PRELIMINARY INVESTIGATION ON THE ROLE OF INDOLE AUXINS IN DAMAGE BY THE GREENBUG, SCHIZAPHIS GRAMINUM (ROND.), TO BARLEY PLANTS

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CHAPTER I

INTRODUCTION

The greenbug (Schizaphis graminum Rond.) is one of the most serious pests of small grains in the central and southwestern states. It causes some damage every year, and several severe outbreaks have occurred. Since 1882, when it was first reported in the United States from Virginia, there have been 15 outbreaks, the most serious in 1907, 1942, 1950, and 1951. Each of these outbreaks caused a loss estimated at more than 50 million bushels of grains (1,2).

A common belief has been that plant damage by aphids is caused primarily by the extraction of plant sap which robs the plant of essential food, water, and minerals. Recent evidence has shown that certain aphids are capable of inducing damage by injecting toxic substances into plants with their saliva. It has also been suggested that the salivary sheaths produced by most aphid species during their penetration into plant cells may be involved in the transmission of plant viruses (3). Some types of injuries to plant cells such as plasma-streaming, polyploidy and gall formation in the grape vine, were thought to be caused by the unutilized plant-derived amino acids and amides absorbed into the hemolymph and excreted by the salivary glands of aphids on feeding (4).

The mechanism of greenbug feeding and the difference in injury to plant cells of barley, oats and wheat was studied by Chatters and

Schlehuber (10). They found that 1) greenbug damage varies from lysis in barley to cell-wall modification in oats, and a combination of lysis and cell-wall modification in wheats; 2) greenbug stylets tend to enter tissues inter-cellularly with the phloem the ultimate feeding site. These workers indicated that the injection of saliva, rather than the intake of food, appears to be the primary cause of tissue damage.

Auclair et al.(5) suggested that the plant growth hormones might be involved in the mechanism of toxicity, and that toxins injected into various hosts by aphids may have their origin from plant substances extracted during feeding. Some evidence indicated that the toxins may be auxins or plant growth inhibitors concentrated in sufficient quantity to be phytotoxic (6).

Maxwell and Painter(7,8,9) determined the free auxin content in the ether extracts of the host plants infested by greenbug, greenbugs (both after starvation and after feeding on host plants), and the honeydew excreted by greenbugs. From this research they concluded that auxins present in aphids were plant-derived rather than synthesized by the aphids and that aphids were effective in removing auxins from the plant sap and concentrating them in the honeydew. They suggested that determination of growth substances present in the salivary glands of aphids would provide information as to whether the toxin in salivary fluids are plant-derived auxins or growth inhibitors in toxic concentrations.

This investigation was undertaken to determine whether or not auxins are involved in the toxic effects of greenbugs feeding on host plants. Two approaches to the problem were utilized. The first involved the use of radioactive greenbugs feeding on susceptible barley and subsequent

assay of labeled indole compounds in the damaged plants. The second was concerned with quantative evaluation of changes in auxin content in susceptible and resistant varities of barley resulting from greenbug feeding.

CHAPTER II

LITERATURE REVIEW

The earlier work on injuries to plants caused by the feeding of aphids was first reviewed by Carter in 1939 (11), and then supplemented by the same author in 1952 (12). In aphids, the mouth parts have evolved and are specialized for the ingestion of a liquid diet. The parts that penetrate into plant tissues are called stylets. As described by Smith (13), the stylet of aphids is formed by four grooves which are arranged in two parts, and the grooves of each pair come together and form two channels. During feeding, saliva flows down along one channel and plant sap flows up the other. Consequently, it has been thought that the plant damage by aphid feeding is caused primarily by the ingestion of plant sap by the aphids. However, recent evidence has shown that toxic substances in the saliva may be responsible for the tissue damage. It was suggested by Schaller (14) that most of the salivary constituents are unutilized dietary products absorbed into the hemolymph and excreted by the salivary glands. Auclair (3) also mentioned that in aphids their excretory functions are probably carried out in part by the gut, especially the rectum, and to some extent, by the salivary glands.

Bradley (15) observed saliva flowing from the tip of the stylets as these progressed through cells. The saliva rapidly sets into a gel which remains within the plant tissue after withdrawal of the stylet.

Sylvester (16) suggested that this sheath participates in the transmission of plant viruses. Marek(17) found several amino acids and amides in aphid saliva and attributed host plant injury to these compounds. Effects on plants (e.g., distortions, galls) attributable to growth hormones such as indoleacetic acid from whole aphid extracts were reported by Nuorteva (18) and Nysterakis (19). Nuorteva (20) also found indoleacetic acid in the salivary glands of nymphs of Stenodema calcaratum Lap. and a substance from the salivary glands of Cinara piceae which inhibited plant growth. Nysterakis (21) reported that root and leaf galls in vines due to Phylloxera feeding are the result of injection of indoleacetic acid into the plant by the insects. Later, the transfer of indoleacetic acid from plant sap to the saliva glands of Stenodema calcaratum Lap, and thence back into the plant was observed by Nuorteva (22). Felt (23) thought that stimulation by insect-injected substances was a fundamental principle in gall formation.

The relationship between insect toxins and plant hormones in the stimulatory development of certain plant tissues on infestation with insects was reviewed by Maxwell and Painter (9). Production of stem galls in sugar cane after injection of macerated leafhoppers, paralled development of insect galls and adventitious buds, and concomitant development of spindling sprout with feeding by Psyllid yellows (gall insect) suggest a relationship between insect, plant host, and plant hormones (auxins).

Various unexplained growth effects often follow the feeding of aphids. Went (24) suggested that aphids might destroy the auxin in the phloem and thus retard growth. Allen (25) reviewed the relationship

between insect damage and plant hormones and suggested that insects may effect a response either by injection or by withdrawal of growth substances. Nysterakis (26) explained differences between healthy plum tree stem and those infested by Aphis helichrysi as being due to indoleacetic acid inoculated by the insects.

The abnormal distribution of plant growth substance caused by parasitic microorganisms has been described by several workers. Excessive levels of indoleacetic acid occurring in that infected plants has been found by Gruen (27). Pate (28) reported the indoleacetic acid is the dominant auxin in pea root nodules and that levels of extractable auxins are much lower in root tissues than in nodules. In a review by Fawcell (29) it was reported that the formation of tumors initiated by the fungus in potatoes resulted in the accumulation of indole auxins in the peripheral fast growing parts, and that the tumor tissue contained more auxin than normal tissue. In the Peronospora-infected plant levels of indole-acetic acid and indoleacetonitrile higher than normally presenthave been reported. Higher auxin content in the extracts from tumor tissue initiated by the crown-gall organism was reported by McDonnell (30). More recently, by using a chromatogram-bioassay technique, Pegg and Selman (31) found that the extracts from leaves and stem of Verticillium infected tomato plants contained approximately twice as much activity in the indoleacetic acid zone as healthy plant tissues.

The injury to oats caused by greenbugs (Toxoptera graminum) has been observed by Wadley (32), who suggested that this was due to the injection of enzyme-like substance by the aphids. The mechanics of feeding of the same aphid on barley, oats, and wheat was studied by Chatters and Schlehuber (10). They found that, upon insertion of the

stylets the greenbugs injected considerable amounts of saliva into the host tissue. The phloem appeared to be the ultimate goal of the stylets in these cereals, although in barley and wheat some feeding occurred in the parenchymatous tissues as well. In carefully prepared sections, it was observed that saliva introduced into the phloem moves along in the phloem elements, altering the cells and their contents as it progresses. A lateral diffusion of the saliva from the phloem appeared to result in an alteration of the cell contents through plasmolysis. In barley a considerable breakdown in the parenchyma tissue was also observed. This indicated that the injected saliva produces plasmolysis in the mesophyll layer.

The effects of greenbug feeding on the free auxin levels in tolerant and susceptible wheat and barley were studied by Maxwell and Painter (9). They found that the free auxins in host plant were removed physically by greenbugs during the feeding process. In another study(7) these authors detected free auxins in the honeydews excreted by greenbugs fed on susceptible barley. Indoleacetic acid, indolepyruvic acid, ethyl-indoleacetate and two unknown growth substances were found. It was suggested that aphids are effective in removing auxins from the plant sap and concentrating them in the honeydews. Maxwell and Painter(8) also detected plant growth hormones in ether extracts of greenbugs fed on susceptible barley. Indoleacetic acid and indolebutyric acid were found in significant amounts. Auxins present in greenbugs were greatly reduced by a short starvation period prior to analysis. This result strongly indicated that auxins in the aphid and aphid honeydews were obtained from the food material (plant sap) in the aphids. It was suggested that these unutilized auxins were concentrated in some

specific area in the aphid such as the salivary glands and excreted in honeydews. No evidence that insects can produce auxins was found.

