Growth of *Sclerotinia minor* on Media Containing Chlorothalonil and Benomyl

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ABSTRACT

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Radial growth of *Sclerotinia minor* was strongly inhibited on potato-dextrose agar containing benomyl at 1–2 μg/ml. This sensitivity of *S. minor* to benomyl was not affected by the physiologic age of mycelium when transferred to amended media. Radial growth was inhibited on media containing chlorothalonil at 0.5–128 μg/ml, but sectors of rapidly growing mycelium developed at irregular points on the periphery of colonies. Inhibition of radial growth on media amended with chlorothalonil was directly related to physiologic age of mycelium at time of transfer. Growth was inhibited when mycelium was transferred from colonies of *S. minor* that had begun to differentiate sclerotia, but growth was not inhibited when mycelium was transferred from colonies that had not begun to differentiate sclerotia. The insensitivity of vegetative mycelium of *S. minor* to chlorothalonil may help to explain the increased severity of Sclerotinia blight in peanuts treated with this fungicide.

Sclerotinia blight, caused by *Sclerotinia minor* (Jagger) Kohn (9), has recently become a serious disease of peanut (*Arachis hypogaea* L.) in Virginia (3,16), North Carolina (3), and Oklahoma (18). This disease was first observed in Virginia in 1971 (14). Under favorable conditions, the disease colonizes and kills branches of the plant in contact with the soil. Pod yields of infected plants are greatly reduced (15).

Several fungicides, including DCNA (2,6-dichloro-4-nitroaniline), PCNB (pentachloronitrobenzene), and benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate], were partially effective in controlling Sclerotinia blight of peanuts in field experiments (3). However, at one site where Sclerotinia blight was severe, chlorothalonil (tetrachloroisophthalonitrile) was ineffective in controlling the blight. The disease was more severe in plots treated with chlorothalonil than in untreated plots. In field experiments, four applications of chlorothalonil (at 0.56, 1.12, 1.68, and 2.24 kg/ha) increased the severity of Sclerotinia blight and significantly reduced pod yields (12). Four applications of benomyl (at either 1.12 or 1.68 kg/ha) decreased disease incidence and significantly increased pod yields. Later field experiments verified these findings (13).

We do not know why Sclerotinia blight is more severe when peanut plants are treated with chlorothalonil. This study investigated the toxicity of benomyl and chlorothalonil incorporated in culture media against *S. minor* and examined cultures growing on amended media for unusual growth characteristics that might account for the increased severity of Sclerotinia blight in peanut plants treated with chlorothalonil. Portions of the report were previously presented (10,11).

MATERIALS AND METHODS

We measured the toxicity of chlorothalonil and benomyl to *S. minor* by observing the inhibition and characteristics of mycelial growth on nonamended potato-dextrose agar (PDA) and on PDA containing each fungicide. Chlorothalonil (75WP or 6F) and benomyl (50WP) were each suspended in water, and desired quantities were transferred by pipette into flasks containing as appropriate volume of partially cooled PDA. The contents of each flask were stirred while the fungicide was added, shaken for a minute, then poured into sterile plastic

Table 1. Radial growth of isolates of *Sclerotinia minor* on potato-dextrose agar with and without benomyl and chlorothalonil

Isolate	Fungicide	Concentration[a] (μg/ml)	Radial growth (mm)[b] 3 days	6 days	9 days	12 days
P-4-123	None	0	37.5[c]	37.5	37.5	37.5
	Benomyl	1	19.0	37.5	37.5	37.5
	Benomyl	2	9.0	15.0	22.5	37.5
	Chlorothalonil	1	0.0	2.5	9.5	34.0
	Chlorothalonil	2	0.0	1.0	2.5	19.5
P-4-136	None	0	14.0	37.5	37.5	37.5
	Benomyl	1	0.0	2.0	20.0	30.0
	Benomyl	2	0.0	0.0	6.5	18.5
	Chlorothalonil	1	0.0	0.0	7.0	35.0
	Chlorothalonil	2	0.0	0.0	0.0	9.5
P-4-182	None	0	22.0	37.5	37.5	37.5
	Benomyl	1	2.0	13.5	25.0	34.0
	Benomyl	2	0.0	5.5	10.5	21.5
	Chlorothalonil	1	0.0	1.5	8.0	16.0
	Chlorothalonil	2	0.0	0.0	2.0	6.0
P-4-199	None	0	37.5	37.5	37.5	37.5
	Benomyl	1	15.0	32.5	35.0	37.5
	Benomyl	2	2.5	14.5	21.5	30.5
	Chlorothalonil	1	17.5	37.5	37.5	37.5
	Chlorothalonil	2	6.5	22.5	28.0	37.5
P-4-209	None	0	37.5	37.5	37.5	37.5
	Benomyl	1	10.0	15.5	21.5	29.0
	Benomyl	2	4.5	6.5	9.5	17.5
	Chlorothalonil	1	3.0	21.0	37.5	37.5
	Chlorothalonil	2	1.0	8.5	30.5	35.0

