# Effect of metam sodium, peanut genotype and inoculum density on incidence of Cylindrocladium black rot[1]

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**ABSTRACT**

Metam sodium was applied as an in-row preplant fumigant in field microplots planted with three peanut genotypes. Preplant inoculum densities of *Cylindrocladium crotalariae* were reduced 67% as compared to untreated controls. End-of-season inoculum densities were reduced an average of 32%. Across-plot soil sampling revealed no microsclerotia in treated row centers. Plantings of CBR-susceptible cultivar Florigiant with metam sodium treatments resulted in partial control of pod and root rot (caused by *C. crotalariae),* but high final inoculum densities. Metam sodium with moderately-resistant NC 8C resulted in reduced pod and root rot and intermediate levels of inoculum density. Lowest levels of pod rot and root rot occurred with metam sodium and highly resistant genotype NC 18016. Inoculum density did not increase where NC 18016 was used. With all genotypes, resistance was the dominant factor in maintaining low final inoculum levels.

Key Words: *Arachis hypogaea, Cylindrocladium crotalariae,* metam sodium, root rot resistance.

Cylindrocladium black rot (CBR) caused by *Cylindrocladium crotalariae* (Loos) Bell & Sobers is a major disease of peanut *(Arachis hypogaea* L.) in North Carolina (3). Microsclerotia (ms) are the primary source of inoculum as well as survival and dispersal units (9). Inoculum distribution in fields is clustered, resulting in "hot spots' of disease in infested fields (9).

Current recommendations for control include the use of resistant cultivar NC 8C in combination with in-furrow fumigation using metam sodium (1). Rotation with nonleguminous crops such as corn may also aid in reducing CBR (5). Natural reduction of the pathogen populations can occur with sustained low winter temperatures (13).

Metam sodium is a soil fumigant that releases methylisothiocyanate (MIT) after injection into soil (17). MIT-liberators are effective fumigants, dispersing by volatilization. Water sealing of soil surface improves dispersal and longevity, and release of MIT is facilitated by contact with soil water. Soil type, percent organic matter, and percent soil moisture are important in determining the effective rate. Phytotoxicity occurs if planting follows fumigation too closely (17). Metam sodium injected into irrigation water can be used as a soil drench. Injection of metam sodium in irrigation water is successful as a preplant fumigant for other peanut pathogens (10) in regions where climate and soil type permit.

Major yield losses with susceptible genotypes in CBR-infested fields have led to development and use of resistant genotypes (12). Heritability of resistance to CBR is additive (7) and is facilitated by the formation of wound response structures which block further ingress by the pathogen (8). Although partially resistant cultivars reduce yield losses, additional strategies may be needed to reduce inoculum density (6).

The objectives of this study were to determine the initial effects of metam sodium on in-row ms levels and to evaluate any possible long-term effects of fumigation. The relative effects of metam sodium and host resistance on initial and final inoculum densities of *C. crotalariae* were also studied.

## Materials and Methods

Microplots (76 cm dia x 60 cm deep, fiberglass walled) were established in April 1977 in methyl bromide treated Norfolk loamy sand at the North Carolina State University (NCSU) Central Crops Research Station at Clayton (2). Soil was infested with *C. crotalariae* microsclerotia (ms) in 1979 for studies on effects of crop rotation on Cylindrocladium root rot of peanut (5,6).

In November 1982, 90 microplots were grouped into five blocks and soil from all 18 plots in each block was removed to 20 cm depth, uniformly mixed and replaced in plots. Approximately 500g of soil was sampled from each microplot using a soil probe (cores 15x2 cm). Samples were sieved (2.38 mm opening) mixed 1 min by shaking and stored at approximately 25 C in closed polyethelene bags until assayed (4). Subsamples were taken from each sample and assayed for *C. crotalariae* using elutriation and plating on semi-selective medium (15). Inoculum densities in ms/g soil were calculated for each microplot. Additional pretreatment samples were taken and assayed in March 1983.

A pre-plant treatment of metam sodium (sodium N-methyldithiocarbamate) was applied in-row in selected plots in April 1983. Trenches were made in soil (15 cm deep) in a circular "row" 19 cm from the microplot wall. Metam sodium was applied at 12.8 mL/plot (10 gal/acre equivalent rate). The 12.8 mL of metam sodium was diluted with water to 200 mL and dispensed in each of the 45 trenches to be treated using a separatory funnel. The remaining 45 microplots were treated similarly using water only. All trenches were closed and 200 mL of water was applied on top to seal each trench.

