Evaluation of Biological Seed Treatment for Controlling Root Diseases of Pea

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ABSTRACT

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Of 100 bacteria and fungi isolated from roots and seeds of peas, and tested for antagonism to *Fusarium solani* and *Rhizoctonia solani*, 37 of 41 fungi and 22 of 59 bacteria proved antagonistic to one or both pathogens in culture. These 59 organisms were tested further against nine isolates of four root rot pathogens (*Aphanomyces euteiches, Fusarium oxysporum, F. solani,* and *Rhizoctonia solani*). They also were coated onto seeds and tested again in field soil in a rolled paper towel test, and in the greenhouse and field. Several organisms improved stand over no seed treatment. None of the laboratory or greenhouse screening tests predicted performance in the field, probably because of variation in weather and in pathogen activity during the 3 yr of field testing (1975-1977). Performance of one of the best fungal antagonists (*Penicillium* spp.) was enhanced by selection and by increase of inoculum on Czapek-Dox and malt agar media. In the greenhouse, *P. oxalicum* was as effective as captan and significantly better than no treatment, and in the field in 1977 it improved stand and pod number over no treatment.

Several reviews treat the application of organisms to seeds (3) or to soil at planting (10, 16). The organisms described are mostly bacteria and fungi. More papers deal with experiments made in the laboratory and greenhouse than in the field. Application of bacteria to seed (bacterization) has been done with carrots (15), cereals (5), corn (4, 11, 13), cotton (18), peas (12), pigeon pea (23), soybean (19, 20), and tomato (9, 17). Similarly, fungi have been applied to seeds of alfalfa (6), corn (4, 11), cotton (18), oats (24), table beet (14), and white mustard (26).

Organisms normally are present on or under seed coats and they may persist there and have some influence on seeds during germination. Although the role of these organisms is not clear, they can have either a neutral, pathogenic, or beneficial effect on germination and plant growth (7). Surface disinfestation of seeds may render the seeds more vulnerable to seedling blight (5, 13). By coating seeds with beneficial organisms capable of growing on the germinating seed and in the rhizosphere, naturally occurring biological control can be enhanced (4, 7).

Beet seeds coated with *Trichoderma viride* or *Penicillium frequentans* resulted in protection from preemergence, but not postemergence, damping-off (14). Protection was thought to be effective for 3-4 wk in corn (3) and for 2-3 wk in tomato (17). After that the normal rhizosphere microflora apparently becomes reestablished (3).

Based on a review of literature, it appeared that biological seed treatment was feasible and experiments were made (i) to select effective antagonists that could be applied to pea seeds and evaluated by using laboratory, greenhouse, and field tests, and (ii) to ascertain methods for improving performance of the selected species.

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MATERIALS AND METHODS

Bacteria and fungi were isolated from pea seeds and pea plant rhizospheres. Four methods were used to test for antagonists: (i) inhibition of pea root pathogens on agar media, (ii) seed germination in rolled paper towels, (iii) seedling stand in the greenhouse, and (iv) plant stand and yield in the field.

In the petri dish test, candidate antagonists were tested against each of four pathogens: *Aphanomyces euteiches* Brechs. (two Minnesota isolates), *Fusarium oxysporum* Schl. emend. Snyd. & Hans. f. sp. *pisi* race 1 (three isolates from Wisconsin) and race 2 (one isolate from Minnesota), *F. solani* (Mart.) App. & Wr. emend. Snyd. & Hans. f. sp. *pisi* (one Minnesota and one Wisconsin isolate), and *Rhizoctonia solani* Kühn (one Minnesota isolate).

An agar disk (5 mm diameter) of the pathogen, grown on commercial potato-dextrose agar (PDA), was placed in the center of a petri dish containing 19 ml PDA. Four equidistant streaks (3 cm long) of the test antagonist were made 3 cm from the pathogen. In tests of *F. oxysporum* and *F. solani,* the pathogen and antagonist were added to the medium on the same day but for *R. solani* and *A. euteiches* the antagonist was streaked on the medium 2 days before the pathogen was added. Antagonist efficacy was ascertained by the presence or absence and size of a zone of inhibition or the degree of overgrowth and sporulation of the antagonist on the pathogen. Rarely was there a zone of inhibition between a fungal antagonist

and a pathogen. Tests were made at 24 C under fluorescent lamps (5,300 lux for 16 hr/day).

The rolled paper towel test was essentially the same as that used by Hoppe (8). In our procedure, a sheet of wax paper was covered first with a wet paper towel and then a layer of soil about 1 cm thick was added, on which 50 organism-coated seeds were placed. Another wet paper towel then was laid over the seeds and the entire unit was loosely rolled into a cylinder and held together with rubber bands. These units were stacked vertically in a container and incubated at 24 C. After 7-8 days, the rolled-towel units were opened and the germination data were recorded.

In the greenhouse test, seeds were coated with propagules of the antagonist, sown in soil contained in metal flats, and kept at 21-24 C. Plants were dug after 4 wk and the final stand and root rot were evaluated. In both the rolled paper towel and greenhouse tests, soil was taken from a field "disease nursery" where peas had been grown each season for several decades and in which pathogens of pea roots were present.

