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WILT RESISTANCE IN TOMATOES

Pectic Enzyme Formation by *Fusarium oxysporum f. lycopersici* on Susceptible and Resistant Tomato Stems

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Because of the adaptive nature of pectic enzymes and their implied function in Fusarium wilt, enzyme formation by *F. oxysporum* grown on resistant and susceptible tomato stem tissues was studied. Fungal growth and pectin methyl esterase formation were about the same; pectic depolymerase activity was three to four times greater when the fungus grew on susceptible tissues as compared to the resistant. High depolymerase may cause vascular plugging and browning associated with this wilt. Apparently pectic enzymes degrade vascular pectin into soluble pectates which form gels or plugs in the small vessels. Wilt susceptibility in tomato is associated with an increase in pectic depolymerase activity of the fungus, whereas the suppression of pectin-splitting enzymes, especially pectic depolymerase, is correlated with single-gene resistance to Fusarium wilt.

MANY ECONOMICALLY IMPORTANT WILT DISEASES of plants are caused by parasitic fungi that grow in the vascular system of the plant. Particularly important in this group of plant diseases are the vascular fusaria, among which is a fungus, *Fusarium oxysporum f. lycopersici* (Sacc.) Snyder and Hansen, which has long been known to be the incitant of the Fusarium wilt disease of tomato. It is an important field disease in many of the warmer regions throughout the world as well as in the United States (*33*). The most effective and practical means of control is to use varieties which are resistant to the fungus.

The fungus becomes established readily in many soils which remain infested. In the field the disease may appear at any time that conditions are favorable. The first symptoms are a yellowing of the lower leaves and a browning of the vascular system.

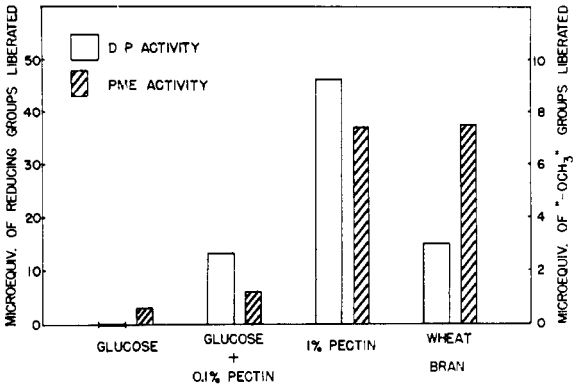
Wilt symptoms have been ascribed to the interaction of specific toxins produced by the parasite (*8, 10, 23*) and to a mechanical blockage of the vascular system by substances liberated by the host-parasite interaction (*22*). Gothoskar *et al.* (*13, 14*) were the first to describe the role of pectic enzymes in Fusarium wilt of tomato. These workers were able to duplicate the wilt symptoms in tomato cuttings by using filtrates from very young cultures of the fungus, particularly when the fungus was grown on wheat bran. The active material in the culture filtrates that was responsible for this wilt was identified as pectic enzymes.

The pectin-splitting enzymes of many fungi are adaptive or inducible enzymes that are formed only when the organism is grown on certain substrates and not when it is grown on others. Waggoner and Dimond (*32*) report that pectin methyl esterase is and polygalacturonase is not excreted by *Fusarium oxysporum* when grown on glucose medium but both enzymes are produced by the fungus on a pectin medium. They presented evidence to show that pectin methyl esterase was present in the vascular stream of diseased plants.

In view of the adaptive nature of pectic enzymes and their function in Fusarium wilt it seemed desirable to study pectic enzyme formation by *Fusarium oxysporum* when the parasite is grown on stem tissues from both susceptible and resistant tomato varieties. Stem tissue was selected because true resistance to Fusarium wilt is a characteristic of the stem (*29, 30*) and use of stems was most practical for these studies. The work reported here further shows that the pectic enzymes of *Fusarium oxysporum* are adaptive enzymes and that much more pectic acid depolymerase is produced when the parasite is grown on tissue from a susceptible variety as compared to a resistant one.