Attempts by Chatters and Schlehuber (10) to correlate host plant resistance to Toxoptera graminum Rond. with structural differences related to leaf thickness, amounts of mechanical leaf tissue, number of stomata, and stylet feeding sites, were mostly negative. They concluded that on the basis of cytological, entomological, and agronomic observations, the implications are that resistance and susceptibility are expressions of physiological differences. The possible relationship between the tolerance exhibited by certain plants toward the greenbug and auxin content was investigated by Maxwell and Painter(9) who studied changes in auxin levels in tolerant and susceptible varieties (wheat and barley) associated with feeding activity of greenbugs. Their results suggested that auxins may be concerned primarily with the tolerance component of resistance, which is closely associated with the free auxin content of host plants, and the ability of certain aphids to extract and concentrate these growth substances.

Consideration has been given to the literature associated with the plant growth hormones and aphid plant injury. In view of the number of known observations and theoretical suggestions, it seems likely that indole auxins may play an important role in the expression of the effects on host barley by greenbug feeding and in the different resistance exhibited by susceptible and tolerant varieties.

Isolation and Identification of Indole Compounds

Many indole derivatives are active components of the metabolism in plants. It now seems likely that most auxins are indole in nature.

However, reliable techniques for the unambiguous identification of certain indoles in physiological concentration are still lacking.

During the early twentieth century, a plant hormone was isolated by Went (33) using an agar extraction method. Liquid-liquid partition chromatographic methods were employed in auxin research in the 1940's. By using successive extractions with alcohol, chloroform and water, the total extraction of free auxins and auxin precursors from plant tissue was successfully accomplished by Avery et al. (34). During the 1950's indole compounds were usually isolated from plant tissues by extraction with a series of appropriate solvents and subsequently separated by paper chromatography. Quantitative estimation was then accomplished by a chemical method or bioassay. Using paper chromatography in conjunction with a modified Avena coleoptile bioassay method, Maxwell and Painter (7,8,9) detected plant growth hormones in ether extracts of the greenbug, a variety of host plants, and the honeydew of the greenbugs.

Stahl (35) showed excellent separation of twenty simple indole derivatives by two-dimensional chromatography on silica gel G thin layer plates. Silica gel thin-layer chromatographic technique as applied to the separation of auxins from plants was also employed by Bayer (36). Thin layer chromatography on polyamide was used in the separation of indole derivatives by Wang (37).

Silica gel columns were first successfully employed to separate a number of synthetic indole derivatives and naturally occurring plant growth regulators by Powell (39). Later, Powell (40) employed a silica gel column in conjunction with gas-liquid chromatography as a preliminary purification technique. A modification of the spectrophotogluorometric

assay described by Bowman et al. (38) was used to assay submicrogram quantities of indoles.

For effective separation of indole auxins by gas-liquid chromatography, it is necessary to separate them into acidic and neutral indoles. The neutral indoles are volatile and may be chromatographed directly. Dedio and Zalik (41) successfully chromatographed the neutral indoles with silicone substrates, SE-30 or SE-52, columns of 5' x 1/8", 6 1/2' x 1/4", respectively. After esterification by diazomethane or BF_3 catalysis, excellent separation of acid indole esters was also obtained with the silicones, particularly SE-52, on a 5 foot column. Recently, Brook et al. (42) demonstrated that trifluoracetylation of the methyl or ethyl ester derivatives of indole compounds accompanied by gas chromatography and electron capture detections offers a method of detecting these compounds which is about 1,000 times as sensitive as the use of hydrogen flame ionization detection of methylated indoles. They concluded that the detection procedure used for trifluoracetyl derivatives of indoles was advantageous for the determination of minute amounts of certain indole compounds that are auxins or are involved in auxine metabolism.

CHAPTER III

EXPERIMENTAL PROCEDURES

Plant and insect materials

Pure varieties of barley, Hordeum vulgare, were used in this investigation. The variety Rogers was used as a representative of barleys susceptible to greenbug attack while the variety Will was employed as representative of resistant varieties. In most experiments 9-day old seedlings were used as starting material for infestation and/or analytical experiments.

Greenbugs (Schizaphis graminum, Rond.) were supplied by the Department of Entomology, Oklahoma State University, and were the Biotype designated as Biotype B.

Plant growth and infestation procedures

For experiments involving the use of ^{14}C, barley seeds were germinated and planted in small pots. The plants were then grown in a growth chamber made by Percival, Boone, Iowa, at 27°C, 50% relative humidity. On the 9th day the plants were infested with greenbugs and transferred to an all-glass photosynthesis chamber for growth in an atmosphere containing $^{14}CO_2$. For the experiments concerned with the transfer of radioactive materials to "cold" barley plants by radioactive greenbugs, 5 mg of $Ba^{14}CO_3$ with a total activity of 0.15 mc were placed in the side arm of the apparatus. The release of $^{14}CO_2$ was effected by

addition of 10 ml of 1% H_2SO_4 injected by a hypodermic syringe inserted through the rubber cap of the side arm. The apparatus was illuminated at a level of about 1000 ft-c and exposure to the $^{14}CO_2$ was for 5 days. The radioactive greenbugs were then transferred to non-radioactive 9-day old barley seedlings and allowed to feed for 5 days. The bugs were removed and the leaf tissue harvested for subsequent analysis.

The procedure used in the experiment in which the radioactive greenbugs themselves were analysed was the same except that 15 mg of $Ba^{14}CO_3$, with a total activity of 0.45 mc, were used and the exposure period was for only 2 days. After this time the bugs were collected for analysis of radioactive components.

Barley seedlings for all other experimental work were grown in 36 x 48 cm flats under greenhouse conditions (30-32°C, 40-45% relative humidity). In the initial comparisons of auxin levels in infested and uninfested seedlings, Rogers was used. Four flats of 9-day seedlings were infested with greenbugs at a rate of 100 bugs per flat with the aphids distributed as evenly as possible over the flat. Each infested flat was covered with a gauze-covered frame to prevent migration of aphids to uninfested flats. Four uninfested flats of seedlings were grown alongside the infested ones to serve as controls. At intervals of 7, 10, 14 and 20 days after infestation the seedlings of one infested and one control flat were harvested.

For the study of conditions for extraction of auxins from barley leaf tissue, two flats of Will seedlings were grown under greenhouse conditions and harvested at 16 days. This time corresponded to a period of 7 days post-infestation of 9-day old plants.

Four flats each of Rogers and Will barley were planted for the

experiment comparing auxin levels in infested and uninfested seedlings of these two varieties. Two flats of each variety were infested at 9 days as before, and one flat each of the uninfested and infested seedlings of each variety was harvested after 7 days infestation and again after 10 days infestation.

The above-ground parts of the plants (hereafter called leaves) were harvested in all of the above experiments and placed in a freezer at -10°c immediately after cutting and held in the frozen state until analyzed.

