

## ABSTRACT

**Partridge, Darcy Erin.** Potential for Management of Sclerotinia Blight of Peanut (*Arachis hypogaea* L.) caused by *Sclerotinia minor* with the Biological Control Agent *Coniothyrium minitans*. (Under the direction of Drs. Turner B. Sutton and David L. Jordan.)

Sclerotinia blight of peanut (*Arachis hypogaea* L.), caused by *Sclerotinia minor* (Jagger) Kohn, is an important disease in North Carolina and Virginia. Sclerotia are the main overwintering propagules of *S. minor* and serve as the primary source of inoculum for Sclerotinia blight. The effectiveness of the fungal mycoparasite *Coniothyrium minitans*, which is capable of colonizing sclerotia of *Sclerotinia* spp., was evaluated in a 5-year field study and in eight short-term field studies in northeastern North Carolina. Control of Sclerotinia blight was highest when *C. minitans* was applied for 3 consecutive years. However, application of *C. minitans* for 1 or 2 years also reduced disease in the long-term study. A single application of *C. minitans* was less effective when applied 4 to 6 months prior to planting and sclerotia numbers were only reduced in two of the eight short-term field studies. Sclerotia used as baits placed in the long-term field study as well as the sclerotia isolated from soil were infected by *C. minitans*, and the number of sclerotia was reduced where *C. minitans* was applied. Moderate resistance in the cultivar Perry and application of the fungicide fluazinam provided adequate control of Sclerotinia blight in all plots.

The integration of *C. minitans* with current peanut management practices is needed for successful biological control of Sclerotinia blight. Laboratory experiments evaluated the effects of nine pesticides commonly used in peanut production on mycelial growth, conidia germination, and mycoparasitic activity of *C. minitans* on sclerotia of *S. minor*. The commercial formulations of the fungicides azoxystrobin, chlorothalonil, fluazinam, pyraclostrobin, and tebuconazole, and the herbicide flumioxazin reduced mycelial growth

and conidia germination of *C. minitans*. Eight of nine pesticides, azoxystrobin, chlorothalonil, fluazinam, pyraclostrobin, tebuconazole, diclosulam, flumioxazin, and pendimethalin applied to soil plates reduced but did not inhibit the mycoparasitic activity of *C. minitans* on sclerotia of *S. minor*. Temperature and moisture effects on mycoparasitism were also evaluated to determine optimum conditions for infection of sclerotia of *S. minor* by *C. minitans*. Optimum temperatures for infection of sclerotia of *S. minor* by *C. minitans* ranged from 14 to 22° C and soil moisture  $-0.33$  to  $-1 \text{ kPa} \times 10^2$ . These results indicate that *C. minitans* should remain active throughout most of the year in North Carolina, except during the hot summer months of June, July and August.

Soil fauna such as collembola may aid in the reduction of sclerotia through direct predation and the movement of inocula of mycoparasites from infected to noninfected sclerotia. Collembola diversity and abundance were compared in four peanut fields. The most prevalent collembola families were Isotomidae, Smithurididae, Poduridae, and Hypogastruridae, with Isotomidae isolated most frequently from all sites. Abundance and diversity of collembola increased from August to October with sampling date and location having the greatest effect on the composition of the population. Determining the abundance and diversity of collembola in the field can help increase our understanding of the soil community structure.

These studies show that *C. minitans* is able to persist and infect sclerotia of *S. minor* in peanut fields of North Carolina when applied in the fall or early winter across crop residue and incorporated into the top layers of the soil. *C. minitans* will not eradicate infestations by *S. minor*, but over time has the potential to reduce inoculum levels and ultimately decrease the incidence of the disease.

**POTENTIAL FOR MANAGEMENT OF SCLEROTINIA BLIGHT OF PEANUT  
(*ARACHIS HYPOGAEA* L.) CAUSED BY *SCLEROTINIA MINOR* WITH THE  
BIOLOGICAL CONTROL AGENT *CONIOTHYRIUM MINITANS***

by

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## **DEDICATION**

**Jack E. Bailey**

(September 16, 1951- April 12, 2002)

I am dedicating my dissertation to Dr. Jack E. Bailey. Dr. Bailey, a long-time Professor and Extension Specialist in the Department of Plant Pathology, at North Carolina State University, was passionate about teaching and mentoring students. He first made contact with me back in 2001 when he relentlessly pursued and convinced me that his lab was the place for me to start my PhD graduate research. Upon my arrival in Raleigh, Jack eagerly welcomed me and instantly sparked my interest in diseases of peanut. He was involved in both applied and basic research aimed at solving disease problems for growers. He was a man with many dreams and not enough time to complete them. He loved to debate philosophical ideas and opened his home to students regularly for discussions and socials. His friendship and guidance is greatly missed. I hope that in the future I may show as much passion and dedication in my research as Jack had in all his accomplishments.

## BIOGRAPHY

Darcy Erin Partridge was born on June 17, 1977, in Batavia, New York. Agriculture and plant sciences were introduced to her as a child growing up on a dairy farm and working in her parents' vegetable stand. In 1999, Darcy obtained her Bachelor of Science in Plant Biology with a concentration in Botany from Cornell University in Ithaca, NY. She then received her Master of Science degree in Plant, Soil and General Agriculture in 2002 from Southern Illinois University, Carbondale, IL under the guidance of Dr. John S. Russin. Her master's thesis research involved studying the effects of two soilborne fungi, *Fusarium solani* f.sp. *glycines* and *Macrophomina phaseolina*, on soybean nodulation and nitrogen fixation. In the summer of 2001 she began work on her degree of Doctor of Philosophy in Plant Pathology and Crop Science at North Carolina State University under the direction of Drs. Jack E. Bailey and David L. Jordan. Dr. Turner B. Sutton graciously joined her committee as Co-chair upon the sudden death of Dr. Bailey. Darcy completed her degree studying the potential for management of Sclerotinia blight of peanut (*Arachis hypogaea* L.) caused by *Sclerotinia minor* with the biological control agent *Coniothyrium minitans* in the spring of 2005 under the direction of Drs. Turner B. Sutton and David L. Jordan.

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## INTRODUCTION

Peanut (*Arachis hypogaea* L.) is produced in subtropical regions throughout the world. In the United States harvested area was estimated at 564,000 ha in 2004, with production limited to the southern states of Virginia, North Carolina, South Carolina, Georgia, Florida, Alabama, Texas, Oklahoma, New Mexico, and Arizona (9). Virginia market-type peanuts dominate the Virginia and North Carolina market whereas runner market-type peanuts are more commonly grown in Alabama, Georgia, and Florida, Spanish market-type peanuts in Oklahoma and Texas; Valencia market-type peanuts are grown in New Mexico (70). Peanut is primarily grown in the eastern counties of North Carolina. In 2004 harvested area was 42,500 ha with an average statewide yield of 3860 kg ha<sup>-1</sup>(9, 12).

The Farm Security Act of 2002 eliminated the two-tier price support system to a single loan rate reducing the guaranteed price for quota peanuts from \$610 per ton to a national loan rate average of \$355 per ton (12). Average prices in Virginia and the Carolinas for 2004 was \$500 per ton (12). Reduced returns and environmental concerns have placed a greater need to limit production costs while maintaining high pod quality and yield (16). The major costs for peanut production include seed, fertilizer, herbicides, and fungicides (16). Peanut is susceptible to a number of diseases including early leaf spot caused by *Cercospora arachidicola* S. Hori, late leaf spot caused by *Cercosporidium personatum* (Berk. & M. A. Curtis) Deighton, web blotch caused by *Phoma arachidicola* Marsas et al., limb rot caused by *Rhizoctonia solani* Kühn, southern stem rot caused by *Sclerotium rolfsii* Sacc., *Cylindrocladium* black rot caused by *Cylindrocladium parasiticum* and Sclerotinia blight caused by *Sclerotinia minor* (Jaggar) Kohn. Peanut diseases are controlled through timely

applications of fungicides beginning in late June and applied bi-weekly through September (67). Pesticides used to manage diseases in peanut include azoxystrobin, boscalid, chlorothalonil, copper-containing fungicides, fluazinam, flutoloniil, mancozeb, metam sodium, propiconazole, pyraclostrobin, tebuconazole, and trifloxystrobin (67). If diseases are not properly managed pod yield and quality can be significantly reduced (61).

### **Biology of *Sclerotinia minor* and Disease Cycle on Peanut.**

**Biology of *S. minor*.** *S. minor* Jagger is a soilborne pathogen of peanut that causes Sclerotinia blight, one of the major diseases of peanut in Virginia and North Carolina. *S. minor* is one of three designated species in the family Sclerotiniaceae (42) which encompasses inoperculate discomycetous fungi of the order Helotiales within the phylum Ascomycota (6). Helotiales are fungi that have cup or disk-shaped apothecia producing asci with only slightly thickened apices and ascospores that are spherical, elongate to filiform and have one to several septa (5). The family Sclerotiniaceae have medium, generally brown apothecia that are borne on long stalks that arise from stromata or sclerotia. Apothecia of Sclerotiniaceae produce hyaline, one-celled, oval or somewhat elongated ascospores (5). Hyphae are hyaline, septate and multinucleate. The fungus reproduces asexually and sexually (42, 78). Sclerotia are small (0.5-3 mm in diameter), irregularly shaped, tuberoid, black structures of condensed mycelium with a hardened rind (42, 78).

*S. minor* occurs worldwide and can infect plants in more than 100 species belonging to 21 families and 66 genera (30, 54). It is the main causal agent of a number of diseases commonly referred to as drop, cottony rot, white mold and blight. Sclerotia are the main

overwintering propagules of *S. minor*. Sclerotia are formed when environmental conditions become unfavorable or when nutrients are lacking (18). The hardened rind of the sclerotia provides resistance to desiccation, extreme temperatures, and other adverse environmental conditions (18). Sclerotia germinate at temperatures of 17 to 21°C and with 95% or greater relative humidity (60). Sclerotia can infect a susceptible host myceliogenically through eruptive or hyphal germination. Sclerotia can germinate carpogenically to produce apothecia. Meiosis within asci produce ascospores (63). Myceliogenic germination is considered more important to the life cycle of *S. minor* since carpogenic germination is rarely seen in nature (1). Once inside the plant the mycelium rapidly attacks plant cells causing them to collapse. De Bary (4) showed that the fungus *Sclerotinia* induces rotting of vegetables by secreting substances such as enzymes and toxins in advance of the fungus. *S. sclerotiorum* has been shown to acidify its ambient environment by producing oxalic acid (64). Oxalic acid production has been implicated in aiding in the pathogenicity of *Sclerotinia* spp. and *Sclerotinia minor* (28, 45). The regulation of pH has been implicated as a cue for processes linked to pathogenicity, development, and virulence in *S. sclerotiorum* (64). White fluffy mycelium can grow on the surface of the infected plant tissue, continuing to invade the healthy tissue surrounding the initial infection site. Sclerotia form on the surface or in cavities of the plant and eventually reach the soil where they remain until a susceptible host and favorable environmental conditions become available, completing the disease cycle.

**Disease on Peanut.** Sclerotinia blight on peanut was first reported in the United States in Virginia in 1971 and in North Carolina in 1972 (11, 59, 76). The disease is currently found in most major peanut producing counties in North Carolina (11) and is

present in New Mexico, Oklahoma, and Texas (11, 71, 76). Sclerotinia blight is also found in most peanut producing countries around the world (43). Fields heavily infested with *S. minor* have resulted in severe outbreaks of disease causing a reduction of yield up to 50% in peanut (60).

Infection of the peanut does not usually occur until canopy closure when environmental conditions favor fungal growth (60). The sclerotia serve as the primary inoculum of Sclerotinia blight (60). Volatile substances released by peanut leaves may induce germination of the sclerotia (29), which predominantly germinate myceliogenically. An infection cushion forms and penetration pegs enter into the plant through stomata or by mechanical force (84). These infection cushions allow *S. minor* to directly penetrate peanut tissue including branches, pegs, pods, and leaflets near or contacting the soil surface (60). White, fluffy mycelium may be seen in advance of the lesions during periods of high humidity (43). This mycelium rapidly colonizes the succulent tissues as enzymes such as pectinase, cellulase, proteinase, and other degrading enzymes damage plant cells in advance of the fungus with distinct zones forming between infected and healthy tissue (23, 45, 84). A rapid flagging or wilting of the tips of peanut branches may be the first observed symptoms of Sclerotinia blight (43). Initial disease symptoms on the stem begin as small light green, water soaked lesions turning bleached or straw colored with age and eventually shredding as the tissues are degraded (59). Foliage on an infected branch will turn chlorotic and ultimately brown and wither. Underground pods and pegs can become necrotic. Sclerotia are produced in and on all infected tissue, fall free to the soil or remain in plant debris, and serve as inoculum for next susceptible host (60).

Sclerotia of *S. minor* are disseminated by several means. The sclerotia overwinter in the soil and can be spread by anything that moves soil from one place to another. The sclerotia of *S. minor* are able to survive and remain viable after being ingested by cattle that eat peanut hay infested by the fungus (53). Peanut seed is also thought to be a means in which *S. minor* may be disseminated into a non-infested field (77).

**Control of Sclerotinia Blight.** Crop rotation, moderately resistant cultivars, and fungicides are currently used to manage Sclerotinia blight (43). Long-term rotations with non-host crops such as corn (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) have shown limited effectiveness in reducing this disease on peanut since the sclerotia are able to remain viable for as many as 4 years in the soil (3, 54, 60). Moderately resistant Virginia-type cultivars are available to growers in Virginia and North Carolina for use in fields infested with *S. minor*. VA 93B, a partially resistant cultivar, was the first Virginia-type cultivar to be registered for use by growers (19), and was followed by the release of VA 98R and Perry (36, 56). Currently the fungicides fluazinam (Omega 500F; Syngenta Crop Protection, Greensboro, NC) and boscalid (Endura; BASF Corp. Research Triangle Park, NC), have been shown to be very effective in controlling Sclerotinia blight if the proper rate and application timing are used (46, 68, 69). These management practices for of Sclerotinia blight are mainly aimed at protecting the plant from infection and not at reducing the numbers of sclerotia in the soil.

A wide range of microorganisms have been found to parasitize sclerotia of *Sclerotinia* spp. Mycoparasites of sclerotia include *Gliocladium virens* Miller and Foster (74), *Clonostachys rosea* (Link:Fr.) Schroers, et al(24, 47, 66), *Coniothyrium minitans* Campbell

(33, 34, 75), *Trichoderma* spp. (*T. harzianum* Rifai, *T. viridae* Pers., *T. koningii* Rifai, and *T. pseudokoningii* Rifai) (22, 37, 86), *Paecilomyces lilacinus* (Thom) Samson (24, 40), *Mucor* spp., *Penicillium* spp., and *Sporodesmium sclerotivorum* (2). The microarthropods Collembola (springtails) and the Acarina (mites) are the most abundant of the soil inhabiting animals excluding the nematodes and protozoa (41). Some of these insects are known to consume fungi and decaying plant material and can carry fungal propagules in their gut and on their cuticle (10, 41). Larvae of *Bradysia coprophila* Winnertz (dark-winged fungus gnat) feed on sclerotia of *S. sclerotiorum* leading to increased susceptibility of the sclerotia to infection by *Trichoderma viride* Pers. (7). Germination of *S. sclerotiorum* sclerotia is also inhibited by salivary secretions of *B. coprophila* during feeding (8). Collembolan species *Proisotoma minuta* Tullberg (Isotomidae) and *Onychiurus encarpatus* Denis (Poduridae) were found to suppress colony growth of *Rhizoctonia solani*, *Verticillium dahliae* Kleb., *Fusarium oxysporum* f. sp. *vasinfectum* Synder and Hansen and *Macrophominia phaseolina* and reduced sclerotial germination of *M. phaseolina* by consuming the hyphae of germinating sclerotia rendering the propagule ineffective (20). Sclerotial parasitism by both fungi and microarthropods may reduce numbers of sclerotia in peanut fields heavily infested with *S. minor*.

### **Biology of *Coniothyrium minitans* and potential as a mycoparasite of sclerotia.**

*Coniothyrium minitans* Campbell was first described for biocontrol of *Sclerotinia sclerotiorum* (Lib.) in California in 1947 (17). *C. minitans* is an ascomycete that is found in 29 countries on all continents except South America (65, 81). Since its discovery, *C.*

*minitans* has been shown to infect and degrade sclerotia in soil and has the potential to control *S. sclerotiorum* by decreasing carpogenic germination, and viability of sclerotia (32, 38, 72, 73, 80).

*C. minitans* hyphae are 2-6  $\mu\text{m}$  in diameter, smooth, simple with numerous septa in larger hyphae, and darken and become roughened with age (57, 81). Pycnidia of *C. minitans* are usually superficial on the sclerotia of *S. sclerotiorum*, sometimes immersed, brown to black, subglobose, become carbonaceous with age, 150-700  $\mu\text{m}$  in diameter, and ostiolate (62). Pycnidiospores are dark brown in mass, individual conidia are yellowish brown to dark brown, ovoid to ellipsoid or shortly cylindrical or nearly globose, smooth to roughened, and 4-7 x 2.5-4  $\mu\text{m}$  (62).

*C. minitans* is a mycoparasite of sclerotia of phytopathogenic fungi within the ascomycota. Hosts of *C. minitans* include *S. sclerotiorum*, *S. trifoliorum* (75). Under laboratory conditions it can infect *S. minor*, *Botrytis cinerea*, *B. fabae*, *B. narcissicola* and *Sclerotium cepivorum* (75). Germinating spores and mycelium of *C. minitans* can infect and kill the mycelium and sclerotia of *Sclerotinia* spp. (34). *C. minitans* directly penetrates the cell walls of *S. sclerotiorum* without the formation of an infection cushion (33). Hyphae then invade sclerotia resulting in the destruction and disintegration of sclerotium tissues; the dark-pigmented rind is more resistant to invasion than the unpigmented cortical and medullary tissues (34). *C. minitans* produces four metabolites inhibitory to fungal growth, including one identified as macrosphelide A (51). Sclerotia that are infected become soft, disintegrate and fail to germinate (58).

A number of studies have shown that sclerotia of *Sclerotinia* spp. in soil are readily infected and colonized by *C. minitans* (32, 35, 50, 72, 80). Control of *S. sclerotiorum* in several crops has been achieved both in the greenhouse and field with the application of *C. minitans* (13, 15, 32, 44, 48, 52, 82, 83). *C. minitans* grown on maize meal-perlite is more effective than a spore suspension in infecting sclerotia and reducing their number, viability, and caropogenic germination in greenhouse systems (38, 39). Fall application of a maize meal-perlite formulation of *C. minitans* to oilseed rape was more effective than applications made in the spring (52), whereas *C. minitans* applied in the spring effectively reduced disease on bean (49). *C. minitans* infects sclerotia produced in and on roots of sunflower and those produced in the base of the stem (31, 32). Sclerotial populations in the soil also are decreased and sclerotinia wilt of sunflower is reduced (31, 32). *C. minitans* applied as a spray to crops infected with *S. sclerotiorum* infected 90% of sclerotia, lowered the number of apothecia produced, and decreased the final sclerotia population (26). Foliar application of *C. minitans* to alfalfa blossoms also suppressed sclerotinia blossom blight of alfalfa (44). *C. minitans* has the potential to keep contamination of soil with *S. sclerotiorum* sclerotia low when crop rotation includes a number of susceptible hosts (26).

The antagonistic activity of *C. minitans* isolates on *S. sclerotiorum* is relatively uniform (25) and only a single conidium of *C. minitans* is needed to infect a sclerotia of *S. sclerotiorum* (27). The optimum temperature for spore germination, infection and destruction of sclerotia of *S. sclerotiorum* by *C. minitans* is 20°C (73). High temperatures inhibit infection of sclerotia of *S. sclerotiorum* by *C. minitans*, but *C. minitans* is able to survive and will resume activity once the temperature returns to optimum (39).

Temperatures < 7°C are not favorable resulting in much slower rates of germination and infection (73). Eighty percent spore germination occurred after 7 days at temperatures ranging from 7 to 20°C, with spore germination occurring at temperatures as low as 4°C and as high as 30°C (73). Inocula containing conidia of *C. minitans* survived better at 5 and 15°C than 30°C for 64 weeks (50). Growth rate of *C. minitans* on various media decreased with decreasing water potential (79). The optimum relative humidity for the mycoparasitism of *S. sclerotiorum* sclerotia is > 95% (73).

*C. minitans* was found to survive in the soil and infected sclerotia equally in all pesticide-treated and untreated soil in glasshouse lettuce (14). *C. minitans* is highly sensitive to iprodione (7-18 µg a.i. ml<sup>-1</sup>), moderately sensitive to thiram (106 µg a.i. ml<sup>-1</sup>) and less sensitive to mancozeb, metalaxyl plus thiram, tolclofos-methyl, and zineb in *in vitro* studies (14). Mycelial growth of *C. minitans* is also reduced by three sclerotial inhibitors, thioglycolic acid, mercaptoethanol and mercaptoethylamine, but is not inhibited by N-thioglycolate (21). Phloxine B, an insecticidal photoactive dye, inhibits growth of *C. minitans* on potato dextrose agar (55).

*C. minitans* has been found to infect sclerotia in non-treated control plots several meters from the treated areas, but it cannot grow saprophytically in the soil (13). It has been suggested that soil fauna might play a role in the dispersal of *C. minitans* (73, 75). Slugs, collembola and mites were reported to be important in the dispersal of *C. minitans* to uninfected sclerotia (73). *Acarus siro* L. (flour mite) and *Folsomia candida* Willem (Isotomidae) can transmit *C. minitans* from infected to non-infected sclerotia of *S.*

*sclerotiorum* in soil and may be important in the dissemination of *C. minitans* in the field (85). *F. candida* and *Bradysia* sp. also can spread *C. minitans* between sclerotia (82).

*C. minitans* may provide a way to reduce sclerotia numbers in peanut fields heavily infested by *S. minor* as long as it can survive and remain active in the soil when integrated with current peanut production practices. This research was initiated to:

1. Determine if *C. minitans* applied as the commercial formulation, Contans WG, is a viable control measure for Sclerotinia blight of peanut.
2. Evaluate the sensitivity of *C. minitans* growth, spore germination, and mycoparasitic activity to pesticides commonly used in peanut production.
3. Identify optimal temperature and moisture requirements for *C. minitans* infection of sclerotia of *S. minor*.
4. Evaluate the diversity and abundance of collembola in peanut fields of northeastern North Carolina.

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## **CHAPTER 1**

# **MANAGEMENT OF SCLEROTINIA BLIGHT IN PEANUT FIELDS WITH THE BIOLOGICAL CONTROL AGENT *CONIOTHYRIUM MINITANS***

## ABSTRACT

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Management of Sclerotinia blight in peanut fields with the biological control agent *Coniothyrium minitans*. To be submitted to Plant Disease.

Sclerotinia blight of peanut (*Sclerotinia minor*) is an important disease found in all major peanut producing counties in the Virginia and North Carolina peanut belt. The effect of the fungal mycoparasite *Coniothyrium minitans*, which is capable of colonizing sclerotia of *Sclerotinia* spp., was studied in a 5-year field experiment and in eight short-term experiments in northeastern North Carolina. The 5-year experiment was initiated in November 1999 to evaluate the effectiveness of repeated soil applications of *C. minitans* (commercial formulation, Contans WG) at 2 and 4 kg ha<sup>-1</sup> in reducing Sclerotinia blight when integrated with current disease management practices. Subsequent applications were made in February 2001 and November 2001. In addition, individual commercial peanut fields were selected in 2001 and 2002 to evaluate the effect of a single application of *C. minitans* at 4 kg ha<sup>-1</sup>.

No differences were found between the 2 and 4 kg ha<sup>-1</sup> rates of *C. minitans* in reducing Sclerotinia blight. In 2002 there was less disease in plots receiving an application of *C. minitans* for either 1 or 3 years compared to the non-treated control, while in 2003 there was less disease in plots receiving *C. minitans* application for 1, 2, or 3 years. Sclerotia baits placed in the field as well as sclerotia isolated from soil were infected by *C. minitans*, and there were reduced numbers of sclerotia in plots where *C. minitans* was applied. Moderate resistance and applications of the fungicide fluazinam (Omega 500) controlled Sclerotinia

blight in the short-term field plots. However, a single application of *C. minitans* was less effective than the multiple applications in the long-term field study. Numbers of sclerotia were reduced in two of the eight fields receiving single applications of *C. minitans*. The mycoparasitic activity observed on sclerotia of *S. minor* in 2003 demonstrates that *C. minitans* is able to establish itself and remain active in peanut fields in North Carolina. A management program integrating moderate cultivar resistance, the fungicide fluazinam applied according to the Sclerotinia advisory forecasting system, and consecutive years of soil application of *C. minitans* at 2 kg ha<sup>-1</sup> may provide increased control of Sclerotinia blight in peanut.

