

# Evaluation of Crucifer Green Manures for Controlling *Aphanomyces* Root Rot of Peas

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## ABSTRACT

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Several different species of crucifers were tested as soil amendments for the control of *Aphanomyces* root rot of peas. In a 2-yr field study, crucifers were planted the first year as full season crops or as fall cover crops following peas. The crucifers were incorporated as green manures. The following spring, peas were planted in all the plots, and stand counts and fresh pea yields were determined. Significant treatment effects on stand loss were found. Average yields were not significantly different. A fall crop of white mustard (*Sinapis alba* L.) following peas appeared most promising and was chosen for further study. In subsequent field studies, a fall crop of white mustard significantly reduced root rot severity ratings of pea plants grown the following year. After two successive cycles of peas followed by white mustard, an increase in pea yield and a decrease in root rot severity were observed. In a growth chamber bioassay, white mustard amendment reduced the number of infective propagules of *Aphanomyces euteiches* Drechs. in infested soil.

*Aphanomyces euteiches* Drechs. is the most economically important pathogen of peas (*Pisum sativum* L.) in the Great Lakes states, where processing peas are grown (7). Annual losses caused by the pathogen are estimated at 10%. *A. euteiches* can also cause damage to beans (*Phaseolus vulgaris* L.) (23) and alfalfa (*Medicago sativa* L.) (3,28). On pea plants, the pathogen causes a severe rotting of the root cortex, hypocotyl, and epicotyl. Infection is greatest in wet soil (20), but damage may only become apparent under dry conditions, when reduction in water transport due to root rot becomes critical.

Although the fungus was first recognized as a pathogen of peas in 1925 (4,9), there is still no economically

feasible control for *Aphanomyces* root rot other than avoidance of heavily infested fields identified by the disease severity index assay (29). Cultural and chemical strategies used against pea root rot caused by *A. euteiches* and other fungi have been reviewed (31). The pathogen appears to be able to survive in soil for over 10 yr without a crop of peas (10). No fungicides that control the disease are commercially available. Dinitro-aniline herbicides have been shown to adversely affect *A. euteiches*, and may be useful in delaying inoculum increases in pea fields (5,6,8,30). Soil treatment with fumigants that release the sulfur-containing compound methyl isothiocyanate has been shown to reduce pea root rot (21), but it is not economically feasible for pea growers.

Research has been conducted on the effects of incorporated crucifer tissues on activity of the soilborne pathogens *Rhizoctonia solani* Kühn (14), *Thielaviopsis basicola* (Berk. & Br.) Ferr.

(1,19,22), *Fusarium oxysporum* Schlecht. f. sp. *conglutinans* (Wr.) Snyd. & Hans. (26,27), *Aphanomyces cochlioides* Drechs. (12), and *A. euteiches* (2,15-18,21). Researchers have speculated that sulfur-containing compounds released during the breakdown of glucosinolates in crucifer tissues may act as soil fumigants and be responsible for effects on soilborne pathogens (11,13).

Some of the most promising work with crucifer amendments has dealt with *Aphanomyces* root rot of peas. In greenhouse studies, Papavizas (17) and Papavizas and Lewis (21) found that air-dried leaves and stems of cabbage (*Brassica oleracea* L. var. *capitata* L.), kale (*Brassica oleracea* L. var. *acephala* DC.), mustard (*Brassica nigra* L.), and turnip (*Brassica rapa* L.) reduced pea root rot when incorporated into soils infested with *A. euteiches*, whereas Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* Zenk.) and radish (*Raphanus sativus* L.) were less effective. Recent greenhouse studies by Chan and Close (2) also demonstrated the effectiveness of some crucifers against *Aphanomyces* root rot and the large differences in effectiveness between different crucifer species. They found cabbage to be the most effective of the amendments tested, followed by fodder radish (*Raphanus sativus* L.), rapeseed (*Brassica napus* L.), white mustard (*Sinapis alba* L.), and kale.

The ability of crucifer green manures to control *Aphanomyces* root rot of peas has not been satisfactorily demonstrated in the field. Trials with pots of infested soil buried in the field have been successful (21), but no control has been demonstrated in uncontained field plots.