Three peanut genotypes were selected to represent varying susceptibilities to root rot caused by *C. crotalariae:* Florigiant (highly susceptible), NC 8C (moderately resistant) and NC 18016 (highly resistant) (12). Seeds were planted (May 27) along the line of metam sodium application and thinned to four plants per plot. Genotypes and metam sodium treatments were arranged in a factorial design with five randomized complete blocks.

In June 1983, five microplots treated with metam sodium were chosen at random to determine spatial effects of metam sodium application. Soil cores were collected at 5 cm intervals in a straight line across each microplot. Each of 15 cores/plot was individually elutriated and assayed for inoculum density. Five randomly chosen plots not treated with metam sodium were sampled and assayed similarly. Bulk samples were assayed from the remaining plots.

Microplots were harvested in November 1983. The four plants in each plot were washed and CBR root rot was rated on a scale of 0-5 (0 = healthy, 5 = severe rot) (14). Pod rot symptoms were characteristic of CBR (16) and *C. crotalatiae* was confirmed as the causal pathogen by isolation on Cylindrocladium selective medium (15). Pod rot was determined as percent of pods rotted. Data from the four plants in each plot was combined to give an average root rot and pod rot rating for each plot.

In November 1983, bulk soil samples were taken and assayed from all plots. Microplots were grouped into high (<23.5), and low (>7.5) categories based on inoculum density (ms/g soil). Soil from the 30 plots in each group was removed, uniformly mixed and returned to plots to insure inoculum uniformity within groups. Cylindrocladium inoculum density was introduced into the experimental design in 1984, with all 18

[1]Paper No. 10444 of the Journal Series of the North Carolina Agriculture Research Service, Raleigh, N. C. 27695-7601. Use of trade names in this publication does not imply endorsement by the North Carolina Agriculture Research Service of the products named nor criticism of similar ones not mentioned.

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treatments re-randomized in five blocked replications. Plots were sampled before treatment in March 1984. Plots were fumigated and planted as previously described. Post-plant soil samples were taken and assayed in June 1984.

In October 1984, eight plots were selected from each of the five blocks to include all combinations of treatments: metam sodium vs no metam sodium, susceptible vs resistant, and high vs low inoculum density. Soil samples were taken systematically from each plot by sampling in five concentric rings at approximately 6.3 cm intervals from the center of each plot to the outer edge. Soil cores were composited for each ring from each plot. Samples were then elutriated and assayed for inoculum density. Plots were harvested in November and disease was evaluated as previously described.

## Results

*Effect of metam sodium on inoculum density.* Treatment with metam sodium reduced inoculum density in all plots by an average of 72.8% in June 1983, and 61.4% in June 1984, as compared to nontreated plots (Table 1). End-of-season inoculum densities averaged over all plots (genotypes) were reduced by 57.5% in November 1983, and 6.1% in November 1984, as compared to nontreated plots.

**Table 1. Effect of metam sodium on inoculum density levels at various periods after application.**

| Dates:[a] | Mar 83 | Jun 83 | Nov 83 | Mar 84 | Jun 84 | Nov 84 |
|---|---|---|---|---|---|---|
| Metam sodium[b] | (25.39a)[c] | 5.99b | 11.45b[d] | (16.58a)[c] | 4.60b | 39.48a[d] |
| Non-treated | 26.46a | 22.02a | 26.91a | 16.71a | 11.93a | 42.04a |

[a] Mean values on a given date with the same letter are not different using Fisher's least significant difference (LSD) at p=0.05.

[b] Metam sodium applied in-row (preplant) in mid-April of each year at 12.8 mL/plot (10 gal/acre equivalent).

[c] Mean values for March represent inoculum densities prior to application of metam sodium for that year. Inoculum density = microsclerotia/g soil.

[d] Mean values for Nov represent inoculum densities averaged over plots for all genotypes.

Across-plot sampling in June 1983 revealed no ms of *C. crotalariae* surviving to a sample depth of 15 cm in the centers of rows treated with metam sodium. Microsclerotia populations were reduced in a band along the line of metam sodium application with ms concentrations increasing with distance from the center of the row (Fig 1). Plots not treated with metam sodium showed variable concentrations of *C. crotalariae* with means consistently higher than treated plots.