Culturol Methods

The fungus used was *Fusarium oxysporum f. lycopersici* (Sacc.) Snyder and Hansen, R5-6, a highly pathogenic strain. The stock culture of the organism was incubated at 28° C. in test tubes on potato-dextrose agar. After growth of the fungus, the tubes were stored at 4° C. For use as inoculum, the organism was grown in 500-ml. Erlenmeyer flasks containing 100 ml. of a Czapek-Dox nutrient medium of the following composition (grams per liter): sodium nitrate, 2; potassium phosphate (monobasic), 1; potassium chloride, 0.5; magnesium sulfate, 0.5; ferrous sulfate, 0.01; and glucose, 30, as the carbon source. The nutrient medium was inoculated directly from the stock test tubes and the flask was placed on a rotary shaker in a room maintained at 25° C. After 3 days on the shaker, the spore suspension was centrifuged and the supernatant replaced by sterile water. To remove metabolites formed in the medium, this latter operation was repeated three times under aseptic



Open bars. Pectic depolymerase (DP) activity as reducing groups liberated from sodium polypectate solution by 1 ml. of culture filtrate in 6 hours. Shaded bars. Pectin methyl esterase (PME) activity as methoxyl groups liberated from pectin solution by 1 ml. of filtrate in 3 hours

Figure 1. Production of pectic enzymes by *Fusarium oxysporum* grown on glucose, pectin, or wheat bran as the carbon source

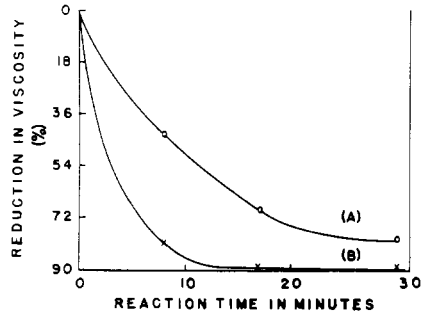


Figure 2. Pectic acid depolymerase activity in filtrates from *Fusarium oxysporum* cultured on resistant (*A*) and susceptible (*B*) tomato stem tissue

conditions. Five milliliters of the final suspension of washed spores served as the inoculum.

To study the adaptive nature of the pectic enzymes further, the fungus was grown in a modified Richard's solution (*34*) or in the Czapek-Dox medium above, in which pectin replaced all or part of the glucose as the carbon source. Each flask was inoculated with 5 ml. of the spore suspension and the fungus allowed to grow at 28° C. on the sterile cultures for 5 days. The pectic enzymes were then assayed as described below.

In addition to cultures in liquid synthetic media, experiments were also completed with the organism grown on wheat bran, a crude source of pectic substances, and stem sections from tomato plants. Bran cultures were prepared by sterilizing 5 grams of wheat bran containing 5 ml. of water and inoculating as described below for the plant material.

Preparation of Plant Material

Growth of Noninfected and Infected Plants. Two tomato varieties, Bonny Best, a very susceptible variety, and Jefferson, which is highly resistant to the Fusarium wilt disease, were grown in vermiculite in the greenhouse at 24° to 27° C. and watered with Hoagland's solution. After 2 weeks, the tomato seedlings were transplanted either into quartz sand or into soil, depending on their intended use. To obtain infected tissue, the seedlings were removed from the quartz sand after 3 weeks, and the roots were immersed in a spore suspension of the wilt fungus and planted back in sand (root-dip method). Wilt symptoms were usually observed in 2 weeks.

Infected stem tissues were prepared from plants inoculated by the method of Scheffer and Walker (*30*). Cuttings of 5-week-old tomato plants were placed in 30 ml. of spore suspension with an inoculum concentration of 400,000 cells per ml. After roots had reformed, the plants were potted in soil and placed in the greenhouse under favorable conditions for disease development. The material was harvested 3 weeks after inoculation.

Sterilization of Stem Tissue. Stems from rather uniform tomato plants were washed free of debris and cut into approximately 4-mm. sections. To eliminate contaminants and yet preserve the tissues as near as possible to those in the living plants, the surface of these stem sections was sterilized by treatment with gaseous propylene oxide (*15*). This was done by placing the stem sections in a large desiccator containing an open vessel of cold liquid propylene oxide and leaving the tissue in the fumes for 8 to 12 hours. In later experiments sterilizing the stems for as little as 3 to 4 hours was found to be effective. The desiccator was then evacuated through a sterile cotton filter to remove gaseous propylene oxide.

