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Measures to Control Fusarium and Phialophora Wilt Pathogens of Carnations

Carnation plant losses due to the vascular wilt pathogens *Fusarium oxysporum* f. sp. *dianthi* and *Phialophora cinerescens* (Fig. 1) were reduced in greenhouses in the United States many years ago with the advent of pathogen-free operations producing massive quantities of vegetatively propagated material. Because wilt pathogens were disseminated within the vascular system, the "cultured cutting" technique was used to obtain pathogen-free propagative material. In the 1960s, however, procedures for growing gnotobiotic or even germfree plants from shoot tips were adopted in the industry (1). For the first time, rooted cuttings free of all known pathogens of carnation could be supplied to commercial growers. Eventually, advanced methods of tissue culture provided even more efficiency in rapid multiplication of "clean" propagative material (5).

In the past, growers transplanted these pathogen-free cuttings into raised benches. Between crops, the benches and soil were steamed according to rigorous protocols, and any inoculum remaining from the previous crop was eradicated. Integrated control measures involving

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Fig. 1. Symptoms of the vascular wilt diseases of carnation: (A) Fusarium wilt. External symptoms often develop on one side. Stems and leaves on the afflicted side are yellow, with a light brown vascular discoloration at the base of the stem. The vascular system on the upper part of the stem is chalky white. (B) Phialophora wilt. Plants gradually wilt, and leaves fade and become straw color. The vascular system is chocolate brown, much darker than with Fusarium wilt. (From original paintings by Eleanor J. Baker highlighting the differences in symptom expression between the two diseases.)

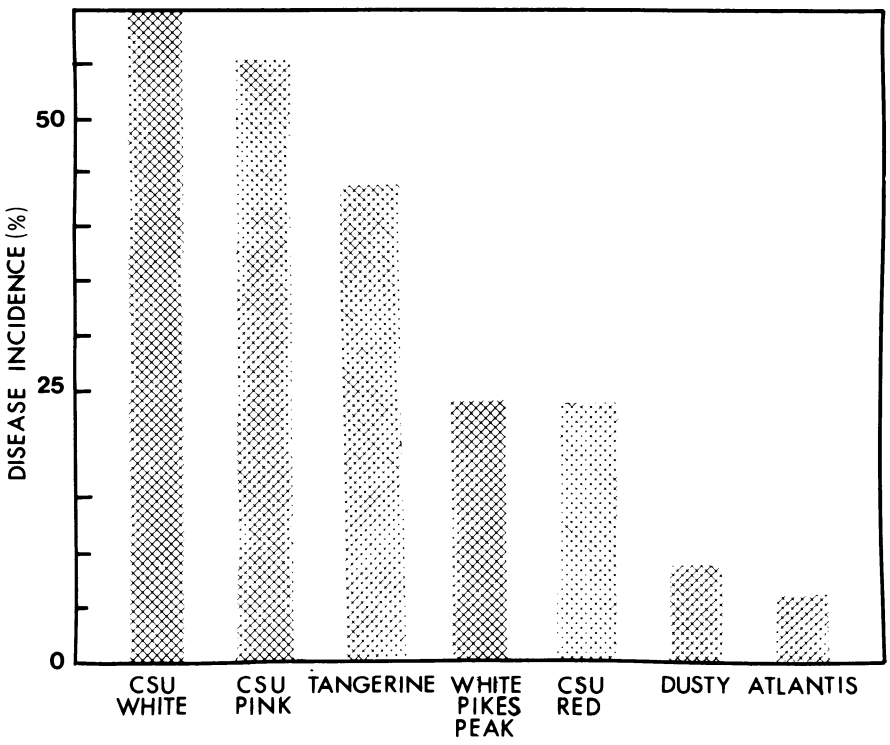


Fig. 2. Disease incidence among carnation cultivars planted in soil infested with *Fusarium oxysporum* f. sp. *dianthi* after 1 year; 90 plants of each cultivar were divided into three replicates.

