

**Plant Residue Decomposition Products and Their Effects
on Host Roots and Fungi Pathogenic to Roots**

R. G. Linderman

Research Plant Pathologist, Crops Research Division, ARS, USDA, Beltsville, Maryland 20705.

Plant residues and their decomposition products are important components of soil. Crop residues, green manure crops, and weeds are added to arable soils by farmers, just as in nature leaves, needles, and roots are continually added to uncultivated soils. The influence of these residues is felt, either directly or indirectly, by the multitude of living components of that soil, including soil fungi and plant roots. Recent reviews (1, 7, 10, 11) have surveyed the available information concerning plant residues and their influence on plant roots, with special emphasis on biological control. The authors of these reviews, however, have generally concluded that we are still groping for answers to many of the most basic problems concerning existence, identity, and mode of action of biologically active compounds under natural field conditions. We need to know how plant residues and their decomposition products alter or influence the microenvironment of the plant root and its rhizosphere. One of the reasons for the many unanswered questions about what occurs in the soil-root microenvironment is the technical difficulty which lies between the questions and answers. One must experimentally reconstruct this microworld and find some way of examining it.

Such problems should be approached with the assumption that the natural balance or equilibrium (both physical and biological) of our soil microworld must be upset in order that we may observe some response by the living components. Many environmental changes will elicit no directly observable response by some of the soil organisms. But plant residues or residue by-products added to soil often stimulate detectable biological responses. In such cases, the residues probably upset the soil balance to such an extent that organisms are forced to respond until a new equilibrium is established. Fungus pathogen propagules existing in soil as resistant structures often respond by becoming vegetatively active. It is this activity that can be detected by direct observation. Once a detectable response is observed, we must then attempt to determine how and why that response came about. The responsible compound(s) must be identified, and their activity in the natural soil environment established.

I have been working to determine the response of pathogenic soil fungi and host roots to this upsetting of the soil equilibrium with plant residues, and I will restrict myself to a description and discussion of these studies.

Phytotoxic decomposition products.—In investigations of the biological significance of phytotoxic crop residue decomposition products, an excess amount of green plant residues (usually barley) was decomposed under waterlogged soil conditions. After appropriate intervals of decomposition, water extracts were prepared from the soil-residue mixtures. A tobacco seed bioassay was used to indicate when phytotoxic decomposition products became the dominant components of the crude extracts. By combining the results of these bioassays with solvent (ether) extraction and paper and gas chromatography, we were able to partially purify and identify the major phytotoxic components of these extracts. They were benzoic, phenylacetic, 3-phenylpropionic (hydrocinnamic), and 4-phenylbutyric acids (17). These laboratory results were supported by extraction of the same toxic organic acids from field-decomposed barley separated from field soil. These compounds were also extracted from soybean, cowpea, and cotton decomposed in soil. We felt, therefore, that these aromatic acids exist in nature following decomposition under wet conditions of a wide variety of plant materials. And, given appropriate decomposition conditions, they could be produced in sufficient quantities to be phytotoxic to the next crop plants.

Predisposition to root rot by crop residue phytotoxins.—The widespread occurrence of phytotoxins in nature and reports on their apparent involvement in the etiology of root diseases prompted us to study the effect of these aromatic acids on pathogenic fungi in soil and on their influence on disease severity. Black root rot of tobacco (9) and bean (15), caused by *Thielaviopsis basicola* (Berk. & Br.) Ferr., were increased in the presence of phytotoxic residue extracts. *T. basicola* was therefore selected as a test pathogen. Germination tests on chlamydo-spores, added to field soil and observed on soil smears following treatment, showed that neither the crude barley phytotoxin extract nor the known phytotoxins stimulated chlamydo-spore germination. Cotton roots were treated for 2 hr with the same solutions at subtoxic (no discoloration or loss of turgor) concentrations before planting in inoculation chambers, then inoculated with chlamydo-spore-infested soil. Disease on roots treated with crude barley extract or hydrocinnamic acid was increased (5). When root tissue which had been treated with hydrocinnamic acid was microscopically examined 27 hr after inoculation, many more chlamydo-spores had germinated and established infection sites than on root tissue treated with water. In addition, the rate of proliferation of the fungus on and in the root tissue was much greater than on water-treated roots. In some way, then, this residue decomposition product had altered the plant's resistance mechanism(s). Could hydrocinnamic acid interfere with a genetically inherited resistance mechanism? We tested this hypothesis with three cotton (*Gossypium arboreum* L.) varieties, A-32 (susceptible), and A-25 and A-26 (resistant or tolerant). These experiments indicated that hydrocinnamic acid could render resistant varieties susceptible to *T. basicola*. Again, the increase or occurrence of root lesions was due to an increased number of chlamydo-

