PHYTOALEXIN TYPE RESPONSE OF SUNFLOWER STEMS TO SCLEROTINIA SCLEROTIORUM

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SUMMARY

Preformed and induced phenolic compounds were implicated initially in the resistance of sunflower stem tissue to S. sclerotiorum (Lib.) de Bary. They accumulated in inoculated stem tissues and up to 12 cm above the lesion within 3 days and at fourfold or more concentrations more than uninoculated tissues. Field-resistant varieties produced more than susceptible ones. The constitutive phenolics were not fungitoxic. An increase in phenylalanine ammonia lyase activity preceded the accumulation of phenols. Five antifungal compounds were synthesized in sunflower in response to S. sclerotiorum, but only one seems to be a phenolic compound, probably isochlorogenic acid. The other four are unidentified.

Inoculation of the stem with S. sclerotiorum induced resistance within 3 days at least 12 cm above the lesion. This was confirmed by challenge inoculations with S. sclerotiorum. Induced fungal inhibitory compounds were located in the tissues with induced resistance. Expression of resistant lesions was favored by removal of inoculum and any food base 24 hours after inoculum exposure.

The development of resistance and accumulation of induced antifungal inhibitors was dependent upon temperature. The development of a restricted lesion seems to depend upon periodic temperatures near 30 C that inhibit fungal development sufficiently to allow an accumulation of the antifungal materials.

INTRODUCTION

Diseases of sunflower caused by S. sclerotiorum (Lib.) de Bary include seedling rots, root rots, stalk rot, wilt, basal stem canker, and head rot. These are serious problems in most of the major sunflower production areas of the world. The broad host range of the pathogen, the long passive soil survival of the sclerotia, and the aggressive, primitive mode of pathogenesis, which can overwhelm plant defenses, have made control of S. sclerotiorum very difficult in intensive commercial sunflower culture.

Genetic resistance to S. sclerotiorum in commercial sunflower hybrids has been difficult to achieve, yet several hybrid varieties with some level of field resistance have been developed. The mechanism of any resistance has not been established, although there is evidence to support suggestions that phenolic compounds (Bazzalo et al. 1985), lignification (Orellana 1975), and Ca^{++} (Antonova et al. 1984) may be involved. Sunflower plants produce a large array of secondary metabolites, many known to be toxic. Chlorogenic acid, isochlorogenic acid, neochlorogenic acid, 4-0-caffeoylquinic acid, scopolin, scopoletin, and ayapin are examples of phenolics and coumarins in sunflower. Many chalcones, flavonoids, sesquiterpenes, and terpenoids are also found in sunflowers as constitutive components. Some of the phenolics and coumarins have been shown to increase in concentration after fungal infection (Bazzalo et al. 1985; Tal and Robeson 1986a), but the phytoalexin-like coumarins, scopoletin and ayapin, were degraded by sunflower pathogens, e.g., Alternaria helianthi (Tal and Robeson 1986b).

Another member of the Compositae, safflower, is known to produce polyacetylenic phytoalexins (Thomas and Allen 1970). In phytoalexin studies on sunflower (Bazzalo et al. 1985; Tal and Robeson 1986a,b), other unidentified induced compounds were observed, but not studied or tested for fungitoxicity. This report summarizes several studies on phenolics and phytoalexin-like materials produced by sunflower in response to S. sclerotiorum.

MATERIALS AND METHODS

Sunflower plants were grown in the greenhouse and controlled-environment chambers. Plants were inoculated at the cotyledonary node with a plug from a fresh culture of S. sclerotiorum, and moist rolled oat grains were applied as a food base. Then, the inoculation site was enclosed with moist cotton and plastic bag. In some instances, the inoculum source and food base were removed after 24 or 48 hours. Tests of disease reaction were also made in vitro. Stem sections about 6 cm long were surface-sterilized in ethanol and placed in test tubes that had a 2-day-old culture of S. sclerotiorum on PDA in the bottom. Lesion development on the stem sections was followed then at different temperatures.

Stem tissue above a 5-day-old lesion was sterilized and serially sectioned into three 2.5-cm pieces that were challenge inoculated in vitro with S. sclerotiorum. Subsequent lesion development was followed on these and stem pieces from nondiseased plants.

Extracts of diseased stems (lesions and tissues above the lesion) were made in hot 80% ethanol, and total phenols were determined with Folin and Dennis reagent. Ethanolic extracts were dried in vacuo and redissolved in acetone for spotting on silica gel thin-layer chromatography (TLC) plates (either with or without 254μm UV indicator). A second extraction procedure involved grinding of stem tissue in diethyl ether, extraction for 24 hours, filtration, drying, evaporation, and redissolving the residue in diethyl ether.