In the field tests, seeds were coated with antagonists and planted in the pea "disease nursery." Peas were sown in 1975 in 120-cm-long rows of 50 seeds per row, in 1976 in 240-cm-long rows of 100 seeds per row, and in 1977 in 240-cm-long rows of 50 seeds per row. Stand counts were taken at emergence and daily thereafter until they no longer increased. Final stand, vine weight (wet and dry), and pod and seed weights were recorded at harvest in 1975 and 1976. However, in 1977, data were recorded on final stand and number of pods.

Inoculum applied to seed was prepared by growing fungi on PDA for 1-2 wk at 24 C and bacteria on PDA for 1 wk or in potato-dextrose broth (PDB) at 24 C on a rotary shaker for 1 wk. To coat seeds with fungi, seeds were placed on the culture in the petri dish or added to a vessel with harvested spores and shaken 40-100 times to ensure coverage of seeds. The procedure used depended upon the number of seeds to be coated. To coat seeds with bacteria, seeds and sterile water were added to the culture in the petri dish and shaken 40-100 times, or seeds were added to a flask of PDA; after 1-2 min, broth was poured from flasks, and the peas were dried on paper towels. The number of spores or cells per seed was not determined although we tried to apply approximately the same quantity of propagules of each antagonist to each seed.

Several controls were used in all tests, including (i) nontreated seed (where seeds were handled similarly to treated seeds but without organisms), (ii) a captan (*N*-trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide) treatment applied by introducing an excess of the fungicide to a container with the seeds, shaking the container 40-100 times (depending on how the organisms had been applied) and then screening off the excess, or (iii) placing seeds in sterile water or in flasks of PDB for 1-2 min because bacteria were applied to seeds in sterile water or in broth. Treated seeds were stored at 5 C until planted (1-6 days).

In all experiments one cultivar was used: *Pisum sativum* L. 'Little Marvel.' This cultivar was selected because of its high susceptibility to the common pea root pathogens.

Analyses of variance were performed, and means were compared by using the Student's *t*-test ($P = 0.05$) and occasionally by using Duncan's new multiple range test ($P = 0.05$).

RESULTS

Antagonism to pathogens on agar media.—About 100 microorganisms (58 bacterial and 42 fungal isolates) were tested first on PDA for antagonism to an isolate of *Fusarium solani* f. sp. *pisi* (Minnesota isolate) and *Rhizoctonia solani* (Minnesota isolate). Cultures of bacteria and fungi were saved if they were antagonistic to either or both pathogens. Later, 59 organisms (22 bacteria, 37 fungi) were tested further against nine isolates of four pathogens (*Aphanomyces euteiches, Fusarium oxysporum, F. solani,* and *Rhizoctonia solani*). Petri dishes were examined after 1 wk for bacterial antagonists and after 2 wk for fungal antagonists.

Of the 22 bacterial isolates, 21 inhibited growth in culture of a Minnesota isolate of *F. solani* but none were inhibitory to a Wisconsin isolate. Although 18 and 19 of the bacterial isolates, respectively, inhibited growth in culture of two isolates of *F. oxysporum* f. sp. *pisi* race 1, only three inhibited growth of a third isolate of race 1; however, 16 of the bacterial isolates inhibited growth of race 2. Also, 21 of the isolates inhibited growth of each of two isolates of *A. euteiches*. All 22 isolates inhibited growth of *R. solani*.

Of the 22 bacterial antagonists tested against the nine isolates of four root pathogens, none inhibited all nine isolates, three inhibited eight isolates, 12 inhibited seven isolates, three inhibited six isolates, two inhibited five isolates, one inhibited four isolates, and one inhibited only three isolates (the two isolates of *A. euteiches* and one of *R. solani*).

Thus, it appeared that in culture there was some specificity of bacterial isolates to races or isolates of *F. solani* and *F. oxysporum* and less specificity to isolates of *A. euteiches* and *R. solani*.

The behavior of 37 candidate fungal antagonists was more uniform than that of the bacterial antagonists. Of 37 fungi, 35 inhibited growth of a Minnesota isolate of *F. solani* f. sp. *pisi* and 32 inhibited a Wisconsin isolate, and the degree of inhibition was about the same for all antagonists to both isolates. Against *F. oxysporum* f. sp. *pisi,* 27 of the 39 fungi inhibited growth of one isolate of race 1 and 36 inhibited each of two other isolates of race 1 and the one isolate of race 2, indicating a relative uniformity of antagonism to the four isolates of this species. All fungal antagonists inhibited the two isolates of *A. euteiches* and the single isolate of *R. solani*.

Of the 37 fungi tested for antagonism to the nine isolates of the four pathogens, 17 were inhibitory to all nine isolates, 11 were inhibitory to eight isolates, four were inhibitory to seven isolates, two were inhibitory to six isolates, two were inhibitory to five isolates and one was inhibitory to three isolates, and those three were *R. solani* and the two isolates of *A. euteiches*.

Screening antagonists in rolled-paper-towel germination tests.—Seedlings produced in the paper-towel test were rated as strong if the seed germinated vigorously, and weak if the seed germinated slowly. Two weak seedlings were counted as one strong seedling. Of the 20 bacterial isolates, five increased germination

percentage over no treatment by 5 to 9%, but this was below the percentage increase (62%) in germination obtained for captan-treated seed. The PDB control resulted in poorer germination (26%) than did the nontreated control (32%) indicating that the PDB may render the seeds vulnerable to seedling blight or root rot. The soil moisture content was 16% (v/w) and might have been low for effective control by bacterial antagonists. Of 39 fungi, 32 increased germination (23-48%) over nontreated seed (22%), but none was better than captan-treated seed (64%).

