A System for the Growth and Delivery of Biological Control Agents to the Soil

P. A. Backman and R. Rodriguez-Kabana

Assistant Professor and Associate Professor, respectively, Department of Botany and Microbiology, Auburn University, Agricultural Experiment Station, Auburn, AL 36830.

This study was supported in part by grants from the Alabama Peanut Producers Association.

Accepted for publication 10 March 1975.

ABSTRACT

A diatomaceous earth granule impregnated with a 10% molasses solution was found suitable for growth and delivery of *Trichoderma harzianum* to peanut fields. *Trichoderma* was grown on the sterile granules for 4 days and applied 70 and 100 days after planting. Significant reductions ($P \leqslant 0.01$) in *Sclerotium rolfsii* damage and increases in yield were recorded over the 3-year test period. Disease control using 140 kg/hectare Trichoderma granules per hectare was equivalent to that achieved using 10% PCNB granules at 112 kg/ha. The biological control granule system has the advantages of low bulk, no residues, and applicability to other pathogen-antagonist systems.

Phytopathology 65:819-821

Additional key words: *Trichoderma, Sclerotium rolfsii,* peanuts.

For many years research workers have been aware of antagonistic fungi which affect plant pathogens (8). To date, however, successful control of these pathogens by antagonists has relied either on differential susceptibility to a selector compound that favors the antagonist (1,2), the addition of huge quantities of organic residues to the soil (3,4), or the addition of organic inoculum containing the antagonist (9). The first system, when successful, utilized costly chemicals and procedures, and is itself subject to the problem of chemical residues. The latter two systems required the addition of large quantities of organic matter to increase the natural antagonists, or to deliver the antagonist and supply it with the needed food base. Wells et al. (9) were the first to report field control of a soil-borne plant pathogen (*Sclerotium rolfsii* Sacc.) by the addition of an antagonist. The disadvantage of their method was the great bulk of material (4,200 kg/hectare) required to obtain control. In addition, their system is difficult to standardize in order that the same effective dose may be delivered on successive dates.

The ideal carrier should have uniform, but small, particle size (preferably granules) that can be delivered with conventional farm machinery and can penetrate the foliar canopy. For economy of supplies, storage and delivery costs, no more than 100-200 kg/hectare should be required to achieve control. With these considerations, a study was made to: (i) find a granule that can be impregnated with an aqueous growth medium while maintaining its integrity through sterilization, growth, and drying; (ii) develop a suitable growth medium for impregnation of the granule and growth of the antagonist *Trichoderma harzianum* Rifai; and (iii) evaluate the *Trichoderma*-colonized granules for viability and for antagonism to *S. rolfsii* in peanut (*Arachis hypogaea* L.) fields.

MATERIALS AND METHODS.—Granules composed of attapulgous clay (30/60-mesh, Englehard Corp., Attapulgous, GA) and diatomaceous earth (Celatom MP-78, Eagle-Picher Corp., Cincinnati, OH) were compared for their water-holding properties and physical properties when wet. Twenty-five, 50, and 75 ml of water were added to a 100-ml volume of each granule. Granule swelling, disintegration, and water retention characteristics were observed. The granule with superior physical properties was impregnated with the optimal rate of a 10% solution (v/v) pH 5.0 of blackstrap molasses (sp. gr. 1.300, feed grade) containing 3g KNO_3 and 3g KH_2PO_4 per liter of solution. Physical properties of the granules were observed before and after autoclaving at 121 C for 15 minutes. Sterile granules were spread in shallow pans in a layer 3-5 cm deep, autoclaved, and inoculated after cooling with a minced 3-day-old potato-dextrose agar (PDA)-grown culture of *T. harzianum* (Isolate 70-3A, from H. D. Wells). Granules were incubated 4-7 days at 25 C until sporulation was profuse. Clumps then were broken-up and granules air-dried with frequent stirring. The dried preparations were stored at 5 C until testing, when the viability of *Trichoderma* was verified by plating granules on water agar.

