Effects of Rapeseed Meal Soil Amendments on Microsclerotia of *Cylindrocladium crotalariae* in Naturally-Infested Soil

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ABSTRACT

Cylindrocladium black rot (CBR) of peanut, caused by *Cylindrocladium crotalariae*, is most often controlled through the use of metham sodium (Vapam), a commercial soil fumigant. Several plant species, including rape, produce glucosinolates which decompose to form isothiocyanates which are closely related to methyl isothiocyanate the active ingredient of metham sodium. Such plants may be useful in CBR control strategies. This research was conducted to determine the fungicidal effect of the glucosinolate in rapeseed meal on microsclerotia of *C. crotalariae*. Rapeseed meal containing 13 mmol of glucosinolate kg^{-1} was added to a soil column 50 mm in diameter and 325 mm long. Meal was either placed at the 150 mm depth or mixed with the top 150 mm of soil. An untreated control and metham sodium at a rate equivalent to 190 L ha^{-1} injected 150 mm deep were included. Metham sodium was much more effective in reducing microsclerotial populations of *C. crotalariae* than rapeseed meal. However, the amount of glucosinolate in the metham sodium treatment was three times as great as that found in the rapeseed meal. Rapeseed meal treatments usually significantly reduced the number of soilborne microsclerotia when comparisons were made with untreated soil.

Key Words: glucosinolates, Cylindrocladium black rot, *Brassica napus*, isothiocyanates.

Cylindrocladium black rot (CBR) of peanut (*Arachis hypogaea* L.) is caused by the soilborne fungus *Calonectria crotalariae* (Loos) Bell and Sobers [*Cylindrocladium crotalariae* (Loos) Bell and Sobers] (Bell and Sobers, 1966). This disease is one of the most damaging diseases of peanut in the Virginia-North Carolina (VC) peanut growing area. Cylindrocladium black rot was first reported in the VC area in 1970 and reached epidemic proportions by 1975 (Garren *et al.*, 1971; Garren and Coffelt, 1976). Annually, peanut plants grown on 4 to 6% of the peanut acreage in the VC area exhibit symptoms of CBR.

Partial control of *C. crotalariae* can be achieved with resistant varieties (Wynne *et al.*, 1990), planting date (Sidebottom and Beute, 1989), cultural practices (Krigsvold *et al.*, 1977), and nematode control (Diomonde and Beute, 1981). Soil fumigation with sodium N-methyldithiocarbamate (metham sodium) also provides excellent control (Phipps, 1990). Metham sodium decomposes in the soil to form methyl isothiocyanate, a biocide which is toxic to soil biota at concentrations as low as 0.04 mg kg^{-1} (Lewis and Papavizas, 1971).

Cruciferous plants such as rape (*Brassica napus* L.) and other *Brassica* species contain the class of compounds, called glucosinolates. Glucosinolates also can decompose to form isothiocyanate compounds (VanEtten and Tookey, 1978). Traditionally, rape has been grown in many countries as an oilseed crop (Shahidi, 1990). It is also often used as a green manure crop. Growers in Europe long have used rape as a break crop in cereal rotations (Ward *et al.*, 1985). Several studies have shown that a green manure crop of rape may reduce disease incidence in subsequent crops and increase yields (Avazov, 1974; Diercks *et al.*, 1980; Mannapova, 1976). Other studies have shown that cruciferous amendments containing glucosinolates reduced the levels of *Aphanomyces* root rot in infested soils (Papavizas, 1966; Papavizas, 1967; Papavizas and Lewis, 1971). Growing rape in soils infested with *Aphanomyces euteiches* (Dreches) significantly reduced disease severity in pea (*Pisum sativum*) (Chan and Close, 1987). The observed fungicidal effects were thought to have been due to the presence of glucosinolates contained in the amendment of plant materials.

This research was conducted to determine the fungicidal effect of glucosinolate in rapeseed meal on microsclerotia of *C. crotalariae* in naturally infested soil.

Materials and Methods

Soil (top 50 to 100 mm) was collected from two fields in Suffolk, Virginia, having a history of CBR when peanuts were planted in a two-year rotation (peanut/corn). Soil one was a Nansemond loamy fine sand (Aquic Hapludult) and was planted to corn (*Zea mays* L.) at the time soil was collected. Soil two was a Eunola loamy fine sand (Aquic Hapludult). The field was sampled shortly after peanuts exhibiting typcial CBR symptoms had been harvested. Soils one and two were sieved (soil passed through a 2 mm stainless steel sieve) and then stored in sealed containers. Soil one was used as the microsclerotia-infested soil for trials one through four and soil two was used as the microsclerotia-infested soil for trials five and six. In trials one and five, moist soil was used for the microsclerotia infested soil. In the other trials the microsclerotia infested soil was air dried prior to use. The soil was dried 6, 38, 120, and 4 days before use for trials 2, 3, 4, and 6, respectively.

