A BIOCHEMICAL STUDY OF RESISTANCE IN BARLEY TO THE GREENBUG, *SCHIZAPHIS GRAMINUM*

By

TAK K. CHAN

Bachelor of Arts
Simpson College
Indianola, Iowa
1964

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1968

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
July, 1971

A BIOCHEMICAL STUDY OF RESISTANCE IN BARLEY TO THE GREENBUG, SCHIZAPHIS GRAMINUM

Thesis Approved:

George H. Odell
Thesis Adviser

Glenn W. Todd

Roger E. Koeppe

George R. Waller

Earl D. Mitchell

O. Duncan
Dean of the Graduate College

ACKNOWLEDGEMENTS

The author gratefully acknowledges the guidance and advice of his major professor, Dr. Donald C. Abbott, during the course of this investigation and the preparation of the thesis, and of Dr. George V. Odell during Dr. Abbott's absence.

Thanks are extended to Drs. George R. Waller, Roger E. Koeppe and Glenn W. Todd, for their valuable time spent as members of the advisory committee and for their contributions to the preparation of this dissertation.

Appreciation is expressed to Dr. Donald C. Cress for conducting the feeding experiments.

The author is indebted to the U. S. Department of Agriculture, and to the Oklahoma State University Biochemistry Department for facilities and financial assistance.

The author would also like to thank his wife, Co-Ming, and his parents, Dr. and Mrs. P. K. Chan, for their encouragement and sacrifices during these years of graduate studies.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

CHAPTER I

INTRODUCTION

The greenbug, Schizaphis graminum (Rond.), is an aphid which causes great damage to wheat, barley and other small grains over large areas of Europe, part of Asia, and North and South America. In the United States, the greenbug causes damage in the central and southeastern states each year and is considered as one of the most serious pests. It has been reported that damages of up to 50 million bushels of grain have occurred in a year.

There are winged and wingless forms of the greenbug. All the wingless forms are females and they give birth to living young. Greenbugs reproduce continuously throughout the year in the southern states. Farther north, on the approach of cold weather, winged males and females are produced, and after mating these females lay eggs. The eggs pass the winter on the leaves of plants on which they were laid and hatch into wingless females early in the spring. Greenbugs are pale green when newly hatched, and develop a dark green stripe on their backs when fully grown. The females start to give birth to living young from 6 to 30 days after hatching, and continue to reproduce for 20 to 30 days, each producing 50 to 60 progeny. In Oklahoma and Texas greenbugs may have more than 20 generations a year.

The use of conventional insecticides has been the primary means for control of the greenbug. Parathion and certain other phosphorus-contain-

ing insecticides have been most effective for this purpose. However, a growing concern exists about the persistence of these conventional synthetic insecticides in soil and water. Their tendency to become incorporated into biological systems has been a major drawback for the use of these synthetic insecticides in recent years. The careful use of new synthetic chemicals which have been developed to minimize these characteristics may not solve these problems associated with pesticides. Insects may still be able to develop resistance to the chemicals. New insecticides may still be toxic to man and livestock and might kill other harmless and beneficial insects. Furthermore, the rapid rate of reproduction of greenbugs and the high cost for the application of insecticides have made this control method less practical.

Another method for the control of insects is the development of resistant varieties of crop plants by breeding. The resistant varieties have characteristics which enable a plant to evade, tolerate or recover from the attacks of insects under conditions that would result in greater injury to other plants of the same species. This is one of the most economical insect control measures because no special effort or expense is necessary on the part of the grower. Only limited progress has been made in this area for the control of greenbugs in crop plants. The appearance of new biotypes of greenbugs which the resistant varieties developed earlier do not have resistance has also slowed advances in this field. The search for a biochemical factor responsible for greenbug resistance in crop plants is necessary in order to make advances in this area. More definite approaches for selection of resistant varieties can be made once these factors are elucidated.

The objectives of the present study are two fold: first, to charac-

terize the biochemical differences between the resistant and susceptible varieties, and to determine if these differences are responsible for the resistance to the greenbug; and second, to determine the difference in effects of greenbugs on resistant and susceptible varieties since the physiological response of the plant following insect attack has a bearing on varietal resistance.

CHAPTER II

REVIEW OF LITERATURE

Early Investigations on the Mechanism of Insect Resistance

The internal condition of the plant has long been recognized as a factor affecting the plant-insect relationship. Biochemical constituents of the plant may render the plant unattractive or repellent or unsuited to the food requirements of the insects. As early as 1916, Comes (1) suggested that acidity might be the plant's defense against its enemies. The receptivity to insects in a given organ increased with the quantity of reducing sugars, and resistance increased with organic acids. The level of organic acids decreased in the vegetative tissues as the quantity of reducing sugar increased. The reducing sugars in the plant tissue were thought to be the major food sought by insects. Bruce (2), in 1920, considered that the odor and taste of the plant might be attractants to insects. Oligophagous insects feeding on various species of Cruciferae, Umbelliferae and Compositae were found to be associated with these factors. Bruce also suggested that there was some attribute of plants perhaps an odor which the insect could recognize in a general way and not as a specific characteristic of some single plant species or genus. The 'botanical instinct' of insects was thought to be just an extreme power of recognition of this sort. Davidson (3) suggested that the varying constitution and concentration of cell sap in different plants was an important factor for the biology and physiology of aphids.

The more favorable development and reproduction of aphids on certain plants was probably associated with the nature of cell sap. Lees (4) cited 14 cases in which the internal condition of the plants was correlated with the susceptibility to insect attack, and he suggested that the susceptibility of some plums to aphid attack might be due to the high nitrogen content in these plums. The higher supply of nitrogenous food was considered likely to give a higher reproduction power to the insect. Chemicals in woods was considered by Fullaway (5) as a factor for the resistance of certain woods to termites. The hard woods were found to be attacked by termites as freely as the soft woods, and examinations indicated that it was chemical rather than physical factors which made some woods resistant. Andrews (6) reported that the high ratio of potash to phosphoric acid in the leaves of some tea plants might be responsible for their resistance to the attack of the tea mosquito. Resistance might be due to the dislike of taste of these tea leaves by the insects since the leaves were sampled by the insects but feeding was not repeated. Osmotic concentrations of host plants was suggested by Carter (7) to be related to the susceptibility to the attack by the sugar-beet leafhopper. This insect avoided extremely high sap concentrations if more suitable food was available. It was found that plants having a higher concentration were abandoned for those having a lower concentration by the insects in a small area where the two growth forms of the same species were present. Carter's data also indicated that, should another host plant appear which has a more suitable concentration, the original host is then abandoned and the new host supports the greater population.

Withycombe (8) observed that susceptible sugar cane was more attrac-

tive to the sugar cane froghopper and suggested that the water content of plant was involved in insect resistance.

Cane leaves with subnormal water content contained carbohydrates in a less advanced stage of condensation and consequently more water-soluble and more easily available to the insect than those of normal cane leaves. Other conditions such as reduced amount of lignified tissue, less tannin content and reduced acidity might be favorable to the froghopper; however, all these factors had been reported to be unrelated to plant resistance to this insect (8). Mumford (9) suggested that amount of reducing sugar was related to the resistance of sugar-cane to froghopper. The excess sugar was stored in the leaf of the sugar-cane in the form of starch during the day, and the starch was converted into reducing sugar at night. It was at night, when these reducing sugars were most abundant, that the froghopper fed. Disturbance in the metabolism of the plant, caused by conditions such as drought, resulted in an increase of reducing sugar as degradation products; it was suggested that this might be the reason why diseased or damaged canes were said to be more attractive to the froghopper. The susceptibility of the cotton plant to the attack of sap-feeding insect pests such as various species of thrips was reported by Mumford (10) to be related to the water balance of the plant. It was observed that plants suffering from water shortage were definitely more attractive to the attacking thrips.

Mumford (11), later in 1930, studied the curly top disease of sugar beets. This disease is transmitted by the beet leafhopper. It was found that sugar beet strains which were more susceptible to the curly top disease were also more susceptible to the attack of beet leafhopper. Physical-chemical measurements of the sap showed that the refractive

index was invariably greater in the resistant strain. The freezing point depression of sap was greater in the young leaves and roots of the susceptible strain than in those of the resistant strain. The results suggested that the sap from the resistant beet was lower in total solids and non-electrolytes, and more concentrated in electrolytes than the sap from the susceptible beet. The observed differences in pH were small, but there was a general tendency towards greater acidity in the leaves of the resistant as compared with the susceptible strain. This observation was in harmony with Comes' suggestion (1) that acidity was the plant's defense against its insect enemies. A comparison of the nitrogen content of the sap in the susceptible and resistant strain of both healthy and diseased plants showed that the amount of nitrogen in the sap of plants of the susceptible strain was greater, both in the young leaves and in the roots, than in the corresponding portions of healthy and diseased plants of the resistant strain. In both strains the nitrogen content was increased markedly by the disease. Concentrations of reducing sugars, sucrose, and alcohol-soluble solids in the leaves of healthy susceptible plants were found to be higher than those in the resistant strains. The starch content was lower in the susceptible plant. Insect infestations and the resulting curly top disease had different effects on the chemical contents of the susceptible and resistant strains. In the susceptible strain, the disease increased the quantity of sugars in the leaves and reduced the quantity in the roots-the loss in one case possibly accounting for the gain in the other; whereas in the resistant strain the disease decreased the quantity of sugar in both leaves and roots. Such an accumulation of sugars in the exposed parts of the susceptible plant would render the plant a more favorable host to

the leafhopper. Susceptible strains also contained alcohol-soluble solids in greater concentration than the resistant strain in the leaves and roots of healthy and diseased plants. The leafhopper was said to be attracted to these alcohol-soluble solids, and in the leaves of the susceptible strain these solids increased with a severe attack of the disease.

Chemical Stimulants or Repellents in Food Plants as a Mechanism of Resistance

The response of insects to chemical attractants and repellents in plants has been considered to constitute the chief mechanism of preferences as part of insect resistance. Organs suitable for the reception of odors are known to occur on various parts of the insects' bodies but are most common on the antennae and, to lesser extent, on the maxillary and labial palpi. Organs of taste are located about the mouth, especially on the palpi, but also on the tarsi of the front legs and less often on the antennae (12).

Fraenkel (13) proposed that the main function of secondary plant substances, such as glucosides, saponins, tannins, alkaloids, essential oils and organic acids was to attract or repel insects. The role of these substance in the metabolism of plants had never been satisfactorily explained; but in view of their infrequent occurrence and of differences in their chemical constitution, it was almost inconceivable that they play a function in the basic metabolism of plants. The basic food requirements for all insects were considered very similar. These include amino acids, carbohydrates, vitamins and minerals. Most living cells including those of plant tissues contain these substances. Therefore,

Fraenkel assumed that all plants could provide sufficient food for insects, and the insects could develop equally well on any leaves if they eat a sufficient quantity. The differences in chemical composition with respect to the primary substance could not be considered responsible for the choice of food plant on the part of the insect. It was suggested that the food specificity of insects was based solely on the presence or absence of the secondary substances in plants which served as repellents or attractants to insects. The resistance or susceptibility within plant families such as Cruciferae, Umbelliferae, Solanaceae, Leguminosae, Moraceae and Gramineae to insects was attributed to the presence or absence of these secondary substances in the plant.

Kennedy (14) later proposed a "dual discrimination" theory of aphid host selection. He postulated that in addition to specific stimulatory substances (secondary plant substances of no nutritive value but governing botanical preferences), primary plant substances such as amino acids which are of fundamental metabolic (nutritional) importance to both plant and insects also played a major part in aphid host relations. These primary plant substances might serve as feeding stimulants for the aphid. Carbohydrates, free amino acids and other dietary components were found to serve as feeding stimulants for the milkweek bug (15). Maintenance of feeding was primarily dependent on the sensory input from chemoreceptors situated in the stylets. Sugars and amino acids in the milkweek seed coat played a role in determination of feeding.

The resistance of Solanum polyadenum to the attack of potato flea beetle was shown by Sleesman (16) to be due to the disagreeable odor of an oil or ester on the upper and lower leaf surface and also on the stem parts. The odor is very strong on the younger leaves but much less pro-

nounced on the lower, mature leaves. This odor may be the restricting factor for the slight amount of feeding on the younger foliage of this species. When adult beetles were confined in laboratory cages, they fed more readily upon younger leaves washed with soap and water to remove the oil than on the mature leaves which normally are the more attractive.

Geraniol and eugenol were found to be the primary attractants for the Japanese beetle (17). Other compounds found also to be attractive to the Japanese beetle were: alcohols, such as citronellol and phenyl ethyl alcohol; fatty acids, such as caproic acid, propionic acid and valeric acid; essential oils, such as pimento oil, citronella, bay oil, coriander, cloves, sassafras, rose geranium, lemon grass and linaloe; esters and aldehydes, such as methyl salicyate and benzaldehyde. All These were of plant origin. Mixtures of these attractants were on the whole, more attractive than any single component. Plants immune to the attack of Japanese beetle have a characteristic repellent odor which keeps the beetle away (18).

Leafhoppers were found to be attracted to cranberries (19). The susceptibility of cranberry varieties can be classified according to their attractiveness to this insect. Sugar-cane varieties susceptible to froghoppers were also more attractive to this insect due to the occurrence of an attractive odor (20). Analysis of the plant extract revealed two attractants, both of which occurred in varying quantities in the plant. One of the attractants was sucrose while the other was responsible for the attractive "grassy" odor of the plant.

European corn borer was attracted to the host corn plant by some odorous constituent elaborated within the plant (21). Steam distillation of the corn plant provided a substance to which the adult insect showed

a marked attraction when tested in an olfactometer. Research indicated that this attractant may be an essential oil of the corn plant produced in the small epidermal cells found in greatest numbers over the vascular bundles of the corn leaves. Silkworms were found to be attracted by the extracts of their host plant (22). The plant extracts contained three stimulants. The first stimulant was identified as β,γ-hexenol which attracted the larvae onto the plant leaves. The second stimulant found in the methanol soluble fraction of the extract induced the biting response of the larvae and was composed of β-sitosterol and isoquercitrin. The third factor stimulated the continuous swallowing of the larvae and was present in the water soluble fraction but was insoluble in methanol. This stimulant was found to consist of cellulose, sucrose, inositol, inorganic phosphate and silica (23).

The food plant selection of the diamond-back moth was determined by the presence of a feeding stimulant, mustard oil glucoside, and feeding inhibitors in the plant. The free mustard oil was also a very important factor for the host selection of this insect. The odor of this essential oil may inhibit the dispersive tendencies of the larvae and prevent them from wandering away from the food plant (24). Isothiocyanate was found to be the most significant oviposition stimulant for the diamond-back moth (25). More eggs were deposited on plants that contained isothiocyanate. Depletion of isothiocyanate content by sulfur-deficient plant nutrition reduced attractiveness of host plants as egg substrates.

Folsom (26), using a chemotropometer, tested the attractiveness of cotton to boll weevil. The boll weevil reacted differently to different varieties of cotton. It was found that Sea Island cotton was more susceptible to boll weevil attack than American upland cotton, and Egyptian

cotton still greater. Analysis of the steam distillate from cotton plants showed that ammonia and trimethyamine were the principal volatile constituents. These two chemical substances proved to be strong attractants of the boll weevil. Water extracts of all cotton-plant parts were found to contain a chemical substance which caused the ceasation of locomotion together with a feeding stimulation for the boll weevil (27). This chemical substance was defined as an arrestant and feeding stimulant since no apparent attraction was evident unless the insect came in contact with the extract. The reproductive tissue of the cotton plant contains the highest concentration of this arrestant and feeding stimulant. This compound was also found to be present in high concentration in the exterior layers of the plant tissue. The weevil must actually come into contact with this chemical substance before the arresting and feeding response is initiated. This substance is specific to cotton because boll weevils were not arrested or stimulated to feed on water extracts from other plants. A repellant was found in the volatile fraction of cotton extracts after the arrestant and feeding stimulant had been evaporated (28). This substance was an oily material and fairly heat stable, retaining considerable activity after being heated at 110°C for ten minutes. Repellency appeared to be associated with the highly piquant odor of this material since physical contact of the material was not necessary.

A mustard oil glucoside, sinigrin, was found to be a specific stimulant for host selection of the cabbage aphid (29). Differences in waxiness of plants also occurred between species in the genus Brassica and might be correlated with relative susceptibility to cabbage aphids. Qualitative and quantitative differences in cytoplasmic wax contents

might also be of some importance. Susceptible plants contained either 15 - nonacosanone or 15-nonacosanol, the ketone and alcohol from n-nonacosan. Resistant plants did not contain these compounds (29).

Plant phospholipids were found to be feeding stimulants for grasshoppers (30). These plant phospholipids were isolated from wheat germ oil by chromatographic techniques. The lecithins and phosphatidyl inositol were shown to evoke striking feeding activity from older nymphs and adults fo two species of grasshoppers. This feeding activity was much more pronounced in the male nymphs than in the female nymphs.

The resistance of cacao to thrips was reported to be a preference phenomenon (31). Food preference tests with leaf discs in the laboratory indicated that thrips larvae showed a significant preference for feeding on leaves of the standard control type rather than on those of the resistant type. Obligatory food tests in the laboratory with individual leaves showed, in comparison with the standard types, thrips larvae and adults found the resistant type unpalatable and few larvae were able to complete their development.

In a study of the wireworm's response to chemical stimulation, Thorpe and Crombie (32) found that aqueous plant extracts of potato tubers, carrots, sugar-beet tap roots and wheat stimulated the biting of this insect. The aqueous extracts contained glucose, fructose and sucrose. Other chemical substances causing biting were fats and fatty acids such as triolein, oleic acid, linolic acid and linolenic acid. The orientation response of the wireworm was found to be elicited by glucose, sucrose, asparagine, glutamine and peptone.

A stimulant was isolated from potato and related plants of the family Solanaceae by Yamamoto and Fraenkel (33). Preliminary characteri-

zation indicated the material to be a glycosidic substance, but its complete chemical identity was not elucidated. In the presence of this substance the potato hornworm attacked filter paper, lettuce leaves or artificial agar diets but only when certain nutrients, particularly sugars, were also present. The material was also active as a feeding stimulant for the Colorado potato beetle which feeds on plants of the same family.

Silica depositions in wheat plant tissue may be one of the factors related to resistance of certain varieties to Hessian fly attack (34). The resistant varieties appeared to have a much more complete and even distribution of silica deposits in their surface than those of the susceptible varieties. Studies on the asiatic rice borer also revealed that silica content was related to the resistance of rice varieties to this insect (35). High silica content in the plant seemed to interfere with feeding and boring of the larvae and could cause defacing of their mandibles. The silica deposits in various parts of barley plants were determined by Lanning (36) in an attempt to find the factors responsible for greenbug resistance. The results showed no direct relationship between total silica content in barley and resistance. Refai et al. (37) found a direct relationship between the hemicellulose content in the wheat plant stem and the degree of resistance to attack by the Hessian fly. In resistant varieties the tissues were tough enough, due to the presence of a large amount of hemicellulose, to prevent normal feeding of the larvae. Resistance to the insect might be associated with inability of the insect to obtain juices from the plant.

Presence of Toxic Substances in Food Plants as a Mechanism of Resistance

Some specific chemicals present in resistant plants may act as metabolic inhibitors and cause deleterious effects on the insects feeding on them. This has been suggested as the basis for high insect mortality and other related phenomena. Beck et al. (38) studied the resistance of corn plants to European corn borer and concluded that the resistance was due to the presence of some toxic substances in the resistant plant. Addition of both the juice and ether soluble portion of the aqueous extract from resistant to susceptible corn plant reduced larval survival to approximately half of that found on plants treated with distilled water. Addition of juice or extract from susceptible plants to resistant plants had no effect on larval survival (39). An ether-soluble substance termed Resistant Factor A (RFA) was isolated from corn plants and identified as 6-methoxy-2(3)-benzoxazolinone (6MBOA). This substance was found to have inhibitory effect on the growth of the corn borer larvae. Corn leaves contained the highest level of the factor and the mature portion of the stem contained very low levels. Incorporation of this substance into synthetic diets inhibited completely the growth of larvae. Another growth inhibitor was found in the ether-insoluble fraction of the aqueous extract. This chemical substance was termed Resistant Factor B (RFB) and accounted for the other half of growth inhibitory activity of the aqueous extract. The precursor of RFA, 2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3-one, was also isolated from the corn plants. The inhibitory activity of this precursor was found to be half of RFA (40). Correlation was found between the amount of RFA produced by 11 inbred strains of maize at the

whorl stage of plant development and the field rating of resistance of the inbred strains to the larvae of the European corn borer. Highly resistant inbred lines yielded about 10 times more RFA than the highly susceptible inbred strains. The content of RFA of inbred strains of maize may serve as an indicator of the degree of resistance a given inbred strain may express in the field (41).

Resistance of pine tree to the needle-eating pests, sawfly larvae and butterfly caterpillars, was found to be due to the presence of an essential oil in the oleoresin excreted from pine needles (42). This essential oil was toxic to the growth of these pests, and the physiologically healthy trees excreted much more of this oleoresin than the weakened ones. Saponins were considered as possible factors for resistance of bean, to insects (43). A mixture of saponins isolated from a resistant variety of bean inhibited the development of beetles while those from the susceptible variety showed no effect. Alkaloid-glycosides in the Solanum species were found to have toxic effects on the Colorado beetle larvae. It was assumed that these alkaloid-glycosides induced a blocking of the steroid metabolism and affected the resorption of the phytosterins that were indispensable for the insects. Disturbance in feeding and fertility was observed when larvae were feeding on the Solanum varieties (44). The major alkaloid constituents secreted by Nicotiana species were identified as anabasine and nornicotine (45). Resistance of these plants to the green peach aphid was due to the toxic activity of these alkaloids on insect growth (46). Lupine varieties resistant to pea aphid also contained alkaloids toxic to the aphid, while the susceptible varieties lacked these substances (47). Infiltration of alkaloids isolated from resistant varieties into the leaves of suscepti-

ble varieties caused a nearly complete death of adult aphids and larvae feeding on the infiltrated leaves. The mortality for the control was found to be negligible.

Lichtenstein et al. (48,49) isolated 2-phenyethylisothiocyanate from the edible part of turnips and 5-allyl-1-methoxy-2,3-methylenedioxy-benzene from parsnips. These chemicals were found to have insecticidal properties and occur naturally in these plant tissues. Derivatives of benzoic acid and salicylic acid which are toxic to the larvae of rice stem borer were found in rice plants (50). Phaseolunatin and related cyanogenetic glycosides were isolated from leaves of bush and lima beans (51). At high concentrations, phaseolunatin acted as an inhibitor for Mexican bean beetle, and was suggested as a possible factor for resistance against this insect.

Paper chromatographic separation of compounds in the extracts of the potato plant showed that the Heterodera rostochiensis Woll resistant varieties had more total phenols, O-dihydrophenols and flavenols than those of the susceptible plants (52). Deposits of calcium oxalate in the phloem parenchyma cells of tea plants was credited for the basis of red spider resistance (53). Other examples of insect resistance due to the presence of toxic substances in the host plants were found in the resistance of wheat to pale western cutworm (54), rutabaga to the cabbage maggot (55), and asparagus to nematodes (56).

Nutritional Deficiencies as a Factor in Resistance

The food requirements of insects for growth and reproduction differ greatly among different species of insects; but generally they include protein, carbohydrate, free amino acids, and certain vitamins or vitamin-

like substances. Different varieties of a single host species may differ in quality, quantity or availability of food for the insect; and they do not all serve equally well for food when measured by the maintenance of the insect population. The length of life, death rate, size and fecundity of insects are affected by nutritional factors. The life of the insect is usually shortened and death most frequently occurs before reaching the adult stage when it feeds on resistant plants. The size and fecundity of the insects reared on resistant plants are also smaller and lower than those on the susceptible ones.

In an investigation of European corn borer resistance Beck et al. (57) reported that growth and survival of this insect was improved by treating corn leaves with sucrose solution prior to feeding to the larvae. The decrease in larval mortality approximate paralleled the increase in percentage of sucrose used. A saccharotropic behavior response of the corn borer larvae was also found (58). The borer tended to concentrate its feeding on the tissue containing the highest level of sugar. In addition, a number of amino acids were found to have significant effects on the feeding behavior of the larvae. Average feeding duration was increased by L-alanine, DL-α-amino-n-butyric acid, L-serine, and L-threonine. A negative effect on feeding was observed with L-tryptophan, L-arginine and β-alanine (59). Concentrations of B-vitamins or inorganic salts had no effect on the feeding behavior of the larvae. However, a weak response to increasing level of protein and corn oil was observed, the larvae tending to select diets high in either of these substances (60). These findings suggested that amounts and kinds of food material available in the host corn plant might be important in resistance to the European corn borer.

Different parts of the same plant may have different quantities of food available either in total amount or in respect to certain kinds. Plant-feeding insects frequently feed on meristem tissues of plant which are richer in certain proteins than are other parts of the plant. Dahms and Painter (61) found that reproduction of pea aphids reared on flowering stems of alfalfa was greater than those on the vegetative branches of the same plant, and they suggested that nutritional factors might be related to resistance. Kennedy (14) stated that Fraenkel's assumption (13) that all plant leaves can provide uniform nutritional substances for insect growth cannot be applied to the aphids. Aphids do not eat leaves; the only nutrients directly available to them are those dissolved in the sap or in transit from one part of the plant to another. It has been shown that contents of the sap varied greatly through the cycle of growth, maturity and senescence of the foliage on the plant. Growth of aphids was found to be related to the nutrients in the sap (14). Maltais and Auclair (62) determined the nitrogen and sugar content of three susceptible pea varieties and three resistant varieties at various stages of growth. The susceptible varieties contained more nitrogen and less sugar than the resistant varieties, and the sugar-nitrogen ratio was 23.4 to 63.4 per cent higher in resistant varieties. Aphids reared on the susceptible varieties of pea had a higher growth rate than those reared on the resistant varieties. The rate of feeding on the susceptible varieties was found to be higher and therefore contributed to the faster rate of aphid growth. Honeydew from aphids feeding on susceptible varieties usually contained a slightly higher concentration of free amino acids (63). Using paper chromatography, Auclair et al. (64) analyzed the amino acids in juice extracts of two varieties of peas and

of pea aphids feeding on these varieties. Eleven free amino acids were found in the plant juice extracts, and 15 in the pea aphid extracts. Amino acids with higher molecular weights such as tryptophan and tyrosine were absent or present only in traces in the plants analyzed, but they were found in aphids feeding on these plants. Quantitative differences in the amino acids were observed in the two varieties of peas. The susceptible variety contained a higher concentration of free amino acids than the resistant one, with the exception of proline. Proline was present in a higher concentration in the resistant variety. The quantitative differences in amino acid content were suggested as factors in resistance or susceptibility to infestation by aphids. Aphids feeding on resistant varieties may not obtain enough of each essential amino acid per unit of time to sustain optimal growth and reproduction. This would cause the total aphid population on resistant varieties to be usually smaller than that on susceptible varieties (65). In the list of amino acids identified, glutamine, asparagine and homoserine were consistently in higher concentration in all susceptible varieties tested. Pea leaves treated with these three amino acids increased aphid growth significantly and to a greater extent than with most other single amino acids. They appeared to play a key role in influencing the degree of susceptibility or resistance of pea varieties to aphid attack (66).

The glucose content in a tobacco variety susceptible to green peach aphid was found to be much higher than that in the resistant variety, but the significance of this difference to plant resistance was uncertain (67). Marble et al. (68) found that the spotted alfalfa aphid contained 1.5 to 5.5 times the quantity of free amino acids as alfalfa. Aphids feeding on susceptible alfalfa varieties accumulated 7 to 10

times more β-alanine and ethanolamine than that was found in the alfalfa tissue, while aphids feeding on resistant alfalfa varieties contained little or none of these compounds. No conclusive differences in amino compounds were apparent that distinguished resistant from susceptible plants. The effects of environment on amino acid content were significant. Resistance of corn to the corn leaf aphid was found to be related to the carotene content of the corn plant (69). Susceptible corn varieties contained greater amounts of carotene than the resistant varieties.

One of the factors for resistance of plants to nematodes is also nutritional (56). Resistant plants were not suitable for the growth of nematodes as they failed to supply some nutrient necessary for survival of the parasites. Knapp et al. (70) examined the free and bound amino acids from silks of corn earworm resistant and susceptible varieties of corn plant. Equal numbers of free and bound amino acids were found in all varieties tested; however, a greater concentration of bound amino acids and a higher percentage of reducing sugar were found in the susceptible varieties.

Resistance to the wheat stem sawfly was attributed to differences in amino acid content in wheat varieties (71). Resistance of wheat to Hessian fly attack was found to be related to the allulose content in the plants (72). This compound was present in the susceptible varieties, absent in the resistant ones and present to a lesser extent in the semi-resistant varieties. Sorbitol was found in the susceptible varieties only. A low level of linoleic acid in the host plant was found to be associated with the sterility of female sugar beet webworms, and thus contributed to the resistance against this insect (73).

CHAPTER III

METHODS AND MATERIALS

Plant Materials

Barley varieties, Will and Rogers (Hordeum vulgare L.), which were resistant and susceptible respectively to the attack of greenbugs were used for the analysis of volatiles and for biological assays. For the determination of hydrogen ion concentrations, Will, Rogers and isogenic lines of five groups consisting of resistant and susceptible varieties were used. These plants were harvested when they were 14 days old.

Isogenic A (resistant) and Isogenic B (susceptible) as well as Will and Rogers were used for the analysis of auxins. One-half of the plants in each variety were infested with greenbugs when the plants reached the age of 7 days. Both uninfested and infested plants were harvested when they were 14 days old.

For the analyses of free amino acids, sugars and organic acids, and the determinations of water content, Will, Rogers, Isogenic A and Isogenic B were used. Some of the plants in each variety were infested with greenbugs when they were 7 days old. Plant samples were collected at ages 7 days, 14 days, 21 days and 28 days. The greenbug-infested plants as well as the uninfested controls were harvested.

All plant materials used in this study were grown in a greenhouse.

Gas-Liquid Chromatography

A modified Barber-Colman Model 5000 gas chromatograph equipped with a hydrogen flame detector was used for all the gas-liquid chromatographic analyses in this study. The modification consisted of an oven and injection port of the same dimensions as used on the gas chromatograph-mass spectrometer combination instrument (74,75).

Volatile Compounds in the Susceptible and Resistant Varieties of Barley

Steam Distillation of Volatile Compounds

Five hundred grams of fresh leaves were cut into short lengths and put into a 3000 ml Pyrex round-bottom flask. The distillation was carried out at 20mm pressure and at a temperature not greater than 40°C. The vapors passed through a spiral glass condenser, two flasks immersed in an ice-salt bath, and finally into a trap cooled with dry ice. The condensates from the flasks were combined and extracted three times with diethyl ether. The ether was anhydrous analytical grade. The ether extracts were combined, saturated with anhydrous sodium sulfate and concentrated by passing a stream of nitrogen gas onto the extracts. Volatile components in the extracts were then ready to be analyzed by the gas-liquid chromatograph and the combination gas chromatograph-mass spectrometer.

Gas-Liquid Chromatography of the Volatile Components

The column used for gas-liquid chromatography was packed according to the technique developed by Horning *et al.* (76). Acid-washed 60-80

mesh Chromosorb G purchased from Applied Science Laboratories, Philadelphia was used as the supporting material for the liquid phase. Carbowax 20M, also purchased from Applied Science Laboratories, was employed as the stationary phase. One hundred milliliters of 25% solution of Carbowax 20M in chloroform were added to 20 g of Chromosorb G. Vacuum was applied and, after 30 minutes, the excess solution was removed by filtration. The packing material was spread on a filter paper to air dry, then dried in the oven at 80°C. A 5 ft. x 1/4 in. coiled glass column designed to fit the mass spectrometer was carefully washed and silanized before packing. The coated support was packed into the column with light tapping and the aid of a water aspirator connected to the opposite end of the column. The packed column was then conditioned at 150°C for 72 hours.

The samples were chromatographed with an initial column temperature of 60°C and an initial isothermal period of 30 min., and thereafter programmed at 1°C/min. to 150°C. Other parameters were: injection port at 150°C, detector at 200°C and carrier gas at 55ml/min. The sample volume was 4 microliters. Volatile compounds in the ether extracts were identified by their retention times. For quantitative determination of the compounds, peak area was measured by a planimeter and compared with the area given by a known amount of the respective standard.

