STUDIES ON THE NATURE OF RESISTANCE OF PLANTS TO DISEASE. ALTERATIONS IN THE NITROGEN AND KETO ACID METABOLISM OF RESISTANT AND SUSCEPTIBLE WHEAT VARIETIES ASSOCIATED WITH INFECTION BY PUCCINIA GRAMINIS TRITICI ERIKS. AND HENN.

A

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TABLE OF CONTENTS

LIST OF TABLES

Page

Page

LIST OF FIGURES

INTRODUCTION

Because stem rust of wheat is of great economic importance, much attention has been given to its control, especially by means of resistant varieties. However, there is very little information upon the nature of this resistance. At the same time, there are several reasons for believing that the differences in resistance between varieties are due to differences in their chemical composition. So far, attempts to find a correlation between the resistance of plants and the presence of certain compounds in them have failed. This failure may possibly be explained by the dependence of resistance on the presence of compounds which may be difficult to detect owing to either their low concentration or their instability. Conceivably, differences in concentration of any such compounds may lead to easily detectable differences in the rate of certain metabolic processes. As there is considerable evidence in the literature that the organic nitrogenous components of the plant may have a bearing upon its resistance to rust, it seemed obvious that experiments designed to determine the metabolic rate of nitrogenous compounds and their precursors, the keto-acids, of resistant and susceptible plants might make very useful contributions to our understanding of the nature of rust resistance and susceptibility.

LITERATURE REVIEW

Introduction

The literature on the physiology of parasitism and the nature of disease resistance in plants has been reviewed by Brown (1,2,3), Rice (4,5), Brown et al. (6), Walker (7,8), Chester (9), Gäumann (10,11), Wingard (12), Eide (13), Wynd (14), Hart (15), Kern (16), Walker and Stahmann (17), Christensen and DeVay (18), Dimond (19), and Allen (20). These reviews contain a vast amount of information of great interest to all who are interested in the nature of parasitism and disease resistance in plants. One cannot read these reviews without fully realizing the intricate and complex nature of the host-parasite relations.

Brown (1) in his review of the physiology of parasitism divides the host-parasite relationships into three stages: pre-penetration, penetration, and post-penetration. The scope of this work and hence the literature review are concerned with only the phenomena of post-penetration stages.

Ample evidence may be found scattered throughout numerous publications indicating that in most cases the post-penetration interactions between the host and its parasite determine the resistance or susceptibility of the former. The studies of Ward (21) with

Puccinia rubigo-vera, of Gibson (22) with Uredinae, of Salmann (23) with powdery mildews, of Stakmann (24) with Puccinia graminis, of Allen (25) with Puccinia glumarum, and of others whose works have been reviewed by Rice (4,26) and Gäumann (11) all indicate that an active mechanism of defense is present in resistant varieties. This resistance has been called protoplasmic or physiologic resistance.

Histological Studies

As early as 1902, Ward (21) showed that Puccinia penetrated both the resistant and the susceptible varieties of Bromus in a similar manner. The fungus caused no immediate apparent injuries in the cells of susceptible varieties and maintained a symbiotic-like relationship with the host cells. On the other hand, the invaded cells of resistant varieties were killed and the infection hyphae disintegrated or grew slowly but never produced pustules. Later, Allen (27,28,29) extended Ward's findings to rust on wheat.

Similar host parasite relations could have been found also in certain facultative parasites. For example, it has been observed (30) that Phytophthora infestans caused no immediate apparent disturbance to the invaded host cells on susceptible plants. The mycelium progressed for some distance before obvious damage to the host ensued.

Recently, a study of the reaction of barley to the obligate parasite Erysiphe graminis was made by White (31) and White and Baker

(32). The behavior of the cell penetrated by a haustorium of the mildew in a susceptible host is very similar to that found in a susceptible-host-rust association. However, in resistant varieties the behavior was quite different. Contrary to the evidence of previous workers (33,34,35), White and Baker found that normal haustoria were formed in all varieties of barley including susceptible, semi-resistant, resistant, to highly resistant ('immune') ones. In the highly resistant varieties the completion of the first haustorial formation in infected epidermal cell was immediately followed by the collapse of the mesophyll beneath. However, on resistant and semi-resistant varieties the effect on the mesophyll occurred later, so that about five and ten haustoria, respectively, were produced underneath each colony on these varieties before the death of the mesophyll halted the fungus. When non-host plants such as oats, rye, and certain Kenya varieties of wheat were inoculated with Erysiphe graminis var. hordei (32), penetration of the epidermal cells either did not occur or was not followed by formation of haustoria. These results confirmed the findings of earlier workers (33,34). An exception was the wheat variety Federation, whose behavior was similar to highly resistant varieties of barley.

The type of resistance in barley to Erysiphe graminis var. hordei described by White and Baker appears then to depend mainly on the sensitivity of mesophyll cells, which react necrogenically to the

presence of haustoria in epidermal cells, rather than to death of epidermal cells (33,35), or to aborted and distorted development of the haustoria (33,34). According to White and Baker (32) the collapse of the mesophyll is important in determining the resistance of the host, whereas the part played by the collapse of the infected and surrounding epidermal cells appears to be minor, but cannot be entirely ignored.

The type of resistance described above has been referred to as a necrogenic defense reaction by White and Baker. The type of resistance showed in cereals other than barley and Federation wheat, however, might represent a different type of resistance as pointed out by Eide (13). The concept of super-sensitivity of host cells as a basis for resistance due to necrogenic defense reactions was first elaborated by Müller (36) in his work on host-parasite relations of Phytophthora infestans on potato. In his work, the development of a necrogenic defense reaction which confers resistance to the tissues of the potato was considered to be the decisive factor. The more sensitive the cells, the more speedily they were killed by the presence of the parasite. Müller suggested that infected cells release substances which bring about the death of super-sensitive host cells adjacent to the infection court, and in turn substances released from these killed host cells inhibit further development of the pathogen. Thus, the speed with which the necrogenic reaction occurred determined

the success or failure of the parasite.

Hirata (37) recently reported that the penetrating hypha of Erysiphe graminis on resistant barley varieties was surrounded by a callus and killed within a day or two. He suggested that the fungus was killed by substances produced near the point of entry and not mechanically by callus, since a few days after inoculation both living and dead haustoria were seen within the same epidermal cell. He further noted that if infected leaves were detached and kept in a moist dish, haustoria might remain alive in dying epidermal cells.

The literature reviewed up to this point may create the impression that no apparent disturbances occur in the invaded cells of the susceptible host in the early stages of infection, and especially so in the case of obligate parasites; but this is not so. Many changes in the host cellular constituents have been observed. Evidently, where penetration occurs, the parasite elicits marked responses from the invaded cell, but occasionally this also happens where there is no complete penetration. Changes in the staining reactions of both host and parasite occur at once, whether the host is congenial or not.

Smith (35) reported that penetration of susceptible hosts by red clover powdery mildew was accompanied by the ingrowth of the cell wall which formed a collar around it. This collar was, in turn, surrounded by a thick sheath containing a considerable amount of

dark-staining, somewhat granular material. In highly resistant hosts, the cytoplasm of the host stained heavily and appeared to undergo some degree of disorganization around the point of infection, in some cases even before penetration into the cell was complete. Also, Corner (34) demonstrated an alteration of the cellulose around the point of penetration, which was indicated by histochemical tests.

It is quite clear that resistance to obligate parasites, referred to above, operate after post-penetration and during the process of intercellular establishment and the intracellular development of haustoria in the host. Various hypotheses have been proposed to explain this resistance to invasion, e.g. 'starvation theory', 'toxin-antitoxin theory', etc. Evidences obtained were based on histological studies. These evidences are, however, disputable. Therefore, new evidence is necessary in order to explain this complex problem. Many investigators have sought new evidence by means of studies on the physiology of disease.

Physiological Studies

Published physiological studies have shown that obligate parasites in the earlier phases of their development in the susceptible host frequently do not have harmful effects. In fact, this may also be the case in certain facultative parasites, such as crown gall caused by Agrobacterium tumefaciens. In spite of the seemingly harmonious associ-

ation between the host and its parasite, changes in various metabolic processes, such as accumulation of metabolites, changes in nitrogen or other compounds, in auxin concentration, in photosynthetic rate, in respiratory rate, or in enzymatic activities, etc., have been reported by many workers.

There seems to be two types of changes involved, although it is difficult, if not impossible, to separate them. One is the change resulting in response to the invader, the other resulting from the interaction between the host and its parasite. The first type is frequently referred to as the defense reaction of the host. Most of the important studies of the changes deal with photosynthesis, carbohydrates and nitrogen metabolism, and respiration.

Photosynthesis

The photosynthetic rate of diseased plants is either decreased (39,40,41) or increased (42,43) relative to that of healthy plants. There is no general agreement in the findings of the various workers. The discrepancies may be attributed to the difference in strain of the organisms used, stage of infection at which readings were made, method of calculation, age of the plant, and possibly many other factors. Certain of these discrepancies will be shown by comparing the results of different authors and may be illustrated in part by the works of Sempio and others.

Sempio (44) reported a sharp increase in photosynthesis during the first two or three days after the inoculation of wheat leaves with Erysiphe graminis. In the following days, during the expansion of the mycelium, there was a definite decrease in photosynthesis, usually down to values below control values. Photosynthesis then rose above normal during conidial formation and again fell to values less than half for healthy plants. On the other hand, respiration increased steadily. He interpreted an increase of photosynthesis over that of respiration in the earliest phase as an expression of metabolic resistance, which was, however, overcome by the parasite subsequently. Allen (41) obtained comparable results and found that the decrease in photosynthesis started sooner with heavily infected than with lightly infected plants and continued while the carbohydrate content was still increasing. The decrease in photosynthesis was attributed to the destruction of chlorophyll and the mesophyll cells beneath the courts of infections as White and Baker (32) indicated histologically. Allen (41) found also that there was a correlation between the destruction of chlorophyll and the decrease in photosynthesis in the mildewed leaves during the period of disease development, with the exception of a few days prior to the death of the leaves. During this period, there was a slight increase in chlorophyll content without a corresponding increase in photosynthesis.

Gassner and Goeze (45) noted that in susceptible wheat leaves inoculated with Puccinia glumarum photosynthesis remained normal during incubation and decreased afterwards, but in resistant wheat the photosynthetic rate did not change.

Increases in carbohydrate content appear to be characteristic of the early phases of infection in susceptible plants. Accumulation and respiratory studies carried out by many workers showed that in the infected leaf the metabolic activity is greatest at and around the locus of infection.

The accumulation of starch and chemicals fed to the plant at areas infected by the parasite was observed by Halsted (see 46) with Puccinia podophylli, by Allen (28,47) with Puccinia triticina, by Humphrey and Dufrenoy (48) with Puccinia coronata, by Gottlieb and Garner (49) with Puccinia graminis tritici, by Allen (41) with powdery mildew, and by Yarwood (50,51) and Shaw and his associates (52,53) with a variety of pathogens (viruses, mildews, rusts, leaf spot fungi).

Gottlieb and Garner (49) were the first ones to employ a radioactive compound to demonstrate the nature of accumulation at the sites of rust infection. In their experiments all leaves of the seedlings except the first ones were removed as soon as they appeared. The radioactive phosphate was supplied to the roots of plants in culture solutions. They found that accumulation occurred at infection courts of

Puccinia graminis tritici, but the specific activity of the spores was less than that of either healthy or infected leaf tissues. They concluded that phosphorus accumulated in the host tissue as well as in the fungus. The form in which P^{32} was accumulated was not determined. Later, in mildew-infected leaves, it was stated by Allen (54) that the phosphorus was not in the form of soluble organic phosphates. Yet, in sweet potato tissue infected with Cerotostomella fimbriata, inorganic phosphate was found to decrease with a concomitant increase in acid soluble organic phosphate according to Akazawa and Uritani (55). Cutter (56) proposed that phosphorylated compounds originating in the host tissue may be utilized by obligate parasites. The positive test of acid phosphatase on or in the haustoria of mildew on barley led Atkinson and Shaw (57) to suggest that this enzyme might play a role in the utilization of organic phosphates by the parasite.

Shaw and Samborski (53) reported that when excised infected leaves were fed with solutions of labeled compounds or exposed to radioactive carbon dioxide, accumulation appeared at uredial and conidial colonies of the obligate parasites Puccinia and Erysiphe, respectively. Allen (54,41) suggested that such an accumulation originated in metabolic changes rather than in a permeability increase of the type described by Thatcher (58). However, Allen (41) considered the increased respiration to be a result of accumulation of carbohydrate. This accumulation was regarded by Shaw and Samborski (53) as an 'active'

process, since they could inhibit it by treating the plant with certain metabolic inhibitors, such as azide and 2,4-dinitrophenol, but they cautioned that the effect of these substances may be merely upon cell permeability.

The results obtained by Yarwood and Jacobson (51) on rust and mildew are in full agreement with the findings of Shaw and Samborski (53). There are, nevertheless, discrepancies in the results obtained by these two groups of workers on tobacco mosaic virus. Shaw and Samborski found that young local lesions on leaves fed with glucose-1-C^{14} were strikingly less radioactive than the surrounding healthy tissue, although veins crossing the lesions were strongly radioactive. On the other hand, detectable accumulations were observed in those fed with radioactive phosphate and calcium. In contrast to the above, Yarwood and Jacobson observed a striking accumulation of sulphur from hydrogen sulphide at local lesions of tobacco mosaic virus on Nicotina glutinosa. This discord might be attributed to the different compounds employed or different ages of infection. Besides, Shaw and Samborski pointed out that the accumulation might be the result of the formation of sulphides of heavy metals, since hydrogen sulphide is known as a respiratory inhibitor.

On the whole, there is agreement among workers that an accumulation of fed chemicals takes place at the sites of infection of obligate and some facultative parasites. Mechanical injuries play

a minor role only in accumulation (51,53).

Shaw and Samborski considered the results they obtained with Puccinia and Erysiphe to be in full accord with the hypothesis that a diffusible substance or substances, produced at the incipient infection, either by the fungus, or by the host cell or cells under attack, or both, stimulates the metabolic activity of the host tissue in the environs of the infections (54).

The possibility that carbohydrate may play an important role in the process of obligate parasitism has been given consideration. Numerous workers designed experiments to show the indispensability of carbohydrate to the development of rusts and mildews by means of either floating detached inoculated leaves on solutions containing carbohydrates (46,59,44,60) or placing intact inoculated leaves in continuous darkness (61,62,63). When detached susceptible leaves, inoculated with rust or mildew, were floated on water, a continued development of the parasites occurred in light with or without supplemented carbohydrate. But no such development took place in continuous darkness, unless soluble carbohydrate was provided. Sempio (64) showed that sorbose was effective for maintaining the healthy green color of detached bean leaves, but they could not support any development of rust (Uromyces appendiculatus). Later, Sempio (44) concluded that resistance could be imparted by the formation by the rust-infected plant of certain carbohydrate isomers which can be metabolized by the plant but not by the fungus.

Since the development of obligate parasites seems to be closely related to the carbohydrate metabolism of its host, many workers think that knowledge of this metabolism may be helpful in understanding the process of parasitism and the nature of disease resistance. Although higher amounts of carbohydrates have been reported in diseased leaves, several workers have found lower levels as well (65,66). These discrepancies again may be attributed to some of the factors discussed previously (p. 8). In 1942, Sempio (67) studied the sugar content of bean, Phaseolus vulgaris, during the development of Uromyces appendiculatus, from the initial infection to spore maturity. He found that during the first 4 to 6 days after infection the sugar content of the juices was notably higher in infected plants than in controls. Then a rapid decline occurred and at about 10 to 12 days after infection the sugar content became considerably lower than that in the healthy controls.

Novikov (68) studied the effect of rust, Uromyces striatus, on the metabolism of lucerne. He found that, with 50 per cent infection, there were qualitative as well as quantitative differences in individual carbohydrate components in healthy and infected leaves, in spite of the fact that no appreciable difference existed in total carbohydrate. Infected leaves had more sucrose and hemicelluloses, but less cellulose than normal leaves. They also contained disaccharides, other than sucrose, not found in healthy leaves. However,

with 100 per cent infection, the amount of sucrose was reduced to less than one-half of that found in healthy leaves, and that of cellulose decreased even further; hemicellulose content remained higher than in healthy plants, but lower than that observed in leaves with 50 per cent infection, whereas other disaccharides doubled in amount. Novikov concluded that the decrease in carbohydrate content of infected leaves in advanced stages of infection was a result of declining anabolic processes coupled with intensified respiratory processes, and that cellulose was converted to hemicelluloses.

In 1942, Allen (41) made a study of the changes in host metabolism resulting from mildew infection. He showed that in heavily infected plants there was an accumulation of soluble sugars which reached a maximum simultaneously with respiration, then the sugar content declined. Allen (41) divided the changes in host metabolism brought about by mildew infection into two phases: a first phase which is characterized by an increase in the rate of oxidation of carbohydrate; by an increase in glucose, sucrose, and starch; by a constant glucose respiration ratio; and by a destruction of chlorophyll which is closely followed by a decrease in photosynthesis but not by a decrease in photosynthetic efficiency. The second phase is characterized by rather abrupt changes in all of the above mentioned relationships. In this phase, glucose, sucrose, and starch decreased

in quantity, the latter two eventually disappearing completely. He interpreted the increase in carbohydrate in the first phase as a result of prevention by the parasite of their removal from the leaf. The decrease in the second phase was attributed to increased respiration resulting from the accumulation of carbohydrate which, together with a decreasing rate of photosynthesis, eventually led to the depletion of the host carbohydrate reserves.

