TRITROPHIC EFFECT OF *CONIDIOBOLUS THROMBOIDES* WITH THREE CEREAL ENTRIES AND THE RUSSIAN WHEAT APHID, *DIURAPHIS NOXIA*

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CHAPTER I

INTRODUCTION AND REVIEW OF THE LITERATURE

INTRODUCTION

The objective in biological control is to manage insects without damaging the environment. Insecticidal studies in the past have indicated that resistant plants can increase or decrease the insect's susceptibility to insecticides (van Edem, 1990). Therefore, if plant resistance can make the insects more susceptible to insecticides, plant resistance may make the insects more susceptible to their fungal pathogens.

Experiments were conducted with Conidiobolus thromboides against Russian wheat aphid, Diuraphis noxia (Mordivilko) (Homoptera: Aphididae) (RWA). The plant entries were PI 386148, a resistant triticale that has antibiosis against RWA, PI 372129, a tolerant wheat entry with no antibiotic effect, and TAM W-101, a susceptible wheat entry (Reed et al., 1991). The tritrophic relationship between the plant (first trophic level), the aphid (second trophic level), and the fungal pathogen (third trophic level) was studied. Spore germination, optimum spore dose, and fungal infection of the nymphs was also studied.

The main objective of these experiments was to determine if resistance in the plant entries enhances or decreases the ability of the fungal pathogens to kill the aphids. If

resistance could enhance the action of the fungal pathogen, this approach could efficiently reduce the aphid numbers in the field or greenhouse below the economic threshold. This could result in the reduction or elimination of the use of chemical pesticides. Conversely, if fungal activity is inversely impacted by the action of antibiosis in the plant, this will add to the warnings against indiscriminate usage of resistance by plant breeders (Reed et.al, 1991).

The second objective was to determine if the fungal pathogens were transferred maternally to the nymphs. In this experiment, all or most of the F1 generation, nymphs should die soon after birth if infected by the fungal pathogen prior to birth. If the nymphs are exposed to the pathogen after birth then only a small number of the nymphs should exhibit signs of mycosis.

The last objective was to determine if the viable spores could be produced on a large scale. In order for fungal pathogens to be useful, the spores must readily germinate in vitro and in vivo, and the dosage applied must be known. Application of a low dose may not infect the aphids and too high a dose may result in competition among the spores.

LITERATURE REVIEW

The Russian wheat aphid, Diuraphis noxia (Mordivilko), has been a serious threat to grain production in the United States since first discovered in Texas in 1986 (Burton, 1990). Fungi are the most common and significant microbial pathogens of insects, mites, and spiders, and the only significant microbial pathogens of the Homoptera and Hemiptera (Humber, 1990). The importance of fungi as pathogens of these insects is underscored by the fact that more fungal genera and species attack these two insect orders than any other orders. These pathogens primarily affect scales and to a lesser degree aphids. Typically, scales and aphids have dense, sessile populations that facilitates the dispersal of the pathogen and results in the rapid spread of epizootics (Humber, 1990). Fungal pathogens almost always infect the host by direct penetration of the cuticle or exoskeleton (Humber, 1990). This mode of infection is unique and characteristic of fungi since all other entomopathogens, viruses, and microsporidia enter the host by ingestion. Bacteria can enter the host either by ingestion or into the hemocoel via cuticular wounds. (Sampson et al., 1988). Most fungal disease development can be divided into nine steps: (1) attachment of infective units (either conidia or zoospores) to the insect cuticle, (2) germination of the infective unit on the cuticle, (3) penetration of the cuticle by germ tubes or by infection pegs

from appressoria, (4) multiplication in the yeast phase (hyphal bodies) in the hemocoel, (5) production of toxic metabolites, (6) death of the host, (7) growth in the mycelia phase with invasion of virtually all host organs, (8) penetration of hyphae from the interior through the cuticle to the exterior of the insect, and (9) production of infective units on the exterior of the insect. Fungi which fail to complete the first four steps will have low virulence regardless of high toxin biosynthetic capability (Roberts, 1981).

Environmental factors govern the production of conidia, penetration of spores that germinate, and fungal sporulation. These, in turn, affect the rate of the epizootic (Carruthers and Soper, 1987). Many species of entomopathogenic fungi are highly dependent on moisture for the production of conidia and sporulation (Wilding, 1969). The fungal pathogens appear to have more potential for aphid control on irrigated cereals because of the high humidity in the crop environment (Feng et al., 1990, 1991). Temperature is a factor that affects the rate the disease progresses in the insect. The optimum temperature for the fungal pathogens is different for each species but is usually around the optimum temperature for insect development (Benz, 1987).

Since the dispersal of conidia is necessary for the spread of disease, the majority of species of Entomophthorales produce air-borne conidia as infective units, but the conidia

may also be dispersed by rain or movement of infected hosts (Hall and Dunn, 1958). Fungi can also produce resting spores or overwintering structures. Resting spores overwinter in cadavers of hosts, on foliage, in feces of scavengers, and in the soil where they can then germinate and become infective units in suitable conditions (Li et al., 1988).

Other important factors to consider when looking at natural diseases for insect control is the virulence and pathogenicity of the disease. Virulence is the intensity of the disease caused by the pathogen. Pathogenicity is the organisms ability to cause disease. Fungi are usually highly virulent because of their ability to produce numerous conidia as infectious units. The most successful fungal pathogens are also highly pathogenic, thus alternate hosts are used when the primary host population are low (Tanada, 1963). Some insects can develop resistance to diseases just as they do to insecticides, although not as often. Milner (1983, 1985) characterized a biotype of pea aphid, Acyrthosiphon pisum, as resistant to 23 of 40 strains or isolates of Pandora neoaphidis occurring in Australia. All seventeen of the strains capable of infecting the resistant aphid were observed to occur in the fields.

Conidiobolus thromboides (Drechsler) is also known as Entomophthora virulentia (Hall and Dunn). C. thromboides is in the division Amastigomycota, class Zygomycetes, order Entomophthorales (Alexopoulos and Mims, 1979), and family

Ancylistaceae (Humber, 1990). C. thromboides is a saprophytic species and may be more infective than C. obscurus (Papierok, 1986). C. thromboides can be isolated from aphids, small diptera, and moths (Waterhouse and Brady, 1982). The invasive process of C. thromboides is not known. Within the genus Conidiobolus, the spores are spherical, and the nuclei are small with a prominent nucleolus (Latge et al., 1988).

Physical factors influence the growth and sporulation of C. thromboides. Sporulation was found to be greater in the darkness on a medium with pH 6.5. However, a mildly acidic pH helped control the growth of bacteria in cultures. Growth in unagitated liquid cultures was very slow because oxygen is needed for sporulation (Latge et al., 1978). The optimum temperature range for C. thromboides is 30°C with a minimum of 6°C and a maximum of 36°C (Yendol and Hamlen, 1973). Resting spores can be found in the species C. thromboides, and the resting spores have been shown to maintain high viability (90%) after laboratory storage for 12 years at 4°C (Fuxa and Tanada, 1987). The mature resting spores of C. thromboides are binucleate (McCabe et al., 1984).

Usually, the fungal pathogens colonize all the host tissue, and the host will die completely filled with fungal material 3-6 days after infection. C. thromboides can kill its host within one day after penetration of the hemoceoel with limited fungal growth (Latge et al., 1980), with death probably resulting from the production of fungal toxins. Two

toxins were extracted from two strains of C. thromboides: 4'-hydroxymethylazoxybenzene-4-carboxylic acid and azoxybenzene-4,4'-dicarboxylic acid, with the first being responsible for the insecticidal activity. The second toxin did not show insecticidal activity. Adult blowflies, Calliphora erythrocephala, were used to test these compounds by injecting the toxins into the hemocoel. The adults died in less than four days with a minimal amount of fungal development in the host (Claydon and Grove, 1977, 1978). The fungal toxins do not appear to have any effect on lepidopterans (Yendol et al., 1968). These toxins have a structural similarity to insecticides in the DDT group and are produced by many entomopathogenic fungi both in vitro and in vivo (Roberts, 1981).

