Title: Enhancing Alfalfa Yields and Stand Life by Improving Management of Seed Rot and Seedling Damping Off

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Abstract

Seed rot and damping off of alfalfa is a soilborne disease caused by multiple pathogens. Damage to seeds and plant roots results in thin initial stands of alfalfa and continued damage by pathogens during wet soil conditions decreases forage yield and winter survival. This project took a two-pronged approach to combat the disease, testing alternative seed treatments and developing disease resistant germplasm. The fungicide Evergol Energy was active against all alfalfa seed rot and damping off pathogens tested: four species of *Pythium*, four strains of *Aphanomyces euteiches*, three strains *of Phytophthora medicaginis*, and three species of *Fusarium*. Intego Solo was active against all pathogens except *Fusarium* species. When used as a seed treatment, Evergol Energy performed similarly to ApronXL in assays with infested soil. Evergol Energy is registered for use on alfalfa seeds. Biological seed treatments were not effective against seed rot and damping off pathogens. A single cycle of selection for resistance to one strain of *Pythium irregulare* resulted in a significant increase in resistance to multiple strains and species of *Pythium*. A second cycle of selection using three strains generally did not improve the percentage of resistant plants. The results of this project provide alfalfa growers and plant breeders with new tools to reduce damage from this disease and increase forage yield and stand life.

Introduction

Rapid and uniform seedling emergence is critical for obtaining a productive and persistent stand of alfalfa. In many locations, alfalfa seeds are planted into cold, wet soil conditions that are ideal for seed rot and damping off to occur. A complex of soil-borne pathogens including *Pythium* spp., *Rhizoctonia solani*, *Phytophthora medicaginis*, and *Fusarium* spp. cause seed rot and damping-off of alfalfa seedlings (1, 2). Infection of mature plants during wet spring weather causes destruction of fine feeder roots, which interferes with nitrogen fixation, nutrient uptake from the soil, and water absorption. Injury to the root may cause root "forking" in which shallow adventitious roots form above the damaged primary root (2). Cumulatively, these seedling and adult diseases result in reduced yields, decreased winter survival, and shortened stand life. New tools are needed for producers to manage these diseases and obtain higher yields per acre over a longer period of time.

Damping-off of alfalfa is managed by over-seeding to offset seed and seedling losses and by use of fungicide seed treatments. However, recent experiments indicate that seed treatments could be improved. Apron (active ingredient: metalaxyl) and the related fungicide Apron XL (active ingredient: mefanoxam), are widely used as a seed treatment and offer protection from *Pythium* spp. and *Phytophthora* spp. but are not effective against the other organisms causing seed rot and damping off or the seedling disease Aphanomyces root rot. Aggressive *Pythium* strains have been identified from Minnesota alfalfa fields that were not controlled by Apron XL or Stamina seed treatment and highly aggressive *Fusarium* strains causing alfalfa seed rot were identified (3). Furthermore, a recent survey of seedling damping off and root rot pathogens of soybean found highly aggressive *Pythium* isolates throughout the soybean producing areas (4). Many *Pythium* species attack both alfalfa and soybean. Newer fungicides labeled for use on soybean are available and may have a greater spectrum of activity than Apron XL for managing seed rot and damping off of alfalfa.

Most diseases of alfalfa are managed using resistant cultivars. Large gains in alfalfa yields were obtained in the 1970s and 1980s by developing disease resistant germplasm and stacking resistance to multiple diseases and pests. Previously, it was shown that alfalfa seedlings can be selected for resistance to seed rot and damping off in a culture plate assay (5) and that significant progress can be made in a single cycle of selection (6). A cultivar with resistance to this pathogen would have greater potential seedling establishment and improved adult plant root health, which will increase crop productivity. The goals of this study were to: (i) test soybean fungicides for their activity against seed rot and seedling damping off pathogens of alfalfa to identify new seed treatments for alfalfa; and (ii) evaluate resistance of alfalfa germplasm selected for resistance to *Pythium* species causing seed rot and damping off.

Materials and Methods

Culture plate assay for fungicide sensitivity. Each pathogen was tested for sensitivity to nine commercial fungicide preparations. The active ingredients and manufacturers are listed in Table 1. All metalaxyl containing fungicides (Evergol Energy, Rancona V RTU FS, Rancona Summit, and Rancona Dimension) were assessed at 0, 0.01, 0.1, 1, and 10 µg metalaxyl/ml. Vibrance and Intego Solo were assessed at 0, 0.01, 0.1, 1, 10, and 100 µg active ingredient/ml. The strobilurin-containing fungicides (Trilex and Dynasty) were assessed at 0, 0.01, 0.1, 1, 10, and 100 µg active ingredient/ml. For the strobilurin fungicides, salicylic hydroxamate (SHAM) was added to the media to inhibit the alternative oxidase respiration pathway, along with the fungicides. A concentration of 50 µg/ml SHAM was added to the media for all pathogens except those for *A. euteiches*, where 12.5 µg/ml SHAM was used. These SHAM concentrations were found to cause 20-40% mycelial growth inhibition. A set of control plates with SHAM but without fungicide was included for each pathogen strain.