[a] Concentration of active ingredient.
[b] Mean of five replications with 10 plates each.
[c] Mycelium covered entire plate.

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petri plates. When the medium had solidified, each plate received a 6-mm agar plug of mycelium taken from the periphery of a 3-day-old colony of *S. minor* growing on PDA. Plates were incubated at 22 C.

The diameter of the *S. minor* colony was measured from 1 to 12 days after incubation on nonamended PDA and on PDA containing benomyl and chlorothalonil. Cultures were examined daily for unusual growth characteristics. The 33 isolates of *S. minor* used were obtained from infected peanut plants in fields in Virginia. We present data for representative isolates.

RESULTS

Within 3 days, luxuriant mycelial growth of most isolates of *S. minor* covered the surface of nonamended PDA (Table 1). Sclerotial initials developed shortly after the mycelium covered the agar surface; within 6–8 days, several hundred sclerotia were produced randomly over the surface (Fig. 1A). Sclerotia were small, black, and irregularly shaped, resembling those normally associated with *S. minor* (14).

Most isolates produced some growth on PDA containing low concentrations of benomyl (Table 1), particularly after 3 days on PDA containing 1 μg/ml. At the higher concentration of benomyl, the inhibition of radial growth was more apparent. Mycelia grew evenly in all directions around the mycelial plugs and sclerotia were produced randomly over the surface of the colony. Insensitive sectors were not noted on media amended with benomyl.

The radial growth of *S. minor* was also inhibited by low concentrations of chlorothalonil (Table 1). Some isolates were less sensitive to chlorothalonil than others. Sclerotia were produced in concentric rings around the mycelial plug rather than randomly, as on nonamended PDA (Fig. 1B).

Sectors insensitive to chlorothalonil developed along the margins of colonies on media containing chlorothalonil at concentrations of 0.5–128 μg/ml. Frequency of occurrence ranged from 10 to 40%. Sectoring occurred along colony margins of 10-day-old cultures of *S. minor* growing on PDA containing 2