## **INTRODUCTION**

Sclerotinia blight, caused by the soilborne fungus *Sclerotinia minor* (Jaggar) Kohn (20), is an important disease of peanut (*Arachis hypogaea* L.). This disease was first reported in Virginia in 1971 and in North Carolina 1972 (31) and since then has spread to all the major peanut producing counties in North Carolina (5). During favorable conditions for Sclerotinia blight, peanut farmers can lose up to 50% of yield as a result of the disease (30).

Overwintering sclerotia of *S. minor* in the soil provide the primary inoculum for Sclerotinia blight on peanut. Sclerotia of *S. minor* germinate myceliogenically producing a mass of mycelium that directly penetrates peanut stem, leaf, peg, and pod tissues, initiating disease (21, 33). As Sclerotinia blight develops in the peanut canopy, numerous sclerotia are produced in and on diseased tissue. Sclerotia are then shed into the soil and increase the inoculum density of the pathogen for future growing seasons.

Rotation sequence is very important in managing diseases in peanut (29). *Cylindrocladium* black rot [caused by *Cylindrocladium parasiticum*] (CBR) was found to increase in peanut fields when crop rotation included soybean [*Glycine max* (L.)Merr.] or when peanut was cropped continuously, but not when a non-host crop was used in the rotation (18, 36). Long-term rotations with non-host crops such as corn (*Zea may* L.) and cotton (*Gossypium hirsutum* L.) have shown limited effectiveness in controlling *Sclerotinia* blight on peanut (33) since the sclerotia are able to remain viable for as many as 4 years in the soil (1, 26). Partial resistance and cultural methods provide limited control, while the fungicides fluazinam (Omega 500F; Syngenta Crop Protection, Greensboro, NC), and boscalid (Endura; BASF Corp. Research Triangle Park, NC), are effective if applied properly (29, 37, 38). These fungicides and host resistance are aimed at reducing disease development but not at reducing the population of sclerotia in soil.

A number of microorganisms have been reported to parasitize sclerotia of *Sclerotinia* spp., including *Coniothyrium minitans* Campbell. *C. minitans* was first identified as a mycoparasite of sclerotia of *Sclerotinia* spp. by Campbell in 1947 (9). A number of studies have shown that sclerotia of *Sclerotinia* spp. in soil are readily infected and colonized by *C. minitans* (14, 15, 24, 39, 43). Control of *S. sclerotiorum* in several crops has been achieved both in the greenhouse and field with applications of solid-state inocula of *C. minitans* (6, 7, 14, 23, 25, 44, 45). *C. minitans* applied to field crops infected by *S. sclerotiorum* reduced the population of sclerotia in soil at the end of a 7-year period even when susceptible crops were planted (12). *C. minitans* may provide a way to reduce sclerotia numbers in peanut fields heavily infested by *S. minor*.

The objective of this study was to determine the effectiveness of soil applications of *C. minitans*, applied as Contans WG (Prophyta Biologischer Pflanzenschutz GmbH, Germany), in reducing the sclerotial population of *S. minor* and subsequent disease in peanut while integrating it with current cultivar, chemical, and cultural practices.

## MATERIALS AND METHODS

**Long-term field site.** A commercial peanut field near Gatesville, North Carolina with a long-term history of peanut production and presence of Sclerotinia blight was selected for this study in 1999. The same field and the plots defined within were used through the 2003 season. The rotation sequence in this field from 1998 to 2003 was peanut, cotton, cotton, peanut, peanut, and peanut.

*Coniothyrium minitans*, commercial formulation Contans WG strain CON/M/91-08 with a minimum of  $1 \times 10^9$  cfu g<sup>-1</sup> (Prophyta Biologischer Pflanzenschutz GmbH, Germany), was applied at 2 and 4 kg ha<sup>-1</sup> each for 1, 2, or 3 consecutive years (Table 1.1). Soil application was made with a CO<sub>2</sub>-pressurized tractor-mounted sprayer at 276 kPa and 142 L aqueous suspension per ha using hollow cone nozzles spaced 31 cm apart. The first application of *C. minitans* was made after cotton harvest on 3 December 1999 and repeated on 9 February 2001 establishing the 1 and 2 consecutive year soil treatments. *C. minitans* was applied again on 12 November 2001 following the first year of peanut, establishing the final 1, 2, and 3 consecutive year treatments (Table 1.1).

Peanut cultivars NC V-11 (susceptible to Sclerotinia blight) (35) and Perry (moderately resistant to Sclerotinia blight) (35) were planted on 2 May 2001, 16 May 2002,

and 18 June 2003. Fluazinam at 0.625 kg a.i. ha<sup>-1</sup> was applied based on the weather based Sclerotinia advisory warning system (27, 28, 29) using the CO<sub>2</sub>-pressurized tractor-mounted sprayer described above. The interval between fluazinam sprays was no shorter than 3 weeks, no more than three applications were made per year, and a 30-day pre-harvest interval was used (Table 1.3). Standard cultural and pest management practices such as tillage, fertilization, herbicide and insecticide applications, and fungicide applications to control early leaf spot (*Cercospora arachidicola*) and southern stem rot (*Sclerotium rolfsii*) were used as recommended by the North Carolina Cooperative Extension Service (18).

The experimental design was a split plot. Main-plot factors consisted of *C. minitans* application at 2 and 4 kg ha<sup>-1</sup> each applied 1, 2 or 3 consecutive years and a non-treated control (Table 1.1). Main plots were 29 m wide by 41 m long with 4.6 m alleys. Subplots factors consisted of combinations of two Virginia-type cultivars (NC-V11 and Perry) and two fluazinam rates (0 and 0.625 kg a.i. ha<sup>-1</sup>) (Table 1.1). Subplots were approximately 9 m wide by 18 m long, the two center rows were used for disease and pod yield evaluations.

**Disease and yield assessment.** Disease ratings were initiated in July each year, and taken biweekly with a final disease assessment taken just prior to harvest. The center two rows in each subplot were each divided into 60, 30-cm long increments per row. Sclerotinia blight incidence was estimated by counting the number of 30-cm increments showing signs of *S. minor* or symptoms of Sclerotinia blight (8). Area under disease progress curve (AUDPC) for each treatment was calculated as a measure of the cumulative incidence of Sclerotinia blight during the season (34).

Plants were dug based on mesocarp color determination (46) and vines inverted. Peanuts were combined with conventional harvesting equipment from the two center rows of each plot. A 500-g sample from each plot was collected in 2002 to determine the percentages of extra large kernels (% ELK), total sound mature kernels (% TSMK), and the fancy kernels (% FP) using United States Department of Agriculture Grading Service Guidelines (42).

Data were subjected to analysis of variance using the PROC GLM procedure of SAS (Version 8, SAS Institute Inc. Cary, NC 1999-2001) for AUDPC, pod yield, %TSMK, %ELK, and %FP. Means for appropriate treatment main effects and interactions were separated using Fisher's Protected Least Significant Difference Test at  $P \leq 0.05$ . Contrast statements were used to separate rate and yearly effects of *C. minitans* treatments at  $P \leq 0.05$ .

**Sclerotia isolation and mycoparasitism.** Soil samples were taken across main plots to determine populations of *S. minor* on 11 September and 10 October in 2002 and 16 September and 16 October in 2003. Soil samples were taken under the peanut canopy in each plot. The top 12 cm of soil was sampled with a shovel that removed soil cores (~10 cm in diameter) from four locations in each plot, which were then combined into one sample. Five hundred gram samples were subjected to a modified soil elutriation technique to trap sclerotia on a 425  $\mu\text{m}$  sieve placed in a four-unit semiautomatic elutriator and allowed to sieve for 8 min (32). Sieves used included a U. S. standard number 10 sieve (2 mm opening) nested on top of U. S. number 35 sieve (425  $\mu\text{m}$  opening). The organic matter collected on the sieve was dried at room temperature (~22° C) for 24 h and then placed in 5° C until enumeration of sclerotia. Sclerotia in each sample were isolated from organic matter and

enumerated under a stereomicroscope. The sclerotia were then surface disinfested in 15 % NaClO for 30 s, rinsed in sterilized distilled water and plated on potato dextrose agar (PDA) amended with tetracycline (25 mg L<sup>-1</sup>). The number of sclerotia showing mycelial growth (viable) and/or infection by *C. minitans* were assessed after 14 d incubation at 18° C.

**Sclerotia baiting.** An isolate of *S. minor* [# 20 obtained from Dr. Barbara Shew, North Carolina State University (13)] originally obtained from a diseased peanut plant in Gates County, North Carolina, was used to produce sclerotia required for baiting. The sclerotia, produced on carrot disks, were separated and dried in room temperature (~22° C) prior to placement in terylene net bags. Approximately 0.03 g (~20 sclerotia) of sclerotia were placed in bags, which were then was buried 10 cm below the surface of the soil underneath the peanut canopy. Four bags were buried in each main plot in the long-term field study on 23 July 2002 and 25 July 2003. Following burial for 2, 4, 8, or 12 weeks, the sclerotia baits were recovered on 7 and 21 August, 9 September, and 10 October in 2002 and 5 and 21 August, 16 September, and 6 October in 2003. The sclerotia remaining in each bag were surface disinfested and plated on PDA amended with tetracycline as described previously for determination of viability and/or infection by *C. minitans* and other fungi.

**Short-term field studies.** Experiments examining single year applications of *C. minitans* were conducted at five locations in 2001 and at three locations in 2002 in North Carolina (Table 1.3). Eight treatments were established at each location to examine the interactions between soil incorporation of *C. minitans*, cultivar (Perry or NC-V11), and fungicide on the development of Sclerotinia blight in peanut, except at two locations where only the cultivar VA98R was planted (Table 1.2). Assessments of disease incidence,

sclerotial isolation, viability and infection by *C. minitans*, and peanut yield and quality were the same as described for the long-term field site. Six of the short-term field sites had a split plot experimental design with six replicates. *C. minitans* treatments were main plots and fungicide x cultivar treatments were subplots. Only one peanut cultivar (VA 98R) was planted in plots in Tyner and Hertford in 2001 resulting in a randomized complete block design of *C. minitans* treatment by fungicide. Data for AUDPCs, sclerotial number, viability and infection, and peanut yield and market grade characteristics were subjected to analysis of variance appropriate for each factorial treatment arrangement as described previously. Means for appropriate treatment main effects and interactions were separated using Fisher's Protected Least Significant Difference Test at  $P \leq 0.05$ .

## RESULTS

**Long-term field site.** Interactions among whole plot treatments of *C. minitans* and subplot treatments of fungicide and cultivars on Sclerotinia blight disease development were not significant (Table 1.4). There were several significant interactions between the main effects on peanut yield in 2001 and 2002 (Table 1.4). Further examination of the simple effects on yield found they were not significant, therefore the mean yield data are presented for each main effect only. Contrasts were used to separate the effects of *C. minitans* rate and years of application (Table 1.4). Less disease, as reflected by the AUDPC, developed in the moderately resistant cultivar Perry than in very susceptible cultivar NC-V11 in both 2001 and 2002 (Table 1.5). In 2003 only the cultivar Perry was planted due to heavy rains, which flooded the plots and necessitated replanting. Seed of the cultivar NC-V11 was not

commercially available for replanting. Fluazinam applied according to the Sclerotinia blight advisory reduced disease in all years (Table 1.5). In 2001, there was no significant difference between *C. minitans* rates of 2 or 4 kg ha<sup>-1</sup>, but a slight reduction in disease occurred when *C. minitans* was applied (Fig. 1.1). The application of *C. minitans* did not affect yield in 2001 (Table 1.5). *C. minitans* at 2 or 4 kg ha<sup>-1</sup> reduced disease across both cultivars in 2002 and on Perry in 2003 (Fig. 1.1). Peanut pod yield was highest in plots treated with *C. minitans* at 4 kg ha<sup>-1</sup> in 2002, but in 2003 all plots treated with *C. minitans* had lower yield compared to the non-treated control (Table 1.5).

Yearly application of *C. minitans* reduced disease in all 3 years, but it had no effect on peanut yield (Fig. 1.2, Fig.1.3). In 2001 the plots receiving a single application of *C. minitans* had less disease than the non-treated control, but disease incidence in plots receiving two applications was not significantly different from the non-treated control (Fig. 1.2). In 2002 three consecutive years of *C. minitans* applications greatly reduced the amount of disease, while both one and two applications of *C. minitans* also reduced disease over the non-treated control (Fig. 1.2). By 2003 applications of *C. minitans* for 1, 2, and 3 years significantly reduced disease when compared to the non-treated plots but there was no difference between the number of years *C. minitans* was applied and the amount of Sclerotinia blight that developed (Fig. 1.2). There was no effect of *C. minitans* or the application of fluazinam on peanut quality as measured by %ELK and %FP (data not shown) consistent with previous research. The cultivar Perry has a significantly larger number of extra larger kernels and fancy pods than NC-V11 (data not shown).

**Isolation of sclerotia and mycoparasitism.** There were no significant interactions between *C. minitans* treatment and the time of soil sampling so the number of sclerotia isolated from soil was averaged across sampling times (Table 1.6). Two and three consecutive applications of *C. minitans* reduced recovery of sclerotia from soil in 2002 and in 2003 one, two, and three consecutive applications of *C. minitans* resulted in reduced recovery of sclerotia from soil (Fig. 1.4). There were significantly fewer sclerotia in all treatments than the non-treated control (Table 1.6). There was no effect of *C. minitans* treatment on the sclerotial viability or the percentage of sclerotia infected by *C. minitans* (Table 1.6). *C. minitans* was isolated from sclerotia in all treated plots and found in the non-treated control plots (Table 1.6). Soil sampled on 11 September 2002 had significantly fewer sclerotia when *C. minitans* was applied for 2 and 3 consecutive years and by 2 October 2002 soil in all three treatments of *C. minitans* had significantly fewer sclerotia than the non-treated control (Table 1.6). When soil was sampled on 16 September 2003, there was no difference between the *C. minitans* treatments and the control but the number of sclerotia was lower than in the previous sample taken in 2002 (Table 1.6). By 16 October 2003 the plots treated with *C. minitans* had slightly fewer sclerotia while the number of sclerotia isolated in the control increased (Table 1.6).

**Sclerotial baits.** There were no significant interactions between the length of burial and treatment on viability and/or infection by *C. minitans* of the sclerotia in baits placed in the long-term field plot. Therefore the data were combined across recovery dates for 2002 and 2003. In both 2002 and 2003 there were no significant treatment effects on the percent of sclerotia infected by *C. minitans* (Table 1.7). *C. minitans* was isolated from the sclerotial

baits in all plots treated with the biological control agent and from the control (Table 1.7). In 2003 the plot treated with one application of *C. minitans* at 2 kg ha<sup>-1</sup> showed a significant reduction of sclerotial viability as compared to the other treatments and the non-treated control (Table 1.7). Several other fungi were also isolated from the sclerotial baits including species in the following genera: *Fusarium*, *Trichoderma*, *Penicillium*, *Aspergillus*, *Sporidesmium*, and *Mucor*.

**Short-term field sites.** Development of Sclerotinia blight occurred in six of the eight locations selected in 2001 and 2002 to evaluate the *C. minitans* treatment. There were no significant interactions between *C. minitans*, fluazinam, and cultivar treatment effects for all locations and dates. Moderate resistance and fungicide provided adequate control of Sclerotinia blight at the six locations (Table 1.8, Table 1.9). Fungicide treatments did not have any effect on peanut quality (data not shown), but the %ELK was higher where Perry was planted in Windsor, Jackson, Scotland Neck, and Gatesville compared with NC-V11 (data not shown). *C. minitans* slightly reduced disease in four locations: Scotland Neck, Hertford and Tyner in 2001 and at Corapeake in 2002, though these reductions were not significant (Table 1.8, Table 1.9, Fig 1.5). There was no effect of *C. minitans* application on peanut yield in all locations and years (Table 1.8, Table 1.9). A higher %ELK was found in plots treated with *C. minitans* in Windsor, Scotland Neck, and Gatesville plots; there was no effect of *C. minitans* on the %FP (data not shown). In 2002 *C. minitans* reduced number of sclerotia recovered at both Gatesville and Corapeake where disease pressure was high (Table 1.10, Fig 1.6). Subsequently only one of these plots had slightly less disease where *C. minitans* was applied. *C. minitans* infected a small number of the sclerotia isolated from soil

samples in both Gatesville and Corapeake short-term field plots but not in Tyner (Table 1.10).

## **DISCUSSION**

*Coniothyrium minitans* reduced the incidence of Sclerotinia blight of peanut and the number of sclerotia in the long-term field plot. However this activity was not observed until at least one year after *C. minitans* was introduced. This activity was observed during favorable environmental conditions and continuous planting of peanut that favored the disease. Both the 2 and 4 kg ha<sup>-1</sup> rate of *C. minitans* applied to the soil decreased the number of sclerotia recovered and disease incidence. The lack of increased control with the higher application of *C. minitans* supports the hypothesis that there is a carrying capacity for this mycoparasite in the soil we examined (17). Once a certain population size is achieved in the soil the addition of additional biological control agent will not reduce disease further as seen often with chemical application.

In our long-term study no significant reduction in disease was observed until approximately 18 months after the first application of *C. minitans* in December of 1999. Many sclerotia may have escaped infection by *C. minitans* and were available to germinate and infect the first peanut crop in 2001 resulting in the disease spreading quickly through the canopy in both treated and non-treated plots. However, in 2002 disease was reduced in all plots that received one or more applications of *C. minitans*. In 2002, three consecutive years of application was most effective in reducing Sclerotinia blight, followed by two and one year of application. By 2003 all three yearly treatments of *C. minitans* effectively reduced disease.

These results suggest that more than one year is needed for the mycoparasite to lower the inoculum density enough for a subsequent reduction of disease. This was also observed in our short-term plots where the sclerotial numbers were reduced by *C. minitans*, but subsequent reduction in disease was not observed. Contact between the spores of the mycoparasite and the sclerotia is required for sclerotial infection (43). Movement of *C. minitans* spores can be augmented by the movement of soil in field preparation and by water or by soil fauna (40, 41, 48, 49, 50) increasing the chance of sclerotial exposure to spores of *C. minitans*, since *C. minitans* does not grow saprophytically through soil (47). *C. minitans* will produce conidia after infection of sclerotia, so that secondary spread of the mycoparasite can also contribute to increased distribution in the soil. The longer *C. minitans* is present in the soil the more likely it will come in contact with, infect, and reduce the inoculum density of sclerotia, ultimately decreasing disease incidence (6, 12).

Where the effects of *C. minitans* on reducing numbers of sclerotia recovered in soil was promoted with longer exposure, disease incidence of Sclerotinia blight increased each year. This was expected since planting of peanut 3 consecutive years greatly increases inoculum density as a result of sclerotia which are shed into the soil from diseased tissue. Continuous cropping was used in our study since we wanted to examine the long-term effect of *C. minitans* on Sclerotinia blight. Current production practices of rotating peanut with corn or cotton, non-hosts of *S. minor*, take this in account limiting the number of consecutive years peanut is planted in a field (26, 33). Long-term rotation has been shown to help reduce disease and allows the sclerotia to be broken down by various soil microorganisms during periods when there is no host to infect (29, 40, 41).

The current control measures used for Sclerotinia blight, including moderate resistance and fungicide applications, did not interact with *C. minitans*. Application of fluazinam to the peanut canopy significantly reduced disease compared to non-treated plots. Fluazinam is a very effective fungicide against *S. minor* when applied at proper times protecting the peanut canopy from infection (10, 11, 37, 38). Fluazinam does not reduce the numbers of sclerotia, but its integration with *C. minitans* would aim to further disrupt the disease cycle. Laboratory experiments have shown that fluazinam limits spore germination, mycelial growth, and mycoparasitic activity of *C. minitans* (D. E. Partridge, *unpublished*). Even though we detected no treatment interactions of fluazinam and *C. minitans* in the field, applications of *C. minitans* should be made cautiously when fluazinam, azoxystrobin, chlorothalonil, pyraclostrobin, and tebuconazole are being used in the field. These fungicides may compromise the performance of *C. minitans*. Application of *C. minitans* over the peanut canopy is not recommended for peanut production as it is in horticultural crops (12). Less disease was also seen in plots planted with the cultivar Perry. Perry has partial resistance that limits the amount of infection by *S. minor* in the peanut canopy (16). Integration of the fungicide fluazinam and moderate resistance with the mycoparasite will help further manage this disease by reducing initial inoculum and rate of infection, thereby slowing the disease epidemic in peanut.

Sclerotia were isolated from soil each month during the peanut growing season. The ability to predict disease incidence by enumerating sclerotia has been difficult for Sclerotinia blight. Numbers of sclerotia tended to fluctuate throughout the growing season as the sclerotia germinate to infect peanuts and as they shed back into the soil from diseased tissue.

Averaging samples taken in September and October did show a reduction of sclerotial numbers with *C. minitans* treatment. Four to 29 % of the sclerotia recovered in samples from each treatment were infected by *C. minitans*. These percentages only represent those sclerotia that were isolated from the soil, while the sclerotia that were degraded by *C. minitans* would not be represented in the sample. *C. minitans* was also isolated from sclerotia placed in the soil during the 2002 and 2003. Reduced sclerotial numbers and their colonization demonstrate the ability of *C. minitans* to infect sclerotia and persist in peanut fields. The baits also showed that there are a number of other fungi present in the soil that can aid in the destruction of sclerotia including species of *Fusarium*, *Trichoderma*, *Penicillium*, *Aspergillus*, *Sporidesmium*, and *Mucor*. These genera have all been shown to be mycoparasites of *Sclerotinia* spp., so potential exists to reduce sclerotial numbers in soil if natural mycoparasitic populations are promoted (2, 3, 4, 19, 22, 51).

Results from these field studies indicate that soil applications of *C. minitans* may be most useful in peanut fields that are heavily infested with *S. minor*. *C. minitans* can easily be integrated with moderately resistant cultivars and fungicide applications in managing *Sclerotinia* blight on peanut. *C. minitans* is able to persist and infect sclerotia of *S. minor* in peanut fields of North Carolina. The biological control agent can be applied in the fall across crop residue and incorporated into the top layers of the soil where it will most effectively parasitize the sclerotia of *S. minor*. It will not provide a rapid cure to fields heavily infested by *S. minor*, but over time has the potential to reduce inoculum levels and ultimately increase the health of peanut by reducing *Sclerotinia* blight.

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**Table 1.1.** Main plot and subplot treatments applied in long-term study located in Gatesville, North Carolina.

<i>Coniothyrium minitans</i> <sup>y</sup> treatment in main plots				
Name	Rate kg ha <sup>-1</sup>	Fall 1999	Fall 2000	Fall 2001
<i>C. minitans</i>	2	yes	yes	yes
<i>C. minitans</i>	4	yes	yes	yes
<i>C. minitans</i>	2	no	yes	yes
<i>C. minitans</i>	4	no	yes	yes
<i>C. minitans</i>	2	no	no	yes
<i>C. minitans</i>	4	no	no	yes
Control	na	no	no	no

Subplot treatments (cultivar by fungicide)	
Cultivar	Fungicide treatments
Perry	fluazinam <sup>z</sup>
NC-V11	fluazinam
Perry	none
NC-V11	none

<sup>y</sup> *C. minitans* applied as Contans WG (Prophyta Biologischer Pflanzenschutz GmbH, Germany).

<sup>z</sup> fluazinam formulated as Omega 500F (Syngenta Crop Protection, Greensboro, NC) and applied at 0.625 kg a.i. ha<sup>-1</sup>.