Culture of Fungus Spores on Sterile Stem Tissue. Twenty or 25 grams of infected or uninfected sterile stem tissue from each variety was transferred aseptically to 500-ml. Erlenmeyer flasks and the tissue was rinsed three times with sterile water. To each flask were added 5 ml. of sterile water and 5 ml. of the spore suspension described above. Uninoculated flasks served as controls to check on the efficiency of the gaseous sterilization and to detect the presence of pectic enzyme in uninfected stem tissue. The fungus was grown on the stem sections for 5 days in an incubator maintained at 28° C.

Preparation of Culture Filtrates. Ninety milliliters of sterile water was added to extract the extracellular enzymes formed by the fungus grown on the stems; four drops of toluene were also added and the flasks placed on a slow shaker. Ten hours later the mixture was filtered through two layers of cheesecloth, the filtrate was diluted to 100 ml. and centrifuged. Filtrates from Fusarium-inoculated tomato stems harvested from noninfected plants were centrifuged at 3700 × g for 30 minutes, whereas filtrates from Fusarium-inoculated stem tissue of previously infected plants were centrifuged at 13,600 × g. The culture filtrates were stored at 4° C. for immediate assay or frozen and maintained at −16° C. for later analyses.

Pectic Enzyme Determination. The methods for assaying the extracellular pectic enzymes in the culture fluids were similar to those used by Gothoskar *et al.* (*14*), which are based on the method described by Kertesz (*20, 21*). Pectin methyl esterase activity (PME) was estimated by electrometric titration of the carboxyl groups liberated from 1.0% pectin solution by culture filtrates. For these experiments, 4 ml. of culture filtrate previously adjusted to pH 7 was incubated with 15 ml. of 1.5% (w./v.) pectin solution, pH 4.5 or 5.5, and 1 ml. of 0.5*M* acetate buffer, pH 4.5 or 5.5, all at 30° C. At the end of 3 hours the reaction mixture was titrated to pH 7.0 with 0.1*N* sodium hydroxide solution, using a magnetic stirrer. Reaction mixture containing culture filtrate heated in boiling water for 15 minutes served as the heated control. The microequivalents of methoxyl groups hydrolyzed by 1 ml. of culture filtrate were calculated. A correction was made for adjustment of the filtrate to pH 7. Pectic depolymerase activity (DP) was determined by measuring the increase in reducing power or reduction in viscosity resulting from the partial hydrolysis of 1.0% sodium polypectate or pectin by the culture filtrate; monogalacturonic acid is not produced as a reaction product. The reaction mixture was usually composed of 15 ml. of 1.0% sodium polypectate or pectin, 1 ml. of 0.5*M* acetate buffer, pH 4.0 or 4.5, and 5 ml. of culture filtrate; this was incubated at 30° ± 0.5° C. Five-milliliter aliquots were withdrawn at intervals and pipetted into glass-stoppered flasks containing 2 ml. of 1*M* sodium carbonate solution to stop the enzymatic reaction.

The reducing groups liberated were estimated iodometrically by the hy-

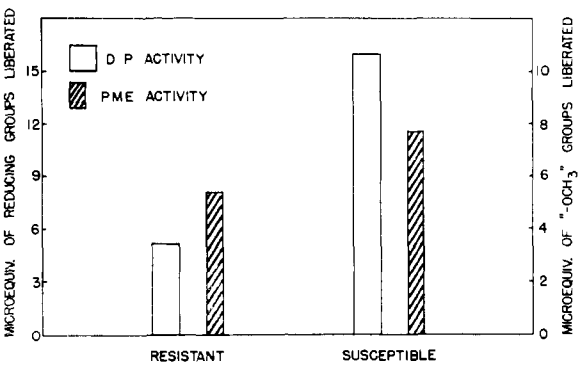


Figure 3. Production of pectic enzymes by *Fusarium oxysporum* grown on tomato stem tissues

