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# Population Dynamics of Sugar Beets, *Rhizoctonia solani*, and *Laetisaria arvalis*: Responses of a Host, Plant Pathogen, and Hyperparasite to Perturbation in the Field†

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***Rhizoctonia solani*** **causes crown rot of sugar beets, a severe disease that has destroyed up to 60% of the plants in a test field in western Nebraska. *Laetisaria arvalis*, a natural hyperparasite of *Rhizoctonia* spp., was isolated from fields in western Nebraska. To test for the potential for biological control of *R. solani*, in November 1980 (following harvest) we applied various combinations of a nematicide (Telone II; Dow Chemical Co.), a nutrition source (sugar beet pulp), and an inoculum of *L. arvalis* in a randomized block design. Populations of *R. solani*, *L. arvalis*, and sugar beets were monitored monthly through October 1981 (just after harvest). In control and nematicide plots, the *R. solani* population did not change significantly through time. In plots inoculated with *L. arvalis*, the *R. solani* populations declined through March, concomitant with an increase in *L. arvalis*. *L. arvalis* then declined with a corresponding increase in the *R. solani* populations. Beet plant numbers declined significantly in all treatments. We suggest that reduction of the *R. solani* populations with the hyperparasite *L. arvalis* is possible but that a stable equilibrium naturally exists.**

Biological control of soil-borne plant pathogens by antagonists has gained widespread acceptance as a potential tool in optimizing agricultural productivity. As with many ecology-based ideals, however, few succeessful applications in the field have been reported. This is in part due to the complexity of factors regulating interactions among plants, pathogens, and antagonists (8).

Sugar beets (*Beta vulgaris* L.) are cultivated in irrigated fields in the western United States. Crop rotation is often practiced, in part to reduce devastating losses to crown rot, a disease caused by *Rhizoctonia solani* Kuhn (11). We have previously observed that crown rot causes up to 60% plant mortality and serious yield decline in some fields (Allen, Boosalis, and Kerr, unpublished data). Thus, practices that promote control of this disease could enhance yields substantially.

We initiated a series of experiments to test for long-term biological control of *R. solani*. Our approach was to apply artificially an inoculum of a natural antagonist to an infested field to determine if a new pathogen-antagonist population equilibrium at a lowered pathogen density would result. As an early step toward this goal, we inoculated a sugar beet field with the hyperparasite *Laetisaria arvalis* Burdsall (previously reported as *Corticium* sp. [9]) present initially in low densities. This fungus was originally isolated from western Nebraska sugar beet fields (4) and caused reduction of *R. solani* populations in greenhouse experiments (9). We then observed population levels of the two fungi and potential alterations in disease.

## MATERIALS AND METHODS

The study site was a flood-irrigated sugar beet field that had been in corn-beet rotation for a number of years. The field was located 5 km north of Morrill, Nebr. The soil was an Alliance fine sandy-loam (fine-silty, mixed, mesic Aridic Argiustoll) with a 0 to 3% slope. Field observations were made between June 1980 and October 1981. Total precipitation at the Mitchell Station (10 km east of the study site) during the 17-month study period was 464 mm, peaking at 113 mm in July 1981. Air temperatures ranged from a mean daily maximum of 32°C for July 1980 to a mean daily minimum of −10°C for February 1981. Production procedures included an April planting, a June thinning to four plants per 1-m row, and an October harvesting.

Plots were treated by adding various combinations of the hyperparasite *L. arvalis*, an artificial nutrient source (sugar beet pulp [10]), and a nematicide (Telone II; Dow Chemical Co.). The nematicide is commonly used in western Nebraska, and some nematicides act as potent fungicides (12). *L. arvalis* inoculum was increased in a still culture of potato dextrose broth as previously described (9). After air drying, the inoculum (consisting of hyphae and sclerotia) was ground in a Wiley mill (20-mesh screen). Telone II (92% active ingredient) was supplied and applied by Jirdon Agrichemicals, Inc., Morrill, Nebr.