**Table 1.2.** General treatments for short-term field studies conducted in 2001 and 2002.

Cultivar	<i>Coniothyrium minitans</i> <sup>y</sup>	Fungicide treatment <sup>z</sup>
Perry	4 kg ha <sup>-1</sup>	fluazinam
NC-V11	4 kg ha <sup>-1</sup>	fluazinam
Perry	4 kg ha <sup>-1</sup>	none
NC-V11	4 kg ha <sup>-1</sup>	none
Perry	none	fluazinam
NC-V11	none	fluazinam
Perry	none	none
NC-V11	none	none

<sup>y</sup> *C. minitans* applied as Contans WG (Prophyta Biologischer Pflanzenschutz GmbH, Germany).

<sup>z</sup> fluazinam formulated as Omega 500F (Syngenta Crop Protection, Greensboro, NC) and applied at 0.625 kg a.i. ha<sup>-1</sup>.

**Table 1.3.** Field experiments, locations, cultivars, and dates of planting, *Coniothyrium minitans* soil incorporation, fluazinam application dates, and harvest date.

Experiment	Location and year	Cultivar	Planting date	Dates of <i>C. minitans</i> application	Dates of fluazinam application			Harvest date
					1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	
<b>Long-Term</b>								
	<i>Gatesville</i>			03 Dec. 99				
	2001	NC-V11, Perry	02 May	09 Feb. 01	26 July	29 Aug.	22 Oct.	22 Oct.
	2002	NC-V11, Perry	16 May	12 Nov. 01	30 July	12 Sept.	-----	08 Nov.
	2003	Perry	18 June	-----	21 Aug.	10 Sept.	-----	10 Nov.
<b>Short-Term</b>								
	<i>2001</i>							
	Windsor	NC-V11, Perry	10 May	15 Jan. 01	25 July	23 Aug.	-----	05 Oct.
	Tyner	VA 98R	12 May	15 Jan. 01	25 July	23 Aug.	-----	02 Oct.
	Scotland Neck	NC-V11, Perry	08 May	15 Jan. 01	25 July	21 Aug.	-----	16 Oct.
	Jackson	NC-V11, Perry	07 May	15 Jan. 01	24 July	30 Aug.	-----	03 Oct.
	Hertford	VA 98R	10 May	15 Jan. 01	25 July	23 Aug.	-----	02 Oct.
	<i>2002</i>							
	Tyner	NC-V11, Perry	08 May	21 Nov. 01	31 July	04 Sept.	-----	-----
	Corapeake	NC-V11, Perry	15 May	12 Nov. 01	30 July	11 Sept.	-----	08 Nov.
	Gatesville	NC-V11, Perry	28 May	22 Nov. 01	31 July	06 Sept.	-----	29 Nov.

**Table 1.4.** Summary of analyses of variance for main effects of *Coniothyrium minitans* treatment, fungicide, and cultivar, their interactions, and contrasts of *C. minitans* treatment on Sclerotinia disease incidence and peanut yield in long-term field study for 2001, 2002 and 2003<sup>x</sup>.

Source	df	2001 AUDPC <sup>y</sup>		2001 Yield		2002 AUDPC		2002 Yield		2003 AUDPC		2003 Yield	
		F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F
Rep	3	0.97	ns	6.06	**	0.48	ns	0.36	ns	7.48	***	7.48	***
Contans Treatment	6	1.46	ns	1.34	ns	7.74	**	1.20	ns	2.62	ns	2.62	ns
Fungicide (Fung.)	1	8.79	**	7.77	**	26.12	****	1.45	ns	295.73	****	295.73	****
Cultivar (Cult.)	1	25.08	****	2.05	ns	91.42	****	1.40	ns	-	-	-	-
Fungicide*Cultivar	1	0.80	ns	0.13	ns	0.32	ns	4.88	*	-	-	-	-
Contans*Fungicide	6	0.77	ns	3.01	*	0.48	ns	2.14	ns	0.86	ns	0.86	ns
Contans*Cultivar	6	0.91	ns	0.75	ns	1.99	ns	2.29	*	-	-	-	-
Contans*Fung.*Cult.	7	0.37	ns	2.86	*	1.70	ns	1.27	ns	-	-	-	-
<b>Contrasts for <i>C. minitans</i> treatment</b>													
Contans year	1	0.41	ns	1.09	ns	19.04	***	0.39	ns	38.84	****	38.84	****
Control vs. 3 years	1	-	-	-	-	26.35	***	0.15	ns	23.61	****	23.61	****
Control vs. 2 years	1	0.04	ns	1.43	ns	3.66	ns	2.32	ns	27.62	****	27.62	****
Control vs. 1 year	1	4.05	*	0.06	ns	6.71	*	1.52	ns	36.89	****	36.89	****
1 year vs. 2 years	1	3.67	ns	0.69	ns	0.46	ns	0.09	ns	0.67	ns	0.67	ns
1 year vs. 3 years	1	-	-	-	-	6.47	*	2.63	ns	1.48	ns	1.48	ns
2 years vs. 3 years	1	-	-	-	-	10.36	**	3.67	ns	0.16	ns	0.16	ns
Contans rate	1	1.46	ns	0.47	ns	14.35	**	1.88	ns	42.20	****	42.20	****
Control vs. 2 kg/ha	1	0.34	ns	1.28	ns	14.21	**	0.08	ns	35.65	****	35.65	****
Control vs. 4 kg/ha	1	1.50	0.2267	ns	0.7651	10.68	**	4.02	ns	34.23	****	34.23	****
2 kg/ha vs. 4 kg/ha	1	0.30	0.5835	ns	0.4745	0.32	ns	6.51	*	0.02	ns	0.02	ns

<sup>x</sup> \*, \*\*, \*\*\*, \*\*\*\* Significant at the 0.05, 0.01, 0.001, and <0.0001 probability levels respectively and ns = not significant at P≤0.05.

<sup>y</sup> AUDPC =area under disease progress curve, calculated using disease incidence taken from 2 center rows of each plot.

**Table 1.5.** Mean area under disease progress curve (AUDPC) and yield (kg ha<sup>-1</sup>) of main effects in the long-term field study for 2001, 2002, and 2003 peanut growing seasons.<sup>w</sup>

Main Effect	Treatment	2001		2002		2003	
		AUDPC <sup>x</sup>	Yield <sup>y</sup> (kg ha <sup>-1</sup> )	AUDPC	Yield (kg ha <sup>-1</sup> )	AUDPC	Yield (kg ha <sup>-1</sup> )
<i>C. minitans</i> Rate	0	188	5810	842 a	3050 b	874 a	1950 a
	2 kg ha <sup>-1</sup>	167	5640	591 b	3000 b	642 b	1430 b
	4 kg ha <sup>-1</sup>	152	5770	625 b	3370 a	646 b	1440 b
Years of <i>C. minitans</i> <sup>z</sup>	0	181 a	5810	842 a	3050	874 a	1950 a
	1	133 b	5780	653 b	3270	615 b	1370 b
	2	188 ab	5630	702 ab	3310	650 b	1450 b
	3	...	...	469 c	2980	667 b	1490 b
Fluazinam	0	207 a	5660 b	749 a	3050	1089	2430
	0.625 kg a.i. ha <sup>-1</sup>	132 b	5850 a	584 b	3260	313	700
Cultivar	NC-V11	234 a	5810	826 a	3080	...	...
	Perry	106 b	5700	507 b	3220	701	1560

<sup>w</sup> Contrasts were used to separate rate and yearly effects of *C. minitans* treatment, values followed by the same letter are not significantly different at a P≤0.05. There were no significant interactions between main effects in all years for disease.

<sup>x</sup> AUDPC =area under disease progress curve, calculated using disease incidence taken from 2 center rows of each plot.

<sup>y</sup> Yield estimated from two center rows.

<sup>z</sup> Only 1 and 2 years of application of *C. minitans* were made in 2001 and 2003 is the year following the yearly treatments.

**Table 1.6.** Average number, viability, and infection by *Coniothyrium minitans* of sclerotia recovered from soil in the long-term field study.<sup>y</sup>

<i>C. minitans</i> treatment	11 Sept. 2002			10 OCT. 2002			16 Sept. 2003			16 Oct. 2003			Average of dates		
	Sclerotia	% Viable	% Infected	Sclerotia	% Viable	% Infected	Sclerotia	% Viable	% Infected	Sclerotia	% Viable	% Infected	Sclerotia	% Viable	% Infected
Control	9 a <sup>z</sup>	53.4	14.2	21 a	39.1	1.9	9	51.0	14.3 ab	13	64.1	21.0	13 a	51.9	12.8
1 Year	7 ab	62.0	11.1	13 b	23.3	10.4	8	59.1	3.1 b	8	58.4	10.1	9 b	50.7	8.7
2 Years	4 b	65.2	28.9	13 b	38.8	4.3	9	50.0	31.5 a	8	51.3	12.8	8 b	51.4	19.0
3 Years	5 b	49.6	29.4	11 b	38.0	9.4	9	58.7	13.5 b	8	66.8	14.8	8 b	53.3	16.7
F-value (P)	3.69 (0.0281)	NS	NS	2.66 (0.0747)	NS	NS	NS	NS	3.87 (0.0257)	NS	NS	NS	3.19 (0.0264)	NS	NS
'Control vs. <i>C. minitans</i> '	7.77 (0.0111)	NS	NS	7.81 (0.0109)	NS	NS	NS	NS	NS	2.00 (0.1716)	NS	NS	9.53 (0.0025)	NS	NS

<sup>y</sup> Sclerotia isolated from 500 g dried soil, % viable and % infected by *C. minitans* determined from sclerotia isolated from soil.

<sup>z</sup> Values followed by the same letter in each column are not significantly different at a  $P \leq 0.05$  according to Fisher's protected least significant difference.

**Table 1.7.** Percentage of sclerotia that were viable and/or infected by *Coniothyrium minitans* from baits placed in long-term field study during 2002 and 2003.<sup>y</sup>

<i>C. minitans</i> treatment	2002	2003	
	Infection (%)	Viable sclerotia (%)	Infection (%)
4 kg/ha for 3 years	27.18	22.85 a <sup>z</sup>	12.14
2 kg/ha for 3 years	16.59	18.97 a	10.44
4 kg/ha for 2 years	9.78	26.68 a	16.06
2 kg/ha for 2 years	14.16	28.09 a	5.69
4 kg/ha for 1 year	23.37	20.89 a	9.29
2 kg/ha for 1 year	20.12	4.82 b	6.32
Non-treated Control	11.82	27.24 a	6.23
F-Value (P)	1.49 (0.1935)	2.76 (0.0171)	0.78 (0.5856)
Contrast Years	0.42 (0.5207)	0.02 (0.8848)	1.36 (0.2477)
Contrast Rate	1.08 (0.3022)	1.22 (0.2728)	0.97 (0.3270)
Contrast Contans vs. Control	0.92 (0.3415)	1.71 (0.1941)	0.66 (0.4184)

<sup>y</sup> Sclerotia baits buried under peanut canopy 2, 4, 8, 12 weeks. Data were combined across length of burial for each year.

<sup>z</sup> Values followed by the same letter in each column are not significantly different at a  $P \leq 0.05$ .

**Table 1.8.** Mean area under disease progress curve (AUDPC) and yield (kg ha<sup>-1</sup>) of main treatments applied to short-term field studies located in peanut production areas of North Carolina in 2001.<sup>x</sup>

Treatment	2001									
	Windsor		Jackson		Scotland Neck		Hertford		Tyner	
	AUDPC <sup>y</sup>	Yield <sup>z</sup> (kg ha <sup>-1</sup> )	AUDPC	Yield (kg ha <sup>-1</sup> )	AUDPC	Yield (kg ha <sup>-1</sup> )	AUDPC	Yield (kg ha <sup>-1</sup> )	AUDPC	Yield (kg ha <sup>-1</sup> )
Contans	224	4215	19	2782	1	4437	357	4730	0	3950
No Contans	237	4332	12	2503	6	4351	436	4595	3	3645
F- Value (P)	0.02 (0.8830)	0.01 (0.9440)	1.70 (0.2487)	2.27 (0.1921)	0.29 (0.6109)	0.13 (0.7354)	0.35 (0.5819)	3.70 (0.1123)	1.65 (0.2548)	4.72 (0.0819)
Fluazinam	136 b	4363	9	2728	0	4541 a	235b	4869 a	0.3	3875
No Fuazinam	326 a	4211	23	2557	2	4247 b	557a	4456 b	0	3719
F-Value (P)	38.88 (<.0001)	4.28 (0.0564)	2.80 (0.1049)	1.36 (0.2519)	3.46 (0.0726)	4.40 (0.0445)	11.95 (0.0062)	6.44 (0.0294)	0.07 (0.7979)	1.27 (0.2858)
Perry	233	4269	7	2573	0	3949 b	...	...	...	...
NCV11	228	4283	24	2712	2	4839 a	...	...	...	...
F-Value (P)	0.02 (0.8780)	0.13 (0.7286)	4.32 (0.0463)	0.89 (0.3529)	3.46 (0.0726)	40.46 (<.0001)				

<sup>x</sup> Values followed by the same letter are not significantly different at a P≤0.05.

<sup>y</sup> AUDPC =area under disease progress curve, calculated using disease incidence taken from two center rows of each plot.

<sup>z</sup> Yield estimated from two center rows.

**Table 1.9.** Mean area under disease progress curve (AUDPC) and yield (kg ha<sup>-1</sup>) of main treatments applied to short-term field studies located in peanut production areas of North Carolina in 2002.<sup>x</sup>

Treatment	2002					
	Tyner		Gatesville		Corapeake	
	AUDPC <sup>y</sup>	Yield <sup>z</sup> (kg ha <sup>-1</sup> )	AUDPC	Yield (kg ha <sup>-1</sup> )	AUDPC	Yield (kg ha <sup>-1</sup> )
Contans	65	...	799	2900	434	1222
No Contans	66	...	774	3040	467	1217
F- Value (P)	0.00 (0.9940)		0.07 (0.7990)	0.55 (0.4912)	1.03 (0.3569)	0.01 (0.9359)
Fluazinam	11 a	...	676 a	2989	323 a	1372 a
No Fuazinam	119 b	...	898 b	2952	556 b	1068 b
F-Value (P)	96.12 (<.0001)		15.17 (0.0005)	0.11 (0.7479)	15.07 (0.0005)	22.73 (<.0001)
Perry	67	...	505 a	3077	434	1224
NCV11	64	...	1069 b	2864	464	1216
F-Value (P)	0.10 (0.7587)		98.20 (<.0001)	3.42 (0.0742)	0.31 (0.5821)	0.75 (0.5912)

<sup>x</sup> Values followed by the same letter are not significantly different at a P≤0.05.

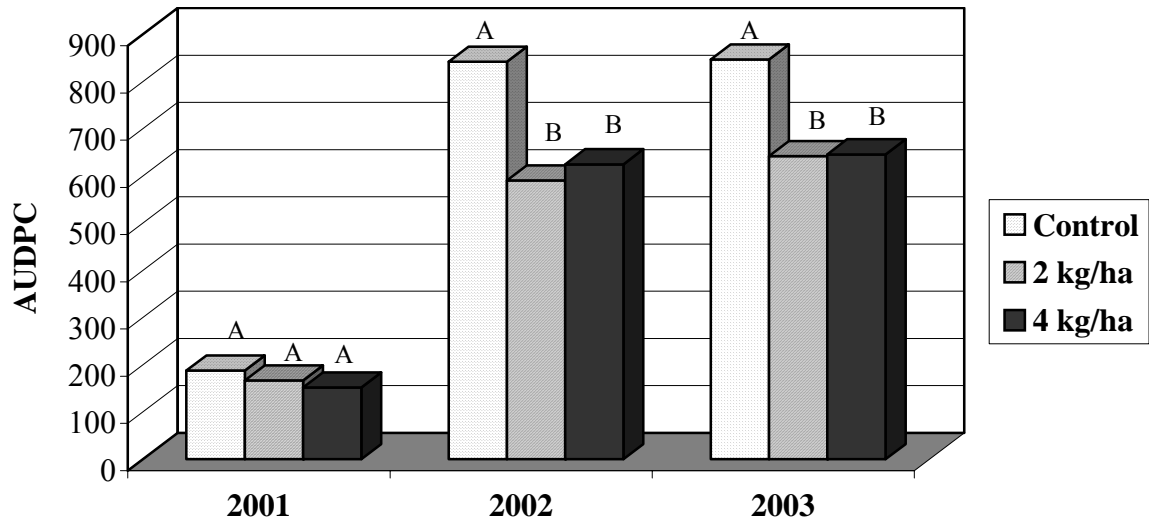
<sup>y</sup> AUDPC =area under disease progress curve, calculated using disease incidence taken from two center rows of each plot.

<sup>z</sup> Yield estimated from two center rows.

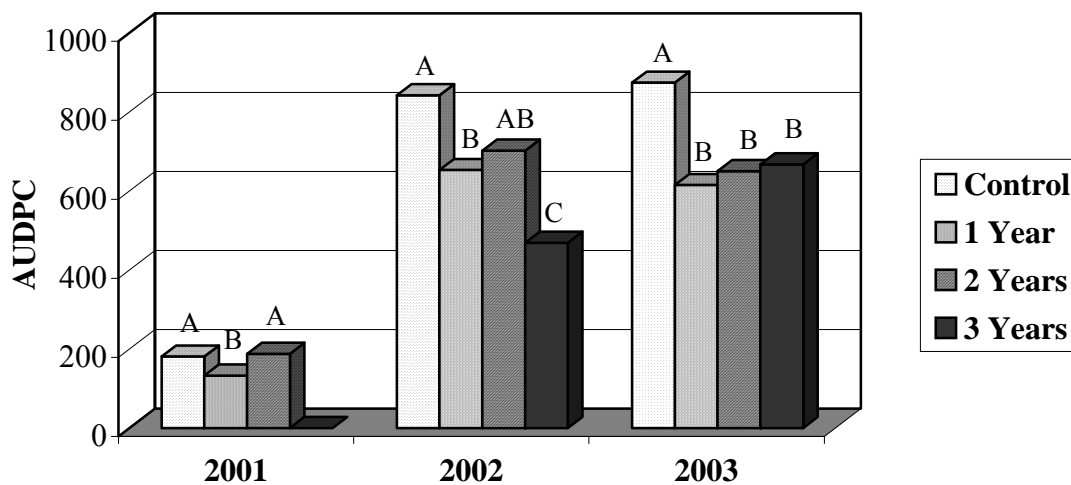
**Table 1.10.** Sclerotia isolated from 500 g dry soil in short-term field studies.<sup>z</sup>

Treatment	Gatesville			Corapeake		Tyner	
	13 Aug. 2002	11 Sept. 2002	9 Oct. 2002	13 Aug. 2002	11 Sept. 2002	13 Aug. 2002	11 Sept. 2002
<i>C. minitans</i>	4 b	6	7 a	10	3	1	2
Control	8 a	9	5 b	11	6	1	2
F-Value (P)	18.83 (0.0049)	0.47(0.5193)	8.98 (0.0241)	0.07 (0.7963)	1.58 (0.2551)	1.28 (0.3004)	0.38 (0.5618)
Fluazinam	4 b	10	4 b	13	2	1	2
No fluazinam	8 a	5	8 a	8	7	1	3
F-Value (P)	13.48 (0.0104)	0.98 (0.3594)	81.57 (0.0001)	1.04 (0.3472)	3.50 (0.1105)	1.43 (0.2765)	1.04 (0.3462)

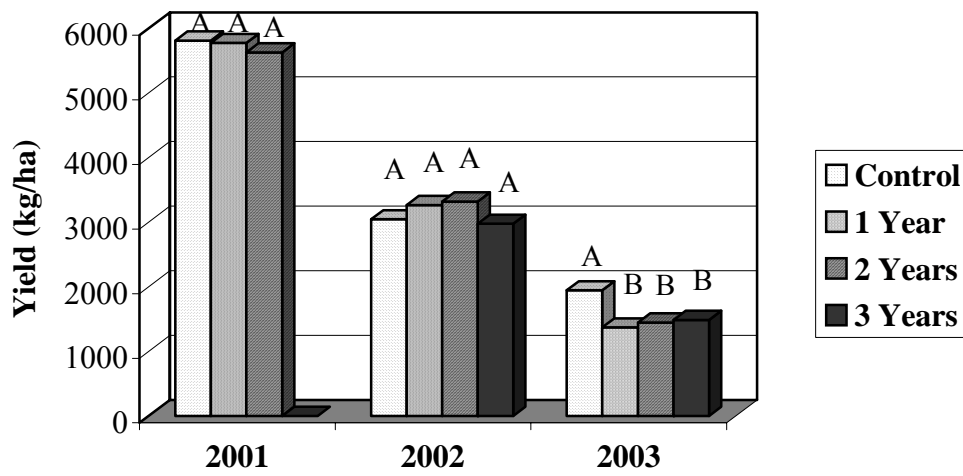
<sup>z</sup> Values followed by the same letter in each column are not significantly different at a  $P \leq 0.05$  according to Fisher's protected least significant difference.



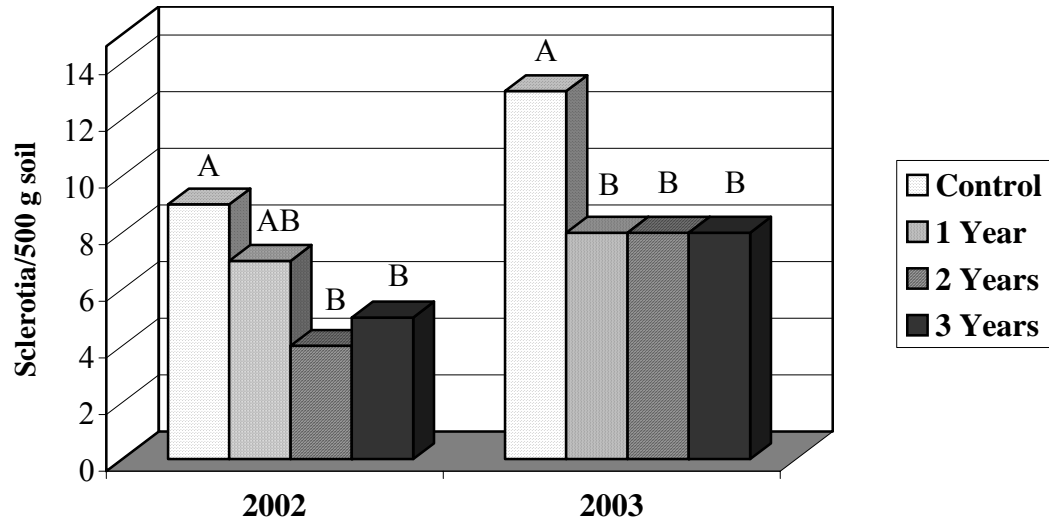
**Figure 1.1.** Effect of 2 and 4 kg ha<sup>-1</sup> soil applications of *Coniothyrium minitans* in the long-term field study on Sclerotinia blight as measured by the area under disease progress curve (AUDPC) for 2001, 2002, and 2003. Treatments followed by the same letter for each year are not significantly different at P≤0.05 according to Fisher’s protected least significant difference.



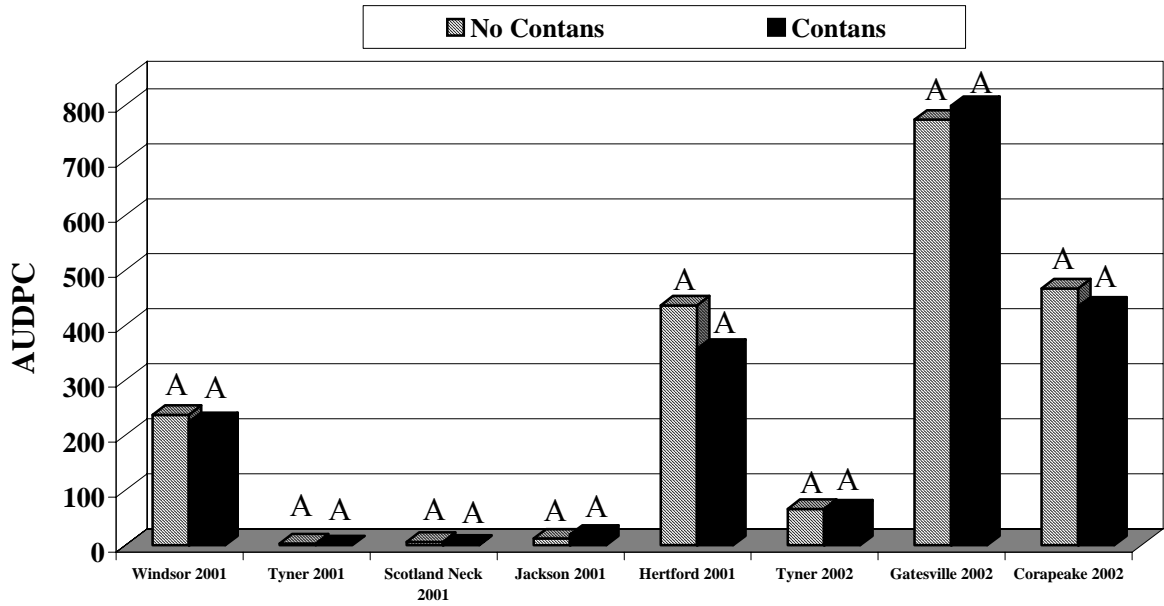
**Figure 1.2.** Effect of 1, 2, and 3 years of *Coniothyrium minitans* soil application in long-term field study on *Sclerotinia* blight as measured by area under disease progress curve (AUDPC). Treatments followed by the same letter for each year are not significantly different at  $P \leq 0.05$  according to Fisher's protected least significant difference.