Table 1. Relative resistance among selections of carnation cultivars to *Fusarium oxysporum* f. sp. *dianthi*

Color	Selection number[a]	Disease incidence[b] (%)
Red	R 1	67
	R 7	63
White	WS 8	33
	WP 9	70
Pink	P 8	77
	P 11	90

[a] Specific clone.
[b] Plants with symptoms after 1 year from 30 plants of each clone in two replicates.

clean stock, soil treatment, and sanitation virtually eliminated the diseases, and they became oddities. Recently, however, this sublime situation has deteriorated. Indeed, "Du sublime au ridicule il n'y a qu'un pas" (Napoleon).

Conventional raised greenhouse benches made from redwood inevitably deteriorated, and replacement materials were either no longer available or expensive. The same problems arose in new construction, but with proper floricultural practices, plants could be grown in the soil on the greenhouse "floor"—in the so-called ground beds. This procedure was much less expensive, and a better quality crop could be produced. In many cases, however, inoculum from the era before adequate control measures survived in the soil on the greenhouse floor. Surface steaming of the ground beds with the conventionally used Thomas method (11) did not eradicate inoculum at depths below heat penetration or in the compacted aisles between beds. Therefore, transplanting pathogen-free propagative material to such beds no longer insured a continuing healthy crop. The same difficulty was experienced with other greenhouse crops.

The vascular wilts have thus become the most important diseases in ground beds with carnations in the United States, in the recently developing industry in Colombia, and in Great Britain. At Colorado State University over the last decade, innovative control measures and those reported in and out of the literature have been tested for their potential in rectifying this situation. This article reviews the progress of those attempts.

Resistance as a Strategy

Development of carnation cultivars resistant to the wilt pathogens provides an opportunity for disease control. The commercial cultivars appear to have varying degrees of resistance, and attempts to breed for resistance have met with some success (3). A fortunate situation, which may be more than a coincidence, is that resistance in a breeding selection operates for both Phialophora and Fusarium wilt (19).

Fig. 2 gives an example of the range of host response to infection by *F. oxysporum* f. sp. *dianthi* encountered in cultivars grown in commercial greenhouses. These ratings confirm grower observations that among the most commonly grown flower colors, pink cultivars are most susceptible, red most resistant, and white intermediate or variable. Even within selections of the same color, however, evidence of variability suggests that resistance differs slightly among clones of the same cultivar (Table 1).

This situation is interesting from the standpoint of the genetic mechanisms contributing to resistance or susceptibility. Most standard carnation cultivars currently in commercial production here and elsewhere were selected directly or indirectly from a single parent, the cultivar William Sim. (The lineage of these cultivars was contributed by Stack et al [20].) All the cultivars listed in Fig. 2 and Table 1 are Sim cultivars. Thus, mutations not only alter growth habit and flower color but also, in more subtle ways, influence resistance. This situation becomes even more intriguing because cultivars derived from William Sim are periclinal chimeras (11). Presumably, all Sim cultivars have similar genetic composition in interior tissues, including the vascular system, and differ in external tissues responsible for flower color.

In spite of evidence that disease control through resistance is of potential benefit, this strategy has not been adopted by commercial carnation growers. The first

reason advanced by a plant pathologist might be the evidence for the existence of various races of the pathogens (10) that could overcome resistance. In a practical sense, however, the reasons are related to floricultural rather than pathological considerations. Management of commercial pathogen-free floricultural programs considers the most important operational factor to be maintenance of quality selections of cultivars (11). Selections that do not adhere to the rigorous standards of plant and flower quality are constantly being eliminated; routinely, 50% or more are eliminated each year. By the time resistant selections are detected, they may no longer exist in the trade. Thus, development and detection of resistant cultivars and clones, a time-consuming and expensive operation, in most cases is wasted effort. Even if breeding succeeds in developing resistant lines, floricultural characteristics must meet or exceed the quality of existing cultivars, an extremely difficult objective even for breeders not selecting for resistance.

At present, the use of resistance as a control strategy for extensive application in the industry is not practical. Fortunately, the vascular wilt diseases are usually distributed in a relatively small area within a greenhouse range. Where disease is a problem, the grower can be advised to plant red-flowered cultivars, because the incubation period for disease development is longer for red than for pink or white cultivars.

