Effects of Chlorothalonil on the Virulence and Physiology of a Nontargeted Pathogen, *Sclerotinia minor*

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Journal Series Paper 8188 of the North Carolina Agricultural Research Service, Raleigh, NC 27650.

This research was supported in part by a grant from the Diamond Shamrock Corporation.

The use of trade names in this article does not imply endorsement by the North Carolina Agricultural Research Service of the products named, or criticism of similar ones not mentioned.

Accepted for publication 4 October 1982.

ABSTRACT

Hau, F. C., and Beute, M. K. 1983. Effects of chlorothalonil on the virulence and physiology of a nontargeted pathogen, *Sclerotinia minor*. Phytopathology 73:475-479.

Inoculum of *Sclerotinia minor* produced in the presence of 0.2–0.4 μg of chlorothalonil (CTL) per milliliter induced larger stem lesions on peanut after 24 hr of incubation than did inoculum similarly produced in the absence of the fungicide. Lesions induced by *S. minor* inoculum grown in the presence of 0.2 μg of CTL per milliliter released more electrolytes into a bathing solution than lesions induced by *S. minor* inoculum grown without fungicide present. Four-day-old cultures of *S. minor* amended with 0.2 μg of CTL per milliliter yielded filtrates that contained more oxalic acid than did otherwise similar culture filtrates from cultures grown without the fungicide. Low concentrations (0.2–0.8 μg/ml) of CTL consistently inhibited the production of pectolytic enzymes by *S. minor* in cultures.

Sclerotinia blight of peanut (*Arachis hypogaea* L.) was first described in Virginia and North Carolina in 1971 and 1972, respectively (24). The disease, which is caused by the soilborne fungus *Sclerotinia minor* Jagger (13), has become more prevalent in the peanut growing areas of Virginia, North Carolina, and Oklahoma (5,30). Porter et al (26) reported that yield loss in infected peanut is highly correlated with severity of Sclerotinia blight.

Most pesticides have a wide range of biological activities, some of which may extend beyond the specific function intended by the manufacturers. The nontarget effects of certain pesticides may disrupt the balance of the existing ecosystem and result in altered levels of disease severity or incidence (1,12,21,27). Reported disease

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enhancements induced through nontarget effects involve chemically different pesticides with different modes of action, diverse crops, and a broad spectrum of pathogens.

Cases in which plant diseases caused by nontargeted pathogens either occur more frequently or are more severe following the application of fungicides generally are associated with the use of highly selective compounds. For example, application of pentachloronitrobenzene and systemic benzimidazole derivatives have enhanced several plant diseases (2,11,28,31).

Chlorothalonil (CTL) (2,4,5,6,tetrachloroisophthalonitrile), a broad-spectrum fungicide widely used to control leafspot diseases on peanut (six or seven applications per season at 0.8–1.2 kg/ha), increased the severity of Sclerotinia blight in research plots in Virginia when applied twice at 2.24 kg/ha (5). Additional tests in Southampton County, VA, in 1977 and 1978 showed that CTL-treated peanut plots (four applications at 1.25 kg/ha) had a higher Sclerotinia blight disease index than did nontreated plots (23). In field experiments, four applications of CTL increased the disease index when rated 32 days after the last application (22). The disease enhancement was most pronounced later in the growing season and in heavily infested fields. In most cases, disease severity increased, but disease incidence was not affected (22,23). Recently, Porter and Lankow (25) reported that *S. minor* mycelia are relatively insensitive to low concentrations of CTL in vitro. The rate of radial growth of mycelia was indistinguishable from that of *S. minor* on nonamended PDA. However, mycelia taken from colonies that had begun to differentiate into sclerotia were strongly inhibited on PDA amended with CTL (25).

Preliminary studies indicated that CTL applied as recommended for control of peanut leafspot diseases (1.2 kg/ha) did not increase susceptibility of peanut tissues to Sclerotinia blight (*unpublished*). Similarly, no consistent effect of CTL on microbial populations (fungi, bacteria, and actinomycetes) in soil or on organic debris on the soil surface (beneath the foliar canopy of field-grown peanuts) was observed, which could be correlated with enhancement of Sclerotinia blight. This report considers a third possible mechanism for disease enhancement, namely, the direct effect of CTL on the physiology and related virulence factors of *S. minor*.

MATERIALS AND METHODS

The isolate of *S. minor* used in this study was obtained in September 1979 from a diseased peanut plant in Northampton County, NC. The fungus was maintained on potato-dextrose agar (PDA) and transferred to fresh PDA every 30 days.

