same rates released in the slow-rotating pairs of traps in field tests. The release of monoterpenes was by the first method only, although the compounds were also dispensed neat from the 5-μL capillary. In method 1, release rates of chemicals from the 5-μL capillary were dependent on the evaporation of the solvent diethyl ether; that is, 2.2 μL of ether was released per min and thus the release rates were about 2.2 times the concentration (in mass per μL) for each dilution tested as indicated in Tables 1, 2, and 3.

The monoterpenes tested were (+)-α-pinene, (−)-α-pinene, (+)-3-carene, terpinolene, and (−)-β-pinene as described above. The two aggregation pheromone components (G1 and cV) were tested together without monoterpenes or together with monoterpenes and compared in some cases to a diethyl ether control in three sets of bioassays (4 May 1999, 7 June 1999, and 21–23 May 2000). At least 40 beetles of each sex were tested for each release rate of the compounds under conditions of 22°C and 1700 lux during 13 : 00–17 : 00. Both sexes were tested at the lowest chemical concentrations initially each day and then additional beetles were tested at increasingly higher concentrations as indicated in the tables. The release rates were chosen based on behavioral results with other bark beetles in previous studies with the same olfactometer [14–16, 19] as well as to correspond to rates used in the field (which represent natural release rates from Scotch pine logs or bark beetles) [9, 14–18]. The release of neat monoterpenes in the open-arena bioassay were determined precisely in 2012 under the same olfactometer conditions by measuring changes in meniscus volume of the capillary as monitored by a time-lapse webcam and computer (Byers unpublished). Statistically significant differences in the percent responding between various release rates and compound combinations were determined by a chi-square test.

3. Results

3.1. Inhibition of Attractive Response of Flying P. bidentatus to Aggregation Pheromone Components by Monoterpenes. In tests of rotating pairs of aggregation pheromone-baited traps, the single trap of each pair dispensing vapor of individual monoterpenes generally caught fewer *P. bidentatus* than its paired control trap (Figure 2). Myrcene was not tested in the earlier studies (Figure 1) and was inhibitory to *P. bidentatus* response (Figure 2). Also, the enantiomers of limonene had not been tested earlier and, contrary to other monoterpenes, these did not appear to reduce response of either sex of the bark beetle (Figure 2). In tests that showed a significant reduction in total catch by monoterpenes (Figure 2), attraction of each sex was inhibited in flight in

Table 1: Percent of walking *Pityogenes bidentatus* females and males responding in a laboratory olfactometer (4 May 1999) to conifer monoterpenes and aggregation pheromone components (G1 = grandisol, cV = (*S*)-*cis*-verbenol).

Chemicals[a]	Percent responding[b]	95% B. C. L.[c]	*N*
Females			
Diethyl ether control	10.0a	4.0–23.1	40
A = G1 + cV at 5×10^{-10} g/μL	42.5b	28.5–57.8	40
A + monoterpenes[d] at 5×10^{-9} g/μL	37.5b	24.5–53.0	40
A + monoterpenes[d] at 5×10^{-8} g/μL	30b	18.1–45.4	40
A + monoterpenes[d] at 5×10^{-7} g/μL	32.5b	20.1–48.0	40
A + monoterpenes[d] at 5×10^{-6} g/μL	37.5b	24.2–53.0	40
Females			
B = G1+ cV at 5×10^{-9} g/μL	72.5a	49.5–77.9	40
B + monoterpenes[d] at 5×10^{-7} g/μL	52.5a	37.5–67.1	40
B + monoterpenes[d] at 5×10^{-6} g/μL	57.5a	42.2–71.5	40
Females			
C = G1+ cV field dispensers	45a	34.6–55.9	80
C + monoterpenes[d] at 5×10^{-5} g/μL	48.75a	38.1–59.5	80
Males			
C	33.33a	22.7–45.9	60
C + monoterpenes[d] at 5×10^{-5} g/μL	38.33a	27.1–51.0	60

[a] Chemicals dispensed by evaporation from 5 μL micropipette at indicated concentration each/μL diethyl ether (release rate approximately 2.2 × concentration/min).
[b] Percentages followed by same letter were not significantly different ($\alpha = 0.05$, chi-square) within a test series for each sex.
[c] Binomial confidence limits for proportions.
[d] Major host Scotch pine monoterpenes: (+)-α-pinene, (−)-α-pinene, (+)-3-carene, and terpinolene were dispensed in diethyl ether solution at concentrations indicated in table.

Table 2: Percent of walking *Pityogenes bidentatus* females and males responding in a laboratory olfactometer (7 June 1999) to conifer monoterpenes and aggregation pheromone components (G1 = grandisol, cV = (*S*)-*cis*-verbenol).

Chemicals[a]	Percent responding[b]	95% B. C. L.[c]	*N*
Females			
B = G1+ cV at 10^{-9} g/μL	68.3a	55.8–78.7	60
B + monoterpenes[d] at 10^{-5} g/μL	65.0a	52.4–75.8	60
B + monoterpenes neat[e]	70.0a	57.5–80.1	60
B + (−)-α-pinene neat[e]	75.0a	62.8–84.2	60
Males			
B	65.0a	52.4–75.8	60
B + (−)-α-pinene neat[e]	71.7a	59.2–81.5	60

[a] Chemicals dispensed by evaporation from 5 μL micropipette at indicated concentration each/μL diethyl ether (release rate approximately 2.2 × concentration/min except when neat).
[b] Percentages followed by same letter were not significantly different ($\alpha = 0.05$, chi-square).
[c] Binomial confidence limits for proportions.
[d] Major host Scotch pine monoterpenes: (+)-α-pinene, (−)-α-pinene, (+)-3-carene, and terpinolene were each at indicated concentration as dispensed in diethyl ether solution.
[e] Chemicals were dispensed neat from 5 μL micropipettes; (−)-α-pinene, (+)-3-carene, and terpinolene were each released at 28, 9.4, and 4.8 μg/min, respectively, according to capillary measurements over time in the olfactometer.

nearly all cases ($P < 0.01$), except male response to (−)-α-pinene ($P = 0.02$) and to (+)-α-pinene ($P = 0.012$) were only marginally significant due likely to low numbers. The inhibition of the beetle by (+)-3-carene ($P < 0.01$) was mainly due to females ($P < 0.01$) because male catches were not significantly different ($P = 0.56$), but again the numbers of males caught were low (Figure 2). However, males and females were inhibited in an earlier study [7] by (+)-3-carene (Figure 1, both sexes $P < 0.01$). In the reanalysis of previous studies catches of each gender (Figure 1), both sexes were significantly inhibited in flight by non-host Norway spruce and host Scotch pine odors as well as by several monoterpenes: α-pinene, β-pinene, 3-carene, and terpinolene tested singly or as a combination (all $P < 0.01$).

Table 3: Percent of walking *Pityogenes bidentatus* females and males responding in a laboratory olfactometer (21–23 May 2000) to conifer monoterpenes and its aggregation pheromone components (G1 = grandisol, cV = *S*-*cis*-verbenol).

Chemicals[a]	Percent responding[b]	95% B. C. L.[c]	*N*
Females			
Diethyl ether control	10.0a	4.0–23.1	40
A = G1 + cV at 5×10^{-10} g/μL	25.0ab	14.2–40.2	40
B = G1 + cV at 5×10^{-9} g/μL	35.0b	22.1–50.5	40
G1 + cV at 5×10^{-8} g/μL	42.5bc	28.5–57.8	40
D = G1 + cV at 5×10^{-7} g/μL	62.5cd	47.0–75.8	40
D + (−)-β-pinene at 10^{-5} g/μL	72.5d	57.2–83.9	40
D + (−)-α-pinene at 10^{-5} g/μL	65.0cd	49.5–77.9	40
Males			
Diethyl ether control	5.0a	1.4–16.5	40
A = G1 + cV at 5×10^{-10} g/μL	17.5a	8.7–32.0	40
B = G1 + cV at 5×10^{-9} g/μL	42.5b	28.5–57.8	40
G1 + cV at 5×10^{-8} g/μL	40.0b	26.3–55.4	40
D = G1 + cV at 5×10^{-7} g/μL	50.0b	35.2–64.8	40
D + (−)-β-pinene at 10^{-5} g/μL	47.5b	32.9–62.5	40
D + (−)-α-pinene at 10^{-5} g/μL	55.0b	39.8–69.3	40

[a] Chemicals dispensed by evaporation from 5 μL micropipette at indicated concentration each/μL diethyl ether (release rate approximately 2.2 × concentration/min).
[b] Percentages followed by same letter were not significantly different ($\alpha = 0.05$, chi-square).
[c] Binomial confidence limits for proportions.

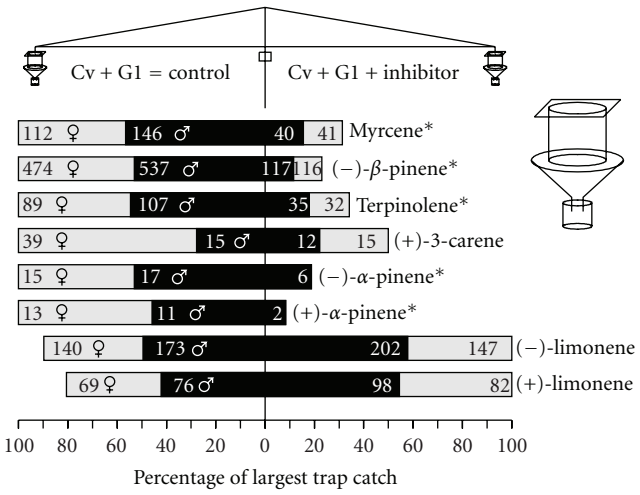


Figure 2: Attraction of flying *Pityogenes bidentatus* to pairs of slowly-rotated traps releasing aggregation pheromone (*cis*-verbenol = Cv and grandisol = G1) when individual monoterpenes were released from one of the two traps. Tests were performed at different dates and times during May 2001 in Sjöbo/Veberöd, Sweden. Numbers inside light and black bars represent catch of each sex. Treatments with asterisks caught significantly fewer beetles (sexes summed) in the treatment than the control trap in the same pair ($P < 0.01$, chi-square test).

In one study [7], however, males were not inhibited by (+)-α-pinene ($P = 0.20$) possibly due to low numbers trapped (10 and 5 males). In a second study [8], both sexes were inhibited by either enantiomer of α-pinene (all $P < 0.01$). In a third study [9], (+)-3-carene was not significantly inhibitory for either sex (both $P > 0.2$), although low numbers were caught as in the second study (Figure 1).

Reanalysis of a similar published study [9], in which one trap of each pair had exact releases of four host monoterpenes representing 0.01 to 10 pine log equivalents, revealed that the dose-response data best fit a logistic regression ($R^2 = 0.86$; equation in Figure 3). The common logarithmic regression, $Y = 24.824 - 5.087 \cdot \ln(X)$, also fits well ($R^2 = 0.84$).

3.2. Tests for Inhibition of the Attractive Response of Walking P. bidentatus to Aggregation Pheromone Components in a Laboratory Open-Arena Olfactometer. The responses of walking female *P. bidentatus* to aggregation pheromone components grandisol (G1) and *cis*-verbenol (cV) at 5×10^{-10} g/μL concentration was 42.5% (Table 1). An increasing dosage of four host Scotch pine monoterpenes (each compound ranged from 10 to 10,000 times the concentration of the pheromone components) did not significantly affect the attraction of walking females to this concentration of G1 and cV (Table 1), as would be expected from the field trapping experiments above. In another bioassay series, a stronger aggregation dose of 5×10^{-9} g/μL caused 72.5% of females to respond, and the addition to this dosage of the four monoterpenes at 1000 to 10,000 times higher concentrations caused a slightly lower response, but this difference was not significant (Table 1). The addition of monoterpenes at the strongest dosage of 10^{-5} g/μL had no effect on attraction in either sex to the field dispensers of G1 and cV (Table 1).

In a second series of bioassays, a dosage of 10^{-9} g/μL G1+cV that alone attracted 68.3% females was combined

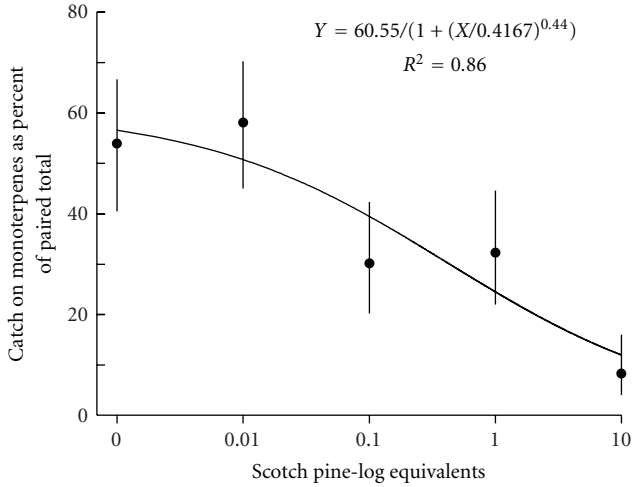


Figure 3: Inhibition of *P. bidentatus* response by increasing release rates of a mixture of Scotch pine monoterpenes ((−)-α-pinene, (+)-α-pinene, (+)-3-carene, and terpinolene) each released in proportion to release of 1.0 Scotch pine log-equivalent (10 μg/min) in hexane with the piezoelectric sprayer from one of a pair of slowly-rotated, pheromone (*cis*-verbenol and grandisol)-baited traps (Veberöd, Sweden, 19 May 1999; data from [9]). Error bars represent 95% binomial confidence limits for the proportion trapped in the monoterpene-releasing trap relative to the total catches by the pair.

with the strongest dosage tested of monoterpenes (10^{-5} g/μL or neat monoterpenes), but their response was not reduced (Table 2). Walking males responded similarly as females to the aggregation pheromone components and there was no evidence of any inhibition by host tree monoterpenes released neat from the 5-μL pipette (Table 2). The releases of neat monoterpenes in the open-arena olfactometer for α-pinene, 3-carene, and terpinolene were 28, 9.4, and 4.8 μg/min, respectively.

A third series of bioassays a year later was performed in which the release rate of the aggregation pheromone components was increased over several orders of magnitude from 5×10^{-10} to 5×10^{-7} g/μL, which resulted in an increasing response of walking females and males to the source (Table 3). But again, a strong release of 10^{-5} g/μL of monoterpenes, either (−)-β-pinene (a major monoterpene of non-host Norway spruce but not of host pine) or (−)-α-pinene (a major monoterpene in both Scotch pine and Norway spruce), did not decrease response to an optimal dosage (5×10^{-7} g/μL) of the aggregation pheromone components (Table 3).

4. Discussion

The results of selected tests from the three previous studies [7–9] showed strong to moderate inhibition of the flight orientation of both sexes of *P. bidentatus* to synthetic aggregation pheromone components in the field (Figure 1). Norway spruce bark was highly inhibitory as well as spruce needles, but even the host tree Scotch pine bark or needles were strongly inhibitory (Figure 1). Mixtures of monoterpenes as well as five individual host monoterpenes clearly cause flying *P. bidentatus* of both sexes to avoid landing on traps releasing aggregation pheromone when compared to traps releasing the same rate of pheromone without inhibitory volatiles (Figures 1 and 2). The current field tests (Figure 2), show that *P. bidentatus* of both sexes avoid landing in areas with aggregation pheromone if the monoterpenes myrcene, (−)-β-pinene, terpinolene, (−)-α-pinene, and (+)-α-pinene are also released. Only females were significantly inhibited by (+)-3-carene, while neither sex appeared to avoid either enantiomer of limonene when orienting to aggregation pheromone (Figure 2).

In the laboratory bioassay with walking beetles, however, several monoterpenes released with aggregation pheromone components had no apparent effect on attraction (Tables 1–3). The large range of monoterpene release rates over three orders of magnitude in the laboratory bioassay are estimated to correspond to natural release rates from small wounds to broken limbs of conifers [14, 15], and were similar to the rates that elicited behavioral activity (attraction) from *Tomicus piniperda* (L.) and *T. minor* (Hart.) in the same laboratory olfactometer [14–16]. The highest concentration of ether-diluted monoterpenes tested in the laboratory olfactometer (10^{-5} g/μL) had a maximum estimated release rate of 1300 μg/h, which is comparable to the release rate in field trials (i.e., 1000 to 1400 μg/h) that caused inhibition of attraction [7]. The actual release rates of monoterpenes dissolved in diethyl ether from the 5-μL capillary, however, were probably different due to differing vapor pressures of the monoterpenes and diethyl ether [41]. However, the highest release rates of neat monoterpenes in the laboratory walking bioassay were 1680 μg/h for each enantiomer of α-pinene, 565 μg/h for 3-carene, and 289 μg/h for terpinolene, and these rates were estimated to be equivalent to the release of the major monoterpenes from three freshly cut logs of Scotch pine (30 cm × 13 cm diameter) [9, 14, 15]. In contrast to walking beetles in the olfactometer, attraction by flying *P. bidentatus* to aggregation pheromone in the field was significantly reduced by these same monoterpenes at 0.1 log-equivalent (60 μg/h) or 1 log-equivalent (600 μg/h) dispensed from a piezoelectric sprayer (Figure 3) [9]. Thus, release rates of monoterpenes that failed to inhibit attraction to pheromone by walking beetles in the laboratory were equivalent [7] or higher [9] than rates that inhibited attraction of flying beetles in the field.

Natural selection should favor conifer-infesting bark beetles that find their host tree by keying on pheromones and/or host volatiles of which the monoterpenes are the most abundant. Some bark beetles such as *T. piniperda* and to a lesser extent *T. minor* are strongly attracted to monoterpene vapors emanating from resinous wounds incurred when the trees fall during winter and spring storms [14–16]. Monoterpenes are also weakly attractive to secondary bark beetles that follow the tree-killing beetles after the tree succumbs [17]. (−)-α-Pinene weakly attracted *Ips grandicollis* (Eichhoff) and *Dendroctonus valens* LeConte but not *I. avulsus* (Eichhoff) or *I. calligraphus* (Germar) [23, 25]. Bark beetles that kill their host tree in a mass attack in the genera *Ips* and

Dendroctonus are weakly or not attracted by monoterpenes alone ([23, 25, 44], Byers unpublished) although in some cases specific monoterpenes can enhance responses to aggregation pheromone, as occurs in *D. brevicomis* LeConte, *D. pseudotsugae* Hopkins, *D. valens*, *D. frontalis* Zimmermann, and *I. grandicollis* [20–25]. This also seems to be the case for tree-killing *P. chalcographus* that attack Norway spruce; both sexes were more attracted to aggregation pheromone components when either enantiomer of α-pinene, $(-)$-β-pinene, or camphene was coreleased [18].

The aggressive bark beetles generally are not attracted to susceptible hosts by means of monoterpenes, but are believed to select hosts by a process of randomly landing on trees and determining their resistance level by boring through the outer bark [45, 46]. If the tree produces enough resin, then the beetle must leave or be killed. If the beetle succeeds in feeding due to low host resistance, then aggregation pheromone is released that attracts many more individuals to overcome the tree. Thus, the vast majority of individuals in the tree-killing bark beetle species find their host tree by orienting to aggregation pheromone [46, 47]. *P. bidentatus* appears to be much less aggressive, preferring to colonize only broken or weakened limbs of pine in which resin production as a defense is greatly reduced or nonexistent [1–4]. As the tree becomes colonized and begins to degrade, higher rates of verbenone and ethanol are released that reduce attraction of many of the bark beetle species to pheromone or host monoterpenes [11, 15, 17, 33, 48–50]. In *P. bidentatus*, however, ethanol reduced, while verbenone did not reduce, response to aggregation pheromone in tests with trap pair slow-rotation [7].

The open-arena olfactometer used here is a bioassay that has been tested on several bark beetle species, and individuals of both sexes commonly behave in a way that is consistent with trap captures in the field. For example, walking *I. paraconfusus* are increasingly attracted to higher doses of aggregation pheromone components in the laboratory [39, 51], but at the higher release rates the males are increasingly less attracted, just as in the field [51]. In *D. brevicomis*, walking females and males are increasingly attracted to their aggregation pheromone components in the olfactometer, and release of male-produced verbenone causes both sexes to avoid the aggregation pheromone source; the same behaviors that occur when flying beetles respond to baited traps in the field [40, 41]. Both sexes of *D. brevicomis* produce *trans*-verbenol during feeding in ponderosa pine [52], and only females are inhibited by higher concentrations of $(-)$-*trans*-verbenol either when walking in the olfactometer or when entering holes in carton traps in the field [42]. Another study with the olfactometer has shown that walking *T. piniperda* are attracted to host monoterpenes as well as similarly attracted when flying to host logs or monoterpenes in sticky traps [14]. Walking *P. chalcographus* are increasingly attracted in the laboratory olfactometer to increasing release of their pheromone components, and subtraction of the monoterpene fraction from an odor collection of a male-infested log caused a moderate decrease in attraction [19]. This is in agreement with a field study showing monoterpenes increasing flight attraction and proportionally greater entry of walking *P. chalcographus* through small holes into traps releasing aggregation pheromone components [18].

Thus, in all cases except the present study, bark beetle behavior in the open-arena olfactometer has been in general agreement with the behavior observed in the field with traps baited with semiochemicals. If there were different bioassay operators, this might explain the discrepancy between flight and walking behaviors in *P. bidentatus*; however, the same person (Byers) performed all laboratory bioassays discussed above [14, 18, 19, 39–42, 51, 52]. Rather, the differences appear to be explained by the ecology of *P. bidentatus* that appears less aggressive and colonizes unhealthy branches, in contrast to the pest bark beetles mentioned above that often kill trees. Six possible combinations of behavior could have evolved in *P. bidentatus* when responding to aggregation pheromone in regard to whether to respond, ignore, or avoid monoterpene odors while flying or walking. The best adaptation for a less aggressive bark beetle such as *P. bidentatus* that prefers to colonize weakened and diseased branches or smaller unhealthy trees would be to avoid monoterpenes when flying (so as to not land in resin and more efficiently select suitable hosts from among resistant hosts and nonhosts) but not when walking (so as to enter holes made by mates or excavate entrance holes where concentrations of monoterpenes are higher). Other more aggressive bark beetles may not avoid monoterpenes in combination with aggregation pheromone while flying because these species are suited to tolerate resin when overcoming the tree's resistance [11].

The monoterpenes were not tested alone in the rotating traps in the field or in the laboratory bioassay so it is not known if they could be attractive to *P. bidentatus* at some concentration. However, Byers [17] tested monoterpenes (enantiomers of α-pinene, 3-carene, and terpinolene, each at 104 μg/h to 583 μg/h) or a combination of ethanol and monoterpenes in the field during the spring in the same location as the present study; neither *P. bidentatus* nor any other *Pityogenes* species were caught even though five other bark beetle species were attracted.

It is proposed that pheromone-producing bark beetles that are not attracted to monoterpenes or other host-associated odors may initially land on trees at random in response to the visual silhouette of the trunk [45, 51]. Once a beetle bores into a suitable tree and is able to produce aggregation pheromone, a mass colonization ensues. The presence of aggregation pheromone indicates an ongoing colonization by *P. bidentatus* and thus is a cue to the likely presence of a weakened host and a valuable resource to be exploited. Conifers usually produce resinous wounds in response to mechanical damage from storms or other causes, and the exposed resin poses a hazard to bark beetles orienting to and attempting to colonize these trees. The visual acuity of bark beetles, with about 200 facets per eye [11], probably does not allow beetles to differentiate resin globules and resinous patches on a tree trunk or branch before they land. *T. piniperda*, *I. typographus* L., and *P. chalcographus* beetles walking on the bark of wounded conifers in Sweden that happened to contact resin globules were seen to back away and turn to find a path free of resin (personal observations).

It is probable that *P. bidentatus* similarly avoid resin while walking. It is also expected that fitness of *P. bidentatus* would be enhanced when responding to aggregation pheromone if they could avoid both potentially fatal landings in resinous patches and bark unsuitable for colonization due to a tree's vigorous resin defenses. In addition, flying beetles would save time and energy by avoiding monoterpenes of fresh host trees as well as nonhost trees in the vicinity of a suitable host. Healthy trees capable of exuding resin from wounds probably would be unsuitable hosts for *P. bidentatus*, since they typically attack diseased and dying branches possessing compromised resin defenses. After landing, the beetles may seek out the source of aggregation pheromone while relying on their ability to back away from any sticky resin they encounter while walking.

It is likely that no species of conifer-infesting bark beetle would be repelled by monoterpenes while walking on the bark surface and orienting to pheromone, since they must encounter high concentrations of monoterpenes either when they enter the gallery of a mate or initiate a new gallery. To my knowledge, no tests of bark beetles walking in laboratory olfactometers and orienting to aggregation pheromone have shown monoterpenes to be repellent. *P. bidentatus* is not the only bark beetle that is inhibited by host volatiles in flight since *I. avulsus* response to aggregation components was inhibited by high releases of loblolly pine turpentine (undefined mixture of monoterpenes at 150,000 μg/h) [25] and *I. pini* (Say) flight response to its aggregation components was inhibited by racemic α-pinene at high rates (23,000 μg/h) [24]. It is not known how *I. pini* or *I. avulsus* respond to monoterpenes and aggregation pheromone when walking.

Further work with *P. bidentatus* is needed to understand the conditions and benefits of ignoring monoterpene odors while walking but avoiding these odors when flying. It appears remarkable that this tiny insect is able to exhibit two types of behavior in regard to aggregation pheromone and monoterpenes. The first behavior of avoiding monoterpenes when flying seems adaptive in that a beetle averts plunging into sticky resin that could entrap them and saves time/energy during searches for suitable hosts. The second behavior of ignoring monoterpene vapors when walking also seems adaptive to find colonization areas and entrance holes of mates on the bark, and escaping any encountered resin by backing away. The possibility of dichotomy in behaviors when flying and walking needs to be investigated in other species of bark beetles to understand the adaptive benefits.

Acknowledgments

This study was supported by grants from the Swedish Agricultural and Forestry Research Council (SJFR) to the Swedish University of Agricultural Sciences, Alnarp, Sweden.

References

[1] B. Lekander, B. Bejer Peterson, F. Kangas, and A. Bakke, "The distribution of bark beetles in the Nordic countries," *Acta Entomologia Fennica*, vol. 32, pp. 1–36, 1977.

[2] I. Amezaga and M. A. Rodríguez, "Resource partitioning of four sympatric bark beetles depending on swarming dates and tree species," *Forest Ecology and Management*, vol. 109, no. 1–3, pp. 127–135, 1998.

[3] P. Martikainen, J. Siitonen, L. Kaila, and P. Punttila, "Intensity of forest management and bark beetles in non-epidemic conditions: a comparison between Finnish and Russian Karelia," *Journal of Applied Entomology*, vol. 120, no. 5, pp. 257–264, 1996.

[4] K. Tunset, A. C. Nilssen, and J. Andersen, "Primary attraction in host recognition of coniferous bark beetles and bark weevils (Coleoptera: scolytidae and curculionidae)," *Journal of Applied Entomology*, vol. 115, no. 2, pp. 155–169, 1993.

[5] V. E. J. Baader, "*Pityogenes* spp. (Col., Scolytidae): untersuchungen über verhaltenssteuernde Duftstoffe und deren Anwendung im Waldschutz," *Journal of Applied Entomology*, vol. 107, no. 1, pp. 1–31, 1989.

[6] W. Francke, J. Bartels, H. Meyer et al., "Semiochemicals from bark beetles: new results, remarks, and reflections," *Journal of Chemical Ecology*, vol. 21, no. 7, pp. 1043–1063, 1995.

[7] J. A. Byers, Q. H. Zhang, and G. Birgersson, "Strategies of a bark beetle, *Pityogenes bidentatus*, in an olfactory landscape," *Naturwissenschaften*, vol. 87, no. 11, pp. 503–507, 2000.

[8] J. A. Byers, Q. H. Zhang, and G. Birgersson, "Avoidance of nonhost plants by a bark beetle, *Pityogenes bidentatus*, in a forest of odors," *Naturwissenschaften*, vol. 91, no. 5, pp. 215–219, 2004.

[9] A. M. El-Sayed and J. A. Byers, "Inhibitory effect of monoterpenes on response of *Pityogenes bidentatus* to aggregation pheromone released by piezoelectric sprayer for precision release of semiochemicals," *Journal of Chemical Ecology*, vol. 26, no. 8, pp. 1795–1809, 2000.

[10] J. H. Tumlinson, D. D. Hardee, R. C. Gueldner, A. C. Thompson, P. A. Hedin, and J. P. Minyard, "Sex pheromones produced by male boll weevil: isolation, identification, and synthesis," *Science*, vol. 166, no. 3908, pp. 1010–1012, 1969.

[11] J. A. Byers, "Host tree chemistry affecting colonization in bark beetles," in *Chemical Ecology of Insects 2*, R. T. Cardé and W. J. Bell, Eds., pp. 154–213, Chapman and Hall, New York, NY, USA, 1995.

[12] J. A. Byers and G. Birgersson, "Pheromone production in a bark beetle independent of myrcene precursor in host pine species," *Naturwissenschaften*, vol. 77, no. 8, pp. 385–387, 1990.

[13] V. Thoss and J. A. Byers, "Monoterpene chemodiversity of ponderosa pine in relation to herbivory and bark beetle colonization," *Chemoecology*, vol. 16, no. 1, pp. 51–58, 2006.

[14] J. A. Byers, B. S. Lanne, J. Löfqvist, F. Schlyter, and G. Bergström, "Olfactory recognition of host-tree susceptibility by pine shoot beetles," *Naturwissenschaften*, vol. 72, no. 6, pp. 324–326, 1985.

[15] J. A. Byers, B. S. Lanne, and J. Löfqvist, "Host tree unsuitability recognized by pine shoot beetles in flight," *Experientia*, vol. 45, no. 5, pp. 489–492, 1989.

[16] B. S. Lanne, F. Schlyter, J. A. Byers et al., "Differences in attraction to semiochemicals present in sympatric pine shoot beetles, *Tomicus minor* and *T. piniperda*," *Journal of Chemical Ecology*, vol. 13, no. 5, pp. 1045–1067, 1987.

[17] J. A. Byers, "Attraction of bark beetles, *Tomicus piniperda*, *Hylurgops palliatus*, and *Trypodendron domesticum* and other insects to short-chain alcohols and monoterpenes," *Journal of Chemical Ecology*, vol. 18, no. 12, pp. 2385–2402, 1992.

[18] J. A. Byers, G. Birgersson, J. Löfqvist, and G. Bergström, "Synergistic pheromones and monoterpenes enable aggregation and host recognition by a bark beetle, *Pityogenes chalcographus*," *Naturwissenschaften*, vol. 75, no. 3, pp. 153–155, 1988.

[19] J. A. Byers, G. Birgersson, J. Löfqvist, M. Appelgren, and G. Bergström, "Isolation of pheromone synergists of bark beetle, *Pityogenes chalcographus*, from complex insect-plant odors by fractionation and subtractive-combination bioassay," *Journal of Chemical Ecology*, vol. 16, no. 3, pp. 861–876, 1990.

[20] W. D. Bedard, P. E. Tilden, D. L. Wood, R. M. Silverstein, R. G. Brownlee, and J. O. Rodin, "Western pine beetle: field response to its sex pheromone and a synergistic host terpene, myrcene," *Science*, vol. 164, no. 3885, pp. 1284–1285, 1969.

[21] R. A. Werner, "Response of the beetle, *Ips grandicollis*, to combinations of host and insect produced attractants," *Journal of Insect Physiology*, vol. 18, no. 7, pp. 1403–1412, 1972.

[22] J. A. Rudinsky, M. M. Furniss, L. N. Kline, and R. F. Schmitz, "Attraction and repression of *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae) by three synthetic pheromones in traps in Oregon and Idaho," *Canadian Entomologist*, vol. 104, no. 6, pp. 815–822, 1972.

[23] N. Erbilgin and K. F. Raffa, "Opposing effects of host monoterpenes on responses by two sympatric species of bark beetles to their aggregation pheromones," *Journal of Chemical Ecology*, vol. 26, no. 11, pp. 2527–2548, 2000.

[24] N. Erbilgin, J. S. Powell, and K. F. Raffa, "Effect of varying monoterpene concentrations on the response of *Ips pini* (Coleoptera: Scolytidae) to its aggregation pheromone: implications for pest management and ecology of bark beetles," *Agricultural and Forest Entomology*, vol. 5, no. 4, pp. 269–274, 2003.

[25] R. F. Billings, "Southern pine bark beetles and associated insects: effects of rapidly-released host volatiles on response to aggregation pheromones," *Journal of Applied Entomology*, vol. 99, no. 5, pp. 483–491, 1985.

[26] J. C. Dickens, R. F. Billings, and T. L. Payne, "Green leaf volatiles interrupt aggregation pheromone response in bark beetles infesting southern pines," *Experientia*, vol. 48, no. 5, pp. 523–524, 1992.

[27] L. M. Schroeder, "Olfactory recognition of nonhosts aspen and birch by conifer bark beetles *Tomicus piniperda* and *Hylurgops palliatus*," *Journal of Chemical Ecology*, vol. 18, no. 9, pp. 1583–1593, 1992.

[28] I. M. Wilson, J. H. Borden, R. Gries, and G. Gries, "Green leaf volatiles as antiaggregants for the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae)," *Journal of Chemical Ecology*, vol. 22, no. 10, pp. 1861–1875, 1996.

[29] A. Guerrero, J. Feixas, J. Pajares, L. J. Wadhams, J. A. Pickett, and C. M. Woodcock, "Semiochemically induced inhibition of behaviour of *Tomicus destruens* (Woll.) (Coleoptera: Scolytidae)," *Naturwissenschaften*, vol. 84, no. 4, pp. 155–157, 1997.

[30] J. H. Borden, L. J. Chong, A. Savoie, and I. M. Wilson, "Responses to green leaf volatiles in two biogeoclimatic zones by striped ambrosia beetle, *Trypodendron lineatum*," *Journal of Chemical Ecology*, vol. 23, no. 11, pp. 2479–2491, 1997.

[31] J. H. Borden, I. M. Wilson, R. Gries, L. J. Chong, H. D. Pierce, and G. Gries, "Volatiles from the bark of trembling aspen, *Populus tremuloides* Michx. (Salicaceae) disrupt secondary attraction by the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae)," *Chemoecology*, vol. 8, no. 2, pp. 69–75, 1998.

[32] E. K. Deglow and J. H. Borden, "Green leaf volatiles disrupt and enhance response to aggregation pheromones by the ambrosia beetle, *Gnathotrichus sulcatus* (Coleoptera: Scolytidae)," *Canadian Journal of Forest Research*, vol. 28, no. 11, pp. 1697–1705, 1998.

[33] J. A. Byers, Q. H. Zhang, F. Schlyter, and G. Birgersson, "Volatiles from nonhost birch trees inhibit pheromone response in spruce bark beetles," *Naturwissenschaften*, vol. 85, no. 11, pp. 557–561, 1998.

[34] Q. H. E. Zhang, G. Birgersson, J. Zhu, C. Löfstedt, J. Löfqvist, and F. Schlyter, "Leaf volatiles from nonhost deciduous trees: variation by tree species, season and temperature, and electrophysiological activity in *Ips typographus*," *Journal of Chemical Ecology*, vol. 25, no. 8, pp. 1923–1943, 1999.

[35] Q. H. Zhang, F. Schlyter, and P. Anderson, "Green leaf volatiles interrupt pheromone response of spruce bark beetle, Ips typographus," *Journal of Chemical Ecology*, vol. 25, no. 12, pp. 2847–2861, 1999.

[36] D. P. W. Huber, R. Gries, J. H. Borden, and H. D. Pierce Jr., "Two pheromones of coniferophagous bark beetles found in the bark of nonhost angiosperms," *Journal of Chemical Ecology*, vol. 25, no. 4, pp. 805–816, 1999.

[37] T. M. Poland and R. A. Haack, "Pine shoot beetle, *Tomicus piniperda* (Col., Scolytidae), responses to common green leaf volatiles," *Journal of Applied Entomology*, vol. 124, no. 2, pp. 63–69, 2000.

[38] J. A. Byers, F. Schlyter, G. Birgersson, and W. Francke, "*E*-myrcenol in *Ips duplicatus*: an aggregation pheromone component new for bark beetles," *Experientia*, vol. 46, no. 11-12, pp. 1209–1211, 1990.

[39] J. A. Byers, D. L. Wood, L. E. Browne, R. H. Fish, B. Piatek, and L. B. Hendry, "Relationship between a host plant compound, myrcene and pheromone production in the bark beetle, *IPS paraconfusus*," *Journal of Insect Physiology*, vol. 25, no. 6, pp. 477–482, 1979.

[40] J. A. Byers and D. L. Wood, "Interspecific inhibition of the response of the bark beetles, *Dendroctonus brevicomis* and *Ips paraconfusus*, to their pheromones in the field," *Journal of Chemical Ecology*, vol. 6, no. 1, pp. 149–164, 1980.

[41] J. A. Byers and D. L. Wood, "Interspecific effects of pheromones on the attraction of the bark beetles, *Dendroctonus brevicomis* and *Ips paraconfusus* in the laboratory," *Journal of Chemical Ecology*, vol. 7, no. 1, pp. 9–18, 1981.

[42] J. A. Byers, "Bark beetle conversion of a plant compound to a sex-specific inhibitor of pheromone attraction," *Science*, vol. 220, no. 4597, pp. 624–626, 1983.

[43] L. E. Browne, M. C. Birch, and D. L. Wood, "Novel trapping and delivery systems for airborne insect pheromones," *Journal of Insect Physiology*, vol. 20, no. 1, pp. 183–193, 1974.

[44] F. Schlyter, G. Birgersson, J. A. Byers, J. Löfqvist, and G. Bergström, "Field response of spruce bark beetle, *Ips typographus*, to aggregation pheromone candidates," *Journal of Chemical Ecology*, vol. 13, no. 4, pp. 701–716, 1987.

[45] H. A. Moeck, D. L. Wood, and K. Q. Lindahl, "Host selection behavior of bark beetles (Coleoptera: Scolytidae) attacking *Pinus ponderosa*, with special emphasis on the western pine beetle, *Dendroctonus brevicomis*," *Journal of Chemical Ecology*, vol. 7, no. 1, pp. 49–83, 1981.

[46] J. A. Byers, "Effects of attraction radius and flight paths on catch of scolytid beetles dispersing outward through rings of pheromone traps," *Journal of Chemical Ecology*, vol. 25, no. 5, pp. 985–1005, 1999.

[47] J. A. Byers, "An encounter rate model of bark beetle populations searching at random for susceptible host trees," *Ecological Modelling*, vol. 91, no. 1–3, pp. 57–66, 1996.

[48] A. Bakke, "Inhibition of the response in *Ips typographus* to the aggregation pheromone: field evaluation of verbenone and ipsenol," *Zeitschrift für Angewandte Entomologie*, vol. 92, no. 2, pp. 172–177, 1981.

[49] D. Klimetzek, J. Kohler, J. P. Vite, and U. Kohnle, "Dosage response to ethanol mediates host selection by "secondary" bark beetles," *Naturwissenschaften*, vol. 73, no. 5, pp. 270–272, 1986.

[50] J. A. Byers, "Avoidance of competition by spruce bark beetles, Ips typographus and *Pityogenes chalcographus*," *Experientia*, vol. 49, no. 3, pp. 272–275, 1993.

[51] J. A. Byers, "Sex-specific responses to aggregation pheromone Regulation of colonization density in the bark beetle *Ips paraconfusus*," *Journal of Chemical Ecology*, vol. 9, no. 1, pp. 129–142, 1983.

[52] J. A. Byers, D. L. Wood, J. Craig, and L. B. Hendry, "Attractive and inhibitory pheromones produced in the bark beetle, *Dendroctonus brevicomis*, during host colonization: regulation of inter- and intraspecific competition," *Journal of Chemical Ecology*, vol. 10, no. 6, pp. 861–877, 1984.

Hindawi Publishing Corporation
Psyche
Volume 2012, Article ID 532652, 14 pages
doi:10.1155/2012/532652

Research Article

Pheromone Production, Attraction, and Interspecific Inhibition among Four Species of *Ips* Bark Beetles in the Southeastern USA

Göran Birgersson,[1] Mark J. Dalusky,[2] Karl E. Espelie,[2] and C. Wayne Berisford[2]

[1] *Division of Chemical Ecology, Department of Plant Protection Biology, Swedish University of Agricultural Sciences, P.O. Box 102, 230 53 Alnarp, Sweden*
[2] *Department of Entomology, University of Georgia, Athens, GA 30602, USA*

Correspondence should be addressed to Göran Birgersson, goeran.birgersson@slu.se

Received 9 December 2011; Accepted 26 January 2012

Academic Editor: John A. Byers

Hindgut volatiles from attacking, unmated males of *Ips avulsus, I. calligraphus, I. grandicollis*, and *I. pini* were analyzed by combined gas chromatography and mass spectrometry. Based on the quantitative identifications of hindguts and subsequent individual aerations, baits were formulated and a combined species-specific subtractive field bioassay was set up for the four bark beetle species. The bioassays were subtractive for the compounds identified in the hindgut analysis of each species, and volatiles identified in sympatric species were added as potential inhibitors alone and in combination. The trap catches from this bioassay revealed strong interspecific inhibition. The subtractive assays showed that *I. grandicollis* and *I. calligraphus* share (–)-(4*S*)-*cis*-verbenol as one pheromone component, while their second, synergistic pheromone component, (–)-(*S*)-ipsenol in *I. grandicollis* and (±)-ipsdienol in *I. calligraphus*, acts as an interspecific inhibitor to the other species. *I. avulsus* and *I. pini* were found to have very similar production of hindgut volatiles, and both use ipsdienol and lanierone as synergistic pheromone components. No beetle-produced interspecific inhibitor was identified between these two species. Lanierone was found to be an interspecific inhibitor for both *I. calligraphus* and *I. grandicollis*.

1. Introduction

The bark beetle genus of *Ips* is circumpolar, and different species range all over the northern hemisphere. In North America, there are 25 species of *Ips* [1], and of these, four species are indigenous to the Southeastern USA [2–4]. *Ips calligraphus* (Germar) has its range predominantly in the coastal plain, *I. pini* (Say) is limited to the Appalachian mountain range, while *I. avulsus* (Eichhoff) and *I. grandicollis* (Eichhoff) both have a wider range and are found in the entire area. The ranges of *I. calligraphus* and *I. pini* never overlap, while *I. avulsus* and *I. grandicollis* are sympatric with both *I. calligraphus* and *I. pini*. All four species utilize several species of pine (*Pinus*) as their host trees, but there is a slight difference in host preferences. Eastern white pine (*P. strobus* L.) is the major host tree for *I. pini* in the Southeastern USA, while the other three *Ips* species below the mountain range use several species of pine, such as loblolly pine (*P. taeda* L.), shortleaf pine (*P. echinata* Miller), slash pine (*P. elliotii* Engelmann), longleaf pine (*P. palustris* Miller), and Virginia pine (*P. virginiana* Muller).

Bark beetles, like many insects, utilize semiochemicals to find a mate and to concentrate their attacks on suitable host plants [5–7]. As a widespread genus, *Ips* spp. beetles were among the first to be investigated for their pheromones [8]. Vité et al. [9] found that many *Ips* species produced ipsenol, ipsdienol, or *cis*-verbenol as their aggregation pheromones either alone or in combination. At this time, a single compound was often regarded as the entire pheromone of a species [10, 11]. Subsequently, the pheromones of several bark beetle species have been reinvestigated, and new compounds have been identified and shown to be synergistic pheromone components of the aggregation pheromone blends [12–14], increasing trap catches up to more than 25 times. All these reinvestigations clearly showed that bark beetle aggregation pheromones very rarely are found to be

a single component; instead most are based on two or more beetle-produced compounds.

The male-produced pheromone compounds that were initially identified in the four *Ips* species in the southeastern USA were ipsdienol in *I. avulsus*, *I. calligraphus*, and *I. pini*; ipsenol in *I. grandicollis*; and *cis*-verbenol in *I. calligraphus* [9]. Based on range overlap in these species, the three identified compounds will not provide sufficient adequate species isolation on a pheromone basis. The aim of our study was to reinvestigate for the presence of new, male-produced pheromone candidates for all the four species and test them in subtractive trap bioassays in the field. In addition, we tested interspecific attraction and inhibition, the latter being common among sympatric bark beetle species.

2. Materials and Methods

2.1. Biological Material. Beetles were collected as brood in host pine logs and brought in to the laboratory. *Ips avulsus*, *I. calligraphus*, and *I. grandicollis* were all collected in forests around Athens, Georgia, while beetles of *I. pini* were collected in the Nanthahala National Forest, North Carolina. The emerging broods were then allowed to attack fresh pine bolts in the laboratory and kept at ambient temperature and humidity. Georgia beetles were established on loblolly pine, while *I. pini* was reared on Eastern white pine. The logs were predrilled with holes (∅ 2.5 mm) to spread the attacks over the log surface. The attacking *I. avulsus*, *I. calligraphus*, and *I. grandicollis* beetles were cut out of the bark after 36 hours, and single males in nuptial chambers were stored on dry ice until dissection and chemical analysis.

The *I. pini* beetles were collected during August and needed a diapause to produce pheromones. Without this diapause, the male beetles did not produce any pheromone components. In order to mimic a natural diapause, the beetles were allowed to attack a cut bolt of Eastern white pine, and the wood was put in a cold storage (5°C, 80% RH) for three weeks. The beetles were then removed from the bolt and reintroduced on new bolts of Eastern white pine. The reattacking beetles were cut out of their host tree after 48 and 96 hours and kept on dry ice until dissection and chemical analysis.

In order to estimate the pheromone release rates during the second day after the initiation of the attack, beetle attacks were also individually induced to provide sites for aerations. These males were put in predrilled holes in the bark (∅ 2.5 mm) and covered with gelatin capsules (No. 000; Eli Lilly & Co., Indianapolis, IN, USA) that fit into a groove made by a cork borer (∅ 9.5 mm) centered over an attack hole. The gelatin capsule prevented the males from escaping during the initiation of the attack. During the aeration, the gelatin capsule was removed and a cut piece of a Pasteur pipette was fitted over the entrance hole and was held firmly to the bark with a rubber band. A Teflon tube (55 mm × ∅ 3 mm) filled with Porapak Q (65 mg, mesh 60–80; Supelco, Bellefonte, PA, USA) fitted in the constraints of the Pasteur pipette, modified from Birgersson and Bergström [15]. Airflow of 50 mL/min was achieved with battery-operated pumps (Gilian HFS 513A; Gilian Instrument Corp., West Caldwell, NJ, USA), and collections continued for 3 hours. Similar aerations were also collected in the field from individual attacks, to compare the release rates of pheromone in the laboratory with those in the field. Aeration columns were kept in Nalgene cryogenic vials (Nalge Nunc Internat., Rochester, NY, USA) on dry ice or in a –83°C freezer until extraction and chemical analysis.

2.2. Chemical Analysis. Beetles kept on dry ice were allowed to thaw and immediately the hindgut was dissected, using a pair of sharp forceps, and transferred to a 0.3 mL Reacti-Vial (Pierce Chem. Comp., Rockford, IL, USA) chilled on dry ice, according to Birgersson et al. [16]. The sex of each beetle was absolutely determined at dissection, by the presence of aedeagus or spermatheca. Extracts were made in batches of 8 to 20 male beetles in 10 μL of redistilled pentane with 10 ng/μL of heptyl acetate (C_7Ac) for extracts of *I. avulsus* and *I. grandicollis*, and 100 ng/μL of C_7Ac for extracts of *I. calligraphus* and *I. pini*, giving 100 ng and 1000 ng of C_7Ac, respectively, as a quantification standard. The hindgut extracts were concentrated to less than 5 μL before the chemical analysis.

2.3. Aerations. The aeration columns were allowed to equilibrate at room temperature and were then eluted with 500 μL diethyl ether into tapered vial inserts (Agilent Technologies), and 1000 ng of C_7Ac was added to each extract as a quantification standard. The extracts were allowed to concentrate in a fume hood to the volume of around 20 μL prior to chemical analysis.

2.4. Chemical Identification and Quantification. All chemical analyses were performed on a combined gas chromatograph and mass spectrometer (GC-MS): Hewlett-Packard (HP) 5890 GC and a HP 5970 MS (nowadays Agilent Technologies, Palo Alto, CA, USA). The GC was equipped with either a 30 m × 0.25 mm fused silica column coated with HP-1 (100% methyl siloxane, df = 0.5 μm: Agilent Technologies) or a 50 m × 0.25 mm fused silica column, coated with HP-FFAP (nitroterphthalic acid modified polyethylene glycol, df = 0.5 μm; Agilent Technologies). Temperature programming was 50°C for 5 minutes, 8°C/min to 225°C, followed by isothermal at 225°C for 10 minutes for both columns. Injector temperature was 200°C and the transfer line was kept at 225°C. Helium was used as mobile phase, at 35 cm/s, and the electron impact (EI mode) mass spectra were obtained at 70 eV. All samples, 2 μL each, were injected manually and splitless for 0.5 minutes. Compounds were identified by their GC retentions times and obtained mass spectra and compared with authentic samples of synthetic references. Mass spectra were also compared to both commercially available MS libraries (NBS and NIST) and to our own MS database.

For chemical analyses two different fused silica columns were used, each with stationary phases of different polarity, to avoid the possible coelution of unknown compounds rendering detection, and identification difficult. Compounds that might coelute on a nonpolar stationary phase, such

Table 1: Compounds used in field bioassays: abbreviation, purity, and source.

Compound	Abbreviation	Purity	Purchased from
2-methyl-3-buten-2-ol	MB	98%	Aldrich Chemical Company Inc., Milwaukee, WI, USA
(±)-ipsdienol	Id	99%	PheroTech Inc., Delta, BC, Canada
(±)-ipsenol	Ie	99%	PheroTech Inc., Delta, BC, Canada
E-myrcenol	EM	99%	Dr. W. Francke, Universität Hamburg, Hamburg, Germany
Geraniol	Ger	99%	Aldrich Inc., Milwaukee, WI, USA
Lanierone	Ln	98%	Dr. H.-E. Högberg, Mid-Swedish University, Sundsvall, Sweden
(−)-(4*S*)-*cis*-verbenol	cV	99%	Aldrich, Milwaukee, WI, USA

as HP-1, most often will separate on a medium polar to polar stationary phase, like HP-FFAP, and *vice versa*. The dual analyses did not show any new compounds on either column. During the manual analysis of the obtained GC-MS chromatograms (HP Standalone data analysis workstation), every single peak in every analysis was checked, in order to identify and confirm the identity of new compounds. In addition, the extracted ion current profiles (EICPs) [17] allowed us to search for compounds identified in the analyses of one species, in the analytical run of another species. The use of EICP also lowered the limit of detection for the compounds to far below 1 ng injected on the GC-MS, and even less per analyzed beetle. This thorough search for new compounds probably allowed us to identify all ecologically relevant compounds in the hindgut extracts. Compounds present in one single analysis, and never found in other analyses, were defined as contaminants, since several analyses were made on each species on each column. Control analyses of extracting solvents also helped us to rule out contaminants.

Quantification of the identified compounds in the analyses was based on standard curves of selected oxygenated monoterpenes. The standard curve covered four orders of magnitude, from 1 ng to 10 μg. For identified compounds, not included in the standard curve, their response factors were assumed to be similar to related compounds in the standard mixture. In aeration analyses, with hundreds of compounds from the host trees, the quantifications were based on prominent MS fragments in the selected male beetle-produced compounds and in the quantification standard, according to Garland and Powell [17] and Dobson [18]. This method gives extracted ion current profiles (EICPs), which increases the signal-to-noise ratio tenfolds, depending on which MS fragment extracted. The EICP-method also allowed us to search for compounds identified from one analysis, in all the other analyses. As controls, both the hindgut extract solvent and the aeration extracting solvent were analyzed for contaminants.

Chiral analyses of ipsdienol, ipsenol, and *cis*-verbenol were made without derivatization of pooled remains of hindgut extract on an HP 5890A GC-FID equipped with a fused silica column (30 m × 0.25 mm) coated with methylated β-cyclodextrin (df = 0.25 μm; cylodex-B J&W Scientific Inc., Folsom, CA, USA), according to König et al. [19]. Injector and detector temperatures were 175°C and 225°C, respectively, and column temperature was held constant at 125°C, with N_2 as carrier gas at 15 cm/s. All samples were injected manually, 2 μL each, and injected splitless for 0.1 minutes.

2.5. Field Bioassays. Multiple subtractive field bioassays [20] were carried out, and the compounds used in these bioassays were selected based on the hindgut analyses made on the attacking males of each species. Compounds identified in sympatric species were added as tentative inhibitors. Release rates of the selected compounds were based on the individual entrance hole aeration analyses, made both from laboratory introduced and naturally attacking bark beetles. The release rates of the baits corresponded to the estimated amounts similar to 500–1000 male attacks on a host tree. All compounds used in the bioassays are listed in Table 1.

The compounds of each bait were mixed and dissolved in nonane and released at the listed hourly rate through 5 cm × 1.5 mm Teflon tubing, lined with a cotton yarn wick, inserted through a hole drilled in the screw top of a 2 mL glass vial [21]. These "wick-baits" were attached to the middle of 12 unit Lindgren multifunnel traps (Phero Tech Inc., Delta, BC, Canada) with binder-clips. The traps within each set were separated by at least 8 meters, twice the distance between attacked and nonattacked trees in the area. Several sets of traps, separated by at least 50 meters, were used in each bioassay. The trapsets were installed on recent clear-cuts within the range of each bark beetle species. The traps were emptied and randomized regularly, either several times a day when any trapping bait had ≥50 beetles, or on a weekly basis, depending on the population density.

The first bioassays, made in July 1990, comprised four "species-specific" pheromone blends and a subtractive assay of six volatiles identified in male hindgut extracts (Table 2). Traps were set up in Francis Marion National Forest, South Carolina, to trap *I. avulsus*, *I. calligraphus*, and *I. grandicollis*, and in Nantahala National Forest, North Carolina, to trap *I. pini*. The results from the subtractive part of this assay clearly showed very strong interspecific inhibition, and that further

Table 2: Pilot study, Francis Marion National Forest, SC, and Nantahala National Forest, NC, July 1990: pheromone candidates released from Wick-baits: for abbreviations, see Table 1. Release rates are given in μg/h.

	MB	Id	Ie	EM	Ger	cV
avulsus-bait	20	20	—	—	—	2.5
calligraphus-bait	—	100	—	—	5.0	50
grandicollis-bait	—	1.0	50	5.0	2.5	2.5
pini-bait	—	250	—	20	10	20
Subtractive assay						
Total blend (TB)	20	250	50	20	10	50
TB-MB	—	250	50	20	10	50
TB-Id	20	—	50	20	10	50
TB-Ie	20	250	—	20	10	50
TB-EM	20	250	50	—	10	50
TB-Ger	20	250	50	20	—	50
TB-cV	20	250	50	20	10	—
Blank	—	—	—	—	—	—

bioassays had to be based on species-specific subtractive assays, with addition of possible interspecific inhibitors.

The total set of the *I. grandicollis* subtractive and additive assay (Table 3) was performed in Oconee National Forest, GA, April 1992. The full blend for *I. avulsus* (Table 3) was bioassayed at Fort Benning, AL, September 1991, in conjunction with the bioassay of *I. calligraphus* (Table 3), but at different sites. The bioassay for *I. pini* (Table 3) was conducted in Rabun Co, GA, June and July 1992, and was checked and randomized on a weekly basis.

2.6. Statistical Design. Completely randomized, Latin square designs were used to position the traps in each field bioassay. Male and female beetle responses were analyzed separately, with the exception of *I. avulsus* trapped on *I. pini* bait (too many beetles trapped for sexing). Trap catches, as percentage catch to each bait in each replicate, were analyzed by ANOVA as square root $(X + 0.5)$ and arcsin square root $(\%X)$ transformations. Treatment means were separated using the Fisher's protected LSD option at $\alpha = 0.01$ when the entry F-statistic was significant at the 0.05 level. All analyses were performed using SAS-PC (SAS-Institute, Carey, NC).

3. Results

3.1. Chemical Analyses. In total, 15 bark beetle-produced, oxygenated compounds were identified and quantified in hindgut extracts from unmated males of the four *Ips* species investigated (Table 4, Figure 1). All the species had either ipsdienol or ipsenol and *cis*-verbenol, which are the most common pheromone components in the genus [9]. *E*-myrcenol was identified in *I. grandicollis* and *I. pini* male hindguts. In addition, geraniol was identified in all species. Lanierone was identified in *I. pini* and *I. avulsus*, and 2-methyl-3-buten-2-ol was identified in *I. avulsus*. Several oxygenated monoterpenes, regarded as detoxification products of host tree monoterpene hydrocarbons, were identified in all species, especially in *I. calligraphus* and *I. pini*. 2-Phenyl ethanol was identified in all species.

The males of *I. pini* were in preparation for their overwintering diapause, when they were collected in August, and produced only trace amounts of oxygenated monoterpenes when put on new logs, of their host tree, Eastern white pine (Figure 2(a)). After the cold stratification, they still did not produce any pheromone components when placed on new logs, but their production of host related oxygenated monoterpenes had increased substantially (Figure 2(b)). This cohort of beetles, when excised and put on new logs did not produce any pheromone for the first days. After 48 hours in new logs they still only contained the oxygenated monoterpenes, related to host tree resin (Figure 2(c), Table 4). However, after 96 hours following cold treatment, they produced the pheromone components ipsdienol and lanierone in large amounts. At this time only trace amounts of host tree-related, oxygenated monoterpenes were detected (Figure 2(d), Table 4).

Chiral analyses were made on ipsdienol, ipsenol, and *cis*-verbenol. All species produced (−)-(*S*)-*cis*-verbenol. Ipsenol in *I. grandicollis* was enantiomerically pure, with 100% (−)-(*S*)-isomer, while the enantiomeric compositions of ipsdienol varied widely among the three species (Table 5). The aerations of male beetles in nuptial chambers showed that the average hourly release of pheromone components closely approximates the average hindgut amounts (Table 4). Based on these results, the field bioassays were set up to release the amount of each compound equal to 500–1000 male attacks.

3.2. Field Bioassays. The results from the pilot bioassay clearly showed that there were too much interspecific inhibitions to do subtractive bioassays of all the identified compounds in one assay. This pilot study was followed by species-specific subtractive bioassays, with compounds identified in sympatric species added as tentative inhibitors.

When ipsenol was subtracted in the *I. grandicollis* subtractive assay, the trap catches was reduced to that of the blank, clearly indicating ipsenol as the key pheromone component in this species (Figure 3(a)). When *cis*-verbenol was omitted, the trap catches of both males and females dropped significantly, but not as much as when ipsenol was excluded. The trap catch of females was significantly lower when *E*-myrcenol was subtracted from the total blend, while the males were not significantly affected. Geraniol does not seem to affect the attraction of either sex. The addition of ipsdienol showed an inhibition to *I. grandicollis*. Lanierone added alone also has a significant negative impact on the trap catches. When both ipsdienol and lanierone were added in combination to the full *I. grandicollis* blend, trap catches were significantly lower, and especially so for males, where the number of beetles trapped was as low as the blank.

Table 3: Subtractive bioassay: pheromone candidates released from Wick-baits:—for abbreviations, see Table 1. Release rates are given in μg/h.

Ips grandicollis bait, used in Oconee National Forest, GA, April 1992.						
	Ie	EM	Ger	cV	Id	Ln
Total blend	250	65	65	250	—	—
– ” – – Ie	—	65	65	250	—	—
– ” – – EM	250	—	65	250	—	—
– ” – – Ger	250	65	—	250	—	—
– ” – – cV	250	65	65	—	—	—
– ” – + Id	250	65	65	250	500	—
– ” – + Ln	250	65	65	250	—	65
– ” – + Id + Ln	250	65	65	250	500	65
Blank	—	—	—	—	—	—
Ips avulsus bait, used at Fort Benning, AL, September, 1991.						
	MB	Id	Ln	cV	Ie	EM
Total blend	500	500	65	250	—	—
– ” – – MB	—	500	65	250	—	—
– ” – – Id	500	—	65	250	—	—
– ” – – Ln	500	500	—	250	—	—
– ” – – cV	500	500	65	—	—	—
– ” – + Ie	500	500	65	250	250	—
– ” – + EM	500	500	65	250	—	65
– ” – + Ie + EM	500	500	65	250	250	65
Blank	—	—	—	—	—	—
Ips calligraphus bait, used at Fort Benning, AL, September 1991.						
	Id	Ger	cV	Ie	EM	Ln
Total blend	500	65	250	—	—	—
– ” – – Id	—	65	250	—	—	—
– ” – – Ger	500	—	250	—	—	—
– ” – – cV	500	65	—	—	—	—
– ” – + Ie	500	65	250	250	—	—
– ” – + EM	500	65	250	—	65	—
– ” – + Ln	500	65	250	—	—	65
– ” – + Ie + EM + Ln	500	65	250	250	65	65
Blank	—	—	—	—	—	—
Ips pini bait, used in Rabun county, GA, June and July 1992.						
	Id	EM	Ger	Ln	cV	Ie
Total blend	500	65	65	65	250	—
– ” – – Id	—	65	65	65	250	—
– ” – – EM	500	—	65	65	250	—
– ” – – Ger	500	65	—	65	250	—
– ” – – Ln	500	65	65	—	250	—
– ” – – cV	500	65	65	65	—	—
– ” – + Ie	500	65	65	65	250	250
Blank	—	—	—	—	—	

There was no attraction by *I. avulsus* to the *I. grandicollis* blend. When ipsdienol was added, there was a weak attraction, while addition of lanierone alone showed no attraction to *I. avulsus*. However, when both ipsdienol and lanierone were added to the full *I. grandicollis* blend, the trap catches of *I. avulsus* peaked (Figure 3(b)). The number of *I. calligraphus* trapped in the bioassay with *I. grandicollis* baits was too low for statistical analysis, even though most *I. calligraphus* were trapped when ipsdienol was added alone to the *I. grandicollis* blend.