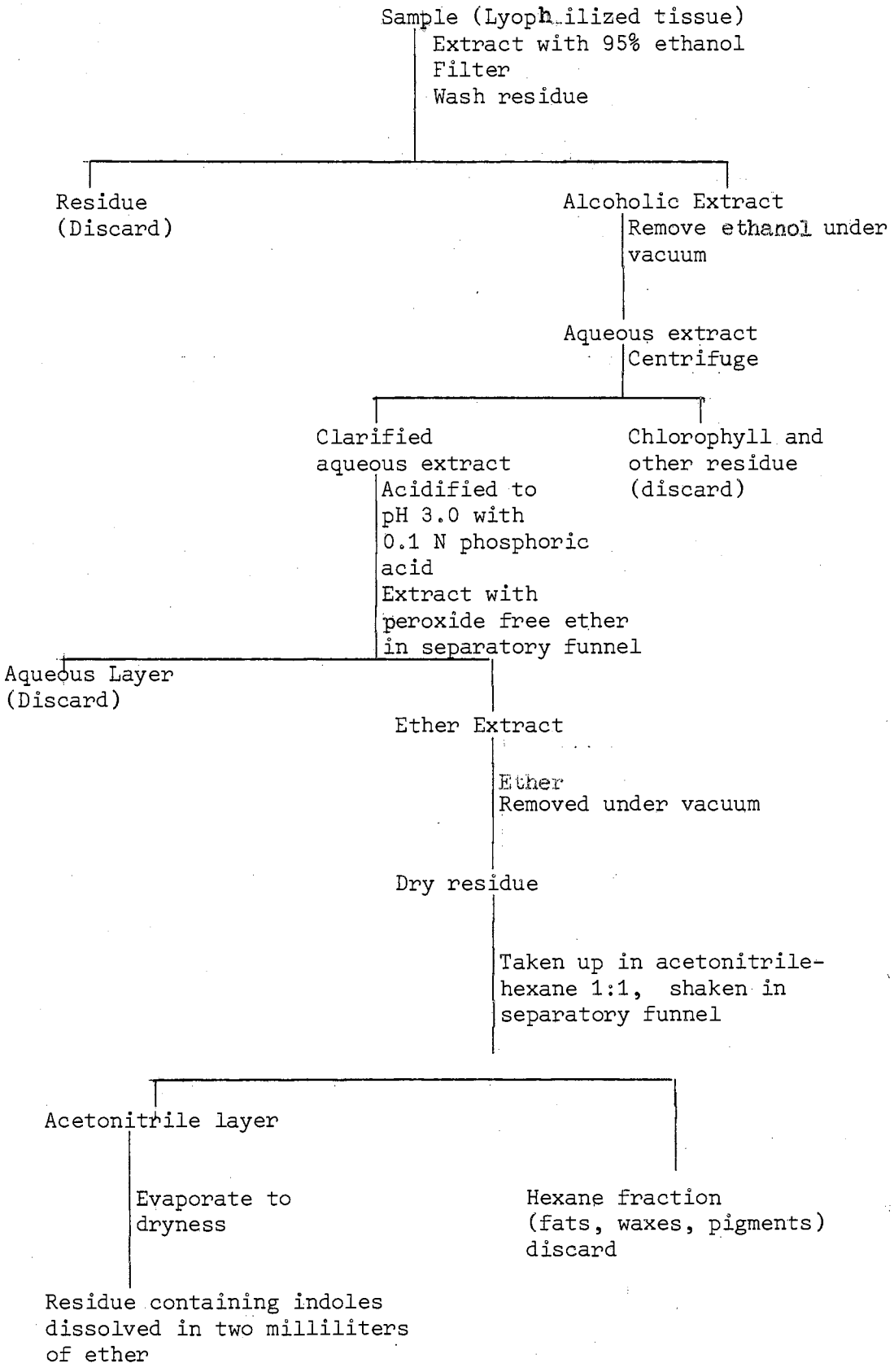
Sample preparation and extraction

In order to facilitate extraction of indole compounds from leaf tissue, all tissue was first homogenized in a Waring blendor in chilled distilled water. The homogenate was then lyophilized and used for subsequent extraction. A constant amount of water was used with varying amounts of leaf tissue ranging from 5 g to 100 g depending upon the particular experiment. All lyophilized tissue was stored in the freezer until used.

The general procedure employed for extraction and partial purification of the indole auxins from barley leaves or greenbugs followed those methods described by Maxwell and Painter (9) and Larsen (43). A schematic diagram of the procedure is shown in Figure 1. The initial extraction was with 95% ethanol rather than absolute ethanol and the extraction times and temperatures were changed in some experiments as described below. After the initial ethanol extraction step, however, the procedure shown was employed for all experiments.

For the extraction of radioactive auxins from greenbugs, 0.86 g of the aphids from radioactive plants was homogenized in 20 ml of 95%

Figure 1 Schematic Diagram of Procedure for Extraction of Indoles from Barley Leaves.


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

ethanol by crushing with a glass stirring rod. The extractions was allowed to continue for 16 hours. The extract was then filtered and the residue washed three times with equal portions of ethanol. Extraction of auxins from the ethanolic extract was then according to the diagram in Figure 1. In the case of the experiments in which radioactive auxins were extracted from barley leaves fed on by labeled greenbugs and also in the first experiments comparing auxin levels in infested and uninfested Rogers barley, weighed amount of the lyophilized leaf tissue was extracted in 95% ethanol at 2°C for 48 hours with occasional shaking. The suspension was then filtered and the residue washed with three equal portions of ethanol, the washings combined with the filtrate, and concentrated as shown in the diagram.

During the course of the investigation a question arose concerning the extractability of "free" and "bound" auxins. Therefore, in one pair of experiments this problem was investigated. For this purpose 400 g of Will barley leaves were harvested at 16 days, homogenized and lyophilized as before. After thorough mixing of the lyophilized tissue, nine 7 g portions were weighed out for subsequent extraction.

For the first extraction experiment, one 7 g sample of lyophilized tissue was extracted with successive 200 ml portions of ethanol for various time periods at room temperature. The extraction periods used for successive extractions were 30 minutes, 60 minutes, 1½ hours, 3 hours, 6 hours, 12 hours, 24 hours, and 48 hours giving cumulative extraction times of 30 min., 90 min., 3 hours, 6 hours, 12 hours, 24 hours, 48 hours, and 72 hours. After each extraction period, the suspension was filtered, the filtrate saved, and the residue further extracted with a fresh portion of ethanol for the indicated time. The

successive extracts were then further extracted according to Figure 1.

In the second extraction experiment eight 7 gram samples of tissue were individually extracted for specific time periods. For the different samples periods of 30 min., 90 min., 3 hours, 6 hours, 12 hours, 24 hours, 48 hours, and 72 hours were employed. Each sample was filtered after the specified time, the residue discarded, and the alcoholic extract further treated according to Figure 1.

On the basis of results of the above extraction experiments, the final experiments concerning comparison of "free" and "bound" indoles in uninfested and infested Rogers and Will barley utilized an initial 3 hour extraction with ethanol at room temperature, filtration of the ethanolic suspension to give a filtrate containing the "free" indoles, and a second extraction of the residue for 45 hours with subsequent filtration to yield an extract containing "bound" indoles. Both extracts were further treated according to Figure 1.

Isolation and assay of indole compounds

During the course of the investigation a variety of methods of isolation and estimation of indole compounds were employed. Some were used because of their particular suitability for a specific experiment while others were improvements of earlier methods.

A. In the experiments involving assay of radioactive indoles, paper chromatography was employed to separate the individual components present in plant or insect extracts. Ascending chromatography on a cylinder of 28 x 30 cm Whatman chromatography paper was the technique used and the solvent system was isopropyl alcohol-ammonia (28%)-water (8:1:1). Six authentic indoles and a

mixture of the six were chromatographed in this system and the location of the individual components determined by spraying with a 2% solution of 0.5M $FeCl_3$ in 5% $HC10_4$. The authentic compounds used were indole-3-acetic acid (IAA), indole-3-Pyruvic Acid (IPyA), indole-3-butyric acid (IBA), indole-3-acetonitrile (IAN), indole-3-propionic acid (IPA), and indole-3-aldehyde (I3H). The average R_f values determined for these compounds were subsequently used to locate the corresponding compounds in barley leaf or greenbug extracts. Leaf or insect extracts were chromatographed in the same system but the position of compounds was located by their ultraviolet fluorescence rather than with the color reagent. The spots were circled and R_f values calculated and compared to the knowns. The spots were then cut out and counted for radioactivity.

Wang and Jones (44) demonstrated that scintillation counting in the presence of paper gave counting efficiencies comparable to those obtained in the absence of paper. Therefore, the activity of auxins in the spot cut from chromatograms was determined directly without elution. Counting was by the liquid scintillation method using the POPOP scintillation system in scintillation grade toluene. A Tri-Carb liquid scintillation spectrometer was employed for this purpose.

B. The initial studies comparing auxin levels in infested and uninfested barley leaves employed a preliminary separation of components by partition chromatography on silica gel followed by analysis of fractions by gas-liquid chromatography.

For the preparation of silica gel columns, silicic acid was thoroughly washed in distilled water, freed of fines, and dried to

constant weight at 100°C. Eight grams of the dried silicic acid was then hydrated in 5.0 ml of 0.5 M formic acid; the hydrated gel was suspended in a small volume of the first eluting solvent and packed under air pressure into a 1.4 x 30 cm glass column. The final gel column was approximately 20 cm in height.

Ether solutions of indoles extracted from leaves or of authentic indoles were pipetted directly on the top of the gel column and allowed to enter the gel. The first eluting solvent was then added and collection of 10 ml fractions at a rate of 2 to 3 ml per minute was begun. A series of four solvents was employed with increasing concentrations of 1-butanol saturated with 0.5M formic acid in hexane. One hundred fifty ml of solvent containing 0.2% butanol was the first eluant. This was followed by 150 ml of 3% butanol eluant, then 100 ml containing 10% butanol, and finally 100 ml of 20% butanol solvent.

The elution volumes for known indoles in this system was determined by chromatography of a mixture of the six authentic indoles described previously. Their position in the elution pattern was determined by the use of the Salkowski reagent as modified by Gordon and Weber (45). This reagent contained 2.7 g $FeCl_3$ per liter of 35% (v/v) perchloric acid. Two ml of reagent was added to 1 ml of eluate and the absorbance at 530 mu read after 30 minutes.

Chromatography of solutions of unknown plant indoles was conducted in the same manner as for the knowns, but treatment with the Salkowski reagent was omitted. Tubes of eluate corresponding to elution volumes for the knowns were pooled, the solvent

evaporated, and the residues were converted to suitable derivatives and then analyzed by gas-liquid chromatography.

GLC Analysis of fractions containing IAN or I3H or IPyA was conducted on trifluoroacetate derivatives of these compounds. IAA, IPA, and IBA were first converted to methyl or ethyl esters by the procedure described by Schlenk and Gellerman (46) and then trifluoracetylated by the method suggested by Brook et al.(42). Analysis was then conducted with these derivatives. A Barber Coleman gas chromatograph equipped with a hydrogen flame ionization detector was employed for these analyses. The columns used and the technique for packing were as described by Horning et al.(47). Particulars of operation are given in the legend of Figure 3. Quantitation of indoles in plant extracts was based on the triangulation method of James and Wheatley (48) for estimation of area under peaks with known amounts of authentic compounds or their derivatives being used to establish the relation between concentration and area under the peak.

