BENEFIT OF TACHIGAREN–TREATED SUGARBEET SEED IN SOILS WITH DIFFERENT APHANOMYCES SOIL INDEX VALUES

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Aphanomyces cochlioides (= *A. cochlioides* or *Aphanomyces*) is a soilborne fungus that causes seedling stand loss and root rot when soil is warm and wet. Once a field is infested with *A. cochlioides,* the pathogen persists in soil indefinitely as oospores (thick-walled survival spores). Severe Aphanomyces root rot on sugarbeet has been reported in fields that were abandoned because of the disease 10 to 20 years earlier. Thus, it is important to integrate all options to maximize control of this potentially devastating disease on sugarbeet. A primary management strategy is to plant seed of a variety with partial resistance or tolerance to *Aphanomyces* that has been treated with the fungicide Tachigaren (= hymexazol). Other control measures, however, also are necessary and include early planting to avoid warm and wet soil conditions favorable for disease, tillage and cultivation to improve soil drainage and drying, and weed control to eliminate alternate hosts.

Fields can be indexed to determine the potential for Aphanomyces root rot. Soil samples are collected to a 6-inch depth within an area of the field where disease is suspect or where problems have been observed (about 1 gallon of soil is needed). Sugarbeet seeds are planted in the soil and then placed in a greenhouse or controlled environment chamber set at conditions favorable for *Aphanomyces*. Sugarbeet seedlings become infected and "bait" *A. cochlioides* from soil. About 4 weeks after planting, seedlings are rated for root rot and an Aphanomyces soil index is calculated where values range from 0 to 100. An index value of 0 means that *Aphanomyces* was not detected in soil (all seedlings were healthy) and an index value of 100 means that soil is severely infested with *A. cochlioides* (all seedlings died and/or were severely rotted). The higher the soil index value, the greater the risk of the field to develop Aphanomyces root rot on seedlings and older plants when weather is wet and warm. Soil samples can be indexed at the University of Minnesota, Department of Plant Pathology, Plant Disease Clinic in St. Paul (on a fee basis). The American Crystal Sugar Cooperative also offers this service for their growers.

Since late 1995, Tachigaren has been registered in the United States for use on pelleted sugarbeet seed at the rate of 45 to 90 grams of product per 100,000 seed. This fungicide is highly effective in protecting young seedlings against infection by *A. cochlioides* and also has some activity against species of *Pythium* and *Rhizoctonia***.** All commercially sold sugarbeet seed is pretreated with the fungicides Apron and Thiram (to control seed rot and damping-off caused by *Pythium* and *Rhizoctonia solani*), so producers who purchase Tachigaren-treated seed (45 and 75g rates are available) bear an additional cost. With recent drops in sugar prices, producers are trying to reduce input costs. This has raised a question about the need for planting Tachigaren-treated seed in soils with a low Aphanomyces soil index value.

OBJECTIVES

The purpose of this research was to evaluate the benefit of two rates of Tachigaren (45 and 75 g/100,000 seed) on two commercially available sugarbeet varieties (susceptible and partially resistant to *Aphanomyces*) planted into soils spanning a range of Aphanomyces soil index values, and from these data, to determine the Aphanomyces soil index values where Tachigaren provides a significant benefit to both varieties.

MATERIALS AND METHODS

To create a range of low to high Aphanomyces soil index values, an *A. cochlioides*-free soil was collected from the University of Minnesota, Northwest Research and Outreach Center field plots (Wheatville loam). Oospores of *A. cochlioides* were grown on oatmeal broth for 4 weeks, extracted, counted, and mixed into vermiculite. Then, *Aphanomyces*-infested vermiculite was mixed into the field soil to produce seven batches of soil with oospore densities of 1, 5, 10, 15, 20, 25, and 50/gram (g) dry weight. The control soil was not inoculated with oospores.

Each of the inoculated and control soils was planted with two commercially available sugarbeet varieties. The susceptible variety has a high root rot rating in the Aphanomyces Betaseed Nursery in Shakopee, MN and is the standard variety used by American Crystal Sugar Company to index producers' field soils for *Aphanomyces*. The partially resistant variety is among the best performers in evaluations at the Aphanomyces Betaseed Nursery. Seeds of each variety were treated with 0, 45, and 75 g of Tachigaren (70% wettable powder)/100,000 seed. All seed also had been pretreated with the fungicides Apron and Thiram. Plastic pots (4 x 4 x 4 inches) were filled about 2.5 inches deep with soil, planted (25 seeds/pot, 6 pots/treatment), and covered with 1 inch of soil. Pots were placed in a growth chamber in a randomized block design. Soil was kept moist and incubated at 74 ?F during the day and 68 ?F at night until seedlings emerged. Then the temperature was increased to 83 ?F during the day and 77 ?F at night to favor disease, and day length was set for 16 hours. The experiment was repeated.