All pathogen strains (Table 2) were previously isolated from soils collected in Minnesota using a seedling baiting technique (3). *Pythium* strains were grown on corn meal agar (CMA; Becton-Dickinson and Company, Sparks, MD) at 21°C for 2 to 3 days in darkness. A 6.5 mm-diameter plug from the colony edge was placed in the middle of a 90 mm petri dish containing CMA amended with each concentration of fungicide. Each fungicide concentration was evaluated in triplicate with each strain. Plates were incubated at 21°C in darkness for 48 hours and then growth was determined by measuring colony diameters in two perpendicular directions on each culture plate. Relative growth reduction was calculated for each fungicide concentration

and the concentration inhibiting growth by 50% (EC_{50}) was estimated by plotting the percent inhibition against the log-scale of fungicide concentrations. The same protocol was used to evaluate sensitivity of the other pathogens except that *A. euteiches* cultures were grown initially on CMA at 21°C for 3 to 4 days in darkness and radial growth was measured after 5 days at 21°C. *Fusarium* strains were grown initially on potato dextrose agar (PDA; Becton-Dickinson and Company) at room temperature for 5 days in darkness and radial growth measured after 6 days of growth. The *P. medicaginis* strains were initially grown on clarified V8 juice agar at room temperature for 5 days and radial growth measured after 7 days.

Efficacy of fungicides as seed treatments. Three fungicides with the broadest range of activity, ApronXL, Evergol Energy and Intego Solo, were used to treat 'Vernal' alfalfa seeds at the manufacturer's recommended product rates (Table 1). To coat the seeds, 25 g seeds were placed in a 1-quart zip top plastic storage bag. A 1 ml solution consisting of fungicide, 100 µl Red PRO-IZED colorant (Bayer CropScience), and water was added to the bag and the bag massaged until seeds were evenly coated. The control consisted of seeds treated with colorant and water. The treated seeds were transferred to a plastic weighing boat and allowed to air dry in a fume hood at room temperature.

An inoculum was prepared using *Pythium* strains BEC56, WAS53, L3; *A. euteiches* strains MF-1 and Mer-4, and *P. medicaginis* strains Pm2019 and A1A2. The pathogens were grown for 7 days at room temperature on agar media. The plates were homogenized in 500 ml water with a blender. The inoculum was mixed with 4 kg pasteurized soil. The control soil was mixed with water without pathogens. Approximately 50 cm^3 of soil was placed into 5.7 x 5.7 cm cells of horticultural tray inserts (Standard Insert 2401, T. O. Plastics, Clearwater, MN) and 25 seeds were planted in each cell. Seeds were covered with 10 cm^3 of control soil and flats placed in a growth chamber at 18°C with a 16-hour photoperiod. Seedling counts were made 7 days after planting. There were six replications and the experiment was done two times. Results of the two experiments were combined and an ANOVA test was performed with significance set at P= 0.05. Means were separated using Tukey's HSD. The experiment was repeated in which the soil of one set of seeds was saturated (flooded) with water immediately after planting for 24 hours and a second set saturated at 2 days after planting for 24 hours. Seedling counts were at 7 days after planting and analyzed as above.

Efficacy of biological seed treatments. Five proprietary biological seed treatments in development by Lallemand Inc. were used for coating 'Vernal' alfalfa seeds by Summit Seed Coatings, Caldwell, ID. Seeds treated with ApronXL treatment and a control treatment without fungicide or biological were also provided. The seeds were used in agar-plate based assays to test their efficacy against *Pythium* strains L3 and BEC56 as described previously (3). Briefly, 3 mm-diameter plugs from the edge of the colony were transferred to the centers of 90 mm diameter plates of 1.5% water agar. Plates were incubated at 21°C for 3 days and then 25 seeds were placed on the surface of each plate. Uninoculated plates with 25 seeds served as controls. The plates were incubated in a growth chamber for 5 days at 21°C, with a 16-hour photoperiod. The experiment was arranged as a randomized complete block with three replicates per strain. Seeds and seedlings were rated for disease severity on a 1 (healthy) to 5 (dead) scale. Seeds were also tested in infested soil. To prepare the soil, 250 cm^3 of a peat-based potting mix (Sungro LC8, SunGrow Horticultural Distribution, Agawam, MA) was mixed with 5 g corn meal, and autoclaved for 20 min. Approximately 50 cm^3 of soil mix was placed in 6 x 6 cm pots. Each

strain of *Pythium* was cultured on a 15 x 100 mm CMA plate at 21°C for 7 days, homogenized in a blender in a total of 1,000 ml sterile water, and 25 ml of homogenate added to each pot. Twenty-five alfalfa seeds were placed in each pot, covered with uninfested potting mix, and placed in a lighted growth chamber at 21°C. The experiment was arranged as a randomized complete block with four replicates per isolate with each replicate as a block. After 5 days, percent seed germination was assessed.