Open and shaded bars as in Figure 1

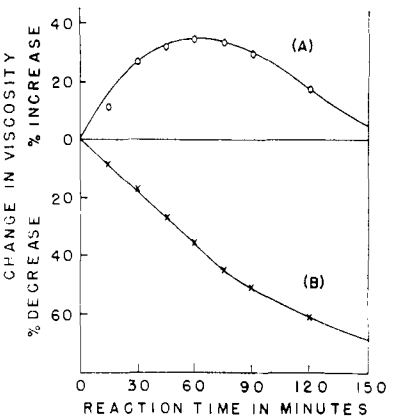


Figure 4. Pectic acid depolymerase activity in filtrates from *Fusarium oxysporum* cultured on preinfected resistant (A) and susceptible (B) tomato stem tissues

poiodite procedure of Jansen and MacDonnell (*18*) as described by Kertesz (*20*). The DP activity is expressed as microequivalents of reducing groups liberated by 1 ml. of culture filtrate. In some experiments both reduction in viscosity and increase in reducing power by filtrates from infected as well as uninfected stem tissues were measured on admixture of equal volumes of filtrate and substrate; each component was first allowed to come to temperature equilibrium in a water bath before mixing.

Viscosity-reducing activity of 5 ml. of reaction mixture was measured in a water bath at 30.0 ± 0.1° C. with a Fenske-Ostwald viscometer, size 100. The per cent viscosity change was calculated as follows (*27*, *28*):

$$A_n \text{ min.} = \frac{t_i - t_a}{t_i - t_o} \times 100$$

where A_n min. is per cent loss in viscosity at n minutes; t_i, initial flow time or flow time in seconds of 1% sodium polypectate solution plus inactivated enzyme; t_a, flow time in seconds at reaction time n minutes, of sodium polypectate solution plus active enzyme; and t_o, flow time in seconds of water plus inactivated enzyme. Other workers have used v for t in the above formula (*27*).

The reaction mixtures were tested for the presence of monogalacturonic acid by paper chromatography (*14*) using benzidine and aniline phthalate (*25*) as spray reagents. Under aseptic conditions these mixtures were sampled at 24-hour intervals up to 5 days.

Results and Discussion

The important role of pectic enzymes in Fusarium wilt of tomato was first pointed out by Gothoskar *et al.* (*13*, *14*), who showed that the thermolabile, nondialyzable factor in replacement culture filtrates which caused vascular browning and wilt typical of diseased tomato plants contained pectin methyl esterase and a polygalacturonase (PG). Furthermore, they indicated that filtrates obtained by growing the fungus on sterile wheat bran were more active in producing the wilt symptoms than those of the organism grown on other media. These workers postulated that PME was the most important single factor in producing typical symptoms of tomato wilt, since it was found in relatively large quantities in Fusarium filtrate from wheat bran (*13*). However, when PME free from PG was subsequently tested on tomato cuttings, no wilt was observed (*14*). Later work showed that the principal pectin-splitting enzyme was a pectic depolymerase rather than polygalacturonase and that this depolymerase may be an important initial causal agent of the wilt disease. The recent finding of pectic acid depolymerase in infected tomato plants (*5*) strengthens these results and substantiates the importance of DP in Fusarium wilt of tomato.

The term "pectic depolymerase" described in these studies is similar to "endopolygalacturonase" as classified by Demain and Phaff (*6*). Other terminology of pectic enzymes acting upon the various pectic substances has also been extensively discussed by Deuel and Stutz (*7*).

The pectin-splitting enzymes seem to degrade the insoluble pectin of the vascular system, so that large soluble pectin fragments pass into the vascular stream where, in the presence of ions like Ca^{+2} and possibly Mg^{+2}, they may form pectin gels which increase the viscosity to the point where mechanical plugging occurs. That the vascular plugs found in diseased plants are apparently pectin was shown by Pierson *et al.* (*26*). These workers followed by histological study the development of vascular plugs in infected plants; they showed by a characteristic staining reaction that the plugs were apparently due to an accumulation or release of pectic materials into the vascular system of the stem. These plugs or gels block the transport of water and nutrients in the stem to cause the typical wilt of tomato plants. Various phenols (*4*) and plant phenol oxidases are also released into the vascular stream and these produce the characteristic browning associated with the disease.

Adaptive Nature of Pectic Enzyme Formation. Kern (*19*) has pointed out in a review that pectin-splitting enzymes are formed as both constitutive and/or inducible (adaptive) enzymes by many fungi or bacteria. The data presented in Figure 1 show that in the case of *Fusarium oxysporum*, depolymerase (DP) is an inducible or adaptive enzyme and pectin methyl esterase (PME) may be both a constitutive and an inducible enzyme.