Gas Chromatography-Mass Spectrometry of the Volatile Components

A prototype of the LKB 9000 combination gas chromatograph-mass spectrometer instrument was used (74). The 25% Carbowax 20M column used in the gas-liquid chromatographic analyses was also used here. The column temperature was initially at 60°C, and programmed to 150°C at 1°/min.

after 30 min. of an initial isothermal period. The molecular separator was kept at 200°C; the ion source, 310°C; and the flash heater, 200°C. The ionizing current was maintained at 20 amp. with the ionization voltage at 70 eV and the electron multiplier voltage at 1.7 kV. The accelerating voltage was 3.5 kV.

Mass spectra were obtained as the peaks appeared on the TIC[1] recorder serving as the monitor for the GLC unit. Background mass spectra derived mainly from the column liquid phase were always recorded and subtracted from the sample spectra. Volatile components were identified by their molecular ions, fragmentation patterns and GLC retention times.

Free Amino Acids, Sugars and Organic Acids in the Susceptible and Resistant Varieties of Barley

Extraction

Twenty grams of fresh leaves were cut into small pieces, treated with liquid nitrogen and ground to fine powder with a pestle in a mortar. The plant material was then extracted with 80% aqueous ethanol at room temperature for 10 min. The extract was centrifuged and the supernatant liquid collected. The residues were re-extracted and the fractions bulked. The final volume of 80% ethanol was 200 ml. The alcohol was removed in vacuo on a rotary evaporator, and the aqueous residue was filtered. The filtrate was a clear yellowish-green solution.

---

[1] TIC = Total Ion Current (proportional to total no. of ion formed and consequently to amount of compound).

Fractionation and Purification

Two ion-exchange columns were arranged in series. The first column was packed with cation-exchange resin, Dowex 50Wx8 (H^+), 50 to 100 mesh, and the second one with anion-exchange resin, Dowex 1x10 (Cl^-), 200-400 mesh. The columns were 1.5 cm in diameter and 25 cm in length. Aliquots of the aqueous plant extract were passed through the columns with the flow rate adjusted to 5 ml per minute. After the extract had passed through, the columns were washed with distilled water with the neutral effluent from the second column being collected along with the distilled water washings. This neutral fraction contained the sugars. The columns were than separated and the amino acids were displaced from the Dowex 50 column with 2 N NH_4OH. The organic acids were eluted from the Dowex 1 column with 0.1N HCl. The three groups of compounds separated in this way were dried on a rotary evaporator at 40°C and made up to the standard volume (5 ml) (77).

Thin-layer Chromatography of Amino Acids, Sugars and Organic Acids

Thin-layer plates of 0.5 mm in thickness were prepared according to Stahl (78). Glass plates of 20 x 20 cm were coated with a suspension of specific supporting material and solvent using a commercial apparatus (Brinkmann and Co.). The plates were allowed to air dry, and then activated in an oven at 110°C - 120°C for three hours before use.

Two dimensional thin-layer chromatography was used to analyze the amino acids. Thin-layer plates were prepared by mixing 10 g of MN 300 Cellulose (Macherey, Nagel and Co., Germany), 4 g of Silica Gel G (Brinkmann and Co.) and 80 ml of distilled water, blending for 30 seconds in a Waring blender and spreading the resulting uniform suspension over

the glass plates in the usual manner (79). The sample was applied to the origin with a micropipette. Each sample application was dried at a temperature not exceeding 40°C. Plates were developed once in phenol-water (80:20, w/w) in the first dimension (6 hr), and then dried overnight at 40°C to insure adequate removal of phenol. In the second dimension the plates were developed twice in butanol- 96% acetic acid-water (5:1:4, v/v/v) (4 hrs in each run).

Amino acids were detected by spraying with 0.5% ninhydrin in 95% ethanol, air drying the plate and then heating it at 105°C for 5 min.

Sugars were analyzed by thin-layer chromatography on Silica Gel G impregnated with sodium monohydrogen phosphate in phosphoric acid. Plates were prepared with a suspension of 50 g Silica Gel G and 110 ml 0.3M Na_2HPO_4 in 0.1M H_3PO_4 was coated on 20 x 20 cm glass plates (80). Sugar samples and standards (10 - 20 µg) were applied on the plate with micropipettes. The plates were developed twice by the ascending technique in a solvent system containing formic acid-methy ethyl ketone-t-butanol-water (15:30:40:15, v/v/v/v). Freshly prepared solution of 0.5 ml anisaldehyde in 9 ml of 95% ethanol with addition of 0.5 ml concentrated sulfuric acid was used to detect the sugars on the plates. This detecting reagent was very sensitive (0.05µg of sugar) and gave characteristic colors for the sugars.

Organic acids were separated and identified by thin-layer chromatography on Silica Gel G plates. The solvent system used contained n-butanol-formic acid-water (6:1:1, v/v/v). The spraying reagent for the organic acids contained 0.04% alcoholic solution of bromocresol green adjusted to pH 5.5 (81).

Quantitative Determination of the Total Soluble Sugars

The total soluble sugars in the purified extracts were determined by the procedure described by Nalewaja et al. (82). An aliquot of the extract was diluted to obtain a sugar concentration of approximately 50µg per 2 ml of solution. A 2 ml portion of the diluted extract was pipetted into a test tube, and 0.125 ml of 80% aqueous phenol (w/w) was added. Five milliliters of concentrated sulfuric acid were then added rapidly with the acid stream being directed to the liquid surface in the test tube. After approximately 10 minutes, each tube was shaken and the rack of tubes were placed in a water bath at 28°C for 20 minutes. The resulting color was read in a spectrophotometer at a wave length of 490mµ.

Gas-Liquid Chromatography of the Monosaccharides

Monosaccharides in the sugar fraction of the extracts were determined quantitatively by gas-liquid chromatography using the method described by Sawardeker et al. (83). Alditol acetates of the monosaccharides were prepared according to the method of Abdel-Akher et al. (84). One ml 1% aqueous solution of sodium borohydride was added to 1 ml of 5% aqueous solution of sugar, and the mixture was held at room temperature for 3 hrs. Excess borohydride was neutralized with acetic acid (glacial), and the solution evaporated to dryness. The dry residue was refluxed for 4 hours with a mixture containing equal amounts of acetic anhydride and pyridine (1 ml/100 mg of sugar). The solution was cooled, concentrated to a standard volume (1 ml) and injected directly in the gas chromatograph for analysis.

A 20 ft. x 1/4 in. coiled glass column packed with 5% ECNSS-M

(cyanoethyl silicone) coated on 100 - 120 mesh Gas-Chrom Q, both purchased from Applied Science Laboratories, State College, Pa., was employed for the analyses. The samples were chromatographed with a column temperature at 195°C, injector temperature at 225°C and detector temperature at 250°C. The carrier gas was kept at 55 ml/min. The volume of samples injected was 2 microliters.

Arabinol acetate was chosen as an internal standard. Linear response was obtained for the ratio of peak area to the amount of arabinol acetate when varying amounts of arabinol acetate were chromatographed. Amount of a monosaccharide in the extract was determined by direct comparison of peak area of the unknown with that of the corresponding standard of known amount. The peak area was measured by a planimeter.

Quantitative Analysis of the Free Amino Acids

The quantity of each free amino acid present in the amino acid fraction of the extracts was determined by automatic amino acid analysis. Because of their high concentration present in the extracts, the amounts of aspartic acid, threonine, serine, asparagine and glutamine were determined by a Beckman Model 120 C Amino Acid Analyzer. Norleucine was used as an internal standard. The other amino acids were determined by a Technicon Amino Acid Autoanalyzer.

Free and Bound Auxins in the Susceptible and Resistant Varieties of Barley

Extraction

The general procedure for the extraction and purification of indoles outlined by Maxwell and Painter (85) and Larsen (86) was followed. A

schematic diagram of the procedure is shown in Figure 1. The "free" indoles were isolated from plant materials after 3 hours of extraction with 95% ethanol. The residual plant materials were further extracted with 95% ethanol for 45 hours to isolate the "bound" indoles. Both groups of indoles were further purified according to the scheme in Figure 1.

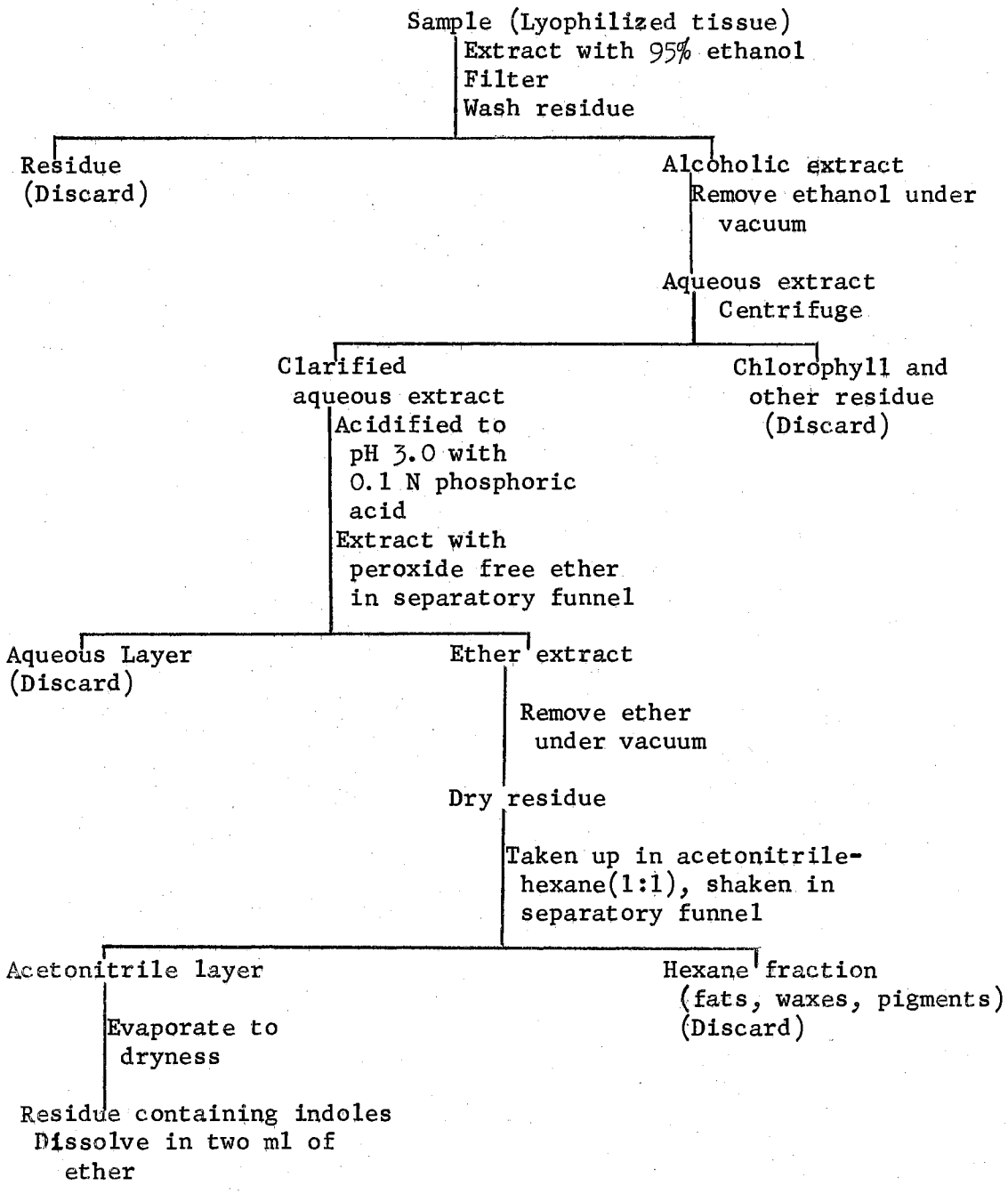
Thin-Layer Chromatography of the Indoles

Thin-layer plates were prepared by coating the 20 x 20 cm glass plates with a slurry of 50 g Silica Gel G and 110 ml 0.3M Na_2HPO_4 in 0.1M H_3PO_4. The plates were allowed to stand at room temperature for 1 hour, and then dried in an oven at 110°C for 3 hours. Samples of indole extracts and standards were applied on the plate with micropipettes. The size of sample used was 10 to 20µg. The plates were developed by ascending technique in a solvent system containing chloroform-96% acetic acid (95:5, v/v). Indoles were detected by spraying the plates with Ehrlich reagent (1% solution of p-dimethylaminobenzaldehyde in 96% ethanol) and placing for 3 to 5 min. in a vessel saturated with hydrochloric acid vapor (87).

Gas-Liquid Chromatography of the Indoles

Quantitative determination of the indoles was accomplished by gas-liquid chromatography. Indoles were first methylated with diazomethane, and their trifluoroacetyl derivatives were prepared according to the method described by Brook et al. (88). One mg of indole was dissolved in 3 ml of anhydrous ethyl acetate in a test tube, and 0.5 ml of trifluoroacetic anhydride (TFA) and a small amount of anhydrous sodium

Figure 1. Scheme of Extraction and Purification of Indoles from Plant Material


Sample (Lyophilized tissue)
Extract with 95% ethanol
Filter
Wash residue
Residue
(Discard)
Alcoholic extract
Remove ethanol under vacuum
Aqueous extract
Centrifuge
Clarified aqueous extract
Acidified to pH 3.0 with 0.1 N phosphoric acid
Extract with peroxide free ether in separatory funnel
Chlorophyll and other residue
(Discard)
Aqueous Layer
(Discard)
Ether extract
Remove ether under vacuum
Dry residue
Taken up in acetonitrile-hexane(1:1), shaken in separatory funnel
Acetonitrile layer
Evaporate to dryness
Residue containing indoles
Dissolve in two ml of ether
Hexane fraction
(fats, waxes, pigments)
(Discard)

sulfate were added. The tube was capped, and the solution agitated by a magnetic stirrer for one hour at room temperature. The solvent and the excess TFA were then removed under reduced pressure, and the residue was dissolved in 1 ml of ethyl acetate.

A 10 ft. x 1/4 in. coiled glass column packed with 3% QF-1 (trifluoropropyl methyl silcone) liquid phase coated on 100 - 200 mesh Gas-Chrom Q, both purchased from Applied Science Laboratories, State College, Pa., was used for the analysis. The samples were analyzed with the column temperature at 165°C, injector temperature at 180°C, and detector temperature at 200°C. The volume of samples injected was 2 microliters.

Water Content of the Resistant and Susceptible Varieites of Barley

Determination of Water Content

Fresh leaves and stems were cut into small pieces and placed in a platinum container of known weight. The plant material and the container were weighed, and dried in an oven at 100°C for 24 hours. The dry plant material and container were weighed again, and the water content of the plant was determined.

Hydrogen Ion Concentrations in the Resistant and Susceptible Varieties of Barley

Preparation of Cell Saps and Determination of pH

Fresh leaves of the plants were cut into short lengths. Cell saps were obtained by disintegrating plant material with a homogenizer without addition of any liquid. Hydrogen ion concentrations of the cell

saps were determined with a Sargent Model DR pH meter.

Secretions of Greenbug to the Host Plant During Feeding

Materials

Radioactive greenbugs were produced by infesting a small pot of Rogers barley plants with greenbugs and placing them inside a photosynthetic chamber in a $^{14}CO_2$ atmosphere produced by adding $Ba^{14}CO_3$ and 1N H_2SO_4 to the side arm. After 48 hours, the plants were taken out of the chamger, and the greenbugs removed from the plants. These greenbugs were then transferred to another pot of barley plants. The plants and the greenbugs were then kept inside a dark chamber in order to stop the photosynthesis occurring in the plant. Greenbugs were removed from the plants after 24 hours, and the necrotic areas on the plant leaves resulting from the feeding of these radioactive greenbugs were excised and extracted with 80% ethanol at room temperature.

Extraction and Fractionation

The method used for the extraction of free amino acids and sugars was followed. The extracts were fractionated into free amino acids and sugars fractions following the procedure previously described.

Identification of Radioactive Substances in the Extracts

Components in the free amino acids fraction of the extract were separated and identified by two-dimensional thin-layer chromatography on plates coated with a mixture of cellulose and Silica Gel G (5:2). Method for the preparation of the thin-layer plates was described in

page 26. The plates were developed once in phenol-water (80:20,w/w) in the first dimension and then twice in n-butanol- 96% acetic acid- water (5:1:4, v/v/v) in the second dimension. Radioactive compounds on the thin-layer chromatogram were detected by autoradiography.

Similar methods were employed to identify the radioactive substances in the sugar fraction of the extract. One dimensional thin-layer chromatogram was used. Thin-layer plates were developed in a solvent system containing formic acid:methylethyl ketone:terbutanol:water (15:30:40:15, v/v/v/v). Autoradiography was also used to detect radioactive compounds on the thin-layer chromatograms.

Comparison of Chemical Substances in the Resistant and Susceptible Barley Varieties by the Technique of Biological Assay

Extraction and Fractionation

The procedure employed for the extraction and fractionation of the plant materials was the same as the one described for the free amino acids, sugars and organic acids.

Biological Assay

The biological assay experiments using greenbugs were performed by the Entomology Department. Different fractions of the plant extracts were incorporated into the standard synthetic diets for the greenbug developed by Dr. Donald C. Cress of the Entomology Department (89). The growth of greenbugs feeding on the modified synthetic diets was measured by their weight and number of progeny they produced.

CHAPTER IV

RESULTS AND DISCUSSION

Volatiles of Greenbug-Resistant and Susceptible Barley Varieties

Several methods of collecting the volatiles of the barley plants have been studied. Steam distillation at atmospheric pressure had been a conventional method used for collecting volatiles of plant materials for many years. More recently, this method has been found unsuitable, because of the formation of artifacts due to thermal decomposition of less stable compounds. The lower alcohols and ketones are formed quite readily from comparatively simple and common precursors, such as pectin, amino acids and sugars, which are present in all plant materials (90). The straight chain, saturated, and unsaturated aldehydes are formed readily from oxidative degradation of the lipids (91). Aeration of fresh plant materials by sweeping with a gas or vapor stream and condensation in cold traps was suggested. This method of sample collection was found to be satisfactory, but also time consuming, more than 24 hours were required to collect enough samples for analysis, and for efficient collection a large number of traps were necessary. In the present study, steam distillation at reduced pressure was found to be the most satisfactory method. Thermal decomposition of the less stable compounds was prevented by working at low temperature. Gas-liquid chromatographic analysis showed this method collected the same number of

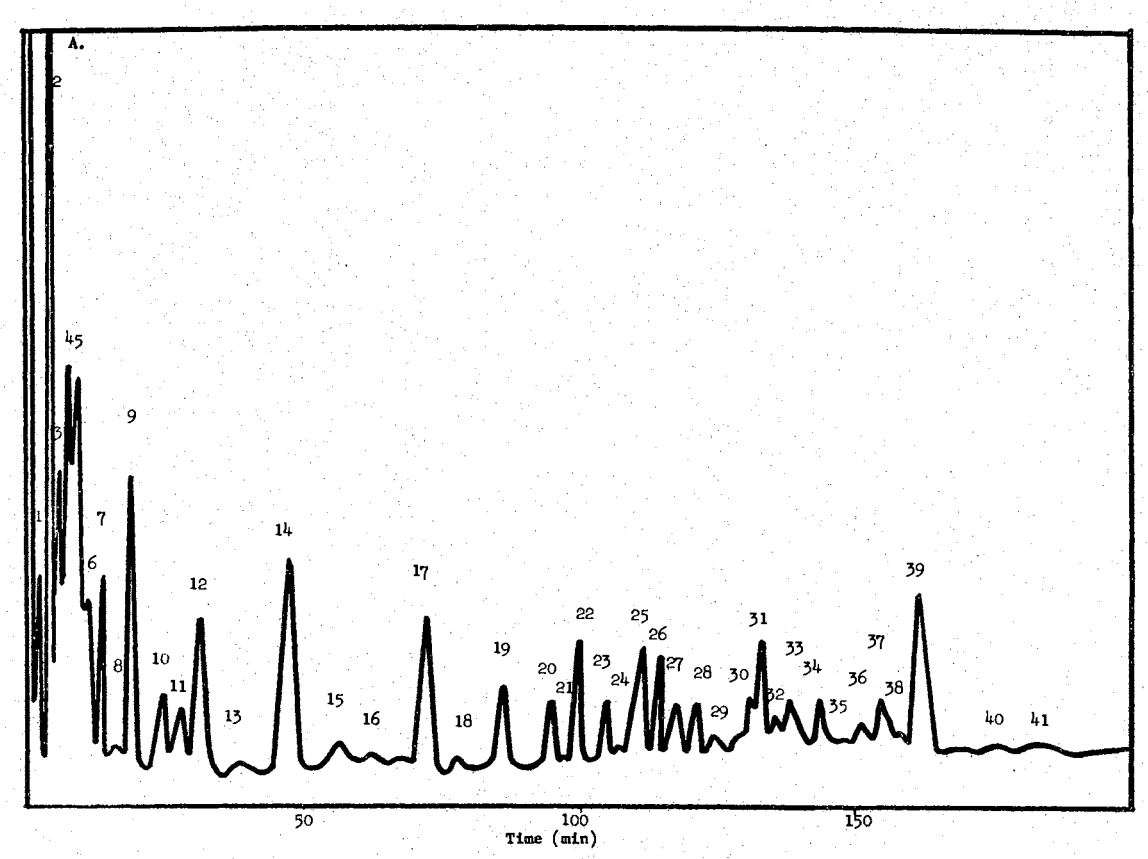
volatiles as the aeration method, but with greater intensity quantitatively. Some studies were made with different liquid stationary phases for gas-liquid chromatography. The liquid phases, Ucon Polar, Apiezon L and Carbowax 20M were employed. The best conditions of operations were obtained using a 5 ft. x 1/4 in. 25% Carbowax 20M column. It was also found in these studies that isothermal operation was not sufficient to provide the degree of resolution necessary for identification purposes. Analysis of the volatiles under isothermal conditions showed the resolution of only 20 component peaks. Comparisons between runs made under isothermal and temperature programmed conditions showed great improvements in resolution and separating power in the temperature programmed conditions. Under temperature programming conditions the number of resolved components was more than 40. Typical chromatograms of volatiles of Will and Rogers barleys run under temperature programmed conditions were shown in Figure 2. Comparisons of the Will and Rogers barley samples showed that they were qualitatively similar, both containing 41 component peaks.

Table I lists the retention time and the approximate relative percentage abundance of each component peak in Will and Rogers samples. The quantitative estimation was made by relating individual peak area to the total area under the curve. Of the 41 peaks present, 11 were greater than 4% in relative abundance, and were arbitrarily defined as major volatiles. Among these, component peaks 3, 5, 12 and 14 were found to have significant higher percentage in Will barley than in Roger barley, whereas component peaks 19, 39 and 41 were higher in Rogers barley than in Will barley. This result would indicate that, although the volatiles of Will and Rogers were found to be similar qualitatively,

Figure 2. Gas-Liquid Chromatography of Volatiles of Resistant and Susceptible Barley Varieties on a 5 Ft. x 1/4 In. 25% Carbowax 20M Column with Initial Isothermal Temperature of 60°C for 30 Min. and Programming at 1°C/Min. to Final Temperature of 150°C

A. Volatiles of Will (resistant)

B. Volatiles of Rogers (susceptible)


A.
50
100
150
Time (min)

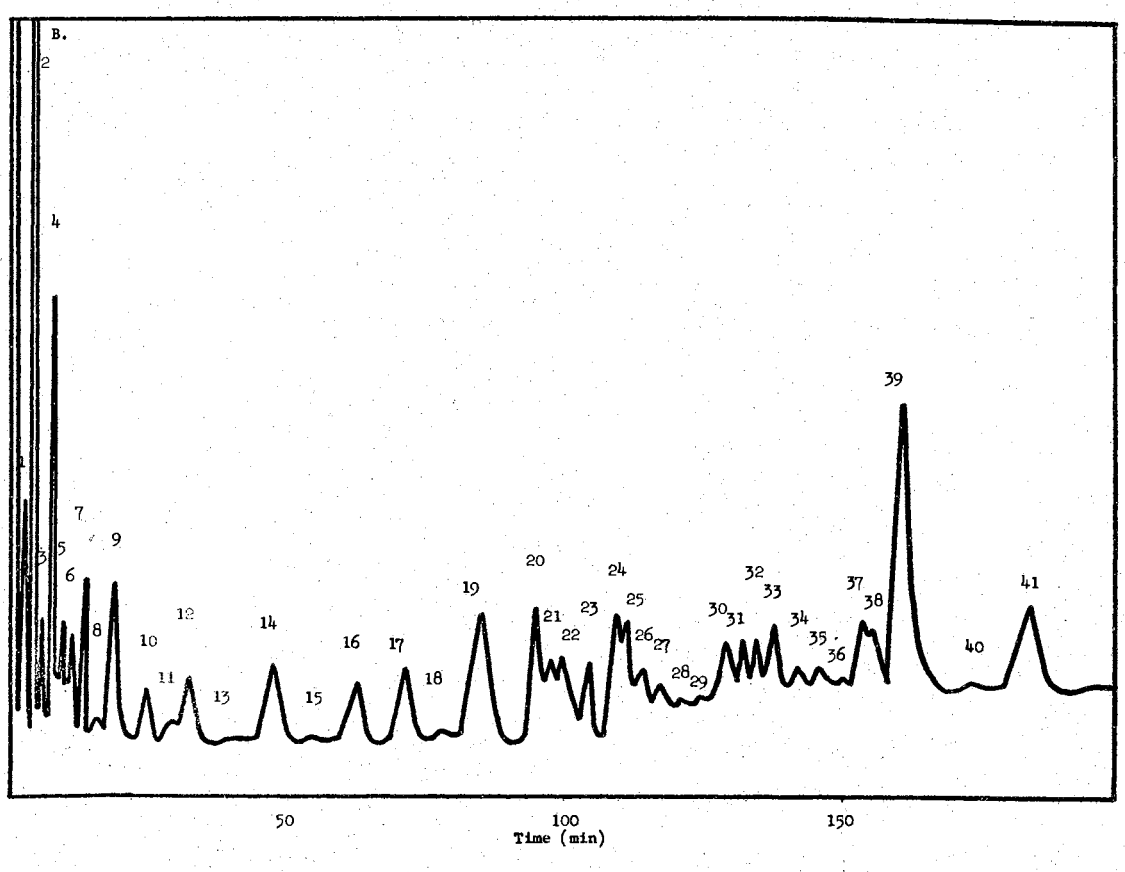

B.
50
100
150
Time (min)

TABLE I

RETENTION TIME AND RELATIVE ABUNDANCE OF VOLATILE COMPONENTS OF WILL AND ROGERS VARIETIES

Peak No.	Retention Time	Relative Abundance		Peak No.	Retention Time	Relative Abundance	
		Will	Rogers			Will	Rogers
	(min)	(per cent)			(min)	(per cent)	
1.	3	0.71	1.4	21.	97.5	0.18	1.7
2.	4.8	8.8	10.1	22.	99.5	2.1	2.4
3.	6.2	5.3	2.4	23.	104	1.4	1.4
4.	7.7	9.9	7.4	24.	108.5	0.18	2.7
5.	9.5	8.5	2.0	25.	111	2.5	2.7
6.	11	3.5	1.7	26.	114	1.8	1.01
7.	13.5	2.8	2.4	27.	117.5	1.1	1.01
8.	15.5	0.71	1.01	28.	121	1.1	0.34
9.	18.5	5.3	3.4	29.	124	1.1	0.67
10.	24	2.5	2.7	30.	129	1.1	1.4
11.	29	2.1	1.01	31.	132.5	2.1	1.0
12.	32	4.6	2.7	32.	135	0.71	1.0
13.	39	1.1	0.67	33.	138	1.4	1.7
14.	47	7.8	3.4	34.	142.5	1.4	0.67
15.	56	1.4	0.67	35.	146.5	0.18	0.67
16.	62.5	1.1	2.4	36.	150	0.71	0.17
17.	71	4.2	3.0	37.	154	1.1	2.7
18.	77.5	0.71	0.34	38.	156	0.35	2.0
19.	84.5	1.8	5.1	39.	161.5	4.6	11.8
20.	95	1.4	3.4	40.	175	1.1	1.01
				41.	184	0.35	5.1

they were different quantitatively. Volatiles that might act as repellents or as attractants may be present in both Will and Rogers barleys but in different concentrations. Repellents may exist in larger quantities in Will barley than in Rogers barley while attractants may be present in larger quantities in Rogers barley than in Will barley.

Identification of some of the volatile components and their approximate concentrations in Will and Rogers barleys were shown in Table II. The approximate concentrations were determined by direct comparisons of peak areas of unknown samples with those of the reference samples of known amounts. Of these identified volatiles, only ethyl acetate appeared to be appreciably different in concentration in Will and Rogers barleys. It has been found recently that the occurrence of ethanol in the samples can be at least partially attributed to the presence of this compound in trace amount in the ether used for the extraction of the steam distillates (92). The mass spectra of these identified volatiles and their corresponding reference compounds are shown in Figures 3, 4, 5, 6 and 7. A number of compounds found were not characterized, even though mass spectra were obtained.

Figure 8 shows the effects of these identified volatile components on the growth of greenbugs. Volatile components at concentrations approximately equivalent to that in the Rogers barley were incorporated individually into the standard synthetic diet for the greenbug, and the growth of greenbugs was measured by their weights and the number of progeny they produced. None of these volatiles were found to have significant effects.