spores germinating and giving rise to infection sites as well as to a reduced hypersensitive reaction (6). Two mechanisms of resistance (or susceptibility) appeared to be altered: a prepenetration mechanism controlling the degree of root stimulation of chlamydo-spore germination, and a postpenetration hypersensitive reaction. The prepenetration mechanism seemed to involve the rate or degree of root exudation. Toussoun & Patrick (15) postulated that crude phytotoxic residue extracts altered cell permeability, allowing more fungus-stimulating compounds to be exuded. This possibility was examined by treating excised root tips of cotton seedlings with the known phytotoxins and determining their subsequent rate of electrolyte leakage by measuring the conductivity of the post-treatment bathing solution. Increased permeability occurred with toxin-treated roots as compared to water controls, but hydrocinnamic acid was no more effective than any of the other phytotoxins. One must conclude that if changes in root cell permeability due to hydrocinnamic acid treatment accounted for increased chlamydo-spore germination and thus increased disease, such changes must be qualitatively selective.

Stimulation of fungus propagules by plant residues.—Under certain conditions of decomposition of plant residues, materials may be released into the soil which stimulate germination and growth of fungus pathogens in the soil. If this stimulation occurred before a susceptible host was present and lysis of resulting hyphae followed, the pathogen might be reduced in numbers. If it occurred in the presence of susceptible host roots, disease might be increased. These possibilities were studied with several soil-borne pathogenic fungi. Toussoun et al. (16) reported that residue extracts stimulated germination of chlamydo-spores of *Fusarium solani* (Mart.) Appel & Wr. f. sp. *phaseoli* (Burkh.) Snyd. & Hans. in soil, and bean root rot caused by this organism increased following treatment with such extracts (15). Another case involved the decline of *Fusarium oxysporum* Schlecht from the roots and rhizosphere of sugar pine (*Pinus lambertiana* Dougl.) seedlings following transplanting from an infested nursery into a natural forest soil environment. Smith (12) first reported this decline or disappearance of the fungus from pine roots. Others (8, 13) have also reported a distinct absence of fusaria from forest soils while meadow soils adjacent to forest stands contained fusaria. What sort of environmental pressure(s) could account for this difference? The most obvious difference between the meadow and forest soil was the presence of a heavy needle duff layer on the forest soil. When water leachates from the duff layer were concentrated and added to soils containing high numbers of *F. oxysporum* chlamydo-spores, nearly all (95-98%) of these spores germinated within 24 hr (14). Such germination, however, generally resulted in the production of abnormal germ tubes which lysed before new chlamydo-spores could form. This response to the duff extracts then could account, at least in part, for the observed decline of *F. oxysporum* introduced into forest soils in or on pine seedlings. The identity of the stimulatory compounds is unknown.