Soil from a disease-free site was obtained from a fallow field of Kenansville loamy sand (Arenic Hapludult). This soil was air dried, sieved to pass 2 mm and stored dry. The soil was dry for over 360 days before use.

Rapeseed meal (cv. Dwarf Essex) containing 13 mmol kg^{-1} of glucosinolate from which oil had been expeller extracted was obtained from the University of Idaho (D. L. Auld). The cultivar used is high in glucosinolates compared to other varieties available. The glucosinolate concentration of the meal was determined by gas chromatograph after water extraction as described by Davis (1988). The application rate for all rapeseed meal treatments was equivalent to 990 g m^{-2}.

Columns (325 mm long) were made from schedule 40 PVC pipe having an inside diameter of 50 mm. Microsclerotia-free soil from a disease-free site was packed in a column to a depth of 150 mm and a bulk density of 1.6 g mL^{-1}. One of four treatments was then applied. In the untreated check, 150 mm of infested soil packed to a bulk density of 1.6 g mL^{-1} was placed on top of the microsclerotia-free soil. For the second treatment, Vapam, a commercial formulation of metham sodium, was added to the top of the microsclerotia-free soil at a rate equivalent to 19 mL of Vapam m^{-2}. Then 150 mm of microsclerotia-infested soil packed to a bulk density of 1.6 g mL^{-1} was added. In the third treatment, rapeseed meal at a rate equivalent to 990 g m^{-2} was added to the top of the microsclerotia-free soil and 150 mm of microsclerotia-infested soil at a bulk density of 1.6 mL^{-1} was placed on top of the meal. This arrangement simulated a deep banding application method. In the fourth treatment, the rapeseed meal at a rate equivalent to 990 g m^{-2} was mixed with the microsclerotia-infested soil and

[1]Mention of firm names or trade products in this paper does not constitute a recommendation by the USDA nor does it imply registration under FIFRA.

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then 150 mm of the soil-meal mixture at a bulk density of 1.6 g m^{-2} was placed on top of the microsclerotia-free soil. Water was then added to bring the soil columns to field capacity and the columns were covered with a piece of cheesecloth. This arrangement allowed free diffusion of gases into and out of the soil. The treatments were replicated six times.

The columns were incubated 14 d at room temperature. After 7 d water lost by evaporation was replaced (15 mL/column). Following incubation, the upper 150 mm layer of soil was removed from each column and divided into two depths (depth 1: 0 to 75 mm (top portion) and depth 2: 75 to 150 mm). After mixing, a subsample was taken from each sample for moisture determination and a second for microsclerotia assay. Soil moisture content was determined gravimetrically after oven drying at 110 C for 48 h and the number of microsclerotia of *C. crotalariae* in each sample was determined by extraction with an elutriater and counting the number of viable microsclerotia after 10 d of incubation on a *C. crotalariae* specific media (Phipps, 1976).

Data were subjected to statistical analyses using the GLM procedure (general linear model) of SAS for personal computers (SAS Institute, 1987).

Results and Discussion

Depth of soil in columns affected survival of microsclerotia of *C. crotalariae* in most cases. Microsclerotia per gram of dry soil at depth one, the uppermost soil layer in columns, was usually not influenced by treatment (Tables 1, 2 and 3). Slight differences, however, were noted in Trial 1 (Table 1). The lack of response may be due to inadequate concentration of isothiocyanates in the upper soil layer of the columns.

At depth two, microsclerotia numbers were usually significantly lower in Vapam-treated soil than in soil receiving rapeseed meal. In trials 1, 2, 4, and 6 (Tables 1, 2, and 3), microsclerotia numbers were significantly lower in rapeseed meal-treated soil than in the untreated soil. In the four trials with soil one, microsclerotial densities in Vapam-treated soil were near zero, supporting the conclusions of Phipps (1990) and demonstrating the efficacy of the commercial treatment. Vapam was almost as effective in reducing microsclerotial populations in soil two where populations were higher than soil one (Table 3). High microsclerotial populations may account in part for the lack of complete control of CBR with Vapam in certain peanut fields. In this study, soils one and two were similar both physically and chemically. There were no obvious differences between these two soils which would account for the differences noted in reduction of microsclerotia in Vapam-treated soil.

The method of rapeseed meal application (unmixed vs. mixed) did not differ significantly in the effect of the amendment on microsclerotial populations (Tables 2 and 3). Numbers of microsclerotia in mixed soil were often less than those in unmixed soil but differences were not significant. Differences were more pronounced in soils containing high microsclerotia populations. Chan and Close (1987) have reported similar reductions of infestations of *Aphanomyces* in soil treated with rapeseed plant parts.