Evaluation of candidate antagonists in the greenhouse.—*Screening bacteria and fungi.*—Seven of the 22 bacteria improved seedling stands over no treatment in the greenhouse. At least one bacterium was as effective as captan (Fig. 1). Twenty-six of the 39 fungi improved stand and resulted in better root development than did nontreated seed (Fig. 2). Some fungi and bacteria applied to seeds resulted in no emergence or in poorer emergence than no treatment.

Based on results from petri dish, paper-towel, and greenhouse screening tests, five fungal and one bacterial isolate were selected for the paper-towel (eight replicates of 50 seeds/replicate) and greenhouse (eight replicates of 25 seeds/replicate) tests. The overall development of the roots was also a factor in selection. In the paper-towel tests, three of the fungi increased percentage of seed germination slightly and one of these increased germination 7% over that from nontreated seed (40%). None of the isolates improved germination as much as that from captan-treated seeds (62%), which significantly increased stand ($P = 0.05$) over the control.

By 3 wk after planting in the greenhouse (set at 21 C), two fungi had increased germination 10% over that of the control, and two other fungi had increased germination only slightly more than that in the control (20%). None of the isolates improved germination equal to that from captan-treated seeds (81%), which significantly increased stand ($P = 0.05$) over the control.

Improvement of antagonist by selection.—Culture 74 (*Penicillium* sp.) was selected for further study because it had performed well in petri dish tests, and it was one of the best organisms in the paper-towel and greenhouse tests. However, this culture, being a mass isolate, appeared to be a mixture and three isolates, representing three colony types, were tested to find the most effective antagonist. These isolates (A, B, C) were identified by D.

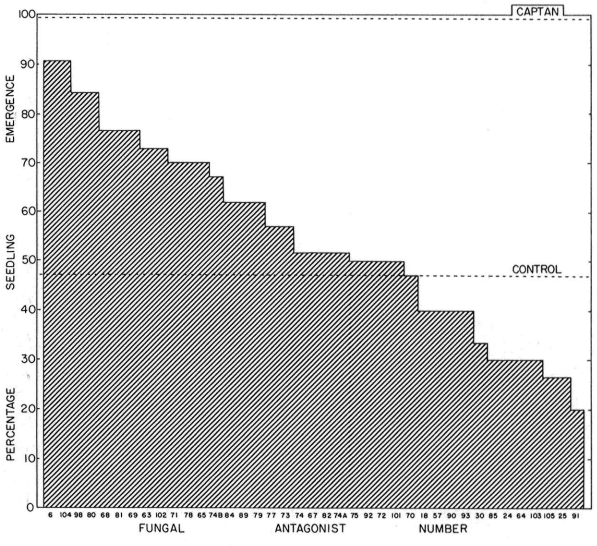

Fig. 2. The effect of 39 candidate fungal antagonists applied to pea seeds on plant stand at 3 wk when sown in pea field soil in the greenhouse set at 21 C, based on 15 seeds per treatment. Two weakly germinated seedlings were counted as one strong one.

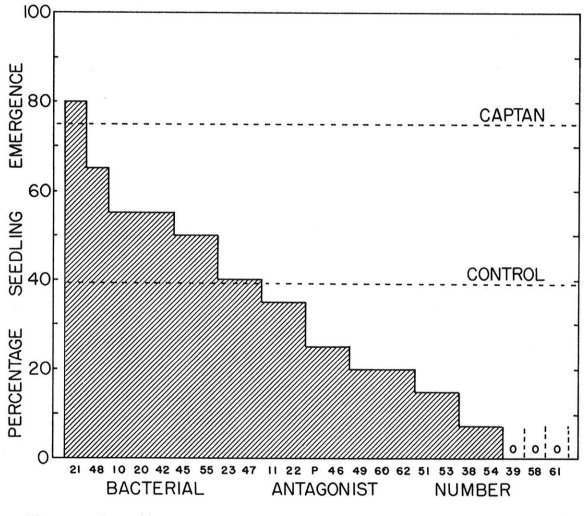

Fig. 1. The effect on pea seedling stand at 3 wk of 22 candidate bacterial antagonists applied to pea seeds sown in pea field soil in the greenhouse set at 21 C, based on 10 seeds per treatment. Two weakly germinated seedlings were counted as one strong one. (P = potato-dextrose broth control.)

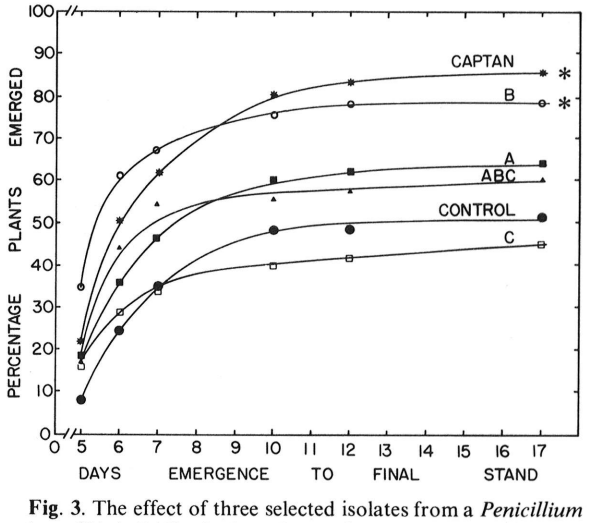

Fig. 3. The effect of three selected isolates from a *Penicillium* culture (74-A, B, C), singly and as a mixture, as seed coatings, on stands of pea plants grown in the greenhouse set at 21 C. Each treatment consisted of eight replicates of 50 seeds per replicate. An asterisk denotes a significant difference (t-test, $P = 0.05$) from the control.