The exact life cycle of C. thromboides is not known, but the life cycle of C. obscurus has been characterized by Latteur and Godefroid (1983). With C. obscurus, aphids are infected from resting spores in the soil. The conidia develop in infected aphids and sporulate to infect other aphids, or they may form hyphal bodies, yeast like structures which will germinate to form conidia or asexual spores to infect other aphids. Hyphal bodies may also form resting spores in the aphid cadavers and overwinter in the soil (Figure 1).

The resistance in the triticale entry PI386148 to the Russian wheat aphid has been attributed to an antibiotic effect (Frank et al., 1989, Webster et al., 1987, Webster,

1991). The rye, Secale cereale L., is thought to be the background for the triticale. Kindler and Springer (1989) reported that Russian wheat aphid populations were reduced by 85% and 95% on cereal rye when compare to a susceptible wheat and barley. Van Edem (1990) reported that insects on resistant entries tended to have lower birth weights, lower reproductive rates, and increased restlessness. Burd (1991) found that population levels were greatly reduced in the resistant triticale, primarily due to decreased reproduction of the Russian wheat aphid, rather than to poor nymphal survival. Aphids on the resistant triticale were also found not to aggregate on the new growth and were thus more widely dispersed on the plant.

Aphids on resistant varieties appear to be more susceptible to insecticides (van Edem, 1990). This is possibly related to reductions in the size of the aphid and stress caused by secondary plant substances. The greater restlessness of the aphids on resistant varieties might increase their contact with the fungal pathogens (van Emden, 1990).

Ecdysis may have an effect on the rate of infection of the fungal pathogens. In Leptinotarsa decemlineata, the pathogen, Beauveria bassiana, could be shed with the cuticle during molting, could penetrate the old cuticle and scar the newly forming cuticle without causing infection, or could cause a secondary infection by damaging the newly formed

cuticle, resulting in bacterial contamination of the wound leading to septicemia (Vey and Fargues, 1974). Hare and Andreadis (1983) found the effectiveness of the fungal pathogen, Beauveria bassiana, varied among the host plants of the Colorado potato beetle, Leptinotarsa decemlineata. When the insects feeding was supported by good growth of the plant, the insects were more resistant to the fungal pathogen, indicating suboptimal hosts would increase the effectiveness of the pathogen. Allelochemicals received from host plants may also cause inhibition of the fungal pathogens (Ramoska and Todd, 1985). Reed et al. (1991 & 1992), Campbell et al., (1992) reported plant entries resistant to the Russian wheat aphid through antibiosis caused a detrimental effect on the third trophic level (parasitoids). The parasitoids on resistant triticale were smaller, took longer to develop, and were fewer in number than those on susceptible or tolerant entries. The sex ratio of the emerging parasitoids on the resistant triticale was higher for females. The combination of the two means of control together reduced the number of aphids on wheat, possibly due to the fact that the leaves on the resistant wheat did not roll as they did on the susceptible entry. Entomopathogenic fungi, as the third trophic level, may also be affected adversely or may be enhanced by the plant entry. Poprawski et. al, (1992) found a greater reduction in the number of Russian wheat aphids when the fungal pathogen Zoophthora radicans was used together with

the aphid parasitoid Aphelinus asychis. The parasitoids were also susceptible to fungus, but it is believed they may have helped transmit the pathogen.

Chemicals, in most cases, do more damage to natural enemies than to the insect they are meant to control (van Edem, 1990). Hall and Dunn (1959) found that some insecticides and fungicides affected the germination and growth of entomopathogenic fungus C. thromboides. The fungus was able to grow well with the insecticides parathion and DDT and the fungicides wet sulfur and Dithane Z-78. With the insecticides malathion, demeton, and trithion, and the fungicides Ferbam, Bordeaux 5-5-50, and captan, the fungus was not able to germinate and grow. Therefore, chemicals that may be used in the field to suppress insect pests and plant diseases may also suppressed the naturally occurring fungal pathogens.

For integrated pest management (IPM) to work, IPM programs need to effectively combine host plant resistance with biological control. In the past, plant breeders bred for resistance without concern on the effect resistance would have on biological control. This could possibly set up situations where host plant resistance may eliminate the effectiveness of biocontrol. Compatibility of pest tolerant cultivars with microbial pathogens is desirable. Herzog and Funderburk (1985) and van Eden (1990) reported that insect pathogens are density-dependent. Therefore, the ability of a tolerant crop

to support substantial populations of the pest should allow a pathogen sufficient substrate on which to develop devastating epizootics.

Fungal pathogens may never be commercially feasible for large scale production because of the cost to culture the pathogens on a commercial level. The weakness of entomopathogenic fungi when compared to chemical insecticides are: (1) the fungal pathogens may act too slowly for those insects conditioned to the pesticides "rapid kill," and (2) fungi cannot suppress viral transmission by any viruliferous aphid, although some chemical pesticides may have this same weakness; however, depressing the overall aphid population might affect the rate of viral transmission. The advantages of fungal pathogens over chemical pesticides, which makes them more suitable in an IPM program are: (1) fungal pathogens may be less costly to develop and register than new pesticides, (2) most fungal pathogens are highly host specific, especially for aphids; therefore, safer than chemicals for the manufacturers, applicatators, and non-target organisms in and near the application sites, and (3) fungi successfully introduced into a area can survive from year to year, protecting surrounding areas as they are dispersed. While chlorinated hydrocarbons may be persistent in the environment, they may leach away from their application site and have a negative impact on non-target organisms including humans. Fungal pathogens are extremely effective in checking and

controlling their natural host in undisturbed environments where they work with other microbes, parasites, and predators to keep the organism as they have for millions of years (Humber, 1990).

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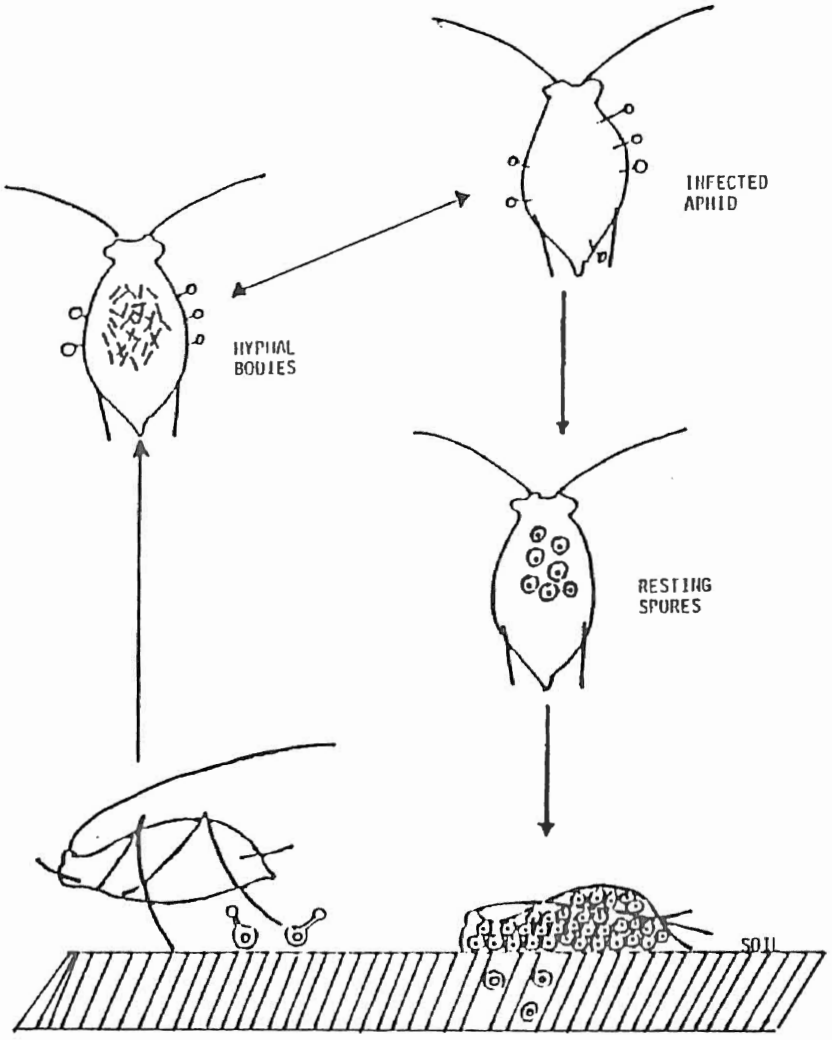