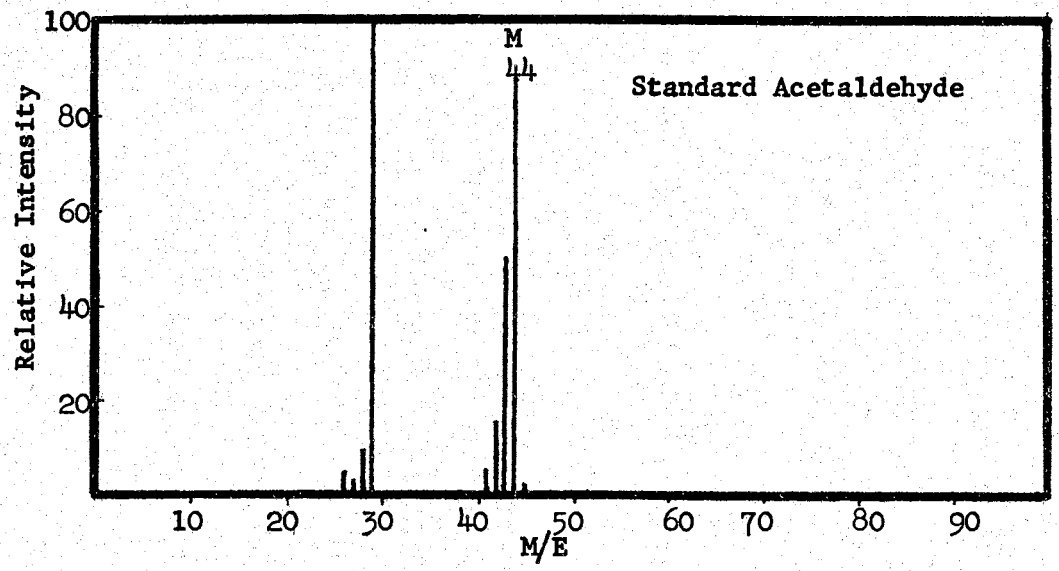
Effects of steam distillates containing all the volatile components from Will and Rogers barleys on the growth of greenbugs are shown in

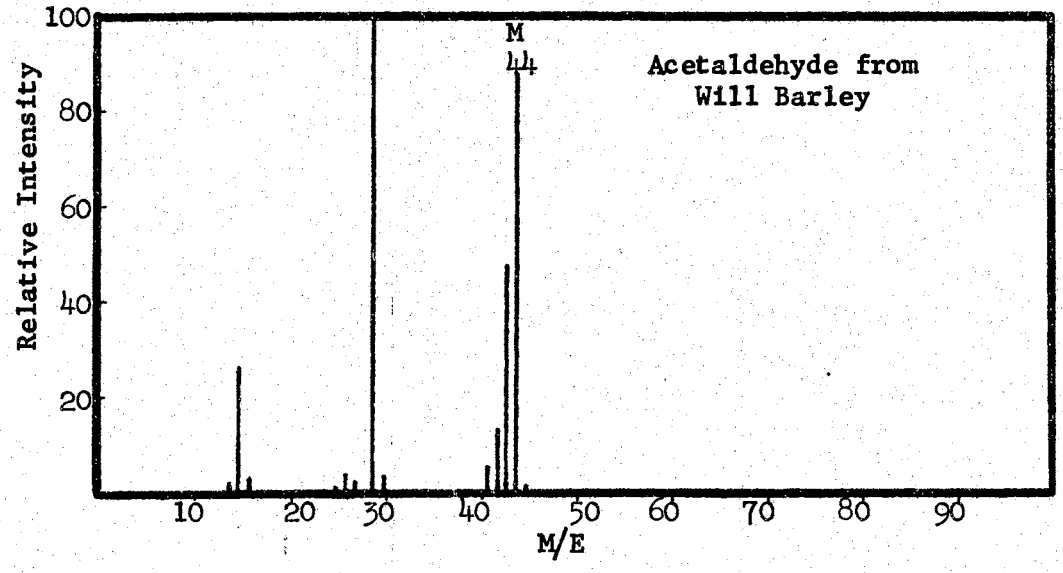
TABLE II

IDENTIFICATION AND APPROXIMATE CONCENTRATIONS OF SOME VOLATILE COMPONENTS OF WILL AND ROGERS VARIETIES

Sample Peak No.	Identification	Sample Peak Retention Time	Reference Compound Retention Time	Approximate Concentration	
				Will	Rogers
		(min)	(min)	(µg/g dry weight)	
1.	acetaldehyde	3	3	0.2	0.3
2.	ethyl formate	4.8	4.8	1.8	2.0
3.	ethyl acetate	6.2	6.2	1.0	0.5
4.	ethanol	7.7	7.7	2.0	1.5
10.	n-butanol	24	24	0.5	0.5

Figure 3. Mass Spectra of Standard Acetaldehyde, Acetaldehyde from Will Barley and Acetaldehyde from Rogers Barley


Relative Intensity
100
80
60
40
20
M
44
Standard Acetaldehyde
10
20
30
40
50
60
70
80
90
M/E


Relative Intensity
100
80
60
40
20
M
44
Acetaldehyde from
Will Barley
10
20
30
40
50
60
70
80
90
M/E

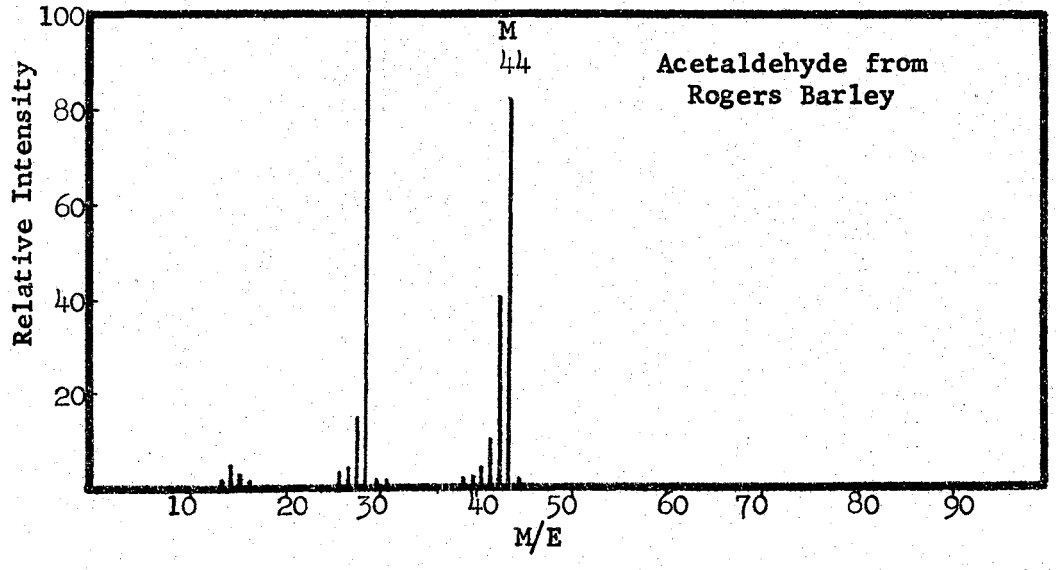
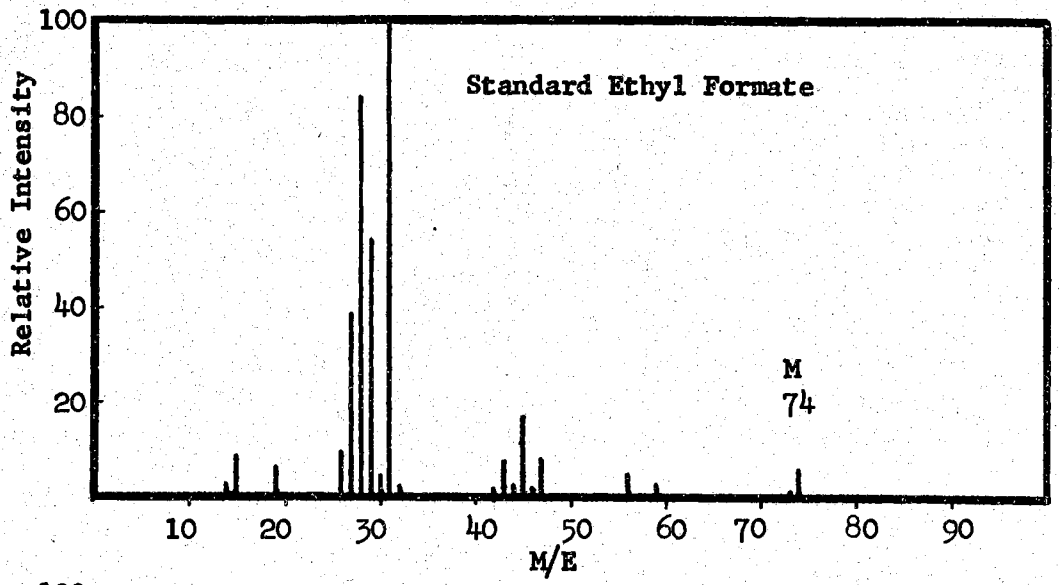
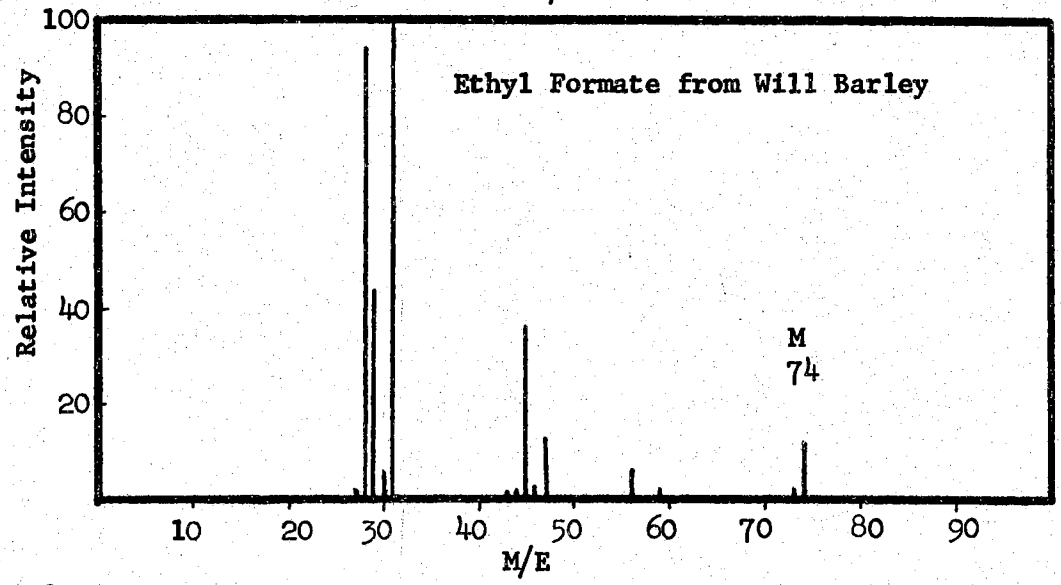

Relative Intensity
100
80
60
40
20
M
44
Acetaldehyde from
Rogers Barley
10
20
30
40
50
60
70
80
90
M/E

Figure 4. Mass Spectra of Standard Ethyl Formate, Ethyl Formate from Will Barley and Ethyl Formate from Roger Barley


Standard Ethyl Formate
Relative Intensity
100
80
60
40
20
M
74
10
20
30
40
50
60
70
80
90
M/E


Ethyl Formate from Will Barley
Relative Intensity
100
80
60
40
20
M
74
10
20
30
40
50
60
70
80
90
M/E

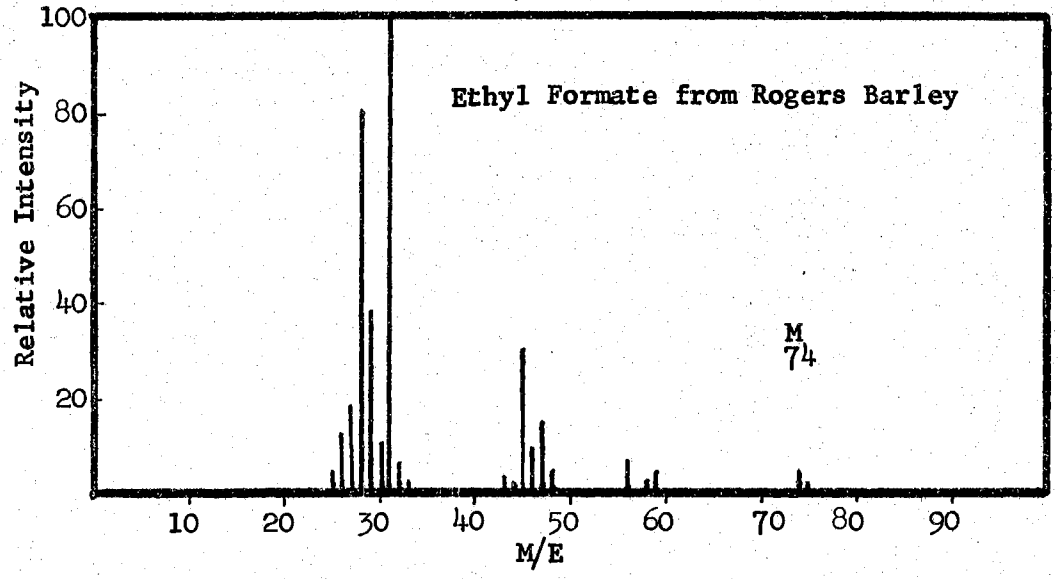
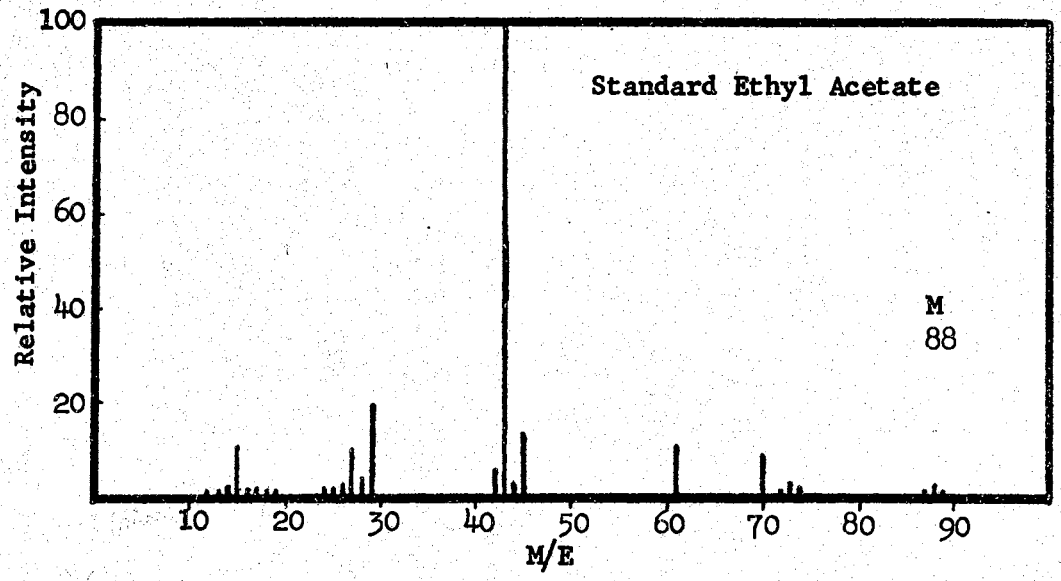
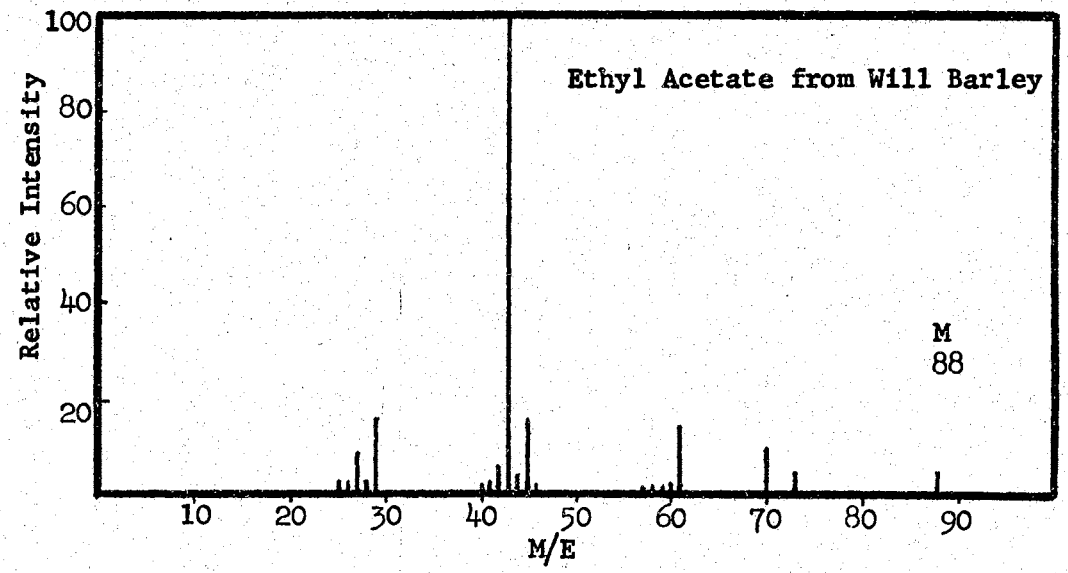

Ethyl Formate from Rogers Barley
Relative Intensity
100
80
60
40
20
M
74
10
20
30
40
50
60
70
80
90
M/E

Figure 5. Mass Spectra of Standard Ethyl Acetate, Ethyl Acetate from Will Barley and Ethyl Acetate from Rogers Barley


Standard Ethyl Acetate
Relative Intensity
100
80
60
40
20
M
88
10
20
30
40
50
60
70
80
90
M/E


Ethyl Acetate from Will Barley
Relative Intensity
100
80
60
40
20
M
88
10
20
30
40
50
60
70
80
90
M/E

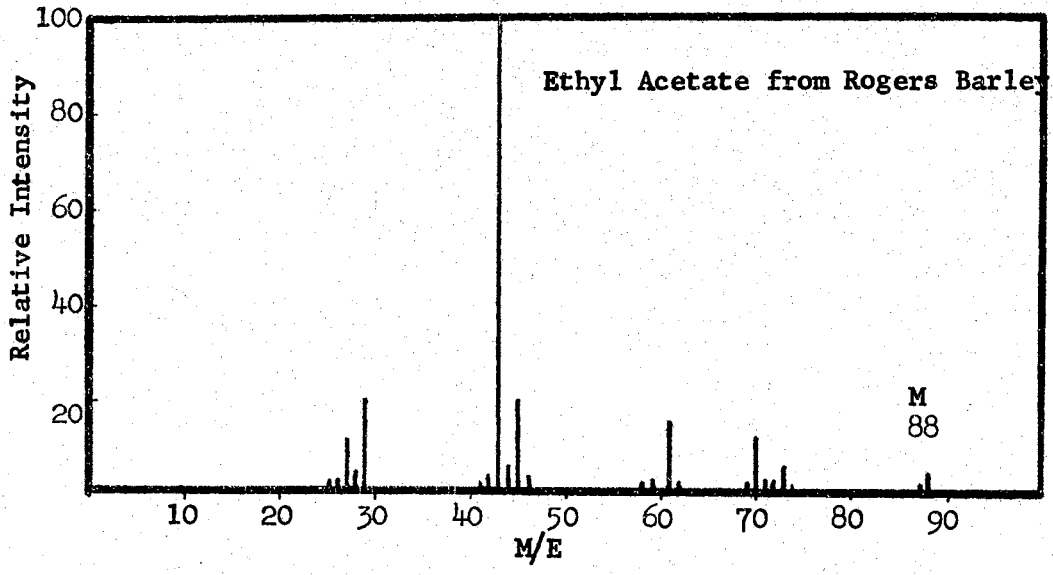
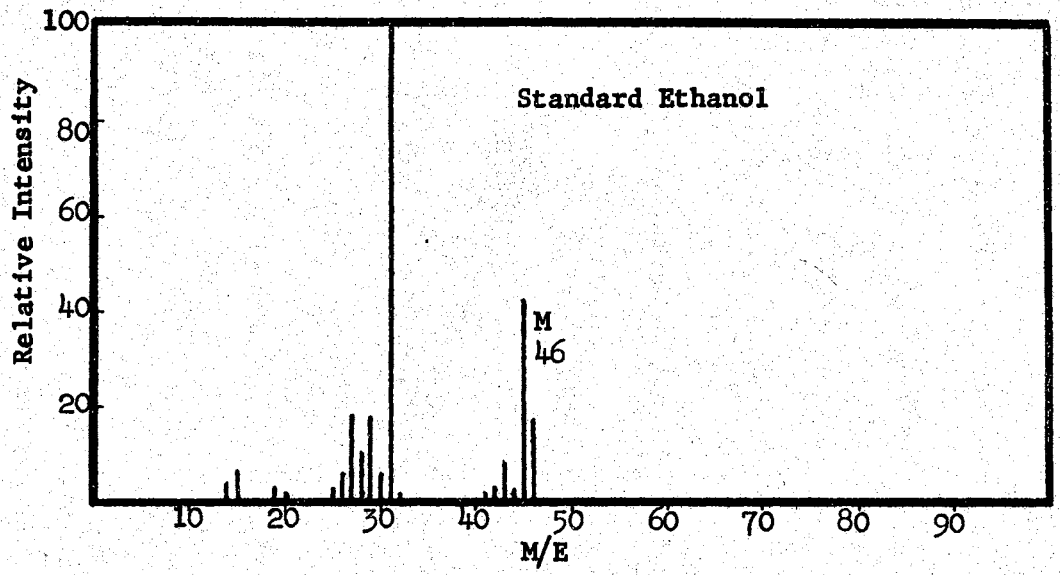
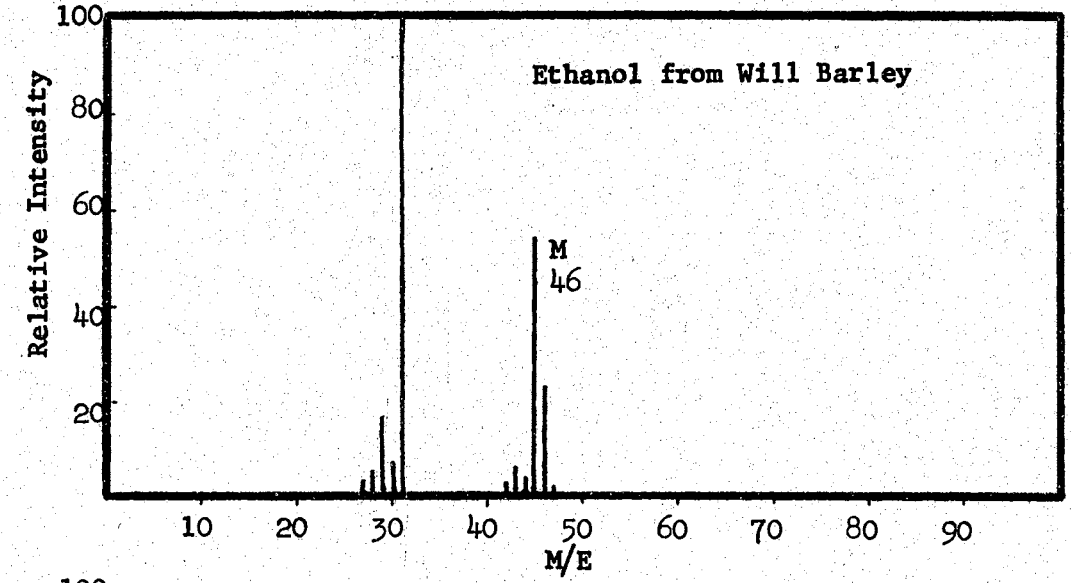

Ethyl Acetate from Rogers Barley
Relative Intensity
100
80
60
40
20
M
88
10
20
30
40
50
60
70
80
90
M/E

Figure 6. Mass Spectra of Standard Ethanol, Ethanol from Will Barley and Ethanol from Rogers Barley


Standard Ethanol
Relative Intensity
100
80
60
40
20
M
46
10
20
30
40
50
60
70
80
90
M/E


Ethanol from Will Barley
Relative Intensity
100
80
60
40
20
M
46
10
20
30
40
50
60
70
80
90
M/E

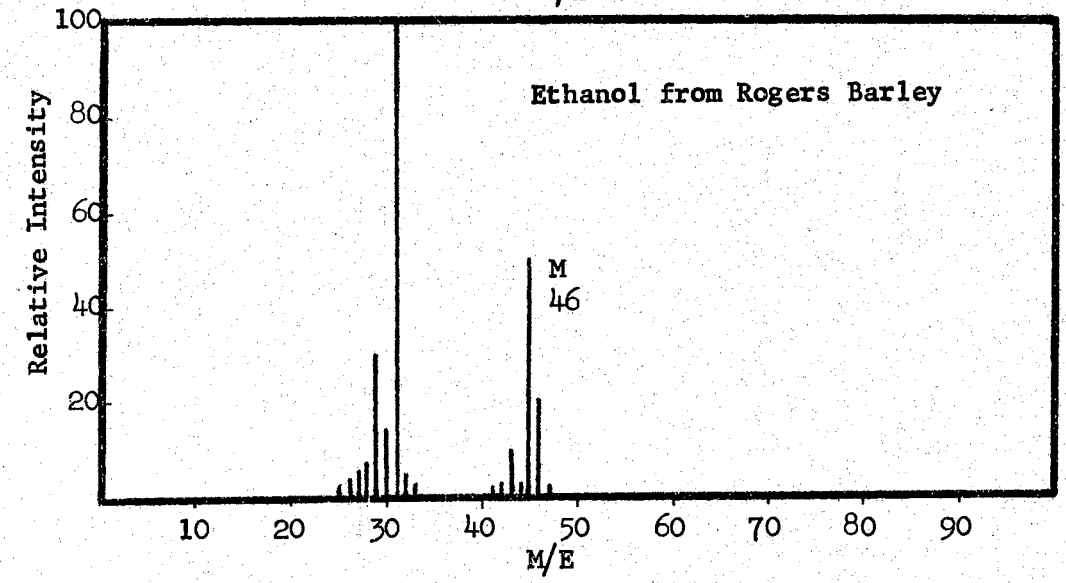
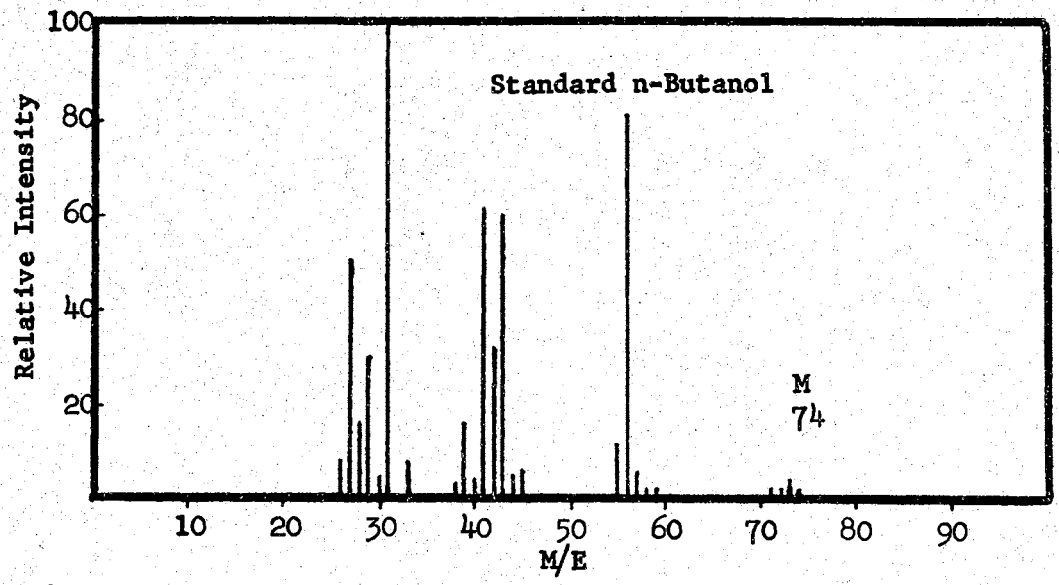
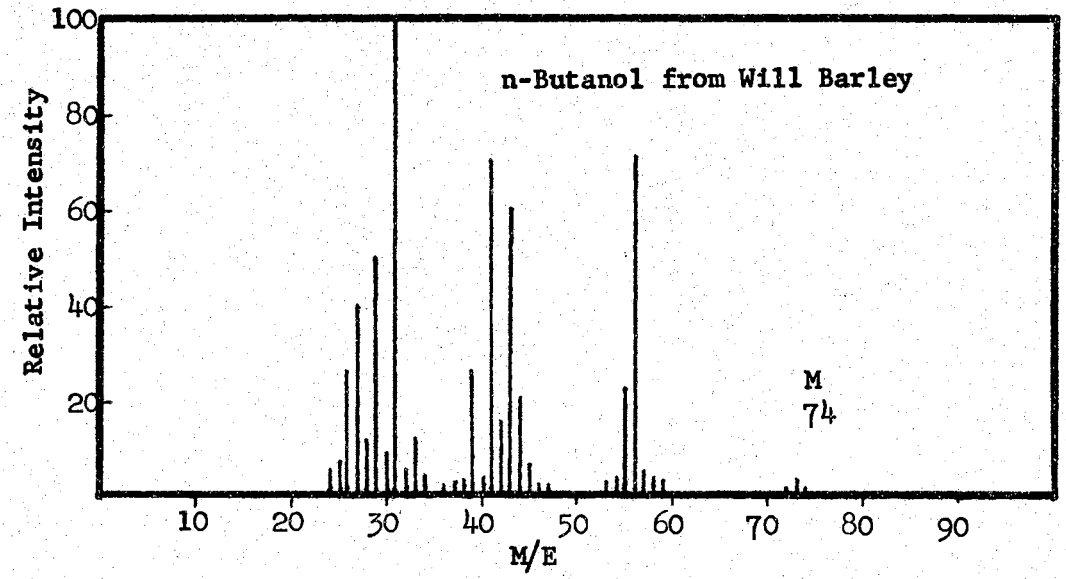

Ethanol from Rogers Barley
Relative Intensity
100
80
60
40
20
M
46
10
20
30
40
50
60
70
80
90
M/E

Figure 7. Mass Spectra of Standard n-Butanol, n-Butanol from Will Barley and n-Butanol from Rogers Barley


Standard n-Butanol
Relative Intensity
100
80
60
40
20
M
74
10
20
30
40
50
60
70
80
90
M/E


n-Butanol from Will Barley
Relative Intensity
100
80
60
40
20
M
74
10
20
30
40
50
60
70
80
90
M/E

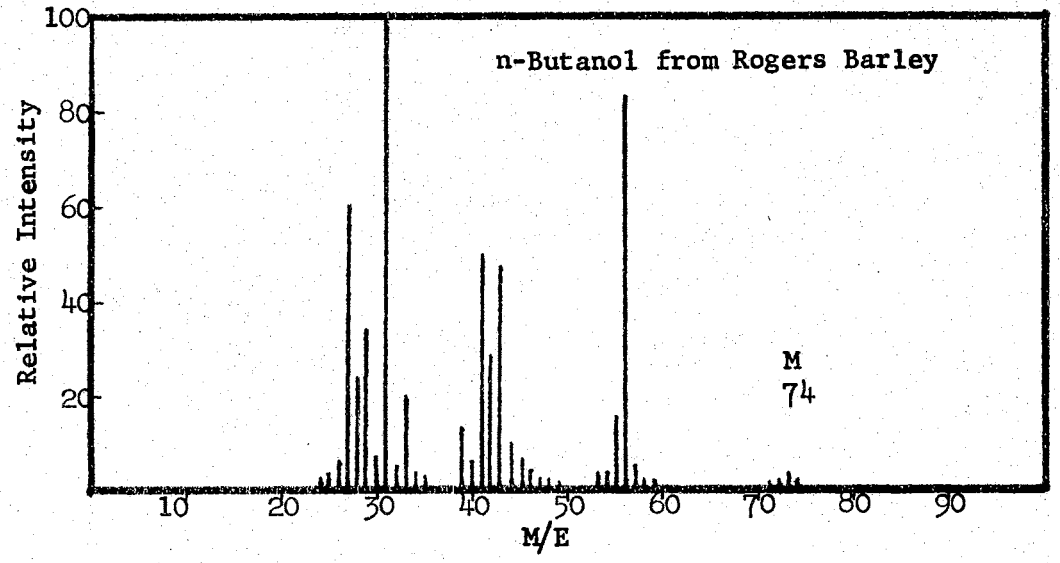
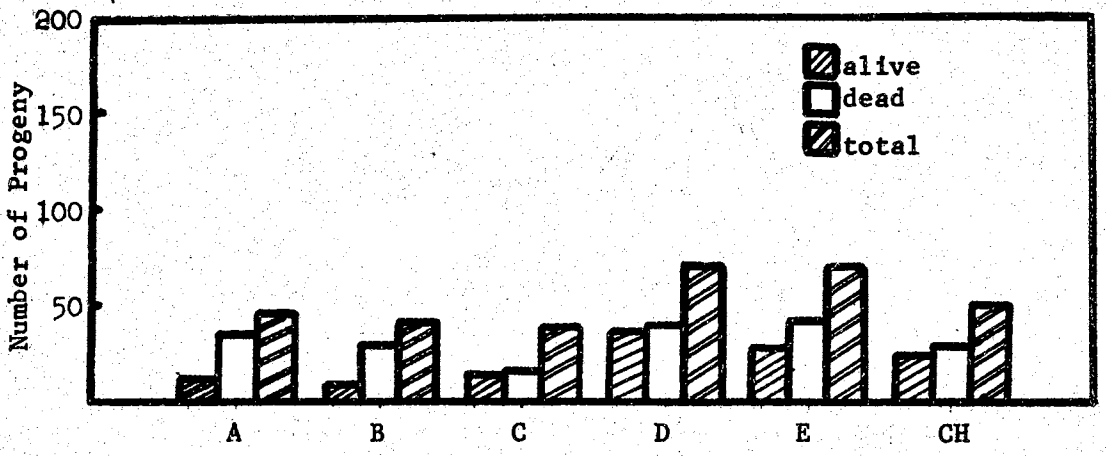

n-Butanol from Rogers Barley
Relative Intensity
100
80
60
40
20
M
74
10
20
30
40
50
60
70
80
90
M/E

Figure 8. Effect of Volatile Compounds on the Growth of Greenbugs Feeding on Artificial Diets for Twenty Days

Alphabets represent the following: A = acetaldehyde, B = ethyl formate, C = ethyl acetate, D = ethanol, E = n-butanol, CH = control.

Concentrations of compounds used were equivalent to the levels observed in Rogers Barley.