I. Fennell (Northern Regional Research Center, Peoria, IL) as: A = *Penicillium verruculosum* Peyronel, B = *P. oxalicum* Currie & Thom, and C = a mixture of *P. lividum* Westling and *P. trzebinskii* Zaleski. Isolates A, B, and C were applied to pea seeds individually and as a mixture of the three isolates and planted in the greenhouse in soil taken from a pea field.

On the 17th day after treated seeds were sown, isolate B and captan resulted in a significantly higher stand than the nontreated control (Fig. 3). Isolates A and B, and captan resulted in greater pea fresh weight than did the nontreated control, and isolate B and captan resulted in greater pea oven-dry weight than did the nontreated control (Table 1). The experiment was repeated and similar results were obtained, except when the isolates A, B, and C were mixed. The mixture proved to be one of the better seed treatments (Table 1).

In the second experiment, peas also were rated for root rot using the system devised by Sherwood and Hagedorn (22). Isolates A, B, ABC, and captan gave lower root rot indices than the nontreated control. However, even the lowest root rot index of 80 was very high because an index rating of 70-100 means that fields should not be planted to peas (22).

Similarly, in another test (based on three replicates of 10 seeds/replicate) six of nine fungi gave significantly lower disease indices (range 75-87) than the nontreated control (= 97), but none was as good as captan (= 70) (*t*-test, $P = 0.05$). None of the bacterial seed treatments reduced the disease index below that of nontreated seed.

Improvement of antagonist by choice of substrate.—Penicillium oxalicum was cultured on Czapek-Dox agar (CDA), malt agar (MA), and PDA. Spores were harvested from each medium, applied to pea seed, and compared to captan-treated or nontreated seed. Inoculum produced on CDA or MA resulted in a significantly higher stand than did the nontreated control whereas inoculum produced on PDA gave increased emergence over the control (Fig. 4). This difference was not statistically significant using the *t*-test ($P = 0.05$) but was statistically significant using a Duncan's new multiple range test ($P = 0.05$). In later experiments (Windels and Kommedahl, *unpublished*), inoculum from PDA was as effective as that from CDA. However, *P. oxalicum* sporulated more abundantly on CDA than on PDA.

Effect of antagonists in the field.—*Effect on plant stand.*—Sixty organisms were tested in the field in 1975 for effectiveness in improving pea stands over no treatment. Nine days after planting seeds, those treated with 10 organisms (eight fungi and two bacteria) gave a significantly higher emergence than did nontreated seeds

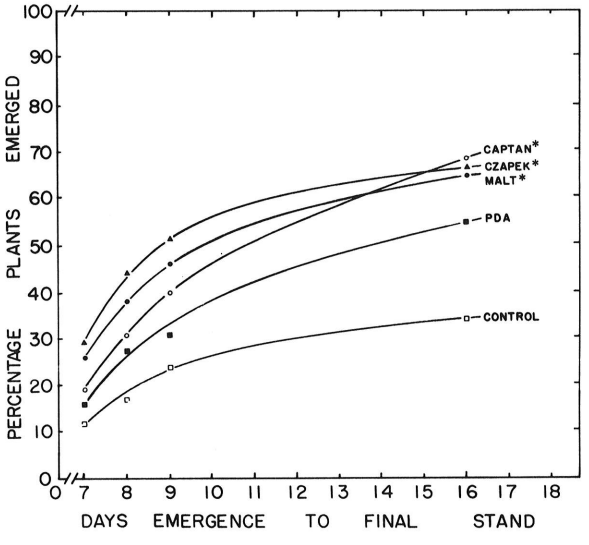

Fig. 4. The effect of three substrates on inoculum increase of *Penicillium oxalicum* (isolate 74-B), used as a seed treatment, on emergence and final stand of pea plants grown in the greenhouse maintained at 21 C. Data are based on five replicates of 50 plants per replicate. An asterisk denotes a significant difference (*t*-test, $P = 0.05$) from the control.

TABLE 1. The effect of three isolates of *Penicillium* species (74-A, B, and C), singly and in combination, on final stand, fresh and dry weights, and root rot of peas planted as two experiments (I and II) in the greenhouse set at 21 C compared with the control

Seed treatment[a]	Final stand[b]		Fresh weight[c]		Oven-dry weight[d]		Root rot index[e]
	Exp. I (%)	Exp. II (%)	Exp. I (g)	Exp. II (g)	Exp. I (g)	Exp. II (g)	Exp. II
Penicillium spp.							
Isolate 74-A	64	61*	44.2*	34.2*	4.57	4.15*	87*
Isolate 74-B	76*	67*	47.4*	34.7*	4.95*	4.19*	87*
Isolate 74-C	45	52	27.0	27.4	2.97	3.41	91
Isolates 74-ABC	57	73*	36.7	37.9*	4.16	4.54*	86*
Captan	84*	85*	57.5*	46.8*	5.82*	5.75*	80*
Control	50	40	30.0	20.1	3.34	2.46	94

[a]Each treatment consisted of eight replicates of 50 seeds per replicate in each experiment.
[b]Final stand was the 17th day in experiment I and the 23rd day in experiment II.
[c]Weight of plants from 50-plant row in greenhouse flats.
[d]Wet weight basis.
[e]Root rot index on scale of 0-100, in which higher values indicate more disease (See Sherwood, R. T., and D. J. Hagedorn 1958. Wisc. Agric. Exp. Stn. Bull. 531).
[f]The asterisks (*) indicate means significantly different from the control (*t*-test, $P = 0.05$).