Figure 1. Life Cycle of Fungal Pathogen Conidiobolus Obscurus

CHAPTER II

TRITROPHIC INTERACTIONS OF THE FUNGAL PATHOGEN *CONIDIOBOLUS THROMBOIDES* WITH THREE CEREAL ENTRIES AND THE RUSSIAN WHEAT APHID, *DIURAPHIS NOXIA*

INTRODUCTION

The Russian wheat aphid, Diuraphis noxia (Mordivilko), has been a serious economic pest to wheat and barley producers since its introduction into the United States in 1986. The estimated economic damage is in the millions of dollars (Burton, 1992). The combination of the naturally occurring fungal pathogen, Conidiobolus thromboides, with resistant and tolerant plants should compliment each other in controlling the Russian wheat aphid (RWA).

C. thromboides is in the division Amastigomycotina, class Zygomycetes, and order Entomophthorales (Alexopoulus and Mims, 1979). Fungal pathogens are a particularly good means of biological control since they are neither harmful to humans nor to the plants the insect pest is found on (Goettel et al., 1990). The fungal pathogens are host specific so that danger to natural enemies is also reduced. The species of Homoptera and Hemiptera are attacked by more species of fungal pathogens than any other insect orders (Humber, 1990). The fungal pathogens are unique in that they infect the host via cuticular penetration. All other entomopathogens, viruses and microsporidia infect the host via ingestion (Humber, 1990);

however, bacteria can enter the host via cuticular wounds causing septicemia (Sampson et al., 1988).

C. thromboides has been shown to produce two different chemicals. One, 4'hydroxymethylaxzobenzene-4-carboxylic acid, has insecticidal activity (Claydon, 1977, 1978). In adult blowflies, this compound was injected directly into the hemoceol, and the flies died within 4 days with limited fungal growth (Claydon and Groves, 1978). Brobyn and Wilding (1977) observed that four different species of fungal pathogens in the order Entomophthorales needed at least 5-8 days for fungal mediated mortality.

Aphids on resistant varieties of plants have been shown to be more susceptible to insecticides, probably due to secondary plant substances (van Edem, 1990). Resistant plant entries have been shown to have an effect on the third trophic level in tests conducted with the parasitoid, Diaeretiella rapae, and the RWA (Reed et al., 1991 & 1992, Campbell et al. 1992). In our experiments, C. thromboides was tested against the RWA on susceptible, resistant, and tolerant plant entries to determine any interactions of the host plants and RWA susceptibility to the pathogen.

MATERIALS AND METHODS

The resistant entry used was PI386148, a resistant triticale which has been found to reduce the total number of aphids through the mechanism of antibiosis. The tolerant wheat entry was PI372129 which is able to sustain a large number of aphids for a long period of time without substantial damage to the plants. The control plant in this test was TAM W-101 which is highly susceptible to the RWA.

The plants were grown in cone-tainers using a fritted clay medium and hand watered daily (Van Bavel et al., 1978). After the plants were thirteen centimeters high, the plants were caged with a ventilated polyvinyl cylinder and placed in a growth chamber for two days until the inoculated aphids were placed on the plants. Also at this point, the plants were watered with a solution of Peter's fertilizer (25g/l).

The fungal pathogens were grown in liquid culture of Sabouraunds's agar (40% dextrose, 10% yeast extract, and 10% peptone per liter of distilled water) with the pH adjusted to 6.5 and autoclaved at 250°C for twenty minutes to sterilize the agar according to Poprawski (1992). Tween 80 (2.5ml/l) and chloramphenicol (25g/l) were added to the agar to increase the spreading of the aqueous material and to eliminate bacterial contamination. The cultures were maintained on a shaker table at 150rpm for two weeks at room conditions to allow for maximum growth and sporulation.

The inoculum was prepared by filtering the liquid culture through #5 Whatman filter paper to remove the larger pieces of mycelia and hyphae, leaving the spores in the agar. The agar and spores (about 150ml) were diluted to 250ml with distilled water. A 200mm by 130mm polyvinyl cylinder was placed over a petri dish containing the aphids on leaf sections on moist filter paper. The suspension was delivered directly onto the aphids with a Sigma aerosol dispenser using the cylinder as a settling tower to obtain optimum distribution. A 12mm by 12mm cover slide was placed in the dish as well so that the number of spores delivered could be counted. The cover slide was removed, stained with lacto-fushia, and read under a phase contrast microscope equipped with an ocular micrometer.

After inoculation, the petri dishes were sealed with parafilm, and the dishes were placed in a dark area for 12h at room temperature. After the time had elapsed, the aphids were transferred to test plants in the cone-tainers. Untreated aphids were placed on similar plants as controls. The plants with the aphids were placed in the growth chamber ar 24°C, L:D 16:8, and RH 85%. The aphids, alive and dead, were counted daily and the numbers recorded. The dead aphids were removed and placed on microscope slides with lacto-fushia stain, and viewed under phase contrast microscopy to detect if the aphids had died from mycosis and these data were recorded for the removal test. For the non-removal test, the procedure was the

same as the removal test except that the aphids were counted but otherwise not disturbed for a ten day period.

RESULTS

In the control plants, there were no aphid deaths attributed to mycosis. There were no significant differences in the number of dead in the controls versus the number of dead in the treated. There were significant differences ($P < 0.05$) in the number of aphids dead per entry per day, cumulative dead, and cumulative loss and death for the control between PI372129 and the PI386148 and TAM W-101 over a ten day period. The resistant and susceptible entries had significantly more dead than did the PI372129, which is the tolerant variety, although total population numbers did not differ between entries. In the treatment, there were no significant differences in the total population (Figures 1 and 2), cumulative dead, cumulative dead mycosis, or cumulative loss and death. The only significant difference was in the mean number of aphids over days for the treated test . The PI372129 showed a trend of more aphids per plant per day in both the treatment and control than did the PI386148, resistant triticale, which was expected due to the antibiotic effect of the triticale. The antibiotic effect of the PI386148 was seen to start around day three in the control and the treatment (Figure 3). The points lie almost exactly on

top one another. This indicates that the secondary plant substances were having an effect on the aphid before the fungal pathogen could develop.

The second test (Table 1), where the aphids were not disturbed for a ten day period after treatment,had much different results. There were significant differences between the control and the treatment when the aphids were not removed. In analyzing the overall difference between the control and the treatment, there was a higher proportion of aphids alive and dead in the treatment than in the control. There was a significant difference in the proportion of aphids alive and dead in the entries. There was no difference between the TAM W-101 and PI372129, but there was a difference between those two entries and PI386148 for the proportion of aphids alive and dead. In PI386148, there was a lower proportion of aphids alive and a higher proportion of aphids dead than in the other two entries. There was significant differences in the proportion of aphids where death was attributed to mycosis between all the entries. PI386148 had the highest proportion of death attributed to mycosis, followed by TAM W-101, while PI372129 had the lowest proportion of death due to mycosis (Figure 4). There was a greater number of aphids alive in the treatment than in the control. In the control, there was also a significant difference in the proportion of deaths between the entries PI386148 and TAM W-101 and PI372129, (Table 1). The

proportion of aphids dead on PI386148 was higher than the TAM W-101 and PI372129. There was no significant difference in proportion of death on the TAM W-101 and the PI372129.

CONCLUSIONS

The tests with the fungal pathogen Conidiobolus thromboides, the Russian wheat aphid, and resistant, tolerant, and susceptible plants did not indicate that the aphids on resistant plants were more susceptible to the pathogen than those aphids on susceptible plants when the dead aphids were removed daily, but the significant differences were observed when the dead aphids were left on the plants (Figure 4).