Greenhouse inoculation. Oat grains were used as a substrate for inoculum production. Twenty grams of oat grains were soaked in 20 ml of distilled water for 15 min. Excess water was decanted and the grain was autoclaved for 45 min at 121 C. After 24 hr, the medium was again autoclaved for 15 min. The oat grain medium was then amended with appropriate amounts of CTL to yield the desired concentrations. The flasks were gently shaken so that the added CTL solutions were dispersed among the oat grains. The medium was inoculated with a 6-mm-diameter plug cut from the periphery of advancing mycelium from a PDA culture of *S. minor*. Cultures were incubated for 5 days in laboratory conditions at about 24 C. After the incubation period, individual oat grains were used for inoculum.

Two-month-old cultivar Florigiant peanut plants were used throughout the study. Plants were grown in Norfolk sandy loam soil in 15-cm-diameter pots. A single oat grain was placed in contact with the base of the stem. The plants were placed in a humidity chamber at 17–19 C. Lesion expansion and wilting were recorded at 24-hr intervals. Each trial consisted of 18 inoculated peanut plants.

Conductivity assay. A method modified from that described by Newton et al (20) was used to study electrolyte loss from infected tissues into a bathing solution of sterile deionized water. Two-centimeter-long stem pieces were cut from the main stem of 2-mo-old peanut plants, surface disinfested in 0.5% NaOCl solution for 10 min, and rinsed thoroughly with sterile distilled water. Five stem pieces were placed on moist, sterilized Whatman No. 1 filter paper in petri dishes. One inoculum grain was placed on the top of each stem piece. The petri dishes were sealed with paraffin and incubated at 21–24 C under continuous fluorescent light. After 2 days, stem pieces were placed in an acid-washed, sterilized test tube containing 10 ml of sterilized deionized water, with constant stirring. Conductance of the bathing solution was measured at 10-min intervals for 70 min. Electrolyte loss was estimated by the increase in conductance of the bathing solution measured with a conductivity bridge (model RC 16B2, Beckman Instruments, Inc., Norcross, GA 30092). Treatments included stems infected by oat grain inoculum, stems infected by oat grain inoculum amended with CTL, seedling stems exposed to a sterile oat grain amended with CTL, stems dipped in CTL solution (0.2 μg/ml), or stems dipped in distilled water. The experiment was repeated twice with two replicated petri dishes each time.

Mycelial dry weight determination and oxalic acid production. *S. minor* was grown in a glucose succinate yeast salt (GSYS) liquid medium (25 ml per 125-ml Erlenmeyer flask) (18). The medium was adjusted to pH 5.8 with diluted hydrochloric acid and was autoclaved for 13 min at 121 C. The medium was then amended with 0.2–0.8 μg/ml CTL in water and allowed to stand overnight. Each flask was seeded with a 6-mm-diameter mycelial plug and incubated under continuous fluorescent light.

At harvest, mycelial mats were collected on a Whatman No. 1 filter paper, washed, and dried to constant weight at 80 C. Culture filtrates were collected under suction, and immediately measured for pH and for oxalic acid content as estimated by potassium permanganate titration (3).

Polygalacturonase (PG) production. Production of PG by *S. minor* was studied over an 8-day period in a basal medium (4) supplemented with 1% citrus pectin (Sunkist Growers, Inc., Ontario, CA 91764). The medium was adjusted to pH 6.0 prior to autoclaving for 15 min at 121 C. The medium was dispensed in 30-ml aliquots into 125-ml flasks, amended with CTL, and seeded with 6-mm-diameter plugs. The culture filtrate was harvested daily. At each harvest, cultures were centrifuged in 50-ml plastic tubes at 20,000 *g* for 10 min. Supernatants were kept for enzyme activity assay. Dry weights of the mycelium mat were obtained as described earlier. The supernatants were then dialyzed against distilled water at 4 C for 24 hr and used immediately or stored at 4 C. Protein content was measured with the Folin phenol reagent (14). PG activity was estimated viscometrically with a size 300 Ostwald-Fenske viscometer at 30 C. Reaction mixtures consisted of 3 ml of culture filtrate and 3 ml of 0.6% sodium polypectate (ICN Pharmaceuticals, Inc., Plainview, NY 11803) in 0.05 M citrate buffer, pH 4.5. The relative PG activity was recorded as percent decrease in flow time (PDFT) per milliliter of culture filtrate or enzyme preparation. The release of reducing sugar, a measure of exo-Pg, was studied by incubating a reaction mixture consisting of 2 ml of 1% polygalacturonic acid (ICN Pharmaceuticals) in 0.05 citrate buffer (pH 4.5) and 1 ml of culture filtrate. After 2 hr of incubation at 30 C, reducing sugar was measured by using a modified Somogyi method (19).