The efficacy of seed treatments against *A. euteiches* and *P. medicaginis* was tested as described previously (8). Briefly, seeds were planted in medium grade vermiculite in 5.7 x 5.7 cm cells of horticultural tray inserts (Standard Insert 2401, T. O. Plastics), the vermiculite moistened with water, and placed in a growth chamber with a 24°C/19°C day/night temperature regime, and a 16-hour photoperiod. *P. medicaginis* strain A2A1 was grown on V8 juice agar at room temperature for 7 days. A 90 mm culture plate was homogenized in 1,000 ml sterile water. *A. euteiches* strain MF-1 was cultured for 7 days on CMA and then a single plate was homogenized in 1,000 ml water. At 7 days after planting each cell was inoculated with 25 ml comminuted mycelium of either *A. euteiches* or *P. medicaginis* then the root zone was flooded for 2 days. Disease symptoms were rated on a 1 to 5 scale at 14 days after inoculation.

Development and evaluation of alfalfa germplasm. Three germplasm sources were evaluated for resistance to *Pythium irregulare* BEC56 using the agar plate-based assay (7). This strain was identified as a moderately aggressive seed rot and damping off pathogen from Minnesota and had a high EC_{50} to mefanoxam (3). The germplasm UMN2804 was developed from plants selected for resistance to Phytophthora root rot, Aphanomyces root rot, and the root-lesion nematode (*Pratylenchus penetrans*) under field conditions in Minnesota and Wisconsin (6). UMN2841 was developed by one cycle of selection for resistance to Pythium seed rot and damping off from BIC-7, as broad-based composite germplasm (6,9). The germplasm UMN3988 was developed for biomass energy with strong nonlodging stems and previously selected for resistance to Phytophthora root rot (10). BEC56 was cultured for 2 days on CMA then a 6.5 mm-diameter plug from the edge of the culture was placed in the center of a 1.5% water agar plate and cultured for 3 days at room temperature. Alfalfa seeds were surface sterilized by rinsing in 70% ethyl alcohol for ~1 min., 10% household bleach for 5 min, and rinsing with sterile water for ~1 min for three times. From 30 to 40 seeds were placed on each plate, plates were sealed with parafilm, and then incubated at 21°C in a growth chamber with a 16 h photoperiod for six days. Seedlings with a disease severity rating of 1 = (resistant) healthy seedling with no symptoms, 2 = (resistant) root tip discolored but root has elongated, or 3 = (moderately susceptible), root tip soft and rotted, were transferred to soil. A total of 53, 54, and 52 plants were retained from UMN 2804, UMN 284,1 and UMN 3988, respectively for a selection intensity of approximately 5%. Plants were randomly intermated by hand pollination within each germplasm source and seeds were collected by female parent. Resistance to *Pythium* strain BEC56 was evaluated using seeds from each line using the plate assay as described above except that seeds were scarified and surface sterilized by soaking in concentrated sulfuric acid for 5 min, then rinsed three times with sterile water. From each germplasm, lines with >31% resistant plants were randomly intercrossed by hand pollination within each germplasm (cycle 1_{syn1}). There were 15, 13, and 16 plants from UMN2804, UMN2841, and UMN3988, respectively. A second cycle of selection was done by mixing equal amounts of seed from all lines within a germplasm source and selecting resistant plants using the plate assay with strains BEC56, WAS53 and L3. Strains WAS53 and L3 were more aggressive seed rot pathogens than BEC56. A selection intensity of

10% was used and the resulting plants (40-50 per germplasm source) were transferred to soil and randomly intermated by hand within germplasm source. Seed from parental populations were generated in the greenhouse at the same time. Equal amounts of seeds from each female parent of the parental populations (cycle 0), cycle 1, and cycle 2 were mixed and tested for resistance to BEC56, WAS53, and L3 using the plate assay.

Project objectives:

1. Evaluate sensitivity of seed rot and damping-off pathogens and *Sinorhizobium meliloti* used for inoculation of alfalfa to fungicides and biological agents.
2. Test efficacy of fungicides and biologicals when used as seed treatments for control of seed rot and damping off of alfalfa.
3. Measure disease resistance in experimental germplasm that has undergone one cycle of selection for resistance to *Pythium* species causing seed rot and damping off.