In October 1984, late season across-plot sampling showed high concentrations of ms in row centers (Fig 2). Highest (P = 0.05) concentrations of ms occurred in the tap root area of plants in nontreated plots. Average inoculum densities, however, were 18% lower in metam sodium treated plots as compared with nontreated plots.

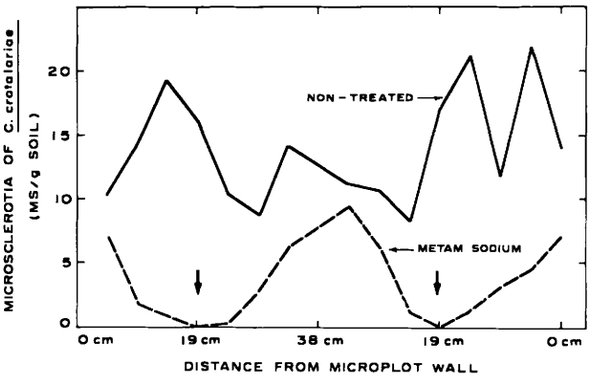


**Fig. 1. Concentrations of *Cylindrocladium crotalariae* in metam sodium-treated vs nontreated microplots in June 1983. Metam sodium applied at 19 cm from the microplot wall at a rate of 12.8 mL/plot (10 gal/acre equivalent) in mid-April ( ↓ ) indicates fumigation site.**

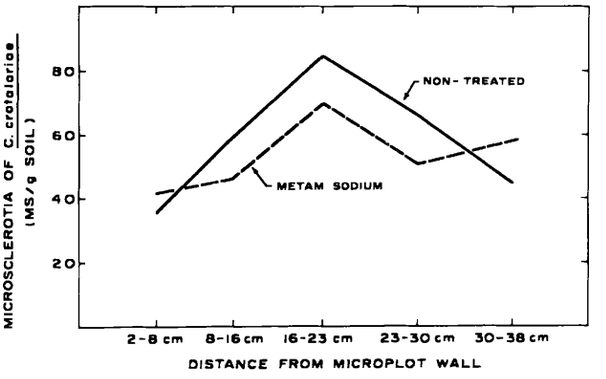


**Fig. 2. End-of-season concentrations of *Cylindrocladium crotalariae* in metam sodium-treated microplots versus non-treated microplots. Metam sodium applied at a rate of 12.8 mL/plot (10 gal/acre equivalent) in April. Peanut were planted 19 cm from microplant wall and soil was sampled in concentric rings from center (38 cm) of plots.**

*Genotype effects on inoculum density.* Plots planted with Florigiant (susceptible) had consistently higher end-of-season inoculum densities than either of the resistant genotypes. Planting either NC 8C or NC 18016 resulted in lower (P = 0.05) inoculum densities than in plots planted with Florigiant in November 1983 and 1984. Plots planted with NC 18016 had lower (P = 0.05) inoculum densities than those planted with NC 8C in 1984 (Table 2). Host resistance used in combination with metam sodium further reduced (P = 0.02) inoculum density (Fig. 3).

In 1983, end-of-season inoculum densities with all genotypes were reduced about 50% by the use of metam sodium. In 1984, large reductions in final inoculum density only occurred in plots planted with resistant genotype NC 18016.

*Reduction of pod and root rot.* Reduction in inoculum density was accompanied by corresponding reuctions (P = 0.01) in pod rot and root rot. Sustained low inoculum

Table 2. The effect of genotype on inoculum levels at various periods of time during the growing season.

| Dates:[a] | Mar 83 | Jun 83 | Nov 83 | Mar 84 | Jun 84 | Nov 84 |
|---|---|---|---|---|---|---|
| Florigiant | (25.21a)[b] | 15.20a | 28.20a | (17.87a)[b] | 8.42a | 67.74a |
| NC 8C | (27.93a) | 13.13a | 16.26b | (15.90a) | 8.59a | 43.24b |
| NC 18016 | (24.64a) | 13.68a | 13.07b | (16.17a) | 7.79a | 11.31c |

[a] Mean values in the same column with different letters are different according to Fisher's least significant difference (LSD) at p=0.05.

[b] Mean values for March represent pre-plant inoculum densities (ms/g soil). Soil in plots was combined and mixed to established initial inoculum densities each year. Peanut genotypes were planted May 27 and May 24 in 1983 and 1984, respectively.

