# CONTROL OF APHANOMYCES DAMPING-OFF OF SUGARBEET BY TACHIGAREN SEED TREATMENT WHEN DISEASE ONSET OCCURS AT DIFFERENT PLANT AGES

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*Aphanomyces cochlioides* is a soilborne pathogen that causes root diseases (damping-off and root rot) of sugarbeet throughout the growing season, depending on when warm, wet soil conditions favorable for infection occur. Infection of sugarbeet by *A. cochlioides* occurs at temperatures above 55 °F, but is favored by warm temperatures (68 to 86 °F) and high soil moisture (3).

*Aphanomyces* is difficult to control because the pathogen survives for several years between sugarbeet crops and infection occurs below the soil surface where fungicides can not be directly applied. Resistance of varieties has improved but is not very effective during the seedling stage when plants are most susceptible (1). Treatment of seed with the fungicide Tachigaren® (hymexazol) is effective in controlled environments when conditions favorable for disease occur immediately after planting. Results have been more variable under field situations where onset of environmental conditions favorable for disease are highly variable. Tachigaren decomposes after planting, so more needs to be known about the duration of its activity in soil.

## OBJECTIVES

Our objective was to determine efficacy of Tachigaren seed treatment (20 and 45 g product per unit [100,000 seed]) compared to non-Tachigaren-treated seed in controlling Aphanomyces damping-off and root rot under conditions of moderate and high levels of inoculum (disease pressure) when onset of disease occurs at different seedling ages (1, 2, 3, and 4 weeks after planting).

## MATERIALS AND METHODS

Sugarbeet variety Beta 1305R (resistant to *Aphanomyces* and rhizomania) was treated with 0, 20 (minimum build-up), or 45 g (pelleted) of Tachigaren (70 WP) per unit (100,000 seed). All seed was treated with standard rates of Apron (metalaxyl) and Thiram (tetramethylthiuram disulfide), which control seed rot caused by species of *Pythium* and *Rhizoctonia solani* but do not affect damping-off and root rot caused by *A. cochlioides*. In this report, "untreated seed" or "control" seed is treated with Apron + Thiram but <u>not</u> with Tachigaren.

Three sources of field soil were used to evaluate efficacy of Tachigaren seed treatment. One soil had a history of moderate Aphanomyces root rot (Hillsboro, ND) and another was severely infested (Breckenridge, MN). An *Aphanomyces*-free soil was collected from the University of Minnesota, Northwest Research and Outreach Center, Crookston as a control. Sixteen seed of each treatment were sown in plastic pots (4.5 x 4.5 x 3.6 inches) at a 0.75-inch depth in the three field-collected soils (six replicates/soil source/seed treatment/seedling age at time of disease onset). Pots were arranged in a randomized block design, with 14 hr of light per day. Soil was kept slightly moist and incubated at 64 °F to inhibit infection of seedlings by *A. cochlioides*.

Planting was staggered so seedlings at 1, 2, 3, and 4 weeks after planting were simultaneously exposed to increased temperatures of 75-78 °F and increased soil moisture to favor infection by *A. cochlioides*. Four days later, temperature was decreased to 72-74 °F for 10 days. Pots also were watered as needed to keep soil wet.

Emergence was recorded at 5, 6, 7, and 8 days after planting. After temperatures were increased to favor disease, stand counts were made three times per week for 2 weeks, when the experiment ended. At each stand count, dead seedlings were removed and assayed to verify infection by *A. cochlioides.* A 1-inch length of each hypocotyl (region between seed and cotyledons) was excised, gently cleaned in tap water, surface-disinfected in 0.5% bleach, rinsed twice with sterile deionized water, and placed in a section of a quad plate containing 5 ml sterile deionized ultra-filtered water (one hypocotyl per section). After 24 to 48 hours, hypocotyls were microscopically examined for diagnostic structures of *A. cochlioides* (zoosporangia with encysted zoospore clusters).
Two weeks after inoculation, surviving seedlings were removed from soil, washed, and rated on a 0 to 3 scale (0 = no disease, 3 = hypocotyl completely constricted and plant dead). Disease ratings and numbers of seedlings that died during the evaluation were used to calculate a root rot index (RRI) value on a 0 to 100 scale, where 0 = no disease and 100 = severe disease (all seedlings dead and roots severely rotted).