200
150
100
50
Number of Progeny
alive
dead
total
A
B
C
D
E
CH

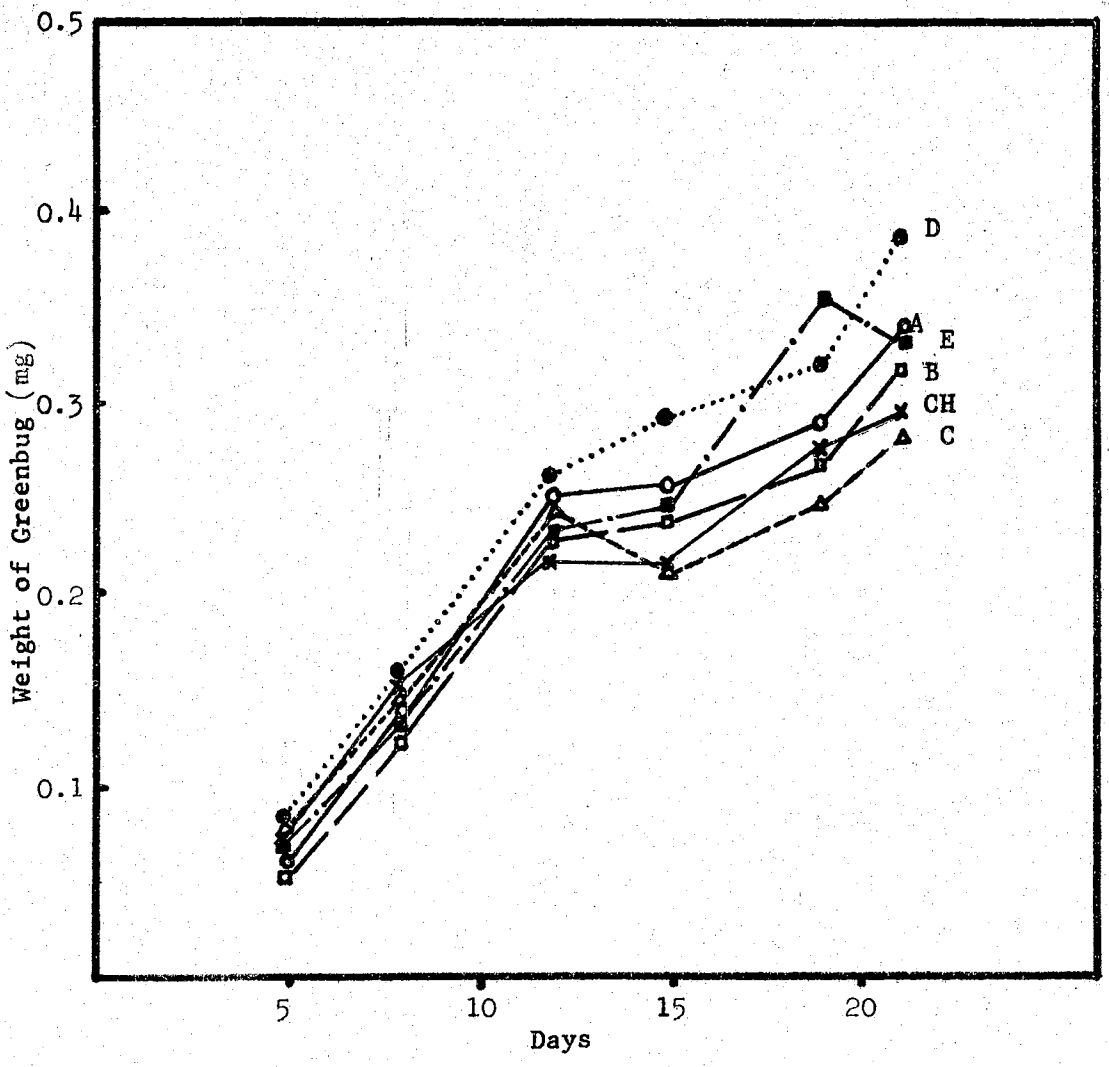

0.5
0.4
0.3
0.2
0.1
Weight of Greenbug (mg)
D
A
E
B
CH
C
5
10
15
20
Days

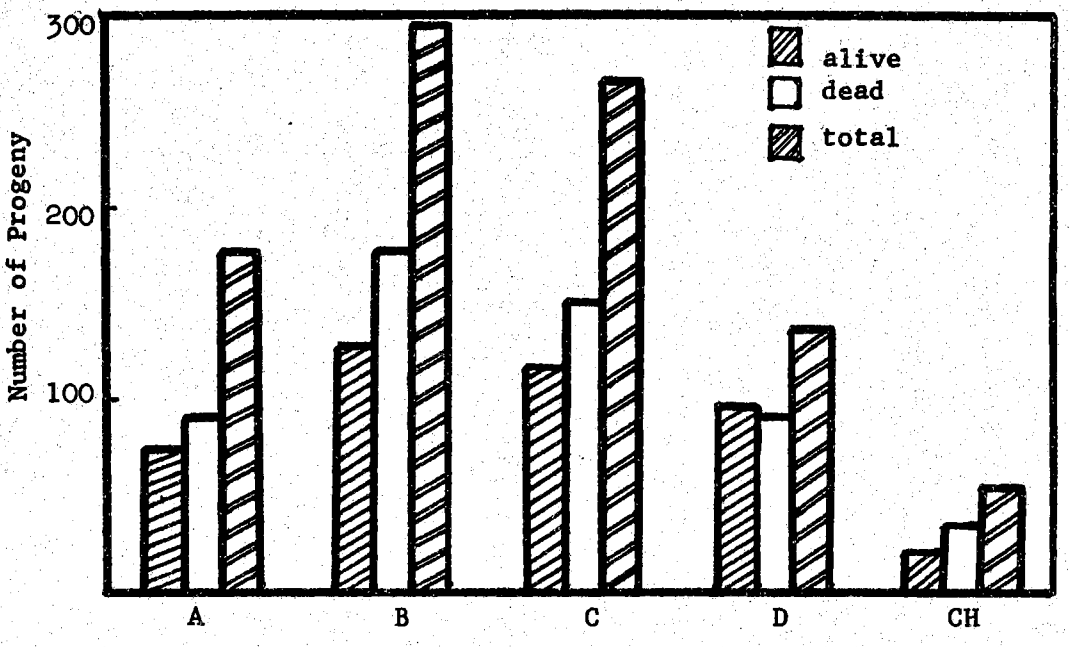
Figure 9. Steam distillate from Rogers barley stimulated the growth of greenbugs much more than that from Will barley, but at 50% dilution of the original concentrations, the Will volatiles increased the growth much more effectively than the Rogers volatiles. This observation may be explained by the unknown volatiles which were present in different concentrations in Will and Rogers varieties. If the volatiles present in higher concentrations in Will are repellents, the decrease of these concentrations in the diluted Will volatiles used in the artificial diet would probably result the increase of stimulatory effect on the growth and reproduction of the greenbugs; and conversely, if the volatiles present in higher concentrations in Rogers are attractants, the decrease of these concentrations in the diluted Rogers volatiles used in the artificial diet would cause the decrease of stimulatory effect on the growth and reproduction of the greenbugs.

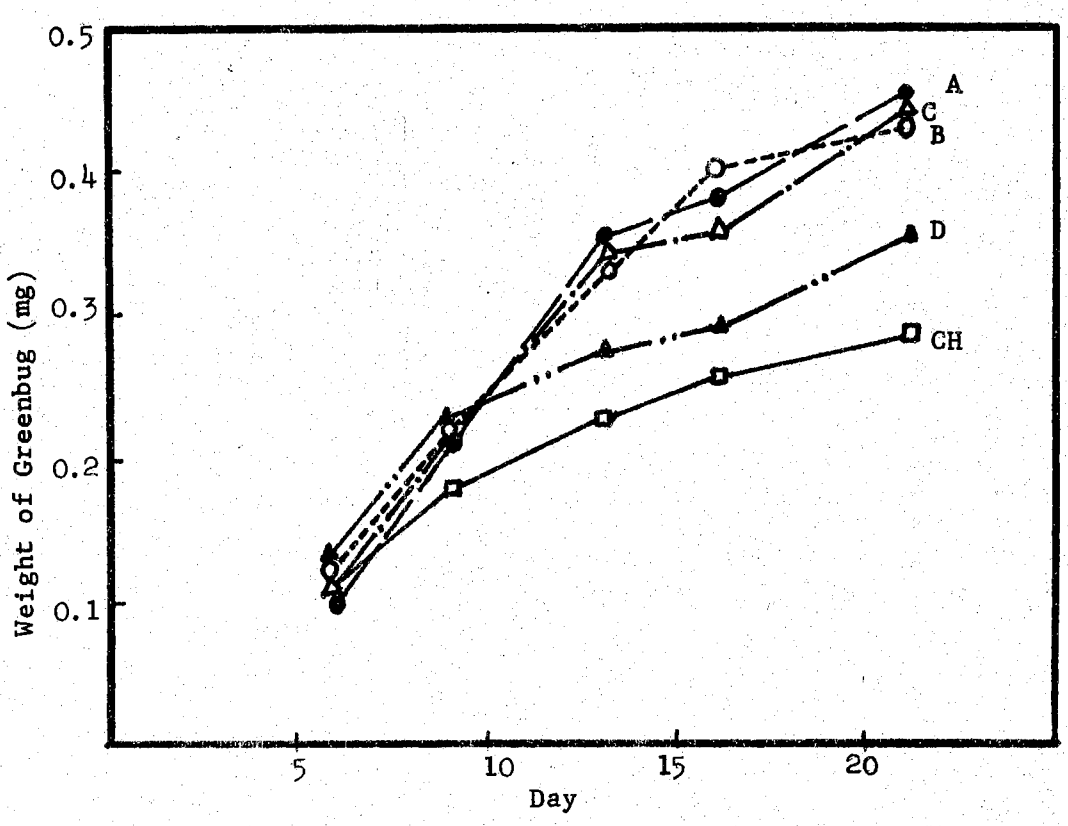
Water Content of the Resistant and Susceptible Barley Varieties

Correlations between aphid behavior and plant water status had been observed by other workers. It is generally agreed that the feeding and reproduction of aphids are influenced by the water content of the host plant. Response of aphids to the shortage of water in the host plant varied with the species of aphid as well as the species of the host plant (93). Water shortage had beneficiary effects on some aphids owing to the increase of concentration of solutes in the sap. Nevertheless, on the insects which depend considerably on the turgor pressure for feeding, water shortage had harmful effects (94). These aphids became restless, and their fecundity was diminished, because the assistance in

Figure 9. Effect of Volatiles Extracted from Resistant and Susceptible Barley Varieties on the Growth of Greenbugs Feeding on Artificial Diets for Twenty Days

Alphabets represent the following: A = volatiles from Will (resistant), B = volatiles from Rogers (susceptible), C = volatiles from Will at 50% concentration of A, D = volatiles from Rogers at 50% concentration of B, CH = control.


300
200
100
Number of Progeny
alive
dead
total
A
B
C
D
CH


0.5
0.4
0.3
0.2
0.1
Weight of Greenbug (mg)
A
C
B
D
CH
5
10
15
20
Day

food uptake provided by the sap pressure was reduced. Greenbugs were found to be favored by the low water content of host plants resulting from low rainfall during drought periods (95). Therefore, it was suspected that the greenbug-resistant varieties may have a higher water content than the susceptible varieties.

The water content of the uninfested susceptible and resistant barley varieties as well as the greenbug-infested ones is listed in Table III. The water content is expressed on a fresh weight basis. No significant difference was observed between the greenbug-resistant and susceptible varieties. A drastic reduction of water content of the susceptible barley after two weeks of greenbug infestation was found. All the susceptible plants were completely dead after 3 weeks of greenbug infestation. Resistant varieties showed a small decrease of water content after 3 weeks of infestation. The more mature plants of all varieties contained less water than the younger ones probably owing to an increase of thickness of cell wall and dry matter in these maturing plants.

Hydrogen Ion Concentration of the Resistant and Susceptible Barley Varieties

Hydrogen ion concentration in the cell sap of plants has long been considered to affect the insect-host plant relationship. Acidity of the plant has been suggested as the plant's defense against its enemies (1). Resistance of the plant to insect attack increased with increasing organic acid content of the plant. Leaves of the sugar beets resistant to leafhoppers were found to be more acidic than those of the susceptible ones (11). Studies of the feeding habits of the leafhoppers showed the leafhoppers concentrated on artifically prepared food that was alka-

TABLE III

WATER CONTENT OF UNINFESTED AND INFESTED RESISTANT AND SUSCEPTIBLE VARIETIES

Age of Barley	Will		Rogers		Isogenic A		Isogenic B	
	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.
	(per cent / g fresh weight)							
1 Week Uninf.	90.5		91.2		90.3		90.2	
2 Weeks Uninf.	91.1		90.4		90.5		90.8	
Inf. 1 Wk		90.8		90.7		90.2		91.0
3 Weeks Uninf.	90.0		90.1		90.2		90.1	
Inf. 2 Wk		89.9		70.3		90.0		71.2
4 Weeks Uninf.	89.5		89.3		88.9		88.7	
Inf. 3 Wk		84.9		dead		86.1		dead

line in reaction (96). The pea aphid was found to be capable of discriminating between as little as 0.3 to 0.6 of a pH unit when feeding on artificial diets (97). Cress (89) observed the best greenbug development when the greenbugs were reared on an artificial diet having a pH of 7.6 compared to those with a more acidic pH.

Table IV shows the result of the present study on pH in the cell saps of several lines of barley plants. Small differences in pH were observed between the greenbug-resistant and greenbug-susceptible varieties; however, a statistical analysis of the results by the t test showed that these differences were not significant.

Auxin Content of Resistant and Susceptible Barley Varieties

Earlier workers have shown correlations between auxin content in the plant and aphid resistance. Maxwell and Painter (85) observed that wheat, barley and alfalfa varieties resistant to greenbugs contained lower concentrations of free auxins than the corresponding susceptible varieties. The susceptible varieties had at least one more neutral auxin present than did the resistant ones. The honeydew and ether extracts of the aphids feeding on the susceptible varieties also had larger amounts of auxins than those of aphids feeding on the resistant varieties (98,99). The same workers found that short periods of aphid infestation significantly reduced the acid auxin content of the susceptible varieties but had very little effect on the total auxin content of the resistant varieties. The aphids were said to be able to remove significant amount of auxins from the susceptible varieties and were unable to do so from the resistant plants. It was suggested that this

TABLE IV

COMPARISON OF HYDROGEN ION CONCENTRATIONS BETWEEN RESISTANT AND SUSCEPTIBLE VARIETIES

Group	Line	Resist. or Suscep.	(1) pH	(2) pH	Ave.	t Test
			Isogenic Lines			
	654832-2	(s)	5.94	6.01	5.98	no
I	654832-3	(r)	5.65	5.84	5.75	sign.
	654832-8	(r)	5.94	5.79	5.87	diff.
II	654833-2	(s)	5.98	5.84	5.91	no sign.
	654833-7	(r)	5.91	5.74	5.83	diff.
	654835-1	(s)	5.87	5.79	5.83	
	654835-2	(s)	5.99	5.81	5.90	no
III	654835-3	(s)	5.91	6.01	5.96	sign.
	654835-4	(r)	5.87	5.73	5.80	diff.
	654835-5	(r)	5.79	5.98	5.84	
	654835-6	(r)	6.19	5.91	6.06	
	654843-3	(s)	5.87		5.87	no
IV	654843-8	(r)	5.95	5.86	5.91	sign.
	654843-9	(r)	5.73	6.01	5.87	diff.
V	654844-1	(s)	5.76	5.73	5.75	no sign.
	654844-8	(r)	5.76		5.76	diff.
			non-Isogenic Lines			
	5-20-2	(r)	5.97	5.91	5.93	
Will	5-20-4	(r)	5.89	5.71	5.80	no
	5-20-7	(r)	6.02	5.84	5.93	sign.
	5-20-10	(s)	5.79	5.91	5.83	
Rogers	5-20-11	(s)	5.87	5.87	5.87	diff.
	5-20-15	(s)	5.81	5.82	5.82	

result indicated that the difference between the resistant and susceptible plants may be related to the availability of phloem material to the aphids. Auxins might exist in greater concentrations in the phloem. Recently, King (100) observed that uninfested greenbug-resistant and susceptible barley varieties had the same level of "free" and "bound" indoles, and that greenbug infestation increased the amount of total indoles significantly in both varieties. The levels of "bound" indoles were about the same in these two greenbug-infested varieites; however, the level of the "free" indoles in the infested susceptible variety was much higher than that of the infested resistant variety, and the resulting total indoles in the former was about 1.5 times as great as the total of the latter.

In the present study the auxin content of two uninfested greenbug-resistant and two uninfested greenbug-susceptible barley varieties as well as the greenbug-infested plants of these varieties were analyzed by thin-layer chromatography and gas-liquid chromatography. The results of thin-layer chromatography and the identification of the auxins are shown in Figures 10 and 11. Indoleacetonitrile, indolebutyric acid, indolepropionic acid, indoleacetic acid, indole-3-aldehyde and indole were found in the uninfested "free" and "bound" fractions of all varieties tested. Quantitative composition of auxins were determined by gas-liquid chromatography. Auxins were analyzed as their methylated trifluoroacetyl derivatives (TFA). The liquid stationary phase used was 3% QF-1. The best resolution was obtained when the column temperature was maintained at 165^{o}C. Typical chromatograms of methylated trifluoroacetates of indoles of uninfested Isogenic A and Isogenic B varieties are shown in Figure 12. The concentrations of the individual

Figure 10. Thin-Layer Chromatogram of "Free" and "Bound" Indoles of Uninfested Resistant and Susceptible Barley Varieties

Abbreviations: IND = indole, IAN = indoleacetonitrile, IBA = indolebutyric acid, IPA = indolepropionic acid, IAA = indoleacetic acid, I3H = indole-3-aldehyde, IpyA = indolepyruvic acid.

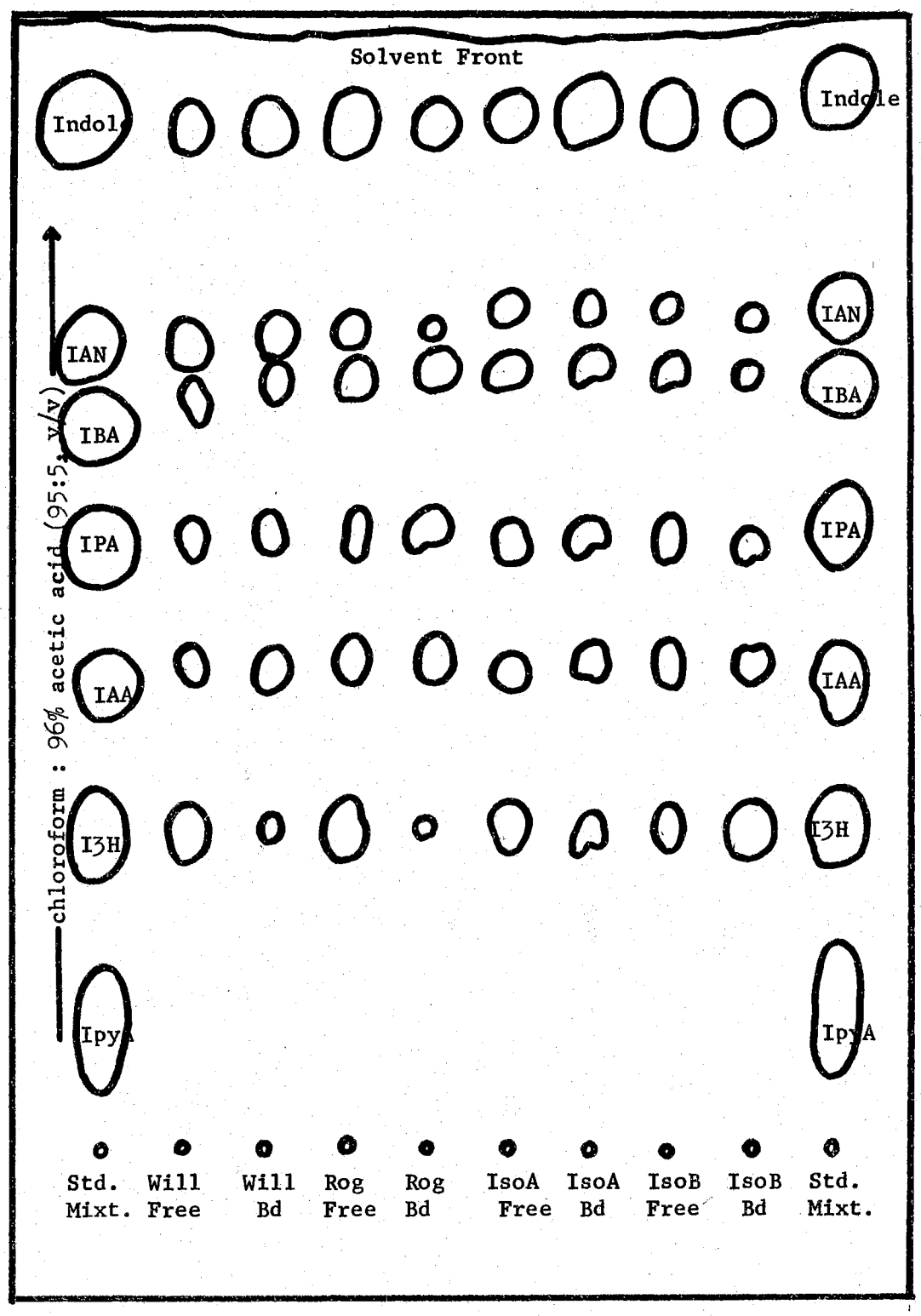

Solvent Front
Indole
Indole
IAN
IBA
IPA
IAA
I3H
IpyA
IAN
IBA
IPA
IAA
I3H
IpyA
chloroform : 96% acetic acid (95:5, v/v)
Std. Mixt.
Will Free
Will Bd
Rog Free
Rog Bd
IsoA Free
IsoA Bd
IsoB Free
IsoB Bd
Std. Mixt.

Figure 11. Thin-Layer Chromatogram of "Free" and "Bound" Indoles of Greenbug-Infested Resistant and Susceptible Barley Varieties

Abbreviations: IND = indole, IAN = indoleacetonitrile, IBA = indolebutyric acid, IAA = indoleacetic acid, I3H = indole-3-aldehyde, IPA = indolepropionic acid, IpyA = indolepyruvic acid.

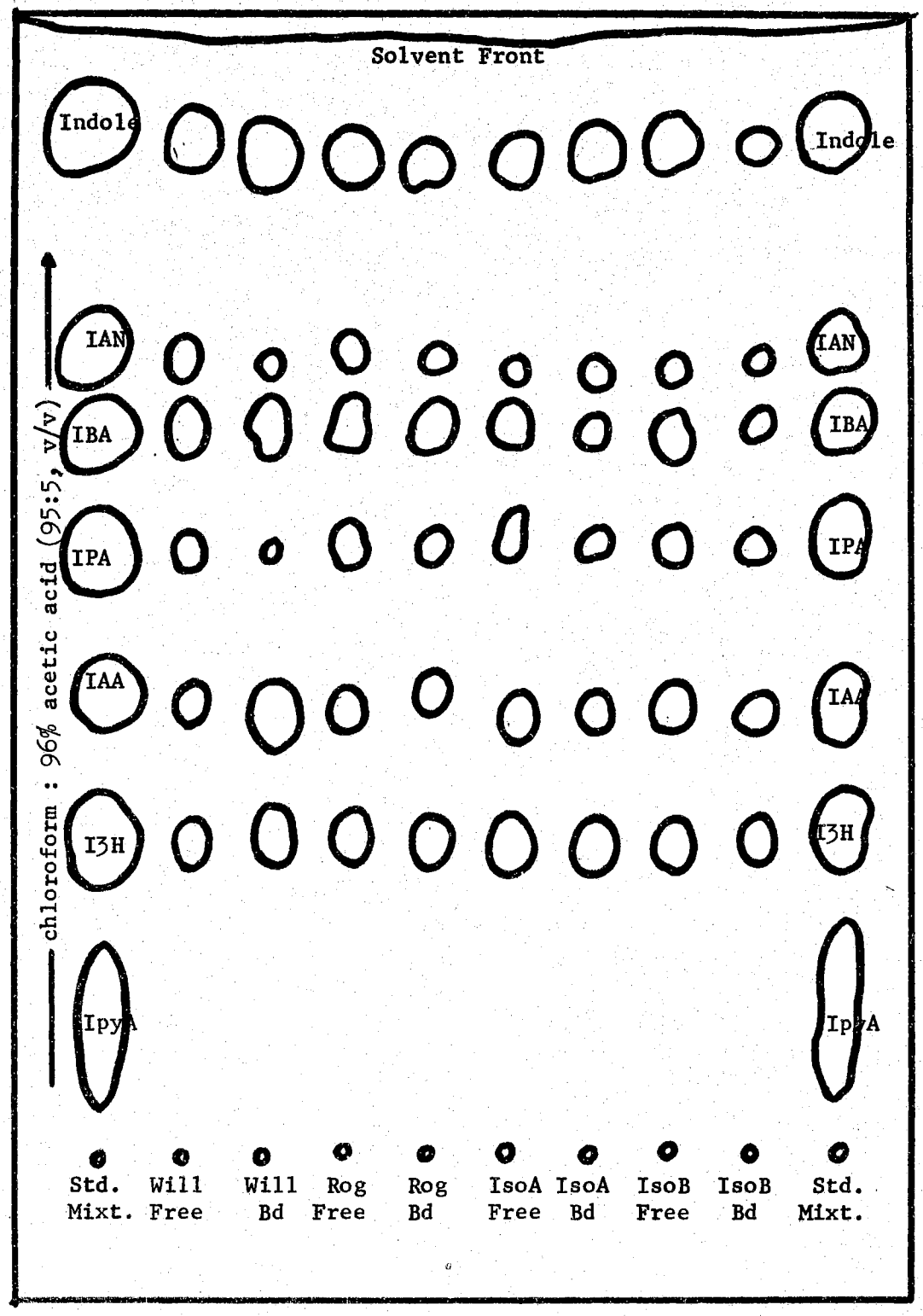
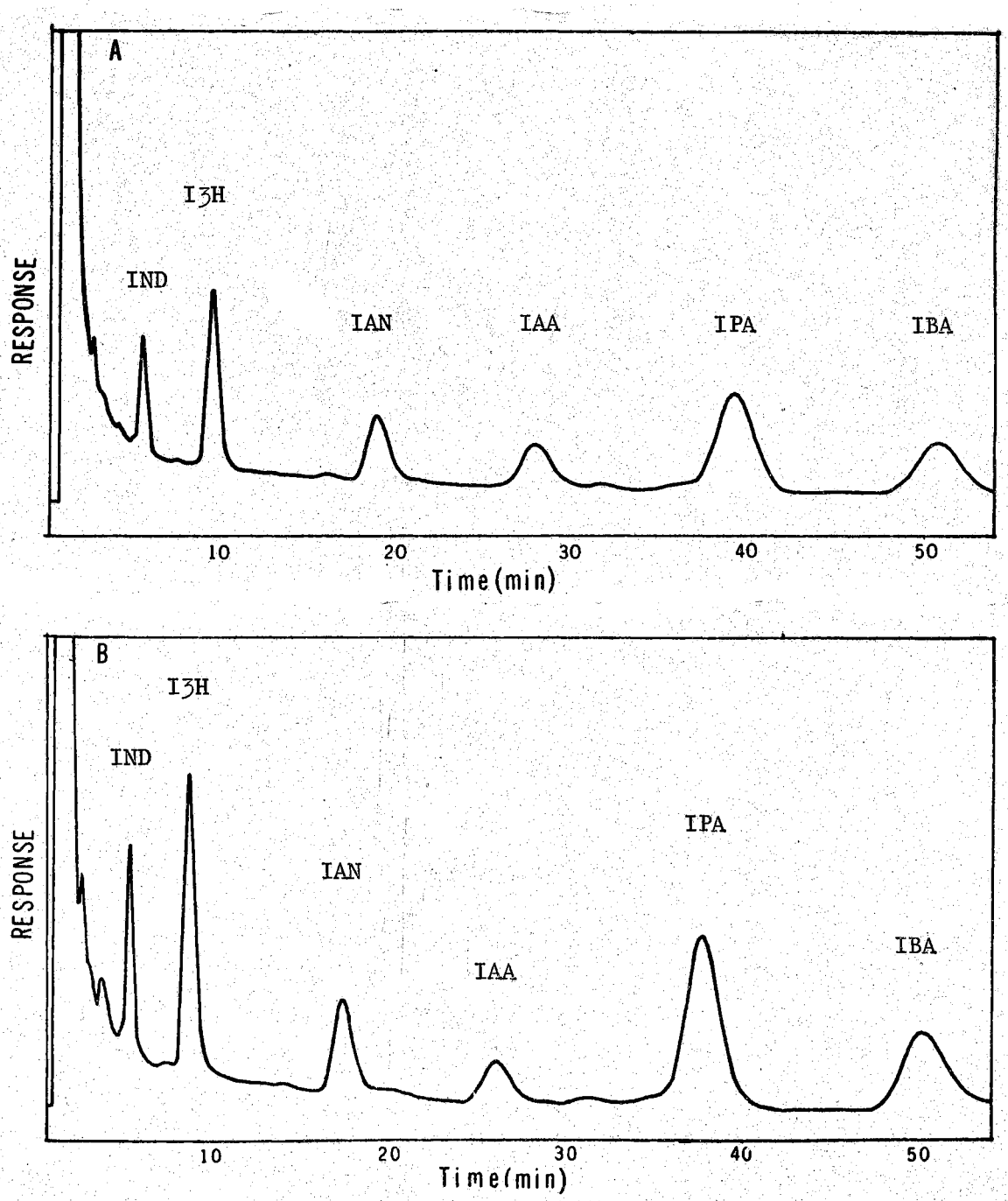

Solvent Front
Indole
Indole
IAN
IAN
IBA
IBA
IPA
IPA
IAA
IAA
I3H
I3H
IpyA
IpyA
chloroform : 96% acetic acid (95:5, v/v)
Std. Mixt.
Will Free
Will Bd
Rog Free
Rog Bd
IsoA Free
IsoA Bd
IsoB Free
IsoB Bd
Std. Mixt.

Figure 12. Gas-Liquid Chromatography of Methylated Trifluoroacetates of Indoles on a 20 Ft. x 1/4 In. 3% QF-1 column at 165°C

A. Indoles of uninfested Isogenic A (resistant)

B. Indoles of uninfested Isogenic B (susceptible)

Abbreviations: IND = indole, IAN = indoleacetonitrile, IBA = indolebutyric acid, IPA = indolepropionic acid, IAA = indoleacetic acid, I3H = indole-3-aldehyde.


A
I3H
IND
IAN
IAA
IPA
IBA
RESPONSE
10
20
30
40
50
Time (min)
B
I3H
IND
IAN
IAA
IPA
IBA
RESPONSE
10
20
30
40
50
Time(min)

components present in the samples analyzed are shown in Table V. The indole concentrations are expressed on dry weight basis. As can be seen in the table the contents of "free" and "bound" indoles of the uninfested susceptible varieties were similar to those of the uninfested resistant varieties. Greenbug infestation was found to increase the amount of total indoles significantly in all varieties as reported by King (100); however, the increases of "free" and "bound" indoles in the infested susceptible varieties were not different from those of the infested resistant varieties, and the resulting total indoles were at the same level in all the varieties. The increase of auxin levels in the infested plant had been explained as the result of increased production of decreased destruction of auxins in the plant. Substances secreted into the plant by the greenbugs might act as inducers to inactivate the inhibitor of the enzymes involved in auxin production, or they might act as inhibitors to inhibit the enzymes responsible for auxin destruction (101).

The high level of "free" auxins found in the susceptible plants after greenbug infestation was suggested as the reason for the damage in the host plant caused by the aphids (100), since high auxin concentration was found to be deleterious to plant growth (102). This explanation of the greenbug damage to the susceptible plants is considered inadequate, because the present study demonstrated that the greenbug-infested resistant plants have the same level of "free" auxins as the infested susceptible plants. The results obtained by Libbert et al. (103) in the studies of auxin metabolism would suggest that the large amount of "free" auxins found in the infested plants may not be related to the damage to the plants caused by aphid infestation. Libbert et al.