Data were collected on stand at emergence and then twice weekly. Dying seedlings were removed at each stand count and assayed microscopically to confirm infection by *A. cochlioides*. At 4 weeks after planting, surviving seedlings were removed from soil, washed, and rated for root rot based on condition of roots and discoloration of hypocotyls (region of stem between seed and cotyledonary leaves) with a 0-3 category rating scale. A category of 0 = hypocotyl and root healthy; 1 = hypocotyl light brown and some root loss; 2 = hypocotyl brown and rot advancing along root; and 3 = hypocotyl and root completely rotted (or seedling died earlier in the experiment). Then, an Aphanomyces soil index value was calculated for each pot of soil with the formula:

$$\frac{\text{Sum of (Number of plants in disease category x Disease category)}}{\text{Total number of emerged plants x 3}} \times 100$$

Aphanomyces soil index values range from 0 to 100 and each value includes stand loss and/or root rot. The Aphanomyces soil index values are obtained from rating roots of a specific susceptible variety not treated with Tachigaren (the same variety used by American Crystal Sugar Cooperative to index growers' fields). For this reason, the Aphanomyces <u>soil</u> index value and the Aphanomyces <u>root rot</u> index value are used interchangeably for the susceptible variety.

Data were subjected to arcsine transformations to correct for heterogeneity of error and then analyzed by analysis of variance, regression analysis, and orthogonal contrasts.

RESULTS

Main treatment effects for variety, seed treatment, and number of oospores/g of soil are shown for both experiments in Table 1. The partially resistant variety resulted in a significantly greater stand than the susceptible variety in one of two experiments and in significantly less root rot than the susceptible variety in both experiments. In both experiments, the 45 and 75g rates of Tachigaren seed treatment resulted in equal and significantly higher stands and less root rot compared to seed not treated with Tachigaren. An increase in the number of oospores/g of soil decreased seedling stands and increased root rot index values. The lowest levels of inoculum were somewhat inconsistent in initiating disease. In experiment 1, 5 oospores/g of soil resulted in a root rot index of 8.8 but in the second experiment, the same amount of inoculum resulted in a root rot index of 18.8. Low root rot index values in soil not inoculated with oospores are not unusual and occurred because of slight discoloration of a couple of roots by an unknown cause (and was not caused by *A. cochlioides*).

There was a significant interaction between variety and seed treatment at 4 weeks after planting for percent stand and root rot. Orthogonal contrasts revealed that the two varieties responded similarly for percent stand and root rot index values when seed was treated with the 45 and 75 g rates of Tachigaren, but when no Tachigaren was applied, they responded differently. When seed was not treated with

Table 1. Main effect of sugarbeet variety, seed treatment, and number of oospores of *Aphanomyces cochlioides*/gram dry weight of soil on percent stand and root rot at 4 weeks after planting (WAP) in each of two experiments conducted in controlled environment chambers.

	% Stand WAP[y]		Root Rot Index[y, z]	
Main treatment	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Variety[v]				
Partially resistant	91 a	89 a	16.5 a	17.5 a
Susceptible	88 a	82 b	20.5 b	26.1 b
Seed treatment[w]				
0	69 a	58 a	40.5 a	52.3 a
45 g	99 b	100 b	8.7 b	7.1 b
75 g	99 b	99 b	6.3 b	6.1 b
Number of oospores[x]				
0	100	100	2.0	2.5
1	99	98	3.6	3.0
5	98	87	8.8	18.8
10	94	83	13.9	24.8
15	89	79	22.5	28.7
20	89	79	21.4	29.0
25	76	84	35.2	30.8
50	69	74	40.6	36.8

[v] Each value is based on planting 3,600 seeds (averaged across 3 seed treatments and 8 concentrations of oospores).

[w] All seed was pretreated with Apron and Thiram; Tachigaren was applied at 45 and 75 grams/100,000 seeds; each value is based on planting 2,400 seeds (averaged across 2 varieties and 8 concentrations of oospores).