Project results:

1. Doses of nine commercial fungicides causing 50% reduction in pathogen growth were determined for 16 pathogen strains. Evergol Energy and Intego Solo had the broadest range of activity.
2. None of the five proprietary biological seed treatments tested were effective against seed rot and damping off pathogens. Evergol Energy and ApronXL had similar protective action as seed treatments.
3. Germplasm resulting from one cycle of selection had 29-62% resistant plants depending on germplasm source and *Pythium* isolate.

Results and Discussion

Identification of alternative fungicide seed treatments. Seed rot and damping off of alfalfa is a disease that is difficult to control because pathogens contributing to the disease are in two orders of Oomycetes (Peronosporales and Saproleginales) and also include fungi. The fungicides that are active against one group of organisms are typically not active against others due to differences in basic metabolism. Commercial formulations of nine fungicides used as seed treatments that had a single or mixture of up to three active ingredients were tested against alfalfa seed rot and damping off pathogens in agar plated based assays to determine the EC_{50}, the concentration causing 50% inhibition of growth (Table 3). The EC_{50} values were rated as proving excellent (<0.05 – 0.1 µg/ml), very good (0.11 - 0.99 µg/ml), good (1.0 – 9.9 µg/ml), fair (10 – 99.9 µg/ml), or poor (>100 µg/ml) growth inhibition.

Apron and ApronXL are currently the most widely used alfalfa seed treatments. Apron is a mixture of two enantiomers, R and S of metalaxyl, the active ingredient, which interferes with RNA synthesis of Oomycetes in the order Peronosporales such as *Pythium* species and *Phytophthora* species. ApronXL is composed of primarily the R enantiomer of metalaxyl, which is the more active form. As expected, ApronXL had excellent or very good EC_{50} values against the *Pythium* and *Phytophthora medicaginis* strains but was not effective against *Fusarium* species or *A. euteiches* (Table 3). Rancona Summit and Rancona Dimension are a combination of metalaxyl with ipconazole, a sterol biosynthesis inhibitor, with ipconazole at either 0.9% or 2.3%. Both fungicides provided excellent to very good control of *Fusarium* species, in addition to control of *Pythium* and *Phytophthora*, but had no effect on *A. euteiches.* Rancona V RTU FS combines carboxin, a mitochondrial respiration inhibitor that targets succinate dehydrogenase, with metalaxyl and ipconazole. Addition of carboxin did not alter the spectrum of fungicidal activity compared to the other Rancona products. Evergol Energy combines metalaxyl,

prothioconazole, a sterol biosynthesis inhibitor, and penflufen, a succinate dehydrogenase inhibitor. This combination resulted in growth inhibition of all pathogens: excellent to very good EC_{50} values against *Pythium* species, excellent values against *P. medicaginis*, fair to good values against *Fusarium* species, and good values against *A. euteiches*. Penflufen and prothioconazole have been shown to have activity against ascomycete and basidiomycete fungi but have not been previously reported to have activity against an oomycete. Evergol Energy is currently labeled for use on alfalfa seed and due to the large range of activity against alfalfa pathogens, would most likely be an effective alternative to Apron or ApronXL.

The two strobilurin class fungicides that inhibit mitochondrial cytochrome c, Trilex (active ingredient trifloxystrobin) and Dynasty (active ingredient azoxystrobin) had different spectrums of activity against the alfalfa pathogens. Trilex had poor activity against all pathogens while Dynasty had fair to good EC_{50} values against *Pythium* and *Phytophthora*, good values against *Aphanomyces* and poor values against *Fusarium* species. A third strobilurin fungicide, Stamina (active ingredient pyraclostrobin) is labeled for use on alfalfa seed and was shown previously to have excellent activity against *A. euteiches* but good to poor activity against *Pythium* species (3).

The fungicide Vibrance (active ingredient sedaxane), which is widely used on seeds of many field crops for control of damping off, had poor activity against the alfalfa damping off pathogens. Previous research indicated that it has high EC_{50} values against *Pythium ultimum*, *Phytophthora infestans*, and *Fusarium species* (11).

Intego Solo (active ingredient ethaboxam) has previously been shown to have broad activity against the Peronosporales oomycete plant pathogens (12) and to have excellent EC_{50} values for *Pythium* species and *Phytophthora sojae* attacking soybean seeds (13, 14). Against alfalfa pathogens it had very good values against *Pythium* species, excellent values against *A. euteiches*, excellent to very good values against *P. medicaginis* and poor activity against *Fusarium* species. This is the first report of ethaboxam EC_{50} values for *A. euteiches*. A recent report evaluated ethaboxam seed treatment against Aphanomyces root rot of pea caused by *A. euteiches* under greenhouse and field conditions (15). The seed treatment was moderately effective in greenhouse conditions but not in field conditions with additional pathogens present. Valent suggests that Rizolex be used in combination with Intego Solo for control of fungal pathogens. However, Rizolex had high EC_{50} values against the alfalfa *Fusarium* pathogens tested.

