present. It is suggested that a low temperature procedure, such as the one proposed by Yee and Davis (*5*) be considered.

The natural organics contribute significantly to the apparent ammoniacal nitrogen. It is not known whether ammoniacal nitrogen actually exists in these products or is liberated during the analytical distillation. The above error is partially offset by the tendency for part of the ammoniacal nitrogen in ammoniated superphosphates not to appear as such.

Inspection of all the results shows that, because of errors in preparation, sampling, or analysis, results for any particular form of nitrogen will frequently differ from the calculated ones by 0.2% and more.

Acknowledgment

The authors wish to acknowledge the help given by A. W. Redding, who prepared the mixtures described in Table II and supervised much of the analytical work.

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BIOCHEMICAL CHANGES IN PLANT DISEASE

Effect of *Fusarium Oxysporum f. Lycopersici* and Its Metabolites on Leaf Constituents of Susceptible and Resistant Tomatoes

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Extracts from leaves of inoculated and uninoculated tomato plants of varieties susceptible or resistant to Fusarium wilt and of cuttings from plants treated with pectic enzymes, fusaric acid, and lycomarasmin solutions were examined by paper chromatography and compared as to relative amounts of free amino acids, sugars, some acidic components, and phenols present. Healthy plants of both varieties were very similar in respect to these constituents. Following infection, a great number of components changed in concentration in plants of the susceptible variety; little or no change was observed in resistant plants. Many changes were nonspecific and secondary because of dehydration of the tissue by the wilting agents. In contrast, some were specifically induced by the primary action of the parasite or its metabolites. Fusaric acid produced changes characteristic of those in inoculated susceptible plants. An acidic component of the host tissue, obviously an organic phosphate, was affected in a way that suggests it is related to resistance.

Plant diseases are commonly recognized by the characteristic morphological or cytological symptoms associated with them. These symptoms are a reflection of changes in the chemical and biochemical constituents of the plant cells, arising from the interaction of the parasite and the host. This interaction must result in changes in many different cell constituents. Some of these changes cause visible symptoms; other changes must occur which cannot be so readily seen. It is therefore of interest to investigate the biochemical symptoms of infection. The development of paper chromatography has provided a convenient tool for studying such changes in a number of plant constituents and relating these changes to the disease syndrome.

Pectolytic enzymes (*6, 12*), as well as the toxic metabolites fusaric acid (*2*) and lycomarasmin (*4*) produced by *Fusarium oxysporum f. lycopersici* (Sacc.) Snyder and Hansen, play important roles in the development of tomato wilt. The purpose of the present investigation was to determine by paper chromatographic methods changes in leaf constituents of inoculated and uninoculated tomato plants and in cuttings from such plants exposed to the enzyme and toxin preparations.

Materials and Methods

Plants of the susceptible variety Bonny Best and the monogenic resistant variety Jefferson were used. Seeds were sown in vermiculite and at 8 days uniform seedlings were transplanted singly to sand in 4-inch clay pots. At 20 days after transplanting uniform plants of both varieties were inoculated by the root-dipping technique (*13*) with an 8-day still culture of the pathogen grown on modified Richard's medium. Control plants were similarly treated, except that the roots were not dipped in the inoculum. Plants in vermiculite and in sand were watered on alternate days with Hoagland's nutrient solution and distilled water, respectively. The plants were grown during April and May 1957 in a greenhouse at Madison, Wis., which was maintained at approximately 28° C. Water and nutrient solution were allowed to come to this temperature before use. The inoculated, susceptible plants showed severe stunting and distortion of young leaves at 12 days, while the only symptom in resistant plants was a slight stunting.

Cuttings, which included the four uppermost leaves of the plant, were made on the twelfth day after inoculation from certain of the healthy plants of both varieties and were divided into the several treatment groups. Twenty-five plants were used in each treatment group.

Pectinase Treatment (bP, jP). The stems of cuttings were placed in 70% ethanol for 2 minutes and the ends were removed with a sterile razor blade. The cuttings were then placed in Erlenmeyer flasks containing a 0.5% solution of pectolytic enzymes prepared from a culture of the pathogen by S. S. Gothos-

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Table I. Treatments of Resistant and Susceptible Tomato Plants Prior to Sampling of Leaf Tissue for Paper Chromatographic Assay

Source of Leaf Tissue	Treatment of Plants of Cuttings Previous to Harvesting	Text Symbol of Leaf Tissue	
		Bonny Best, susceptible	Jefferson, resistant
Noninoculated plants	None	BH	JH
Inoculated plants	None	BI	JI
Cuttings from uninoculated plants	Nutrient solution	bH	jH
Cuttings from inoculated plants	Nutrient solution	bI	jI
Cuttings from healthy plants	Pectolytic enzymes	bP	jP
	Fusaric acid	bF	jF
	Lycomarasmin	bL	jL
	Water shortage	bW	jW

kar according to his method (*5*), and sterilized by passing through a Seitz filter. The cuttings were held in place by cotton plugs and the ends were removed twice daily. They were treated for 2 days, during which period the original enzyme solution was replaced three times. The leaf tissues from treated susceptible and resistant plants were labeled by symbols bP and jP, respectively (Table I).