C. In the experiment concerned with extraction of indoles for various periods of time, both colorimetric and biological assay procedures were used. The extracted indoles were taken up in 3 ml of ether and separate assays carried out on 1 ml portions. IAA and IPyA were determined together on one aliquot by colorimetric assay using a modified Gordon-Weber Salkowski reagent (1.62g $FeCl_3$ per liter of 35% perchloric acid) with absorbancy readings taken at 530 mμ. For determination of IAN and IBA together, a second aliquot was treated with the nitrous acid reagent described by Wightman (49) and absorbance read at 394 mμ.

Standard curves based on authentic indoles were used to calculate concentrations of these indoles in the unknowns. The sum of indoles by these two colorimetric assays was taken as an approximate total of indoles in the extracts.

The third aliquot of unknown indole solutions was subjected to biological assay for auxin activity by the oat coleoptile test as outlined by Nitsch and Nitsch (50). IAA was used as the standard and results were expressed in terms of ug IAA.

D. The studies involving separation and assay of indole auxins in infested and uninfested Rogers and Will barleys utilized an ion-exchange chromatography system for purification of the individual indoles as a substitute for the silica gel system employed in earlier experiments. Furthermore, specific color reactions for the different indoles were used for assay purposes instead of the time consuming derivatization and gas-liquid chromatography. The ion-exchange procedure was one developed by Chen (personal communication) and utilized DEAE-cellulose as exchanger and formate buffer as eluting solvent. The color reagents employed for assay included the modified Salkowski reagent for IAA and IPyA, the nitrous acid reagent for IBA, IPA, and IAN, and 2,4-dinitrophenylhydrazine for I3H.

DEAE-cellulose was prepared by treating first with 0.5N HC1, washing thoroughly with water, treating with 0.5N NaOH, washed by water and finally packing a large column (5 x 65 cm) and washing with water until the pH of the effluent was 4.0. The washed exchanger was then packed in a 1.4 x 35 cm glass column to give an exchanger column 33 cm high.

Preliminary experiments with authentic indole auxins showed that the neutral indoles, IAN and I3H, were eluted and separated with water as eluant while elution and separation of IAA, IBA, IPA, and IPyA required a linear gradient of formate buffer from 0.02 M (sodium formate) to 0.04 M (sodium formate). The elution order of the knowns were established for use later in identifying unknowns.

Knowns and unknowns were added to the column in 3 ml aqueous solutions. For IAN and I3H 2 ml fractions were collected at an elution rate of 8 ml/hr while 5 ml fractions were collected for the other indoles at a rate of 10 ml/hr. Tubes containing indoles were detected by absorbance measurements at 280 mμ. The fractions containing either IAA or IPyA were then quantitatively assayed by use of the modified Salkowski reagent with absorbance measurements at 530 mμ. Fractions containing IBA, or IAN were quantitated by absorbance measurement at 394 mμ after treatment with the nitrous acid reagent while absorbance at 376 mμ was used for determination of IPA after treatment with this reagent. I3H was assayed by treatment of fractions containing this compound with 2,4-dinitrophenylhydrazine and taking absorbance readings at 550 mμ. For each compound individual standard curves were established with knowns and used to convert absorbancies to μg of indole.

Chemical reagents and meterials

Scintillation grade POPOP (1,4-bis. 2-5 phenyloxazolyl-benezene), purchased from Packard Instrument Company, Inc., Box 428, La Grange, Illinois, was used.

Silica gel columns were prepared from silicic acid (100 Mesh), analytical grade made by Mallinckrodt Chemical works of St. Louis, Missouri.

Gas chrom Q (silanized), 100-120 Meah purchased from Applied Science Laboratories, Philadelphia, Pennsylvania was used as the supporting material for the liquid phase. The stationary liquid phase, SE-30, was also purchased from Applied Science Laboratories.

DEAD-cellulose employed was cellex-D from Bio-Rad Laboratories, Richmond, California.

Authentic indoles: IAN, IBA, IPA, IPyA, I3H were purchased from K & K Laboratories, Plainview, New York. IAA was obtained from Mann Research Laboratories, New York, New York.

Other chemicals (reagent grade) were used without further purification, except that ether was redistilled over ferrous sulfate and calcium oxide in a small amount of water before use.

CHAPTER IV

RESULTS AND DISCUSSION

Transfer of radioactive indoles from greenbugs to host.

Earlier workers suggested that toxic effects resulting from aphid feeding might be due to the removal and concentration of auxins from the plant by the insect followed by re-injection of toxic amounts of these auxins via the saliva. It seemed desirable to determine whether the feeding greenbug actually removed indoles from the barley plant and if so, whether these compounds were transferred back into the plant. Preliminary research employing autoradiography of barley leaves which had been infested with radioactive greenbugs showed that radioactivity was present and was localized in small spots in these leaves, presumably at sites of aphid feeding. The nature of the radioactive materials, however, was not determined. Experiments were, therefore, conducted to ascertain whether indoles were among the labeled compounds present in the greenbugs and in leaves which had been fed upon by radioactive greenbugs. In the experiment in which labeled indoles in greenbugs were assayed, Will barley seedlings were used as the "hot" host plant while Rogers barley was used as host in the experiments concerned with the identification of labeled indoles transferred from greenbug to plant. The results of these experiments are presented in Table I.

These results clearly demonstrate that greenbugs grown on radioactive barley contain radioactive indoles. Since efforts to detect

TABLE I

RADIOACTIVITY DISTRIBUTION OF INDOLE AUXINS IN EXTRACTS FROM INFESTED BARLEY[1] AND GREENBUGS

COMPOUND	EXTRACTS FROM INFESTED BARLEY				EXTRACTS FROM GREENBUGS			
	R_f Value[2]		Radioactivity CPM[2]	Percentage in Labeled Auxin	R_f Value[2]		Radioactivity CPM[2]	Percentage in Labeled Auxin
	Sample	Authentic			Sample	Authentic		
3-Indoleaceto Nitrile (IAN)	0.95	0.95	1,960	36.4	0.97	0.97	8,135	49.2
3-Indolealdehyde (I3H)	0.83	0.83	1,080	20.0	0.86	0.86	3,864	23.4
3-Indoleacetoaldehyde (IAH)	0.61	0.60[3]	375	7.0	----	----	-----	----
3-Indolebutyric Acid (IBA)	0.56	0.55	356	6.6	0.66	0.66	887	5.3
3-Indolepyruvic Acid (IPyA)	0.44	0.44	305	5.7	0.51	0.51	507	3.1
3-Indoleacetic Acid (IAA)	0.38	0.37	604	11.2	0.38	0.57	1,246	7.5
Unknown I	0.27	----	358	6.6	0.34	----	771	4.7
Unknown II	0.16	----	348	6.5	0.18	----	1,100	6.6
Total CPM for Indole Auxins			5,386				16,510	

[1]Average of data from two experiments.

[2]The total indole fraction from 5 g of lyophilized leaf tissue or 0.82 g of greenbugs was chromatographed. Paper chromatography developing system: isopropanol: ammonia: H_2o (8:1:1 v/v); ascending technique. Radioactivity determined by liquid scintillation counting.

[3]Tentative identification by literature R_f value and characteristic ultraviolet fluorescence.

an indole-synthesizing capability in greenbugs have been unsuccessful, it seems reasonable to conclude that auxins present in greenbugs were derived from the host plant. The presence of labeled indoles in non-radioactive plants infested with radioactive greenbugs indicates that these compounds came from the feeding aphids.

Five identifiable indoles (3-indole acetonitrile, IAN; 3-indole aldehyde, I-3-H; 3-indole butyric acid, IBA; 3-indole pyruvic acid, IPyA; 3-indole acetic acid, IAA) were found in both extracts. Two additional components with R_f values of about 0.17 and 0.30 were not identified. However, based on literature R_f values it would appear that these may be 3-indole acetyaspartic acid (R_f 0.18) and 3-indole carboxylic acid (R_f 0.34). In addition the chromatograms of the leaf extracts exhibited a radioactive spot corresponding in R_f and ultraviolet fluorescence characteristics to literature values for 3-indole acetaldehyde. Although the presence of this component in the leaf extracts and its absence in the aphid extracts may reflect a difference between Rogers and Will barleys, this particular finding appears to be of doubtful significance since this compound was not observed in any subsequent experiments.

Although the actual concentrations of labeled indoles were not determined in these experiments, the results show that the relative proportions of the various labeled indoles are of the same order of magnitude for the indoles found in plants infested with radioactive greenbugs as for the indoles present in the greenbugs themselves. This finding would appear to support the suggestions of other authors that the saliva is one route of excretion for the greenbug.

These experiments involving the use of radioactive greenbugs

establish that the feeding aphid transfers labeled compounds to the host plant and that among these compounds are the indole auxins or auxin precursors, presumably obtained from the radioactive host plant fed upon first. The question of whether or not amounts of auxins re-injected into the plant by the feeding greenbug are sufficient to produce toxic effects will require further investigations concerned with assay of auxin levels at feeding sites as compared to normal areas of tissue.

Determination of indole auxins in infested and uninfested Rogers barley.