TABLE V

AUXIN CONTENT OF UNINFESTED AND INFESTED RESISTANT AND SUSCEPTIBLE VARIETIES

Compounds	Will				Rogers				Iso-A				Iso-B			
	Uninfested		Infested		Uninfested		Infested		Uninfested		Infested		Uninfested		Infested	
	Free	Bound	Free	Bound	Free	Bound	Free	Bound	Free	Bound	Free	Bound	Free	Bound	Free	Bound
	(µg / g dry weight)															
IAA	1.6	2.0	4.7	15.3	2.7	3.0	7.6	5.3	1.8	0.7	5.6	3.3	1.7	0.9	6.7	2.5
IPA	1.4	2.0	1.9	0	1.6	0.9	4.0	1.4	1.5	0.3	4.8	1.4	1.9	0.5	2.0	1.8
IAN	1.6	2.3	4.2	1.1	0.5	0	3.3	2.0	0.1	0.2	1.8	2.6	0.3	0.4	3.1	0.7
IBA	6.6	5.0	6.6	6.6	6.9	2.2	8.5	6.4	3.2	1.2	4.0	2.7	3.5	0.2	5.4	2.6
IND	0.7	0.9	1.8	4.9	1.4	1.3	2.2	1.2	1.0	0.4	2.6	1.1	1.5	0.4	3.2	0.7
I3H	4.9	0	2.3	12.0	10.8	0	16.2	10.3	7.6	9.0	17.8	18.1	9.0	6.1	15.2	10.3
TOTAL	16.8	12.2	41.0	39.9	23.9	7.3	41.7	26.6	15.2	11.7	36.6	29.2	17.9	8.1	35.6	18.6

IAA = indole acetic acid; IPA = indole propionic acid; IBA = indole butyric acid; IAN = indole acetonitrile; IND = indole; I3H = indole-3-aldehyde; Iso-A = isogenic A (resistant); Iso-B = isogenic B (susceptible)

(103) found that the epiphytic bacteria which usually live at the plant surface of the higher plants had the greatest ability to form indoleacetic acid from tryptophan among all plant organisms. The conversion of tryptophan to indoleacetic acid in the plant by plant enzymes was very slow, and the efficiency was far less than that by the epiphytic bacteria. Sterile plants grown from seeds that had been sterilized with ethanol and bromine water contained indoleacetic acid in a quantity very much less than the non-sterile plants. The major part of auxins which was found in the extracts of non-sterile plants was not produced by the plant but by the epiphytic bacteria. It was suggested also that part of the auxins found in the extracts of non-sterile plants was produced by the epiphytic bacteria during extraction, because the extraction of non-sterile plants in presence of antibiotics (chloramphenicol or streptomycin) yielded less indoleacetic acid than the one in absence of antibiotics. High amounts of bacteria-produced auxins were shown to be normally present on higher plants; however, the effect of these auxins on the plant was not known. In view of these reports, it would not be unreasonable to suggest that the large amount of auxins found in the extracts of the greenbug-infested plants may be the auxins produced in greater quantities by the epiphytic bacteriae, stimulated perhaps by the greenbug's infestation. These auxins may not necessarily be involved with the aphid's damage in the plant.

Free Amino Acid Content of Susceptible and Resistant Barley Varieties

The free amino acid composition of alcoholic extracts of the four barley varieties, Will (resist.), Rogers (suscept.), Isogenic A (resist.)

and Isogenic B (suscept.) were analyzed by thin-layer chromatography. Twenty amino acids were found in all the varieties. No significant qualitative difference was found between the resistant and susceptible varieties, with the exception that two unknown compounds found in Rogers barley were not detected in Will, Isogenic A or Isogenic B. The same twenty amino acids were found in the greenbug-infested barleys. Nevertheless, the chromatograms of the infested barleys, especially the susceptibles ones, were not as intense as the uninfested ones. This indicates that there was a decrease of free amino acid concentration after greenbug infestation and that this decrease occurred to a greater extent with the susceptible varieties. The reconstructed typical 2-dimensional thin-layer chromatograms of Will and Rogers barleys are shown in Figures 13 and 14.

Amino acid content was also determined quantitatively by automatic amino acid analysis. Figure 15 shows a reconstructed typical chromatogram of free amino acids of uninfested one-week old Rogers barley. The change of free amino acid composition of the uninfested barley varieties during the growth period from 1 week to 4 weeks as well as those of the greenbug-infested barleys at this period are shown in Tables VI and VII. In all varieties tested, the concentration of total amino compounds was found to be higher in the younger plants, and it decreased as the plants grew. The total amino compounds was also found to be higher in the susceptible varieties than in the resistant varieties at all stages of growth. The greenbug-infested barleys, especially those susceptible, were found to exhibit a sharp reduction of total amino compounds following 1 week of infestation. Figure 16 shows the amount of total amino compounds present in the uninfested and infested barley varieties at

Figure 13. A Typical Two-Dimensional Thin-Layer Chromatogram of Free Amino Acids of Will Barley (Resistant)

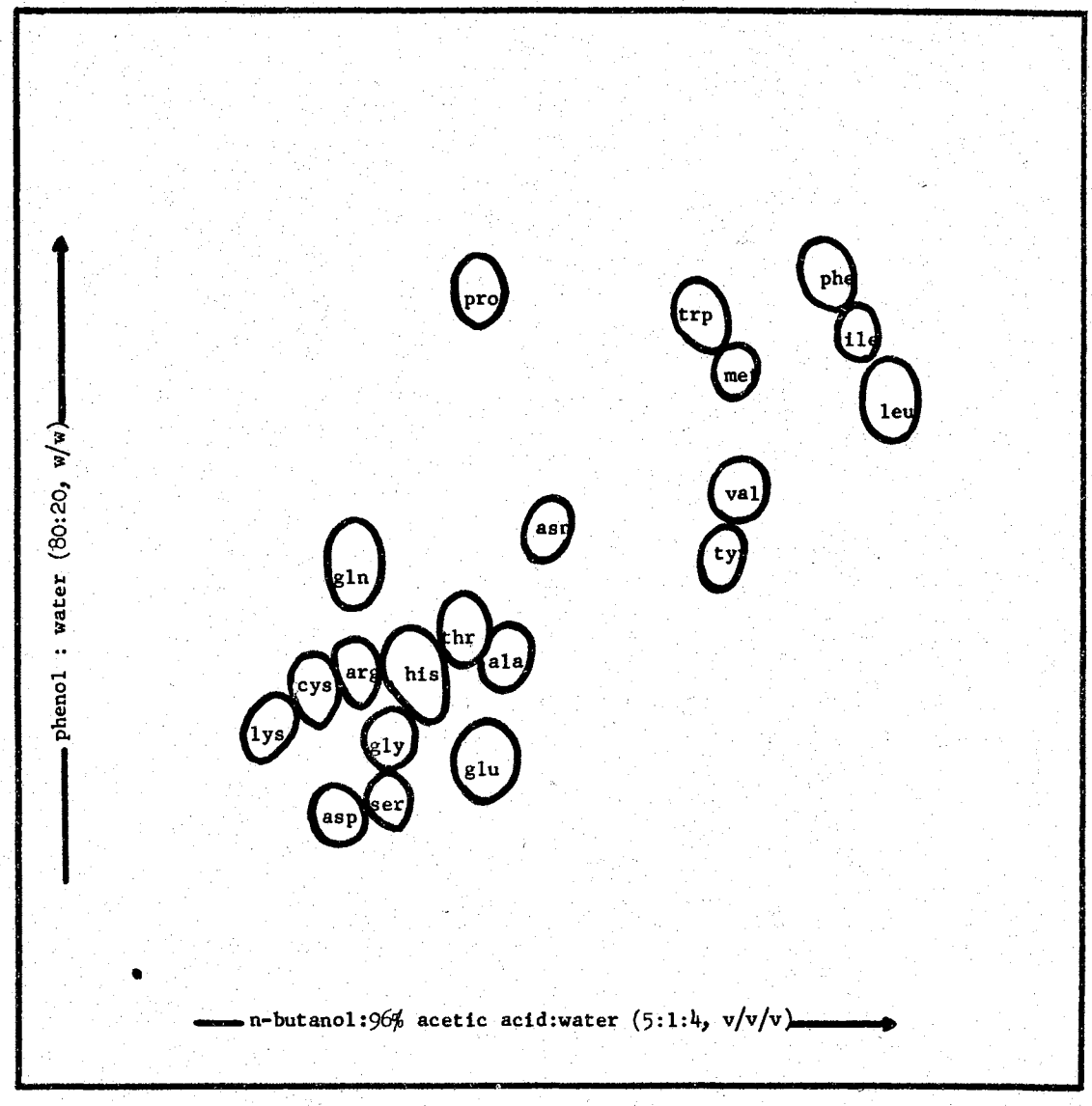

pro
trp
phe
ile
met
leu
val
asn
tyr
gln
thr
ala
cys
arg
his
lys
gly
glu
asp
ser
phenol : water (80:20, w/w)
n-butanol:96% acetic acid:water (5:1:4, v/v/v)

Figure 14. A Typical Two-Dimensional Thin-Layer Chromatogram of Free Amino Acids of Rogers Barley (Susceptible)

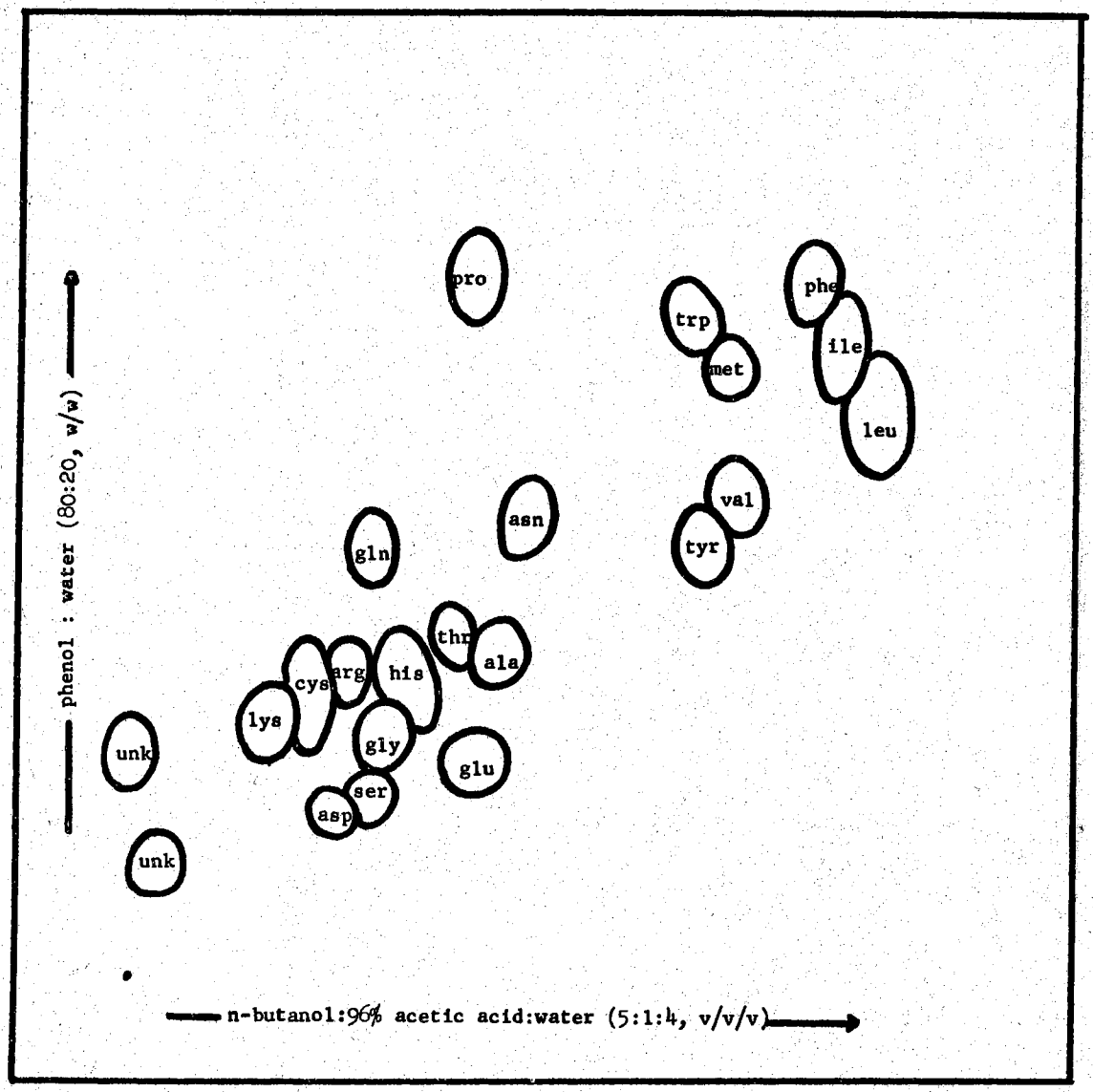

pro
trp
met
phe
ile
leu
val
tyr
asn
gln
thr
ala
cys
arg
his
lys
gly
glu
ser
asp
unk
unk
phenol : water (80:20, w/w)
n-butanol:96% acetic acid:water (5:1:4, v/v/v)

Figure 15. A Reconstructed Typical Chromatogram of Free Amino Acids of Uninfested Rogers Barley (One Week Old) Analyzed by Automatic Amino Acid Analyzers

The first five amino acids, aspartic acid, threonine, serine, asparagine and glutamine were analyzed by a Beckman Model 120 C Amino Acid Analyzer. The other amino acids were analyzed by a Technicon Amino Acid Autoanalyzer. Parameters for the Beckman Amino Acid Analyzer were:

column size - 0.9 x 69 cm
resin type - Beckman Type AA-15
height of resin column - 53 cm
column flow rate - 50 ml/hr
column back pressure - 290 psi
buffer - pH 3.25
temperature - 30°C.

Parameters for the Technicon Amino Acid Autoanalyzer were:

column size - 0.9 x 133 cm
resin type - Type B Chromo-Beads
height of resin column - 129 cm
column flow rate - 30 ml/hr
column back pressure - 300 psi
buffer - pH 2.785 to pH 5.00
temperature - 60°C.

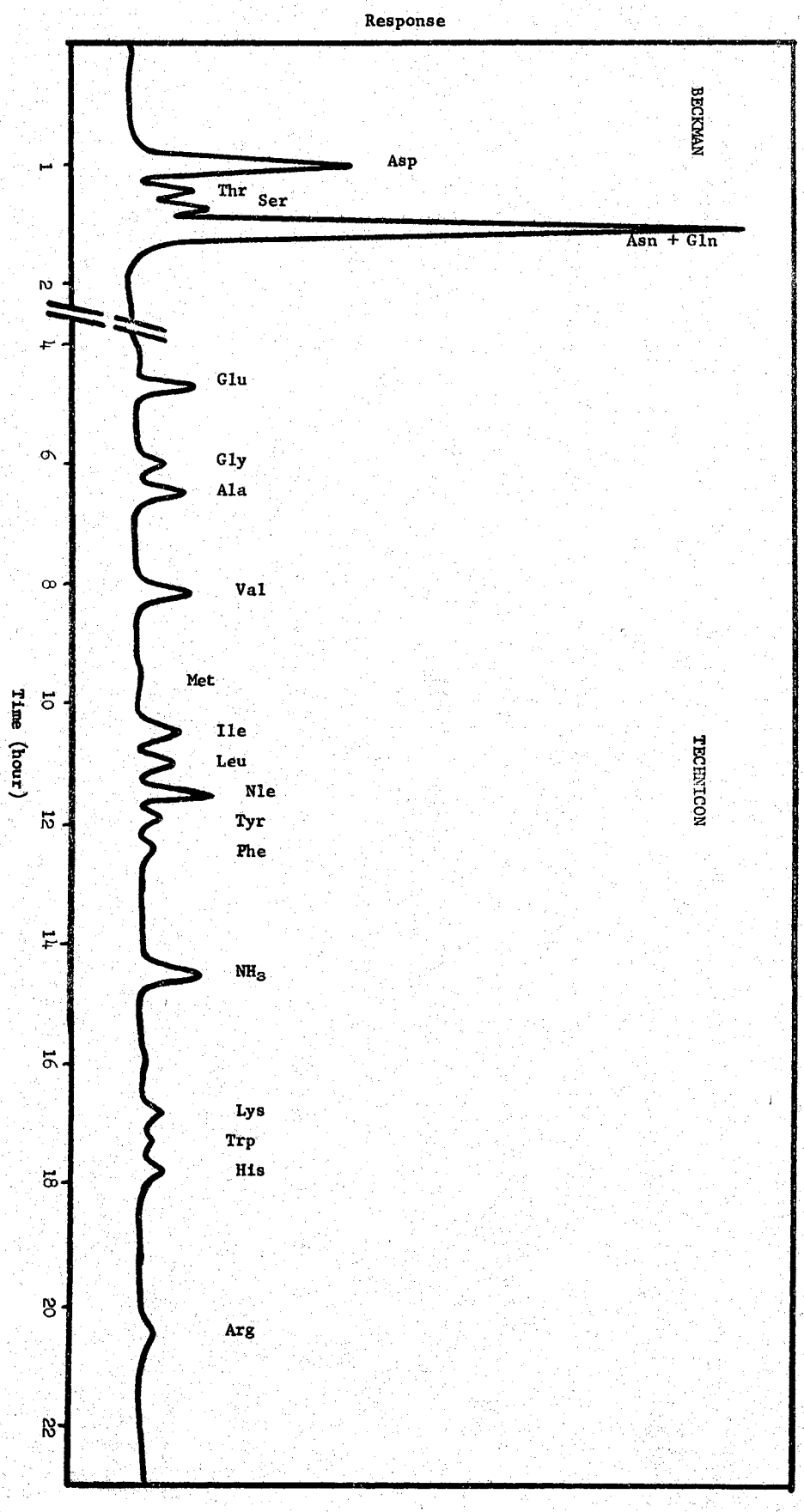

Response
BECKMAN
Asp
Thr
Ser
Asn + Gln
Glu
Gly
Ala
Val
Met
Ile
Leu
Nle
Tyr
Phe
NH_3
Lys
Trp
His
Arg
TECHNICON
Time (hour)
1
2
4
6
8
10
12
14
16
18
20
22

TABLE VI

FREE AMINO ACID COMPOSITION OF UNINFESTED AND INFESTED WILL AND ROGERS VARIETIES

Amino Acid		Uninfested								Infested					
		Will				Rogers				Will			Rogers		
	Age:	1 Wk	2 Wk	3 Wk	4 Wk	1 Wk	2 Wk	3 Wk	4 Wk	2 Wk	3 Wk	4 Wk	2 Wk	3 Wk	4 Wk
	Time Inf:	--	--	--	--	--	--	--	--	1 Wk	2 Wk	3 Wk	1 Wk	2 Wk	3 Wk
		(μ mole/ g dry weight)													
Asp		65.2	56.2	96.6	30.6	30.8	68.9	180.0	51.1	62.4	74.2	27.7	38.0	23.2	dead
Thr		5.5	7.2	4.5	4.6	6.1	9.4	13.9	7.6	8.8	5.2	3.6	4.1	1.8	dead
Ser		6.3	13.0	6.9	11.0	7.4	22.1	12.6	14.2	12.6	4.9	4.4	9.2	4.4	dead
Asn + Gln		90.2	159.7	91.6	48.2	158.4	182.2	123.6	62.8	80.3	86.1	40.6	46.8	32.6	dead
Glu		4.6	4.8	2.5	5.4	6.0	6.7	4.5	9.5	4.5	2.9	5.2	4.5	1.6	dead
Gly		2.8	2.5	2.5	1.5	3.0	2.4	1.7	1.1	2.4	2.4	1.1	1.7	1.3	dead
Ala		5.4	5.0	10.9	11.9	4.2	4.7	4.0	8.2	4.4	8.9	8.7	3.9	5.2	dead
Val		7.8	9.8	7.4	4.7	5.1	14.8	7.6	5.7	8.8	5.4	2.9	7.4	3.7	dead
Met		trace	trace	trace	trace	trace	trace	trace	trace	--	--	--	--	--	dead
Ile		3.4	3.7	2.7	2.0	2.5	4.6	3.3	2.4	3.3	2.2	1.1	3.2	1.0	dead
Leu		2.4	2.3	2.5	2.9	2.1	3.6	2.5	3.4	1.8	1.8	1.5	1.7	1.1	dead
Tyr		1.3	1.3	1.0	1.5	1.0	1.5	1.5	1.5	1.3	1.2	0.7	1.0	1.0	dead
Phe		2.1	10.3	5.4	3.6	1.6	13.3	3.4	3.8	8.3	4.5	2.7	8.1	3.3	dead
NH_3		7.6	10.9	8.0	14.9	7.7	16.7	11.1	16.1	7.9	6.2	7.0	7.3	4.9	dead
His		2.1	3.5	2.1	2.0	3.0	6.0	3.2	2.6	3.4	2.8	1.5	4.5	2.2	dead
Arg		1.1	3.3	2.7	1.5	1.1	3.5	4.4	2.2	3.1	2.6	1.3	2.2	2.1	dead
Lys		2.8	3.8	3.7	2.7	1.9	4.3	1.8	2.8	3.8	2.7	1.7	1.8	1.7	dead
Trp		trace	3.4	trace	trace	trace	2.6	trace	trace	2.6	trace	trace	2.4	0.6	dead
Total		210.3	300.4	250.8	148.8	241.6	367.2	379.4	195.0	219.1	213.7	111.5	147.5	91.4	dead

TABLE VII

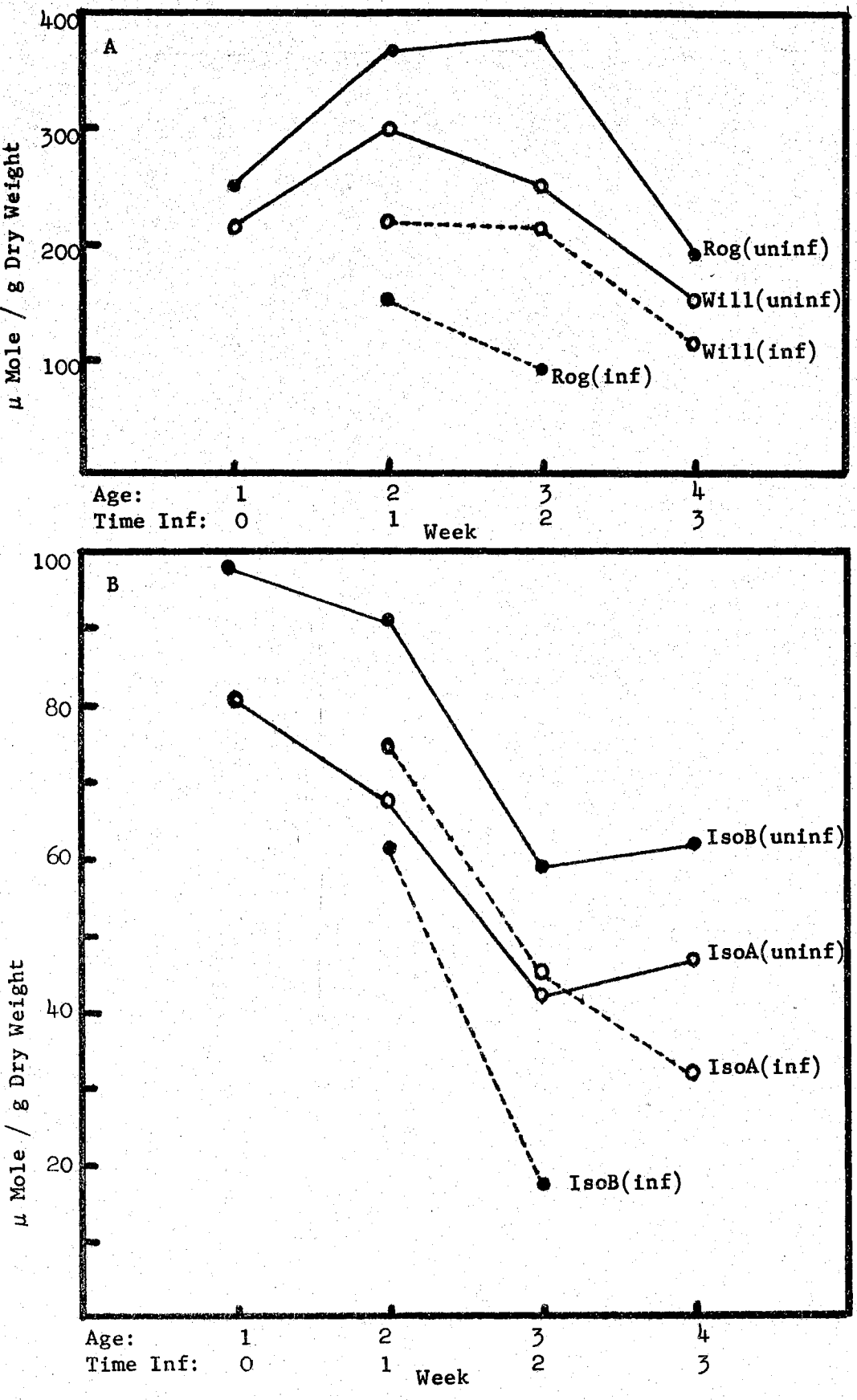
FREE AMINO ACID COMPOSITION OF UNINFESTED AND INFESTED ISOGENIC A AND ISOGENIC B VARIETIES

Amino Acid		Uninfested								Infested					
		Isogenic A				Isogenic B				Isogenic A			Isogenic B		
	Age:	1 Wk	2 Wk	3 Wk	4 Wk	1 Wk	2 Wk	3 Wk	4 Wk	2 Wk	3 Wk	4 Wk	2 Wk	3 Wk	4 Wk
	Time Inf:	--	--	--	--	--	--	--	--	1 Wk	2 Wk	3 Wk	1 Wk	2 Wk	3 Wk
		(μ mole/ g dry weight)													
Asp		21.7	18.1	8.7	8.2	21.1	20.4	10.6	9.9	18.0	8.3	5.1	10.4	3.0	dead
Thr		4.7	4.5	3.2	2.6	8.6	5.9	4.7	2.9	4.2	2.1	1.6	3.3	0.8	dead
Ser		7.4	6.7	4.5	4.4	16.3	11.6	6.5	5.6	6.8	4.4	2.6	4.9	1.2	dead
Asn + Gln		16.0	6.6	5.0	5.3	33.3	15.4	14.5	11.3	6.5	6.2	2.2	8.0	3.8	dead
Glu		1.3	3.4	3.2	2.4	1.7	6.0	4.4	4.4	4.0	3.3	2.4	4.5	1.4	dead
Gly		trace	1.7	0.3	0.6	0.6	1.1	0.6	0.7	1.8	0.9	0.5	1.1	0.3	dead
Ala		6.4	5.1	3.8	7.7	2.9	3.1	2.9	6.8	4.7	4.0	4.0	2.4	1.2	dead
Val		3.6	3.3	2.2	1.9	1.6	3.0	1.7	3.2	3.2	2.6	1.2	2.1	0.5	dead
Ile		1.9	1.9	1.5	1.2	1.7	1.8	1.5	1.6	2.1	1.0	0.6	1.3	0.2	dead
Leu		2.3	3.0	1.6	1.8	2.0	3.0	1.9	1.9	3.7	1.0	1.0	3.1	0.3	dead
Tyr		0.7	1.0	0.7	0.8	0.8	1.2	0.9	0.9	1.5	1.0	0.1	1.2	0.2	dead
Phe		1.3	2.0	1.4	1.3	1.7	3.0	1.4	2.6	3.0	2.0	0.6	3.0	0.2	dead
NH_3		9.6	6.0	4.5	5.7	3.2	8.5	4.6	5.6	9.2	4.7	8.0	10.6	1.8	dead
Lys		2.0	2.3	0.8	1.5	1.3	3.9	0.6	2.6	3.9	1.6	1.2	3.6	1.7	dead
His		0.1	trace	trace	0.4	0.4	0.5	0.2	1.1	trace	0.1	0.2	0.2	trace	dead
Arg		1.2	2.1	0.9	1.0	1.6	2.4	1.6	1.5	2.2	1.4	0.7	2.1	0.5	dead
Total		80.2	67.7	42.1	46.6	98.6	90.8	58.6	61.8	74.7	44.5	31.4	61.7	17.0	dead

Figure 16. Change of Concentration of Total Free Amino Compounds in Uninfested and Infested Susceptible and Resistant Barley Varieties at Different Ages

A. In Will (resistant) and Rogers (susceptible)

B. In Isogenic A (resistant) and Isogenic B (susceptible)


A
400
300
200
100
μ Mole / g Dry Weight
Rog(uninf)
Will(uninf)
Will(inf)
Rog(inf)
Age: 1 2 3 4
Time Inf: 0 1 2 3
Week
B
100
80
60
40
20
μ Mole / g Dry Weight
IsoB(uninf)
IsoA(uninf)
IsoA(inf)
IsoB(inf)
Age: 1 2 3 4
Time Inf: 0 1 2 3
Week

different growth periods. Among the list of amino acids identified; aspartic acid, threonine, serine, asparagine + glutamine, glutamic acid, histidine and arginine were found to be present in higher concentrations in the susceptible varieties than in the resistant varieties. The concentration of alanine was found to be higher in the resistant varieties. Aspartic acid together with asparagine and glutamine were the major components of the amino compounds. The concentrations of these amino acids found in the uninfested and infested barleys at the different growth periods are shown in Figures 17 and 18. The decreases of these amino acids in the older plants and in the infested ones were quite parallel to those of the total amino compounds. These results are comparable to those of Auclair and Maltais (64) who found the free amino acid concentrations were higher in the pea varieties susceptible to pea aphid than in the resistant ones. Concentrations of glutamine, asparagine and homoserine were found to be consistently lower in the resistant pea plants. The growth of pea aphid was increased significantly when pea leaves were treated with these three amino acids. They concluded that free amino acids were nutritional requirements for the pea aphids, and that the low concentration of the amino acids in the resistant plants caused the slower rate of growth and reproduction of the aphid. It was also suggested that glutamine, asparagine and homoserine may play a key role in influencing the degree of susceptibility or resistance of pea varieties to aphid attack (66). Marble et al. (68) determined the free amino acid concentration in several alfalfa varieties, some susceptible and some resistant to the spotted alfalfa aphid. He found that the susceptible varieties contained the higher concentrations. He also observed that asparagine, which ranged from 30 to 70% of the total amino

Figure 17. Change of Concentration of Aspartic Acid in Uninfested and Infested Susceptible and Resistant Barley Varieties at Different Ages