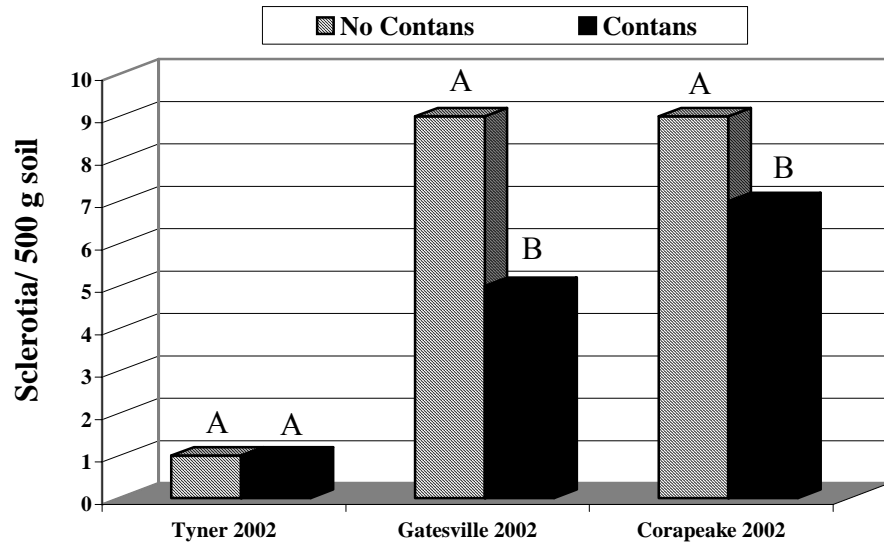
**Figure 1.3.** Effect of 1, 2, and 3 years of *Coniothyrium minitans* soil application in long-term field study on peanut yield (kg ha<sup>-1</sup>). Treatments followed by the same letter for each year are not significantly different at a  $P \leq 0.05$  according to Fisher's protected least significant difference.



**Figure 1.4.** Effect of 1, 2, and 3 consecutive years of *Coniothyrium minutans* soil application in long-term field study on number of sclerotia recovered from 500 g of dry soil. Treatments followed by the same letter for each year are not significantly different at a  $P \leq 0.05$  according to Fisher's protected least significant difference.



**Figure 1.5.** Effect of *C. minitans* application on short-term field studies in 2001 and 2002 on area under disease progress curve (AUDPC). There was no significant differences between treatments for any location for either year.



**Figure 1.6.** Effect of *Coniothyrium minitans* application in short-term field studies for 2001 and 2002 on number of sclerotia recovered from 500 g of dry soil. Treatments followed by the same letter for each year are not significantly different at  $P \leq 0.05$  according to Fisher's protected least significant difference.

## **CHAPTER 2**

# **SENSITIVITY OF *CONIOTHYRIUM MINITANS* MYCELIAL GROWTH, CONIDIA GERMINATION, AND MYCOPARASITIC ACTIVITY ON SCLEROTIA OF *SCLEROTINIA MINOR* TO PESTICIDES COMMONLY USED IN PEANUT PRODUCTION**

## ABSTRACT

Partridge, D. E., Sutton, T. B., Jordan, D. L. 2005. Sensitivity of *Coniothyrium minitans* mycelial growth, conidia germination, and mycoparasitic activity on sclerotia of *Sclerotinia minor* to pesticides commonly used in peanut production. To be submitted for publication in Plant Disease.

Nine pesticides commonly used in peanut production (five fungicides and four herbicides) were evaluated for their effect on mycelial growth, conidia germination, and mycoparasitic activity of *Coniothyrium minitans* on sclerotia of *Sclerotinia minor*. The effective concentration (EC<sub>50</sub>) that reduced mycelial growth of *C. minitans* by 50 % was < 1 µg a.i. ml<sup>-1</sup> for chlorothalonil, fluazinam, pyraclostrobin, tebuconazole, and flumioxazin and > 10 µg a.i. ml<sup>-1</sup> for azoxystrobin, diclosulam, S-metolachlor, and pendimethalin. EC<sub>50</sub>s for conidia germination of *C. minitans* were < 1 µg a.i. ml<sup>-1</sup> for chlorothalonil, fluazinam, pyraclostrobin, tebuconazole, and flumioxazin, and > 10 µg a.i. ml<sup>-1</sup> for azoxystrobin, diclosulam, S-metolachlor, and pendimethalin. A modified soil plate technique was used to assess the pesticides for their effect on infection of *S. minor* sclerotia by *C. minitans*. *C. minitans* survived and infected sclerotia of *S. minor* in the presence of all nine pesticides, but mycoparasitic activity was reduced compared to the nontreated control by all pesticides but S-metolachlor. The ability of *C. minitans* to parasitize sclerotia of *S. minor* even in the presence of the nine pesticides demonstrates their compatibility with the biological control agent. However, high sensitivity of mycelial growth, conidia germination, and reduced mycoparasitic activity to the five fungicides and the herbicide flumioxazin indicates that *C.*

*minitans* should not be applied during times in which there is a high risk of it contacting these pesticides before it becomes established in the soil.

## INTRODUCTION

*Coniothyrium minitans* Campbell is a fungal mycoparasite of sclerotia formed by *Sclerotinia* spp. and has been shown to be an effective biological control agent in several crops (10, 15, 21, 22, 27, 28). Sclerotinia blight of peanut, caused by the soilborne fungus *Sclerotinia minor* (Jagger) Kohn (17), is an important disease in the peanut growing regions in Virginia and North Carolina. Recent field studies have shown that *C. minitans* is able to colonize sclerotia of *S. minor* and reduce disease in peanut when applied for consecutive years (Partridge, *Chapter 1*). However, various agrichemicals used in peanut production may affect the mycoparasitic activity of *C. minitans*. Twenty three fungicides, twelve insecticides, and twenty herbicides are registered for use on peanut in North Carolina (29). Previous studies have examined the interaction of *C. minitans* with pesticides used in lettuce production, including the fungicides iprodione, and thiram, which were found to reduce mycelial growth and spore germination, but not mycoparasitism of sclerotia of *S. sclerotiorum* (9). *C. minitans* growth and spore germination were also found to be highly sensitive to benomyl and vinclozolin ( $EC_{50} < 1 \mu\text{g a.i. mL}^{-1}$ ) (19).

Among the most widely used pesticides in peanut production are the fungicides azoxystrobin, chlorothalonil, fluazinam, pyraclostrobin, and tebuconazole, and the herbicides diclosulam, flumioxazin, S-metolachlor, and, pendimethalin, (25). The herbicides selected are those that are soil applied (PPI or PRE), and therefore are most likely to interact with *C.*

*minitans* in the soil early in the season, while most Post herbicides very often do not contact a high percentage of the soil when applied. Azoxystrobin and pyraclostrobin are strobilurin fungicides that inhibit respiration and are known to suppress spore germination in a number of filamentous fungi (7, 12). Chlorothalonil is a broad spectrum aromatic hydrocarbon fungicide that inhibits sulfur containing enzymes, disrupts energy production, and inhibits spore germination in fungi (12). Fluazinam is a pyridinamine fungicide that inhibits fungal respiration and energy production and is currently used in the management of *S. minor* on peanut (5, 13, 23). Tebuconazole is a triazole fungicide that inhibits sterol production in a wide range of foliar and soilborne fungi that cause plant diseases (18, 26). Diclosulam, a triazolopyrimidine herbicide that is an acetolactate synthase inhibitor (20), and flumioxazin, a *N*-phenyl phthalimide herbicide that is an inhibitor of protoporphyrinogen (4, 30); both provide control of broadleaf weeds in peanut (16). *S*-metolachlor, a chloroacetanilide herbicide inhibits synthesis of long-chain fatty acids and is used to control a number of grasses in peanut (16, 20). Pendimethalin, a dinitraniline herbicide inhibits microtubule assembly in annual grasses and small-seeded broadleaf weeds (16, 20). These pesticides are commonly used in weed and disease management in peanut and would potentially interact with *C. minitans* in the field during the growing season.

For *C. minitans* to become widely accepted as a management tool it must be successfully integrated with the current production practices. The objective of this research was to evaluate the sensitivity of *C. minitans* to nine pesticides commonly used in peanut production and to determine if they interfere with mycoparasitic activity on sclerotia of *S. minor*.

## MATERIALS AND METHODS

**Isolate information and production of sclerotia.** An isolate of *C. minitans* (CON/M/91-08) obtained by plating out the commercial formulation Contans WG (Propyta Biologischer Pflanzenschutz GmbH) was grown on potato dextrose agar (PDA) in an incubator at 18° C. A spore suspension of *C. minitans* was made by flooding the culture dishes with sterilized water and gently scraping the colony surfaces to free conidia from the pycnidia. The spore suspension was collected and adjusted to approximately  $1 \times 10^6$  conidia  $\text{ml}^{-1}$  with a haemocytometer.

An isolate of *S. minor* [#20 obtained from Dr. Barbara Shew, North Carolina State University (14)] originally isolated from a diseased peanut plant in Gates County, North Carolina was used to produce sclerotia. Sclerotia were produced at 18° C on sterilized oat kernels infested with *S. minor* (1). After 5 weeks, oats were dried for 2 w at room temperature (~22° C) and then stored in a refrigerator at 5° C until used in experiments.

**Pesticide information.** Commercial formulations of all pesticides were used in this study. Fungicides used and the maximum labeled rate for peanuts were: azoxystrobin (Abound Flowable, Syngenta Crop Protection, Greensboro, NC; maximum labeled application rate (MLAR)  $450 \text{ g a.i. ha}^{-1}$ ); chlorothalonil (Bravo Weather Stik, Syngenta Crop Protection, Greensboro, NC; MLAR  $1.26 \text{ kg a.i. ha}^{-1}$ ); fluazinam (Omega 500F, Syngenta Crop Protection, Greensboro, NC; MLAR  $875 \text{ g a.i. ha}^{-1}$ ); pyraclostrobin (Headline, BASF Corp., Research Triangle Park, NC; MLAR  $275.4 \text{ g a.i. ha}^{-1}$ ); and tebuconazole (Folicur 3.6 F, Bayer Crop Science, Research Triangle Park, NC; MLAR  $228.6 \text{ g a.i. ha}^{-1}$ ). Herbicides

included: diclosulam (Strongarm, Dow AgroSciences LLC, Indianapolis, IN; MLAR 26.5 g a.i. ha<sup>-1</sup>); flumioxazin (Valor, Valent U.S.A. Corp., Walnut Creek, CA; MLAR 107 g a.i. ha<sup>-1</sup>); S-metolachlor (Dual Magnum, Syngenta Crop Protection, Greensboro, NC; MLAR 2.14 kg a.i. ha<sup>-1</sup>); and pendimethalin (Prowl H2O, BASF Corp., Research Triangle Park, NC; MLAR 1.07 kg a.i. ha<sup>-1</sup>).

**Mycelial growth and conidia germination.** Stock suspensions of each pesticide were prepared using sterile distilled water. The stock suspension of each pesticide was then adjusted to appropriate concentrations to achieve final concentrations of 0.01, 0.1, 1, 10 µg a.i. ml<sup>-1</sup> when added to molten PDA. PDA was autoclaved and cooled to 55°C before amending with each pesticide. Fifteen ml of the pesticide-amended medium was then added to standard 9-cm petri dishes. A 5-mm plug taken from the margin of an actively growing 7-day-old culture of *C. minitans* was placed upside down in the center of each test dish. Dishes were incubated at 18° C and radial growth of *C. minitans* was measured in two axes every 48 h for 14 days. There were three replicate dishes for all pesticide treatments and the experiments were repeated twice. Percentage of mycelial growth inhibition (PMGI) was calculated by the formula  $PMGI = 100 \times [(D_{PDA} - D_F) / D_{PDA}]$ , where  $D_{PDA}$  is the average colony diameter of *C. minitans* after 14 days of growth on PDA and  $D_F$  is the colony diameter of *C. minitans* after 14 days of growth on PDA amended with each of the pesticides.

*C. minitans* conidia germination was measured in the presence of the pesticides at 0.01, 0.1, 1, and 10 µg a.i. ml<sup>-1</sup> by placing three drops of conidia suspension (5x10<sup>5</sup> spores per ml) on surface of each agar dish. The dishes were incubated at 18°C for 32 h at which time 85% lactic acid was added to each dish to stop conidia germination. Conidia

germination was assessed by counting the number of germinated conidia out of 100 per spot on each dish using a compound light microscope (9). A conidium was considered germinated when the germ tube length was equal to or greater than half the length of the conidium. There were three replicate dishes for all pesticide treatments and the experiments were repeated once. Percentage of conidia germination inhibition (PCPI) was calculated by the formula  $PCGI = 100 \times [(C_{PDA} - C_F)/C_{PDA}]$ , where  $C_{PDA}$  is the number of conidia that germinated on PDA after 32 h and  $C_F$  is the number of conidia after 32 h that germinated on PDA amended with each pesticide.

**Soil-plate experiments.** Pesticide effects on the mycoparasitism of sclerotia of *S. minor* by *C. minitans* were examined using a modification of a soil plate technique described by Smith et al. 1991(24). A sample of a Rains fine sandy-loam soil, was collected from a peanut field in Gates County, North Carolina, where *Sclerotinia* blight is a common problem in peanut production. The field soil was air-dried then sifted through a U. S. standard number 12 sieve (1.7 mm opening) to produce uniform sized soil particles and remove debris. The soil was autoclaved for 30 min (121° C at 103 kPa) allowed to cool and then 30 g of soil was dispensed into 9-cm plastic petri dish and 10 ml of sterile distilled H<sub>2</sub>O was added to each dish. Twenty sclerotia were placed on the soil surface and sprayed with a suspension of spores of *C. minitans* (~ 1 x 10<sup>6</sup> spores ml<sup>-1</sup>) using an airbrush sprayer. The dishes were placed for 4 h on the laboratory bench to allow for spores to settle, after which an airbrush sprayer was used to apply the maximum recommended field concentration of each pesticide across each dish. The maximum rates per ha were converted to maximum rates per dish using surface area of the dish equal to 63.6 cm<sup>2</sup>. The dishes were incubated at 18° C for 14

days. After 14 days, sclerotia were removed from the soil plate, surface disinfested in 15% NaClO for 30 s, rinsed in sterilized distilled H<sub>2</sub>O and plated on PDA amended with tetracycline (25 mg L<sup>-1</sup>). The number of sclerotia with mycelial growth (viable) and/or infection by *C. minitans* were assessed after 7 d incubation at 18 °C. Each pesticide treatment plus a non-treated control replicated three times and the experiment was repeated once.

**Data analyses.** For each experiment the experimental design was a randomized complete block. Analysis of variance (ANOVA) (Version 8, SAS Institute Inc., Cary, NC 1999-2001) was used to separate the treatment effects on sclerotia viability and infection by *C. minitans*. Significant differences between treatments in each experiment were assessed using Fisher's protected least significant difference at P≤0.05 level. For calculation of EC<sub>50</sub>, percentage of mycelial growth inhibition or conidia germination inhibition was plotted against the log<sub>10</sub>-transformed concentration of each pesticide to determine the linear relationship between the two parameters. The EC<sub>50</sub>s for each pesticide were then determined using the linear equation given by the general linear model (Version 8, SAS Institute Inc., Cary, NC 1999-2001).

## RESULTS

**Mycelial growth.** Of the nine pesticides evaluated, azoxystrobin, chlorothalonil, fluazinam, pyraclostrobin, tebuconazole, and flumioxazin reduced radial mycelial growth of *C. minitans* on PDA (Table 2.1). At a concentration of 0.01 µg a.i. ml<sup>-1</sup> in PDA inhibition of *C. minitans* mycelial growth ranged from 0 to 50% among tested pesticides (Fig. 2.1). The

fungicides azoxystrobin, chlorothalonil, fluazinam, and pyraclostrobin, and the herbicide tebuconazole suppressed mycelial growth 9 to 50% at a concentration of  $0.01 \mu\text{g a.i. ml}^{-1}$  in PDA (Fig. 2.1). At  $0.1 \mu\text{g a.i. ml}^{-1}$  in PDA flumioxazin reduced mycelial growth 13 %, whereas the activity of diclosulam, *S*-metolachlor, and pendimethalin reduced mycelial growth of *C. minitans* 0 to 4 % at concentrations ranging from  $0.01$  to  $10 \mu\text{g a.i. ml}^{-1}$  (Fig. 2.1). Radial mycelial growth was most sensitive to fluazinam and flumioxazin with  $\text{EC}_{50}\text{s} < 0.01 \mu\text{g a.i. ml}^{-1}$  (Table 2.3). Chlorothalonil and pyraclostrobin also inhibited radial growth with  $\text{EC}_{50}\text{s} < 0.1$  (Table 2.3).

**Conidia germination.** Reduction in conidia germination ranged from 88% on PDA amended with pyraclostrobin to 27% on PDA amended with tebuconazole at  $0.01 \mu\text{g a.i. ml}^{-1}$  (Fig. 2.2). Increasing the concentration of azoxystrobin, chlorothalonil, fluazinam, pyraclostrobin, tebuconazole, and flumioxazin to  $10 \mu\text{g a.i. ml}^{-1}$  resulted in  $< 10$  % of *C. minitans* conidia germinating (Table 2.2). The fungicides azoxystrobin, chlorothalonil, fluazinam, and pyraclostrobin were the most active pesticides tested on conidia germination with  $\text{EC}_{50} < 0.01 \mu\text{g a.i. ml}^{-1}$  (Table 2.3). Tebuconazole was the least active fungicide with an  $\text{EC}_{50}$  of  $0.25 \mu\text{g a.i. ml}^{-1}$  (Table 2.3). Flumioxazin was the only herbicide that significantly affected conidia germination with an  $\text{EC}_{50}$  of  $0.03 \mu\text{g a.i. ml}^{-1}$  (Table 2.3). Conidia germination was reduced by diclosulam, *S*-metolachlor, and pendimethalin but  $\text{EC}_{50}\text{s}$  could not be reliably estimated since they were greater than concentrations used in the experiment (Table 2.3).

**Mycoparasitic activity.** All fungicides and the herbicides, diclosulam, flumioxazin, and pendimethalin reduced infection of *S. minor* sclerotia by *C. minitans* compared to non-

treated control (Table 2.4). None of the pesticides completely prevented *C. minitans* from infecting sclerotia (Table 2.4). However, as the percentage of sclerotia infected decreased, the percent viable sclerotia increased (Table 2.4). Azoxystrobin, chlorothalonil, fluazinam, pyraclostrobin, and flumioxazin treatments had the greatest effect on infection of the sclerotia by *C. minitans* (Table 2.4). Approximately 51% of sclerotia of *S. minor* in the control were infected by *C. minitans* and 61% of the sclerotia remained viable as compared to the chlorothalonil treatment in which only 14% of the sclerotia were infected by *C. minitans* and 90% of the sclerotia remained viable (Table 2.4).

## **DISCUSSION**

In this study, mycelial growth, spore germination, and mycoparasitic activity of *C. minitans* were inhibited by most fungicides and some herbicides commonly used in peanut production. *C. minitans* was most sensitive to the fungicides azoxystrobin, fluazinam, chlorothalonil, pyraclostrobin, and tebuconazole, and the herbicide flumioxazin. These fungicides all have broad spectrum activity against basidiomycota, ascomycota, and deuteromycota (2). Azoxystrobin, chlorothalonil, and pyraclostrobin have good activity on web blotch of peanut caused by *Phoma arachidicola* (11). *Phoma* and *Coniothyrium* are both anamorphs of fungi in the family Leptosphaeriaceae making them close relatives, so it is not surprising *C. minitans* is sensitive to the same fungicides used to control *P. arachidicola* and leaf spot (3).

The herbicide flumioxazin, used to control a number of problematic weeds in peanut including Florida beggarweed (*Desmodium tortuosum* (Sw.) DC.), common ragweed

(*Ambrosia artemisiifolia* L.), and eclipa (*Eclipta prostrata* L.), was found to have the same mode of action as many of the fungicides in reducing fungal growth and mycoparasitic activity *C. minitans* (6). The herbicide lactofen, an inhibitor of protophyrinogen oxidase, has been found to reduce leaf spot on peanut by reducing spore germination and decreasing sporulation in lesions (8). Since lactofen has fungicidal activity its not surprising that flumioxazin, a herbicide that also inhibits protophyrinogen oxidase like lactofen, showed similar fungicidal activity in reducing *C. minitans* growth, spore germination, and mycoparasitic activity.

Even though *C. minitans* was very sensitive to some pesticides tested, sufficient inocula survived in all soil experiments to infect at least 11% of the sclerotia of *S. minor*. The activity observed may be a result of escapes or failure to sufficiently contact and distribute the chemical across the sclerotia, either of which could have allowed the spores of *C. minitans* to germinate and infect sclerotia. Budge and Whipps (2001) found that *C. minitans* growth and spore germination were sensitive to *in vitro* pesticides including the fungicides iprodione, thiram, mancozeb, metalaxyl, tolclofos-methyl, and zineb along with a number of insecticides and the herbicide chloropopham plus cetrimide used in lettuce production. They found *C. minitans* was highly sensitive to iprodione ( $EC_{50} = 7-18 \mu\text{g a.i. ml}^{-1}$ ), moderately tolerant to thiram ( $EC_{50} = 52-108 \mu\text{g a.i. ml}^{-1}$ ), and less sensitive to mancozeb, metalaxyl, tolcofos-methyl, and zineb ( $EC_{50} > 200 \mu\text{g a.i. ml}^{-1}$ ) (9). However, *C. minitans* was able to infect and destroy sclerotia of *S. sclerotiorum* in soil when iprodion (Rovral) was applied to the lettuce crop until run-off (9). Li et al. (2002) also found that *C. minitans* was highly sensitive to benomyl and vinclozolin ( $EC_{50} < 1 \mu\text{g a.i. ml}^{-1}$ ). In our

study, mycelial growth and spore germination of *C. minitans* was highly sensitive to chlorothalonil, fluazinam, pyraclostrobin, tebuconazole, and flumioxazin but less sensitive to azoxystrobin, diclosulam, *S*-metolachlor, and pendimethalin.

Although most pesticides tested have some activity on *C. minitans*, the mycoparasite still exhibited activity on sclerotia when sclerotia were inoculated directly with spores of *C. minitans*. In the field, these pesticides are applied to the soil surface or the peanut plant and residuals would be broken down relatively quickly. Therefore the potential for them to detrimentally affect *C. minitans* activity would be relatively limited. However, since growth, spore germination, and mycoparasitic activity of *C. minitans* has the potential to be reduced in the presence of these pesticides, care should be taken in the timing of the applications of the biological control. *C. minitans* should not be applied with or during times when there is a high risk of it coming in direct contact with azoxystrobin, chlorothalonil, fluazinam, pyraclostrobin, tebuconazole, and flumioxazin. Field experiments have shown the effectiveness of late fall and early winter soil application of *C. minitans* on reducing sclerotia of *S. minor* and subsequent disease (Partridge, *Chapter 1*). Therefore, if *C. minitans* is applied in the fall and has a chance to become established in the soil, by spring and summer months when herbicides and fungicides are used in peanut production, the effects on *C. minitans* mycoparasitic activity will be minimized.