Eradicating Inoculum in Soil

Eradication of inoculum from ground beds is no small problem. In situations similar to those encountered in the carnation industry, *F. oxysporum* f. sp. *lycopersici* was recovered from soil at depths to 90 cm (8).

Soil fumigation with methyl bromide controlled vascular wilt disease of carnation (2), but bromide residues in soil are toxic to carnations and must be leached out with copious amounts of water. Although the research was not formal, English growers reported favorable results from treating "wilt patches" with metham sodium followed by steaming (7). These possibilities for controlling vascular wilts have been evaluated during the past 6 years in commercial greenhouses in Colorado. Using the method of the English growers, metham sodium was applied as a soil

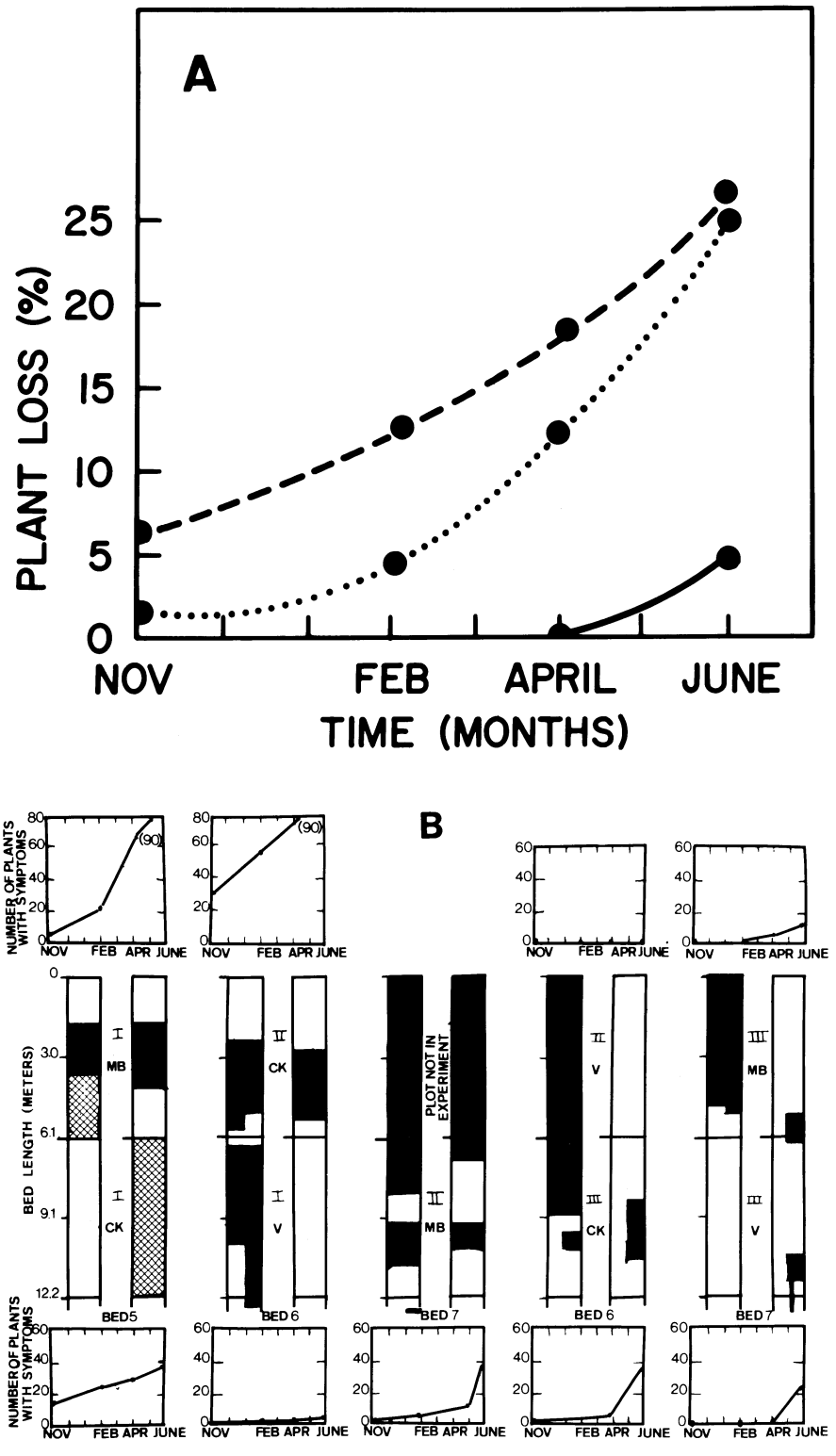


Fig. 3. (A) Losses in carnations due to Fusarium wilt in the second year after transplanting when beds were treated with fumigants. All plots were steamed after fumigation. Each plot contained 210 plants, with three replicates. •---• steam only, •···• methyl bromide (1.36 kg/6.51 m^2) and steam, •—• metham sodium (946 ml/9.3 m^2) and steam. (B) Diagrammatic representation to scale of data obtained in plots in A. Loss over time is presented above or below each plot. Figures in parentheses are total losses in the methyl bromide-treated plot in replicate I and in the control (steamed only) plot in replicate II by June. In each case, dark areas on the bench diagram to the left represent dead plants in the preceding crop and those to the right, dead plants after 2 years, at the end of the experiment. Checkered areas indicate that disease was scattered through that section of the plot. CK = control, V = metham sodium, MB = methyl bromide.

drench (946 ml/9.3 m^3) to plots (6.1 m ×1.06 m) at the ends of ground beds in which losses from Fusarium wilt had been extensive. Carnations from the preceding planting, transplanted 2 years before, were still in the beds. Treated areas were watered thoroughly to aid penetration of the fumigant and provide a seal for trapping evolved volatiles. Then, 5 days later, the plants were uprooted and removed, the beds were cultivated, and preparations were made for steaming.