It is apparent that much attention has been given to various phases of carbohydrate metabolism in diseased plants; also, as we will see in the next section, to those of nitrogen metabolism. However, no reference was found on the effect of pathogens on the metabolism of keto acids which link the carbohydrate metabolism with that of nitrogen.

Nitrogen Metabolism

In spite of the conflicting findings and lack of direct evidence, the view generally held is that nitrogen metabolism of the host possibly plays an important role in the host-parasite relations and in the determination of the resistance of the host. Doak (69) found that excess nitrogen increased the susceptibility of wheat varieties to leaf rust, Puccinia rubigo-vera tritici. Gassner and Hassebrauk (70,71) and Hassebrauk (72) observed that the rust reaction of wheat varieties, showing intermediate rust reaction, could be modified by changing the N, K, and P ratio. When potassium was higher in relation

to other ions, resistance was increased. When phosphorus was lower, resistance was decreased. Gassner and Hassebrauk, however, attributed this increased resistance to phosphorus. Recently, Samborski (73) attributed an increase in resistance of barley to powdery mildew to potassium, since the greatest increase in resistance was observed when combinations of low nitrogen and high potassium levels were employed. Results obtained by Smith and Blair (74) from field trial showed that phosphate enhanced mildew infection while nitrogen had no effect.

There are other recent reports (33,75) indicating that mildew development is not favored by high levels of nitrogen even when supplied as nitrate. Gassner and Hassebrauk (76) showed that ammonium nitrate was unfavourable to rust development in weak light but was favourable in stronger light or when carbohydrates were fed with the ammonium salt. Similar results were obtained by Daly (75), who found that when Thatcher variety was supplied with ammonium nitrogen it was completely resistant to Puccinia graminis tritici, race 56, at temperature between 65° and 75° F., but more susceptible when supplied equivalent amounts of nitrate nitrogen. Thatcher was susceptible to race 56 at temperatures below or above this range, regardless of the type of nitrogen supplied. It was also found that at lower temperatures, the ammonia content of leaves of plants supplied with ammonium sulphate was approximately four times as high as that of plants supplied with

nitrate nitrogen. High ammonia and urea contents found in infected resistant leaves were considered to be associated with rust resistance (77,78). Samborski (73) was also inclined to interpret this increased resistance as a result of an accumulation of ammonia which is toxic to living cells, and suggesting that cells of the fungus were probably more sensitive than those of the host.

Recently Colotelo (79) observed higher ammonia contents in relation to total nitrogen in infected rust-susceptible wheat and oats than in resistant ones. He also showed that the ratio of free amino acid nitrogen to ammonia nitrogen in infected leaves of the susceptible Little Club was approximately 10 : 1 to 12 : 1 at advanced stages, but in controls the ratio varied from 2 : 1 to 4 : 1. In infected and healthy leaves of the resistant Khapli, the ratio was approximately the same, i.e., 2 : 1. He was inclined to believe that ammonia had been used in the formation of glutamine in infected leaves of Little Club at a higher rate than in Khapli. He also found a greater amount of ammonia in rust infected leaves of susceptible varieties at advanced stages of infection than in resistant ones. This could be a result of the reduction in glutamine synthesis caused by increased rate of protein synthesis and low carbohydrate content.

Further evidence that the growth responses of the host plant are of more importance in determining resistance than the nature of the

element supplied was furnished by Last (80) working with powdery mildew. He obtained a close correlation between mildew development (number of colonies/cm^2 of leaf surface/day) and the rate of formation of new leaf surface, when growth was induced by new addition of nitrogen to deficient plants or to plants with various levels of nitrogenous supply.

It was shown that grains, leaves and straw from rusted wheat plants contained more protein than non-rusted materials (81,82). Yet, lower values of nitrogen in infected leaves have been observed (83,68). The percentage of nitrogen in leaves of six barley varieties infected with Puccinia hordei was found by Newton et al. (83) to be lower than that of healthy leaves. Novikov (68) made a comparative study of the effect of Uromyces striatus on the total, protein, and non-protein nitrogen in both infected and non-infected lucerne leaves. He found that leaves with either 50 per cent or 100 per cent infection had lower percentages of these three fractions of nitrogen than controls, and that leaves with 100 per cent infection had lower values than those with 50 per cent infection.

The extensive analyses of Gassner and his associates gave a rather clear picture of the effect of leaf rust on the nitrogen metabolism of its host. Gassner and Franke (84) found no significant differences in the ratio of protein nitrogen to soluble nitrogen between healthy and infected leaves of both resistant and susceptible

plants during the first two weeks after inoculation. After this period, however, protein and soluble nitrogen were higher in the leaves of infected susceptible and resistant plants than in uninfected controls. This increase was particularly striking in the susceptible variety.

Samborski and Shaw (85) found that the total nitrogen per unit dry weight remained constant in heavily rust-infected leaves of Little Club, but the total nitrogen content of heavily infected leaves of Khapli was lower than that of healthy leaves. This lower value of total nitrogen in the infected resistant Khapli was attributed to the rapid losses in dry weight and total nitrogen as a result of rapid proteolysis followed by transport of the soluble nitrogen to competing sinks in other parts of the growing plants.

Colotelo (79) obtained higher total protein and nitrogen contents in infected Little Club, and lower total protein and nitrogen contents in infected Khapli than in healthy leaves as rust infection progressed. His results for Little Club wheat are not in accord with those of Samborski and Shaw (85). The discrepancies are obviously due to the fact that they calculated their results on different basis. Colotelo calculated his nitrogen on the basis of per unit area, whereas Samborski and Shaw on per unit dry weight. The differences might be leveled off if calculated on the same basis, since Colotelo indicated that lower values of total nitrogen in

infected leaves of Little Club than in controls would result, if calculated on a dry weight basis.

There seems to be a general agreement between the results of Gassner et al. and Samborski et al. for susceptible varieties. However, there are discrepancies for resistant varieties. The cause for this difference is probably the heavier infection in plants used by Samborski et al. than that in plants used by Gassner et al.

Barrett and McLaughlin (86) studied the effect of leaf rust on the water soluble proteins of resistant and susceptible wheat varieties. They found that seedlings of the susceptible variety Michigan Amber upon infection by rust showed a change in the electrophoretic mobility of its water soluble proteins, which in diseased plants was more negative than in healthy plants. On the other hand, no detectable changes in electrophoretic mobility or amino acid constituents of the aqueous proteins in the resistant variety Pawnee were observed. The relative amounts of amino acids in hydrolyzates of the aquous protein from diseased seedlings of Michigan Amber possessed more dicarboxylic amino acids than that from healthy ones. They conjectured that the susceptibility of wheat to rust might be associated with a ratio of amino to carboxyl groups in the aqueous protein.

It is interesting to note that changes in protein synthesis in plants were also found to be associated with infections by

facultative parasites. In the study of nitrogen metabolism in sweet potato infected with black rot organism, Cerotostomella fimbriata, Akazawa and Uritani (55) demonstrated an activated synthesis of acid-insoluble nitrogen (proteins) at the expense of the acid soluble nitrogen (amino acids) in the adjacent uninvaded parts of an infected tuber. The acid-insoluble nitrogen was shown to be functional proteins -- mitochondrial enzymes -- which contributed to the protein recovered in the parts adjacent to the infected areas. They suggested that fungal growth might stimulate synthetic activity in tissues adjacent to the infected areas (87).

Chemical analyses of free and bound amino acids have been carried out by several investigators, in the hope of finding either quantitative or qualitative changes or both as a result of disease. The addition or complete removal of any amino acid resulting from parasitism is interesting. For example, Pseudomonas tabaci, which causes wildfire of tobacco, produces tabtoxin, an alpha-amino acid (a diaminodihydroxy pimelic acid), in the host, acting as a competitive inhibitor for l-methionine. Thus, tabtoxin interferes with the normal nitrogen metabolic processes of the host and causes the latter to show the typical symptom, a chlorotic halo around infections (88). However, almost all of the evidence in the literature on obligate parasites indicates that the difference in amino acids, if any, is in quantity rather than quality.

Samborski (73) could not find any apparent qualitative or quantitative differences in free amino acids between leaves of susceptible and resistant barley varieties. Upon infection by powdery mildew, marked changes appeared in the infected leaves. In the susceptible variety, the dicarboxylic acids decreased while the level of the amides increased as compared with healthy leaves. Leaves of the resistant variety showed a drop in dicarboxylic acids but little change in the corresponding amides.

With wheat rust, Samborski (73) found that infection was accompanied by a decrease in bound amino acids. Free amino acids increased in rust-free areas of Khapli leaves. The infected portion of Little Club leaves showed a similar increase in most amino acids while in the basal, rust-free areas of the same leaves, free amino acid content became lower. The diseased areas of Little Club leaves were higher in glutamine and lower in glutamic acid than corresponding areas of healthy leaves. He concluded that protein hydrolysis occurred in all parts of rusted leaves in both varieties and that the decrease in Little Club rust-free basal part was at least partially due to more movement of free amino acids into the rusted areas of this variety.

Colotelo (79) could not find any qualitative difference in non-protein and protein amino acids for rust-infected and uninfected leaves of Little Club and Khapli. He showed, however, that the

non-protein amino acid contents were higher in infected leaves of Little Club, and lower in Khapli, than in healthy leaves. Among the amino acids, glutamine and tryptophan were present in greater amounts in infected than in uninfected leaves of both varieties. The amounts found in infected leaves of Little Club were considerably higher than in infected leaves of Khapli. Colotelo concluded that increases in amino acid contents were associated with protein synthesis, thus the increases in Little Club were due to increased translocation and accumulation of the nitrogenous constituents. It is assumed by the writer that the references to translocation and accumulation made by Colotelo are based on the results of feeding experiments of Shaw and Samborski (85). Some of Colotelo's results appear difficult to reconcile with Samborski's findings.

Isotopic nitrogen has been used extensively in animal physiology. There are, however, only few references on the use of N^{15} as a tracer to study nitrogen metabolism in plants. Studies with heavy nitrogen, $N^{15}H_4Cl$, as a tracer element in infected tobacco leaves (89,90) showed for the earlier stages of infection that the virus protein had a higher relative content of N^{15} than the normal leaf protein. This implies a more direct route from NH_4Cl to virus protein than from the normal leaf protein to the virus. The suggestion was made with the assumption that the virus protein, once formed, is not further broken down and resynthesized which is

actually the case as was shown by Menighini et al. (90). All the information available suggests that the host metabolism is somehow altered under the influence of virus to synthesize the viral protein precursors, since the virus itself is not equipped with synthetic mechanisms, and that probably the final assemblage of viral protein is carried out by the viral ribonucleic acids.

Respiration

Respiration is a process which provides the main source of available energy and certain intermediates for synthesis and maintenance. Observations on respiratory rate are important in many diverse types of physiological studies. Their significance lies in the fact that almost any change in metabolism is reflected in the amount of energy involved, and this is detectable usually in the respiratory rates. However, they do not signify the factors affecting the oxygen consumed or the carbon dioxide eliminated, for we are dealing with a very complex system. Our knowledge of the biochemical processes involved in respiration is still too limited to allow us to present any specific explanation.

Respiratory changes as a result of disease have been observed by many investigators. Almost always an increase in the respiratory rate has been reported. Augmented respiration for rust-infected leaves was observed by Reed and Crabill (39), Yarwood

(59), Gretchusknikov (77), Sempio (91,92,93), Farkas and Király (94), Shaw and Samborski (53), and Samborski and Shaw (85), and for leaves infected by powdery mildew by Allen (41), Allen and Goddard (95), Sempio (96,97,91,92,93), Millerd and Scott (98), and Samborski and Shaw (85).

As a result of a series of studies on the metabolism of a variety of host-parasite complexes conducted over the entire cycle of the disease, Sempio (91,92,93,96,97) clearly demonstrated the fluctuations in photosynthesis and aerobic and anaerobic respirations, and particularly in 'wheat-Erysiphe complex'. During the first phase of parasitism, there was a marked increase in photosynthesis, compared to respiration. In the final phase of the disease, however, the situation was completely reversed. The respiratory activity attained very high values, while photosynthesis was much below normal. The ratio of photosynthesis : glycolysis : respiration was 1.71 : 1.23 : 1.19 in the initial phase parasitism, and 0.45 : 0.56 : 3.82 in the final phase. Whereas, the ratio found in the respective controls was arbitrarily taken as 1 : 1 : 1. Sempio (44) considered the dynamic balance of these metabolic functions in the host to be most important in determining its resistance or metabolic receptivity. He referred to the abnormal increase in photosynthesis over respiration in the first phase of infection as a defensive reaction and the enormous functional disequilibrium

at the end of the disease cycle as a result of the breakdown of resistance in the host.

The influence of mildew infection on the respiratory rate of wheat has been studied by a number of other investigators (99,100,101,95,41). They all obtained respiratory drifts similar to Sempio's. Pratt (100) reported that the respiratory intensity increased rapidly and reached a maximum 9 days after infection, with values almost triple the normal. After an interval of a few days, however, the respiratory rate decreased. Allen and Goddard (95) found that the respiratory intensity was at a maximum 6 days after inoculation, with values three to four times normal ones. They noted also a small increase in anaerobic respiration.

Sempio (44,102) reported that the peak of the respiration, sometimes four times the normal rate, coincided with the conidial formation of Erysiphe graminis. The time required to attain the peak and the extent of increased respiration above that of the controls were determined by the amount of infection. The increase in respiration in heavily infected leaves was reported as great as 650 per cent on fresh weight basis (95). It has been demonstrated by several workers (99,100,95) that the considerable increase in respiratory activity of the diseased tissues is not directly due to the respiratory activity of the parasite, but largely to the stimulation it exerts on the metabolism of the host. It was found

(99,100) that dusting leaves infected with Erysiphe graminis, with sulfur, which kills mildew, did not appreciably alter the respiration of the infected leaves. Allen and Goddard (95) showed by mechanical removal of the mildew mycelium, by differential poisoning of the mildew respiration with sodium azide, and by measurement of the respiration of the intact mildew isolated on epidermal strips, that most of the increased respiration was occurring in the non-invaded tissues of the leaf. The differentially inhibitory action of azide is, however, disputable, since the respiration of wheat leaves was shown by Farkas and Király to be highly sensitive to azide, but Allen and Goddard did not obtain any appreciable inhibition of respiration in mildew-infected leaves. Samborski and Shaw (85) reported that the increase in the rate of respiration of rust-infected wheat leaves, decreased rapidly with increasing distance from the center of the infection site. They regarded the increased respiration in rust-infected tissues as being similar to that found in mildew-infected tissues; in the sense that the respiration of the mycelium of the rust fungus was not directly responsible to any considerable extent for the observed respiratory increase in diseased tissue, but rather that the host tissues at and in the environs of infection were mainly responsible. This, however, has to be verified.

More recently, Millerd and Scott (98) carried out comparative studies on respiratory rates of four barley varieties affected by

Erysiphe graminis var. hordei. The varieties they used included susceptible, semi-resistant, resistant and highly resistant ones. They found that the presence of the pathogen resulted in augmented respiration in all cases. The increased respiratory rate of the four varieties, however, varied with host resistance to the parasite. In highly resistant barley strains there was a very rapid response to the invading parasite. As a result, a particularly rapid rise in respiration occurred, followed immediately by a return to normal levels. The respiratory rate of resistant plants commenced to increase one day after inoculation and a small increased respiration was maintained until three days after inoculation. With semi-resistant plants, the respiratory drift was quite similar to that of the resistant variety, with the difference that the increase did not begin until two days after inoculation, and reached a peak three days later, followed by a gradual decrease. A similar delay was observed in susceptible varieties. After the rate of respiration reached the maximum, it was maintained at this level for the rest of the experimental period (7 days).

Concerning the possible mechanism of the augmented respiration in mildew-infected leaves, Allen (54), in 1953, advanced a hypothesis based on data from Sempio's work, also on the results obtained by other investigators on the mode of action of certain antibiotics and the uncoupling agent 2,4-dinitrophenol. According

to Allen, the parasite produces substances, somewhat similar to the uncoupling agent 2,4-dinitrophenol, which diffuse into the non-invaded cells, and remove the Pasteur effect and also increase the rate of oxygen uptake. Upon calculation of Sempio's data, Allen found that the aerobic carbon dioxide production was more than three times the anaerobic in mildew-infected leaves, whereas the aerobic carbon dioxide production was less than three times the anaerobic in healthy controls. These results suggested to him that the Pasteur effect was abolished in diseased tissues under the complete uncoupling action of toxins produced by Erysiphe graminis. However, with complete uncoupling resulting in large reduction in the amount of energy-rich phosphate, very little energy is available to drive endergonic reactions, such as carbohydrate synthesis. Moreover, there are many indications that synthetic processes are accelerated in plants infected with obligate parasites. These indications led Allen to revise his original assumption, so that in 1954, he stated that the increase of phosphorus in the infection court and the stimulation of host cell growth by many obligate parasites and of synthesis of various substances by others, are evidence for an increase in the utilization of energy-rich phosphate as the cause of increased respiration (20). It would seem that an uncoupling action is not the primary or exclusive cause of the augmented respiration. He, however, believes that partial uncoupling may be possible.