A. In Will (resistant) and Rogers (susceptible)

B. In Isogenic A (resistant) and Isogenic B (susceptible)

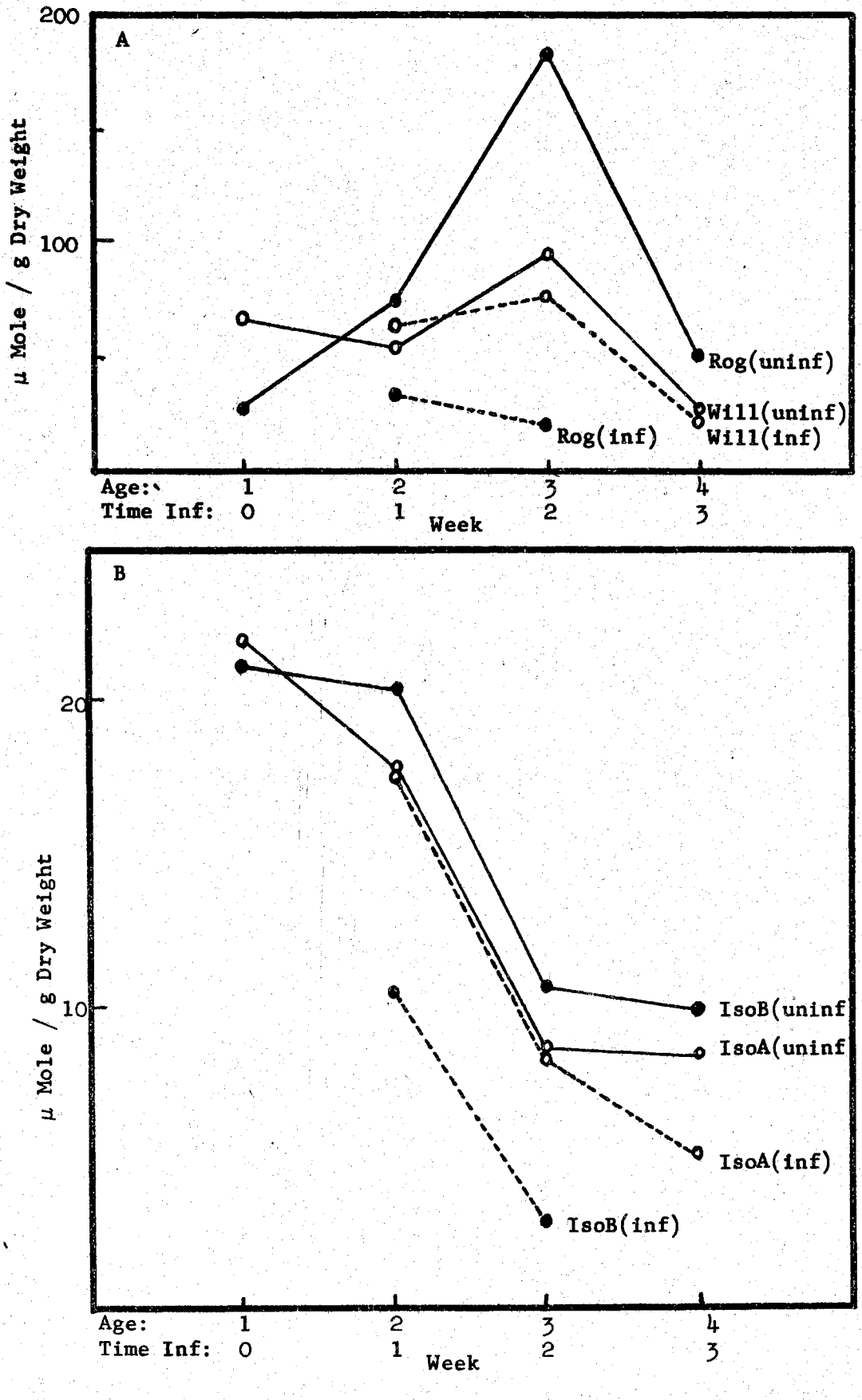
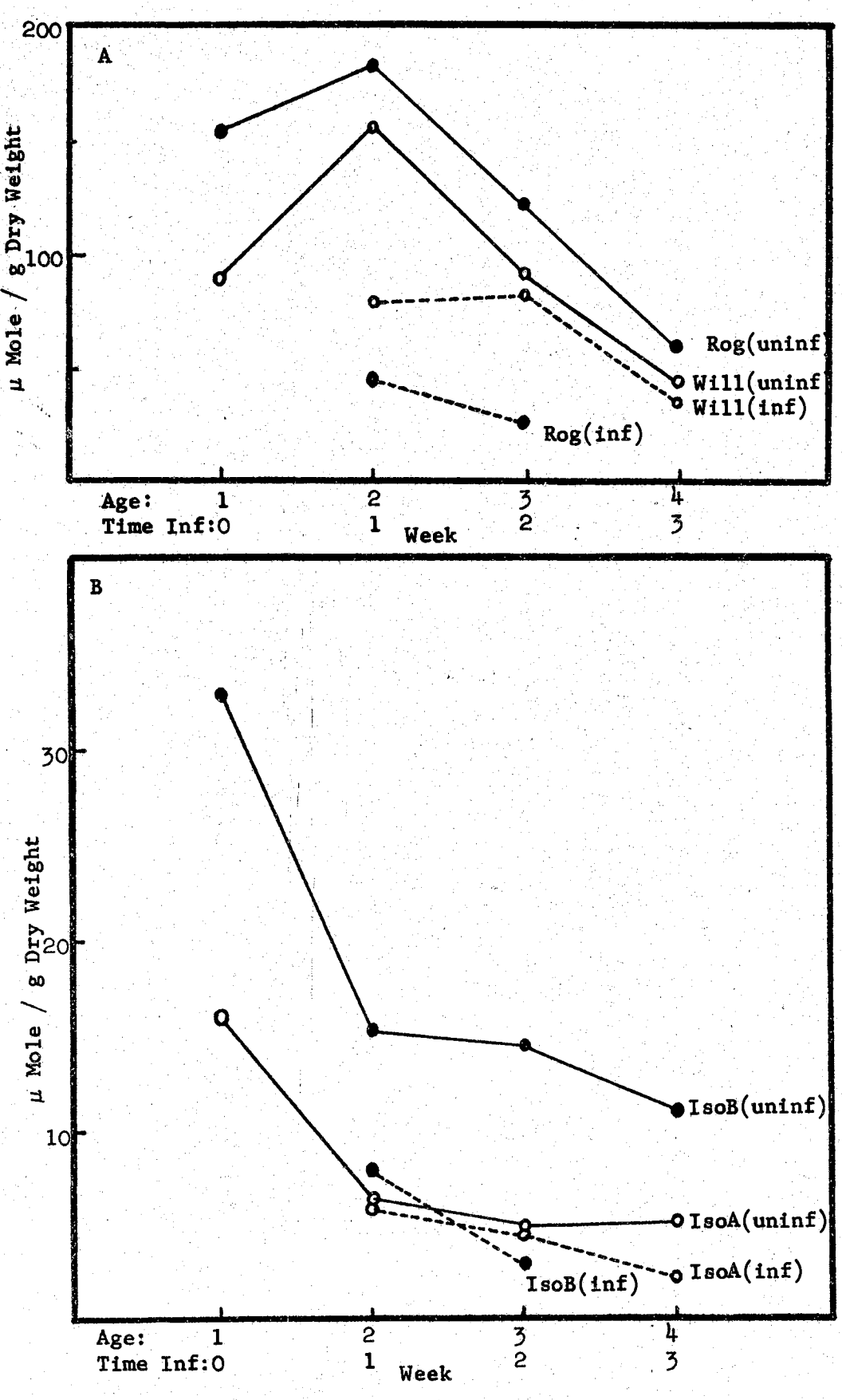

A
200
100
μ Mole / g Dry Weight
Rog(uninf)
Will(uninf)
Rog(inf)
Will(inf)
Age: 1 2 3 4
Time Inf: 0 1 2 3
Week
B
20
10
μ Mole / g Dry Weight
IsoB(uninf
IsoA(uninf
IsoA(inf)
IsoB(inf)
Age: 1 2 3 4
Time Inf: 0 1 2 3
Week

Figure 18. Change of Concentration of Asparagine + Glutamine in Uninfested and Infested Resistant and Susceptible Barley Varieties at Different Ages

A. In Will (resistant) and Rogers (susceptible)

B. In Isogenic A (resistant) and Isogenic B (susceptible)


A
200
100
μ Mole / g Dry Weight
Rog(uninf
Will(uninf
Will(inf)
Rog(inf)
Age: 1 2 3 4
Time Inf:0 1 2 3
Week
B
30
20
10
μ Mole / g Dry Weight
IsoB(uninf)
IsoA(uninf)
IsoA(inf)
IsoB(inf)
Age: 1 2 3 4
Time Inf:0 1 2 3
Week

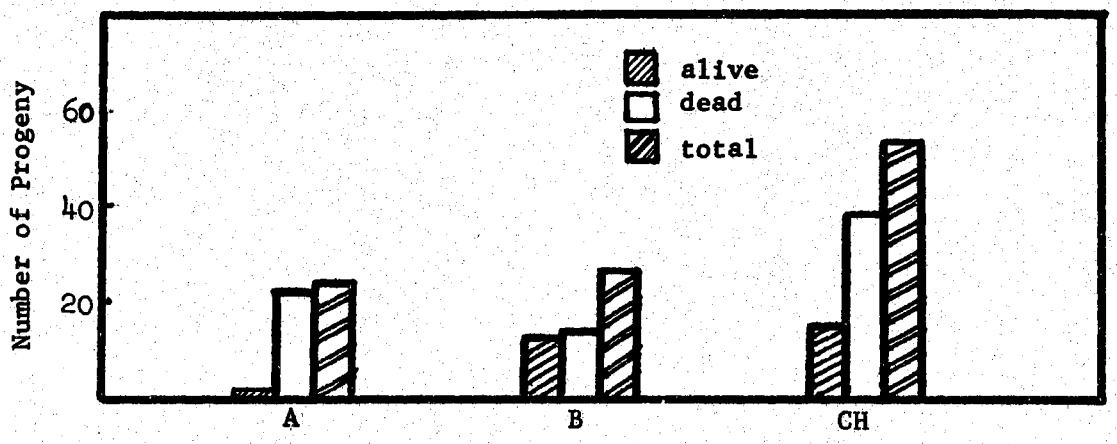
nitrogen, was the principal source of free amino acid nitrogen in alfalfa. Dadd et al. (104) found that growth of the aphid, Myzus pericae (Sulzer), fell sharply when the amino acid level was reduced from 3% to 2% on the artificial diet.

Based on the results of the present study, it would appear that the concentration of the total amino compounds in the barley plants may be related to the susceptibility of barley varieties to greenbug attack. However, the balance of the proportions of individual amino acids should be considered also as another possible factor. Correct proportions were found necessary within some classes of nutrients especially amino acids (105). Banks and Macaulay (106) suggested that composition of the nitrogen fraction in two varieties of bean might account for the different fecundities of the aphid, Aphis fabae.

The effects of the free amino acids extracted from Will and Rogers on the growth and reproduction of greenbugs are shown in Figure 19. Portions of amino acids in the standard artificial diet for the greenbug were substituted with equivalent amounts of amino acids extracted from Will and Roger barley. The growth of greenbugs on the modified diets was measured by their weight and the number of progeny they produced. It was found that amino acids from both varieties had slight inhibitory effects. Figure 20 shows the growth of greenbugs on modified diets in which the concentration of each amino acid was different from that normally used in the standard diet. The concentration of amino acids normally used in the standard diet was estimated to be approximately 40 times of that found in the leaf tissue of the barley plant; therefore the following 3 different concentrations of each amino acids were used: 1) the same concentration as found in leaf tissue of Rogers, 2) ten

Figure 19. Effect of Free Amino Acids Extracted from Will and Rogers on the Growth of Greenbugs Feeding on Artificial Diets for Twenty Days

Alphabets represent the following: A = free amino acids from Will (resistant), B = free amino acids from Rogers (susceptible), CH = control.


Number of Progeny
60
40
20
alive
dead
total
A
B
CH

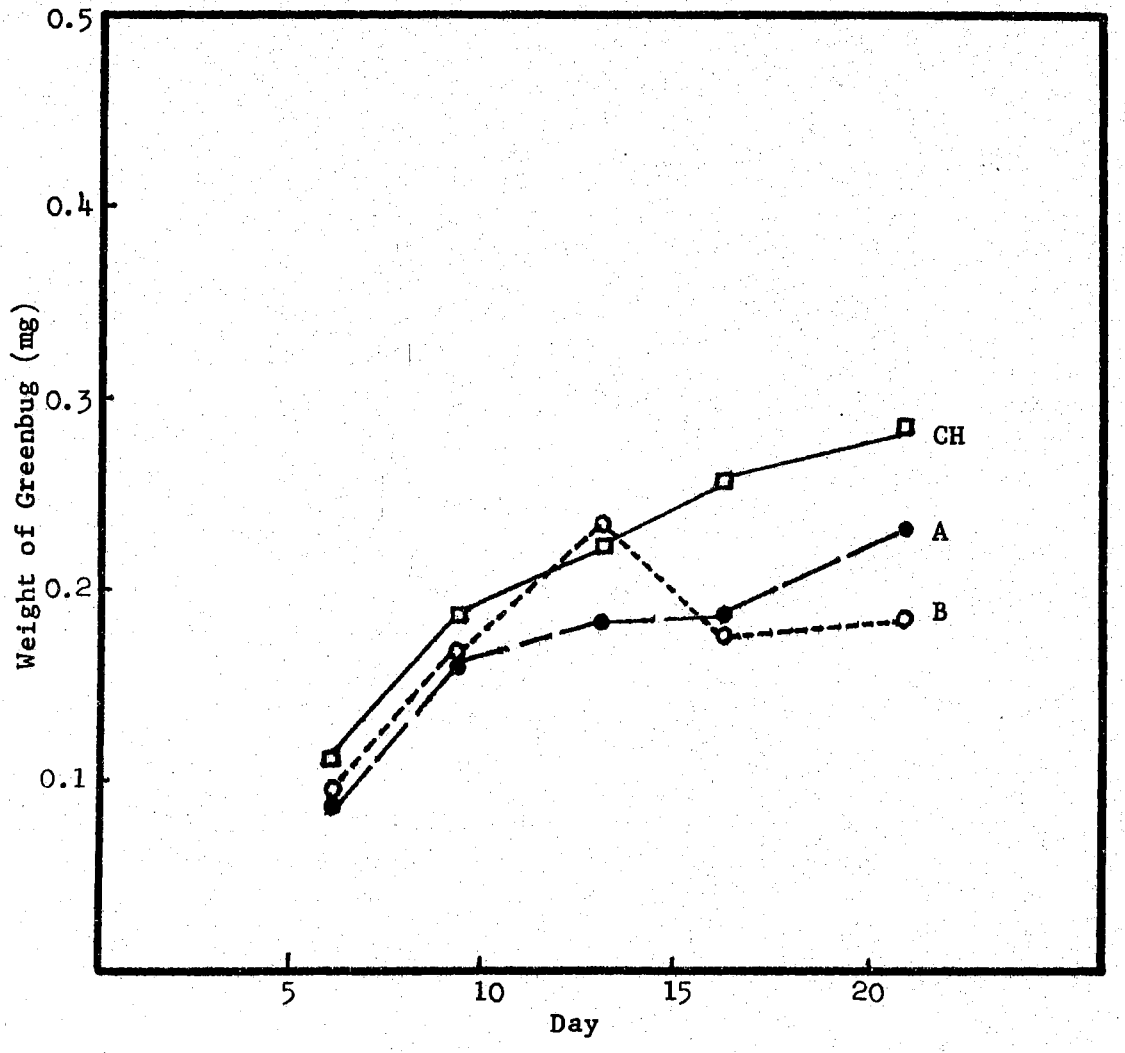
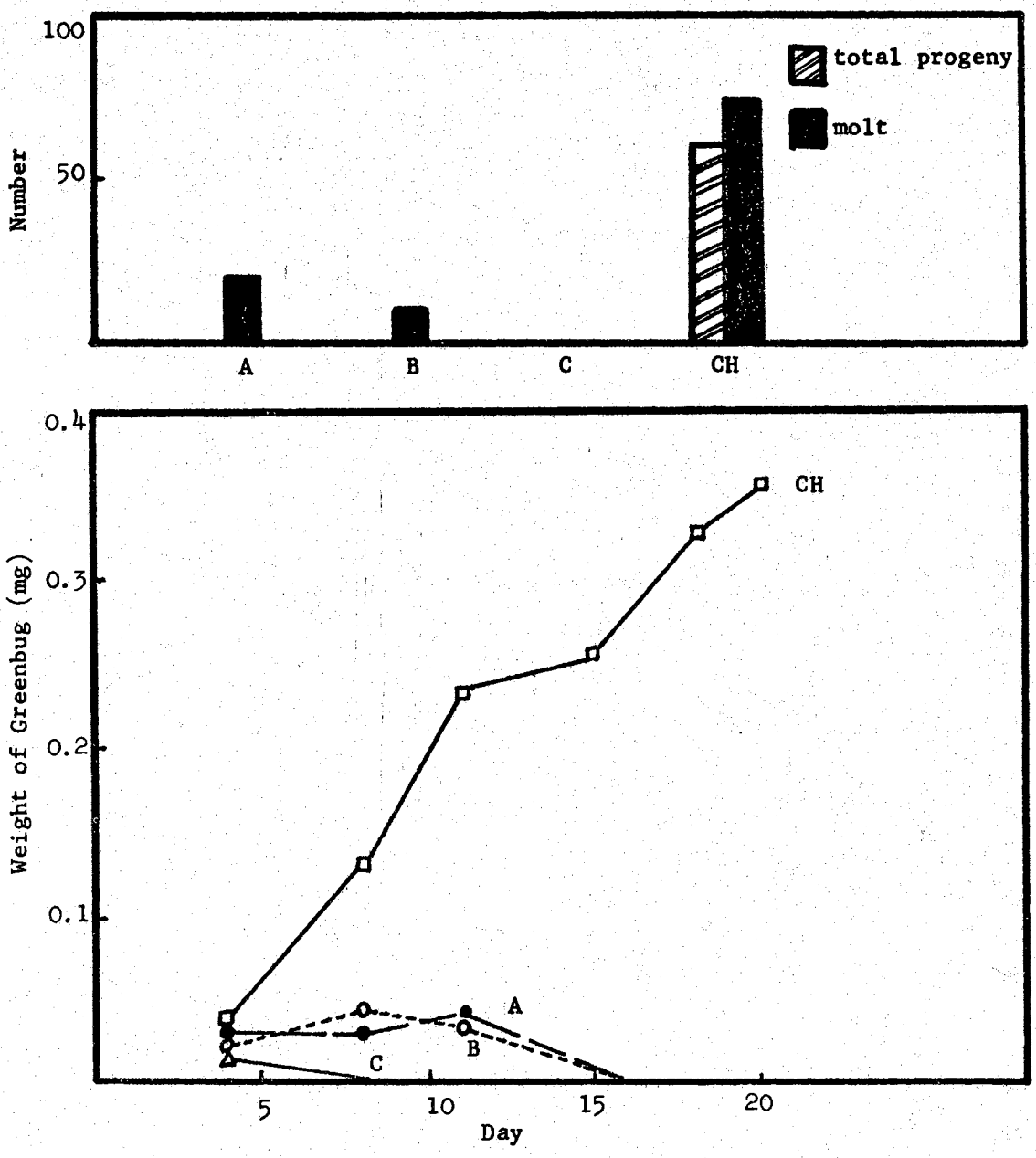

Weight of Greenbug (mg)
0.5
0.4
0.3
0.2
0.1
CH
A
B
5
10
15
20
Day

Figure 20. Effect of Different Amino Acids Concentrations on the Growth of Greenbugs Feeding on Artificial Diets for Twenty Days

Alphabets represent the following: A = amino acids concentration used equivalent to that observed in Roger leaf tissue, B = amino acids concentration used equivalent to 10 x that observed in Roger leaf tissue, C = amino acids concentration used equivalent to 100 x that observed in Roger leaf tissue, CH = control.


100
50
Number
total progeny
molt
A
B
C
CH
0.4
0.3
0.2
0.1
Weight of Greenbug (mg)
CH
A
B
C
5
10
15
20
Day

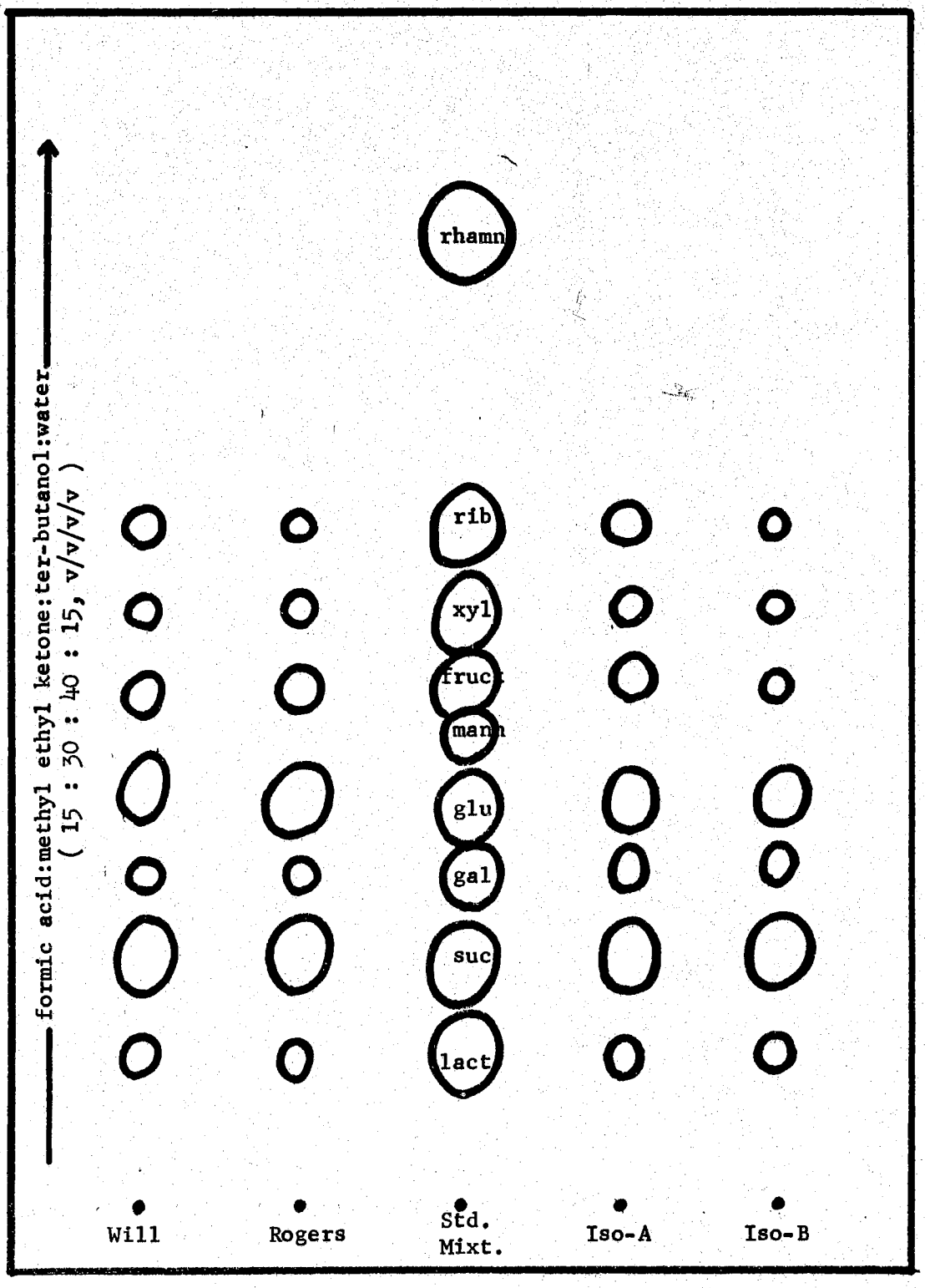
times the concentration found in Rogers leaf tissue and 3) one hundred times the concentration found in Rogers leaf tissue. All of these modified diets were found to inhibit the growth of the greenbug significantly, and the inhibitory effect was increased with the increase in concentration of the amino acids. The results indicated that the poor growth of the greenbug may not be caused by low concentrations of the amino acids. It may be that amino acid imbalance is responsible for the poor growth of the greenbugs, and thus may be related to resistance.

Besides being required as nutrients the free amino acids have been shown to act as feeding stimulants. Amino acids either alone or in combination with other chemicals were found to stimulate feeding activity of insects (107). Beck and Hanec (59) found that free amino acids increased the feeding duration of the European corn borer. An "amino acids receptor" which enables the insect to perceive these compounds had been found in Crustaceae (108).

Sugar Content of Susceptible and Resistant Barley Varieties

The composition of sugars in the resistant and susceptible barley varieties was determined qualitatively by thin-layer chromatography. Ribose, xylose, fructose, glucose, galactose, sucrose and lactose were found in all the varieties tested. There was no qualitative difference between the susceptible and resistant varieties. The same sugars were found in the greenbug-infested barley plants. The intensities of the chromatograms indicated only slight difference of concentrations between uninfested and infested barleys. Figure 21 shows a typical thin-layer chromatogram of sugars of the susceptible and resistant barley varieties.

Figure 21. A Typical Thin-Layer Chromatogram of Sugars of Uninfested Resistant and Susceptible Barley Varieties (Two Weeks Old)


formic acid:methyl ethyl ketone:ter-butanol:water
(15 : 30 : 40 : 15, v/v/v/v)
rhamn
rib
xyl
fruct
mann
glu
gal
suc
lact
Will
Rogers
Std. Mixt.
Iso-A
Iso-B

The total soluble carbohydrates present in the plant extracts were determined by the phenol-sulfuric acid method. Table VIII and Figure 22 show the amount of total soluble carbohydrates in the resistant and susceptible varieties and the changes during growth and greenbug infestation. In the younger plants, only small differences were found between the susceptible and resistant varieties; however, in the older plants, the susceptible varieties had nearly double the amount of soluble carbohydrate than the resistant ones. Greenbug infestation caused a decrease of soluble carbohydrates in both resistant and susceptible varieties but to a greater extent in the susceptible ones.

Monosaccharide composition was determined by gas-liquid chromatography. The monosaccharides were converted to their alditol acetyl derivatives before analysis. The best resolutions were obtained when 5% ECNSS-M was used as stationary liquid phase. Typical chromatograms of alditol acetates of monosaccharides of uninfested Will and Rogers varieties (4 weeks old) are shown in Figure 23. The concentrations of monosaccharides in the uninfested resistant and susceptible barley varieties at different growth periods as well as the greenbug-infested ones are shown in Tables IX and X. The changes of total monosaccharide concentrations of uninfested and infested barley during growth were plotted and are shown in Figure 24. Only slight differences between resistant and susceptible varieties appeared in the 1-week and 2-week old samples. In contrast to the amino acid concentration, the concentration of sugars in the two uninfested susceptible varieties, Rogers and Isogenic B, started to increase at the 3-week stage and increased very rapidly at the period from 3-weeks to 4-weeks. The concentration in one of the resistant varieties, Will, remained quite constant during the growth

TABLE VIII

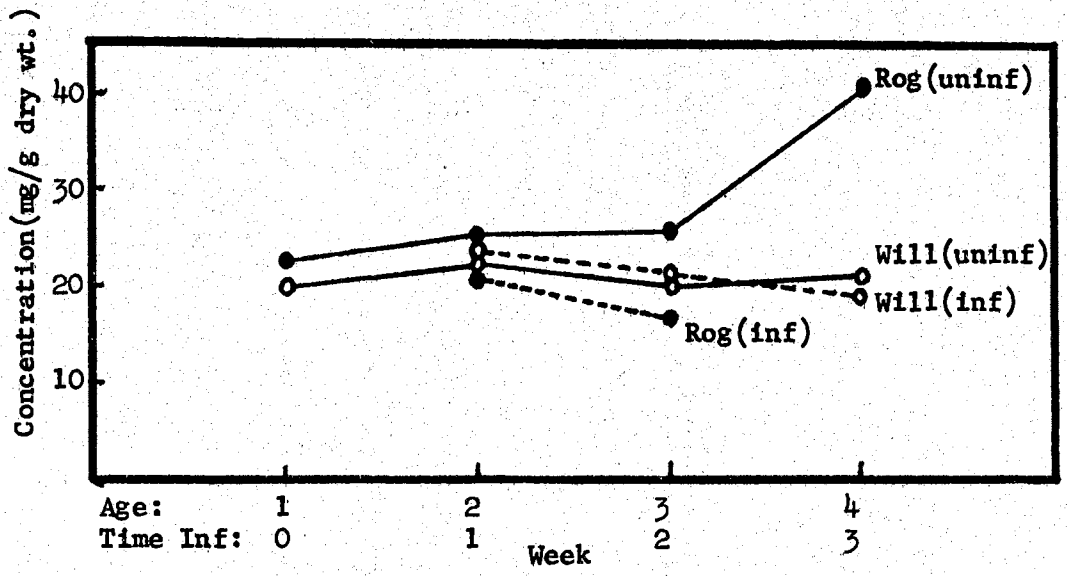
AMOUNT OF TOTAL SOLUBLE CARBOHYDRATE IN UNINFESTED AND INFESTED RESISTANT AND SUSCEPTIBLE VARIETIES

Age of Barley	Will		Rogers		Isogenic A		Isogenic B	
	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.	Uninf.	Inf.
				(mg / g dry weight)				
1 Week Uninf.	20.5		22.5		12.5		10	
2 Weeks Uninf.	22.0		25.0		10.5		10.5	
Inf. 1 Wk		22.5		20.0		9.0		12.5
3 Weeks Uninf.	20.0		25.0		16.1		16.5	
Inf. 2 Wk		22.1		16.5		17.1		14.3
4 Weeks Uninf.	20.1		40.2		34.3		63.4	
Inf. 3 Wk		19.6		dead		26.6		dead

Figure 22. Change of Concentration of Total Soluble Carbohydrates in Uninfested and Infested Susceptible and Resistant Barley Varieties at Different Ages

A. In Will (resistant) and Rogers (susceptible)

B. In Isogenic A (resistant) and Isogenic B (susceptible)


Concentration(mg/g dry wt.)
40
30
20
10
Rog(uninf)
Will(uninf)
Will(inf)
Rog(inf)
Age: 1 2 3 4
Time Inf: 0 1 2 3
Week

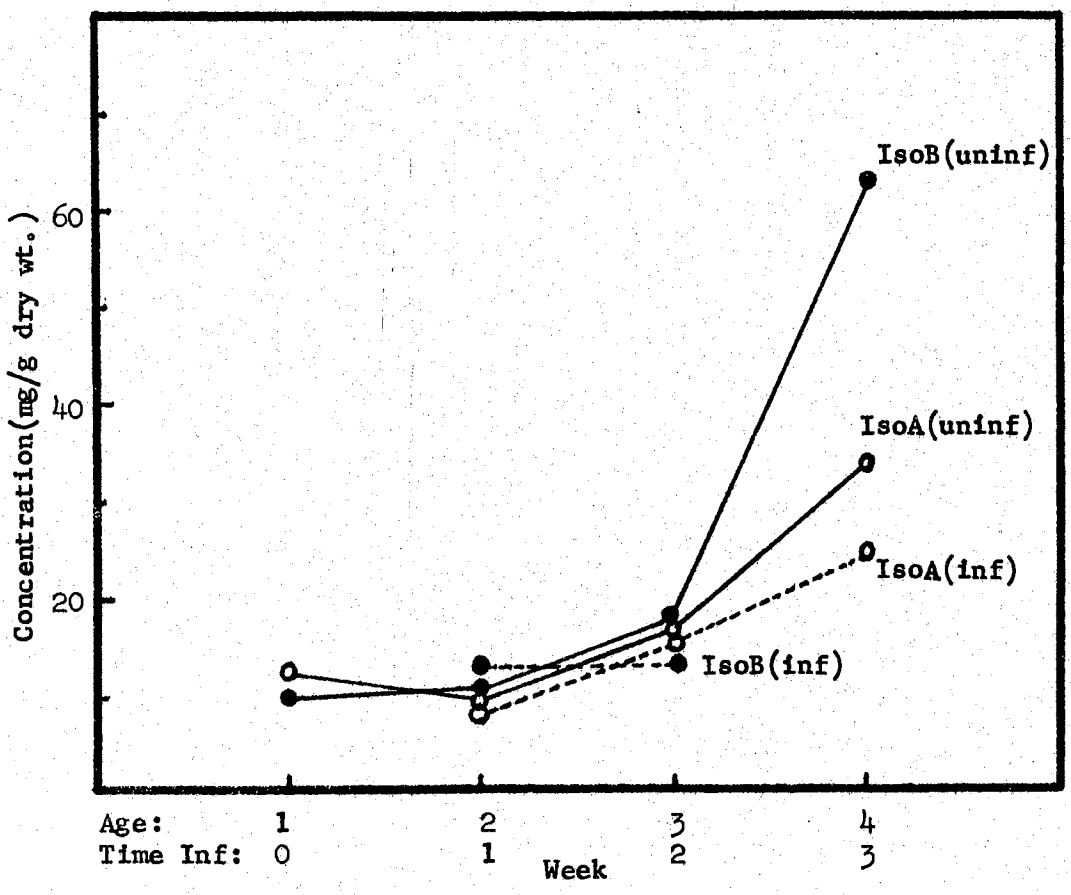

Concentration(mg/g dry wt.)
60
40
20
IsoB(uninf)
IsoA(uninf)
IsoA(inf)
IsoB(inf)
Age: 1 2 3 4
Time Inf: 0 1 2 3
Week

Figure 23. Gas-Liquid Chromatography of Alditol Acetates of Monosaccharides on a 20 Ft. x 1/4 In. 5% ECNSS-M Column at 195°C