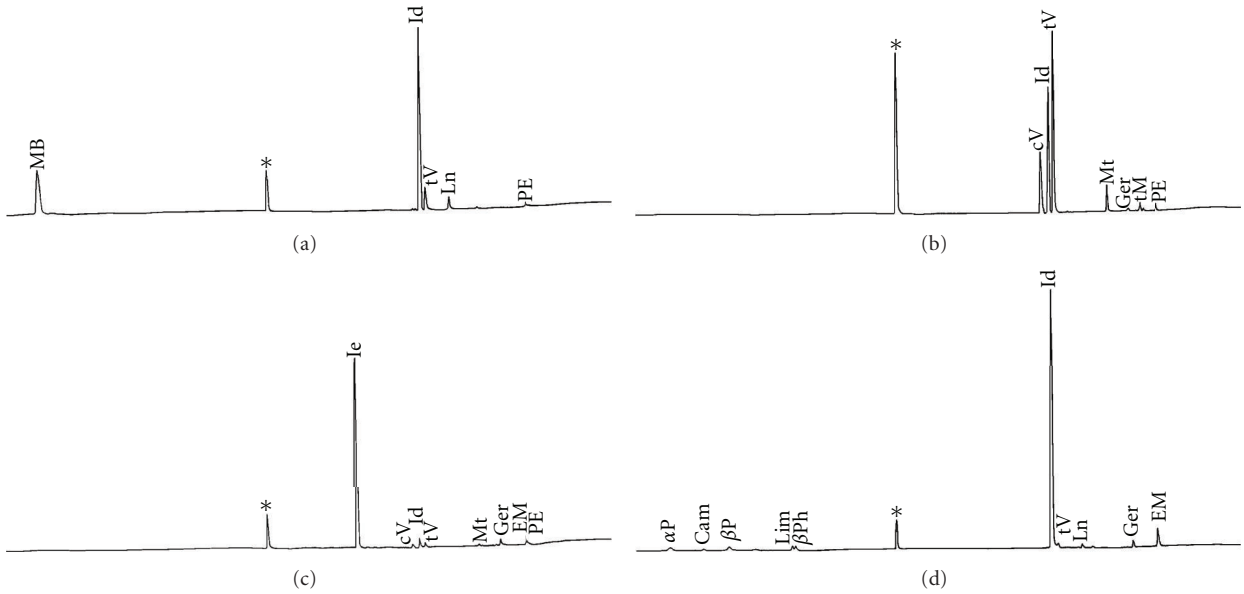


Figure 1: GC chromatograms from GC-MS analyses of male hindgut extracts. MB: 2-methyl-3-buten-2-ol; Id: ipsdienol; Ie: ipsenol; cV: *cis*-verbenol; tV: *trans*-verbenol; Ln: lanierone; Mt: myrtenol; Ger: geraniol; tM: *trans*-myrtanol; EM: *E*-myrcenol; PE: 2-phenyl ethanol; ∗: internal quantification standard, heptyl acetate (C_7Ac) amount varies between sample—see the following (for full list of identified compounds and quantities, see Table 4). (a) *I. avulsus* 15 males: C_7Ac is 100 ng. (b) *I. calligraphus* 4 males: C_7Ac is 1000 ng. (c) *I. grandicollis* 5 males: C_7Ac is 100 ng. (d) *I. pini* 10 males: C_7Ac is 1000 ng.

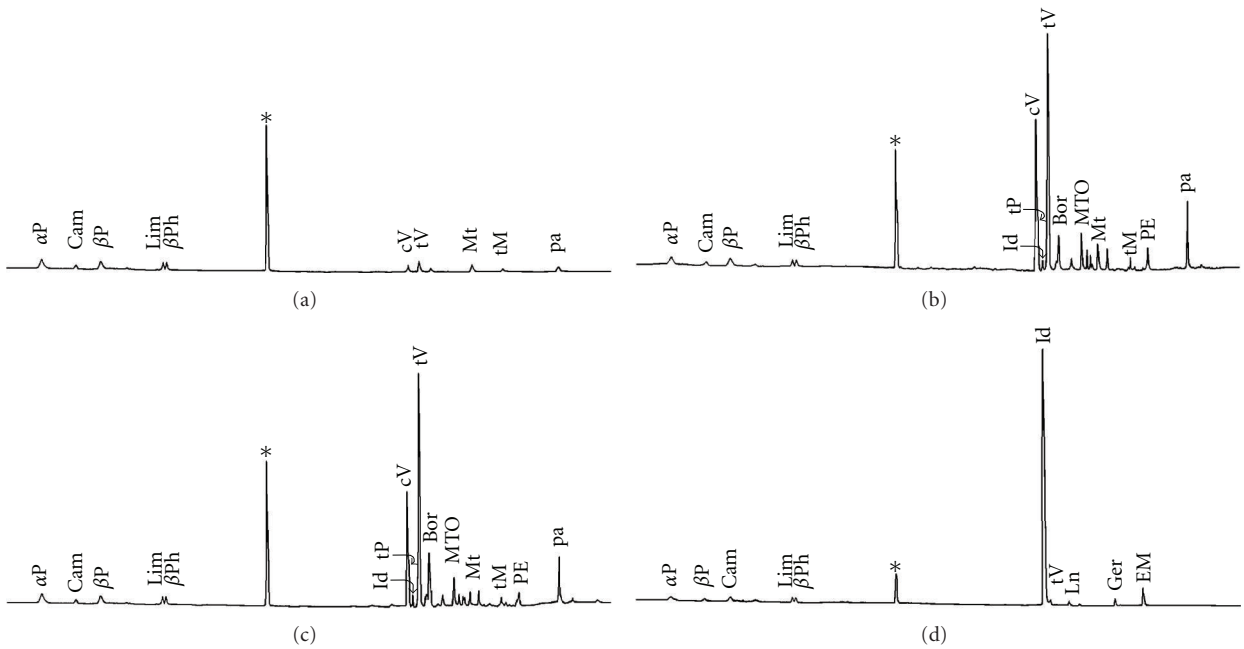


Figure 2: GC chromatograms from GC-MS analyses of *I. pini* male hindgut extracts at different overwintering/diapause status. αP: α-pinene; Cam: camphene; βP: β-pinene; Lim: limonene; βPh: β-phellandrene; Id: ipsdienol; cv: *cis*-verbenol; tP: *trans*-pinocarveol; tV: *trans*-verbenol; Bor: borneol; MTO: oxygenated monoterpenes; Mt: myrtenol; Ger: geraniol; tM: *trans*-myrtanol; EM: *E*-myrcenol; Pa: perilla alcohol; Ln: lanierone; PE: 2-phenyl ethanol ∗: internal quantification standard, heptyl acetate (C_7Ac) amount varies between sample—see the following. (a) Late fall; in diapauses, 5 males; C_7Ac is 100 ng. (b) Overwintering, cut out of bolts; 8 males; C_7Ac is 1000 ng. (c) Overwintering, cut out of bolts, on new bolts 48 hrs; 5 males; C_7Ac is 1000 ng. (d) Overwintering, cut out of bolts, on new bolts 96 hrs; 10 males; C_7Ac is 1000 ng.

Table 4: Average amounts of volatiles identified in hindgut extracts: ng/unmated male.

Compound	*I.avulses*	*I. calligraphus*	*I. grandicollis*	*I. pini*	
				48 h	96 h
232-metylbutenol	20	—	—	—	—
Ipsenol	—	—	120	—	—
ipsdienol	20	275	~0.5	8.1	725
E-myrcenol	—	—	6.0	5.6	61
geraniol	(+)	6.5	3.0	—	20
lanierone	2.0	—	—	7.4	23
cis-verbenol	2.5	135	~0.5	87	6.0
trans-verbenol	7.5	400	~1.0	170	10
myrtenol	1.0	90	~0.5	11	—
trans-pinocarveol	—	11	—	27	—
trans-myrtanol	—	30	—	4.5	—
cis-myrtanol	—	2.0	—	2.5	—
perilla alcohol	—	—	—	34	—
2-phenylethanol	1.0	15	3.0	11	—
borneol	—	—	—	47	—

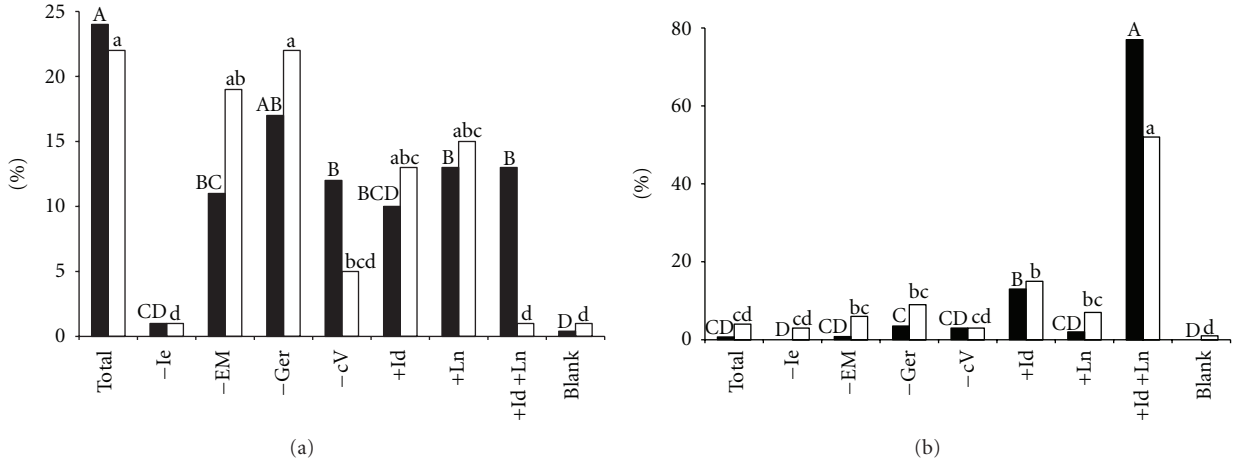


Figure 3: Percentage trap catches in a subtractive and additive field bioassay of *I. grandicollis*-bait: (a) *I. grandicollis* and (b) *I. avulses,* (for bait information, see Table 3). ■ females; □ males. Bars with same letter are not significantly different. $n = 6$; total trap catches: *I. grandicollis* 363 ♀♀, 235 ♂♂; *I. avulsus* 341 ♀♀, 229 ♂♂.

Table 5: Chiral analysis of ipsdienol in three species of *Ips.*

	Ipsdienol	
	(+)-(*S*)	(−)-(*R*)
I. avulsus	85.4%	14.6%
I. pini	61.4%	38.6%
I. calligraphus	21.0%	79.0%

The chemical analysis of hindgut volatiles in unmated males of *I. avulsus* revealed two new pheromone component candidates, 2-methyl-3-buten-2-ol and lanierone. Both of these compounds were included in the field subtractive bioassay, together with ipsdienol and *cis*-verbenol, also identified in the hindgut extracts (Table 4). The results clearly show that ipsdienol is the key pheromone component in this species, as the number of trapped beetles was as low as the blank when this compound was omitted (Figure 4(a)). Lanierone did prove to be a pheromone component of this species, since the trap catch of both sexes were significantly lower without this compound, compared to the full blend. When methylbutenol was excluded, the trap catches were reduced somewhat, but not significantly different from the full blend. The exclusion of *cis*-verbenol had no effect on the trap catch, which suggests that, in *I. avulsus*, this compound is only a detoxification product of the host resin monoterpene (−)-(*S*)-α-pinene, which is unusual for beetles in the genus *Ips* [5, 6]. None of the added compounds, ipsenol and/or *E*-myrcenol, hypothesized to be inhibitory

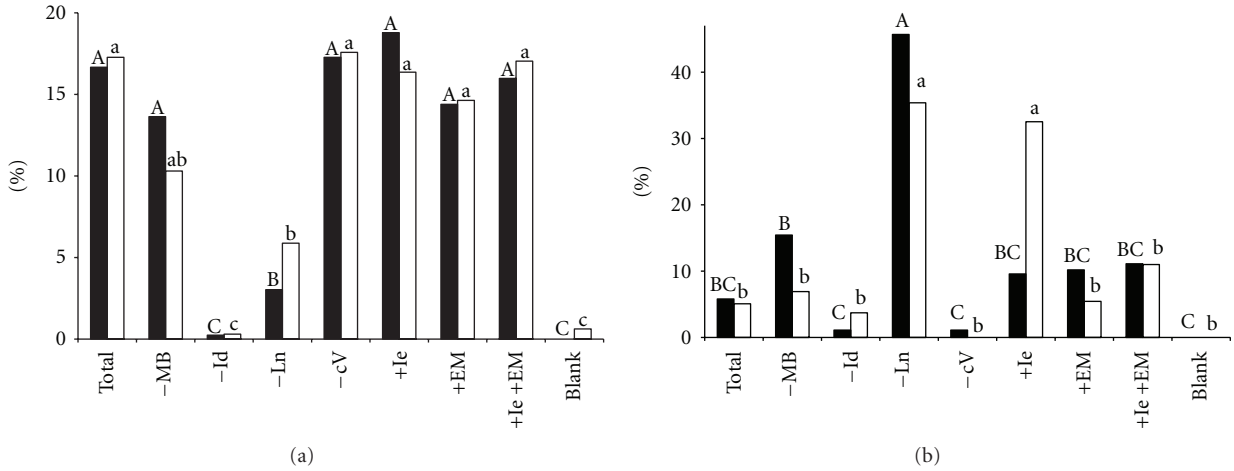


Figure 4: Percentage trap catches in a subtractive and additive field bioassay of *I. avulsus*-bait: (a) *I. avulsus* and (b) *I. calligraphus*. (for bait information, see Table 3). ■ females; □ males. Bars with same letter are not significantly different. $n = 7$; total trap catches: *I. avulsus* 1673 ♀♀, 795 ♂♂; *I. calligraphus* 209 ♀♀, 135 ♂♂.

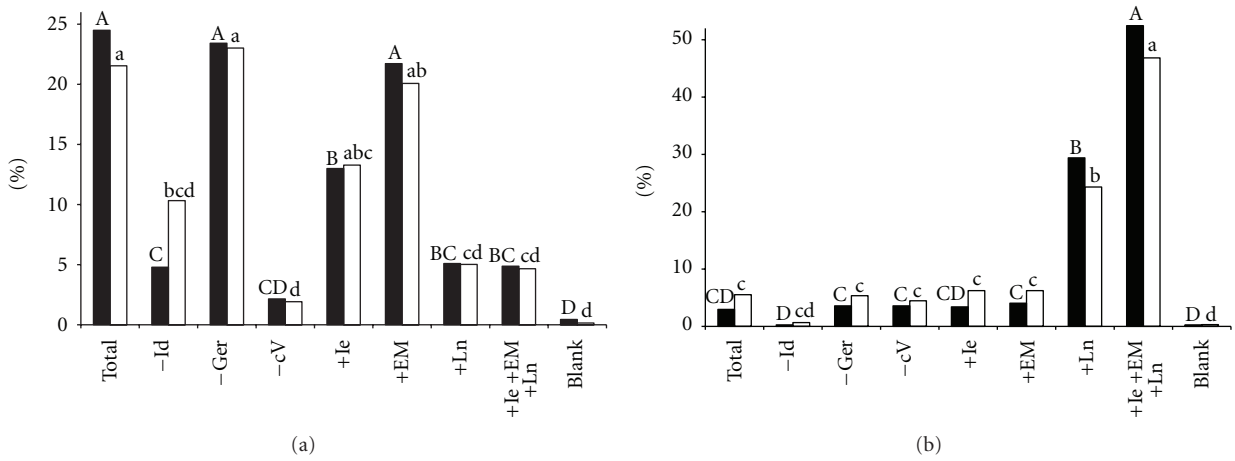


Figure 5: Percentage trap catches in a subtractive and additive field bioassay of *I. calligraphus*-bait: (a) *I. calligraphus*, and (b) *I. avulsus*. (for bait information, see Table 3). ■ females; □ males. Bars with same letter are not significantly different. $n = 6$; total trap catches: *I. calligraphus* 523 ♀♀, 378 ♂♂; *I. avulsus* 225 ♀♀, 183♂♂.

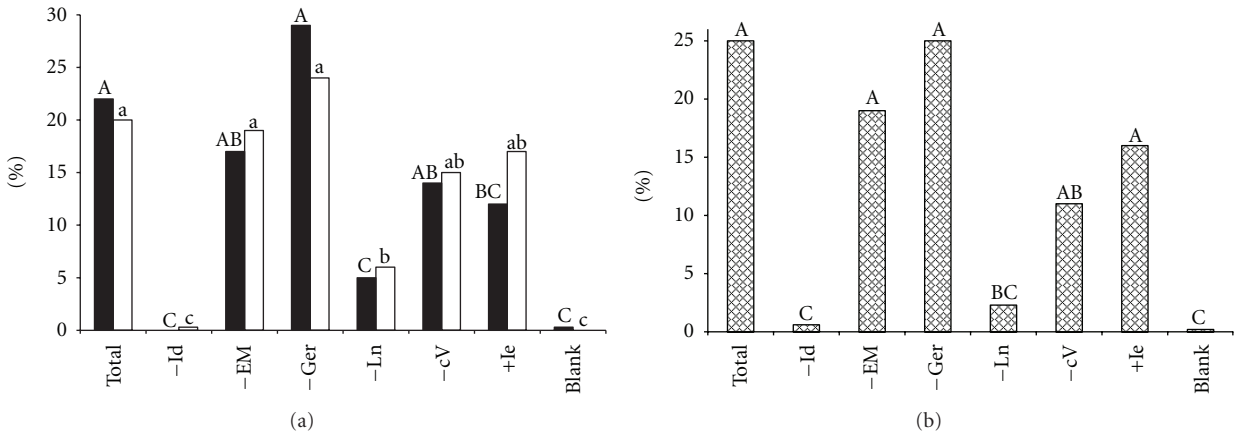


Figure 6: Percentage trap-catches in a subtractive and additive field bioassay of *I. pini*-bait: (a) *I. pini* (b) *I. avulsus*. (for bait information, see Table 3). ■ females; □ males. Bars with same letter are not significantly different. $n = 5$; total trap catches: *I.pini* 602 ♀♀, 296 ♂♂; *I. avulsus* 7,000 ♀♀ + ♂♂.

to this species, had any effect on the trap catches on either sex. Most *I. calligraphus* were trapped when lanierone was omitted from the total blend of the *I. avulsus* bait. This indicates that lanierone is an inhibitor to *I. calligraphus* (Figure 4(b)). No other compound in the *I. avulsus* bait had any significant effect on *I. calligraphus*, even though exclusion of methylbutenol gave a higher percentage trap catch than the total bait, however not significantly. Surprisingly, when ipsenol was added to the bait, the number of trapped *I. calligraphus* males increased significantly, compared to the total blend.

The major pheromone candidates in *I. calligraphus* from the hindgut analyses were ipsdienol and *cis*-verbenol. Both these compounds together with geraniol were tested in the subtractive field bioassay, with ipsenol, *E*-myrcenol, and lanierone as possible interspecific inhibitors (Table 3). Both of the suggested pheromone components proved to be necessary for the pheromone blend of this species, while geraniol had no effect on the trap catches (Figure 5(a)). In this species, *cis*-verbenol seems to be the key pheromone component, with very low trap catches for both sexes, which were not significantly different from the blank, when omitted. Exclusion of ipsdienol significantly reduced the trap catches for both sexes, more for females than for males, but not as much as the exclusion of *cis*-verbenol. The addition of *E*-myrcenol did not affect the trap catches, while both ipsenol and lanierone reduced the number of beetles trapped, significantly so by lanierone for both sexes and by ipsenol for females. When all the inhibitory candidates were added, the result was the same as when lanierone was added alone. *Ips avulsus* shares ipsdienol with *I. calligraphus*, but very few *I. avulsus* beetles were trapped on the full bait (Figure 5(b)), and logically the trap catches were even lower when ipsdienol was omitted. The number of trapped *I. avulsus* increased only when lanierone was added to the *I. calligraphus* bait, and it increased even more when all three putative *I. calligraphus* inhibitors, lanierone, ipsenol, and *E*-myrcenol, were added to the full blend.

Ips pini in Georgia is at its southernmost range and is only found at higher elevations. This species also has a diapause, and beetles in the fall are not supposed to be attracted to pheromone. Therefore the subtractive bioassay of this species was undertaken during the early summer. The bait for the subtractive assay was based on the compounds identified in the hindgut extracts, after the beetles had gone through a cold treatment in the laboratory, to mimic a diapause, and were put on new logs for 96 hours (Table 4; Figures 1(d) and 2(d)). Ipsenol was added as a candidate inhibitor. The key pheromone component of this species is ipsdienol, with almost no beetles trapped when this compound was omitted (Figure 6(a)). The exclusion of lanierone from the full blend resulted in low trap catches, significantly less than the full blend, making this compound a pheromone component, synergistically active with ipsdienol. When *cis*-verbenol was omitted, there was a slight but nonsignificant reduction in the trap catch. The subtraction of either *E*-myrcenol or geraniol had no effect on the trap catches, when compared to the total blend. The addition of ipsenol significantly lowered trap catch of females, while the reduction in trapped males was not significant. *Ips avulsus* was also trapped in this assay. However, the number of trapped *I. avulsus* was too high for sexing the beetles (~7000); therefore only results from combined sexes are presented. The trap catch pattern for *I. avulsus* is very similar to that for *I. pini* (Figure 6(b)). When ipsdienol is omitted, almost no beetles were trapped, and the subtraction of lanierone also gave significantly lower trap catches, compared to the total blend. Reduced trap catch due to exclusion of *cis*-verbenol was not significant. Addition of ipsenol did not have any significant effect on the number of beetles trapped.

4. Discussion

The chemical analyses revealed new pheromone component candidates in most species. However, these compounds have earlier been identified and found active in other species of *Ips*. 2-Methyl-3-buten-2-ol in *I. avulsus* was first identified in *I. typographus* by Bakke et al. [22], was found to be a synergist to *cis*-verbenol for that species, and was used in mass trapping programs in Scandinavia and Germany [23–25]. *E*-Myrcenol in *I. grandicollis* and *I. pini* was first identified as a bark beetle pheromone component in *I. duplicatus* [13]. The compound was inactive by itself but increased the trap catches of *I. duplicatus* 25-fold compared to ipsdienol alone. Teale et al. [14] identified lanierone in *I. pini*, in a rigorous aeration, fractionation, and bioassay study. Lanierone was present in *I. avulsus* male hindgut extracts, on the average 2 ng per beetle. When the males of *I. pini* produced their pheromone after cold treatment, there were 10 times more lanierone than found in *I. avulsus*. Besides the new pheromone components, geraniol was identified in all four species of *Ips*, but no pheromonal activity could be found. Therefore, this compound must be regarded as a precursor to the *de novo* produced ipsenol, ipsdienol, and *E*-myrcenol.

The decision regarding which of the identified compounds to include in the subtractive assay of each species was based on three factors. First, each subtractive bioassay should only include compounds that are produced by the species to test. Second, compounds not included in the specific subtractive assay of one species, but included as a pheromone candidate in another species-specific assay, can be added as an inhibitor candidate and should be added individually and in combination. Third, the basis for exclusion of compounds was those regarded as host tree resin detoxification products, that is, oxygenated monoterpenes. This relationship was partly based on the compounds present in *I. pini* during the period after cold treatment and the start of their pheromone production (Table 4 and Figures 2(c) and 2(d)) and in part on experiences with analyses of *Ips* species and other bark beetles [21, 26]. Large quantities of these monoterpene alcohols reflect the amount of resin the beetles have encountered during their excavation of their nuptial chambers. The only exception in this group is *cis*-verbenol, which has been proven as a pheromone component for several species of *Ips* [5, 6], and was therefore included in the bioassays. The last compound excluded was 2-phenyl

ethanol, which has been identified in male hindgut extracts of several genera and species of bark beetles. The activity of this compound is still very unclear, as it has been reported to be an attractant synergist [27], or to have no behavioral impact [26], or even reduce the attraction to pheromone components [28, 29].

4.1. Subtractive Bioassays of Aggregation Pheromones. For *I. grandicollis*, the major result is that ipsenol and *cis*-verbenol are necessary for the pheromone of this species (Figure 3(a)). The subtraction of *E*-myrcenol gave different results in males and females. The subtraction had no significant effect on the trap catches of males but significantly reduced the trap catches of females. Therefore, *E*-myrcenol might act more as a sex pheromone, rather than an aggregation pheromone component.

Two new compounds were identified in *I. avulsus*: 2-methyl-3-buten-2-ol and lanierone. The subtractive bioassay (Figure 4(a)) clearly shows that the aggregation pheromone is made up of ipsdienol and lanierone. When either of these two compounds was excluded, the trap catches dropped significantly for both sexes. However, when the methylbutenol was subtracted, neither of the sexes showed significantly reduced attraction to the bait, even though overall trap catches were reduced. Therefore, we cannot rule out the possibility that methylbutenol might be a behaviorally active compound. 2-Methyl-3-buten-2-ol in *I. typographus* has been found to have a close range attractance [30], apparently promoting landing, as hypothesized by Dickens [31], based on electrophysiological studies. The exclusion of *cis*-verbenol had no effect on the number of beetles trapped, indicating that *cis*-verbenol is not included in the pheromone of *I. avulsus* (Figure 4).

Males of *I. calligraphus* produced very few compounds besides the oxygenated monoterpenes related to the host tree resin. The only two compounds that had an effect on the trap catches were ipsdienol and *cis*-verbenol. Both these compounds are needed for attraction, since subtracting either of them reduces the trap catches dramatically, especially so for *cis*-verbenol (Figure 5(a)).

Ips pini has a wide range in North America, covering the western and northern states of the USA and the southern parts of the Canadian provinces, and following the Appalachian range into the southeastern USA [2]. This species has been reported to be attracted to different pheromone blends in different regions within its range. For many years, this species was thought to have a one compound pheromone, ipsdienol [32, 33]. A decade later, *E*-myrcenol was identified [34] and reported to be behaviorally active in British Columbia [35]. Further investigations on the aggregation pheromone in the New York population of this species resulted in the identification of lanierone another year later [14]. The male beetles analyzed in this study produce, besides ipsdienol, both *E*-myrcenol and lanierone, together with *cis*-verbenol. The subtractive bioassay clearly showed ipsdienol and lanierone to be critical compounds for the aggregation pheromone of *I. pini*, in the present Southeastern population. Lanierone alone does not appear to be attractive alone, as the exclusion of ipsdienol reduced catches to the level of the blank. The exclusion of either *E*-myrcenol or *cis*-verbenol did not have any significant effect on the trap catches, even though somewhat fewer beetles were trapped when *cis*-verbenol was omitted (Figure 6).

4.2. Interspecific Attraction and Inhibition. The results from the pilot subtractive bioassay showed that interspecific inhibition among the four species of *Ips* is very strong, as the most beetles were trapped when one of the compounds was omitted from the total subtractive blend. This was the major reason for performing subtractive bioassays for each species and addition of compounds from sympatric species alone and together to test their effects as inhibitors.

The major difference among the species is the presence of ipsdienol or ipsenol. *Ips grandicollis* is the only species that utilizes ipsenol in its aggregation pheromone, while all the other species produce ipsdienol, as one major aggregation pheromone component. When ipsdienol or lanierone was added individually to the *I. grandicollis* bait, the trap catches were reduced similar to when *cis*-verbenol was omitted (Figure 3(a)). When both ipsdienol and lanierone were added together to the total blend, there were significant reductions in trap catches for both sexes of *I. grandicollis*, and especially so for males. Therefore, male *I. grandicollis* beetles are less likely to land on a host tree from which ipsdienol and/or lanierone are emitted.

In all species-specific bioassays, sympatric beetles were attracted as well as the target bark beetle species, sometimes to the subtractive part, sometimes to the additive part. As the most abundant species, *I. avulsus* was always attracted to the same treatment in the bioassays. In the *I. grandicollis* assay, *I. avulsus* was only trapped when ipsdienol was added (Figure 3(b)), and especially so in combination with lanierone. This clearly indicates that there is no cross-attraction from *I. avulsus* to the pheromone of *I. grandicollis*. In addition, *I. calligraphus* was only attracted in the *I. grandicollis* assay when ipsdienol was added alone.

The three other species of *Ips*, excluding *I. grandicollis*, all have ipsdienol in common. Therefore, more of interspecific attraction will be likely. Accordingly, few beetles of *I. calligraphus* were attracted to the full bait of *I. avulsus*. Only when lanierone was omitted, a significant number of *I. calligraphus* were attracted to the bait (Figure 4(b)). Furthermore, when lanierone were added to the *I. calligraphus* blend, there was a significantly reduced number of *I. calligraphus* attracted to the traps (Figure 5(a)). When ipsenol was added to the *I. calligraphus* bait, the reduction in trapped *I. calligraphus* was lower, but still significant. On the other hand, when *I. avulsus* was exposed to the *I. calligraphus* blend, a low number of beetles were trapped (Figure 5(b)), independent of which compound was subtracted. This indicates that there is no strong attraction to "the total blend", nor is there any inhibitor to *I. avulsus* in the *I. calligraphus* aggregation pheromone blend. When ipsdienol was omitted, the trap catches were even lower. However, when lanierone was added to the *I. calligraphus* bait, significantly more *I. avulsus* were attracted, and, surprisingly, to an even higher level

of significance when all three proposed inhibitors to *I. calligraphus*, ipsenol, *E*-myrcenol, and lanierone, were added to the full blend.

Two species, *I. avulsus* and *I. pini*, were found to have very similar aggregation pheromone blends, and their responses were also very similar. Unfortunately, no *I. pini* was trapped in the *I. avulsus* bioassay, since it was conducted on the coastal plain, outside the range of *I. pini*. However, large numbers of *I. avulsus* were trapped in the bioassay for *I. pini*. In fact, the number of trapped *I. avulsus* outnumbered the *I. pini*, 7000 to 900. The trapping pattern for the *I. avulsus* beetles is almost identical to the pattern for *I. pini* in its bioassay, indicating that these two species behave similarly to the compounds tested in this study. In addition, these two species both belong to the *avulsus* group (group IV) of the genus *Ips*, according to Hopping [2].

Ips avulsus and *I. pini* have overlapping ranges only in the southern Appalachians [36, 37], and they may use spatial separations on the host tree. *Ips avulsus* is usually on the limbs and the top, along with *Pityogenes hopkinsi* Swaine, and *I. pini* is usually on the trunk. Neither of these *Ips* species is attracted to the pheromone of *P. hopkinsi* [38].

4.3. Production of "Inactive Compounds". Why do bark beetles produce compounds not included in their aggregation pheromone? There are different reasons why some compounds identified in male hindgut extracts are not used in their aggregation pheromone. Even though most of the compounds identified in the analyses were monoterpene alcohols, their biosynthetic backgrounds differ. Some are detoxification products of toxic monoterpene hydrocarbons in the host tree resin. Very few detoxification products are used by bark beetles as pheromone components. For *Ips* beetles only *cis*-verbenol of all the detoxification products has been proven to be a component of their aggregation pheromones. The presence of this compound is tightly linked to the amount of (−)-(*S*)-*α*-pinene in the host resin [39, 40]. The chirality of *cis*-verbenol in *Ips* beetles is reported to be the (−)-(4*S*)-isomer. On the other hand, the (+)-(*R*)-enantiomer of *α*-pinene is always hydroxylated to (+)-(4*S*)-*trans*-verbenol, with no behavioral effect in *Ips* bark beetles [26]. All other cyclic and bicyclic monoterpene alcohols identified in this study (Table 4) have direct connections to monoterpene hydrocarbons in the host tree resin (Bergquist and Birgersson, unpublished).

On the other hand, the noncyclic monoterpene alcohols identified in this study are not detoxification products of host tree monoterpene hydrocarbons. Instead, geraniol, ipsdienol, ipsenol, and *E*-myrcenol are all produced *de novo* through the mevalonic pathway by the beetles [41–43]. The three compounds earlier found to be active in several *Ips* species are all produced via geraniol as a precursor. While either ipsdienol or ipsenol was found to be active in the species investigated here, no strong activity could be assigned to *E*-myrcenol, other than reduce attraction of female *I. grandicollis* when omitted (Figure 3(a)), even though it was identified in both *I. grandicollis* and *I. pini*. In British Columbia, *I. pini* were found to produce and use *E*-myrcenol as a pheromone component [34, 35]. However, the activity of this compound is still not clear, as trap catches were reduced when it was added to ipsdienol, but attacks increased when the compound was applied to pine logs [35]. It is not known if all populations of *I. pini* produce and are able to perceive *E*-myrcenol. The southeastern *I. pini* produce *E*-myrcenol, but they apparently do not use it as an aggregation pheromone component. This is probably not a by-product from the production of ipsdienol since *I. calligraphus*, which produces large amounts of ipsdienol, does not produce any *E*-myrcenol, while *I. grandicollis*, which produces ipsenol, was found to also produce *E*-myrcenol.

The small amounts of ipsdienol found in *I. grandicollis* are probably a by-product from the production of ipsenol, as ipsdienol is supposedly an intermediate between geraniol and ipsenol in the biosynthetic pathway [44]. Besides the noncyclic monoterpene alcohols, the 2-methyl-3-buten-2-ol, identified in *I. avulsus*, is also produced *de novo* via the mevalonic pathway [45, 46]. This compound was first identified in *I. typographus* by Bakke et al. [22] and is used by this species as a pheromone component [10, 26]. The biosynthetic pathway for lanierone is not yet elucidated.

4.4. Chirality of Compounds. Several bioassayed compounds are chiral, that is, having two enantiomeric isomers. Ipsenol and *cis*-verbenol have been identified as only one enantiomer in *Ips* bark beetles, (−)-(*S*)-ipsenol and (–)-(4*S*)-*cis*-verbenol, and the opposite enantiomers have never been shown to have any effect on the attraction. The chirality of ipsdienol, on the other hand, varies among species and populations within the same species [47, 48]. The chirality of ipsdienol varied between the three species of *Ips* (Table 5), but no species produced enantiomerically pure ipsdienol, nor did any species have racemic ipsdienol, similar to analyses by Kohnle et al. [49] and Seybold et al. [48]. However, all bioassays were done with racemic ipsdienol. There could have been different trap catches if the enantiomeric composition found in each species had been used in the subtractive assays, but at the time the main focus was to identify which compounds each species used in their aggregation pheromones. We do not think that the opposite enantiomer in the racemic ipsdienol had an inhibitory effect on the response, as none of the species had enantiomeric pure ipsdienol, and *I. avulsus*, which was farthest from racemic mixture (Table 5), was trapped in very high numbers on *I. pini* bait (Figure 6). Now that the active pheromone components are identified, the most attractive chiral composition of ipsdienol can be identified.

Ips pini is known to have a wide variation in the chiral composition of ipsdienol between different populations throughout its range [30, 31, 50, 51]. There is a correlation between ipsdienol chirality and attractivity of lanierone over the geographic range, with lower response to lanierone with a higher percentage of (–)-(*R*)-ipsdienol. However, more chiral analyses [50, 51] than bioassays have been done [52]. California populations of *I. pini* do not respond to lanierone [51, 52]. This shift from lanierone as pheromone component

Table 6: Compounds active in field bioassays to four species of *Ips,* P: produced by males; PC: pheromone component; I: inhibitor.

Compound	P	PC	I	P	PC	I	P	PC	I	P	PC	I
		grandicollis			*calligraphus*			*avulsus*			*pini*	
232-metylbutenol							X	?				
ipsenol	X	X				X						♀
ipsdienol			X	X	X		X	X		X	X	
E-myrcenol	X	♀								X		
geraniol	X			X			X			X		
lanierone			X			X	X	X		X	X	
cis-verbenol	X	X		X	X		X			X	?	

Table 7: Baits suggested for monitoring bark beetles in the genus *Ips* in southeastern USA.

I. grandicollis	Ipsenol, *cis*-verbenol
I. calligraphus	Ipsdienol, *cis*-verbenol
I. avulsus and *I. pini*	Ipsdienol, lanierone

is possibly due to a selection pressure by a clerid predator [51].

4.5. Conclusions. Only a few compounds have been identified as aggregation pheromone components in the genus *Ips*. The four species of *Ips* in the southeastern USA investigated in this study are no exception, as they share most of their compounds with other species. The production of compounds and their use as pheromone components and/or interspecific inhibitors for each species are summarized in Table 6. The only species that has a unique compound is *I. grandicollis*, which is the only one of the species studied to use (−)-(*S*)-ipsenol as a pheromone component. Compounds not produced by one species, but by a sympatric species, can act as an interspecific inhibitor. Therefore it is not possible to make a common bait for monitoring populations of the four southern species of *Ips*. In order to avoid inhibitors, key pheromone components will be left out. If all identified attractants are included, then inhibition for some species will occur.

To monitor the four species of *Ips* in the southeastern USA, three different baits will be necessary: one for *I. grandicollis*, one for *I. calligraphus*, and one for both *I. avulsus* and *I. pini*. These baits (Table 7) will not only be highly attractive to the target species but will also keep the other beetles out, with exception for *I. avulsus* from the *I. calligraphus* bait.

Acknowledgments

The authors would like to thank Tim McClure for sorting the trap catches, Bob Larimore, Charles Ford (in memoriam), Fort Benning, and Joe Dinkens, U.S. Forest Service, for providing field sites, Professor Hans Erik Högberg, Mid-Sweden University, for the synthesis of lanierone; and Dr. Dan R. Miller, Dr. Brian T. Sullivan, and Dr. Fredrik Schlyter for reviewing the manuscript. Results from this study were presented at the IUFRO Joint Conference February 6-11, 1994, Maui, Hawaii, and a brief summary was published in the proceedings as G. Birgersson, M. J. Dalusky, and C. W. Berisford in 1995. Interspecific attraction and inhibition among four species of southeastern *Ips* bark beetles; *in* F. P. Hain, S. M. Salom, W. F. Ravlin, T. L. Payne, and K. F. Raffa (eds.), Behavior, population dynamics and control of forest insects, proceedings of the IUFRO Joint Conference, February, 1994, pp 12–18. The authors of this paper do not have any financial relation with any commercial identity mentioned in this paper.

References

[1] S. L. Wood, "The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph," *Great Basin Naturalist Memoirs*, no. 6, p. 1359, 1982.

[2] G. R. Hopping, "The North American species in groups IV and V of *Ips* de Geer (Coleoptera: Scolytidae)," *Canadian Entomologist*, vol. 96, no. 7, pp. 970–978, 1964.

[3] G. R. Hopping, "The North American species in groups IX of *Ips* de Geer (Coleoptera: Scolytidae)," *Canadian Entomologist*, vol. 97, no. 4, pp. 422–434, 1965.

[4] G. R. Hopping, "The North American species in groups X of *Ips* de Geer (Coleoptera: Scolytidae)," *Canadian Entomologist*, vol. 97, no. 8, pp. 803–809, 1965.

[5] J. H. Borden, "Aggregation pheromones," in *Bark Beetles in North American Conifers*, J. B. Mitton and K. B. Sturgeon, Eds., 1984.

[6] J. H. Borden, "Aggregation pheromones," in *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*, G. A. Kerkut and L. I. Gilbert, Eds., 1985.

[7] F. Schlyter and F. G. Birgersson, "Forest beetles," in *Pheromones of Non-Lepidopteran Insect Pests of Agricultural Plants*, R. J. Hardie and A. K. Minks, Eds., pp. 113–148, CABI Publishers, 2000.

[8] R. M. Silverstein, J. O. Rodin, and D. L. Wood, "Sex attractants in frass produced by male *Ips confusus* in ponderosa pine," *Science*, vol. 154, no. 3748, pp. 509–510, 1966.

[9] J. P. Vité, A. Bakke, and J. A. A. Renwick, "Pheromones in *Ips* (Coleoptera: Scolytidae): occurrence and production," *Canadian Entomologist*, vol. 104, no. 12, pp. 1967–1975, 1972.

[10] A. Bakke, "Aggregation pheromone in the bark beetle *Ips duplicatus*," *Norwegian Journal of Entomology*, vol. 22, no. 1, pp. 67–69, 1975.

[11] W. Francke, V. Heemann, and B. Gerken, "2 Ethyl 1,6 dioxaspiro[4.4]nonane, principal aggregation pheromone of *Pityogenes chalcographus*," *Naturwissenschaften*, vol. 64, no. 11, pp. 590–591, 1977.

[12] J. A. Byers, G. Birgersson, J. Löfqvist, and G. Bergström, "Synergistic pheromones and monoterpenes enable aggregation and host recognition by a bark beetle, *Pityogenes chalcographus*," *Naturwissenschaften*, vol. 75, no. 3, pp. 153–155, 1988.

[13] J. A. Byers, F. Schlyter, G. Birgersson, and W. Francke, "*E*-myrcenol in *Ips, duplicatus*: an aggregation pheromone component new for bark beetles," *Experientia*, vol. 46, no. 11-12, pp. 1209–1211, 1990.

[14] S. A. Teale, F. X. Webster, A. Zhang, and G. N. Lanier, "Lanierone: a new pheromone component from *Ips pini* (Coleoptera: Scolytidae) in New York," *Journal of Chemical Ecology*, vol. 17, no. 6, pp. 1159–1176, 1991.

[15] G. Birgersson and G. Bergström, "Volatiles released from individual spruce bark beetle entrance holes Quantitative variations during the first week of attack," *Journal of Chemical Ecology*, vol. 15, no. 10, pp. 2465–2483, 1989.

[16] G. Birgersson, F. Schlyter, J. Löfqvist, and G. Bergström, "Quantitative variation of pheromone components in the spruce bark beetle *Ips typographus* from different attack phases," *Journal of Chemical Ecology*, vol. 10, no. 7, pp. 1029–1055, 1984.

[17] W. A. Garland and M. L. Powell, "Quantitative selected ion monitoring (QSIM) of drugs and/or drug metabolites in biological matrices," *Journal of Chromatographic Science*, vol. 19, no. 8, pp. 392–434, 1981.

[18] H. E. M. Dobson, "Analysis of flower and pollen volatiles," in *Modern Method of Plant Analysis, Eessential Oils and Waxes*, H. F. Linskens and J. F. Jackson, Eds., vol. 12 of *New series*, Springer, Berlin, Germany, 1991.

[19] W. A. König, R. Krebber, and G. Wenz, "Enantioselective capillary gas chromatography on the basis of host-guest interactions with modified cyclodextrins," *High Resolution Chromatography*, vol. 12, no. 10, pp. 641–644, 1989.

[20] J. A. Byers, G. Birgersson, J. Löfqvist, M. Appelgren, and G. Bergström, "Isolation of pheromone synergists of bark beetle, *Pityogenes chalcographus*, from complex insect-plant odors by fractionation and subtractive-combination bioassay," *Journal of Chemical Ecology*, vol. 16, no. 3, pp. 861–876, 1990.

[21] C. Lejfalk and G. Birgersson, "Wick-baits – a novel delivery system for testing potential semiochemicals," in *Proceedings of the 14th Annual Meeting of the International Society of Chemical Ecology*, Vancouver, Canada, 1997.

[22] A. Bakke, P. Frøyen, and L. Skattebøl, "Field response to a new pheromonal compound isolated from *Ips typographus*," *Naturwissenschaften*, vol. 64, no. 2, pp. 98–99, 1977.

[23] A. Bakke, "The utilization of aggregation pheromone for control of the spruce bark beetle," in *Insect Pheromone Technology: Chemistry and application*, B. A. Leonhart and M. Beroza, Eds., ACS Symposium Series, no. 190, Washington, DC, USA, 1982.

[24] A. Bakke, "The recent *Ips* typographus outbreak in Norway experiences from a control program," *Holarctic Ecology*, vol. 12, no. 4, pp. 515–519, 1989.

[25] J. P. Vite, "The European struggle to control *Ips typographus*: past, present and future," *Holarctic Ecology*, vol. 12, no. 4, pp. 520–525, 1989.

[26] F. Schlyter, G. Birgersson, J. A. Byers, J. Löfqvist, and G. Bergström, "Field response of spruce bark beetle, *Ips typographus*, to aggregation pheromone candidates," *Journal of Chemical Ecology*, vol. 13, no. 4, pp. 701–716, 1987.

[27] J. A. A. Renwick, G. B. Pitman, and J. P. Vité, "2-Phenylethanol isolated from bark beetles," *Die Naturwissenschaften*, vol. 63, no. 4, p. 198, 1976.

[28] N. Tomescu, B. B. Kis, I. Opreanu, and L. Tautan, "Modifications of the response of *Ips typographus* (Coleoptera: Scolytidae) to the aggregation pheromone in mixture with other substances," *Revue Roumaine de Biologie Serie de Biologie Animale*, vol. 27, no. 1, pp. 77–80, 1982.

[29] D. S. Pureswaran, R. Gries, J. H. Borden, and H. D. Pierce, "Dynamics of pheromone production and communication in the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, and the pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae)," *Chemoecology*, vol. 10, no. 4, pp. 153–168, 2000.

[30] F. Schlyter, J. Lofqvist, and J. A. Byers, "Behavioural sequence in the attraction of the bark beetle *Ips* typographus to pheromone sources," *Physiological Entomology*, vol. 12, no. 2, pp. 185–196, 1987.

[31] J. C. Dickens, "Behavioural and electrophysiological responses of the bark beetle, *Ips* typographus, to potential pheromone components," *Physiological Entomology*, vol. 6, no. 3, pp. 251–261, 1981.

[32] M. C. Birch, D. M. Light, D. L. Wood et al., "Pheromonal attraction and allomonal interruption of *Ips* pini in California by the two enantiomers of *Ips*dienol," *Journal of Chemical Ecology*, vol. 6, no. 3, pp. 703–717, 1980.

[33] G. N. Lanier, A. Classon, T. Stewart, J. J. Piston, and R. M. Silverstein, "*Ips* pini: the basis for interpopulational differences in pheromone biology," *Journal of Chemical Ecology*, vol. 6, no. 3, pp. 677–687, 1980.

[34] G. Gries, H. D. Pierce Jr., B. S. Lindgren, and J. H. H. Borden, "New techniques for capturing and analyzing semiochemicals for scolytid beetles (Coleoptera: Scolytidae)," *Journal of Economic Entomology*, vol. 81, no. 6, pp. 1715–1720, 1988.

[35] D. R. Miller, G. Gries, and J. H. Borden, "*E*-myrcenol: a new pheromone for the pine engraver, *Ips* pini (Say) (Coleoptera: Scolytidae)," *Canadian Entomologist*, vol. 122, no. 5-6, pp. 401–406, 1990.

[36] D. R. Miller, C. Asaro, and C. W. Berisford, "Attraction of southern pine engravers and associated bark beetles (Coleoptera: Scolytidae) to *Ips*enol, *Ips*dienol, and lanierone in Southeastern United States," *Journal of Economic Entomology*, vol. 98, no. 6, pp. 2058–2066, 2005.

[37] USDA Forest, "Insects of eastern conifers," USDA Forest Service Miscellaneous Publications, no. 1426. 1985.

[38] G. Birgersson, M. J. Dalusky, and C. W. Berisford, "Identification of an aggregation pheromone for *Pityogenes hopkinsi* (Coleoptera: Scolytidae)," *Canadian Entomologist*, vol. 132, no. 6, pp. 951–963, 2000.

[39] D. Klimetzek and W. Francke, "Relationship between the enantiomeric composition of α-pinene in host trees and the production of verbenols in *Ips*-species," *Experientia*, vol. 36, no. 12, pp. 1343–1345, 1980.

[40] M. Lindström, T. Norin, G. Birgersson, and F. Schlyter, "Variation of enantiomeric composition of α-pinene in norway spruce, *Picea abies*, and its influence on production of verbenol isomers by *Ips typographus* in the field," *Journal of Chemical Ecology*, vol. 15, no. 2, pp. 541–548, 1989.

[41] P. Ivarsson, F. Schlyter, and G. Birgersson, "Demonstration of de *novo* pheromone biosynthesis in *Ips duplicatus* (Coleoptera: Scolytidae): inhibition of *Ips dienol* and *E*-Myrcenol production by compactin," *Insect Biochemistry and Molecular Biology*, vol. 23, no. 6, pp. 655–662, 1993.

[42] P. Ivarsson and G. Birgersson, "Regulation and biosynthesis of pheromone components in the double spined bark beetle

Ips duplicatus (Coleoptera: Scolytidae)," *Journal of Insect Physiology*, vol. 41, no. 10, pp. 843–849, 1995.

[43] S. J. Seybold, D. R. Quilici, J. A. Tillman, D. Vanderwel, D. L. Wood, and G. J. Blomquist, "*De novo* biosynthesis of the aggregation pheromone components *Ips enol* and *Ips dienol* by the pine bark beetles *Ips* paraconfusus lanier and *Ips pini* (Say) (Coleoptera: Scolytidae)," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 18, pp. 8393–8397, 1995.

[44] R. H. Fish, L. E. Browne, D. L. Wood, and L. B. Hendry, "Pheromone biosynthetic pathways: conversions of deuterium labelled *Ips dienol* with sexual and enantioselectivity in *Ips paraconfusus* lanier," *Tetrahedron Letters*, vol. 20, no. 17, pp. 1465–1468, 1979.

[45] B. S. Lanne, P. Ivarsson, P. Johnsson, G. Bergström, and A. B. Wassgren, "Biosynthesis of 2-methyl-3-buten-2-ol, a pheromone component of *Ips typographus* (Coleoptera: Scolytidae)," *Insect Biochemistry*, vol. 19, no. 2, pp. 163–167, 1989.

[46] P. Ivarsson, *The pheromone systems of the spruce bark beetles, Ips duplicatus and I. typographus: biosynthesis and regulations*, Ph.D. thesis, Göteborg University, Sweden, 1995.

[47] S. Seybold, *The role of chirality in the olfactory-directed aggregation behavior of pine engraver beetles in the genus Ips (Coleoptera: Scolytidae)*, Ph.D. thesis, Department of Entomology, University of California, Berkeley, Calif, USA, 1992.

[48] S. J. Seybold, T. Ohtsuka, D. L. Wood, and I. Kubo, "Enantiomeric composition of *Ips dienol*: a chemotaxonomic character for north American populations of *Ips* spp. in the *Ips* subgeneric group (coleoptera: Scolytidae)," *Journal of Chemical Ecology*, vol. 21, no. 7, pp. 995–1016, 1995.

[49] U. Kohnle, J. P. Vite, H. Meyer, and W. Francke, "Response of 4 American engraver bark beetles, *Ips* spp. (Col, Scolytidae), to synthetic racemates of chiral pheromones," *Journal of Applied Entomology*, vol. 117, no. 5, pp. 451–456, 1994.

[50] D. R. Miller, J. H. Borden, and K. N. Slessor, "Enantiospecific pheromone production and response profiles for populations of pine engraver, *Ips pini* (Say) (Coleoptera: scolytidae), in British Columbia," *Journal of Chemical Ecology*, vol. 22, no. 11, pp. 2157–2172, 1996.

[51] S. J. Seybold, S. A. Teale, D. L. Wood et al., "The role of lanierone in the chemical ecology of *Ips pini* (Coleoptera: Scolytidae) in California," *Journal of Chemical Ecology*, vol. 18, no. 12, pp. 2305–2329, 1992.

[52] D. R. Miller, K. E. Gibson, K. F. Raffa, S. J. Seybold, S. A. Teale, and D. L. Wood, "Geographic variation in response of pine engraver, *Ips pini*, and associated species to pheromone, lanierone," *Journal of Chemical Ecology*, vol. 23, no. 8, pp. 2013–2031, 1997.

Hindawi Publishing Corporation
Psyche
Volume 2012, Article ID 539624, 10 pages
doi:10.1155/2012/539624

Research Article

Host-Tree Monoterpenes and Biosynthesis of Aggregation Pheromones in the Bark Beetle *Ips paraconfusus*

John A. Byers[1] and Göran Birgersson[2]

[1] *US Arid-Land Agricultural Research Center, USDA-ARS, 21881 North Cardon Lane, Maricopa, AZ 85138, USA*
[2] *Chemical Ecology, Protection Biology, Swedish University of Agricultural Sciences, 230-53 Alnarp, Sweden*

Correspondence should be addressed to John A. Byers, john.byers@ars.usda.gov

Received 12 January 2012; Accepted 15 March 2012

Academic Editor: Qing-He Zhang

A paradigm developed in the 1970s that *Ips* bark beetles biosynthesize their aggregation pheromone components ipsenol and ipsdienol by hydroxylating myrcene, a host tree monoterpene. Similarly, host α-pinene was hydroxylated to a third pheromone component *cis*-verbenol. In 1990, however, we reported that amounts of ipsenol and ipsdienol produced by male *Ips paraconfusus* (Coleoptera: Scolytinae) feeding in five host pine species were nearly the same, even though no detectable myrcene precursor was detected in one of these pines (*Pinus sabiniana*). Subsequent research showed ipsenol and ipsdienol are also biosynthesized from smaller precursors such as acetate and mevalonate, and this *de novo* pathway is the major one, while host tree myrcene conversion by the beetle is the minor one. We report concentrations of myrcene, α-pinene and other major monoterpenes in five pine hosts (*Pinus ponderosa*, *P. lambertiana*, *P. jeffreyi*, *P. sabiniana*, and *P. contorta*) of *I. paraconfusus*. A scheme for biosynthesis of ipsdienol and ipsenol from myrcene and possible metabolites such as ipsenone is presented. Mass spectra and quantities of ipsenone are reported and its possible role in biosynthesis of aggregation pheromone. Coevolution of bark beetles and host trees is discussed in relation to pheromone biosynthesis, host plant selection/suitability, and plant resistance.

1. Introduction

The California five-spined engraver, *Ips paraconfusus* (Lanier) (Coleoptera: Scolytinae), is an important pest of young pine forests in California and Oregon. Struble and Hall [1] state that "all pine species within the range of this beetle are attacked", although the beetle occurs most frequently on ponderosa pine (*Pinus ponderosa* Laws) at elevations from 600 to 1,400 m in California. Due to the pest status of this insect, extensive studies have been conducted to elucidate the pheromone signal concerning the biosynthetic, behavioral, and ecological aspects [2–4].

The aggregation pheromone produced by males has been identified as a synergistic blend of three components, (*S*)-(−)-ipsenol, (*S*)-(+)-ipsdienol, and (4*S*)-*cis*-verbenol [5–7]. Ipsenol and ipsdienol are produced only in males when exposed to vapors of the host plant monoterpene, myrcene [8], and the quantitative relationships between precursor vapor concentration and pheromone products have been reported [9]. Hendry et al. [10] used D_2-labelled myrcene to demonstrate that it can be converted in the male to ipsenol and ipsdienol under vapor exposure conditions. Unexposed control males contained no pheromone components, nor did females, even when exposed to myrcene vapors [9]. Another host monoterpene, (−)-α-pinene, in the vapor phase is converted to *cis*-verbenol in both sexes [11], and the relationship between increasing (−)-α-pinene vapor concentration and increasing *cis*-verbenol production in both sexes has been quantified [12]. Based on the above studies and others, a paradigm was established that *I. paraconfusus*, and probably most other *Ips* species, use myrcene and α-pinene in their host tree as precursors to ipsenol and ipsdienol and to *cis*-verbenol, respectively.

However, this paradigm began to be questioned when Byers and Birgersson [13] reported that males of *I. paraconfusus* that had fed in five different host pine species produced almost identical amounts of the pheromone components ipsenol and ipsdienol, regardless of the concentration of

myrcene in the host species fed upon. In fact, gray pine, *Pinus sabiniana*, had so little myrcene that it could not be detected by gas chromatography and mass spectrometry (GC-MS). Thus, a beetle would need to eat at least eight times its weight in oleoresin in order to have any chance of obtaining the required amounts of myrcene [13]. This appears unrealistic since males were observed to ingest phloem alone. Therefore, coevolution of host myrcene and bark beetle pheromone production in regard to host selection and suitability appears unlikely. On the other hand, the conversion of host α-pinene to *cis*-verbenol appears to be the major pathway, and so in this case coevolution could occur. Here, we present a more complete analysis of the host pine monoterpenes in phloem and oleoresin from pines in 1985 presented in part in Byers and Birgersson [13], as well as additional data from 1986, and the mass spectra of ipsenone. We will discuss in more detail our previous findings in relation to knowledge about the biosynthesis of aggregation pheromone components in relation to behavior, physiology, and coevolution of host tree monoterpenes and bark beetle ecology.

2. Materials and Methods

Ips paraconfusus were reared from ponderosa pine (*P. ponderosa*) and introduced into five host species of pine: ponderosa, sugar (*P. lambertiana*), Jeffrey (*P. jeffreyi*), gray (*P. sabiniana*), and lodgepole (*P. contorta*) as reported earlier [13]. The latter species, however, is not listed as a primary host probably because it generally occurs at elevations above the range of *I. paraconfusus* [1]. Males were dissected from their nuptial chambers after five days, and the posterior two thirds of the alimentary canal was extracted in groups of eight in 150 μL diethyl ether with 10 ng heptyl acetate per μL as an internal standard. Three samples of phloem (dry weight of each about 22 $\pm$ 7 mg, $\pm$SD, $n = 15$) not affected by beetle galleries (not oxidized) from each of the infested pine species were each extracted in 250 μL diethyl ether with internal standard. Pheromone components and ipsenone in the hindgut extracts were identified and quantified by gas chromatography (GC) on a Hewlett-Packard model 5880 and by GC-MS on a Finnigan model 4021. GC analysis used a fused silica column (0.2 mm i.d. $\times$ 12.5 m) coated with SE-54 CL (General Electric, 1% vinyl-, 5% phenyl-, 94% methylpolysiloxane) on a temperature program of 60°C for 3 min, rising to 220°C at 5°C/min, and isothermal for 15 min. Nitrogen, 20 cm/s, was used as carrier gas. GC-MS used a column of fused silica (0.15 mm i.d. $\times$ 25 m, df = 0.3 μm) coated with Superox FA (Alltech, TPA-treated PEG, df = 0.3 μm) on a temperature program of 50°C for 4 min, rising to 200°C at 8°C/min and isothermal for 10 min and helium carrier gas at 35 cm/s. Synthetic chemical standards of ipsenol, ipsdienol, and *cis*-verbenol were obtained from Borregaard (Norway). Ipsenone was prepared by oxidation of ipsenol in Jones reagent [14].

The phloem extracts described above were analyzed by GC on the fused silica column of SE-54 above. GC-MS used the SE-54 column on a program of 50°C for 1 min, rising to 220°C at 5°C/min and isothermal for 10 min. Carrier gas was as described above. Synthetic monoterpenes used for reference spectra were obtained from Sigma-Aldrich. There was some question as to the species and chemical identification for Jeffrey and/or gray pine. This was because Jeffrey pine phloem contained large quantities of α-pinene and myrcene relative to some of the other pines while Jeffrey pine was expected to contain mostly n-heptane [15, 16]. Also, gray pine had virtually none of the monoterpene hydrocarbons. Therefore, phloem samples were collected Oct. 17, 1986, from four trees of each of the five species. Also one tree each of sugar pine and ponderosa pine were sampled in four cardinal directions to determine the variation in monoterpene hydrocarbon content between samples. Oleoresin was collected from each of the species except sugar pine in which resin flow was insufficient for collection. Chemical analyses were as described above.

3. Results

Extracts of the hindguts of the male *I. paraconfusus* that had fed on the five host pines contained only a few major components, with ipsenol and ipsdienol dominating (Figure 1). Ipsenone, the ketone of ipsenol, was observed (Figure 1) in *I. paraconfusus* males fed in ponderosa pine, sugar pine, Jeffrey pine, gray pine, and lodgepole pine at 161 $\pm$ 124 (ng/male $\pm$ SD), 115 $\pm$ 83, 111 $\pm$ 85, 86 $\pm$ 41, and 87 $\pm$ 36, respectively.

The quantities of ipsenol and ipsdienol in fed males in each of the pine species were reported previously [13]. The quantities of these two pheromone components were similar and not significantly different; while it appeared that males from Jeffrey and lodgepole pines had more *cis*-verbenol than those from the other species where it could not be detected [13]. Here, we report that correlations between ipsenol and ipsdienol were consistently high within host species (R^2 from 0.64 to 0.97), and an overall $R^2 = 0.85$ ($N = 25$) for all species. However, correlations between ipsenone and ipsenol ($R^2 = 0, N = 25$) or ipsdienol ($R^2 = 0.14, N = 25$) were low.