A similar explanation has been proposed by Millerd and Scott (98), but without experimental support, except their finding that 2,4-dinitrophenol but not sucrose, glucose, succinate, nor citrate increased the respiratory rate of healthy leaves. No experiment on the effect of 2,4-dinitrophenol on infected leaves was carried out.

In their attempt to elucidate the possible mechanism of the respiratory increase in the uninvaded tissues of sweet potato infected with black rot, Akazawa and Uritani (55) and Akazawa (87) examined phosphate and nitrogen metabolisms as well as activities of mitochondrial enzymes. Akazawa and Uritani found that there was a decrease of inorganic phosphate with a concomitant increase of acid-soluble organic phosphate in diseased tissues, whereas both fractions of the control remained almost constant. Also, it was found that the protein nitrogen of mitochondria, microsomes, and supernatant fraction of the tissue was higher in the uninvaded parts of infected slices than in controls. Furthermore, the rate of oxygen uptake by mitochondria from the uninvaded parts of infected slices was about double that from uninfected slices, and phosphorylative activity was greater also. From this they concluded that as a result of the penetration of the fungus the protoplasm of the uninvaded tissue next to the infected one is stimulated to synthesize organic phosphate and mitochondrial enzymes, and

concomitantly the reaction

$$ATP \longrightarrow ADP + P_i$$

is accelerated, resulting in an increase in the respiratory rate. However, they regarded this mechanism as responsible in part only for the respiratory increase in the infected tissues; other factors involved would be: the uncoupling agent ipomeamarone, which is present in large amounts in infected tissues; polyphenol oxidase and cytochrome oxidase which are activated by the parasitic invasion (103,104,55,87).

Recently, Farkas and Király (94) advanced another hypothesis in attempting to explain the augmented respiration in rust infected tissues of wheat. Their idea is based on comparative studies of respirations in healthy and infected leaves under the influence of specific respiratory inhibitors. They found that the respiration of infected leaves and healthy controls was fairly sensitive to malonate, azide, and glycolytic inhibitors, such as iodoacetate and fluoride. On the other hand, healthy leaves were resistant to the action of cyanide, while the infected ones were sensitive. They agreed in part with Allen's suggestion of 'prevention of Pasteur effect', and further suggested, (a) the existence of an aerobic oxidation of the end products of glycolytic process without the participation of the tricarboxylic acid cycle, and (b) the partial elimination of a protective mechanism for the metal-containing oxidase in the infected leaves.

Conclusion

The intricacy and complexity of the phenomena of host-parasite association and disease resistance were made quite obvious in the review of the literature. Many hypotheses have been proposed by various workers to explain these phenomena, but so far none of these are satisfactory. However, among these hypotheses, that postulating metabolic resistance appears to be more promising than the others. Much more evidence in support of this hypothesis is needed in order to make it generally accepted by the critical plant pathologists.

MATERIALS AND METHODS

Preparation of Plant Materials

(1) Culture of Wheat

Apparently sound wheat seeds of uniform size of varieties Little Club, Golden Ball, Khapli, and Gabo were selected, and surface sterilized by immersing them for 10-15 min. in a solution containing three per cent chlorine and a trace of wetting agent. Then, the seeds were rinsed with generous amounts of water and placed on sterile moist filter paper in a glass vessel providing conditions suitable for germination. During germination the seeds were kept in darkness at room temperature. Water was added whenever it was necessary. After reaching a height of approximately two inches, the seedlings were transplanted to nutrient solution contained in pint-size jars and placed in the greenhouse.

Half strength of number 2 Hoagland solution, with ferric citrate and other microelements added, was employed in these studies. The culture solution was changed every three days, thus eliminating the need for aerating the solution.

Some of the plant material was obtained from plants grown from seed in greenhouse soil (one part sand and three parts loam) in pots or flats.

(2) Culture of Rust

The rust fungus (*P. graminis tritici* race 15B-1, for most experiments and 34 for a few) was cultured on Little Club wheat seedlings to

provide uredospores as required for inoculating experimental plants. The spores were collected from infected leaves and stored dry in a refrigerator at about 24°F.

(3) Inoculation of Wheat Leaves

At the two-leaf stage, the seedlings were inoculated with a urediospore suspension. The inoculated and uninoculated control plants were placed for 48 hours in a moist chamber with transparent plastic walls, located in a greenhouse. During incubation the humidity in the chamber was maintained at 100 per cent with an electrically operated humidifier. Temperature in the chamber varied between 65° to 90°F. At the end of 48 hours, all plants were transferred onto a bench in the greenhouse. The resultant infection in every case was light to moderate. The seedlings remained in the greenhouse until the lesions attained a diameter of about 0.5 mm. and then they were exposed to C-14-labelled carbon dioxide or treated with N^{15}-enriched potassium nitrate.

Treatment of Plants and Methods of Analyses

Procedures followed in treating plants with isotopic elements and methods of chemical analyses are described later with the results of each experiment in which they were used.

EXPERIMENTAL RESULTS

Effect of Puccinia graminis tritici on the Nitrogen Metabolism of Susceptible, Semi-resistant, and Resistant Wheat Seedlings

Attempts to find an explanation for the resistance of plants to disease have been made in recent years by studying the effect of disease on the presence and concentration of various constituents of susceptible and resistant plants (see Literature Review). In nearly every case, the differences which were found were quantitative rather than qualitative ones. These differences appear to be not correlated well enough with resistance to be the basis for a satisfactory explanation for the resistance of a plant to a parasite. However, no attempts had been made to correlate resistance to the metabolic activity of nitrogenous compounds. Therefore, experiments were performed in which the effect of rust on both concentration and metabolic activity of nitrogen in a susceptible, a semi-resistant, and a resistant variety was studied.

Materials and Methods

About 120 wheat seedlings of each of the varieties Little Club, Golden Ball, and Khapli, which are susceptible, semi-resistant, and resistant, respectively, were grown in nutrient solution. When the seedlings were at the two-leaf stage half of them were inoculated with urediospores of *Puccinia graminis tritici* race 15B-1. The different varieties were not grown at the same time: Little Club and Khapli were sown on May 6 and

Golden Ball on April 21, 1956.

(1) Treatment of Plants with Isotopic Nitrogen

Approximately 10 days after inoculation, signs of pustules appeared on the leaves. The plants were then transferred to 200 ml of nutrient solution containing 0.25 g. per liter of N^{15}-enriched potassium nitrate (30 per cent N^{15} atom excess) as the sole source of nitrogen and placed 2 feet below an incandescent bulb of 500 W. as a source of continuous illumination for a period of 24 hours. About 6-8 hours after the seedlings were placed in the solution, it was necessary to add water to the vessel in which they stood, to restore the volume. Before and after the treatment with N^{15} the roots of the plants were washed with tap water for two hours in order to remove the nitrogen adsorbed on the root surface.

(2) Killing and Drying Plant Materials

At the end of isotopic-nitrogen feeding, the plants were killed by placing them for one hour in an oven at 100° C. and drying in another at 70° C.

(3) Preparation of Plant Materials for Analysis

Each seedling was divided into three parts: namely, the first two leaves (oldest leaves), the younger remaining leaves, and the roots. Each of these parts for each of three seedlings was used for the determination

of total nitrogen and the isotopic ratio. For fractionation to alcohol soluble and alcohol insoluble nitrogen, a 0.5 g. sample of the dry tissue of 20 seedlings ground together in a Wiley mill with a 20-mesh screen was used. The sample was ground in a mortar with 80 per cent alcohol. It was then transferred quantitatively into a ground joint Erlenmeyer flask using further additional quantity of alcohol, which gave a final volume of approximately 60 ml. After shaking gently, the mixture was allowed to stand for 10 hours or overnight. The supernatant was transferred in successive small portions into a beaker. Each portion was evaporated rapidly until dry under a stream of air before another was transferred. The tissue was extracted twice again then refluxed for an hour, in each case with about 60 ml. of 80 per cent alcohol. The dry extract (alcohol soluble substance) in the beaker was dissolved in about 60-80 ml. of water and the chlorophyll was extracted twice in a separatory funnel with a total of 40 ml. of chloroform. The chloroform extract was washed with two small portions of water. The washings were added to the aqueous solution of the alcohol soluble substances. The chloroform was let to evaporate to a smaller volume in a beaker, the aqueous solution was concentrated in vacuo in a Roto-Vap. The volume of each fraction was then brought to 5 ml.

(4) Determination of Total Nitrogen

Tissue samples weighing from 0.035 to 0.2 (depending on the part of the plant) and 2 ml. aliquots of the fractions were digested using the

reductive semi-micro Kjeldahl (zinc dust) method described by the Association of Official Agricultural Chemists (105). At the end of the recommended period of digestion, the digest was cooled, few crystals of $KMnO_4$ were added, and digestion continued for three additional hours. The digest was made alkaline and distilled in an all glass distillation apparatus of the Kemmerer-Hallet type. The distillate (about 25 ml.) was collected in a flask containing 10 ml. of N/10 H_2SO_4. The total nitrogen of each sample was determined colorimetrically by nesslerization of a small aliquot of the distillate. The remaining portion of the distillate was boiled down to a small volume and kept in a deep-freeze until it was used for isotopic ratio determination.

(5) Determination of Isotopic Nitrogen

The conversion of ammonia in the distillate to elementary nitrogen was carried out by reaction with alkaline hypobromite <u>in vacuo</u> in an apparatus similar to that described by Rittenberg (106). The gaseous nitrogen collected was introduced into a Consolidated Nier isotope-ratio Mass spectrometer and the ratio of $N^{14}N^{14}$ and $N^{14}N^{15}$ was determined. The atom per cent N^{15} was calculated and from it was subtracted the N^{15} concentration in air or normal nitrogen sample to obtain atom N^{15} per cent excess.

(6) Statistical Analysis

The atom N^{15} per cent excess was determined on each part of each of three seedlings, and on duplicates of the alcohol soluble and insoluble fractions of samples (0.5 gr.) of tissues from twenty seedlings. An analysis of variance was performed on the results obtained.

Results of Preliminary Experiment on the Effect of Puccinia graminis tritici on the Nitrogen Metabolism of Little Club Wheat Seedlings

In a preliminary experiment, nitrogen metabolism of healthy and rust-infected seedlings of only one variety, namely Little Club, was studied. This variety is highly susceptible to the rust race (15B-1) used. The first two leaves possessed each an average of five pustules, 0.5-1.0 mm. in diameter. Table 1 gives the results on the N^{15} atom per cent excess in the different parts of healthy and diseased seedlings, 10 days after the latter were inoculated on their first two leaves with race 15B-1 of Puccinia graminis tritici. The concentration of N^{15} in the infected first two leaves and non-infected younger leaves of diseased plants is 36.7 and 107 per cent higher, respectively, than in corresponding leaves of healthy plants. However, the N^{15} concentration in the roots of healthy plants is 7.1 per cent higher than that in diseased ones. All these differences are

Table 1. N^{15} atom per cent excess in different parts of healthy and diseased Little Club seedlings, 10 days after the latter were inoculated on their first two leaves with P. graminis tritici (race 15B-1).

Plant Part	Healthy	Diseased	H-D
First two leaves	0.862±0.038(a)	1.178±0.038	-0.316**
Younger leaves	0.406	0.841	-0.436**
Roots	2.079	1.931	+0.148**
Mean	1.116±0.022(b)	1.317±0.022	-0.201**

(a) Mean of 2 replicates and standard error. L.S.D. ± 0.093 (P=0.05) ± 0.142(P=0.01);
(b) L.S.D. ± 0.053(P=0.05); ±0.081(P=0.01)
** significant at 1 per cent level.

highly significant statistically. Differences in N^{15} concentration between the different parts of each plant, although significant in all cases, are much greater in the case of healthy plants than in diseased ones.

The concentration of Kjeldahl nitrogen in different parts of healthy and diseased Little Club seedlings is given in Table 2. Apparently, there is no significant difference between the values obtained for healthy and diseased plant parts. The first two leaves and younger leaves have about the same nitrogen concentration while roots have a relatively lower concentration.

Table 2. Concentration of Kjeldahl nitrogen in different parts of healthy and diseased Little Club seedlings, 10 days after the latter were inoculated on their first two leaves with P. graminis tritici (race 15B-1).

Plant Part	Healthy (mg N/g dry wt)	Diseased (mg N/g dry wt)	H-D
First two leaves	60.4	59.8	+ 0.6
Younger leaves	59.6	61.5	- 1.9
Roots	52.2	50.1	+ 2.1

In Table 3 are given the N^{15} atom per cent excess in different fractions of the first two leaves of healthy and diseased

Table 3. N^{15} atom per cent excess in different fractions of the first two leaves of healthy and diseased Little Club seedlings, 10 days after the latter were inoculated on their first two leaves with P. graminis tritici (race 15B-1).

Fraction	Healthy	Diseased	H-D
Chlorophyll	0.764±0.01019(a)	0.953±0.01019	-0.189**
Alcohol soluble (minus chlorophyll)	1.109	1.547	-0.438**
Alcohol insoluble	0.088	0.135	-0.047**
Mean	0.654±0.0058(b)	0.878±0.0058	-0.224**

(a) Mean of duplicates and standard error. L.S.D. ± 0.0244 (P=0.05); ±0.0377 (P=0.01);
(b) L.S.D. ± 0.014 (P=0.05); ±0.021 (P=0.01).

seedlings. The concentration of N^{15} in the chlorophyll, alcohol soluble (minus chlorophyll), and alcohol insoluble nitrogen fractions of diseased leaves is 24.7, 39.5, and 53.4 per cent higher, respectively, than in corresponding fractions of healthy leaves. These differences are highly significant. In the case of both healthy and diseased plants, the alcohol soluble (minus chlorophyll) fraction has the highest N^{15} concentration, chlorophyll has 30-40 per cent less, and alcohol insoluble about 90 per cent less. Urediospores collected from infected plants immediately after they were allowed to absorb N^{15} contained 0.597 N^{15} atom per cent excess.

Results of an Experiment with a Susceptible, a Semi-resistant and a Resistant Variety.

Kjeldahl nitrogen concentration in different parts of seedlings.

Following the preliminary experiment reported above, a more complex one was performed. It included Little Club, Golden Ball, and Khapli which are susceptible, semi-resistant, and resistant, respectively, to race 15B-1. It was found for both Little Club (Table 4) and Golden Ball (Table 5) that the concentration of total nitrogen in the various parts of healthy seedlings is not significantly different from that of corresponding parts of diseased seedlings. In the case of the resistant variety Khapli, there is significantly less nitrogen in each part of the diseased plants

Table 4. Concentration of Kjeldahl nitrogen in different parts of healthy and diseased Little Club seedlings, 10 days after the latter were inoculated on their first two leaves with P. graminis tritici (race 15B-1).

Plant Part	Healthy (mg N/g dry wt)	Diseased (mg N/g dry wt)	H-D
First two leaves	47.3±2.65(a)	49.6±2.65	- 3.3
Younger leaves	47.9	48.7	- 0.8
Roots	39.0	38.9	+ 0.1
Mean	44.7±1.24(b)	45.7±1.24	- 1.0

(a) Mean of 3 replicates and standard error. L.S.D. ± 5.77 (P=0.05); ±8.09 (P=0.01);
(b) L.S.D. ±2.7 (P=0.05)

Table 5. Concentration of Kjeldahl nitrogen in different parts of healthy and diseased Golden Ball seedlings, 10 days after the latter were inoculated on their first two leaves with P. graminis tritici (race 15B-1).

Plant Part	Healthy (mg N/g dry wt)	Diseased (mg N/g dry wt)	H-D
First two leaves	41.1±1.725(a)	41.6±1.725	- 0.5
Younger leaves	44.7	43.4	+ 1.3
Roots	42.3	38.9	+ 3.4
Mean	42.7±0.81(b)	41.3±0.81	+ 1.4

(a) Mean of 3 replicates and standard error. L.S.D. ± 3.75 (P=0.05);
(b) L.S.D. ± 2.57 (P=0.05)

than in corresponding parts of healthy plants (Table 6). Roots of all three varieties, although probably not significantly so in Golden Ball, have a lower concentration of nitrogen than leaves. Of each part of the three varieties, those of resistant Khapli have lowest concentration of nitrogen.

Table 6. Concentration of Kjeldahl nitrogen in different parts of healthy and diseased Khapli seedlings, 10 days after the latter were inoculated on their first two leaves with P. graminis tritici (race 15B-1).

Plant Part	Healthy (mg N/g dry wt)	Diseased (mg N/g dry wt)	H-D
First two leaves	38.1±0.205(a)	37.2±0.205	+ 0.9**
Younger leaves	42.0	40.9	+ 1.1**
Roots	32.0	28.4	+ 3.6**
Mean	37.4±0.97(b)	35.4±0.97	+ 2.0

(a) Mean of 3 replicates and standard error. L.S.D. ± 0.45 (P=0.05); ± 0.63 (P=0.01);
(b) L.S.D. ± 2.11 (P=0.05)

N^{15} atom per cent excess in different parts of seedlings.

The concentration of N^{15} in the first two leaves of healthy and diseased seedlings of Little Club (susceptible), Golden Ball (semi-resistant), and Khapli (resistant) are presented in Table 7.

Table 7. N^{15} atom per cent excess in the first two leaves of healthy and diseased seedlings of varieties differing in their reaction to P. graminis tritici (race 15B-1).