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**Table 2.1.** Colony diameter of *Coniothyrium minitans* isolate CON/M/91-08 on potato dextrose agar amended with fungicides or herbicides commonly used in peanut production<sup>y</sup>.

Pesticide	Pesticide concentration ( $\mu\text{g a. i. ml}^{-1}$ )					R <sup>2</sup>	Slope <sup>z</sup>
	0	0.01	0.1	1	10		
<b>Fungicides</b>							
Azoxystrobin	76.8	55.6	50.8	47.1	44.4	0.979	2.20
Chlorothalonil	76.8	60.1	33.8	19.3	16.4	0.890	8.28
Fluazinam	76.8	38.5	21.1	16.4	14.4	0.814	4.32
Pyraclostrobin	76.8	51.0	47.6	34.5	29.9	0.947	4.24
Tebuconazole	76.8	69.8	48.7	25.6	10.8	0.993	11.26
<b>Herbicides</b>							
Diclosulam	76.8	77.4	71.4	78.9	78.1	0.173	0.772
Flumioxazin	76.8	76.9	66.8	35.5	33.1	0.911	9.32
S-Metolachlor	76.8	79.8	78.2	74.1	77.0	0.428	0.72
Pendimethalin	76.8	78.9	74.5	77.5	76.7	0.071	0.20

<sup>y</sup> Growth (mm) after 14 days of incubation. Data represents the mean of nine replicates.

<sup>z</sup> Slope determined from the regression of percent inhibition of mycelial growth verses pesticide concentration.

**Table 2.2.** Percent conidia germination of *Coniothyrium minitans* isolate CON/M/91-08 after 32 hours at 18° C on potato dextrose agar amended with pesticides commonly used in peanut production<sup>y</sup>.

Pesticide	Pesticide concentration ( $\mu\text{g a.i. ml}^{-1}$ )					R <sup>2</sup>	Slope <sup>z</sup>
	0	0.01	0.1	1	10		
<b>Fungicides</b>							
Azoxystrobin	69.7	40.3	16.5	6.3	3.8	0.863	7.53
Chlorothalonil	69.7	33.8	5.7	1.8	0.7	0.724	6.47
Fluazinam	69.7	49.0	8.5	2.0	0.3	0.737	9.63
Pyraclostrobin	69.7	8.3	5.3	2.0	0.7	0.972	1.64
Tebuconazole	69.7	50.3	40.0	13.7	6.8	0.947	9.85
<b>Herbicides</b>							
Diclosulam	69.7	48.0	47.3	47.8	48.8	0.423	-0.23
Flumioxazin	69.7	46.3	23.0	12.8	10.3	0.857	7.24
S-Metolachlor	69.7	45.2	46.2	46.3	40.3	0.408	0.88
Pendimethalin	69.7	48.2	52.5	51.2	50.2	0.112	-0.31

<sup>y</sup> Percentage of germinated spores out of 100. Data represents the mean of six replicates.

<sup>z</sup> Slope determined from the regression of percent inhibition of conidia germination verses pesticide concentration.

**Table 2.3.** The effect of fungicides and herbicides commonly used in peanut production on mycelial growth and conidia germination of *Coniothyrium minitans* isolate CON/M/91-08<sup>y</sup>.

Pesticide	Maximum application rate (g a.i. ha <sup>-1</sup> )	Mycelial growth	Conidia germination
		ED <sub>50</sub> (µg a.i. ml <sup>-1</sup> )	ED <sub>50</sub> (µg a.i. ml <sup>-1</sup> )
<b>Fungicides</b>			
Azoxystrobin	450	> 10 (-) <sup>z</sup>	<0.01
Chlorothalonil	1260	0.12	< 0.01
Fluazinam	875	< 0.01	0.01
Pyraclostrobin	275	0.61	< 0.01
Tebuconazole	229	0.33	0.25
<b>Herbicides</b>			
Diclosulam	26.5	> 10 (-)	(-)
Flumioxazin	107	< 0.01	0.03
S-Metolachlor	2140	> 10 (-)	> 10 (-)
Pendimethalin	1070	> 10 (-)	(-)

<sup>y</sup> Data averaged across trials. Pesticides used were from commercial formulations.

<sup>z</sup> (-) = No effect within confines of experiment.

**Table 2.4.** Mycoparasitic activity of *Coniothyrium minitans* on sclerotia of *Sclerotinia minor* in soil plates in the presence of the maximum recommended field concentration of pesticides commonly used in peanut production<sup>w</sup>.

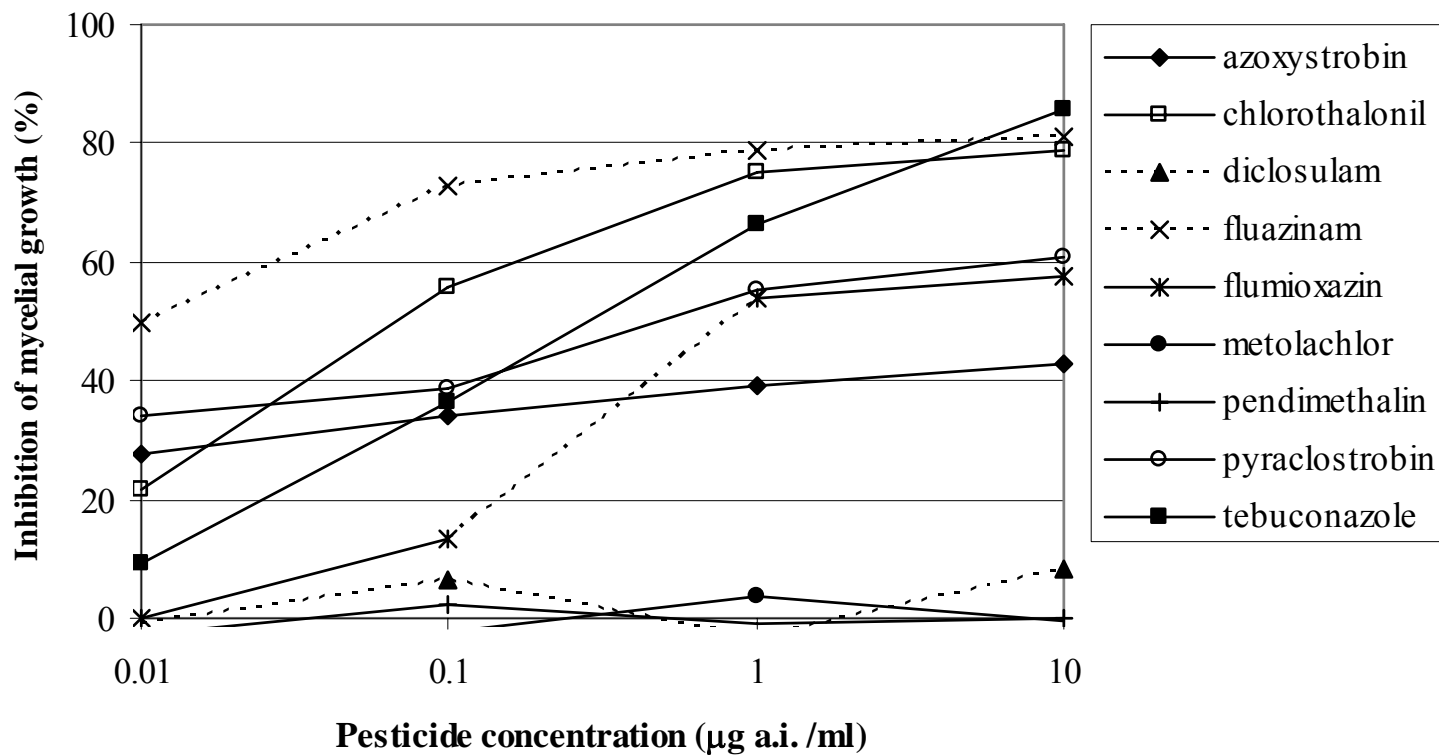
Pesticide	Maximum recommended formulated application rate (g a.i. ha <sup>-1</sup> )	<i>S. minor</i> sclerotia	
		Infected by <i>C. minitans</i> <sup>x</sup>	Viable <sup>y</sup>
Non-treated control		50.8 a <sup>z</sup>	60.8 c
<b>Fungicides</b>			
Azoxystrobin	450	12.5 d	83.3 ab
Chlorothalonil	1260	14.2 d	90.0 a
Fluazinam	875	21.7 cd	80.8 ab
Pyraclostrobin	275	10.8 d	90.0 a
Tebuconazole	229	35.8 b	73.3 cb
<b>Herbicides</b>			
Diclosulam	26.5	31.7 bc	70.8 cb
Flumioxazin	107	18.3 d	80.0 ab
S-Metolachlor	2140	38.3 ab	64.2 c
Pendimethalin	1070	37.5 b	71.7 cb
	F (Pr>F)	9.29 (<.0001)	3.97 (0.0008)
	LSD	12.62	14.31

<sup>w</sup> Pesticides used were from commercial formulations

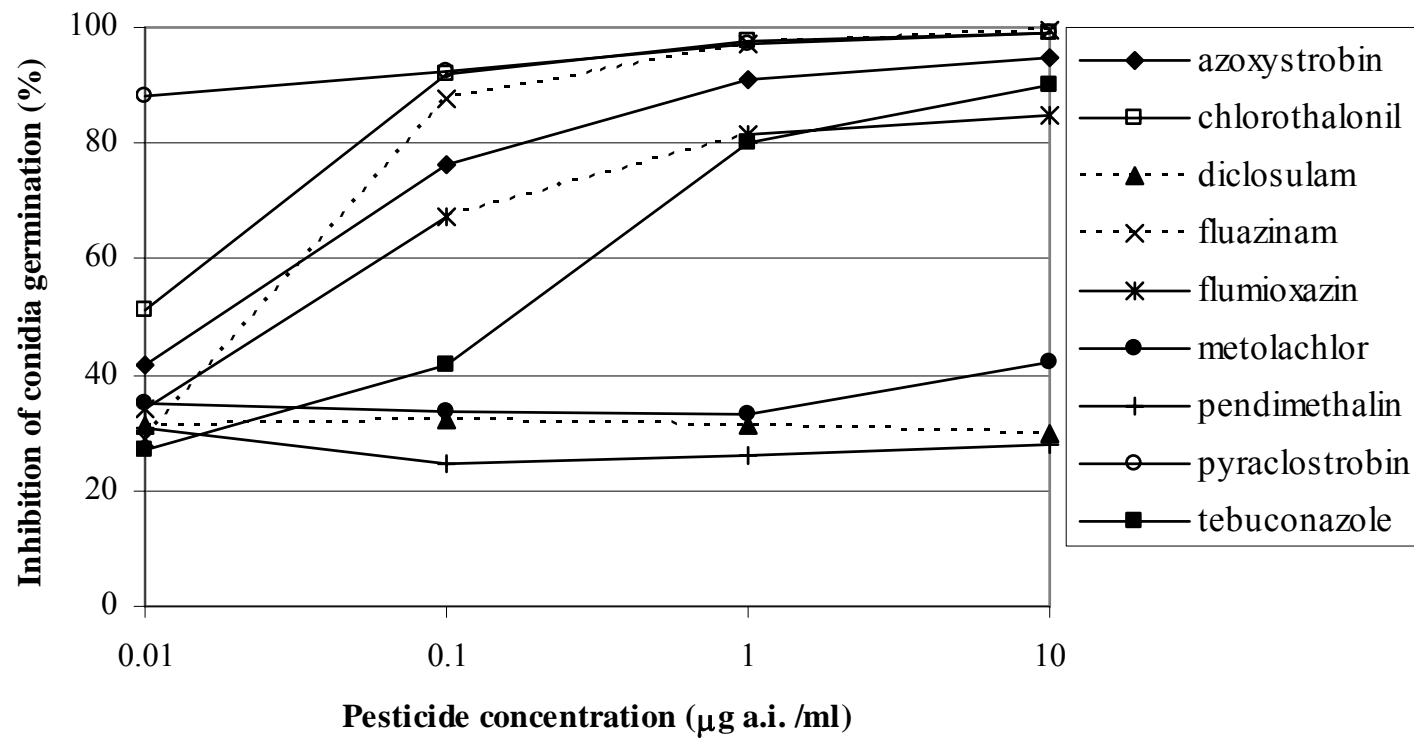
<sup>x</sup> Percent sclerotia infected by *C. minitans* after 14 days. Data represents average results from two trials with 20 sclerotia each.

<sup>y</sup> Percentage sclerotia germinating after 14 days. Data represent average results from two trials with 20 sclerotia each.

<sup>z</sup> Values followed by the same letter in each column are not significantly different at a P≤0.05 according the Fisher's protected least significant difference.



**Figure 2.1.** Influence of azoxystrobin, chlorothalonil, diclosulam, fluazinam, flumioxazin, *S*-metolachlor, pendimethalin, pyraclostrobin, and tebuconazole in potato dextrose agar medium on inhibition of mycelial growth of *Coniothyrium minutans* isolate CON/M/91-08 after a 14 day incubation period at 18°C. Each value is the mean of 3 replicate dishes from three experiments.



**Figure 2.2.** Influence of azoxystrobin, chlorothalonil, diclosulam, fluazinam, flumioxazin, *S*-metolachlor, pendamethalin, pyraclostrobin, and tebuconazole in potato dextrose agar medium on inhibition of conidia germination of *Coniothyrium minutans* isolate CON/M/91-08 after a 32 hour incubation period at 18°C. Each value is the mean of 3 replicate dishes from two experiments.

## CHAPTER 3

### EFFECT OF TEMPERATURE AND MOISTURE ON THE MYCOPARASITISM OF SCLEROTIA OF *SCLEROTINIA MINOR* BY *CONIOTHYRIUM MINITANS*

## ABSTRACT

Partridge, D. E., Sutton, T. B., and Jordan, D. L. 2005. Temperature and moisture effects on the mycoparasitism of *Sclerotinia minor* sclerotia by *Coniothyrium minitans*. To be submitted for publication in plant disease.

Sclerotinia blight of peanut (*Arachis hypogaea*), caused by *Sclerotinia minor*, is an important disease in Virginia and North Carolina. Field experiments have shown that multiple years of soil application of *Coniothyrium minitans*, a mycoparasite of sclerotia, can reduce sclerotial numbers and Sclerotinia blight on peanut. In order to be a successful biocontrol agent, *C. minitans* must parasitize sclerotia of *S. minor* under the variable environmental conditions in a peanut field. In this study, effects of temperature and moisture effects on the mycoparasitism of sclerotia of *S. minor* by *C. minitans* were examined. Mycoparasitic activity of *C. minitans* remained high (98% sclerotia parasitized) at temperatures ranging from 14 to 22° C, but decreased at temperatures > 28° C. Mycoparasitic activity decreased as soil moisture increased from -1 kPa x 10<sup>2</sup> to field capacity (0 kPa x 10<sup>2</sup>). The highest level of activity, with 45 to 53% of the sclerotia parasitized, occurred between -0.33 and -1 kPa x 10<sup>2</sup>. The activity then declined dramatically as soil moisture was reduced to -15 kPa x 10<sup>2</sup>. The viability of sclerotia was directly related to the proportion infected by *C. minitans*; as infection increased the number of viable sclerotia decreased. Optimum conditions for infection were temperatures from 14 to 22°C and soil moisture from -0.33 to -1 kPa x 10<sup>2</sup>. These results indicate that *C. minitans* would remain active throughout most of the year in peanut fields in North Carolina, except during the hot summer months of June, July, and August. During these months

mycoparasitic activity will decline, but may resume upon return to optimum temperature and moisture conditions.

## **INTRODUCTION**

Sclerotinia blight is one of the major diseases of peanut in Virginia and North Carolina. The disease is caused by the soilborne fungus *Sclerotinia minor* (Jaggar) Kohn (11). Sclerotinia blight was first reported in the United States in Virginia in 1971 and in North Carolina 1972 (17). Since then it has been reported in New Mexico, Oklahoma, and Texas (3, 17, 24). Overwintering sclerotia of *S. minor* in the soil provide the primary inoculum for Sclerotinia blight of peanut. Sclerotia of *S. minor* germinate myceliogenically by producing a mass of mycelium that can directly penetrate peanut tissue to initiate infection (12, 18). As peanut is infected by *S. minor*, numerous sclerotia are produced in and on the diseased tissue and are shed into the soil as peanut tissues decompose.

Management of Sclerotinia blight is mainly aimed at protecting the plant from infection. Long-term rotation with a non-host crop such as corn or cotton has shown limited effectiveness in controlling this disease since the sclerotia of *S. minor* are able to remain viable for as many as 4 years in the soil (2, 16, 18). Partial resistance and cultural methods provide limited control, while the fungicides fluazinam and boscalid are effective protectants when applied properly (19, 20).

*Coniothyrium minitans* Campbell is a mycoparasite of sclerotia of *Sclerotinia* spp.(6). A number of studies have shown that *C. minitans* can infect and colonize sclerotia in the soil (8, 9, 14, 21, 26). Control of *S. sclerotiorum* has been achieved in several crops in both the

greenhouse and field by the application of a solid-state inocula of *C. minitans* (5, 8, 13, 15, 27, 28). Recent field studies have shown that *C. minitans* is able to colonize the sclerotia of *S. minor* and reduce Sclerotinia blight in peanut when applied for at least 2 consecutive years (Partridge, Chapter 1).

For *C. minitans* to be an effective biological control agent of *S. minor* it must be able to persist in soil in the peanut field under varying environmental conditions. The environment can also greatly influence the mycoparasitic activity of *C. minitans* on sclerotia of *S. minor*. The optimum temperature for *C. minitans* spore germination, infection and destruction of sclerotia of *S. sclerotiorum* is 20°C (22). Temperatures < 7°C are not favorable for *C. minitans* fungal growth resulting in much slower rates of spore germination and infection (22). The optimum relative humidity for the mycoparasitism of *S. sclerotiorum* sclerotia is > 95% (22). *In vitro* studies found that 80% spore germination occurs after 7 days with temperatures ranging from 7 to 20°C, and spore germination occurs at temperatures as low as 4°C and as high as 30°C (22).

The objective of this study was to determine the optimum temperature and moisture requirements for *C. minitans* infection of sclerotia of *S. minor*. Knowledge of the requirements will allow us to determine the potential for *C. minitans* to persist, infect, and manage *S. minor* under field conditions in North Carolina.

## **MATERIALS AND METHODS**

**Isolate information and production of sclerotia.** An isolate of *Sclerotinia minor* [#20 obtained from Dr. Barbara Shew, North Carolina State University (7)] originally

obtained from a diseased peanut plant in Gates County, North Carolina was used to produce sclerotia used in the temperature and moisture experiments. Sclerotia were produced on sterilized oat kernels infested with *S. minor* at 18°C (1). After 5 weeks, the oat kernels were dried for 2 weeks at room temperature (~22°C) and stored at 5°C until used in experiments. One standard isolate of *Coniothyrium minitans* (CON/M/91-08) obtained from plating out the commercial formulation Contans WG (Prophyta Biologischer Pflanzenschutz GmbH, Germany) was used in all of the experiments. The isolate was grown on potato dextrose agar (PDA) at 18°C. A spore suspension of *C. minitans* was made by flooding the culture dishes with sterilized distilled H<sub>2</sub>O and gently scraping the colony surface to free spores from pycnidia. The spore suspension was collected and diluted to ~ 1 x 10<sup>6</sup> spores ml<sup>-1</sup> with the aid of a haemocytometer.

**Preparation of soil plates.** Temperature and moisture effects on mycoparasitism of sclerotia of *S. minor* were studied using a modification of a soil plate technique described by Smith (19). A sample of a Rains fine sandy-loam soil was collected from a peanut field in Gates County, North Carolina with a history of Sclerotinia blight of peanut. The field soil was air-dried and sifted through a U. S. standard number 12 sieve (1.7 mm opening) to produce uniform sized soil particles and to remove organic debris.

In the temperature experiments, soil was amended with commercial cornmeal (5% w/w), thoroughly mixed, and 50 g of the amended soil was placed in 9-cm glass petri dishes. The amended soil was moistened with 20 ml of distilled H<sub>2</sub>O, autoclaved for 30 min at 121°C (103 kPa) (19), and allowed to cool. Twenty sclerotia of *S. minor* were placed on the soil surface of each dish and sprayed uniformly with a suspension of spores of *C. minitans*

( $\sim 1 \times 10^6$  spores  $\text{ml}^{-1}$ ) using an airbrush sprayer. Dishes with soil and sclerotia were then placed in growth chambers at 14, 18, 22, 28, or 30° C for 14 days, after which time the sclerotia were removed from the soil dish. The sclerotia were surface disinfested in 15% NaClO for 30 s, rinsed in sterilized distilled H<sub>2</sub>O and plated on PDA amended with tetracycline (25 mg L<sup>-1</sup>). The number of sclerotia showing mycelial growth (viable) and/or infection by *C. minitans* (infected) was assessed after 7 d incubation at 18 °C. For each temperature treatment there were four replications plus a control with non-inoculated sclerotia. The experiment was repeated once.

Thirty-year average temperature data were obtained from the State Climate Office of North Carolina (SCONC) for comparison to results of temperature trials. Daily temperature data were collected at the Peanut Belt Research Station, Lewiston, NC and at the weather station at the Northeastern Regional Airport, Edenton. Daily soil temperatures collected from 1997 to 2004 at Peanut Belt Research Station, Lewiston, NC were also examined. These sites were chosen because of their proximity to the areas where a majority of peanut production occurs in North Carolina with Lewiston located in the heart of North Carolina peanut production.

In the soil moisture experiments, field soil was dried for 24 h at 60° C to completely remove moisture. The soil was allowed to cool and 30 g of soil was dispensed into 9-cm plastic petri dishes. A sample of the field soil was submitted to the Soil Physical Properties Laboratory, North Carolina State University, for a soil water retention analysis under pressures of 0, -0.1, -0.33, -0.5, -1, -8, -15 kPa  $\times 10^2$ . Data from the soil water retention analysis were used to develop a soil release curve (Fig. 2.1). Sterile distilled water was

added by weight to each soil dish to obtain the moisture treatments of approximately 0, -0.10, -0.33, -1, -15 kPa x 10<sup>2</sup>. Sclerotia were inoculated by dipping them into a *C. minitans* spore suspension (~1 x 10<sup>6</sup> spores ml<sup>-1</sup>). The sclerotia were removed from the spore suspension and placed on sterilized filter paper to remove excess water. Twenty sclerotia were then placed on the soil surface of each petri dish. Static Guard<sup>®</sup> (Alberto-Culver Co., IL) was applied to the top of each petri dish to prevent condensation on the lid and the dishes were sealed with Parafilm to maintain constant moisture. Preliminary experiments showed that Static Guard<sup>®</sup> did not interact with the mycoparasitic interaction, but did inhibit the condensation of water on the lid. The weight of each dish was recorded to monitor soil moisture levels. The dishes were then placed in an incubator at 18° C for 14 days, after which time a final weight of the plates were recorded and sclerotia were removed. The sclerotia were surface disinfested and plated on PDA as described earlier. The number of sclerotia with mycelial growth (viable) and/or infection by *C. minitans* was assessed after 7 d incubation at 18 °C. Each moisture treatment was replicated four times and included a non-inoculated control. The experiment was repeated once.

**Data analysis.** Experiments were a randomized complete block design and data for percent sclerotia viable and percent infected by *Coniothyrium minitans* were subjected to analysis of variance (ANOVA) (Version 8, SAS Institute Inc., Cary, NC 1999-2001). Treatment means were separated using Fisher's protected least significant difference at P≤0.05 level in each experiment.

## RESULTS

A moisture release curve was developed using data obtained from the soil retention analysis of the field soil for pressures -0.01, -0.33, -0.5, -1, -8, -15 kPa x 10<sup>2</sup> (Fig 3.1). Infection of sclerotia of *Sclerotinia minor* by *C. minitans* decreased as soil moisture level increased from -1 kPa x 10<sup>2</sup> to 0 kPa x 10<sup>2</sup> (Fig. 3.2). The highest level of infection, 45 to 53%, occurred between -0.33 and -1 kPa x 10<sup>2</sup>. Infection declined as soil moisture was reduced to -15 kPa x 10<sup>2</sup> (Fig 3.2). Viability of *S. minor* sclerotia varied directly with proportion of sclerotia infected by *C. minitans*. As the percentage of sclerotia infected by *C. minitans* increased from 0 to -1 kPa x 10<sup>2</sup> after 14 days, viability of sclerotia decreased from 98 to 90% (Fig. 3.2). Viable sclerotia then increased to 98% at -15 kPa x 10<sup>2</sup> while sclerotial infection was reduced (Fig. 3.2).