The monoterpene hydrocarbons, myrcene and α-pinene, in the infested logs, were found in the largest amounts in phloem of Jeffrey pine, with significant amounts in lodgepole pine, lower amounts in ponderosa and sugar pine, and undetectable levels in gray pine. These phloem samples showed a large variation (within tree) in monoterpene hydrocarbons (Table 1). Phloem samples from several trees of each of the five species taken in October 17, 1986, showed an even larger variation (between tree) in monoterpene hydrocarbons (Table 2), but the relative amounts were consistent with those of the previous year (Table 1). These results are in agreement with field observations of the phloem during the dissection of the logs where Jeffrey pine was observed to contain "many 1 mm diam. resin pockets", lodgepole as "resinous," gray as "not resinous," and sugar and ponderosa as "slightly resinous." The relative amounts of the major monoterpenes in oleoresin of four of the pine species (sugar pine oleoresin could not be obtained) were found in percentages similar to those for the phloem

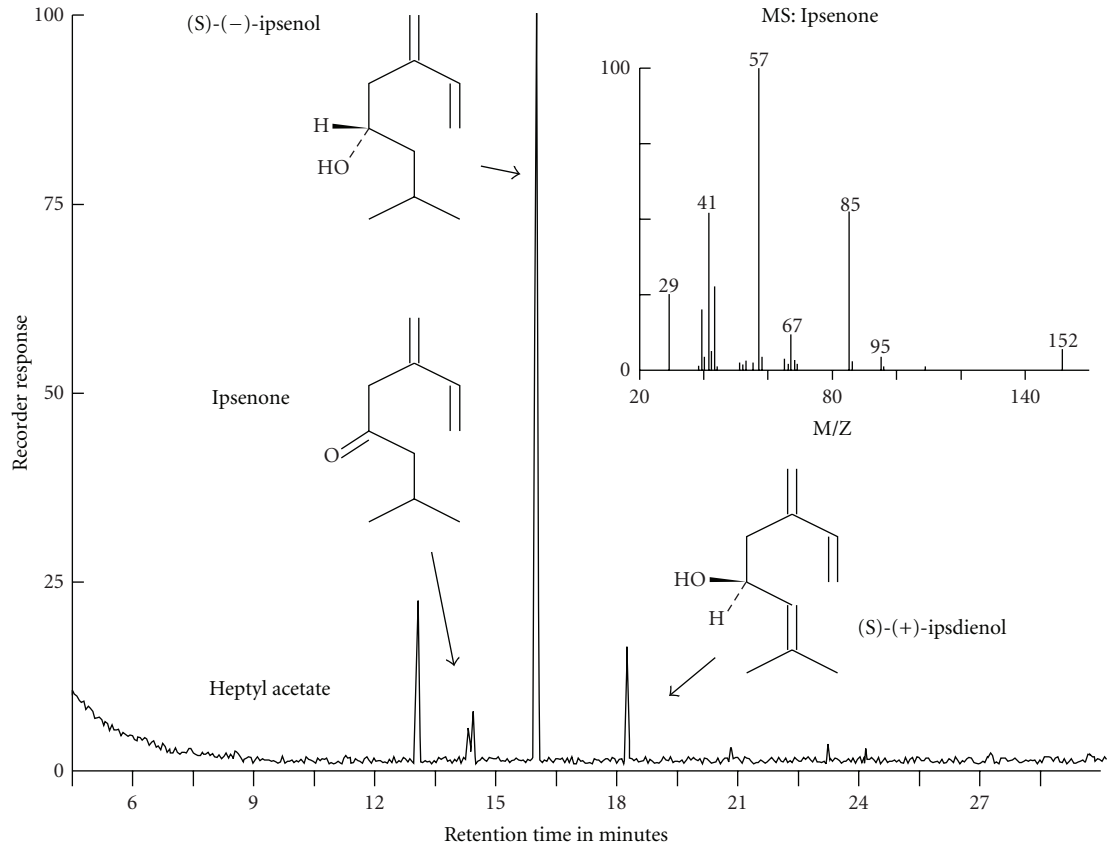


Figure 1: Gas chromatogram (Superox FA) of extract of hindguts of eight male *Ips paraconfusus* that had fed in Jeffrey pine. Heptyl acetate was used as an internal standard to quantify the pheromone components ipsenol and ipsdienol. Ipsenone, a related analog, eluted immediately before diacetone alcohol in a doublet peak. The mass spectrum of ipsenone is shown in the inset.

Table 1: Amounts of monoterpene hydrocarbons in phloem samples (15–25 mg dry weight) from five species of pine that were fed on by *Ips paraconfusus* (near Bass Lake, California, USA, September 3, 1985).

	Monoterpene hydrocarbons (μg) per g phloem (dry weight)				
Pine species	α-pinene mean ± SD (range)	β-pinene mean ± SD (range)	Myrcene mean ± SD (range)	3-Carene Mean ± SD (range)	Limonene mean ± SD (range)
Ponderosa	6.0 ± 9.0	1.1 ± 0.5	3.53 ± 2.69	21.7 ± 32.7	13.5 ± 21.0
$N = 3$[1]	(<1.3–16.4)	(<1.3–1.7)	(1.6–6.6)	(<1.3–59.3)	(<1.9–37.7)
Sugar	5.4 ± 2.2	<1.5	2.5 ± 0.1	<1.9	<1.9
$N = 3$	(3.8–7.9)	(<1.5)	(2.4–2.6)	(<1.9)	(<1.9)
Jeffrey	335.7 ± 160	116.9 ± 52.2	36.0 ± 18.7	246.3 ± 107	16.0 ± 7.4
$N = 3$	(152–445)	(56.6–147)	(15.8–52.6)	(129–338)	(7.5–20.8)
Gray	<1.0	<1.0	<1.0	<1.0	<1.0
$N = 3$	(<1.0)	(<1.0)	(<1.0)	(<1.0)	(<1.0)
Lodgepole	50.5 ± 22.5	8.5 ± 3.4	26.4 ± 11.0	18.6 ± 8.4	695.3 ± 297.8
$N = 3$	(35.0–76.3)	(6.0–12.4)	(18.6–39.0)	(12.5–28.2)	(479–1035)

[1] Number of samples from each tree.

(Tables 1–3). However, the percentage of the oleoresin that consisted of monoterpene hydrocarbons was much higher in ponderosa (83.5%) and lodgepole pine (89.9%) than in Jeffrey pine (2.9%); and gray pine oleoresin was only 0.08% monoterpene hydrocarbons (of those in Table 3).

4. Discussion

The similarity of chemical structure between the major host monoterpene, myrcene, and ipsenol and ipsdienol led Hughes [8] to propose that the tree's myrcene was a

Table 2: Amounts of monoterpene hydrocarbons in phloem samples (15–25 mg dry weight) from five species of pine (near Bass Lake, California, USA, 17 October 1986).

	Monoterpene hydrocarbons (μg) per g phloem (dry weight)				
Pine species	α-pinene mean ± SD (range)	β-pinene mean ± SD (range)	Myrcene mean ± SD (range)	3-Carene mean ± SD (range)	Limonene mean ± SD (range)
Ponderosa	1076 ± 1904	1179 ± 1921	428 ± 338	2747 ± 2483	2345 ± 3997
$N = 4$[1]	(<20–3930)	(<50–4060)	(<50–1015)	(237–6750)	(<50–8335)
Sugar	395 ± 378	180 ± 224	54 ± 55	121 ± 145	<15
$N = 4$	(97–1015)	(425–577)	(<15–131)	(<15–307)	(<15)
Jeffrey	1665 ± 1612	1216 ± 1692	272 ± 291	1924 ± 1563	4410 ± 3673
$N = 4$	(601–3520)	(124–3165)	(75–606)	(283–3395)	(1105–8365)
Gray	<20	<20	<20	<20	<20
$N = 4$	(<20)	(<20)	(<20)	(<20)	(<20)
Lodgepole	1400 ± 1811	3261 ± 4595	780 ± 1034	2413 ± 3096	14284 ± 14566
$N = 4$	(<25–4060)	(<25–10075)	(<25–2300)	(98–6965)	(1095–35000)

[1] Number of trees.

Table 3: Amounts of monoterpene hydrocarbons in oleoresin samples from four species of pine (near Bass Lake, California, USA, 17 October 1986).

	Monoterpene hydrocarbons (μg) per μL oleoresin					
Pine species	α-pinene	β-pinene	Myrcene	3-Carene	Limonene	Monoterpene Percent of Oleoresin
Ponderosa[1]	43.5 ± 6.4	102.6 ± 14.3	120.5 ± 19.6	498.5 ± 79.2	70.0 ± 12.7	83.5
Jeffrey	1.32	1.00	3.37	16.35	6.93	2.9
Gray	0.68	<0.06	<0.06	<0.06	<0.06	0.08
Lodgepole	43.2	39.7	23.7	69.5	723.0	89.9

[1] Four samples from cardinal directions of one tree, mean ± SD.

precursor of these pheromone components in *Ips*. Evidence for this theory was based on exposure of *Ips paraconfusus* males to myrcene vapor and the subsequent production of compounds with GC retention times identical to ipsenol and ipsdienol [8]. Byers et al. [9] confirmed the identifications using GC-MS and behavioral assays and reported a male-specific increasing relationship between precursor vapor concentration and pheromone products. Hendry et al. [10] labeled myrcene with deuterium and established the direct conversion of myrcene vapor to the pheromone components. Hughes [8] suggested that ipsdienol was directly converted to ipsenol since topical application of ipsdienol on males resulted in ipsenol production. Fish et al. [17] supported this by using deuterium-labeled ipsdienol (64% D) that was converted in males to labeled ipsenol (25% D). Some deuterium at carbon 4 was lost suggesting that an alternate pathway to ipsdienone (ketone at carbon 4) and back again to ipsdienol was occurring before conversion to ipsenol. However, *I. paraconfusus* contained no detectable ipsdienone, although it may occur in small proportions accounting for the loss of deuterium on the recovered ipsdienol (59% D). Fish et al. [17] showed that males could convert synthetic ipsdienone to ipsdienol, which was then converted to ipsenol.

In the present study, we did not find ipsdienone but instead ipsenone (also ketone at carbon 4) in feeding males (Figure 2) and this compound could explain the loss of deuterium in ipsenol (25% D) by a reversible pathway. Ipsenone can also explain the observed loss of deuterium in the recovered ipsdienol since it would be expected that a reversible pathway exists between ipsdienol and ipsenol. In fact, until ipsdienone is found in beetles naturally, it is more logical to assume that ipsenone rather than ipsdienone is involved in the deuterium loss observed earlier by Fish et al. [17]. Later work by Ivarsson et al. [18] found that when ^{3}H-ipsdienone was injected into males, radiolabel was incorporated into both ipsenol and ipsdienol, found mainly in the metathorax, while incubation of male tissues with ^{3}H-ipsdienone did not produce radiolabel in these components. *In vitro* incubation of tissues from *I. paraconfusus* with ^{14}C-acetate gave radiolabeled ipsenone/ipsdienone, but these were not chromatographically separated.

Hughes [8, 20] hypothesized that aggregating pheromones in *Dendroctonus* and *Ips* are "waste products from the metabolism of terpenes that have secondarily been utilized as chemical messengers." According to this hypothesis one would expect no differences between the *cis*-verbenol and ipsenol/ipsdienol production in regard to vapor exposure and feeding conditions or between the sexes—but great differences are evident [12, 21]. The *cis*-verbenol system appears to be a detoxification process in part, although males produce about twice as much *cis*-verbenol, and the ratios with other metabolites are different than in females [12]. The ipsenol/ipsdienol system has clearly evolved beyond that of a simple detoxification process

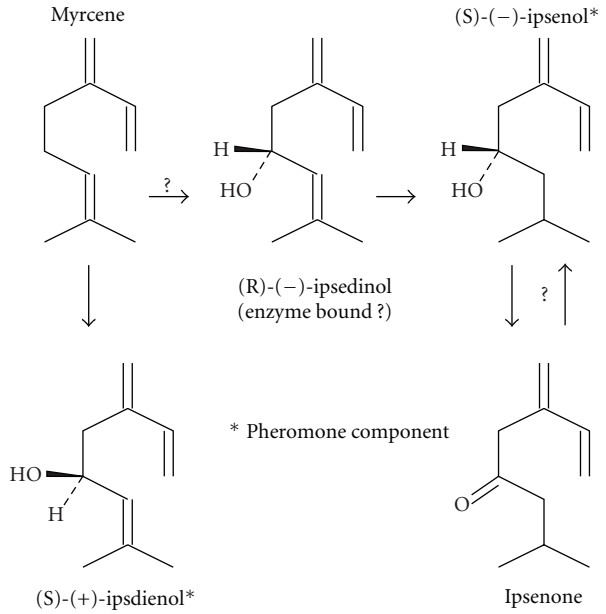


Figure 2: Proposed scheme for the conversion of the host tree compound, myrcene, to the pheromone components (*S*)-(−)-ipsenol and (*S*)-(+)-ipsdienol in *Ips paraconfusus* based on radiolabelling experiments and enantiomers found in the male [5, 7–12, 17, 19]. Conversion arrows with question marks have not been proven. (*R*)-(−)-ipsdienol does not accumulate in the hindgut but may occur as an enzyme-bound intermediate. However, contrary to the scheme, the amounts of ipsenone, (*S*)-(−)-ipsenol and (*S*)-(+)-ipsdienol in males were not correlated with myrcene titres in the host trees.

since this system (a) is sex-specific [9], (b) specifically influenced by juvenile hormone (JH) [22, 23], and (c) selectively inhibited by the antibiotic streptomycin [19]. The *cis*-verbenol system, on the other hand, is not affected by JH or streptomycin, and *cis*-verbenol is produced in both sexes, although females produced about half the amounts as males. Another difference between the ipsenol/ipsdienol and the *cis*-verbenol systems is that the male reduces his production of ipsenol and ipsdienol while feeding if he is joined by several females in his nuptial chamber [24]. The inhibition of pheromone production (and release) is physiological since males with females produced very little ipsenol and ipsdienol even when exposed to myrcene vapors, compared to males alone. In contrast, the production of *cis*-verbenol from α-pinene vapors in males was not affected by females [24].

Earlier work provided intriguing suggestions that symbiotic microorganisms may convert myrcene to pheromone components. Byers and Wood [19] fed males in a diet of powdered cellulose and ground phloem (22%) with and without streptomycin antibiotic. The males were removed from both diets and exposed to vapors of myrcene and α-pinene whereupon only those in diets without streptomycin-produced ipsenol and ipsdienol (there was no affect of antibiotic on *cis*-verbenol production). However, Conn et al. [25] reported that axenically reared *I. paraconfusus* can produce their aggregation pheromones "completely in the absence of the normal, extracellular complement of symbiotic microorganisms." Their data show that five axenic beetles produced half as much ipsenol as five feral (wild) beetles when feeding in logs and that axenic beetles without yeast as adults produced only about 10% the normal amounts. Hunt and Borden [26] repeated these tests and also found no significant statistical differences between axenic and control males, but again the axenic males produced only 36% as much ipsenol and ipsdienol. They also fed streptomycin to males and then introduced them into a ponderosa pine log. The ipsenol production in these males was reduced to only 2% indicating that both the feeding and aeration pathways are inhibited by the antibiotic [19, 26]. No further work has implicated microorganisms, but in any case, it seems that the ipsenol/ipsdienol system is peculiarly sensitive to streptomycin.

According to the paradigm when our experiments were conducted (1985-1986), catches of *I. paraconfusus* on five species of host pines infested with conspecific males should be correlated with quantities of aggregation pheromone components ipsenol, ipsdienol, and *cis*-verbenol that were converted directly from myrcene and α-pinene in the host trees. The attractions of *I. paraconfusus* to each of the five pine species of infested logs were similar except for an approximate doubling of catch on the Jeffrey pine log, as reported previously [13]. The sex ratios of catch (females per male) on four of the species were also similar (2.5 to 3.9) with more females than males, but the catch on Jeffrey pine was the most female biased (15.6) and this ratio was significantly different from the others [13]. The generally similar attraction to each of the pine species agrees with the similar amounts of the pheromone components, ipsenol and ipsdienol, found in the hindguts of the feeding males. However, there was no correlation between the widely varying amounts of myrcene in the host pines and the uniform amounts of ipsenol and ipsdienol in the males.

The increased catch on Jeffrey, and to a lesser extent on lodgepole, can be explained by the higher amounts of α-pinene in the phloem that was converted to the third pheromone component, *cis*-verbenol [13]. Detection of *cis*-verbenol in hindguts of feeding males is difficult [12], and Silverstein et al. [27] found that *cis*-verbenol occurred in quantities of only 2.5% the amount of ipsdienol in male frass. We found *cis*-verbenol to be 1% or 0.05% the amount of ipsdienol in hindguts of males feeding in Jeffrey and lodgepole pines, respectively. *cis*-Verbenol was presumably present in sufficient quantities in the males feeding in the other pines (although we could not quantify the amounts) as to be synergistically active with ipsenol and ipsdienol, since the latter two components have low activity without *cis*-verbenol in the field [28].

The content of ipsenol and ipsdienol in groups of eight males (within or between species) was rather consistent [13] with a total ($n = 25$) coefficient of variation (CV) of 26% for both ipsenol and ipsdienol. In comparison, the variations of the precursors α-pinene and myrcene in phloem were much larger (Tables 1–3), and the total CV for α-pinene was 185% and for myrcene 126%. Even within a tree the variation in α-pinene and myrcene in phloem could be large (ranges

in Table 1), which was probably the result of the rather small sample units (15–25 mg dry weight). Resin pockets are probably not evenly distributed in phloem so smaller samples would tend to vary more in the numbers of pockets. However, the sample unit was equivalent to about 80% of a nuptial chamber and thus indicates that beetles could ingest large differences between individuals in monoterpene hydrocarbons (calculation based on [12, 29]). The amounts of myrcene and α-pinene reported earlier [13] in the pine species as well as the other three major monoterpenes (Table 1) were considerably lower in phloem sampled in 1985 than they were in 1986 (Table 2). We are not sure why this was apparently the case unless the log's phloem had lost monoterpenes during the week-long behavioral tests in the field (1985) compared to immediate extraction of phloem cut from trees in 1986. Byers [12] showed that monoterpene vapors in male nuptial chambers in logs remain constant for about a week before declining rapidly in concentration.

Could males obtain enough myrcene in host phloem or oleoresin to account for the quantities of ipsenol and ipsdienol found in the hindguts? The male does not eat the entire contents of the nuptial chamber (fecal pellets appear to be a minor component of the frass), and it is doubtful that he selectively eats the "toxic" oleoresin [30–33]. The headspace concentration of myrcene in a nuptial chamber of ponderosa pine (2.8×10^{-8} g/mL) [12] is expected to account for only 1.6% at most of the ipsenol in feeding males (by linear interpolation between lowest value and 0, Figure 1 in Byers et al. [9], note: equations should be $Y = 2.72 + 1.05\ \ln X$ and $Y = 0.62 + 0.26\ \ln X$). Also, a feeding beetle must produce and release several times over the amounts found in hindguts at the end of the feeding period. The gut turnover rate (pheromone content of gut release per time) can be estimated from the airborne collection of components and gut contents. Studies with *D. brevicomis* [34, 35] can be used to calculate that females release *exo*-brevicomin at 16 gut contents per day at the peak of mass attack. *P. chalcographus* males release chalcogran at 18 gut contents per day [36, 37], and *I. typographus* males release 2-methyl-3-buten-2-ol at about 240 gut turnovers/day and *cis*-verbenol at 48 turnovers/day [38, 39].

Assuming conservatively that gut turnover rates above are just 10 per day, then based on the quantities of myrcene in ponderosa pine phloem (fresh weight is 3.87 $\times$ dry weight) [29] or oleoresin (Tables 1–3), a male would need to eat a minimum of from 99 to 413 nuptial chambers in the 1985 experiment (Table 1), or from 0.6 to 13 chambers in the 1986 samples (Table 2, 111 μL at 0.895 g/mL) to account for pheromone amounts [12]. However, only 0.14 μL oleoresin is needed (1986 samples) to produce the estimated amounts of ipsenol and ipsdienol released over two days. Thus, *I. paraconfusus* would need to eat some oleoresin to account for pheromone production based on the myrcene precursor theory, as suggested earlier [12]. However, assuming amounts of myrcene in gray pine oleoresin of at most 0.06 μg/μL (our quantification limit), then at least 280 μL of oleoresin from gray pine would be required (again assuming 100% conversion). Thus, a beetle would need to eat more than 28 times its weight in oleoresin to have any possibility of producing the observed amounts of ipsenol and ipsdienol from eating gray pine. Even higher amounts of oleoresin would be required to replace pheromone released. Therefore, another biosynthetic pathway (*de novo*) is indicated since beetle's guts contain mostly phloem, and oleoresin is toxic to bark beetles (*I. paraconfusus* and *D. brevicomis*) [12, 30–33]. Because small quantities of *cis*-verbenol are produced and required for attraction, it is probable that sufficient α-pinene precursor is available from the host.

It is apparent that all five species of pine are about equally suitable as hosts, at least in terms of adult survival, nuptial chamber construction, pheromone production, and attraction [13]. Sugar pine is a soft pine (subgenus *Haploxylon*) while the others are hard pines (*Diploxylon*). However, sugar pine had monoterpene hydrocarbon characteristics more similar to ponderosa pine than these two species had with Jeffrey and gray pines. The Jeffrey pine with a low titer of α-pinene and myrcene in the oleoresin is consistent with earlier reports [16] but the large amounts of oleoresin in its phloem were unexpected.

I. *paraconfusus* feeding in *P. monticola* and *P. monophylla* also appear to produce at least some of their pheromone components since *I. montanus* and *I. confusus* were significantly attracted [40]. *I. paraconfusus* can also produce attractant (pheromone) when boring in nonhosts Douglas fir in the laboratory [41] and white fir in the field [42]. In the latter species, however, it was shown that the amounts of ipsenol and ipsdienol were only one or two percent of the amounts produced in beetles feeding in ponderosa pine [42]. Differences in attractiveness of *I. pini* boring in two host species have also been observed [43], but it is not known which semiochemicals were responsible.

Elkinton et al. [42] proposed that evolution of host selection behavior by *Ips* bark beetles could have been influenced by the amounts of α-pinene and myrcene in the tree needed for pheromone biosynthesis. Since α-pinene in the tree appears to be converted to *cis*-verbenol, beetles may select trees high in this monoterpene. A related hypothesis is that tree genotypes lower in pheromone precursor monoterpenes may have evolved through natural selection [2]. This is doubtful since in the case of the ipsenol/ipsdienol system there does not appear to be any limitation in pheromone production when feeding in the wide variation of myrcene-containing trees [13]. Thus coevolution of host selection and insect resistance does not seem to be occurring, except possibly with respect to α-pinene. There does seem to be coevolution of detoxification genes for monoterpenes and tree genotypes, which has a major impact on host selection by Ips [44].

Assuming the detoxification theory was the first evolutionary stage of pheromone biosynthesis as proposed [8], then why was myrcene selected as the pheromone precursor instead of another monoterpene like limonene or 3-carene? Our results for ponderosa pine in 1985 show that myrcene and α-pinene were found in four of the five pine species while sugar pine did not have detectable amounts of β-pinene, 3-carene, and limonene (Table 1). Myrcene had the least variation among the five monoterpenes in ponderosa pine in 1986 (Table 2). These data are limited, but Smith

[45] sampled 74 areas across California and western USA and Canada and found that most areas had lower variation for myrcene and α-pinene, while variation in 3-carene, limonene, and β-pinene was higher (his Figure 7). In another study of 64 ponderosa pines, he reported that myrcene in oleoresin varied from 4.6 to 27.5% and α-pinene from 1.5 to 13.3%, while variation of limonene, β-pinene, and 3-carene varied from 0 to 31, 57, and 82%, respectively [46].

After the initial use of myrcene vapor as a precursor to ipsenol/ipsdienol in an Ips species, later speciation events appear to have evolved a *de novo* biosynthesis that now predominates in *Ips* species (at least in *I. pini*, *I. paraconfusus*, *I. typographus*, and *I. duplicatus*). Seybold et al. [47] state the benefits of "redundancy" would result by adding *de novo* biosynthesis and thus provide "assurance" of producing pheromone. Byers [2] argued that a *de novo* system would be advantageous to an individual since he could control the quantity of pheromone for optimal benefit and not be dependent on the host tree for precursor. The *de novo* system would be especially beneficial when a species radiates to use other host pines or when a particular host tree had unusually low amounts of precursor such as to limit pheromone production and fitness. In *I. paraconfusus*, a *de novo* system seems especially beneficial when colonizing host pines of species with little or no myrcene such as in gray pine as reported here and earlier [13]. The *de novo* systems of *I. pini* and *I. paraconfusus* could have become different with evolutionary time as the two species are moderately separated phylogenetically [48]. This is indicated by findings of Tillman et al. [49, 50], who showed that JH III from the corpora allata and by injection induced pheromone production in *I. pini*, but not as much in *I. paraconfusus*, compared to amounts in both species after feeding in host logs.

The aggregation pheromone components are essential to reproductive success, and thus, it may be too "risky" to rely on either levels of precursor in the tree or on generally available microorganisms —but rather generate the components *de novo* from acetate or mevalonate using the beetle's enzymatic systems [51]. As early as 1969, studies had shown that *I. paraconfusus* produced ipsenol and ipsdienol after application of JH analogues without feeding in hosts or exposure to myrcene [22, 23]. This indicated that the corpora allata released JH due to feeding, which then stimulated *de novo* biosynthesis of the two aggregation pheromone components from energy reserves. Lanne et al. [52] showed that *I. typographus* can convert radiolabelled mevalonate to one of its two aggregation pheromone components, 2-methyl-3-buten-2-ol, indicating that *de novo* pheromone biosynthetic pathways exist in *Ips*. Following this, Ivarsson et al. [53] injected an inhibitor of mevalonate biosynthesis into *I. duplicatus* and then allowed the beetles to feed in host Norway spruce. The accumulation of aggregation pheromone components *E*-mrycenol and ipsdienol were reduced 40 to 70%, indicating these components are synthesized *de novo* when feeding via mevalonate. Although ipsdienol and *E*-mrycenol were found "to be produced *de novo* and not from myrcene" [51], exposure to myrcene did cause more of these two components to accumulate than controls, but only about 10 to 20% as much as application of JH analog or feeding in host alone.

Seybold et al. [54] provided further evidence that *I. paraconfusus* produces ^{14}C-labeled ipsenol and ipsdienol (and traces of amitinol) *de novo* from injected ^{14}C-labelled acetate prior to feeding in host logs. Similarly, in 1995, the same group showed that *I. pini* synthesized ^{14}C-labeled ipsdienol (and large amounts of amitinol) from labeled acetate [54]. Interestingly, amitinol has not been reported as a major constituent of *I. pini* or *I. paraconfusus* aggregation pheromones, although its presence was noted in *I. paraconfusus* frass extracts [6]. JH III induced expression of regulatory enzymes (probably 3-hydroxy-3-methylglutaryl-CoA reductase = HMG-R) in *I. paraconfusus* metathoraxes to begin *de novo* isoprenoid pathways resulting in ipsenol and ipsdienol [18, 55]. Hall et al. [56] localized the pheromone biosynthesis in *I. pini* to the anterior midgut (region just after the proventriculus). The HMG-R expression was in the anterior midgut, and when these and other tissues were incubated *in vitro* with radiolabeled acetate, then only the anterior midgut produced radiolabeled ipsdienol. The involvement of microbial symbionts was discounted since anterior midgut tissues when cut open and washed still incorporated radioactivity in ipsdienol. However, internal cell symbionts are still possible, if unlikely. Byers [21] found most ipsenol and ipsdienol in the rectum of *I. paraconfusus*; however, he dissected and extracted only the alimentary canal that is posterior to the anterior midgut. It is likely that although these components are produced in the anterior midgut epithelia, they migrate with the alimentary flow and accumulate in the rectum. Nardi et al. [57] provided electron micrographic evidence that the digestive secretory cells are interspersed with the pheromone-secreting cells in the anterior midgut. The pheromone-secreting cells are distinguished by many highly ordered arrays of smooth endoplasmic reticula. There was no evidence of internal symbiotic bacteria in this region [57].

Seybold and Tittiger [4] point out that JH III stimulated HGM-R enzyme activity in male *I. pini*, but not in male *I. paraconfusus*. Feeding in both species, however, induces HMG-R and pheromone production. It was found earlier that decapitated *I. paraconfusus* treated with JH were inhibited from producing pheromone, possibly due to a brain hormone from corpora cardiaca [23] that is not important in *I. pini* [4]. Mature (emerged) and callow (pre-emerged) adults of both sexes of *I. paraconfusus* do not contain detectable aggregation pheromone components, but after exposure to myrcene and α-pinene vapors only the mature males produced ipsenol and ipsdienol, indicating certain "detoxification" enzyme systems become functional after maturity in males [21]. It is not known if HMG-R can be induced by JH in *Ips,* but in *D. jeffreyi* there is a weak activity compared to mature adults [4]. HMG-R is involved in the early (upstream) steps of isoprenoid biosynthesis that then diverges at isopentenyl diphosphate and geranyl diphosphate in scolytids [4]. Somehow, it seems that these diphosphate precursors are converted to myrcene, which is then hydroxylated by novel enzymes of each *Ips* species [4]. The question remains whether myrcene vapors play any role

in pheromone biosynthesis or are merely artifacts of the manipulated near-saturation concentrations, since these can be about 70 times higher than in nuptial chambers [12]. Dietary myrcene could play a role, but in the case of *I. paraconfusus* feeding in gray pine with undetectable myrcene, the amounts of ipsenol and ipsdienol were similar to that produced when males fed in other host pines [13]. Seybold et al. [54] showed that the enantiomeric composition of ipsenol and ipsdienol is racemic when exposed to myrcene vapor, but specific enantiomers result when feeding. This shows that the *de novo* system is by far the major pathway.

Sandstrom et al. [58] isolated an NADPH-cytochrome P450 reductase that converted myrcene to the appropriate natural enantiomer (4*R*)-(−)-ipsdienol in male *I. pini*. They concluded that this was a myrcene hydroxylase functioning near the end (downstream) of the pheromone biosynthetic pathway. A second report found that *I. confusus* in pinyon pine also had a cytochrome P450 enzyme that hydroxylated myrcene in males to about 85% (−)-ipsdienol, similar to that in *I. pini* [59]. However, since *I. confusus* has a natural ipsdienol of >90% (4*S*)-(+)-ipsdienol, they state there are still additional enantio-specific enzymes that regulate the end product that have yet to be identified [59]. Since various species of *Ips* have different ratios of enantiomers of ipsenol and ipsdienol, then there are likely species-specific enzymes in the different species [59].

Further work is needed to determine the importance of the host tree monoterpene pathways that appear quantitatively minor (and more primitive?) compared to the major *de novo* pathways (derived?). It would also be interesting to determine when the biosynthetic pathways evolved in the various *Ips* species by using molecular clocks [60] and phylogenetic relationships of the biosynthetic genes (as done for other genes in *Ips* [61]).

Acknowledgments

The authors would like to thank D.L. Wood, University of California, Berkeley, and W. D. Bedard, P. E. Tilden, and M. I. Haverty, USFS, Berkeley and Oakhurst, Calif, for providing facilities and support for research in the field during 1985-1986. Support during this time was also provided by grants from the Swedish Agricultural and Forestry Research Council (SJFR) to Lund University, the Swedish University of Agricultural Sciences, and Göteborg University.

References

[1] G. R. Struble and R. C. Hall, "The California five-spined engraver. Its biology and control," *USDA Circular*, no. 964, pp. 1–21, 1955.

[2] J. A. Byers, "Chemical ecology of bark beetles," *Experientia*, vol. 45, no. 3, pp. 271–283, 1989.

[3] J. A. Byers, "Host tree chemistry affecting colonization in bark beetles," in *Chemical Ecology of Insects 2*, R. T. Cardé and W. J. Bell, Eds., pp. 154–213, Chapman and Hall, New York, NY, USA, 1995.

[4] S. J. Seybold and C. Tittiger, "Biochemistry and molecular biology of *de novo* isoprenoid pheromone production in the Scolytidae," *Annual Review of Entomology*, vol. 48, pp. 425–453, 2003.

[5] R. M. Silverstein, J. O. Rodin, and D. L. Wood, "Sex attractants in frass produced by male *Ips confusus* in ponderosa pine," *Science*, vol. 154, no. 3748, pp. 509–510, 1966.

[6] R. M. Silverstein, J. O. Rodin, D. L. Wood, and L. E. Browne, "Identification of two new terpene alcohols from frass produced by *Ips confusus* in ponderosa pine," *Tetrahedron*, vol. 22, no. 6, pp. 1929–1936, 1966.

[7] D. L. Wood, L. E. Browne, W. D. Bedard, P. E. Tilden, R. M. Silverstein, and J. O. Rodin, "Response of *Ips confusus* to synthetic sex pheromones in nature," *Science*, vol. 159, no. 3821, pp. 1373–1374, 1968.

[8] P. R. Hughes, "Myrcene: a precursor of pheromones in *Ips* beetles," *Journal of Insect Physiology*, vol. 20, no. 7, pp. 1271–1275, 1974.

[9] J. A. Byers, D. L. Wood, L. E. Browne, R. H. Fish, B. Piatek, and L. B. Hendry, "Relationship between a host plant compound, myrcene and pheromone production in the bark beetle, *Ips paraconfusus*," *Journal of Insect Physiology*, vol. 25, no. 6, pp. 477–482, 1979.

[10] L. B. Hendry, B. Piatek, L. E. Browne et al., "*In vivo* conversion of a labelled host plant chemical to pheromones of the bark beetle *Ips paraconfusus*," *Nature*, vol. 284, no. 5755, p. 485, 1980.

[11] J. A. A. Renwick, P. R. Hughes, and I. S. Krull, "Selective production of *cis*- and *trans*- verbenol from (-) and (+) α pinene by a bark beetle," *Science*, vol. 191, no. 4223, pp. 199–201, 1976.

[12] J. A. Byers, "Pheromone biosynthesis in the bark beetle, *Ips paraconfusus*, during feeding or exposure to vapours of host plant precursors," *Insect Biochemistry*, vol. 11, no. 5, pp. 563–569, 1981.

[13] J. A. Byers and G. Birgersson, "Pheromone production in a bark beetle independent of myrcene precursor in host pine species," *Naturwissenschaften*, vol. 77, no. 8, pp. 385–387, 1990.

[14] L. F. Fieser and M. Fieser, *Reagents for Organic Synthesis*, John Wiley and Sons, New York, NY, USA, 1967.

[15] N. T. Mirov, "Composition of gum turpentines of pines," USDA For Service Technical Bulletin 1239, 1961.

[16] R. H. Smith, "Variations in the monoterpene composition of the wood resin of Jeffrey, Washoe, Coulter and lodgepole pines," *Forest Science*, vol. 13, pp. 246–252, 1967.

[17] R. H. Fish, L. E. Browne, D. L. Wood, and L. B. Hendry, "Pheromone biosynthetic pathways: conversions of deuterium labelled ipsdienol with sexual and enantioselectivity in *Ips paraconfusus* lanier," *Tetrahedron Letters*, vol. 20, no. 17, pp. 1465–1468, 1979.

[18] P. Ivarsson, C. Tittiger, C. Blomquist et al., "Pheromone precursor synthesis is localized in the metathorax of *Ips paraconfusus* lanier (Coleoptera: Scolytidae)," *Naturwissenschaften*, vol. 85, no. 10, pp. 507–511, 1998.

[19] J. A. Byers and D. L. Wood, "Antibiotic-induced inhibition of pheromone synthesis in a bark beetle," *Science*, vol. 213, no. 4509, pp. 763–764, 1981.

[20] P. R. Hughes, "*Dendroctonus*: production of pheromones and related compounds in response to host monoterpenes," *Journal of Applied Entomology*, vol. 73, no. 3, pp. 294–312, 1973.

[21] J. A. Byers, "Influence of sex, maturity and host substances on pheromones in the guts of the bark beetles, *Ips paraconfusus* and *Dendroctonus brevicomis*," *Journal of Insect Physiology*, vol. 29, no. 1, pp. 5–13, 1983.

[22] J. H. Borden, K. K. Nair, and C. E. Slater, "Synthetic juvenile hormone: induction of sex pheromone production in *Ips confusus*," *Science*, vol. 166, no. 3913, pp. 1626–1627, 1969.

[23] P. R. Hughes and J. A. A. Renwick, "Neural and hormonal control of pheromone biosynthesis in the bark beetle, *Ips paraconfusus*," *Physiological Entomology*, vol. 2, no. 2, pp. 117–123, 1977.

[24] J. A. Byers, "Effect of mating on terminating aggregation during host colonization in the bark beetle, *Ips paraconfusus*," *Journal of Chemical Ecology*, vol. 7, no. 6, pp. 1135–1147, 1981.

[25] J. E. Conn, J. H. Borden, D. W. A. Hunt et al., "Pheromone production by axenically reared *Dendroctonus ponderosae* and *Ips paraconfusus* (Coleoptera: Scolytidae)," *Journal of Chemical Ecology*, vol. 10, no. 2, pp. 281–290, 1984.

[26] D. W. A. Hunt and J. H. Borden, "Terpene alcohol pheromone production by *Dendroctonus ponderosae* and *Ips paraconfusus* (Coleoptera: Scolytidae) in the absence of readily culturable microorganisms," *Journal of Chemical Ecology*, vol. 15, no. 5, pp. 1433–1463, 1989.

[27] R. M. Silverstein, J. O. Rodin, and D. L. Wood, "Methodology for isolation and identification of insect pheromones with reference to studies on California five-spined *Ips*," *Journal of Economic Entomology*, vol. 60, no. 4, pp. 944–949, 1967.

[28] D. L. Wood, R. W. Stark, R. M. Silverstein, and J. O. Rodin, "Unique synergistic effects produced by the principal sex attractant compounds of *Ips confusus* (LeConte) (Coleoptera: Scolytidae)," *Nature*, vol. 215, no. 5097, p. 206, 1967.

[29] F. W. Cobb Jr., D. L. Wood, R. W. Stark, and P. R. Miller, "Effect of injury upon physical properties of oleoresin, moisture content, and phloem thickness," *Hilgardia*, vol. 39, pp. 127–134, 1968.

[30] R. H. Smith, "The fumigant toxicity of three pine resins to *Dendroctonus brevicomis* and *D. jeffrei*," *Journal of Economic Entomology*, vol. 54, no. 2, pp. 365–369, 1961.

[31] R. H. Smith, "Effect of monoterpene vapors on the western pine beetle," *Journal of Economic Entomology*, vol. 58, no. 3, pp. 509–510, 1965.

[32] R. H. Smith, "A physiological difference among beetles of *Dendroctonus ponderosae* (= *D. monticolae*) and *D. ponderosae* (= *D. jeffreyi*)," *Annals of the Entomological Society of America*, vol. 58, no. 4, pp. 440–442, 1965.

[33] R. H. Smith, "Resin quality as a factor in the resistance of pines to bark beetles," in *Breeding Pest-Resistant Trees*, pp. 189–196, Pergamon Press, New York, NY, USA, 1966.

[34] L. E. Browne, D. L. Wood, W. D. Bedard, R. M. Silverstein, and J. R. West, "Quantitative estimates of the western pine beetle attractive pheromone components, *exo*-brevicomin, frontalin, and myrcene in nature," *Journal of Chemical Ecology*, vol. 5, no. 3, pp. 397–414, 1979.

[35] J. A. Byers, D. L. Wood, J. Craig, and L. B. Hendry, "Attractive and inhibitory pheromones produced in the bark beetle, *Dendroctonus brevicomis*, during host colonization: regulation of inter- and intraspecific competition," *Journal of Chemical Ecology*, vol. 10, no. 6, pp. 861–877, 1984.

[36] J. A. Byers, G. Birgersson, J. Löfqvist, M. Appelgren, and G. Bergström, "Isolation of pheromone synergists of bark beetle, *Pityogenes chalcographus*, from complex insect-plant odors by fractionation and subtractive-combination bioassay," *Journal of Chemical Ecology*, vol. 16, no. 3, pp. 861–876, 1990.

[37] G. Birgersson, J. A. Byers, G. Bergström, and J. Löfqvist, "Production of pheromone components, chalcogran and methyl (E,Z)-2,4-decadienoate, in the spruce engraver *Pityogenes chalcographus*," *Journal of Insect Physiology*, vol. 36, no. 6, pp. 391–395, 1990.

[38] G. Birgersson, F. Schlyter, J. Löfqvist, and G. Bergström, "Quantitative variation of pheromone components in the spruce bark beetle *Ips typographus* from different attack phases," *Journal of Chemical Ecology*, vol. 10, no. 7, pp. 1029–1055, 1984.

[39] G. Birgersson and G. Bergström, "Volatiles released from individual spruce bark beetle entrance holes quantitative variations during the first week of attack," *Journal of Chemical Ecology*, vol. 15, no. 10, pp. 2465–2483, 1989.

[40] G. N. Lanier and D. L. Wood, "Specificity of response to pheromones in the genus *Ips* (Coleoptera: Scolytidae)," *Journal of Chemical Ecology*, vol. 1, no. 1, pp. 9–23, 1975.

[41] D. L. Wood and R. W. Bushing, "The olfactory response of *Ips confusus* (LeConte) (Coleoptera: Scolytidae) to the secondary attraction in the laboratory," *Canadian Entomologist*, vol. 95, pp. 1066–1078, 1963.

[42] J. S. Elkinton, D. L. Wood, and L. B. Hendry, "Pheromone production by the bark beetle, *Ips paraconfusus*, in the nonhost, white fir," *Journal of Chemical Ecology*, vol. 6, no. 6, pp. 979–987, 1980.

[43] J. L. Piston and G. N. Lanier, "Pheromones of *Ips pini* (Coleoptera: Scolytidae). Response to inter populational hybrids and relative attractiveness of males boring in two host species," *Canadian Entomologist*, vol. 106, pp. 247–251, 1974.

[44] V. Thoss and J. A. Byers, "Monoterpene chemodiversity of ponderosa pine in relation to herbivory and bark beetle colonization," *Chemoecology*, vol. 16, no. 1, pp. 51–58, 2006.

[45] R. H. Smith, "Local and regional variation in the monoterpenes of ponderosa pine wood oleoresin," USDA Forest Service Research Paper, PSW-56, 1969.

[46] R. H. Smith, "Variation in the monoterpenes of Pinus ponderosa laws," *Science*, vol. 143, no. 3612, pp. 1337–1338, 1964.

[47] S. J. Seybold, J. Bohlmann, and K. F. Raffa, "Biosynthesis of coniferophagous bark beetle pheromones and conifer isoprenoids: evolutionary perspective and synthesis," *Canadian Entomologist*, vol. 132, no. 6, pp. 697–753, 2000.

[48] A. I. Cognato, "Phylogenetic analysis reveals new genus of Ipini bark beetle (Scolytidae)," *Annals of the Entomological Society of America*, vol. 93, no. 3, pp. 362–366, 2000.

[49] J. A. Tillman, G. L. Holbrook, P. L. Dallara et al., "Endocrine regulation of *de novo* aggregation pheromone biosynthesis in the pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae)," *Insect Biochemistry and Molecular Biology*, vol. 28, no. 9, pp. 705–715, 1998.

[50] J. A. Tillman, F. Lu, L. M. Goddard et al., "Juvenile hormone regulates *de novo* isoprenoid aggregation pheromone biosynthesis in pine bark beetles, *Ips* spp., through transcriptional control of HMG-CoA reductase," *Journal of Chemical Ecology*, vol. 30, no. 12, pp. 2459–2494, 2004.

[51] P. Ivarsson and G. Birgersson, "Regulation and biosynthesis of pheromone components in the double spined bark beetle *Ips duplicatus* (Coleoptera: Scolytidae)," *Journal of Insect Physiology*, vol. 41, no. 10, pp. 843–849, 1995.

[52] B. S. Lanne, P. Ivarsson, P. Johnsson, G. Bergström, and A. B. Wassgren, "Biosynthesis of 2-methyl-3-buten-2-ol, a pheromone component of *Ips typographus* (Coleoptera: Scolytidae)," *Insect Biochemistry*, vol. 19, no. 2, pp. 163–167, 1989.

[53] P. Ivarsson, F. Schlyter, and G. Birgersson, "Demonstration of *de novo* pheromone biosynthesis in *Ips duplicatus* (Coleoptera: Scolytidae): inhibition of ipsdienol and *E*-myrcenol production by compactin," *Insect Biochemistry and Molecular Biology*, vol. 23, no. 6, pp. 655–662, 1993.

[54] S. J. Seybold, D. R. Quilici, J. A. Tillman, D. Vanderwel, D. L. Wood, and G. J. Blomquist, "*De novo* biosynthesis of the aggregation pheromone components ipsenol and ipsdienol by the pine bark beetles *Ips paraconfusus* lanier and *Ips pini* (Say) (Coleoptera: Scolytidae)," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 18, pp. 8393–8397, 1995.

[55] C. Tittiger, G. J. Blomquist, P. Ivarsson, C. E. Borgeson, and S. J. Seybold, "Juvenile hormone regulation of HMG-R gene expression in the bark beetle *Ips paraconfusus* (Coleoptera: Scolytidae): implications for male aggregation pheromone biosynthesis," *Cellular and Molecular Life Sciences*, vol. 55, no. 1, pp. 121–127, 1999.

[56] G. M. Hall, C. Tittiger, G. L. Andrews et al., "Midgut tissue of male pine engraver, *Ips pini*, synthesizes monoterpenoid pheromone component ipsdienol *de novo*," *Naturwissenschaften*, vol. 89, no. 2, pp. 79–83, 2002.

[57] J. B. Nardi, A. G. Young, E. Ujhelyi, C. Tittiger, M. J. Lehane, and G. J. Blomquist, "Specialization of midgut cells for synthesis of male isoprenoid pheromone components in two scolytid beetles, *Dendroctonus jeffreyi* and *Ips pini*," *Tissue and Cell*, vol. 34, no. 4, pp. 221–231, 2002.

[58] P. Sandstrom, W. H. Welch, G. J. Blomquist, and C. Tittiger, "Functional expression of a bark beetle cytochrome P450 that hydroxylates myrcene to ipsdienol," *Insect Biochemistry and Molecular Biology*, vol. 36, no. 11, pp. 835–845, 2006.

[59] P. Sandstrom, M. D. Ginzel, J. C. Bearfield, W. H. Welch, G. J. Blomquist, and C. Tittiger, "Myrcene hydroxylases do not determine enantiomeric composition of pheromonal ipsdienol in *Ips* spp.," *Journal of Chemical Ecology*, vol. 34, no. 12, pp. 1584–1592, 2008.

[60] F. J. Ayala, "Molecular clock mirages," *BioEssays*, vol. 21, no. 1, pp. 71–75, 1999.

[61] A. I. Cognato, S. J. Seybold, D. L. Wood, and S. A. Teale, "A cladistic analysis of pheromone evolution in *Ips* bark beetles (Coleoptera: Scolytidae)," *Evolution*, vol. 51, no. 1, pp. 313–318, 1997.

Hindawi Publishing Corporation
Psyche
Volume 2012, Article ID 529573, 9 pages
doi:10.1155/2012/529573

Research Article

A Population Genetic Model of Evolution of Host-Mate Attraction and Nonhost Repulsion in a Bark Beetle *Pityogenes bidentatus*

John A. Byers

US Arid-Land Agricultural Research Center, USDA-ARS, 21881 North Cardon Lane, Maricopa, AZ 85138, USA

Correspondence should be addressed to John A. Byers, john.byers@ars.usda.gov

Received 12 December 2011; Accepted 14 January 2012

Academic Editor: Qing-He Zhang

Studies have shown that the bark beetle *Pityogenes bidentatus* (Coleoptera, Curculionidae, Scolytinae) avoids volatiles of nonhost trees (Norway Spruce, birch, and oak) and healthy host Scotch Pine when orienting to aggregation pheromone. A population genetic model of two behavioral genes was hypothesized where AA, Aa, and aa were allele combinations regulating orientation to host tree and pheromone odors, and BB, Bb, and bb were combinations allowing avoidance of nonhost and unsuitable host odors. The nine possible genotypes were assigned different survival factors that remained constant during simulation. The initial proportion of aabb genotype (little aggregation/host response and little avoidance of nonhosts) was ~1.0 when a mutation was hypothesized that caused better orientation to host/beetle odors (Aabb) and another mutation causing more efficient avoidance of nonhosts (aaBb). After these initial mutations, the model used indiscriminate mating of genotypic proportions and subsequent survival as input for each successive generation. The results indicate that AABB eventually fixates in the populations in some scenarios, while AABB and other genotypes reach stable equilibriums in other models depending on genotypic survival values supported by ecologically sound assumptions. The models indicate how development of insecticide resistance in pest insects may proceed.

1. Introduction

Individuals of *Pityogenes bidentatus* (Herbst) (Coleoptera, Scolytinae) are fairly small (2–3 mm long) bark beetles that only colonize Scotch Pine, *Pinus sylvestris* L. during a yearly mating flight that occurs in April-May depending on the latitude [1]. These beetles are common in mixed deciduous and conifer forests of northern Europe where they prefer to feed on smaller diameter trunks and limbs of weakened hosts [1]. By 1989, the beetle had become established in the northeastern United States [2]. The males in flight appear to find weakened hosts by a combination of attraction to aggregation pheromone, (*S*)-*cis*-verbenol and grandisol [3–7], and by avoiding odors from nonhost deciduous trees [such as birch, *Betula pendula* Roth; Rowan (mountain ash), *Sorbus aucuparia* L.; English oak, *Quercus robur* L.; alder buckthorn (glossy buckthorn), *Frangula alnus* Mill.], and conifers Norway Spruce, *Picea abies* L., and fresh/healthy host Scotch Pine [5, 6]. In these studies, aggregation components, (*S*)-*cis*-verbenol and grandisol, were placed inside each of a pair of barrier traps separated 6 m apart at 1.2 m height and revolved slowly at 2 rph to even out any trap position effects [5–8]. One of the traps in the pair also had an inhibitory source, either monoterpenes (1 mg/h) or other synthetic plant volatiles, or a fine screen cage containing freshly cut bark chips or twigs with leaves/needles (80 to 200 g) of hosts or nonhosts.

Odors from unsuitable hosts and nonhosts have not been tested alone without aggregation pheromone, so it is not certain that the plant odors can act alone during the beetle's dispersal and search for hosts. However, it was observed that the plant volatiles did repel the beetles in flight as they approached to within 1 m from a source of aggregation pheromone [5]. Many individual monoterpenes and blends released at rates comparable to that released from physical wounds of trees also inhibited attraction to their aggregation pheromone [5–7]. Earlier, a body of evidence had accumulated that attraction responses of conifer-infesting bark beetles in several genera are reduced by volatiles from nonhost angiosperm trees (e.g., *Betula*, *Populus*, *Acer*) [9–20]. Conifers such as pines and spruce usually produce resin, consisting of about 80% of mildly toxic monoterpenes, in order to

defend against the penetrations of the attacking bark beetles [21, 22].

Once a male finds suitable host pine bark, he releases an aggregation pheromone that probably assists most individuals in finding suitable host and breeding habitat [23, 24]. The avoidance of nonhost volatiles may aid the pioneer males in finding suitable hosts during extensive searches as well as aid individuals while landing on colonized bark to avoid nearby nonhosts. On the other hand, little or nothing is known about the behavioral responses needed to select the appropriate host substrate, but it can be hypothesized that there is some attraction to host volatiles that might occur at close range after landing. Interestingly, host pine monoterpenes were only repellent to *P. bidentatus* during flight when responding to aggregation pheromone [5–7] and not when walking (Byers unpublished). Bolts cut from standing Scotch Pine placed in the forest were not colonized by *P. bidentatus* for several weeks during the same time that the beetles were caught in the hundreds on pheromone-baited traps (personal observations). However, several weeks later these bolts became infested, suggesting either a random landing after avoiding nonhosts or a weak attraction to fermenting host volatiles. Various monoterpene blends could indicate to arriving beetles that the trunk was the appropriate host since different tree species have different sets of monoterpenes [25, 26]. In a few cases, bark beetles in the genus *Tomicus* are significantly attracted to Scotch Pine and to its monoterpenes, especially α-pinene (both enantiomers), 3-carene, and terpinolene [27–30]. Several studies have found that certain monoterpenes enhance the attraction to pheromone components in some of the more "aggressive" bark beetles that kill standing trees [31–34].

The objective was to construct a population genetic computer model of evolution with selection of hypothetical genotypes of *P. bidentatus* with two genes each with two alleles, one gene for attraction to host/beetle semiochemicals (A and a) and the second for repulsion by nonhost semiochemicals (B and b). This means that there would be nine possible genotypes conferring special survival or reproductive benefits for each genotype that remained constant throughout the simulation of a specified number of generations. Throughout the population and in every generation, mating was assumed indiscriminate and proportional to each genotype currently present [35, 36]. The nine-by-nine pairings of genotypes gives 81 possible pairings resulting in certain proportions of the nine genotypes, each generation based on the preceding population's proportions of each genotype. The initial proportion of aabb (little or no repulsion by nonhosts and little attraction to hosts) was the prevailing genotype except that one individual would have a mutation of a $\rightarrow$ A, and a second individual would have a mutation of b $\rightarrow$ B to begin the simulations. At each generation the proportions of each genotype were calculated and used as input for the next generation. Survival factors were set initially for the nine genotypes based on logical assumptions. For example, genotype aabb would have a low survival compared to AABB since the latter's individuals would avoid toxic nonhosts (BB) and be attracted to hosts (AA); heterozygous (Aa or Bb) would be intermediate in survival. The results of the models following an evolutionary mutational event would reveal the dramatic to gradual genotypic changes that might be expected during a number of generations resulting in gene fixation or gene equilibrium depending on the survival benefits of the mutated alleles. The same processes illustrated by the models help in understanding the population dynamics of pest insects that overcome crop plant resistance or develop resistance to insecticides [36–38].

2. Materials and Methods

A genetic model of evolution with two alleles, A and a, for attraction to hosts and two alleles, B and b, for repulsion from nonhosts was developed. This two-gene model has nine possible combinations of alleles AABB, AABb, AAbb, AaBB, AaBb, Aabb, aaBB, aaBb, and aabb that can be found in male and female beetles. Mating proceeds according to the proportion of each genotype (pan mixing) giving 81 possible pairings as shown in Table 1. However, the genotype offspring in the table's boxes in the lower left of the diagonal line of the outlined boxes are replicated in the upper diagonal half. Thus, the number of unique pairings is reduced to 9 + 32/2 = 45 as shown in Algorithm 1. This algorithm takes the proportion (P_1 to P_9) of each of the male and female mated genotypes (progeny are equally female and male) and multiplies it by the indicated proportions (1, 2, 4, 8, or 16) times 2 for those not in the diagonal line of the boxes or as indicated if in the diagonal line. Each such value is multiplied by the survival factor (S_1 to S_9) for the appropriate genotype. For example, reasonable survival factors that are relative to each other might be $S_1 = 1$ for AABB, $S_2 = 0.9$ for AABb, $S_3 = 0.5$ for AAbb, $S_4 = 0.8$ for AaBb, $S_5 = 0.6$ for AaBb, $S_6 = 0.3$ for Aabb, $S_7 = 0.4$ for aaBB, $S_8 = 0.3$ for aaBb, and $S_9 = 0.2$ for aabb. These survival factors can just as well be any values as long as they are relative in magnitude (e.g., 10, 9, 5, 8, 6, 3, 4, 3, and 2) since the sums of all multiplications for each of the nine genotypes (G_1 to G_9) are then expressed as a proportion of the total sum of the nine genotypes (P_1 to P_9) according to the following:

$$P_1 = \frac{G_1}{\sum_{k=1}^{9} G_k}. \quad (1)$$

The updated P_1 to P_9 values then serve as the mating proportions of the genotypes for the next generation, iterating until the last generation is attained to obtain the ending genotypic frequencies.

The initial population number based on the initial frequencies would be 10^7. The population would have an initial proportion of almost all aabb ($P_9 = 0.9999998$), except one individual would mutate to Aabb ($P_6 = 0.0000001$) and another would mutate to aaBb ($P_8 = 0.0000001$), and then the model would proceed as described above for at least 100 generations. The model can accommodate any population size by adjusting the initial proportions of the genotypes. The survival factor of each genotype determines the ultimate proportion of each genotype, and as such the possibilities appear unlimited. However, the relative survival of the nine genotypes is constrained as will be evident in four examples

Table 1: Nine genotypes of each sex and the 81 possible crossings and their proportions.

	AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb
AABB[1]	**16AABB**	8AABB 8AABb	16AABb	8AABB 8AaBB	4AABB 4AABb 4AaBB 4AaBb	8AABb 8AaBb	16AaBB	8AaBB 8AaBb	16AaBb
AABb	8AABB 8AABb	**4AABB** **8AABb** **4AAbb**	8AABb 8AAbb	4AABB 4AABb 4AaBB 4AaBb	2AABB 4AABb 2AAbb 2AaBB 4AaBb 2Aabb	4AABb 4AAbb 4AaBb 4Aabb	8AaBB 8AaBb	4AaBB 8AaBb 4Aabb	8AaBb 8Aabb
Aabb	16AABb	8AABb 8AAbb	**16AAbb**	8AABb 8AaBb	4AABb 4AAbb 4AaBb 4Aabb	8AAbb 8Aabb	16AaBb	8AaBb 8Aabb	16Aabb
AaBB	8AABB 8AaBB	4AABB 4AABb 4AaBB 4AaBb	8AABb 8AaBb	**4AABB** **8AaBB** **4aaBB**	2AABB 2AABb 4AaBB 4AaBb 2aaBB 2aaBb	4AABb 8AaBb 4aaBb	8AaBB 8aaBB	4AaBB 4AaBb 4aaBB 4aaBb	8AaBb 8aaBb
AaBb	4AABB 4AABb 4AaBB 4AaBb	2AABB 4AABb 2AAbb 2AaBB 4AaBb 2Aabb	4AABb 4AAbb 4AaBb 4Aabb	2AABB 2AABb 4AaBB 4AaBb 2aaBB 2aaBb	**1AABB** **2AABb** **1AAbb** **2AaBB** **4AaBb** **2Aabb** **1aaBB** **2aaBb** **1aabb**	2AABb 2AAbb 4AaBb 4Aabb 2aaBb 2aabb	4AaBB 4AaBb 4aaBB 4aaBb	2AaBB 4AaBb 2Aabb 2aaBB 4aaBb 2aabb	4AaBb 4Aabb 4aaBb 4aabb
Aabb	8AABb 8AaBb	4AABb 4AAbb 4AaBb 4Aabb	8AAbb 8Aabb	4AABb 8AaBb 4aaBb	2AABb 2AAbb 4AaBb 4Aabb 2aaBb 2aabb	**4AAbb** **8Aabb** **4aabb**	8AaBb 8aaBb	4AaBb 4Aabb 4aaBb 4aabb	8Aabb 8aabb
aaBB	16AaBB	8AaBB 8AaBb	16AaBb	8AaBB 8aaBB	4AaBB 4AaBb 4aaBB 4aaBb	8AaBb 8aaBb	**16aaBB**	8aaBB 8aaBb	16aaBb
aaBb	8AaBB 8AaBb	4AaBB 8AaBb 4Aabb	8AaBb 8Aabb	4AaBB 4AaBb 4aaBB 4aaBb	2AaBB 4AaBb 2Aabb 2aaBB 4aaBb 2aabb	4AaBb 4Aabb 4aaBb 4aabb	8aaBB 8aaBb	**4aaBB** **8aaBb** **4aabb**	8aaBb 8aabb
aabb	16AaBb	8AaBb 8Aabb	16Aabb	8AaBb 8aaBb	4AaBb 4Aabb 4aaBb 4aabb	8Aabb 8aabb	16aaBb	8aaBb 8aabb	**16aabb**

explored here. In the first, it is hypothesized that AABB survives best ($S_1 = 1$) since AA confers a strong attraction to the host, while BB allows the beetle to avoid feeding in the nonhost that would kill the individual. AABb survives well ($S_2 = 0.8$) for the same reasons although Bb, being intermediate, causes some attacks on nonhosts and mortality. AAbb has considerably lower survival ($S_3 = 0.5$) due to bb causing nonhost feeding and mortality, but it does allow many AA to find hosts. AaBB ($S_4 = 0.9$) can be given higher survival than AABb because individuals of the former avoid nonhosts that is slightly more important than a specific attraction to hosts. Heterozygous AaBb ($S_5 = 0.6$) has intermediate survival, while Aabb ($S_6 = 0.3$) and aaBb ($S_8 = 0.3$) are of equally low survival. The aaBB ($S_7 = 0.4$) has slightly more survival due

AABB: $P_1 = 0$: $S_1 = 1$: AABb: $P_2 = 0$: $S_2 = 0.8$: AAbb: $P_3 = 0$: $S_3 = 0.5$
AaBB: $P_4 = 0$: $S_4 = 0.9$: AaBb: $P_5 = 0$: $S_5 = 0.6$: Aabb: $P_6 = 0.0000001$: $S_6 = 0.3$
aaBB: $P_7 = 0$: $S_7 = 0.4$: aaBb: $P_8 = 0.0000001$: $S_8 = 0.3$: aabb: $P_9 = 0.9999998$: $S_9 = 0.2$

For generation = 1 to 100
Row 1: AABB: $G_1=16*P_1*P_1+16*P_1*P_2+16*P_1*P_4+8*P_1*P_5$
AABb: $G_2=16*P_1*P_2+32*P_1*P_3+8*P_1*P_5+16*P_1*P_6$
AaBB: $G_4=16*P_1*P_4+8*P_1*P_5+32*P_1*P_7+16*P_1*P_8$
AaBb: $G_5=8*P_1*P_5+16*P_1*P_6+16*P_1*P_8+32*P_1*P_9$
Row 2: AABB: $G_1=G_1+4*P_2*P_2+8*P_2*P_4+4*P_2*P_5$
AABb: $G_2=G_2+8*P_2*P_2+16*P_2^*P_3+8*P_2*P_4+8*P_2*P_5+8*P_2*P_6$
AAbb: $G_3=4*P_2*P_2+16*P_2*P_3+4*P_2*P_5+8*P_2*P_6$
AaBB: $G_4=G_4+8*P_2*P_4+4*P_2*P_5+16*P_2*P_7+8*P_2*P_8$
AaBb: $G_5=G_5+8*P_2*P_4+8*P_2*P_5+8*P_2*P_6+16*P_2*P_7+16*P_2*P_8+16*P_2*P_9$
Aabb: $G_6=4*P_2*P_5+8*P_2*P_6+8*P_2*P_8+16*P_2*P_9$
Row 3: AAbb: $G_3=G_3+16*P_3*P_3+8*P_3^*P_5+16*P3*P_6$
AABb: $G_2=G_2+16*P_3*P_4+8*P_3*P_5$
AaBb: $G_5=G_5+16*P_3*P_4+8*P_3*P_5+32*P_3*P_7+16*P_3*P_8$
Aabb: $G_6=G_6+8*P_3*P_5+16*P_3*P_6+16*P_3*P_8+32*P_3*P_9$
Row 4: AABB: $G_1=G_1+4*P_4*P_4+4*P_4*P_5$
AaBB: $G_4=G_4+8*P_4*P_4+8*P_4*P_5+16*P_4*P_7+8*P_4*P_8$
aaBB: $G_7=4*P_4*P_4+4*P_4*P_5+16*P_4*P_7+8*P_4*P_8$
AABb: $G_2=G_2+4*P_4*P_5+8*P_4*P_6$
AaBb: $G_5=G_5+8*P_4*P_5+16*P_4*P_6+8*P_4*P_8+16*P_4*P_9$
aaBb: $G_8=4*P_4*P_5+8*P_4*P_6+8*P_4*P_8+16*P_4*P_9$
Row 5: AABB: $G_1=G_1+P_5*P_5$: AABb: $G_2=G_2+2*P_5*P_5+4*P_5*P_6$
AAbb: $G_3=G_3+P_5*P_5+4*P_5*P_6$: AaBB: $G_4=G_4+2*P_5*P_5+8*P_5*P_7+4*P_5*P_8$
AaBb: $G_5=G_5+4*P_5*P_5+8*P_5*P_6+8*P_5*P_7+8*P_5*P_8+8*P_5*P_9$
Aabb: $G_6=G_6+2*P_5*P_5+8*P_5*P_6+4*P_5*P_8+8*P_5*P_9$
aaBB: $G_7=G_7+P_5*P_5+8*P_5*P_7+4*P_5*P_8$
aaBb: $G_8=G_8+2*P_5*P_5+4*P_5*P_6+8*P_5*P_7+8*P_5*P_8+8*P_5*P_9$
aabb: $G_9=P_5*P_5+4*P_5*P_6+4*P_5*P_8+8*P_5*P_9$
Row 6: AAbb: $G_3=G_3+4*P_6*P_6$: Aabb: $G_6=G_6+8*P_6*P_6+8*P_6*P_8+16*P_6*P_9$
aabb: $G_9=G_9+4*P_6*P_6+8*P_6*P_8+16*P_6*P_9$
AaBb: $G_5=G_5+16*P_6*P_7+8*P_6*P_8$ aaBb: $G_8=G_8+16*P_6*P_7+8*P_6*P_8$
Row 7: aaBB: $G_7=G_7+16*P_7*P_7+16*P_7*P_8$: aaBb: $G_8=G_8+16*P_7*P_8+32*P_7*P_9$
Row 8: aaBB: $G_7=G_7+4*P_8*P_8$: aaBb: $G_8=G_8+8*P_8*P_8+16*P_8*P_9$
aabb: $G_9=G_9+4*P_8*P_8+16*P_8*P_9$
Row 9: aabb: $G_9=G_9+16*P_9*P_9$

total = $G_1*S_1+G_2*S_2+G_3*S_3+G_4*S_4+G_5*S_5+G_6*S_6+G_7*S_7+G_8*S_8+G_9*S_9$
$P_1 = G_1*S_1$/total: $P_2 = G_2*S_2$/total: $P_3 = G_3*S_3$/total: $P_4=G_4*S_4$/total
$P_5 = G_5*S_5$/total: $P_6 = G_6*S_6$/total: $P_7 = G_7*S_7$/total: $P_8 = G_8*S_7$/total: $P_9 = G_9*S_9$/total
Next generation

Algorithm 1: General code for algorithms to calculate the proportions P_1 to P_9 of the nine mated genotypes (AABB to aabb, see Table 1) for 200 generations based on initial proportions and nine constant survival factors S_1 to S_9 for individuals of these genotypes (an asterisk denotes multiplication).

to the importance of avoiding toxic nonhosts, while the genotype with the least survival would be aabb ($S_9 = 0.2$). Thus, the order of survival was $S_1 > S_4 > S_2 > S_5 > S_3 > S_7 > S_6 = S_8 > S_9$ (Table 2).

In the second example, the initial proportions were the same, and the survival factors were similar: S_1 (1) > S_4 (0.8) > S_7 (0.7) > S_2 (0.6) > S_5 (0.5) > S_8 (0.4) > S_3 (0.3) > S_6 (0.2) > S_9 (0.1). However, S_7 had significantly higher survival, as did S_8, than in the first example (Table 2). The justification was that the BB of aaBB would allow avoidance of toxic nonhosts better than Bb of AABb, and this advantage outweighs the benefits of a better attraction to hosts (AA versus aa). In the third example, the survival factors were S_2 (1) > S_1 (0.9) > S_5 (0.8) > S_4 (0.7) > S_8 (0.5) > S_7 (0.4) = S_3 (0.4) > S_6 (0.3) > S_9 (0.2). The rationale for this order was that the BB gene caused these bark beetles to be somewhat repelled from forests with nonhost trees [39] so these beetles found hosts less often than Bb, thus Bb > BB > bb and AA > Aa > aa in survival. In the fourth model (Table 2), the order was affected by the preceding rationale for BB, but in addition AA caused too much attraction and competition, while Aa allowed less attraction to crowded hosts and increased survival [40–43].

Table 2: Survival factors (S_1 to S_9) of the nine genotypes used in the five example models.

Example	S_1 AABB	S_2 AABb	S_3 AAbb	S_4 AaBB	S_5 AaBb	S_6 Aabb	S_7 aaBB	S_8 aaBb	S_9 aabb
1	1	0.8	0.5	0.9	0.6	0.3	0.4	0.3	0.2
2	1	0.6	0.3	0.8	0.5	0.2	0.7	0.4	0.1
3	0.9	1	0.4	0.7	0.8	0.3	0.4	0.5	0.2
4	0.8	0.9	0.4	0.4	1	0.5	0.1	0.5	0.2
5	1	0.98	0.95	0.99	0.96	0.93	0.94	0.93	0.92

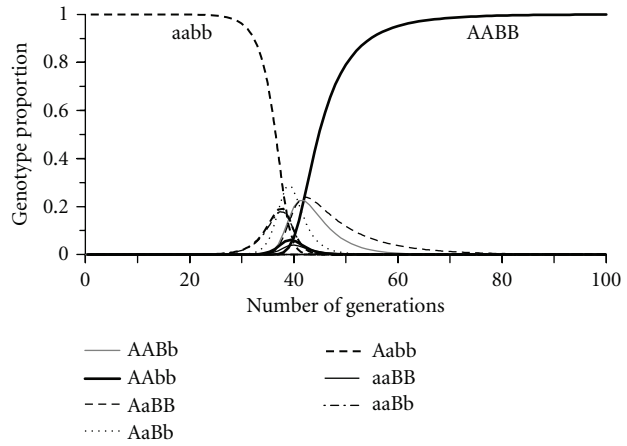


Figure 1: Change in proportions of the nine genotypes during 100 generations (example 1) with indiscriminate mating in which there were initially about 10^7 (0.9999998 aabb) individuals and initially one mutation of a to A (0.0000001 Aabb) and one of b to B (0.0000001 aaBb) resulting in 0.9994339 AABB after 100 generations. Relative survival of each genotype was $S_1 = 1$ (AABB), $S_2 = 0.8$ (AABb), $S_3 = 0.5$ (AAbb), $S_4 = 0.9$ (AaBB), $S_5 = 0.6$ (AaBb), $S_6 = 0.3$ (Aabb), $S_7 = 0.4$ (aaBB), $S_8 = 0.3$ (aaBb), and $S_9 = 0.2$ (aabb).

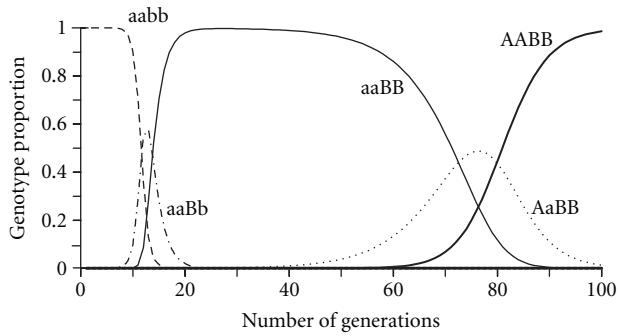


Figure 2: Change in proportions of the nine genotypes during 100 generations (example 2) with indiscriminate mating in which there were initially about 10^7 (0.9999998 aabb) individuals and initially one mutation of a to A (0.0000001 Aabb) and one of b to B (0.0000001 aaBb) resulting in 0.986431 AABB (four genotypes never achieved any significant proportion). Relative survival of each genotype was $S_1 = 1$ (AABB), $S_2 = 0.6$ (AABb), $S_3 = 0.3$ (AAbb), $S_4 = 0.8$ (AaBB), $S_5 = 0.5$ (AaBb), $S_6 = 0.2$ (Aabb), $S_7 = 0.7$ (aaBB), $S_8 = 0.4$ (aaBb), and $S_9 = 0.1$ (aabb).

Thus, Aa > AA > aa, giving an order of S_5 (1) > S_2 (0.9) = S_4 (0.9) > S_1 (0.8) > S_8 (0.5) = S_6 (0.5) > S_3 (0.4) > S_9 (0.2) > S_7 (0.1).

The effect of smaller relative differences in the survival factors was tested in a fifth example where the first model's factors (Table 2) were altered S_1 (1) > S_4 (0.99) > S_2 (0.98) > S_5 (0.96) > S_3 (0.95) > S_7 (0.94) > S_6 (0.93) = S_8 (0.93) > S_9 (0.92), and the number of generations was noted at which P_1 (AABB) > 0.01 or P_9 (aabb) < 0.99. The model (Algorithm 1) was programmed in QuickBASIC 4.5 (Microsoft Corp., Redmond, WA, USA) with results graphed using PostScript 2.0 language (Adobe Systems Inc., San Jose, CA, USA). The model was also implemented in Java 6.0 code (Oracle, Redwood City, CA, USA) for general demonstration on the Internet with a web browser (http://www.chemical-ecology.net/java2/aabb.htm).

3. Results

The predominate initial genotype aabb, with no significant attraction to host volatiles and no avoidance of nonhost volatiles, appears stable for almost 30 generations before plummeting rapidly to near zero by generation 42 (Figure 1). Concomitantly, the dominant genotype AABB logistically grows to 1.0 from generations 38 to 60 and reaches 0.9994 by generation 100. The other seven genotypes rise and fall in approximate normal curves with some skews during generations 25 to 80 (Figure 1). It is apparent that AABB will fixate to 100% eventually.

In the second example, the initial aabb genotype also declines precipitously after near constancy for about 10 generations and approaches zero by generation 15 (Figure 2). However, AABB does not increase above zero for a considerable time until about generation 60 whereupon AABB rises logistically to 0.9864 by 100 generations. It is again clear that AABB fixates. For a number of generations aaBB rises after generation 10 and approaches fixation by generation 22 but then declines gradually until about generation 60 when the genotype then falls to zero (the same period when AABB increases). Only aaBb and AaBB genotypes rise and fall (as Gaussian-like curves) substantially during the fall of aabb and rise of AABB, respectively (Figure 2). The fixation occurs on a time scale that is similar to that found for pesticide resistance in insects and nematodes [37, 38, 44] and indicates that the survival factors chosen here are reasonable for strong selection.

In example 3 (Table 2), the initial aabb is again stable for about 12 generations and then falls when aaBb begins to increase (Figure 3). Other genotypes, aaBB, AaBb, and Aabb

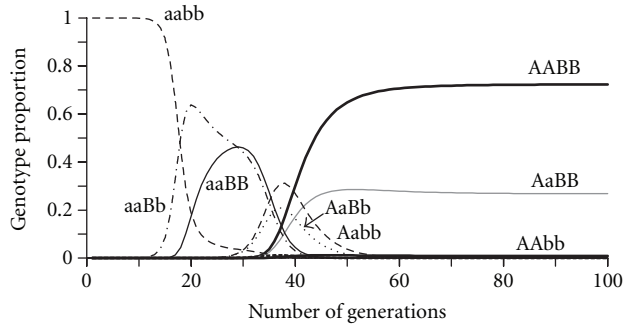


Figure 3: Change in proportions of the nine genotypes during 100 generations (example 3) with indiscriminate mating in which there were initially about 10^7 (0.9999998 aabb) individuals and initially one mutation of a to A (0.0000001 Aabb) and one of b to B (0.0000001 aaBb) resulting in 0.7230 AABB, 0.2681 AABb, and 0.09 AAbb (AABb never achieved any significant proportion). Relative survival of each genotype was S_1= 0.9 (AABB), S_2 = 1 (AABb), S_3 = 0.4 (AAbb), S_4 = 0.7 (AaBB), S_5 = 0.8 (AaBb), S_6 = 0.3 (Aabb), S_7 = 0.4 (aaBB), S_8 = 0.5 (aaBb), and S_9 = 0.2 (aabb).

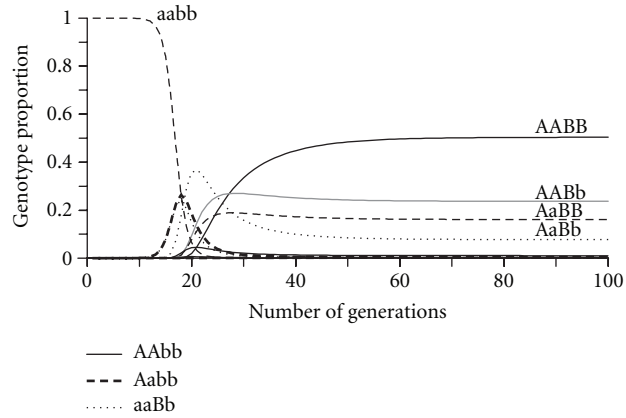


Figure 4: Change in proportions of the nine genotypes during 100 generations (example 4) with indiscriminate mating in which there were initially about 10^7 (0.9999998 aabb) individuals and initially one mutation of a to A (0.0000001 Aabb) and one of b to B (0.0000001 aaBb) resulting in 0.5037 AABB, 0.2376 AABb, and 0.1614 AaBB. Relative survival of each genotype was S_1= 0.8 (AABB), S_2 = 0.9 (AABb), S_3 = 0.4 (AAbb), S_4 = 0.9 (AaBB), S_5 = 1 (AaBb), S_6 = 0.5 (Aabb), S_7 = 0.1 (aaBB), S_8 = 0.5 (aaBb), and S_9 = 0.2 (aabb).

rise, and fall during the rise of AABB and AaBB around generation 33. It is remarkable that AABB appears to reach equilibrium at 0.7230, as does AABb at 0.2681, accounting for most of the population's genotypes at 100 generations. AAbb reaches a low level of equilibrium at 0.0895 (9 percent). Running the model to 1000 generations did not appreciably change these results (AABB = 0.7232, AABb = 0.2679, and AAbb = 0.0893).

In example 4 (Table 2), the initial aabb genotype is stable until about generation 12 and falls rapidly to near 0 by generation 22 while three genotypes (Aabb, aaBb, and AAbb) rise after generation 10 and then decline around generation 20, reaching near 0 levels asymptotically (Figure 4). A few generations before 20, four genotypes rise, with AaBb falling gradually but then reaching a constant equilibrium of about 0.0779. The dominant genotype AABB appears to rise logarithmically to a stable equilibrium that was 0.5037 by generation 100. Similarly, AABb and AaBB rose and then fell slightly to stable equilibriums at 0.2374 and 0.1614, respectively (Figure 4). Running the model to 1000 generations did not change the results (AABB = 0.5039, AABb = 0.2373, AaBB = 0.1614, AaBb = 0.0779, AAbb = 0.0110). The other genotypes, Aabb, aaBB, aaBb, and aabb, also became stable but below 0.0050 proportion. In example 5 (Table 2), the survival parameters were compressed but related to example 1. In this case, the genotypic frequencies were identical but spread out over more generations (not shown). In example 1, the initial population of aabb began to decline significantly when the proportion fell below 0.99 on generation 27 while the AABB proportion began to significantly increase above 0.01 on generation 39. Using the compressed survival factors that caused less selection in example 5, aabb fell below 0.99 on generation 1001, and AABB rose above 0.01 on generation 1394.

4. Discussion

Bark beetles that are termed "aggressive" are among the tree-killing pest species thought to find trees in either of two ways. The first is through a nondirected flight and landing on trees at random whereupon the beetle, a male if in tribe Ipini (e.g., *Pityogenes* or *Ips*) or a female if in tribe Tomicini (e.g., *Dendroctonus* or *Tomicus*), must determine whether the tree is its host and probably whether the tree is acceptable [24, 45]. A beetle that lands on a tree and attempts to enter by boring through the outer bark is termed a "pioneer," especially if there are few others present. Pioneers were presumed to encounter significant host resistance and resin when attacking compared to later arrivals ("joiners") when the tree has succumbed [21, 22, 24, 46]. The hypothesis was that since pioneers must attack the tree and survive to produce pheromone before the rest of the population can exploit the resource, pioneers must be the largest and most vigorous of the population. Byers [24] questioned this paradigm since an individual would undertake a pioneer strategy only if no pheromone was encountered during the dispersal, or after leaving unsuitable colonization areas [41, 43], so that eventually its fat reserves became low [47]. In this "desperate" state, the beetle attempts to bore into any tree and may fortuitously find a tree of low resistance. Thus, smaller beetles that have suffered severe larval competition, or beetles regardless of size that have used up their fat reserves in flight, are hypothesized to be the pioneers.

The second way a beetle finds a host is by orienting to aggregation pheromone. It is evident from host finding models using EAR (effective attraction radius), representing trees and hosts under colonization, that the vast majority of beetles find hosts by orientation to aggregation pheromone [23]. This still means that many beetles perish as pioneers or

simply in the search for hosts; however, most find their host by means of aggregation pheromone. As mentioned earlier, some species in the genus *Tomicus* (e.g., *T. piniperda*) appear not to use a long-range aggregation pheromone but rely instead on volatile monoterpenes predominant in the hosts of their region (in Sweden: α-pinene, 3-carene, and terpinolene) [27–29]. *P. bidentatus* has a strong aggregation pheromone, and thus most individuals would seem to find hosts by the use of these semiochemicals. However, fresh hosts, even with synthetic aggregation pheromone, are repellent, while aged logs in the field may be attractive or located through a random landing process. Avoidance of nonhost volatiles has evolved in a number of bark beetles as stated earlier including *P. bidentatus*. All bark beetles colonize a thin layer (often only 2-3 mm) of cambium/phloem that causes both intraspecific and interspecific competition for food resources [48]. Thus, bark beetles have evolved avoidance of verbenone and aggregation pheromone in order to reduce competition [21, 22, 28, 29, 40, 43, 49].

The attraction to host odors, aggregation pheromone, and avoidance of volatiles indicative of crowding can be implemented in the survival factors of the hypothetical (A, a) gene. The avoidance of nonhost odors, both at the tree and forest stand level, and semiochemicals from unsuitable hosts was incorporated in the assumptions about the survival factors conferred by the (B, b) gene. The relative magnitudes of the survival factors used in the five examples were ecologically reasonable, but many other relative rankings are possible. The speed of evolution can be greatly affected based on the survival factors that represent selection pressures. In example 1 (Figure 1), 39 generations transpired before the first sign of an increase in AABB. It took until generation 45 for 50% of the populations to become AABB. In example 5 with the same order but less difference in relative survival factors, it took 1394 generations before AABB began to increase, and this genotype did not reach 50% until generation 1498 (about 104 generations to increase to 50%).

These results demonstrate that eventually there is a relatively rapid change in the genotype frequencies with fixation of the dominant alleles that are the most beneficial. This evolution is analogous to a mutation for resistance to an insecticide [36–38, 44, 50–52]. It supports why resistance may remain hidden phenotypically for many years (1 generation per year) before suddenly appearing to become widespread. Resistance to insecticides from a mutation that commonly shows up in several to tens of years would have survival factors similar to those used in the present study. According to example 5, resistance in a pest insect could remain hidden for hundreds of years before becoming established. In examples 3 and 4, in which the beneficial genes are heterozygous (Figures 3 and 4), it is evident that phenotypic changes can also take many generations before intermediate gene frequencies result that are stable thereafter. On the other hand, if the population already has an allele that is common that confers insecticide resistance, then there can be an immediate and rapid change favoring this gene, which fixates or reaches equilibriums as in the examples. The population genetic models show how only two loci with two alleles can result in complex genotypic frequencies. More genes are probably involved in both choosing a host and avoiding nonhosts in *P. bidentatus*, which makes the models, undoubtedly, exceedingly complex.

References

[1] B. Lekander, B. Bejer Peterson, F. Kangas, and A. Bakke, "The distribution of bark beetles in the Nordic countries," *Acta Entomologia Fennica*, vol. 32, pp. 1–36, 1977.

[2] E. R. Hoebeke, "*Pityogenes bidentatus* (Herbst), a European bark beetle new to North America (Coleoptera: Scolytidae)," *Journal of New York Entomological Society*, vol. 97, no. 3, pp. 305–308, 1989.

[3] V. E. J. Baader, "*Pityogenes* spp. (Col., Scolytidae): Untersuchungen über verhaltenssteuernde Duftstoffe und deren Anwendung im Waldschutz," *Journal of Applied Entomology*, vol. 107, no. 1, pp. 1–31, 1989.

[4] W. Francke, J. Bartels, H. Meyer et al., "Semiochemicals from bark beetles: new results, remarks, and reflections," *Journal of Chemical Ecology*, vol. 21, no. 7, pp. 1043–1063, 1995.

[5] J. A. Byers, Q. H. Zhang, and G. Birgersson, "Strategies of a bark beetle, *Pityogenes bidentatus*, in an olfactory landscape," *Naturwissenschaften*, vol. 87, no. 11, pp. 503–507, 2000.

[6] J. A. Byers, Q. H. Zhang, and G. Birgersson, "Avoidance of nonhost plants by a bark beetle, *Pityogenes bidentatus*, in a forest of odors," *Naturwissenschaften*, vol. 91, no. 5, pp. 215–219, 2004.

[7] A. M. El-Sayed and J. A. Byers, "Inhibitory effect of monoterpenes on response of *Pityogenes bidentatus* to aggregation pheromone released by piezoelectric sprayer for precision release of semiochemicals," *Journal of Chemical Ecology*, vol. 26, no. 8, pp. 1795–1809, 2000.

[8] J. A. Byers, F. Schlyter, G. Birgersson, and W. Francke, "*E*-myrcenol in *Ips duplicatus*: an aggregation pheromone component new for bark beetles," *Experientia*, vol. 46, no. 11-12, pp. 1209–1211, 1990.

[9] J. C. Dickens, R. F. Billings, and T. L. Payne, "Green leaf volatiles interrupt aggregation pheromone response in bark beetles infesting southern pines," *Experientia*, vol. 48, no. 5, pp. 523–524, 1992.

[10] L. M. Schroeder, "Olfactory recognition of nonhosts aspen and birch by conifer bark beetles *Tomicus piniperda* and *Hylurgops palliatus*," *Journal of Chemical Ecology*, vol. 18, no. 9, pp. 1583–1593, 1992.

[11] I. M. Wilson, J. H. Borden, R. Gries, and G. Gries, "Green leaf volatiles as antiaggregants for the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae)," *Journal of Chemical Ecology*, vol. 22, no. 10, pp. 1861–1875, 1996.

[12] A. Guerrero, J. Feixas, J. Pajares, L. J. Wadhams, J. A. Pickett, and C. M. Woodcock, "Semiochemically induced inhibition of behaviour of *Tomicus destruens* (Woll.) (Coleoptera: Scolytidae)," *Naturwissenschaften*, vol. 84, no. 4, pp. 155–157, 1997.

[13] J. H. Borden, L. J. Chong, A. Savoie, and I. M. Wilson, "Responses to green leaf volatiles in two biogeoclimatic zones by striped ambrosia beetle, *Trypodendron lineatum*," *Journal of Chemical Ecology*, vol. 23, no. 11, pp. 2479–2491, 1997.

[14] J. H. Borden, I. M. Wilson, R. Gries, L. J. Chong, H. D. Pierce, and G. Gries Jr., "Volatiles from the bark of trembling aspen, *Populus tremuloides* Michx. (Salicaceae) disrupt secondary attraction by the mountain pine beetle, *Dendroctonus pondevosae* Hopkins (Coleoptera: Scolytidae)," *Chemoecology*, vol. 8, no. 2, pp. 69–75, 1998.

[15] E. K. Deglow and J. H. Borden, "Green leaf volatiles disrupt and enhance response to aggregation pheromones by the ambrosia beetle, *Gnathotrichus sulcatus* (Coleoptera: Scolytidae)," *Canadian Journal of Forest Research*, vol. 28, no. 11, pp. 1697–1705, 1998.

[16] J. A. Byers, Q. H. Zhang, F. Schlyter, and G. Birgersson, "Volatiles from nonhost birch trees inhibit pheromone response in spruce bark beetles," *Naturwissenschaften*, vol. 85, no. 11, pp. 557–561, 1998.

[17] Q. H. E. Zhang, G. Birgersson, J. Zhu, C. Löfstedt, J. Löfqvist, and F. Schlyter, "Leaf volatiles from nonhost deciduous trees: variation by tree species, season and temperature, and electrophysiological activity in *Ips typographus*," *Journal of Chemical Ecology*, vol. 25, no. 8, pp. 1923–1943, 1999.

[18] Q. H. Zhang, F. Schlyter, and P. Anderson, "Green leaf volatiles interrupt pheromone response of spruce bark beetle, *Ips typographus*," *Journal of Chemical Ecology*, vol. 25, no. 12, pp. 2847–2861, 1999.

[19] D. P. W. Huber, R. Gries, J. H. Borden, and H. D. Pierce, "Two pheromones of coniferophagous bark beetles found in the bark of nonhost angiosperms," *Journal of Chemical Ecology*, vol. 25, no. 4, pp. 805–816, 1999.

[20] T. M. Poland and R. A. Haack, "Pine shoot beetle, *Tomicus piniperda* (Col., Scolytidae), responses to common green leaf volatiles," *Journal of Applied Entomology*, vol. 124, no. 2, pp. 63–69, 2000.

[21] J. A. Byers, "Chemical ecology of bark beetles," *Experientia*, vol. 45, no. 3, pp. 271–283, 1989.

[22] J. A. Byers, "Host tree chemistry affecting colonization in bark beetles," in *Chemical Ecology of Insects 2*, R. T. Cardé and W. J. Bell, Eds., pp. 154–213, Chapman and Hall, New York, NY, USA, 1995.

[23] J. A. Byers, "An encounter rate model of bark beetle populations searching at random for susceptible host trees," *Ecological Modelling*, vol. 91, no. 1–3, pp. 57–66, 1996.

[24] J. A. Byers, "Effects of attraction radius and flight paths on catch of scolytid beetles dispersing outward through rings of pheromone traps," *Journal of Chemical Ecology*, vol. 25, no. 5, pp. 985–1005, 1999.

[25] J. A. Byers and G. Birgersson, "Pheromone production in a bark beetle independent of myrcene precursor in host pine species," *Naturwissenschaften*, vol. 77, no. 8, pp. 385–387, 1990.

[26] V. Thoss and J. A. Byers, "Monoterpene chemodiversity of ponderosa pine in relation to herbivory and bark beetle colonization," *Chemoecology*, vol. 16, no. 1, pp. 51–58, 2006.

[27] J. A. Byers, B. S. Lanne, J. Löfqvist, F. Schlyter, and G. Bergström, "Olfactory recognition of host-tree susceptibility by pine shoot beetles," *Naturwissenschaften*, vol. 72, no. 6, pp. 324–326, 1985.

[28] J. A. Byers, B. S. Lanne, and J. Löfqvist, "Host tree unsuitability recognized by pine shoot beetles in flight," *Experientia*, vol. 45, no. 5, pp. 489–492, 1989.

[29] B. S. Lanne, F. Schlyter, J. A. Byers et al., "Differences in attraction to semiochemicals present in sympatric pine shoot beetles, *Tomicus minor* and *T. piniperda*," *Journal of Chemical Ecology*, vol. 13, no. 5, pp. 1045–1067, 1987.

[30] J. A. Byers, "Attraction of bark beetles, *Tomicus piniperda*, *Hylurgops palliatus*, and *Trypodendron domesticum* and other insects to short-chain alcohols and monoterpenes," *Journal of Chemical Ecology*, vol. 18, no. 12, pp. 2385–2402, 1992.

[31] W. D. Bedard, P. E. Tilden, D. L. Wood, R. M. Silverstein, R. G. Brownlee, and J. O. Rodin, "Western pine beetle: field response to its sex pheromone and a synergistic host terpene, myrcene," *Science*, vol. 164, no. 3885, pp. 1284–1285, 1969.

[32] R. A. Werner, "Response of the beetle, *Ips grandicollis*, to combinations of host and insect produced attractants," *Journal of Insect Physiology*, vol. 18, no. 7, pp. 1403–1412, 1972.

[33] J. A. Rudinsky, M. M. Furniss, L. N. Kline, and R. F. Schmitz, "Attraction and repression of Dendroctonus pseudotsugae (Coleoptera: Scolytidae) by three synthetic pheromones in traps in Oregon and Idaho," *Canadian Entomologist*, vol. 104, no. 6, pp. 815–822, 1972.

[34] J. A. Byers, G. Birgersson, J. Löfqvist, and G. Bergström, "Synergistic pheromones and monoterpenes enable aggregation and host recognition by a bark beetle," *Naturwissenschaften*, vol. 75, no. 3, pp. 153–155, 1988.

[35] J. Roughgarden, "Population genetics with multiple loci," in *Theory of Population Genetics and Evolutionary Ecology: An Introduction*, J. Roughgarden, Ed., pp. 111–133, MacMillan, New York, NY, USA, 1979.

[36] D. W. Onstad and C. A. Guse, "Concepts and complexities of population genetics," in *Insect Resistance Management: Biology, Economics, and Prediction*, D. W. Onstad, Ed., pp. 69–88, Academic Press, London, UK, 2008.

[37] J. Mallet, "The evolution of insecticide resistance: have the insects won?" *Trends in Ecology and Evolution*, vol. 4, no. 11, pp. 336–340, 1989.

[38] P. C. Lopes, É. Sucena, M. E. Santos, and S. Magalhães, "Rapid experimental evolution of pesticide resistance in *C. elegans* entails no costs and affects the mating system," *PLoS One*, vol. 3, no. 11, Article ID e3741, 2008.

[39] Q. H. Zhang and F. Schlyter, "Olfactory recognition and behavioural avoidance of angiosperm nonhost volatiles by conifer-inhabiting bark beetles," *Agricultural and Forest Entomology*, vol. 6, no. 1, pp. 1–19, 2004.

[40] J. A. Byers and D. L. Wood, "Interspecific inhibition of the response of the bark beetles, *Dendroctonus brevicomis* and *Ips paraconfusus*, to their pheromones in the field," *Journal of Chemical Ecology*, vol. 6, no. 1, pp. 149–164, 1980.

[41] J. A. Byers, "Sex-specific responses to aggregation pheromone: regulation of colonization density in the bark beetle *Ips paraconfusus*," *Journal of Chemical Ecology*, vol. 9, no. 1, pp. 129–142, 1983.

[42] J. A. Byers, "Nearest neighbor analysis and simulation of distribution patterns indicates an attack spacing mechanism in the bark beetle, *Ips typographus* (Coleoptera: Scolytidae)," *Environmental Entomology*, vol. 13, pp. 1191–1200, 1984.

[43] F. Schlyter, J. A. Byers, and J. Löfqvist, "Attraction to pheromone sources of different quantity, quality, and spacing: density-regulation mechanisms in bark beetle *Ips typographus*," *Journal of Chemical Ecology*, vol. 13, no. 6, pp. 1503–1523, 1987.

[44] F. R. Groeters and B. E. Tabashnik, "Roles of selection intensity, major genes, and minor genes in evolution of insecticide resistance," *Journal of Economic Entomology*, vol. 93, no. 6, pp. 1580–1587, 2000.

[45] H. A. Moeck, D. L. Wood, and K. Q. Lindahl, "Host selection behavior of bark beetles (Coleoptera: Scolytidae) attacking *Pinus ponderosa*, with special emphasis on the western pine beetle, *Dendroctonus brevicomis*," *Journal of Chemical Ecology*, vol. 7, no. 1, pp. 49–83, 1981.

[46] A. A. Berryman, "Dynamics of bark beetle populations: towards a general productivity model," *Environmental Entomology*, vol. 3, no. 4, pp. 579–585, 1974.

[47] P. F. Botterweg, "Dispersal and flight behaviour of the spruce bark beetle *Ips typographus* in relation to sex, size and fat

content," *Zeitschrift für Angewandte Entomologie*, vol. 94, no. 1–5, pp. 466–489, 1982.

[48] D. M. Light, M. C. Birch, and T. D. Paine, "Laboratory study of intraspecific and interspecific competition within and between two sympatric bark beetle species, *Ips pini* and *Ips paraconfusus*," *Zeitschrift für Angewandte Entomologie*, vol. 96, no. 2, pp. 233–241, 1983.

[49] J. A. Byers, "Avoidance of competition by spruce bark beetles, *Ips typographus* and *Pityogenes chalcographus*," *Experientia*, vol. 49, no. 3, pp. 272–275, 1993.

[50] P. J. Daborn, J. L. Yen, M. R. Bogwitz et al., "A single P450 allele associated with insecticide resistance in *Drosophila*," *Science*, vol. 297, no. 5590, pp. 2253–2256, 2002.

[51] P. Labbé, C. Berticat, A. Berthomieu et al., "Forty years of erratic insecticide resistance evolution in the mosquito *Culex pipiens*," *PLoS Genetics*, vol. 3, no. 11, pp. 2190–2199, 2007.

[52] E. Gazave, C. Chevillon, T. Lenormand, M. Marquine, and M. Raymond, "Dissecting the cost of insecticide resistance genes during the overwintering period of the mosquito *Culex pipiens*," *Heredity*, vol. 87, no. 4, pp. 441–448, 2001.

Hindawi Publishing Corporation
Psyche
Volume 2012, Article ID 414508, 7 pages
doi:10.1155/2012/414508

Research Article

2-methyl-3-buten-2-ol: A Pheromone Component of Conifer Bark Beetles Found in the Bark of Nonhost Deciduous Trees

Qing-He Zhang,[1,2] Fredrik Schlyter,[1] and Göran Birgersson[1]

[1] *Chemical Ecology, Department of Plant Protection Biology, Swedish University of Agricultural Sciences, P.O. Box 102, 230 53 Alnarp, Sweden*
[2] *R&D Department, Sterling International, Inc., 3808 N. Sullivan Road Building 16, Spokane, WA 99216, USA*

Correspondence should be addressed to Qing-He Zhang, qing-he@rescue.com

Received 30 December 2011; Accepted 9 February 2012

Academic Editor: John A. Byers

Volatiles from bark of aspen, *Populus tremula* L. and two species of birch: silver birch (*Betula pendula* Roth.) and common birch (*B. pubescens* Ehrh.), were collected by direct solvent extraction and aeration of both newly cut bark chips and undamaged stems in June 1998 and subjected to GC-MS analysis. The results showed the presence of 2-methyl-3-buten-2-ol (MB), one of the two principal aggregation pheromone components of the spruce bark beetle, *Ips typographus*, in bark extraction samples of all the three deciduous tree species tested. In addition, one more oxygenated hemiterpene, 3-methyl-3-buten-2-one, and (*E*)-3-penten-2-ol were also found in the bark extracts. Only trace amounts of MB were detected in some aeration samples of the fresh bark chips, and no MB was found from the aeration samples of undamaged stems at detectable levels. The occurrence of this compound was also confirmed in the bark of four exotic birch species: *B. albosinensis* Schneid., *B. ermanii* Cham., *B. jacquemontii* Spach, and *B. maximowicziana* Regel, but not yet in the European pines/spruces and the common yeasts. Our results raise major questions regarding the evolution, the tropospheric chemistry, and the ecological role of this hemiterpene alcohol. They also suggest that comparative studies on the biosynthetic pathways for MB in different sources would be of considerably evolutionary interest.

1. Introduction

Aspen, *Populus tremula* L. and two birch species, silver birch (*Betula pendula* Roth.) and common birch (*B. pubescens* Ehrh.), are the most common deciduous trees in Norway spruce forests of Scandinavia [1]. Studies showed that volatiles from leaves and bark of nonhost aspen/birch trees strongly inhibit pheromone attraction in spruce bark beetles, *Ips typographus* L. and *Pityogenes chalcographus* L. [2, 3]. In order to determine what kind of volatiles are responsible for the inhibition effect, volatiles from these nonhost trees were collected by headspace aerations and solvent extraction and analyzed by GC-MS [1, 4, 5]. Further electrophysiological and field bioassay studies showed that green leaf alcohols (GLVs) from leaves and bark and some specific compounds like *trans*-conophthorin, from bark of the nonhost deciduous trees, disrupt the secondary attraction response of sympatric coniferophagous bark beetles [4, 5]. Such inhibitory effects by angiosperm nonhost volatiles have been shown on many other conifer-inhabiting bark beetles throughout the world [3]. In the present paper, we report our finding of 2-methyl-3-buten-2-ol (here abbreviated as MB [6], while in atmospheric chemistry often as MBO), one of the principal aggregation pheromone components of *I. typographus,* in the bark of its nonhost trees *P. tremula, B. pendula*, and *B. pubescens*, which poses questions regarding the evolution and ecological role of this semiochemical in the natural habitat.

2. Materials and Methods

Volatiles from the nonhost bark were collected by direct extraction with diethyl ether and by aerations of both newly cut bark chips and undamaged stems in June 1998, Asa, Småland, Sweden. One cm^2 of bark cut into 2×5 mm pieces taken at 1.5 m height of standing trees from each species was extracted in 1 mL of diethyl ether. Fresh bark chips (size: 3×6 cm, with total area of ca. 1000 cm^2) from each individual

tree at breast height were aerated in the laboratory within 15–30 min after bark sampling. They were enclosed in a plastic-cooking bag (35 × 43 cm) with an activated charcoal filter tube at the air inlet. The volatiles in the bag were trapped on Porapak Q (30 mg, mesh 50–80 (Supelco), in Teflon tube: ID 3 mm × 35 mm) for 2 h at airflow rate of 300 mL/min and recovered by extraction with diethyl ether [1]. The same aeration setup was also used for the volatile collection from the undamaged stems at 1.3–1.7 m height for 1.5 h with battery-operated pumps. Air temperatures inside and outside of sampling bags were recorded during the aerations with a Min-Max reading thermometer. Additional fresh bark extraction samples of four exotic birch species, *B. albosinensis* Schneid., *B. ermanii* Cham., *B. jacquemontii* Spach, and *B. maximowicziana* Regel, using the same sampling approach as described previously for the three native Scandinavian species were taken from the Alnarp Botanical Garden, Skåne, Sweden in February 1999. All extracts were kept at −20°C before the GC-MS analysis. After collection, the bark samples were dried at 65°C for 72 h and weighed. The detailed information about the bark sampling is shown in Table 1.

The chemical analyses were made by a combined HP 5890 series II gas chromatography and HP 5972 mass selective detector (GC-MSD). The GC was equipped with a 25 m × 0.25 mm × 0.30 μm fused silica column, coated with CP-Wax no. 58 (FFAP CB) (Agilent Technologies). All samples were injected by a HP 7673 autoinjector (2 μL each). Helium was used as the carrier gas at an electronically controlled constant flow of 31 cm/s. The injector temperature was 200°C, and oven temperature was at 30°C for the first 3 min, then programmed to 200°C at 10°C/min, where it remained for 2 min.

Volatiles were identified by comparison of the retention indices and mass spectra with those of authentic compounds, with computerized data library, NBS75K, and with custom produced library (KE1995). Absolute amounts were obtained by comparison to the internal standard, the stabilizer, butylated hydroxytoluene (BHT) of diethyl ether.

3. Results

GC-MS analyses of bark solvent extracts clearly showed the presence of 2-methyl-3-buten-2-ol (MB) in the bark of all the three native Scandinavian deciduous tree species tested (Figure 1). This identification was proven by comparison of retention time and mass spectrum to the authentic compound and computer data libraries (NBS75K and KE1995) (Figure 1). The amounts of MB in the extracts were estimated ca 4.5, 2.5, and 10.7 μg/g dw, for *B. pendula*, *B. pubescens* and *P. tremula*, respectively (Table 2). In addition to the MB, one more oxygenated hemiterpene, 3-methyl-3-buten-2-one, and (*E*)-3-penten-2-ol were identified in the bark extracts, with their average amounts being lower than that of MB (Table 2). The occurrence of MB as a minor component was also confirmed in the bark samples of four exotic birch species: *B. albosinensis, B. ermanii, B. jacquemontii*, and *B. maximowicziana* by GC-MS.

GC-MS analyses of some aeration samples of the fresh bark chips did also indicate the presence of MB, but only in trace amounts which might be due to the major breakthrough of this highly volatile alcohol through the Porapak Q trap [7]. No MB was found in the aeration samples of undamaged stems at detectable levels.

4. Discussion

4.1. Insect Sources. MB was first identified as one of the principal aggregation pheromone components of spruce bark beetle, *Ips typographus* [8], and was also observed in emissions from the entrance holes made by this species on the trunks of both live [7] and cut [9] spruce trees [10], with emission rates per bore hole being significantly larger than the average content of hindgut. It has been reported as a pheromone component or male-specific compound of several other conifer bark beetles in Eurasia, *Ips* (*Orthotomicus*) *erosus* Woll. [11], *I. nitidus* Eggers [12], *I. shangrila* Cognato and Sun [13], *Pteleobius vittatus* (F.) [14], and *Pityogenes* spp. [15]. MB was found to be produced by females of *Ips amitinus* (Eichhoff) as well and seemed to be inhibitive [16]. It is reportedly an alarm pheromone of the European hornet, *Vespa crabro* L. [17].

4.2. Plant Sources. This is the first report on the presence of MB in bark of deciduous trees. In addition to the three major native angiosperm deciduous tree species (*P. tremula, B. pendula*, and *B. pubescens*), MB was also detected in bark samples (taken in February) of several exotic birch species, including *B. albosinensis, B. ermanii, B. jacquemontii* and *B. maximowicziana*. Thus, its natural occurrence in plants might be much more common than we ever realized. In fact, the emission of this isoprene alcohol from plants had been observed before; the orchid *Aerides lawrenceae* produces MB [18]. Interestingly, MB is also a hop constituent with sedative hypnotic activity [19] and formed from humulones and lupulones by reaction with OH radicals in the presence of atmospheric oxygen [20]. Further study suggested that the same reaction with OH radicals may occur *in vivo*. For instance, it induced the murine cytochrome P4503A and ethylmorphine N-demethylation (a functional marker for P4503A) in mice [21]. Both 2-methyl-3-buten-2-ol (MB) and 3-methyl-3-buten-2-one are parts of volatile composition from the headspace of five lima bean plants infested with two-spotted spider mites (*Tetranychus urticae* Koch) [22]. MB is also a fragrance ingredient used in cosmetics, fine fragrances, shampoos, toilet soaps, and other toiletries as well as in noncosmetic products such as household cleaners and detergents [23].

Zimmerman et al. (1991) noted the presence of MB in samples taken from enclosures placed around branches of Loblolly pine (*Pinus taeda* L.) [24]. Goldan et al. (1993) characterized the trace gas composition of ambient air in a small clearing in a predominantly lodgepole pine forest with a significant admixture of aspen and occasional Colorado blue spruce (3050 m elevation) in Colorado in June 1991 [25]. They found MB to be the dominant volatile organic compound (VOC), with a concentration 4–7 times higher than that of isoprene. Based on the fact that diurnal changes in

Table 1: Background information on bark samples of nonhost deciduous trees, Sweden.

Samples						Tree dimension			
Scientific name	Common name	Location	Date	Time	No. of trees	Height (m)	DBH (cm)	Dry weight or sampling area	Temperature (inside/outside) °C
Bark extractions of the key species									
B. pendula Roth	Silver Birch	Asa, Småland	1998-06-11	15:00–15:10	2	10–13	10–14	0.16–0.20 g	
B. pubescens Ehrh.	Downy Birch	Asa, Småland	1998-06-11	15:35–15:50	3	12–20	10–18	0.13–0.26 g	
P. tremula L.	Aspen	Asa, Småland	1998-06-11	15:10–15:25	3	8.5–9.0	8–10	0.13–0.20 g	
Bark extractions of additional birch species									
B. albosinensis var. *septentrionalis* Schneid.	Northern Chinese red birch	Alnarp, Skåne	2/24/1999	10:00–10:20	2	9–12	8–12	NA*	
*B. ermanii*Cham.	Erman's birch	Alnarp, Skåne	2/24/1999	10:21–10:40	2	8–10	8–11	NA	
B. jacquemontii Spach	White barked Himalayan Birch	Alnarp, Skåne	2/24/1999	10:41–10:55	2	8–14	7–12	NA	
B. maximowicziana Regel	Monarch Birch	Alnarp, Skåne	2/24/1999	11:10–10:25	2	15–20	15–22	NA	
Aerations of fresh bark chips									
B. pendula Roth	Silver Birch	Asa, Småland	1998-06-09	9:30–10:00	4	13–16	14–16	147–152 g	21.9–22.7/21–21.7
B. pubescens Ehrh.	Downy Birch	Asa, Småland	1998-06-08	17:00–17:30	4	15–20	24–30	140–210 g	22.8–23.4/22.5–23
P. tremula L.	Aspen	Asa, Småland	1998-06-09	9:30–10:00	4	12–17	11–20	106–194 g	21.9–22.7/21–21.7
Aerations of undamaged stems									
B. pendula Roth	Silver Birch	Asa, Småland	1998-06-29	12:50–14:20	2	15–20	18–20	0.22–0.24 m^2	17.5–31.4/15.5–21.3
B. pubescens Ehrh.	Downy Birch	Asa, Småland	1998-06-29	13:55–15:25	2	15–20	12–20	0.11–0.16 m^2	19.5–20.0/18.3–19.2
P. tremula L.	Aspen	Asa, Småland	1998-06-29	12:15–13:45	2	13–15	14–18	0.18–0.22 m^2	17.5–31.4/15.5–21.3

*No fresh/dry weights were measured, and the amounts of 232 MB in the samples were not quantified.

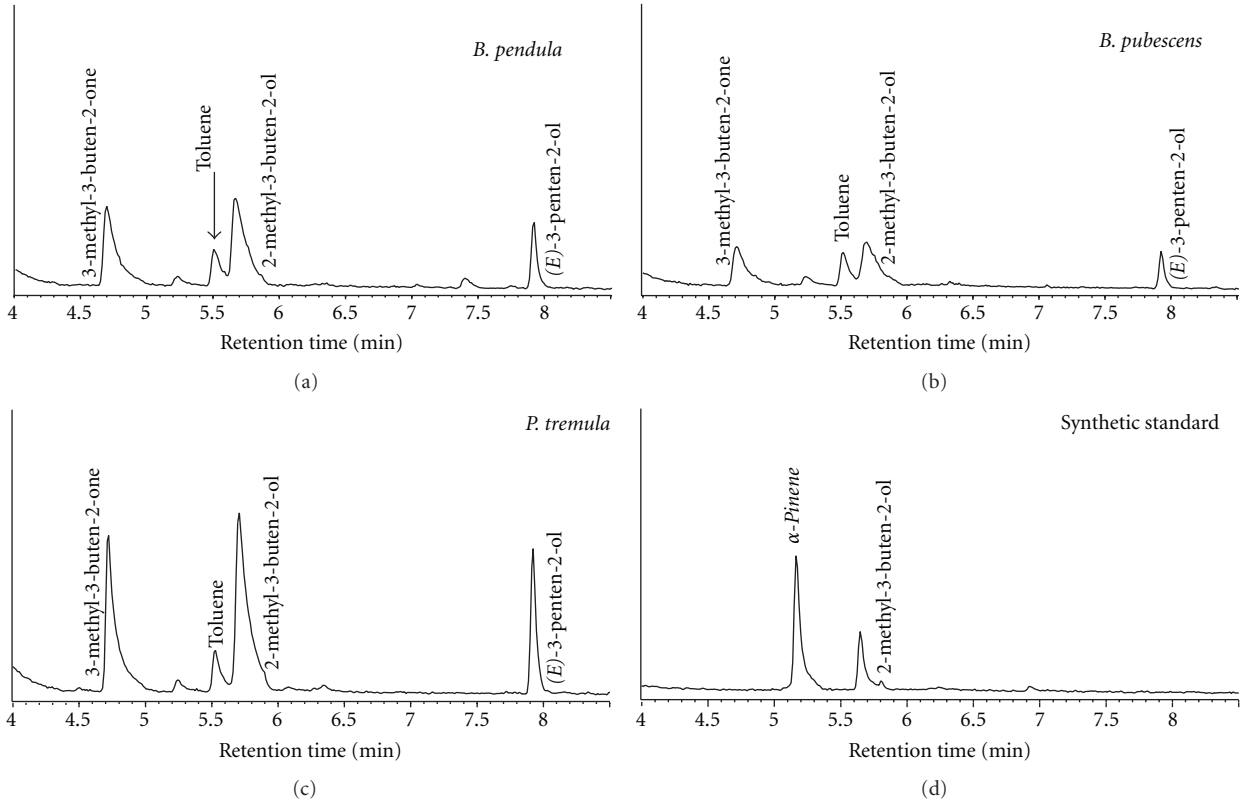


Figure 1: Gas chromatograms from bark extracts of *B. pendula, B. pubescens*, and *P. tremula,* and synthetic standard of 2-methyl-3-buten-2-ol (MB).

Table 2: Amounts of oxygenated hemiterpenes and (*E*)-3-penten-2-ol present in the bark extracts of deciduous trees, Asa, Sweden, June 11, 1998.

Compounds	Mean ± SD (μg/g DW bark)		
	B. pendula ($n = 2$)	*B. pubescens* ($n = 3$)	*P. tremula* ($n = 3$)
2-methyl-3-buten-2-ol (MB)	4.53 ± 1.37	2.51 ± 2.80	10.68±5.63
3-methyl-3-buten-2-one	3.83 ± 1.13	1.66 ± 2.06	7.42 ± 4.33
(*E*)-3-penten-2-ol	1.37 ± 0.46	0.61 ± 0.81	4.04 ± 2.02

ambient MB concentrations were very similar to those of isoprene, with known biogenic sources, and on the fact that MB concentrations did not correlate well with those of benzene, an indicator of anthropogenic source, they concluded that there was likely to be a large local biogenic source of MB, probably the lodgepole pine forest. Harley et al. (1998) successfully detected and measured the emission of MB from needles of several North American pine species, confirming MB as a biogenic VOC (BVOC) [26]. MB emissions from *Pinus ponderosa* were absent in the dark and strongly dependent on incident light, behaving similarly to net photosynthesis. The fact that MB emissions rapidly fall to near zero in darkness suggested that MB is being emitted immediately upon production, rather than stored in any specialized structures of pine needles. Their further screening study on 34 species of pines for MB emission in California showed that 11 species exhibited high emissions of MB (>5 μg C/g/h), and 6 emitted small but detectable amounts. All the emitting species are of North American origin, and most are restricted to western North America. Their results from both intact and severed branches indicated that MB emissions from pines may constitute a significant source of reactive carbon and a significant source to the atmosphere of acetone, a product of MB oxidation [26]. Interestingly, we find no MB records from European pines, and MB is not detected, based on its characteristic base ion $m/z = 71$, in the host tree *Picea abies* L. neither from bark extracts (C. Schiebe, unpubl.) nor emitted from foliage (M. Binyameen, unpubl.).

4.3. Microbial Sources. A plethora of short-chained BVOC is produced by yeasts and other microorganisms, including methyl butenol isomers [27, 28]. Somewhat surprisingly, the insect- and plant-produced 2-methyl-3-buten-2-ol is not reported in the microbial-related literatures, and is not found among VOCs analyzed with GC-MS from different types of cultured yeasts (M. Proffit, unpubl results).

4.4. Atmospheric Chemistry. Following the observation of MB emission in pine forest by Goldan et al. (1993) [25], several studies on the atmospheric chemistry of this BVOC alcohol have been actively carried out [29–35]. Harley et al. (1998) claimed that the major photochemical sink for MB

during daylight hours is assumed to be with OH^- [26]. The rate coefficients with similar values for the OH^- reaction with MB were reported by Rudich et al. (1995) and Ferronato et al. (1998), which suggested a relatively short atmospheric lifetime of ca. 2 hours [30, 34]. However, given typical atmospheric values of O_3 and NO_3, the rate constants for MB reaction with O_3 [32] or with NO_3 [33, 34] imply significantly longer MB lifetimes with respect to these destruction processes. Further reaction chamber experiments [29, 30] indicate that the MB–OH reaction leads to the production of acetone, glycol aldehyde, formaldehyde, and presumably 2-hydroxy-2-methylpropanal. The reaction with O_3 appears to yield the same major products, though in different proportions [29, 32]. Recently, Chan et al. (2009) suggested that photooxidation of MB might be a potential but minor source of secondary organic aerosol (SOA) [35]. Despite its structural similarity to isoprene, photooxidation of MB is not expected to make a significant contribution to SOA formation [35].

By using a model considering landscape average emission potential (μg C $g^{-1} h^{-1}$), total foliar density (g m^{-2}) (estimated by the available data on forest biomass and species composition), and emission activity factor, Harley et al. (1998) were able to compare the ambient concentrations of MB observed by Goldan et al. (1993) with their own enclosure rates of MB emission and found a reasonable agreement [25, 26]. Recent estimate of global MB emission is about 9.6 Tg per year [36, 37].

4.5. Potential Semiochemical Functions. MB seems to have multiple functions, including semiochemical, flavor, and pharmacological roles, and strong impact on atmospheric chemistry, which are dependent on its sources. The role of MB in the semiochemical system of *I. typographus* has been intensively studied. On the basis of dose-response curves from electroantennograms (EAGs), Dickens (1981) suggested that MB might act as a close-range/landing substance as it had a higher threshold (100 μg on filter paper) and very steep dose-response profile [38]. By using specially designed trap groups, Schlyter et al.(1987) clearly showed that MB does act as a close-range landing (or entering of trap holes) stimulus in the field [39]. However, our combined gas chromatographic-electroantennographic detection (GC-EAD) analysis of the bark extract samples of these three tree species showed no antennal responses by *I. typographus* to the existing MB (Zhang et al. unpubl.). It is mainly due to the fact that the amounts of MB in the extracts are much lower than the response threshold of *I. typographus* [38]. Furthermore, no MB was detected in the aeration samples of the undamaged stems, which might be caused by either the breakthrough of this compound through the Porapak Q trap or the minor amounts of release. Thus, the amounts of MB produced by the bark of nonhost birch or aspen, or emitted from the undamaged stems if any, most probably have no significant impact on the host selection behavior of *I. typographus* in the natural habitat. Goldan et al. (1993) doubted the source of MB from *I. typographus* and speculated an extra-insect source from host spruce trees [25]. In contrast, our GC-MS analyses of aeration samples of bark chips and cut branches with fresh needles of Norway spruce, *Picea abies*, did not find any MB at detectable levels [1]. In fact, no MB emitting trees had been discovered in Europe where the two bark beetles (*I. typographus* and *Ips* (*Orthotomicus*) *erosus*) use MB as parts of their pheromone systems prior to our current study.

Inclusion of MB in a trap consisting of a mixture of pheromone attractants for the spruce bark beetle, *Dendroctonus rufipennis*, was shown to reduce the number of *D. rufipennis* trapped, suggesting a possible antiattractant role of this alcohol [40]. However, MB exhibited no repellent properties when tested alone nor did it appear to have any effect on the aggregation response of two North American conifer bark beetles (*Ips paraconfusus* and *Dendroctonus brevicomis*) and their predators (Trogositidae and Cleridae) to their pheromones [41].

4.6. Biosynthesis. A biosynthetic study by Lanne et al. (1989), using radiolabeled precursors, clearly showed that MB is produced *de novo* by *I. typographus* through the mevalonic pathway [9]. The biosynthetic pathway in other insects (*Ips* (*Orthotomicus*) *erosus*, *I. nitidus* and *I. shangrila*, and *Vespa crabro*) and the Eurasian angiosperm trees (bark of birch and aspen) still remains unknown. However, the gene for MB synthase was recently identified from *Pinus sabiniana*, the MB producing pine species, and the protein encoded was functionally characterized by Gray et al. (2011) [42]. MB synthase is a bifunctional enzyme which produces both MB and isoprene in a ratio of ca. 90 : 1 [42] via dimethylallyl diphosphate (DMADP) [43]. Another oxygenated hemiterpene, 3-methyl-3-buten-2-one, was also found constantly from our bark extracts. It is not clear if this oxygenated hemiterpene is involved in the biosynthetic pathway of MB. Our results raise major questions regarding the evolution, tropospheric chemistry, and ecological role of this short, branched alcohol. They also suggest that comparative studies on the biosynthetic pathways for MB in different natural sources would be of considerable evolutionary interest.

Acknowledgments

The authors thank their younger colleagues at Chemical Ecology, Alnarp, for sharing their unpublished GC-MS results from Norway spruce (C. Schiebe, M. Binyameen) and yeasts (Dr. M. Proffit), respectively. Their data collection was supported by Grants from the Swedish Council for Forestry and Agricultural Research (SJFR, no. 23.0521/96 and no.24.0293/98) and an EU-INCO project ("TATRY", CT 98-0151). F. Schlyter and G. Birgersson are supported by the Linnaeus program "Insect Chemical Ecology, Ethology and Evolution" (ICE^3).

References

[1] Q. H. E. Zhang, G. Birgersson, J. Zhu, C. Löfstedt, J. Löfqvist, and F. Schlyter, "Leaf volatiles from nonhost deciduous trees: variation by tree species, season and temperature, and electrophysiological activity in *Ips typographus*," *Journal of Chemical Ecology*, vol. 25, no. 8, pp. 1923–1943, 1999.

[2] J. A. Byers, Q. H. Zhang, F. Schlyter, and G. Birgersson, "Volatiles from nonhost birch trees inhibit pheromone response in spruce bark beetles," *Naturwissenschaften*, vol. 85, no. 11, pp. 557–561, 1998.

[3] Q. H. Zhang and F. Schlyter, "Olfactory recognition and behavioural avoidance of angiosperm nonhost volatiles by conifer-inhabiting bark beetles," *Agricultural and Forest Entomology*, vol. 6, no. 1, pp. 1–19, 2004.

[4] Q. H. Zhang, F. Schlyter, and P. Anderson, "Green leaf volatiles interrupt pheromone response of spruce bark beetle, *Ips typographus*," *Journal of Chemical Ecology*, vol. 25, no. 12, pp. 2847–2861, 1999.

[5] Q. H. Zhang, F. Schlyter, and G. Birgersson, "Bark volatiles from nonhost angiosperm trees of spruce bark beetle, *Ips typographus* (L.) (Coleoptera: Scolytidae): chemical and electrophysiological analysis," *Chemoecology*, vol. 10, no. 2, pp. 69–80, 2000.

[6] G. Birgersson, F. Schlyter, J. Löfqvist, and G. Bergström, "Quantitative variation of pheromone components in the spruce bark beetle *Ips typographus* from different attack phases," *Journal of Chemical Ecology*, vol. 10, no. 7, pp. 1029–1055, 1984.

[7] G. Birgersson and G. Bergström, "Volatiles released from individual spruce bark beetle entrance holes quantitative variations during the first week of attack," *Journal of Chemical Ecology*, vol. 15, no. 10, pp. 2465–2483, 1989.