(t-test, $P = 0.05$). However, one of the two bacteria was significantly better than the PDB control. Captan-treated seeds did not give a significantly greater emergence than nontreated seeds.

Twelve days after planting, about 70% of the peas had emerged. At this time seeds treated with 18 organisms (14 fungi and four bacteria), captan and the PDB control gave significantly greater emergence than the nontreated seeds (t-test, $P = 0.05$). All but one organism that had increased emergence at 9 days also increased emergence at 12 days.

At harvest, total plant stands varied from 74 to 96% and averaged 91% for the 60 organisms. The average of 37 fungi produced a slightly higher average stand than the average of 23 bacteria. However, only one bacterial seed treatment gave a significantly higher stand than the nontreated control, but not over the PDB control (t-test, $P = 0.05$). This isolate had given a high emergence at 12, but not at 9 days after planting.

By harvest, nearly every plant sustained severe root rot due to warm, moist conditions and nearly half of the plants died. *Aphanomyces euteiches* was the main pathogen. The stand of live plants at harvest varied from 24 to 60% and averaged 47% for the organism-treated seeds. Organism-treated seeds were not better than nontreated or captan-treated seed (57 and 59% stand, respectively).

In 1976 disease incidence was low and treatment effects could not be discerned. Of the 10 organisms tested in 1976, the average final stand was 61% (range: 55-66%) vs. 63% for captan and 64% for the nontreated control.

Pea seeds were sown about 2 wk earlier in 1976 than in 1975 because of the early spring, yet it took 3 days longer for seedlings to appear in 1976 than in 1975 (Fig. 5). Moreover, total emergence in 1976 never reached that of 1975 and it took about 3 wk longer to attain the greatest emergence. Low moisture probably accounted for reduced emergence or could have predisposed germinating seeds to pathogens.

In 1977, severe disease occurred early in the season as evidenced by low emergence (Table 2). Of nine organism seed treatments, *P. oxalicum* (74-B) gave the highest emergence which was significantly better than the nontreated control using the Duncan's new multiple range test ($P = 0.05$), but was not nearly as good as captan-treated seeds. Captan was the only treatment significantly better than the nontreated control when the data were analyzed by a t-test ($P = 0.05$).

Effect on vine weight.—In both 1975 and 1976, organism- and captan-coated seeds did not yield a significantly greater vine weight than the nontreated control (t-test, $P = 0.05$). The average vine weight (fresh) for 1975 was 244 g (range: 116-308 g) for 60 organisms, 303 g for captan treatment, 263 g for PDB treatment, and 273 g for the nontreated seed. The average vine weight for 1976 was 615 g (range: 555-662 g) for 10 organisms, 596 g for captan treatment, 607 g for PDB treatment, and 651 g for nontreated seed.

Effect on yield.—In 1975, one fungus and one bacterium significantly (t-test, $P = 0.05$) increased pod weight when compared to the nontreated control but not for the bacterium over the PDB control (Fig. 6). These two organisms had also accounted for a greater number of plants 12 days after planting but did not result in a significantly greater number of plants than the control at harvest. Captan was not a significantly better treatment than the control.

In 1976, seed treatments did not improve pea pod yields. Pea pod yields were more variable in 1975 than in 1976 with treatment (Fig. 7), probably because disease situations were different in the succeeding year. Fewer plants emerged in 1976 which allowed plants to branch

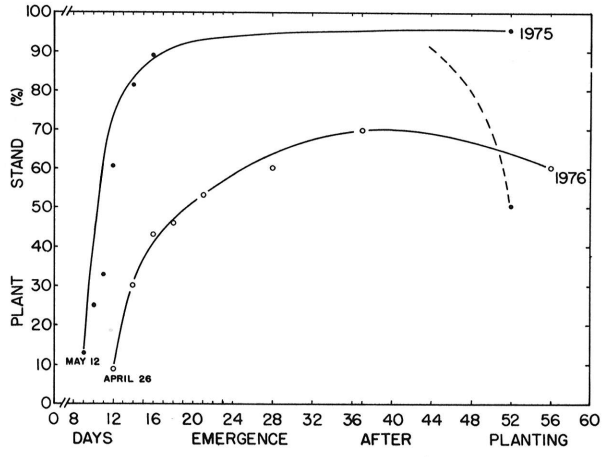

Fig. 5. The stand of pea plants in the field in 1975 and 1976 from the dates of emergence to harvest for two different planting dates: 12 May 1975 and 26 April 1976. The dashed line for 1975 designates plants that died late in the season and it is not connected because the exact dates when plants died are not known. The values given are averages of all 63 treatments in 1975 and all 13 treatments in 1976. The curve for any single treatment closely approximates values given for the average.