Fusaric Acid Treatment (bF, jF). Cuttings were weighed to the nearest 0.1 gram and placed in a 5×10^{-3} *M* solution of the toxin at pH 6.5 (*3*). They were allowed to take up the solution until each cutting had received 225 mg. of the toxin per kg. of fresh weight. They were then removed to Hoagland's solution for the remainder of an over-all treatment period of 48 hours. The leaf tissues from susceptible and resistant plants were labeled bP and jP, respectively (Table I).

Lycomarasmin Treatment (bL, jL). Cuttings were weighed and placed in a 5×10^{-3} *M* solution of lycomarasmin at pH 6.5. They were allowed to take up 300 mg. of the toxin per kg. of fresh weight of cutting and were then removed to Hoagland's solution for the remainder of an over-all 48-hour treatment period. The leaf tissues of susceptible and resistant plants were labeled bL and jL, respectively (Table I).

Water Shortage Treatment (bW, jW). Because the cuttings in the previously described three treatments wilted within 48 hours, a control series was included in which cuttings were allowed to wilt as a result of water shortage only. Cuttings were placed in flasks containing just enough Hoagland's solution to keep the cut surface moist, but not enough to maintain turgor in the leaves. These cuttings had wilted at the end of the 48-hour period. The leaf tissues of susceptible and resistant plants from this treatment were labeled bW and jW, respectively (Table I).

Nutrient Solution Treatment (bH, bI, jH, jI). Cuttings from uninoculated and inoculated plants were made 12 days after the time of inoculation and placed in Hoagland's solution for 48 hours. The cuttings in each of the first four treatments listed above had wilted at the end of the 48-hour treatment period. By contrast the cuttings from uninoculated plants and from the inoculated, resistant plants remained turgid in Hoagland's solution. The inoculated, susceptible plants, although showing signs of wilt when made, recovered in Hoagland's solution and at the end of the 48-hour treatment period were completely turgid. The leaf tissues from susceptible and resistant uninoculated plants in this series were labeled bH and jH, respectively; those from susceptible and resistant inoculated plants were labeled bI and jI, respectively (Table I).

Untreated Plants (BH, BI, JH, JI). On the fourteenth day after the time of inoculation, leaf tissues were removed from uninoculated and inoculated potted plants of both varieties. The leaf tissues from susceptible and resistant uninoculated plants were labeled BH and JH, respectively; those from susceptible and resistant inoculated plants were labeled BI and JI, respectively (Table I).

Harvest, Preservation, and Preparation of Leaf Tissue for Analysis

After the 48-hour period of the various treatments the four uppermost leaves of plants in each group were removed between 10 A.M. and 3 P.M. and plunged immediately into liquid air. The tissue was then transferred to dry ice and stored at −25° C. for future use. The frozen material was ground in a mortar with solid carbon dioxide to a fine powder and then freeze-dried. The freeze-dried material was stored in the dark under refrigeration in a desiccator containing phosphorus pentoxide in an atmosphere of nitrogen. Six hundred milligrams of each freeze dried sample was refluxed for 4 hours with 100 ml. of acetone-water (1 to 1) mixture at 60° C. according to the method of Rohringer (*9*). Following extraction each sample was filtered through Hexagon brand filter paper (Schaar and Co.), the residue was washed with 50 ml. of acetone-water (1 to 1) mixture, and the extracts were stored under refrigeration, until they were concentrated to dryness in vacuo at approximately 40° C. Each residue was then taken up with 3 ml. of acetone-water (1 to 1) mixture, glass beads being used to aid in formation of solution or suspension. The insoluble material was removed by centrifugation in the cold and the supernatant liquid was stored at −25° C., until used for chromatographic analysis on paper.