Herzog and Funderbank (1985) observed that in many cases resistant plants had a negative effect in combination with biological control methods. It is possible that secondary plant chemicals affect the aphids in such a manner that the fungal pathogen is inhibited, but the fungal pathogen may allow the aphid to survive longer on the resistant plant than non-treated aphids on similar plants. The results of the test where the dead aphids were removed daily indicate that secondary chemical in the plant are having a detrimental effect on the aphid before the fungal pathogen has time to develop and kill the aphids. This may be a result of a lower amount of inoculum present. The nonremoval tests also indicated that when the fungal pathogen is applied to aphids

on resistant plants, the rate increase for the population is the greater than rate of increase with non-treated aphids (Table 1). The tests also confirm the ability of PI386148 to reduce aphid numbers.

One hypothesis for the low death rate due to the fungus in the PI372129 and TAM W-101 is that the aphids had enough suitable plant material that the aphids were able to "out grow" the pathogen. Vey and Fargues (1974) and Hare and Andreadis (1983) found that larvae of the Colorado potato beetle, Leptinotarsa decemlineata, fed an adequate diet, had enhanced resistance to the fungal pathogen, Beauveria bassiana, because the spores were sloughed off prior to invasion as the larvae molted. Another experiment that would be of value would be the combination of a fungal pathogen and bacteria. Although the aphids may be able to shed the fungal spores before the spores can penetrate the cuticle, the invading hyphae may scar the new cuticle before being shed, thus leaving the aphids more susceptible to secondary infection by the bacteria. Further experiments should be done to completely evaluate the effect of host plant resistance and biological control methods in combination. The two methods could work together to more efficiently control insect pests in the field.

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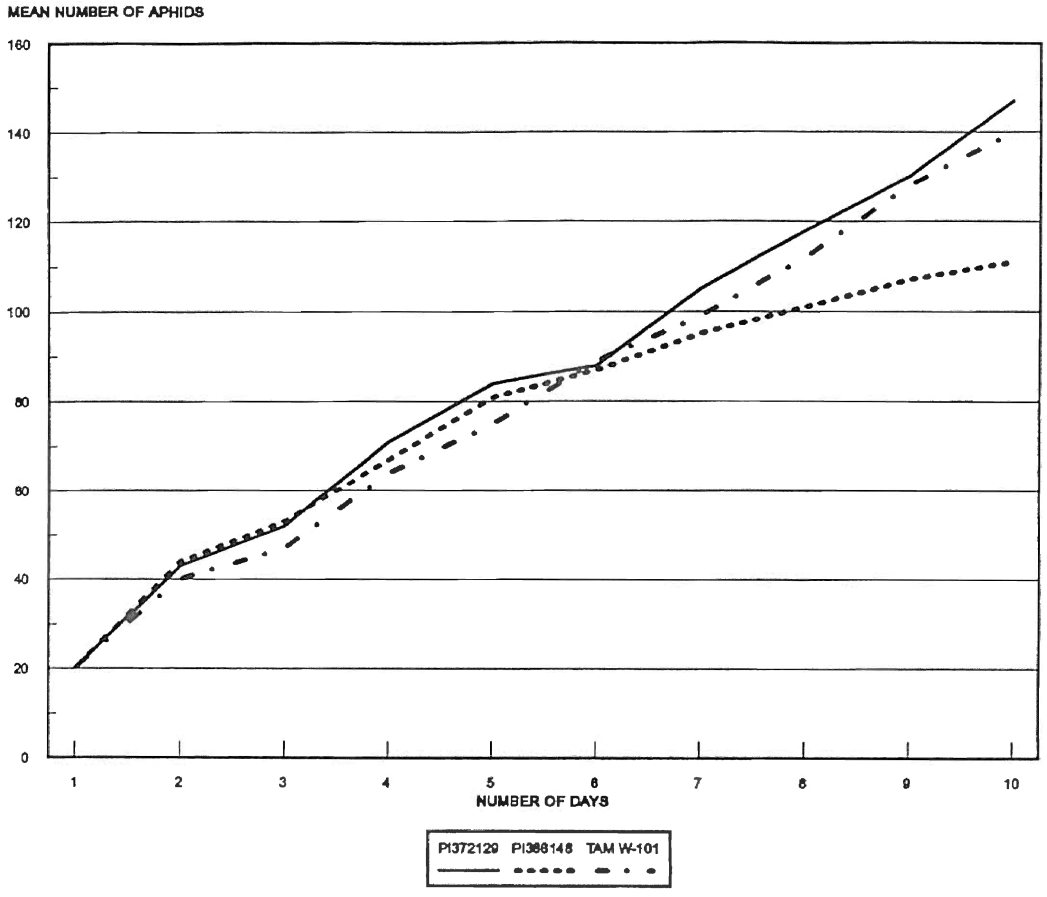

Figure 1. Relationship of mean number of aphids per day for the three plant entries (PI372129, PI386148, and TAM W-101) treated with Conidiobolus thromboides.

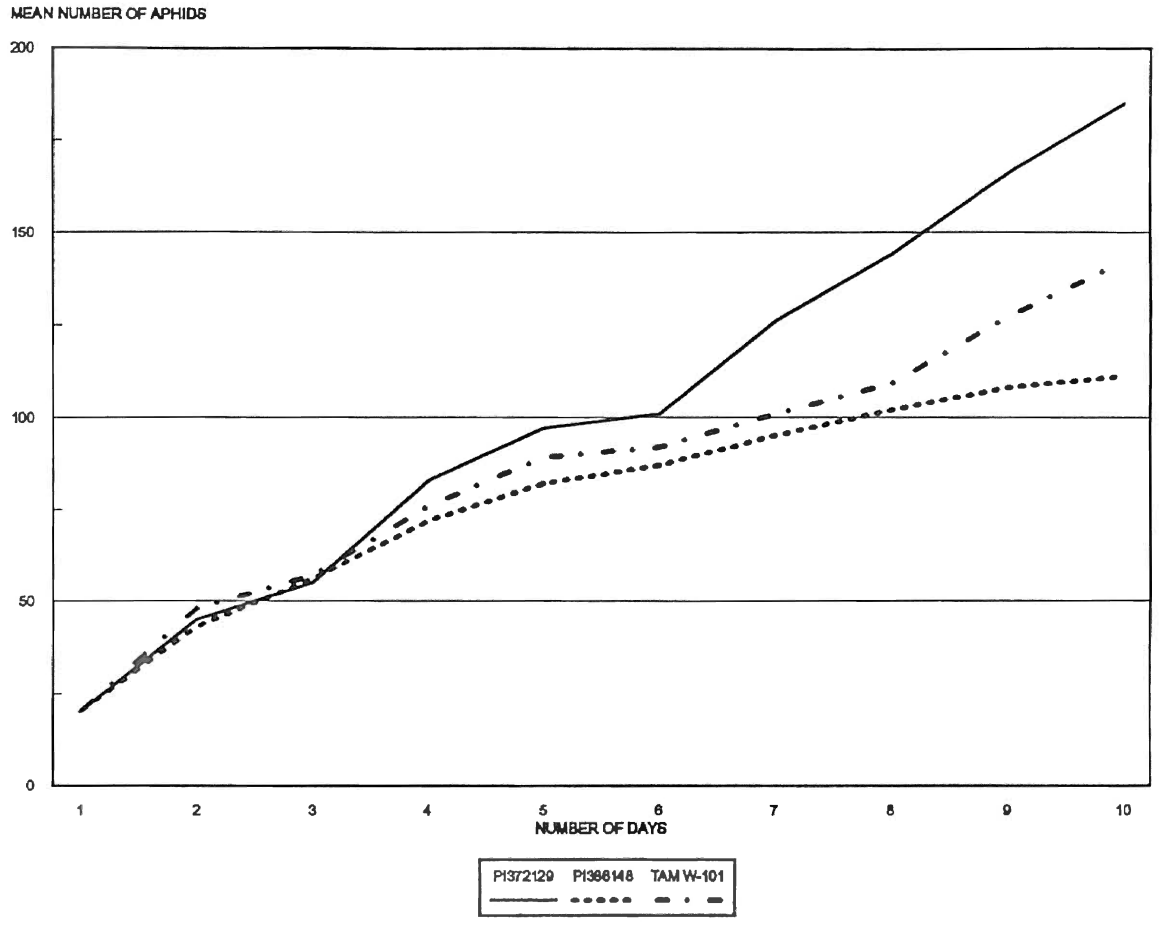

Figure 2. Relationship of mean number of aphids per day for the three plant entries (PI372129, PI386148, and TAM W-101) not treated with Conidiobolus thromboides.