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Mitchell and Hagedorn (15) attempted to control pea root rot in an infested field through the incorporation of fresh cabbage tissue brought to the site. No difference was found in disease between amended and unamended plots, probably because the amount of tissue incorporated was not enough to reduce disease (20). The purpose of the present research was to examine the potential of crucifer green manures to control *Aphanomyces* root rot in the field.

## MATERIALS AND METHODS

**Comparison of crucifer green manures.** Cabbage (*Brassica oleracea* L. var. *capitata* L. 'Brunswick'), curled mustard greens (*B. juncea* L. 'Southern Giant Curled'), yellow seed mustard (*B. campestris* L. ssp. *trilocularis* (Roxb.) Olsson), rapeseed (*B. campestris* L. 'Candle'), white mustard (*Sinapis alba* L. 'Emergo'), and oilradish (*Raphanus sativus* L. 'Resal') were tested in the field for their ability to suppress root rot of peas. Corn (*Zea mays* L.) was included as a noncrucifer crop often grown in rotation with peas. The treatments included each crop as a full season crop and (except for corn) as a fall cover crop following peas (cv. Perfection 8221, treated with captan). Treatments of fallow and peas followed by fallow were also included. Fallow treatments were kept free of all weeds by hand-weeding. Each crop was grown in 1.2-m (4-ft) square plots in rows 20 cm apart for peas and crucifers, and 30 cm apart for corn. Crucifer seeds were planted 7.5 cm apart, peas 5 cm apart, and corn 12.5 cm apart. Five plots of each treatment were planted in a randomized complete block design.

Plots were planted in late April 1986 at a pea root rot nursery at the Arlington Agricultural Research Station. The soil was a Plano silt loam that had been planted continuously to peas. It had, per hectare, a pH of 6.9, 76 t of organic matter, 7.5 t of nitrogen, 213 kg of phosphorus, and 330 kg of potassium. Rainfall was supplemented once during the season with overhead sprinkler irrigation. Weeding was done by hand. Crucifers were sprayed twice with carbaryl (60 ml per plot, 60 g of a.i. per liter) and twice with rotenone (60 ml per plot, 25 cm<sup>3</sup> of 1% rotenone per liter) during May and June to control flea beetle populations.

Plants were chopped and incorporated into soil at a time when each crop was judged to have maximum biomass (mid-July to early August). Cabbage and curled mustard did not produce seeds. Cabbage was starting to form heads and curled mustard was approximately 60 cm high and very leafy at the time of incorporation. Seeds from the other crucifers were removed before the plants were incorporated. These latter crops were approximately 1 m high at the time of incorporation. Pea plants were incorpor-

ated in early August, after pod formation. Plant material was chopped into pieces measuring about 15 cm and turned under with a spade to a depth of about 20 cm. Corn stalks were cut off to a height of 30 cm and removed in early fall, and the residues were turned under in mid-October. This treatment was judged comparable to that of a machine-harvested corn crop.

Fall crucifer crops were planted in mid-August. They were sprayed with rotenone once in late August and once in early September to control flea beetles. Plants were chopped and dug under in mid-October.

The following spring, all the plots were rototilled and planted to peas (Perfection 8221, captan treated) at the beginning of May. Emergence percentages were determined 18 days after planting, and stand counts were taken weekly, beginning 2 wk after the initial emergence count. To avoid destructive sampling, root rot severity ratings were not determined, although disease symptoms were evident in the field. The plots were irrigated once. Weeding was again done by hand.

In late June, peas were hand-harvested from each plot at the processing stage. The peas from each plot were shelled and weighed. Pea yields, germination and subsequent stand counts, and percent stand losses were analyzed by two-way analysis of variance and compared by LSD comparisons. A Spearman's rank correlation coefficient was calculated for comparison of the yield and stand loss data.