Paper Chromatography of Extracts. All chromatograms were run ascending on 28.5 × 28.5 cm. Whatman No. 1 filter paper in cylindrical glass jars at approximately 20° C. Amino acids were chromatographed one-dimensionally in *sec*-butyl alcohol–formic acid–water mixture (75–13–12). After the first run in this solvent system (*11*), the papers were air-dried and run a second time in this solvent system in the same direction. Two-dimensional chromatograms of amino acids were run twice in *sec*-butyl alcohol–formic acid–water mixture (75–13–12) as the first solvent system and in citrate-phosphate buffered phenol as the second solvent (*11*). For detection of the amino acids the papers were sprayed with 0.2% ninhydrin in a mixture of *n*-butyl alcohol–2*N* acetic acid (19–1).

The sugars were run one-dimensionally with citrate-phosphate buffered phenol (*11*). Glucose, fructose, and other reducing sugars were detected with aniline-phthalate spray (*7*). Sucrose and other ketohexose-containing

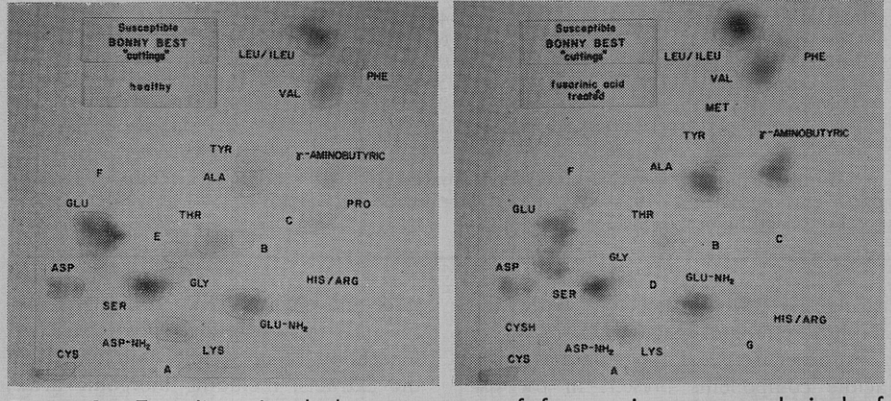

Figure 1. Two-dimensional chromatograms of free amino compounds in leaf extracts of healthy tomato cuttings and cuttings treated with fusaric acid

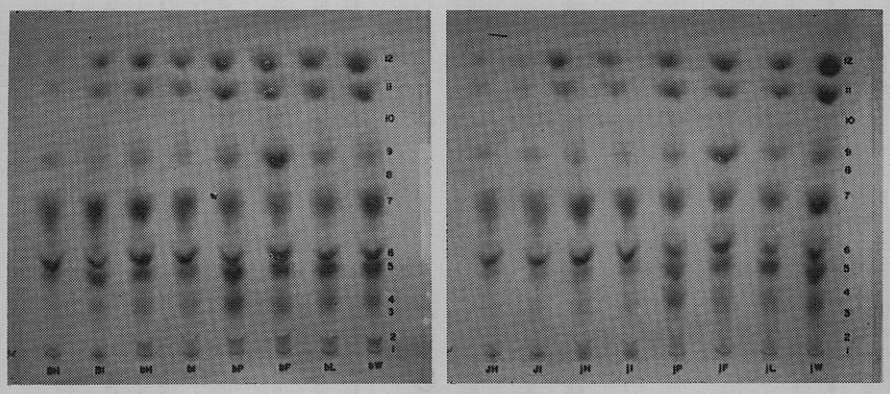

Figure 2. One dimensional chromatograms of free amino compounds in leaf extracts of healthy and Fusarium infected susceptible (left) and resistant (right) tomato plants and of cuttings treated with various wilting agents, listed in Table I

Identity of components

1. Cystine
2. A
3. Cystine, lysine, G
4. Asparagine
5. Glutamine, histidine-arginine
6. Aspartic acid, serine, glycine
7. Glutamic acid, threonine, B, D, E
8. C, F
9. Tyrosine, alanine, proline, γ-aminobutyric acid
10. Methionine
11. Valine, phenylalanine
12. Leucine-isoleucine

Table II. Free Amino Acids and Other Ninhydrin-Positive Compounds in Tomato Leaf Tissue of Susceptible and Resistant Plants

(Following infection with *Fusarium oxysporum f. lycopersici* or treatment with pectinase, fusaric acid, and lycomarasmin)