When the fungus was grown on 3% glucose as a carbon source, no depolymerase activity could be detected although a small amount of pectin methyl esterase, sufficient to liberate 0.6 μeq. of methoxyl groups, was present. When 0.1% pectin was added to the glucose medium, pectic acid depolymerase was formed, as shown by the liberation of 13.2 μeq. of reducing groups. Pectin methyl esterase activity was only slightly increased. However, when the fungus was grown on 1% pectin as the sole carbon source, depolymerase activity greatly increased. Forty-six microequivalents of reducing groups were liberated by 1 ml. of filtrate when pectin was the substrate. With wheat bran as the growth medium, considerable amounts of both enzymes were formed.

In another experiment fungus spores preadapted to 1% pectin and to 1% glucose were subsequently grown on sterile wheat bran. One-milliliter volumes of bran filtrate from the former liberated 28.6 μeq. of methoxyl groups

Table I. Pectic Acid Depolymerase Activity in Filtrates from *Fusarium oxysporum* Cultured on Wheat Bran and on Susceptible and Resistant Tomato Stem Tissues

Reaction Time, Hours	Enzyme Activity of Filtrates[a]		
	Wheat bran	Bonny Best, susceptible	Jefferson, resistant
2.00	11.0	12.8	1.6
3.00	12.0	14.0	4.0
4.00	13.0	14.4	4.0
6.00	14.8	16.0	5.6

[a] Microequivalents of reducing groups liberated from sodium polypectate, pH 4, by 1 ml. of culture filtrate at 30° C.

Table II. Pectinic Acid[a] and Pectic Acid[b] Depolymerase Activity in Filtrates from *Fusarium oxysporum* Cultured on Stem Tissue of Susceptible and Resistant Infected Tomato Plants

Reaction Time, Hours	Enzyme Activity of Filtrate			
	Bonny Best, Susceptible		Jefferson, Resistant	
	a	b	a	b
0.75	5.0	6.8	1.5	2.0
1.50	9.5	10.0	2.8	3.0
3.00	12.0	13.2	4.0	4.2
6.00	16.5	21.0	5.5	7.8

[a] Microequivalents of reducing groups liberated from pectin, pH 4, by 1 ml. of culture filtrate at 30° C.

[b] Microequivalents of reducing groups liberated from sodium polypectate, pH 4, by 1 ml. of culture filtrate at 30° C.

from pectin, pH 4.5, in 3 hours; the latter released only 2.5. Thus pectin methyl esterase activity in wheat bran culture filtrate from pectin-grown spores increased 11-fold over that from the glucose-grown fungus. Growth of the fungus was approximately the same on these media.

Therefore, the data indicate that depolymerase is an adaptive enzyme which is not formed in the absence of pectin. However, pectin methyl esterase appears to be both an adaptive and a constitutive enzyme, for its activity is increased in the presence of pectin.

Later work from our laboratory showed that when wheat bran is inoculated with *Fusarium oxysporum f. lycopersici* spores grown on 2.5% glucose plus 0.5% pectin medium, the filtrate from this fungus bran culture can liberate a small amount of monogalacturonic acid from polypectate solution (*16*). In contrast, the bran filtrate from glucose-grown Fusarium spores does not produce the monomer (*14*). The latter filtrate contained DP activity; the former, a small amount of PG activity. These studies lend additional strong support to the importance of the substrate in growth of the fungus and pectic enzyme production.

Pectic Enzyme Production on Uninfected Stem Tissue. In view of the pronounced effect of the nature of the substrate on pectin enzyme formation and its importance in producing wilt symptoms, it seemed desirable to study the formation of these enzymes when the fungus is grown on tomato tissue. Waggoner and Dimond (*32*) have reported the presence of fungal pectin methyl esterase in the vascular stream of susceptible, diseased tomato plants. We wanted to study the relationship of varietal resistance to pectic enzyme formation, for when the fungus penetrates resistant tomato plants, mild disease symptoms appear but the plants always recover. In contrast, susceptible plants develop severe disease symptoms. Bohn and Tucker (*1*) demonstrated that this type of resistance is controlled by a single dominant gene which is found in such varieties as Pan America and Jefferson but not in the susceptible variety, Bonny Best.