Field tests were conducted in 1972, 1973, and 1974 at the Wiregrass Substation of Auburn University in southeastern Alabama. Tests were conducted on Dothan sandy loam soil which had been planted with peanuts for the previous 2 years and had a history of severe damage from *Sclerotium rolfsii*. Peanuts (cultivar-Florunner) were planted in single-rows with a 0.9 m spacing and a plot size of four 9-m rows. Treatments were replicated seven times in a randomized complete block design. The 1972 test was irrigated during periods of moisture stress to supplement natural rainfall, while the 1974 test was conducted during a summer of extensive rainfall when no supplemental irrigation was necessary; both experiments were considered 'wet.' The two sites selected for testing in

TABLE 1. Mean numbers of dead peanut plants (per 20 m of row) by experiment and moisture levels in *Sclerotium rolfsii* control tests comparing *Trichoderma harzianum* introduced on granules of diatomaceous earth and application of a granular formulation of pentachloronitrobenzene (PCNB)

	Dead peanut plants (no. per 20 m row)[x]						
	Wet experiments[y]			Dry experiments[z]			
Treatment and rate	1972	1974	Mean	1973a	1973b	Mean	Grand mean
Trichoderma granules 140 kg/ha	2.86 ab	3.57 ab	3.21 Ab	5.28 b	6.43 a	5.85 Ab	4.53 Bb
PCNB 10% granules 112 kg/ha	1.71 b	1.29 b	1.50 Bb	6.43 ab	8.14 a	7.28 Aab	4.39 Bb
Nontreated control	5.86 a	6.00 a	5.93 Aa	9.86 a	9.71 a	9.78 Aa	7.85 Aa

[x]Column values followed by the same letter-case are not significantly different ($P = 0.05$ lower case; $P = 0.01$ upper case) using Duncan's multiple range test.
[y]Wet: 38.1-50.8 cm (15-20 inches) of rain or irrigation water during pod development (no moisture stress).
[z]Dry: <17.8 cm (7 inches) of rain during pod development (frequent moisture stress).

TABLE 2. Mean yields (kg per plot and kg/hectare) of dry Florunner peanuts from *Sclerotium rolfsii* control plots treated with *Trichoderma harzianum* on granules of diatomaceous earth and a granular formation of pentachloronitrobenzene (PCNB) and maintained under wet and dry conditions.

	Yield of dry peanuts (kg/plot and kg/ha)[x]							
Treatment	Wet experiments[y]			Dry experiments[z]			Grand mean	
and rate	1972	1974	Mean	1973a	1973b	Mean	kg/plot	kg/ha
Trichoderma granules 140 kg/ha	32.6 B	36.4 B	34.6 B	21.2 a	19.5 a	20.3 a	27.4 B	3,336
PCNB 10% granules 112 kg/ha	32.8 B	39.1 B	36.0 B	19.7 a	18.9 a	19.3 a	27.6 B	3,360
Nontreated control	27.5 A	31.9 A	29.7 A	18.2 a	18.5 a	18.5 a	24.0 A	2,928

[x]Column values followed by the same letter-case are not significantly different ($P = 0.05$ lower case; $P = 0.01$ upper case) using Duncan's multiple range test.
[y]Wet: 38.1-50.8 cm (15-20 inches) of rain or irrigation water during pod development (no moisture stress).
[z]Dry: <17.8 cm (7 inches) of rain during pod development (frequent moisture stress).

1973 were dry-land farmed, and were frequently under moisture stress.

Trichoderma granules were removed from cold storage and mixed (1:1, v/v) with sterile dry granules that had previously been impregnated with 10% molasses solution. This material was hand-distributed over the peanut row-centers in a 45-cm-wide band at a rate of 112 kg/ha. The first application was made 70 days after planting, followed by a second treatment at the same rate 100 days after planting. Trichoderma granules were compared for performance to an untreated control and the standard PCNB chemical program. In 1973, tests of air-dried molasses-impregnated granules (112 kg/ha) were included as an additional control. PCNB (Terraclor 10G) was applied in a 45-cm-wide band over-the-row at a rate of 2.5 kg/ha 70 and 100 days after planting. Fourteen days before harvest the number of dead plants in the center pair of rows of each plot showing signs of *S. rolfsii* were counted (7). The same two rows were harvested for yield approximately 150 days after planting. Statistical comparisons of treatments over the 3-year study were made and their performance compared under wet and dry conditions.

RESULTS AND DISCUSSION.—Laboratory evaluations of candidate granules revealed that attapulgous clay granules swelled excessively, and lost their integrity if stirred while moist. Saturated diatomaceous earth granules did not swell significantly, withstood autoclaving, and remained firm and intact even after frequent stirring. This granule showed excellent absorptive capacity, absorbing aqueous solutions in quantities equal to at least 50% of its volume (v/v).