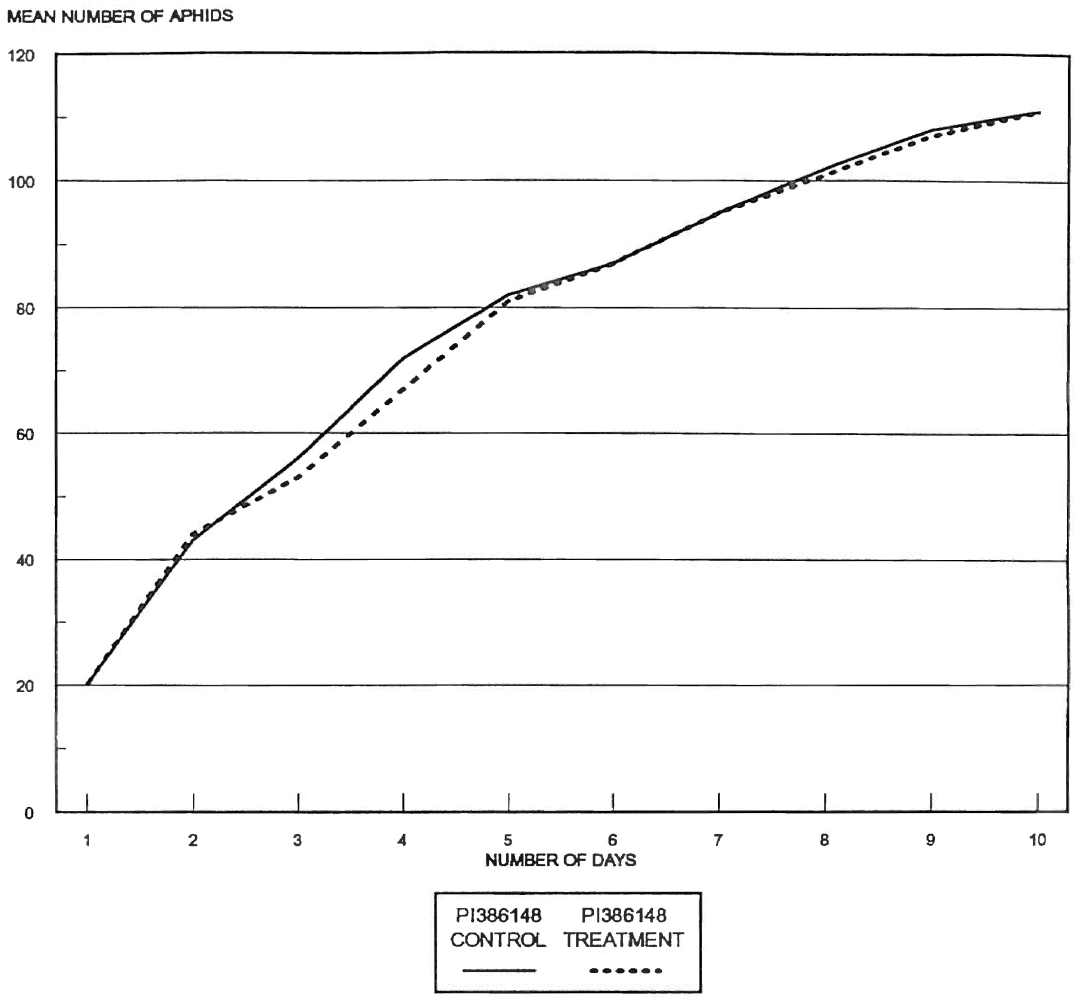

Figure 3. Relationship of treated and untreated Russian wheat aphids on the resistant triticale, PI386148 over a ten day period.

TABLE 1

PERCENTAGE OF APHIDS ALIVE AND DEAD AFTER TEN DAYS

TESTING PATHOGEN AND NONREMOVAL OF DEAD APHIDS

ENTRY	TREATMENT	PERCENTAGE[1] ALIVE	PERCENTAGE[1] DEAD
PI372129	CONTROL	89.5% ± 0.77 A	10.5% ± 0.77 A
PI386148	CONTROL	25.5% ± 0.89 B	74.4% ± 0.89 B
TAM W-101	CONTROL	89.7% ± 0.21 A	10.2% ± 0.21 A
PI372129	TREATMENT	97.1% ± 0.37 A	2.8% ± 0.37 A
PI386148	TREATMENT	43.6% ± 0.24 B	56.4% ± 0.24 B
TAM W-101	TREATMENT	86.4% ± 0.69 C	13.5% ± 0.69 C

[1] REGWQ Grouping significantly different at $\alpha = 0.05$

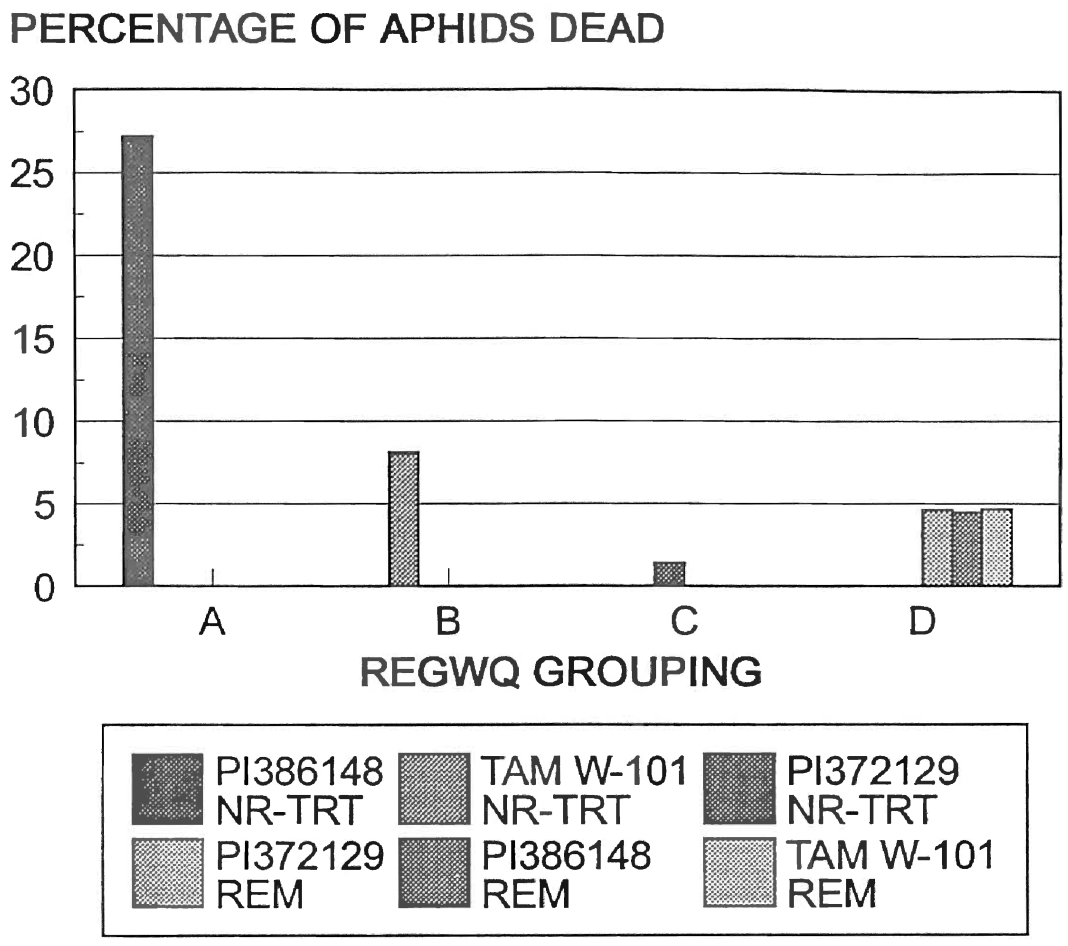

FIGURE 4. Difference in Death Attributed to Mycosis in the Removal and Nonremoval Tests.