When *Fusarium oxysporum* was grown on stem sections from healthy, susceptible plants, 1 ml. of the filtrate released 7.7 μeq. of methoxyl groups from pectin in 3 hours; that from stem sections of healthy, resistant plants liberated only 5.2 μeq. Pectin methyl esterase activity from wheat bran cultures was approximately the same as that from the susceptible stem cultures. In contrast, there was a greater difference in the pectic depolymerase activity when the organism was grown on the susceptible as compared to the resistant variety.

The data in Table I show that the pectic acid depolymerase activity from the fungus cultures grown on stem sections of healthy, susceptible Bonny Best tomato plants is about the same as that formed on wheat bran. However, the pectic depolymerase activity from the cultures on the resistant Jefferson tissues is much less than that from the susceptible tissue. Only about one third as much pectic depolymerase activity is found when the organism is grown on the resistant tissue.

To detect the earlier stages of pectin acid hydrolysis, the change in viscosity of the substrate is a more sensitive method. The curves in Figure 2 show that at 8 minutes of reaction time, the reduction in viscosity of polypectate solution by fungus culture filtrates of the susceptible tissue (curve *B*) is about twice that caused by the fungus filtrate from resistant stems (curve *A*).

Figure 3 further illustrates this difference in amount of pectin-splitting enzymes formed by *Fusarium oxysporum* when grown on stem tissues from resistant and susceptible tomato varieties. There is about three times more pectic acid depolymerase formed by this pathogen on susceptible stem tissue, but only slightly higher pectin methyl esterase activity on the susceptible tissue as compared to the resistant. Moreover, no monogalacturonic acid was detected on paper chromatographic analysis of aliquots from the reaction mixtures kept under sterile conditions up to 5 days: this confirmed that the enzyme was a pectic depolymerase. Although the visual growth of the fungus was the same on both varieties, the resistant plant in some way suppressed the formation of the depolymerase enzyme.

Pectic Enzyme Formation on Infected Stem Tissue. Additional experiments were carried out to compare the depolymerase activity using both pectic acid and pectin as enzyme substrates. In these experiments the organism was grown on stem tissue sections from plants which had been inoculated with the fungus 2 weeks earlier. This was done to evaluate enzyme formation more closely under conditions in which the disease had developed in the plant. When fungus culture filtrates of preinfected tomato stems were examined for extracellular pectic enzymes secreted by the organism, both pectin methyl esterase and pectic depolymerase activities were found.

Pectin methyl esterase activity in culture filtrate from diseased susceptible stem (24.7 μeq. per ml. of filtrate) was twice that in filtrate from preinoculated resistant stem (12.7 μeq.). The difference in PME activity of these culture filtrates is larger than that observed for fungus filtrates from uninfected stem tissue. The ratio of PME activity of susceptible to the resistant was 1.3 to 1 for the uninfected stem cultures compared to 2 to 1 for the respective two infected stem cultures.

When pectin was used as the enzyme substrate, the difference in depolymerase activity of the filtrates from cultures of the two varieties was larger than the PME activity in the same filtrate.

The data in Table II show that the susceptible Bonny Best culture contains about three times more pectinic acid depolymerase than that from the resistant Jefferson culture. This, again, points to the greater importance of depolymerase in wilt symptoms produced by the fungus. Slightly more DP activity was found in the filtrate of the fungus culture from infected, susceptible Bonny Best stems when polypectate was substituted for pectin as substrate; less DP was present in filtrate from the resistant stems. For these measurements both viscosity change and increase in reducing groups were determined.

At first, the earlier stage of pectic acid hydrolysis was followed. In Figure 4 the culture filtrate from the preinfected susceptible stem tissue (curve *B*) caused a steady decrease in viscosity of polypectate solution, whereas the filtrate from preinfected resistant tis-

sue (curve *A*) produced not a decrease but rather an increase in viscosity for the first 60 minutes before it began to drop slowly.

The reducing groups liberated by the culture filtrates used in the viscosity determinations discussed above are presented in Table II. These data show that three times more pectic acid depolymerase was formed when the organism was grown on the stem tissue from diseased, susceptible Bonny Best than on inoculated resistant Jefferson plants.