Based on EC_{50} values, ApronXL, Evergol Energy, and Intego Solo were tested as seed treatments with infested soil. As shown in Figure 1, the introduction of pathogens into the soil was effective in causing seed rot and damping off with the nontreated seeds having a significantly lower seedling counts than the control seeds in uninfested soil. The three seed treatments significantly increased seedling counts. ApronXL and Evergol Energy were the most effective seed treatments. When the soil was flooded immediately after planting, seedling counts were reduced. Apron XL was the most effective treatment under these conditions. When the soil was flooded two days after seeding, ApronXL and Evergol Energy performed similarly to the control. Evergol Energy appears to be an effective alternative to ApronXL with additional activity against *A. euteiches*. Additional experiments are needed to validate these results by testing the seed treatments under field conditions in multiple locations.

Biological seed treatments were not effective against alfalfa seed root and damping off pathogens. Biological seed treatments are of interest due to the low risk of developing

resistance compared to chemical seed treatments, longer windows of activity, and the potential for multiple benefits such as nutrient acquisition. Seed treatments used with alfalfa need to be active in cool temperatures under which alfalfa seeds germinate in spring seasons. The five proprietary treatments tested did not show activity against *Pythium* in the culture plate assay or infested soil assay (Fig. 2), or against *P. medicaginis* or *A. euteiches* in growth chamber assays (Fig. 3).

Development of alfalfa germplasm resistant to Pythium seed rot and damping off. Two cycles of selection were done to increase resistance in three germplasm sources. The first cycle was done with strain BEC56, which is moderately aggressive, and the second cycle was done with BEC56, WAS53, a highly aggressive isolate, and L3, a moderately aggressive isolate. Each germplasm responded to the two cycles of selection differently, but resistance generally increased with selection. UMN2804 had the highest amount of resistance in the parental population with 12% resistant plants to WAS53 and 36% resistant plants to BEC56 and L3 (Fig. 4). This germplasm had been selected previously under wet field conditions for resistance to soil borne pathogens, which likely resulted in increased resistance to *Pythium* species. Selection did not significantly increase resistance to BEC56 but resistance did increase significantly in cycle 1 to WAS53 and L3. Cycle 2 resulted in a decrease in percent resistant plants to WAS53 and L3. As observed previously (6), selection using a single strain of *Pythium* increased resistance to other more aggressive strains of the pathogen. Plants from cycle1 had greater resistance to WAS53 and L3 than the parental material, although selection was carried out with BEC56. The parental germplasm of UMN2841 had undergone one cycle of selection for resistance to a strain of *P. ultimum* that increased resistance from 2% in the base germplasm to 25% after selection (6). The plants resulting from the first cycle of selection in this experiment were significantly more resistant to all three *Pythium* strains with 58% resistant plants to WAS53, a highly aggressive strain. A second cycle of resistance using multiple strains for selection did not increase resistance to BEC56 or L3 and had a negative effect on resistance to WAS53. Plants from the UMN3988 germplasm showed gains in disease resistance to each strain with each cycle of selection. The greatest gain was in resistance to WAS53. The parental germplasm had no resistance to this strain but resistance increased to 32% after cycle 1 and 72% after cycle 2. All germplasm developed in this project will be deposited in the USDA-National Plant Germplasm System.

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Table 1. Fungicides evaluated for activity against alfalfa seed rot and damping off pathogens.

Fungicide	Company	Active Ingredient	Active Ingredient (g/L)	Application Rate[1]
ApronXL	Syngenta	Mefanoxam	360	0.64
Rancona Summit	Arysta	Ipconazole	9.6	
		Metalaxyl	15.4	
Rancona Dimension	Arysta	Ipconazole	25.0	
		Metalaxyl	20.0	
Rancona V RTU FS	Arysta	Carboxin	133.0	
		Metalaxyl	13.3	
		Ipconazole	5.0	
Evergol Energy	Bayer	Prothioconazole	76.8	3.0
		Penflufen	38.4	
		Metalaxyl	61.4	
Trilex Flowable	Bayer	Trifloxystrobin	239.0	
Dynasty	Syngenta	Azoxystrobin	99.5	
Vibrance	Syngenta	Sedaxane	515.0	
Intego Solo	Valent	Ethaboxam	3.2	0.6
Rhizolex	Valent	Tolclofos-methyl	503.3	

[1] Application rate was fluid ounces/100 pounds of seed

Table 2. Pathogens used in this research.