CHAPTER II

MATERNAL TRANSMISSION OF THE FUNGAL PATHOGEN *CONIDIOBOLUS THROMBOIDES* in the RUSSIAN WHEAT APHID, *DIURAPHIS NOXIA* (MORDIVILKO)

INTRODUCTION

Several viruses have been shown to be transmitted transovarially in insects (Fuxa and Tanada, 1987), but there are no known incidences where fungal pathogens have been transmitted to the nymphs of the Russian wheat aphid, Diuraphis noxia. The Russian wheat aphid reproduces parthenogenically, without mating, and are viviparous, producing a living nymph. This type of reproduction may result in a slightly enhanced rate of increase in number of offspring produced, improved chances of survival, and an earlier start to independent growth (Dixon, 1987). The experiments conducted were to determine if maternal infection of the nymph prior to birth occurred with Conidiobolus thromboides. The aphids were closely monitored for ten days using treated and untreated controls to determine the effect of the fungus on the aphids and nymphs. The results gave more information than was expected in terms of reproductive rates and life span of the Russian wheat aphid when treated with this particular pathogen.

MATERIALS AND METHODS

The fungal pathogens were cultured using Sabouraund's agar (40% dextrose, 10% yeast extract, 10% peptone, and 25gm chloramphenicol per liter of distilled water. The pH was adjusted to 6.5, and the media was autoclaved at 250°C for twenty minutes to sterilize the agar according to Poprawski (1992). The cultures were placed on a liquid shaker table at 150 rpm at room temperature. The plants, TAM W-101, were grown in the greenhouse in cone-tainers in fritted clay, (Van Bavel, 1978) and watered from the bottom.

One adult aphid was placed on a TAM W-101 leaf section in a 35mm X 10mm petri dish with water soaked cotton. The aphids were then treated with the fungal pathogen using a Sigma aerosol sprayer for dispersal, and a 12mm X 12mm coverslide to determine the spore dose. The petri dishes were placed in a growth chamber at 24°C, L:D 16:8, and RH 90%. The aphids were counted each day and the number of offspring produced was recorded as well as the number of days until the first offspring were produced. The offspring were placed in separate petri dishes on leaf sections and placed in the growth chamber. Dead aphids were then counted, removed, and stained with lacto fushia and viewed with phase contrast microscopy to determine if death was due to the fungal pathogen. The aphids were monitored for ten days. At this

time, most of the parents had died and the first offspring or F1 had started to reproduce.

The data collected were analyzed using SAS and the Weibull probability function. The Weibull function is a three-parameter model which is successful in predicting the failure among test materials or events that occur or will probably occur at any time. x is given as the time period since the observation began, the instantaneous probability is given as the probability density function:

$$f(x) = \{(c/b)[(x - a)/b]^{c-1}\}e^{\{-[(x-a)/b]c\}}$$

The parameter a is the earliest time when $f(x) > 0$. The parameter b is a location parameter. The shape of the graph of f(x) is defined by c. When c is 3.4, f(x) is symmetrical. As c increased above 3.4, f(x) is skewed to the right and when c is greater than 2 but less than 3.4, f(x) is skewed to the left. When the function is used to predict birth rates of the Russian wheat aphid, about 63% of the births occur before (a + B) (Bonner and Dell, 1976, Williams and Popham, 1983).

The Weibull cumulative distribution function is defined by integrating f(x). The resulting function is:

$$f(x) - 1 - e^{\{-[(x-a)/b]c\}}$$

Figures 1 and 2 were fitted using the maximum likelihood and not the least squares as is most commonly found in regressions.

RESULTS

Data collected in the experiment were examined initially to determine if the fungal pathogen could possibly be transmitted to the nymphs before birth. An ANOVA was also run to compare the lifespan of the offspring in the treatment and control. No significant differences were noticed, indicating that transovarial infection was not occurring. If the fungal pathogen was being transmitted transovarially, there should be differences in the lifespan of the aphids produced from parents treated with the pathogen. Although some of the offspring died as a result of fungal infection, the infection was most likely transmitted as the nymph came in contact with a spore from the parent after birth.

It was found that the fungal pathogen had a significant effect on the reproductive rates of the aphids. In the control, the aphids produced more offspring than did the aphids which were treated with the pathogen. Although this is expected because of the death of the treated parents, 95% of the offspring from treated parents were produced by day 7 whereas in the control, 95% of the aphids were produced by day 10 (Figures 1 and 2).

An ANOVA was run to determine if there were significant differences between the treatment and control reproduction rates at the 25th, 50th, 75th, 90th, and 95th percentiles. It was found that at each of the different percentiles were significantly different at $P < 0.05$ (Table 1).

CONCLUSIONS

In this experiment, it was determined that the fungal pathogen is not transmitted transovarially, but that the fungal pathogen does significantly affect the reproduction rates of the aphid. The proportion of aphids is fairly constant and there is no significant difference between the replications in the control (Figure 1). After 10 days, 95% of the aphids were born. In the treatment, 95% of the aphids were born by day six (Figure 2). This indicates that while the parents were dying as a result of the pathogen, reproductive rate were increased. This may be a result of stress due to the presence of the pathogen, or possibly a mechanism to insure the survival of the species.

There were significant differences in birth rates at the different percentiles depending on the treatment (Table 1). In the control, the mean number of aphids produced is higher than in the treatment. The significance is that the fungal pathogen is affecting the birth rates at the different

percentiles as well as overall affecting the birth rates of the treated aphids.

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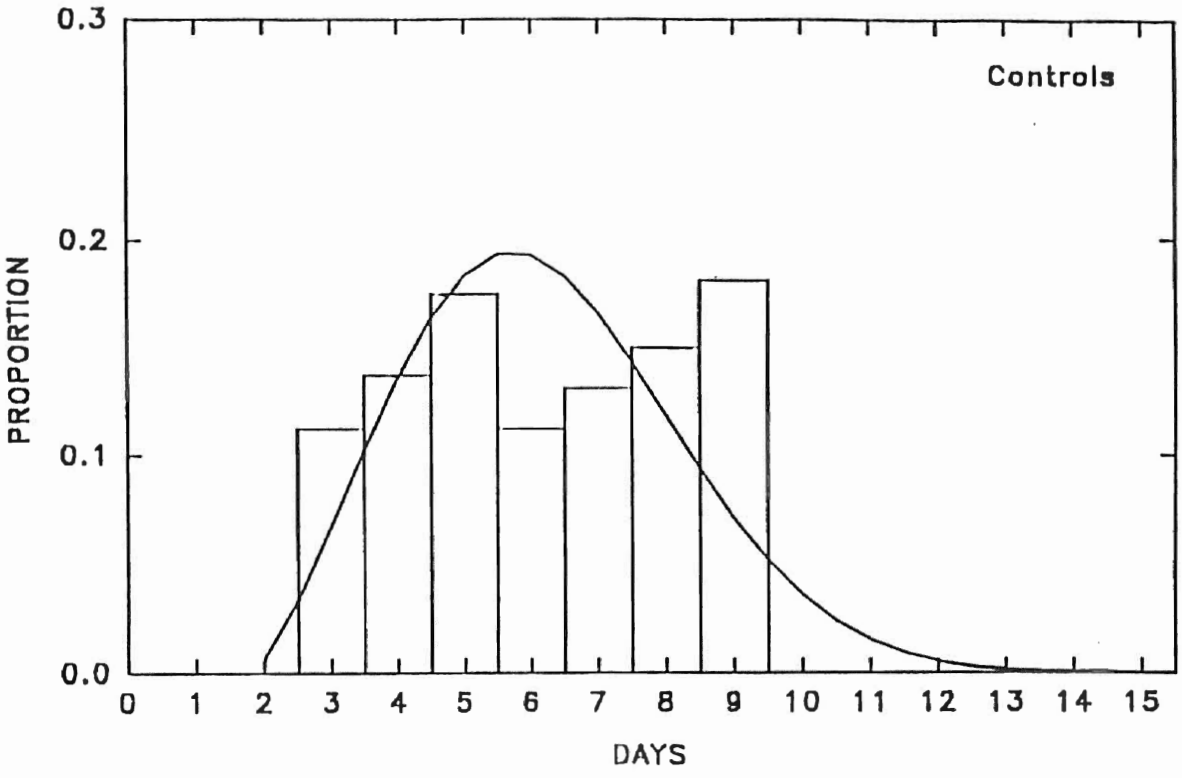