Ascending TLC involved irrigating systems of toluene:ethyl formate:formic acid (5:4:1) (TEF), dichloromethane:hexane (82:18), and glacial acetic acid:chloroform (1:9). Standards of suspected compounds were included when available. Visualization of the compounds on TLC was done under UV at 254 and 350μm, and materials that fluoresced or quenched fluorescence were noted. Reagents employed for characterization were potassium iodoplatinate, antimony pentachloride, ferric chloride in 2 N HCl, 1% aluminum chloride in ethanol, 10% vanillin in concentrated HCl, 1% potassium permanganate in 2% sodium carbonate, 0.4% isatin in concentrated sulfuric acid, and Folin-Ciocalteu reagent.

The compounds separated on TLC plates were bioassayed on the plates by asceptically spraying the plates with a suspension of 1 to 5 x 10^6 spores/ml of Cladosporium herbarum or C. cucumerinum. The spores and plates were oversprayed with sterile Czapeks-Dox broth, and sometimes the spores were applied in Czapeks-Dox broth. The plates were incubated in a sterile moist chamber until growth of the test fungus was evident. Zones of fungal growth inhibition were marked and compared with zones viewed under UV light.

RESULTS

Phenylalanine ammonia lyase (PAL) activity increased within 24 hours after inoculation of stem tissues with S. sclerotiorum. An increase in total phenols (sevenfold after 5 days) followed the increase in PAL. Phenol content in the upper stem and leaves did not increase. Total phenol content in the lesion area doubled by 3 days and then decreased to the original level by 5 days. The phenols in the tissue contiguous with the lesion and 12.5 cm above the lesion increased steadily over the 5 day period. The phenol content in a resistant variety (PI 431560) was about 50-100% greater than in a susceptible variety (PI 431515) over the entire experiment. The lesions on both varieties were very large and did not reflect differences in resistance observed in the field.

The duration of inoculum exposure was directly related to lesion size; the susceptible variety developed lesions 3.6, 5.7, and 6.1 cm long after 5 days when exposed to inoculum and food base for 1, 2, and 5 days, respectively. The resistant variety had only a fleck reaction with one day of inoculum exposure and 0.8- and 4.4-cm lesion lengths with 2 and 5 days of inoculum exposure, respectively. The highest concentration of total phenols occurred in the resistant variety with 2 days of inoculum exposure.

no evidence that ayapin and scopoletin were involved in the S. sclerotiorum-sunflower system. The fungitoxic compounds reported herein were synthesized de novo and, except for isochlorogenic acid, seem to be new to sunflower. Isochlorogenic acid accumulated at a low level at 25 C while the other four fungitoxic compounds did not. Bazzalo et al. (1985) previously found that isochlorogenic acid was synthesized in response to S. sclerotiorum infection. Toxicity of the phytoalexins to S. sclerotiorum has not been confirmed because a suitable bioassay with S. sclerotiorum has not been developed.

The elevated concentrations of phenols detected at least 12.5 cm above the lesion is unusual for phytoalexins because they normally increase only in those tissues near the pathogen activity. Stimulation of PAL activity and production of phenolics may result from some systemic signal from S. sclerotiorum, possibly oxalic acid which can be systemic (Noyes and Hancock 1981). Kuć, University of Kentucky, (personal communication) indicates that oxalic acid can elicit systemic-induced resistance in cucurbits.

The role of temperature in the S. sclerotiorum-sunflower system remains a mystery. Temperature has affected phytoalexin production in Phaseolus vulgaris (Bailey et al. 1980), soybean (Classen and Ward 1985), and lucerne (Khan and Milton 1979), but these were all quantitative effects. The decrease of phenolics within lesions and the absence of the antifungal compounds in tissues aggressively colonized by S. sclerotiorum would support an opinion that the fungus can degrade the phytoalexins. This is consistent with current concepts of basic host-pathogen compatibility; e.g. the degradation of scoletin and ayapin in sunflower by A. helianthi (Tal and Robeson 1986b).

The resistant response of sunflower plants to S. sclerotiorum at 30 C may have resulted from insufficient temperature control by the growth chamber. A fluctuating temperature could have allowed sufficient fungal growth to induce infection yet temperature inhibition of the fungus might have permitted the accumulation of phytoalexins.

The response of sunflower plants to S. sclerotiorum by the production of phytoalexins is worthy of further research, especially to characterize and identify the compounds. It remains to be determined whether or not they function in any resistance response of the plant to this pathogen.

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