Field plots were established in a randomized complete block design with four blocks of six treatment plots (3 by 3 m [9 $m^2$]). Within each block, two rows of plots were separated by a 3-m-wide border, with 1-m-wide borders separating plots. In addition, each block was separated by a 3-m-wide border. The 6 soil treatments were untreated control, sugar beet pulp, sugar beet pulp plus *L. arvalis*, nematicide, nematicide plus sugar beet pulp, and nematicide plus sugar beet pulp plus *L. arvalis*. Nematicide applications were made immediately after beet harvest in 1980 and were applied at a standard rate of 120 ml per plot. Other treat-

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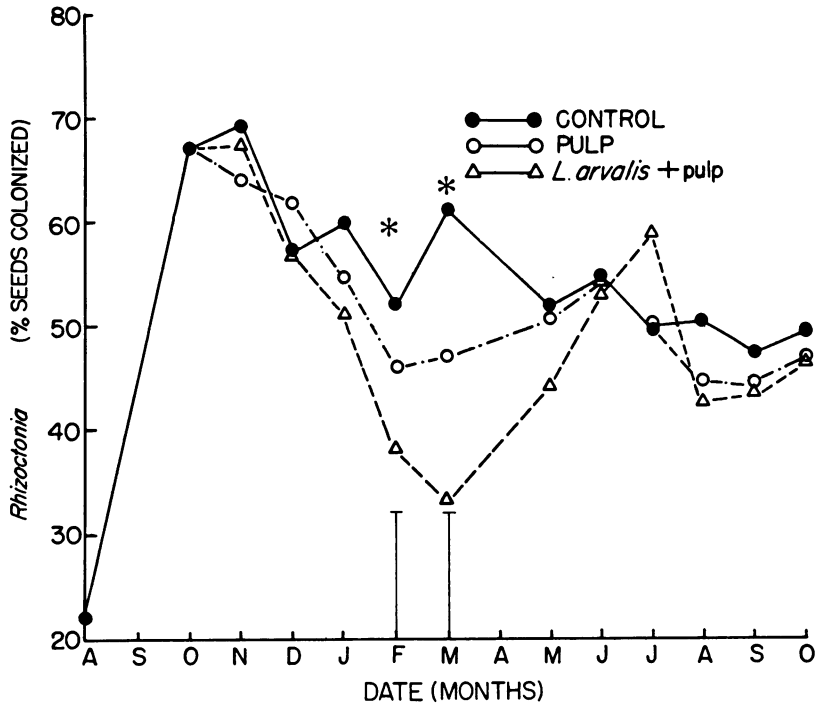


FIG. 1. Influence of biological control practices on the population of *R. solani*. Values are means for each month measured. *, Dates at which significant differences ($P \geq 0.95$) among treatments exist with the least significant difference (0.05) (1).

ments were applied 5 days later. Sugar beet pulp was applied by hand at a rate of 1,800 g per plot (2,000 kg/ha), and *L. arvalis* was applied at 200 g per plot (222 kg/ha). All treatments (including the control) were hand raked to approximately 5 cm to incorporate applied materials.

Plots were sampled in June, August, and October 1980 (before treatment establishment) and monthly (except April) thereafter until harvest in October 1981. During April, the plots were tilled to 20 cm and planted. A total of five soil samples (approximately 200 g each) were taken at random to a depth of 5 cm for each plot and were bulked in the field to provide a soil sample of approximately 1 kg for each plot at each sample date. Soil samples were taken in the rows adjacent to the plants when sugar beets were present in the field. These soil samples were used to estimate the relative populations of *R. solani* and *L. arvalis* by use of the modified sugar beet seed colonization method (9). Relative populations were recorded as percentage of sterile sugar beet seeds colonized. In 1980, these counts were compared with *R. solani* counts estimated by dilution plating (as per Christensen [5]). Plant density was counted within the plots both as total numbers (before thinning to 60 plants per plot in June) and as percentage of thinned plants surviving.

Standard statistical tests were run (14). Percentages were transformed by arc-sine transformations to normalize data. Variance-to-mean ($s^2/\bar{x}$) ratios were used to determine dispersion patterns; an $s^2/\bar{x}$ of $<1$ indicates a regular dispersion, 1 indicates a random dispersion, and $>1$ indicates a clumped dispersion pattern.

## RESULTS

Fungal populations estimated by the beet seed assay and soil dilution plating methods were contrasted with percentage of beets showing crown rot in August 1980 before field plot establishment. Spearman's rank correlation test showed no correlation between populations of *R. solani* as measured by dilution plating versus beet seed assay ($r_s = 0.01$). Also, no correlation was found between the dilution plate count of *R. solani* and plant survival ($r_s = 0.36$). *R. solani* populations estimated by the beet seed assay, however, showed a significant negative correlation ($r_s = -0.60$) with plant survival. Thus, subsequent estimates of populations capable of causing infections were made by using the beet seed assay.