**Effects of white mustard.** Using information gained from the first field experiment, a second experiment was designed to further examine the effects of a crucifer green manure on pea root rot. A fall crop of white mustard following a spring crop of peas, the most promising treatment in the initial field trial, was chosen as the sole crucifer treatment. This was compared with the standard practice of not following peas with a fall crop. An area of the Arlington root rot nursery was chosen that had been planted to peas in spring 1987. The field was plowed and cultivated, and in late July white mustard was planted in half of the plots. The remaining plots were kept fallow and weed-free. There were 30 1.2-m-square plots in all, arranged in a paired design. Plants were watered once, 1 wk after planting, and sprayed with rotenone three times, 5 days apart, starting 12 days after planting. In late September, the plants were approximately 75 cm high and were beginning to flower. The plants were removed from the soil, weighed, chopped, and then dug under as in the previous year. An average of 11 kg of fresh plant material (shoots and tap roots) was dug into each plot.

In 1988, peas were planted in early May in all plots. Live plants were

counted after emergence. To determine whether the white mustard amendment directly affected disease, obtaining pea root rot severity ratings was judged important enough to warrant some destructive sampling in this experiment. Seven weeks after planting, the center row (not included in stand counts) of each plot was removed and the plants were rated for root rot severity using a scale where 0 = healthy plant and 4 = dead plant (29). An average root rot severity rating was calculated for each plot. Fresh pea weights for each plot were determined at harvest. Emergence counts, root rot severity ratings, and pea fresh weights were compared in mustard and fallow plots using paired-sample Student's *t* tests.

After the pea harvest, the plots were rototilled in late July, and white mustard was planted in the same plots as in the previous year. At the same time, a new set of plots in the Arlington root rot nursery was planted to white mustard in a repeat of the previous year's experiment. These plots were irrigated twice to supplement rainfall. In early October, just as the plants began flowering, they were incorporated as previously described.

In the spring of 1989, *Rhizobium*-treated peas were planted in all the plots. Plants were counted after emergence. Plots were irrigated with approximately 2.5 cm of water each week, beginning 3 wk after planting. Root ratings were determined twice during the season, at 6 and 8 wk after planting. Isolations from these roots were made on MBV (metalaxyl, benomyl, vancomycin) agar (24) to confirm the presence of the pathogen. At harvest, fresh pea yields were determined as before. Comparisons were made using paired Student's *t* tests.

**Bioassay for *Aphanomyces euteiches*.** A growth chamber study was conducted to determine the effects of a crucifer amendment on the population density of *A. euteiches*. White mustard plants grown in the greenhouse were ground in a food processor (Cuisinart DLC-7, Greenwich, CT) and used to amend soil at a rate of 150 g of fresh tissue per 500 cm<sup>3</sup> of air-dry soil. Naturally infested field soil from the Arlington root rot nursery was used, both fresh and pasteurized (at 70 C for 1 hr). Unamended soil served as a control. Both treatments were moistened as necessary to achieve a gravimetric water content of 34%. The soil was left for 1 wk in open bags and was then used for a most-probable-number bioassay of infective *Aphanomyces* propagules per aliquot of soil (25). The soils to be tested were diluted to 80, 60, 40, and 20% with pasteurized soil.

For the bioassay, peas (Perfection 8221 treated with captan and *Rhizobium*) were grown in 60-cm<sup>3</sup> cones (Ray Leach Conetainer Nursery, Canby, OR) at 24 C in a growth chamber with a 12-

hr photoperiod. Each cone contained a synthetic cosmetic puff in the bottom (to prevent vermiculite leakage), 25 cm<sup>3</sup> of vermiculite, and 4 cm<sup>3</sup> of the test soil below two pea seeds covered with 5 cm<sup>3</sup> of additional vermiculite. Ten cones were planted for each soil dilution. Cones were top-watered until emergence, and then the water level was maintained at 11 cm below the soil level by inserting the rack of cones in pans of deionized water. Three weeks after planting, the plants were harvested and rated as positive or negative for symptoms of *Aphanomyces* root rot. A cone was rated as positive if either or both of the plants in a cone showed symptoms. Ratings were compiled, and the most probable number of infective propagules in the soils was calculated (25). The growth chamber experiment was repeated once.

## RESULTS

In the 1986-1987 field trial, in which several different crucifers were compared for their effectiveness in reducing *Aphanomyces* root rot, no significant differences were found in emergence counts of pea plants among the treatments. Significant stand count differences between treatments only occurred at the final count, just before harvest (data not shown). An analysis of the percent stand reduction in each plot between the emergence count and the final count similarly revealed significant differences among treatments (Table 1). Analysis of variance of fresh pea yields for each plot did not reveal any significant differences between treatments (Table 1). Yields were, however, correlated with percent stand loss ( $r_s = -0.681$ ,  $P < 0.01$ ).