Figure 1. Proportion of Aphids Born Per Day in Untreated Controls

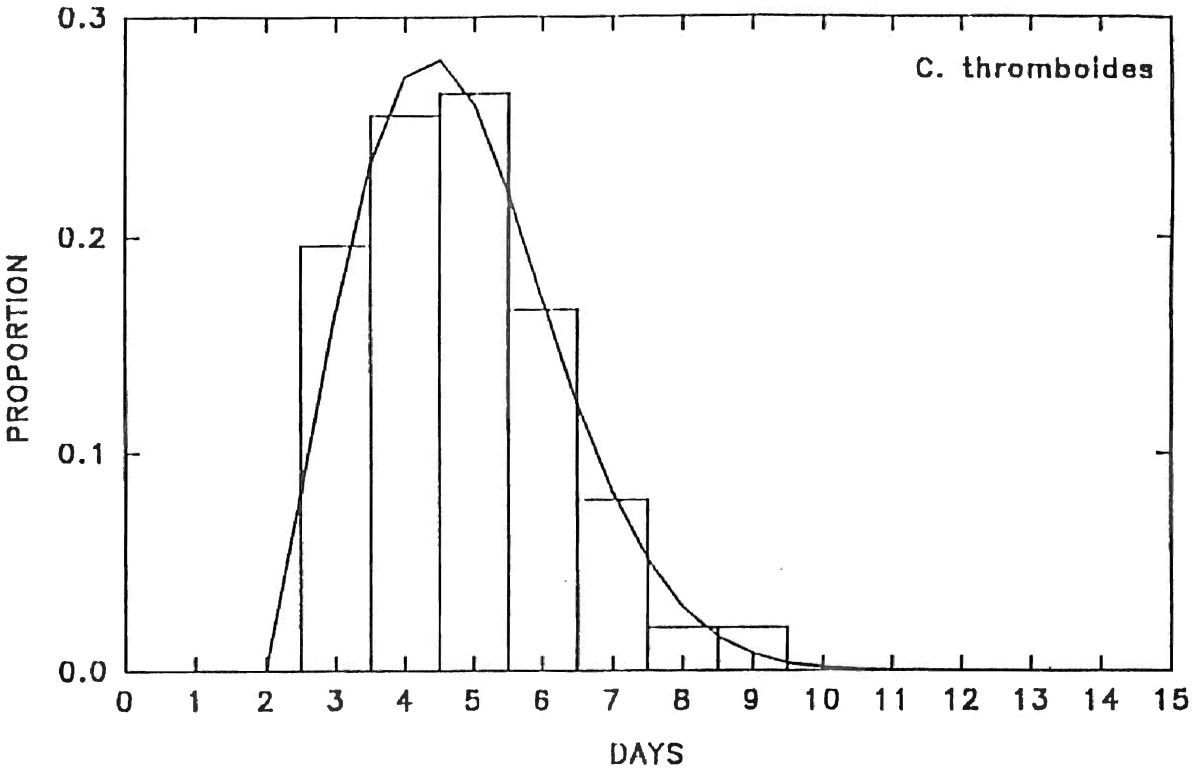

Figure 2. Proportion of Aphids Born Per Day When Treated with the Fungal Pathogen Conidiobolus thromboides.

TABLE 1.

Mean Births At Five Different Percentiles

TREATMENT	p25	p50	p75	p90	p95
C. thromboides	3.86 ± 0.365	4.66 ± 0.436	5.52 ± 0.603	6.34 ± 0.845	6.83 ± 1.02
CONTROL	4.81 ± 0.592	6.09 ± 0.612	7.46 ± 0.604	8.75 ± 0.653	9.53 ± 0.74

CHAPTER III

GERMINATION TEST OF THE FUNGAL PATHOGEN *CONIDIOBOLUS THROMBOIDES* AND OPTIMUM SPORE DOSE OF THE PATHOGEN FOR THE RUSSIAN WHEAT APHID

INTRODUCTION

To determine the effectiveness a fungal pathogen, the percentage of germination of the spores from the culture, the optimal spore dose, and spore storage life must be determined. Conidiobolus thromboides conidia that had been desiccated were found to remain 90% viable or 90% of the spores germinated after cold storage at 4°C for 12yr (Fuxa and Tanada, 1987). This was the premise for the study with cold storage of the fungal pathogen except that a glycerol suspension was used.

Fungal pathogens are mainly dispersed by air (Hall and Dunn, 1958), and the optimal number of spores to cause an epizootic needs to be known. Claydon and Grove (1977), Claydon, (1978) and Latge et al. (1978) found that C. thromboides can kill the host in one day after penetration of the hemoceol by the production of mycotoxin. Therefore, theoretically, one spore which lands on the host and germinates should kill the host as the pathogen reproduces.

The purpose of these two studies was to determine the optimal age for maximum germination of spores and the optimal dose of conidia of C. thromboides for maximum death rate for the Russian wheat aphid, Diuraphis noxia.

MATERIALS AND METHODS

The fungal pathogens were cultured using Sabouraund's agar (40% dextrose, 10% yeast extract, 10% peptone, and 25gm chloramphenicol per liter of distilled water. The pH was adjusted to 6.5, and the media was autoclaved at 250°C for twenty minutes to sterilize the agar according to Poprawski (1992). The cultures were placed on a liquid shaker table at 150rpm at room temperature to allow for maximum growth and sporulation.

Two methods were employed to test for germination. In the first, the fungal pathogens were diluted to 1 X 10^{-6} and 0.1ml was placed in a microscopic well. The slide was placed in a 130mm petri dish with a water soaked filter paper to maintain the humidity. The petri dish was sealed with parafilm and placed in an environmental chamber at 24°C, L:D 16:8 for 24h. The slide was then viewed under a phase contrast microscope equipped with an optical micrometer. Five different fields of the well were counted. The total number of spores counted and total number of spores germinated were recorded. In the second method, a suspension of the spores was sprayed directly onto a water agar plate treated with chloramphenicol using a Sigma aerosol sprayer and a spore tower. A 12mm X 12mm slide was placed on the plate so the spore count per mm^2 could be ascertained. The plate was then sealed with parafilm and placed in L:D 16:8 and 24°C. After

36h, five different fields were counted using the phase contrast microscope, and the total number of spores and total number of germinated spores was recorded. The percentage germination was calculated by dividing the total number germinated by the total number of spores in the field.

Three germination tests were completed. One test involved the germination of a two week old culture, a four week old culture, a six week old culture, and a two week old culture suspended in a 2 parts glycerol and 1 part distilled water and placed in a freezer at 0°C for eight weeks. After eight weeks, this cultures was thawed and centrifuged and the pelleted spores removed. The spores were washed three times with the media by adding 1ml of the liquid media and centrifuging for 1 minute and removing the supernatant. The spores were then placed in 150ml of the media and placed on the shaker table at 150 rpm for 2 weeks. The germination test was conducted using a serial dilution and the microscopic well slides.