Earlier work by Maxwell and Painter (9) had indicated a possible relationship in barley and wheat between "free" auxin levels and tolerance to greenbugs, and these investigators reported lower levels of "free" auxins in infested plants than in uninfested plants. It was of interest in the present investigation to determine the effects of infestation of the "total" auxin levels in barley. Auxin levels were determined in infested Rogers barley leaves at various times after infestation and in uninfested leaves taken at corresponding times. For these experiments an extraction time of 48 hours was used to extract "total" auxins in contrast to the 16-hour extraction period employed by Maxwell and Painter for "free" auxins. After preliminary separation by partition chromatography on silica gel, appropriate derivatives of the extracted indoles were analyzed by gas-liquid chromatography. The elution patterns for authentic indoles are shown in Figure **2** while patterns for indoles from infested and uninfested barley leaves are presented in Figure **3**. The calculated amounts of indole auxins present in the barley leaf tissue are shown in Table II. These values must be considered as approximate because of losses during formation of

Figure 2. Gas-Liquid Chromatogram of a Mixture of Trifluoroacetylated Methylated and Ethylated Authentic Indole Derivatives.

[1]Abbreviations see Table II.

[2]Chromatographic Conditions: Column 8 ft. 0.25 in. o.d. coiled glass column packed with 15% S.E. 30 on 60 to 80 mesh, acid washed, silane treated chromosorb W. Injection: 3.5 µl. 230°c for injection port, 180°c for the column oven, and 190°c for the detector. Flow rate, 60 ml/min. Helium was used as carrier gas.

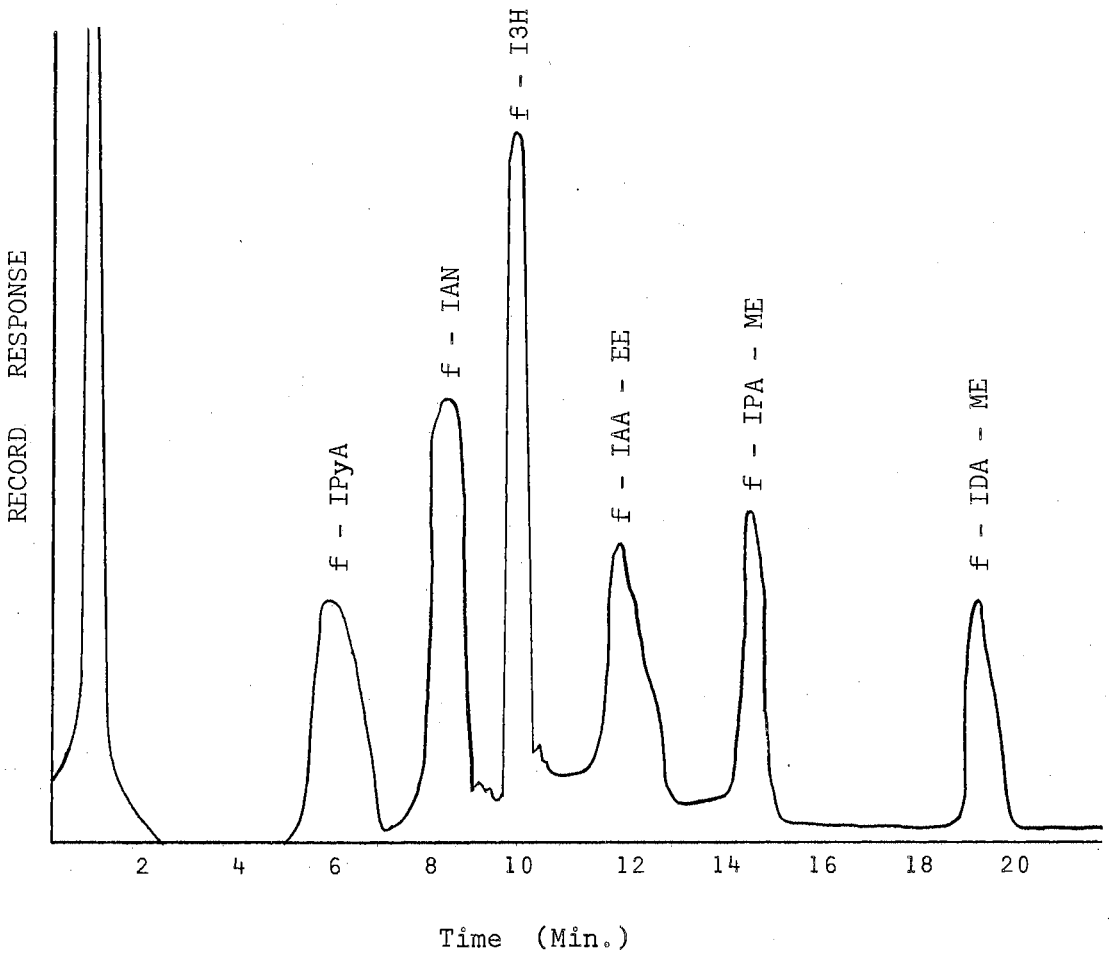

RECORD RESPONSE
f - IPyA
f - IAN
f - I3H
f - IAA - EE
f - IPA - ME
f - IDA - ME
2
4
6
8
10
12
14
16
18
20
Time (Min.)

Figure 3. Gas-Liquid Chromatogram of Trifluoroacetylated Methylated and Ethylated Indoles from Extracts of Uninfested and Infested Barley.

[1]Chromatography conditions, see Figure 2.

[2]Ru_7, Ru_{10}, Ru_{14}, Ru_{20} -- Rogers Uninfested 7, 10, 14 and 20 days control.
RI_7, RI_{10}, RI_{14}, RI_{20} -- Rogers 7, 10, 14 and 20 days after infestation.

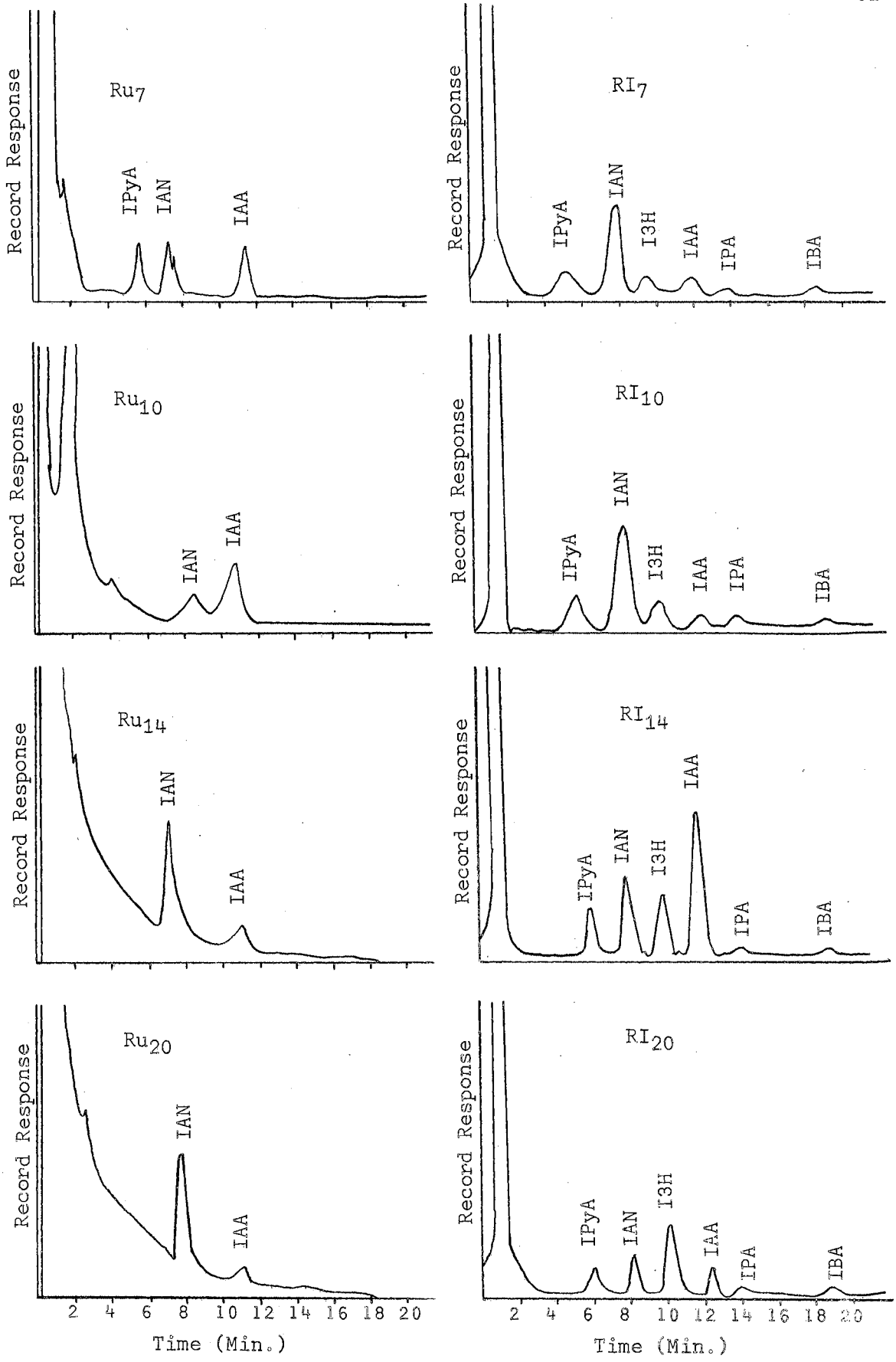

Ru7
IPyA
IAN
IAA
RI7
IPyA
IAN
I3H
IAA
IPA
IBA
Ru10
IAN
IAA
RI10
IPyA
IAN
I3H
IAA
IPA
IBA
Ru14
IAN
IAA
RI14
IPyA
IAN
I3H
IAA
IPA
IBA
Ru20
IAN
IAA
RI20
IPyA
IAN
I3H
IAA
IPA
IBA
Record Response
2 4 6 8 10 12 14 16 18 20
Time (Min.)