TABLE 2. The effect of organism-treated seed on the average final stand and the average number of pods per treatment in the field in 1977

Seed treatment[a]	Stand[b] (%)	Pods[b] (no.)
74-B	19.2 b	34.1 b
105	18.4 bc	33.1 bc
82	18.1 bc	34.9 b
30	15.1 bcd	29.8 bcd
101	14.9 bcd	26.7 bcde
65	14.7 bcd	25.5 bcde
72	12.0 cde	22.8 bcde
B-10	8.7 de	19.1 cde
B-39	6.5 e	13.8 e
Captan	48.0 a	66.3 a
PDB	10.3 de	17.6 de
Control	10.8 de	18.8 cde

[a]Each treatment consisted of 15 replicates of 50 seeds per replicate. B (prefix) = bacteria; others are fungi. No. 30 and 65 are *Trichoderma* spp., 74-B is *Penicillium oxalicum*, and the other fungi are *Aspergillus* and *Penicillium* spp. PDB = potato-dextrose broth.

[b]Means in columns followed by the same letter(s) are not significantly different at $P = 0.05$ according to Duncan's new multiple range test.

early and thereby compensate in yield for the stand losses whereas more plants emerged in 1975 only to succumb late in the season.

In 1977, plants from seeds treated with *P. oxalicum* and another fungus gave a higher number of pods than the nontreated control according to Duncan's new multiple range test, $P = 0.05$, but none of the seed treatments was as good as captan (Table 2). Captan was the only seed treatment significantly better than the nontreated control when data were analyzed by a *t*-test, $P = 0.05$. Although *P. oxalicum* had always been a reliable antagonist in greenhouse tests, this was the first field test in which it was one of the best antagonists. The two organisms that resulted in a significantly higher pea yield in 1975 (the fungus is No. 72, and the bacterium B-10), were not better than the nontreated control in 1977.

Evaluation of methods for selecting antagonists.—Twenty-two bacteria and 40 fungi were selected originally from 100 cultures isolated from pea roots and seeds. Only three of the 10 best bacterial antagonists in petri dishes were among the 10 best antagonists in the field (Fig. 8). The paper-towel and greenhouse tests each predicted five but failed to predict the other five of the 10 best bacterial antagonists in the field; four of these five that were predicted were the same bacteria. Six bacteria were among the 10 best antagonists in three of four tests.

The 10 fungus cultures that were most antagonistic to pea root pathogens in petri dish tests, or which performed best in improving emergence of plants in the paper-towel, greenhouse, and field tests are shown in Fig. 9. The test in petri dishes predicted four fungi among the 10 best antagonists in the field. The paper-towel test predicted only one and the greenhouse test predicted three of the 10 best antagonists in the field (Fig. 9). No single method seemed effective in predicting performance in the field. Four fungi were on the list of the 10 best antagonists in three of the four tests.

In the paper-towel, greenhouse, and field tests there were usually more than 10 organisms that performed better than the nontreated control but they were not the same 10 organisms in each test. Although some organisms were not among the 10 best organisms, they performed better than no treatment. For instance, in the paper-towel test, 32 of the 39 fungi increased germination over nontreated seed. Twenty-six of the 32 fungi that increased percentage germination in the paper-towel test also increased percentage germination over nontreated seed in the greenhouse.

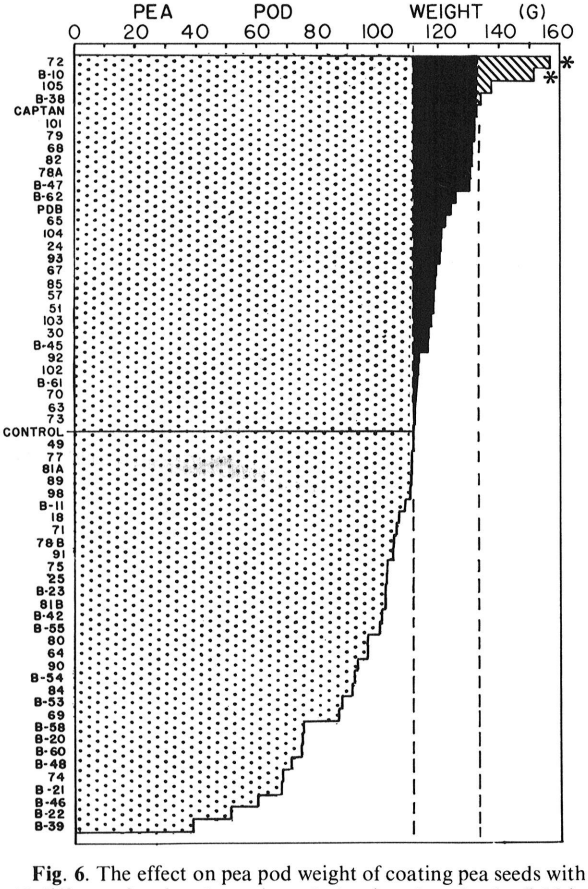

Fig. 6. The effect on pea pod weight of coating pea seeds with 60 different fungi or bacteria and planting them in the field in 1975, based on four replicates per treatment and 50 seeds sown per replicate. Crosshatching designates organisms better than captan as seed treatments and solid black designates organisms better than the nontreated control. Two organisms (72 and B-10) were significantly better as seed treatments than the nontreated control using the *t*-test ($P = 0.05$).