Reaction (Variety)	Healthy	Diseased	H-D
Susceptible (Little Club)	1.080±0.159(a)	0.916±0.159	+ 0.164
Semi-resistant (Golden Ball)	0.658	1.159	- 0.501**
Resistant (Khapli)	1.813	1.110	+ 0.705**

(a) Mean of 3 replicates and standard error. L.S.D. ±0.346 (P=0.05); ±0.485 (P=0.01)

The N^{15} concentrations in the first two leaves of healthy and diseased Little Club plants are not significantly different. This result is different from that obtained in the preliminary experiment (Table 1) in which the N^{15} concentration in rust-infected leaves was higher than that in healthy plants. The cause of this discrepancy is not known with certainty, but several possible factors which may have contributed to this difference are: (a) the plants for the two experiments were grown in the greenhouse at different times of the year and undoubtedly they were subjected to different environmental conditions; (b) the amount of

infection was not the same in the two experiments; in the first one infected leaves had an average of five pustules each, while in the second they had an average of nine; and (c) the stage of infection was slightly different; in the first experiment the epidermis over a large percentage of the pustules was ruptured, whereas in the second experiment, it was unruptured over the majority of the pustules. The percentage of N^{15} atoms was 76 per cent greater in the rust-infected first two leaves of the semi-resistant variety Golden Ball than in healthy controls. In the case of the resistant variety Khapli, the difference is in the opposite direction, since in the infected leaves the N^{15} concentration is 39 per cent less than in the first two leaves of healthy plants. These differences are highly significant. The N^{15} concentration in healthy plants was highest in the resistant variety, intermediate in the susceptible, and lowest in the semi-resistant one, whereas in diseased plants, the differences between the varieties are not significant.

The N^{15} atom per cent excess in the younger leaves of healthy and diseased seedlings of varieties differing in their resistance to rust race 15B-1 is given in Table 8. The concentration of N^{15} in the non-infected remaining leaves of diseased seedlings of both susceptible and semi-resistant varieties is 31.7 and 41 per cent higher, respectively, than in corresponding leaves on the healthy plants. In contrast with this, the -

concentration of N^{15} in the non-infected leaves of diseased plants of the resistant variety is 21.4 per cent lower than it is in the healthy plants of the same variety. In the healthy plants, there is a direct correlation between resistance and concentration of

Table 8. N^{15} atom per cent excess in the younger leaves of healthy and diseased seedlings of varieties differing in their reaction to P. graminis tritici (race 15B-1).

Reaction (Variety)	Healthy	Diseased	H-D
Susceptible (Little Club)	0.445±0.037(a)	0.586±0.037	- 0.141**
Semi-resistant (Golden Ball)	0.506	0.714	- 0.208**
Resistant (Khapli)	0.571	0.449	+ 0.122**

(a) Mean of 3 replicates and standard error. L.S.D. ± 0.044 (P=0.05); ±0.115 (P=0.01)

N^{15} in the younger leaves, while in the diseased plants, the semi-resistant variety has the highest, the susceptible an intermediate, and the resistant the lowest concentration of N^{15} in all three varieties.

The concentration of N^{15} in the roots of healthy and diseased seedlings of a susceptible, a semi-resistant, and a resistant is shown in Table 9. The concentration of N^{15} is

significantly higher in the roots of diseased seedlings of both the susceptible (Little Club) and the semi-resistant (Golden Ball) varieties. The increase in N^{15} concentration as a result of disease is 12.7 per cent in the case of Little Club and 38.2 per cent in the case of Golden Ball. There is no significant difference in the N^{15} concentration in the roots of healthy and diseased seedlings of the resistant Khapli variety. Healthy Little Club has a

Table 9. N^{15} atom per cent excess in the roots of healthy and diseased seedlings of varieties differing in their reaction to P. graminis tritici (race 15B-1).

Reaction (Variety)	Healthy	Diseased	H-D
Susceptible (Little Club)	1.421±0.022(a)	1.601±0.022	- 0.180**
Semi-resistant (Golden Ball)	1.258	1.738	- 0.480**
Resistant (Khapli)	1.271	1.256	+ 0.150

(a) Mean of 3 replicates and standard error. L.S.D. ±0.049 (P=0.05); ±0.069 (P=0.01)

higher concentration of N^{15} in the roots than the other two varieties which are not significantly different in this respect. For diseased plants the decreasing order in which the three varieties can be placed with respect to the N^{15} concentration in their roots is

Golden Ball, Little Club, and Khapli. This order is the same as that in which they fall with respect to their N^{15} concentration in the younger leaves.

The preceding results on the N^{15} atom per cent excess in the three different parts of healthy and diseased seedlings of the three different varieties are presented graphically in Fig. 1. The histograms show in a striking manner, the difference in N^{15} concentration in each of the different parts of healthy and diseased seedlings. They show that, except for the first two leaves of Little Club for which there is no difference, for both Littlbe Club and Golden Ball the N^{15} concentration is higher in the parts of diseased seedlings than in the corresponding parts of healthy seedlings. In the case of Khapli, the parts of a healthy seedling have a higher N^{15} concentration than corresponding parts of a diseased seedling, except for the roots which are not significantly different in this respect.

N^{15} atom per cent excess in different fractions of the first two leaves.

The N^{15} atom per cent excess in chlorophyll fraction of the first two leaves of healthy and diseased seedlings of a susceptible, a semi-resistant, and a resistant variety is given in Table 10. The chlorophyll fraction of the first two leaves of the diseased susceptible plants has significantly higher concentration

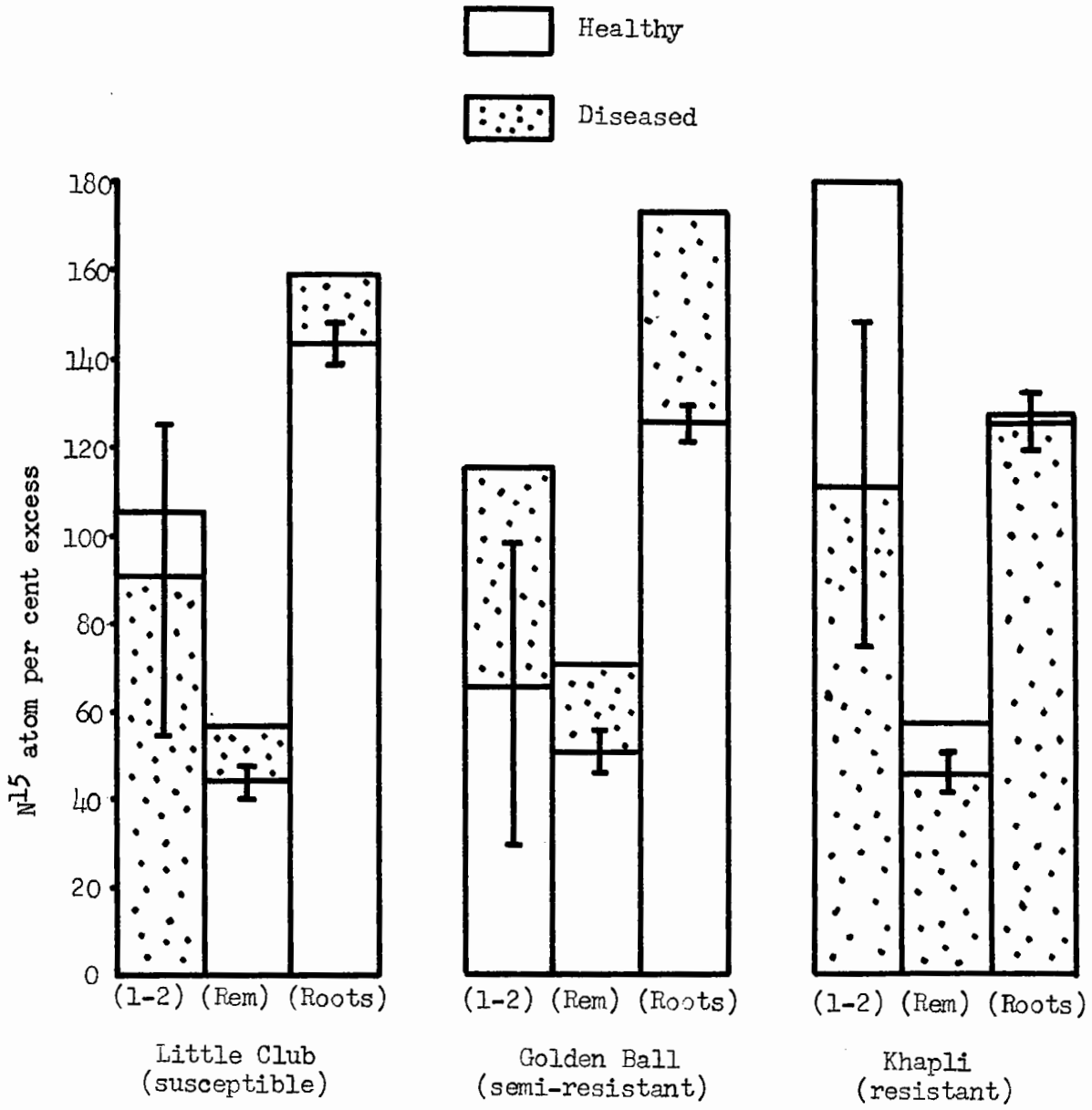


Fig. 1. N^{15} atom per cent excess in the first 2 leaves (1-2), the remaining leaves (Rem), and roots of healthy and diseased seedlings of a susceptible, a semi-resistant and a resistant wheat variety. (The L.S.D. (P = 0.05) is indicated in each bar)

of N^{15} than that of healthy plants. On the other hand, the chlorophyll fraction of diseased semi-resistant and resistant plants is significantly lower, 32.5 and 41.7 per cent, respectively, in N^{15} concentration than that of the corresponding healthy plants. A comparison between the results obtained for the

Table 10. N^{15} atom per cent excess in the chlorophyll fraction of the first two leaves of healthy and diseased seedlings of varieties differing in their reaction to P. graminis tritici (race 15B-1).

Reaction (Variety)	Healthy	Diseased	H-D
Susceptible (Little Club)	0.090±0.0034(a)	0.103±0.0034	- 0.013*
Semi-resistant (Golden Ball)	0.203	0.137	+ 0.066**
Resistant (Khapli)	0.199	0.116	+ 0.083**

(a) Mean of duplicates and standard error. L.S.D. ± 0.0084 (P=0.05); ±0.0127 (P=0.01)
* Significant at 5 per cent level

healthy seedlings of the three varieties show that the resistant and the semi-resistant varieties are not significantly different, while the susceptible has significantly lower values. The results for the diseased seedlings of the three varieties, are significantly different one from the other, the semi-resistant variety showing the

highest, the resistant, an intermediate, and the susceptible, the lowest concentration.

It should be noted that this and subsequent analyses of plant fractions were not replicated, and that the standard error was calculated from results of duplicate determinations which are probably much less variable than replicates.

The concentration of N^{15} in the alcohol soluble fraction (minus chlorophyll) of the first two leaves of healthy and diseased seedlings of varieties differing in their reaction to Puccinia graminis tritici (race 15B-1) is shown in Table 11. Among the healthy plants, the susceptible variety contains the lowest concentration of N^{15} in its alcohol soluble fraction, the resistant variety the highest, while the semi-resistant variety shows an intermediate value between the other two varieties. The concentration of N^{15} in the alcohol soluble fraction of healthy susceptible plants is not significantly changed as a result of disease, while that of semi-resistant and resistant plants is decreased by 10.6 and 27.9 per cent, respectively, and consequently their N^{15} concentration in this fraction is not significantly different.

Results on the concentration of N15 in the alcohol insoluble fraction of the first two leaves of healthy and diseased seedlings of varieties differing in their reaction to rust race 15B-1 are presented in Table 12. The concentration of N^{15} in the

Table 11. N^{15} atom per cent excess in the alcohol soluble fraction of the first two leaves of healthy and diseased seedlings of varieties differing in their reaction to P. graminis tritici (race 15B-1).

Reaction (Variety)	Healthy	Diseased	H-D
Susceptible (Little Club)	0.605±0.02(a)	0.589±0.02	+ 0.016
Semi-resistant (Golden Ball)	0.757	0.677	+ 0.080**
Resistant (Khapli)	0.936	0.675	+ 0.261**

(a) Mean of duplicates and standard error. L.S.D. ± 0.0489 (P=0.05); ±0.0741 (P=0.01)

Table 12. N^{15} atom per cent excess in the alcohol insoluble fraction of the first two leaves of healthy and diseased seedlings of varieties differing in their reaction to P. graminis tritici (race 15B-1).

Reaction (Variety)	Healthy	Diseased	H-D
Susceptible (Little Club)	0.057±0.0034(a)	0.069±0.0034	- 0.012*
Semi-resistant (Golden Ball)	0.056	0.054	+ 0.002
Resistant (Khapli)	0.097	0.090	+ 0.007

(a) Mean of duplicates and standard error. L.S.D. ± 0.0084 (P=0.05); ±0.0128 (P=0.01)

alcohol insoluble fraction of the rust-infected susceptible plants is 21.1 per cent higher than that of healthy plants. There is no significant difference in this respect between the healthy and diseased seedlings of both semi-resistant and resistant varieties. The N^{15} concentration in this fraction for both diseased and healthy resistant plants is the highest one of all obtained for the three varieties. The N^{15} concentration in healthy seedlings of susceptible and semi-resistant plants are equal. But because the N^{15} concentration in healthy susceptible plants is increased as a result of disease, it becomes significantly higher than that of diseased semi-resistant plants.

N^{15} atom per cent excess in different fractions of the younger leaves

Results on the concentration of N^{15} in the chlorophyll fraction of the younger leaves of healthy and diseased seedlings of a susceptible, a semi-resistant, and a resistant variety to rust race 15B-1 are given in Table 13. Among the healthy plants of all three varieties, those of the susceptible variety have the lowest concentration of N^{15} in the chlorophyll fraction of their younger leaves, those of the semi-resistant variety the highest, while those of the resistant variety have an intermediate value between the other two varieties. In the diseased susceptible

Table 13. N^{15} atom per cent excess in the chlorophyll fraction of the younger leaves of healthy and diseased seedlings of varieties differing in their reaction to P. graminis tritici (race 15B-1).

Reaction (Variety)	Healthy	Diseased	H-D
Susceptible (Little Club)	0.178±0.0113(a)	0.192±0.0113	- 0.014
Semi-resistant (Golden Ball)	0.405	0.370	+ 0.035*
Resistant (Khapli)	0.291	0.195	+ 0.096**

(a) Mean of duplicates and standard error. L.S.D. ± 0.0276 (P=0.05); ±0.0418 (P=0.01)
* Significant at 5 per cent level

plants, the non-infected leaves are not significantly different from corresponding leaves on healthy plants, while in those of diseased semi-resistant and resistant plants the N^{15} concentration is 8.6 and 33 per cent less, respectively, than in corresponding leaves of healthy plants.

The N^{15} atom per cent excess in the alcohol soluble fraction (minus chlorophyll) of the younger leaves of healthy and diseased seedlings of varieties differing in their reaction to rust race 15B-1 is given in Table 14. The concentrations of N^{15} in the alcohol soluble fraction of the younger leaves of healthy seedlings of semi-resistant and resistant plants are equal, that of the

Table 14. N^{15} atom per cent excess in the alcohol soluble fraction of the younger leaves of healthy and diseased seedlings of varieties differing in their reaction to P. graminis tritici (race 15B-1).

Reaction (Variety)	Healthy	Diseased	H-D
Susceptible (Little Club)	0.969±0.0112(a)	0.994±0.0112	- 0.025
Semi-resistant (Golden Ball)	1.071	1.155	- 0.084**
Resistant (Khapli)	1.071	0.739	+ 0.332**

(a) Mean of duplicates and standard error. L.S.D. ± 0.0276 (P=0.05): ±0.0418 (P=0.01)

susceptible plants is significantly lower. Compared to the healthy controls, the value obtained for the diseased susceptible plants is not significantly different, while that for semi-resistant plants is significantly higher. In contrast to these results, the resistant plants show a reduction of 31 per cent in the concentration of N^{15} as a result of disease. Diseased resistant plants have the lowest values of the three varieties, while the semi-resistant plants have the highest.

The concentration of N^{15} in the alcohol insoluble fraction of the younger leaves of healthy and diseased seedlings of a susceptible,

a semi-resistant, and a resistant variety to rust race 15B-1 are presented in Table 15. Among the healthy plants, the susceptible ones contain the lowest concentration of N^{15} in

Table 15. N^{15} atom per cent excess in the alcohol insoluble fraction of the younger leaves of healthy and diseased seedlings of varieties differing in their reaction to P. graminis tritici (race 15B-1).

Reaction (Variety)	Healthy	Diseased	H-D
Susceptible (Little Club)	0.202±0.0092(a)	0.286±0.0092	- 0.084**
Semi-resistant (Golden Ball)	0.297	0.337	- 0.040**
Resistant (Khapli)	0.329	0.282	+ 0.047**

(a) Mean of duplicates and standard error. L.S.D. ±0.0226 (P=0.05); ±0.0342 (P=0.01)

its alcohol insoluble fraction, the resistant plants the highest, while the semi-resistant plants give an intermediate value between the other two varieties. The concentration of N^{15} in this fraction of all three varieties are significantly changed as a result of rust infection. The concentration of N^{15} in the alcohol insoluble fraction of diseased seedlings of the susceptible and semi-resistant varieties is 41.6 and 13.5 per cent higher, respectively, than that of the

corresponding healthy controls, while that of the diseased seedlings of the resistant variety is 14.3 per cent lower than that of the healthy plants of the same variety. All these differences are highly significant.