RESULTS

Growth characteristics. On PDA, mycelium of *S. minor* covered the 8.1-cm-diameter petri dishes in 4 days. Growth of *S. minor* after 3 days on PDA was greater ($P < 0.05$) than on CTL-amended PDA at all concentrations tested (0.5–8.0 μg/ml). Although growth of *S. minor* on CTL-amended (0.5 μg/ml and 1.0 μg/ml) PDA was inhibited for up to 3 days, growth did not differ from that on nonamended PDA after four days. At the fourth day of growth, brown sclerotial initials formed on PDA. Mature sclerotia were black and nearly spherical.

The arrangement of sclerotia on PDA was random over the entire surface of the agar. On PDA amended with low concentrations of CTL, sclerotia formed around the site of the agar disk and, as the colony grew, sclerotia formed in concentric rings. After 12 days of growth there was an average of 512, 616, 460, 440, 380, and 186 sclerotia per plate in plates containing 0, 0.5, 1.0, 2.0, 4.0, and 8.0 μg of CTL per milliliter (F-test significant, $P = 0.05$;

least significant difference = 231 sclerotia). Average sclerotial sizes were 0.95, 0.64, 1.69, 1.73, 1.53, and 2.90 mm in diameter on PDA with 0, 0.5, 1.0, 2.0, 4.0, and 8.0 μg of CTL per milliliter, respectively (F-test significant, $P = 0.05$ level; least significant difference = 0.4 mm).

Sectors developed along the margin of colonies of PDA amended with low concentrations of CTL. These sectors were usually well developed after 8 days. Sclerotia formed in the sectors, but in contrast to their parental mycelium on the same plate, sclerotia formed randomly as on nonamended PDA. The sectors had the same sclerotial size and abundance as their parental type. Frequency of sectoring varied considerably, but sectoring usually occurred at CTL concentrations less than 6 μg/ml and after at least 8 days of incubation.

Greenhouse inoculation. Lesions formed within 24 hr when 2-mo-old peanut plants were inoculated with oat grain inoculum. Lesion size was measured at 24-hr intervals. One-day-old lesions (average length, 7.8 mm) on plants inoculated with inoculum grown on nonamended medium were smaller ($P = 0.05$) than those of plants inoculated with CTL-treated inoculum (average length, 10.3 mm) (Fig. 1). While lesions induced by inoculum grown on CTL-amended (0.2 μg/ml) medium were not statistically longer than those induced by inoculum grown on nonamended medium after the first day of incubation, the initial enhancement of lesion size was obvious for the duration of the test (6 days). The experiment was repeated twice with similar results.

Conductivity assay. The electrolyte loss from lesions induced by 2 days of incubation with CTL-amended (0.2 μg/ml) inoculum was greater ($P = 0.01$) than that from lesions induced by nontreated inoculum of *S. minor* (Fig. 2). After 70 min, the conductivity readings were 157 and 46 μmhos in the bathing solutions containing lesions induced by CTL-amended and unamended inoculum, respectively (Fig. 2). Without the presence of *S. minor*, neither CTL-dipped oat grain nor CTL-dipped stem pieces contributed to electrolyte leakage. The experiment was repeated twice with similar results and the results of one trial are presented in Fig. 2.

Oxalic acid production. On nonamended and CTL-amended GSYS medium, mycelial weight increased linearly over time (Fig. 3A). Autolysis of *S. minor* occurred after 12 and 16 days in unamended and CTL-amended (0.2 μg/ml) medium, respectively. Thick puffs of mycelium of *S. minor* were frequently observed in CTL-amended medium.

In media containing no fungicide, the oxalic acid production was linearly correlated with growth (Fig. 3A and B). Accumulation of oxalic acid was 12.95 mg per flask at the 14th day. On CTL-amended (0.2 μg/ml) medium, oxalic acid production by *S. minor* was not correlated with mycelial dry weight (Fig. 3B). The production of oxalic acid in CTL-amended medium had a lag phase and then increased rapidly to a peak of 27.4 mg per flask at the eighth day. The increase in oxalic acid was reflected by a decrease in pH of the media with the pH often dropping to as low as 3.5.