[x] Number of oospores/gram dry weight of soil; each value is based on planting 900 seeds (averaged across 2 varieties and 3 seed treatments).

[y] For each column of each main treatment, numbers followed by the same letter are not significantly different ($P \leq 0.05$).

[z] Root rot index values based on a 0 - 100 scale; 0 = plant healthy, 100 = all plants dead or severely rotted at 4 weeks after planting.

Tachigaren, the partially resistant variety had stands of 75% and 69% in experiments 1 and 2, respectively, compared to the susceptible variety which had stands of 63% and 47% in experiments 1 and 2, respectively. Root rot index values of the partially resistant variety were 34 and 42 in experiments 1 and 2, respectively, compared to the susceptible variety, where root rot index values were 48 and 62 in experiments 1 and 2, respectively.

There was a significant interaction between seed treatment and number of oospores in soil at 4 weeks after planting for percent stand and root rot. Results were similar for both experiments, so data are shown for experiment 1. When seed was not treated with Tachigaren, there was a dramatic decrease in percent stand (Figure 1A) and increase in root rot (Figure 1B) as oospore concentrations increased in soil (R^2 = 0.9282, R^2 = 0.9575, respectively). Orthogonal contrasts indicated that both varieties responded similarly, and equally, for percent stand and root rot indices when treated with the 45 or 75 g rates of Tachigaren. Seed treatment with Tachigaren resulted in consistently high stands across oospore concentrations (Figure 1A) and also resulted in low root rot indices that increased slightly, but significantly (R^2 = 0.8223), as inoculum concentrations increased (Figure 1B).

