Effect of Dibromochloropropane Fumigation on the Growth of Sclerotium rolfsii and on the Incidence of Southern Blight in Field-Grown Peanuts

R. Rodríguez-Kábana, M. K. Beute, and P. A. Backman

First and third authors, professor and associate professor, respectively, Department of Botany and Microbiology, Auburn University, Auburn, AL 36830; second author, professor, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27650. The research was supported in part by the Alabama Peanut Producers Association. Accepted for publication 25 May 1979.

ABSTRACT


The fumigant nematicide 1,2-dibromo-3-chloropropane (DBCP) was applied to soil infested with Sclerotium rolfsii contained in standard petri dishes. DBCP at concentrations less than 9.6 mg/dish (11.5 L/ha) resulted in stimulation of mycelial growth and production of sclerotial initials by S. rolfsii as measured 4 days after application; higher concentrations of DBCP resulted in either no change or less mycelial growth, and in fewer initials than those for the control. Counts of mature sclerotia at the 10th day were in indirect proportion to DBCP concentration and the number of colonies of Trichoderma spp. found on colonies of S. rolfsii increased. When sclerotia produced on nonsterile soil were exposed to vapor from 1 ml of emulsions containing 0-4% (v/v) DBCP in 24-cm-diameter desiccators with moist BaO, O supply, sclerotial germination was significantly stimulated by the vapor from emulsions containing 1-4% DBCP. Natural sclerotia on soil amended with decomposed peanut crop residue produced significantly more new sclerotia when exposed to DBCP vapor from 1 ml of a 1% (v/v) emulsion in the desiccators than those in desiccators without DBCP. Incidence of disease caused by S. rolfsii generally increased in Florunner peanut fields treated with DBCP over a 5-yr period.

Additional key words: nontarget effects.

Halogenated hydrocarbons have been the traditional fumigants for crops in soil infested with plant parasitic nematodes. The use of DBCP (1,2-dibromo-3-chloropropane) for preplant soil fumigation in commercial peanut production has increased exponentially in the southeastern USA in the past decade and has contributed to significant increases in peanut yields and quality. Widespread use of this fumigant in Alabama raised questions about its effects on nontarget organisms, and specifically its effect on Sclerotium rolfsii Sacc. which causes southern blight. DBCP has antifungal activity against Phytophthora ultimum (2), Rhizoctonia solani (1), and other pathogens (9). The possibility that DBCP results in more southern blight of tomatoes has been suggested (8). We decided to determine the effects of DBCP on S. rolfsii and on the incidence of southern blight in peanuts. This paper presents data on the effects of DBCP on cultures of S. rolfsii on nonsterile soil, its effect on the germination of sclerotia, and on the incidence of southern blight in fields planted to Florunner peanuts in southern Alabama.

MATERIALS AND METHODS

The isolate of S. rolfsii that was used in these experiments was obtained from infected peanuts (cultivar Florunner) collected at the Wiregrass Substation at Headland, Alabama. The isolate was grown on potato-dextrose agar (PDA) and transferred to autoclaved oat grains (4). After 8 days of growth on the grains, the oats were air dried and kept in the dark at 4 C.

Production of sclerotia. Out inoculum was spread on the flattened surface of moist (17% [w/w]; 1/3 bar) sandy loam (pH 6.1; organic matter <1%) from a soybean field; the 35 X 50 cm trays were filled with soil to a depth of 8 cm. The oats were added at the rate of one grain per cm² and the trays were covered with a layer of Saran® (Dow Chemical Co., Midland, MI 48640) wrap to reduce...
moisture loss. After 3 wk in the greenhouse, mature sclerotia were collected, air dried, and kept in a vial at room temperature (27 C) until used.

**Studies with soil plates.** The direct effect of DBCP on growth of *S. rolfsii* was studied in plates filled with nonsterile field soil. Soil plates were prepared with the sandy loam as previously described (10). Five infested oats were placed on the soil surface in 9-cm-diameter petri plates. Each plate was sprayed with 0.3 ml of the appropriate DBCP emulsion or water, and covered with the lid. Emulsions of DBCP were prepared from the 86 EC formulation of Fumazone® (Dow Chemical Co., Midland, MI 48640) formulation and contained the fungicide at the following concentrations by volume: 0, 0.125, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 4.0, or 8.0%. These concentrations when sprayed provided, respectively, 0, 0.6, 1.2, 2.4, 3.0, 4.8, 7.2, 9.6, 19.3, or 38.6 mg DBCP/plate which were equivalent to 0, 0.7, 1.4, 2.9, 4.3, 5.7, 8.6, 11.5, 23.0, or 46.0 L/ha, respectively. Six replicate soil plates were used for each concentration. Treated plates were incubated at 27 C for 10 days. Numbers of sclerotial initials, maturing initials, sclerotic, and degree of mycelial growth were determined at 4, 5, and 10 days after infestation of the plates. The degree of mycelial growth was estimated using a scale (10) in which 0 = no growth and 10 = maximal development. The number of colonies of *Trichoderma* spp. growing on colonies of *S. rolfsii* also was recorded.