The production of oxalic acid was also studied in media amended with 0.4, 0.6, and 0.8 μg CTL/ml. A similar pattern of oxalic acid production was observed at 0.2, 0.4, and 0.6 μg CTL/ml. However, at lower concentrations of CTL, *S. minor* produced more oxalic acid with a shorter lag period than at higher concentrations. Production of oxalic acid was inhibited at 0.8 μg CTL/ml.

Polygalacturonase (PG) production. In preliminary studies, *S. minor* produced small amounts of PG in a few of the synthetic media tested. In the basal medium employed by Bateman et al (4), *S. minor* grew well and the dry weight reached 12 mg in 7 days (Fig. 4A). Mycelial growth was significantly ($P = 0.05$) inhibited in the presence of 0.2 μg CTL/ml. The endo-Pg activity was low (3.7–9.2 PDFT) in the culture filtrate (Fig. 4B). Endo-PG activity was inhibited ($P = 0.05$) after 2 days incubation in the presence of CTL, but not at 3–6 days. Reducing sugars released by exo-Pg were less ($P = 0.05$) after 1 day of incubation in the presence of CTL (Fig. 4C). Cellulase (C_x) production by *S. minor* was also studied in the same manner as that of polygalacturonase. Results showed that CTL did not significantly affect the C_x production of the fungus.

DISCUSSION

Katan and Eshel (12) postulated four possible mechanisms whereby disease could be increased by a pesticide: direct stimulation of the pathogen, increased virulence of the pathogen, increased host susceptibility, and suppression of microorganisms antagonistic to the pathogen. These mechanisms of disease enhancement due to pesticides have been reported and reviewed by several individuals (1,12,21,27). Among these mechanisms, increase in virulence of the pathogen is the one least discussed. Katan and Eshel (12), however, emphasized that pathogens induce disease by means of sequential metabolic processes involving

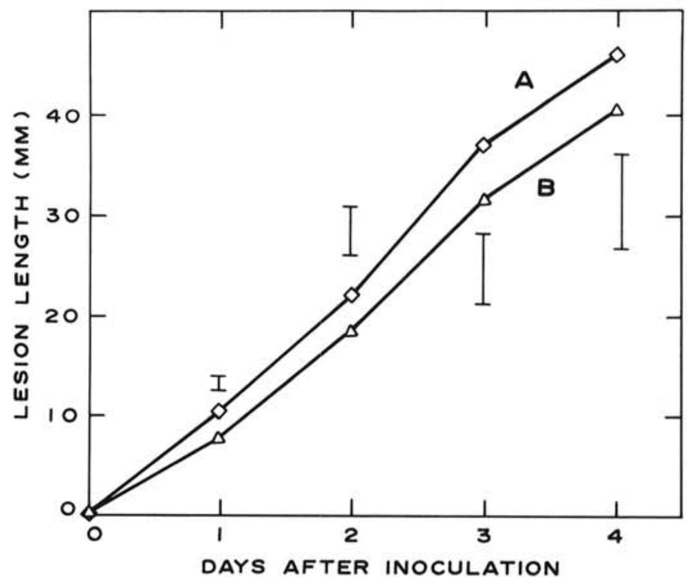

Fig. 1. Disease severity rated by lesion length measured 4 days after inoculation of peanut stems with inoculum of *Sclerotinia minor* grown on either **A**, medium amended with 0.2 μg of chlorothalonil per milliliter, or **B**, unamended medium. Each data point represents an average of lesion lengths in 18 plants. Vertical bars represent least significant difference at $P = 0.05$.