In other plots, 1 day before steaming, methyl bromide was applied at a rate of 1.36 kg/6.51 m^2 of soil surface and confined with a plastic cover. The cover was left in place for 24 hours.

All plots, including nontreated controls, were steamed with the Thomas method. Disease losses were observed over a 2-year period (Fig. 3A). Steaming may have reduced bromide residue to below toxic levels, since no injury was observed in plots treated with methyl bromide. Losses from Fusarium wilt were not as great initially in plots with this treatment; at the end of 2 years, however, little difference was found in comparison with controls given only conventional steam treatment. Disease was not seen in plots treated with metham sodium and steam until the end of the experiment.

Experiments such as this one have the advantage of being performed under commercial conditions, and results may be readily applicable to the industry as a whole. Some parameters cannot be adjusted to uniformity, however. For instance, even distribution of inoculum is difficult to insure. Obviously, growers will not allow the inclusion of nontreated inoculated controls. Such disadvantages can be at least partially overcome by plotting, in diagrammatic form, loss distribution before treatments were applied and disease incidence at the end of the experiment (Fig. 3B). Study of the diagram suggests that 1) inoculum was eradicated consistently when metham sodium and steam were applied and 2) a large proportion of the loss with this treatment (Fig. 3A) originated from disease in bed 7 (third relicate) from an adjacent nontreated area. In some plots, steam alone or combined with methyl bromide apparently eradicated inoculum.

Another experiment that essentially repeated the one described but omitted methyl bromide treatment also indicated good control of Fusarium wilt with the