[8] A. Bakke, P. Froyen, and L. Skattebol, "Field response to a new pheromonal compound isolated from Ips typographus," *Naturwissenschaften*, vol. 64, no. 2, pp. 98–99, 1977.

[9] B. S. Lanne, P. Ivarsson, P. Johnsson, G. Bergström, and A. B. Wassgren, "Biosynthesis of 2-methyl-3-buten-2-ol, a pheromone component of *Ips typographus* (Coleoptera: Scolytidae)," *Insect Biochemistry*, vol. 19, no. 2, pp. 163–167, 1989.

[10] T. Zhao, A.-K. Borg-Karlson, N. Erbilgin, and P. Krokene, "Host resistance elicited by methyl jasmonate reduces emission of aggregation pheromones by the spruce bark beetle, *Ips typographus*," *Oecologia*, vol. 167, no. 3, pp. 691–699, 2011.

[11] H. Giesen, U. Kohnle, J. P. Vité, M.-L. Pan, and W. Francke, "Das Aggregationspheromon des mediterranen Kiefernborkenkäfers *Ips (Orthotomicus) erosus*," *Journal of Applied Entomology*, vol. 98, no. 1, pp. 95–97, 1984.

[12] Q. H. Zhang, J. H. Ma, F. Y. Zhao, L. W. Song, and J. H. Sun, "Aggregation pheromone of the qinghai spruce bark beetle, *Ips nitidus* eggers," *Journal of Chemical Ecology*, vol. 35, no. 5, pp. 610–617, 2009.

[13] Q. H. Zhang, L. W. Song, J. H. Ma, F. Z. Han, and J. H. Sun, "Aggregation pheromone of a newly described spruce bark beetle, *Ips shangrila* Cognato and Sun, from China," *Chemoecology*, vol. 19, no. 4, pp. 203–210, 2009.

[14] D. Klimetzek, J. Bartels, and W. Francke, "Das Pheromon-System des Bunten Ulmenbastkäfers *Pteleobius vittatus* (F.) (Col., Scolytidae)," *Journal of Applied Entomology*, vol. 107, no. 5, pp. 518–523, 1989.

[15] E. J. Baader, "*Pityogenes* spp. (Col., Scolytidae): investigations on semiochemicals and their application in forest protection," *Journal of Applied Entomology*, vol. 107, no. 1, pp. 1–31, 1989.

[16] M. Zuber, "Racemate and enantiomers of ipsdienol for attracting *Ips amitinus* (Eichh.) (Col., Scolytidae)," *Anzeiger für Schaedlingskunde Pfanzenschutz Unweltschutz*, vol. 67, no. 5, pp. 92–93, 1994.

[17] H. J. Veith, N. Koeniger, and U. Maschwitz, "2-methyl-3-butene-2-ol, a major component of the alarm pheromone of the hornet *Vespa crabro*," *Naturwissenschaften*, vol. 71, no. 6, pp. 328–329, 1984.

[18] R. Kaiser, *The Scent of Orchids*, Elsevier, Amsterdam, The Netherlands, 1993.

[19] R. Wohlfart, R. Hansel, and H. Schmidt, "Sedative hypnotic principles in hops. Part 4. Pharmacology of the hop constituent 2-methyl-3-buten-2-ol," *Planta Medica*, vol. 48, no. 2, pp. 120–123, 1983.

[20] R. Wohlfart, G. Wurm, R. Hansel, and H. Schmidt, "edative hypnotic principles in hops. Part 5. degradation of humulones and lupulones to 2-methyl-3-buten-2-ol, a hop constituent possessing sedative hypnotic activity," *Archiv der Pharmazie*, vol. 316, no. 2, pp. 132–137, 1983.

[21] G. J. Mannering and J. A. Shoeman, "Murine cytochrome P4503A is induced by 2-methyl-3-buten-2-ol, 3-methyl-1-pentyn-3-ol(meparfynol), and tert-amyl alcohol," *Xenobiotica*, vol. 26, no. 5, pp. 487–493, 1996.

[22] M. Dicke, "Behavioural and community ecology of plants that cry for help," *Plant, Cell and Environment*, vol. 32, no. 6, pp. 654–665, 2009.

[23] D. McGinty, J. Scognamiglio, C. S. Letizia, and A. M. Api, "Fragrance material review on 2-methyl-3-buten-2-ol," *Food and Chemical Toxicology*, vol. 48, supplement 3, pp. S97–S100, 2010.

[24] P. Zimmerman, W. Pollack, A. Guenther, and H. Westberg, "Field deployment of a GC-MS system for determination of biogenic hydrocarbons in vegetation enclosures and ambient air (abstract)," *Eos Transactions, American Geophysical Union*, vol. 72, p. 88, 1991.

[25] P. D. Goldan, W. C. Kuster, F. C. Fehsenfield, and S. A. Montzka, "The observation of a C5 alcohol emission in a north American pine forest," *Geophysical Research Letters*, vol. 20, no. 11, pp. 1039–1042, 1993.

[26] P. Harley, V. Fridd-Stroud, J. Greenberg, A. Guenther, and P. Vasconcellos, "Emission of 2-methyl-3-buten-2-ol by pines: A potentially large natural source of reactive carbon to the atmosphere," *Journal of Geophysical Research D*, vol. 103, no. 19, pp. 25479–25486, 1998.

[27] A. M. El-Sayed, V. J. Heppelthwaite, L. M. Manning, A. R. Gibb, and D. M. Suckling, "Volatile constituents of fermented sugar baits and their attraction to Lepidopteran species," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 4, pp. 953–958, 2005.

[28] J. Stökl, A. Strutz, A. Dafni et al., "A deceptive pollination system targeting drosophilids through olfactory mimicry of yeast," *Current Biology*, vol. 20, no. 20, pp. 1846–1852, 2010.

[29] G. Fantechi, N. R. Jensen, J. Hjorth, and J. Peeters, "Mechanistic study of the atmospheric degradation of isoprene and MBO," in *Proceedings of the EUROTRAC symposium*, pp. 397–340, Computational Mechanics, 1996.

[30] C. Ferronato, J. J. Orlando, and G. S. Tyndall, "Rate and mechanism of the reactions of OH and Cl with 2-methyl-3-buten-2-ol," *Journal of Geophysical Research D*, vol. 103, no. 19, pp. 25579–25586, 1998.

[31] D. Grosjean and E. Grosjean, "Carbonyl products of the ozone-unsaturated alcohol reaction," *Journal of Geophysical Research*, vol. 100, no. 11, pp. 22815–22820, 1995.

[32] E. Grosjean and D. Grosjean, "Rate constants for the gas-phase reactions of ozone with unsaturated aliphatic alcohols," *International Journal of Chemical Kinetics*, vol. 26, no. 12, pp. 1185–1191, 1994.

[33] M. Hallquist, S. Langer, E. Ljungström, and I. Wängberg, "Rates of reaction between the nitrate radical and some unsaturated alcohols," *International Journal of Chemical Kinetics*, vol. 28, no. 6, pp. 467–474, 1996.

[34] Y. Rudich, R. Talukdar, J. B. Burkholder, and A. R. Ravishankara, "Reaction of methylbutenol with hydroxyl radical: mechanism and atmospheric implications," *Journal of Physical Chemistry*, vol. 99, no. 32, pp. 12188–12194, 1995.

[35] A. W. H. Chan, M. M. Galloway, A. J. Kwan et al., "Photooxidation of 2-methyl-3-buten-2-ol (MBO) as a potential source of secondary organic aerosol," *Environmental Science and Technology*, vol. 43, no. 13, pp. 4647–4652, 2009.

[36] T. M. Fu, D. J. Jacob, F. Wittrock, J. P. Burrows, M. Vrekoussis, and D. K. Henze, "Global budgets of atmospheric glyoxal and methylglyoxal, and implications for formation of secondary organic aerosols," *Journal of Geophysical Research D*, vol. 113, no. 15, Article ID D15303, 17 pages, 2008.

[37] A. Guenther, T. Karl, P. Harley, C. Wiedinmyer, P. I. Palmer, and C. Geron, "Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature)," *Atmospheric Chemistry and Physics*, vol. 6, no. 11, pp. 3181–3210, 2006.

[38] J. C. Dickens, "Behavioral and electrophysiological responses of the bark beetle, *Ips typographus*, to potential pheromone components," *Physiological Entomology*, vol. 6, no. 3, pp. 251–261, 1981.

[39] F. Schlyter, J. Lofqvist, and J. A. Byers, "Behavioural sequence in the attraction of the bark beetle Ips typographus to pheromone sources," *Physiological Entomology*, vol. 12, no. 2, pp. 185–196, 1987.

[40] R. A. Werner and E. H. Holsten, "Current status of research with the spruce beetle *Dendroctonus rufipennis*," in *Application of Semiochemicals for Management of Bark Beetle Infestations—Proceedings of an Informal Conference USDA For Ser Gen Tech Rep INT-GTR-318*, S. M. Salom and K. R. Hobson, Eds., pp. 23–29, 1995.

[41] D. W. Gray, "Field response of Ips paraconfusus, *Dendroctonus brevicomis*, and their predators to 2-methyl-3-buten-2-ol, a novel alcohol emitted by ponderosa pine," *Journal of Chemical Ecology*, vol. 28, no. 8, pp. 1583–1597, 2002.

[42] D. W. Gray, S. R. Breneman, L. A. Topper, and T. D. Sharkey, "Biochemical characterization and homology modeling of methylbutenol synthase and implications for understanding hemiterpene synthase evolution in plants," *The Journal of Biological Chemistry*, vol. 286, no. 23, pp. 20582–20590, 2011.

[43] A. J. Fisher, B. M. Baker, J. P. Greenberg, and R. Fall, "Enzymatic synthesis of methylbutenol from dimethylallyl diphosphate in needles of *Pinus sabiniana*," *Archives of Biochemistry and Biophysics*, vol. 383, no. 1, pp. 128–134, 2000.

Hindawi Publishing Corporation
Psyche
Volume 2012, Article ID 321084, 7 pages
doi:10.1155/2012/321084

Research Article

Declining Bark Beetle Densities (*Ips typographus*, Coleoptera: Scolytinae) from Infested Norway Spruce Stands and Possible Implications for Management

Alexander Angst,[1] Regula Rüegg,[2] and Beat Forster[1]

[1] *Research Unit Forest Dynamics, Swiss Federal Research Institute WSL, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland*
[2] *Department of Environmental System Sciences, Swiss Federal Institute of Technology ETH, ETH-Zentrum, 8092 Zürich, Switzerland*

Correspondence should be addressed to Beat Forster, beat.forster@wsl.ch

Received 15 September 2011; Revised 22 December 2011; Accepted 28 December 2011

Academic Editor: John A. Byers

The eight-toothed spruce bark beetle (*Ips typographus*) is the most serious insect pest in Central European forests. During the past two decades, extreme meteorological events and subsequent beetle infestations have killed millions of cubic meters of standing spruce trees. Not all the infested stands could be cleared in time, and priorities in management had to be set. Natural or man-made buffer zones of about 500 meters in width are frequently defined to separate differently managed stands in Central Europe. While the buffer zones seem to be effective in most of the cases, their impact has not been studied in detail. Beetle densities were therefore assessed in three case studies using pheromone traps along transects, leading from infested stands into spruce-free buffer zones. The results of the trap catches allow an estimation of the buffer zone influence on densities and the dispersal of *Ips typographus*. Beetle densities were found to decrease rapidly with increasing distance from the infested spruce stands. The trap catches were below high-risk thresholds within a few hundred meters of the infested stands. The decrease in catches was more pronounced in open land and in an urban area than in a broadleaf stand. Designed buffer zones of 500 m width without spruce can therefore very probably help to reduce densities of spreading beetles.

1. Introduction

The eight-toothed or European spruce bark beetle (*Ips typographus* L.) is, from an economic and ecological point of view, one of the most serious forest pests in Europe [1, 2]. It mainly attacks Norway spruce (*Picea abies* (L.) Karst.). Poorly textured, even-aged, pure stands of spruce are particularly vulnerable. Suddenly exposed spruce on the edges of stands are highly attractive to bark beetles. The pest outbreaks, such as after windstorms or droughts, are likely to trigger dieback of host trees on a large scale within several years. This happened for example after the years 1983 and 1984, when heavy thunderstorms swept over the Bavarian National Park, resulting in an abundance of uncleared wind-felled trees. The following bark beetle outbreak spreads rapidly to the surrounding stands due to favourable weather conditions. Managed spruce stands bordering the park were also affected [3]. In wide parts of Central Europe, the infestations in the years after the storm "Lothar" in 1999 and again after the hot and dry summer of 2003 were similarly impressive. In Switzerland, the spruce bark beetle killed a volume corresponding to 40% of the spruce increment that grew in the period from 1999 to 2005 [4].

The mass attacks posed a great challenge for the forest services and forest owners. Logistic problems were often the reason why conventional control measures could not be organized in time. A certain amount of wind-felled spruces and infestation spots had to remain uncleared. That is why forest owners and the local authorities have to set management priorities according to what functions the forest has and what type of landscape is involved [5]. A considerably high level of *Ips typographus* infestation can often be observed in disturbed and unmanaged spruce stands such as those in protected areas [6], which leads to a greater beetle pressure on neighbouring managed stands. If managed stands border unmanaged ones, the edges of

cleared infestation spots often become reinfested with bark beetles, due to a high beetle pressure from the uncleared spots. A clever choice of natural spruce-free borders like mountains, meadows, villages, lakes, or broadleaf stands help to minimize such buffer-zone problems between managed and unmanaged spruce stands.

Because the distance of *I. typographus*' active dispersal flights is believed to vary within a few hundred meters, a buffer zone of 500 meters between uncleared infested stands and managed forests is recommended in forest practice. If a spruce-free belt cannot be selected, intense monitoring takes place within the buffer zone and beetle attacked trees will be felled and removed or debarked. Within such a zone, no infested spruce is tolerated.

The safety distance of 500 meters is based on the experience of field foresters and the results of several studies. The GIS-based study of Wichmann and Ravn [7] showed that the short distance dispersal of beetles is less than 500 meters since at a distance of more than 500 meters an area with wind-thrown or infested trees has no significant influence on a beetle population. Similar findings were obtained in the Bavarian National Park. Despite heavy infestation pressure, Heurich et al. [3] found that a reinfestation in the peripheral zone could be limited to a distance of 500 meters. Becker and Schröter [8] showed that standing attacks markedly decreased 500 meters away from the primary infestation spot. On the other hand, Duelli et al. [9, 10], who investigated the migrational behaviour of the spruce bark beetle and their flight patterns outside forests or outside spruce stands in situations with a low beetle impact, did not observe such a clear decrease. In contrast to these dispersal studies in areas with a low beetle density, in the present study, we analyzed distance-dependent beetle captures emanating from stands with high beetle densities and infestations on standing spruce. In particular, we wanted to clarify whether the postulated minimal width of a buffer zone of 500 meters is reasonable and if it can be recommended for control strategies in Switzerland. What is relevant in this case is that the impact decreases sufficiently within this distance to reduce the infestation risk for neighbouring stands. So the goal was not to ascertain the origin of all the captured beetles, but rather to find out whether there is a distance-dependent decrease in beetle pressure emanating from known infestation spots.

2. Materials and Methods

2.1. Study Areas. For our study, we focused particularly on forest districts with a high beetle infestation in the previous year, which had resulted in considerable compulsory fellings, infestations spots, and beetle catches in pheromone traps. The research areas were chosen to include Norway spruce stands bordering large open areas or pure broadleaf stands. No experiments within spruce stands were conducted because it would not have been possible to specify how many of the beetles caught in the traps spread from the initial infestation spots without marking and releasing beetles.

All transects started at spots or stands that had been infested by *Ips typographus* in the previous year. In all three study areas, the *I. typographus* populations were bivoltine as the altitude varies between 400 and 700 m a.s.l. The preliminary transect of 2006 led to a city (Figure 1(a)) and the 2008 transects led to an open area (Figure 1(b)), and a broadleaf stand (Figure 1(c)). The transect in the city of Zurich (8°33′51.56″/47°23′4.35″) was approximately 2,000 meters long. It started on the Zurichberg (Figure 1(a)) in a mixed stand with several uncleared infestation spots within a few hundred meters and then went southwest towards the city centre (main station). It crossed an urban area with green spaces and gardens, where some scattered Norway spruce occur as ornamentals.

In Oberbüren (9°10′36.24″/47°26′23.74″) in Canton St. Gallen, transect b was nearly 1,000-meter long (Figure 1(b)). It led from west to east with an uphill slope of just 3%, away from a formerly infested Norway spruce forest edge to an open area (9°13′44.1″/47°26′3.06″). The traps were set up along the talus of a motorway several dozen meters away from traffic. The Norway spruce stand had scattered infestations in the previous years on an area of nearly 20 hectares.

The 1,320-meter-long transect c at Renedaal near Reutenen (9°2′47.65″/47°39″8.32″) in Canton Thurgau (Figure 1(c)) led northwest with a downward slope of 7% into a broadleaf stand (9°2′0.52″/47°39′34.87″) of about 45 hectares. This transect started in a recently cleared infestation spot within a mixed stand of roughly 2.5 hectares containing Norway spruce. The transect went through an almost pure broadleaf stand (mainly European beech, *Fagus sylvatica*) with only a few single spruce trees, silver firs (*Abies alba*), and Scots pines (*Pinus sylvestris*), all without any bark beetle infestations. The second half of transect c followed a forest road.

2.2. Arrangement of the Traps. Four to seven pairs of black slit traps were set per transect. The lure in all traps was Pheroprax of BASF, a synthetic aggregation pheromone, based on (S)-*cis*-verbenol and methylbutenol. The traps were arranged pairwise to average out the influences of the microlocations and eventual lure irregularities. The trap arrangement in Zurich was somewhat different from the others. Only four pairs of traps were used. The distance between the single traps within a pair varied in this case between 10 and 90 meters, with the traps often set up on buildings (roofs, terraces) to prevent vandalism.

In the two 2008 experiments, a pair of traps was positioned at each sampling point with the upper end of the trap at breast height (1.3 meter). The trap pairs had a gap of approximately 10 meters between the single traps. The front side of the trap was arranged so as to have as much antemeridian and midday sun as possible. Furthermore, care was taken to place the traps at homogeneous microlocations to ensure catch conditions were as uniform as possible.

The preliminary experiment in Zurich (transect a) was performed in a shortened period from June 19 to August 15, 2006. It did not cover the whole flight activity of the overwintered *Ips typographus* generation. The traps in transects b and c were installed on April 23 and 24, 2008, and immediately baited with the attractants. The traps were last emptied and then removed on September 11, 2008. Exactly

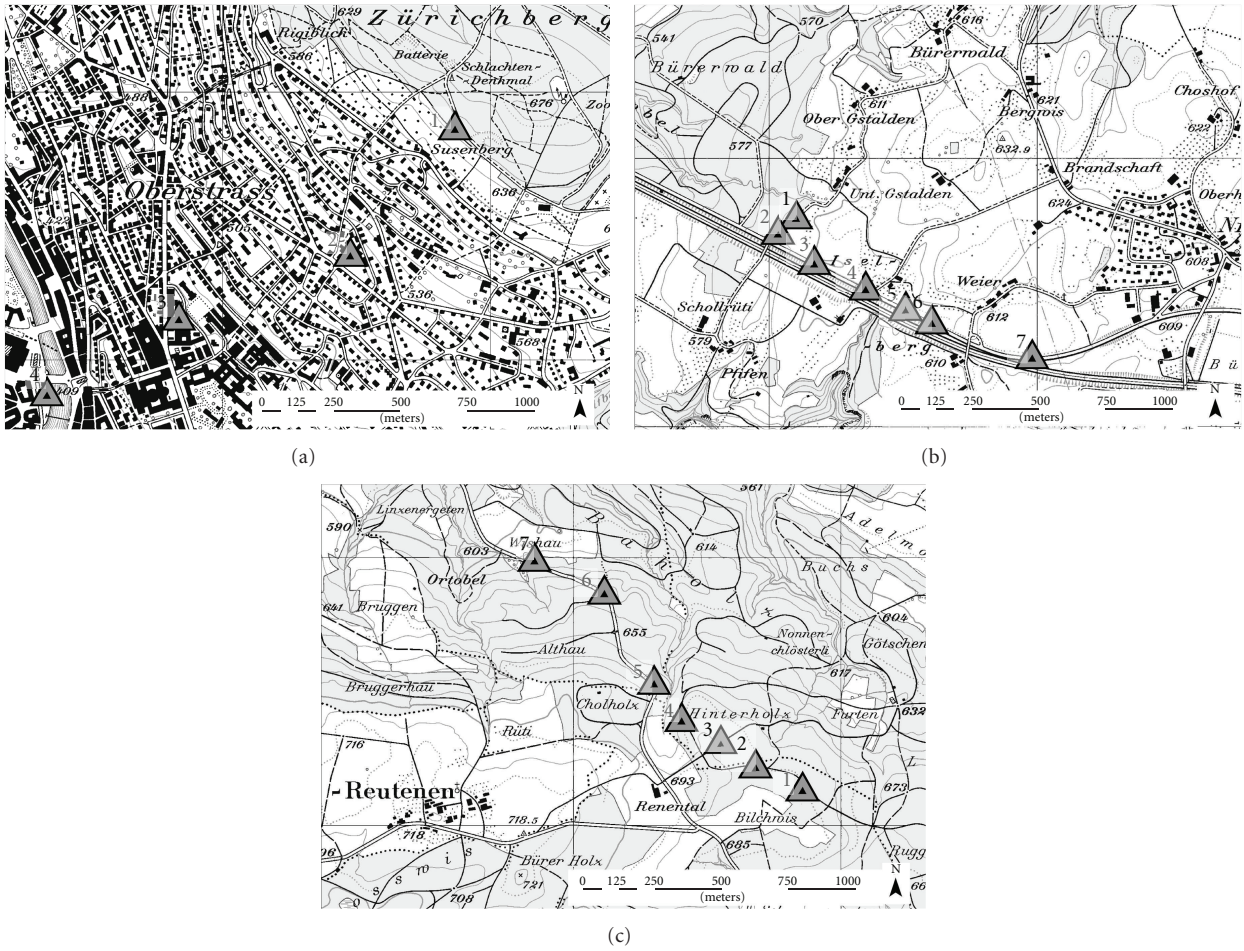


Figure 1: The design of the trap transects in (a) the city, (b) the open land, and (c) the broadleaf stand. All transects start in stands with infestations spots. (Maps: VECTOR 25, swisstopo (JA100118), 2010.)

every 14 days, the traps were inspected, emptied, and the beetles counted.

2.3. Data Analysis. The data were plotted with the program DataDesk (Version 6.3, Data Description, Inc., Ithaca, NY), and a curve was fitted to the points. For the "fall-off of density with distance," the equation of the type $y = a + bx$ was applied, or rather an improvement on it: $y = e(a + bxc)$, according to Southwood [11], where x is the number of beetles and y is distance from the infestations. The curve with the best fit was that of Hawkes [12] of the type $y = e(a + b\sqrt{x})$. Duelli et al. [10] used this formula to represent the spread of freshly emerged beetles. This equation assumes that the lengths of individual moves are not haphazard and random [11]. The coefficient of determination R^2 was calculated to describe the correlation. For the whole analysis, the sample pairs were not pooled. Instead, the single traps within the pairs were used.

3. Results

3.1. Number of Beetles Caught. All pheromone traps caught at least a few specimens of *Ips typographus,* and the catches decreased with the distance to the infested stands (Figure 2). The average number of the catches per trap and transect ranged between 2,500 beetles in the city (transect a), 3,600 beetles in the open area (transect b), and 11,300 beetles in the broadleaf stand (transect c). Because all transects lead away from former infested stands or spots, it makes more sense to compare the trap catches in or near the infestation area only. This means the catches at the starting points of the transects are higher and demonstrate a considerable infestation level. At the starting point of transect c in the broadleaf stand, the catches (25,300) are about twice as high as at the starting point of transect b in the open area (11,200). The catches at the starting point of the city transect a (9,400) are below this value, but they were caught during a shorter trapping period that excluded the first flight in spring, which is when a high flight density of beetles is usual [13].

3.2. Beetle Densities in the City, Open Land, and Mixed Deciduous Stand. Along all transects, a clear decrease in the numbers of bark beetles individuals was monitored (Figure 2). In the city of Zurich, along transect a, the calculated curve of the catches declines quickly: after 40 meters, the beetle density was only half that in the forest and after 680 meters

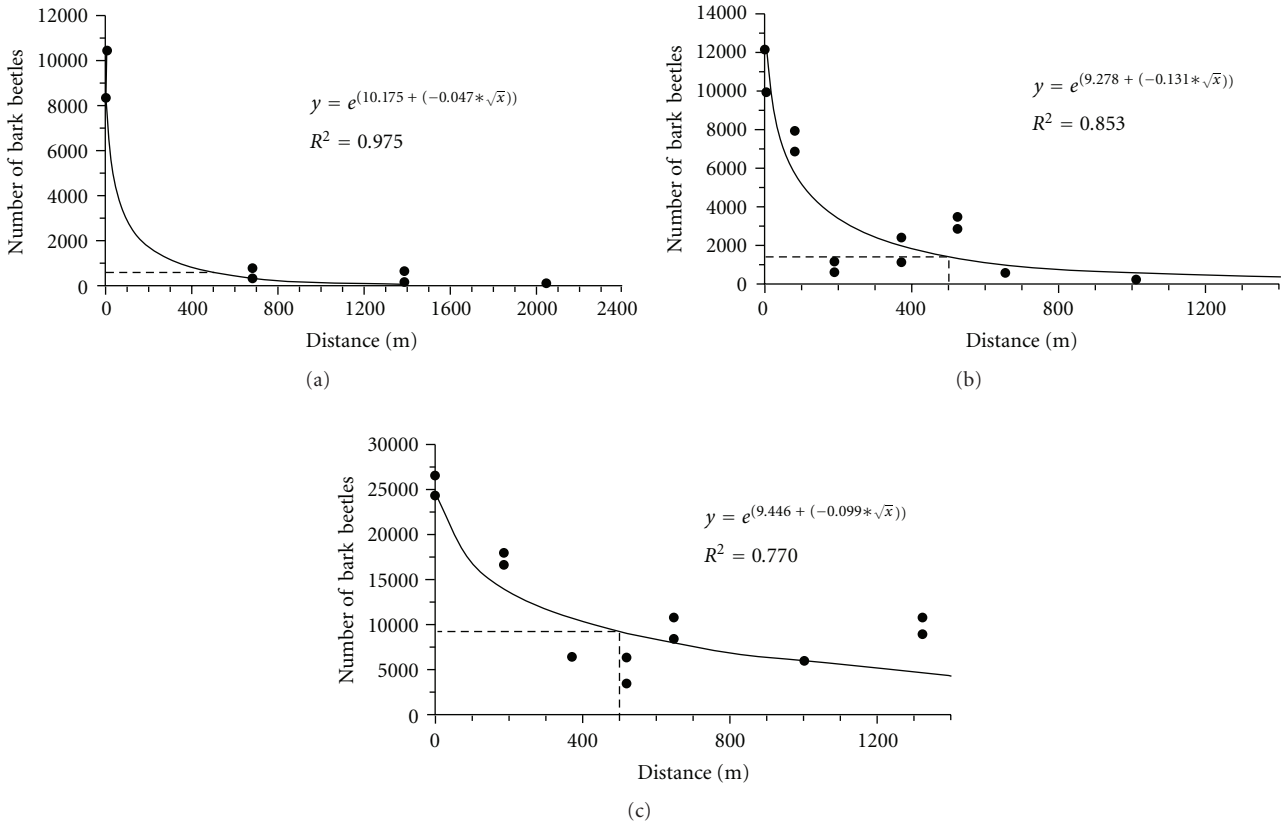


Figure 2: Trap catches of *Ips typographus* in the city (a), in the open land (b), and in the broadleaf stand (c), starting from infested spruce. The calculated beetle density 500 meters from the infestation spot is marked.

only 4%. At a distance of 500 meters, it was calculated that 572 beetles would have been caught per trap, that is, only about 7% of the initial trap catches in the forest (Figure 2(a) and Table 1). In this preliminary study in 2006, the fast decrease in beetle density was possibly a consequence of having no traps between the infested stand with the first trap pair and the second trap pair already 680 meters away. Therefore, a goal of the other two case studies from 2008 was to fill in these gaps by including results from traps set closer to the infested stands.

The numbers of catches in the open area (transect b) declined also rather fast. The density dropped after just 200 meters to only one-fourth (Figure 2(b)). A second tiny peak at about 500 meters distance from the infestation point was probably due to a small and isolated spruce group bordering the open area to the south. This could have been a source of irregularity, which resulted in an increased number of catches at this trapping point. According to the applied regression formula by Hawkes [12], we estimated that 1,383 beetles would have been caught at a distance of 500 meters.

After the first 400 meters, the catches in the broadleaf stand (transect c) reached only one-fifth of the number of catches of the first trap pair. But at a distance of 500 meters, we calculated there would have been still 9,173 beetles. Towards the end of the transect, the catches even out to a level of about 7,500 beetles (Figure 2(c)). It can be assumed that this reflects the basic flight density level in this 45 hectare broadleaf stand that bordered forests with infested spruce. The population density seems to have been much higher in the broadleaf stand (c) than in the open area (b).

Table 1: Calculated values for the catches (beetles per trap) in (a) the city, (b) the open land, and (c) in the broadleaf stand transect.

	(a) City	(b) Open land	(c) Broadleaf stand
Catches at 500 m (n)	572	1,383	9,173
Catches at 500 m (%)	7%	12%	36%
50% catches at	40 m	70 m	250 m
5% catches at	570 m	990 m	4,160 m

The differences between the trap catches in the three investigation areas are shown in Figure 2. Whereas the number of beetles caught per trap in the broadleaf stand was more than twice that in the open land and in the city, the "broadleaf" curve does not decrease as fast as the other two. The beetle density in the deciduous stand was at least twice as high as in the open land or the city.

In the open area, a marked decline was observed after just 200 meters. The basic population evened out to a low level of about 1,000 beetles per trap. In the broadleaf stand, the gradient also declined rapidly but evened out to a considerable level of 7,500 beetles per trap. The population density halved at approximately 250 meters, much further than in the open land or in the city. At a distance of

500 meters, the catches still added up to 36% that at the beginning of the transect.

4. Discussion

4.1. Beetle Pressure and Frequency of Ips typographus. Altogether, a minimum of 61 beetles per trap were caught over the whole period in the city and a maximum of 26,405 beetles per trap at the beginning of the broadleaf stand transect. These figures indicate that *I. typographus* was present in all the studied areas and support the conclusion of Piel et al. [14], who claimed that this species is able to spread over large areas, even though the host tree is relatively rare. According to Duelli et al. [9], who studied the beetle flight in a low impact situation, considerably more beetles can be found in the forest than outside. Sanders [15] used pheromone traps to demonstrate that bark beetles pass through broadleaf forests in their dispersal flight during the latent phase. Gugerli et al. [16] found that the basic beetle population, and consequently also the mass reproduction, does not differ much genetically in space. This may indicate that there is a constant and significant gene exchange by migrating beetles.

It should be remembered that trap catches do not necessarily represent the actual population density, flight activity, and the migration of the spruce bark beetle, as described by Zolubas and Byers [17]. Zumr [18] and Duelli et al. [10] performed recapture experiments and found that, besides the released beetles, many other feral individuals were trapped. It is always difficult to interpret trap catches without considering the attractiveness of the host trees and the potential breeding material. Nevertheless, the above experiments and the present research show that the spread and area-wide distribution of *Ips typographus* are impressive. It would be possible to build up new mass attacks nearly anywhere in the forest if suitable breeding material is present and the weather conditions are favourable.

Under our study conditions, however, the population quickly thinned within 300 to 400 meters of an infestation spot (Figure 2), particularly outside the forest. Since beetles have very little chance of breeding in the conditions along the chosen transects, the numbers of beetles caught along the transects reflect the impact of the beetle populations rather well.

It was striking that three times more beetles were caught in the broadleaf stand than in the open area. There are several possible reasons for the relatively high number of catches in the deciduous forest. First, beetles probably prefer to swarm in forests. As already mentioned, Duelli et al. [9] found considerably more beetles in the forest than outside. In Norway, Botterweg [19] observed that *I. typographus* spreads homogenously throughout forested areas. Second, we were not able to find an absolutely pure deciduous forest with an isolated infected spruce stand nearby. Hence, the stand with a very few sporadically scattered spruce trees had to be accepted for the experiment, and these could have influenced the number of catches slightly, even though the trees were in good sanitary condition. Healthy spruce trees are not in strong competition with pheromone traps, and it can be assumed that the traps in our study were highly attractive and thus reflected the spreading behaviour of *I. typographus*. On the open land transect, a small strip of woodland may have interfered with the traps at 512 m distance. The small stand, which lies south of the traps across the motorway, contains some Norway spruce. It cannot be excluded that some of the bark beetles caught originated or were influenced by this stand.

The results from study-site a in the city should, however, be treated with caution, as the second trap pair was 680 meters away from the infestation spot and no data were collected between the first and the second trap pair.

The research areas were chosen to include infested Norway spruce stands that bordered directly onto an adequate open or urban area and onto a preferably pure broadleaf stand. We consciously did not choose transects within spruce stands because, without releasing marked beetles, it would not have been possible to identify the proportion of the trap captures that did not originate from the initial infestation spots. To simulate high beetle pressure artificially, several hundred thousand marked beetles would be necessary, which would pose an impractical logistical challenge. It is not, in fact, clear that all individuals caught in the traps have a common origin. Nevertheless, on all transects, the population thinned quickly within only a few hundred meters, regardless of the origin of the beetles.

4.2. Buffer Zones. Up to now, forest managers have tended to use an empirically derived rule that specifies that a combat and/or buffer zone of 500 meters around an infested stand prevents a substantial invasion into the adjacent forests [20–22]. Byers [13] maintained that a border area of 500 meters width is justifiable under epidemic conditions. This rule has often been applied in Central Europe but until now has never actually been tested experimentally. On the basis of a GIS analysis, Wichmann and Ravn [7] concluded that epidemical attacks only spread out across short distances of less than 500 meters. The present study of high beetle impact indicates that a clear decline in the beetle population takes place within the first 300 meters away from the infestation spot. It then evens out to a level that does not pose an imminent danger to surrounding Norway spruce stands. If the buffer zone is an open area, the population may even decline considerably within the first 200 meters. The findings of our study indicate therefore that open areas may provide very suitable border zones between managed and unmanaged stands and help lower high beetle pressure from a control-free infested stand.

Infestations of living spruce trees are likely to occur in Switzerland when there are over 12,000 beetles caught per pheromone trap and year as mentioned by Forster and Meier [23]. Above this threshold, there is a considerable risk that the spruce bark beetle will locally spread and attack new trees. Various authors have reported similar thresholds: in Swedish spruce forests between 10,000 [24] and 15,000 [25] beetles per trap and year and between 8,000 [26] and 10,000 [27] beetles per trap and year in Northern Italy. Thus, the estimate of 12,000 beetles for Swiss spruce forests seems reasonable. In most of the cases, the beetles spreading from infestation spots will very likely thin out within the first 300 meters without spruce and densities will drop under the above threshold. This means that a buffer zone 500 meters in

width [13] is probably on the safe side. This study therefore confirms the hypothesis that the bark beetle density declines considerably within the first 500 meters of a spruce free buffer zone (Table 1). For all these reasons, such a buffer zone can be recommended.

Whether such buffer zones inhibit a further enlargement of beetle-infested stands should be always considered critically. If the host tree resistance is low, new trees will be successfully colonized by a smaller quantity of migrating beetles. However, it seems possible to considerably reduce the spread of bark beetles through implementing forest protection strategies and well-timed and consistent control measures, at least where conditions are similar to those at the sites we studied.

However, some beetles can be transported by wind much further, even though there are usually no active and directed immigration flights over long distances. The population in such cases thins out quickly, and a new breeding locality is chosen more or less random. To spread geographically, the beetles have to build up first a new population at a distant infestation spot.

4.3. Consequences for Bark Beetle Management. The findings of this study should be used in bark beetle management practices, especially under difficult conditions where there is a need for buffer zones between managed and unmanaged spruce stands. What is crucial is that management priorities should be defined on a large enough scale on the basis of well-defined landscape compartments. Compartments for the interventions should be chosen to be at least 100 hectares in size. At the same time, natural area borders (mountains, meadows, villages, lakes) 500 meters in width will also help to improve the effectiveness of selected buffer zones [28]. Setting such spatial priorities in bark beetle management and clearly defining and separating areas can reduce the spread of beetle infestations and infestation pressure.

The present study does not refer to buffer zones between managed and unmanaged, differently infested spruce stands that directly border each other. Experience has shown that under such conditions, stand edges of cleared spots within selected buffer zones are often reinfested. In the worst case, infestations may continue until all spruce trees in the zone have been attacked and subsequently removed. Under such high-risk conditions, the flight behaviour and beetle pressure of *Ips typographus* may be different. This still needs to be studied in detail.

Acknowledgments

The authors are grateful to Professor Dr. Peter Duelli, Dr. Andreas Rigling and Dr. Beat Wermelinger for their helpful suggestions for improving the paper. They also thank the forest services of the Cantons Thurgau, St. Gallen and Zurich, especially their forest protection agents and field foresters, for their cooperation, Thomas Reich for lending a helping hand setting up the traps, Franz Meier for valuable discussions, and Dr. Silvia Dingwall for her linguistic corrections. This study received financial support from the Federal Office for the Environment (FOEN) in Berne.

References

[1] F. Lieutier, K. R. Day, A. Battisti, J.-C. Grégoire, and H. F. Evans, *Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis*, Kluwer Academic Publishers, Dordrecht, The Netherlands, 2004.

[2] B. Wermelinger, "Ecology and management of the spruce bark beetle *Ips typographus*—a review of recent research," *Forest Ecology and Management*, vol. 202, no. 1–3, pp. 67–82, 2004.

[3] M. Heurich, A. Reinelt, and L. Fahse, "Die Buchdruckermassenvermehrung im Nationalpark Bayerischer Wald," in *Waldentwicklung im Bergwald nach Windwurf und Borkenkäferbefall*, M. Heurich, Ed., vol. 14 of *Bayer. Staatsforstverwaltung, Wiss. Reihe*, pp. 9–48, Grafenau, Germany, 2001.

[4] B. Forster, F. Meier, and U. B. Brändli, "Deutlicher Rückgang der Fichte im Mittelland—Vorratsabbau auch durch Sturm und Käfer," *Wald Holz*, vol. 89, no. 3, pp. 52–54, 2008.

[5] B. Forster, F. Meier, R. Gall, and C. Zahn, "Erfahrungen im Umgang mit Buchdrucker-Massenvermehrungen (*Ips typographus* L.) nach Sturmereignissen in der Schweiz," *Schweizerische Zeitschrift für Forstwesen*, vol. 154, no. 11, pp. 431–436, 2003.

[6] H. Schröter, "Ausbreitung des Borkenkäferbefalls in Bannwäldern Baden-Württembergs," in *Forstschutzprobleme in Nationalparken und Naturschutzgebieten*, A. Wulf and K. H. Berendes, Eds., vol. 362 of *Mitteilungen Biologische Bundesanstalt Land- Forstwirtschaft*, pp. 63–79, Berlin, Germany, 1999.

[7] L. Wichmann and H. P. Ravn, "The spread of *Ips typographus* (L.) (Coleoptera, Scolytidae) attacks following heavy windthrow in Denmark, analysed using GIS," *Forest Ecology and Management*, vol. 148, no. 1–3, pp. 31–39, 2001.

[8] T. Becker and H. Schröter, ""Ausbreitung von rindenbrütenden Borkenkäfern nach Sturmschäden," *Allgemeine Forstzeitung*, vol. 55, no. 6, pp. 280–282, 2000.

[9] P. Duelli, M. Studer, and W. Näf, "Der Borkenkäferflug ausserhalb des Waldes," *Zeitschrift für Angewandte Entomologie*, vol. 102, pp. 139–148, 1986.

[10] P. Duelli, P. Zahradnik, M. Knizek, and B. Kalinova, "Migration in spruce bark beetles (*Ips typographus* L.) and the efficiency of pheromone traps," *Journal of Applied Entomology*, vol. 121, no. 1–5, pp. 297–303, 1997.

[11] T. R. E. Southwood, *Ecological Methods with Particular Reference to the Study of Insect Populations*, Chapman & Hall, London, UK, 1978.

[12] C. Hawkes, "The estimation of the dispersal rate of the adult cabbage root fly (*Erioischia brassicae* (Bouché)) in the presence of a brassica crop," *Journal of Applied Entomology*, vol. 9, no. 2, pp. 617–632, 1972.

[13] J. A. Byers, "Wind-aided dispersal of simulated bark beetles flying through forests," *Ecological Modelling*, vol. 125, no. 2-3, pp. 231–243, 2000.

[14] F. Piel, M. Gilbert, A. Franklin, and J. C. Grégoire, "Occurrence of *Ips typographus* (Col., Scolytidae) along an urbanization gradient in Brussels, Belgium," *Agricultural and Forest Entomology*, vol. 7, no. 2, pp. 161–167, 2005.

[15] W. Sanders, "A contribution to the behaviour of the Scolytid *Ips typographus* L. during the flight period," *Anzeiger für Schädlingskunde Pflanzenschutz Umweltschutz*, vol. 57, no. 7, pp. 131–134, 1984.

[16] F. Gugerli, R. Gall, F. Meier, and B. Wermelinger, "Pronounced fluctuations of spruce bark beetle (Scolytinae: *Ips typographus*) populations do not invoke genetic differentiation," *Forest Ecology and Management*, vol. 256, no. 3, pp. 405–409, 2008.

[17] P. Zolubas and J. A. Byers, "Recapture of dispersing bark beetle *Ips typographus* L. (Col., Scolytidae) in pheromone-baited traps: regression models," *Journal of Applied Entomology*, vol. 119, no. 2, pp. 285–289, 1995.

[18] V. Zumr, "Dispersal of the spruce beetle *Ips typographus* (L.) (Col., Scolytidae) in spruce woods," *Forest Ecology Management*, vol. 114, no. 1–5, pp. 348–352, 1992.

[19] P. F. Botterweg, "Dispersal and flight behaviour of the spruce bark beetle *Ips typographus* in relation to sex, size and fat content," *Zeitschrift für Angewandte Entomologie*, vol. 94, no. 1–5, pp. 466–489, 1982.

[20] H. Schröter, "Borkenkäferproblematik im Nationalpark Bayerischer Wald," in *Borkenkäferproblematik im Nationalpark Bayerischer Wald*, C. Wild, J. Metzger, and C. Strauss, Eds., pp. 16–19, Bayerische Landesanstalt für Wald und Forstwirtschaft, Freising Germany, 1998.

[21] Nationalparkverwaltung Berchtesgaden, *Nationalparkplan*, Bayer. Staatsministerium für Landesentwicklung und Umweltfragen, München, Germany, 2001.

[22] G. Lobinger, "Rindenbrütende Borkenkäfer an Fichte," *LWF Merkblatt*, vol. 14, p. 6, 2004.

[23] B. Forster and F. Meier, "Die Borkenkäfersituation 1989/90, Buchdrucker (*Ips typographus*)," *PBMD-Bulletin*, vol. 7, p. 18, 1990.

[24] J. Weslien, "Monitoring *Ips typographus* (L.) populations and forecasting damage," *Journal of Applied Entomology*, vol. 114, no. 1–5, pp. 338–340, 1992.

[25] Å. Lindelöw and M. Schroeder, "Spruce bark beetle, *Ips typographus* (L.), in Sweden: monitoring and risk assessment," *Journal of Forest Science*, vol. 47, pp. 40–42, 2001.

[26] M. Faccoli and F. Stergulc, "A practical method for predicting the short-time trend of bivoltine populations of *Ips typographus* (L.) (Col., Scolytidae)," *Journal of Applied Entomology*, vol. 130, no. 1, pp. 61–66, 2006.

[27] P. Ambrosi, D. Angheben, and C. Salvadori, "Tecniche di difesa e di controllo delle popolazioni di scolitidi in boschi di conifere," *Monti e Boschi*, vol. 3, pp. 22–26, 1990.

[28] B. Forster and F. Meier, "Sturm, Witterung und Borkenkäfer. Risikomanagement im Forstschutz," *Merkblatt für die Praxis*, vol. 44, p. 8, 2008.

Hindawi Publishing Corporation
Psyche
Volume 2012, Article ID 363767, 6 pages
doi:10.1155/2012/363767

Research Article

Performance of *Tomicus yunnanensis* and *Tomicus minor* (Col., Scolytinae) on *Pinus yunnanensis* and *Pinus armandii* in Yunnan, Southwestern China

Tao Zhao[1,2] and Bo Långström[3]

[1] *Division of Forest Protection, Yunnan Academy of Forestry, Kunming 650224, China*
[2] *Department of Chemistry, Royal Institute of Technology, 10044 Stockholm, Sweden*
[3] *Department of Ecology, Swedish University of Agricultural Sciences, 75007 Uppsala, Sweden*

Correspondence should be addressed to Tao Zhao, taozhao@kth.se

Received 30 September 2011; Revised 16 December 2011; Accepted 14 January 2012

Academic Editor: John A. Byers

Pine shoot beetles, *Tomicus yunnanensis* Kirkendall and Faccoli and *Tomicus minor* Hartig (Col., Scolytinae), have been causing substantial mortality to Yunnan pine (*Pinus yunnanensis* Franch) in Yunnan, southwestern China, whereas only a few Armand pine (*Pinus armandii* Franch) were attacked by the beetles. In order to evaluate the suitability of *P. armandii* as host material for the two *Tomicus*, adults of both *Tomicus* were caged on living branches and felled logs of the two pines during shoot feeding and trunk attack phase, respectively. More beetles survived on the living branches of *P. yunnanensis* than on *P. armandii*. *Tomicus yunnanensis* and *T. minor* produced similar progeny in the logs of the two pines. The sex ratio and developmental period were not affected by host species, but the brood beetles emerging from Armand pine weighed less than those from Yunnan pine, suggesting that *P. armandii* are less suitable to be host of *T. yunnanensis* and *T. minor*.

1. Introduction

As the most important forest pests in southwestern China, pine shoot beetles, *Tomicus yunnanensis* Kirkendall and Faccoli and *Tomicus minor* Hartig (Col., Scolytinae), have killed more than 200 000 ha of Yunnan pine (*Pinus yunnanensis* Franch) in Yunnan province since early 1980s [1], of which *T. yunnanensis* is considered as a more serious species [2–4]. Since the morphology and gallery system of *T. yunnanensis* are very similar to *Tomicus piniperda* L., *T. yunnanensis* had long been confused with *T. piniperda*. Molecular and taxonomic studies have, however, demonstrated that *T. piniperda* is absent in Yunnan [3, 4]. Thus, the aggressive *Tomicus* species in Yunnan was a new undescribed species, and consequently *T. yunnanensis* was finally described and named in 2008 [3].

Like the pine shoot beetles in Europe, the life cycle of *T. yunnanensis* and *T. minor* is univoltine and contains two phases, a reproduction phase and a maturation feeding phase [5–7]. In Yunnan, adults of the two *Tomicus* species mate and lay eggs in the inner bark of trunks and large branches of living trees from November to May [6–8]. Larvae and pupae subsequently complete their development there. After emergence, the young adults fly to the crowns of host pine trees where they feed in the shoots and become sexually mature [2, 5, 9]. The main shoot-feeding phase lasts from May to November in Yunnan [5–7]. However, *T. minor* usually initiates its flight one or two weeks later than *T. yunnanensis* [1, 2]. Since *T. minor* usually attack trees that previously have been attacked by *T. yunnanensis*, it is regarded as a more secondary species in Yunnan [1–3].

P. yunnanensis and Armand pine (*Pinus armandii* Franch) are distributed at similar elevations. Armand pine often grows together with *P. yunnanensis* in Yunnan province, but *P. armandii* has rarely been attacked by *T. piniperda* and *T. minor* [7]. The reason for this is poorly understood, but it would be valuable to know if *P. armandii* really is more resistant to pine shoot beetle damage. In this study, we

compared the suitability of *P. armandii* and *P. yunnanensis* for maturation feeding and breeding of the two *Tomicus* species.

2. Materials and Methods

2.1. Origins of Pine Shoot Beetles. The pine shoot beetles were collected from Yunnan pine forests in Yiliang or Shilin County. We recognized the shoots containing maturation feeding beetles by their yellowish needles and then cut off the shoots 5–10 cm below the entrance holes by scissors. The shoots with beetles were collected from the forest a few days before the experiment started, and the beetles were peeled out of the shoots and identified to species under a stereoscope (Nikon SMZ). The *Tomicus* population in Shilin consisted of mostly *T. yunnanensis*, but a relatively high proportion of *T. minor* was found in Yiliang.

2.2. Shoot Cage Experiment. In order to assess the effect of tree species on the maturation feeding of pine shoot beetles, the shoot cage experiment was carried out in a mixed stand of *P. yunnanensis* and *P. armandii* in Kunming Tree Garden (25°07′′ N, 103°00′′ E, 1953 m above sea level). Most of the trees there were 4-5 m high and exhibited a healthy appearance. No damage history had been recorded. A total of 18 similar trees (nine *P. yunnanensis* and nine *P. armandii*) were selected. For each tree, a mid-crown branch with 20–30 current shoots was enclosed within a 2.0 m × 1.5 m net cage. On July 28, 2000, *T. yunnanensis* adults (10 beetles/cage) were released in six cages for each host species. *T. minor* adults were released in three cages for each pine species in the same way on October 24, 2000. The ends of all the cages were closed and fastened by string. On December 25, 2000, all the cages were cut off the trees and taken to the laboratory. The number of attacked shoots in each cage was counted. The numbers of beetles alive, killed by resin, dead from other reasons or missing were recorded. For each attacked shoot, the shoot diameter at entrance hole, the distance from entrance hole to the shoot tip, as well as the length of feeding tunnel (defined as the length from entrance hole to the end of the tunnel) were measured by ruler.

2.3. Breeding Experiments. On December 26, 2000, three *P. yunnanensis* and three *P. armandii* trees of similar size were felled in a naturally generated mixed stand in Yiliang County. The lower stem section of each tree was cut into four 50 cm-long logs and placed in 80 × 70 cm net cages. A total of 12 cages were used, six of these each contained two logs of *P. yunnanensis* or *P. armandii*, and were divided into two groups, each group containing three *P. yunnanensis* cages and three *P. armandii* cages. On December 27, twenty pairs of unsexed *T. yunnanensis* adults were released to each cage in the first group, and an unsexed mixture of the two *Tomicus*-species (11 pairs of *T. minor* and nine pairs of *T. yunnanensis*) was released to the second group of cages.

After beetles were released, all the logs were checked during the entire experimental period. The new entrance holes were marked and counted twice a week. On Mar. 30, 2001, the new brood adults started emerging. Then the emerging beetles were collected at different intervals ranging from one day to several days. We measured the diameter of each log on April 20. After measurement, we carefully peeled off the outer bark of each log and counted the number of egg galleries constructed by *T. yunnanensis* and *T. minor,* respectively. For each egg gallery, we measured gallery length and counted the number of larvae, pupae, and adults remaining in the phloem. The brood production was obtained by summing up the number of emerged adults and brood remaining in the galleries. The brood adults from the second group were identified to species under stereoscope. On April 28, the collected beetles of *T. yunnanensis* or *T. minor* were separately dried for each sampling occasion at 60°C for 8 hours, and the dry body weight of the beetles collected at different dates was weighed in 10-beetle samples using an electronic balance.

2.4. Data Analysis. Data were analyzed using the statistics program "StatPac for windows" (StatPac Inc., 1999). All the means were given as mean ± SD (standard deviation) and compared by Student's *t*-test. The percentage data was compared by Chi-square test.

3. Results

3.1. Maturation Feeding. The status of pine shoot beetles that were caged in the branches of two pine species was identified as alive, killed by resin, dead from other reasons, or missing at the inspection. Although a few beetles of both species survived on both host species (Table 1), the *Tomicus* species performed differently on the two hosts. The survival rate of both *Tomicus* species on *P. yunnanensis* was two-fold higher than on *P. armandii*. Correspondingly, the mortality of *T. yunnanensis* and *T. minor* on *P. armandii* (63% and 73% resp.) was somewhat higher than on *P. yunnanensis* (42% and 50% resp.). Significantly more beetles feeding on *P. armandii* were killed by resin than that feeding on *P. yunnanensis* (X^2 = 5.44–9.31, $P < 0.05$). In addition, *T. yunnanensis* excavated twofold more tunnels on *P. yunnanensis* than on *P. armandii* ($t = 4.45$, $P < 0.01$), and the tunnels by both *Tomicus* species on *P. yunnanensis* were longer than on *P. armandii* (t = 4.28–5.61, $P < 0.01$) (Table 2), indicating that the beetles were more adapted to their principal host than to *P. armandii* in the shoot feeding phase.

3.2. Oviposition and Brood Production. During reproduction phase, the female adults of either *T. yunnanensis* or *T. minor* excavated similar numbers of egg galleries in the logs of *P. yunnanensis* and *P. armandii* (Table 3). The brood production of the two beetles was also similar in the two hosts. These results suggest that the oviposition and brood production of *T. yunnanensis* and *T. minor* do not differ that much in the logs of the two hosts.

Tomicus minor seemed to be in an inferior position in the competition with *T. yunnanensis.* After the mixture of two beetle species, composed of 55% *T. minor* and 45% *T. yunnanensis*, was released in the cages, the resulting egg galleries of *T. minor* occupied only 24.4% and 19.0% of the

Table 1: The performance of *Tomicus yunnanensis* and *T. minor* in the caged shoots of *Pinus armandii* and *P. yunnanensis* in Kunming. 60 *T. yunnanensis* adults were released into the 6 shoot cages on July 28, and 30 *T. minor* adults were released into 3 cages on October 24, for each tree species. The performances of the beetles were checked on December 25, 2000.

Tomicus species	*Pinus* species	Total	Number of beetles (percentage)			
			Alive	Killed by resin	Dead from other reasons	Missing
T. yunnanensis	*P. yunnanensis*	60	13 (21.7)	1 (1.7)	24 (40.0)	22 (36.6)
	P. armandii	60	7 (11.7)	12 (20.0)	26 (43.3)	15 (25.0)
Chi-square analysis of 2 × 4 contingency table				$X^2 = 12.5$, d.f. = 3, $P = 0.006$		
T. minor	*P. yunnanensis*	30	8 (26.7)	1 (3.3)	14 (46.7)	7 (23.3)
	P. armandii	30	4 (13.3)	8 (26.7)	14 (46.7)	4 (13.3)
Chi-square analysis of 2 × 4 contingency table				$X^2 = 7.60$, d.f. = 3, $P = 0.055$		

Table 2: Feeding tunnels by *Tomicus yunnanensis* and *T. minor* in the caged shoots of *Pinus armandii* and *P. yunnanensis* in Kunming. Ten *T. yunnanensis* adults were released into each cage on July 28, and 10 *T. minor* adults were released into each cage on October 24. The data were collected on December 25, 2000 and are expressed as means ± 1SD. Means followed by the different letters in a column are significantly different at $P < 0.05$ by *t*-test.

Tomicus species	Cages	*Pinus* species	Tunnel no cage^{-1}	Tunnel length (mm) cage^{-1}
T. yunnanensis	6	*P. yunnanensis*	16.5 ± 3.7 a	24.5 ± 19.5 a
	6	*P. armandii*	7.5 ± 3.3 b	7.9 ± 5.2 b
T. minor	3	*P. yunnanensis*	12.0 ± 2.0 a	16.1 ± 8.7 a
	3	*P. armandii*	11.0 ± 6.4 a	9.3 ± 3.3 b

total attack density in *P. yunnanensis* and *P. armandii,* respectively. Correspondingly, the brood production of *T. minor* was only 18.9% and 13.7% of the total brood production in *P. yunnanensis* and *P. armandii* logs, respectively (Table 3).

3.3. Developmental Period. To investigate the influence of host species on the developmental period of the *Tomicus*, we estimated the speed of brood development of *T. yunnanensis* under laboratory conditions by counting the days from median attacking date to the median date of emergence (Table 4). The developmental period of *T. yunnanensis* was 89 days on *P. yunnanensis*, and 93 days on *P. armandii*, demonstrating that the developmental period of *T. yunnanensis* was nearly similar in the logs of two host species.

3.4. Size of Emerging Beetles. In addition to developmental period, we also investigated the effect of host species on the size of emerging beetles, by comparing the dry weight of *T. yunnanensis* emerging from the logs of the two hosts. The result indicated that *T. yunnanensis* adults reared on *P. yunnanensis* were heavier than those reared on *P. armandii* (data not shown). In addition, the dry weight of *T. yunnanensis* brood adults was strongly related to the date of emergence. The weights of brood adults bred on both *P. yunnanensis* and *P. armandii* decreased with time after the initial brood emergence date, indicating an effect of intraspecific competition or deteriorating food quality (Figure 1).

4. Discussion

The pine shoot beetles *T. piniperda* and *T. minor* have been reported from a large number of pine species and other conifers as well [10], but the principal host for them in Europe is Scots pine (*Pinus sylvestris* L.). Since the accidental introduction into North America, *T. piniperda* has been reported from a number of North American pines since the 1990s [11–13]. Experiments in Sweden and France have shown successful development in several exotic pine hosts [14]. Although both *T. piniperda* and *T. minor* occur on the exotic host lodgepole pine (*Pinus contorta* Douglas ex Loudon) in Sweden [15], they perform less well in this host [16].

There is another pine shoot beetle species, *Tomicus destruens* Woll., in the Mediterranean area which biologically is more similar to *T. yunnanensis* than *T. piniperda* [3], and this species did better on local maritime than on boreal pine species in northern Italy [17]. In Portugal, Vasconcelos et al. found different host preferences between local populations of *T. piniperda* and *T. destruens,* that is, that northern populations preferred Aleppo pine (*Pinus halepensis* Miller) whereas southern populations preferred Italian stone pine (*Pinus pinea* L.) [18].

The shoot cage experiments showed that *T. yunnanensis* and *T. minor* are capable of feeding in the shoots of *P. armandii*, but more beetles died due to resin and other reasons on *P. armandii* than on *P. yunnanensis*. The resistance of conifers against invaders is mainly based on their ability to produce resin [19–22]. The resin of *P. armandii* was more abundant, and its concretionary speed was slower than *P. yunnanensis* [23]. In addition, the terpene compositions of the two pine species were also different. The shoot piths of *P. armandii* trees contain a lower proportion of α-pinene but a higher proportion of β-pinene than *P. yunnanensis* (Borg-Karlson, A.-K., unpublished data). The observation that more beetles were killed by resin on *P. armandii* might be due to the stronger physical repellency and sticky property of its resin and reflected a higher resistance of *P. armandii* to pine shoot beetles. In addition, the small shoot diameter of *P.*

Table 3: Oviposition and brood development of *Tomicus yunnanensis* and *T. minor* in the logs of *Pinus armandii* and *P. yunnanensis* in laboratory condition, after 11 pairs of *T. minor* and 9 pairs of *T. yunnanensis* were introduced to each cage with two logs of *P. yunnanensis* or *P. armandii*. Gallery and brood production data were collected from three replicates. Larval tunnel gallery^{-1} and gallery length were the mean from all the egg galleries appeared in the logs (number in the bracket). Data are expressed as means ± 1SD. Means followed by the different letters in a column are significantly different at $P < 0.05$ by t-test.

Pinus species	*Tomicus* species	Galleries m^{-2}	Brood production m^{-2}	Larval tunnel gallery^{-1}	Gallery length cm
P. yunnanensis	*T. yunnanensis*	83.4 ± 12.4 a	1449.9 ± 96.3 a	29.1 ± 6.9 a	6.75 ± 4.2 a (68)
	T. minor	36.80 ± 14.6 b	338.6 ± 36.2 b	9.2 ± 3.1 b	6.18 ± 3.59 a (30)
P. armandii	*T. yunnanensis*	86.8 ± 9.1 a	1693.2 ± 166.7 a	22.4 ± 4.5 a	5.02 ± 2.50 a (68)
	T. minor	20.43 ± 7.6 b	268.2 ± 23.2 b	13.1 ± 2.2 b	4.08 ± 1.85 a (16)

Table 4: Developmental periods of *Tomicus yunnanensis* in logs of *Pinus armandii* and *P. yunnanensis* in laboratory condition. The developmental periods were estimated from the median date of entering (50% entrance holes existed) to the median date of emergence (50% of new generation emerging from brood logs).