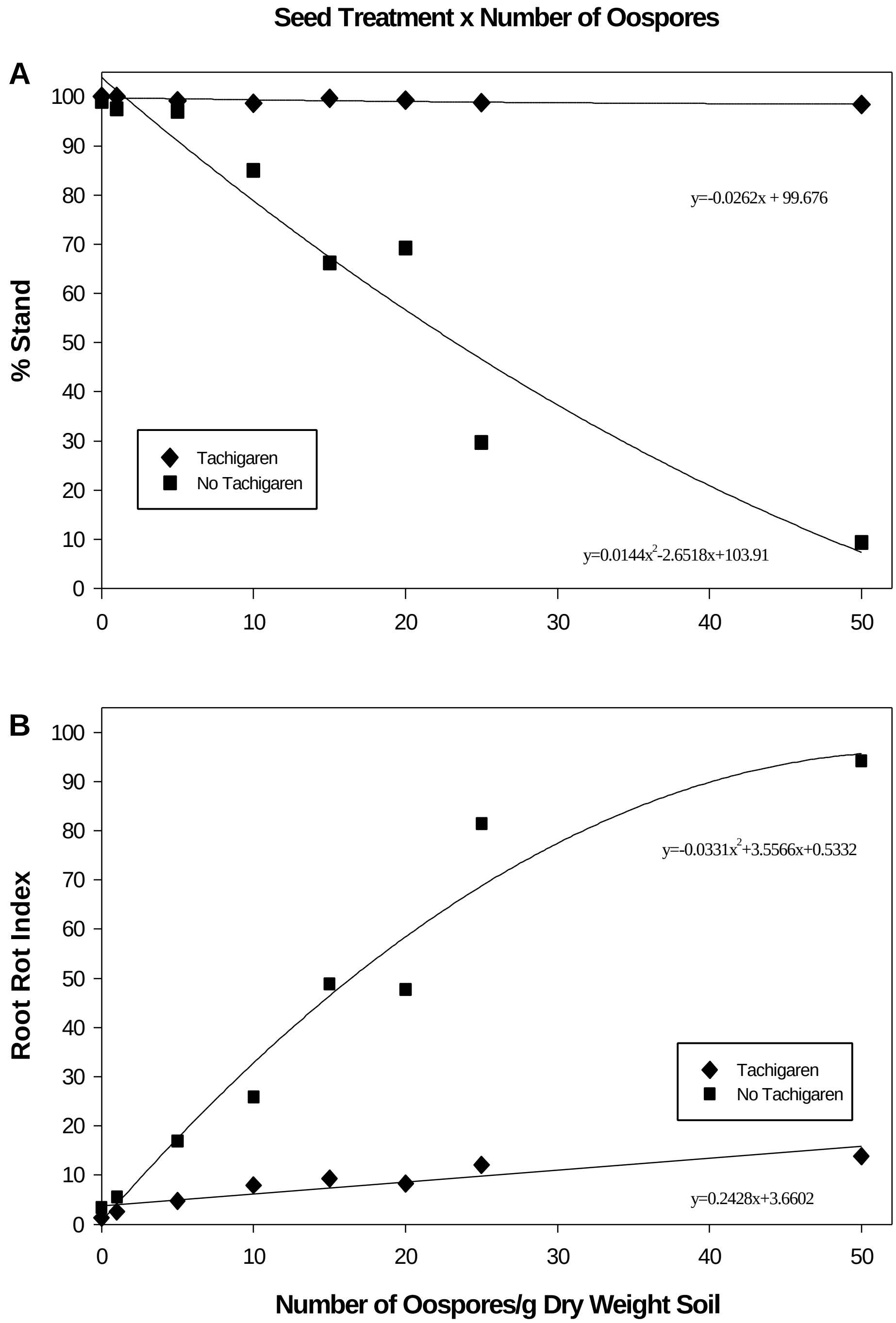

Figure 1. Interaction between seed treatment and number of oospores of *Aphanomyces cochlioides* in soil. Regression lines are shown for **A.)** percent stand and **B.)** root rot index verses number of oospores/g dry weight of soil. Values are averaged across a susceptible and parti ally resistant variety treated with Tachigaren (45 and 75 g rates) and not treated with Tachigaren.

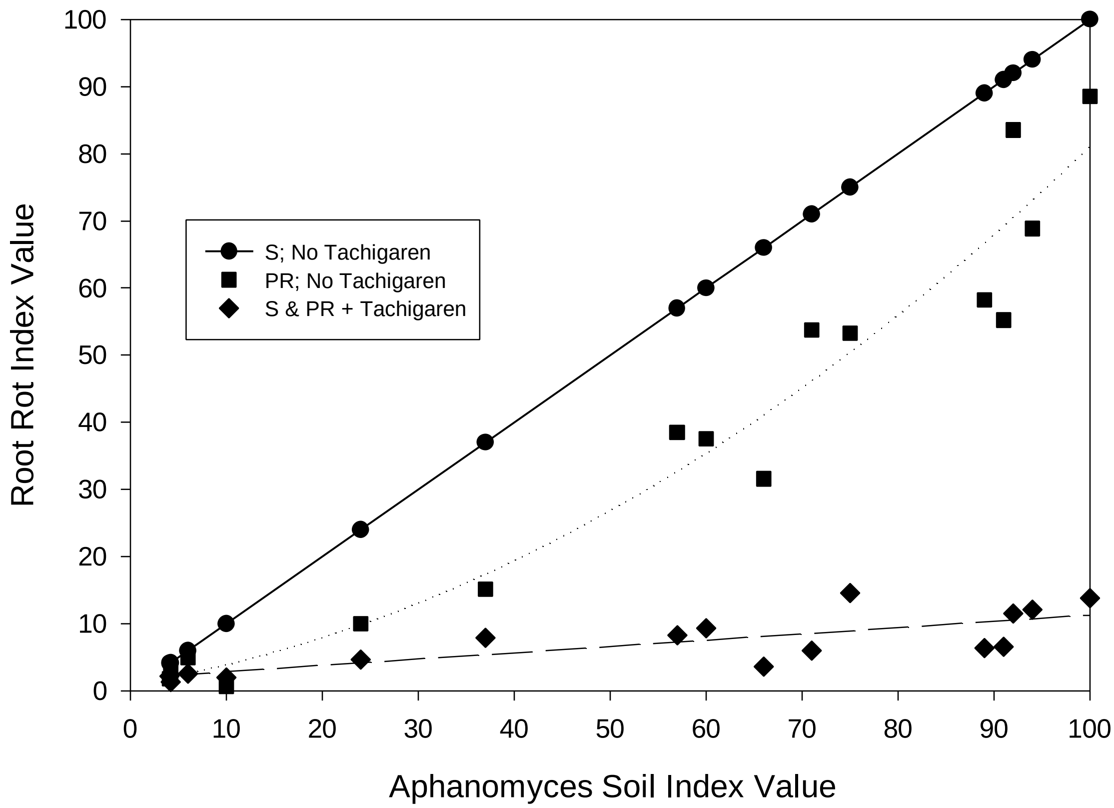

Figure 2. Relationship between root rot index values and Aphanomyces soil index values for a susceptible (S) variety not treated with Tachigaren (provided as a baseline, $R^2 = 1.0$), a partially resistant (PR) variety not treated with Tachigaren ($R^2 = 0.9448$), and Tachigaren seed treatment (averaged across both varieties and the 45 and 75 g rates, $R^2 = 0.6033$).