Several other fungi have been tested with leachates from conifer needles as well as deciduous oak leaves. Ascospores of several species of *Chaetomium* and chlamydospores of several clones of *T. basicola* are stimulated to germinate by these extracts (Linderman & Toussoun, unpublished data). The germination of *T. basicola* chlamydospores warrants comment in that it may, like the germination of *F. oxysporum* chlamydospores, result in a decline in number of chlamydospores in that soil. *T. basicola* chlamydospores germinate in response to the litter extracts, and usually immediately form a small phialide conidiophore which produces numerous small conidia that readily lyse in the soil. Occasionally, chlamydospore germ tubes produced what appeared to be secondary "chlamydospore initials". But these structures were not resistant, and rapidly lysed. It appears, then, that these fungus stimulants may be widespread. And potentially, at least, they may activate resistant fungus structures which could ultimately result in the elimination of that propagule from the soil.

Volatiles.—Up to this point, the plant residue decomposition products mentioned were active while in the water-soluble state. The distance and rate of diffusion of such compounds into the soil solution from residues may limit their effectiveness in activating pathogenic fungus propagules. The effective distance of diffusion of residue by-products in their gaseous state, however, may be greater. Gilbert et al. (2) reported that volatile components from alfalfa hay increased the microbial respiration in soil. Gilbert et al. (3) also showed an initial increase, followed by a decline and subsequent elimination of *Verticillium dahliae* Kleb. in soil. The behavior of the *V. dahliae* propagules in soil, however, was not directly observed. Linderman & Gilbert (4) investigated the behavior of sclerotia of *Sclerotium rolfsii* Sacc. during exposure to vapors of an alfalfa distillate, showing that sclerotia placed on moist field soil are stimulated to germinate and grow when exposed to volatiles from alfalfa. Exposing soil to the volatiles for 1 week also increased its antagonistic potential to sclerotia placed on the soil and given a secondary exposure to the distillate vapors. Such exposures caused a striking increase in soil respiration. Parallel soil population studies indicated corresponding increases in total numbers of microorganisms, primarily bacteria. This increase in soil organisms undoubtedly played a role in inhibiting sclerotial germination and growth. The greatest amount of sclerotial inhibition, however, occurred on soil previously exposed to distillate vapors from which *S. rolfsii* sclerotia and mycelium had been removed. When new sclerotia and distillate vapors were added, sclerotial germination and growth were almost completely inhibited, even when the soil had been exposed only to water vapors. Thus, antagonism was increased by both the presence of the fungus sclerotia and mycelium and exposure to the distillate. Following exposure to the distillate vapors, the sclerotium and its attached mycelium were readily colonized by soil antagonists and saprophytes. And, unless a new sclerotium was produced by the mycelium, that sclerotium of the pathogen was eliminated. The respiration stimulants in the distillate, i.e., methanol,

acetaldehyde, and isovaleraldehyde, and a reconstituted mixture of these compounds similarly stimulated *S. rolfsii*.

Studies such as those briefly mentioned here stimulate further consideration of the efficacy of plant residues in manipulating the ecology of soil fungi. An environment which favors the increase of organisms antagonistic to pathogens in soil is a desirable goal. By understanding the biological response of fungus propagules in soil to natural plant by-products, one might create a hostile environment by culturally favoring the production of such compounds and thus effectively working against the pathogen. This could be accomplished by forcing the pathogen to become vegetative and thus vulnerable to microbial attack, or by increasing the antagonistic potential of the soil to prevent the pathogen from ever becoming vegetative, even in the presence of a susceptible host plant. In this way one might restrict the activity of, or eliminate, existing pathogen propagules as well as prevent subsequent recontamination. Nature with time has accomplished this, as evidenced by cases such as the decline and elimination of *F. oxysporum* introduced into forest soils. In addition to increasing our understanding of specific pathogen propagule response to residue additives, we might also consider the importance of identifying the compounds responsible for that response. The known compound might be added to soil instead of the residue from which it came during decomposition. In that way, the increase of specific segments of the microflora might be favored, or compounds might be specifically aimed at pathogen propagules. The use of natural plant by-products to control soil diseases would also eliminate the present concern over the increase in harmful pesticide residues in soil. Research is needed to further elucidate the responses of pathogenic soil fungi to plant residues.

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