Competitive Saprophytic Ability of *Laetisaria arvalis* Compared With *Sclerotium rolfsi*

Vijaya Bobba and Kenneth E. Conway
Department of Entomology & Plant Pathology, Oklahoma State University, Stillwater, OK 74078-3032

The interaction of the biocontrol fungus *Laetisaria arvalis* with the pathogen *Sclerotium rolfsii* as competitors for either autoclaved beet or okra seeds in a white - quartz sand matrix was examined. Densities of each fungus resulting in 100% colonization of 10 seeds were determined separately. In competitive colonization experiments, *L. arvalis* colonized 80 to 83% of the seeds compared with 17 to 20% for *S. rolfsii*, suggesting competition for organic matter as a possible mechanism for the interaction between these two fungi.

INTRODUCTION

Cook and Baker (1983) cite the work by Hartley (1921) as one of the first attempts at direct application of biological control agents (BCA) to control plant pathogens. Yet, 67 years later, Jutsum (1988) noted that only 5 % of deliberate releases of BCA had actually achieved control of the organisms that they were released to suppress. Most of these failures have been attributed to inappropriate formulations and a lack of understanding of the mode of action and ecology of the biological control organisms (Baker, 1986; Deacon, 1991). Formulation science now considers which propagules are most efficacious for control and whether amendments are needed to stimulate the BCA. Antagonism exterior to the host is classically divided into three types: antibiosis, parasitism, and competition (Baker, 1986).

Laetisaria arvalis Burds., a soilborne basidiomycete, was first isolated and identified as a *Corticium* sp by Odvody et al (1980). The fungus was later identified as *L. arvalis* by Burdsall et al (1980). Odvody et al (1980) observed hyphal interactions between *L. arvalis* and *Rhizoctonia solani* in culture and suggested that the mode of action of *L. arvalis* was hyperparasitism. We have shown that *L. arvalis* reduces the incidence of southern blight of apple seedlings caused by *Sclerotium rolfsii* Sacc. in the field (Conway 1995; Conway et al 1996). In addition, *L. arvalis* has been used to reduce seedling damping - off caused by *Pythium ultimum* in sugar beets (Burdsall et al 1980) and *Phoma betae* in table beets (Burdsall et al 1980). Bowers et al (1986) isolated laetisaric acid from *L. arvalis* and suggested that antibiosis was the mode of action against *P. ultimum*. However, we could not confirm either antibiosis or hyperparasitism as a mode of action using standard agar-coated microscope slides as described by Fisher (1984) and cultural techniques (Bell et al 1982). Therefore, we initiated this research to evaluate competition for organic matter as a possible mechanism of the biocontrol activity of *L. arvalis*.

MATERIALS and METHODS

Optimum temperature for mycelial growth of *L. arvalis*. In order to establish the parameters of the competitive interaction, the optimum temperature for mycelial growth of *L. arvalis* had to be determined. Mycelial plugs (0.5 cm diam.) were removed from the margins of cultures of *L. arvalis* actively growing on potato dextrose agar (PDA) amended with 300 µg/L streptomycin sulfate and were placed in the center of Petri dishes containing PDA. Ten cultures were incubated in growth chambers (12h/12h light/dark, constant temperature for both light/dark cycles) at each of the following temperatures: 15, 20, 25, 30, and

35°C. Colony diameters were measured every 24 h until growth covered the agar surface completely. This experiment was repeated twice with similar results. Data from all temperatures were analyzed with an ANOVA. When appropriate, means were separated with a Student-Newman-Keuls mean separation test ($P \leq 0.05$). Regression analysis was performed to analyze growth over a 72 h period.