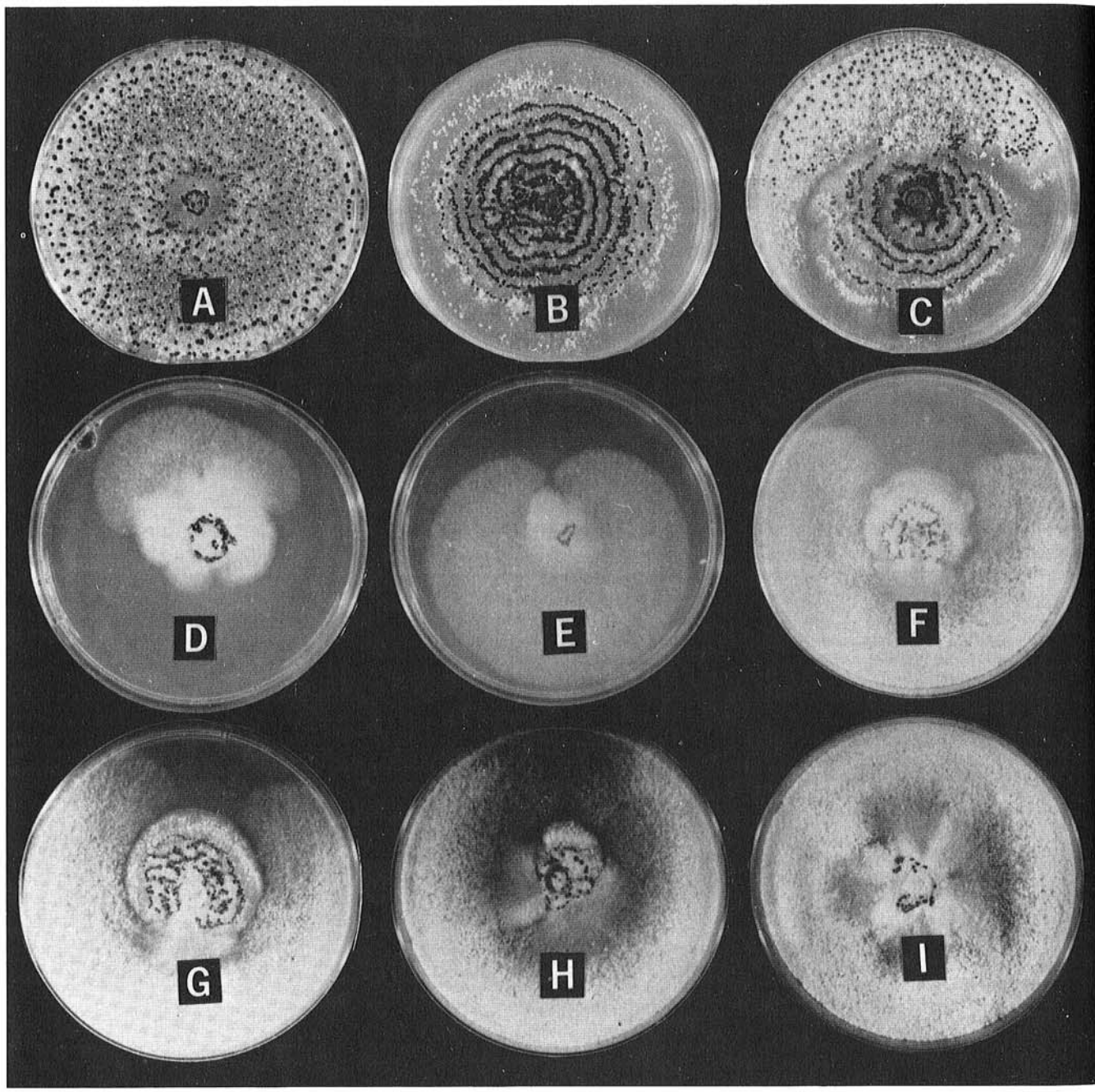

Fig. 1. *Sclerotinia minor*: 12-day-old cultures growing **(A)** on potato-dextrose agar and **(B,C)** on potato-dextrose agar containing chlorothalonil (1 μg/ml). **(D-I)** Sectors insensitive to chlorothalonil are shown after plate inoculation on potato-dextrose agar containing the fungicide (2 μg/ml).

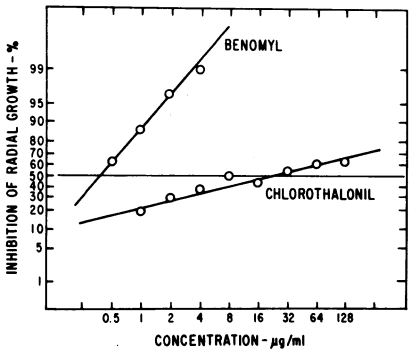

Fig. 2. Log-probability plots of the inhibition by chlorothalonil and benomyl of radial growth of four isolates of *Sclerotinia minor*.

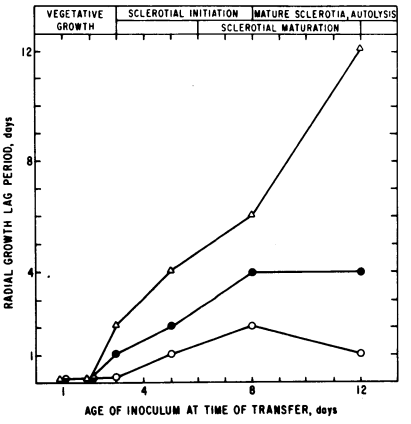

Fig. 3. Effect of chlorothalonil and inoculum age on the initiation of radial growth after transfer of *Sclerotinia minor* to new agar medium: o = no chlorothalonil, ● = chlorothalonil at 1 µg/ml, and Δ = chlorothalonil at 10 µg/ml.

µg/ml chlorothalonil (Fig. 1C). The insensitive sectors developed on both young and old colonies at a single point and spread rapidly to surround the original colony and coalesce on the opposite side of the inoculum plug. Multiple sectoring occurred occasionally (Fig. 1I). Humpherson-Jones and Cooke (6,8) have pointed out that *S. sclerotiorum* colonies displayed regions with different biochemical activities. The loci from which vegetative growth resumed may represent portions of the colony having lower levels of active chlorothalonil-sensitive biochemical pathways.