A. Monosaccharides of uninfested Will (resistant, 4 weeks old)

B. Monosaccharides of uninfested Rogers (susceptible, 4 weeks old)

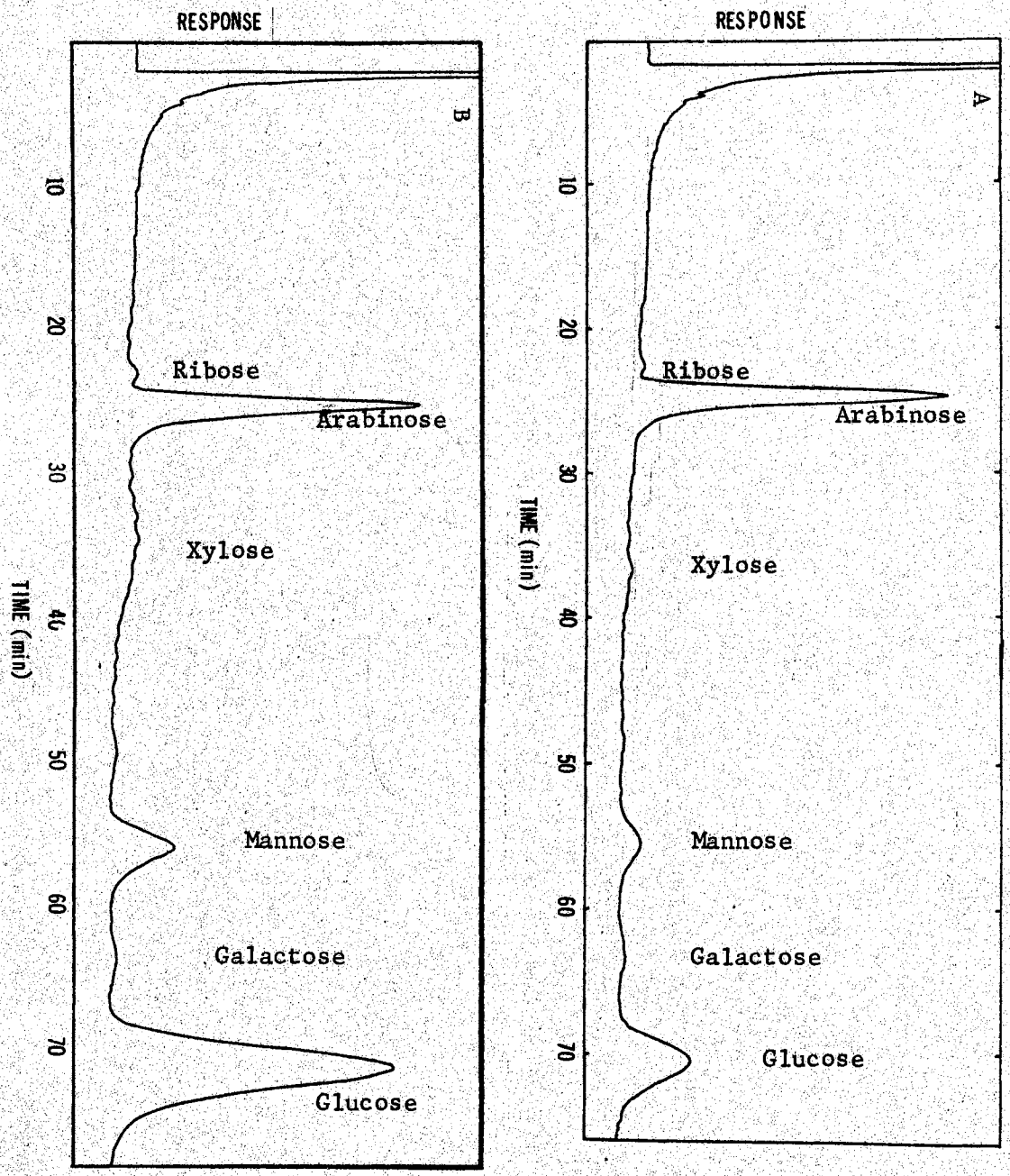

RESPONSE
B
Ribose
Arabinose
Xylose
Mannose
Galactose
Glucose
TIME (min)
10
20
30
40
50
60
70
RESPONSE
A
Ribose
Arabinose
Xylose
Mannose
Galactose
Glucose
TIME (min)
10
20
30
40
50
60
70

TABLE IX

MONOSACCHARIDE COMPOSITION OF UNINFESTED AND INFESTED WILL AND ROGERS VARIETIES

Compound		Uninfested								Infested					
		Will				Rogers				Will			Rogers		
	Age:	1 Wk	2 Wk	3 Wk	4 Wk	1 Wk	2 Wk	3 Wk	4 Wk	2 Wk	3 Wk	4 Wk	2 Wk	3 Wk	4 Wk
	Time Inf:	--	--	--	--	--	--	--	--	1 Wk	2 Wk	3 Wk	1 Wk	2 Wk	3 Wk
										(mg / g dry weight)					
Ribose		trace	trace	trace	trace	trace	trace	trace	trace	trace	0.21	0.12	trace	0.07	dead
Xylose		0.32	0.35	0.19	0.17	0.52	0.38	0.26	0.18	0.22	0.17	0.10	0.22	0.17	dead
Fructose		2.90	1.38	1.30	2.78	2.32	0.30	2.32	9.72	0.56	3.24	2.84	1.52	5.4	dead
Galactose		trace	0.44	0.36	0.44	0.33	0.24	0.44	0.22	trace	0.44	0.24	trace	0.07	dead
Glucose		3.33	3.28	5.70	3.19	4.89	2.12	7.35	14.05	2.03	2.64	3.53	0.68	4.12	dead
Total		6.55	5.45	7.55	6.75	8.06	3.4	10.36	24.36	2.81	6.46	6.82	2.42	9.76	dead

TABLE X

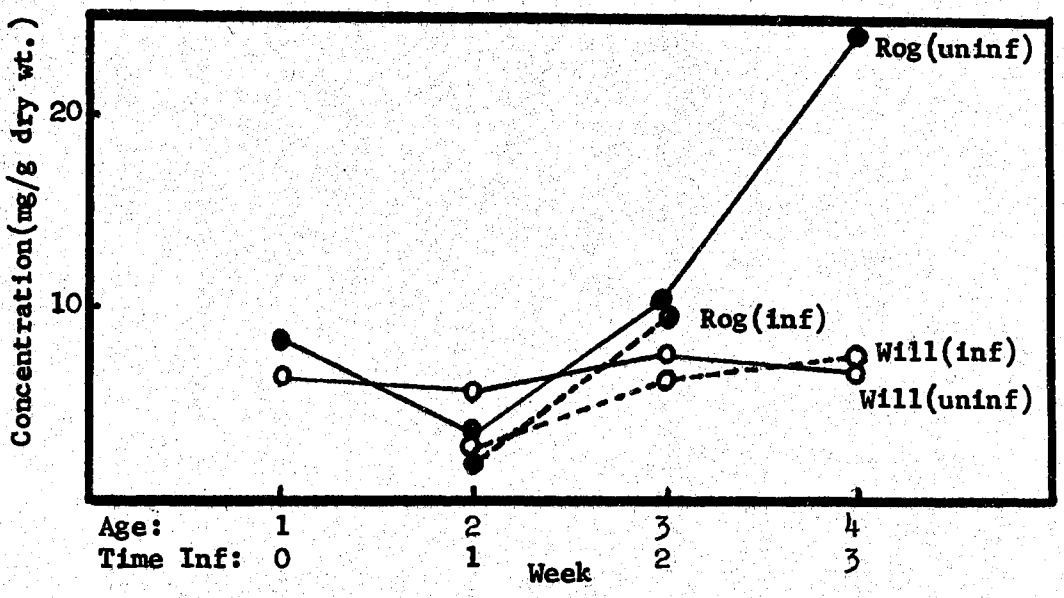
MONOSACCHARIDE COMPOSITION OF UNINFESTED AND INFESTED ISOGENIC A AND ISOGENIC B VARIETIES

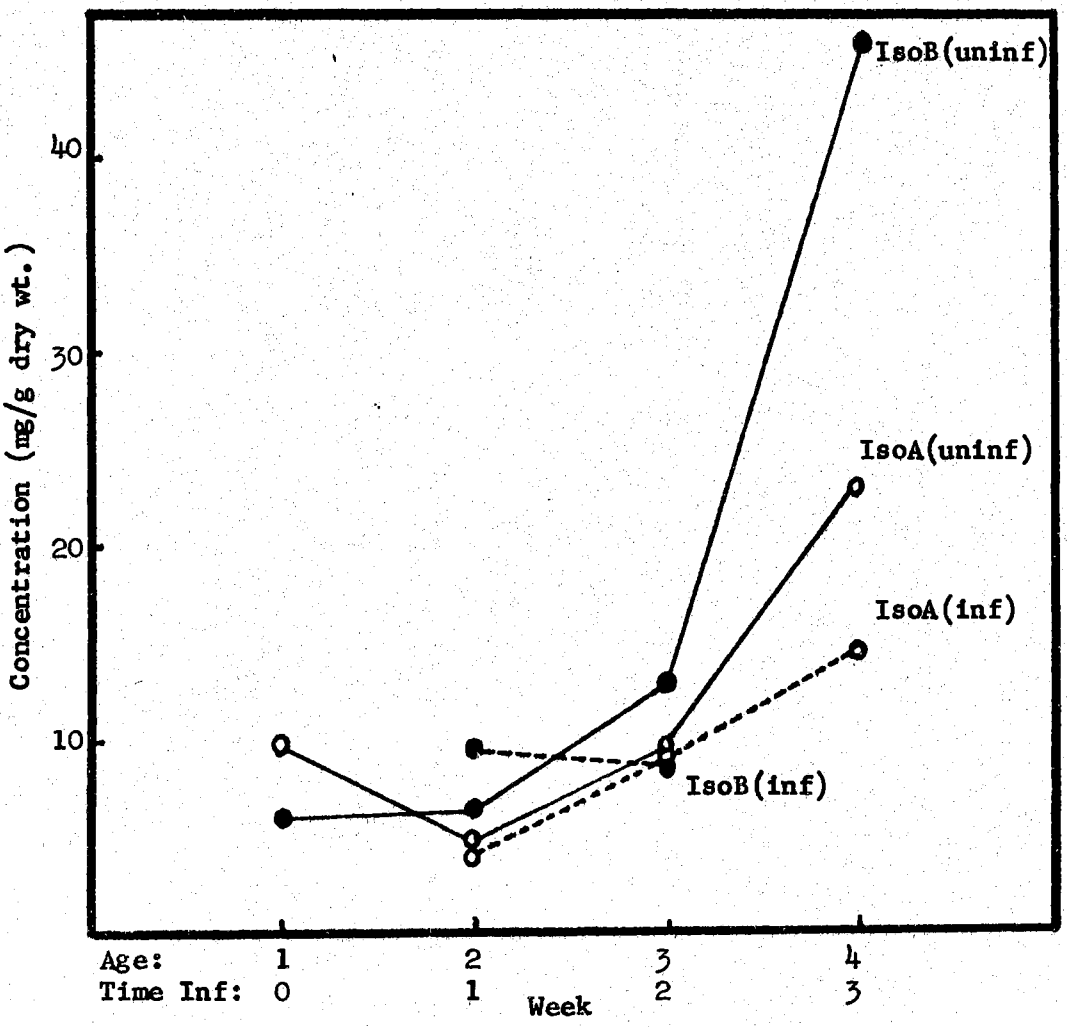
Compound		Uninfested								Infested					
		Isogenic A				Isogenic B				Isogenic A			Isogenic B		
	Age:	1 Wk	2 Wk	3 Wk	4 Wk	1 Wk	2 Wk	3 Wk	4 Wk	2 Wk	3 Wk	4 Wk	2 Wk	3 Wk	4 Wk
	Time Inf:	--	--	--	--	--	--	--	--	1 Wk	2 Wk	3 Wk	1 Wk	2 Wk	3 Wk
		(mg / g dry weight)													
Ribose		trace	trace	trace	0.21	0.17	trace	trace	0.18	trace	trace	0.09	trace	0.16	dead
Xylose		1.07	0.18	0.16	0.60	0.42	0.48	trace	0.18	0.48	0.40	trace	0.44	0.16	dead
Fructose		2.64	1.38	6.14	8.60	3.62	3.50	5.2	15.24	2.20	2.62	5.62	3.18	2.78	dead
Galactose		0.22	0.33	0.11	0.74	trace	0.44	0.26	0.63	0.22	0.33	trace	trace	trace	dead
Glucose		6.05	2.70	3.28	12.98	1.78	1.97	7.46	29.35	0.87	6.57	8.76	5.99	5.00	dead
Total		9.98	4.60	9.68	23.13	5.98	6.39	12.94	45.58	3.77	9.93	14.48	9.61	8.10	dead

Figure 24. Change of Total Monosaccharide Concentration in Uninfested and Infested Susceptible and Resistant Barley Varieties at Different Ages

A. In Will (resistant) and Rogers (susceptible)

B. In Isogenic A (resistant) and Isogenic B (susceptible)


Concentration(mg/g dry wt.)
20
10
Rog(uninf)
Rog(inf)
Will(inf)
Will(uninf)
Age: 1 2 3 4
Time Inf: 0 1 2 3
Week


Concentration (mg/g dry wt.)
40
30
20
10
IsoB(uninf)
IsoA(uninf)
IsoA(inf)
IsoB(inf)
Age: 1 2 3 4
Time Inf: 0 1 2 3
Week

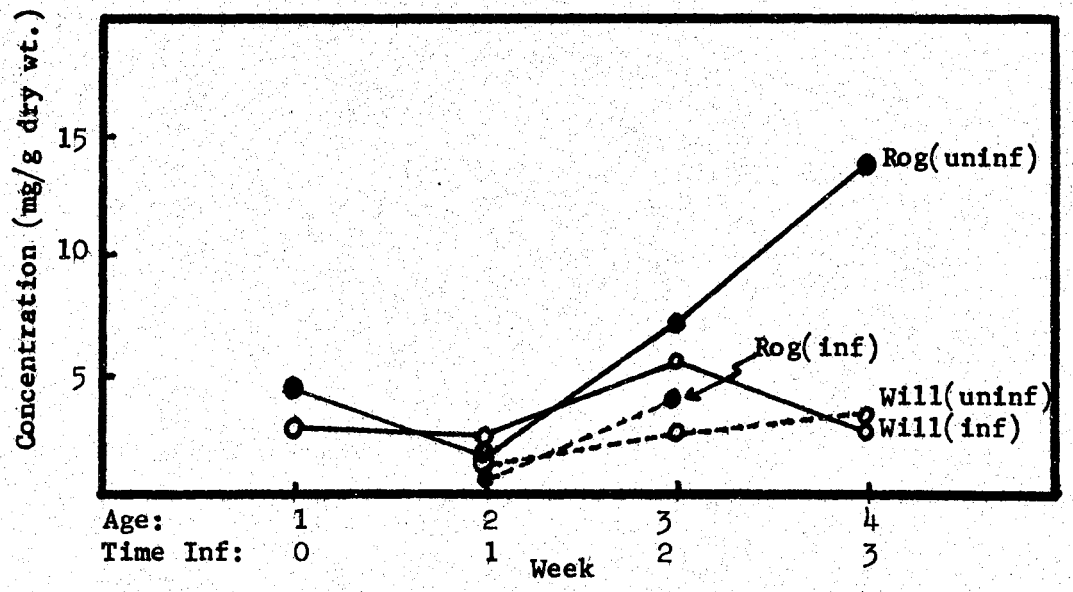
period from one to four weeks. In the other one, Isogenic A, the concentration of sugars increased gradually. The concentrations in the 4-week sample were much higher in the susceptible varieties than in the resistant varieties. There were some decreases of concentrations in the greenbug-infested samples. Glucose and fructose were the main components in the monosaccharides. However, the concentrations of these two sugars shown in Table IX and X are approximate quantities, since fructose was detected as mannose (TLC showed an absence of mannose from the samples) and glucose in this gas chromatographic system. Reduction of fructose yielded mannitol and glucitol; and that of glucose, glucitol. Equal amounts of mannitol and glucitol were assumed to have resulted from fructose, and therefore, the actual amount of fructose present in the sample was calculated as twice the amount of mannitol detected; and the actual amount of glucose, as the amount of glucitol detected minus the amount of mannitol detected. Figures 25 and 26 show the concentrations of glucose and fructose in the samples. The changes of concentrations of these 2 sugars in the uninfested resistant and susceptible barleys during growth as well as in the infested plants were quite comparable to those of the total monosaccharides and the total soluble carbohydrates. The uninfested susceptible varieties had also much higher concentrations of glucose and fructose than the uninfested resistant varieties.

The effects of sugars extracted from resistant and susceptible barley plants on the growth of greenbugs are shown in Figure 27. The greenbugs were grown on modified artificial diets in which part of the sucrose normally used was replaced with equivalent amounts of sugars extracted from Will and Rogers barleys. Sugars from both varieties had

Figure 25. Change of Glucose Concentration in Uninfested and Infested Resistant and Susceptible Barley Varieties at Different Ages

A. In Will (resistant) and Rogers (susceptible)

B. In Isogenic A (resistant) and Isogenic B (susceptible)


Concentration (mg/g dry wt.)
15
10
5
Rog(uninf)
Rog(inf)
Will(uninf)
Will(inf)
Age: 1 2 3 4
Time Inf: 0 1 2 3
Week

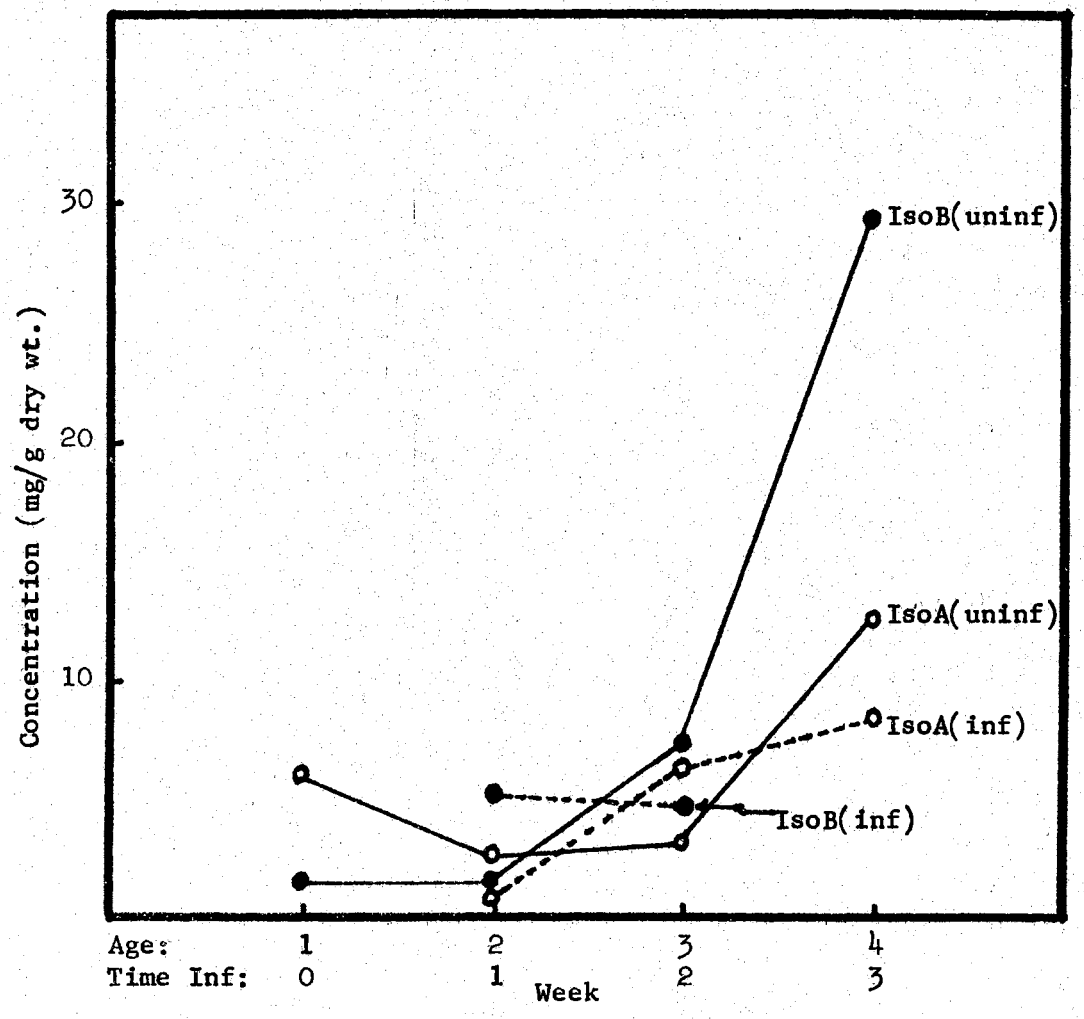
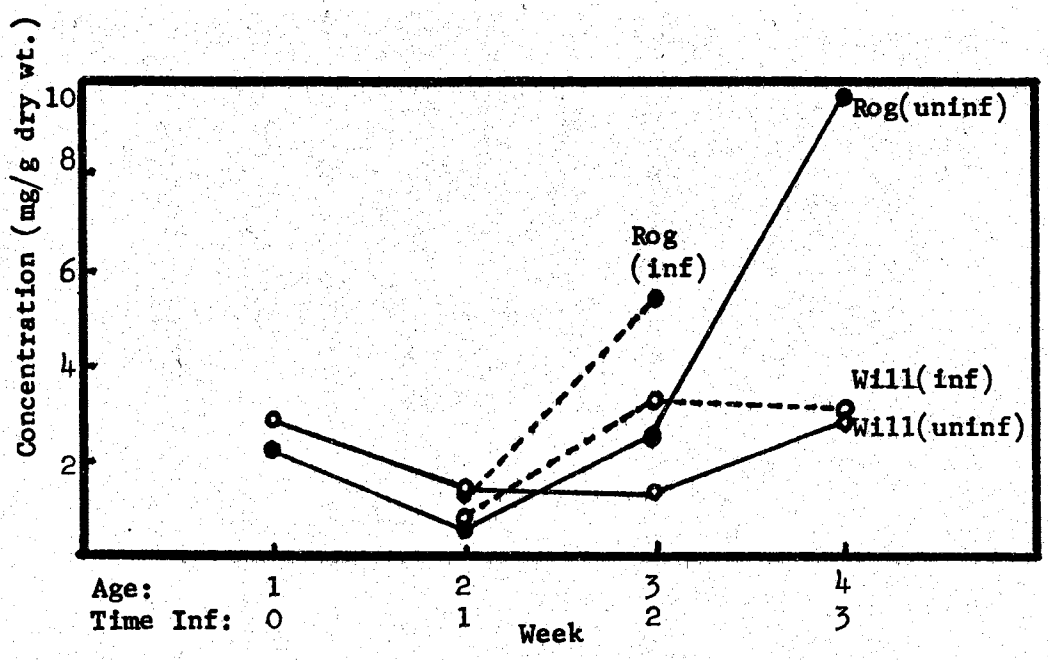

Concentration (mg/g dry wt.)
30
20
10
IsoB(uninf)
IsoA(uninf)
IsoA(inf)
IsoB(inf)
Age: 1 2 3 4
Time Inf: 0 1 2 3
Week

Figure 26. Change of Fructose Concentration in Uninfested and Infested Resistant and Susceptible Barley Varieties at Different Ages

A. In Will (resistant) and Rogers (susceptible)

B. In Isogenic A (resistant) and Isogenic B (susceptible)


Concentration (mg/g dry wt.)
10
8
6
4
2
Rog(uninf)
Rog
(inf)
Will(inf)
Will(uninf)
Age: 1 2 3 4
Time Inf: 0 1 2 3
Week

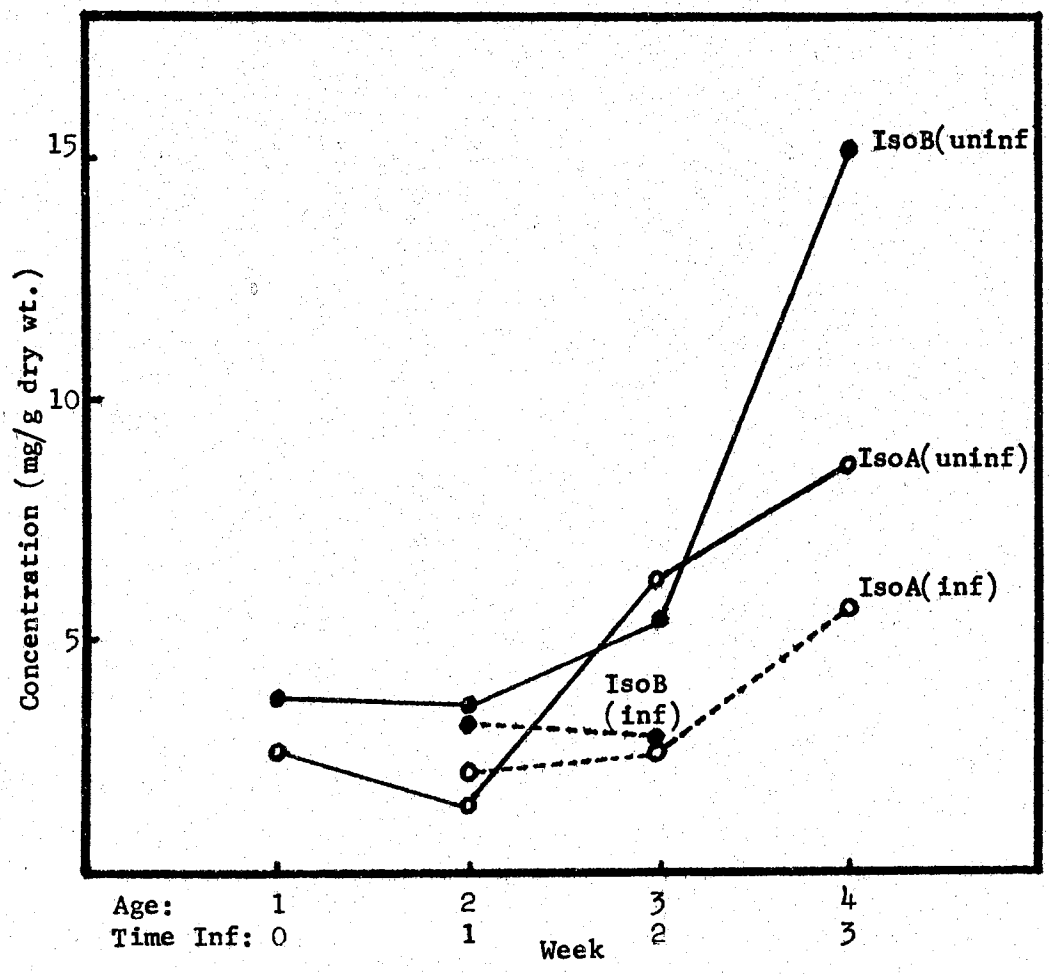
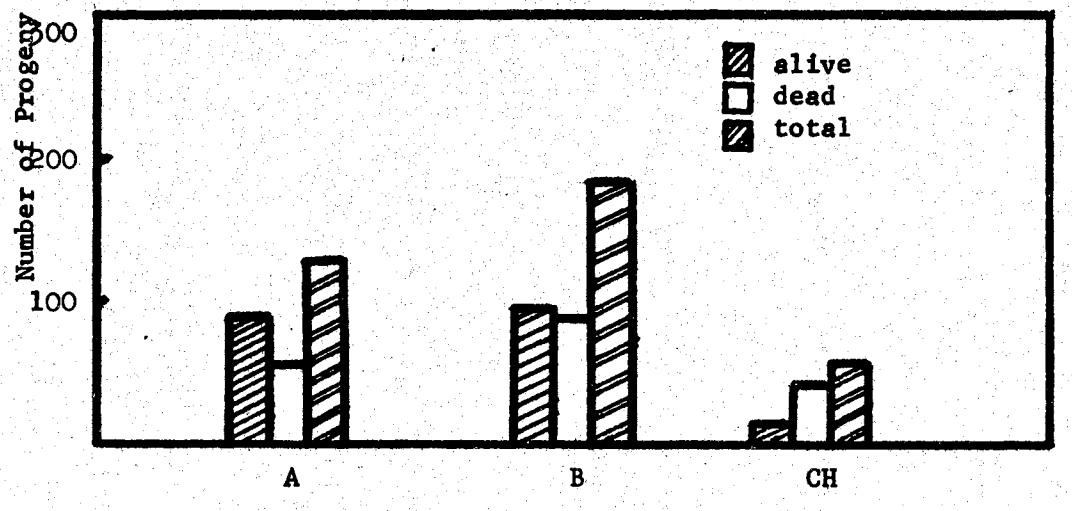
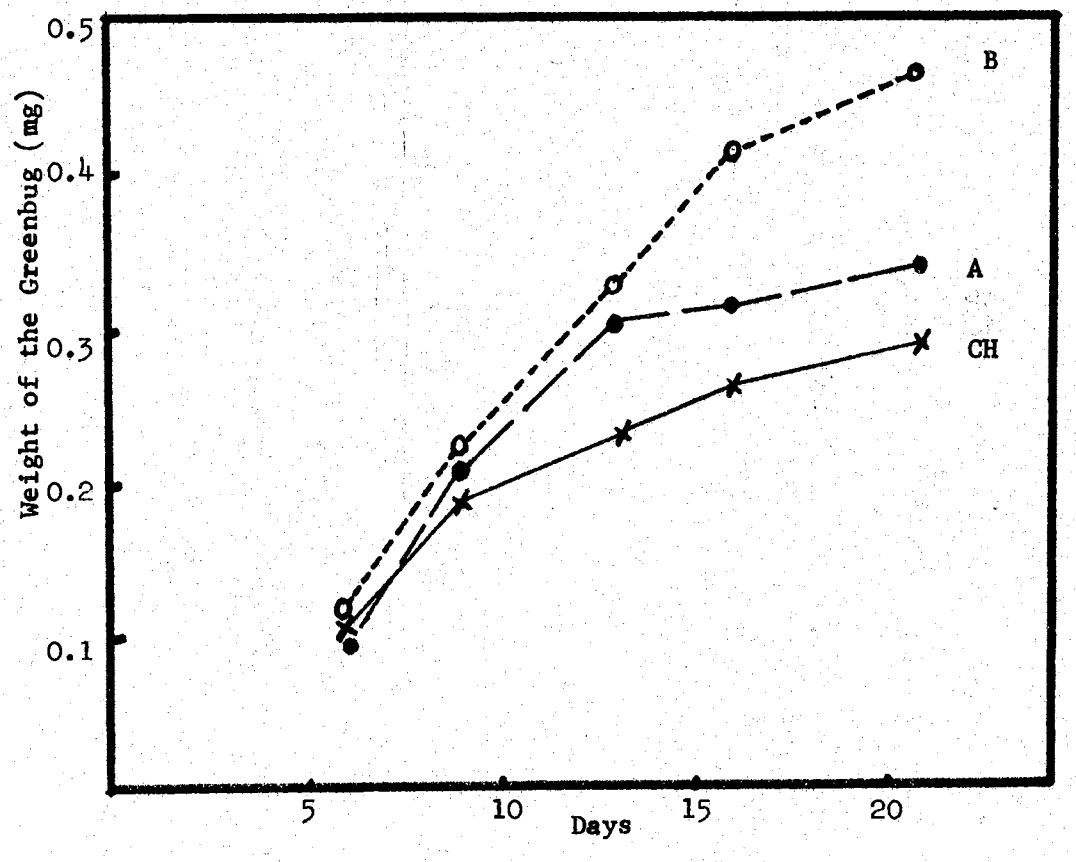

Concentration (mg/g dry wt.)
15
10
5
IsoB(uninf
IsoA(uninf)
IsoA(inf)
IsoB
(inf)
Age: 1 2 3 4
Time Inf: 0 1 2 3
Week

Figure 27. Effect of Sugars Extracted from Resistant and Susceptible Barley Varieties on the Growth of Greenbugs Feeding on Artificial Diets for Twenty Days

Alphabets represent the following: A = sugars from Will (resistant), B = sugars from Rogers (susceptible), CH = control.


Number of Progeny
300
200
100
alive
dead
total
A
B
CH


Weight of the Greenbug (mg)
0.5
0.4
0.3
0.2
0.1
B
A
CH
5
10
15
20
Days

a stimulatory effect on growth and reproduction of the greenbugs; however, the effect caused by Rogers was greater than that caused by Will. These results gave a strong indication that the differences in sugar may also play a role in greenbug resistance in barley varieties. It is evident that sugars are required nutrients for the greenbug. Since, in the artificial diet for the greenbug, a very high concentration of sucrose, 35%, was needed in order to obtain optimum growth of the greenbugs (89). Carbohydrates have been found to be important also in the growth of Trogoderma granarium (109), and fecundity of Lampetia equestris (F.) (110).

Sugars may also be chemical feeding requirements which act as phagostimulants. Insects feeding on plants were found to be able to perceive sugars. A "glucose receptor" and a "sucrose receptor" was found in the maxillary palpi of the silkworm (111). These two sugars had considerable significance in the regulation of feeding in this insect.