Soil moisture and the length of time that the soil had been dry appeared to influence microsclerotial populations of *C. crotalariae*. In Trials 1 and 5 (Tables 1 and 3), the microsclerotia-infested soil had been stored at the moisture level at which it was collected (approximately 10% water) while in the other four trials the soil had been air dried before it was used (Tables 1, 2, and 3). The amount of time the soils had been dry varied from zero in Trials 1 and 5 to 120 days in Trial 4. In Trials 2 and 6 (Tables 1 and 3), soil was air dried a only a few days before it was used in the columns. In soil one, microsclerotia densities declined as the length of time the soil had been dried increased (Tables 1 and 2). The microsclerotia numbers were very similar in Trials 1 and 2 where the only difference was drying the soil a few days before it was used. In soil two, microsclerotia were more numerous in moist soil than in dry soil (Table 3). This is in agreement with others showing moisture plays an important

Table 1. Survival of *C. crotalariae* microsclerotia in peanut field soil treated with Vapam or rapeseed meal (number per g of dry soil in soil 1, trials 1 and 2).

	Depth	
Treatment	0 to 75 mm	75 to 150 mm
Trial 1 - Soil one, moist condition.		
Untreated	26.4 ab*	29.7 a
Vapam	16.5 b	0.2 c
Rapeseed Meal Unmixed	32.1 a	8.1 b
Trial 2 - Soil one, air dry, stored 6 days after drying.		
Untreated	26.4 a	29.7 a
Vapam	16.6 a	0.3 b
Rapeseed Meal Unmixed	31.5 a	7.8 b

* Values within the same column and trial followed by the same letter are not significantly different by the Waller-Duncan mean separation test with a K-ratio of 100.

Table 2. Survival of *C. crotalariae* microsclerotia in peanut field soil treated with Vapam or rapeseed meal (number per g of dry soil in soil 1, trials 3 and 4).

	Depth	
Treatment	0 to 75 mm	75 to 150 mm
Trial 3 - Soil one, air dry, stored 38 days after drying.		
Untreated	6.2 a*	10.2 a
Vapam	8.3 a	0.4 b
Rapeseed Meal Unmixed	9.1 a	10.0 a
Trial 4 - Soil one, air dry, stored 120 days after drying.		
Untreated	8.8 a	12.3 a
Vapam	6.0 a	0.2 c
Rapeseed Meal Unmixed	8.2 a	4.1 b
Rapeseed Meal Mixed	8.0 a	4.8 b

* Values within the same column and trial followed by the same letter are not significantly different by the Waller-Duncan mean separation test with a K-ratio of 100.

Table 3. Survival of *C. crotalariae* microsclerotia in peanut field soil treated with Vapam or rapeseed meal (number per g of dry soil in soil 2, trials 5 and 6).

	Depth	
Treatment	0 to 75 mm	75 to 150 mm
Trial 5 - Soil two, air dry, moist condition.		
Untreated	120.3 a*	160.2 a
Vapam	136.8 a	112.7 b
Rapeseed Meal Unmixed	137.3 a	151.0 ab
Rapeseed Meal Mixed	133.2 a	128.3 ab
Trial 6 - Soil two, air dry, stored 4 days after drying.		
Untreated	54.2 a	97.5 a
Vapam	55.4 a	8.9 c
Rapeseed Meal Unmixed	51.9 a	61.1 b
Rapeseed Meal Mixed	33.3 a	49.3 b

* Values within the same column and trial followed by the same letter are not significantly different by the Waller-Duncan mean separation test with a K-ratio of 100.

role in the epidemiology of CBR (Phipps and Beute, 1977).

In this study, the amount of Vapam applied was equivalent to the upper limits currently recommended (Phipps, 1990). Based on the dry matter and glucosinolate data of Davis (1988), the amount of rapeseed meal (990 g m^{-2}) and amount of glucosinolate applied was in the range that can be produced with a rape cover crop. The potential concentrations of isothiocyanates were three times higher in the Vapam-treated soil than in the rapeseed meal amended soil and may explain why rapeseed meal was not as effective in reducing microsclerotia of *C. crotalariae* as Vapam. Also, the rate of formation of the isothiocyanate and the fungicidal activity of the specific isothiocyanates formed from glucosinolate in the rapeseed meal may not be as high as that from Vapam. Other factors, such as formation of metabolites from microbial decomposition of the rapeseed meal could affect both the efficacy of the isothiocyanates formed, and the viability of the microsclerotia. Decomposition of the rapeseed meal also could promote the growth of soil organisms that could hinder the development of *C. crotalariae* microsclerotia.

These initial trials with rapeseed meal suggest that glucosinolate containing plant materials may have potential for use in managing CBR infestations in peanut fields. Several other plant species also contain glucosinolates and may be biocidal against microsclerotia. While cover crops, such as rape, may never be totally effective in controlling CBR, their use in crop production may allow use of reduced rates of fumigant, especially when used in conjunction with partially resistant cultivars.

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Accepted May 9, 1992