Discussion

The amount of pectic enzymes formed by the fungus grown on tissue from the susceptible variety (Figure 3) is about the same as that produced when grown on wheat bran (Figure 1). The culture filtrate from wheat bran was similar to that described by Gothoskar *et al.* (*14*) to cause wilt symptoms and to contain pectic enzymes. Culture filtrates from the fungus grown on uninfected resistant stem tissue contained only slightly less pectin methyl esterase activity than that on stem tissue from uninfected susceptible plants. Pectic depolymerase activity in fungus filtrates from uninfected resistant stems was about one third that found in similar filtrates from susceptible tissues (Table I, Figures 2 and 3). This difference and the important role that pectin-splitting enzymes play in production of disease symptoms suggest that the Jefferson variety may be resistant because of a factor which suppresses the formation or activity of these enzymes.

Pectic enzyme production in filtrates from the fungus cultured on preinfected stem tissues further supports the above suggestion. The fungus produced about one half as much pectin methyl esterase on stem tissue from preinfected resistant plants as on that from preinfected susceptible ones. When the ratios of pectin methyl esterase activity of resistant and susceptible stem cultures are compared, PME in cultures from the preinfected resistant tissues is suppressed more than that from uninfected resistant tissues.

Pectic depolymerase activity in filtrates of fungus cultures from preinfected resistant stems was about one third that from infected susceptible stems (Table II). This reduction in pectic acid and pectinic acid depolymerase activity as measured by release of reducing groups (Table II) is about the same as that found in fungus cultures from uninfected resistant stems at 3 and 6 hours (Table I and Figure 3). However, when pectic acid depolymerase was measured by the viscosity method using these same culture filtrates, the filtrate from the resistant stems caused an increase in viscosity rather than a decrease as produced by the filtrate from preinfected susceptible tissues (Figure 4). Preinfection of the resistant tissue thus seemed to suppress the viscometric reduction of pectic acid by the filtrate for about 3 hours. Apparently the pectic depolymerase activity in the resistant filtrate was too low to degrade enough pectic acid to prevent the interaction of Ca and/or Mg ions and the substrate. Moreover, the observed slow increase in viscosity was probably due to a gradual release of the ions bound to the inhibited protein in the filtrate. However, the high DP activity in the filtrate from preinfected susceptible tissue immediately reduced the viscosity of polypectate (Figure 4, *B*). Two other possibilities for low DP activity are: the release of a DP inhibitor (*2, 3*) by the injured resistant cells and adsorption of the enzyme to resistant tissue walls as found with PME (*11*). The inhibition or reduction in DP activity and the reaction of multivalent ions with the substrate may logically explain the data on filtrates from preinfected resistant stems. The importance of the host-parasite interaction to the success or failure of pathogenicity and these results strongly suggest a role for pectic enzymes in susceptibility and resistance of tomato plants to Fusarium wilt.

Tissue disintegration by the pathogen (*17*) and histological studies of Fusarium wilt of tomato (*26, 30*) also suggest that the fungus confined to the vascular system sends out hypha which secrete hydrolytic enzymes including pectic enzymes to dissolve the middle lamellae between the vessels and between the vessels and the xylem parenchyma. The parenchymatous tissue, being high in pectic substances, is also attacked by the fungus, resulting in disintegration of host tissue. Although breakdown of pectic substances in the middle lamella and cell walls in wilted plants was shown histologically, severe destruction of the vascular tissue of young plants occurs only in the last stages of disease.

Our results show that the amount or activity of one important hydrolytic enzyme, pectic depolymerase, secreted by the fungus is greatly reduced by some factor in the resistant variety but not in the susceptible variety. If this persists, the pathogen may not obtain hydrolyzed products to allow continued growth and penetration of undamaged xylem tissue; the fungus may starve in the vascular system of resistant stems. The initial wilt symptoms then disappear and the resistant plant continues to grow normally, unaffected by the presence of the fungus. In the susceptible plant, however, the fungus continues to hydrolyze pectin, and undoubtedly other carbohydrates, down to the size where they are used as sources of energy. The fungal pectic depolymerase may degrade insoluble pectin into large soluble pectic fragments which form gels or mechanical plugs by reaction with Ca and Mg ions in the vascular stream. The blocked vascular system cannot accumulate nutrients for repair or synthesis of tissue and the plant soon succumbs to the catabolic action of the fungus.