The field-applied treatments influenced populations of the two fungi only at certain times of the year. The sugar beet pulp had no effect on either organism (Fig. 1 to 3), but *R. solani* was significantly influenced in February and March 1981 by application of *L. arvalis* (Fig. 1). Application of the nematicide had no significant influence on *R. solani* (Fig. 2). *L. arvalis* populations were unaffected by the nematicide or pulp treatments but did respond to field inoculation (Fig. 3), especially in February, March, and May.

There were no differences in seedling emergence ($\bar{x} = 146$) or survival to thinning. Thereafter, mean beet survival declined in all treatments to 15 to 40% of the plants remaining (not significantly different among treatments).

*R. solani* and *L. arvalis* populations were negatively correlated: without the nematicide, $r = -0.46$; with the nematicide, $r = -0.35$ (both significant at a confidence level of $\geq 0.99$). Upon application and subsequent increases in *L. arvalis*, *R. solani* declined rapidly. When *R. solani* populations declined below the 40% seed colonization level, however, *L. arvalis* populations also declined, and *R. solani* populations climbed to the pretreatment level equal to control populations. Thus, in control plots and plots with nematicide only, a stable equilibrium was observed (Fig. 4). With applications of *L. arvalis* inoculum, the populations were shifted significantly for a short time but rapidly returned to the previous equilibrium.

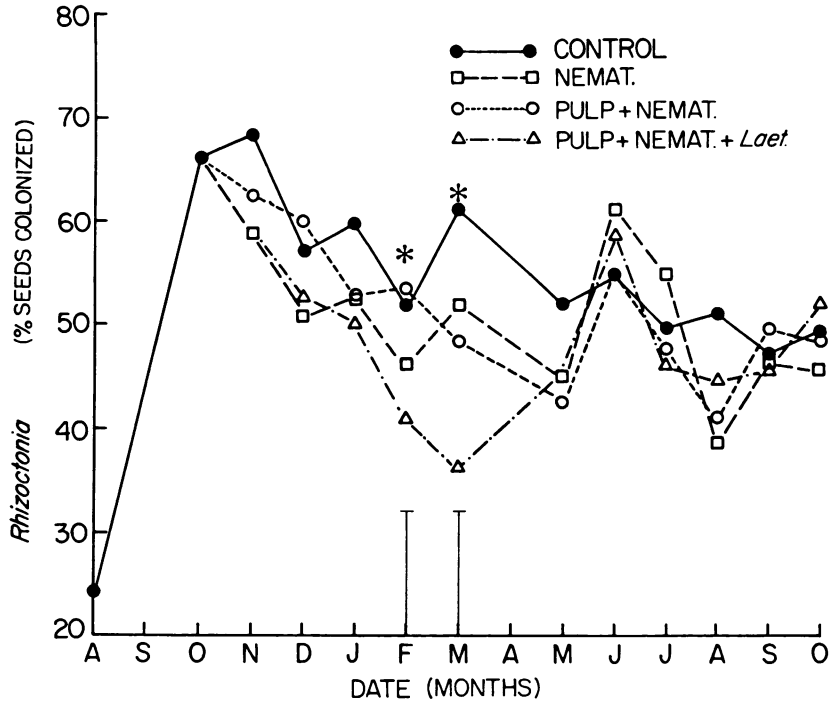


FIG. 2. Influence of chemical and biological control practices on the population of *R. solani*. Values are means for each month measured. *, Dates at which significant differences ($P \geq 0.95$) among treatments exist with the least significant difference (0.05) (I).

To test for the effects of the irrigation water flow or tillage on movement of inoculum across plots, we compared populations of *R. solani* and *L. arvalis* in plots down the row versus across the row with inoculated plots. Using 2-by-2 *t* tests of *R. solani*, *L. arvalis*, and interplot differences, we found no differences between within-row and between-row plots ($P \geq 0.95$). No effects of potential soil dragging with tillage or irrigation could be detected.