N^{15} atom per cent excess in different fractions of the roots

The concentration of N^{15} in the alcohol soluble fraction of the roots of healthy and diseased seedlings of varieties differing in their reaction to rust race 15B-1 are given in Table 16. The alcohol soluble fraction of the roots

Table 16. N^{15} atom per cent excess in the alcohol soluble fraction of roots of healthy and diseased seedlings of varieties differing in their reaction to P. graminis tritici (race 15B-1).

Reaction (Variety)	Healthy	Diseased	H-D
Susceptible (Little Club)	1.963±0.095(a)	2.082±0.095	- 0.119
Semi-resistant (Golden Ball)	2.763	3.079	- 0.316*
Resistant (Khapli)	2.976	2.872	+ 0.104

(a) Mean of duplicates and standard error. L.S.D. ± 0.23 (P = 0.05); ± 0.35 (P = 0.01)

of the diseased seedlings of both susceptible and resistant plants does not differ significantly in concentration of N^{15} from that of corresponding healthy plants. On the other hand, the roots of semi-resistant diseased plants are significantly higher, by 11.4 per cent, in N^{15} concentration than that of the corresponding healthy plants. Among the healthy plants, the susceptible variety has the lowest value, while there is no significant difference between values obtained from semi-resistant and resistant varieties. Different results are obtained with roots of diseased seedlings, in which the highest value is found for the semi-resistant variety, the lowest for the susceptible one, and a value intermediate between the two others, for the resistant variety.

The concentration of N^{15} in the alcohol insoluble fraction of the roots of healthy and diseased seedlings of a susceptible, a semi-resistant, and a resistant variety is presented in Table 17. The concentration of N^{15} in the alcohol insoluble fraction of roots of both healthy and diseased seedlings of the semi-resistant variety is the lowest of the three varieties. Results obtained for healthy seedlings of both susceptible and resistant varieties are equal. The concentration of N^{15} of susceptible variety is increased by 18.7 per cent, while that of resistant variety is increased by only 1.8 per cent, as a result of rust infection. Both increases are highly significant.

The value obtained for this fraction of diseased semi-resistant plants is lower than that of healthy plants of the same variety.

Table 17. N^{15} atom per cent excess in the alcohol insoluble fraction of roots of healthy and diseased seedlings of varieties differing in their reaction to P. graminis tritici (race 15B-1).

Reaction (Variety)	Healthy	Diseased	H-D
Susceptible (Little Club)	0.380±0.0005(a)	0.451±0.0005	- 0.071**
Semi-resistant (Golden Ball)	0.344	0.322	+ 0.022**
Resistant (Khapli)	0.380	0.387	- 0.007**

(a) Mean of duplicates and standard error. L.S.D. ± 0.0013 (P = 0.05); ± 0.002 (P = 0.01)

Kjeldahl nitrogen concentration in fractions of different plant parts

The concentration of Kjeldahl nitrogen in various fractions observed in the healthy and diseased Little Club wheat seedlings is given in Table 18. In healthy plants, the first two leaves have lower values for chlorophyll and alcohol insoluble nitrogen than the younger leaves, but a higher value for alcohol soluble nitrogen.

Table 18. Concentration of Kjeldahl nitrogen in different fractions of healthy and diseased Little Club seedlings, 10 days after the latter were inoculated on their first two leaves with P. graminis tritici (race 15B-1).

Little Club				
Plant Part	Fraction	Healthy (mg N/0.5 g dry wt)	Diseased (mg N/0.5 g dry wt)	Dx100/H
First two leaves	Chlorophyll	0.62(a)	0.60	96.7
	Alcohol soluble (minus chlorophyll)	10.16	9.06	89.2
	Alc. insoluble	12.92	15.14	117.2
Younger leaves	Chlorophyll	0.77	0.54	70.1
	Alcohol soluble (minus chlorophyll)	7.81	7.50	96.0
	Alc. insoluble	15.42	16.36	106.1
Roots	Alcohol soluble	9.27	5.72	61.7
	Alc. insoluble	10.23	13.78	134.7

(a) Mean of duplicates.

Apparently, there is no significant difference between the values obtained for chlorophyll fraction of the first two leaves of healthy and diseased plants. On the other hand, the concentration of chlorophyll nitrogen in non-infected younger leaves of diseased plants is

29.9 per cent lower than that of the corresponding healthy plants. As a result of infection by rust, the concentration of nitrogen in alcohol soluble fraction of the first two leaves is decreased by 10.8 per cent and is increased by 17.2 per cent in alcohol insoluble fraction, and similar changes take place in the younger leaves. In the roots of diseased plants, the nitrogen concentration in the alcohol soluble and in the alcohol insoluble fractions is significantly decreased by 38.3 per cent and increased by 34.7 per cent, respectively. Although there is, as a result of disease, an increase in alcohol insoluble nitrogen in all three parts, the greatest increase takes place in the roots. Also, as a result of disease , the concentration of alcohol insoluble nitrogen in the rust-infected first two leaves reaches a level equal to that in the younger leaves of the same plant.

In Table 19 are given the nitrogen concentration of various fractions of healthy and diseased Golden Ball (semi-resistant) wheat seedlings. In healthy plants, the difference in chlorophyll nitrogen content between the first two and younger leaves is probably not significant, while the alcohol soluble nitrogen concentration is higher by 21.6 per cent and the alcohol insoluble lower by 19.7 per cent in the first two leaves than in the younger leaves. In diseased plants, the concentration of nitrogen in the chlorophyll, the alcohol insoluble and the soluble fractions in the first two leaves is 9 and 11 per cent lower and

Table 19. Concentration of Kjeldahl nitrogen in different fractions of healthy and diseased Golden Ball seedlings, 10 days after the latter were inoculated with P. graminis tritici (race 15B-1).

Golden Ball				
Plant Part	Fraction	Healthy (mg N/0.5 g dry wt)	Diseased (mg N/0.5 g dry wt)	Dx100/H
First two leaves	Chlorophyll	0.75(a)	0.67	89.3
	Alcohol soluble (minus chlorophyll)	7.03	7.19	102.2
	Alc. insoluble	12.75	13.04	102.3
Younger leaves	Chlorophyll	0.74	0.74	100.0
	Alcohol soluble (minus chlorophyll)	5.78	5.78	100.0
	Alc. insoluble	15.88	14.68	92.4
Roots	Alcohol soluble	7.81	9.06	116.0
	Alc. insoluble	13.29	10.44	78.6

(a) Mean of duplicates.

24.4 per cent higher, respectively, than that of the non-infected younger leaves. Compared to that of corresponding fraction from healthy plants, the concentration of nitrogen in the chlorophyll fraction of the infected first two leaves is 11 per cent lower,

while the alcohol soluble and insoluble fractions have concentrations similar to those in healthy plants. There is no difference between the chlorophyll and alcohol soluble nitrogen content of the non-infected leaves of a diseased plant and the corresponding leaves of a healthy one, but the alcohol insoluble nitrogen content in this portion of the diseased plant is 7.6 per cent lower than it is in the healthy plant. As a result of disease, there is in the roots an increase of 16 per cent in alcohol soluble nitrogen, that is accompanied by a decrease of 21.4 per cent in the alcohol insoluble nitrogen in roots.

In Table 20 are given the results obtained for nitrogen content in various fractions of healthy and diseased Khapli (resistant) wheat seedlings. The concentration of nitrogen in the chlorophyll and alcohol soluble fractions of the infected first two leaves is 33.8 and 8.7 per cent lower, respectively, than that of the corresponding fractions from healthy first two leaves, while the alcohol insoluble nitrogen concentration is 6.5 per cent higher. All three fractions of the younger leaves of diseased plants are probably not significantly different from those of healthy controls. In roots, there is a significant increase of 15.6 per cent in alcohol soluble nitrogen and a decrease of 32.3 per cent in alcohol insoluble nitrogen as a result of disease . The effect of rust on the nitrogen concentration of the different fractions of the younger (non-infected) leaves is

Table 20. Concentration of Kjeldahl nitrogen in different fractions of healthy and diseased Khapli seedlings, 10 days after the latter were inoculated with P. graminis tritici (race 15B-1).

Khapli				
Plant Part	Fraction	Healthy (mg N/0.5 g dry wt)	Diseased (mg N/0.5 g dry wt)	Dx100/H
First two leaves	Chlorophyll	0.68(a)	0.45	66.2
	Alcohol soluble (minus chlorophyll)	9.06	8.28	91.3
	Alc. insoluble	9.26	9.87	106.5
Younger leaves	Chlorophyll	0.51	0.52	102.0
	Alcohol soluble (minus chlorophyll)	6.94	6.88	99.1
	Alc. insoluble	13.55	13.10	96.8
Roots	Alcohol soluble	7.03	8.13	115.6
	Alc. insoluble	8.97	6.07	67.7

(a) Mean of duplicates.

about the same as that on the nitrogen concentration of the different fractions of the first two leaves, except for chlorophyll nitrogen.

The results obtained on the effect of Puccinia graminis tritici on the concentration of N^{15} and Kjeldahl nitrogen in each of

three fractions from each of three parts of susceptible, semi-resistant and resistant seedlings can be summerized as follows: The N^{15} concentration in the alcohol insoluble fraction of the different parts of diseased susceptible plants is higher than in healthy plants and an increase in its concentration in this fraction is always accompanied by a higher concentration in total nitrogen. On the other hand, in the leaves of resistant plants, the concentration of N^{15} in the alcohol insoluble fraction is decreased as a result of disease, while the total nitrogen concentration remains about the same or is slightly increased. The alcohol insoluble fraction of roots of resistant plants, however, shows a decrease of 32 per cent in total nitrogen and a slight increase in N^{15} concentration. Another striking difference between the response of susceptible and resistant plants to rust infection is that a decrease in N^{15} concentration takes place in nearly every fraction of diseased resistant plants, while there is an increase in diseased susceptible plants. Golden Ball, a variety semi-resistant to rust race 15B-1, resembles susceptible plants in certain aspects of its behavior and resistant plants in other aspects.

Effect of P. graminis tritici on the Rate of Amino Acid Metabolism

The profound effect of the rust pathogen on the activity of the nitrogen metabolism of the host has been revealed in the preceding experiment in which heavy nitrogen was used as a tracer. Whether or not the effect of rust on the rate of nitrogen metabolism can be detected by measuring the rate of incorporation of C^{14} of $C^{14}O_2$ in the amino acids is not known. Therefore, an experiment of exploratory nature was carried out to study the alterations on amino acid metabolism induced by rust infection.

Materials and Methods

Whole seedlings grown in nutrient solution as described previously and excised leaves from plants grown in soil were used. These seedlings were then placed in the dark for 24 hours to deplete the carbohydrate reserves before exposure to $C^{14}O_2$. The first two leaves of soil-grown seedlings were excised and stood in water for five min. before exposure to $C^{14}O_2$. The amount of tissue used for each treatment was the following: whole seedlings, 5-10 g. fresh weight; excised leaves, 10 first and second leaves.

(1) Exposure to C-14-labelled Carbon Dioxide

Detached leaves which had been placed in a holder (Fig. 2) were quickly inserted into a Perspex photochamber. The system was evacuated, then perchloric acid was injected into the reaction vessel (Fig. 2) which contained 400 microlitres of a sodium carbonate-C^{14} solution containing 500 microcuries per millilitre to release the $C^{14}O_2$. Carbon dioxide-free air was allowed momentarily into the system to sweep $C^{14}O_2$ from the reaction vessel into the photochamber. Two flood lights placed on each side of the photochamber were turned on immediately; each lamp gave a light intensity of 1000 f.c. at the chamber wall facing it. Between the photochamber and each light, a light filter was placed to absorb the heat generated from it. A Soya flask having a 16 1/2 cm. diameter containing a solution of 0.5 g. $CuSO_4$, 100 ml. water, and one drop H_2SO_4 was used as filter. The duration of exposure to $C^{14}O_2$ was light minutes.

In the case of seedlings grown in nutrient solution, the procedure was similar to that described above except that the whole plant was placed in a bell jar for exposure to C-14-labelled carbon dioxide, the system was not evacuated, and 10 minutes preillumination was given to the plant before the introduction of perchloric acid into the reaction vessel. The duration of the exposure to $C^{14}O_2$ was 15 minutes.

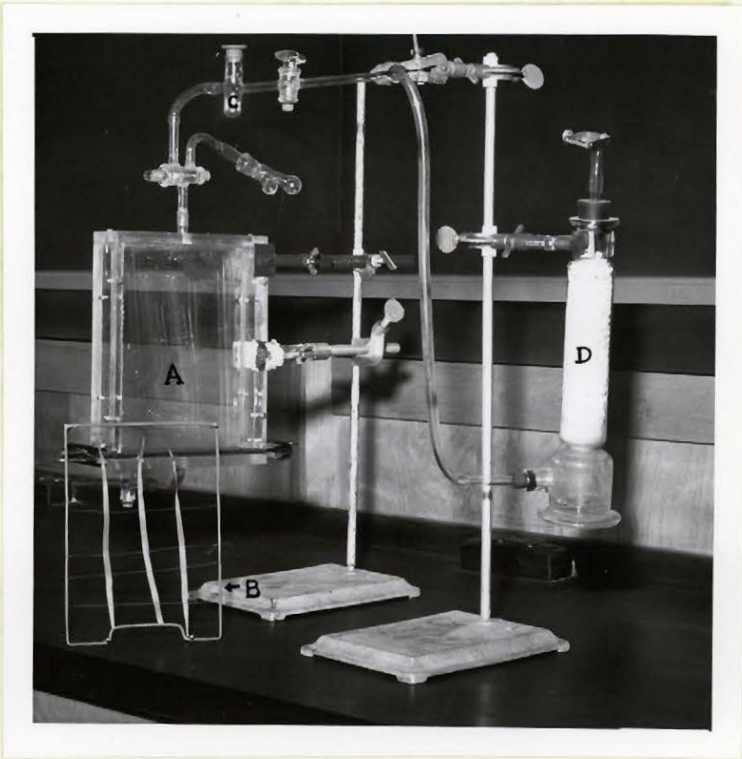


Fig. 2. Apparatus used for $C^{14}O_2$ fixation by excised leaves.
A. photochamber
B. holder for excised leaves
C. reaction vessel
D. soda lime tower

(2) Extraction Procedure

After exposure to $C^{14}O_2$ the leaves were quickly weighed and dropped into boiling 80 per cent alcohol to stop enzymatic action. The leaves were then extracted in a Soxhlet extractor for eight hours. The residue was again extracted with 30 per cent alcohol in a beaker for one hour. The two extracts were combined and chlorophyll was removed with benzene. The volume of the combined extracts was reduced *in vacuo* and made up to volume, 10 millilitres.

(3) Paper Chromatography

Separation of the constituents of the chlorophyll-less alcohol soluble fraction was performed by means of descending two-dimensional paper chromatography. An aliquot, 100 to 200 microlitres, of the extract was spotted, with intervals to allow for drying between applications, on a small area in a corner of a sheet of 18" X 22 1/2" Whatman No. 1 chromatographic filter paper. The sheets of paper were placed in a Shannon chromatographic chamber and equilibrated overnight. It was then developed in the conventional manner with the solvents described by Benson *et al.* (107). The composition of the solvent mixtures was water saturated phenol, for separation in one direction and butanol : propionic acid : water for the other. The latter solvent was freshly prepared before use from equal volumes of the following solutions:

(i) 1, 246 ml. of n-butanol : 84 ml. of water.

(ii) 620 ml. of propionic acid : 790 ml. of water.