Formulation of *L. arvalis*. *Laetisaria arvalis strain* strain OK-206, obtained from the Forest Products Laboratory, Madison, WI 62715 (originally isolated by L. Herr as ZH-1, from soil in Ohio), was used. Inoculum was prepared by the method described by Conway et al (1997). The formulation contained vermiculite (150 g), wheat bran or corn meal (141 g), sterile water (500 ml) and 1 wk-old inoculum from six petri dishes of *L. arvalis* was grown on PDA and the mixture was incubated for two weeks at room temperature. This mixture was dried overnight and ground to a fine powder in a Glen Mills grinder (Glen Mills Co., Maywood, NJ). Uniform particle size was achieved by using nested sieves (500 μm-, 250 μm-apertures). Par-ticles that passed through the 500 μm sieve were collected on the 250-μm-aperture sieve and used in the experiments. The for-mulation contained both sclerotia (400-500 μm diam.) and hyphal fragments. and was stored in sterile glass vials in the refrigerator until used (approximately one month).

Determination of propagule density in the formulation of *L. arvalis*. To estimate the propagule density in the formulation, dilutions (0.5%, 0.25%, 0.15%, 0.1%, 0.07%, and 0.05%) were made with sand (15% moisture), and a multiple-pellet sampler (Henis et al 1978) was used to estimate colony forming units per gram (cfu/g) (Fig. 1). The sampler was calibrated to deliver 15 pellets, each weighing 0.1g, onto an *L. arvalis* semiselective medium (Papavizas et al 1983). The cfu/g of formulation was determined using a multiple infection transformation equation:

$$Ln[\frac{1}{1-y}] \cdot 15 \cdot \textit{correction factor}$$

where y = proportion of pellets colonized and the correction factor is the moisture content and dilution factor (Henis et al 1978).

Formulation of *S. rolfsii*. *Sclerotium rolfsii* strain OK-410, recently isolated from wheat plots in Stillwater, OK, and known to produced abundant sclerotia, was used in this study. Sclerotia were produced on autoclaved corn seeds (30 g seeds/ 250 ml-flask, 16% water w/v). Containers were inoculated with several agar plugs removed from cultures actively growing on PDA and incubated 25 - 30 d until sclerotia formed. Sclerotia were dried on metal trays for 4 d in a laminar flow hood and separated from the corn by sieving.

Preparation of substrate for competitive colonization and inoculum load experiments. Our technique to determine saprophytic ability was modified from that used by Papavizas et al (1975) for *Rhizoctonia solani* in soil. A 2.5 cm layer of white quartz sand (approximately 910 g) was placed in an aluminum pan (23 x 13 x 7 cm). Initially, the organic substrate consisted of either autoclaved, untreated beetseed balls (beets produce two to five flowers in dense clusters and "seeds" consist of two to five seeds held

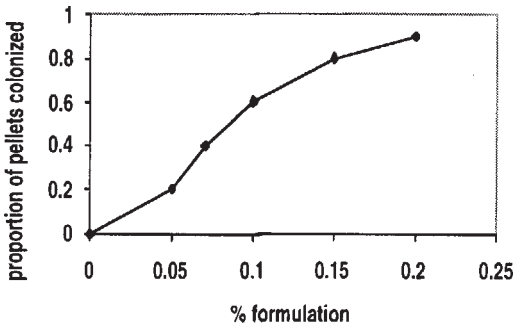

Figure 1. Relationship between % concentration of *Laetisaria arvalis* and the proportion of soil pellets colonized using a multiple pellet soil sampler. Data points can be converted to colony forming units per gram (cfu/g) by using the multiple transformation equation $Ln[\frac{1}{1-y}] \cdot 15 \cdot \textit{correction factor}$ where y= proportion of pellets colonized and the correction factor is the moisture content and dilution.

together in a seedball) cultivar (cv) "Detroit Red", or untreated, autoclaved okra seed, cv "Clemson spineless." All ingredients and inoculum were thoroughly mixed in re-sealable polyethylene bags and then added to the aluminum containers All inoculum load experiments were repeated twice for each seed type to ensure that both were suitable substrates.

Inoculum load determination for competitive experiments. To determine the amounts of formulation of *L. arvalis* or sclerotia of *S. rolfsii* to use for competitive colonization, a series of containers were prepared, each containing sand (910 g) (15% moisture), ten autoclaved beet or okra seeds, and 100 to 125 sclerotia (100, 105, 110, 115, 120, and 125) of *S. rolfsii.* The mixture in each container was thoroughly mixed in resealable polyethylene bags and incubated in containers for 2 wk at 25°C. Seeds were removed from the sand mixtures using a sieve and placed onto PDA; the number of *S. rolfsii* colonies was recorded after 48 h at 25°C. The competition experiment was repeated three times, and the density of sclerotia required for 100% colonization was verified (repeated) six more times.