Pathogen	Strain
Pythium irregulare	BEC56
Pythium sylvaticum	BEC 63
Pythium sylvaticum	STH 33
Pythium ultimum var *ultimum*	WAS 126
Pythium ultimum var *ultimum*	WAS 53
Pythium paroecandrum	L3
Aphanomyces euteiches race 1	MF-1
Aphanomyces euteiches race 2	MER-4
Aphanomyces euteiches race 2	GRE-5
Aphanomyces euteiches race 2	JIM-1
Fusarium verticilliodes	113
Fusarium incarnatum-equiseti	180
Fusarium oxysporum	184
Phytophthora medicaginis	Pm2019
Phytophthora medicaginis	W10
Phytophthora medicaginis	A1A2

Table 3. Growth inhibition of seed rot and damping off pathogens by commercial fungicide preparations. EC_{50} values were calculated for each strain. Excellent (E)= <0.05-0.1 µg/ml, Very Good (VG) = 0.11-0.99 µg/ml, Good (G) = 1.0-9.9 µg/ml, Fair (F) = 10-99.9 µg/ml, Poor (P) = >100 µg/ml. ND= not determined.

Fungicide	***Pythium***	***Aphanomyces***	***Phytophthora***	***Fusarium***
ApronXL	E-VG	P	E	P
Rancona Dimension	E-VG	P	E	E-VG
Rancona Summit	E-G	P	E	VG
Rancona V RTU FS	E-VG	P	E	VG
Trilex	P	P	P	P
Dynasty	G-F	G	G-F	P
Evergol Energy	E-VG	G	E	G-F
Vibrance	P	P	P	P
Intego Solo	VG	E	E-V	P
Rizolex	ND	ND	ND	F-P

Figure 1. Protection of alfalfa seeds from seed rot and damping off pathogens in infested soil by fungicide seed treatments. Seedling counts were made at 7 days after planting in soil infested with cultures of *Pythium*, *A. euteiches*, and *Phytophthora medicaginis*. Plants were grown at 18°C with a 16 h photoperiod. A, moist soil conditions. B, soil saturated for 24 h after planting. C, soil saturated 2 days after planting for 24 h.