	Susceptible Variety, Bonny Best[a]								*Resistant Variety, Jefferson*[a]							
	Whole Plants		*Cuttings*						*Whole Plants*		*Cuttings*					
Amino Acid	*BH*	*BI*	*bH*	*bI*	*bP*	*bF*	*bL*	*bW*	*JH*	*JI*	*jH*	*jI*	*jP*	*jF*	*jL*	*jW*[b]
Group I																
Asparagine	2	4	4	4	6	5	5	5	2	2	3	3	6	5	4	5
Glutamine	4	5	4	4	6	6	6	6	3	3	4	4	5	5	5	5
Leucine-isoleucine	2	4	5	5	6	6	5	6	3	3	5	5	6	6	6	6
Methionine	0	1	0	1	1	1	1	1	0	0	0	0	1	1	1	1
Phenylalanine	1	2	2	2	2	3	2	3	1	1	2	2	2	3	2	3
Valine	2	4	4	4	6	5	5	5	2	2	4	4	5	5	5	5
Cystine	0	0	0	0	1	1	1	1	0	0	0	0	2	1	1	2
C	1	2	2	2	2	2	2	2	1	1	1	1	2	2	2	2
D	2	0	0	1	0	1	1	1	1	1	0	0	0	0	0	0
E	0	1	1	1	0	0	0	0	0	0	0	0	1	1	1	1
G	0	1	0	0	1	1	1	1	0	0	1	1	1	2	1	3
Serine	4	4	6	4	4	4	4	5	4	4	6	5	5	5	5	6
Group 2																
Alanine	3	2	3	3	3	6	4	4	3	3	3	3	3	6	3	4
γ-Aminobutyric	1	2	2	1	3	6	3	1	1	1	1	1	3	6	3	1
Histidine-arginine	0	0	2	2	2	3	2	2	0	0	1	1	0	3	1	2
Lysine	1	1	1	1	1	2	1	1	0	0	1	1	1	2	1	1
F	0	2	2	1	2	3	2	2	0	0	1	1	1	2	1	2
Group 3																
Cystine	1	0	2	1	2	2	2	2	1	1	1	1	2	3	1	1
Glycine	2	3	2	3	2	2	2	2	2	2	1	1	1	1	1	1
Group 4																
Aspartic	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Glutamic	5	6	6	6	6	6	5	6	6	6	6	6	6	6	6	6
Proline	0	2	1	1	1	0	0	1	0	0	0	0	1	0	0	1
Threonine	2	2	3	2	2	3	2	3	2	2	3	3	2	3	3	3
Tyrosine	0	2	2	2	2	3	2	3	0	0	1	1	2	2	1	3
A	1	0	2	1	2	3	3	3	1	1	2	2	2	1	1	2
B	1	0	1	0	1	2	1	1	1	1	1	1	1	1	1	1

[a] Numbers indicate relative amounts estimated from size and color intensity of spots on paper chromatograms. 0 = no spot, 1 to 6 = increasing amounts.
[b] For decoding of symbols see Table I.

saccharides were detected with the naphthoresorcinol reagent (*1*).

In order to reveal and separate all the acidic components present in the extracts, chromatograms were run according to the method described by Jermstad and Jensen (*8*). Although nonspecific, this technique gives excellent separation of all acidic components in the extracts, including inorganic acids, organic phosphates, and acids of the tricarboxylic acid cycle.

The phenolic plant constituents were separated on chromatograms run in *n*-butyl alcohol–acetic acid–water mixture (4–1–5) with diazotized sulfanilic acid used as the spraying reagent (*1*).

The relative amount of each of the constituents under investigation was determined by estimation of the comparative size and intensity of the spots obtained. Only the concentration of malic acid was determined quantitatively (*7*). The relative amount of the amino acids was determined by evaluating the corresponding two-dimensional chromatograms where all ninhydrin positive compounds were separated, except for leucine-isoleucine and histidine-arginine.

Experimental Results

Amino Acids and Other Ninhydrin-Positive Compounds. The paper chromatographical analysis revealed 26 different ninhydrin-positive compounds, 19 of which could be identified as amino acids or amino acid amides. As an example of the separation achieved in the analysis, two two-dimensional chromatograms are shown in Figure 1. The distribution of these components in the different samples as illustrated in Table II and Figure 2 shows that they can be divided into four different categories. The first group consists of asparagine, glutamine, leucine-isoleucine, methionine, phenylalanine, valine, cystine, serine, and the unidentified substances indicated as C, D, E, and G. The changes observed with these components also appear in the water shortage treatment (bW, jW), indicating that these changes are entirely or partially nonspecific, being produced secondarily by the dehydration of the tissue. The fact that all those changes were observed in the leaf tissues of susceptible as well as of the resistant plants renders it even more likely that they are nonspecific in nature and that they might not have anything to do with the primary action of the parasite or of any functions governing resistance.