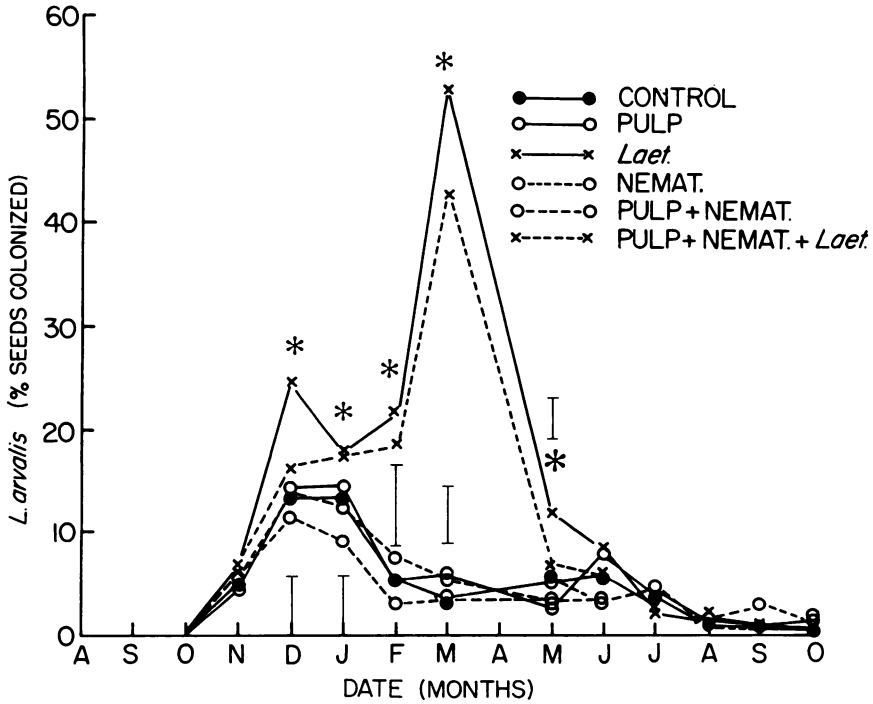


FIG. 3. Effects of treatment on populations of *L. arvalis*. Values are means for each month measured. *, Dates at which significant differences ($P \geq 0.95$) among treatments exist with the least significant difference (0.05) (I).

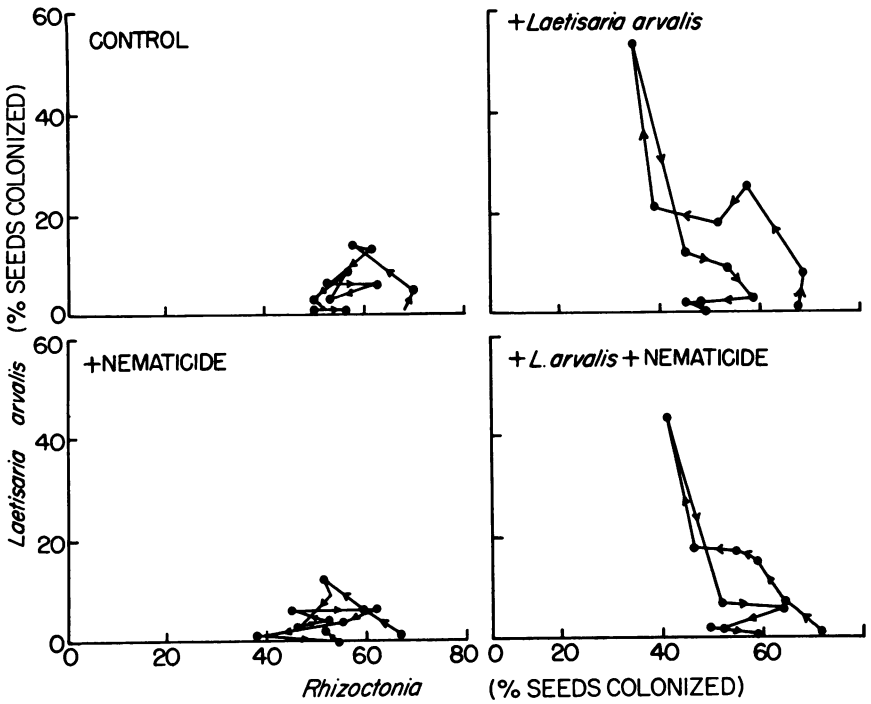


FIG. 4. Population shifts of *R. solani* versus *L. arvalis* under four treatments through time. Arrows represent mean monthly population shifts from October 1980 through October 1981.

Variance-to-mean ratios suggested that the dispersion patterns of the fungi generally ranged from regular to random: *R. solani* ranged from 0.35 to 1.98, and *L. arvalis* (except for February and March) ranged from 0.55 to 1.51 (Table 1). Patterning of *L. arvalis* was in clumps in February and March because of high densities in the inoculated plots ($s^2/\bar{x}$ = 4.19 in February and 14.56 in March). The $s^2/\bar{x}$ ratios of surviving plants after thinning ranged from 6.30 to 9.96, indicating that sugar beet plants were lost to crown rot in clumps.