TABLE II

TABLE II

TOTAL AUXIN CONTENT OF INFESTED AND UNINFESTED ROGER BARLEY
DETERMINED BY GAS CHROMATOGRAPHY

INDOLE COMPOUNDS	Rt Relative Retention Time to IAN	CONTENT OF INDOLE COMPOUNDS* µg/g LYOPHILIZED TISSUE							
		7 Days		10 Days		14 Days		20 Days	
		U	I	U	I	U	I	U	I
Trifluoroacetylate Derivatives									
Indole-3-Acetonitrile (f-IAN)	1.00	0.52	49.6	0.8	69.6	3.2	38.4	3.7	20.0
Indole-3-Aldehyde (f-I3H)	1.25	----	14.4	---	20.0	---	28.0	---	54.4
Indole-3-Pyruvate (f-IPyA)	0.77	092	68.8	---	65.6	---	53.6	---	26.4
Trifluoroacetylate Methylated or Ethylated Derivatives									
Methylindole-3-Butyrate (f-IBA-ME)	2.37	----	7.2	---	4.6	---	4.6	---	4.8
Methylindole-3-Propionate (f-IPA-ME)	1.75	----	9.6	---	5.0	---	3.0	---	4.8
Ethylindole-3-Acetate (f-IAA-EE)	1.50	1.00	41.6	3.8	6.2	1.7	148.0	2.4	31.2
Total		2.42	191.2	4.6	171.0	4.9	275.6	6.1	141.6

* U and I are uninfested and infested Barley, respectively.

derivatives together with some irreversible adsorption of the derivatives to the supporting medium. Nevertheless, the data clearly show that for the indoles extracted under the described conditions there was a marked increase in indole content in infested tissue as compared to uninfested tissue. IAA, IAN, and IPyA were detected in relatively low quantities in the uninfested samples corresponding to 7 day infestation but IPyA did not appear in the 10, 14 and 20 day uninfested samples. High concentrations of these three indoles were present in the infested samples. I3H, IBA, and IPA, absent from uninfested samples, appeared in appreciable amounts in the infested samples. In general the quantities present in infested samples were highest after 7 or 10 days infestation, but IAA concentration was highest after 14 days.

Extraction of "free" and "bound" indoles.

Because of the marked contrast between the effects of infestation on indole levels observed in this investigation and those reported by Maxwell and Painter, seemed advisable to seek an explanation. These investigators employed about 4 days infestation period, a 16 hour extraction of auxins, and a biological assay for evaluation of quantity of indoles present in their extracts. In the experiments described in the preceding section of the present study the infestation period ranged from 7 to 20 days, a 48 hour extraction was used, and assay was chemical rather than biological. It appeared likely that the differences in extraction tiems might have a marked effect on observed indole levels, since the terms "free" and "bound" auxins, although generally recognized as applied to plants, have been variously and ambiguously defined.

Therefore, two different extraction experiments were performed as described in the "Experimental" section. In order to evaluate the effect of assay procedure on apparent levels of indoles extracted, a biological assay was performed on each extract in addition to the chemical assays. The results of these experiments are shown in the curves of Figure 4. The results of the two types of extraction experiments are essentially the same and show a rapid rise in amount of indoles extracted up to about 3 hours. This is followed by a gradual increase in amount extracted with increasing extraction time up to about 48 hours after which the curve levels off. The results with the oat coleoptile assay parallel those with chemical assay. It would appear, therefore, that extraction for 3 hours would remove the easily extractable or "free" indole compounds while re-extraction of the 3-hour residue for about 45 hours would extract the "bound" indoles. These results do not appear to reconcile the conflict between the effects of infestation described in this study and those reported by earlier workers. On the basis of these results, however, further studies on effects of greenbug infestation employed a 3 hour extraction of "free" indoles followed by a 45 hour extraction of "bound indoles.

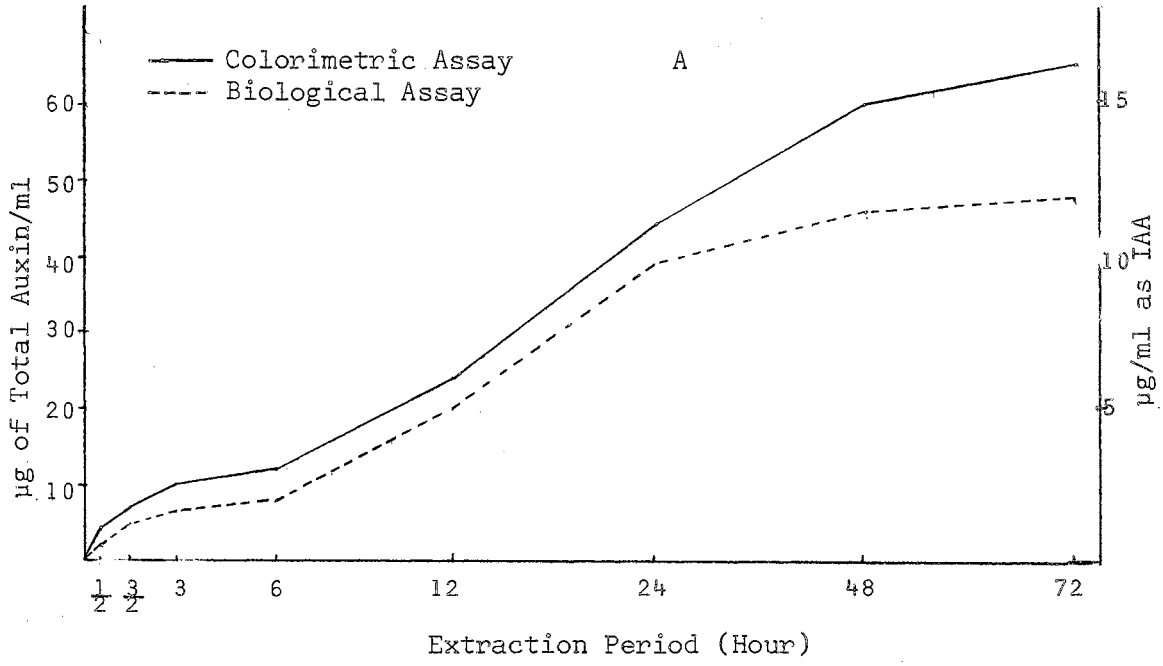
Comparison of effects of greenbug infestation on a susceptible barley (Rogers) and a resistant barley (Will).

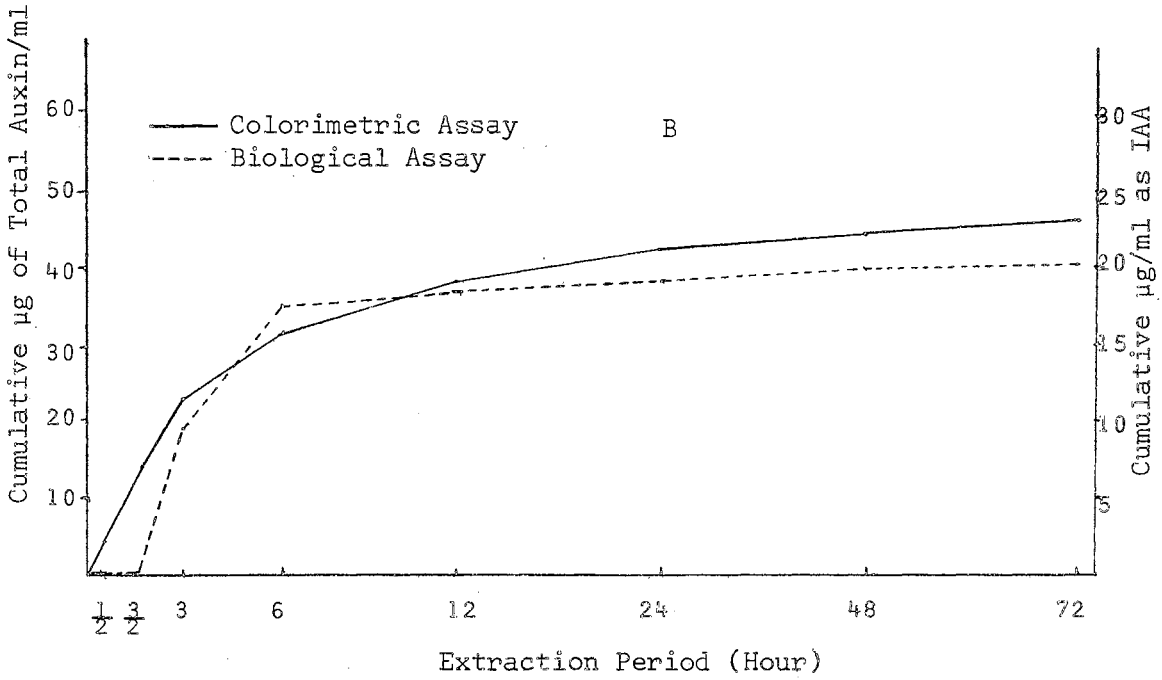
Because of the marked effect of greenbug infestation on indole levels observed earlier for the susceptible variety of barley (Rogers) it was desirable to ascertain if the same effect was produced by infestation of a greenbug-resistant variety (Will). A comparative experiment was, therefore, conducted with these two varieties with

Figure 4. Relation Between Extraction Time and Amount of Indoles Extracted from Lyophilized Barley Tissue.