Similar experiments were conducted to determine the inoculum load of *L. arvalis* formulation needed for 100% seed colonization. Sand and 10 autoclaved seeds were mixed with concentrations of formulation ranging from 0.2 g to 0.7 g/g^{-} sand at 0.1 g intervals. Mixtures were incubated at 25°C for 1 wk, then seeds were removed and placed on PDA to determine the extent of colonization. This experiment was repeated twice with similar results. The concentration resulting in 100% colonization was verified six more times.

Competitive colonization experiment. Ten autoclaved beet or okra seeds were thoroughly mixed with 910 g white quartz sand (15% moisture) in resealable polyethylene bags and 125 sclerotia of *S. rolfsii* and 0.3 g formulation of *L. arvalis* were added, mixed, and incubated in aluminum pans at 25°C for 2 wk. This experiment was replicated six times. Seeds were removed as before, placed on PDA, and the number of colonies of each fungus recovered were counted. The entire experiment was repeated twice to verify the results. Data were analyzed by ANOVA (SAS Institute, Box 8000, Cary, NC 27511), and means were separated when appropriate with a Duncan multiple range test. In addition, a t - test and a test for normality for the two means was conducted using a Sigma Stat program (Jandel Scientific, San Rafael, CA 94912-8920).

RESULTS

Optimum temperature for mycelial growth of *L. arvalis*. Aycock (1966) had reported that *S. rolfsii* had a growth optimum between 25° and 30°C. However, since the growth optimum for *L. arvalis* had not been established in the literature, we needed to determine the average growth of mycelium (colony diameters) at temperatures ranging from 15°C to 35°C. Maximum diameters were produced at 25°C and 30°C. There was no difference ($P \leq 0.05$) between growth at 25° and 30°C, but the difference between growth at 25/30°C and that at other temperatures was significant ($P \leq 0.05$). Regression analysis fit a linear model; Y= - 0.0858X + 3.8085, the coefficient of determination (R^2)$\cong$0.97 and $P \leq 0.0004$.

Determination of propagule density of *L. arvalis* formulation. There was a positive correlation between formulation concentration and the proportion of colonized soil pellets. Decreasing the concentration of formulation in the mix resulted in a decrease in the proportion of soil pellets colonized by *L. arvalis*. The average concentration of the formulation calculated from the multiple infection transformation equation was 2980 cfu/g. (Fig. 1).

Inoculum load determination for competitive experiments. One hundred percent colonization of seeds resulted when 0.3 g or more of *L. arvalis* formulation was added to the heat sterilized (overnight at 120°C) white quartz sand containing 10 seeds. This colonization percentage was consistent in each of the six repeated tests. One hundred percent colonization of seeds with *S. rolfsii* was achieved when 125 sclerotia were

added to the system and this was consistent for each of the six repeated tests.

Competitive colonization experiments. There was a difference ($P \leq 0.05$) between the number of seeds colonized by *L. arvalis* compared with that colonized by S. rolfsii (t test, $P \leq 0.0001$) when 125 sclerotia of *S. rolfsii* and 0.3 g of *L. arvalis* formulation were added to the sand containing 10 autoclaved seeds and incubated at 25°C for a 2 week period. Both fungi grew equally well on PDA but could easily be separated by the color of their mycelia. Recovery of *L. arvalis* from the seeds ranged from 81 to 83% and for *S. rolfsii* only 17 to 20% (SD=1.7). Since colonization of seed could only be confirmed by plating seed onto agar and observing growth of each fungus, seeds colonized by both fungi were recorded as colonized by the first and largest colony to grow from each seed. The t-test and the normality test showed that the differences in the mean values of the two groups (seed colonized by *L. arvalis* and seed colonized by *S. rolfsii*) was greater than would be expected by chance; there was a significant difference between the two groups ($P \leq 0.0001$).