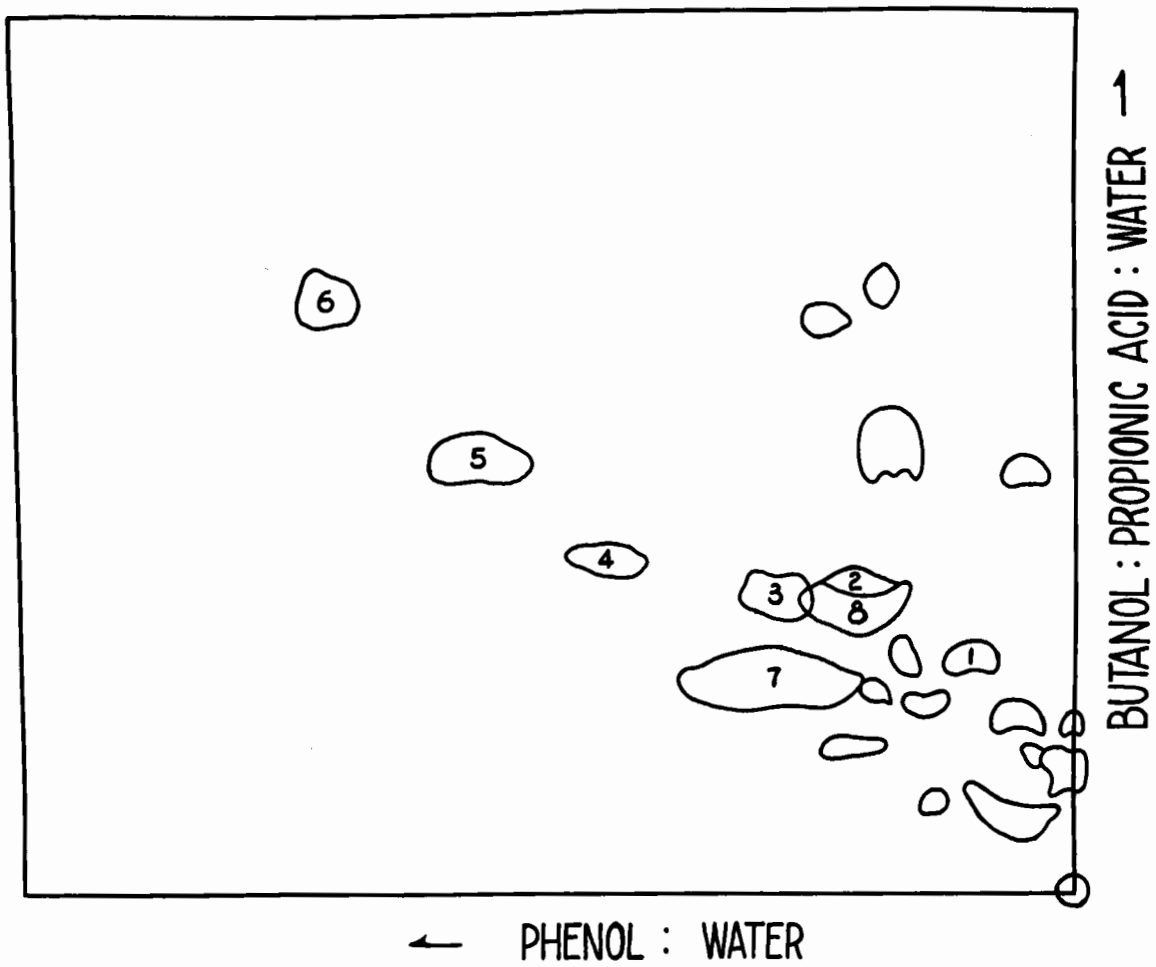
(4) Radioautography

To reveal the presence of radioactive spots radioautograms of the chromatograms were made by placing Kodak No-Screen X-ray film on each paper chromatogram. Then the film and the paper were placed between heavy glass plates and stored in complete darkness for about two weeks. At the end of this time the exposed X-ray film was developed in Kodak D-19b developer and fixed in Agfa 201 fixer.

Results

Fig. 3 represents a map drawn from a radiochromatogram obtained from the alcohol extract of attached wheat leaves of healthy seedlings exposed to C-14-labelled carbon dioxide for 15 minutes. There is no apparent qualitative difference between this radiochromatogram and those obtained from extracts of rusted leaves. It would seem that at this particular stage of development and under the experimental conditions used, rust does not affect carbon assimilation. However, there is a difference between attached and detached leaves. Of the radioactive spots on the chromatograms six were ninhydrin positive (Fig. 3 spot No. 1,2,3,4,5,8) in the detached leaves, and seven in the attached leaves (Fig. 3 spot No. 1,2,3,4,5,6,8). It should be recalled here, that the detached leaves were exposed to $C^{14}O_2$ eight minutes and grown in soil

Fig. 3. Map of a radiochromatogram obtained from an alcohol fraction of excised healthy wheat leaves exposed to $C^{14}O_2$ for eight minutes. Similar radiochromatograms were obtained from extracts of diseased leaves. Spot No. 1 to 6 and 8 are ninhydrin-positive, 7 is a sugar. The nature of the compounds in the unnumbered spots has not been determined.


6
5
4
3
2
8
1
7
BUTANOL : PROPIONIC ACID : WATER
PHENOL : WATER

while the leaves on the plants were exposed 15 minutes and grown in nutrient solution. A sugar (Fig. 3 spot No. 7) with the same Rf value as that of sucrose was found labelled in all treatments.

The Rf values of these ninhydrin positive spots correspond to those of the following amino acids: No. 1, aspartic acid; No. 2, glutamic acid; No. 3, glycine; No. 4, alanine; No. 5, γ-amino butyric acid; No. 6, phenylalanine; and No. 8, serine. Aspartic acid gave a characteristic blue colour when sprayed with five per cent alcoholic dicyclohexylamine.

It is concluded from these results that there is no qualitative difference in the distribution of fixed carbon among the different compounds detected on the radioautograms of alcohol extracts of healthy and rusted wheat leaves.

Effect of Puccinia graminis tritici on Keto-acid Metabolism of Wheat Seedlings

Results of the preceding study on the effect of Puccinia graminis tritici on the nitrogen metabolism of wheat seedlings indicate that this effect can be an important one in determining their resistance or susceptibility. Furthermore, results reported in the 'Literature Review' show that considerable alteration in the carbohydrate metabolism takes place as a result of disease. Since the alpha-keto-acids were known to occupy a central position between the carbohydrate and nitrogen metabolism of plants, it was considered that useful information might be obtained by investigating the effect of P. graminis tritici on keto-acid metabolism of wheat seedlings. Therefore, a series of experiments were performed in which the keto-acid metabolism in healthy, diseased, susceptible and resistant wheat varieties was studied.

Materials and Methods

Little Club seedlings used in this study were grown either in soil or in nutrient solution and were inoculated according to the procedures described previously. Varieties Gabo and Khapli were included also in one experiment. In the experiments where no tracer was employed, only the infected first two leaves were used. The

infection in every case was light or moderate, unless otherwise stated. In experiments in which C^{14} was used as tracer element, the whole top of the plant was used. One of these experiments consisted of an analysis of the keto-acids present in plants at different stages of rust infection; these stages were the following: 6,8,10,12 and 14 days after inoculation. Plants were exposed to labelled carbon dioxide as described under 'Materials and Methods' in the study on the effect of stem rust on the rate of amino acid metabolism. The amount of tissue used varied from experiment to experiment ranging from 2.5 to 10 g. of fresh tissue.

A large sample of urediospores were collected from time to time from rust pustules on plants growing in the greenhouse and stored dry in a refrigerator until they could be analyzed for alpha-keto acids.

The number of times each experiment was carried out is given with the results of each experiment.

(1) Extraction Procedure

Keto-acids were extracted according to the following method which is the one used in the laboratory of the Botany Department at McGill University by Dr. Towers.

After determining the fresh weight of each sample, the plant material was ground with 2,4-dinitrophenylhydrazine (2 ml. of

the reagent per gram of fresh weight of tissue) to convert keto-acids to the more stable phenylhydrazone. After grinding, 100 ml. of alcohol was added, and the mixture was filtered through paper in a Buchner funnel and the residue washed with 30 ml. of alcohol. The filtrate was evaporated to near dryness under a stream of air at room temperature. The concentrate was next diluted with 100 ml. of distilled water and extracted twice in a separatory funnel with a total amount of 150 ml. of ethyl acetate. These two extracts were combined, washed with water and extracted twice with one per cent sodium carbonate solution (150 ml. total volume). The two sodium carbonate extracts were combined and acidified with concentrated hydrochloric acid (about 5 ml.) and again extracted twice with ethyl acetate (150 ml. total volume). After washing with water, the ethyl acetate was evaporated. The residue was finally dissolved in alcohol to obtain a final volume of one millilitre.

(2) Paper Chromatography

An aliquot of the extract was spotted on a 14 1/2" X 18 1/2" sheet of Whatman No. 1 chromatographic paper. Eight equidistant spots were placed along a line two and a half inches from the shorter edge of the paper and parallel to it. Solution of individual known keto-acid phenylhydrazone, mixture of these, and

the extract to which was added a mixture of known keto-acid phenylhydrazones were spotted at intervals along the sheet. The known keto-acid derivatives were used to help in the identification of those in the plant extract. The paper was stapled in the form of a cylinder and irrigated at room temperature in an ascending manner with the following solvent, which is the one used by Dr. Towers, tert-amyl alcohol : ethanol : water in a ratio of 9 : 1 : 4. A small beaker containing ammonium hydroxide was placed in the center of the paper cylinder. Under these conditions it is possible to follow visually the separation of the keto-acid phenylhydrazones since they appear as coloured spots. After irrigation the chromatogram was dried at room temperature. The keto-acid derivatives were identified on the basis of their Rf value and confirmed by the development of their characteristic colours when the chromatogram was sprayed with 10 per cent alcoholic sodium hydroxide solution.

Part of the extract was applied on the chromatographic paper in a five-inch long streak and chromatographed in the manner described above. Certain of the resulting bands were cut out and eluted with alcohol. The resulting solution was later used for hydrogenlysis.

(3) Hydrogenlysis

The eluted keto-acid derivatives were hydrogenated in a Parr hydrogenator with platinum oxide as a catalyst (108). The resulting amino acids were chromatographed in a two-dimensional manner as described on page 71, and autoradiograms made as previously described.

(4) Determination of Relative Radioactivity of the Spots on the Chromatograms

The relative radioactivity of each keto-acid was determined by measuring the optical density of the spots on the X-ray film with a densitometer having a slit one mm-side. The values obtained were plotted on graph paper, using optical density as the ordinate and distance from origin as the abscissa. In this way, the relative amount of radioactivity in each keto-acid for each of the different treatments can be compared.

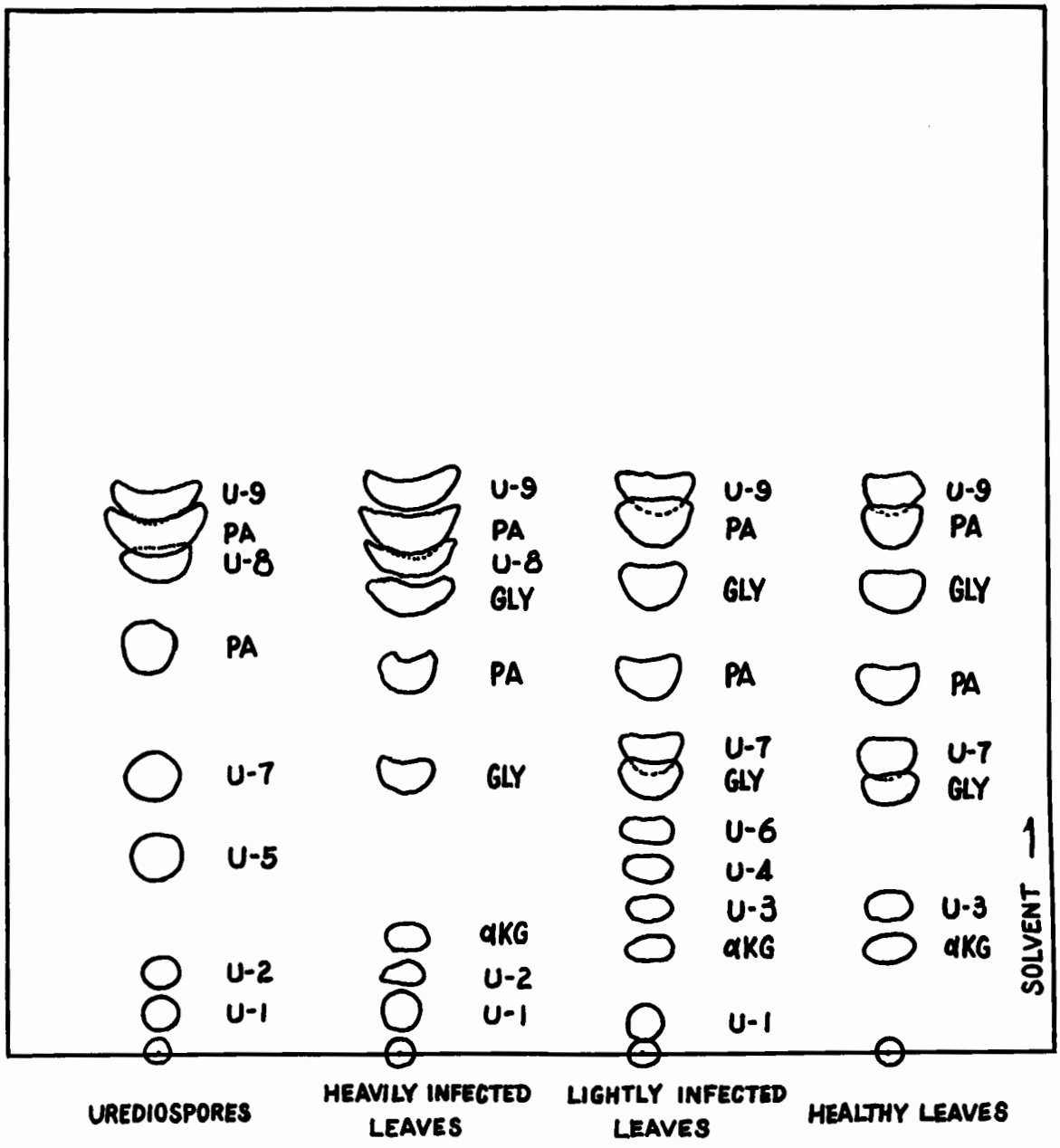
(5) Determination of Radioactivity in the Keto-acid Fraction

An aliquot of the extract was uniformly deposited in an aluminum planchet. Counting was made with a Berkeley decimal scaler (Model 1000-B) equipped with a lead shielded Geiger tube with a one-inch end-window.

Keto-acids found in Healthy and Rust-infected Leaves of Wheat and in Urediospores of Puccinia graminis tritici

In order to obtain consistency in the keto-acid content of healthy and rusted leaves, it was found necessary to perform the experiment a number of times. Figure 4 is a map of representative chromatograms of the 2,4-dinitrophenylhydrazones of keto-acids extracted from urediospores, heavily, and lightly infected leaves and healthy leaves. The keto-acids of urediospores appearing on the chromatogram are evidently different from those of leaves of wheat seedlings. Some of the most commonly occurring alpha-keto-acids in plants, such as alpha-ketoglutaric and glyoxylic acids, are not found in rust urediospores. However, pyruvic acid is present on all the chromatograms. Certain spots were not identified; they are referred to as 'unknowns' and are given a number. Unknowns 7 and 9 are found in urediospores and in healthy and lightly infected leaves. Unknown 9 is present also on the chromatogram obtained from heavily infected leaves. Glyoxylic acid is detected on all the chromatograms except those from rust spores. Unknown 2 is found only on chromatograms from rust spores and heavily infected leaves, while unknown 5 is confined to that from rust spores. As to unknown 1, it is present in large amounts in rust spores, and also in lightly and heavily infected leaves, and occasionally in small amounts in healthy leaves. It appears as a bright yellow coloured spot during the irrigation of

Fig. 4. Map of chromatograms of 2,4-dinitrophenylhydrazones of keto-acids from rust spores, rust-infected, and healthy Little Club wheat leaves. (PA) Pyruvic acid, (α-KG) alpha-ketoglutaric acid, (GLY) glyoxylic acid, and (U-1 to 9), unidentified keto-acid dinitrophenylhydrazone.


U-9
PA
U-8
PA
U-7
U-5
U-2
U-1
U-9
PA
U-8
GLY
PA
GLY
αKG
U-2
U-1
U-9
PA
GLY
PA
U-7
GLY
U-6
U-4
U-3
αKG
U-1
U-9
PA
GLY
PA
U-7
GLY
U-3
αKG
SOLVENT
1
UREDIOSPORES
HEAVILY INFECTED LEAVES
LIGHTLY INFECTED LEAVES
HEALTHY LEAVES

the chromatogram in an atomosphere containing ammonia vapor. Upon evaporating the ammonia and solvent, the colour of this keto-acid derivative disappears. Under ultraviolet light, it gives a greenish-blue fluorescent spot. The yellow colour returns and stays after the chromatogram is sprayed with 10 per cent alcoholic sodium hydroxide solution. Hydrogenlysis of unknown 1 was carried out following elution from the chromatogram. The resulting amino acid was chromatographed two-dimensionally in an ascending manner with alanine as a reference compound. The solvents were phenol : water, and collidine : lutindine : water. A ninhydrin positive spot appeared on the chromatogram having Rf values of 0.42 and 0.14, while alanine gave 0.60 and 0.17. The Rf values of the ninhydrin positive spot given by hydrogenlysed unknown 1 does not correspond to that of any known amino acid.

Keto-acid Metabolism of Healthy and Diseased Leaves of Susceptible and Resistant Wheat Seedlings

In order to understand the nature of resistance, the reactions of both resistant and susceptible varieties to infection have to be known. In the preceding experiments only one variety was used : the susceptible Little Club variety. Therefore, an experiment was performed in which the keto-acid metabolism of healthy and diseased Little Club was compared to that of Gabo. These varieties are susceptible and semi-resistant, respectively, to the rust race used,

namely, race 34.

In this experiment, seedlings were allowed to photosynthesize in an atmosphere containing C-14-labelled carbon dioxide, so that differences in rate of keto-acid metabolism might be measured as differences in the radioactivity of the keto-acid fraction of the plant. The radioactivity of keto-acid fraction of healthy and rust-infected Little Club was 2370 and 2591 counts per minute per gram of tissue, respectively, while that of healthy and rust-infected Gabo, 3715 and 4025 counts per minute per gram of tissue, respectively. Autoradiograms revealed quantitative differences but no qualitative differences in the keto-acids of healthy plants of Gabo and Little Club. No difference in keto-acids was detected between diseased and healthy plants of both varieties.

A similar experiment was performed with Little Club (susceptible) and Khapli (resistant) using race 15B-1 for inoculation. Results obtained are similar to those described above.