Mycoparasitic activity of *C. minitans* remained high (98% sclerotia parasitized) at temperatures ranging from 14 to 22°C, but decreased dramatically at temperatures ≥ 28°C (Fig. 3.5). Sclerotial viability was directly related to proportion of sclerotia infected by *C. minitans*. At all temperatures the non-inoculated sclerotia were 100% viable (Fig. 3.5). At temperatures ranging from 14 to 22°C *C. minitans* infected 98% or more of sclerotia and only 15 to 30% remained viable whereas at 28 and 30°C *C. minitans* infected 9% of sclerotia and 100% remained viable (Fig 3.5).

The 30-year average daily maximum temperatures from 1971 to 2000 were 22.2 at Edenton and 21.8°C at Lewiston, and the average minimum was 10.6 at Edenton and 8.8°C at Lewiston (Fig. 3.3). Maximum average air temperatures for the months of June, July and August exceeded 28°C in both locations (Fig. 3.3). The average soil temperature at Lewiston

from 1997 to 2004 was 18.1°C with a maximum average temperature 21.3°C and minimum average temperature 15.7°C (Fig 3.4). Maximum soil temperatures were 30.2, 32.1, and 31.4°C for June, July, and August respectively (Fig. 3.4).

## DISCUSSION

Soil temperature and moisture influenced the ability of *C. minitans* to infect sclerotia of *S. minor*. Optimum infection of sclerotia by *C. minitans* occurred with soil moisture ranging from  $-0.33$  to  $-1$  kPa  $\times 10^{-2}$  and temperatures ranging from 14 to 22°C. These temperatures and moisture conditions are typical throughout most of the year in peanut fields in North Carolina.

Extremes in soil moisture reduced the ability of *C. minitans* to infect sclerotia of *S. minor*. Sclerotial viability decreased from 98 to 90% and *C. minitans* infection increased from 10 to 45% with increasing soil moisture. Other studies have also shown the importance of moisture on the activity of *C. minitans* (4, 22, 23). Sclerotia of *S. sclerotiorum* in celery and lettuce crops grown in a glasshouse are infected by *C. minitans* at all times of the year, but the sclerotia failed to degrade during the summer months when the soil was dry (4). In culture, mycelial extension of *C. minitans* decreases as PDA is osmotically adjusted from  $-0.7$  MPa to  $-7.0$  MPa (25). Relative humidities  $\geq 90\%$  are required for *C. minitans* spore germination with 95% or above required for maximum germination and infection of *S. sclerotiorum* (22).

The mycoparasitic activity of *C. minitans* on sclerotia of *S. minor* was drastically reduced at temperatures  $\geq 28^\circ\text{C}$ . *C. minitans* was active at temperatures ranging from 14 to

22°C, infecting more than 98% of the sclerotia, significantly reducing their viability. Infection of sclerotia of *S. sclerotiorum* by *C. minitans* occurs between 4 and 25° C with a optimum infection occurring at 20° C (5, 22). We were mainly interested with the effect of high temperatures on the mycoparasitic activity of *C. minitans* on sclerotia of *S. minor*, since spores of *C. minitans* are able to survive in cold storage at 5° C for up 64 weeks (15) and spore germination occurs at temperatures at low as 4° C (22). Also, extremely low temperatures in North Carolina are usually short lived and would probably not be detrimental to *C. minitans* spore survival and germination (minimum average soil temperatures ranged from 5.9 to 26.5 °C).

Spores of *C. minitans* can germinate at 30°C (22), but temperatures exceeding 26°C inhibit infection of *S. sclerotiorum* by *C. minitans* (10). During the summer months in northeastern North Carolina soil can reach as high as 32°C. Both air and soil temperatures consistently exceed 28° C during the months of June, July, and August. In our study infection of sclerotia of *S. minor* by this isolate of *C. minitans* was significantly reduced at temperatures  $\geq 28^{\circ}$  C, but residual activity (9% of sclerotia infected) was still detected at 30°C after 14 days. Therefore, *C. minitans* should persist and infect sclerotia of *S. minor* in the soil throughout most of the year in North Carolina, except during June, July, and August, when mycoparasitic activity would decline with high temperatures. Activity may resume when temperatures become optimum (14-22° C) in the late summer and fall.

Our results suggest that after harvest in the fall or early spring may be the best time to apply *C. minitans* to the soil. After harvest (early-mid October) temperatures remain favorable for infection for at least 6 weeks. However, October tends to be a dry month in

North Carolina so early spring (March) applications may be more effective. However, because *C. minitans* survives cold temperatures application during the winters are also likely to be effective. In a long-term study we found that multiple applications of *C. minitans* made in November, December, and February were effective on reducing sclerotial numbers and Sclerotinia blight of peanut (Partridge, *Chapter 1*).

## **ACKNOWLEDGEMENTS**

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## **LITERATURE CITED**

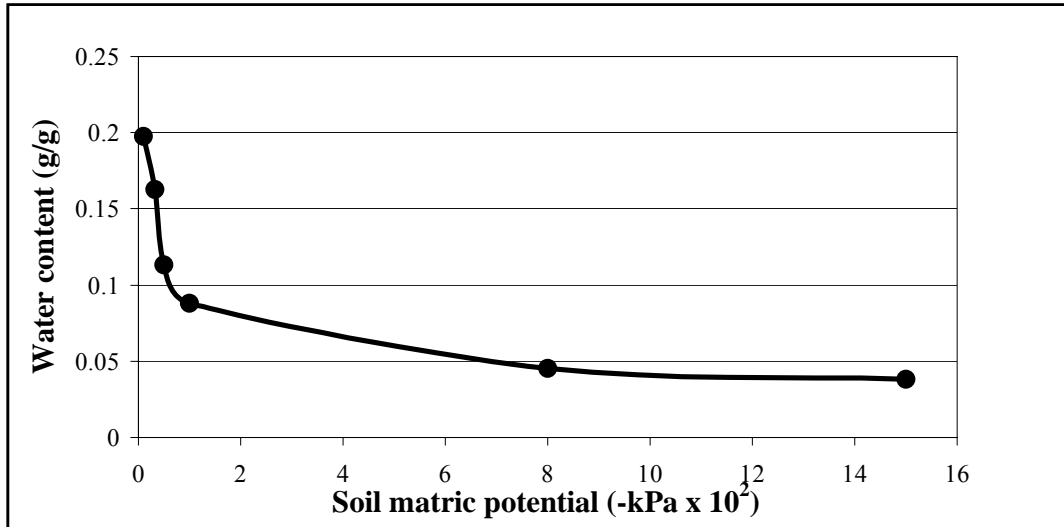
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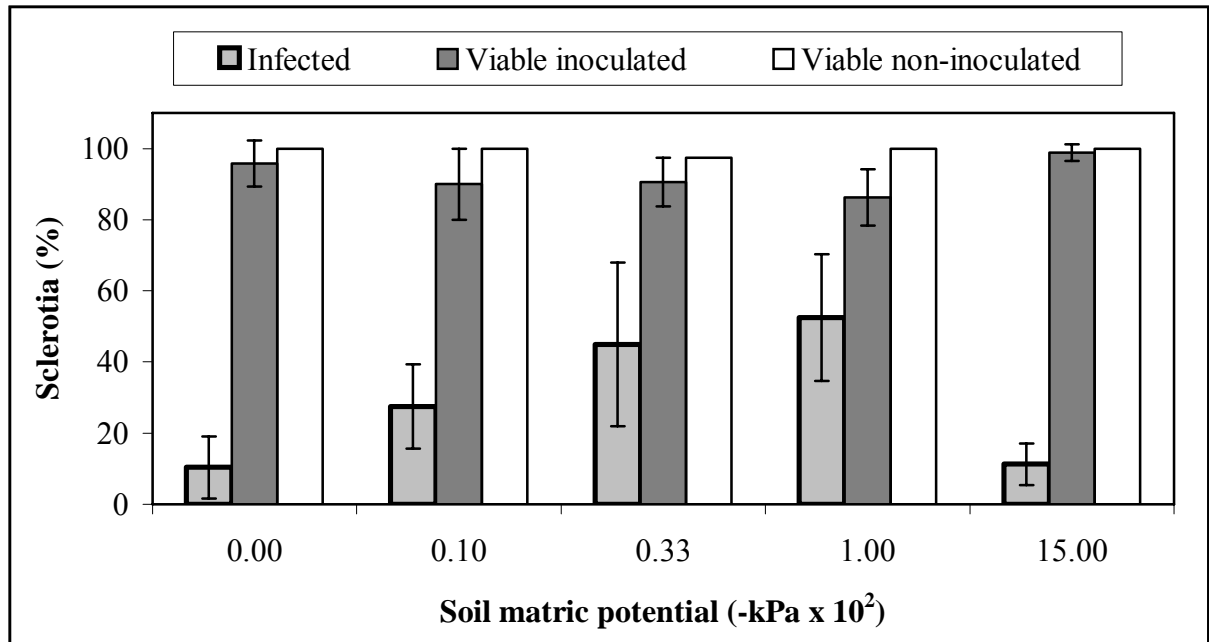
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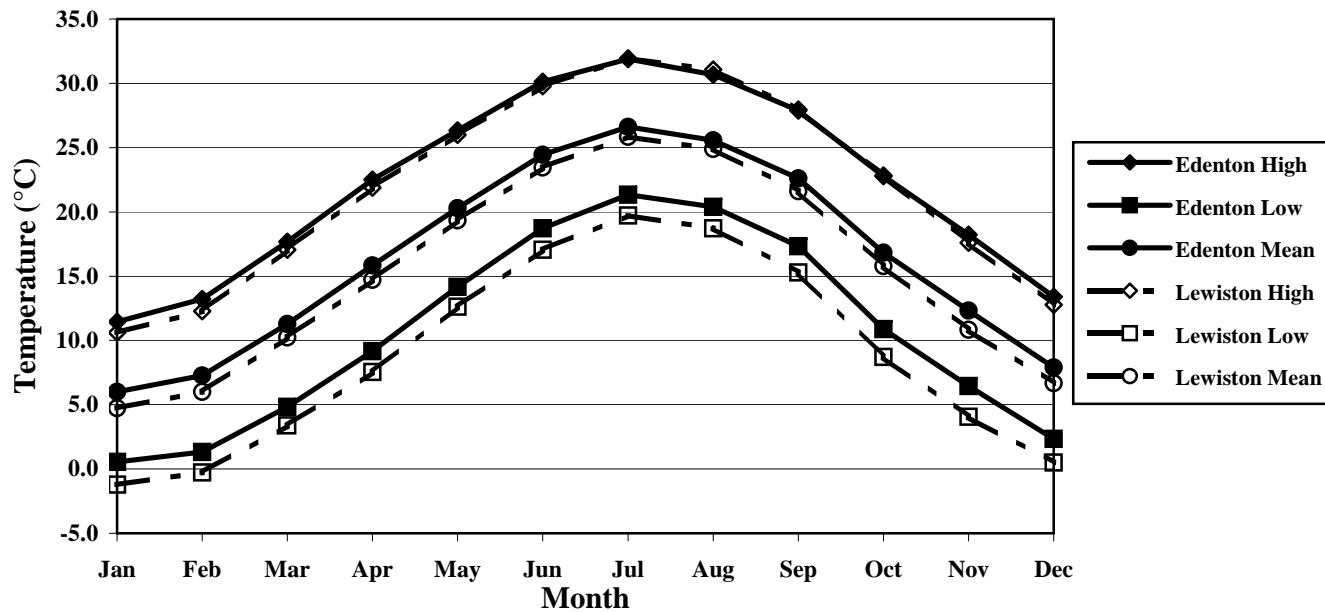
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**Figure 3.1.** Soil moisture release curve for a Rains fine sandy-loam soil collected from a peanut field on the Umphlett Brother's Farm in Gates County, North Carolina.

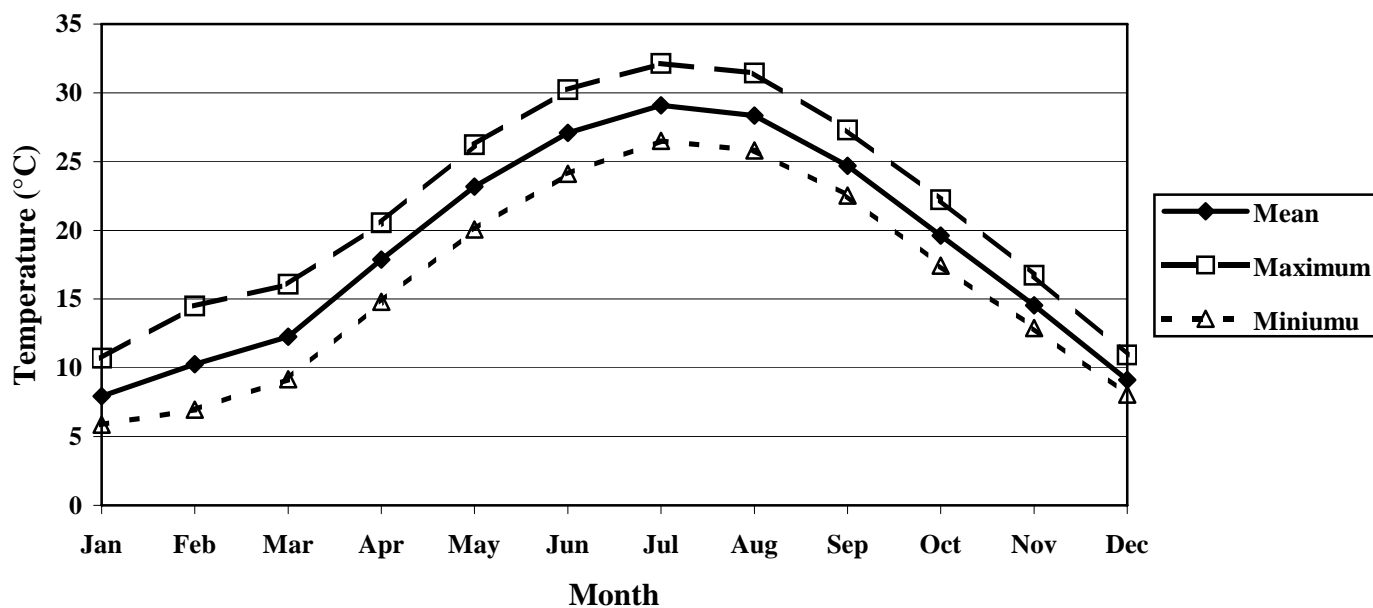


**Figure 3.2.** The effects of soil moisture on infection of *Sclerotinia minor* sclerotia by *Coniothyrium minitans*. Percentage of sclerotia that were viable and/or infected by *C. minitans* after 14 days of incubation at 18° C with soil moistures ranging from 0 to -15 -kPa x 10<sup>2</sup>.



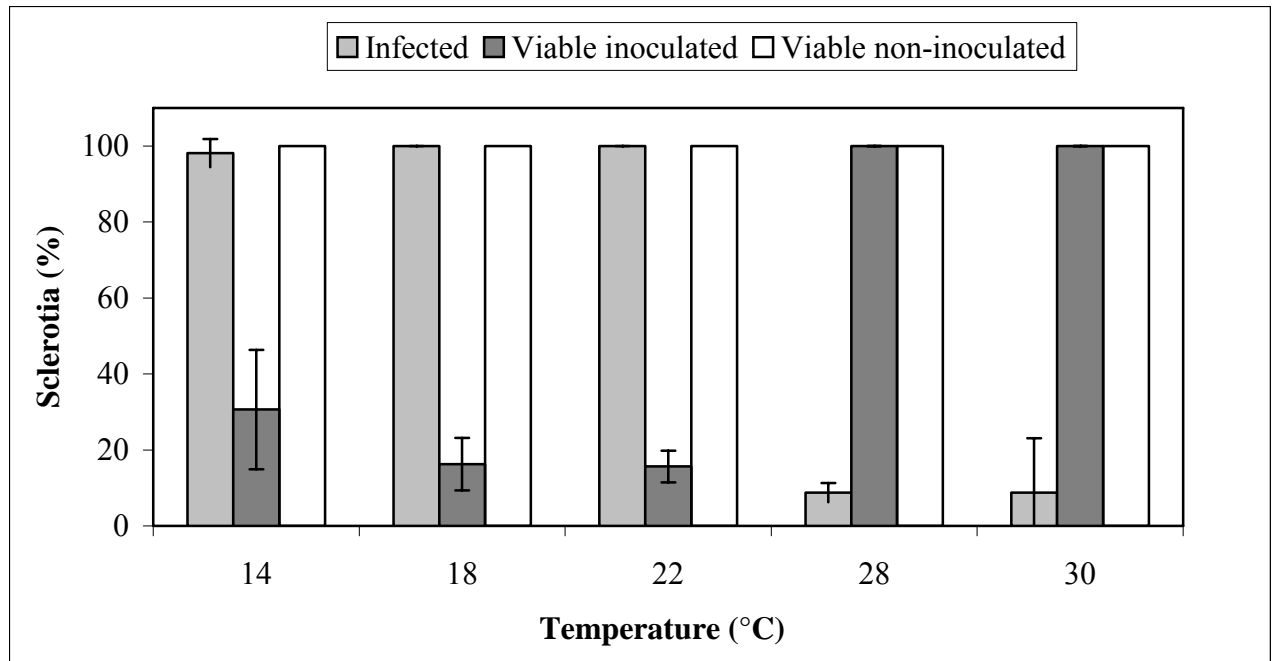
Location	Average (°C)	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Edenton, NC	Maximum	11.4	13.2	17.7	22.5	26.3	30.1	31.9	30.7	27.9	22.8	18.2	13.4
	Mean	6.0	7.3	11.3	15.8	20.3	24.4	26.6	25.6	22.6	16.8	12.3	7.9
	Minimum	0.6	1.3	4.8	9.2	14.2	18.7	21.3	20.4	17.3	10.9	6.4	2.3
Lewiston, NC	Maximum	10.6	12.3	17.1	21.9	26.0	29.8	31.9	31.1	27.9	22.8	17.6	12.8
	Mean	4.7	6.0	10.2	14.7	19.3	23.4	25.8	24.9	21.6	15.8	10.8	6.7
	Minimum	-1.2	-0.3	3.4	7.6	12.6	17.1	19.7	18.7	15.3	8.7	4.1	0.5

**Figure 3.3.** Thirty-year average maximum, mean, and minimum temperatures (°C) for each month in Edenton and Lewiston, North Carolina taken from 1971 to 2000. Data provided by the State Climate Office of North Carolina.



Location	Average soil (°C)	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Lewiston, NC	Maximum	10.7	14.5	16.1	20.5	26.2	30.2	32.1	31.4	27.3	22.2	16.7	10.9
	Mean	7.9	10.3	12.3	17.9	23.2	27.1	29.1	28.3	24.7	19.6	14.5	9.1
	Minimum	5.9	7.0	9.2	14.8	20.1	24.1	26.5	25.8	22.5	17.4	12.9	8.1

**Figure 3.4.** Eight year average monthly maximum, mean and minimum soil temperatures (°C) at the Peanut Belt Research Station, Lewiston, NC (1998 to 2004). Data provided by the State Climate Office of North Carolina.



**Figure 3.5.** The effect of temperature on mycoparasitism of *Sclerotinia minor* sclerotia by *Coniothyrium minitans*. Percentage of sclerotia that were viable and/or infected by *C. minitans* after 14 days of incubation at temperatures ranging from 14 to 30° C.

## **CHAPTER 4**

### **DIVERSITY AND ABUNDANCE OF COLLEMBOLA IN SELECTED PEANUT FIELDS OF NORTHEASTERN NORTH CAROLINA**

## ABSTRACT

Partridge, D. E., Sutton, T. B., Anas, O., and Jordan, D. L. and 2005. Diversity and abundance of collembola in selected peanut fields of northeastern North Carolina.

The collembolan community structure was compared in four peanut fields in North Carolina across treatments of the biological control agent, *Coniothyrium minitans* (commercial formulation, Contans WG), and the fungicide fluazinam during July, August and September of 2002 and 2003. Biotic similarity ( $\beta$ ) was used to determine similarity between locations and treatment effects of *C. minitans* and fluazinam on the collembola. Sampling dates and field locations were dissimilar from each other as indicated by  $\beta$ . In all four fields diversity and abundance of collembolan increased from August to October and was influenced by soil moisture. The most prevalent collembola families isolated were Isotomidae, Smithurididae, Poduridae, and Hypogastruridae with Isotomidae isolated most frequently from all four locations. Field application of *C. minitans* and fluazinam had variable effects on collembola number and composition in these fields. Analysis of collembolan community similarity indicated that collembolan abundance and diversity remained unchanged with multiple applications of *C. minitans* or fluazinam to the soil at the long-term site in Gatesville, NC. However, at three locations with a single application of *C. minitans* communities were found to be dissimilar ( $\beta < 0.5$ ) and the number of collembola were found higher in plots treated with *C. minitans* at two locations and lower at one. Also at one location collembola abundance and diversity increased in plots treated with fluazinam. Determining the abundance and diversity of collembola in the field can increase our understanding of the soil community structure and how pesticides or biological control

agents applied to the soil might promote or inhibit these micro arthropods. The results of this study indicate there is not consistent evidence that applications of *C. minutans* or fluazinam influence the collembola community structure in these four peanut fields during July, August and September of 2002 and 2003.

## INTRODUCTION

The micro arthropods from the Order Collembola (Springtails) and Acarina (mites) are the most abundant of the soil inhabiting animals excluding the nematodes and protozoa (21, 25). Micro arthropods are known to consume fungi and decaying plant material and can carry fungal propagules in their gut and on their cuticle (21, 25). The soil-borne fungus, *Sclerotinia minor*, produces sclerotia that act as survival structures in the soil and are the primary inoculum for Sclerotinia blight of peanut. The survival of sclerotia in soil is affected by many biotic factors (12). A number of microorganisms have been reported to influence the survival of *Sclerotinia* spp. Larvae of *Bradysia coprophila* Winnertz (dark-winged fungus gnat) feed on sclerotia of *S. sclerotiorum* leading to increased susceptibility of the sclerotia to infection by *Trichoderma viride* Pers. (1). Germination of *S. sclerotiorum* sclerotia is also inhibited by salivary secretions of *B. coprophila* during feeding (2). The collembolan species *Proisotoma minuta* Tullberg (Isotomidae) and *Onychiurus encarpatus* Denis (Poduridae) were found to suppress colony growth of *Rhizoctonia solani*, *Verticillium dahliae*, *Fusarium oxysporum* f. sp. *vasinfectum* and *Macrophominia phaseolina* and reduced sclerotial germination of *M. phaseolina* by consuming the hyphae of germinating sclerotia

rendering the propagule ineffective (13). Soil fauna may also aid in the movement of inoculum to susceptible hosts promoting disease incidence (25).

In addition to micro arthropods, many fungi have been identified as mycoparasites of sclerotia including *Coniothyrium minitans* Campbell (9). Soil applications of solid-state inocula of *C. minitans* has been a successful biocontrol against *S. sclerotiorum* in both the greenhouse and field (7, 8, 19, 22). *C. minitans* applied to crops infected with *S. sclerotiorum* reduced the population of sclerotia in field at the end of a 7-year period even when susceptible crops were planted (18). Multiple years of soil application of *C. minitans* to peanut fields were shown to reduce sclerotial number of *S. minor* and Sclerotinia blight on peanut (Partridge, *Chapter 1*). During greenhouse trials the mycoparasites have been found to spread several meters and infect sclerotia in non-treated control plots (7). Soil fauna may play a role in the dispersal of *C. minitans* (27). Slugs, collembola and mites were reported to be important in the dispersal of *C. minitans* to noninfected sclerotia (27). *Acarus siro* L. (mite) and *Folsomia candida* Willem (Isotomidae) can transmit *C. minitans* from infected to noninfected sclerotia of *S. sclerotiorum* in soil and may be important in the dissemination of *C. minitans* in the field (31). *F. candida* and *Bradysia* sp. (sciarid larvae) also can spread *C. minitans* between sclerotia (28).