There was no significant interaction between variety and number of oospores at 4 weeks after planting for percent stand or root rot (data not shown). When seed was not treated with Tachigaren, however, the partially resistant variety had a better stand and less root rot than the susceptible variety across oospore concentrations (data not shown).

Inoculation of soil with different concentrations of oospores generated a range of root rot index values from 0 to 100 for the susceptible variety without Tachigaren, which are the same as the Aphanomyces soil index values. The baseline regression line for the susceptible variety not treated with Tachigaren is shown in Figure 2; since root rot index values and Aphanomyces soil index values are the same, the relationship is a straight line ($R^2 = 1.0$). When the partially resistant variety not treated with Tachigaren was planted in soils with the same potential for disease as the susceptible variety (based on the Aphanomyces soil index value), root rot was reduced (Figure 2). For instance, planting a partially resistant variety not treated with Tachigaren into soil with an Aphanomyces soil index value of 20, 40, 60, 80, or 100 would mean that root rot ratings at 4 weeks after planting would be about 8, 19, 35, 56, and 81, respectively.

For both varieties, seed treated with Tachigaren resulted in less root rot than when seed was not treated with Tachigaren (Figure 2). Reduction of root rot for Tachigaren-treated seed of both the susceptible and partially resistant varieties became statistically different from untreated seed at Aphanomyces soil index values of 37 and greater (according to 95% confidence intervals for predicted values from the regression lines plotting root rot index verses oospore density, data not shown). For the susceptible variety, Tachigaren-treated seed began to result in less root rot than untreated seed at Aphanomyces soil index values around 10 (root rot index values were 2 with Tachigaren seed treatment and 10 without Tachigaren seed treatment, Figure 2). For the partially resistant variety, Tachigaren-treated seed began to result in less root rot than untreated seed at Aphanomyces soil index values around 25 (root rot index values were 4.5 with Tachigaren seed treatment and 10 without Tachigaren seed treatment, Figure 2).

DISCUSSION

The results reported here clearly illustrate the importance of Tachigaren seed treatment in reducing damping-off and root rot on seedlings of a variety susceptible and partially resistant to *A. cochlioides* over a range of Aphanomyces soil index values. Although planting seed of the partially resistant variety not treated with Tachigaren reduced Aphanomyces diseases, performance was greatly enhanced by Tachigaren. Partial resistance to *A. cochlioides* is expressed in seedlings, but becomes more active as root tissues mature with age. Tachigaren is highly soluble in water and decomposes in soil, usually within about 4 weeks after planting. Thus, Tachigaren protects seedlings of the partially resistant variety when they are most vulnerable to infection and when they are maturing and becoming more resistant to *A. cochlioides*. By the time Tachigaren has decomposed, varieties with partial resistance are better able to withstand attack by *A. cochlioides* for the rest of the season. Although performance of the susceptible variety was greatly enhanced when Tachigaren-treated seeds were planted in soil over a range of Aphanomyces soil index values, this practice is not recommended. After Tachigaren decomposes, susceptible varieties are vulnerable to infection by *A. cochlioides* for the remainder of the growing season whenever soil is warm and wet.

In previous articles, we reported a complete loss of stand by 4 weeks after planting seed of varieties with partial resistance to Aphanomyces that were not treated with Tachigaren (1996 Minnesota and North Dakota Sugarbeet Research and Extension Reports, 27:228 -238; 1998 Minnesota Sugarbeet Research and Extension Reports, 29:266-267). Thus, previous reports appear to be in conflict with the research reported here where partial resistance to *Aphanomyces* was observed when seed was not treated with Tachigaren. This apparent discrepancy is because the previously reported trials were conducted in soils with Aphanomyces soil index values at or near 100 that were extremely prone to disease ("hot") and plant resistance was overwhelmed. <u>Aphanomyces soil index values of 100 do not mean the same thing for every field that has this rating</u>. An index value of 100 indicates severe disease (all seedlings died and/or were severely rotted by 4 weeks after planting). For some fields rated with an index of 100, however, all seedlings died by 2 ½ or 3 weeks after planting. Although the Aphanomyces soil index value is the same for these fields (100), the <u>rate of seedling death in the bioassay</u> strongly suggests that concentrations of inoculum are higher in soils where all plants die within 3, rather than 4, weeks. There is no way to verify this conclusion, however, because there are no direct methods to quantify inoculum of *A. cochlioides* in soil. In the experiments in this report, soils were inoculated with oospores of *A. cochlioides* to create a range of Aphanomyces soil index values. A maximum index of 100 was attained for one soil but it apparently was not as severely infested as naturally infested soils used in previously reported trials.