DISCUSSION

Garrett (1970) realized the importance of competitive colonization of organic matter and the ability of a fungus to survive in the soil and defined competitive saprophytic ability as an intrinsic characteristic of a fungal species that results in successful competitive colonization of dead organic substrates. However, he later found that this definition was too restrictive and modified it to include substrates of all kinds (i.e. simple sugars, lignocellulose, etc.). He further defined "inoculum potential" as the energy of a fungus available for colonization of a substrate at the surface of the substrate to be colonized. This phrase also includes the endogenous and exogenous growth vigor of propagules. Therefore, the size of a propagule and both the endogenous energy and its exogenous supplies (as substrate amendments) must be considered in competitive colonization of the substrate. We chose to modify the technique of Papavizas, et al (1975) who used a soil matrix in their experimentation, while we used heat sterilized white-quartz sand which lacked any additional organic matter and provided a light background in which it was convenient to locate seeds and even sclerotia.

Deacon (1991) maintained that successful BCAs must be ecologically adapted for the task they are expected to perform and that even a BCA of limited spread could give a worthwhile benefit by creating a *cordon sanitaire* around the base of a plant. In our previous research with southern blight of apple seedlings (Conway, 1995; Conway et al,1996), sclerotia of *L. arvalis* suspended in a gel and applied to the crown and root area of seedlings, protected the trees from crown infection by *S. rolfsii*. This method of protection would support Deacon's idea of a *cordon sanitaire*. It is possible that *L. arvalis* may employ more than one mechanism in its interaction with soilborne pathogenic fungi. In the work presented here, we have shown that competition for organic matter is one possible mechanism utilized by this fungus to exclude pathogens from sites of infection along roots or at the crown of plants.

We chose to conduct the interaction experiments at 25°C, which was optimal for growth of both fungi. Although *S. rolfsii* might have been favored at higher temperatures, we felt a lower temperature would more closely reflect actual temperatures in the field when *L. arvalis* would be deployed and begin to colonize organic matter.

The association of *L. arvalis* with organic amendments has been recognized and exploited in previous research (Martin et al 1984; Lewis and Papavizas 1992). Successful biocontrol in the field has been achieved against several pathogens through the addition of *L. arvalis* on infested seeds/bran 6, 10) colonized sugar beet pulp (Odvody et al 1980) or gel suspensions (Conway et al 1996; Martin et al 1984). Interestingly, Herr (1988) reported limited success in controlling *R. solani* by *L. arvalis* ZH-7 only when it was added as comminuted mycelium. The formulation of *L. arvalis* used in our studies allowed for the

production of sclerotia on the wheat bran. In our previous research, when dried sclerotia was added to the gel matrix, rehydrated sclerotia allowed for quicker germination of sclerotia than non-hydrated sclerotia.

In our competitive colonization experiments, we attempted to account for the difference in sclerotia size [inoculum potential, sensu Garrett (1970] by determining the inoculum densities of each fungus that were needed to colonize 100% of the autoclaved seeds. Sclerotial size would affect how close to the seed a fungal propagule had to lie (spatial arrangement) in order to colonize it. Larger sclerotia would have more internal energy and could infect seeds from a greater distance. Tomasino and Conway (1987) determined that sclerotia of *S. rolfsii* can infect the crowns of apple seedlings from a distance of 3 to 4 cm.

Information concerning the competitive saprophytic ability of *L. arvalis* should lead to better biocontrol formulations and better ecological strategies for deployment of this fungus in the field.

ACKNOWLEDGMENTS

This work was approved for publication by the director of the Oklahoma Agricultural Experiment Station, and supported in part under projects OKLO 2187 and OKLO 2117 provided to the second author. The authors thank Dr. Stephen F. Tomasino who conducted the initial research efforts during his studies at Oklahoma State University We also thank Stacey Blazier and Dr. H. A. Melouk for assistance with regression equations and Dr. Richard Grantham and Les Magee for assistance with graphs. We appreciate the helpful editorial comments of Cindy Conway, Dr. Jacque Fletcher and Dr. Al Filonow. This research represents portions of a special report by the senior author in partial fulfillment for a Masters' degree.