A - Individual extraction of separate samples.

B - Repeated extraction of single sample. Cumulative plot.


Colorimetric Assay
Biological Assay
A
µg of Total Auxin/ml
µg/ml as IAA
60
50
40
30
20
10
15
10
5
1/2
3/2
3
6
12
24
48
72
Extraction Period (Hour)


Colorimetric Assay
Biological Assay
B
Cumulative µg of Total Auxin/ml
Cumulative µg/ml as IAA
60
50
40
30
20
10
30
25
20
15
10
5
1/2
3/2
3
6
12
24
48
72
Extraction Period (Hour)

results summarized in Table IV and presented graphically in Figure 5. Individual indoles in extracts containing "free" and "bound" indoles were resolved by ion-exchange chromatography on DEAE-cellulose, a method found superior to the silica-gel partition chromatography employed earlier. Three colorimetric assay procedures as described in the "Experimental" section were employed for assay of specific indoles rather than the laborious gas-liquid chromatography procedure requiring conversion to volatile derivatives.

Comparison of indole levels in uninfested Rogers and Will shows that for both time periods Will was slightly higher than Rogers in both "free" and "bound" indoles. For the infested samples both varieties showed a marked increase in total auxins but the increase for Rogers was about twice as great as that for Will, with the result that the total indoles in the infested Rogers was about 1.5 times as great as the total for Will. Consideration of changes in "free" and "bound" indoles in the two varieties reveals that bound indoles increased by about the same degree in both varieties, i.e., about 9 - 10 fold for 7 day infested samples and about 5 fold for 10 day samples. But, the free indoles in infested Rogers increased by about the same degree as the bound indoles whereas in Will the free indoles increased only about 1.5 to 2 times the level of the uninfested samples. Thus, it would appear that while both varieties show a marked increase in total indole levels when subject to greenbug attack, the susceptible variety, Rogers, exhibits a greater increase in indole content than does Will and that the difference in response between the two varieties is chiefly in the greater increase in free indoles in Rogers.

Inspection of the particular indoles present in the "free"

TABLE III

WEIGHT OF LYOPHILIZED TISSUE AND DRY MATTER CONTENTS FOR INFESTED AND UNINFESTED BARLEY

Species of Barley	Weight of Sample Extracted (g)	Percent of Dry Matter	Dry Matter Content (g)	Dry Matter In Fresh Tissue (g)
Wu7 [1]	6.0	90.6	5.436	8.0
WI7	6.0	93.2	5.592	8.3
Ru7	6.0	93.4	5.604	9.1
RI7	6.0	91.2	5.472	9.2
Wu10	6.0	92.1	5.526	8.6
WI10	6.0	98.6	5.916	6.6
Ru10	6.0	91.2	5.472	8.4
RI10	6.0	91.3	5.478	6.7

[1] Wu7, WI7, Ru7, RI7 -- Will uninfested 7 days control, will 7 days after infestation, Roger uninfested 7 days control, Roger 7 days after infestation.
Wu10, WI10, Ru10, RI10 -- Will uninfested 10 days control, will 10 days after infestation, Roger uninfested 10 days control, Roger 10 days after infestation.

TABLE IV

AUXIN CONTENT[1] OF INFESTED AND UNINFESTED ROGER AND WILL BARLEY

Species of Barley	Forms of Auxins	INDIVIDUAL INDOLE AUXINS																		TOTAL			Total (free + bound) µg/g Fresh Tissue
		IAN			I3H			IBA			IPA			IAA			IPyA			µg	µg/g of Dried Matter	µg/g of Fresh Tissue	
		µg	µg/g	µg/g	µg	µg/g	µg/g	µg	µg/g	µg/g	µg	µg/g	µg/g	µg	µg/g	µg/g	µg	µg/g	µg/g				
Wu_7[2]	Free	-----	----	----	-----	----	----	19.0	3.5	0.28	-----	----	----	32.0	5.9	0.47	15.0	2.8	0.22	66.0	12.2	0.98	1.51
	Bound	36.0	6.6	0.53	-----	----	----	-----	----	----	-----	----	----	-----	----	----	-----	----	----	36.0	6.6	0.53	
WI_7	Free	-----	----	----	-----	----	----	103.0	19.0	1.52	-----	----	----	92.0	16.9	1.35	-----	----	----	195.0	34.9	2.89	8.66
	Bound	212.5	37.1	3.16	144.0	25.8	2.40	-----	----	----	-----	----	----	34.0	6.1	0.51	-----	----	----	390.5	69.6	5.77	
Ru_7	Free	-----	----	----	-----	----	----	7.0	1.3	0.18	-----	----	----	29.0	5.2	0.47	10.0	1.8	0.16	46.0	8.2	0.75	1.27
	Bound	32.0	5.7	0.52	-----	----	----	-----	----	----	-----	----	----	-----	----	----	-----	----	----	32.0	5.7	0.52	
RI_7	Free	-----	----	----	-----	----	----	121.0	22.1	2.03	-----	----	----	136.0	24.8	2.29	220.0	40.1	3.70	477.0	86.7	7.98	13.44
	Bound	274.0	50.0	4.61	53.0	9.7	0.89	-----	----	----	-----	----	----	-----	----	----	-----	----	----	327.0	59.4	5.46	
Wu_{10}	Free	-----	----	----	-----	----	----	19.0	3.5	0.29	-----	----	----	39.0	7.3	0.67	16.0	2.9	0.25	74.0	14.1	1.21	2.05
	Bound	40.0	7.2	0.63	11.0	1.9	0.19	-----	----	----	-----	----	----	-----	----	----	-----	----	----	51.0	9.7	0.84	
WI_{10}	Free	39.0	6.6	0.44	-----	----	----	110.0	18.6	1.23	-----	----	----	98.0	16.6	1.09	18.0	3.4	0.21	265.0	44.7	2.95	7.56
	Bound	203.0	34.3	2.26	161.0	27.2	1.80	-----	----	----	-----	----	----	50.0	8.5	0.56	-----	----	----	414.0	69.8	4.61	
Ru_{10}	Free	-----	----	----	-----	----	----	10.5	1.9	0.16	12.0	2.2	0.18	36.0	6.6	0.55	-----	----	----	58.5	10.7	0.90	1.57
	Bound	38.5	7.0	0.59	5.0	0.9	0.08	-----	----	----	-----	----	----	-----	----	----	-----	----	----	43.5	7.9	0.67	
RI_{10}	Free	-----	----	----	-----	----	----	156.5	28.6	1.92	124.0	22.6	1.52	241.0	41.9	2.94	-----	----	----	521.5	95.3	6.39	10.69
	Bound	227.0	41.4	2.78	125.0	22.8	1.53	-----	----	----	-----	----	----	-----	----	----	-----	----	----	352.0	64.2	4.30	