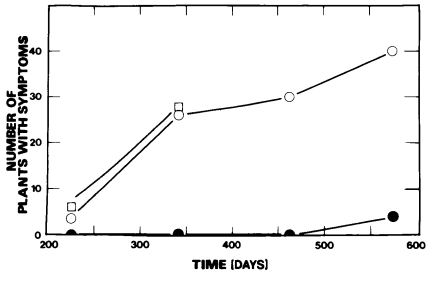
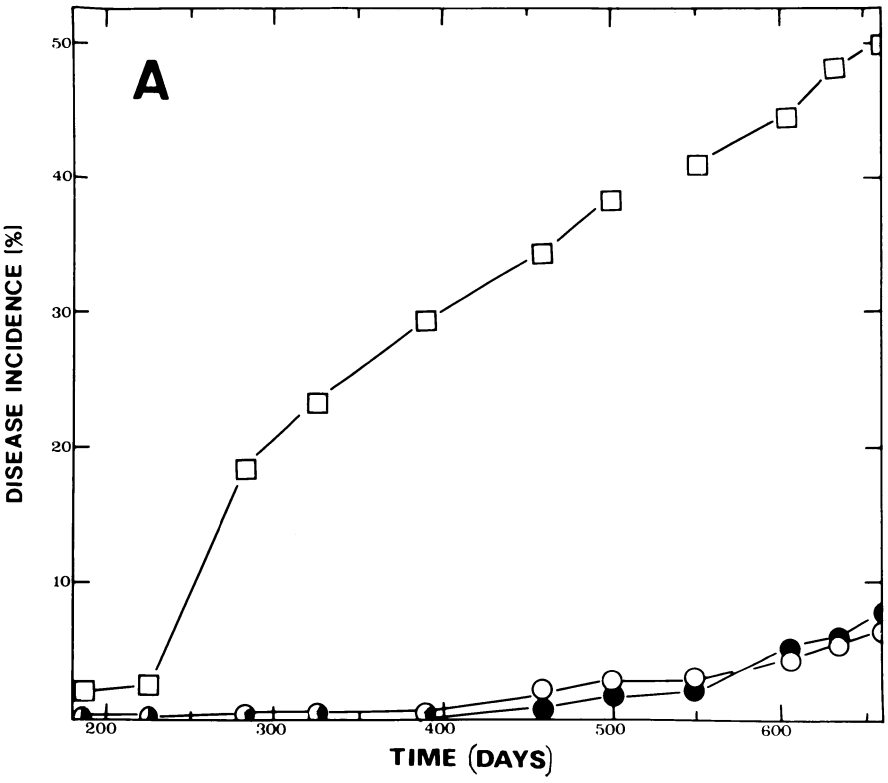


Fig. 4. Losses in carnations due to Fusarium wilt when beds were treated with metham sodium (946 ml/9.3 m^2) or drenched with ethephon (10 mg/plant at monthly intervals). Each plot contained 525 plants and was steamed before beds were planted. Ethephon treatments were discontinued after 350 days because of phytotoxicity. o–o steam only, •—• metham sodium and steam, □—□ steam followed by drenches of ethephon.