**Tests for germination of natural sclerotia.** These tests were designed to determine the effect of DBCP on germination of sclerotia produced on nonsterile soil. Small sieves were made by cementing plastic rings (5.5 cm in diameter and 1 cm deep) to the border of circles of nylon screen (mesh = 1 mm) with silicone rubber. The sieves were filled with moist sandy loam, and the surface was smoothed by packing to form small soil plates. Ten sclerotia were implanted on the soil 1 cm from the edge of the plate. Seven plates with sclerotia were placed on a wire screen (1.5 cm mesh) in a 24-cm-diameter desicator. A 5-cm-diameter petri dish with 5 g BaO, and 10 ml of water also was placed on the wire screen to simultaneously absorb CO₂ and release O₂ (3). One milliliter of test emulsion was delivered to the bottom of the desiccator which was then sealed and kept at room temperature (27 C). Emulsions tested contained 0, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, or 8.0% (v/v) of DBCP as Fumazone 86 EC. After 48 hr, the number of sclerotia with any mycelial growth were counted by direct observation and the amount of growth rated according to a scale in which 1 = no growth and 4 = maximal growth.

A second study was designed to determine the influence of DBCP on the formation of new sclerotia from those implanted in the plates. Soil plates with sclerotia were prepared as described above, except the soil was amended with finely ground decomposed peanut crop debris at rates of 0, 1, 2, 4, and 8 g/500 g soil. The debris was collected in late February, 1976, from the surface of a peanut field and consisted mostly of partially decomposed peanut vines. Plates were divided into two groups; one group of desiccators with 1 ml of 1% (v/v) DBCP. Otherwise the experimental design was as described previously. After 7 days at room temperature, the desiccators were opened and the numbers of sclerotia formed per plate were counted.

**Field studies.** Field experiments with Flourunner peanuts were established in which DBCP was applied as Fumazone 86 EC at 9.35 L/ha (1 gal/acre) and compared with nontreated control. Soils in these experiments were sandy loams (pH 6.1–6.5) and the fields were under continuous peanut culture with winter fallow. Peanut production practices were those recommended for the area and included the use of chlorothalonil (tetrachloroisophthalonitrile) for control of Cercospora leaf spot and the herbicides benzin (N-butyl-N-ethyl-aa-aa-trifluoro-2, 6-dinitro-p-toluamide) and veronolate (5-propyl-dipropyl-thiocarbamate) applied pre-plant followed by alachlor (2-chloro-2, 6-diethyl-N-methoxymethyl acetanilide) at post plant but before peanut emergence for control of weeds. In each experiment, the treatments were represented by eight four-row plots, each measuring 0.9 × 9 m. Plots were part of larger experiments and were arranged in randomized complete blocks. Single experiments were conducted in 1972, 1973, 1974, and 1975; in 1976 there were two experiments. Incidence of southern blight was determined at the peak of its development each year (late August or early September just prior to harvest). Disease incidence was determined by counting the number of disease lori 30 cm or less in length in the two center rows of each plot as described previously (12).

**Statistical analysis.** All data were analyzed following standard procedures for analysis of variance and differences between means were evaluated for significance with the modified Duncan's multiple range test (13).

**RESULTS**

Effects of DBCP on growth of *S. rolfsii*. Numbers of sclerotial initials and mycelial growth 4 days after initiation of the experiment increased sharply above those in the controls in plates.
receiving DBCP at concentrations up to 4.8 mg per plate (Fig. 1A); higher concentrations resulted in reduction in sclerotial initials and growth in inverse proportion to the concentration of the fumigant. The number of immature sclerotia was indirectly proportional to DBCP concentration so that plates with concentrations higher than 2.4 mg contained few or no immature sclerotia. On the 5th day, numbers of sclerotial initials in DBCP-treated plates (Fig. 1B) were higher than those for the control except at the lowest concentration; however, these differences were significant ($P \leq 0.05$) only for plates with 3.0, 9.6, and 19.3 mg of the chemical. The numbers of immature sclerotia were significantly higher than those for the control in all plates with DBCP between 0.6 and 7.2 mg; plates with concentrations higher than 7.2 mg of DBCP per plate contained few or no mature sclerotia. At the final reading 10 days after initiation of the experiment (Fig. 1C), numbers of mature sclerotia had declined in proportion to DBCP concentration while numbers of colonies of Trichoderma spp. in S. rolfsii colonies had increased.

**Effect of DBCP on germination of field-grown sclerotia.** The presence of DBCP in the atmosphere of the desiccator significantly increased the percentage of sclerotia germinating when emulsions contained 1, 2, 3, or 4% DBCP (Fig. 2), but the 8% emulsion prevented germination. A similar response was observed for the growth; emulsions with 0.5, 1.2, and 4% DBCP stimulated growth of sclerotia (Fig. 2).