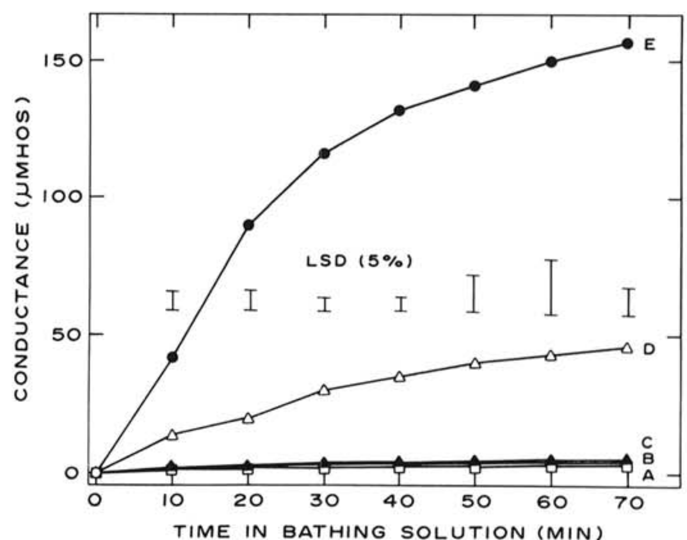

Fig. 2. Electrolyte leakage from peanut stem lesions induced by *Sclerotinia minor* inoculum grown on curve E, medium amended with chlorothalonil (CTL) (0.2 μg/ml), and curve D, by inoculum grown on nonamended medium. Three treatments served as controls: curve C, stems exposed to oat grain only; curve B, stems exposed to CTL-dipped oat grain; and curve A, CTL-dipped stem pieces exposed to sterilized oat grain. Vertical bars represent least significant difference at $P = 0.05$.

enzymatic activity and toxin production, and that environmental factors such as chemical stimuli or nutrients might enhance the processes and increase pathogen virulence. Papavizas and Lewis (21), however, suggested that increased virulence may not occur commonly.

Chlorothalonil (877–1,181 g/ha) may be applied to peanut foliage six to seven times (14-day intervals) during the growing season. Analysis of CTL concentration on debris at the soil surface indicated the presence of approximately 0.2–0.5 ppm of CTL during the months when fungicides were being applied. *S. minor* produced on oat grains amended with CTL (0.2 μg/ml) consistently, although not always significantly, produced larger lesions on peanut than did inoculum produced without CTL present. The effect of CTL on virulence was observed for 24 hr after inoculation with CTL-amended oat grains; however, as the fungus progressively invaded stem tissue distant from the oat grain inoculum, lesion length tended to increase at equal rates for both CTL-amended and nonamended inocula.

The mechanism of fungicidal action of CTL is attributed to reaction with the thiol groups of certain enzymes (29). Although the pathway of oxalate formation in fungi is not well understood, the precursors were shown to be dicarboxylic acid, and the pathways involved several enzyme systems (17). Our studies indicate that oxalic acid concentrations were consistently enhanced in culture filtrates of *S. minor* containing CTL. Porter and Lankow (25) recently reported morphological changes in cultures of *S. minor* when CTL was incorporated into the media. Morphological abnormalities were thought to arise from alteration of normal metabolic pathways by CTL. Excess formation of sclerotia and increased sclerotial size on CTL-amended media was also observed