For the 1987-1988 experiment, analysis by paired-sample Student's *t* test revealed that the mean pea root rot severity in plots treated with white mustard was significantly lower than in non-treated plots (Table 2). Emergence was significantly reduced by the white mustard treatment, but no significant difference was found in fresh pea yields between white mustard-treated and fallow plots. Reduced nodulation was noted on plants from the amended plots.

When the paired experiment was repeated in a different plot in 1988-1989, root rot severity was again significantly reduced by the white mustard amendment (Table 2). As in the previous study, emergence was reduced, and yield was not affected by the crucifer amendment.

After two cycles of peas and white mustard, root rot severity was again significantly lower in the plots amended with white mustard (Table 2). In spite of reduced pea emergence in the treated plots, fresh pea yields were significantly higher in treated than in nonamended plots. Reduced nodulation was noted in both sets of crucifer-amended plots in 1989. The presence of *A. euteiches* was

confirmed by isolation from 87% of symptomatic plants sampled 6 wk after planting.

Results of the growth chamber bioassay indicated that amendment of naturally infested soil with white mustard reduced the number of infective *Aphanomyces* propagules from 0.287 to 0.001 propagules per gram of soil.

## DISCUSSION

In the first field study, stand loss differences between treatments only became apparent at the final stand count, just before harvest. Although none of the fresh pea yields from plots in the first field study were significantly different, the significant correlation between stand loss (which was significantly affected by crucifer treatments) and yield indicates that crucifer amendments could potentially affect yields. A fall crop of white mustard following peas appeared very

promising. A full season of fallow, which resulted in an average yield similar to that of this treatment, is not an economically feasible option for growers. Demonstration of significant differences between the field treatments was prevented by high random variability in yield among the five field plots per treatment. This variability may be due to uneven distribution of pathogen propagules throughout the field at the start of the experiment, uneven watering by overhead sprinklers (particularly if the irrigation came at a critical time for infection), or many other unidentified factors affecting either disease or plant growth. To overcome this random variability, the number of replications per treatment was increased to 15 in the second field experiment.

A fall crop of white mustard following peas was chosen as the crucifer treatment for further field studies. In both repeti-

**Table 1.** Loss in plant stands over the growing season and fresh pea yields in 1987 in field plots planted to different green manure crops in 1986

| Treatment 1986                  | Percent stand loss 1987 <sup>y</sup> | Pea yield (g/plot) 1987 <sup>y</sup> |
|---------------------------------|--------------------------------------|--------------------------------------|
| fallow                          | 31.2 a <sup>x</sup>                  | 428 <sup>y</sup>                     |
| peas/white mustard <sup>z</sup> | 32.8 ab                              | 427                                  |
| white mustard                   | 33.8 abc                             | 390                                  |
| rapeseed                        | 34.2 abc                             | 316                                  |
| peas/fallow                     | 39.8 abcd                            | 299                                  |
| peas/cabbage                    | 41.4 abcd                            | 323                                  |
| peas/rapeseed                   | 41.8 abcd                            | 373                                  |
| corn                            | 42.2 abcd                            | 380                                  |
| peas/oilradish                  | 42.4 abcd                            | 360                                  |
| yellow seed mustard             | 45.6 bcde                            | 336                                  |
| peas/yellow seed mustard        | 46.6 cde                             | 339                                  |
| peas/curled mustard greens      | 47.6 de                              | 310                                  |
| curled mustard greens           | 48.2 de                              | 346                                  |
| cabbage                         | 52.0 de                              | 351                                  |
| oilradish                       | 56.2 e                               | 288                                  |

<sup>y</sup> Percent stand loss = (emergence stand count — harvest stand count)/emergence stand count × 100.

<sup>w</sup> Correlation between stand loss and pea yield (Spearman's rank correlation coefficient,  $r_s = -0.681$ ) was significant at the 0.01 probability level.

<sup>x</sup> Mean stand losses followed by common letters are not significantly different ( $P = 0.05$ ) according to LSD test.