The second category (group 2) includes alanine, γ-aminobutyric, histidine-arginine, lysine, and F. Each of these compounds showed a specific and in some cases a pronounced increase on treatment of the plants with fusaric acid (bF, jF). This change in concentration again appeared in both varieties and therefore cannot be related to disease resistance. Likewise this fusaric acid action could not be detected at all, or significantly so, in infected plants of either variety. Therefore, if fusaric acid plays a role in the disease development, this action on the group of substances in question cannot be demonstrated in leaf tissue of Fusarium infected plants.

Glycine and cystine constitute the third category (group 3). The former

showed an increase following infection in the susceptible variety (BI, bI), whereas the amount of cystine seemed to decrease slightly in the same instance. Neither of these changes could be reproduced or duplicated by any one of the four different treatments. In the resistant variety, however, pectinase treatment (jP) and especially fusaric acid treatment (jF) caused a substantial increase of cystine.

The fourth group consists of aspartic, glutamic, proline, threonine, tyrosine, and the nonidentified compounds A and B. These compounds either did not show any major change or the change observed could not readily be explained or classified. However, tyrosine was detected only in cuttings or plants the vascular systems of which were infected by the fungus. Tyrosine was not found in BH, JH, and JI. Leucine-isoleucine, valine, and phenylalanine, incidentally, showed the same tendency, although in a lesser degree. It can be theorized that the amount of these substances present in the leaf tissue is at least partially affected by the functional capacity and integrity of the vascular system of the entire plant, and that any plugging or occlusion of the vessels or the removal of the root system results in an accumulation of these components in the leaf tissue.

Sugars. In the extracts under investigation, glucose, fructose, and sucrose were detected, and to a lesser amount, two nonidentified sugars or sugar derivatives, K_x and S_x (Table III). Because the substance designated as K_x appeared on the chromatograms only when sprayed with the naphthoresorcinol–trichloroacetic acid reagent, the component either is a ketohexose (other than fructose) or a compound liberating a ketohexose on hydrolysis with the spraying reagent.

Glucose and fructose decreased markedly following infection in the susceptible variety (BI, bI). This decrease is partially nonspecific, because the water-shortage treatment (bW) also resulted in a decrease in these sugars. Fusaric acid treatment (bF), however, seemed to accentuate this change considerably, suggesting that part of the observed decrease in infected susceptible plants might be brought about by the action of this toxin. The results show further that sucrose, too, decreased following infection of the susceptible variety. However, this was true only in leaf tissue of whole plants (BI) and the opposite was true for cuttings of infected plants of the same variety (bI). Treatment of Bonny Best cuttings with fusaric acid also resulted in an increase in sucrose.

The resistant variety, Jefferson, did not show any changes in the sugar content of the leaves of whole plants (JI) and only minor changes in infected cuttings (jI). Cuttings of this variety when wilted by any of the treatments, in most instances, showed a decrease in the sugar content, the decrease in sucrose seemingly being counteracted by fusaric acid treatment (jF).

Carbonic Acids and Other Acidic Compounds. The acid chromatograms revealed eight different spots, two of which definitely could be identified as malic and citric acid (Figure 3). Spot b_1 travels with the same R_f value as sulfate and chloride in the solvent system used. It is believed to be an organic phosphate, because spraying the chromatograms with molybdate (*10*) revealed a distinct spot at the same position on the paper. The spot designated as c_2 was shared by aspartic, glutamic, tartaric acid, and inorganic phosphate. Most of the changes observed here, however, must be attributed to changes in inorganic phosphate. This was established further by running corresponding phosphate chromatograms in the same and different solvent systems.

The results in Table III show that the susceptible variety, Bonny Best (BH, BI) contained almost double the amount of malic acid found in plants of the resistant variety, Jefferson (JH, JI). Cuttings of either variety contained substantially less malic acid than the leaf tissue of the corresponding whole plants. There is a tendency for malic acid to increase in concentration after infection in the susceptible variety (BI, bI). This change apparently is nonspecific, since the water-shortage treatment shows the same trend.