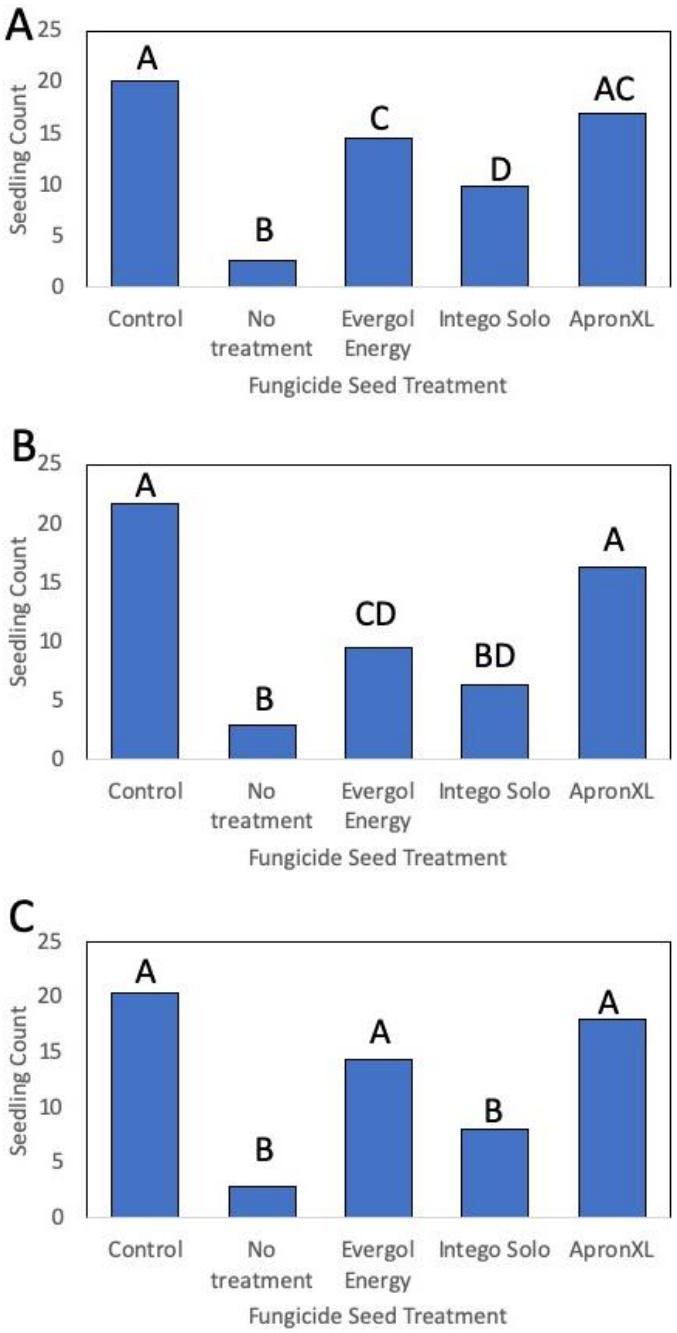


Figure 2. Effect of biological seed treatments on Pythium seed rot and damping off symptoms. Seeds were coated by a commercial process with protectants and a clay coating. Treatment 1=control, no treatment; 2=ApronXL fungicide treatment; 3-7=proprietary biological seed treatments. A, Percent protected plants in the agar plate assay for Pythium seed rot and damping off. B, Percent protected plants in an infested soil assay.

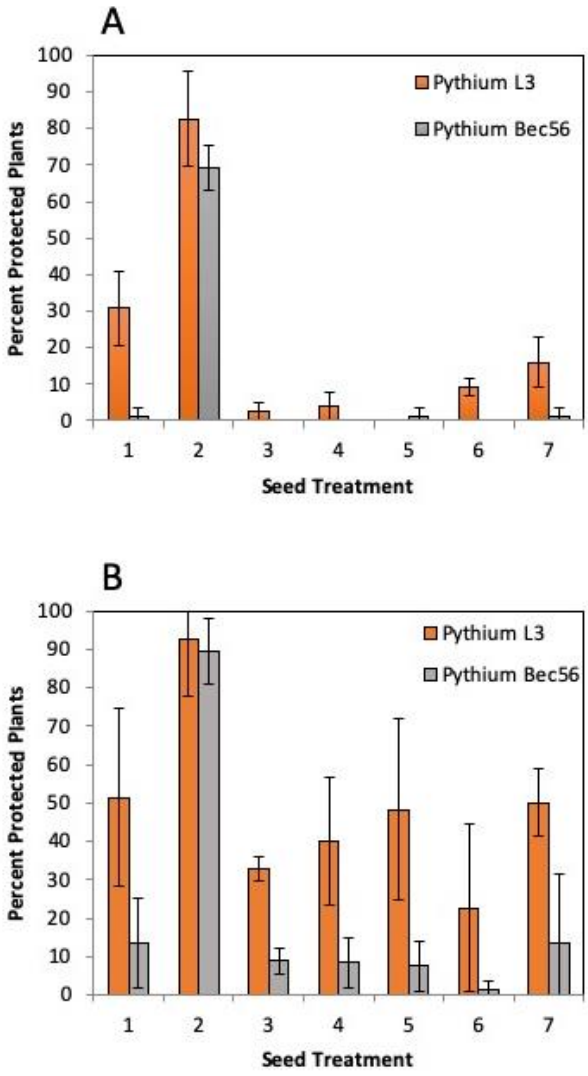


Figure 3. Effect of biological seed treatments on Phytophthora root rot (PRR) and Aphanomyces root rot (ARR) symptoms. Seeds were coated by a commercial process with protectants and a clay coating. Treatment 1=control, no treatment; 2=ApronXL fungicide treatment; 3-7=proprietary biological seed treatments. Severity of symptoms were rated at 14 days after inoculation and averaged across all plants within a treatment. 1=no necrosis of roots or hypocotyl; 2=slight necrosis of roots or hypocotyl; 3= necrosis of roots and lower hypocotyl and moderate stunting of stems; 4=extensive necrosis of roots, hypocotyls and cotyledons, and severe stunting of stem; 5=dead seedling.