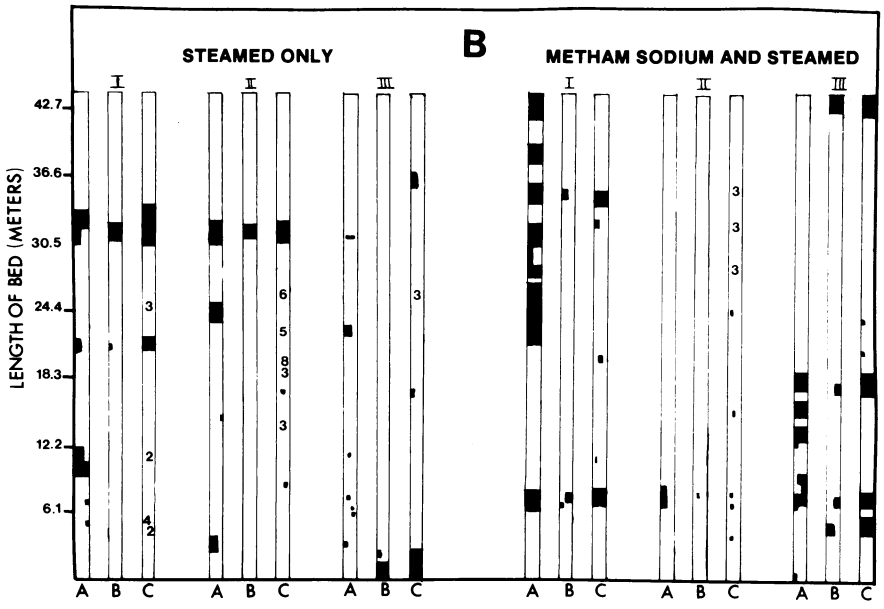


Fig. 5. (A) Losses in carnations due to Phialophora wilt when beds were treated with metham sodium (946 ml/9.3 m^2) and/or steam. The break in the curve between 500 and 550 days for the metham sodium treatment indicates elimination of one replicate. Data for that treatment after 550 days include only two replicates. o–o steam only, •–• metham sodium and steam, □–□ metham sodium only. (B) Diagrammatic representation to scale of data illustrated in A for those plots where metham sodium plus steam and steam alone were applied. Roman numerals indicate replicate numbers. Dark areas are locations where all plants died. Letters at the base of each plot: A, loss in preceding crop; B, loss approximately 1 year after benches were planted; C, loss at completion of experiment approximately 2 years after transplanting. Figures on plots refer to actual numbers of plants with symptoms of Fusarium wilt at the end of the experiment.

metham sodium treatment combined with steaming (Fig. 4).

Another large experiment involving over 14,000 plants was established to test the efficacy of the metham sodium treatment. Losses in the preceding crop were due primarily to *P. cinerescens*. An important element in this experiment, not used in the preceding test, was the introduction of steam through two tiles, each 10 cm in diameter, running the length of all beds and buried approximately 60 cm below the soil surface. The results (Fig. 5A) indicate that treatment with metham sodium alone is not effective. They also suggest that steam applied at a 60-cm depth effectively eradicates a large proportion of the inoculum and that treatment with metham sodium has no additional value.

The graphic presentation (Fig. 5B) suggests that inoculum was apparently eradicated in some areas treated with metham sodium and steam or steam alone but not in others. New centers of infection were apparent when either treatment was used. In areas in which eradication was not complete, subsequent disease proportions were usually as great as or even greater than those in the preceding crop.

These results confirm the experience of British growers (7): combined metham sodium and steam treatment is more effective than steam alone when the Thomas steaming method is used. Steam alone applied at depths sufficient to eradicate inoculum also provides control.

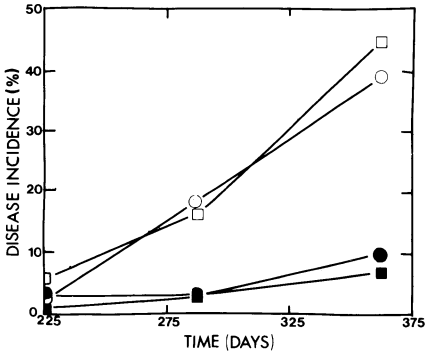


Fig. 6. Control of Phialophora wilt by mixing benomyl (1.15 kg a.i./93.03 m^2) or ethazole/thiophanate-methyl (1.6 kg a.i./93.03 m^2) in soil before transplanting rooted carnation cuttings. o–o inoculated control, •–• benomyl, □–□ ethazole, ■–■ ethazole/thiophanate-methyl.

Using Systemic Fungicides and Growth Regulators

In his elegant review of the status of research on control of the wilt pathogens (6), Erwin defines general principles related to the properties and characteristics of systemic fungicides with specific application to ornamentals. The 2-substituted benzimidazoles are effective in controlling the vascular wilt pathogens. They are insoluble but hydrolyze to methyl-1-benzimidazole carbamate in plant tissue. The fungitoxicant must be in continuous supply in the xylem fluid to keep the parasite suppressed; this is best accomplished by allowing root uptake of the chemical. Thus, soil application of systemic fungicides should be preferred for controlling the vascular wilt pathogens of carnation. Numerous reports from Holland (17), France (15), Germany (4), and Great Britain (7,9) suggest that soil applications of appropriate systemic fungicides control Fusarium and Phialophora wilt diseases of carnation. In the intensive type of agriculture associated with carnations, this control, although expensive, is usually cost-effective.

Typical results of controlling Phialophora wilt with benomyl and thiophanate-methyl are shown in Fig. 6. After 364 days, loss was approximately 40% in control plots or in those treated with ethazole but less than 10% in those treated with benomyl or thiophanate-methyl. Systemic fungicides may also be used for Fusarium wilt; typical results of treatment with benomyl are shown in Fig. 7.

The amount of control achieved in these examples is impressive, especially since the fungicide was incorporated into the soil only once, at the time of transplanting. In practice, drenches should be repeated to maintain an effective concentration of the fungicide in the soil while beds are producing flowers. With carnations, this period is usually 2 years and the total amount of, for example, benomyl applied may be from 4.5 to 6 kg a.i./90 m^3 per year.