The community structure of collembola can be influenced by many factors in the soil, including soil moisture, temperature, and crop residue (21, 26, 29). A number of pesticides are found to affect the abundance of some collembola species and mites (15, 16, 25, 30). The community structure of collembola in the soil may be influenced by applying the biological control agent *C. minitans* (Contans WG) and the fungicide fluazinam during the management

of Sclerotinia blight of peanut. The objectives of this study were to examine the abundance and diversity of collembola in four peanut fields of North Carolina during July, August and September and determine if application of the biological control agent, *C. minitans*, or the fungicide fluazinam promote or inhibit this community in the field.

## **MATERIALS AND METHODS**

**Field sites and sampling.** Four peanut fields were sampled during 2002 and 2003 growing seasons to examine community structure of collembola families and the effects of *Coniothyrium minitans* and fluazinam on these populations. The fields were located in Gatesville, Tyner, and Corapeake, North Carolina. Field soils were classified as Rains fine sandy loam, Goldsboro fine sandy loam, and Dogue fine sandy loam for the Gatesville, Corapeake and Tyner locations respectively.

A long-term field site located in Gatesville was designed to examine the long-term effects of *C. minitans* on Sclerotinia blight of peanut. This field had a history of peanut production and Sclerotinia blight. The rotation sequence for 1998 to 2003 was peanut, cotton, cotton, peanut, peanut, and peanut. *C. minitans*, commercial formulation Contans WG (Prophyta Biologischer Pflanzenschutz GmbH, Germany), strain CON/M/91-08, with a minimum of  $1 \times 10^9$  cfu g<sup>-1</sup>, was applied at 4 kg ha<sup>-1</sup> for 1, 2, or 3 consecutive years. *C. minitans* was applied on 3 December 1999 and again on 9 February and 12 November 2001. The peanut cultivar NCV-11 was planted on 16 May 2002 and 18 June 2003. Fluazinam (Omega 500, Syngenta Crop Protection, Greensboro, NC) was applied based on the weather based Sclerotinia advisory warning system with the interval between fluazinam sprays at

least 3 weeks and no more than three applications in total with a 30-day pre-harvest interval. The experimental design was a split plot. Main-plot factors consisted of *C. minitans* applied 1, 2 or 3 consecutive years at 4 kg ha<sup>-1</sup> and a non-treated control. Subplot factors consisted of two fluazinam rates (0 and 0.625 kg ai ha<sup>-1</sup>). Subplots were approximately 9 x 18 m (Table 4.1).

The remaining three fields were designed to examine short-term effects of *C. minitans*. All fields had a history of peanut production. Four treatments were established at each location to examine the interactions between soil incorporation of *C. minitans* and fluazinam fungicide application. The experimental design was a split plot with six replicates. *C. minitans* treatments were main plots and fungicide treatments were subplots (Table 4.1). Standard cultural and pest management practices such as tillage, fertilizer, herbicide, insecticide, and fungicides to control early leaf spot and southern stem rot were used as recommended by the North Carolina Cooperative Extension Service in all field sites (20).

Soil were removed from each experimental unit on 13 August, 11 September, and 9 October 2002 in all field locations and additional samples were taken on 27 July, 28 August, 16 September, and 6 October 2003 in the long-term site. The top 15 cm of soil under the peanut canopy was sampled using a shovel that removed soil cores (~10 cm diameter) from three locations within each experimental unit and were combined into one soil sample. Soil moisture was determined for each sample by drying the soil (~25 g) at 70°C for 24 h, and percent water content was determined by subtracting the dry weight from the original wet weight of the sample (17). Soil fauna were extracted using a Berlese funnel apparatus in which 500 g of soil from each sample was placed on a mesh sieve under a 60-watt bulb for

72 h and fauna were collected in a jar of 70 % ethanol. A stereomicroscope was used to identify order level of the insects (5, 6) and Collembola were identified to the family level using the taxonomic keys of Christiansen and Bellinger (10).

Community similarity was examined using the index of biotic similarity,  $\beta$  (23, 24). For comparisons of collembola families from different samples,  $\beta$  was calculated using the formula

$$\beta = \frac{1}{k} \sum_{i=1}^k \frac{\text{Min}(X_{ia}, X_{ib})}{\text{Max}(X_{ia}, X_{ib})}$$

where  $k$  is the number of taxa in each sample,  $X_{ia}$ , is the datum for the  $i^{\text{th}}$  taxon in the  $a^{\text{th}}$  sample, and  $X_{ib}$ , is the datum for the  $i^{\text{th}}$  taxon in the  $b^{\text{th}}$  sample. The smaller of  $X_{ia}$  or  $X_{ib}$  forms the numerator while the larger forms the denominator. The  $\beta$  values for all samples and resulting 2-D dendrogram were calculated using a FOTRAN IV program BIOSIM1 (obtained from J. G. Pearson, EPA Environmental Monitoring Systems Laboratory, Las Vegas, NV). All BIOSIM1 analyses were conducted using the methods of Pearson and Pinkman (23), where the similarity index was  $\beta_o$ , all matches were considered, and the unweighted cluster method was used. Clusters were set based on cophenetic correlation of each cluster. Clusters were only considered valid when they occurred at  $\beta$  values less than the  $\beta$  value where all the replications from the sample with the most variability formed a single cluster (23). Data for soil moisture were subjected to analysis of variance (ANOVA) (SAS Institute Inc. Cary, NC 1999-2001) and Fisher's Protected Least Significant Difference at  $P \leq 0.05$  level was used to separate mean sampling date effects at each location.

## RESULTS

Soil moisture varied with sampling date ( $p < 0.0001$ ) in all of the field studies. At all sites soil moisture was the lowest in the samples taken in August. In both Corapeake and Tyner, soil moisture content for August and September was 1.5 and 10.0%, respectively. In the field located in Gatesville soil moisture content was 1.1% in August, 5.8% in September, and 12.2% in October. At the long-term site soil moisture content in 2002 was 1.7% in August, 13.0% in September, and 6.3% in October and in 2003 was 15.0% in July, 5.7% in August, 12.8% in September, and 8.2% in October.

The abundance and composition of collembola in the short-term field studies varied by sampling date and location. Biotic similarity was  $< 40\%$ , except for the August samples from Gatesville and Corapeake which had 65% biotic similarity (Fig. 4.2). The number and diversity of collembolan were lower in August than in September at all sites (Table 4.2). In Gatesville total number of collembola increased from August to October, while diversity was at its highest in September (Table 4.2). Treatments containing soil application of *C. minitans* were grouped within a single cluster with 67% biotic similarity at Corapeake and 51% at Gatesville (Figs. 4.2, 4.3). At Corapeake higher numbers of collembola were isolated from soil treated with *C. minitans*, while in Gatesville the number of Collembola families decreased under the same treatments (Table 4.3). The fluazinam and non-treated control treatments were dissimilar in Corapeake and Tyner, but formed a single cluster at 60% biotic similarity in Gatesville (Figs. 4.2-4.4). In Tyner treatments containing fluazinam formed a

single cluster with 44% biotic similarity, and non-treated and *C. minutans* were grouped with 45% biotic similarity (Fig. 4.4).

Collembola from at least four families were isolated from all experimental sites in the long-term field study (Table 4.4). Cluster analysis of the  $\beta$  values in the long-term field study indicated that the abundance and composition of collembola was similar in all treatments,  $\beta > 0.52$  (Fig. 4.6, 4.7). Total numbers of collembola isolated increased from August to October in both 2002 and 2003 and at least three families of collembola were represented in each sample date (Table 4.4). Abundance and composition of collembola in all samples taken on October 2002 and August and October 2003 had 57% biotic similarity, while in August 2002 and September 2003 they had 49% biotic similarity. Samples taken on September 2002 and July 2003 were least similar (32% biotic similarity) to any of the other sampling dates (Fig. 4.5).

## **DISCUSSION**

Collembola play an important role in the soil ecosystem by preparing organic matter for decomposition and transporting fungal spores, and they have been shown to consume plant pathogens (1, 11, 25, 29). Collembola were isolated soil at all peanut field locations in our study. The most prevalent families were Isotomidae, Smithurididae, Poduridae, and Hypogastruridae. Collembola abundance and diversity increased from August to October at all locations. This was expected since moisture content of the soil also increased during this time. A positive correlation exists between soil moisture and density of soil-dwelling collembola with the density of collembola increasing as soil moisture increased (3, 4, 14).

Arthropod abundance on the soil surface also may increase over time with peanut plant growth (26).

Isotomidae were isolated most frequently. Presence of Isotomidae in these peanut fields may increase effectiveness of the biological control agent *C. minitans*. A species belonging to Isotomidae (*F. candida*) has been shown to distribute spores of *C. minitans* from infected to uninfected sclerotia of *S. sclerotiorum* up to 55 mm in Europe (31). It is possible that these fauna would help to spatially distribute *C. minitans*, thus increasing the likelihood that they mycoparasite would contact uninfected sclerotia of *S. minor*.

In our study, there was no consistent effect of soil application of *C. minitans* on collembola abundance and composition. All yearly application treatments of *C. minitans* at the long-term study in Gatesville were greater >50% similar based on the coefficient of similarity ( $\beta$ ), indicating that the number and diversity of the collembola isolated from soil remained similar even when the soil was treated with *C. minitans*. However, in the short-term field sites at Corapeake, Gatesville, and Tyner  $\beta$ -values comparing plots treated with *C. minitans* and the non-treated control were < 35 % similar. At these sites, there was an increased number of collembola isolated from plots receiving an application of *C. minitans* at Corapeake and Tyner versus the non-treated control, indicating that the soil treatment may promote collembola, but at the Gatesville site the effect was reversed with the plots receiving *C. minitans* having a smaller number of collembolan than the non-treated control.

Pesticides have been found to affect the abundance of some Collembola species and mites (15, 16, 25, 30). The triazole fungicides propiconazole and triadimenol, reduced the abundance of Collembola in wheat while there was no consistent effect of the benzimidazole

or carabendazim on the population (16). In our study, there was no consistent effect of fluazinam on collembola number and composition. Fluazinam did not affect collembolan number or diversity at the long-term site located in Gatesville as  $\beta$ -values comparing these treatments showed > 50% similarity. However in Tyner  $\beta$ -values indicated that there was a treatment effect of fluazinam with <35 % similarity to the non-treated control. In these fluazinam treated plots at Tyner collembolan numbers were actually higher than in the non-treated control.

The results of this study indicate that community structure of collembola isolated from July to September in four peanut fields of North Carolina is most strongly influenced by sampling date. The effect of date on the community of collembola is correlated with soil moisture with collembola increasing in numbers as soil moisture increased from July to September. Consequently the results of this study also indicated there is no consistent evidence that applications of *C. minitans* or fluazinam influence collembola abundance and diversity at these locations.

## **ACKNOWLEDGMENTS**

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**Table 4.1.** Field experiments, locations, cultivars, and dates of planting, Contans WG soil incorporation, fluazinam application dates, and harvest dates.

Experiment	Location and year	Cultivar	Planting date	Date of Contans WG application	Dates of fluazinam application			Harvest date
					1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	
<b>Long-Term</b>								
	<i>Gatesville</i>			03 Dec 99				
	2001	NC-V11	02 May	09 Feb 01	26 July	29 Aug	22 Oct	22 Oct
	2002	NC-V11	16 May	12 Nov 01	30 July	12 Sept	-----	8 Nov
	2003	Perry	18 June	-----	21 Aug.	10 Sept	-----	10 Nov
<b>Short-Term</b>								
	<i>2002</i>							
	Tyner	NC-V11	08 May	21 Nov 01	31 July	4 Sept	-----	-----
	Corapeake	NC-V11	15 May	12 Nov 01	30 July	11 Sept	-----	8 Nov
	Gatesville	NC-V11	28 May	22 Nov 01	31 July	6 Sept	-----	29 Nov

**Table 4.2.** The number of fauna per collembola family extracted from the soil samples taken in peanut fields located in Corapeake, Tyner, and Gatesville, North Carolina on 13 August, 11 September, and 9 October 2002.

Family	Number of fauna <sup>z</sup>						
	<u>Corapeake</u>		<u>Tyner</u>		<u>Gatesville</u>		
	13 Aug.	11 Sept.	13 Aug.	11 Sept.	13 Aug.	11 Sept.	9 Oct.
<b>Onychiuridae</b>	0	1	0	0	0	0	0
<b>Poduridae</b>	0	0	2	0	0	3	107
<b>Hypogastruridae</b>	0	0	1	1	0	2	0
<b>Isotomidae</b>	33	140	73	215	21	252	316
<b>Entomobryidae</b>	0	0	0	4	0	5	0
<b>Smithurididae</b>	0	11	0	24	0	11	3
<b>Total collembola</b>	33	152	76	244	21	273	426
<b>Number of families</b>	1	3	3	4	1	5	3

<sup>z</sup> The number of collembola identified from 500g dry soil samples pooled across 12 replications for each date.

**Table 4.3.** The effects of *Coniothyrium minitans* and fluazinam treatments on the number of fauna per collembola family extracted from soil samples taken from peanut fields located in Corapeake, Tyner, and Gatesville, North Carolina, 2002.

Family	Number of fauna <sup>y</sup>											
	UTC <sup>z</sup>	Corapeake			UTC	Tyner			UTC	Gatesville		
		CM	F	CM+F		CM	F	CM+F		CM	F	CM+F
<b>Onychiuridae</b>	0	0	1	0	0	0	0	0	0	0	0	0
<b>Poduridae</b>	0	0	0	0	0	0	1	1	57	27	10	16
<b>Hypogastruridae</b>	0	0	0	0	53	66	82	87	1	0	1	0
<b>Isotomidae</b>	44	58	28	43	0	3	1	0	208	148	133	100
<b>Entomobryidae</b>	0	0	0	0	5	9	8	2	2	0	3	0
<b>Smithurididae</b>	0	5	3	3	0	0	2	0	3	1	6	4
<b>Total collembola</b>	44	63	32	46	58	78	94	90	271	176	153	120
<b>Number of families</b>	1	2	3	2	2	3	5	3	5	3	5	3

<sup>y</sup> The number of fauna identified from 500g dry soil samples pooled across three replications and two sampling dates.

<sup>z</sup> (UTC) = non-treated control, (CM) = soil application of *C. minitans*, (F) = fluazinam application, (CM+F) = *C. minitans* soil application and fluazinam application.

**Table 4.4.** The number of fauna per collembola family extracted from soil samples taken in the long-term peanut field site located in Gatesville, North Carolina at different dates in 2002 and 2003.

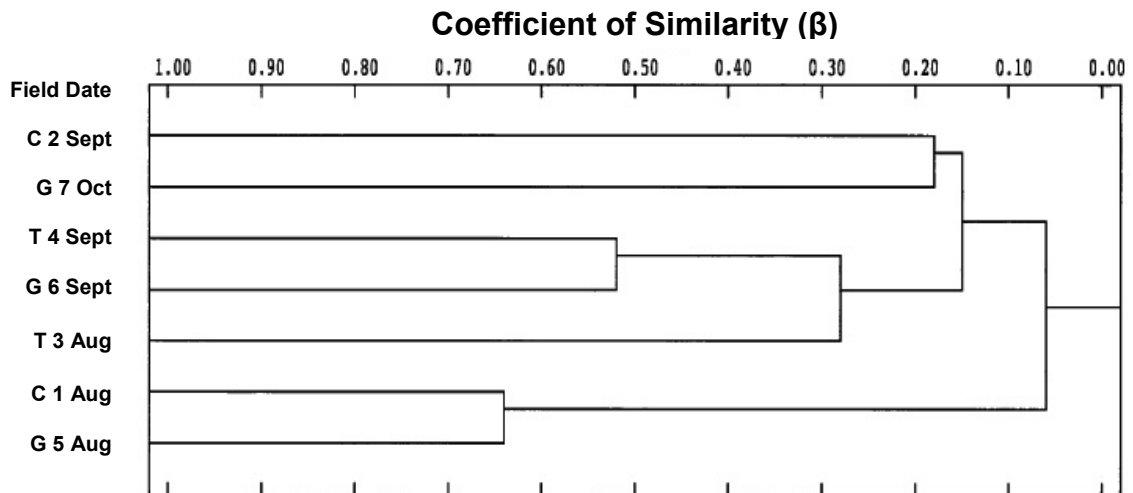
Family	Number of fauna from long-term field plot in Gatesville, NC <sup>z</sup>							
	13 Aug.	<u>2002</u> 11 Sept.	9 Oct.	27 July	<u>2003</u> 28 Aug.	16 Sept.	6 Oct.	
<b>Onychiuridae</b>	0	0	0	0	0	0	0	
<b>Poduridae</b>	1	9	18	0	19	2	17	
<b>Hypogastruridae</b>	2	0	2	5	2	6	1	
<b>Isotomidae</b>	71	349	1054	254	234	581	824	
<b>Entomobryidae</b>	0	6	0	0	0	0	0	
<b>Smithurididae</b>	5	22	14	84	16	5	44	
<b>Total collembola</b>	79	386	1088	343	271	594	886	
<b>Number of families</b>	4	4	4	3	4	4	4	

<sup>z</sup> The number of fauna identified from 500 g dry soil samples pooled across four replications and eight treatments.

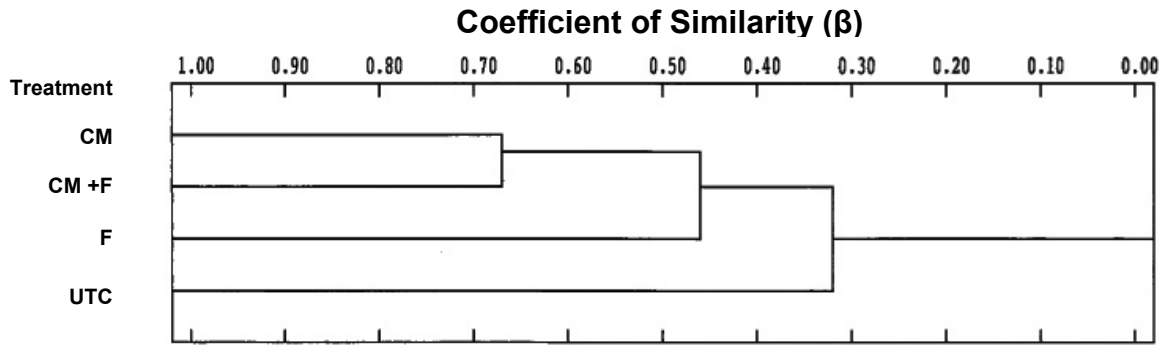
**Table 4.5.** The effects of yearly soil application of *Coniothyrium minitans* and fluazinam on the number of fauna per collembola family extracted from soil samples taken in a peanut field located in Gatesville, North Carolina.

Family	Number of isolates <sup>z</sup>					
	<i>C. minitans</i> number of years				Fungicide	
	0	1	2	3	None	Fluazinam
<b>Onychiuridae</b>	0	0	0	0	0	0
<b>Poduridae</b>	20	25	3	18	23	43
<b>Hypogastruridae</b>	5	5	5	3	7	11
<b>Isotomidae</b>	808	747	950	862	1761	1606
<b>Entomobryidae</b>	0	6	0	0	6	0
<b>Smithurididae</b>	40	64	48	33	63	127
<b>Total collembola</b>	873	847	1006	916	1860	1787
<b>Number of families</b>	4	5	4	4	5	4

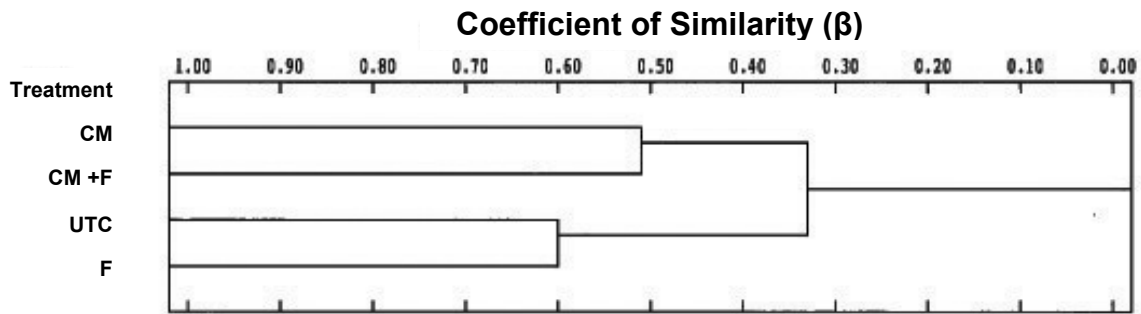
<sup>z</sup> The number of fauna identified from 500 g dry soil samples pooled across four replications and seven dates.



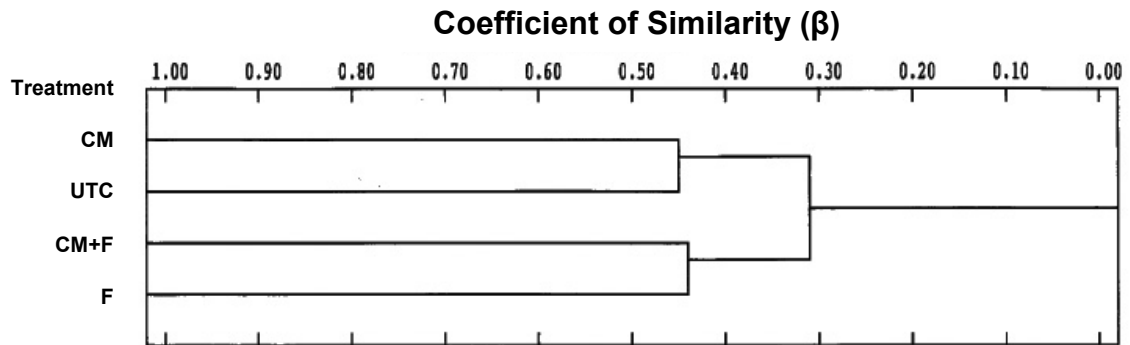
**Figure 4.1.** A 2-D dendrogram of field-date similarity,  $\beta$ , based on the distribution of collembola families among each field-date from (C) Corepeake, (G) Gatesville, and (T) Tyner samples taken 13 August, 11 September, and 9 October in 2002. Clusters were derived from a matrix of  $\beta$  values based on comparisons of all possible pairs, where all matches were considered and the unweighted cluster method was used. Cophenetic correlation was 0.98 with 95% correlation intervals of 0.98 and 0.89.



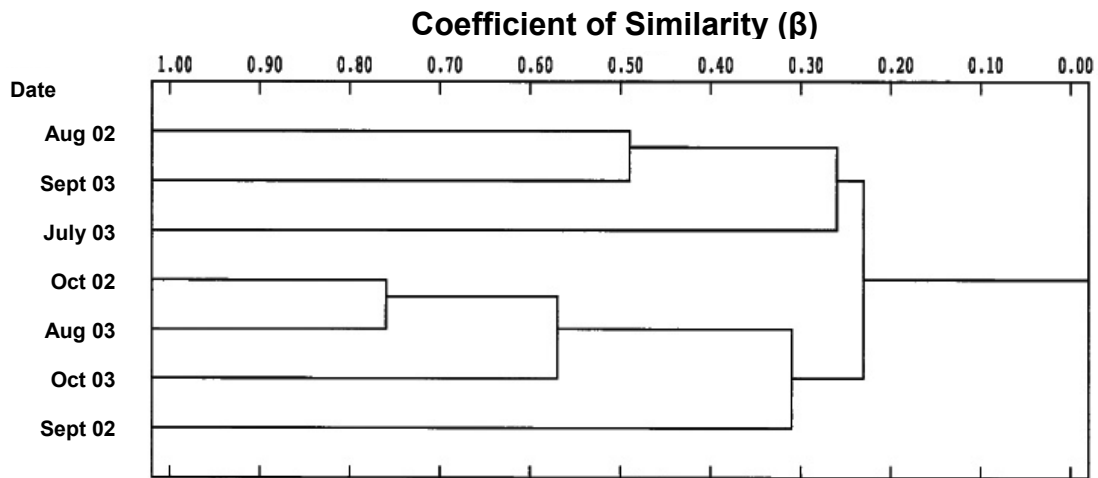
**Figure 4.2.** A 2-D dendrogram of treatment similarity,  $\beta$ , based on the distribution of collembola families among treatment (CM) *Coniothyrium minitans*, (CM+F) *C. minitans* plus fluazinam, (F) fluazinam, and (UTC) non-treated control samples taken at Corapeake in 2002. Clusters were derived from a matrix of  $\beta$  values based on comparisons of all possible pairs, where all matches were considered and the unweighted cluster method was used. Cophenetic correlation was 0.69 with 95% correlation intervals of 0.96 and  $-0.27$ .