Host species	Median date of attack	Median date of emerging	Developmental period, days
P. yunnanensis	Jan. 4	Apr. 3	89
P. armandii	Jan. 8	Apr. 12	93

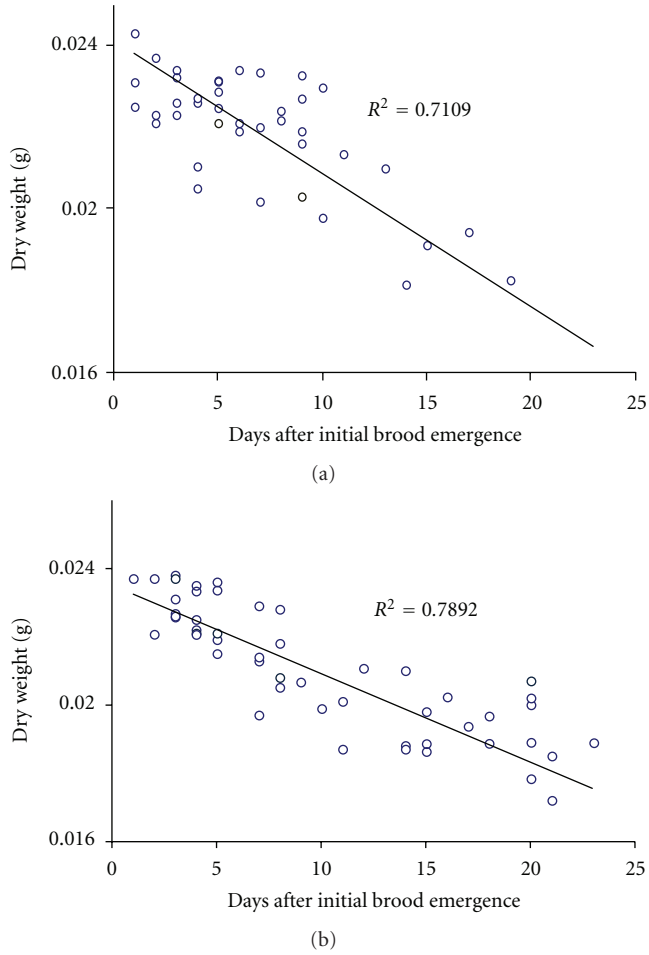


Figure 1: Mean dry weight of emerging *Tomicus yunnanensis* reared on *Pinus yunnanensis* (a) and *P. armandii* (b) related to days post the initiation of brood adult emergence. Each dot represents the average adult weight for a 10-beetle sample.

armandii might also contribute to high mortality of *Tomicus* in this pine species during maturation feeding.

The females of the two *Tomicus*-species accepted *P. armandii* as brood material, and the brood production of the two species was also similar in the two hosts, indicating that *T. yunnanensis* and *T. minor* could reproduce in the logs of *P. armandii* as well. However, *T. yunnanensis* oviposited later, and the brood development was somewhat slower on *P. armandii* than on *P. yunnanensis*, suggesting that this beetle preferred the last host. Similarly, Långström and Hellqvist found no variation on brood production and adult weight between *T. piniperda* beetles reared on lodgepole pine and those reared on Scots pine, but the development time of this beetle was longer on *P. contorta* than on *P. sylvestris* [16]. Führer and Mühlenbrock demonstrated that six-toothed spruce bark beetle (*Pityogenes chalcographus* L.) had similar brood production on its principal and secondary conifer hosts [24]. Differently, Cerezke showed that mountain pine beetle (*Dendroctonus ponderosae* Hopkins) was able to reproduce successfully in some pine species, but with a considerable variation in the brood production [25].

In our experiments the dry weight of *T. yunnanensis* brood adults emerged from Yunnan pine was higher than of those beetles that emerged from Armand pine. This observation might be due to qualitative differences in nutritional value and/or secondary metabolisms in the two hosts or just simply have resulted from the difference in phloem thickness of the two tree species. A similar pattern was found for the spruce bark beetle (*Ips typographus* L.) developing on its native host, Norway spruce [*Picea abies* (L.) Karsten], as compared to beetles emerging from an exotic host, sitka spruce [*Picea sitchensis* (Bong) Carrière] [26]. Since heavier bark beetles survive better than the lighter ones [27], and the fecundity of female bark beetles is related to the fat reserves available [28, 29], the lower body weight for *T. yunnanensis* bred from *P. armandii* could reduce survival of the beetles when they feed in the shoot and lead to less brood production later on. Långström and Hellqvist found that the weights of callow adults bred on both *P. contorta* and *P. sylvestris* decreased over the days following the initiation

of brood emergence [16]. We found the same pattern in the present experiment, both on *P. yunnanensis* and *P. armandii*. This pattern indicates an intraspecific competition and/or a deteriorating food quality.

In conclusion, this study demonstrates that *T. yunnanensis* and *T. minor* can feed in the shoots and reproduce in the logs of *P. armandii.* The performance of the beetle species is, however, somewhat lower in Armand pine than in Yunnan pine, which may explain the beetles' preference for the latter species. We suggest that a stronger defense in Armand pine may be the cause of this difference, and more experiments on host defenses are needed to assess the risk of *T. yunnanensis* and *T. minor* to *P. armandii* forests.

Acknowledgments

The present study was funded by European Union under its INCO program and the Natural Science Foundation of Yunnan, China. The authors sincerely thank Prof. Lisha Li for all of her support and Mr. Jiang Hu for his skilled assistance during experiments.

References

[1] Y. Hui and D. Xue-Song, "Impacts of Tomicus minor on distribution and reproduction of *Tomicus piniperda* (Col., Scolytidae) on the trunk of the living *Pinus yunnanensis* trees," *Journal of Applied Entomology*, vol. 123, no. 6, pp. 329–333, 1999.

[2] B. Långström, L. Lisha, L. Hongpin et al., "Shoot feeding ecology of *Tomicus piniperda* and *T. minor* (Col., Scolytidae) in southern China," *Journal of Applied Entomology*, vol. 126, no. 7-8, pp. 333–342, 2002.

[3] L. R. Kirkendall, M. Faccoli, and H. Ye, "Description of the Yunnan shoot borer, *Tomicus yunnanensis* Kirkendall & Faccoli sp. n. (Curculionidae, Scolytinae), an unusually aggressive pine shoot beetle from southern China, with a key to the species of Tomicus," *Zootaxa*, no. 1819, pp. 25–39, 2008.

[4] Y. Duan, C. Kerdelhué, H. Ye, and F. Lieutier, "Genetic study of the forest pest *Tomicus piniperda* (Col., Scolytinae) in Yunnan province (China) compared to Europe: new insights for the systematics and evolution of the genus Tomicus," *Heredity*, vol. 93, no. 5, pp. 416–422, 2004.

[5] H. Ye, "On the bionomy of *Tomicus piniperda* (L.)(Col., Scolytidae) in the Kunming region of China," *Journal of Applied Entomology*, vol. 112, no. 1–5, pp. 366–369, 1991.

[6] L. S. Li, H. L. Wang, D. S. Yang, and Y. X. Wang, "Study on the biological characteristics of *Tomicus yunnanensis* and its damage," *Forestry Research*, vol. 6, pp. 14–20, 1993 (Chinese).

[7] H. L. Wang, S. W. Chen, Y. WU, and M. G. Pu, "Preliminary studies on the bionomics and management of the pine shoot beetle (Blastophagus piniperda L.) in Kunming district, China," *Journal of Southwestern Forestry University*, vol. 2, pp. 14–19, 1987 (Chinese).

[8] T. Zhao, N. Zhou, and L. S. Li, "The reproduction biology of *Tomicus piniperda* in living *Pinus yunnanensis*," *Journal of Northwestern Forestry University*, vol. 18, pp. 47–49, 2003 (Chinese).

[9] H. Ye, "The distribution of *Tomicus piniperda* (L.) population in the crown of Yunnan pine during the shoot feeding period," *Acta Entomologica Sinica*, vol. 37, no. 3, pp. 311–316, 1994.

[10] F. G. Browne, *Pests and Diseases of Forest Plantation Trees: An Annotated List of the Principal Species Occurring in the British*, Commonwealth Oxford, Oxford, UK, 1968.

[11] R. A. Haack and T. M. Poland, "Evolving management strategies for a recently discovered exotic forest pest: the pine shoot beetle, *Tomicus piniperda* (Coleoptera)," *Biological Invasions*, vol. 3, no. 3, pp. 307–322, 2001.

[12] T. A. Eager, C. W. Berisford, M. J. Dalusky et al., "Suitability of some southern and western pines as hosts for the pine shoot beetle, *Tomicus piniperda* (Coleoptera: Scolytidae)," *Journal of Economic Entomology*, vol. 97, no. 2, pp. 460–467, 2004.

[13] R. A. Haack, R. K. Lawrence, and G. C. Heaton, "Seasonal shoot-feeding by *Tomicus piniperda* (Coleoptera: Scolytidae) in Michigan," *Great Lakes Entomologist*, vol. 33, no. 1, pp. 1–8, 2000.

[14] B. Långström, F. Lieutier, C. Hellqvist, and G. Vouland, "North American pines as hosts for *Tomicus piniperda* (L.) (Col., Scolytidae) in France and Sweden," in *Behavior, Population Dynamics and Control of Forest Insects*, F. P. Hain, S. M. Salom, W. F. Ravlin, T. L. Payne, and K. F. Raffa, Eds., Proceedings of a joint IUFRO working party conference, p. 644, 1995.

[15] Å. Lindelöw and C. Björkman, "Insects on lodgepole pine in sweden - Current knowledge and potential risks," *Forest Ecology and Management*, vol. 141, no. 1-2, pp. 107–116, 2001.

[16] B. L ångström and C. Hellqvist, "*Pinus contorta* as a potential host for *Tomicus piniperda* L. and *T. minor* (Hart.)(Col., Scolytidae) in Sweden," *Zeitschrift für Angewandte Entomologie*, vol. 99, no. 1–5, pp. 174–181, 1985.

[17] M. Faccoli, "Breeding performance and longevity of Tomicus destruens on Mediterranean and continental pine species," *Entomologia Experimentalis et Applicata*, vol. 123, no. 3, pp. 263–269, 2007.

[18] T. Vasconcelos, M. Branco, and F. Lieutier, "Host preference of *Tomicus piniperda* and Tomicus destruens for three pine species," in *Forest Insect Population Dynamics and Host Influences*, Proceedings IUFRO Kanazawa, 2003.

[19] C. I. Keeling and J. Bohlmann, "Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens," *New Phytologist*, vol. 170, no. 4, pp. 657–675, 2006.

[20] K. F. Raffa, B. H. Aukema, N. Erbilgin, K. D. Klepzig, and K. F. Wallin, "Interactions among conifer terpenoids and bark beetles across multiple levels of scale: an attempt to understand links between population patterns and physiological processes," *Recent Advances in Phytochemistry*, vol. 39, pp. 79–118, 2005.

[21] M. A. Phillips and R. B. Croteau, "Resin-based defenses in conifers," *Trends in Plant Science*, vol. 4, no. 5, pp. 184–190, 1999.

[22] T. Zhao, P. Krokene, J. Hu, E. Christiansen, and B. Långström, "Induced terpene accumulation in response to fungal inoculation inhibits bark beetle colonization in a dose dependent manner," *PLoS One*, vol. 6, no. 10, Article ID e26649, 2011.

[23] Y. Y. Zeng, *The Geographic Variation of Monoterpene and Sesquiterpene in Pinus armandii*, Press of Beijing Agriculture University, Beijing, China, 1992.

[24] E. Führer and B. Mühlenbrock, "Brutexperimente mit Pityogenes chalcographus L. an verschiedenen Nadelbaumarten," *Zeitschrift für Angewandte Entomologie*, vol. 96, no. 3, pp. 228–232, 1983.

[25] H. F. Cerezke, "Egg gallery, brood production, and adult characteristic of mountain pine beetle, Dendroctonus ponderosae Hopkins (Coleoptera: Scolytidae), in three pine hosts," *Canadian Entomologist*, vol. 127, no. 6, pp. 955–965, 1995.

[26] B. Økland, N. Erbilgin, O. Skarpaas, E. Christiansen, and B. Långström, "Inter-species interactions and ecosystem effects of non-indigenous invasive and native tree-killing bark beetles," *Biological Invasions*, vol. 13, no. 5, pp. 1151–1164, 2011.

[27] I. Amezaga, "Monterrey pine (*Pinus radiata* D. Don) suitability for the pine shoot beetle (*Tomicus piniperda* L.) (Coleoptera: Scolytidae)," *Forest Ecology and Management*, vol. 86, no. 1–3, pp. 73–79, 1996.

[28] H. Jactel and F. Lieutier, "Effects of attack density on fecundity of the scots pine beetle Ips sexdentatus Boern (Col.; Scolytidae)," *Journal of Applied Entomology*, vol. 104, no. 2, pp. 190–204, 1987.

[29] D. Sauvard, "Reproductive capacity of *Tomicus piniperda* L. (Col., Scolytidae)," *Journal of Applied Entomology*, vol. 116, no. 1, pp. 25–38, 1993.

Hindawi Publishing Corporation
Psyche
Volume 2012, Article ID 149572, 14 pages
doi:10.1155/2012/149572

Review Article

Mechanisms of Odor Coding in Coniferous Bark Beetles: From Neuron to Behavior and Application

Martin N. Andersson

Department of Biology, Lund University, 223 62 Lund, Sweden

Correspondence should be addressed to Martin N. Andersson, martin_n.andersson@biol.lu.se

Received 3 October 2011; Accepted 12 December 2011

Academic Editor: John A. Byers

Coniferous bark beetles (Coleoptera: Curculionidae: Scolytinae) locate their hosts by means of olfactory signals, such as pheromone, host, and nonhost compounds. Behavioral responses to these volatiles are well documented. However, apart from the olfactory receptor neurons (ORNs) detecting pheromones, information on the peripheral olfactory physiology has for a long time been limited. Recently, however, comprehensive studies on the ORNs of the spruce bark beetle, *Ips typographus*, were conducted. Several new classes of ORNs were described and odor encoding mechanisms were investigated. In particular, links between behavioral responses and ORN responses were established, allowing for a more profound understanding of bark beetle olfaction. This paper reviews the physiology of bark beetle ORNs. Special focus is on *I. typographus*, for which the available physiological data can be put into a behavioral context. In addition, some recent field studies and possible applications, related to the physiological studies, are summarized and discussed.

1. Introduction

Bark beetles (Coleoptera: Curculionidae: Scolytinae) constitute some of the most destructive pests of coniferous trees throughout the world, destroying forests of great economic value. Currently, the large-scale outbreak of the mountain pine beetle, *Dendroctonus ponderosae*, in North America has resulted in the loss of hundreds of millions m^3 timber and turned the forests into major sources of carbon release [1]. In Europe and parts of Asia [2, 3], the European spruce bark beetle, *Ips typographus* (Figure 1), is considered the most destructive bark beetle of coniferous forests [4, 5].

Bark beetles, like most insects, locate their hosts mainly by means of olfactory signals. It is clear that they utilize both attractants and antiattractants that emanate from host and nonhost plants, as well as from conspecific and heterospecific bark beetle individuals [3, 6–10]. The odor molecules are transported downwind from their source of release as an odor plume with a complex structure [11–13]. Molecules are picked up by olfactory receptors (ORs) or ionotropic receptors (IRs) [14], located mainly in the antennae and maxillary palps. Specifically, the ORs are present in the cell membrane of olfactory receptor neuron (ORN) dendrites that, in turn, are housed within olfactory sensilla [15]. The ORs are encoded by a large and diverse family of olfactory receptor genes [16]. Each ORN is generally thought to express only one member from this family in addition to the widely expressed coreceptor, Orco [17]. IRs act in combinations of up to three subunits that are comprised of odor-specific receptors and one or two broadly expressed coreceptors [14]. These receptors are expressed in neurons that do not express ORs. When an odor molecule binds to a receptor, the ORN sends a neuronal signal to the primary olfactory center of the brain, the antennal lobe. Typically, the signal that is generated by an ORN is an increase in the firing frequency of action potentials (excitation), but some odorants may instead cause a decrease in firing activity (inhibition). ORNs can be divided into classes based on their odor response profiles. Often, ORNs are fairly specific and activated by only one or a few compounds, but some appear to have a broader tuning. In addition, each compound often activates more than one type of ORN, and thus, the odor input is thought to be constructed as a combinatorial code [18].

Figure 1: The European spruce bark beetle, *Ips typographus*. Photo: Göran Birgersson.

In contrast to the well-studied chemical ecology of bark beetles, until recently, little was known about the physiological responses of individual bark beetle ORNs. Mainly in the 1980s, Single-sensillum recordings (SSR) were carried out, primarily identifying classes of ORNs that responded to various pheromone compounds. Some decades later, comprehensive studies on *I. typographus* have characterized additional ORNs that respond also to host and nonhost plant compounds [19] and have provided novel insights into potential odor coding mechanisms in insects in general [8]. This review summarizes the results from early and recent studies on the physiology of ORNs in conifer-feeding bark beetles. Particular focus is on *I. typographus,* for which a sufficient amount of information has emerged in order to bridge the physiological data with previously recorded behavioral responses to several semiochemicals. In addition, some recent behavioral studies with connections to olfactory physiology are summarized and possible applications discussed. First, however, a brief overview of the semiochemicals that are used by *I. typographus* in host selection is presented.

2. Host Selection by *I. typographus*

The male is the initial host seeking, or "pioneering," sex of *I. typographus*. Once a male has located a suitable host material to colonize, it releases an aggregation pheromone, a mixture of (4*S*)-*cis*-verbenol and 2-methyl-3-buten-2-ol [20], which attracts individuals of both sexes. Although the olfactory-mediated host location behavior of *I. typographus* has been extensively studied, it is not known how the pioneering males locate a suitable host tree, as no primary attraction (in the absence of pheromone) to spruce volatiles has been demonstrated. However, spruce volatiles may modulate the pheromone response [9] or possibly attract beetles to a suitable habitat [21]. It is also possible that pioneering beetles land randomly on trees and assess host quality upon contact [22]. However, apart from the few pioneering males, the aggregation pheromone attracts the majority of individuals to the host.

The attraction to the pheromone is modulated by other semiochemicals that appear in later attack phases. Verbenone and ipsenol are two such compounds that are believed to be used as cues to avoid heavily attacked trees [23]. In addition, volatiles that are particularly abundant in nonhost angiosperm plants (so called nonhost volatiles, NHV), such as green leaf volatiles (GLVs) [24] and compounds from the bark, such as C8-alcohols and *trans*-conophthorin [25, 26], have inhibitory effects on pheromone attraction. Combining these compounds with verbenone produces a strong synergistic effect and a potent antiattractant blend [27]. Possibly, the individual constituents in the synergistic blend represent different levels in the host selection sequence [6]. The GLVs that are common to broad-leaved plants may represent a signal of a nonhost dominated habitat. More specific plant volatiles, such as *trans*-conophthorin, may indicate nonhosts at the tree species level [7], whereas the antiattractive pheromone components may signal unsuitability of individual spruce trees.

3. Olfactory Receptor Neurons of *I. typographus* and Other Bark Beetles

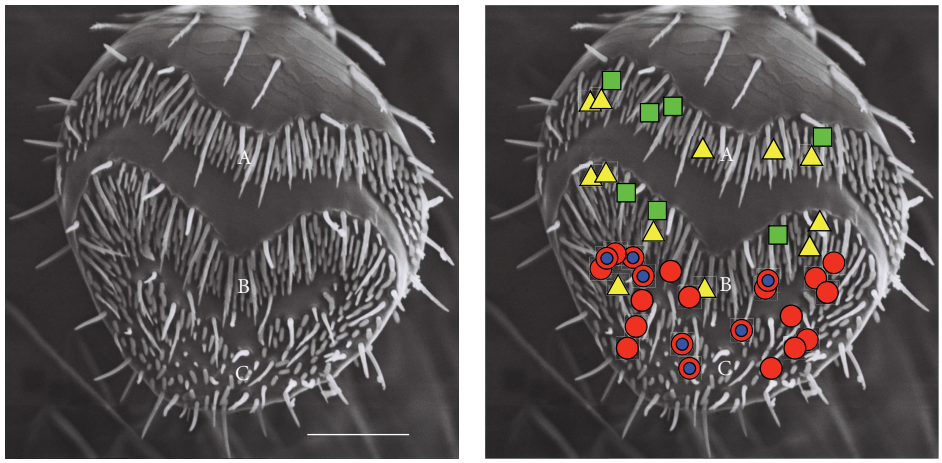
Many compounds that are either attractants or antiattractants for conifer bark beetles have been identified [3, 6, 7]. Single-sensillum recordings from the ORNs of several bark beetle species have shown that many of the behaviorally active compounds elicit responses in different classes of neurons (Table 1). It is obvious that, except for *I. typographus* and the ambrosia beetle *Trypodendron lineatum*, more is known about ORN responses to pheromone components than about responses to plant odors (Table 1). In addition, several of the tested compounds (i.e., ipsdienol, ipsenol, verbenone, *cis*/*trans*-verbenol, *exo*-brecicomin, and α-pinene) elicit strong responses in the majority of species studied. For more details on ORN specificity, sensitivity, and abundance in each species, the reader is referred to the cited literature.

Olfactory sensilla of *I. typographus* are present in three areas (or bands) on the antenna (Figure 2(a)) [41]. Andersson et al. [19] screened 150 olfactory sensilla for responses to an odor panel comprised of similar numbers of synthetic pheromone, host, and nonhost compounds. Strong excitatory responses were obtained from 106 ORNs; 45 responded specifically to various bark beetle pheromone compounds, 37 to host compounds, and 24 to antiattractive nonhost volatiles (NHVs). Based on response spectra, the 106 ORNs were grouped into 17 different classes (Figure 3). Additionally, 26 neurons (divided into 12 ORN classes) responded only weakly to any test odorant, indicating that the most potent compounds for these ORNs were lacking. In addition to the ORN classes described by Andersson et al. [19], three other classes, responding specifically to (+)-*trans*-verbenol, phenylethanol, or campher plus pino-camphone, respectively, had been identified previously (Table 1) [28]. Furthermore, the majority of the ORN classes responding to pheromone compounds was found in both studies. Many ORN classes have been subjected to dose-response trials that indicated that the ORNs, in general, are highly sensitive and specific for only one or a few structurally related pheromone or plant compounds (Figures 4(a)–4(c)) [19, 28]. Response thresholds for the best ligand(s) were normally found around

Table 1: Compounds from different ecological sources that elicit strong responses in olfactory receptor neurons in eight species of Scolytinae.

Species	Beetle-produced compounds	Host compounds	Nonhost compounds	References
Ips typographus	(+)-ipsdienol (−)-ipsdienol (−)-ipsenol (−)-*cis*-verbenol (+)-*trans*-verbenol (−)-verbenone 2-methyl-3-buten-2-ol Amitinol Phenylethanol *exo*-brevicomin (A)* (±)-chalcogran (A)	Myrcene Campher (B) Pino-camphone (B) *p*-cymene 3-carene 1,8-cineole (+)-*α*-pinene (C) (−)-*α*-pinene (C)	Pine bark extract Birch bark extract 1-hexanol (D) *E*2-hexenol (D) *Z*3-hexenol (D) 1-octen-3-ol 3-octanol (*S*,*S*)-*trans*-conophthorin (A)	[19, 28–31]
Ips pini	(+)-ipsdienol (−)-ipsdienol (±)-ipsenol *cis*-verbenol *trans*-verbenol Verbenone	Linalool Camphor Myrcene		[29, 32–34]
Ips paraconfusus	(+)-ipsdienol (−)-ipsdienol (±)-ipsenol			[33]
Dendroctonus pseudotsugae	Frontalin 3-methyl-2-cyclohexenone 3-methyl-2-cyclohexenol 1-methyl-2-cyclohexenol *trans*-verbenol *cis*-verbenol Verbenone Ipsenol	*α*-pinene Limonene		[35, 36]
Dendroctonus frontalis	(−)-frontalin *exo*-brevicomin *endo*-brevicomin Verbenone *trans*-verbenol	*α*-pinene 3-carene		[37, 38]
Dendroctonus micans	(+)-ipsdienol *exo*-brevicomin			[31]
Trypodendron lineatum	(+)-lineatin Phenylethanol	Ethanol Methanol Butanol *α*-pinene *β*-pinene Spruce bark extract	Pine bark extract Birch bark extract	[39]
Tomicus destruens		Compounds in pine extract	Benzyl alcohol	[40]

*Compounds that elicit responses of similar strength in the same ORN class are indicated by the same capital letter. Odorants eliciting secondary responses are omitted for clarity.



(a) (b)

Figure 2: (a) Olfactory sensilla are present in three areas (A, B, and C) on the antennal club of *Ips typographus*. (b) Spatial distribution patterns of four classes of olfactory receptor neurons (ORNs). ORNs responding to green leaf volatile alcohols (nonhost) = green squares, myrcene (host) = yellow triangles, *cis*-verbenol (pheromone) = red circles, 1,8-cineole (host) = blue small circles (from [19], with permission from the publisher). Scale bar = 50 μm.

the 1 ng dose on the filter paper using paraffin oil as solvent [19]. A high specificity, not only among pheromone ORNs, but also among those for plant compounds, seems to be a general rule also in other bark beetle species (see especially [32, 35]).

The ORNs of *I. typographus* are not randomly distributed on the antenna. Instead, ORNs from a particular class are generally found either in both the proximal and medial bands of sensilla, or exclusively in the distal area (Figure 2(b)) [19]. This distribution pattern seems to correspond to the distribution of the two morphological types of single-walled sensilla previously identified [41].

It is common in insects that the pheromone ORNs are numerous on the antenna and that the most common ORN type is tuned to the major (most abundant) component [42, 43]. In *I. typographus*, the most recurrent ORN class was tuned to (−)-*cis*-verbenol [19]. In contrast, there were only few cells specific for 2-methyl-3-buten-2-ol (MB) (Figure 3) [19, 28], an essential pheromone component which is produced, and behaviorally active, in much larger quantities [20, 44]. This suggests that the pattern might be reversed in the bark beetle. However, the MB cells were found in a restricted area on the antenna [19], that is, on the borderline between the medial band and distal area of sensilla, which could have resulted in this cell type being underrepresented among the sampled sensilla. Alternatively, as MB is highly volatile, the low number of cells could be the result of the compound being lost from the stimulus cartridge upon stimulation. Indeed, photoionization detector measurements showed that the airborne amount of MB released from the stimulus pipette drops dramatically upon stimulation (Figure 5) [45]. However, the insect ORN still responded vigorously despite the low concentration, rendering this explanation unlikely. In contrast to Andersson et al. [19], Tømmerås [28] found that the ORNs tuned to ipsdienol were the most common ones

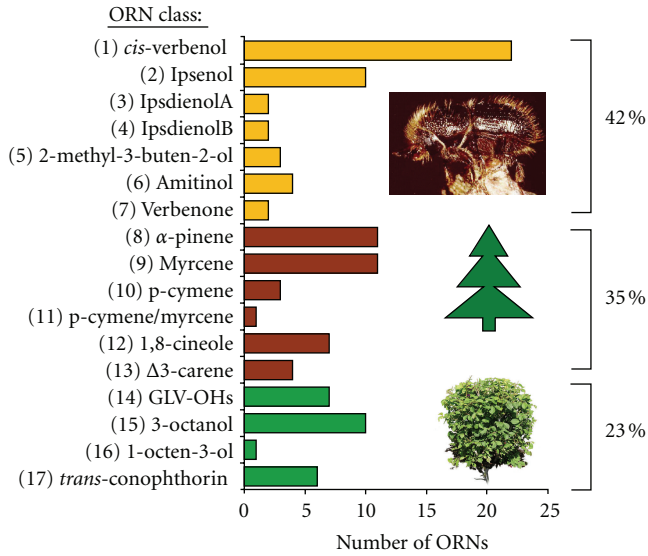


Figure 3: Number of olfactory receptor neurons (ORNs) of 17 strongly responding classes of *Ips typographus* (data from [19]). ORN classes are labeled according to which compound(s) elicited the strongest response. As pure enantiomers were not tested, it is likely that the ipdienol ORN classes A and B correspond to the ORNs responding to (+)- and (−)-ipdienol, respectively [29]. Orange = bark beetle pheromone compounds, brown = conifer compounds, green = nonhost volatiles. GLV-OHs = green leaf volatile alcohols.

in *I. typographus*. This discrepancy may also be explained by the nonrandom localization of ORNs on the antenna (i.e., neurons for *cis*-verbenol are abundant only in the distal part of the antennae, Figure 2(b)).

Although no primary attraction has been demonstrated, the high frequency of ORNs tuned to conifer-related

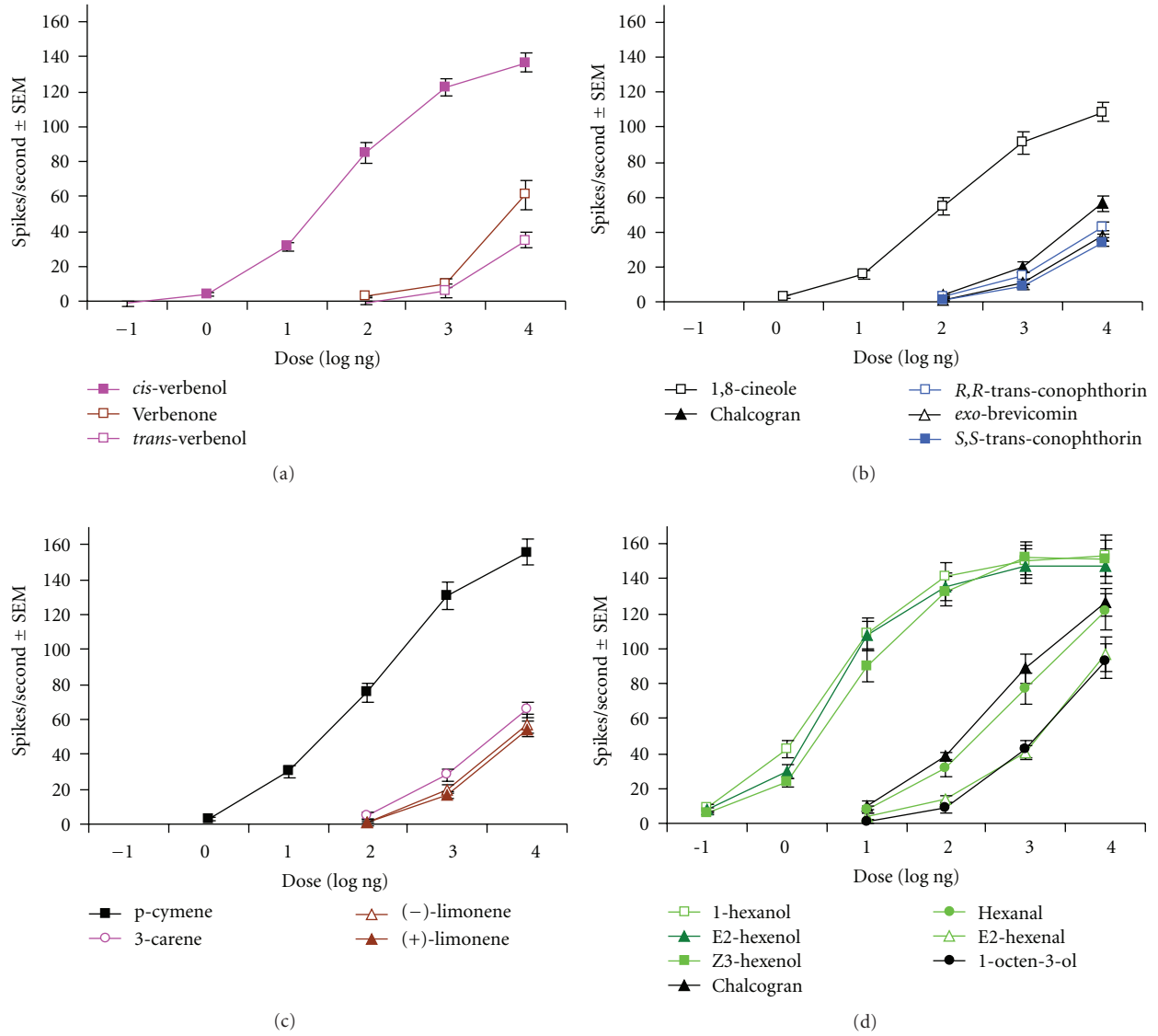


FIGURE 4: Dose-response curves from four receptor neuron classes of *Ips typographus*, demonstrating specific primary responses to (a) the pheromone component *cis*-verbenol, the spruce compounds (b) 1,8-cineole and (c) p-cymene. (d) Indiscriminate response to the three green leaf volatiles 1-hexanol, *E*2-hexenol, and *Z*3-hexenol (modified from [19], with permission from the publisher).

monoterpenes (Table 1, Figure 3) suggests that host kairomone is relevant for host location by *I. typographus*. As mentioned previously, these compounds may serve as habitat-scale attractants [21], or as modulators of pheromone attraction [8, 9]. Perhaps the most striking finding from the bark beetle SSR studies is that almost 25% of the strongly responding ORNs were specifically tuned to anti-attractive NHV (Table 1, Figure 3) [19]. This indicates that insects may devote a lot of olfactory capacity to the detection of compounds from sources that they avoid. Similar results have not been found in any other insect studied so far, however, it is likely that many other bark beetles that show strong GC-EAD responses to NHV also have a large proportion of ORNs tuned to such compounds [7, 46–48].

4. Discrimination of Enantiomers

Most bark beetle pheromone compounds are chiral. Attraction is typically evoked by only one of the enantiomers, while the other sometimes inhibits attraction (e.g., [20, 33]). The enantiospecific behavioral response is reflected in the specificity of the ORNs detecting the compounds (Table 1). For instance, ORNs that are specific for either the (+)- or the (−)-enantiomer of ipsdienol, ipsenol, verbenone, or *cis*- and *trans*-verbenol have been identified in several *Ips* species [28, 33]. Other examples are the ORNs in the Southern pine beetle, *D. frontalis* [37], and in the Douglas-fir beetle, *D. pseudotsugae* [36], that discriminate between the (+)- and (−)-enantiomer of frontalin (Table 1).

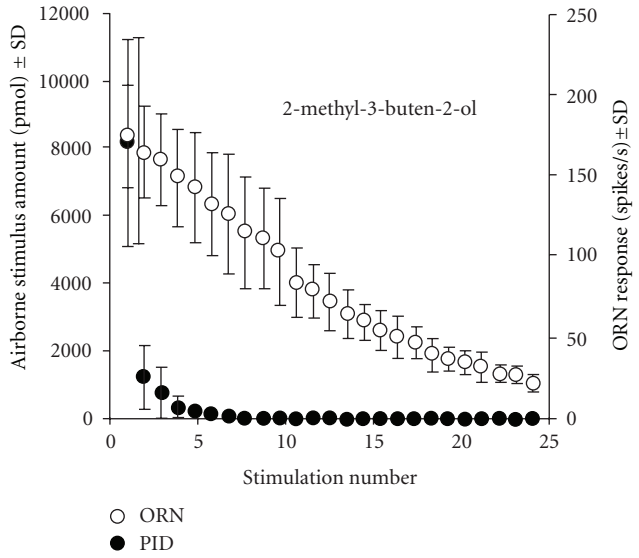


Figure 5: Response of *Ips typographus* olfactory receptor neurons (ORNs) and a photoionization detector (PID) to successive stimulations with 2-methyl-3-buten-2-ol ($N = 4$) (modified from [61]).

In general, the sensitivity of the pheromone ORNs seems to be 10–100-fold higher for the enantiomer that they are tuned to, as compared to the other [28, 33]. In addition, there seems to be a correspondence between the attraction to a specific enantiomer, and the frequency of ORNs on the antenna that responds to it. For instance, *I. pini* is attracted by (−)-ipsdienol and has more of its ORNs tuned to (−)-ipsdienol than to (+)-ipsdienol. Similarly, *I. paraconfusus*, which is attracted by (+)-ipsdienol, has most of its ipsdienol ORNs tuned to the (+)-enantiomer [33].

Enantiospecific responses to plant compounds have been recorded in *I. typographus* [19]. The neuron class that responded most strongly to the nonhost volatile *trans*-conophthorin (Table 1) was >100-fold more sensitive to the (5*S*,7*S*)-enantiomer than to the (5*R*,7*R*)-enantiomer. In fact, other structurally related compounds (racemic *exo*-brevicomin and chalcogran) elicited stronger responses in this ORN than did the (5*R*,7*R*)-enantiomer of *trans*-conophthorin [19]. In another class of ORN, the naturally occurring (−)-1-octen-3-ol elicited a slightly stronger response than the racemic mixture, indicating that the (−)-enantiomer is the key ligand. In contrast, the neuron that is tuned to α-pinene responded similarly to both enantiomers (Table 1) [19].

5. Olfactory Receptor Neuron Responses and Behavior

The results that have been obtained from single sensillum recordings [19, 28] indicate that behavioral responses of *I. typographus* to several compounds can likely be explained by the responses of the ORNs.

Several volatiles from nonhost plants were previously shown to inhibit pheromone attraction of *I. typographus* [24–26]. The three GLVs: 1-hexanol, *E*2-hexenol, and *Z*3-hexenol all reduced pheromone attraction to a similar extent. However, combining the three did not produce a stronger inhibition of attraction, a phenomenon defined as redundancy [27]. Interestingly, the only ORN that was sensitive to any of these volatiles had a more or less identical sensitivity to all three of them (Table 1, Figure 4(d)) [19]. Thus, it appears as if the bark beetle cannot differentiate between the compounds at the physiological level, which agrees well with their behavioral redundancy. In contrast, the compounds verbenone and *trans*-conophthorin that synergize the inhibition are detected by different ORNs [19, 28]. Interestingly, the pheromone component, chalcogran, of the sympatric *Pityogenes chalcographus*, was primarily detected, by *I. typographus*, by the same neuron as *trans*-conophthorin (Table 1). Chalcogran also inhibits pheromone attraction of *I. typographus* [49].

Most insects house their ORNs for pheromone compounds in sensilla that are distinct from the ones that detect plant compounds (e.g., [50, 51]). However, in some sensilla in *I. typographus,* the ORN for the aggregation pheromone component *cis*-verbenol (cV) is colocalized with an ORN that responds to the host plant compound 1,8-cineole (Ci) [8, 19] (Figure 2(b)). This lack of segregation between ORNs detecting pheromones and plant volatiles may suggest that host finding in bark beetles is an integrated process that involves both pheromones and plant volatiles. When the ORN for Ci responded, the colocalized cV cell was inhibited, indicative of interactions between ORNs in the periphery. In addition, Ci was found to be particularly abundant in heavily attacked spruce trees and the compound strongly reduced pheromone attraction (88% reduction in trap catch) in the field [8]. Possibly, Ci is a signal of an unsuitable (crowded) host or a well-defended tree.

6. Peripheral Modulation of ORN Responses

Colocalization of insect ORNs in the same sensillum is thought to improve coincidence detection, which increases the insect's spatiotemporal resolution of odor signals [52] and improves ratio detection of ecologically relevant odor mixtures [53]. In addition, the presence of two or more neurons in the same sensillum may provide opportunities for signal modulation in the periphery. Indeed, in the Douglas-fir beetle, *Dendroctonus pseudotsugae*, two ORNs, each specific for one of the two pheromone components 3-methyl-2-cyclohexenone or 3-methyl-2-cyclohexenol, are colocalized. When either one of the ORNs responded to its specific ligand, the spontaneous activity of the other ORN was reduced. This observation indicated reciprocal interactions, either directly between the two neurons, or between the two ligands and their respective receptors [35]. In addition, when another ORN type that responded to limonene (10 ng dose) was challenged with a binary mixture of limonene and 3-methyl-2-cyclohexenol (10 : 1000 ng), the response to limonene was completely shut down [35].

In *I. typographus*, not all cV neurons (large amplitude A-cell) are colocalized with the neuron for Ci (small amplitude B-cell) (Figure 6(a)). These cV neurons are instead found

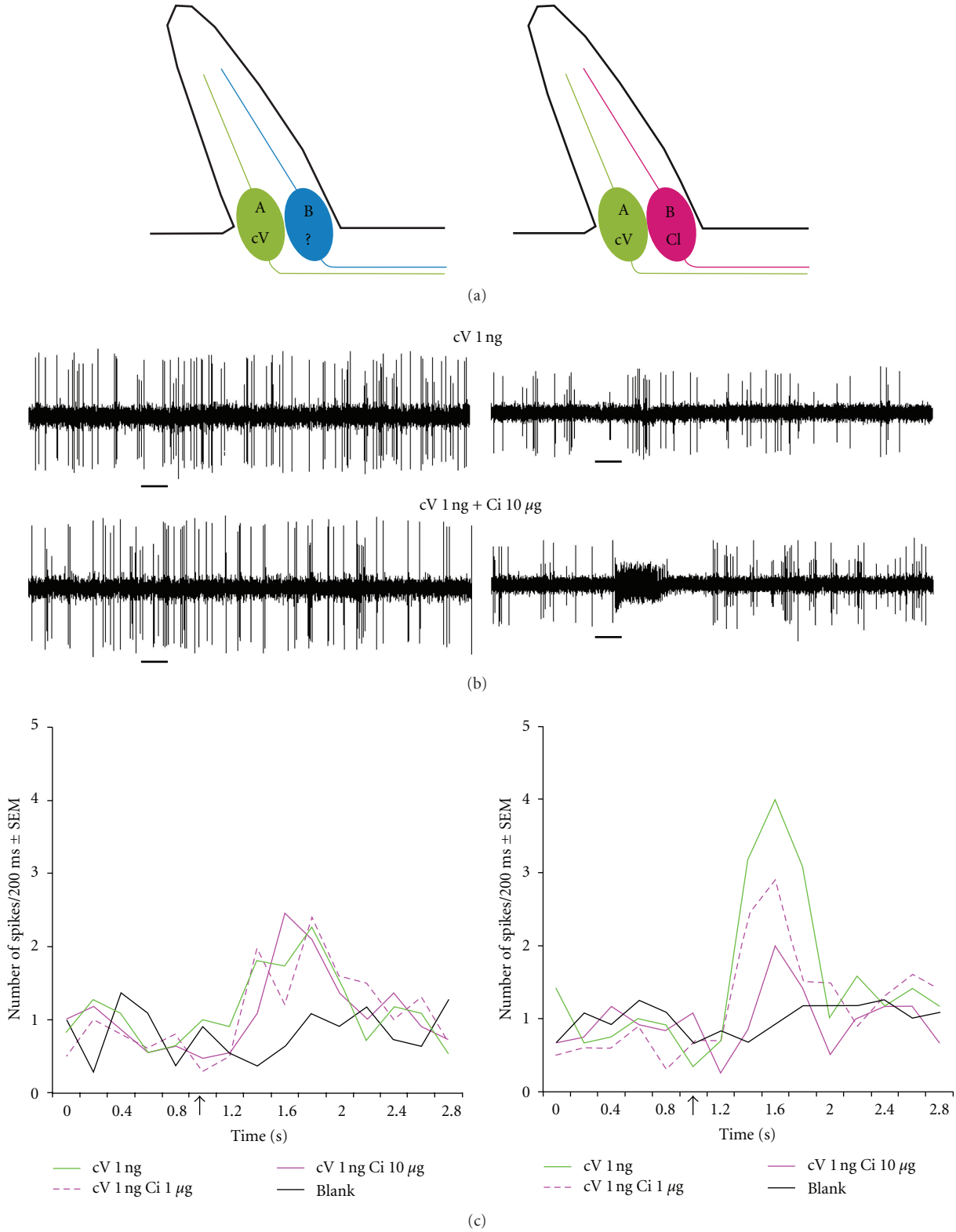


Figure 6: (a) Schematic drawing of the two types of sensilla in *Ips typographus* containing the A cell (large amplitude spikes) for *cis*-verbenol (cV), accompanied either by a nonresponsive (*left column*) B cell (small amplitude), or a B cell for 1,8-cineole (Ci) (*right column*). (b) Responses of both sensillum types to 1 ng cV (*upper traces*), and a binary mixture of 1 ng cV and 10 μg Ci (*lower traces*). Note the inhibition of the cV response during the response to Ci in the B cell. Black horizontal bars indicate the 0.5 s stimulation period. (c) Detailed response curves to cV and binary cV : Ci mixtures showing a Ci dose-dependent inhibition of the cV response only in sensilla that also contain the Ci cell (N = 10–12). Arrows indicate the onset of the 0.5 s stimulation period (modified from [8], with permission from the publisher).

together with another ORN type that does not respond to any odorant tested so far [19]. The Ci inhibited the cV cell only in sensilla in which the two neurons were colocalized, implying that the inhibition might be due to interactions between the ORNs. To test this hypothesis, Andersson et al. [8] recorded both types of cV sensilla (with or without the Ci cell) and tested responses to binary cV/Ci mixtures. They found that not only the spontaneous activity but also the ORN response to the lowest cV dose (1 ng) was inhibited by simultaneous stimulation with high doses of Ci (1–10 μg). This inhibition occurred only in sensilla that also contained the Ci cell (Figures 6(b)-6(c)). In addition, the response to the higher cV dose (10 ng) was more strongly inhibited in sensilla where the Ci cell was colocalized. Thus, it seems plausible that the two ORNs interact, possibly by means of passive electrical interactions [54]. However, if or to which extent the reduction in pheromone trap catches by the presence of Ci [8] can be explained by the inhibition of the cV ORN remains unknown, as the excitatory input from the two ORNs provides the means also for central integration [55]. It seems like similar inhibitory interactions between colocalized ORNs occur also in other insects [51, 56, 57], but the phenomenon has so far only been systematically addressed in bark beetles.

7. Difficulties in Comparing ORN Responses to Compounds with Different Volatility

In most SSR studies, odor stimuli are prepared based on a known amount (e.g., in nano- or microgram) of compound applied to a piece of filter paper that is positioned inside a Pasteur pipette odor cartridge. Upon stimulation, the headspace in the cartridge is blown over the insect preparation. Depending on compound, solvent, and how many times the cartridge has been used, the quantity of molecules reaching the insect can be highly variable and seriously affect the ORN response [58, 59]. Indeed, different stimulation regimes, compound doses, and solvents (mostly hexane and paraffin oil) have been used in the various bark beetle SSR studies (Table 1), making it difficult to directly compare the sensitivity and specificity of ORNs characterized in different species or studies. Furthermore, the physical parameters of the odor-delivery system also affect the integrity of an airborne odor stimulus [60], which may further increase the variability among responses.

Airborne amounts of different compounds have been measured with a photoionization detector [45]. A huge variation among compounds was observed. For the most volatile compounds, such as 2-methyl-3-buten-2-ol (Figure 5), ca 80% of the headspace in the odor cartridge was lost at the first puff, even though paraffin oil was used as solvent. Airborne amounts of heavier compounds, such as linalool, were reduced by only ca 50% after 50 reiterative stimulations. In addition, compounds that were dissolved in pentane were released at a much higher rate than compounds in paraffin [45].

The large variation between compounds, solvents, and successive stimulations could easily bias electrophysiological responses in insects. This was verified by reanalyzing the response of the 3-octanol ORN of *I. typographus* [19] to two C8-alcohols (3-octanol and 1-octen-3-ol) and two C6-alcohols (*Z*3-hexenol and 1-hexanol) using both fresh (not used) and "old" (used 10 times) stimulus pipettes [45]. The ORN response to fresh pipettes was clearly different from the response to the "old" pipettes. In particular the response to the C6-alcohols was clearly lower when old pipettes were used. In fact, the difference in response was so large that it falsely implied that recordings were made from two distinct ORN classes. Such a finding suggests that it is absolutely necessary to use very strict experimental protocols for electrophysiological recordings, and that it sometimes is required to measure airborne odor amounts, especially when compounds of different volatility are used as stimuli [45].

8. Odor Coding in Bark Beetles Compared to Other Insects

In insects in general, neurons that detect pheromone constituents have a narrow tuning. Bark beetles are no exception as the ORNs that respond to aggregation pheromone compounds are, in most cases, sensitive to only one compound. The tuning width of insect ORNs detecting plant volatiles seems to range from narrow to broad, although ORN specificity is strongly correlated to the stimulus concentration tested [18]. Most of the ORNs for plant volatiles in *I. typographus* are narrowly tuned [8]. However, some show more indiscriminate responses, such as the GLV neuron that had similar sensitivity to 1-hexanol, *E*2-hexenol, and *Z*3-hexenol. This is in contrast to the highly specific GLV neurons that have been described in, for instance, scarab beetles [50, 51], and in the Colorado potato beetle [62]. The difference may be related to the fact that these other species feed on angiosperms, which presumably requires a better resolution of angiosperm dominated volatiles (i.e., GLVs) than what is needed for a conifer specialist. Many of the bark beetle ORNs are highly selective for specific enantiomers, both in terms of pheromones and plant compounds. However, this feature is not unique for bark beetles; highly enantioselective neurons have been characterized also in other insects [63–65]. In contrast, no other insect studied so far has a comparable frequency of ORNs tuned to antiattractants as the one found in *I. typographus* [19].

The co-localization of ORNs for pheromone and plant compounds in *I. typographus* is not commonly found in insects. This special type of ORN pairing may be related to the fact that host colonization in bark beetles often involves both pheromone and plant-produced compounds [8]. In addition, the colocalized neurons for plant and pheromone compounds also interacted by inhibiting their neighboring neuron while responding [8]. It is difficult to say whether a similar interaction occurs also in other insects, since it has not been systematically addressed elsewhere. However, inhibition of the spontaneous activity of the large-spiking cell when the small-spiking cell responds seems to be a common phenomenon [35, 51, 56, 57], indicating that the same type of modulation could be present. Indirect evidence for ORN interactions was found previously in the honeybee [66]. The 18–35 ORNs that are housed within honeybee

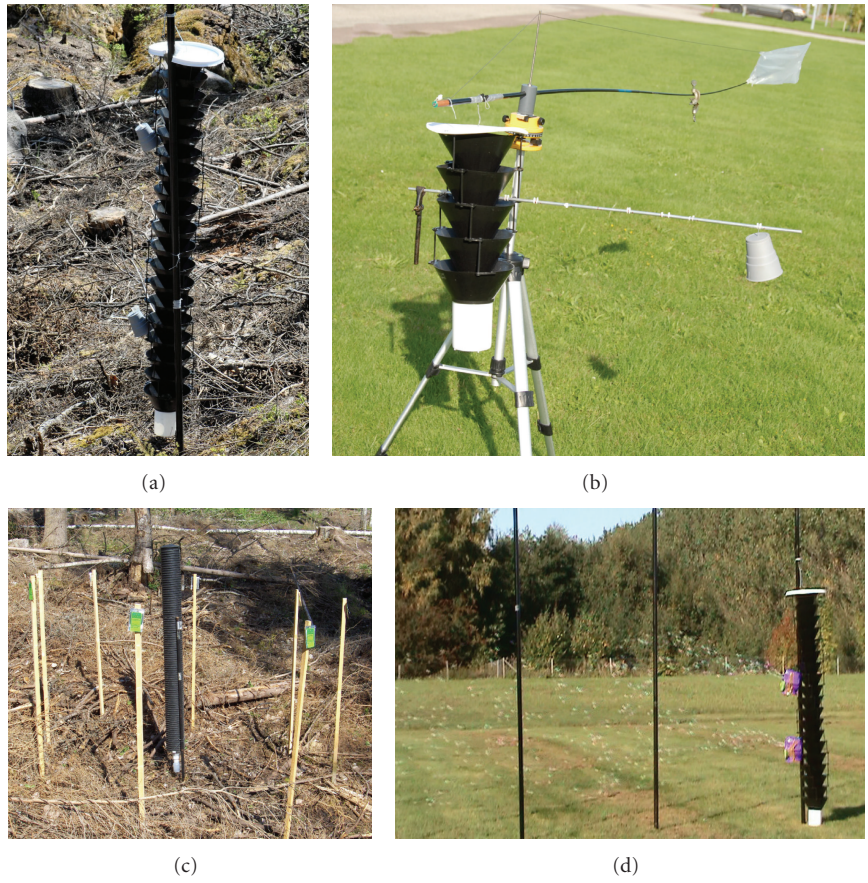


Figure 7: (a) Lindgren funnel traps (19-funnel size) were used in the vertical spacing tests with *Ips typographus*. Dispensers positioned under grey cups. (b) A Lindgren trap (5-funnel size) was attached to a wind vane in the horizontal spacing tests to ensure constant distance between plumes. (c) Pipe trap surrounded by eight nonhost volatile dispensers in the antiattractant background tests. (d) Soap bubble visualization of vertical plume overlap at a spacing distance of 48 cm. Distance between black poles = 1 m (modified from [61]).

sensilla placodea seemed to respond to odors in a coordinated manner, indicating that the individual ORNs do not act as independent response units. However, in that study, it was not possible to keep track of the individual ORNs.

Taken together, odor coding in bark beetles is, in general, similar to odor coding in other insects, but it also exhibits some rare features. The coding principle seems to be consistent with the "combinatorial code" theory, but the olfactory input travels mainly through highly specific channels.

9. Detection and Behavior in Odor-Diverse Habitats

Activation of an ORN by an attractant may cause an upwind flight by the insect towards the odor source. However, if repulsive compounds simultaneously trigger other ORNs to fire, the upwind flight may be aborted. Thus, in environments with a high "semiochemical diversity" [27] where odor plumes from different sources intermix, localization of host plants may be hampered by the presence of odors from nonhosts [67, 68]. Thus, for bark beetles, it may be possible to reduce the risk of attacks by making the environment more semiochemically diverse. Homogenous mixing of odor plumes from different sources is, however, contradicted by the partitioning of plumes into "odor packages" (or filaments) that are interspersed with pockets of "clean air" [11, 12]. This, in turn, is thought to facilitate plume discrimination by insects.

Placing an NHV mixture inside a pheromone trap, that is, next to the pheromone bait, greatly reduces trap catch of *I. typographus* [27]. However, to test the "semiochemical diversity hypothesis," pheromone trap catches in the presence of NHV at different vertical and horizontal distances from the pheromone dispenser (Figures 7(a)-7(b)), were investigated [69]. Trap catches in response to separated pheromone components (*cis*-verbenol and 2-methyl-3-buten-2-ol) were also tested (in the absence of NHV) to further investigate responses to separated baits in general. In addition, the response of the beetle was compared to the response of the Egyptian cotton leaf worm, *Spodoptera littoralis* (Lepidoptera: Noctuidae), to separated sex pheromone components and to separated pheromone and behavioral antagonist. In both species, increased spacing between pheromone and antiattractants led to increased trap catch, whereas, as expected, increased spacing between pheromone components had the opposite effect. However,

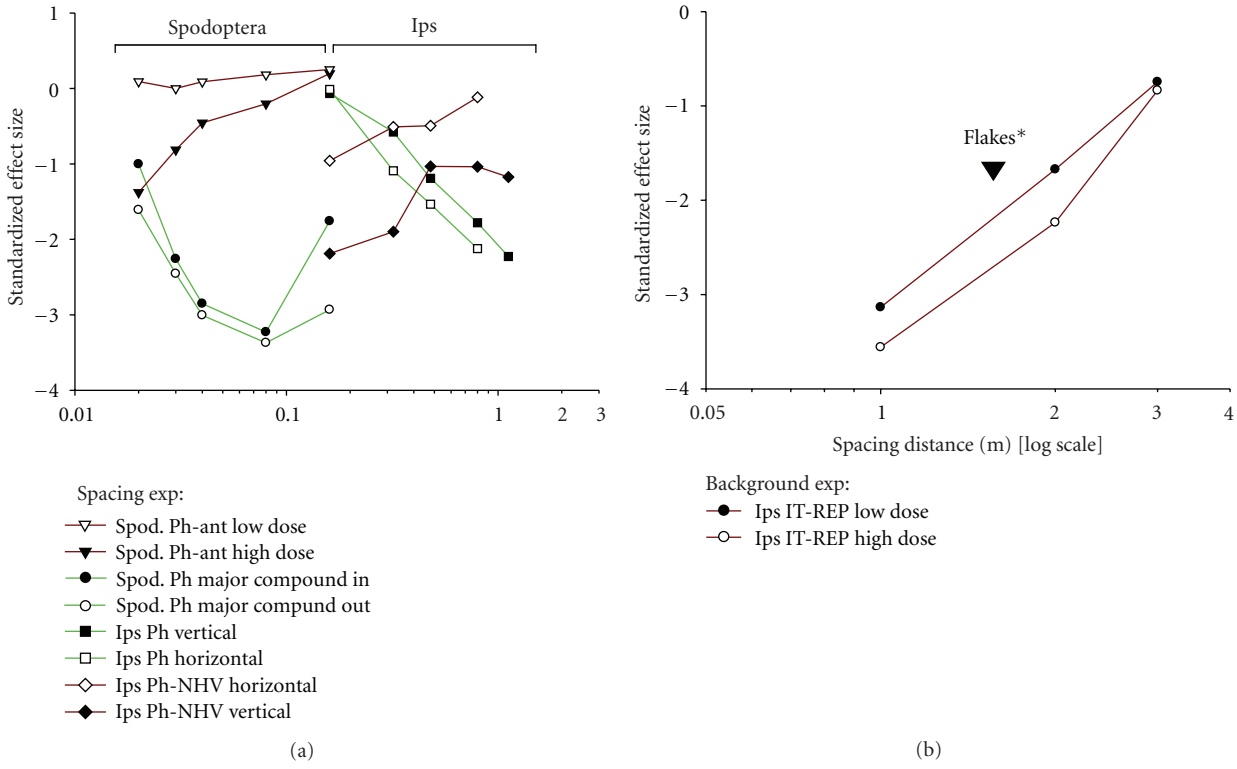


FIGURE 8: (a) The effect of spacing between attractant and antiattractant sources on trap catches of *Ips typographus* and *Spodoptera littoralis*, illustrated by measures of effect size (*Hedges*' unbiased *g*). The effect size provides a measure of a biological treatment effect by scaling the difference between the treatment and the control means, with the pooled standard deviation for those means. Effect sizes further from zero than 0.8 are regarded as strong effects. In all experiments, the pheromone bait alone (zero distance between components) was the control. The zero cm spacing distance in experiments involving antiattractants is omitted for clarity. (b) Effect sizes in the *Ips* antiattractant background experiments using nonhost volatile dispensers at eight positions, or flakes around the trap. *Flakes were evenly distributed on the ground 0–2 m from the trap. Thus, this treatment is "not to scale" on the *x*-axis. Ph = pheromone; Ant = *Spodoptera* pheromone antagonist; NHV = nonhost volatiles; IT-REP = semicommercial *Ips typographus* repellent dispenser (from [69], with permission from the publisher).

the two species differed greatly with respect to the spacing distances that affected their trap catch (Figure 8(a)). While beetle trap catches were affected by separation of some decimeters, trap catches of the moth were affected by separation distances of just a few centimeters [69]. In each species, the spacing distances affecting trap catch did not differ between the pheromone component spacing and the pheromone/antiattractant spacing experiments [69].

The bark beetle pheromone/NHV spacing experiments indicated antiattractive effects of NHV up to a distance of >1 m [69]. To further investigate potential effects of NHV at even longer distances, pheromone attraction was studied in the presence of a synthetic background of NHV, either created by eight NHV point sources positioned in a ring (with 1, 2, or 3 m radius) around a central pheromone trap (Figure 7(c)), or by ca 6000 small (ca 3 × 3 mm) NHV impregnated flakes [70] on the ground around a pheromone trap [69]. With the eight NHV sources, bark beetle attraction was reduced up to the 2 m spacing distance, and there was still a tendency for reduced attraction at the 3 m distance (Figure 8(b)). Similar to the eight point sources, the NHV flakes also reduced pheromone attraction [69]. The active spacing distances are in accordance with the "active inhibitory range" of NHV of at least 2 m that was estimated previously [27]. The pheromone dose used by Andersson et al. [69] was comparable to that released from a mass-attacked tree, which is a very strong signal. Thus, it is striking that volatiles from nonhost plants can inhibit attraction when they are released a few meters away from the pheromone source. This indicates that avoiding not only nonhost species, but also nonhost habitats, likely improves bark beetle fitness.

The different spacing distances that affected trap catches of the beetle and the moth may reflect differences in the size of the natural odor sources (and plumes) the insects orient to [69]. While a male moth orients towards a single calling female, bark beetles may orient to large patches of trees with hundreds of calling males. Furthermore, the moth sex pheromone communication system is highly specialized. A male moth flies towards a calling female for mating only, whereas the bark beetle aggregation pheromone can be used as a signal of mates, food, and oviposition sites. Thus, the different selection pressures that operate on these systems have likely resulted in different degrees of specialization. The ORNs for pheromone compounds in moths are housed in specific sensilla (*trichodea*), distinct from the ones that

detect plant odors [43]. In contrast, *I. typographus* groups the *cis*-verbenol pheromone ORN together in the same sensilla as the ORN for the plant compound 1,8-cineole, although the ORNs themselves are specific in their response [8].

Similar to *I. typographus*, studies on *Dendroctonus* bark beetles indicated synergistic interactions between pheromone components when two baits were separated by several meters [71, 72]. The sharp response of *S. littoralis* to odor source spacing has been observed previously in other moths [73–75]. The most extreme example is provided by Fadamiro et al. [52], who found that 1 mm separation between pheromone and antagonist was sufficient to restore upwind flight to the pheromone by male *Helicoverpa zea*. It was hypothesized that coincident detection of pheromone components and antagonists, achieved by colocalization of the ORNs, was the reason for this amazing ability of the males. Furthermore, synchronous detection of pheromone compounds was shown to improve the temporal spiking pattern by projection neurons in the antennal lobe of *Manduca sexta* moths [76]. Thus, it is clear that coincidence detection is of great importance in the pheromone system of moths. Soap bubble generators were used to visualize plume overlap at the different spacing distances used for *I. typographus* (Figure 7(d)) [69]. The simulations indicated that filaments from different plumes are more likely to overlap and, thus, to be detected coincidently, when the sources are close to each other. Therefore, the lower sensitivity of *I. typographus* to small-scale spatial separation of odor sources might indicate that coincidence detection is of less importance for bark beetles than for moths [69].