REFERENCES

Aycock R. 1966. Stemrot and other diseases caused by *Sclerotium rolfsii*. North Carolina State Univ Tech Bull #174:202.

Baker R. 1986 Biological control: an overview. Can J Plant Path 8:218-221.

Bell DK, Wells HD, Markham CR. 1982. In vitro antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology 72:379-382.

Bowers WS, Hoch H, Evans PH, Katayama M. 1986. Thallophytic allelopathy: isolation and identification of laetisaric acid. Science 1232:105-106.

Burdsall HH, Jr., Hoch H, Boosalis MG, Setliff EC. 1980. *Laetisaria arvalis* (Aphyllophorales, Corticiaceae): A possible Biological control agent for *Rhizoctonia solani* and *Pythium* species. Mycologia 72:728-736

Conway KE. 1995 Comparison of biological and chemical treatments for control of southern blight of apple Seedlings in the Field. Biol and Cult Tests 10:49

Conway KE, Maness NE, Motes JE. 1997. Integration of biological and chemical controls for *Rhizoctonia* blight and root rot of rosemary. Plant Disease 81:705-798.

Conway KE, Tomasino S, Claypool PL. 1996. Evaluation of biological and chemical controls for southern blight of apple rootstock in Oklahoma nurseries. Proc Okla Acad Sci 76:9-15.

Cook RJ Baker K. 1983. The Nature and Practice of Biological Control of Plant Pathogens. The American Phytopathological Society, St. Paul, MN. 539 p.

Deacon JW. 1991. Significance of ecology in the development of biocontrol agents gainst soil-borne plant pathogens. Biocontrol Sci and Tech 1:5-20

Fisher C J. 1984. Fluid drilling: A potential delivery system for *Trichoderma* spp. as biocontrol agents.[M. S. thesis] Stillwater (OK): Oklahoma State University. 50 p. Available from: OSU Library.

Garrett SD. 1970. Pathogenic root-infecting Fungi. London and NY: Cambridge University Press. 294 p.

Hartley C. 1921. Damping-off in forest nurseries. US Dept Agric Bull 934:1-99.

Henis Y, Ghaffar A, Baker R, Gillespie SL. 1978. A new pellet soil - sampler and its use for the study of population

dynamics of *Rhizoctonia solani* in soil. Phytopathology 68:371-376.

Herr LJ. 1988. Biocontrol of *Rhizoctonia* crown and root rot of sugar beet by binucleate *Rhizoctonia* spp. and *Laetisaria arvalis*. Ann Appl Biol 113 (10):7-118.

Jutsum AR. 1988. Commercial application of biological control: status and prospects. Phil Trans Roy Soc London B 318:357-373.

Lewis JA, Papavizas GC. 1992. Potential of *Laetisaria arvalis* for biocontrol of *Rhizoctonia solani*. Soil Biol Bochem 24:1075-1079.

Martin SB, Abawi GS, Hoch HH. 1984. Influence of antagonist *Laetisaria arvalis* on infection of table beet by *Phoma betae*. Phytopathology 74:1092-1096.

Odvody GH, Boosalis MG, Kerr ED. 1980. Biological control of *Rhizoctonia solani* with a soil-inhabiting Basidiomycete. Phytopathology 70:655-658.

Papavizas GC, Adams PB, Lumsden, RD, Lewis, JA, Dow, RL, Ayers, WA, Kantzes, JG. 1975. Ecology and Epidemiology of *Rhizoctonia solani* in field soil. Phytopathology 65:871-877.

Papavizas GC, Morris BB, Marois JJ.1983. Selective isolation and enumeration of *Laetisaria arvalis* from soil. Phytopathology 73:220-223.

Tomasino, SF, Conway, KE. 1987. Spatial pattern, inoculum density - disease incidence relationship, and population dynamics of *Sclerotium rolfsii* on apple rootstock. Plant Disease 71:719-724.

Received: Nov 26, 2002; Accepted May 21, 2003