DISCUSSION

Ideally, an antagonist should be effective against a number of pathogens under a wide variety of conditions. The differences found in the field from 1975 to 1977

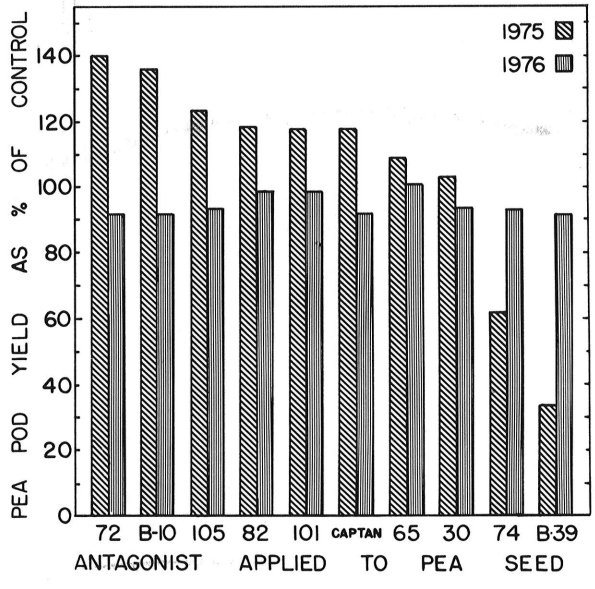

Fig. 7. Comparison of pea pod yields in the field for 1975 and 1976 and expressed as percentage of the control (no treatment). Results are based on four replicates of 50 seeds sown per replicate in 1975 and on 15 replicates of 100 seeds sown per replicate in 1976.

probably were due to differences in weather which favored different pathogens. *Aphanomyces euteiches* was destructive late in 1975 and early in 1977; however, *Fusarium solani*, *F. oxysporum*, and *Rhizoctonia solani* were the predominant pathogens in 1976, based on symptoms and isolations from roots. The antagonists we used were not equally effective against all these pathogens.

Variability in results also can be attributed to different isolates or races of a given pathogen. In petri dish tests more bacterial and fungal isolates inhibited *A. euteiches* and *R. solani* than inhibited either *F. oxysporum* or *F. solani*. Some of these antagonists inhibited some races or isolates of *Fusarium* spp. but not others. Thus, specificity appears to be a greater consideration in the more specialized fungi such as *Fusarium* spp. and this may explain some of the inconsistent behavior in the field, if the isolates or races tested in petri dishes can be assumed as representative of isolates present or active in soil.

Only a few of the 100 bacteria and fungi isolated from seeds and rhizospheres of peas proved to have potential as antagonists. Bacteria coated onto seeds appeared to have less potential as antagonists than fungi; in fact some bacteria appeared to be pathogenic and caused stand reductions in the field. If not pathogenic, bacteria may have inhibited the normal microflora resident on seeds that may have been antagonistic to soil-borne pathogens. Lang and Kommedahl (13) suggested that resident microflora on corn kernels protected them from seedling blight, but NaOCl or *Bacillus subtilis* applied to kernels nullified the slight benefits conferred by resident organisms. Similarly, Giha (5) reported that resident bacteria on wheat grains kept *Helminthosporium rostratum* in check, and that a strong bactericide applied to grains left them vulnerable to subsequent infection. *Bacillus subtilis* also was antagonistic to *Phoma betae* but the influence of this antagonist was cancelled by the presence of other soil bacteria (21). Thus bacteria, even though antagonistic to pathogens can be either beneficial or detrimental ecologically, depending on the combinations of antagonist, pathogen, and seed microflora.

Only a few of about 40 fungi seemed promising as candidates for further testing. Fungal antagonists were

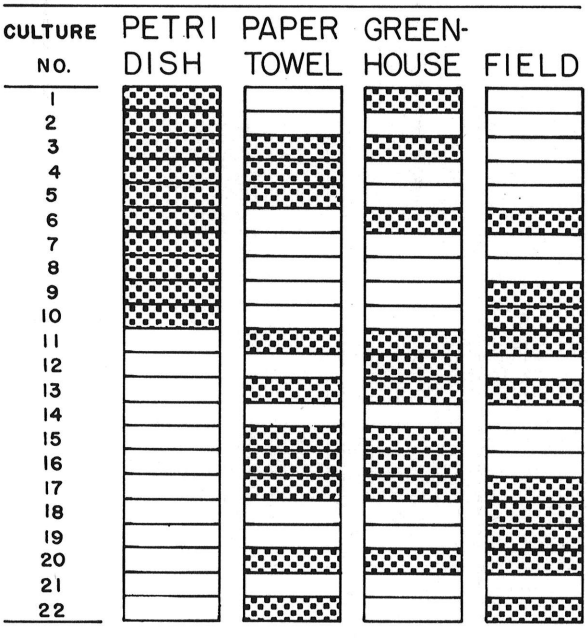

Fig. 8. Comparison of the 10 best bacterial antagonists based on tests made in petri dishes, in rolled paper towels, and in greenhouse and field plantings. Criteria for choosing the 10 best isolates were inhibition in culture (petri dish), germination in soil (paper towel), and plant stand (21 days in the greenhouse and 9 days in the field). The spotted bars designate the cultures that were the 10 best performers in each test.