10. Applications

Conifer pest insect infestations are typically less common in diversified habitats [67], which in part may be due to the presence of antiattractive NHV. The finding that NHV, from a distance of at least 2 m (see also [27]), can reduce attraction to a pheromone dose comparable to that released from a mass-attacked tree suggests a potential for NHV in forest protection. However, pheromone attraction was not completely shut down so it is more likely that, instead of counteracting ongoing mass attacks, synthetic or natural NHV sources may reduce the risk of spruces being attacked in the first place. Indeed, spruces were previously protected by NHV dispensers attached to every second tree, demonstrating a protective effect of ca 2 m [77]. In another study, groups of ten trees were all protected by 20 NHV dispensers, and bark beetle attacks were diverted to trees >15 m away [78].

In addition, the spruce compound 1,8-cineole that strongly reduced pheromone attraction should be further tested in combination with the other active semiochemicals for possible improvement of antiattractant blends. It is possible that the repression of the ORN for *cis*-verbenol by 1,8-cineole, adds another inhibitory mechanism by distorting the "perceived blend ratio" of the aggregation pheromone. If so, it is likely that a more effective antiattractant blend can be obtained than the one that is comprised of GLV alcohols, C8-alcohols, *trans*-conophthorin, and verbenone [27].

11. Conclusions and Future Directions

The recent advances in bark beetle olfactory physiology have provided a connection between the physiological and behavioral responses of *I. typographus* to ecologically relevant compounds. This connection has allowed for a deeper understanding about how bark beetles (and possibly insects in general) may encode, and respond to, the odor environment. However, there are still several ORNs of *I. typographus* (and other species) for which odor ligands have not been identified, meaning that there is yet more to be learned about its olfactory physiology. Identification of active compounds should be achieved by GC-coupled SSR and by testing headspace collections from, for instance, attacked and unattacked or resistant host trees.

At the molecular level, Andersson and collaborators [61, 79] recently sequenced the antennal transcriptome of *I. typographus*, leading to identification of gene sequences for 40 different candidate olfactory receptors (ORs). The amino acid sequences of the receptors were compared, in a sequence similarity tree, with receptors that were previously identified from the genome of the flour beetle, *Tribolium castaneum*. Many of the *Ips* ORs formed a bark beetle-specific branch, indicating an extension of OR function. Possibly, these receptors detect conifer-related volatiles or pheromones that are especially relevant for bark beetles. The other ORs of *Ips* were grouped together with ORs of *T. castaneum*, which may indicate conserved functionality of some sets of ORs within Coleoptera. Functional studies to reveal which compounds the ORs of *Ips* bind will be the next step in the study. Such studies will hopefully extend the connection from behavior, through physiology, all the way to the level of the receptor and gene.

The identification of the bark beetle ORs paves the way for the development of potential novel management strategies in the future. If the receptors for pheromone components and antiattractive NHV can be identified, it might be possible to identify ligands that pharmacologically block the pheromone receptors or hyperstimulate [80] receptors for nonhost volatiles. If such compounds are found, they might be dispensed in the forest to disrupt bark beetle pheromone communication and host tree localization.

One hypothesis why insect colocalize specific ORNs in the same sensilla is that it allows for improved spatiotemporal resolution of odor stimuli [52]. This hypothesis could be tested by comparing trap catches of *I. typographus* in response to spacing between pheromone and 1,8-cineole (ORNs for *cis*-verbenol and 1,8-cineole co-localized), with trap catches in response to spacing between pheromone and verbenone, the latter compound being detected by an ORN that is never colocalized with an aggregation pheromone neuron. Predictably, the beetle should be more "sensitive" to small-scale spacing between pheromone and 1,8-cineole than to spacing between pheromone and verbenone.

In order to put the sensory physiology into a more natural context, a portable single sensillum recording device [81] should be used in the field. The sensillum that contains the ORNs for *cis*-verbenol and 1,8-cineole could be used as a biological detector for measurements of plume filament

overlap. Such measurements would reveal whether filaments from overlapping plumes are detected coincidently or not. It would also provide some indirect clue if beetles temporally integrate filaments from different plumes to a larger degree than moths, which could explain the difference in response to spacing in the two types of insects.

Acknowledgments

The author is grateful to Professor Fredrik Schlyter (SLU, Alnarp) for useful criticism on a previous manuscript draft. Funding was provided by FORMAS Project no. 230-2005-1778 "Semiochemical diversity and insect dynamics," the Linnaeus program "Insect chemical ecology, ethology, and evolution (ICE3)," and by the Department of Plant Protection Biology, SLU, Alnarp.

References

[1] W. A. Kurz, C. C. Dymond, G. Stinson et al., "Mountain pine beetle and forest carbon feedback to climate change," *Nature*, vol. 452, no. 7190, pp. 987–990, 2008.

[2] L. Stauffer, F. Lakatos, and G. M. Hewitt, "Phylogeography and postglacial colonization routes of *Ips typographus* L. (Coleoptera, Scolytidae)," *Molecular Ecology*, vol. 8, no. 5, pp. 763–773, 1999.

[3] J. A. Byers, "Chemical ecology of bark beetles in a complex olfactory landscape," in *Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis*, F. Lieutier, K. R. Day, A. Battisti, J.-C. Grégoire, and H. F. Evans, Eds., pp. 89–134, Kluwer Academic Publishers, Dordrecht, The Netherlands, 2004.

[4] B. Økland and O. N. Bjørnstad, "Synchrony and geographical variation of the spruce bark beetle (*Ips typographus*) during a non-epidemic period," *Population Ecology*, vol. 45, no. 3, pp. 213–219, 2003.

[5] B. Økland and O. N. Bjørnstad, "A resource-depletion model of forest insect outbreaks," *Ecology*, vol. 87, no. 2, pp. 283–290, 2008.

[6] F. Schlyter and G. A. Birgersson, "Forest beetles," in *Pheromones of Non-Lepidopteran Insects Associated with Agricultural Plants*, J. Hardie and A. K. Minks, Eds., pp. 113–148, CAB International, Oxford, UK, 1999.

[7] Q.-H. Zhang and F. Schlyter, "Olfactory recognition and behavioural avoidance of angiosperm nonhost volatiles by conifer-inhabiting bark beetles," *Agricultural and Forest Entomology*, vol. 6, no. 1, pp. 1–19, 2004.

[8] M. N. Andersson, M. C. Larsson, M. Blaženec, R. Jakuš, Q.-H. Zhang, and F. Schlyter, "Peripheral modulation of pheromone response by inhibitory host compound in a beetle," *Journal of Experimental Biology*, vol. 213, no. 19, pp. 3332–3339, 2010.

[9] N. Erbilgin, P. Krokene, T. Kvamme, and E. Christiansen, "A host monoterpene influences *Ips typographus* (Coleoptera: Curculionidae, Scolytinae) responses to its aggregation pheromone," *Agricultural and Forest Entomology*, vol. 9, no. 2, pp. 135–140, 2007.

[10] J. A. Byers and Q.-H. Zhang, "Chemical ecology of bark beetles in regard to search and selection of host trees," in *Recent Advances in Entomological Research*, T.-X. Liu and L. Kang, Eds., pp. 91–113, Higher Education Press, Beijing, China, 2010.

[11] N. J. Vickers, "Mechanisms of animal navigation in odor plumes," *Biological Bulletin*, vol. 198, no. 2, pp. 203–212, 2000.

[12] R. T. Cardé and M. A. Willis, "Navigational strategies used by insects to find distant, wind-borne sources of odor," *Journal of Chemical Ecology*, vol. 34, no. 7, pp. 854–866, 2008.

[13] J. Murlis, M. A. Willis, and R.T. Cardé, "Spatial and temporal structures of pheromone plumes in fields and forests," *Physiological Entomology*, vol. 25, pp. 211–222, 2000.

[14] L. Abuin, B. Bargeton, M. H. Ulbrich, E. Y. Isacoff, S. Kellenberger, and R. Benton, "Functional architecture of olfactory ionotropic glutamate receptors," *Neuron*, vol. 69, no. 1, pp. 44–60, 2011.

[15] L. B. Vosshall and R. F. Stocker, "Molecular architecture of smell and taste in *Drosophila*," *Annual Review of Neuroscience*, vol. 30, pp. 505–533, 2007.

[16] M. de Bruyne and T. C. Baker, "Odor detection in insects: volatile codes," *Journal of Chemical Ecology*, vol. 34, no. 7, pp. 882–897, 2008.

[17] L. B. Vosshall and B. S. Hansson, "A unified nomenclature system for the insect olfactory coreceptor," *Chemical Senses*, vol. 36, no. 6, pp. 497–498, 2011.

[18] E. A. Hallem and J. R. Carlson, "Coding of odors by a receptor repertoire," *Cell*, vol. 125, no. 1, pp. 143–160, 2006.

[19] M. N. Andersson, M. C. Larsson, and F. Schlyter, "Specificity and redundancy in the olfactory system of the bark beetle *Ips typographus*: single-cell responses to ecologically relevant odors," *Journal of Insect Physiology*, vol. 55, no. 6, pp. 556–567, 2009.

[20] F. Schlyter, G. Birgersson, J. A. Byers, J. Löfqvist, and G. Bergström, "Field response of spruce bark beetle, *Ips typographus*, to aggregation pheromone candidates," *Journal of Chemical Ecology*, vol. 13, no. 4, pp. 701–716, 1987.

[21] M. Saint-Germain, C. M. Buddle, and P. Drapeau, "Primary attraction and random landing in host-selection by wood-feeding insects: a matter of scale?" *Agricultural and Forest Entomology*, vol. 9, no. 3, pp. 227–235, 2008.

[22] J. A. Byers, "An encounter rate model of bark beetle populations searching at random for susceptible host trees," *Ecological Modelling*, vol. 91, no. 1-3, pp. 57–66, 1996.

[23] F. Schlyter, G. Birgersson, and A. Leufvén, "Inhibition of attraction to aggregation pheromone by verbenone and ipsenol - Density regulation mechanisms in bark beetle *Ips typographus*," *Journal of Chemical Ecology*, vol. 15, no. 8, pp. 2263–2277, 1989.

[24] Q.-H. Zhang, F. Schlyter, and P. Anderson, "Green leaf volatiles interrupt pheromone response of spruce bark beetle, *Ips typographus*," *Journal of Chemical Ecology*, vol. 25, no. 12, pp. 2847–2861, 1999.

[25] Q.-H. Zhang, F. Schlyter, and G. Birgersson, "Bark volatiles from nonhost angiosperm trees of spruce bark beetle, *Ips typographus* (L.) (Coleoptera: Scolytidae): chemical and electrophysiological analysis," *Chemoecology*, vol. 10, no. 2, pp. 69–80, 2000.

[26] Q.-H. Zhang, T. Tolasch, F. Schlyter, and W. Francke, "Enantiospecific antennal response of bark beetles to spiroacetal (*E*)-conophthorin," *Journal of Chemical Ecology*, vol. 28, no. 9, pp. 1839–1852, 2002.

[27] Q.-H. Zhang and F. Schlyter, "Redundancy, synergism, and active inhibitory range of non-host volatiles in reducing pheromone attraction in European spruce bark beetle *Ips typographus*," *Oikos*, vol. 101, no. 2, pp. 299–310, 2003.

[28] B. Å. Tømmerås, "Specialization of the olfactory receptor cells in the bark beetle *Ips typographus* and its predator *Thanasimus formicarius* to bark beetle pheromones and host tree volatiles," *Journal of Comparative Physiology A*, vol. 157, no. 3, pp. 335–341, 1985.

[29] H. Mustaparta, B. Å. Tømmerås, P. Baeckström, J. M. Bakke, and G. Ohloff, "Ipsdienol-specific receptor cells in bark beetles: structure-activity relationships of various analogues and of deuterium-labelled ipsdienol," *Journal of Comparative Physiology A*, vol. 154, no. 4, pp. 591–595, 1984.

[30] B. Å. Tømmerås and H. Mustaparta, "Chemoreception of host volatiles in the bark beetle *Ips typographus*," *Journal of Comparative Physiology A*, vol. 161, no. 5, pp. 705–710, 1987.

[31] B. Å. Tømmerås, H. Mustaparta, and J. C. Gregoire, "Receptor cells in *Ips typographus* and *Dendroctonus micans* specific to pheromones of the reciprocal genus," *Journal of Chemical Ecology*, vol. 10, no. 5, pp. 759–770, 1984.

[32] H. Mustaparta, M. E. Angst, and G. N. Lanier, "Specialization of olfactory cells to insect-and host-produced volatiles in the bark beetle *Ips pini* (say)," *Journal of Chemical Ecology*, vol. 5, no. 1, pp. 109–123, 1979.

[33] H. Mustaparta, M. E. Angst, and G. N. Lanier, "Receptor discrimination of enantiomers of the aggregation pheromone ipsdienol, in two species of *Ips*," *Journal of Chemical Ecology*, vol. 6, no. 3, pp. 689–701, 1980.

[34] H. Mustaparta, M. E. Angst, and G. N. Lanier, "Responses of single receptor cells in the pine engraver beetle, *Ips pini* (SAY) (Coleoptera: Scolytidae) to its aggregation pheromone, ipsdienol, and the aggregation inhibitor, ipsenol," *Journal of Comparative Physiology. A*, vol. 121, no. 3, pp. 343–347, 1977.

[35] J. C. Dickens, T. L. Payne, L. C. Ryker, and J. A. Rudinsky, "Single cell responses of the Douglas-fir beetle, *Dendroctonus pseudotsugae* hopkins (Coleoptera: Scolytidae), to pheromones and host odors," *Journal of Chemical Ecology*, vol. 10, no. 4, pp. 583–600, 1984.

[36] J. C. Dickens, T. L. Payne, L. C. Ryker, and J. A. Rudinsky, "Multiple acceptors for pheromonal enantiomers on single olfactory cells in the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopk. (Coleoptera: Scolytidae)," *Journal of Chemical Ecology*, vol. 11, no. 10, pp. 1359–1370, 1985.

[37] T. L. Payne, J. V. Richerson, J. C. Dickens et al., "Southern pine beetle: olfactory receptor and behavior discrimination of enantiomers of the attractant pheromone frontalin," *Journal of Chemical Ecology*, vol. 8, no. 5, pp. 873–881, 1982.

[38] J. C. Dickens and T. L. Payne, "Bark beetle olfaction: pheromone receptor system in *Dendroctonus frontalis*," *Journal of Insect Physiology*, vol. 23, no. 4, pp. 481–489, 1977.

[39] B. Å. Tømmerås and H. Mustaparta, "Single cell responses to pheromones, host and non-host volatiles in the ambrosia beetle *Trypodendron lineatum*," *Entomologia Experimentalis et Applicata*, vol. 52, no. 2, pp. 141–148, 1989.

[40] A. Guerrero, J. Feixas, J. Pajares, L. J. Wadhams, J. A. Pickett, and C. M. Woodcock, "Semiochemically induced inhibition of behaviour of *Tomicus destruens* (Woll.) (Coleoptera: Scolytidae)," *Naturwissenschaften*, vol. 84, no. 4, pp. 155–157, 1997.

[41] E. Hallberg, "Sensory organs in *Ips typographus* (Insecta: Coleoptera)—Fine structure of antennal sensilla," *Protoplasma*, vol. 111, no. 3, pp. 206–214, 1982.

[42] T. C. Baker, S. A. Ochieng, A. A. Cossé et al., "A comparison of responses from olfactory receptor neurons of *Heliothis subflexa* and *Heliothis virescens* to components of their sex pheromone," *Journal of Comparative Physiology A*, vol. 190, no. 2, pp. 155–165, 2004.

[43] H. Ljungberg, P. Anderson, and B. S. Hansson, "Physiology and morphology of pheromone-specific sensilla on the antennae of male and female *Spodoptera littoralis* (Lepidoptera: Noctuidae)," *Journal of Insect Physiology*, vol. 39, no. 3, pp. 253–260, 1993.

[44] G. Birgersson, F. Schlyter, J. Löfqvist, and G. Bergström, "Quantitative variation of pheromone components in the spruce bark beetle *Ips typographus* from different attack phases," *Journal of Chemical Ecology*, vol. 10, no. 7, pp. 1029–1055, 1984.

[45] M. N. Andersson and F. Schlyter, "What reaches the antenna? How to calibrate odor flux and ligand-receptor affinities," *Chemical Senses*. In press.

[46] I. M. Wilson, J. H. Borden, R. Gries, and G. Gries, "Green leaf volatiles as antiaggregants for the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae)," *Journal of Chemical Ecology*, vol. 22, no. 10, pp. 1861–1875, 1996.

[47] Q.-H. Zhang, N. Erbilgin, and S. J. Seybold, "GC-EAD responses to semiochemicals by eight beetles in the subcortical community associated with Monterey pine trees in coastal California: similarities and disparities across three trophic levels," *Chemoecology*, vol. 18, no. 4, pp. 243–254, 2008.

[48] D. P. W. Huber, R. Gries, J. H. Borden, and H. D. Pierce, "A survey of antennal responses by five species of coniferophagous bark beetles (Coleoptera: Scolytidae) to bark volatiles of six species of angiosperm trees," *Chemoecology*, vol. 10, no. 3, pp. 103–113, 2000.

[49] J. A. Byers, "Avoidance of competition by spruce bark beetles, *Ips typographus* and *Pityogenes chalcographus*," *Experientia*, vol. 49, no. 3, pp. 272–275, 1993.

[50] B. S. Hansson, M. C. Larsson, and W. S. Leal, "Green leaf volatile-detecting olfactory receptor neurones display very high sensitivity and specificity in a scarab beetle," *Physiological Entomology*, vol. 24, no. 2, pp. 121–126, 1999.

[51] M. C. Larsson, W. S. Leal, and B. S. Hansson, "Olfactory receptor neurons detecting plant odours and male volatiles in *Anomala cuprea* beetles (Coleoptera: Scarabaeidae)," *Journal of Insect Physiology*, vol. 47, no. 9, pp. 1065–1076, 2001.

[52] H. Y. Fadamiro, A. A. Cossé, and T. C. Baker, "Fine-scale resolution of closely spaced pheromone and antagonist filaments by flying male *Helicoverpa zea*," *Journal of Comparative Physiology, A*, vol. 185, no. 2, pp. 131–141, 1999.

[53] T. J. A. Bruce, L. J. Wadhams, and C. M. Woodcock, "Insect host location: a volatile situation," *Trends in Plant Science*, vol. 10, no. 6, pp. 269–274, 2005.

[54] A. Vermeulen and J. P. Rospars, "Why are insect olfactory receptor neurons grouped into sensilla? The teachings of a model investigating the effects of the electrical interaction between neurons on the transepithelial potential and the neuronal transmembrane potential," *European Biophysics Journal*, vol. 33, no. 7, pp. 633–643, 2004.

[55] A. F. Silbering, R. Okada, K. Ito, and C. G. Galizia, "Olfactory information processing in the *Drosophila* antennal lobe: anything goes?" *Journal of Neuroscience*, vol. 28, no. 49, pp. 13075–13087, 2008.

[56] M. M. Blight, J. A. Pickett, L. J. Wadhams, and C. M. Woodcock, "Antennal perception of oilseed rape, *Brassica napus* (Brassicaceae), volatiles by the cabbage seed weevil *Ceutorhynchus assimilis* (Coleoptera, Curculionidae)," *Journal of Chemical Ecology*, vol. 21, no. 11, pp. 1649–1664, 1995.

[57] E. A. Hallem, M. G. Ho, and J. R. Carlson, "The molecular basis of odor coding in the *Drosophila antenna*," *Cell*, vol. 117, no. 7, pp. 965–979, 2004.

[58] J. E. Cometto-Muñiz, W. S. Cain, and M. H. Abraham, "Quantification of chemical vapors in chemosensory research," *Chemical Senses*, vol. 28, no. 6, pp. 467–477, 2003.

[59] T. Tsukatani, T. Miwa, M. Furukawa, and R. M. Costanzo, "Detection thresholds for phenyl ethyl alcohol using serial dilutions in different solvents," *Chemical Senses*, vol. 28, no. 1, pp. 25–32, 2003.

[60] R. S. Vetter, A. E. Sage, K. A. Justus, R. T. Cardé, and C. G. Galizia, "Temporal integrity of an airborne odor stimulus is greatly affected by physical aspects of the odor delivery system," *Chemical Senses*, vol. 31, no. 4, pp. 359–369, 2006.

[61] M. N. Andersson, *Olfaction in the spruce bark beetle,* Ips typographus*: receptor, neuron and habitat*, Ph.D. dissertation, Swedish University of Agricultural Sciences, Alnarp, Sweden, 2011.

[62] R. De Jong and J. H. Visser, "Integration of olfactory information in the Colorado potato beetle brain," *Brain Research*, vol. 447, no. 1, pp. 10–17, 1988.

[63] K. Hansen, "Discrimination and production of disparlure enantiomers by the gypsy moth and the nun moth," *Physiological Entomology*, vol. 9, pp. 9–18, 1984.

[64] G. P. Svensson and M. C. Larsson, "Enantiomeric specificity in a pheromone-kairomone system of two threatened saproxylic beetles, *Osmoderma eremita* and *Elater ferrugineus*," *Journal of Chemical Ecology*, vol. 34, no. 2, pp. 189–197, 2008.

[65] H. Wojtasek, B. S. Hansson, and W. S. Leal, "Attracted or repelled? A matter of two neurons, one pheromone binding protein, and a chiral center," *Biochemical and Biophysical Research Communications*, vol. 250, no. 2, pp. 217–222, 1998.

[66] W. M. Getz and R. P. Akers, "Honeybee olfactory sensilla behave as integrated processing units," *Behavioral and Neural Biology*, vol. 61, no. 2, pp. 191–195, 1994.

[67] H. Jactel and E. G. Brockerhoff, "Tree diversity reduces herbivory by forest insects," *Ecology Letters*, vol. 10, no. 9, pp. 835–848, 2007.

[68] H. Jactel, G. Birgersson, S. Andersson, and F. Schlyter, "Non-host volatiles mediate associational resistance to the pine processionary moth," *Oecologia*, vol. 166, no. 3, pp. 703–711, 2011.

[69] M. N. Andersson, M. Binyameen, M. M. Sadek, and F. Schlyter, "Attraction modulated by spacing of pheromone components and anti-attractants in a bark beetle and a moth," *Journal of Chemical Ecology*, vol. 37, no. 8, pp. 899–911, 2011.

[70] N. E. Gillette, J. D. Stein, D. R. Owen et al., "Verbenone-releasing flakes protect individual *Pinus contorta* trees from attack by *Dendroctonus ponderosae* and *Dendroctonus valens* (Coleoptera: Curculionidae, Scolytinae)," *Agricultural and Forest Entomology*, vol. 8, no. 3, pp. 243–251, 2006.

[71] J. A. Byers, "Interactions of pheromone component odor plumes of western pine beetle," *Journal of Chemical Ecology*, vol. 13, no. 12, pp. 2143–2157, 1987.

[72] B. T. Sullivan and K. Mori, "Spatial displacement of release point can enhance activity of an attractant pheromone synergist of a bark beetle," *Journal of Chemical Ecology*, vol. 35, no. 10, pp. 1222–1233, 2009.

[73] G. H. L. Rothschild, "Problems in defining synergists and inhibitors of the Oriental Fruit Moth pheromone by field experimentation," *Entomologia Experimentalis et Applicata*, vol. 17, no. 2, pp. 294–302, 1974.

[74] P. Witzgall and E. Priesner, "Wind-tunnel study on attraction inhibitor in male *Coleophora laricella* Hbn. (Lepidoptera: Coleophoridae)," *Journal of Chemical Ecology*, vol. 17, no. 7, pp. 1355–1362, 1991.

[75] M. Coracini, M. Bengtsson, L. Cichon, and P. Witzgall, "Codling moth males do not discriminate between pheromone and a pheromone/antagonist blend during upwind flight," *Naturwissenschaften*, vol. 90, no. 9, pp. 419–423, 2003.

[76] T. A. Christensen and J. G. Hildebrand, "Coincident stimulation with pheromone components improves temporal pattern resolution in central olfactory neurons," *Journal of Neurophysiology*, vol. 77, no. 2, pp. 775–781, 1997.

[77] R. Jakuš, F. Schlyter, Q.-H. Zhang et al., "Overview of development of an anti-attractant based technology for spruce protection against *Ips typographus*: from past failures to future success," *Journal of Pest Science*, vol. 76, no. 4, pp. 89–99, 2003.

[78] C. Schiebe, M. Blaženec, R. Jakuš, C. R. Unelius, and F. Schlyter, "Semiochemical diversity diverts bark beetle attacks from Norway spruce edges," *Journal of Applied Entomology*, vol. 135, no. 10, pp. 726–737, 2011.

[79] M. N. Andersson, J. M. Bengtsson, E. Grosse-Wilde et al., "Olfactory receptors in *Ips typographus*. Transcriptome from antenna analysed and preliminary compared to *Dendroctonus ponderosae* and *Tribolium castaneum* (Coleoptera: Curculionidae & Tenebrionidae)," in *Genetics of Bark Beetles and Associated Microorganisms*, F. Lakatos, B. Mészáros, and C. Stauffer, Eds., p. 15, Sopron, Hungary, 2011.

[80] N. Triballeau, E. van Name, G. Laslier et al., "High-potency olfactory receptor agonists discovered by virtual high-throughput screening: molecular probes for receptor structure and olfactory function," *Neuron*, vol. 60, no. 5, pp. 767–774, 2008.

[81] J. N. C. van der Pers and A. K. Minks, "Pheromone monitoring in the field using single sensillum recording," *Entomologia Experimentalis et Applicata*, vol. 68, no. 3, pp. 237–245, 1993.

Hindawi Publishing Corporation
Psyche
Volume 2012, Article ID 794683, 5 pages
doi:10.1155/2012/794683

Research Article

Attraction of *Tomicus yunnanensis* (Coleoptera: Scolytidae) to Yunnan Pine Logs with and without Periderm or Phloem: An Effective Monitoring Bait

Rong Chun Lu,[1,2] Hong Bin Wang,[1] Zhen Zhang,[1] John A. Byers,[3] You Ju Jin,[4] Hai Feng Wen,[2] and Wen Jian Shi[2]

[1] *Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry and The Key Laboratory of Forest Ecology and Environment, State Forestry Administration, Beijing 100091, China*
[2] *College of Urban Construction, University of Shanghai for Science and Technology, Shanghai 200093, China*
[3] *U.S. Arid-Land Agricultural Research Center, USDA-ARS, 21881 North Cardon Lane, Maricopa, AZ 85138, USA*
[4] *College of Plant Sciences, Beijing Forest University, Beijing 100083, China*

Correspondence should be addressed to Hong Bin Wang, wanghb@caf.ac.cn

Received 6 October 2011; Revised 13 December 2011; Accepted 13 December 2011

Academic Editor: Qing-He Zhang

The Yunnan pine shoot beetle, *Tomicus yunnanensis* Kirkendall and Faccoli (Coleoptera: Scolytinae) is an important pest of Yunnan pine (*Pinus yunnanensis* Franch) in China. Experiments with host log baits were done to develop a pest monitoring system using host tree kairomone. Five Yunnan pine logs (each 10–15 cm diam. × 30-cm long) in a trap-log bundle were treated by peeling periderm (outer bark) off to expose the phloem, and half of each log was covered with sticky adhesive to capture any attracted adult beetles. Significantly, more beetles were attracted and caught on the periderm-peeled logs (ca 30 beetles/m^2 log surface/day) than on untreated control logs with adhesive (ca 2.5/m^2/day). No significant differences were observed between catches on logs taken from lower or upper halves of Yunnan pines. *T. yunnanensis* flies mostly during the afternoon according to trap catches throughout the day. Attraction to the periderm-peeled logs decreased considerably when they were peeled further to remove the phloem, indicating phloem volatiles play a role in selection of the host by the beetle. The readily-available log baits appear useful for monitoring pine shoot beetle populations in integrated pest management programs.

1. Introduction

Tomicus yunnanensis (Coleoptera: Scolytinae) is a newly discovered and aggressive species of pine shoot beetle [1, 2]. It was confused with *T. piniperda* (L.) in the past [1–3]. Recent studies show there are clear morphological, genetic, and ecological differences between these two species [2–4]. Compared with *T. piniperda*, *T. yunnanensis* is more harmful because it not only causes great growth losses, but also kills healthy pines by mass attack [4–6]. It has caused extensive mortality of Yunnan pines, *Pinus yunnanensis* Franch, in Yunnan province of China [1, 7]. Since the 1980s, more than 200,000 ha Yunnan pine forests have been killed [3, 4, 7, 8].

T. yunnanensis attacks trunks during maturation feeding in Yunnan province [5, 7]. Trunk attacks last six to seven months from December to May, and peak attacks occur from January to March. Attacks on trunks begin in the crown and then spread down the bole. New adults fly to the shoots starting in March, the peak flight emerging in mid June. Maturation feeding lasts six to eight months [3, 5, 7, 8].

The extensive damage by *T. yunnanensis* indicates that as in other pine systems, effective monitoring and control are necessary [9–11]. However, there are no effective methods to manage the insect so far. It is reasonable to suspect there may be plant compounds from Yunnan pines that are attractive [7, 8, 12]. However, it is not known whether the beetles use a kairomone of Yunnan pines to locate hosts. The most popular method in the past has been to use trap log bundles to see if they are attractive to the beetles. This method is simple to try, but requires detection of odor signals (kairomone) from

host or associated organisms that can be difficult to observe with catches of beetles using many logs [8, 13, 14]. Our objective was to design experiments that tested whether Yunnan pines release an attractive kairomone from various periderm- and phloem-peeled bait logs that could serve as an effective method for monitoring and potential control of *T. yunnanensis*. The research should provide methods that avoid unnecessary work and improve bait log efficiency [15, 16].

2. Materials and Methods

2.1. Study Site Conditions. A series of experiments were conducted in a 300 ha plantation of Yunnan pines located on the mountain near Qujing city in Yunnan province (25°14′N, 103°50′, and 1700–1800 m above sea level). The field site mainly consisted of Yunnan pines, *Pinus yunnanensis* L., and a small proportion (<10%) of broad-leaved trees. Most of the Yunnan pines were infested with *T. yunnanensis*. The trees were 30–45 years old and ranged from 10–15 m in height and 10–15 cm in diameter.

2.2. Experimental Design. Two experiments were conducted from December to February in 2006 and 2007. In the first experiment, to avoid any sex pheromone effects, six healthy Yunnan pines were cut whose trunks were free of bark beetle attacks (no obvious beetle entrance holes). All the tree trunks were cut into 30-cm long logs. Among all the logs, 30 cut logs were randomly selected and the surface area of each log measured, then the periderm (outer bark) of the logs was peeled off leaving the phloem exposed (Figure 1(a)), and every five treated logs were placed in a trap log bundle. Another 30 cut logs were treated the same, but the phloem was also peeled from the logs (Figure 1(b)). The last 30 cut logs had only their surface area measured and were not peeled (Figure 1(c)). Half the surface area of each log (15 cm) was covered with sticky adhesive supplied by Hebei Academy of Forestry to catch any bark beetles that landed. Finally all the cut logs in the 18 bundles were randomly hung at about 1.5–1.7 m height in trees. The distance between one trap log bundle to another was about 100–120 m. In addition, six healthy Yunnan pines were randomly chosen and the trunk periderm was peeled off for 1.5 m of the trunk upward beginning 0.6 m above the ground, but the phloem was left in good condition, then half of the peeled trunk surface was covered with the adhesive every other 15-cm height interval (Figure 1(d)). All six trees remained standing. The distance between each of these trees was also about 100–120 m. The trap catches of the bark beetles were collected daily and species distinguished with a binocular microscope. This experiment was repeated monthly for three months (December to February in 2006 and 2007); with results of all months pooled to obtain a daily mean trap catch.

In the second study, four healthy Yunnan pines were cut down, each tree divided into two sections from the middle (lower half and upper half). Then a 1.5 m long log was cut from each section, and all the periderm peeled off with the phloem remain in good condition. Finally, each log was divided into five 30 cm long bolts and these were put in one trap log bundle; half the surface of each piece was covered with adhesive. The bundles from the top-half section and the bottom-half section were arranged in pairs (to give almost equal diameter) and hung up in trees at 1.5–1.7 m height. The distance between each trap log bundle was about 100–120 m. The experiment was replicated each of three months for two years as in the first experiment. The trap catches of the bark beetles were collected each day and species distinguished with a binocular microscope.

2.3. Data Presentation and Analyses. Data were checked for normality and presented as mean ± standard error. Variance analysis (ANOVA) was conducted with SPSS 11.5 (SPSS Inc., Chicago, IL, USA).

3. Results

The trapping efficiency of log bundles for *T. yunnanensis* was greatly affected by the log treatment method (Figure 2). Logs with the periderm peeled off and the phloem left were most attractive to *T. yunnanensis*, attracting roughly 30 *T. yunnanensis* per day per square meter log surface. There were significant differences between the trap catches of *T. yunnanensis* among the other log treatment methods ($P < 0.05$, Tukey's multiple comparison). The other two methods of treating the trap log bundles attracted much fewer *T. yunnanensis*. However, the logs with both periderm and phloem peeled off attracted slightly, but not significantly, more *T. yunnanensis* than the logs not peeled. The uncut pine trees attracted the fewest *T. yunnanensis*.

Dissection of the trunk (results not shown) indicated that the distribution of the entrance holes of *T. yunnanensis* is mainly in the mid and upper sections of Yunnan pine trees. Whether this pattern is affected by variations in a kairomone of Yunnan pine is unknown. To investigate this, logs from different parts of Yunnan pine trees were compared for attraction of *T. yunnanensis*. We found that logs from the upper half of Yunnan pine trees attracted a little more *T. yunnanensis* than sections from the lower half of the tree, but there was no significant difference ($P < 0.05$, Tukey's paired-samples test, Figure 3).

The trap catches of *T. yunnanensis* during the day show that *T. yunnanensis* was trapped in the afternoon, the peak capture rate occurred from 14:00 to 18:00, while a few were trapped from 7:00 to 14:00. The peak time of capture was strongly affected by weather conditions; occurring a little earlier or later, or not at all under poor weather conditions. Figure 4 shows results under good weather conditions.

4. Discussion

The periderm is important to trees; the outer bark slows the release of water and organic volatiles from inside the tree. Once logs were peeled, their volatiles were released in much larger amounts, allowing the peeled logs to attract more *T. yunnanensis* than the unpeeled ones. Yunnan pine bark is known to contain many different monoterpenes (primarily α-pinene and 3-carene) somewhat similar to Scots pine [17].

(a) (b) (c) (d)

Figure 1: (a) Trap log bundles with periderm peeled off. (b) Trap log bundles with periderm and phloem peeled off. (c) Intact (not peeled) trap log bundles. (d) Standing Yunnan pine tree with periderm peeled off and every other 15 cm section covered with sticky adhesive (Qujing, Yunnan, China).

These volatiles may comprise a kairomone of various monoterpenes that was shown attractive to another *Tomicus* species, *T. piniperda*, in Sweden that colonizes Scots pine [12]. Interestingly, the peeled logs that retained the phloem were much more attractive to *T. yunnanensis* than the logs peeled of both periderm and phloem. This is surprising since monoterpenes should exude from the xylem tissues and cause attraction. However, this was not the case. Observations during the experimental study revealed that all the beetle galleries were found in the phloem, which means the phloem is the main food of the beetle, as in most bark beetles [18, 19]. The results suggest the odors released from this part of the tree are used as the kairomone by the beetles when searching for food. The phloem is the tree's main transport channel of photosynthetic nutrition. Perhaps the energy from phloem is needed to continue releasing resin (and kairomone). In any case, the experimental results from the logs treated by peeling off the phloem shows that once the log has lost its phloem, its ability to attract beetles is greatly weakened.

Trunk attacks by *T. yunnanensis* are a major cause of tree mortality in Yunnan province [20]. It is reasonable to use kairomone of Yunnan pine trees to monitor and aid in control of the beetles. Natural kairomone would be expected to be as effective as the best synthetic blends that require much research to elucidate. As an alternative to synthetic baits, one of the most popular methods is to use trap log bundles to monitor population levels of beetles. This method is simple and uses readily available resources, but does require significant labor and many logs. We peeled logs to improve the quantities of volatile chemical compounds released, making it possible to use fewer logs and trap more bark beetles. In addition, the peeled logs make it easier to count beetles that

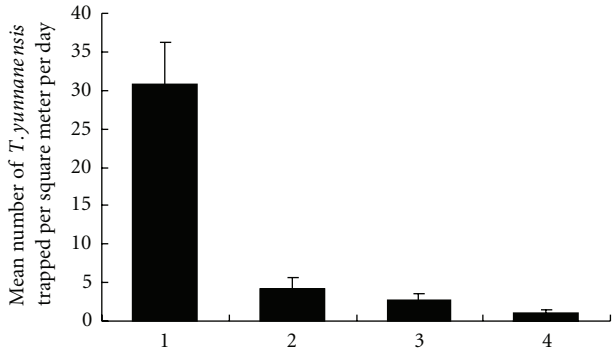


Figure 2: Daily mean trap catches (±SE, $N = 180$) of *T. yunnanensis* attracted to: (1) periderm peeled logs, (2) periderm and phloem peeled logs, (3) intact (unpeeled) logs, and (4) standing periderm-peeled Yunnan pines (Dec.–Feb. 2006, 2007; Qujing, Yunnan, China).

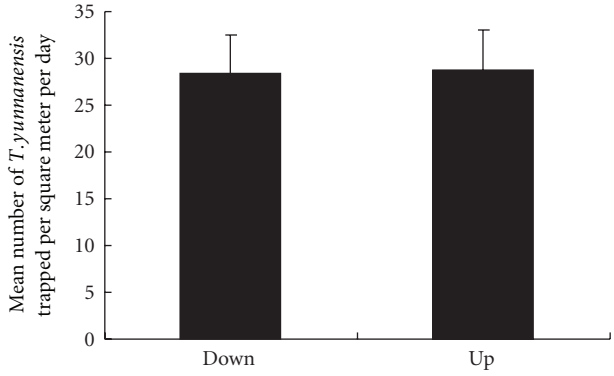


Figure 3: Daily mean trap catches (±SE, $N = 180$) of *T. yunnanensis* on logs from lower-half (Down) and upper-half (Up) sections of Yunnan pines (Dec.–Feb. 2006, 2007; Qujing, Yunnan, China).

are attracted and caught, especially when the surface of the peeled logs is covered with sticky adhesive. Our experimental results show the logs peeled of periderm (outer bark) trapped the most *T. yunnanensis*. While the standing trees peeled in the same way attracted the fewest bark beetles. The standing trees should have not only had constitutive defenses but also inducible defenses [21–24]. The periderm was the most important defensive factor that protected the trunks. When the periderm of trees was peeled off, the constitutive defenses lost effectiveness to some extent, but this damage activated the strong inducible defenses [23]. If the trees were cut down, they lost the inducible defenses. The periderm is important because it not only retards water loss from trunks, but also prevents volatiles from rapidly evaporating from the phloem's resin canals. When the periderm was peeled off, the release rate of volatiles likely increased greatly and enhanced attraction. Logs which were not peeled trapped only a few bark beetles as did the logs peeled to remove phloem. This may happen because most of the kairomone came from phloem, so when the phloem was lost, the attractive ability was reduced, indicating that the phloem was the most important source of the kairomone. However, whether the kairomone is synthesized in phloem is uncertain.

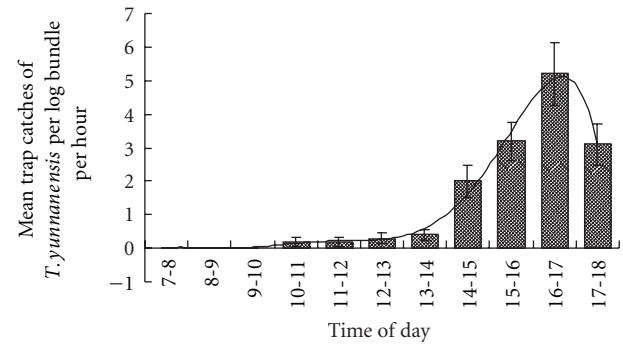


Figure 4: Mean trap catches (±SE) per hour of *T. yunnanensis* on periderm-peeled log bundles during the day (Dec.–Feb. 2006, 2007; Qujing, Yunnan, China).

Most *T. yunnanensis* were trapped in the afternoon; maybe because *T. yunnanensis* was more flight active in the afternoon. Consequently, the afternoon is the best time to study bark beetle behavior in regard to host selection and colonization. In nature, most of the *T. yunnanensis* attacks are found in the top section of the tree [25] but when we used different sections of the tree to attract and trap the beetle, there were no significant differences among the sections. Thus, there appears to be no obvious differences in kairomone release among the different sections from the lower and upper halves of the tree.

Here we present an efficient method to determine whether the kairomone of the host trees can be used to trap pest bark beetles, and whether it is possible to produce attractive baits from the host tree. Pine logs peeled of periderm as baits are easier and more effective compared with traditional log bait methods that use logs with intact bark.

Acknowledgments

The authors thank the Forest Pest Control Station of Qujing City, Yunnan province, for logistical support and field assistance. This study was funded by the Importing International Advanced Agricultural Science and Technology Research (2002-38) and the International Technological Cooperation Research (2006DFA31790).

References

[1] B. Långström, L. Lisha, L. Hongpin et al., "Shoot feeding ecology of *Tomicus piniperda* and *T. minor* (Col., Scolytidae) in southern China," *Journal of Applied Entomology*, vol. 126, no. 7-8, pp. 333–342, 2002.

[2] L. R. Kirkendall, M. Faccoli, and H. Ye, "Description of the Yunnan shoot borer, *Tomicus yunnanensis* Kirkendall & Faccoli sp. n. (Curculionidae, Scolytinae), an unusually aggressive pine shoot beetle from southern China, with a key to the species of *Tomicus*," *Zootaxa*, no. 1819, pp. 25–39, 2008.

[3] Y. Hui and D. Xue-Song, "Impacts of *Tomicus minor* on distribution and reproduction of *Tomicus piniperda* (Col., Scolytidae) on the trunk of the living Pinus yunnanensis trees," *Journal of Applied Entomology*, vol. 123, no. 6, pp. 329–333, 1999.

[4] Y. Duan, C. Kerdelhué, H. Ye, and F. Lieutier, "Genetic study of the forest pest *Tomicus piniperda* (Col., Scolytinae) in Yunnan province (China) compared to Europe: new insights for the systematics and evolution of the genus *Tomicus*," *Heredity*, vol. 93, no. 5, pp. 416–422, 2004.

[5] H. Ye, "Occurrence, distribution and damages of *Tomicus piniperda* in Yunnan, Southwestern China," *Journal of Yunnan University*, vol. 20, pp. 361–363, 1998.

[6] H. Wang, Y. Zhang, and L. Li, "Bionomics of *Tomicus piniperda* in Yunnan," *Forest Pest and Disease*, vol. 25, no. 4, pp. 21–24, 2006.

[7] J. H. Sun, S. R. Clarke, L. Kang, and H. B. Wang, "Field trials of potential attractants and inhibitors for pine shoot beetles in the Yunnan province, China," *Annals of Forest Science*, vol. 62, no. 1, pp. 9–12, 2005.

[8] T. Zhao, N. Zhou, L. Li et al., "*Tomicus piniperda* trapping in damaged *Pinus yunnanensis* stands by trap log and living tree," *Yunnan Forestry Science and Technology*, vol. 95, pp. 53–57, 2001.

[9] R. N. Conner, D. Saenz, D. C. Rudolph, and R. N. Coulson, "Southern pine beetle-induced mortality of pines with natural and artificial Red-cockaded Woodpecker cavities in Texas," *Wilson Bulletin*, vol. 110, no. 1, pp. 100–109, 1998.

[10] M. Dobbertin and A. Rigling, "Pine mistletoe (*Viscum album* ssp. *austriacum*) contributes to Scots pine (*Pinus sylvestris*) mortality in the Rhone valley of Switzerland," *Forest Pathology*, vol. 36, no. 5, pp. 309–322, 2006.

[11] P. Martikainen, J. Kouki, O. Heikkala, E. Hyvärinen, and H. Lappalainen, "Effects of green tree retention and prescribed burning on the crown damage caused by the pine shoot beetles (*Tomicus* spp.) in pine-dominated timber harvest areas," *Journal of Applied Entomology*, vol. 130, no. 1, pp. 37–44, 2006.

[12] J. A. Byers, B. S. Lanne, J. Löfqvist, F. Schlyter, and G. Bergström, "Olfactory recognition of host-tree susceptibility by pine shoot beetles," *Naturwissenschaften*, vol. 72, no. 6, pp. 324–326, 1985.

[13] E. Bois, F. Lieutier, and A. Yart, "Bioassays on *Leptographium wingfieldii*, a bark beetle associated fungus, with phenolic compounds of Scots pine phloem," *European Journal of Plant Pathology*, vol. 105, no. 1, pp. 51–60, 1999.

[14] U. Kohnle, "Host and non-host odour signals governing host selection by the pine shoot beetle, *Tomicus piniperda* and the spruce bark beetle, *Hylurgops palliatus* (Col., Scolytidae)," *Journal of Applied Entomology*, vol. 128, no. 9-10, pp. 588–592, 2004.

[15] C. Chararas, C. Revolon, M. Feinberg, and C. Ducauze, "Preference of certain scolytidae for different conifers—a statistical approach," *Journal of Chemical Ecology*, vol. 8, no. 8, pp. 1093–1109, 1982.

[16] J. A. Byers, "Attraction of bark beetles, *Tomicus piniperda*, *Hylurgops palliatus*, and *Trypodendron domesticum* and other insects to short-chain alcohols and monoterpenes," *Journal of Chemical Ecology*, vol. 18, no. 12, pp. 2385–2402, 1992.

[17] A. K. Borg-Karlson, M. Persson, Å. Christiansson et al., "Relative amounts and enantiomeric composition of monoterpene hydrocarbons in *Pinus yunnanensis* Fr. and *Pinus sylvestris* L.," in *Physiology and Genetics of Tree-Phytophage Interactions*, vol. 90 of *Les Colloques*, pp. 101–110, INRA, 1999.

[18] J. A. Byers, "Chemical ecology of bark beetles," *Experientia*, vol. 45, no. 3, pp. 271–283, 1989.

[19] J. A. Byers and Q. H. Zhang, "Chemical ecology of bark beetles in regard to search and selection of host trees," in *Recent Advances in Entomological Research*, T.-X. Liu and L. Kang, Eds., pp. 88–111, The Higher Education Press, Beijing, China, 2010.

[20] H. Ye, "On the bionomy of *Tomicus piniperda* L. (Coleoptera: Scolytidae) in the Kunming region of China," *Journal of Applied Entomology*, vol. 112, no. 1–5, pp. 366–369, 1991.

[21] B. Langstrom, C. Hellqvist, A. Ericsson, and R. Gref, "Induced defence reaction in Scots pine following stem attacks by *Tomicus piniperda*," *Ecography*, vol. 15, no. 3, pp. 318–327, 1992.

[22] B. Långström, H. Solheim, C. Hellqvist, and P. Krokene, "Host resistance in defoliated pine: effects of single and mass inoculations using bark beetle-associated blue-stain fungi," *Agricultural and Forest Entomology*, vol. 3, no. 3, pp. 211–216, 2001.

[23] D. Wainhouse, D. R. Rose, and A. J. Peace, "The influence of preformed defences on the dynamic wound response in Spruce bark," *Functional Ecology*, vol. 11, no. 5, pp. 564–572, 1997.

[24] V. R. Franceschi, P. Krokene, E. Christiansen, and T. Krekling, "Anatomical and chemical defenses of conifer bark against bark beetles and other pests," *New Phytologist*, vol. 167, no. 2, pp. 353–376, 2005.

[25] R. C. Lu, H. B. Wang, Z. Zhang et al., "Coexistence and competition between *Tomicus yunnanensis* and *T. minor* (Coleoptera: Scolytinae) in Yunnan pine," *Psyche*, vol. 2012, Article ID 185312, 6 pages, 2012.

Hindawi Publishing Corporation
Psyche
Volume 2012, Article ID 590619, 9 pages
doi:10.1155/2012/590619

Review Article

Hylastes ater (Curculionidae: Scolytinae) Affecting *Pinus radiata* Seedling Establishment in New Zealand

Stephen D. Reay,[1] Travis R. Glare,[2] and Michael Brownbridge[3]

[1] *Silver Bullet Forest Research, P.O. Box 56-491, Dominion Rd, Auckland, New Zealand*
[2] *Bio-Protection Research Centre, Lincoln University, P.O. Box 84, Lincoln 7647, New Zealand*
[3] *Vineland Research and Innovation Centre, 4890 Victoria Avenue N., P.O. Box 4000, Vineland Station, ON, Canada L0R 2E0*

Correspondence should be addressed to Travis R. Glare, travis.glare@lincoln.ac.nz

Received 5 August 2011; Revised 20 November 2011; Accepted 22 December 2011

Academic Editor: John A. Byers

The introduced pine bark beetle *Hylastes ater* has been present in New Zealand for around 100 years. The beetle has been a minor pest on pines. Research was undertaken to control the pest in the 1950s–1970s, with a biological control agent introduced with limited success. Following a reasonably long period with minimal research attention, renewed interest in developing a better understanding of the pest status was initiated in the mid to late 1990s. Subsequently, a significant amount of research was undertaken, with a number of studies exploring the role of this pest of exotic forests in New Zealand. These studies ranged from attempting to quantify damage to seedlings, evaluate the role of the beetle in vectoring sapstain fungi, explore options for management, and evaluate the potential for chemical and biological control. From these studies, a number of findings were made that are relevant to the New Zealand exotic forest industry and shed new light onto the role of secondary bark beetles globally.

1. Introduction

The introduced pine bark beetle, *Hylastes ater* (Paykull) (Curculionidae: Scolytinae), is a pest of reestablished *Pinus radiata* D. Don forests in New Zealand. First recorded in New Zealand in 1929 [1], it has become a problem in second and third rotation forests where it breeds under the bark of stumps and other similar logging waste (log sections). Both adults and larvae feed on the phloem. Adults lay eggs in galleries, and larvae may take up to 300 days to develop to maturity. Subsequent emergence of adults from stumps is not necessarily immediate, and some adults continue to feed for longer periods [2]. Emerging adults feed on seedlings that have been planted in the immediate area. This maturation feeding characteristically involves the adult beetle eating the bark around the root collar of a seedling below the ground. In severe cases, seedlings may be completely ring barked and will die. Beetle feeding also commonly causes considerable sublethal damage, and feeding wounds may serve as a point of entry for soil-borne pathogens.

Despite initial concerns, historically *H. ater* was not regarded as a significant forest establishment pest in New Zealand. More recently, surveys have indicated that attacks on *P. radiata* seedlings by *H. ater* may be more common than previously documented and not evenly distributed across forest estates [3]. *Hylastes ater* usually attacks seedlings within the first year after planting [3]. Consequently, mortality surveys that are undertaken much later may fail to detect dead seedlings that are difficult to see, or death is attributed to other causes. In cases where dead seedlings are observed, they must be removed from the soil for inspection around the root collar region to confirm feeding damage by *H. ater* as a potential cause of mortality. Forest establishment practices currently focus on lowering initial stocking rates and planting higher quality (and more expensive) seedling material. This means that low amounts of damage by *H. ater* may be more significant to forest establishment operations than previously experienced [3].

2. Damage of Pine Seedlings by *H. ater*

In New Zealand, large areas of mature *P. radiata* forest are harvested all year round. The resulting stumps create a continuous supply of breeding habitat allowing *H. ater* and/or

other beetle populations to continuously persist at epidemic levels compared with a natural forest environment [4, 5]. Adults emerge from stumps following larval development and may begin maturation feeding on seedlings that were planted following harvesting operations [3].

In New Zealand, *H. ater* does not build up high populations in all areas. Surveys where live and dead seedlings were destructively sampled in 60 compartments in the central North Island showed that seedling mortality due to severe *H. ater* damage in most compartments was less than 5% [3]. However, seedling mortality was occasionally higher (up to 30%). These surveys revealed relatively few dead seedlings without evidence of severe feeding damage (i.e., root collar region completely ring barked), suggesting that seedling mortality due to severe *H. ater* damage was more likely than other factors (e.g., drought, poor planting). These other factors may have contributed to seedling death. There was evidence of some seedling attack by *H. ater* in most compartments. Sublethal damage by *H. ater* was identified by destructively sampling live seedlings along transects and was observed to be greater than 30% in half of all the compartments sampled over the two-year period (and was over 80% in some areas) [3]. Seedlings were occasionally found to have survived severe attacks (multiple feeding attempts or complete girdling of the stem) by *H. ater*. When such seedlings were inspected at a later date (i.e., one year after damage had occurred), they were found to be alive and "growing well", suggesting that if mortality did not occur subsequent to the *H. ater* feeding event, recovery was likely. Overall, this study suggested that considerable amounts of feeding damage might have previously been undetected as surveys were undertaken to identify areas of seedling mortality, and dead trees were often not removed from the ground for inspection. It is unlikely that in such surveys live trees were destructively removed.

The time trees were harvested was identified to be an important factor in determining whether seedlings are likely to be attacked by *H. ater* [3]. Sites harvested during autumn and planted the following winter were at the greatest risk, and the risk of damage decreased with increasing time between harvesting and planting. Sites harvested prior to spring and planted the following winter were seldom found to contain seedlings that were attacked by *H. ater*.

The relationship between harvesting history and the likelihood of seedling damage was related to the life history of *H. ater*, including flight activity and competition for brood sites by other bark beetles. *Hylurgus ligniperda* (Fabricius) (Curculionidae: Scolytinae) was found to be the dominant species in stumps during summer months [6]. *Hylurgus ligniperda* was first discovered in New Zealand in 1974 and, like *H. ater,* breeds in the stumps and logs of *Pinus* spp., and is found throughout New Zealand, but is not a threat to seedlings [7, 8]. Sites harvested during spring to late summer were colonised predominantly by *H. ligniperda* suggesting that this species is able to outcompete *H. ater* during this period. While *H. ater* has a spring flight, sites harvested in late summer-autumn contained the largest populations of *H. ater,* relative to *H. ligniperda.* The subsequent *H. ater* populations resulting from these autumnal colonisation events emerge during the following late spring/summer and attack seedlings [6].

Experimentation in forest establishment practices by some forestry companies in New Zealand resulted in areas being replanted outside of the traditional winter replanting times using containerised tree stocks. Essentially, this means the planting season was extended so re-establishment could, in theory, occur year round. In reality, planting was not undertaken during the driest months of summer. The implications of this replanting approach, with regard to seedling damage by *H. ater,* was not fully assessed, but, in theory, trees could be planted immediately (within a month) following harvesting. While harvested land was traditionally left "fallow" for extended periods to allow weeds to germinate and be controlled, this practice was being challenged by at least one forestry company during the early 2000s in an attempt to minimise the period between harvesting events. Consequently, areas previously considered of low risk due to the emergence of *H. ater* populations before planting occurred may be more at risk if populations of *H. ater* larvae are present in stumps when seedlings are planted.

3. The Relationship between *H. ater* and Sapstain Fungi

Bark beetles are known worldwide as vectors of fungi, largely due to interactions between aggressive bark beetles and fungi. These fungi were thought to play an important role in the tree killing by bark beetles (e.g., members of the genus *Dendroctonus*) [9–13]. Six and Wingfield [13] have recently challenged this view and have presented several arguments against a pathogenic role by these fungi. Firstly, tree-killing bark beetles do kill trees in the absence of virulent pathogens. Secondly, the growth of fungi follows beetles colonisation and is relatively slow until colonisation by beetles has resulted in tree heath deteriorating beyond the point where the tree might survive. Thirdly, virulent fungi are found to be associated with bark beetles that do not typically kill trees, and many tree-killing beetles carry weak or nonpathogenic fungi. Finally, most bark beetles do not kill trees and still carry fungi similar fungi to their tree killing relatives, which indicates that fungi play important roles other than killing trees. Instead, Six and Wingfield [13] suggest that fungal phytopathogenicity may be important for fungi that exhibit this characteristic to compete with other fungi and/or survive in living trees.

Staining fungi are a significant economic concern to the *P. radiata* forest industry [14–17], due to the high susceptibility of *P. radiata* wood to staining [18]. While some saprophytic, pathogenic, and endophytic fungi cause sapstain in wood, it is generally the saprophytic fungi that invade timber after the tree has been harvested [19]. The staining effect only becomes evident when conditions are favourable for fungal growth. In New Zealand, this is normally after harvesting when the sapwood dries and the aerobic sapstain fungi are able to grow in the wood cells. In some instances, wood may have to be discarded prior to processing [15, 19].

Sapstaining fungi are commonly recorded from less aggressive bark beetles. In particular, the fungal species of *Leptographium*, *Graphium,* and *Ophiostoma* have been found on *H. ater* in Britain, South Africa, and Australia [18, 20, 21]. In New Zealand, *Leptographium* sp., *L. lundbergii* Largerburg and Melin, and *Ophiostoma ips* (Rumb.) Nannf. were isolated from *H. ater* [22–24]. The presence of *L. procerum* (Kendrick) Wingfield and *O. huntii* (Robins-Jeff.) DeHoog and Scheffe in New Zealand is likely to be due to its introduction with either *H. ater* or *H. ligniperda* [18, 25]. *Ophiostoma ips* is commonly associated with bark beetles [26–29]. Species of *Hylastes* are known vectors of fungal root diseases in other parts of the world [30–32]. In these cases, *Hylastes* adults attack the roots of stressed or diseased adult trees and vector root disease fungi [30–32].

Reay et al. [24, 34] described a strong relationship between the sublethal attack of *P. radiata* seedlings by *H. ater* and invasion by sapstain fungi. The presence of sapstain fungi was found to increase as severity of damage increased. Half of severely attacked seedlings were found to contain sapstain fungi, indicating the potential for large numbers of seedlings throughout forests to be infected [24, 34]. The sapstain fungi were from the Ophiostomataceae [15, 29]. Most frequently isolated were *O. huntii* and *O. galeiforme* (Bakshi) Math-Käärik [24, 34]. *Ophiostoma huntii* has been isolated from many parts of the world [25] and has been associated with several species of bark beetles, including *H. ater* [25, 26] and *H. porculus* Erichson, and may be an important species in red pine decline [10]. *Ophiostoma galeiforme* is a European species, which has been found with *Hylurgops palliatus* (Gyllehan) on larch in Scotland [35, 36] and *Hylastes cunicularius* (Erichson) in Sweden [37]. Mathiesen-Käärik [37] describes *O. galeiformis* as a "secondary" staining fungus. *Ophiostoma galeiforme* may have been introduced into New Zealand with *H. ater* [24]. The remaining sapstain species isolated from seedlings by Reay et al. [24, 34] are commonly found in New Zealand pine plantations [15, 17].

Fortunately, the fate of fungi following feeding damage appears to be limited. When areas of damaged seedlings were revisited three years following planting, Reay et al. [38] failed to isolate any sapstain fungi species from the previously damaged trees that were sampled. However, *Sphaeropsis sapinea* (Fr.) Dyko and B. Sutton (which was not isolated from seedlings in initial sampling following seedling damage) were isolated from 10–16% of seedlings at the three-year after beetle attack sampling. *Sphaeropsis sapinea* is an important opportunistic fungal pathogen of *P. radiata* (and other conifers) in New Zealand. While there was a possibility that colonisation by the bark beetle vectored fungi may have had some influence on the health, growth, and long-term fate of the trees, this was not investigated [38].

Hylastes ater has been suggested as the mechanism by which a number of species of fungi have been introduced into New Zealand. Therefore, it is possible that future introductions of *H. ater* (or other bark beetles) may establish new fungal species (or other organisms). If new fungal pathogens were introduced into New Zealand by other means, there is potential for *H. ater* to vector these throughout forests. Therefore, continued treatment of bark beetles as biosecurity threats to New Zealand is imperative, despite the establishment of several species.

4. Molecular Characterisation of *Hylastes ater* and Associated Species

Hylastes ater is currently the only example of the genus found in New Zealand, but other Coleoptera can colonise similar environments. *Hylurgus ligniperda* is found under the bark of pine stumps, often with *H. ater.* Another bark beetle beetle, *Pachycotes peregrinus,* (Chapuis) (Scolytinae) and a native pinhole borer, *Platypus apicalis* White (Platypodinae) are also found in pine stumps. *Hylastes ater* may be confused with *P. peregrinus* [2] by inexperienced forest management personnel and is morphologically similar to closely related European species, such as *H. brunneus* Erichson. As biosecurity incursions are a constant threat to New Zealand exotic plantation forestry, identification of new occurrences of bark beetles is important. Larval stages are difficult to identify with morphological characteristics, so we investigated molecular identification of available species. This preliminary data is not intended as a full phylogenetic analysis, but rather to provide, through GenBank, reference sequences for each species for future researchers.