The soil index value measures the potential of a field to develop Aphanomyces stand loss and root rot when soils are wet and warm. The higher the soil index value the greater the risk of disease. Thus, some familiarity with the soil bioassay and disease rating system that determines the Aphanomyces soil index value is important in understanding the meaning of the index value. The bioassay is based on planting a commercial susceptible variety to "bait" *A. cochlioides* from soil (4-6 pots are planted, 25 seeds/pot). After 4 weeks, all seedlings are rated in disease categories on a 0 to 3 scale. Seedlings in the 0 category have no blemishes or rot on hypocotyls (region of stem between the seed and cotyledonary leaves) or roots. The hypocotyl is where *A. cochlioides* initially infects seedlings and rot proceeds from this point down the root and up to cotyledonary leaves. Seedlings in the 1 category have a light brown discoloration on the hypocotyl, indicating they are infected, and there may be a slight loss of the root system (compared to healthy roots). Seedlings assigned to the 2 category have a dark brown discoloration and some constriction of the hypocotyl; the root system also may be deteriorating. Seedlings in the 3 category have a hypocotyl that is dark brown and shriveled to a thin thread; roots are rotting and deteriorating; and rot sometimes is extending into the cotyledonary leaves. Seedlings placed in the 3 category are so severely diseased they will not recover when soil dries or temperatures are cool, and they will die within a day or two. Seedlings that die during the 4 weeks of the bioassay, also are placed in the 3 category. The Aphanomyces soil index value of a field assayed for a sugarbeet producer is an average of index values calculated for each pot of soil planted with the susceptible variety. The Aphanomyces soil index value is calculated from the following equation:

$$\frac{\text{Sum of (Number of plants in disease category x Disease category)}}{\text{Total number of emerged plants x 3}} \times 100$$

Therefore, the number of plants in a disease category are multiplied by the disease category (0, 1, 2, or 3) and the resulting values are summed. The summed number then is divided by a number attained by multiplying the total number of plants that emerged (maximum is 25) by 3. Then, the resulting value is multiplied by 100 to yield the Aphanomyces soil index value. This calculation is illustrated in the following example: 22 seedlings emerge after planting 25 seeds and at 4 weeks after planting, 10 seedlings are in the 0 category, 9 are in the 1 category, 3 are in the 2 category, and 0 are in the 3 category. These numbers are assigned as follows to calculate the Aphanomyces soil index value:

$$\frac{((10 \times 0) + (9 \times 1) + (3 \times 2) + (0 \times 3))}{22 \times 3} \times 100 = 23$$

In another example, 24 seedlings emerge after planting 25 seeds and at 4 weeks after planting, 5 seedlings are in the 0 category, 4 are in the 1 category, 8 are in the 2 category, and 7 are in the 3 category. The Aphanomyces soil index value is calculated as follows:

$$\frac{((5 \times 0) + (4 \times 1) + (8 \times 2) + (7 \times 3))}{24 \times 3} \times 100 = 57$$

These examples illustrate distribution of seedlings into three or four disease categories, which normally occurs when indexing fields that fall into low or mid-range soil index values. Soils with high index values have a large number of plants in the 2 category, and especially in the 3 category.

Based on our research, seed of a susceptible or partially resistant variety treated with Tachigaren resulted in a statistically significant reduction in root rot compared to seed without Tachigaren when planted in soils with an Aphanomyces soil index value of about 37 or greater. It should be noted that there was less root rot when Tachigaren-treated seed was planted into soil with Aphanomyces soil index values as low as 10 for the susceptible variety and as low as 25 for the partially resistant variety, but these benefits were not statistically significant from non-Tachigaren-treated seed. These still may be meaningful differences but they were not determined to be statistically significant at the chosen level of confidence ($P = 0.05$) because of variability in experiments, especially for the susceptible variety.