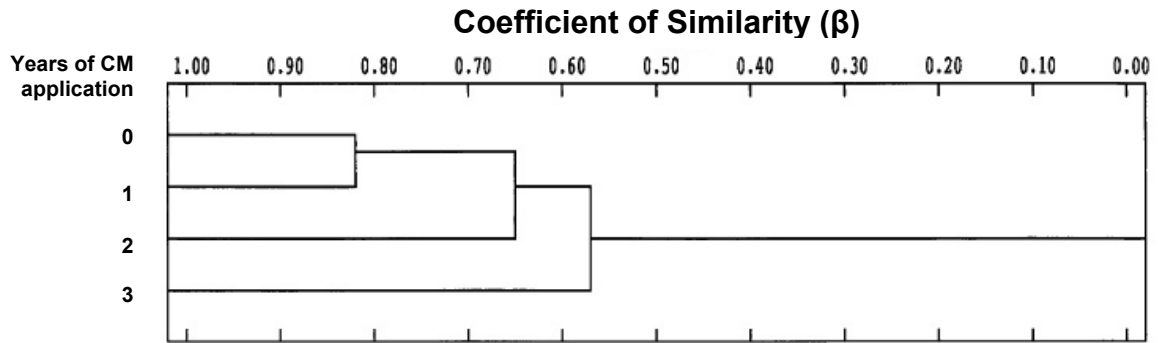
**Figure 4.3.** A 2-D dendrogram of treatment similarity,  $\beta$ , based on the distribution of collembola families among treatment (CM) *Coniothyrium minitans*, (CM+F) *C. minitans* plus fluazinam, (F) fluazinam, and (UTC) non-treated control samples taken at Gatesville in 2002. Clusters were derived from a matrix of  $\beta$  values based on comparisons of all possible pairs, where all matches were considered and the unweighted cluster method was used. Cophenetic correlation was 0.94 with 95% correlation intervals of 0.99 and 0.54.



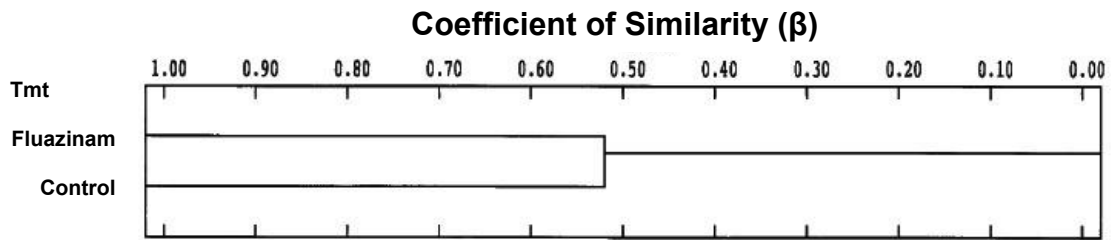
**Figure 4.4.** A 2-D dendrogram of treatment similarity,  $\beta$ , based on the distribution of collembola families among treatment (CM) *Coniothyrium minitans*, (CM+F) *C. minitans* plus fluazinam, (F) fluazinam, and (UTC) non-treated control samples taken at Tyner in 2002. Clusters were derived from a matrix of  $\beta$  values based on comparisons of all possible pairs, where all matches were considered and the unweighted cluster method was used. Cophenetic correlation was 0.45 with 95% correlation intervals of 0.92 and  $-0.57$ .



**Figure 4.5.** A 2-D dendrogram of sampling date similarity,  $\beta$ , based on the distribution of collembola families among samples taken from the long-term field plot location near Gatesville on 13 August, 11 September, and 9 October 2002 and 25 July, 28 August, 16 September, and 6 October 2003. Clusters were derived from a matrix of  $\beta$  values based on comparisons of all possible pairs, where all matches were considered and the unweighted cluster method was used. Cophenetic correlation was 0.87 with 95% correlation intervals of 0.95 and 0.71.



**Figure 4.6.** A 2-D dendrogram of *Coniothyrium minitans* treatment similarity,  $\beta$ , based on the distribution of collembola families among samples taken from 0, 1, 2, and 3 years of *C. minitans* treated plots in the long-term field study near Gatesville, NC. Clusters were derived from a matrix of  $\beta$  values based on comparisons of all possible pairs, where all matches were considered and the unweighted cluster method was used. Cophenetic correlation was 0.77 with 95% correlation intervals of 0.97 and -0.11.



**Figure 4.7.** A 2-D dendrogram of treatment similarity,  $\beta$ , based on the distribution of collembola families among samples taken from the non-treated control and fluazinam plots in long-term field study near Gatesville, NC. Clusters were derived from a matrix of  $\beta$  values based on comparisons of all possible pairs, where all matches were considered and the unweighted cluster method was used. Cophenetic correlation was 1.00 with 95% correlation intervals of 1.00 and 1.00.

**APPENDIX 1.** Peanut quality results from field studies.

**Table A1.1.** Main effects of *Coniothyrium minitans* treatment, fluazinam and cultivar on peanut quality as measured by percent extra large kernal (ELK) and percent fancy pod.<sup>z</sup>

Main Effect	Treatment	2002	
		%ELK	% Fancy
<i>C. minitans</i> rate	Control	24.8	62.2
	2 kg/ha	24.0	64.4
	4 kg/ha	23.5	59.5
	F value (P≤F)	2.84 (0.1112)	0.16 (0.6989)
Years of <i>C. minitans</i>	0	24.8	62.2
	1	23.6	61.7
	2	24.8	63.4
	3	22.9	60.9
	F value (P≤F)	3.01 (0.1018)	0.02 (0.9005)
Fluazinam	Control	23.5	61.3
	1.25 L/ha	24.5	62.8
	F value	1.99 (0.1636)	0.40 (0.5303)
Cultivar	NC-V11	21.8 a	59.2 a
	Perry	26.2 b	64.8 b
	F value (P≤F)	39.82 (<.0001)	13.57 (0.0005)

<sup>z</sup> Values followed by the same letter are not significantly different at a P=0.05 according to Fisher's Protected least significant difference.

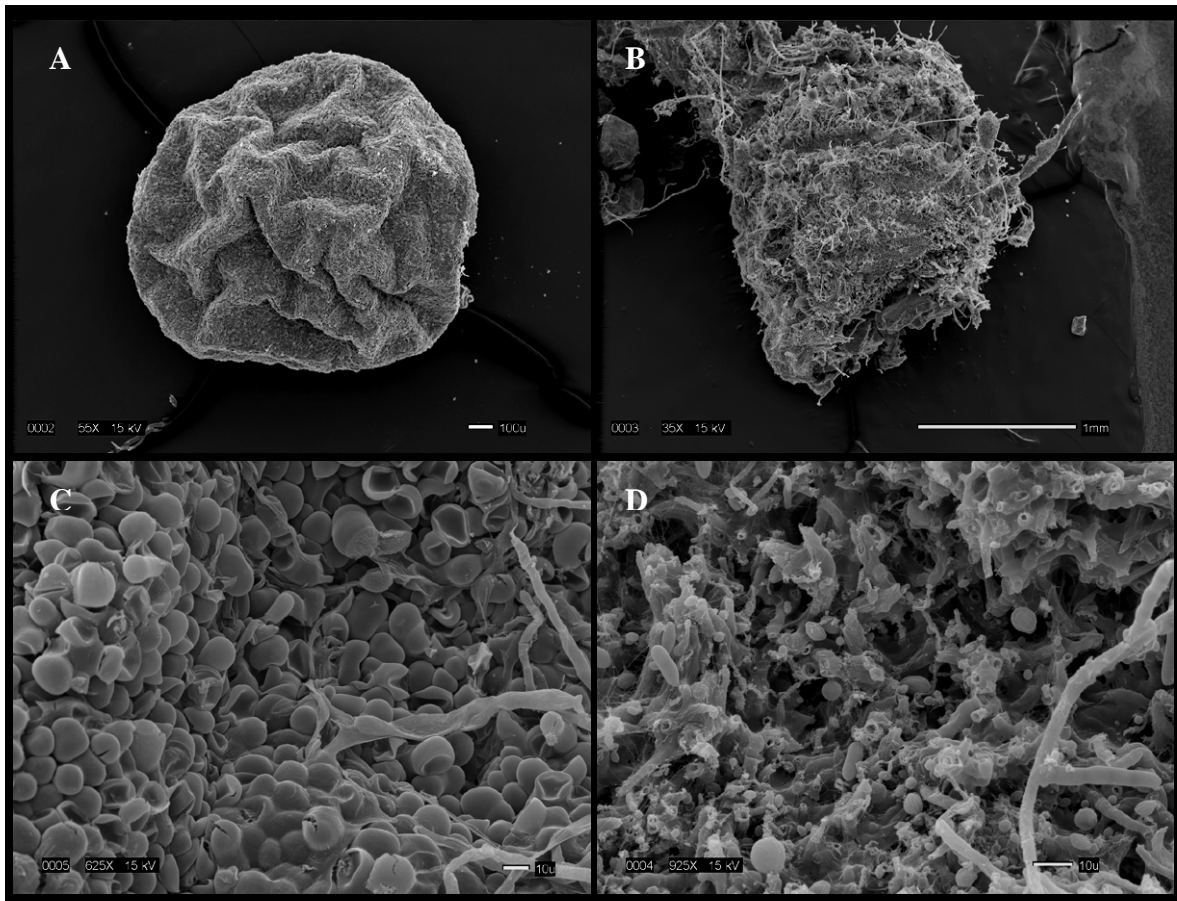
APPENDIX 1. (continued).

Table A1.2. Peanut quality data from short-term field studies<sup>z</sup>.

Treatment	2001						2002							
	Windsor		Jackson		Scotland Neck		Hertford		Tyner		Gatesville		Corapeake	
	%ELK	% Fancy	%ELK	% Fancy	%ELK	% Fancy	%ELK	% Fancy	%ELK	% Fancy	%ELK	% Fancy	%ELK	% Fancy
Contans	35.8	64.2	27	74.4	43.3	70.9	41.8	71.8	27.3	73.3	32.3	70.9	22.5	54.6
No Contans	32.7	67.4	29.3	75.8	40.8	66.8	41.8	70.7	27.5	73.8	30.4	72.5	22.6	51.5
F- Value (P)	18.54 (0.0499)	1.52 (0.3423)	0.17 (0.7208)	0.03 (0.8868)	13.16 (0.0.83)	11.38 (0.0778)	0.00 (1.000)	0.19 (0.7045)	0.00 (0.9576)	0.02 (0.9097)	12.10 (0.0177)	0.53 (0.5011)	0.00 (0.9789)	1.04 (0.3547)
Fluazinam	33.1	66.0	27.9	76.1	42.3	68.8	40.5	70.5	27	73.3	31.0	69.3	23.0	53.5
No Fuazinam	34.4	66.4	28.5	74.3	41.8	68.9	43.2	72.0	27.8	73.8	31.7	74.1	22.1	52.6
F-Value (P)	1.48 (0.2693)	0.01 (0.9421)	0.15 (0.7026)	0.62 (0.4489)	0.35 (0.5669)	0.00 (0.9795)	2.72 (0.1742)	0.20 (6797)	0.06 (0.8310)	0.03 (0.8730)	0.55 (0.4646)	3.55 (0.0693)	0.56 (0.4582)	0.16 (0.6887)
Perry	37.3	69.4	30.9	73.3	46.1 a	69.9	...	...	...	...	33.1	73.2	22.8	50.7
NCV11	29.9	62.6	26.0	76.8	38.0 b	67.8	...	...	...	...	29.5	70.3	22.4	55.4
F-Value (P)	61.73 (0.0002)	6.22 (0.470)	9.38 (0.0120)	1.75 (0.2155)	66.57 (<.0001)	0.43 (0.5246)					13.72(0. 0009)	1.34 (0.2564)	0.10 (0.7497)	3.95(0.0 561)

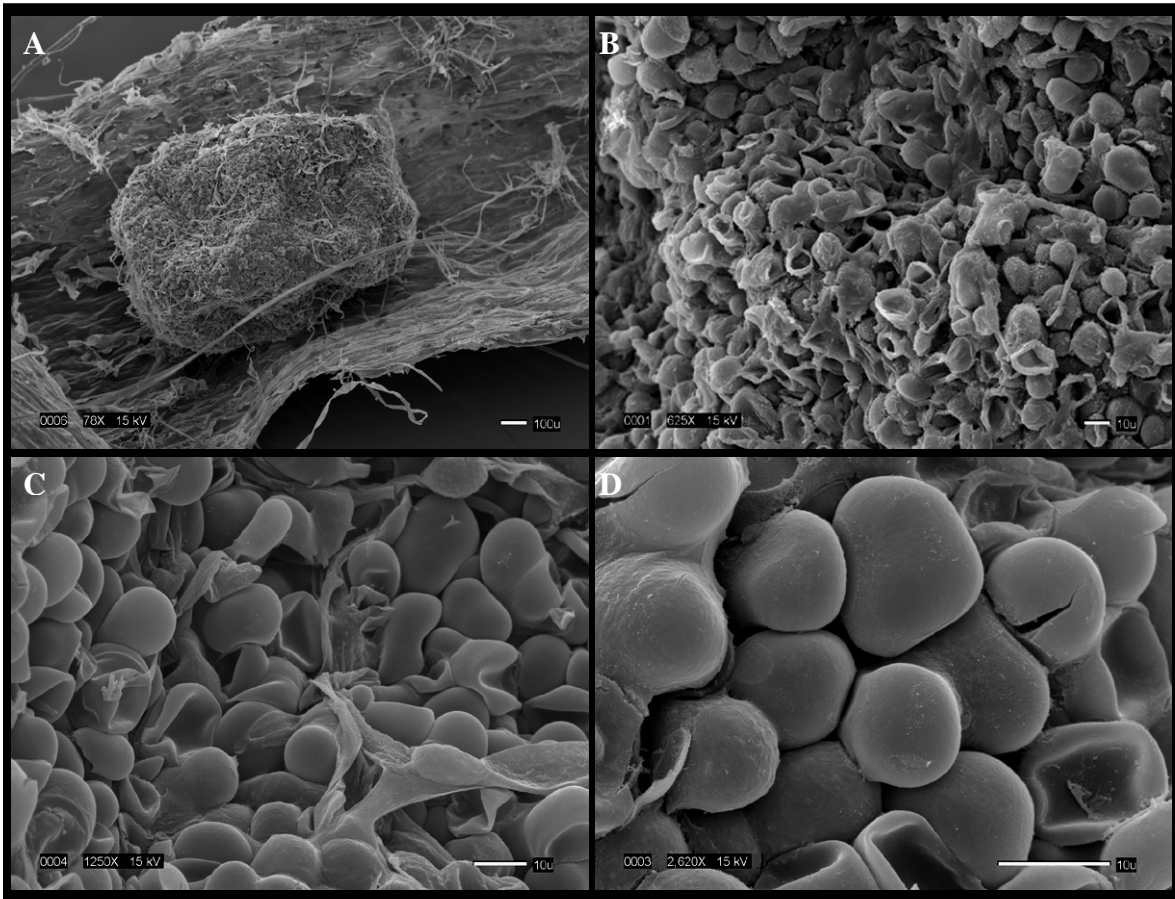
<sup>z</sup> Values followed by the same letter are not significantly different at a P=0.05.

**APPENDIX 2.** Scanning electron microscopy images of *Sclerotinia minor* sclerotia.



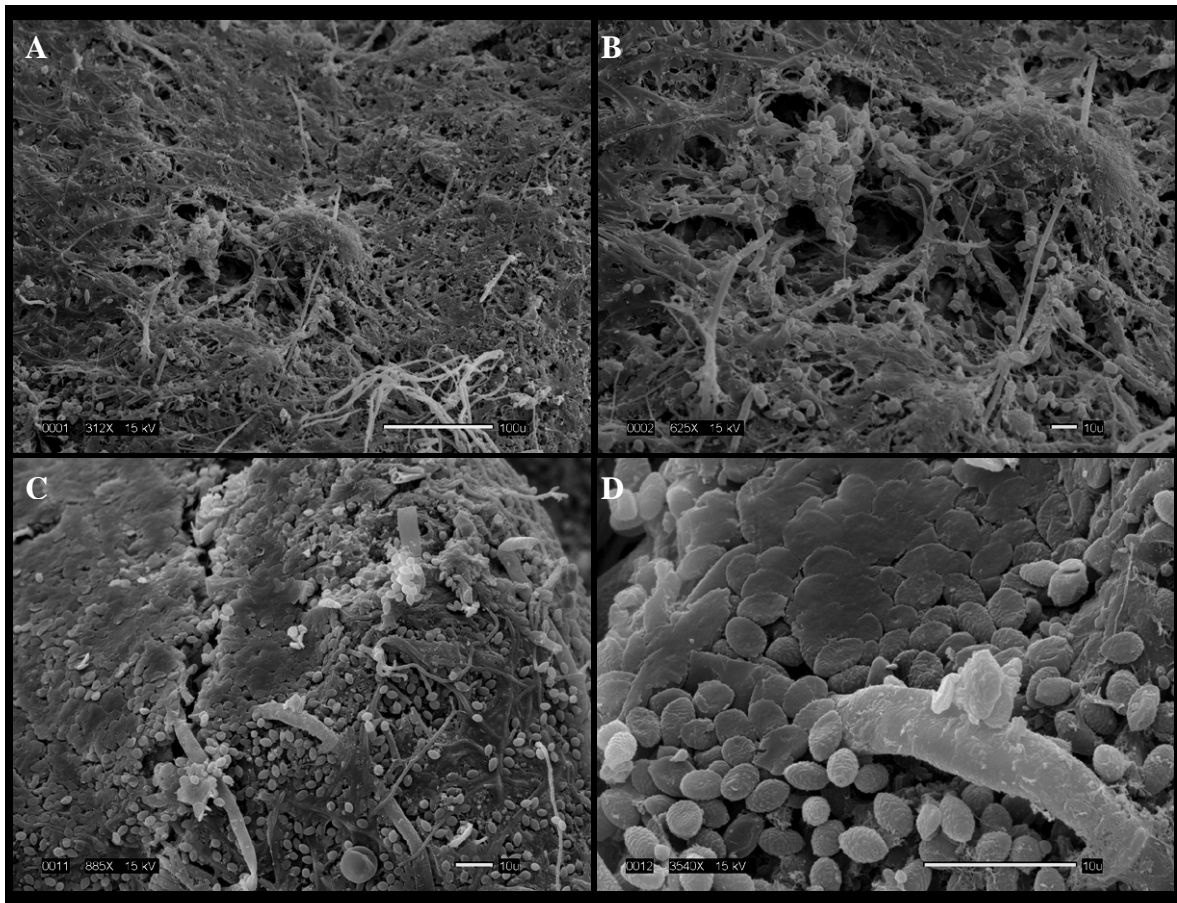
**Figure A2.1.** Scanning electron images of sclerotia of *Sclerotinia minor*. A. Healthy sclerotium (Magnification 55 x). B. Sclerotium infected by *Coniothyrium minutans* after 28 days (35 x). C. Surface of healthy sclerotium showing outer rind cells intact (625 x). D. Sclerotium infected by *C. minutans* after 42 days showing outer rind cells collapsed and perforated (925x). (Images taken with Philips 505T Scanning Electron Microscope at 15 kV).

APPENDIX 2. (continued).



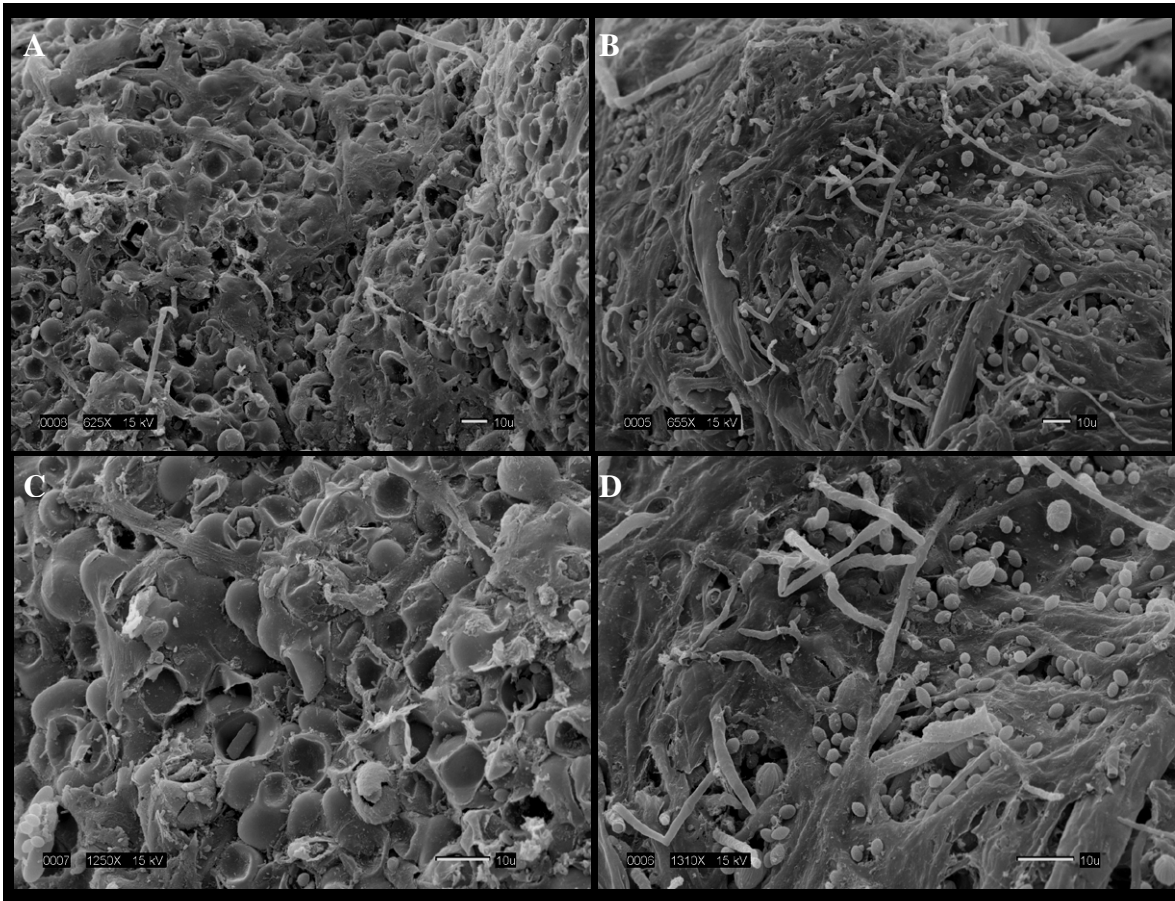
**Figure A2.2.** Scanning electron microscope images of a healthy sclerotium of *Sclerotinia minor* produced on peanut. A. Sclerotium on peanut stem (78 x). B. Surface of sclerotium showing intact rind cells (625 x). C. Surface of sclerotium (1250 x). D. Rind cells on surface of sclerotium of *S. minor* (2620 x). (Images taken with Philips 505T Scanning Electron Microscope at 15 kV).

APPENDIX 2. (continued).



**Figure A2.3.** Surface of *Sclerotinia minor* sclerotium after infection by *Coniothyrium minitans*. A. Surface of sclerotium 14 days after infection (312 x). B. Higher magnification (625 x) at 14 days showing complete breakdown of outer rind and *C. minitans* mycelium and spores. C. Surface of sclerotium 28 days after infection (885 x). D. Magnification of surface at 28 days showing spores of *C. minitans* and dissolution of cells (3450 x). (Images captured with Philips 505T Scanning Electron Microscope).

APPENDIX 2. (continued).



**Figure A2.4.** Surface of *Sclerotinia minor* sclerotium after 42 days of infection by *Coniothyrium minitans* showing collapse and breakdown of outer rind cells. A.625 x. B. 655x. C. 1250 x. D. 1310 x. (Images captured with Philips 505T Scanning Electron Microscope).