<sup>y</sup> Differences between mean fresh pea yields were not statistically significant.

<sup>z</sup> Fall cover crops following peas are listed as peas/crop.

**Table 2.** Emergence, root rot severity, and fresh yield of peas grown after one and two cycles of peas followed by white mustard or fallow

| Crop                      |              |      | Emergence (plants/plot) | Root rating <sup>w</sup> |             | Yield (g/plot)  |
|---------------------------|--------------|------|-------------------------|--------------------------|-------------|-----------------|
| 1987                      | 1988         | 1989 |                         | At 6 wk                  | At 8 wk     |                 |
| Peas/mustard <sup>x</sup> | Peas         | ...  | 120                     | 1.54                     | ...         | 437             |
| Peas/fallow               | Peas         | ...  | 128                     | 2.17                     | ...         | 434             |
|                           |              |      | $P = 0.005^y$           | $P = 0.005$              |             | NS <sup>z</sup> |
| ...                       | Peas/mustard | Peas | 141                     | 0.38                     | 1.63        | 200             |
| ...                       | Peas/fallow  | Peas | 152                     | 0.81                     | 2.45        | 212             |
|                           |              |      | $P = 0.005$             | $P = 0.027$              | $P < 0.001$ | NS              |
| Peas/mustard              | Peas/mustard | Peas | 141                     | 0.68                     | 1.93        | 184             |
| Peas/fallow               | Peas/fallow  | Peas | 150                     | 1.48                     | 2.83        | 154             |
|                           |              |      | $P < 0.001$             | $P < 0.001$              | $P < 0.001$ | $P = 0.002$     |

<sup>w</sup> Rating scale: 0 = healthy plant, 4 = dead plant (29).

<sup>x</sup> A fall crop of white mustard and fall fallow are listed as peas/mustard and peas/fallow, respectively.

<sup>y</sup> Probability values ( $P$ ) based on paired Student's *t* tests.

<sup>z</sup> NS indicates no significant difference ( $P > 0.05$ ) between pairs of means.

tions of the experiment in which peas were examined following one fall crop of white mustard, root rot severity was decreased by the green manure crop. Yield, however, was only significantly affected by two fall crops of white mustard. It is important to note, however, that although the effect was not seen until after two fall crops, a spring crop of peas was planted each year and the two crucifer crops were, therefore, not successive. The stand reduction observed in the amended plots of each experiment indicate that an increased pea seeding rate may be required following the use of a white mustard green manure.

Soil amendment with white mustard appeared to reduce the number of infective propagules of *Aphanomyces*. This was demonstrated in the growth chamber bioassay after infested soils amended with the crucifers were incubated for only 1 wk. According to comparisons of most-probable-number estimates of inoculum density with root rot potential assessments of loam soils (25), a value of 0.287 infective propagules per gram would fall within the range deemed hazardous for peas, whereas 0.001 infective propagules per gram would correspond to soils with low root rot hazard.

Papavizas (17,18) and Papavizas and Lewis (21) did not use white mustard in their crucifer amendment trials. Chan and Close (2) used dried leaves and stems of white mustard in their greenhouse trials and found it to be not as effective as cabbage in reducing pea root rot disease severity. White mustard may be more effective than cabbage only when grown under field conditions, where environmental stresses may alter the growth and development of the two plants, affecting their disease control properties. Different cultivars of cabbage and mustard, different ages of the plants at the time of incorporation, the difference between dry and fresh plant material, and the presence or absence of root tissue may also account for the difference between the results of Chan and Close and the present field studies. Although air-dry tissue was not tested in these trials, the fresh tissue used was effective.

This research indicates that a green manure crop of white mustard does have the potential to be an important part of pea root rot management programs, especially since white mustard can be effectively used as a fall cover crop fol-

lowing peas. The use of fall green manures is a practical, easily implemented control strategy. Their use may even have more far-reaching benefits than the control of *Aphanomyces* root rot of peas. Other pathogens of peas and other crops may be affected, as might weeds and soil-dwelling insects. With the loss of many chemical pesticides from the market and increasing concern over their use, control strategies such as the use of crucifer green manures deserve further investigation.

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