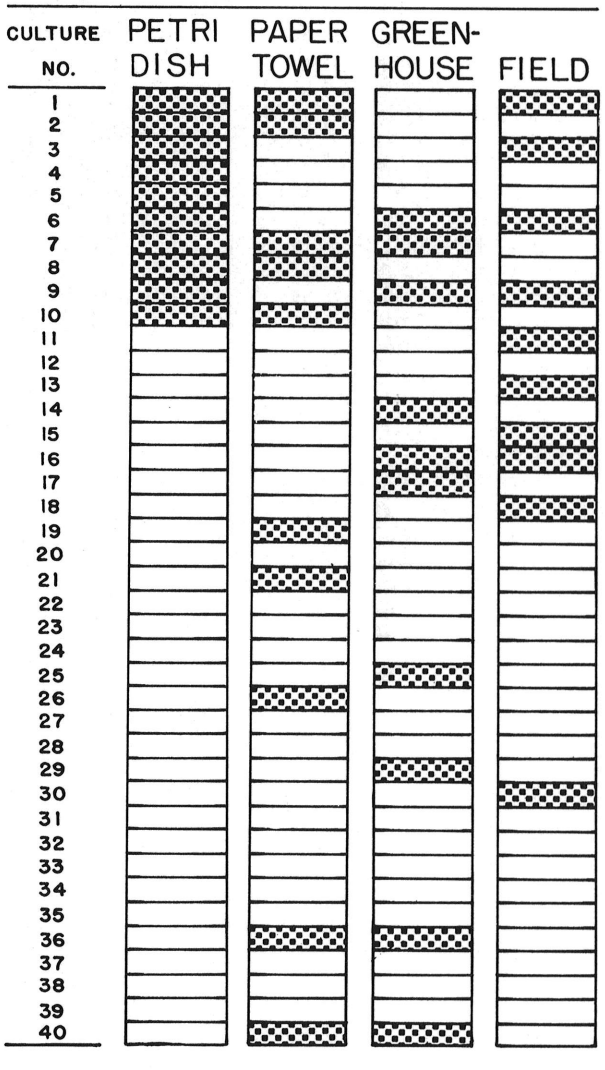

Fig. 9. Comparison of the 10 best fungal antagonists based on tests made in petri dishes, in rolled paper towels, and in greenhouse and field plantings. Criteria for choosing the 10 best isolates were inhibition in culture (petri dish), germination in soil (paper towel), and plant stand (21 days in the greenhouse and 9 days in the field). The spotted bars designate the cultures that were the 10 most effective antagonists in each kind of test.

species of *Aspergillus, Penicillium,*and *Trichoderma.* A *Penicillium* isolate (P-74) was consistently effective in stand improvement when applied to seeds which were sown in the greenhouse. However, in the field, it was not effective in 1975. This isolate (P-74) was a mass isolate, and from it, four species were selected and identified. One of these (74-B) was *P. oxalicum* and in 1977, it was coated onto seeds and planted in the field where it resulted in significantly higher stands than were obtained from nontreated seeds.

At this point, one might have either continued the search for more effective antagonists or attempted to improve the performance of the best antagonist found. Selection within mass cultures improved performance and further selection of strains within species may improve performance still more. Choice of substrate for growth and sporulation of antagonist also was a factor as shown by the apparent superiority of CDA medium over PDA in improving performance. Whether the substrate used increased nutrition or viability of spores was not established, but sporulation was greater on Czapek-Dox agar than on other media. Other factors such as duration and storage temperature of inoculum and of treated seed, nutrients added to inoculum, spore age, and spore load may further enhance performance of candidate antagonists. Some of these factors have been reported briefly (25).

An effective coating material used as a carrier for the antagonist probably would increase the efficacy of the antagonist and be more attractive commercially. The pelleting of seeds enhanced effectiveness of *Trichoderma viride* against *Pythium* seedling blight of alfalfa (6), and of *Bacillus subtilis* in yielding marketable carrots (15) and improvement of pigeon pea stands (23). Several coating and pelleting materials are now available commercially.

Whether screening of antagonists in the laboratory or greenhouse predicts performance in the field is questionable. Such tests made indoors usually are reproducible because conditions are controllable. Field conditions vary by season and location. Therefore, we are not certain that a field test in a given year is an accurate measure of the performance of the antagonist indoors. We illustrated how emergence and final stand of peas can vary in 3 yr of field testing. In 1975, results in the greenhouse did not correlate with results in the field, probably because root disease occurred late in the season. Then, in 1977, greenhouse results were confirmed by field results using *P. oxalicum,* probably because seedling blight occurred early in the season–a situation like that occurred with seedlings in the greenhouse.

Our experience differs in part from those of Broadbent et al. (2) who reported that about 40% of more than 3,500 isolates inhibited one or more of nine pathogens on agar media and about 4% were effective in soil; they also reported that performance of candidate organisms in soil in the laboratory or greenhouse generally agreed with their performance in that same soil in the field. Later (1), they reported that field tests were not as reproducible as greenhouse tests, which agrees with our experience.

Taking all factors into consideration, and the difficulties involved, it appears to be a matter of technology as well as ecology to find ways to make biocontrol effective and competitive with chemical seed treatment.

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