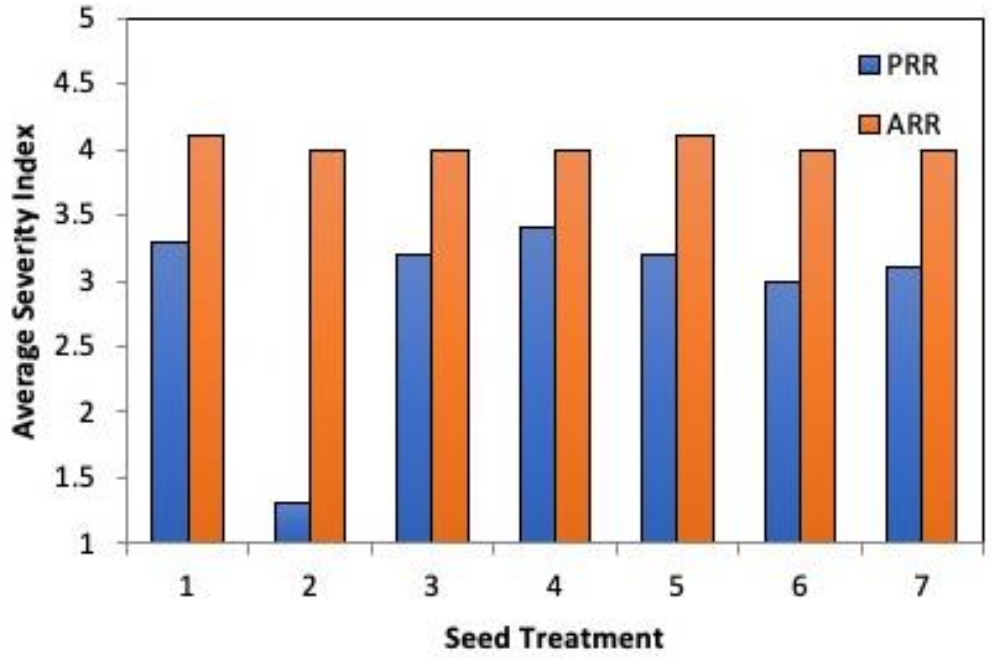
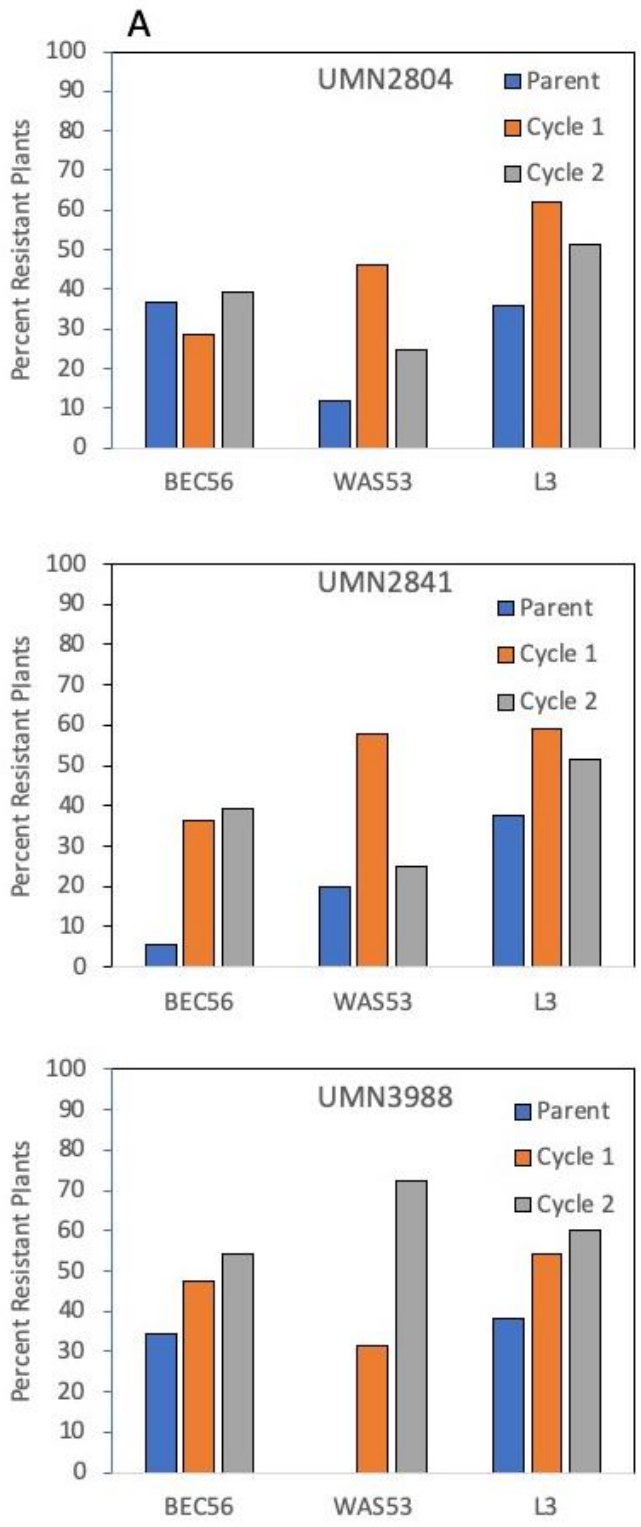


Figure 4. Response of parental and selected populations to three *Pythium* strains. Seeds were tested for resistance to seed rot and damping off in the agar plate-based assay. Percent resistant plants were scored 6 days after inoculation.



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