The numbers of new sclerotia produced from sclerotia implanted in soil was significantly higher in plates exposed to DBCP than in corresponding control plates (Fig. 3). Production of new sclerotia in DBCP-exposed plates increased with the concentration of debris between 0 and 2 g/500 g soil, but no additional increase was observed in higher concentrations. Numbers of new sclerotia in control plates were significantly higher only in plates with the highest concentrations of debris as compared with numbers from nonamended plates.

**Field studies.** Incidence of southern blight in field experiments increased significantly in DBCP-treated plots in all years except 1973 (Table 1). Disease incidence varied from as low as 18% for 1972 to as high as 46–90% in 1976. Combined analysis of the data indicated that the difference between the multiyear averages was significant and that it amounted to an 18.7% increase in southern blight. The environmental conditions during the 6-yr period were typical for the peanut growing area of south Alabama and included years of below normal (25–40 cm/season, 1972) and above normal (greater than 75 cm per season, in 1975) precipitation.

**DISCUSSION**

Our results indicate that DBCP has a direct effect on S. rolfsii. It is difficult, however, to extrapolate field use dosage of DBCP to the amounts used in our laboratory tests. DBCP is chiselled into the soil to a depth of 15–20 cm at rates of 9.3 L/ha (1 gal/acre) at planting. Consequently, concentration of the compound at the point of delivery in soil will be very high and would be equivalent to the highest concentrations used in our tests. As the nematicide diffuses out from the point of injection, a concentration gradient is gradually established so that all concentrations of DBCP used in the test could be expected to occur somewhere in the soil. The actual concentration of DBCP at any point in the soil would depend on the distance from the place of injection, time elapsed after injection, soil temperature, and the position of such a point because the pattern of diffusion is not spherical but pear-shaped (5).

Results of experiments with oat inoculum of S. rolfsii on soil represent the effect of DBCP on mycelial growth of the pathogen. These results point to a stimulatory effect of DBCP on production of disease loci per 30.5 m of row in the year:

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<tr>
<td>Control</td>
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<td>15.2a</td>
<td>6.0a</td>
<td>3.0a</td>
<td>10.28a</td>
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<tr>
<td>DBCP*</td>
<td>10.7b</td>
<td>19.8a</td>
<td>9.3a</td>
<td>18.9b</td>
<td>8.8b</td>
<td>5.7b</td>
<td>12.20b</td>
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<td>Increase (%)</td>
<td>18.9</td>
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<td>20.8</td>
<td>24.3</td>
<td>46.7</td>
<td>90.0</td>
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*Figures are averages of eight replications and represent numbers of disease loci (≤30 cm in length) in 30.5 m of row; figures within a column followed by the same letter were not significantly different ($P = 0.05$).

*Applied at 9.3 L/ha using two chisels per row 15 cm apart and 15–20 cm deep on each side of the seed furrow.
of sclerotial initials, few of which mature. The failure to mature is probably caused by colonization of S. rosfii by Trichoderma spp. in the final stages of growth when nutrients are likely to be limiting for the growth of the pathogen. The resultant reduction in numbers of mature sclerotia would not explain the increased incidence of southern blight observed in peanut fields.

Effects of DBCP on the germination of sclerotia are important in understanding disease development in the field. DBCP is applied at planting time in late April or early May, when sclerotia from the preceding season are dormant in soil. Detectable levels of southern blight do not develop in Alabama until mid-July (12) which indicates that sclerotia from the preceding season are dormant for a period of approximately 2 mo after planting until receiving a stimulus from the host to germinate. There is evidence that certain compounds stimulate sclerotia of S. rosfii to germinate (7). Our results indicate that DBCP is stimulatory for germination of dormant field-grown sclerotia. Once germination has occurred, the mycelium can use available organic debris to produce new sclerotia as demonstrated by our results. This process would lead to a higher inoculum density in DBCP-treated soil than in nontreated soil. Since the number of sclerotia in the soil relates to disease incidence (6,11), an increase in numbers of sclerotia at planting time when DBCP is applied would be expected to result in higher incidence of southern blight later in the season. Our results strongly suggest that this mechanism is operative in peanut fields treated with DBCP. The amount of crop debris present at planting time would be critical for production of new sclerotia from dormant sclerotia stimulated to germinate by DBCP. Dry weight of available crop debris on the surface on peanut fields in Alabama before planting in early April amounts to 4–5 MT/ha (R. Rodríguez-Kábana and P. A. Backman, unpublished). This debris is of the type used in our experiment and is equivalent to concentrations of about 1–4 g/500 g soil. Our results indicate that this debris is sufficient to provide substrate for development of significantly higher numbers of new sclerotia from sclerotia treated with DBCP. The recent ban on the use of DBCP in peanuts probably will result in a shift to other fungimags of similar chemical composition (eg. D-D). It is possible that other halogenated hydrocarbons may stimulate germination of dormant sclerotia of S. rosfii and result in increased disease incidence, as was reported for DD with southern blight in tomatoes (8). We suggest that when these alternative fungimags are used, the incidence of southern blight should be closely monitored.

LITERATURE CITED