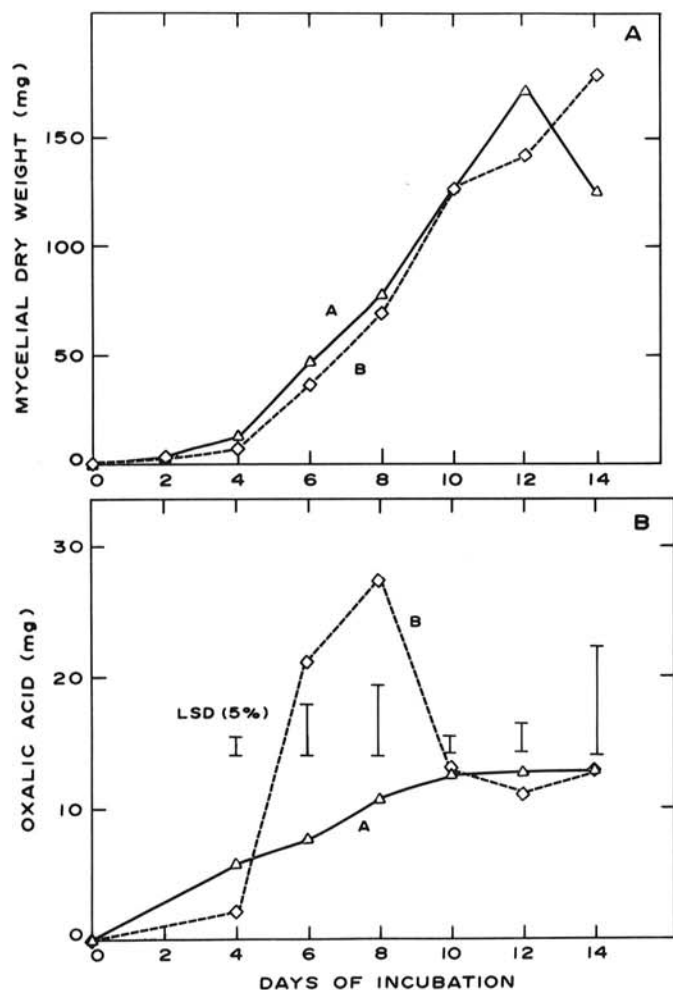

Fig. 3. Growth response and oxalic acid production by *Sclerotinia minor* grown on a glucose succinate yeast salt liquid medium nonamended and amended with chlorothalonil (CTL). **A,** Mycelial dry weight in nonamended medium (curve A) and 0.2 μg/ml CTL-amended medium (curve B). **B,** Oxalic acid production of *S. minor* grown on nonamended medium (curve A) and CTL-amended (0.2 μg/ml) medium (curve B). Vertical bar represents least significant difference (LSD) value at the $P = 0.05$ level.

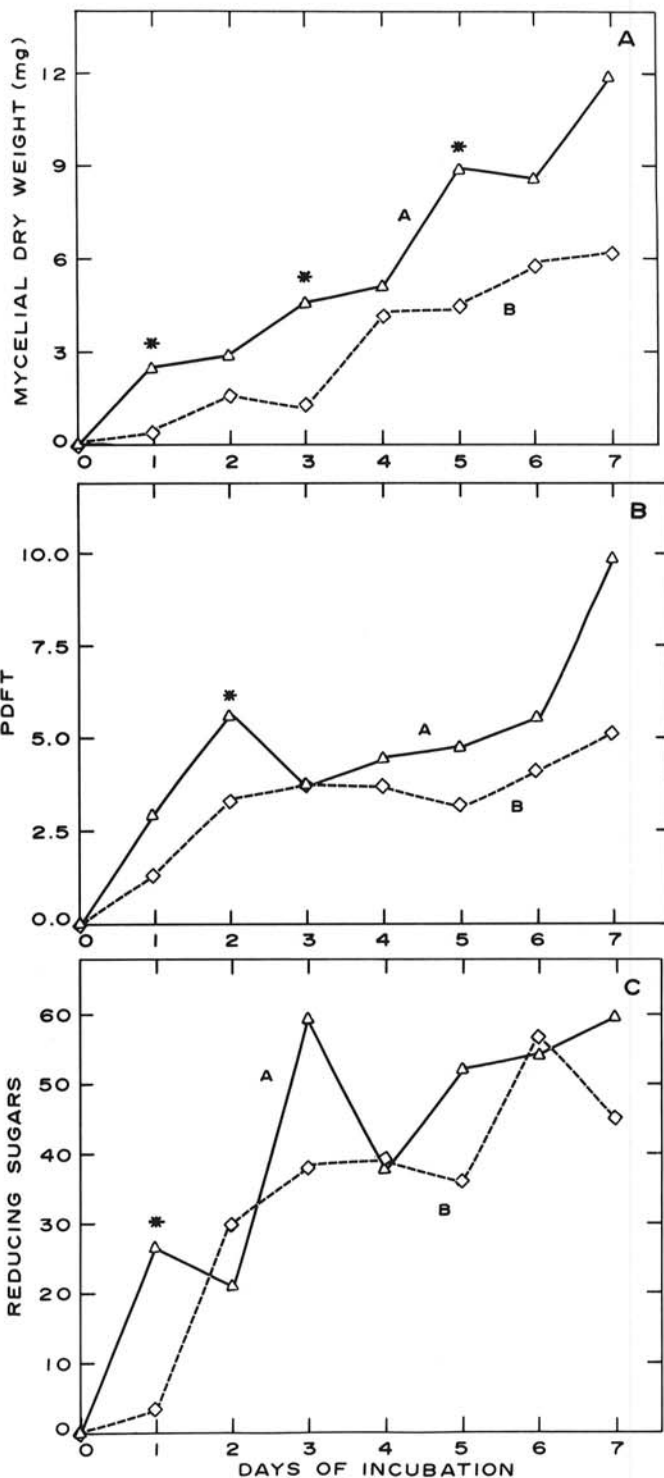

Fig. 4. Growth response and polygalacturonase production of *Sclerotinia minor* grown on Bateman et al (4) basal medium supplemented with 1% pure pectin. Treatments were medium amended with no chlorothalonil (CTL) (curve A) and medium amended with 0.2 μg CTL/ml (curve B). **A,** Mycelial dry weight. **B,** Endopolygalacturonase activity percent decrease in flow time (PDFT). **C,** Exo-polygalacturonase activity in micrograms of reducing sugars per milligram of protein per hour. Each data point represents average of three replicates. An asterisk represents significant difference between treatments at that day as determined by least significant difference at $P = 0.05$.