Table III. Sugars, Some Acidic Compounds, and Phenols in Tomato Leaf Tissue of Susceptible and Resistant Plants

(Following infection with *Fusarium oxysporum f. lycopersici* or treatment with pectinase, fusaric acid, and lycomarasmin)

	Susceptible Variety, Bonny Best[a]								*Resistant Variety, Jefferson*[a]							
	Whole Plants		*Cuttings*						*Whole Plants*		*Cuttings*					
	BH	*BI*	*bH*	*bI*	*bP*	*bF*	*bL*	*bW*	*JH*	*JI*	*jH*	*jI*	*jP*	*jF*	*jL*	*jW*[b]
Sugars																
Glucose	6	4	5	3	3	2	3	4	6	6	4	5	2	3	4	2
Fructose	5	4	4	3	3	2	3	3	5	5	3	4	2	2	3	2
Sucrose	4	3	2	3	1	3	1	1	4	4	3	3	1	3	2	1
K_x	1	1	2	2	2	2	2	2	1	1	2	2	2	2	2	2
S_x	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
Acidic compounds																
a	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
b_1	6	1	4	0	5	0	6	5	6	6	5	5	1	2	2	2
b_2	4	1	4	4	4	4	4	4	4	4	4	4	4	2	4	2
c_1	1	1	1	1	1	1	1	1	0	0	0	0	5	5	5	5
c_1	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0
c_2	1	2	1	1	1	1	1	1	1	1	1	1	5	5	5	5
Malic[c]	43	44	12	19	13	8	9	17	26	25	10	16	11	10	14	11
Citric	0	3	0	1	1	1	0	1	0	0	1	1	2	2	2	2
Phenols																
1	1	3	1	3	2	1	2	1	3	3	3	3	1	1	1	2
2	3	4	1	3	2	1	2	1	3	3	2	2	1	0	1	0
3	1	3	1	2	2	3	2	1	0	0	1	1	2	3	2	1
4	0	2	1	1	2	1	2	1	0	0	1	1	2	1	2	1
5	0	3	1	1	3	2	2	1	0	0	1	1	3	2	3	3

[a] Numbers indicate relative amounts estimated from size and color intensity of spots on paper chromatograms. 0 = no spot, 1 to 6 = increasing amounts.
[b] For decoding of symbols see Table I.
[c] γ/10 cu. mm. extract; maximum error 18%.

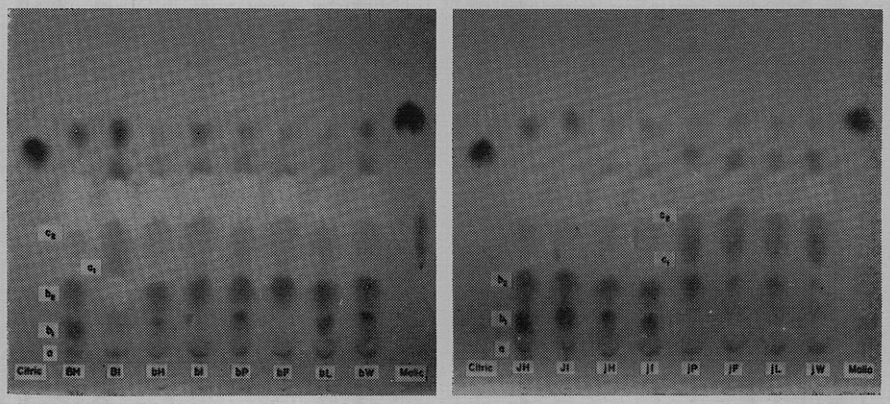

Figure 3. One-dimensional chromatogram of acidic compounds in leaf extracts of healthy and Fusarium infected, susceptible (left) and resistant (right) tomato plants and of cuttings treated with various wilting agents, listed in Table I

The toxins lycomarasmin and fusaric acid on the other hand seem to counteract the accumulation of malic acid in wilted plants (bL, bF). Infected cuttings of the resistant variety (jI) contained more malic acid than the corresponding healthy plants (jH), this increase being specifically reproduced by lycomarasmin treatment of plants of this variety (jL). Citric acid definitely increased in concentration in the susceptible variety following infection (BI, bI). This again, however, seems to be nonspecific, because cuttings wilted by lack of water supply (bW) showed the same tendency. This same kind of nonspecific increase in citric acid was also observed in cuttings of the resistant variety wilted by any of the treatments (jP, jL, jF, jW).

Inorganic phosphate (spot c_1) did not change in concentration in any of the samples from susceptible plants, but it increased tremendously in cuttings of the resistant variety wilted by any of the treatments (jP, jF, jL, jW). The most interesting changes observed concern a substance b_1, which possibly is an organic phosphate. This compound disappeared completely following infection of the susceptible variety (BI, bI) and treatment with fusaric acid (bF). It decreased very much in the resistant plants following treatment with any of the wilting agents (see Figure 3). In the susceptible variety, therefore, the disappearance of this compound is strictly dependent upon infection or upon fusaric acid action; in the resistant variety, however, it disappears nonspecifically on wilting. Indications are that this observation reflects a primary action of the parasite brought about by fusaric acid and it also might have an important bearing on factors governing disease resistance, particularly because the reactions of the two varieties are so strikingly different. It remains to be seen whether or not the reciprocal change observed for the concentration of inorganic phosphate in the resistant variety has any relation to this matter.