The above experiments were not repeated.

Keto-acid Metabolism of Diseased Plants at Different Stages of Infection

The results of a number of experiments on the effect of rust on the keto-acid metabolism of its host revealed that both qualitative and quantitative changes can take place. Table 21 shows the radioactivity of the keto-acid fraction extracted from healthy and

Table 21. Radioactivity of keto-acids in healthy and diseased leaves which were allowed to photosynthesize at different times in an atmosphere containing $C^{14}O_2$, after the latter were inoculated with P. graminis tritici (race 15B-1).

Variety	Number of leaves (a)	Days after inoculation	Counts/min./g.fresh tissue		
			Healthy	Diseased	Dx100/H
Little Club	2-3	6	4057	4152	102.3
	3	8	2700	1941	71.9
	3-4	10	5317	3696	69.5
	4	12	1945	1259	64.7
	4-5	14	4022	3614	89.8
	3-4(b)	9(c)	1800	1133	62.9

(a) Number of leaves on the plant when they were exposed to $C^{14}O_2$ and extracted.
(b) Results from an experiment carried out at a time different from that of the experiment from which the other results in this table were obtained.
(c) Light infection with an average of four pustules on each of the first two leaves; elsewhere, moderate infection (about 10 pustules per infected leaf).

diseased leaves of Little Club wheat seedlings which at different times after inoculation were allowed to photosynthesize in an atomosphere containing C-14-labelled carbon dioxide. Six days after inoculation, there seems to be no difference between healthy and rust-infected plants in the amount of C^{14} assimilated into keto-acids. In later stages the radioactivity of the keto-acid fraction from diseased plants is less than that of healthy plants and is increasing less as rust infection progresses up until 14 days after inoculation, when the radioactivity in diseased plants increases, although remains 11.2 per cent below that of healthy plants. In both healthy and diseased plants there are wide variations in the amount of radioactivity of the keto-acid fraction of plants exposed to C-14-labelled carbon dioxide at different times.

Fig. 5 is an autoradiogram representative of a series of paper chromatograms made at different intervals after inoculation, showing the effect of rust on keto-acid metabolism of its host, Little Club wheat. Spots on this autoradiogram are due to the radioactive derivatives of the keto-acids extracted from healthy and rust-infected plants, six days after inoculation. The infection was moderate. The derivatives of keto-acid which are labelled pyruvic acid, and alpha-ketoglutaric acid in Fig. 5 have been tentatively identified by co-chromatographing them with known keto-acid

Fig. 5. Autoradiogram of a paper chromatogram of the 2,4-dinitrophenylhydrazones of the keto-acids extracted from Little Club wheat seedlings (three-leaf stage) immediately after they were allowed to photosynthesize for 30 minutes in an atmosphere containing $C^{14}O_2$, six days after inoculation with P. graminis tritici (race 15B-1). Infection was moderate.

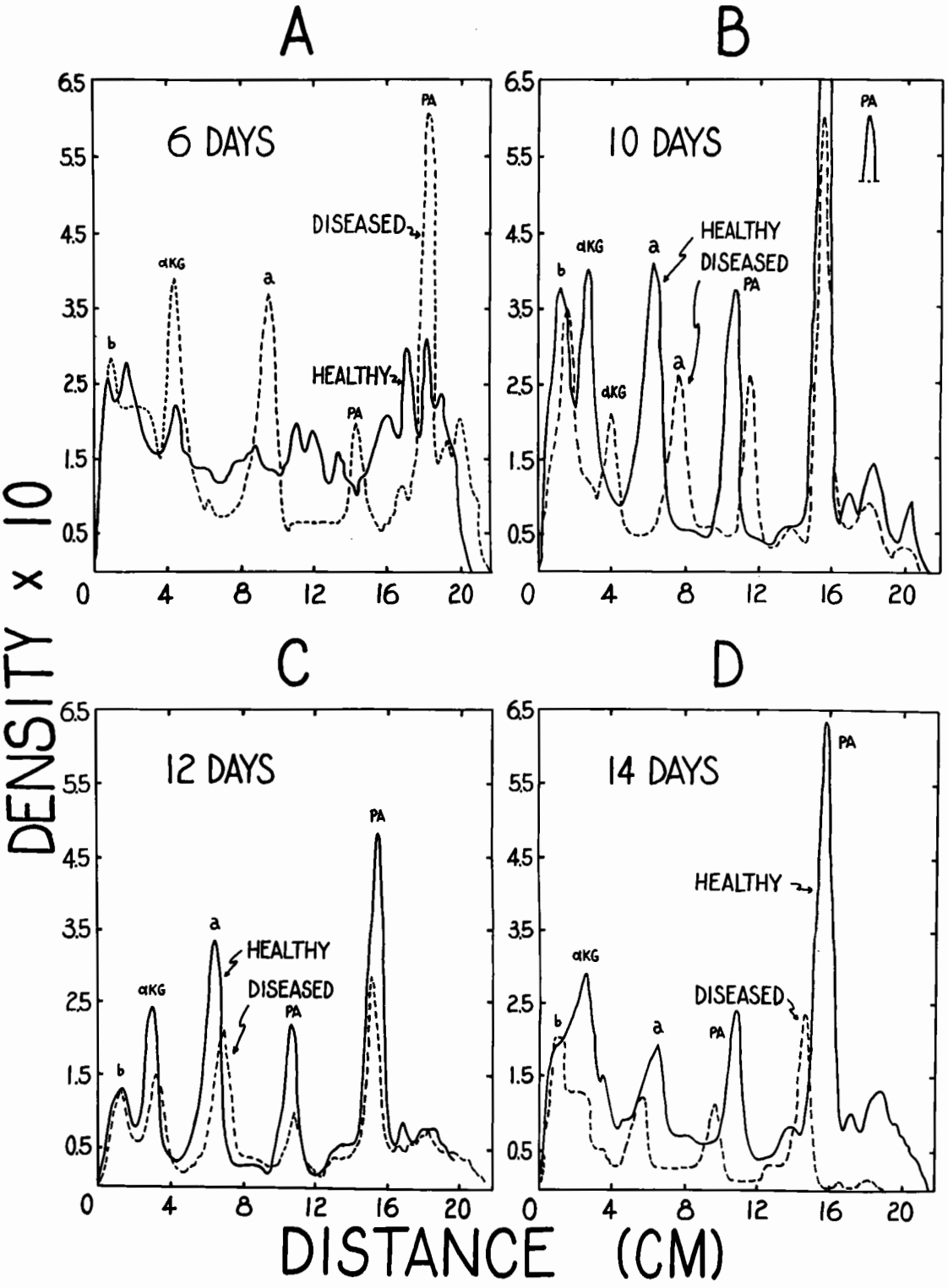

Pyruvic acid
Pyruvic acid
Unknown a
Alpha-ketoglutaric acid
Unknown b
Healthy
Diseased

derivatives and the amino acids resulting from their hydrogenlysis with known amino acids. Spots which are labelled unknown a and b are unidentified keto-acid derivatives. It appears from the autoradiogram that healthy plants contain several keto-acids which are not found in rust-infected plants. Similar autoradiograms were obtained for the later stages of infection.

The optical density of spots on autoradiograms obtained by this method is roughly correlated with its radioactivity on the chromatogram. Use of this fact was made to determine the relative radioactivity of the keto-acids in plants, by measuring the density of the spots on the autoradiogram obtained from chromatograms of keto-acid extracts.

The relative radioactivity of the keto-acids from both diseased and healthy plants at different stages of rust infection are presented graphically in Fig. 6. In diseased plants, nearly all the radioactivity is found in alpha-ketoglutaric, pyruvic and unknown acids a and b at six days after inoculation, while in healthy plants, it is distributed more or less uniformly among the keto-acids mentioned above and other unknowns (Fig. 6 A). Ten days after inoculation, the diseased plants show a decrease in radioactivity in unknown a and alpha-ketoglutaric acids, but an increase in unknown b. The activity in pyruvic acid remains about the same. The pattern obtained for the labelled keto-acids in

Fig. 6. Optical density plotted against distance along film strips of autoradiograms obtained from paper chromatograms of the 2,4-dinitrophenylhydrazones of keto-acids extracted from Little Club wheat seedlings immediately after they were allowed to photosynthesize for 30 minutes in an atomsphere containing $C^{14}O_2$, (A) 6, (B) 10, (C) 12, and (D) 14 days after inoculation with P. graminis tritici (race 15B-1). Fig. 3 is a photograph of the autoradiogram represented graphically here is A.


A
6 DAYS
DISEASED
HEALTHY
αKG
a
b
PA
B
10 DAYS
HEALTHY
DISEASED
αKG
a
b
PA
C
12 DAYS
HEALTHY
DISEASED
αKG
a
b
PA
D
14 DAYS
HEALTHY
DISEASED
αKG
a
b
PA
DENSITY x 10
DISTANCE (CM)
0
4
8
12
16
20
0.5
1.5
2.5
3.5
4.5
5.5
6.5

healthy plants has drastically changed, when compared to the pattern of the preceding stage, to a pattern similar to that of diseased plants. The radioactivity found in each keto-acid in healthy plants is greater than that in diseased. Twelve days after inoculation, the radioactivity in diseased as well as in healthy plants decreased as compared with that of the 10 day interval. This decrease in radioactivity in both diseased and healthy plants is probably due to the overall reduction in the ability of the plants to assimilate C-14-labelled carbon dioxide. At 14 days the radioactivity in diseased plants is about the same as in the preceding stage, yet the radioactivity found in the healthy controls is greater. Thus, the difference in relative radioactivity between diseased and healthy plants becomes greater.

These results show that the distribution of radioactivity among the different keto-acids in young healthy plants is different from that in diseased plants of the same age, but that with time, this distribution in healthy plants becomes similar to that of diseased plants. The results show also that the effect of rust infection on the keto-acid metabolism of wheat seedlings varies with the stage of infection.

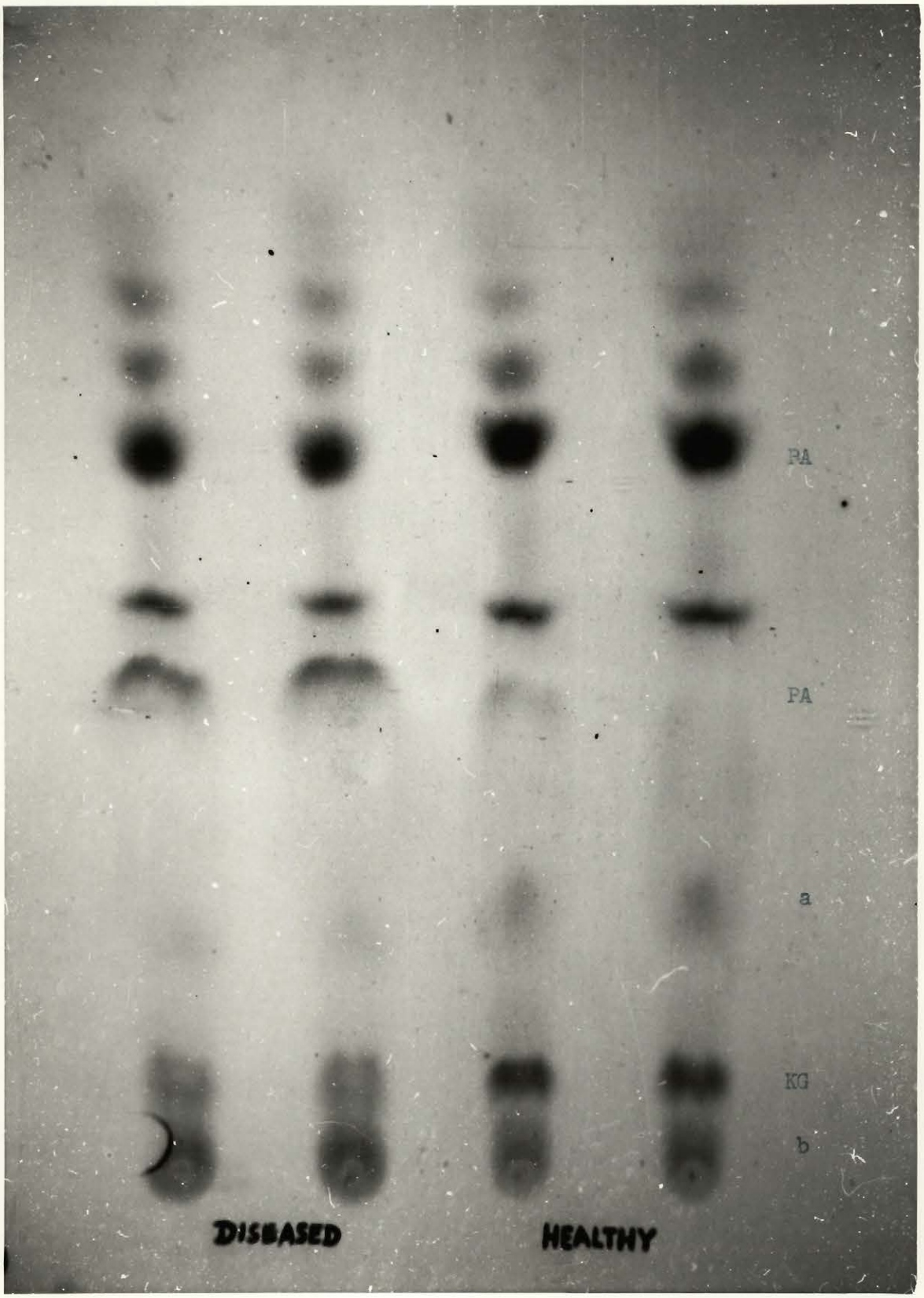
This experiment as a whole was not repeated. However, a similar experiment involving only one stage of infection (nine days after inoculation) was later performed. The results obtained are

reported below.

Keto-acid Metabolism of Diseased and Healthy Little Club Wheat Nine Days After the Latter were Inoculated with P. graminis tritici (race 15B-1)

Fig. 7 shows the effect of rust nine days after inoculation on the host keto-acid metabolism. At this stage alpha-ketoglutaric acid in diseased plants has much less activity than that of healthy plants, while the pyruvic acid has much more. The pattern of the distribution of C^{14} among the keto-acids obtained in this experiment is similar to that found in the preceding experiment in plants 10 days after inoculation (Fig. 5B). However, in this experiment the activity of pyruvic acid in diseased plants is higher than that of healthy plants, while it is lower in the preceding experiment. Spots on the autoradiogram were tentatively identified by the method described previously (page 87).

Fig. 7. Autoradiogram of a paper chromatogram of the 2,4-dinitrophenylhydrazones of the keto-acids extracted from Little Club wheat seedlings (three to four-leaf stage) immediately after they were allowed to photosynthesize for 20 minutes in an atmosphere containing $C^{14}O_2$, nine days after inoculation with P. graminis tritici (race 15B-1). Infection was light (average of four pustules per infected leaf).


PA
PA
a
KG
b
DISEASED
HEALTHY

DISCUSSION AND CONCLUSION

The review of the literature presented above makes it evident that, so far, results obtained in studies on the nature of resistance of plants to invasion by obligate parasites, such as Puccinia graminis tritici, do not explain this phenomenon satisfactorily. Even biochemical studies, which appear to be the most promising ones, have so far failed to supply an explanation. A reason for this failure could be the inadequacy of the analytical methods used which cannot detect the biochemical changes induced in the host by the pathogen in terms of rate of metabolic activity. A useful tool in following such changes in the metabolism of a plant is the employment of isotopic elements.

Data obtained on nitrogen metabolism indicate that there is a difference among normal plants of the three varieties of wheat, Khapli (resistant), Golden Ball (semi-resistant), and Little Club (susceptible). Plants of the resistant variety (Khapli) contain in each of their parts (the first two leaves, the younger leaves, and the roots) a concentration of nitrogen which is lower than that of corresponding parts of plants of other varieties. The susceptible variety (Little Club) has the highest nitrogen concentration in leaves. The parts of the semi-resistant variety (Golden Ball) possess a concentration of nitrogen intermediate between that of the other two varieties, with the exception of the roots which have

a nitrogen concentration higher than that of roots of susceptible plants (Tables 4,5 and 6). The three varieties differ not only in nitrogen concentration, but also in nitrogen metabolic activity, which is indicated by the concentration of N^{15} present in the plants. The roots of healthy resistant and semi-resistant plants have lower metabolic activity than those of susceptible plants (Table 9). The younger leaves of the resistant plant show the highest metabolic activity, the semi-resistant next highest, and the susceptible the lowest (Table 8). In the first two leaves, however, it appears that both susceptible and semi-resistant plants show a much lower metabolic activity as compared with resistant plants (Table 7).

The three varieties used respond differently in their nitrogen metabolic activity to infection by rust (Fig. 1). This response toward infection is probably most significant in determining the susceptibility or resistance of a given plant to a pathogen. In both susceptible and semi-resistant plants, rust does not seem to exert any effect on the nitrogen concentration, but produces a profound influence on the nitrogen metabolic activity of its host. The resistant plants, which possess the lowest nitrogen concentration of the three varieties, show a reduction in nitrogen concentration as a result of rust infection. This response to rust likely has its importance, but more important probably is the fact that resistant plants respond with respect to nitrogen

metabolic activity in all their parts in a way opposed to that of susceptible plants.