Several assumptions should be made by producers who make decisions on whether or not to plant Tachigaren-treated seed based on Aphanomyces soil index values for their fields. These assumptions include the following:

1. The Aphanomyces soil index value is based on the soil sample that was collected and it should represent the field, or portion of the field, in question. Some portions of the sampled field will have higher, and others lower, disease potential than the calculated index value (the value is an average based on combining soil samples or cores). Variability in distribution of inoculum (oospores) of *A. cochlioides* is typical in fields that have low, moderate, or severe disease. We have sampled adjacent 250 ft^2 plots in *Aphanomyces*-infested fields and found adjacent plots with soil index values as low as 30 and as high as 100. Another factor that may affect a soil index value is the laboratory that does the bioassay. Although guidelines and instructions are provided to laboratories, there are different types of controlled environments (greenhouse, growth chambers) and practices (e.g., amount of water added to soil) that could result in different index values for the same soil sample.

2. Field soil should be moist but not excessively wet because this creates conditions overwhelmingly favorable for infection by *A. cochlioides* and also hastens loss of Tachigaren. The fungicide is highly soluble in water so wet soil conditions, especially following heavy precipitation or flooding, can quickly deplete it. Also, when warm soil temperatures accompany moisture, microbial activity is stimulated, which hastens decomposition of Tachigaren. Fields with a high soil index value can be devastated in a couple of weeks when they are excessively wet, even if the variety is partially resistant to Aphanomyces and was treated with Tachigaren. Furthermore, all *Aphanomyces*-infested fields, even those with low soil index values, are at risk of developing higher soil index values, especially in warm seasons when wet soil conditions (from drainage, rainfall, or flooding) persist for several days or continue to reoccur. Sometimes a single substantial rainfall can cause flooding and result in wet soil conditions that prevail for days or weeks. Prolonged or regularly reoccurring wet soil conditions are very favorable for *Aphanomyces* and even low levels of initial inoculum (as suggested by a low soil index value) can increase rapidly. Adverse wet conditions also tend to weaken plants so resistance is more easily overcome. On the other hand, if soil is somewhat dry or well-drained or if temperatures are cool, *Aphanomyces* will cause no disease or a low level of disease, even in soils with a high Aphanomyces soil index value.

3. All available control measures should be practiced to manage *Aphanomyces* diseases, regardless of the Aphanomyces soil index value. These controls include selecting varieties with partial resistance, planting Tachigaren-treated seed, planting early, cultivating and enhancing field drainage, increasing length of rotations, controlling weeds, and avoiding spread of contaminated soil. Producers who do not follow these best management practices in *Aphanomyces*-infested fields (including fields with a low soil index value) are at risk of building up inoculum levels of *A. cochlioides*. Every time a sugarbeet seedling is infected or killed by *A. cochlioides,* the pathogen produces oospores which survive in soil for years, even in the absence of a sugarbeet crop.

We were successful in creating a range of Aphanomyces soil index values by inoculating soil with oospores of *A. cochlioides.* Although the number of oospores mixed into soil was known, it is unknown how many germinated and caused diseased. It has been well documented that oospores produced by *Aphanomyces* and other oospore-forming plant pathogens such as *Pythium* and *Phytophthora*, have very low germination rates of living oospores. This phenomenon allows the fungus to exert minimal energy when field conditions become favorable for disease (a sugarbeet crop and wet, warm soils). Perhaps only 1% or fewer of the oospores added to soil in our experiments germinated. This factor would explain variability in root rot when inoculating soils with low numbers of oospores and also explain variability between experiments inoculated with the same number of oospores. Thus, it is impossible to determine the oospore concentrations of field-collected soils based on the Aphanomyces soil index value.

CONCLUSIONS

- Planting Tachigaren-treated seed of a susceptible and partially resistant variety resulted in <u>statistically significant</u> reductions in root rot compared to seed without Tachigaren at Aphanomyces soil index values of about 37. Planting Tachigaren-treated seed resulted in <u>less</u> root rot than untreated seed at Aphanomyces soil index values as low as 10 for the susceptible variety and as low as 25 for the partially resistant variety.

- Several assumptions should be made by producers who make decisions about planting Tachigaren-treated seed based on the Aphanomyces soil index values for their fields. These assumptions include: an Aphanomyces soil index value represents the field; soil moisture is moderate (and not excessive or abnormally low); and control measures for managing Aphanomyces diseases are implemented to reduce disease and prevent buildup of *A. cochlioides* inoculum in soil.

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