Another acidic component, b_2, decreased in concentration in the susceptible variety after infection, but only in leaf tissue obtained from whole plants (BI). Its concentration remained constant in all the other samples of this variety. In the resistant variety no change was observed following infection (JI). Only treatment with fusaric acid (jF) and water-shortage treatment (jW) resulted in a considerable decline of this component.

Phenolic Compounds. The chromatograms show that most of the samples under investigation contained a large variety of phenolic compounds, five of which are listed in Table III. These compounds have not been identified. After infection of plants of the susceptible variety (BI, bI), an increase of these phenols can be observed in most instances, as anticipated, assuming that some of the phenols responsible for vascular discoloration in infected susceptible plants are transported into the leaf tissue of these plants. This increase was also detected in cuttings treated with pectolytic enzyme solution and lycomarasmin. In addition, an increase in the amount of substances 3 and 5 was noted after treatment with fusaric acid. All these changes must be considered to be more or less specific, since plants wilted by insufficient water supply do not show any changes in respect to the concentrations of these phenols in question. In the resistant variety there were no changes in phenols whatsoever following infection. Compounds 1 and 2 decreased substantially upon treatment of cuttings with any of the wilting agents (jP, jF, jL, jW). Substances 4 and 5 had a distribution similar to those of tyrosine, leucine-isoleucine, phenylalanine, and valine; their concentration apparently depends on the integrity of the vascular system.

Discussion

Leaf tissue of healthy, susceptible Bonny Best tomatoes contains essentially the same constituents as the leaf tissue of healthy, resistant Jefferson plants. Before infection, any differences in the relative amount of the 44 constituents under investigation are negligible. This illustrates that two host varieties with widely different degrees of resistance do not necessarily differ in their chemical composition previous to infection. This fact often appears not to have been recognized in the literature dealing with disease resistance in plants.

In these experiments, a striking difference can be noted between the infected plants of both varieties. Infection of susceptible plants results in a pronounced change of most of the substances under investigation, whereas practically no change can be observed after inoculation of the resistant variety. The extracts of healthy and infected susceptible cuttings (bH, bI) do not show as severe a change as extracts of whole plants (BH, BI), probably because these cuttings (bI) had recovered considerably after detachment and did not show such severe symptoms as the corresponding whole plants (BI). In evaluating the changes observed in the susceptible variety following infection, a comparison was made between the biochemical picture of Fusarium infected plants and plants wilted by withholding water. As a result it was possible to distinguish between nonspecific biochemical changes of host constituents following infection, such as those being brought about secondarily by wilting and necrobiotic degeneration of the tissue, and other changes which obviously directly reflect the primary action of the parasite or its metabolites. The results indicate that changes observed in the concentration of free amino acids are generally nonspecific, because many occur in tissue wilted by physically induced dehydration or water shortage. The majority of the amino acids increases in amount in these cases, apparently because of proteolytic reactions in the wilting and degenerating tissue. This interpretation is supported by the fact that *wilted Fusarium infected leaf tissue from* susceptible tomatoes contains approximately 50% less protein than leaf tissue from healthy plants of the same variety (*10*).

These nonspecific changes show that the physiology of wilting as such has an important bearing on the distribution and amount of plant constituents found in the tissues of these plants, and that careful interpretation must be given of such observations which, in the biochemical syndrome of wilt diseases in general, are probably only secondary in nature. This, incidentally, illustrates the importance of including control plants which are wilted by withholding water in such experiments. Omission of this control would have led to a completely different interpretation of the results and the nonspecific changes probably would have been entirely overlooked.

Many of these plant constituents are substrates for enzymes of either the parasite or the host. Probably most of the changes observed are the result of enzymatic reactions influenced by or characteristic of the pathological condition. These enzymatic reactions may be affected by metabolites or inhibitors of either the parasite or the host. A study of the enzyme systems acting on host plant constituents whose change in concentration is related to the primary action of the fungus may give further information on the biochemistry of such wilt diseases.