in the present study. The morphogenesis of sclerotia of *S. sclerotiorum*, a close relative of *S. minor*, has been recently studied (9). Humpherson-Jones and Cooke (10) showed that excessive sclerotial formation can be induced by acid staling compounds that accumulate in cultures. The formation of abnormal sclerotia observed in this study is thought to be a manifestation of alteration of sclerotial morphogenetic process caused by the effect of CTL on the thiol-containing enzyme system of the oxalate pathway.

Correlation of various enzymes or toxic substances with virulence has been difficult to establish for *Sclerotinia* species (16). Newton et al (20) developed a conductivity assay for measuring virulence based on the assumption that the capability of the pathogen to alter cell membrane permeability correlates with the virulence of the pathogen. The assumption is logical for *S. minor*, a typical necrotrophic pathogen. The present study showed a marked increase in electrolyte loss from lesions induced after incubation for 2 days with CTL-treated (0.2 μg/ml) inoculum of *S. minor*, compared to those lesions induced by nontreated inoculum. The increased electrolyte leakage may reflect an increased amount of oxalic acid production by *S. minor* in the presence of CTL. Electrolyte leakage also was thought to be attributable to membrane damage resulting from oxalic acid produced by the pathogen (16). The increased electrolyte loss could provide exogenous nutrients for the invading mycelium, resulting in an increased inoculum potential of *S. minor* as suggested by Katan and Eshel (12).

Growth of *S. minor* was not inhibited in GSYS medium containing a low amount of CTL. As *S. minor* grew, however, it produced excessive amounts of oxalic acid. During the early stages of disease development and at advancing margins of lesions, oxalic acid may work synergistically with pectinase (18). Oxalic acid is a chelator of calcium that enables the pectate portion of calcium pectate to be readily degraded by pectinase (3). Oxalic acid also affects the pH of the infected tissue. The secretion of oxalic acid would favor the activity of extracellular enzymes such as endo-PG, exo-PG (3,6), cellulase (15), and hemicellulase (7) with pH optima for activity in the range of 3 to 5. The direct effect of drastic change of pH may increase membrane permeability and death of host cells (8). Oxalic acid alone can induce wilting similar to that observed in peanut plants infected with *S. minor* (*unpublished*).

The role of pectolytic enzymes in pathogenesis of diseases caused by *Sclerotinia* species is well documented (16). Due to lack of information on peanut isolates of *S. minor*, experiments were conducted to measure the ability of this fungus to produce pectolytic enzymes. *S. minor* isolated from peanut produced only small amounts of pectolytic enzymes in several synthetic media tested. Pectin methyl trans-eliminase and polygalacturonic acid transeliminase were not detected (*unpublished*).

It has been shown that CTL was not only ineffective in controlling Sclerotinia blight, but it also enhanced disease severity in field research plots (5,22,23). The enhancement effect was not restricted to any particular peanut cultivar and location; however, enhancement was only reported in late season and in heavily infested fields (22,23). Increased virulence of *S. minor* in the presence of CTL within the peanut canopy would account more for an effect on disease severity than on disease incidence.