Growth regulators have been suggested as chemotherapeutic agents for vascular wilt (6). The recommendations of Orion and Hoestra (14) were followed when applying ethephon to the soil as a part of the test illustrated in Fig. 6. In another treatment, plants were sprayed with naphthaleneacetic acid (13). Application of ethephon (see also Fig. 4) or naphthaleneacetic acid had no effect on development of Fusarium wilt.

Apparently, chemical control (other than fumigants) of the vascular wilts of carnation has been feasible only with systemic fungicides applied to soil. Unfortunately, strains of the pathogens are resistant to these agents (12,21).

Antitranspirants–Hypothetical but Not Practical Measure

A unique method of controlling Fusarium wilt of carnations is presented here not because of its application to commercial situations but because it seems to confirm certain features of the

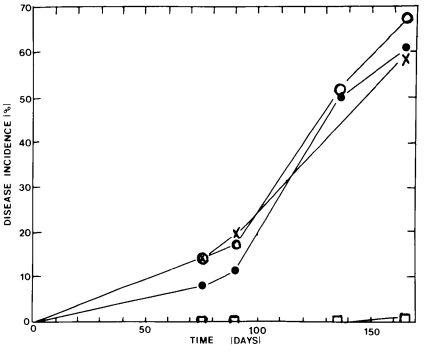


Fig. 7. Control of Fusarium wilt by mixing benomyl in soil before transplanting rooted carnation cuttings into soil infested with *Fusarium oxysporum* f. sp. *dianthi*. Other plots were either treated with sprays (every 10 days) of naphthaleneacetic acid (10 μg/ml of water) or drenched with ethephon solutions (10 mg/plant each month). o–o inoculated control, •–• naphthaleneacetic acid, X–X ethephon, □–□ benomyl.

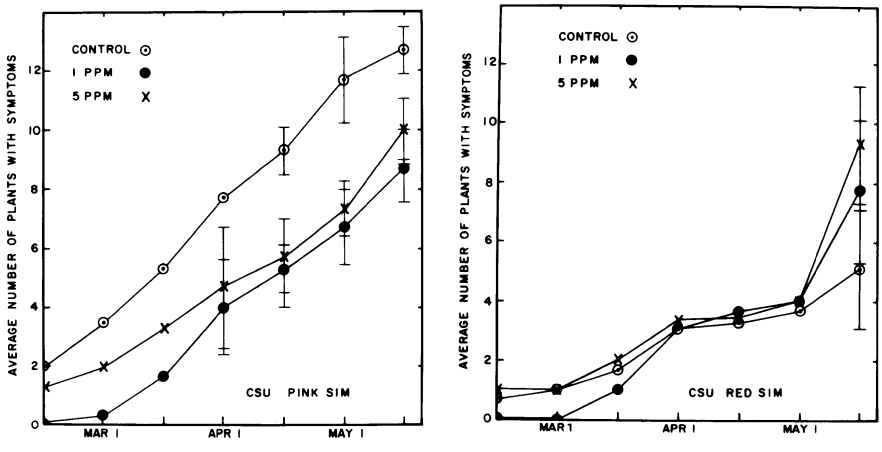
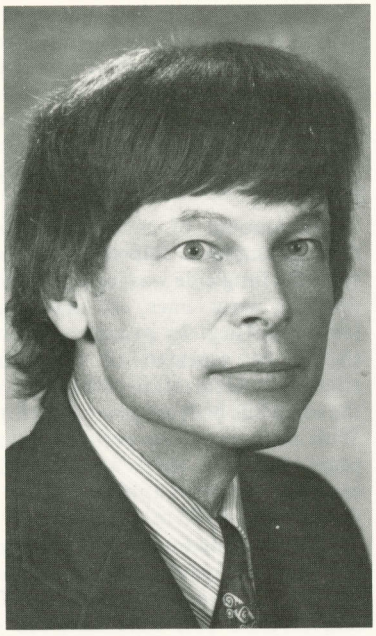


Fig. 8. Incidence of Fusarium wilt the second year after transplanting in two carnation cultivars sprayed to runoff at weekly intervals with phenylmercuric acetate (PMA) at two concentrations. The standard errors shown as vertical brackets were computed from original data. ⊙—⊙ nontreated control, •—• 1 μg/ml PMA, X—X 5 μg/ml PMA.