## DISCUSSION

Understanding the potential use of an antagonist for biological control of a disease depends on the answers to a series of questions regarding the interactions of the host (crop), pathogen, and the antagonist. (i) Are the population interactions density dependent? (ii) Is there a natural equilibrium, and how stable is it? (iii) Can the populations be altered to the needed levels? (iv) What is the dispersion pattern of the three species? (v) What are the environmental factors influencing the organisms and their interactions? If the pathogen-antagonist-host population interactions are density dependent, and if the equilibrium found in control plots can be readily manipulated, mere addition of an antagonist such as a hyperparasite might initiate some degree of biological control. The level to which a parasite population must be reduced for disease control, however, appears to be very low. Beddington et al. (3) proposed that the equilibrium depression ($q$) could be described as a function of the carrying capacity ($K$) and the new equilibrium after addition of the antagonist ($*N$) where $q = *N/K$. Numerous studies have suggested that, in arthropod predator-prey systems, $q$ must be $\leq 0.025$ for biological control to be effective (7).

Dispersion patterns and environmental constraints also can affect the possibility for biological control. If the mass of propagules of the antagonist are not found in conjunction with the desired pest (6) or both have patchy dispersion patterns (7), such reductions in populations necessary to effect control may be impossible. Moreover, tillage, seasonality, nutrition, or other environmental factors may regulate populations more than their potenital interactions can (10).

Our data provide some clues that may answer some of these questions. The population shifts of *R. solani* in response to added inoculum of *L. arvalis* plus the negative correlations between the two species suggest that their interactions are density dependent and, thus, may be amenable to the use of predator-prey models. We still know little, however, about the interactions between *R. solani* and sugar beets from these results. Laboratory studies (9) and the correlatvie data of 1980 suggest that the interaction is density dependent, but more work remains to be done.

The field experiments strongly indicate that there is a stable equilibrium between the two fungi. *R. solani* populations can be depressed, however, by the addition of *L.*

TABLE 1. Variance-to-mean ratios of the principal organisms through time

| Sample Time | $s^2/\bar{x}$ ratio | | |
|---|---|---|---|
| | *R. solani* | *L. arvalis* | *B. vulgaris* |
| Nov. | 1.98 | 0.60 | |
| Dec. | 0.79 | 1.26 | |
| Jan. | 0.42 | 1.28 | |
| Feb. | 1.58 | 4.19 | |
| Mar. | 1.85 | 14.56 | |
| June | 1.00 | 1.22 | 0 |
| July | 1.98 | 0.58 | 6.30 |
| Aug. | 0.73 | 0.78 | 8.35 |
| Sept. | 0.44 | 0.55 | 9.96 |
| Oct. | 0.35 | 0.79 | 8.69 |

*arvalis*. If our population estimates represent infection potential (as indicated by the 1980 correlation data), our population estimates may be compared with actual inoculum density by using a log-log relationship (2). If a slope of 0.27 were used (13), $q$ would be reduced to $\leq 0.060$, which approaches the range possible for biological control. The correlative evidence from 1980 suggested that if the populations of *R. solani* could be maintained at the 30% seed colonization level, only 20% of the beets would have been lost instead of the 60 to 70% loss observed in 1981. Discovery of the characteristics necessary to maintain this depression might then make biological control feasible.

The influence of dispersion patterns and environmental factors on biological control cannot be discerned from this study. Although the incidence of disease in the plants was dispersed in patches, at our scale of sampling the fungi were not. Uneven dispersal of the fungi would affect the ability of *L. arvalis* to find and attack *R. solani* (7). Our scale of sampling may have been too coarse to detect the existence of fungal patches, as fungal dispersal patterns are often oriented around individual plants, not large homogeneous patches (1). The patchy distribution of disease also could be due to a mixture of pathogenic and nonpathogenic genotypes of *R. solani*. The nematicide had only minimal effects on the populations, and recovery was rapid. A more recent study implies that seasonality (spring application) affected timing of peak populations but did not affect beet production (8a). Tillage probably also affected the responses by displacing both *L. arvalis* and *R. solani* from their normal soil surface dispersion. Soil mixing between plots due to the tillage or irrigation may have influenced plant survival. Analyses of adjacent, within-row, and between-row plots, and block comparison, however, should have shown these population mixes. No such patterns were found.

In general, these data suggest that the populations of the pathogen can be manipulated, possibly to a range wherein biological control is feasible. The data also show, however, that the populations of the two are in a stable equilibrium and that maintaining the lowered population of *R. solani* will require a much more comprehensive understanding of the environmental factors (including spatial and temporal responses) affecting the population interactions.

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