Percent data were subjected to an arcsine transformation when appropriate. Percent emergence, percent stand, and RRI data were subjected to analysis of variance and if significant ($P = 0.05$), means were separated by Least Significant Difference. Where interactions occurred between plant age at disease onset and seed treatment, effect of seed treatment was analyzed within each plant age.

## RESULTS

In all three soils, rate of emergence was slightly, but statistically, reduced 5 to 7 days after planting with the 45 g rate of Tachigaren compared with the 20 g rate and untreated seed (Figure 1). By 8 days after planting, however, all seed treatments resulted in equal emergence.

**Aphanomyces-free soil.** In the pathogen-free control soil there were no interactions between plant age at onset of disease-favorable conditions and seed treatment (data not shown). Disease did not occur and there was no effect of Tachigaren seed treatment on final stand or RRI values. For 0, 20, and 45 g of Tachigaren, final stands averaged 97, 98, and 98%, respectively, and RRI values were zero for all seed treatments.

**Moderate Aphanomyces inoculum level.** In soil from Hillsboro, there were significant interactions between plant age and seed treatment for most stand data collected during the trial but not for final stand or root rot index values.

When disease-favorable environmental conditions were applied 1 and 2 weeks after planting (Figure 2A and 2B, respectively), no plants had died from Aphanomyces damping-off. After disease favorable conditions were imposed 1 week after planting, however, stands of seedlings from untreated seed started to decline within a couple of days (Figure 2A). Stands from Tachigaren-treated seed remained stable until 1 week later, when seedlings from the 20 g rate started to slightly decline. Final stands from seed treated with 20 and 45 g of Tachigaren were significantly higher and statistically the same (85 and 95%, respectively) compared to untreated seed (28%). When favorable environmental conditions occurred 2 weeks after planting, stands from untreated seed rapidly declined beginning about 4 days later (Figure 2B). Plant populations remained stable for seedlings treated with 20 and 45 g of Tachigaren seed treatment and final stands averaged 94 and 97%, respectively, compared to the untreated control at 58%.

When disease-favorable environmental conditions began 3 and 4 weeks after planting (Figure 2C and 2D, respectively), there were significant interactions for stand between plant age at onset of disease-favorable temperatures and seed treatment because Aphanomyces damping-off started 8 to 14 days after sowing untreated seed. After disease-conducive environments were applied to seedlings 3 weeks after planting, stands from untreated seed continued to decline; plant populations were significantly higher for both rates of Tachigaren-treated seed and remained stable until 10 days later, when there was a slight decline in stand for the 20 g rate (Figure 2C). Final stands averaged 54, 88, and 95% for seed treated with 0, 20, and 45 g of Tachigaren, respectively. When environmental conditions were favorable for disease 4 weeks after planting, seedlings from untreated seed had been dying between 8 and 14 days after planting, well before the environment was changed to favor disease development (Figure 2D). Seed treated with both rates of Tachigaren had similar and stable stands with slight declines toward the end of the trial that remained significantly higher than the untreated control. Final stands averaged 37, 80, and 87% for 0, 20, and 45 g of Tachigaren, respectively.

Overall, root rot index (RRI) values tended to be lowest for seedlings from Tachigaren-treated seed and highest from untreated seed when plants were exposed to environmental conditions favorable for disease at all ages (Figure 3A).