Since the fungus also penetrates the resistant variety, a postulated mechanism of the ensuing reactions expressing its resistance is given. First of all the initial infected area in the vascular system of the resistant plants apparently activates the release of materials inhibiting or suppressing pectic enzyme activity. Secondly, the observed proliferation of the xylem parenchyma cells around the infected vessels results in a "walling off" of the damaged region. Finally, the plant resumes its normal function when new conductive tissue develops around the affected area (*30*). The latter activated anabolic action occurs fairly soon, since the inoculated highly resistant variety showing limited wilting recovers in 48 hours, and grows well, sometimes at a rate faster than the noninfected, control resistant plants.

The walling off phenomenon or formation of protective barriers is similar to the hypersensitive reaction in which denaturation, coagulation of proteins, and browning occur in certain resistant plants attacked by fungi. These observations are often preceded by the activation of higher polyphenolase activity in resistant tissue. Menon and Schachinger (*24*) were able to show that a strong accumulation of phenolic compounds during the vegetative period and a high polyphenolase activity was a special property of resistant tomato varieties. In infection the amount of phenolic compounds was further augmented and the increase was particularly high in resistant forms. Farkas and Kiraly (*9*) state that these facts may suggest that the polyphenol-polyphenolase system plays a major role in the resistance of tomato to Fusarium but is not concerned with the wilt symptoms.

Our studies of pectic enzymes in Fusarium wilt and the published literature suggest the following: The fungus invades the tissue of the vascular system by secreting pectic enzymes and other hydrolytic enzymes which break down the host tissue. In the susceptible plant this secretion of fungal pectic enzymes continues; it produces the typical wilting symptoms and contributes eventually to the death of the plant. In the resistant plant, limited fungal penetration occurs but the formation or activity of fungal pectic enzymes, especially the important pectic depolymerase, is reduced or inhibited. Limited vascular browning occurs in the injured cells and increased synthetic reactions commence in the adjacent cells, resulting in the proliferation of

parenchyma cells as observed by Scheffer and Walker (*30*). This activation of anabolic processes does not occur when the resistant plant is pretreated with dinitrophenol, since the effectiveness of ATP-generating systems (*31*) is lost by this treatment; then the resistant plant becomes susceptible (*12*). We conclude that the suppression of pectic enzyme activity followed by an accelerated anabolic activity appears to be important defense reactions associated with the single-gene resistant character of tomato plants resistant to Fusarium wilt.

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PARASITICIDE METABOLISM

Residue and Metabolism of Radioactive 4-*tert*-Butyl-2-chlorophenyl Methyl Methylphosphoramidate Administered as a Single Oral Dose to Sheep

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Sheep were given single oral doses of 4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate (Ruelene) parasiticide labeled with P^{32}; the metabolic fate was studied, and the residue in the tissues was determined for periods up to 21 days posttreatment. Ruelene and several hydrolysis products were found in the blood shortly after treatment, but by 2 days the Ruelene had decreased to a low level. Over 85% of the administered P^{32} was recovered in the excreta. The P^{32} in the urine, amounting to 75% of the dose, was primarily in the form of hydrolysis products of Ruelene. Some of the Ruelene was hydrolyzed completely to inorganic phosphate and retained in the animal tissues, in part as natural phosphate esters and in part as inorganic phosphate. Ruelene itself was not found in the tissues after 7 days.

RUELENE BRAND of 4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate (registered trademark, The Dow Chemical Co., Midland, Mich.) possesses a rather broad spectrum of anthelmintic activity in cattle, sheep, and goats (*1, 3, 10, 11*), exhibits good systemic control of cattle grubs (*7, 9, 12*), and controls several types of ectoparasites (*7*). The purpose of this work was to examine the metabolic fate and tissue residue to Ruelene labeled with P^{32}, administered as single oral doses to sheep.

Experimental Methods

Two syntheses of Ruelene labeled with P^{32} (*8*) provided the material for the eight sheep experiments described. The first synthesis batch had a specific activity of 0.64 mc. per gram, and the second had a specific activity of 1.0 mc. per gram, on the dates of administration to the animals.

The animals were wethers of a Hampshire-Southdown cross, weighing 75 to 87 pounds when put on the test. They