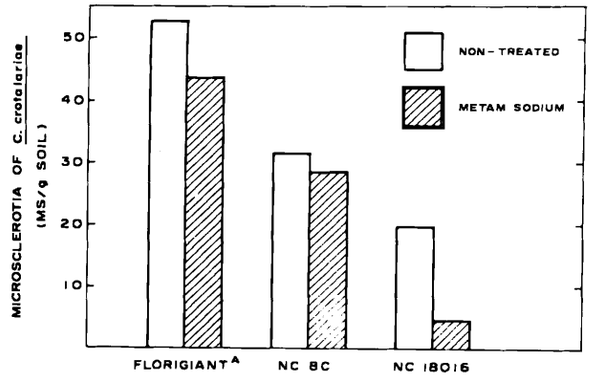


Fig. 3. Effect of peanut genotype and metam sodium on inoculum density of *Cylindrocladium crotalariae* in November. Metam sodium applied at 12.8 mL/plot (10 gal/acre equivalent); data averaged over two years. Significant factors were genotypes (P = 0.01) and IDpf x metam sodium (P = 0.02).

densities due to the use of resistant genotypes plus metam sodium resulted in low pod and root rot severities (Fig 4,5). Pod and root rot decreased with decreasing CBR susceptibility: Florigaint > NC 8C > NC 18016. Metam sodium reduced pot rot (Fig. 4) and root rot (Fig. 5) by about 50% as compared to untreated controls.

*Interaction of initial inoculum density, metam sodium and genotype.* In 1984 a factor of high/low relative inoculum densities was added to the treatments. Regardless of the original inoculum density, use of CBR-susceptible Florigiant resulted in high levels of pod rot, root rot, and high final inoculum density (Table 3). Use of metam sodium with Florigiant gave reductions in pod rot and root rot, but final inoculum density (pf) was not reduced by the use of metam sodium. Reduction of final inoculum density occurred when NC 18016 was used in conjunction with metam sodium. NC 18016 used without metam sodium did not appear to increase inoculum density (Table 3). In all cases, root rot was reduced by metam sodium. Root rot severity consistently reflected final inoculum density (high IDpf = high RR).

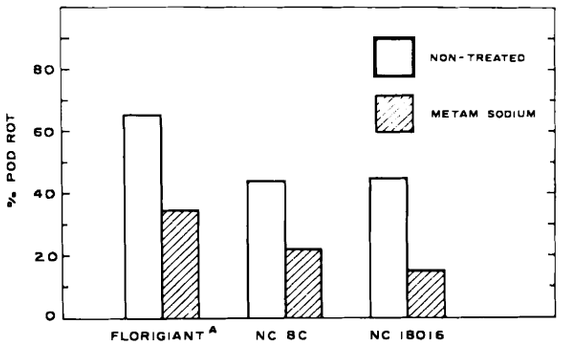


Fig. 4. Effect of peanut genotype and metam sodium on pod rot caused by *Cylindrocladium crotalariae*. Metam sodium applied at 12.8 mL/plot (10 gal/acre equivalent); data averaged over two years. Significant factor were genotypes (P = 0.01) and metam sodium treatment (P = 0.01).

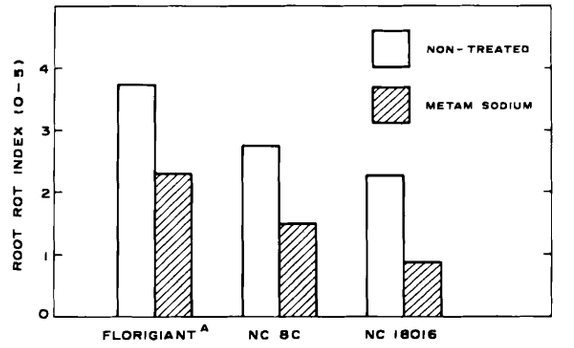


Fig. 5. Effect of peanut genotype and metam sodium on root rot caused by *Cylindrocladium crotalariae*. Metam sodium applied at 12.8 mL/plot (10 gal/acre equivalent); data averaged over two years. Significant factors were genotypes (P = 0.01), metam sodium treatments (P = 0.01) and IDpf x metam sodium (P = 0.01).

Final inoculum density (IDpf) in plots planted to Florigiant ranged from 70 to 80 ms/g soil, regardless of IDpi or metam sodium treatment. Resistant genotype NC 8C also gave increased IDpf in all cases. However, IDpf (25-60 ms/g soil) was always lower than with Florigiant. For plots in which NC 8C was used, both metam sodium and IDpi were capable of reducing IDpf. No increase in ID resulted with resistant genotype NC 18016 (IDpi = IDpf).