To find the optimal spore count for infection, a two week old culture was sprayed directly onto 10 RWA's using varying spore counts with three replications of each dose, and the aphids were incubated for 24h at 25°C and L:D 16:8. The aphids were then transferred to TAM W-101 plants in cone-tainers with a bottom water system. The plants with the aphids were then placed in a growth chamber at 24°C, L:D 16:8, and RH 90% for ten days. The aphids were counted and the dead

aphids were placed on water agar plates treated with chloramphenicol for incubation to determine the number of RWA infected.

RESULTS

To determine the optimal age for harvesting the spores to optimize germination, the non-frozen cultures were tested at two, four, and six weeks. The two week old frozen culture was also evaluated. The frozen culture did not produce enough conidia to test for germination. The two week old spores had a 96.6% germination rate which is the highest of all the time points tested (Table 1). The percentage germination of the spores decreased from the two week cultures, and the four week cultures still had a 90.2% germination rate. The six week old culture had a significantly lower percent germination of 76.6% ($\alpha < 0.05$). There were significant differences between all the fresh cultures.

The spore course test was used to determine the optimal spore dose for the tritrophic tests. A dosage less than 3 spores/mm^2 or greater than 18 spores/mm^2 was not effective in causing a significant amount of mortality in the aphid populations on the plant (Figure 1). The optimal doses appeared to be between 12 - 15 spores/mm^2.

CONCLUSIONS

The fresher the fungal culture, the higher the percentage of germination. If older cultures were used for application, and the germination age is not optimal, the effect of the pathogen will be minimal at best.

The spore course test showed the optimal doses of the fungal pathogen C. thromboides against the Russian wheat aphid to be between 12-14 spores/mm^2. Doses lower than this do not contain enough spores to cause a high percentage of infection. The assumption would be that the more spores applied, the higher the incidence of infection, but this was not found to be the case with this pathogen. After the optimum spores/mm^2 applied to the aphids was reached, number of deaths attributed to the mycosis greatly declined. These results are similar to Poprawski et. al. (1990). They found that in testing the fungal pathogen, Zoophthora radicans with the Russian wheat aphid, that with a spore dose over 16 spore/mm^2, the percentage of deaths due to the fungal pathogen decreased. Brobyn and Wilding (1977) found the optimal dose to be 50 spore/mm^2 for several different pathogens in the order Entomophthorales. However, their method of treating the aphids may be different than the method used in these experiments. Coating the aphids with dry spores (Gustoffson, 1971), direct spraying of the liquid culture (Goettel, 1990), or treating the aphids with other dead aphids (Poprawski et.

al., 1990) are different methods that have been used to apply pathogens to aphids. The method of inoculation may have an effect on the death rate. There is also the possibility that small, immature spores which are unable to germinate, appear as mature spores when counting. Dead spores or spores which are unable to germinate, but are not distinguishable from viable spores may also be included. A test using the three methods of inoculation would possible eliminate the errors in this test. A method of separating the immature spores from the mature spores is needed, but, at this time, no method is known.

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TABLE 1.

PERCENTAGE GERMINATION OF CONIDIA OF *CONIDIOBOLUS THROMBOIDES* OF DIFFERENT AGES

AGE (WEEKS)	REPLICATION	TOTAL SPORES	TOTAL GERMINATION	PERCENTAGE GERMINATION	MEAN PERCENTAGE
2	1	100	95	95.0%	
	2	100	98	98.0%	
	3	100	96	96.0%	
	4	100	96	96.0%	
	5	100	97	97.0%	96.6% ± 1.11
	6	100	98	98.0%	
	7	100	96	98.0%	
	8	100	97	97.0%	
	9	100	96	96.0%	
	10	100	95	95.0%	
4	1	100	89	89.0%	
	2	100	88	88.0%	
	3	100	91	91.0%	
	4	100	92	92.0%	
	5	100	92	92.0%	90.2% ± 1.24
	6	100	90	90.0%	
	7	100	91	91.0%	
	8	100	90	90.0%	
	9	100	89	89.0%	
	10	100	90	90.0%	
6	1	100	63	63.0%	
	2	100	72	72.0%	
	3	100	89	89.0%	
	4	100	53	53.0%	
	5	100	84	84.0%	76.2% ± 10.18
	6	100	79	79.0%	
	7	100	82	82.0%	
	8	100	80	80.0%	
	9	100	81	81.0%	
	10	100	79	79.0%	

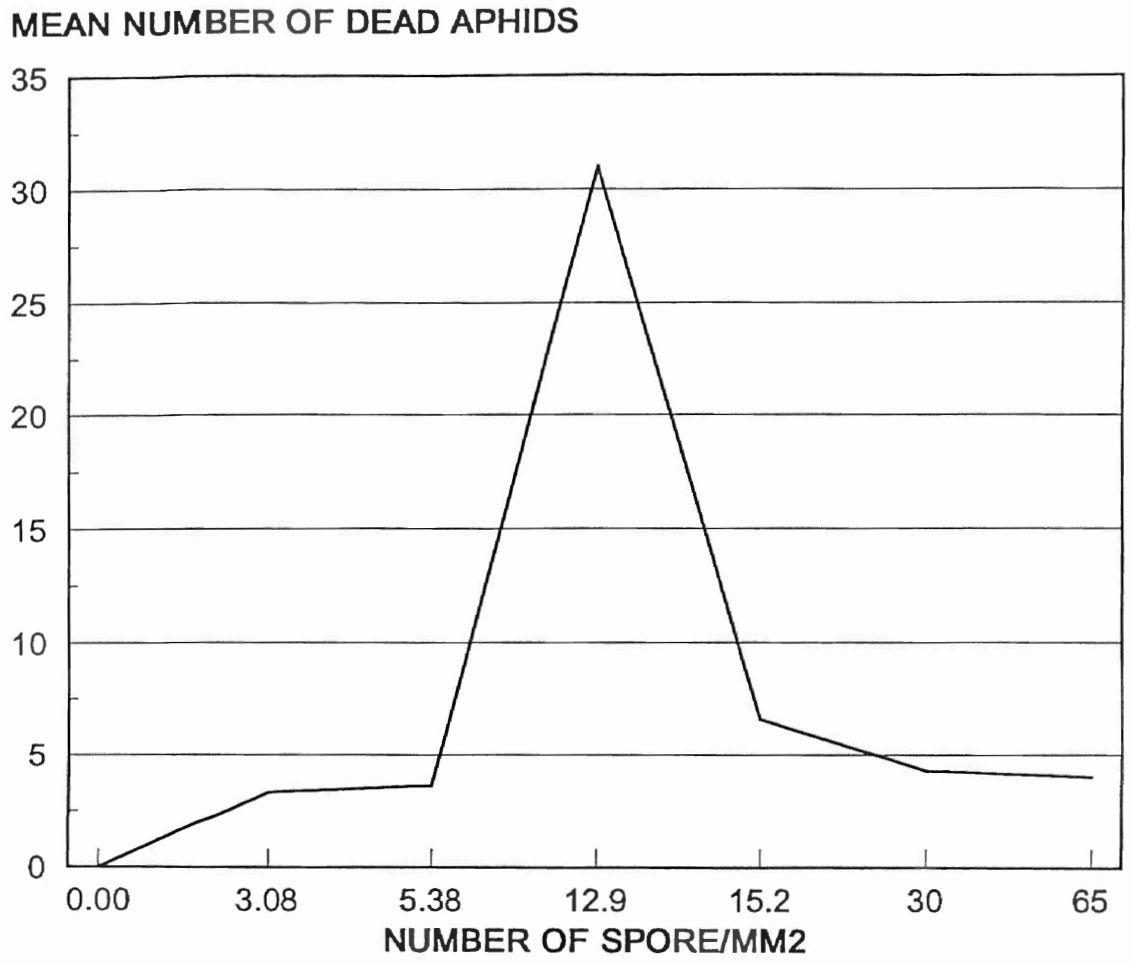

Figure 1. Spore course for the optimum spore dose for Conidiobolus thromboides against the Russian wheat aphids on TAM W-101

VITA

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Master of Science

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