4.1. Methods. A number of individuals of *H. ater*, *H. ligniperda*, *P. peregrinus, Treptoplatypus caviceps* (Broun) (Platypodinae), *Platypus apicalis,* and *P. gracilis* Broun (Platypodinae) were collected from various sites throughout New Zealand (Table 1). In addition, specimens of *H. brunneus*, *H. cunicularius*, *Hylobius abietis* L. (Molytinae), and *Austroplatypus incompertus* (Schedl) (Platypodinae) were obtained from outside New Zealand and were included (Table 1). Sampling and collection of beetles were not systematic. Using DNA extracted from the heads, elytra, and legs (DNeasy Plant Kit, Qiagen), PCR was used to amplify the terminal region of the 28S rRNA domain 2 region was performed using the primers 28S-F (5′-AGAGAGAGTTCAAGAGTACGTG-3′) and 28S-R (5′-TTGGTCCGTGTTTCAAGACGGG-3′) [39]. Amplifications were carried out using 30 cycles of 15 sec at 98°C, 30 sec at 48°C, 40 sec at 72°C. PCR products were cleaned using an Eppendorf Perfect Prep Gel Cleanup Kit and sequenced directly (AWCGS Sequencing Facility, Massey University, New Zealand). Resulting sequences were aligned and compared using Bayesian inference (Figure 1). Sequences were aligned using ClustalX [40]. Phylogenetic analysis using Bayesian inference was conducted using MRBAYES version 3.1.2 [41, 42]. Models of nucleotide substitution were selected using the Akaike Information Criterion (see [43]) in MrModelTest v2 [44] implemented in PAUP*4.0b10 [45]. The model selected was GTR + G, which is a general time reversible model [46, 47] with a gamma-shaped rate variation across sites and a proportion of invariable sites. Two runs of four chains saving trees every 100 generations were conducted. After 1,000,000 generations, the two runs had converged close to the same value (determined by when

Table 1: Beetle isolates and GenBank sequence data used in this study.

Isolate	Species	Location, date, collected by	GenBank number
Hylg 1	*Hylurgus ligniperda*	Auckland, NZ. 2007 Reay	JN544556
Hylg2	*Hylurgus ligniperda*	Auckland, NZ. 2007 Reay	JN544555
Hylg3	*Hylurgus ligniperda*	Auckland, NZ. 2007 Reay	JN544554
Hyls96	*Hylastes ater*	Canterbury, 2004 Reay	JN544548
Hyls99	*Hylastes ater*	New South Wales, Australia 2004 Reay, D Kent	JN544549
UK1	*Hylastes brunneus*	Galway, Ireland 2005 Reay, Walsh	JN544550
UK12	*Hylastes brunneus*	Galway, Ireland 2005 Reay, Walsh	JN544551
UK8	*Hylastes cunicularius*	Northumberland, England 2005 Reay, Glare	JN544552
UK9	*Hylastes cunicularius*	Northumberland, England 2005 Reay, Glare	JN544553
UK6	*Hylobius abietis*	Galway, Ireland 2005 Reay, Walsh	JN544547
Pla90	*Austroplatypus incompertus*	New South Wales, Australia 2004 D Kent	JN544546
Platy1	*Platypus apicalis*	Auckland, NZ. 2007 Reay	JN544557
Platy2	*Platypus apicalis*	Auckland, NZ. 2007 Reay	JN544558
Platy3	*Platypus apicalis*	Auckland, NZ. 2007 Reay	JN544559
Platy4	*Platypus gracilis*	Canterbury, NZ. 2007 Reay	JN544561
Platy5	*Platypus gracilis*	Canterbury, NZ. 2007 Reay	JN544562
Platy6	*Platypus gracilis*	Canterbury, NZ. 2007 Reay	JN544563
Platy7	*Treptoplatypus caviceps*	Canterbury, NZ. 2007 Reay	JN544568
Platy8	*Treptoplatypus caviceps*	Canterbury, NZ. 2007 Reay	JN544569
Platy10	*Platypus gracilis*	Westland, NZ. 2007 Reay	JN544564
Platy20	*Treptoplatypus caviceps*	Westland, NZ. 2007 Reay	JN544570
Pla47	*Platypus gracilis*	Canterbury, NZ. 2007 Reay	JN544560
Pac6	*Pacyhcotes perigrinius*	Dunedin,NZ. 2002 S Reay	JN544566
Pac7	*Pacyhcotes perigrinius*	Tokoroa, NZ. 2004 Reay	JN544567
Pac8	*Pacyhcotes perigrinius*	Tokoroa, NZ. 2004 Reay	JN544565

the standard deviation of split frequencies fell below 0.005) and the first 25% of trees were discarded as burn-in. The consensus tree, with the posterior probabilities for each split and mean branch lengths, was visualised using Treeview 1.6.6 [48].

4.2. Molecular Identification Using 28S rRNA. Using this short sequence of the 28S rRNA, it was possible to distinguish between all species (Figure 1). As stated above, this is not a phylogenetic study and Figure 1 is provided simply for visual reference of the separations seen between species using this DNA segment. Clear separation was achieved between Platypodinae and Scolytinae, as would be expected, but also between the species of Scolytinae. The three *Hylastes* species were separated into a group with the related species, *H. ligniperda*.

The results of this analysis show the potential for the 28S region of RNA to be used for the identification of Curculionidae and may be a useful biosecurity tool, particularly if larvae of the Curculionidae are intercepted at the border.

5. Mitigating Impacts of *H. ater*

Early efforts to reduce the impact of *H. ater* in New Zealand included importation and release of three species of predatory *Rhizophagus* beetles, as no native predators were known [2]. These were originally imported in 1933 from Britain and released but did not establish. Further importations and release from Europe of natural enemies were made in 1975 and 1976. A parasitic wasp, *Rhopalicus tutele* (Walker) (Pteromalidae), and the predatory beetle, *Thanasimus formicarius* L. (Cleridae), were released but had little impact.

Following the work of Reay and Walsh [3, 49], management practices that could reduce likelihood of attack were recommended. As discussed above, high-risk sites could be planted later in the season in spring/early summer (rather than during winter) when late instar larvae are present allowing little time for seedlings to establish and grow prior to beetle emergence and may result in seedlings being more vulnerable to damage.

In New Zealand, chemical insecticides are rarely used in plantation areas to control *H. ater*. A carbosulfan insecticide was shown to protect seedlings from damage by *H. ater* but is not currently in operational use [49].

6. The Potential Role of Biocontrol of *Hylastes ater* Using Insect-Pathogenic Fungi

Currently, site management is the only economically viable option for minimising impacts to regenerative plantings due to *H. ater* damage in commercial operations. This results

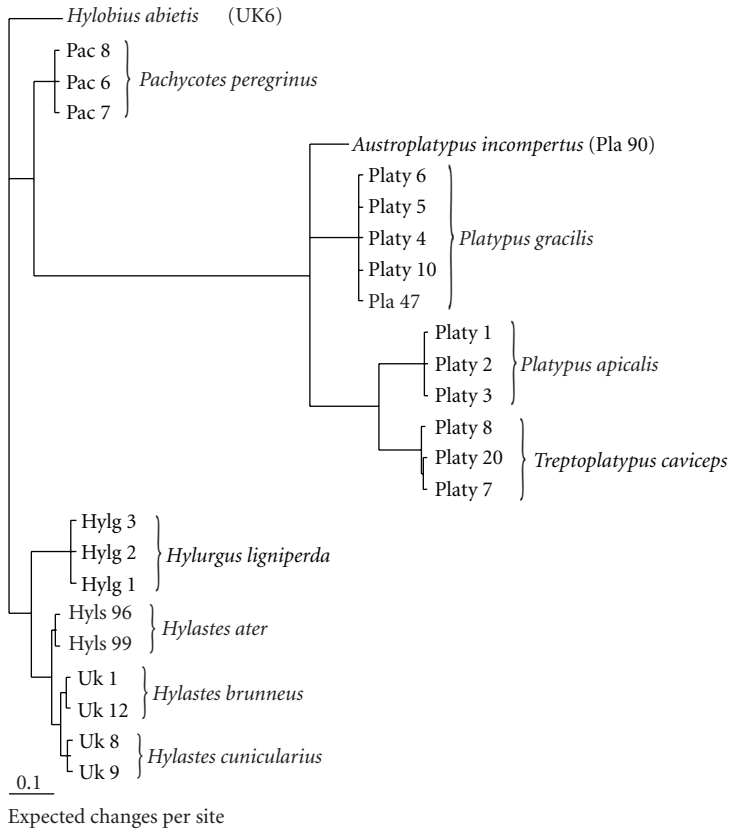


Figure 1: Representation of the species divergence using comparison of partial 28S rDNA sequences.

in land out of production and open to colonisation by weeds and erosion for a significant period. In addition, the increased use of containerised cuttings (in addition to bare root stock) has meant that planting seasons are extended, resulting in more sites at higher risk from *H. ater*. Alternative management options that would protect and promote the overall health and establishment of pine seedlings while reducing pest threat would benefit the forest industry. Moreover, improved control options are needed for use against any new species incursions. Biosecurity incursions are a constant threat to New Zealand plantation forests.

Entomopathogenic microbes have been developed as commercially available biopesticides for some pests. For example, the bacterium *Bacillus thuringiensis* Berl. has been used as the active agent in numerous biopesticides used in forestry for control of lepidopteron pests. Over a number of years, we have been investigating entomopathogens of *H. ater* in New Zealand and the potential for developing biopesticides.

Entomopathogenic fungi are important mortality factors in bark beetle populations, although the natural infection rate and impact on beetle populations is estimated to be relatively low [50]. Fungi in the genus *Beauveria* (Balsamo) Vuillemin are the most common species reported attacking bark beetles [51]. This genus contains a number of species, all of which are pathogenic to arthropods, including insects and Acari [52, 53], and occupy diverse habitats above and below ground [54–56].

Beauveria caledonica Bissett and Widden was isolated from *H. ater* and *H. ligniperda* in New Zealand and subsequently shown to be pathogenic to these two species in laboratory bioassays [57]. Previous to this, *B. caledonica* was not known to be pathogenic to insects. In the UK and Ireland, *B. caledonica* was isolated by concentrating on the major forestry pest, the large pine weevil, *H. abietis* [57]. *Hylobius abietis* is a serious pest of spruce and pine plantation trees, with an average of 33% and up to 100% of new plantings being killed per annum when untreated in some regions [58].

A survey of *Beauveria* spp. in substrates (soil, stumps, bark and grass from insect galleries) associated with bark beetles in *P. radiata* cutover forests was undertaken to identify what fungal isolates might be present in these forest systems [59]. *Beauveria* spp. were commonly isolated from all substrates sampled and were recovered from all but one of the six sites surveyed. However, there was considerable variation within and between sites in the relative prevalence of the fungi across all substrates and within substrate types, and three species of fungi were isolated (*B. caledonica*, *B. bassiana* (Balsamo) Vuillemin and *B. malawiensis* Rehner and Aquino de Muro) [59]. *Beauveria caledonica* was isolated from all substrates in this study, including beetle and larval cadavers. It was not isolated from live insects [59]. In total,

13 *Beauveria* isolates representing the three species recovered were selected and found to be pathogenic to both *H. ater* and *H. ligniperda* in laboratory bioassays [59]. Thus, in spite of the lack of *B. bassiana*-infected cadavers recovered in the field, the fungus is clearly able to infect and kill both bark beetle species and has been demonstrated for other bark beetles in the laboratory [60–65]. However, no epizootics have been reported in field populations. This may simply be due to natural inoculum levels being too low to initiate an epizootic, or due to inhibition of the fungi in the field. *Hylastes ater* was found to be less susceptible to all of the isolates tested than *H. ligniperda*, although the reasons for this are unclear. However, it is likely to have implications for any control programme using fungal entomopathogenic fungi.

While entomopathogenic fungi from *Beauveria* are predominant, fungi from other genera have been recovered from *H. ater* cadavers [66]. These include *Metarhizium flavoviride* var. *pemphigi* Driver and Milner and *Hirsutella guignardii* (Maheu) Samson, Rombach and Seifert. While some *Metarhizium anisopliae* (Metsch.) Sorokin. isolates are known to be pathogenic to bark beetles in laboratory bioassays, *Metarhizium* spp. do not appear to have been previously isolated from field-collected specimens [51]. Similarly for *H. guignardii*, this record is therefore not only a first for *H. ater* in New Zealand but may be the first record from a bark beetle [66]. Recovery of the fungi from the cuticles of bark beetle adults clearly demonstrates the capacity for insect-mediated movement of the fungi in a pine forest [59]. The recent research into entomopathogenic fungi represents recent attempts to obtain new ways to mitigating the impacts of *H. ater* in New Zealand. Differences in the natural prevalence of different species suggests that some isolates may be better suited as biocontrol agents as they persist in the environment better, while the high levels of inoculum detected in frass indicate that virulent, environmentally competent isolates must be selected and formulation and application technologies to efficiently target specific stages of the pest developed to effectively utilize these pathogens in bark beetle management.

The use of entomopathogenic fungi as biopesticides has been considered (e.g., [59]), but our estimates of production costs of *Beauveria* spp. could be prohibitively expensive for broadcast application against an occasional pest in pine plantations. This, coupled with the difficulty of applying fungi to larvae and adults in cryptic habitats, makes use of biopesticides unlikely without a major application development. Interestingly, further investigation of entomopathogenic fungi in New Zealand pine plantations found that *B. bassiana* also exists as an endophyte in some trees. A survey by Reay et al. [67] found that *B. bassiana* could be recovered from needle samples, as well as roots and seed from approximately 15% of 125 trees sampled over the country. Further research has demonstrated that the fungus can be established as an endophyte in seedlings M. Brownbridge et al. (then pers comm). *Beauveria bassiana* has been found as an endophyte of a number of plant species around the world, and the presence of the fungus has been shown to impact feeding in some insects [68]. The fungus, as an endophyte, may also offer protection against phytopathogens [69]. We are currently researching the potential of endophytic *Beauveria* in New Zealand pines as a method to reduce bark beetle populations. Endophytic entomopathogenic fungi would provide cost-effective methods to inoculate trees against bark beetles as we have shown seedlings can be infected with the fungus and *Beauveria* is carried in seed [67].

7. Conclusion

Hylastes ater may not have been considered an important pest of pine plantations in New Zealand during the early years of establishment in New Zealand, but more recently has been acknowledged as a pest of new second-generation plantings.

Investigation of biological control options has included predators, parasites, and entomopathogenic microbes. No introduced predator or parasite has yet had an impact on the populations of the beetles. Entomopathogenic fungi, especially *Beauveria* spp., are common in *H. ater* populations in New Zealand, but development as biopesticides is unlikely to be successful for *H. ater* due to the cost of any product and application to the cryptic environments being difficult. The discovery that the fungi can exist in pines as endophytes may, however, hold some promise for a cheaper method to use the entomopathogenic fungi in *H. ater* control.

Acknowledgments

The authors would like to thank Dr. Cor Vink (AgResearch) for instruction in running Mr. Bayes, Nicola Richards for support with DNA extractions, Dr. Debbie Kent and Dr. Patrick Walsh for help with collection of beetle specimens, and Tracey Nelson.

References

[1] A. F. Clark, "The pine-bark beetle, *Hylastes ater*, in New Zealand," *New Zealand Journal of Science and Technology*, vol. 14, pp. 1–20, 1932.

[2] R. H. Milligan, "*Hylastes ater* (Paykull) (Coleoptera: Scolytidae)," Forest and timber insects in New Zealand 29, Forest Research Institute, New Zealand Forest Service, Rotorua, New Zealand, 1978.

[3] S. D. Reay and P. J. Walsh, "The incidence of seedling attack and mortality by *Hylastes ater* (coleoptera: Scolytidae) in second rotation *Pinus radiata* forests in the Central North Island, New Zealand," *New Zealand Journal of Forestry*, vol. 47, no. 2, pp. 19–23, 2002.

[4] G. Örlander, U. Nilsson, and G. Nordlander, "Pine weevil abundance on clear-cuttings of different ages: a 6-year study using pitfall traps," *Scandinavian Journal of Forest Research*, vol. 12, no. 3, pp. 225–240, 1997.

[5] S. R. Leather, K. R. Day, and A. N. Salisbury, "The biology and ecology of the large pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae): a problem of dispersal?" *Bulletin of Entomological Research*, vol. 89, no. 1, pp. 3–16, 1999.

[6] S. D. Reay and P. J. Walsh, "Observations on the flight activity of *Hylastes ater* (Curculionidae: Scolytinae) and *Hylurgus*

ligniperda (Curculionidae: Scolytinae) in *Pinus radiata* forests in the central North Island, New Zealand," *New Zealand Entomologist*, vol. 24, no. 1, pp. 79–85, 2001.

[7] J. Bain, "*Hylurgus ligniperda* (Fabricius) (Coleoptera: Scolytidae)," Forest and Timber Insects in New Zealand 18, Forest Research Institute, New Zealand Forest Service, Rotorua, New Zealand, 1977.

[8] S. D. Reay, *Aspects of the ecology and behaviour of* Hylastes ater *(Coleoptera: Scolytidae) in second rotation* Pinus radiata *forests in the central North Island, and options for control*, Ph.D. thesis, University of Canterbury, Canterbury, New Zealand, 2001.

[9] M. C. Birch, "Chemical communication in pine bark beetles," *American Scientist*, vol. 66, no. 4, pp. 409–419, 1978.

[10] K. D. Klepzig, K. F. Raffa, and E. B. Smalley, "Association of an insect-fungal complex with red pine decline in Wisconsin," *Forest Science*, vol. 37, no. 4, pp. 1119–1139, 1991.

[11] K. F. Raffa, "Induced defensive reactions in conifer-bark beetle systems," in *Phytochemical Induction by Herbivores*, D. W. Tallamy and M. J. Raupp, Eds., pp. 246–276, John Wiley & Sons, New York, NY, USA, 1991.

[12] T. D. Paine, K. F. Raffa, and T. C. Harrington, "Interactions among scolytid bark beetles, their associated fungi, and live host conifers," *Annual Review of Entomology*, vol. 42, pp. 179–206, 1997.

[13] D. L. Six and M. J. Wingfield, "The role of phytopathogenicity in bark beetle-fungus symbioses: a challenge to the classic paradigm," *Annual Review of Entomology*, vol. 56, pp. 255–272, 2011.

[14] J. Butcher, "The ecology of fungi infecting untreated sapwood of *Pinus radiata*," *Canadian Journal of Botany*, vol. 46, no. 12, pp. 1557–1589, 1968.

[15] R. L. Farrell, S. J. Kay, E. Hadar, Y. Hader, R. A. Blanchette, and T. C. Harrington, "Survey of sapstain in New Zealand—the causes and a potential anti-sapstain solution," in *Biology and Prevention of Sapstain*, B. Kreber, Ed., pp. 25–29, Forest Products Society No. 7273, Madison, Wis, USA, 1998.

[16] J. M. Thwaites, R. L. Farrell, K. Hata, P. Carter, and M. Lausberg, "Sapstain fungi on *Pinus radiata* logs—from New Zealand forest to export in Japan," *Journal of Wood Science*, vol. 50, no. 5, pp. 459–465, 2004.

[17] J. M. Thwaites, R. L. Farrell, S. M. Duncan et al., "Survey of potential sapstain fungi on *Pinus radiata* in New Zealand," *New Zealand Journal of Botany*, vol. 43, no. 3, pp. 653–663, 2005.

[18] M. J. Wingfield and J. N. Gibbs, "*Leptographium* and *Graphium* species associated with pine-infesting bark beetles in England," *Mycological Research*, vol. 95, no. 11, pp. 1257–1260, 1991.

[19] K. A. Seifert, "Sapstain of commercial lumber by species of *Ophiostoma* and *Ceratocystis*," in *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity*, M. J. Winfield, K. A. Seifert, and J. F. Webber, Eds., pp. 141–151, American Phytopathological Society, St. Paul, Minn, USA, 1993.

[20] P. Dowding, "Effects of felling time and insecticide treatment on the interrelationships of fungi and arthropods in pine logs," *Oikos*, vol. 24, no. 3, pp. 422–429, 1973.

[21] J. N. Gibbs, "The biology of Ophiostomatoid fungi causing sapstain in trees and freshly cut logs," in *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity*, M. J. Winfield, K. A. Seifert, and J. F. Webber, Eds., pp. 153–160, American Phytopathological Society, St. Paul, Minn, USA, 1993.

[22] M. MacKenzie and M. Dick, "Verticicladiella root disease," Forest Pathology in New Zealand 6, Forest Research Institute, New Zealand Forest Service, Rotorua, New Zealand, 1984.

[23] M. J. Wingfield, P. Capretti, and M. Mackenzie, "*Leptographium* spp. as root pathogens of conifers: an international perspective," in *Leptographium Root Diseases of Conifers*, T. C. Harrington and F. R. Cobb Jr, Eds., pp. 113–128, American Phytopathological Society, St. Paul, Minn, USA, 1988.

[24] S. D. Reay, P. J. Walsh, A. Ram, and R. L. Farrell, "The invasion of *Pinus radiata* seedlings by sapstain fungi, following attack by the Black Pine Bark Beetle, *Hylastes ater* (Coleoptera: Scolytidae)," *Forest Ecology and Management*, vol. 165, no. 1–3, pp. 47–56, 2002.

[25] K. Jacobs, M. J. Wingfield, B. D. Wingfield, and Y. Yamaoka, "Comparison of *Ophiostoma huntii* and *O. europhioides* and description of *O. aenigmaticum* sp. nov," *Mycological Research*, vol. 102, no. 3, pp. 289–294, 1998.

[26] T. C. Harrington, "*Leptographium* species, their distributions, hosts and insect vectors," in *Leptographium Root Diseases of Conifers*, T. C. Harrington and F. W. Cobb Jr., Eds., pp. 1–39, American Phytopathological Society, St. Paul, Minn, USA, 1998.

[27] T. C. Harrington, "Biology and taxonomy of fungi associated with bark beetles," in *Beetle-Pathogen Interactions in Conifer Forests*, T. D. Schowalter and G. M. Filip, Eds., pp. 37–58, Academic Press, London, UK, 1993.

[28] T. C. Harrington, "Diseases of conifers caused by species of *Ophiostoma* and *Leptographium*," in *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity*, M. J. Winfield, K. A. Seifert, and J. F. Webber, Eds., pp. 161–172, American Phytopathological Society, St. Paul, Minn, USA, 1993.

[29] D. Malloch and M. Blackwell, "Dispersal biology of the Ophiostomatoid fungi," in *Ceratocystis and Ophiostoma: Taxonomy, Ecology, and Pathogenicity*, M. J. Winfield, K. A. Seifert, and J. F. Webber, Eds., pp. 195–205, American Phytopathological Society, St. Paul, Minn, USA, 1993.

[30] M. J. Wingfield and P. S. Knox-Davis, "Root disease, associated with *Verticicladiella alacris*, of pines in South Africa," *Plant Disease*, vol. 64, no. 1, pp. 569–571, 1980.

[31] J. J. Witcosky and E. M. Hansen, "Root-colonising insects recovered from Douglas-fir in various stages of decline due to black-stain root disease," *Phytopathology*, vol. 75, pp. 399–402, 1985.

[32] J. J. Witcosky, T. D. Schowalter, E. M. Hansen, and M. Everett, "*Hylastes nigrinius* (Coleoptera; Scolytidae), *Pissodes fasiatus*, and *Steremnius carinatus* (Coleoptera: Curculionidae) as vectors of black-stain root disease of Douglas-fir," *Environmental Entomology*, vol. 15, no. 5, pp. 1090–1095, 1986.

[33] W. R. Jacobi, "Potential insect vectors of the black stain root disease pathogen on Southern Vancouver Island," *Journal of the Entomological Society of British Columbia*, vol. 89, pp. 54–56, 1992.

[34] S. D. Reay, J. M. Thwaites, and R. L. Farrell, "A survey of *Ophiostoma* species vectored by *Hylastes ater* to pine seedlings in New Zealand," *Forest Pathology*, vol. 35, no. 2, pp. 105–113, 2005.

[35] B. K. Bakshi, "Development of perithecia and reproductive structures in two species of *Ceratocystis*," *Annals of Botany*, vol. 15, no. 1, pp. 53–62, 1951.

[36] B. K. Bakshi, "Studies on four species of *Ceratocystis*, with a discussion on fungi causing sap-stain in Britain," *Mycological Papers*, vol. 35, pp. 1–16, 1951.

[37] A. Mathiesen-Käärik, "Studies on the ecology, taxonomy and physiology of Swedish insect-associated blue stain fungi, especially the genus *Ceratocystis*," *Oikos*, no. 1, pp. 1–25, 1960.

[38] S. D. Reay, J. M. Thwaites, R. L. Farrell, and T. R. Glare, "The lack of persistence of Ophiostomataceae fungi in *Pinus radiata* three years after damage by the bark beetle *Hylastes ater*, and the subsequent colonization by *Sphaeropsis sapinea*," *Forest Ecology and Management*, vol. 233, no. 1, pp. 149–152, 2006.

[39] L. R. Castro, A. D. Austin, and M. Dowton, "Contrasting rates of mitochondrial molecular evolution in parasitic diptera and hymenoptera," *Molecular Biology and Evolution*, vol. 19, no. 7, pp. 1100–1113, 2002.

[40] J. D. Thompson, T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins, "The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools," *Nucleic Acids Research*, vol. 25, no. 24, pp. 4876–4882, 1997.

[41] J. P. Huelsenbeck and F. Ronquist, "MRBAYES: bayesian inference of phylogenetic trees," *Bioinformatics*, vol. 17, no. 8, pp. 754–755, 2001.

[42] F. Ronquist and J. P. Huelsenbeck, "MrBayes 3: bayesian phylogenetic inference under mixed models," *Bioinformatics*, vol. 19, no. 12, pp. 1572–1574, 2003.

[43] D. Posada and T. R. Buckley, "Model selection and model averaging in phylogenetics: advantages of akaike information criterion and bayesian approaches over likelihood ratio tests," *Systematic Biology*, vol. 53, no. 5, pp. 793–808, 2004.

[44] J. A. A. Nylander, *MrModeltest 2.2*, Department of Systematic Zoology, Uppsala University, Uppsala, Sweden, 2004.

[45] D. L. Swofford, *2002 PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0b10*, Sinauer Associates, Sunderland, Mass, USA, 2002.

[46] F. Rodriguez, J. L. Oliver, A. Marin, and J. R. Medina, "The general stochastic model of nucleotide substitution," *Journal of Theoretical Biology*, vol. 142, no. 4, pp. 485–501, 1990.

[47] Z. Yang, N. Goldman, and A. Friday, "Comparison of models for nucleotide substitution used in maximum- likelihood phylogenetic estimation," *Molecular Biology and Evolution*, vol. 11, no. 2, pp. 316–324, 1994.

[48] R. D. M. page, "TreeView: an application to display phylogenetic trees on personal computers," *Computer Applications in the Biosciences*, vol. 12, no. 4, pp. 357–358, 1996.

[49] S. D. Reay and P. J. Walsh, "A carbosulfan insecticide to protect pine seedlings from *Hylastes ater* (Coleoptera: Scolytidae) damage," *New Zealand Plant Protection*, vol. 55, pp. 80–84, 2002.

[50] S. Balazy, "Analysis of bark beetle mortality in spruce forests in Poland," *Ekologia Polska A*, vol. 33, no. 16, pp. 657–687, 1968.

[51] R. Wegensteiner, "Pathogens in bark beetles," in *Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis*, F. Lieutier, K. Day, A. Battisti, J. C. Grégoire, and H. Evans, Eds., pp. 291–313, Kluwer Academic, Dodrecht, The Netherlands, 2004.

[52] D. Chandler, G. Davidson, J. K. Pell, B. V. Ball, K. Shaw, and K. D. Sunderland, "Fungal biocontrol of acari," *Biocontrol Science and Technology*, vol. 10, no. 4, pp. 357–384, 2000.

[53] G. Zimmermann, "Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*," *Biocontrol Science and Technology*, vol. 17, no. 6, pp. 553–596, 2007.

[54] N. V. Meyling and J. Eilenberg, "Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control," *Biological Control*, vol. 43, no. 2, pp. 145–155, 2007.

[55] E. Quesada-Moraga, J. A. Navas-Cortés, E. A. A. Maranhao, A. Ortiz-Urquiza, and C. Santiago-Álvarez, "Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils," *Mycological Research*, vol. 111, no. 8, pp. 947–966, 2007.

[56] F. E. Vega, F. Posada, M. Catherine Aime, M. Pava-Ripoll, F. Infante, and S. A. Rehner, "Entomopathogenic fungal endophytes," *Biological Control*, vol. 46, no. 1, pp. 72–82, 2008.

[57] T. R. Glare, S. D. Reay, T. L. Nelson, and R. Moore, "*Beauveria caledonica* is a naturally occurring pathogen of forest beetles," *Mycological Research*, vol. 112, no. 3, pp. 352–360, 2008.

[58] R. Moore, "Public and private enemy no.1, A new approach to dealing with the large pine weevil (*Hylobius abietis*)," *Forestry and British Timber*, vol. 26, no. 4, pp. 12–15, 1997.

[59] S. D. Reay, M. Brownbridge, N. J. Cummings et al., "Isolation and characterization of *Beauveria* spp. associated with exotic bark beetles in New Zealand *Pinus radiata* plantation forests," *Biological Control*, vol. 46, no. 3, pp. 484–494, 2008.

[60] J. W. Doberski and H. T. Tribe, "Isolation of entomopathogenic fungi from elm bark and soil with reference to ecology of *Beauveria bassiana* and *Metarhizium anisopliae*," *Transactions of the British Mycological Society*, vol. 74, no. 1, pp. 95–100, 1980.

[61] R. Wegensteiner, "Laboratory evaluation of *Beauveria bassiana* (Bals.) Vuill. against the bark beetle, *Ips typographus* (L.) (Coleoptera, Scolytidae)," *IOBC/WPRS Bulletin*, vol. 19, pp. 186–189, 1996.

[62] R. A. Prazak, "Laboratory evaluation of *Beauveria bassiana* (Bals.) Vuill. (Deuteromycotina: Hyphomycetes) against *Trypodendron lineatum*. Oliv. (Coleoptera, Scolytidae)," *Journal of Plant Diseases and Protection*, vol. 104, no. 5, pp. 459–465, 1997.

[63] J. Kreutz, G. Zimmermann, H. Marohn, O. Vaupel, and G. Mosbacher, "Preliminary investigations on the use of *Beauveria bassiana* (Bals.) Vuill. and other control methods against the bark beetle, *Ips typographus* L. (Col., Scolytidae) in the field," *IOBC/WPRS-Bulletin*, vol. 23, pp. 167–173, 2000.

[64] J. Kreutz, O. Vaupel, and G. Zimmermann, "Efficacy of *Beauveria bassiana* (Bals.) Vuill. against the spruce bark beetle, *Ips typographus* L., in the laboratory under various conditions," *Journal of Applied Entomology*, vol. 128, no. 6, pp. 384–389, 2004.

[65] J. Kreutz, G. Zimmermann, and O. Vaupel, "Horizontal transmission of the entomopathogenic fungus *Beauveria bassiana* among the spruce bark beetle, *Ips typographus* (Col., Scolytidae), in the laboratory and under field conditions," *Biocontrol Science and Technology*, vol. 14, no. 8, pp. 837–848, 2004.

[66] M. Brownbridge, S. D. Reay, and N. J. Cummings, "Association of Entomopathogenic fungi with exotic bark beetles in New Zealand pine plantations," *Mycopathologia*, vol. 169, no. 1, pp. 75–80, 2009.

[67] S. D. Reay, M. Brownbridge, B. Gicquel, N. J. Cummings, and T. L. Nelson, "Isolation and characterization of endophytic *Beauveria* spp. (Ascomycota: Hypocreales) from *Pinus radiata* in New Zealand forests," *Biological Control*, vol. 54, no. 1, pp. 52–60, 2010.

[68] B. H. Ownley, M. R. Griffin, W. E. Klingeman, K. D. Gwinn, J. K. Moulton, and R. M. Pereira, "*Beauveria bassiana*:

endophytic colonization and plant disease control," *Journal of Invertebrate Pathology*, vol. 98, no. 3, pp. 267–270, 2008.

[69] B. H. Ownley, K. D. Gwinn, and F. E. Vega, "Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution," *BioControl*, vol. 55, no. 1, pp. 113–128, 2010.

Hindawi Publishing Corporation
Psyche
Volume 2012, Article ID 185312, 6 pages
doi:10.1155/2012/185312

Research Article

Coexistence and Competition between *Tomicus yunnanensis* and *T. minor* (Coleoptera: Scolytinae) in Yunnan Pine

Rong Chun Lu,[1,2,3] Hong Bin Wang,[1,2] Zhen Zhang,[1,2] John A. Byers,[4] You Ju Jin,[5] Hai Feng Wen,[3] and Wen Jian Shi[3]

[1] *Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, Beijing 100091, China*
[2] *The Key Laboratory of Forest Ecology and Environment, State Forestry Administration, Beijing 100091, China*
[3] *College of Urban Construction, University of Shanghai for Science and Technology, Shanghai 200093, China*
[4] *USDA-ARS, U.S. Arid-Land Agricultural Research Center, 21881 North Cardon Lane, Maricopa, AZ 85138, USA*
[5] *College of Plant Sciences, Beijing Forest University, Beijing 100083, China*

Correspondence should be addressed to Hong Bin Wang, wanghb@caf.ac.cn

Received 6 October 2011; Accepted 12 December 2011

Academic Editor: Qing-He Zhang

Competition and cooperation between bark beetles, *Tomicus yunnanensis* Kirkendall and Faccoli and *Tomicus minor* (Hartig) (Coleoptera: Scolytinae) were examined when they coexisted together in living Yunnan pine trees (*Pinus yunnanensis* Franchet) in Yunnan province in Southwest China. *T. yunnanensis* bark beetles were observed to initiate dispersal from pine shoots to trunks in November, while the majority of *T. minor* begins to transfer in December. *T. yunnanensis* mainly attacks the top and middle parts of the trunk, whereas *T. minor* mainly resides in the lower and middle parts of the trunk. The patterns of attack densities of these two species were similar, but with *T. yunnanensis* colonizing the upper section of the trunk and *T. minor* the lower trunk. The highest attack density of *T. Yunnanensis* was 297 egg galleries/m^2, and the highest attack density of *T. minor* was 305 egg galleries/m^2. Although there was significant overlap for the same bark areas, the two species generally colonize different areas of the tree, which reduces the intensity of competition for the relatively thin layer of phloem-cambium tissues where the beetles feed and reside.

1. Introduction

A new species of pine bark beetle, *Tomicus yunnanensis* Kirkendall and Faccoli (Coleoptera: Scolytinae), was recently discovered, and which formerly had been confused with *Tomicus piniperda* (L.) [1]. *T. yunnanensis* is an important forest pest since it has caused extensive mortality of Yunnan pines, *Pinus yunnanensis* Franchet, in the southwest of China [2–5]. More than 200,000 ha of Yunnan pine forests were killed by the bark beetle by 2005 [4–6]. In addition to Yunnan pines, *T. yunnanensis* also feeds on Simao pines, *P. kesiya var. langbianensis,* and Gaoshan pines, *P. densata*, as well as some other pine species [4, 5].

T. yunnanensis is frequently joined by *T. minor* (Hartig) in attack of Yunnan pine trees in the southwest of China, Yunnan province [2, 7]. In most of the cases, the two species live in the same area and feed on the same host trees [5, 7]. Several studies have reported that there is a general competition between *T. minor* and *T. yunnanensis* [2, 5, 7–10]. However, the specific mechanisms and the extent of the competition are unclear. An investigation on the cooperation and competition between these two bark beetle species to obtain food may indicate new approaches of integrated control of pest bark beetles, especially *T. yunnanensis* and *T. minor* which are very difficult to control and monitor [11–14]. Thus, the goals of the present studies were to investigate the changes in distribution on the host tree of the beetles during the autumn dispersal from shoots to trunks and to determine the mutual influence of the two *Tomicus* species on each other's attack distribution and reproduction within the tree. Additional knowledge about the two pest bark beetle species' attack distributions and densities, colonization sequences, migratory movements, and competitive interactions

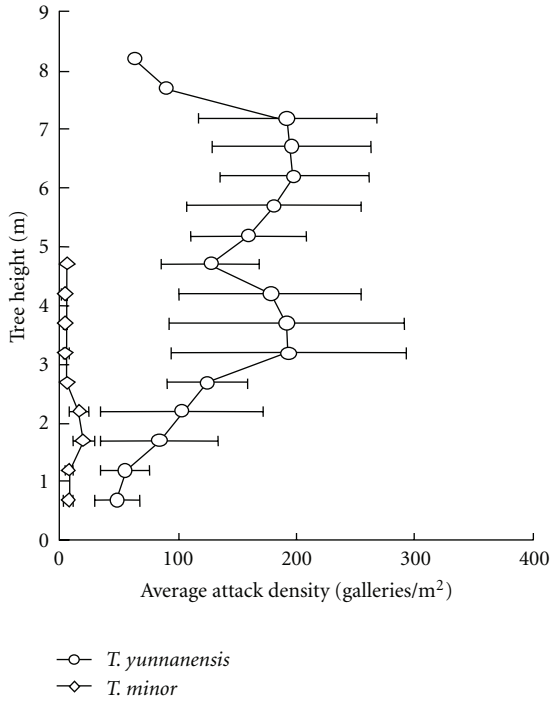


Figure 1: Average attack (egg gallery) density (±SE) and distribution of *T. yunnanensis* and *T. minor* along the trunks of Yunnan pines in November, 2005 (Qujing, Yunnan province, China).

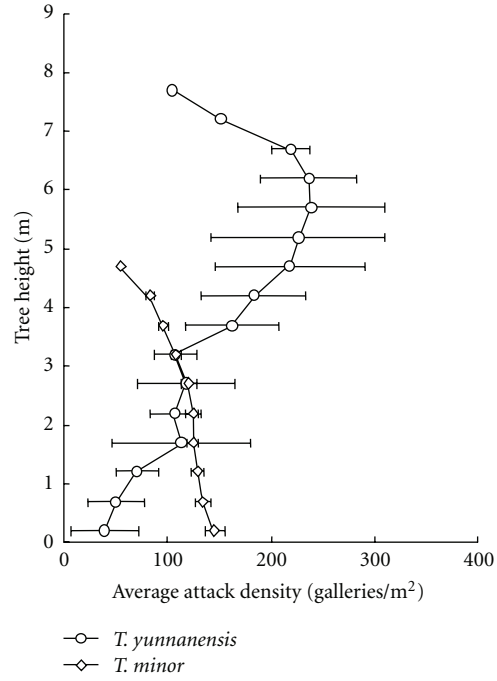


Figure 2: Average attack (egg gallery) density (±SE) and distribution of *T. yunnanensis* and *T. minor* along the trunks of Yunnan pines in December, 2005 (Qujing, Yunnan province, China).

may provide insights into more efficient management practices.

2. Materials and Methods

2.1. Study Site Conditions. A series of field studies were conducted from November to March in 2005-2006 in a 300 ha plantation of Yunnan pines, located in the mountain near Qujing city in Yunnan province (25°14′ N, 103°50′ E, and 1700–1800 m above sea level). Some broad-leaved trees were scattered inside the plantation. Most of the Yunnan pines were infested with both *T. yunnanensis* and *T. minor*. The trees were 30–45 years old and ranged from 10 to 15 m in height and 10 to 15 cm in diameter.

2.2. Experimental Design. Beginning in November 2005, two to three Yunnan pines were selected at random and cut down every week through March, or approximately 44 trees in total. Every shoot was carefully examined, and any bark beetles that were found were collected and distinguished by species with a binocular microscope. In addition, one to two pine trunks that had been colonized by *T. yunnanensis* or *T. minor* were selected and cut down every week, or approximately 17 trees. The tree trunks were divided into 0.5 m long logs, and each log was carefully peeled of bark to reveal egg galleries. The entire trunk was cut into two sections (upper section and lower section) from the middle; and the number and length of all egg galleries over the surface of each log were recorded. The bark beetles within galleries were also collected and distinguished with a binocular microscope. The heights and diameters of all the cut trees were measured.

2.3. Data Presentation and Analyses. Data are presented as mean ± standard error (±SE). The correlations between *T. yunnanensis* and *T. minor* were calculated with bivariate methods. These analyses were conducted using SPSS 11.5 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Egg Gallery Distribution in Trunks. Most *T. yunnanensis* began to move from shoots to trunks in November (Figure 1), and most *T. minor* began in December (Figure 2). In November, there were fewer *T. minor* egg galleries compared with *T. yunnanensis*, and all of the *T. minor* egg galleries were distributed in the lower half of the tree's trunk (Figure 1). *T. yunnanensis* egg galleries were distributed over the entire trunk in some trees, but with different densities in different sections. The highest density of *T. yunnanensis* was in the upper half of the tree trunks and was about 198 galleries/m^2, while the highest density of *T. minor* was only about 19 galleries/m^2.

In December, the attack density of *T. yunnanensis* and *T. minor* was in both higher than in the previous month, with *T. minor* increasing significantly (Figure 2). However, in the lower section of the trunks, the attack density of *T. yunnanensis* decreased a little compared to earlier. *T. minor* was still mostly distributed in the lower section of the trunk.

In January, the attack density (egg galleries) of *T. yunnanensis* remained highest in the upper section of the trunks

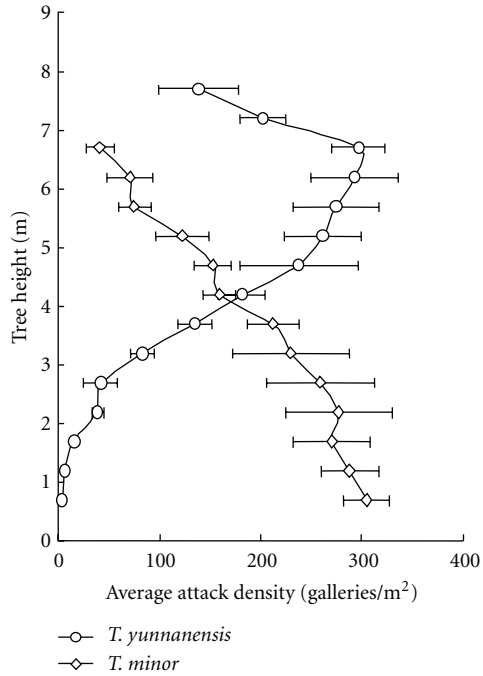


Figure 3: Average attack (egg gallery) density (±SE) and distribution of *T. yunnanensis* and *T. minor* along the trunks of Yunnan pines in January, 2006 (Qujing, Yunnan province, China).

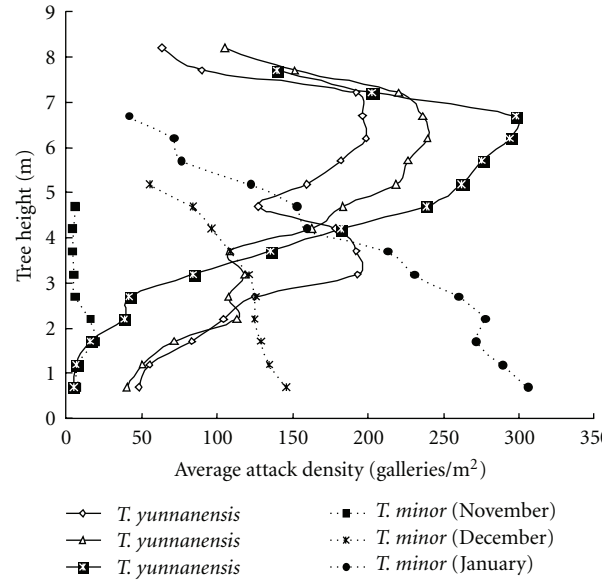


Figure 4: Progression of average attack (egg gallery) density and distribution of *T. yunnanensis* and *T. minor* along the trunks of Yunnan pines from November to January, 2005-2006 (Qujing, Yunnan province, China).

and also remained low in the lower section of the trunk (Figure 3). The highest gallery density of *T. yunnanensis* was about 297 galleries/m^2. The attack density of *T. minor* increased further, especially in the lower section of the trunks; and the egg galleries of *T. minor* were now distributed nearly over all areas of the trunks (Figure 4). The highest attack density of *T. minor* was about 305 galleries/m^2.

The measurements of egg gallery lengths show that in November these lengths for *T. yunnanensis* and *T. minor* were relatively short (Figure 5); *T. yunnanensis* egg galleries were about 3.6–4.9 cm long, while *T. minor*'s egg galleries were about 3.2–4.3 cm long. In December, the egg gallery length increased, especially for *T. minor* (Figure 6). The egg galleries of *T. minor* were about 5.9–6.2 cm long, and *T. yunnanensis* egg galleries were about 4.6–6.0 cm long. In January, egg gallery length continued to increase (Figure 7), *T. minor* egg galleries lengthened to about 8.2–8.9 cm, and *T. yunnanensis* went up to about 5.2–6.6 cm long (Figure 8).

3.2. Bark Beetle Distribution in Tree Crowns. About 11 *T. yunnanensis* were found in shoots per tree in November, and this number decreased to about nine by January, but the differences were not significant. Fewer *T. minor* (about four) were found per tree in November, and the number appeared to increase to five in December and then returned to four in January, but these differences were not significant.

3.3. Relationship between T. yunnanensis and T. minor. The two bark beetle species had different relationships in different parts of the tree during the November to January period

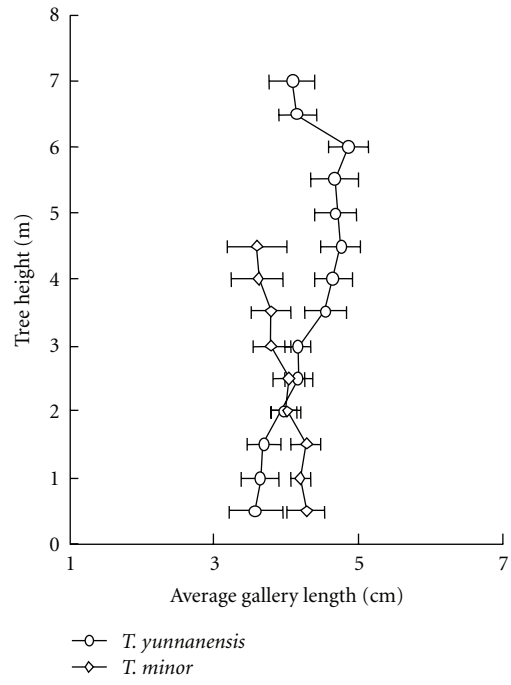


Figure 5: Average egg gallery length (±SE) of *T. yunnanensis* and *T. minor* along the trunks of Yunnan pines in November, 2005 (Qujing, Yunnan province, China).

(Figures 4 and 8). In November, there was no significant correlation between the two bark beetle species either with respect to trunks or to shoots (Table 1). In December, the two bark beetle species in shoots had no significant correlation either, but there was a significant negative correlation ($P \leq 0.01$) between *T. yunnanensis* and *T. minor*

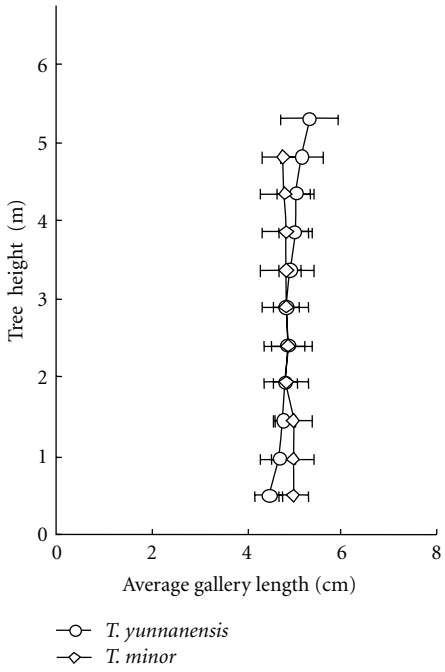


Figure 6: Average egg gallery length (±SE) of *T. yunnanensis* and *T. minor* along the trunks of Yunnan pines in December, 2005 (Qujing, Yunnan province, China).

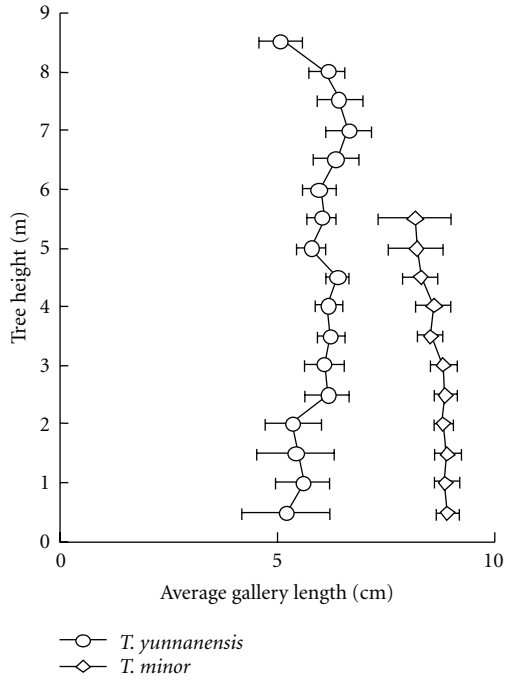


Figure 7: Average egg gallery length (±SE) of *T. yunnanensis* and *T. minor* along the trunks Yunnan pines in January, 2006 (Qujing, Yunnan province, China).

in trunks (Table 1). In January, the two bark beetle species had significant negative correlations in shoots and in trunks (Table 1).

4. Discussion

The appearance of bark beetle galleries show that most *T. yunnanensis* begin to transfer from shoots to trunks in November, a little earlier than *T. minor*. Most of *T. minor* begin to move in December. This result is consistent with earlier reports [15, 16]. The transferring period lasts from November to January, or even longer, and there is no clear peak in transferring time. In Yunnan province, the weather is much drier than other times in winter from November to April. When winter approaches, the weather has little rainfall and the soil becomes dry. Some trees are weakened because of the shortage of water. These weak trees are the first choice for bark beetles to attack and feed on. As dry weather conditions persist, more and more trees are weakened and become suitable for bark beetles to colonize. At the study site, the colonized trees were cut down when they were found. However, as the colonized trees were cut down, newly colonized trees could always be found until February. When *T. minor* begins to transfer from shoots to trunks, and the density of *T. minor* increases in the lower trunk, the newly colonizing *T. yunnanensis* will no longer feed on lower sections, indicating the latter species is avoiding competition with *T. minor* (Figure 4). Thus, the colonized trees cut down later were found to have fewer *T. yunnanensis* in their lower sections. Similarly, *T. minor* were found mainly in the lower trunk in areas with less *T. yunnanensis*, thus both species

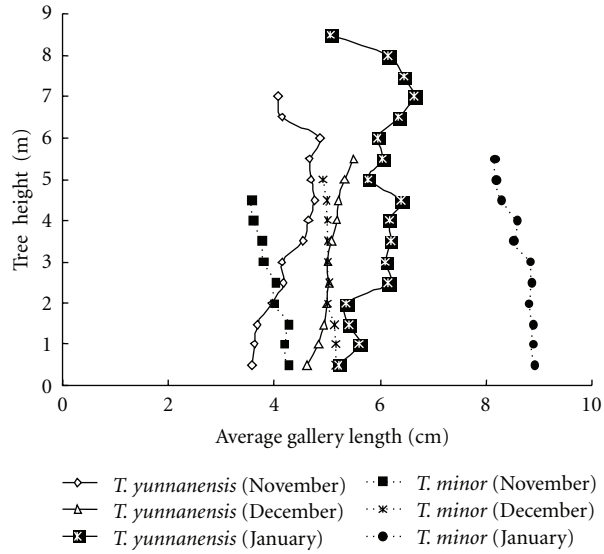


Figure 8: Progression of average egg gallery length of *T. yunnanensis* and *T. minor* along the trunks of Yunnan pines from November to January, 2005-2006 (Qujing, Yunnan province, China).

appear to be avoiding competition by selecting areas of the host with lower densities of the opposite species (Figure 4).

The egg gallery length of *T. yunnanensis* and *T. minor* not only increased during the three-month period, but also changed with the height of the trunks (Figures 5–8). The egg gallery length of *T. yunnanensis* increased, and the egg gallery

Table 1: The correlation between *T. yunnanensis* and *T. minor* in trunks and shoots of Yunnan pines.

	Bark beetles in trunks		Bark beetles in shoots	
Month	Pearson correlation	Sig. (2 tailed)	Pearson correlation	Sig. (2 tailed)
11	−0.491	0.179	−0.126	0.729
12	−0.956	$1.52E-05^{**}$	0.169	0.642
1	−0.981	$3.5E-09^{**}$	0.768	0.009^{**}

** Correlation significant at the 0.01 level (2-tailed).

length of *T. minor* decreased with the height of the trunks in different months.

We found that there are mainly two ways for *T. yunnanensis* and *T. minor* to transfer from shoots to trunks. If the host trees were already weakened, the beetles would feed on the host tree trunks directly; if the host trees were initially more healthy, beetles would feed together on the shoots of the tree to weaken it and then transfer to the trunks of these trees. These insects use this strategy to weaken the tree's defensive ability and allow better survival of the individual bark beetles.

The relationship between *T. yunnanensis* and *T. minor* changed with time and location on the tree. When the time for transferring came, *T. yunnanensis* would feed on the top of the host tree's trunk to make their host weaker or to kill it. Occasionally we found live trees with a dead top that had been killed by *T. yunnanensis*; this indicates that host trees generally have a strong resistance and were not easy to kill [17, 18], but when *T. minor* joined in to feed on the lower section of the host trunk, the resistance of the tree was further weakened which often led to its death. It is well known that the egg galleries of *T. minor* are perpendicular to the trunk length [19]. This would probably cut more transporting tissues and might be more harmful to trees than *T. yunnanensis* which have galleries aligned with the trunk. In November, the attack density was relatively low in trunks, especially for *T. minor*, so the relationship between the two bark beetle species was predominately cooperation. With more and more bark beetles transferring to trunks; however, the competition between the two species intensified, and the cooperation between them diminished. However, they could coexist very well since both species had a separate colonization location on the trunk to avoid competition as much as possible. In fact, we scarcely found two egg galleries of different families joined together, no matter whether the galleries were from the same or the other species, as has been observed in other species [12, 13, 20, 21]. When two egg galleries almost touched, bark beetles would not proceed ahead, and in most of the cases, *T. yunnanensis* would change the egg gallery direction or abandon their egg gallery, thus making some egg galleries of *T. yunnanensis* not straight or with deviations. *T. minor* also would stop when closely approaching another egg gallery and often turned and excavated in the opposite direction, because there are only two directions for *T. minor* to bore their egg galleries perpendicular to the grain of the trunk.

In the tree crowns, the behavioral relationship between the two species was more complicated. It was not uncommon that two or three bark beetles tunneled in one shoot. But in most cases, the beetles were of the same species and usually consisted of one male and one female, or one male and two females. This suggests that there was competition between *T. yunnanensis* and *T. minor* in shoots. At the beginning of the transferring period, their relationships were dominated by competition in shoots; but with more and more bark beetles transferring from shoots to trunks, their relationship changed. In other words, the competitive relationship between the beetles changed as they transferred from branches to trunks. Thus, the competitive relationship between *T. yunnanensis* and *T. minor* occurs during their entire life cycle but alters in intensity and type with the life phase and location on the host tree. Knowledge about the movement from shoots to trunks (transferring) and trunk colonization process, as well as competition between the two species, will be useful in designing control strategies that take advantage of the vulnerability of the beetles and maximize resistance of the tree.

Acknowledgment

The authors thank the Forest Pest Control Station of Qujing City, Yunnan province for logistical support and field assistance. This study was funded by the Importing International Advanced Agricultural Science and Technology Research (2002-38) and International Technological Cooperation Research (2006DFA31790).

References

[1] L. R. Kirkendall, M. Faccoli, and H. Ye, "Description of the Yunnan shoot borer, *Tomicus yunnanensis* Kirkendall & Faccoli sp. n. (Curculionidae, Scolytinae), an unusually aggressive pine shoot beetle from southern China, with a key to the species of *Tomicus*," *Zootaxa*, no. 1819, pp. 25–39, 2008.

[2] B. Långström, L. Lisha, L. Hongpin et al., "Shoot feeding ecology of *Tomicus piniperda* and *T. minor* (Col., Scolytidae) in southern China," *Journal of Applied Entomology*, vol. 126, no. 7-8, pp. 333–342, 2002.

[3] F. Lieutier, H. Ye, and A. Yart, "Shoot damage by *Tomicus* sp. (Coleoptera: Scolytidae) and effect on *Pinus yunnanensis* resistance to subsequent reproductive attacks in the stem," *Agricultural and Forest Entomology*, vol. 5, no. 3, pp. 227–233, 2003.

[4] Y. Duan, C. Kerdelhué, H. Ye, and F. Lieutier, "Genetic study of the forest pest *Tomicus piniperda* (Col., Scolytinae) in Yunnan province (China) compared to Europe: new insights for the systematics and evolution of the genus *Tomicus*," *Heredity*, vol. 93, no. 5, pp. 416–422, 2004.

[5] J. H. Sun, S. R. Clarke, L. Kang, and H. B. Wang, "Field trials of potential attractants and inhibitors for pine shoot beetles in the Yunnan province, China," *Annals of Forest Science*, vol. 62, no. 1, pp. 9–12, 2005.

[6] X. D. Zhou, K. Jacobs, M. Morelet, H. Ye, F. Lieutier, and M. J. Wingfield, "A new *Leptographium* species associated with *Tomicus piniperda* in south-western China," *Mycoscience*, vol. 41, no. 6, pp. 573–578, 2001.

[7] Y. Hui and D. Xue-Song, "Impacts of *Tomicus minor* on distribution and reproduction of *Tomicus piniperda* (Col., Scolytidae) on the trunk of the living *Pinus yunnanensis* trees," *Journal of Applied Entomology*, vol. 123, no. 6, pp. 329–333, 1999.

[8] F. E. Regnier, "Semiochemical—structure and function," *Biology of Reproduction*, vol. 4, no. 3, pp. 309–326, 1971.

[9] A. Borkowski, "Threats to pine stands by the pine shoot beetles *Tomicus piniperda* (L.) and *Tomicus minor* (Hart.) (Col., Scolytidae) around a sawmill in southern Poland," *Journal of Applied Entomology*, vol. 125, no. 9-10, pp. 489–492, 2001.

[10] H. Solheim, P. Krokene, and B. Långström, "Effects of growth and virulence of associated blue-stain fungi on host colonization behaviour of the pine shoot beetles *Tomicus minor* and *T. piniperda*," *Plant Pathology*, vol. 50, no. 1, pp. 111–116, 2001.

[11] M. C. M. De Jong and P. Grijpma, "Competition between larvae of *Ips typographus*," *Entomologia Experimentalis et Applicata*, vol. 41, no. 2, pp. 121–133, 1986.

[12] J. A. Byers, "Behavioral mechanisms involved in reducing competition in bark beetles," *Holarctic Ecology*, vol. 12, no. 4, pp. 466–476, 1989.

[13] J. A. Byers, "Avoidance of competition by spruce bark beetles, *Ips typographus* and *Pityogenes chalcographus*," *Experientia*, vol. 49, no. 3, pp. 272–275, 1993.

[14] G. L. Robins and M. L. Reid, "Effects of density on the reproductive success of pine engravers: is aggregation in dead trees beneficial?" *Ecological Entomology*, vol. 22, no. 3, pp. 329–334, 1997.

[15] H. Ye, "Occurrence, distribution and damages of *Tomicus piniperda* in Yunnan, Southwestern China," *Journal of Yunnan University*, vol. 20, pp. 361–363, 1998.

[16] H. Wang, Y. Zhang, and L. Li, "Bionomics of *Tomicus piniperda* in Yunnan," *Forest Pest and Disease*, vol. 25, no. 4, pp. 21–24, 2006.

[17] B. Langstrom, C. Hellqvist, A. Ericsson, and R. Gref, "Induced defence reaction in Scots pine following stem attacks by *Tomicus piniperda*," *Ecography*, vol. 15, no. 3, pp. 318–327, 1992.

[18] B. Långström, H. Solheim, C. Hellqvist, and P. Krokene, "Host resistance in defoliated pine: effects of single and mass inoculations using bark beetle-associated blue-stain fungi," *Agricultural and Forest Entomology*, vol. 3, no. 3, pp. 211–216, 2001.

[19] M. M. F. Fernández, J. A. Pajares Alonso, and J. M. Salgado Costas, "Shoot feeding and overwintering in the lesser pine shoot beetle *Tomicus minor* (Col., Scolytidae) in north-west Spain," *Journal of Applied Entomology*, vol. 123, no. 6, pp. 321–327, 1999.

[20] J. D. Reeve, D. J. Rhodes, and P. Turchin, "Scramble competition in the southern pine beetle, *Dendroctonus frontalis*," *Ecological Entomology*, vol. 23, no. 4, pp. 433–443, 1998.

[21] B. H. Aukema and K. F. Raffa, "Relative effects of exophytic predation, endophytic predation, and intraspecific competition on a subcortical herbivore: consequences to the reproduction of *Ips pini* and *Thanasimus dubius*," *Oecologia*, vol. 133, no. 4, pp. 483–491, 2002.