Only a few of the changes observed following infection of susceptible variety obviously are specifically induced by, or related to, the primary action of the metabolites of the parasite. Especially one acidic component, believed to be an organic phosphate, is of considerable interest in this connection. Its concentration changes only in susceptible plants following infection and in plants treated with fusaric acid, probably as a result of the action of this toxin. Other changes, such as in the inorganic phosphate content, appear to be correlated with the resistance phenomena. These particular changes seem most worthy of further investigation.

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NEMATOCIDES

Screening Tests on Bromoacetates as Nematocides

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Many of the 53 bromoacetates synthesized and tested as nematocides against *Rhabditis* sp. and *Panagrellus* sp. were found to exhibit high activity. About two thirds of the esters gave an LD_{95} of less than 20 p.p.m., and about one half less than 10 p.p.m. Some were effective at the 1-p.p.m. level, most of these being esters of straight-chain alcohols having 6 to 12 carbon atoms. The effect of structural variations in the alcohol portion of the ester is discussed. Several of the compounds show sufficient promise to warrant further testing. The octyl, 4-bicyclohexylyl, heptyl, decyl, 4-sec-butylcyclohexyl, and hexyl esters gave an LD_{95} of less than 2 p.p.m.

SEVERAL ESTERS of bromoacetic acid were found to exhibit a marked lethal effect, in the course of screening a large group of miscellaneous organic compounds for toxicity to nematodes. To find out which of these compounds would be most effective, 53 bromoacetates were prepared and tested. The results indicated that the chemical structure of the alcohol moiety of the ester had an important effect on the nematocidal activity of the compound.

Preparation of Compounds

The bromoacetates were synthesized by azeotropic esterification of bromoacetic acid with the various alcohols. Benzene was used as a solvent and *p*-toluenesulfonic acid as a catalyst. The physical and chemical data of the esters prepared are presented in Table I. Only four of the esters have had their constants previously recorded in the literature—cyclohexyl (*3*), benzyl (*2*), 2-chloroethyl (*4*), and the diester of ethylene glycol (*1, 5*). The constants agree with those reported here.

Biological Tests

The compounds were screened against mixed populations of *Rhabditis* sp. and *Panagrellus* sp. according to the technique described by Taylor, Feldmesser, and Feder (*6*). In this procedure nematodes in small glass vials filled with sand were exposed to various dosages of each compound in a water-acetone solution or in a water emulsion. A dosage-response curve was plotted for each compound, as illustrated in Figure 1. The curves shown are typical of those obtained in this study. The phenethyl ester was selected because its response was practically a straight line and the 2-methylpentyl ester, because the actual straight-line response had to be approximated between several points. The dosages corresponding to LD_{50} and LD_{95} are the averages of three replicates and are given in Table I, which shows the compounds arranged in order of increasing LD_{95}.

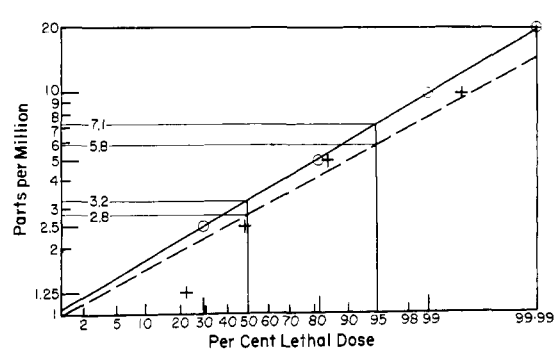

Figure 1. Typical dosage response curves

— Phenethyl bromoacetate
--- 2-Methylpentyl bromoacetate

Discussion

Several interesting facts are evident from the table. Approximately two thirds of the 53 compounds tested give an LD_{95} of less than 20 p.p.m. and about one half of less than 10 p.p.m. This is a high proportion of active compounds for such a series of related substances. There also seems to be a very wide difference between the LD_{95} and LD_{50} figures for many of the compounds, indicating that some of the dosage response curves are steep and others flat.

Among the seven most effective bromoacetates are five straight-chain aliphatic —hexyl, heptyl, octyl, decyl, and dodecyl. Some of the branched aliphatic esters, such as 1 = ethylpropyl, 2-ethylbutyl, 2-methylpentyl, 1-ethylpentyl, and 2-ethylhexyl also show considerable toxicity, but are not quite so effective as the straight chain compounds. A few of the substituted cyclohexyl esters, especially the para-substituted ones, are also toxic—namely, 4-bicyclohexylyl, 4-*sec*-butylcyclohexyl, and 4-isopropylcyclohexyl. The 2-isopropylcyclohexyl is much less effective than the corresponding para compound. The cyclohexyl ester itself is only slightly less active than the 4-methylcyclohexyl.