Ralph Baker

Dr. Baker received his Ph.D. at the University of California (Berkeley) in 1954. Since then, he has taught and done research at Colorado State University, Fort Collins, except for appointments as visiting professor at the University of California (Berkeley) during 1963–1964 and as a National Science Foundation Senior Post Doctoral Fellow at Cambridge University, England, during 1968–1969. His original research responsibility in Colorado was to develop control measures for diseases of ornamentals; this resulted in establishment of large pathogen-free operations for propagating carnations in the state. His other research interests involve the modeling of phenomena associated with the ecology of plant pathogens in soil, especially those connected with biological control. He also dabbles in space biology.

Fig. 9. All the carnations in the plot on the right, planted in a ground bed steamed 18 months previously, have died and shown symptoms of Fusarium wilt. Plants in the plot on the left are without symptoms to the line where 600 gm/m^2 of Metz fine sandy loam, a Fusarium-suppressive soil, had been added at the time of transplanting.

mechanisms involved in disease expression. Wilting is thought to be induced by impediment of water transpiration in the host's vascular system. Therefore, chemicals that reduce transpiration (18) and, hypothetically, could reduce stress on hosts infected with the vascular wilt pathogens were tested for their ability to extend the incubation period required for symptom expression.

Four antitranspirants were screened in preliminary tests. One, phenylmercuric acetate (PMA), exerted some degree of control and was selected for further tests. CSU Pink Sim was lightly sprayed biweekly at 0, 20, and 40 μg a.i. PMA/ml of water. After 1 year, wilt symptoms were seen in 60, 42, and 28% of the plants, respectively. The experiment was repeated, this time using the relatively resistant CSU Red Sim as well as the susceptible CSU Pink Sim. Significant control was obtained with PMA for CSU Pink but not for Red Sim (Fig. 8).

Attempts to detect fungitoxic entities in plants treated with PMA were not successful. Thus, the hypothesis that the mechanism of control was associated with the ability of the antitranspirant to reduce stress on the host was not disproved. The inability of PMA to influence the incubation period of Fusarium wilt on the resistant cultivar suggests another hypothesis. CSU Red Sim may be resistant because the tissue in the periclinal chimera regulating stomatal aperture reduces transpiration—and, correspondingly, stress—of the host. Applications of an antitranspirant, which closes stomata, to such a cultivar provide no additional relief from transpirational stress.

Control with PMA is not spectacular. Also, PMA contains mercury and therefore is not available to agriculturalists. These factors preclude its use as a practical control measure.

Biological Control, a Possibility

The Salinas Valley in California contains a Metz fine sandy loam soil that is suppressive to Fusarium wilt diseases. This soil (600 gm/m^2) was added to ground beds in a commercial greenhouse with a history of loss from Fusarium wilt. Carnations were planted, and losses induced by *F. oxysporum* f. sp. *dianthi* were observed over a 2-year period. At that time, losses were reduced 60% (Fig. 9).

The mechanism apparently is associated with a biocontrol agent in the suppressive soil. Experimental evidence suggests that the factor may be in the bacterial community, and, indeed, certain component *Pseudomonas* spp., added at 10^5 cells/gm, confer suppressiveness to soils that were conducive before treatment (16). The possibility of using this type of biological control in commercial applications is being investigated.

Which Measures to Use

Losses from the Fusarium and Phialophora wilt pathogens of carnations in ground beds may be reduced by employing a number of control measures, singly or combined. Unfortunately, none is 100% effective, and each has disadvantages related to cost or consistent efficiency. The most effective long-term solution may lie in the transfer to conducive soils of antagonistic biological entities responsible for soil being suppressive of the vascular wilt pathogens, but this method is still under development. Although costly, the only completely effective control currently available is steaming the soil in raised benches to eradicate the pathogens and preventing recontamination by using pathogen-free propagative material.

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