## Discussion

Susceptibility of peanut genotypes to CBR consistently affected variables such as pod rot, root rot and final inoculum density (IDpf). Plots planted to Florigiant resulted in high IDpf as well as high pod and root rot ratings. Increase in IDpf with Florigiant occurred regardless of initial inoculum density (IDpi) or metam sodium treatment. Florigiant is not recommended for use in fields infested with *C. crotalariae* because of yield losses due to pod and root rot (4) and the production of high inoculum levels year after year, regardless of control measures (7,16).

Use of the resistant cultivar NC 8C gave root rot, pod rot and IDpf levels which were generally intermediate

Table 3. Interactive effect of metam sodium, genotype and initial inoculum density on root rot and final inoculum density.

| Genotype | Metam sodium | IDpi[a] | IDpf[b] | RR[c] |
|---|---|---|---|---|
| Florigiant | non-treated | high | 70.8 | 4.1 |
| (susc) | | low | 74.8 | 3.8 |
| NC 8C | non-treated | high | 60.4 | 3.7 |
| (m-res) | | low | 29.1 | 2.2 |
| NC 18016 | non-treated | high | 33.2 | 2.7 |
| (h-res) | | low | 3.2 | 1.6 |
| Florigiant | 10 gal/acre | high | 79.9 | 2.7 |
| (susc) | | low | 76.1 | 3.0 |
| NC 8C | 10 gal/acre | high | 34.5 | 1.2 |
| (m-res) | | low | 25.0 | 1.9 |
| NC 18016 | 10 gal/acre | high | 3.7 | 0.9 |
| (h-res) | | low | 5.0 | 0.8 |

[a] Inoculum density level (ms/g soil, high>23.5; low<7.5) as of November 1983. Significant factors were genotypes ($p<0.01$), metam sodium treatment ($p<0.01$) and IDpi x metam sodium ($p<0.01$). NC 8C is moderately resistant (m-res) and NC 18016 is highly resistant (h-res) to CBR, respectively.

[b] IDpf = Final inoculum density level in November 1984.

[c] RR = root rot on a scale of 0-5 (1984).

between Florigiant and NC 18016. Of the three, NC 8C alone resulted in IDpf levels (high vs low). High IDpi resulted in high IDpf (47.5 ms/g soil), whereas low IDpi resulted in lower IDpf (27.0 ms/g soil). NC 8C is currently recommended for planting in CBR-infested areas.

Plots planted to NC 18016 had consistently low IDpf levels as well as low levels of root and pod rot. Final ID levels attained with NC 18016 were comparable to those of a non-host species such as corn (6).

In-row chisel application of metam sodium is currently recommended for CBR control in combination with planting of resistant genotype NC 8C (1). A water seal increases the effectiveness of methylisothiocyanate liberating fumigants (11), and was utilized in our tests. Metam sodium as applied in our tests created a band of microsclerotia-free soil to a depth of at least 15 cm. Microsclerotia were detected on either side of this band in increasing numbers with distance from the line of application.

If peanut seed are able to germinate and establish roots in noninfested areas of the soil, initial infection is delayed until roots grow into adjacent infested areas. When metam sodium is used with resistant genotypes, this 'head start', combined with host resistance, can effectively reduce damage to the crop and subsequent ms inoculum levels in the soil. The use of NC 8C with metam sodium was equivalent to the use of NC 18016 without fumigation. Unfortunately, high levels of resistance are not available in agronomically acceptable cultivars at this time.

Without host resistance the benefits of metam sodium are evident as a partial reduction of pod and root rot. Disease levels, however, were still greater than with resistant genotypes. Inoculum levels of *C. crotalariae* can increase tenfold in the course of a growing season on a susceptible host even when metam sodium is applied preplant.

Initial ID levels are important in determining the amount of disease that will occur, regardless of the increase and spread of CBR during the season. Inoculum density which is high at the beginning of the season will be high at the end of the season, even when highly resistant genotypes are used. The potential exists for long-term control of CBR with resistance, but only if other control measures such as preplant treatment with metam sodium or non-host rotation are used to reduce inoculum concentrations (6) to levels manageable by commercially available levels of resistance.

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Accepted June 11, 1986