The differences between the responses of the three varieties to parasitic infection in their metabolic activity can be further demonstrated by the results of total nitrogen and N^{15} concentration on various fractions of the different parts in which the plants were divided. There is no difference in concentration of nitrogen in the chlorophyll fraction between rust-infected and healthy first two leaves of the susceptible plants, but the N^{15} concentration increases in diseased plants indicating that rust slightly increases the rate of chlorophyll metabolism (Tables 10 and 18). As a result of disease, there is in the chlorophyll nitrogen concentration in the first two leaves of resistant and semi-resistant varieties, a reduction which is not accompanied by an increase in N^{15} concentration (Table 10). This indicates that the rate of chlorophyll synthesis in the infected leaves of both varieties is greatly reduced, while the rate of the chlorophyll breakdown remains the same or is slightly enhanced. However, there is a greater reduction in chlorophyll metabolic activity in the resistant plants than in the semi-resistant ones. This decrease in chlorophyll concentration and metabolic activity in the infected first two leaves of the resistant and semi-resistant plants would mean less chlorophyll available for photosynthesis. On the other hand, the availability of chlorophyll in diseased susceptible plants

remains unchanged, if not increased, since there is an increase in chlorophyll metabolic activity as a result of infection in the first two leaves of susceptible plants without a decrease in total chlorophyll nitrogen.

Rust affects not only the photosynthetic apparatus, but also the protein and soluble nitrogen metabolism of its host. In the first two leaves of susceptible plants, the concentration of alcohol soluble nitrogen is decreased as a result of disease without any change in its metabolic activity, while in the first two leaves of resistant plants the concentration of alcohol soluble nitrogen is only slightly decreased, but the metabolic activity is greatly reduced by rust infection. Among the three varieties, the greatest reduction in soluble nitrogen metabolic activity was found in infected resistant plants. The semi-resistant variety gave a type of response to rust infection intermediate between that of the two other varieties. These results show that the metabolic activity of soluble nitrogen in the different varieties can be affected differently by rust infection (Table 11). Despite the profound effect of rust on chlorophyll and soluble nitrogen metabolism, there seems to be little or no effect on the protein metabolism of the infected first two leaves of both resistant and semi-resistant plants (Table 12). In contrast to this, in susceptible plants, the protein metabolic activity increases greatly as a result of infection by rust. The net increase in protein

concentration and the decrease in that of soluble nitrogen, together with the increased overall nitrogen metabolic activity in the infected first two leaves of the susceptible variety suggest that protein synthesis is activated by rust infection. A similar activation of protein synthesis was observed by Akazawa and Uritani (87,55) in potato tubers infected by Ceratostomella fimbriata. In the first two leaves of the resistant variety Khapli, no increase in protein metabolism has been observed as a result of infection. There is, however, a decrease in both soluble nitrogen metabolic activity and available chlorophyll. These findings would suggest that in the susceptible host conditions suitable for parasitic existence are created as a result of the augmented protein metabolic activity and the increased host proteins (enzymes) available for catalyzing reactions in infected plants. On the other hand, the conditions created in the resistant plants are probably unsuitable for rust development. The semi-resistant plants resemble the resistant ones in their responses in protein and chlorophyll metabolism to rust infection, but differ from them in their responses in soluble nitrogen metabolic activity which is not affected to the same extent by infection.

Rust affects not only the nitrogen metabolic activities of infected leaves, but also that of non-infected leaves and roots of the diseased plant. Besides their original differences in nitrogen metabolism, the non-infected leaves of the three varieties respond

differently to rust established on the older leaves. The concentration of chlorophyll available for photosynthesis is lower in the non-infected leaves of diseased susceptible plants as compared to that of corresponding leaves on healthy plants. However, the nitrogen metabolic activity is greatly enhanced and is accompanied by an increase in nitrogen concentration, resulting in a small net increase in protein. The concentration of alcohol soluble nitrogen in the non-infected leaves of diseased resistant plants is the same as that of corresponding leaves on healthy plants, but the N^{15} concentration is much less (Tables 20 and 14). This response indicates that rust on the older leaves of resistant plants reduces the soluble nitrogen metabolic activity of the younger non-infected leaves. As to the protein metabolism of the younger leaves of resistant plants, it appears that infection on the first two leaves stimulates proteolysis and reduces the rate of protein synthesis of the younger leaves, since the decrease in insoluble nitrogen concentration is accompanied not by an increase in N^{15} concentration, but by a decrease (Tables 20 and 15).

Semi-resistant plants resemble resistant ones in their responses to infection in that there is no change in the concentration of alcohol soluble nitrogen and little or no change in that of alcohol insoluble nitrogen, but resemble susceptible plants in that there is an increase in N^{15} concentration in both soluble and insoluble nitrogen, indicating an increase in the rate of both soluble nitrogen and

protein metabolism. The extent to which these parts of the nitrogen metabolism are changed in semi-resistant plants as a result of infection is intermediate between that of the other two varieties.

In the root system of susceptible plants, there is no change in nitrogen concentration and only a slight increase in N^{15} concentration as a result of infection. The alcohol soluble nitrogen concentration is much lower in roots of diseased plants than in those of healthy controls, but in this fraction the N^{15} concentration is the same (Tables 18 and 16). This might be taken to suggest that the rate of N^{15} uptake by the roots of diseased plants from the outside solution is less than that by the roots of healthy plants. However, this is not likely the case, since the total N^{15} concentration in diseased plants is greater than that in healthy plants. Therefore, another explanation must be sought. It may be explained by assuming that upward translocation in diseased plants is increased as a result of rust infection. That this is so is indicated by a greater N^{15} concentration in the leaves of diseased plants than in those of healthy controls (Tables 7 and 8). There must be an increased uptake of N^{15} by the roots in order to meet the increased demand of N^{15} in the leaves. With higher N^{15} concentration in all fractions of the diseased plants than in those of healthy plants, except for the alcohol soluble fraction of the roots, it would seem that the N^{15} content in roots is diluted by a large amount of

unlabelled nitrogenous compounds from the leaves. Furthermore, there is an increased protein concentration in roots of diseased plants with a concomitantly increased N^{15} concentration (Table 17). Yet, the increase in N^{15} is not proportional to the net increase in insoluble nitrogen, suggesting that there is an increased rate of protein synthesis, but without an increased rate of proteolysis. In the diseased resistant plants there is probably a decreased rate of N^{15} uptake by the roots and also a decreased upward translocation. This is indicated by the fact that there is no change in N^{15} concentration in the soluble fraction of roots (Table 16), while there is a decrease both in total nitrogen and in N^{15} concentration in the leaves (Table 6 and Fig. 1). The alcohol insoluble fraction of the roots of diseased resistant plants shows a great decrease in total nitrogen concentration and a slight increase in N^{15} concentration. This increase in N^{15} as compared with the decrease in total nitrogen is so small that the only possible explanation appears to be that the rate of proteolysis is slightly increased or remains the same while the rate of synthesis is reduced.

The semi-resistant plants resemble susceptible plants in that (a) there is no difference in total nitrogen of the roots of diseased plants as compared with that of healthy controls, and (b) there is an increase in N^{15} concentration as a result of rust infection. They resemble resistant plants in that there is an increase in total alcohol soluble nitrogen and a decrease in total insoluble

nitrogen. They, however, resemble susceptible plants in that there is an increase in N^{15} in soluble fraction, but a decrease in N^{15} in insoluble. Since the semi-resistant plants show a small decrease in N^{15}, and the resistant a slight increase, and the susceptible a marked increase, the N^{15} concentration of the diseased semi-resistant was closer to that of the diseased resistant than that of the diseased susceptible. These results indicate that semi-resistant plants show an increase in the rate of N^{15} uptake and translocation which are slower than those in susceptible plants.

These findings on the altered nitrogen metabolism of the non-infected leaves and the roots of plants with rust lesions on their first two leaves clearly show that rust, even in cases where infection is restricted to a small part of the host, induces systemic changes in the nitrogen metabolism of the whole plant. This was later found to be true also for the keto-acid metabolism.

The obligate nature of rust has suggested that it may require a certain specific protein which is supplied by the living host. However, the concentration of N^{15} found in urediospores, 0.594 atom per cent excess, is much higher than that (0.136 N^{15} atom per cent excess) of host proteins. This implies that the sources of nitrogen of the pathogen have on the average a N^{15} concentration higher than 0.594. This would seem to eliminate the host proteins as a whole as the sole source of nitrogen for the pathogen, but not necessarily certain specific host proteins.

Results of some of the experiments on the effect of rust on keto-acid metabolism of its host revealed that qualitative changes often take place. These changes, however, were not obtained consistantly in the experiments first carried out. The cause of the variation was thought probably to be due to variation in the stage of infection development on the plants employed in the different experiments. In order to check this hypothesis, determinations on the keto-acids were made at intervals as infection progressed. It was found that rust can affect keto-acid metabolism of the host either quantitatively or qualitatively, depending on the stage of infection. The amount of C^{14} from $C^{14}O_2$ assimilated into keto-acids by healthy plants varied considerably with determinations made at different times. This variation was probably due to differences in the physiological condition of the plants and the influence of changes in environmental conditions. There is a good correlation between the intensity and duration of sunlight and the amount of C^{14} incorporated into keto-acids. Low radioactivity was found in the keto-acid fraction of plants allowed to photosynthesize in an atmosphere containing $C^{14}O_2$ after they had been in bright sunlight for several hours. Under such conditions, plants have a higher carbohydrate reserve which probably causes a reduction in the rate of $C^{14}O_2$ fixation. On the other hand, high radioactivity appeared in plants that had been under low light intensity

(cloudy day) before C-14-labelled carbon dioxide fixation.

There seems to be no difference in the amount of C-14 incorporated into keto acids between healthy plants and those involved in an early stage of infection. However, the results indicate that diseased plants in earlier stages of infection may incorporate slightly more, about 2.3 to 11 per cent, C-14 into the keto-acid fraction than the healthy controls. But as the disease progresses, the radioactivity in the keto-acid fraction of diseased plants decreases and eventually becomes much lower than that of healthy plants. The slight increase in radioactivity of the keto-acid fraction in rust-infected seedlings in the early stages of infection, and the decrease in later stages may be considered as an indication that rust increases the rate of photosynthesis in the earlier stages of infection and decreases it in later stages. This interpretation is in accord with that of other investigators (86,89) who, working with powdery mildew, found that the rate of photosynthesis in wheat leaves increased during the first few days after inoculation, but later decreased. They found also that photosynthesis increased at sporulation time. The slight increase in radioactivity of the keto acid in diseased plants fourteen days after inoculation, shown by the present studies, agree with their findings.

Radioactivity measurements on individual keto-acids showed that even in early stages of infection rust affects not only the

radioactivity of the whole fraction, as mentioned before, but also the metabolism of each keto-acid as indicated by an increase in radioactivity in certain keto-acids and a decrease in others. In later stages, both diseased and healthy plants show a distribution of C^{14} among the different keto-acids which resembles that of plants in an early infection stage. This would indicate that rust only hastens the change to a different metabolic pathway which would eventually take place in healthy seedlings at a later physiological age. Evidence exists in the literature which shows that a change from one metabolic pathway to another can take place during the development of a plant. For example, Goddard and Meeuse (109) found that the cytochrome system which functions in the early stages of plant development is replaced by other systems as the plant matures.

Results obtained on keto acid metabolism indicate also that a systemic effect is brought about as a result of rust infection, since the amount of actually invaded tissue, constituting only a fraction of that analyzed, is too small to account for the difference between diseased and healthy plants. This result agrees with that mentioned previously in discussing effects of infection on the nitrogen metabolism for which a systemic effect of disease was also obtained.

Despite the profound influence that rust was found to have on both nitrogen and keto-acid metabolism of its host, it was found to have no effect on the amino acid metabolism. This lack of difference

between the rate of labelling of amino acids in healthy and diseased plants is probably due to (a) a lack of effect on the rate of incorporation of C^{14} at this particular stage of infection into the number of amino acids detected, or (b) to a too short an exposure of the plants to $C^{14}O_2$, resulting in few amino acids becoming labelled and making impossible detection of differences in metabolic activity in the unlabelled ones.

Comparative results on nitrogen metabolism of healthy and diseased plants were obtained for one stage in the development of infection involving only one physiologic race. This leaves certain questions unanswered. What is the effect on nitrogen metabolism in earlier and later stages of infection? How do different physiologic races affect the nitrogen metabolism of different varieties? In order to answer these questions, determinations should be made at suitable intervals covering the entire course of infection, and tissues of plants inoculated with different rust races should be used. The importance of analyzing plants at different times after inoculation was demonstrated in the study on keto-acid metabolism of Little Club wheat seedlings. Also, future studies on the effect of rust on host metabolism could probably best be carried out by using plants of a single variety infected with rust races differing in their virulence to that variety, instead of several varieties differing in their resistance to a single rust race. By the former method, interpretation of any metabolic difference in the host between a susceptible

and a resistant reaction will not be complicated by metabolic differences in non-inoculated healthy controls. Moreover, with this method only one healthy control is needed, instead of one for each variety as in the other method. It is only after information is available on the points mentioned above, that a theory on the nature of resistance could be proposed with a fair degree of plausibility. Nevertheless, it seems useful to speculate on the basis of the results obtained in the present work on the possible mechanism responsible for the resistance and susceptibility of wheat to rust.

Results on nitrogen metabolism showed that the response to rust infection of varieties differing in resistance is definitely quite different. This suggests that nitrogen metabolic activity may play a very important role in determining resistance or susceptibility of a plant. Therefore, the following working hypothesis is proposed: If a plant reacts to the presence of rust by increasing its nitrogen metabolic activity, more proteins (enzymes) are synthesized and made available for catalyzing reactions which bring about in the host conditions suitable for the development of the parasite. Such a plant is susceptible to disease. On the other hand, if the plant responds by decreasing its nitrogen metabolic activity and protein synthesis, conditions would be induced which are unsuitable for the development of the parasite, it is a resistant plant. If a plant shows a type of response intermediate between the two mentioned above, it will be

semi-resistant. This mechanism can determine whether or not a plant is susceptible, semi-resistant, or resistant to a given physiologic race of a pathogen.

SUMMARY

1. In studies on the nitrogen metabolism of healthy and diseased wheat plants of three varieties differing in their resistance to Puccinia graminis tritici Eriks. and Henn., seedlings were allowed to absorb $KN^{15}O_3$ through the roots about 10 days after the diseased ones had been inoculated on their first two leaves, and were showing moderate infection. Kjeldahl nitrogen and N^{15} concentration was determined for each of the parts (the first two leaves, the younger leaves, and the roots) in which seedlings were divided, and also for the alcohol soluble and insoluble fractions of each of these parts. In studies on the keto-acid metabolism, healthy and diseased seedlings were allowed to photosynthesize in an atmosphere containing $C^{14}O_2$. Keto-acids of these seedlings were separated by paper chromatography and their relative radioactivity determined.

2. Rust infection does not have any effect on the concentration of nitrogen in the different parts of both susceptible (Little Club) and semi-resistant (Golden Ball) plants, but decreases that of the different parts of resistant (Khapli) plants.

3. Rust infection induces systemic alterations in the nitrogen metabolism of all three varieties used, and also in the keto-acid metabolism of Little Club.

4. The difference in N^{15} concentration in each of the different parts in which healthy and diseased seedlings were divided shows that, except for the first two leaves of susceptible for which there is no difference, in both susceptible and semi-resistant the N^{15} concentration is higher in the parts of diseased seedlings than in the comparable parts of healthy seedlings. In the case of the resistant variety, different parts of healthy seedlings have a higher N^{15} concentration than comparable parts of diseased seedlings, with the exception of the roots which are not significantly different in this respect.

5. The N^{15} concentration in the alcohol insoluble fraction of the different parts of diseased susceptible plants is higher than in comparable parts of healthy plants, and an increase in its concentration in this fraction is always accompanied by a higher concentration in total nitrogen. On the other hand, in the leaves of resistant plants, the concentration of N^{15} in the alcohol insoluble fraction is decreased as a result of disease, while the total nitrogen concentration remains about the same or is slightly increased. The alcohol insoluble fraction of roots of resistant plants, however, shows a decrease of 32 per cent in total nitrogen and a slight increase in N^{15} concentration. Another striking difference between the response of susceptible and resistant plants to rust infection is that a decrease in N^{15} concentration, in nearly every fraction of diseased resistant plants, takes place, while there is an increase in diseased

susceptible plants. Semi-resistant plants resemble susceptible plants in certain aspects of their behavior and, resistant plants in other aspects.

6. Rust infection does not have any effect on the rate of incorporation of C-14 of $C^{14}O_2$ into the amino acids under the conditions used in the experiment reported here.

7. Rust infection may induce either qualitative or quantitative changes in the keto-acid metabolism of plants of the susceptible variety Little Club depending on the stage of infection. Six days after inoculation, the pattern of keto-acid labelling in healthy and diseased plants is very different. The pattern obtained for healthy plants becomes with age similar to that of diseased plants. The radioactivity in keto acids of diseased plants decreases as the infection progresses up until fourteen days after inoculation. At that time it increases slightly, but remains well below that of healthy plants.

8. Rust urediospores contain a number of keto acids not found in wheat seedlings. Also, rust spores from N^{15}-treated plants have a much higher N^{15} concentration than that of host protein.

9. An hypothesis based on the evidence obtained in this study is proposed as an explanation of the nature of rust resistance in wheat.

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