When the media received mycelial plugs removed from colony margins before sclerotial initials had formed, radial colony growth was much more inhibited on media containing benomyl than on those containing chlorothalonil. After 4 days of growth, combined log-probability plots for four *S. minor* isolates indicated ED_{50} values of 0.4 µg/ml and 25 µg/ml for benomyl and chlorothalonil, respectively (Fig. 2).

When mycelial plugs were removed from colonies that had reached the edge of the petri dish and begun to differentiate sclerotia, subsequent growth on PDA containing chlorothalonil was strongly inhibited. The inhibition was usually temporary, however, and individual plugs of mycelium gave rise to two types of mycelial growth. The first type was restricted and frequently produced sclerotia in concentric rings around the plug (Fig. 1B). This formation was not correlated to such physical effects as light. The second type, which was insensitive to chlorothalonil, grew rapidly from one or more distinct points on the periphery of the colony (Fig. 1C–H). The rate of radial growth of these chlorothalonil-insensitive sectors was indistinguishable on nonamended PDA and on PDA amended with chlorothalonil. The random arrangement of sclerotia on the sectored mycelium shown in Figure 1C was similar to that on the nonamended PDA shown in Figure 1A. The rapidly growing mycelia from PDA containing chlorothalonil were readily returned to a chlorothalonil-sensitive state merely by allowing the colony to reach the edge of the culture dish and initiate sclerotial differentiation.

The age of the colony from which the mycelial plug was transferred to amended media thus influenced the appearance of mycelium insensitive to chlorothalonil. Figure 3 depicts the relationship of colony age to the time required for vegetative growth to resume following transfer of mycelial plugs to media amended with chlorothalonil. Once sclerotial initials were formed, however, inhibition increased as reflected by the increasing time or lag period required for the resumption of growth.

DISCUSSION

Sclerotinia minor differentiates sclerotia when radial growth is restricted on culture media (4,8). Sclerotial differentiation involves the activation of both biochemical and morphogenetic processes (6,7). In this experiment, chlorothalonil inhibited at least one of the processes but did not inhibit the processes active during vegetative growth. Benomyl, on the other hand, inhibited biochemical processes active during both vegetative growth and sclerotial differentiation.

It was recently shown that chlorothalonil is not only ineffective in controlling Sclerotinia blight of peanuts but that it also enhances disease severity (3,11–13). The reasons for this enhancement are not clear, but several explanations are possible. Foliar disease control and management practices allow abundant peanut vine growth, which shades the soil surface. This moist, shaded environment beneath the foliar canopy is thought to be conducive to the growth of *S. minor*, a fungus that does not grow extensively in natural environments unless conditions are ideal. *S. minor* is also considered a poor fungal competitor.

The introduction of chlorothalonil into this environment might favor the growth of *S. minor* provided strains develop that are insensitive to the fungicide. Chlorothalonil could also reduce or eliminate populations of nontarget microorganisms, some of which may be antagonists or strong competitors of *S. minor*. Once these nontarget antagonists are eliminated, *S. minor* may grow unimpeded, especially if mycelia insensitive to chlorothalonil have developed. However, insensitive strains have not been recovered from peanut plants in fields where this fungicide was used.

Chlorothalonil-insensitive mycelia may also be capable of detoxifying the fungicide. The physiology of either the host plant or the pathogen may be altered by the presence of chlorothalonil in a way that would make the host more susceptible or the pathogen more virulent.

Either of these mechanisms could account in part for the increased severity of Sclerotinia blight following the use of chlorothalonil. In other crop diseases, the application of fungicides under field conditions has also favored various nontarget organisms. Soil applications of PCNB for clubroot control increased *Pythium* populations and subsequent postemergence damping-off (1,5). Similarly, Pythium stem rot of cowpeas (17) and southern stem rot of peanuts (2) increased in plots treated with benomyl.

Chlorothalonil is used extensively in Virginia and other regions to control the fungus that causes Cercospora leaf spot of peanuts. Because of the importance of Sclerotinia blight in Virginia, further research is under way to determine the exact nature of the phenomena responsible for the chlorothalonil-induced increase of this disease in peanut fields infested with *S. minor*.

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