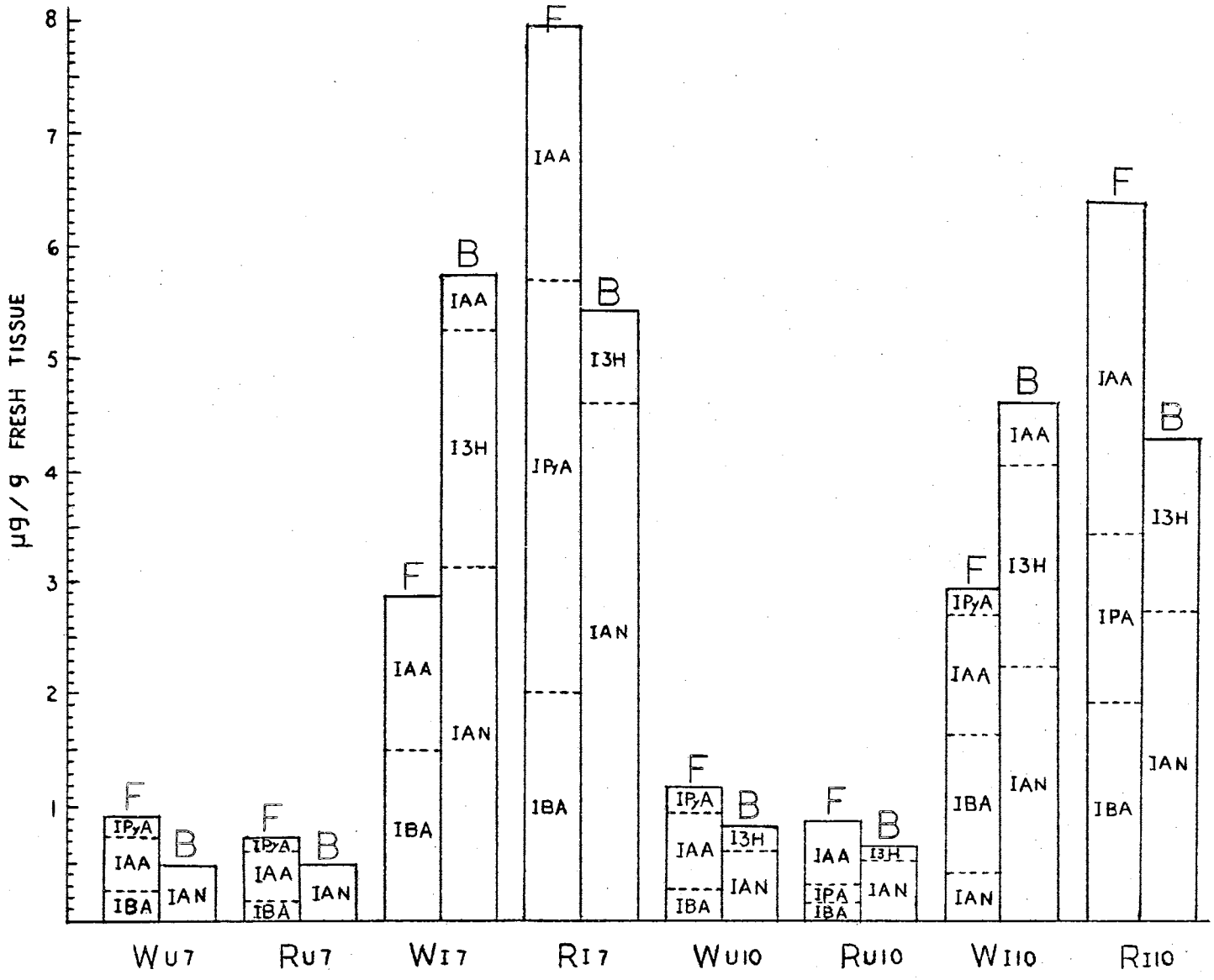
[1]For each individual auxin and for totals, first column is µg of the total amount of auxins detected, second column is µg/gram on dry matter basis, third column is µg/gram of fresh tissue.

[2]Abbreviations, dry matter content and dry matter in fresh tissue: see Table III.

Figure 5. Auxin Content (Fresh Tissue Basis) of Infested And Uninfested Will and Rogers Barleys.

Abbreviations: F - Free Auxin, B - Bound Auxin.

Other abbreviations: Refer to previous Table or Figure.


µg / g FRESH TISSUE
8
7
6
5
4
3
2
1
F
B
IPyA
IAA
IBA
IAN
I3H
WU7
RU7
WI7
RI7
WU10
RU10
WI10
RI10

fraction of Will and Rogers indoles after 7 days infestation shows that while the increase in both IAA and IBA is somewhat greater in Rogers than in Will, the major part of the difference in free indoles is the very high level of IPyA in Rogers. For the corresponding "bound" fractions it is apparent that the major part of the bound indoles in Rogers is IAN with a relatively small amount of I3H. In Will barley these two indoles are present in approximately equal amounts with a low level of IAA also present.

The data for the 10 day infested samples of Will show a general similarity to those for the 7 day samples although the level of indoles is slightly lower. But, although the general picture with regards to amounts of free and bound indoles in the 10 day Rogers samples agrees with the 7 day data for this variety, there is a major discrepancy with regard to one of the indoles present. In the 7 day infested Rogers "free" indoles, IPyA was a major component while in the 10 day sample this indole was absent and IPA was present instead. Although the elution position of IPA and the color tests and chromatographic evidence indicates that this indole was, indeed, present, it was not present in appreciable quantity in the earlier experiment with Rogers. It seems unlikely that all of the IPyA present at 7 days would be converted to IPA at 10 days. Therefore, the actual identification of this particular indole in the 10 day Rogers infested sample must be considered questionable. This point should be checked again in future work.

The results of this investigation clearly show that infestation of barley by greenbugs resulted in marked increases in the levels of auxins or auxin precursors. This occurred in both the susceptible and the resistant varieties of barley. The explanation for these increases is

not clear at present; but at least two mechanisms, functioning alone or together, are possible. The high auxin levels in infested plants may be the result of either increased production or decreased destruction of auxins by the plant or a combination of both. Removal of indoles by the greenbug during feeding and excretion in the honeydew as reported by Maxwell and Painter (8) could stimulate production of these compounds by the plant. However, whether this occurs or not, in order for levels of indoles to increase beyond normal concentrations, a derangement of the control mechanism must occur. It would appear that the aphid must secrete via the saliva some substance which affects the control mechanism. This substance might affect (increase) auxin synthesis by inactivation of inhibitors controlling the synthesis of enzymes involved in indole production. On the other hand the normal degradative pathways for auxin destruction in the plant might be disrupted by inactivation of one or more enzymes involved in these reactions. The latter mechanism has been described in a review by Greun (51) as responsible for increased levels of auxins in fungus-infected plants. The question of which of these mechanisms is involved in the observed increase in auxin levels in infested barley cannot be answered at this time.

It is well known that, while normal plant growth reactions are generally stimulated by low concentrations of auxins, they are inhibited by high concentrations. Marinos (52) showed that exposure of Avena sections to high concentrations of IAA resulted in cell shrinkage together with movement of water and solid constituents out of the cells. He suggested that, since the immediate effect of supra-optimal concentrations of auxin was a stimulation of elongation, cell damage was the result of damage to the cytoplasmic membrane rather than inhibition

of growth. Bennet and Clark (53) explained the progressive toxicity of high auxin concentrations in plants as being the result of increasing solubilization of auxin molecules in the tonoplast, a bimolecular layer of fatty substances. This would result in a progressive reduction of the water-holding capacity of the tonoplast and increased permeability. The eventual result is collapse of the cell due to loss of water. It would appear that such a mechanism would account for the yellowing and stunting observed in infested barley and would explain the mode of cell damage observed by Chatters and Schlehuber (10).

Both susceptible and resistant varieties of barley show damage symptoms when subjected to greenbug infestation. However, the extent of damage and severity of symptoms are much less in resistant varieties. Since both Will and Rogers showed about the same increase in the level of bound indoles when infested, the difference in resistance cannot be associated with this fraction of the indoles. The levels of free indoles, however, are quite different in infested Will and infested Rogers; Rogers contained approximately twice the amount of free indoles as Will. If differences in resistance can be attributed to indoles, these differences must be associated with the free indole levels. It seems reasonable to expect that the easily extractable or "free" indoles would be more readily transported throughout the leaf tissue and would be more effective in causing tissue damage than the bound indoles. If this is true, Rogers would be expected to exhibit more severe damage than Will.

The information revealed by this investigation demonstrates that the metabolism of the barley plant is deranged by greenbug infestation with a resulting increase in levels of indole compounds. Results with

resistant and susceptible varieties suggest that resistance may be, at least in part, associated with the level of free indoles produced as a result of infestation. Further research employing shorter infestation periods and involving analysis of infested parts of leaves rather than whole leaves may provide more conclusive information concerning the role of indoles in resistance of barley to greenbug attack. In addition, effort should be made to identify the factor or factors secreted by the greenbug which are responsible for the observed effects on indole levels in infested plants.

SUMMARY

The results of the present investigation are consistent with the suggestion by Chatter and Schlehuber (10) that the injection of saliva and not the uptake of food appears to be the primary cause of tissue damage to the host plants by greenbug feeding. Marked increases of the auxins or auxin precursors levels in the greenbug-infested susceptible and resistant barleys was observed. The high auxin levels in infested plants may be the result of either increased production or decreased destruction of auxins by the plant or a combination of both. Both susceptible and resistant varieties of barley show damage symptoms when subjected to greenbug infestation. However, the extent of damage and severity of symptoms are much less in resistant variety. If differences in resistance can be attributed to indoles, these differences must be associated with the free auxin levels, because no significant difference in bound auxin level between susceptible and resistant barley was found. A mechanism to explain the progressive toxicity of high auxin concentrations in plants was suggested to account for the yellowing and stunting of infested barley and would also explain the mode of cell damage observed by Chatters and Schlehuber (10).

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