LITERATURE CITED

1. Altman, J., and Campbell, C. L. 1977. Effect of herbicides on plant disease. Annu. Rev. Phytopathol. 15:361-385.
2. Beckman, P. A., Rodriguez-Kabana, R., and Williams, J. C. 1975. The effect of peanut leafspot fungicides on the nontarget pathogen, *Sclerotium rolfsii*. Phytopathology 65:773-776.
3. Bateman, D. F., and Beer, S. V. 1965. Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by *Sclerotium rolfsii*. Phytopathology 55:204-211.
4. Bateman, D. F., Van Etten, H. D., English, P. D., Nevins, D. J., and Albersheim, P. 1969. Susceptibility to enzymatic degradation of cell walls from bean plants resistant and susceptible to *Rhizoctonia solani* Kuehn. Plant Physiol. 44:641-648.
5. Beute, M. K., Porter, D. M., and Hadley, B. A. 1975. Sclerotinia blight of peanut in North Carolina and Virginia and its chemical control. Plant Dis. Rep. 59:697-701.
6. Hancock, J. G. 1966. Degradation of pectic substances associated with pathogenesis by *Sclerotinia sclerotiorum* in sunflower and tomato stem. Phytopathology 55:975-979.
7. Hancock, J. G. 1967. Hemicellulose degradation in sunflower hypocotyls infected with *Sclerotinia sclerotiorum*. Phytopathology 57:203-206.
8. Hancock, J. G. 1972. Changes in cell membrane permeability in sunflower hypocotyls infected with *Sclerotinia sclerotiorum*. Plant Physiol. 49:358-364.
9. Humpherson-Jones, F. M., and Cooke, R. C. 1977. Morphogenesis in sclerotium-forming fungi. Part 2. Rhythmic production of sclerotia by *Sclerotinia sclerotiorum* (Lib.) deBary. New Phytol. 78:181-187.
10. Humpherson-Jones, F. M., and Cooke, R. C. 1977. Induction of sclerotium formation by acid staling compounds in *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*. Trans. Br. Mycol. Soc. 68:413-420.
11. Katan, J., and Lockwood, J. L. 1970. Effect of PCNB on colonization of alfalfa residues by fungi and streptomycetes in soil. Phytopathology 60:1578-1582.
12. Katan, J., and Eshel, Y. 1973. Interaction between herbicides and plant pathogens. Residue Rev. 45:145-177.
13. Kohn, L. M. 1979. A monographic revision of the genus *Sclerotinia*. Mycotaxon 9:365-444.
14. Lowry, O. H., Rosenbrough, N. J., Farr, A. I., and Randall, E. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193:265-275.
15. Lumsden, R. D. 1969. *Sclerotinia sclerotiorum* infection of bean and the production of cellulase. Phytopathology 59:653-657.
16. Lumsden, R. D. 1979. Histology and physiology of pathogenesis in plant diseases caused by *Sclerotinia* species. Phytopathology 69:890-896.
17. Maxwell, D. P. 1973. Oxalate formation in *Whetzelinia sclerotiorum* by oxaloacetate acetylhydrolase. Physiol. Plant Pathol. 3:279-288.
18. Maxwell, D. P., and Lumsden, R. D. 1970. Oxalic acid production by *Sclerotinia sclerotiorum* in infected bean and in culture. Phytopathology 60:1395-1398.
19. Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 133:375-380.
20. Newton, H. C., Maxwell, D. P., and Sequeira, L. 1973. A conductivity assay for measuring virulence of *Sclerotinia sclerotiorum*. Phytopathology 63:424-428.
21. Papavizas, G. C., and Lewis, J. A. 1979. Side-effects of pesticides on soil-borne pathogens. Pages 484–505 in: Soil-borne Plant Pathogens. B. Schipper and W. Gams, eds. Academic Press, London. 686 pp.
22. Porter, D. M. 1977. The effect of chlorothalonil and benomyl on the severity of Sclerotinia blight of peanut. Plant Dis. Rep. 61:995-998.
23. Porter, D. M. 1980. Increased severity of Sclerotinia blight in peanut treated with captafol and chlorothalonil. Plant Dis. 64:394-395.
24. Porter, D. M., and Beute, M. K. 1974. Sclerotinia blight of peanuts. Phytopathology 64:263-264.
25. Porter, D. M., and Lankow, R. K. 1981. Growth of *Sclerotinia minor* on media containing chlorothalonil and benomyl. Plant Dis. 65:591-594.
26. Porter, D. M., Powell, N. L., and Cobb, P. B. 1977. Severity of Sclerotinia blight of peanut as detected by infrared aerial photography. Peanut Sci. 4:475-477.
27. Rodriguez-Kabana, R., and Curl, E. A. 1980. Nontarget effects of pesticides on soilborne pathogens and diseases. Annu. Rev. Phytopathol. 18:311-320.
28. Spalding, D. H. 1970. Postharvest use of benomyl and thiobendazole to control blue mold rot development in pears. Plant Dis. Rep. 54:774-775.
29. Vincent, P. G., and Sisler, H. D. 1968. Mechanism of antifungal action of 2,4,5,6,tetrachloroisopthalonitrile. Physiol. Plant. 21:1249-1264.
30. Wadsworth, D. F. 1979. Sclerotinia blight of peanuts in Oklahoma and occurrence of the sexual stage of the pathogen. Peanut Sci. 6:77-79.
31. Warren, C. G., Sanders, P. I., and Cole, H., Jr. 1976. Increased severity of Pythium blight associated with the use of benzimidazole fungicides on creeping bentgrass. Plant Dis. Rep. 60:932-935.