Viability of *Trichoderma* on air-dried infested granules was excellent, with virtually 100% of granules displaying mycelial growth. *Trichoderma* remained viable in the granule even after 1 month of cold storage. Field plots treated with these granules had significantly fewer ($P \leqslant 0.01$) dead plants showing signs of *S. rolfsii* when compared to nontreated controls (Table 1). When efficacy of PCNB and Trichoderma granules was compared, both PCNB and Trichoderma granules reduced plant losses under wet conditions ($P \leqslant 0.05$), but did not under dry conditions. Yield response was consistent with plant loss (Table 2), although neither product resulted in significant yield increases under dry conditions. Molasses granules were not different from the nontreated control in either disease or crop yield.

Field results with Trichoderma granules, indicated an average yield increase of 408 kg/ha, with an increase in peanut crop value of $80.00 per hectare when translated to 1974 prices. Although comparisons between Trichoderma granules and the standard PCNB chemical treatment were very similar for both disease control and peanut yield response, Trichoderma granules appeared to reduce *S. rolfsii* damage somewhat more than PCNB under dry conditions, while PCNB was slightly superior when conditions were wet (Table 1).

Trichoderma granules have several major advantages when compared to those with PCNB: (i) there is no residue of synthetic chemicals remaining in the soil; (ii) the mode of action of *Trichoderma* relies on a shift of soil ecology after a take-over of colonized soil organic matter; this is facilitated by an induced pH shift and production and activation of proteolytic enzymes (6), not on chemical toxicity (i.e., PCNB) to the pathogen; (iii) since *Trichoderma* is a natural inhabitant of peanut soil, registration and labeling may be easier than for chemicals.

Use of the granular carrier rather than an organic food base-carrier has several immediate advantages: (i) the weight of material delivered per acre is reduced 30-fold; (ii) standard agricultural granule machinery can be used for application; (iii) since there are no complex organic molecules in the granule, enzymatic methods (5) can be used to standardize the activity of the retail product; (iv) growth responses of *Trichoderma* are repeatable since the medium is of relatively standardized composition; and (v) the inorganic carrier system can be adapted to other pathogen-antagonist systems, by selecting the proper isolate and growth medium for infusion into the granule. To our knowledge, the granular delivery system reported here is the first economical method for biological control of a soil-borne pathogen under field conditions.

LITERATURE CITED

1. BLISS, D. E. 1951. The destruction of Armillaria mellea in citrus soils. Phytopathology 41:665-683.
2. KELLEY, W. D., and R. RODRIGUEZ-KABANA. 1974. Sodium azide as a soil fumigant in forest nursery beds. Auburn Univ. Agric. Exp. Stn., Highlights of Agric. Res. 21(4):12.
3. MIXON, A. C., and E. A. CURL. 1967. Influence of plant residues on Sclerotium rolfsii and inhibitory soil organisms. Crop Sci. 7:641-644.
4. PAPAVIZAS, G. C. 1973. Status of applied biological control of soil-borne plant pathogens. Soil Biol. Biochem. 5:709-720.
5. RODRIGUEZ-KABANA, R. 1969. Enzymatic interactions of Sclerotium rolfsii and Trichoderma viride in mixed soil culture. Phytopathology 59:910-921.
6. RODRIGUEZ-KABANA, R. 1974. The use of enzymatic activities of development of mixed culture models to study microbial interactions in soil. Pages 81-85 *in* The relationship of soil microorganisms to soilborne plant pathogens. South. Coop. Series Bull. 183. Virginia Polytechnic Inst., Blacksburg. 98 p.
7. RODRIGUEZ-KABANA, R., and P. A. BACKMAN. 1973. Determination of linear regression equations to estimate yield losses to white mold in peanut fields. J. Am. Peanut Res. Educ. Assoc. 5:199-200 (Abstr.).
8. WEINDLING, R. 1932. Trichoderma lignorum as a parasite of other soil fungi. Phytopathology 22:837-845.
9. WELLS, H. D., D. K. BELL, and C. A. JAWORSKI. 1972. Efficacy of Trichoderma harzianum as a biocontrol for Sclerotium rolfsii. Phytopathology 62:442-447.