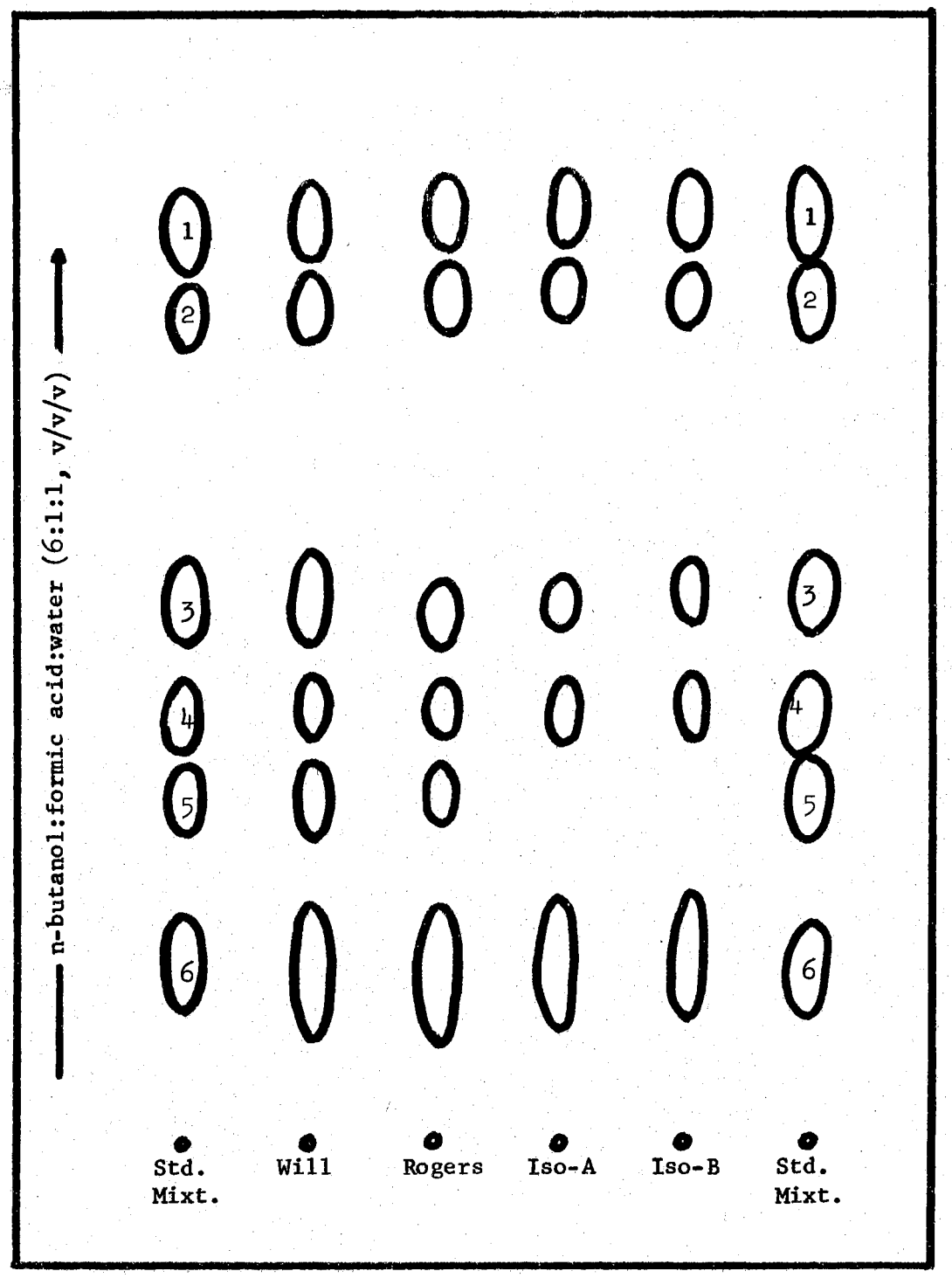
In studies of resistance of pea varieties to pea aphids, Maltais and Auclair (62) found the sugar content of the resistant varieties were higher than in the susceptible varieties. They concluded that sugars would apparently be considered unnecessary or slightly utilizable substances for pea aphid development. However, the nutritional requirements of the pea aphid may not necessarily be the same as of the greenbug.

Organic Acids of the Resistant and Susceptible Barley Varieties

The composition of organic acids in the resistant and susceptible barley varieties was determined by thin-layer chromatography. Figure 28

Figure 28. A Typical Thin-Layer Chromatogram of Organic Acids of Uninfested Susceptible and Resistant Barley Varieties (Two Weeks Old)

Numbers represent the following: 1 = aconitic acid, 2 = succinic acid, 3 = malic acid, 4 = citric acid, 5 = tartaric acid, 6 = quinic acid.


n-butanol:formic acid:water (6:1:1, v/v/v)
1
2
3
4
5
6
Std. Mixt.
Will
Rogers
Iso-A
Iso-B
Std. Mixt.

shows a typical chromatogram of the organic acids in the uninfested 2-week old barleys. Qualitative differences were not found between the resistant and susceptible varieties, and judging from the intensities of the chromatograms there were also no quantitative differences. Aconitic acid, succinic acid, malic acid, citric acid, tartaric acid and quinic acid were found in Will and Rogers barleys; however, tartaric acid was not detected in Isogenic A and Isogenic B. Malic acid appeared to be the major component in all varieties. The same organic acids were detected in the greenbug-infested plants. No significant difference in organic acid concentration between the infested and uninfested barleys was observed since the density of the chromatograms appeared to be similar. Therefore, the organic acids are unlikely to be important in influencing the susceptibility or resistance of barley to greenbug attack.

Secretions of Greenbug to the Host Plant During Feeding

Three radioactive substances were found by autoradiography on the thin-layer chromatogram of the sugar fraction from plants that had been infested by radioactive greenbugs. Only one radioactive substance was found in the amino acid fraction of these plant materials. The radioactive compounds in the sugar fraction were identified as sucrose, galactose and erythrose, and the one in the amino acids fraction was identified as L-cysteic acid by additional thin-layer chromatography. Identifications of these compounds by thin-layer chromatography are shown in Figures 29 and 30. The sucrose and galactose may originate from the plant in which these two sugars were also present. Erythrose was not found in the plant; however, it may be a product from the degradation of glucose is present in the plant in great amounts. Cysteic acid

Figure 29. Two-Dimensional Thin-Layer Chromatogram of Radioactive Substance in the Free Amino Acids Fraction and a Standard L-Cysteic Acid

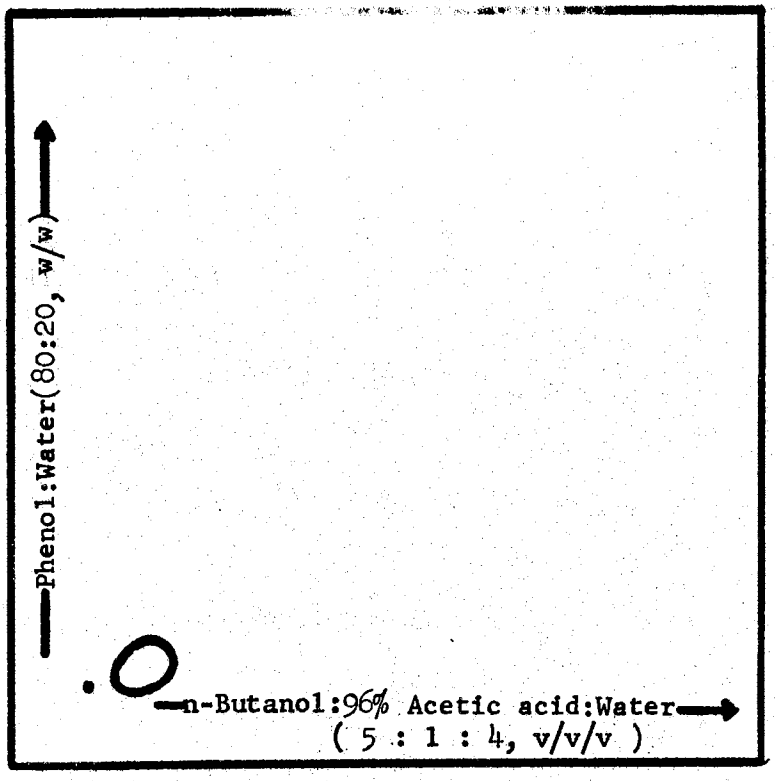
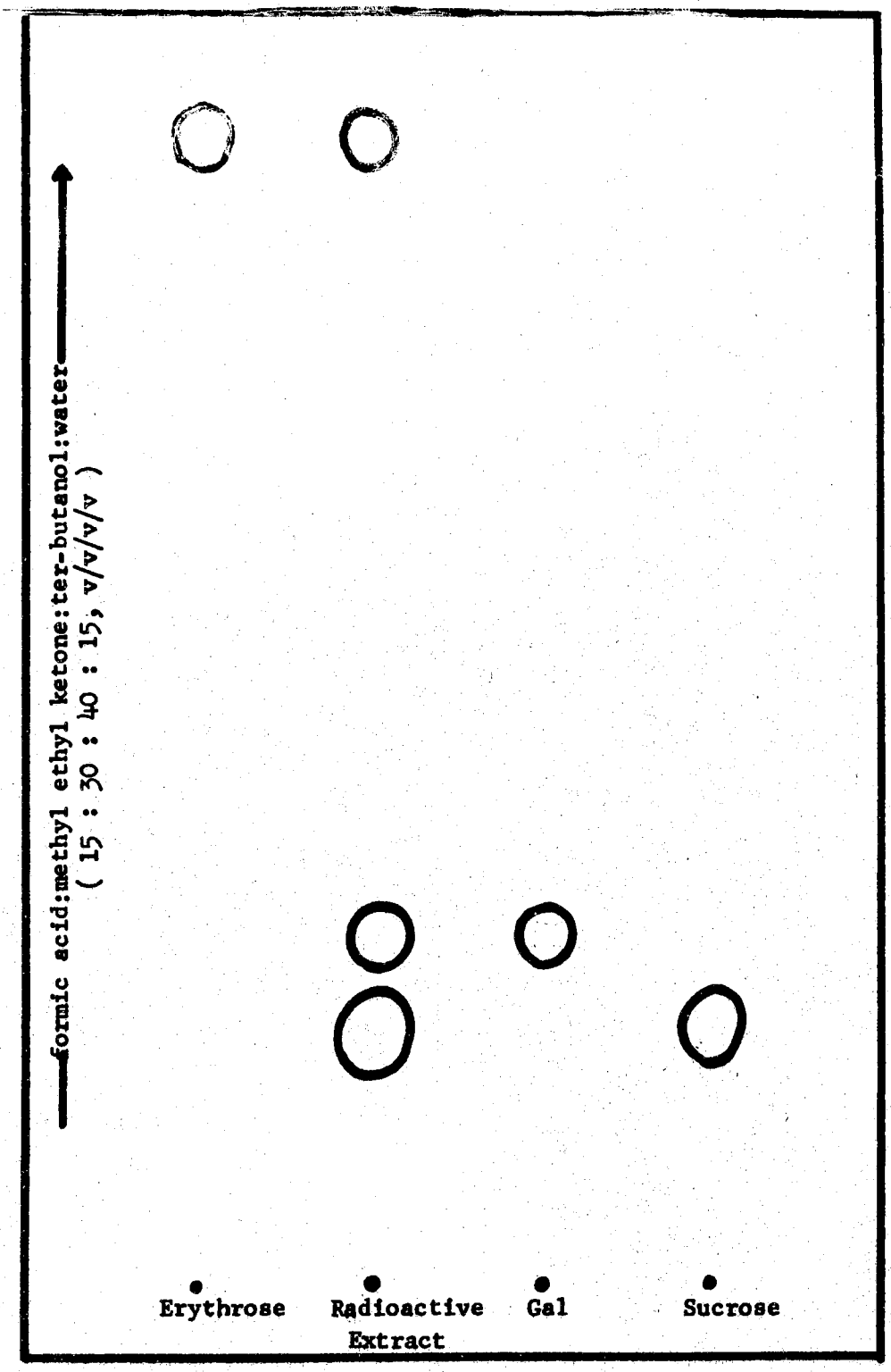

Phenol:Water(80:20, w/w)
n-Butanol:96% Acetic acid:Water
(5 : 1 : 4, v/v/v)

Figure 30. Thin-Layer Chromatogram of Radioactive Substances in the Sugar Fraction and Standard Sugars


formic acid:methyl ethyl ketone:ter-butanol:water
(15 : 30 : 40 : 15, v/v/v/v)
Erythrose
Radioactive Extract
Gal
Sucrose

was not found in the plant, but it is reported in the literature (112) that cysteic acid can be formed from cysteine, and cysteine from methionine. Both methionine and cysteine were found in the barley plants and were used in the artificial diet for the greenbug. Therefore, it might be suggested that cysteic acid was formed from methionine and cysteine by the greenbug and secreted into the plant during feeding. The effect of cysteic acid on plant is not known. Kloft (113) found the amino acids, glycine, alanine, asparagine and glutamic acid in the saliva of the aphid, M. ascalonicus, and attributed host injury to these amino acids. The piercing of plant cells by the stylets of the aphids was found to cause a shortening of the time of plasmolysis in cells lying close to the point of injection and an increase of streaming in the cell plasma. The increased cell plasma-streaming was thought to be caused by the amino acids in the aphids' saliva. Feeding of these compounds to uninfested plants caused some disturbance of water uptake and traspiration. The presence of amino acids has also been confirmed in the saliva of other aphids (114,115).

Conclusions

Information obtained from this investigation suggest some general conclusions about the factors which may be involved in greenbug resistance in the barley plant, and the effects of the greenbug on the host plant.

Quantitative differences of the volatiles between susceptible and resistant varieties indicated that preference and non-preference may be one of the mechanisms for greenbug resistance in barley. Susceptible varieties might possess high concentrations of volatile chemical attract-

ants and low concentrations of repellents while the resistant varieties may contain high concentrations of repellent and low concentrations attractants. The volatile components which differed quantitatively in susceptible and resistant varieties were not identified in this investigation.

The higher concentrations of free amino acids and sugars found in the susceptible varieties suggest that nutritional factors may be involved in resistance. Free amino acids and sugars may be required for the nutrition of the greenbug. Poor growth of greenbugs on the resistant varieties may be caused by the low concentrations of these nutrients in these varieties. The balance between these two groups of compounds as well as the balance within a single group, especially amino acids, might also be important. Sugars and amino acids may have synergistic interaction. A plant may therefore, elicit more intense sensory reactions in an insect than merely the sum of the reactions to each of its constituents. Maltais and Auclair (62) found a relationship between the ratio of total sugar to total nitrogen in the pea plant and pea aphid resistance. Sugars and amino acids may also act as chemical feeding requirements as well as being nutritional requirements. "Sugar receptor" and "amino acid receptor" are known to be present in some insects.

It is doubtful if the presence of toxic substances in the resistant barley can be involved in greenbug resistance, since greenbugs can feed on the resistant variety for a period of time and occasionally mature on the resistant plants. These greenbugs may have reduced size and fecundity, but it would not appear to be the result of poisons. Auclair (116) showed that pea aphids feeding on resistant pea varieties were comparable with aphids starved for 10 hours daily, and disproved the hypothesis

that substances toxic to aphid are present in the resistant host. However, Todd et al. (118) demonstrated the toxicity of phenolic and flavonoid compounds to the greenbug, and suggested the presence of these compounds in barley plants may be involved in the resistance of some barley varieties to greenbugs.

The stunting effect on host plants caused by greenbug feeding may result from the removal of essential food, water and minerals from the plant. Reductions of sugar and free amino acid concentrations in susceptible barleys resulting from greenbug infestation were observed in the present investigation. Carter (117) had also observed depletion of carbohydrates in the plants following aphid infestation. Depletion of carbohydrate caused the reduction of root yield, and this was suggested as the main cause of the early wilting of plant attacked by aphids. The secretion of substances which may be toxic to plant growth into the plant by the aphids during feeding remains as another possibility for the aphids stunting of host plants. Maxwell and Painter (99) proposed that the stunting effect in the plant might be caused by the reduction of critical levels of plant hormones extracted and excreted by aphids during feeding. King (100) suggested the stunting effect was caused by the drastic increase of "free" auxins in the susceptible plants following infestation. These two hypotheses would seem unlikely since the present investigation indicated that auxin levels in both of the susceptible and resistant varieties increased following greenbug infestation, and the increases of "free" auxins in the susceptible varieties were not significantly different from those in resistant varieties.

This proposal of mechanism for the greenbug resistance in barley plants is in harmony with Kennedy's "dual discrimination" theory (14)

which suggested that, in addition to specific stimulatory substances of no nutritive value which govern botanical preferences, primary substances such as amino acids which are of nutritional importance to plant and insect alike, also played a major role in aphid-host relations.

CHAPTER V

SUMMARY

Qualitative and quantitative comparisons of chemical substances existing in the greenbug-resistant and susceptible varieties of barley were made in order to characterize the differences between these two varieties. Volatiles were collected by steam distillation of the barley leaves. Gas-liquid chromatography and gas chromatography-mass spectrometry were employed for the separation and identification of the components. More than 40 compounds were observed. No qualitative difference of these compounds was found between the susceptible and resistant varieties; however, quantitative differences were observed.

Hydrogen ion concentrations of cell saps from several varieties of barley were determined, but no significant difference was obtained between the resistant and susceptible varieties. Water content of the barley plants was also determined. The uninfested barleys were not significantly different, but the greenbug-infested susceptible varieties had reduced water content following greenbug infestation.

Comparisons of the "free" and "bound" indoles of the susceptible and resistant barleys were also made. Thin-layer chromatography and gas liquid chromatography were used for the analyses. Qualitative and quantitative differences were not found between the two varieties. The greenbug-infested plants had increased concentrations of indoles, but the increases were also the same for the 2 varieties.

Free amino acids were extracted from the plant with 80% ethanol and purified through a cation-exchange column. Thin layer chromatography was used for qualitative determination. The quantitative compositions were determined by automatic amino acid analyses. Only quantitative differences were found between susceptible and resistant varieties. The susceptible varieties had much higher concentrations than the resistant varieties. Greenbug-infested susceptible varieties showed a sharp reduction of free amino acids.

Sugars were extracted with 80% ethanol and purified by eluting through an cation-exchange column connected to an anion-exchange column. Thin-layer chromatography showed no qualitative difference between the resistant and susceptible varieties, but gas-liquid chromatography indicated that the concentrations of sugars were much higher in the susceptible varieties than in the resistant varieties in the more mature plants. Greenbug-infested susceptible varieties were also found to have decreased amount of sugars.

Analysis of the organic acids of the resistant and susceptible varieties by thin-layer chromatography did not show any qualitative or quantitative difference between the two varieties.

Secretions of greenbugs into the host plants during feeding were studied by radioactive tracing method. Three sugars and one amino acid were detected by thin-layer chromatography and autoradiography. Effects of some of these compounds on plants are not known.

The mechanism of greenbug resistance in barley plants is suggested to be preference and non-preference caused by the difference of concentrations of volatile components in the resistant and susceptible varieties, and malnutrition owing to the low concentrations of the nutrients

such as amino acids and sugars present in the resistant varieties. The balance between the free amino acids and sugars may also be important in resistance in barley to the greenbug.

REFERENCES

(1) Comes, O., Rev. Appl. Entom., 6, 55 (1918).

(2) Bruce, C. F., Am. Naturalist, 54, 313 (1920).

(3) Davidson, J., Ann. Appl. Biol., 10, 35 (1923).

(4) Lees, A. H., Ann. Appl. Biol., 13, 506 (1926).

(5) Fullaway, D. T., Hawaiian Forester and Agriculturalist, 23, 68 (1926).

(6) Andrew, E. A., J. Ind. Tea Assoc., 10, 75 (1923).

(7) Carter, W., Ecology, 8, 350 (1927).

(8) Withycome, C. L., Ann. Appl. Biol., 13, 64 (1926).

(9) Mymford, E. P., Rev. Appl. Entomol., 14, 619 (1926).

(10) Mumford, E. P., Nature, 125, 411 (1930).

(11) Mumford, E. P., Ann. Appl. Biol., 17, 28 (1930).

(12) Wigglesworth, V. B., The Principles of Insect Physiology, 3rd ed., Methuen and Co., London, 1947, p. 434.

(13) Fraenkel, G., Science, 129, 1466 (1959).

(14) Kennedy, J. S., Entomol. Exptl. Appl., 1, 50 (1958).

(15) Feir, D. and Beck, S. D., Ann. Entomol. Soc. Am., 56, 224 (1963).

(16) Sleesman, J. P., Am. Potato J., 17, 9 (1942).

(17) Richmond, E. A., Entomol. Soc. Wash. Proc., 29, 36 (1927).

(18) Metzger, F. W. and Grant, D. H., U.S. Dept. Agr. Tech. Bull., 299, 21 (1932).

(19) Wilcox, R. B. and Beckwith, C. S., J. Agr. Res., 47, 583 (1933).

(20) Fennah, R. G., Trop. Agr., 16, 233 (1939).

(21) Moore, R. H., Proc. Okla. Acad. Sci., 8, 16 (1928).

(22) Hamamura, Y., Nature, 183, 1746 (1959).

(23) Hamamura, Y., Hayashiya, K., Naito, K., Matsura, K. and Nishida, J., Nature, 194, 754 (1962).

(24) Gupta, P. D. and Thorsteinson, A. J., Entomol. Exptl. Appl., 3, 241 (1960).

(25) Gupta, P. D. and Thorsteinson, A. J., Entomol. Exptl. Appl., 3, 305 (1960).

(26) Folsom, J. W., J. Econ. Entomol., 24, 827 (1931).

(27) Maxwell, F. G., Jenkins, J. N., Keller, J. C. and Parrott, W. L., J. Econ. Entomol., 56, 449 (1963).

(28) Maxwell, F. G., Jenkins, J. N. and Keller, J. C., J. Econ. Entomol., 56, 894 (1963).

(29) Thompson, K. F., Nature, 198, 209 (1963).

(30) Thorsteinson, A. J. and Nayar, J. K., Can. J. Zool., 41, 931 (1963).

(31) Callan, E. M., Trop. Agr., 20, 127 (1943).

(32) Thorpe, W. H. and Crombie, A. C., J. Exptl. Biol., 23, 234 (1947).

(33) Yamamoto, R. T. and Fraenkel, G., Ann. Entomol. Soc. Am., 53, 499 (1960).

(34) Miller, B., J. Econ. Entomol., 53, 995 (1960).

(35) Djamin, A. and Pathak, M. D., J. Econ. Entomol., 60, 347 (1967).

(36) Lanning, F. C., J. Agr. Food Chem., 14, 636 (1966).

(37) Refai, F. Y., Jones, E. T., and Miller, B. S., Cereal Chem., 32, 437 (1955).

(38) Loomis, R. S., Beck, S. D. and Stauffer, J. F., Plant Physiol., 32, 379 (1957).

(39) Scott, G. E., Guthrie, W. D., Tipton, C. L., Crop Sci., 6, 359 (1966).

(40) Kristiansen, K., "Insect Resistance Factors in Corn Plants". (Ph.D. dissertation, University of Kansas, 1962).

(41) Klun, J. A. and Brindley, T. A., J. Econ. Entomol., 59, 711 (1966).

(42) Grimal'skii, V. I., Zool. Zhur., 45, 551 (1966).

(43) Applebaum, S. W., J. Agr. Food Chem., 17, 618 (1969).

(44) Wilde, J. D., Entomol. Exptl. Appl., 1, 14 (1958).

(45) Thurston, R., Smith, W. T. and Cooper, B. P., Entomol. Exptl. Appl., 9, 428 (1966).

(46) Thurston, R. and Webster, J. A., Entomol. Exptl. Appl., 5, 233 (1962).

(47) Wegorek, W. and Krzymanska, J., Pr. Nauk. Inst. Ochr. Roslin, 10, 7 (1969).

(48) Lichtenstein, E. P. and Casida, J. E., J. Agr. Food Chem., 11, 410 (1963).

(49) Lichtenstein, E. P. and Strong, F. M., J. Agr. Food Chem., 10, 30 (1962).

(50) Munakata, K., in Proceedings to a Symposium on the Major Insect Pests of the Rice Plant, Johns Hopkins Press, Baltimore, Md., 1967, p. 419.

(51) Nayar, J. K. and Fraenkel, G., Ann. Entomol. Soc. Am., 56, 174 (1963).

(52) Wilski, A., Giebel, J. and Glowinkowska, A., Pr. Nauk. Inst. Ochr. Roslin, 9, 25 (1968).

(53) Wright, W. and Bora, P.C., Current Sci., 29, 395 (1960).

(54) McGinnis, A. J. and Kasting, R., Entomol. Exptl. Appl., 5, 313 (1962).

(55) Swailis, G. E., Can. Entomologist, 92, 958 (1960).

(56) Rohde, R., Phytopathology, 55, 1159 (1965).

(57) Beck, S. D. and Lilly, J. H., Iowa State Coll. J. Sci., 23, 249 (1949).

(58) Beck, S. D., Ann. Entomol. Soc. Am., 49, 582 (1956).

(59) Beck, S. D. and Hanec, W., J. Insect. Physiol., 2, 85 (1958).

(60) Beck, S. D., Ann. Entomol. Soc. Am., 49, 399 (1956).

(61) Dahms, R. G. and Painter, R. G., J. Econ. Entomol., 33, 482 (1940).

(62) Maltais, J. B. and Auclair, J. L., Can. Entomologist, 89, 365 (1957).

(63) Auclair, J. L., Entomol. Exptl. Appl., 2, 279 (1959).

(64) Auclair, J. L. and Maltais, J. B., Can. Entomologist, 82, 175 (1950).

(65) Auclair, J. L., Maltais, J. B. and Cartier, J. J., Can. Entomologist, 89, 457 (1957).

(66) Maltais, J. B. and Auclair, J. L., J. Ins. Physiol., 8, 391 (1962).

(67) Thurston, R., J. Econ. Entomol., 54, 946 (1961).

(68) Marble, V. L., Meldeen, J. C., Murray, H. C. and Zscheile, F. P., Agronomy J., 54, 741 (1962).

(69) Coon, B. F., Miller, R. C. and Aurand, L. W., Penn. Agr. Expt. Sta. Dept. Zool. and Entomol. Multilith Rpt., 8p (1948).

(70) Knapp, J. L., Hedin, P. A., and Douglas, W. A., Ann. Entomol. Soc. Amer., 58, 401 (1965).

(71) Kasting, R. and McGinnis, A. J., Can. J. Zool., 39, 273 (1961).

(72) Miller, B. S. and Swain, T., J. Sci. Food Agr., 11, 344 (1960).

(73) Peppers, J. H. and Hasting, E., Mont. Agr. Expt. Sta. Tech. Bull., 413, (1943).

(74) Waller, G. R., Proc. Okla. Acad. Sci., 47, 271 (1968).

(75) Ryhage, R., Arkiv Kemi, 26, 305 (1967).

(76) Horning, E. C., Vanden Hennel, W. J. A. and Creech, B. G., in Glick, D. (ed.), Method of Biochemical Analysis, Vol. XI, Interscience, New York, 1963, p. 69.

(77) Cruickshank, I. A. M., J. Sci. Food Agr., 8, 26 (1957).

(78) Stahl, E., in Stahl, E. (ed.), Thin-Layer Chromatography, Academic Press, Inc., Publishers, New York, 1965, p. 97.

(79) Turner, N. A. and Redgwell, R. J., J. Chromatog., 21, 129 (1966).

(80) Ovodov, Y. S., Evtushenko, E. V., Vaskovsky, V. E., Ovodov, R. G. and Soloveve, T. F., J. Chromatog. 26, 111 (1967).

(81) Miller, B. S. and Swain, T., J. Sci. Food Agric., 11, 344 (1960).

(82) Nalewaja, J. D. and Smith, L. H., Agron. J., 55, 523 (1963).

(83) Sawardeker, J. S., Sloneker, J. H. and Jeanes, A., Anal. Chem., 37, 1602 (1965).

(84) Abdel-Akher, M., Hamilton, J. K. and Smith, F., J. Am. Chem. Soc., 73, 4691 (1951).

(85) Maxwell, F. G. and Painter, R. H., J. Econ. Entomol., 55, 46 (1963).

(86) Larsen, P. in Paeck, K. and Tracey, M. (editors), Modern Method of Plant Analysis, Vol. III, 565 (1955).

(87) Randerath, K., Thin-Layer Chromatography, Academic Press, New York, 1964, p. 86.

(88) Brook, J. L., Biggs, R. H., St. John, P. A. and Antony, D. S., Anal. Bioch., 18, 453 (1967).

(89) Cress, D. C., "Development and Utilization of a Synthetic Diet for the Greenbug, Schizaphis graminum (Rond.), for Use in Determining the Factor or Factors Responsible for Resistance in Barley and Wheat." (Ph.D. dissertaion, Oklahoma State University, 1969).

(90) Maclead, A. J. and Maclead, J. Sci. Food Agric., 19, 273 (1968).

(91) Buttery, R. G., Seifert, R. M., Guadagni, D. G. and Ling, L. C., J. Agr. Food Chem., 19, 524 (1971).

(92) Gholson, R. K. (personal communication).

(93) Kennedy, J. S. and Booth, C. O., Entomol. Exptl. Appl., 2, 1 (1959).

(94) Wearing, C. H., Nature, 213, 1052 (1967).

(95) Walker, P. T., Empire J. Exptl. Agr., 22, 293 (1954).

(96) Fife, J. M. and Frampton, V. L., J. Agr. Res., 53, 581 (1936).

(97) Auclair, J. L., Ann. Entomol. Soc. Amer., 58, 855 (1965).

(98) Maxwell, F. G. and Painter, R. H., J. Econ. Entomol., 55, 57 (1962).

(99) Maxwell, F. G. and Painter, R. H., Ann. Entomol. Soc. Amer., 55, 299 (1962).

(100) King, R. L., "Preliminary Investigation on the Role of Indole Auxins in Damage by the Greenbug, Schizaphis graminum (Rond.), to Barley Plants." (unpub. M.S. thesis, Oklahoma State University, 1969).

(101) Greun, H. E., Ann. Rev. Plant Physiol., 10, 405 (1959).

(102) Bennet, L. and Clark, J., in Wain, R. L. and Wight, F. (editors), The Chemistry and Mode of Action of Plant Growth Substance (1956), p. 310.

(103) Libbert, E., Wichner, S., Schiewer, U., Risch, H. and Kaiser, W.,

Planta, 68, 327 (1966).

(104) Dadd, R. H. and Mitter, T. E., J. Inst. Physiol., 11, 717 (1965).

(105) Friend, W. G., Salkeld, E. H. and Stevenson, I. L., Ann. N.Y. Acad. Sci., 77, 379 (1954).

(106) Banks, C. J. and Macaulay, E. D. M., Ann. Appl. Biol., 55, 207 (1965).

(107) Thorsteinson, A. J., Ann. Rev. Entomol., 5, 143 (1960).

(108) Hodgson, E. S., Viewpoints Biol., 4, 83 (1965).

(109) Pant, N. C. and Uberoi, N. K., Experientia, 14, 71 (1958).

(110) Doucette, C. F., and Eide, P. M., Ann. Entomol. Soc. Am., 48, 343 (1955).

(111) Ishikawa, S., J. Cellular Comp. Physiol., 61, 99 (1963).

(112) White, A., Handler, P. and Smith, E. L., Principles of Biochemistry, McGraw-Hill, New York, 1964, p. 543.

(113) Kloft, W., Z. Angew. Entomol., 45, 377 (1960).

(114) Anders, F., Experientia, 14, 623 (1958).

(115) Schäeler, G., Entomol. Exptl. Appl., 3, 128 (1960).

(116) Auclair, J. L., Entomol. Exptl. Appl., 3, 229 (1960).

(117) Carter, W., Boton. Rev., 18, 680 (1952).

(118) Todd, G. W., Getachun, A. and Cress, D. C., Ann. Entomol. Soc. Am., 64, 718 (1971).

VITA

Tak K. Chan

Candidate for the Degree of

Doctor of Philosophy

Thesis: A BIOCHEMICAL STUDY OF RESISTANCE IN BARLEY TO THE GREENBUG, SCHIZAPHIS GRAMINUM

Major Field: Biochemistry

Biographical:

Personal Data: Born in Macao, June 11, 1941, the son of Dr. and Mrs. P. K. Chan.

Education: Attended grade school and high school in Hong Kong; received the Bachelor of Arts degree in Biology from Simpson College, Indianola, Iowa, in June, 1964, and the Master of Science degree in Biochemistry from Oklahoma State University, Stillwater, Oklahoma, in May, 1968.

Professional Experience: Laboratory technician, Department of Biochemistry, Oklahoma State University, October, 1966 to September, 1967; Graduate Assistant, Department of Biochemistry, Oklahoma State University, October, 1967 to May, 1971.

Professional Organizations: Beta Beta Beta Biological Society, the Society of Sigma Xi.