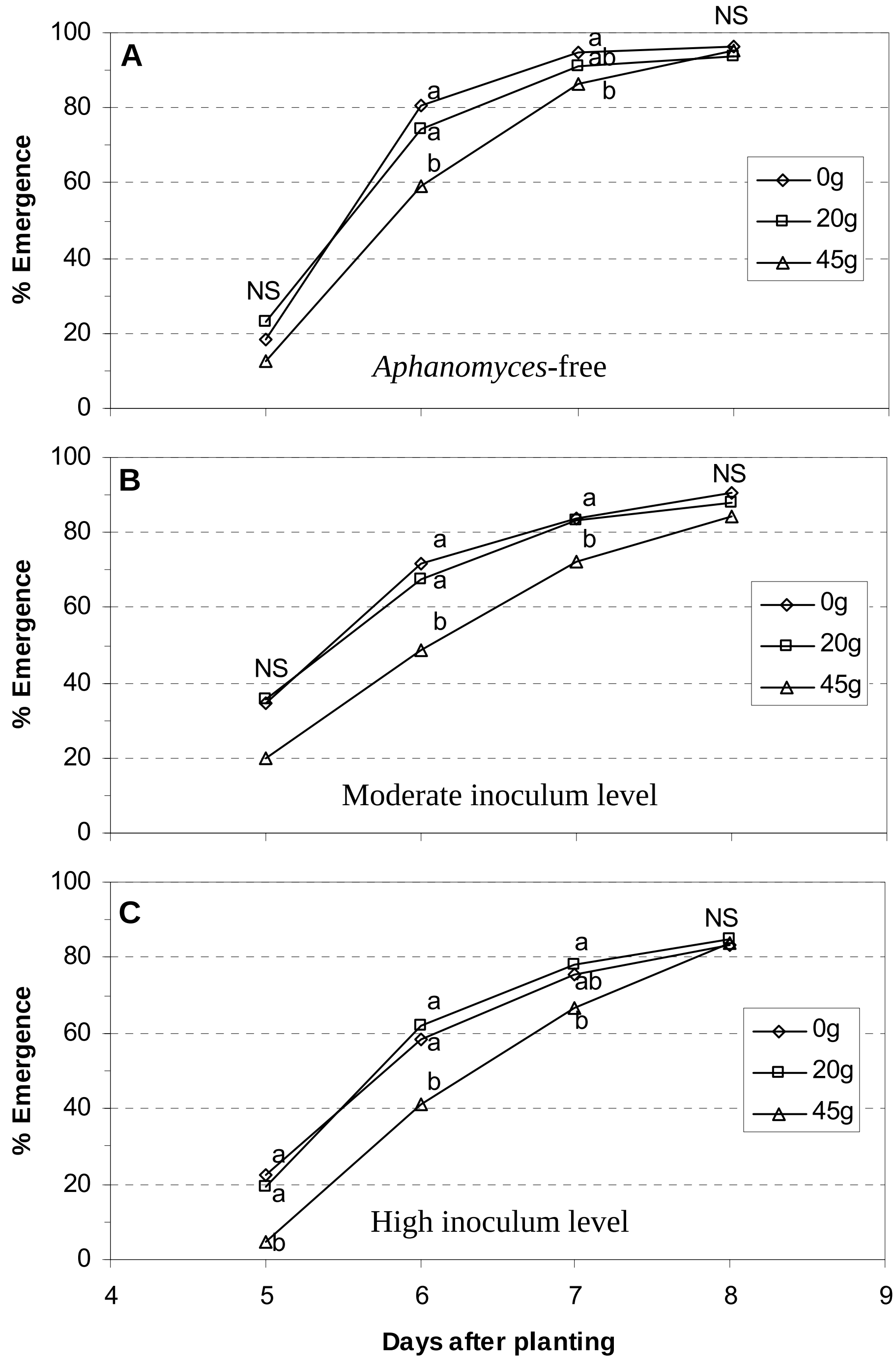


**Figure 1.** Emergence of sugarbeet Beta 1305R when seeds were treated with 0, 20 g (minimum build-up pellet), or 45 g (standard pellet) of Tachigaren (70 WP) per unit (100,000 seed). All seed also was treated with standard rates of the fungicides Apron + Thiram to control seed rot caused by species of *Pythium* and *Rhizoctonia solani*. Seed was sown in field soil that was **A.)** *Aphanomyces*-free (=control, University of Minnesota, Northwest Research and Outreach Center, Crookston) and two fields naturally infested with *A. cochlioides* with **B.)** moderate levels of inoculum (Hillsboro, North Dakota) and **C.)** high levels of inoculum (Breckenridge, Minnesota). Plants were

grown at 64 ºF in a controlled environment chamber. Each data point is an average of 24 replicates across four planting dates. For each day after planting, points marked with a different letter are significantly different ($P$ = 0.05); NS = not significantly different.

**Soil Naturally Infested with Aphanomyces: Moderate Inoculum Level**

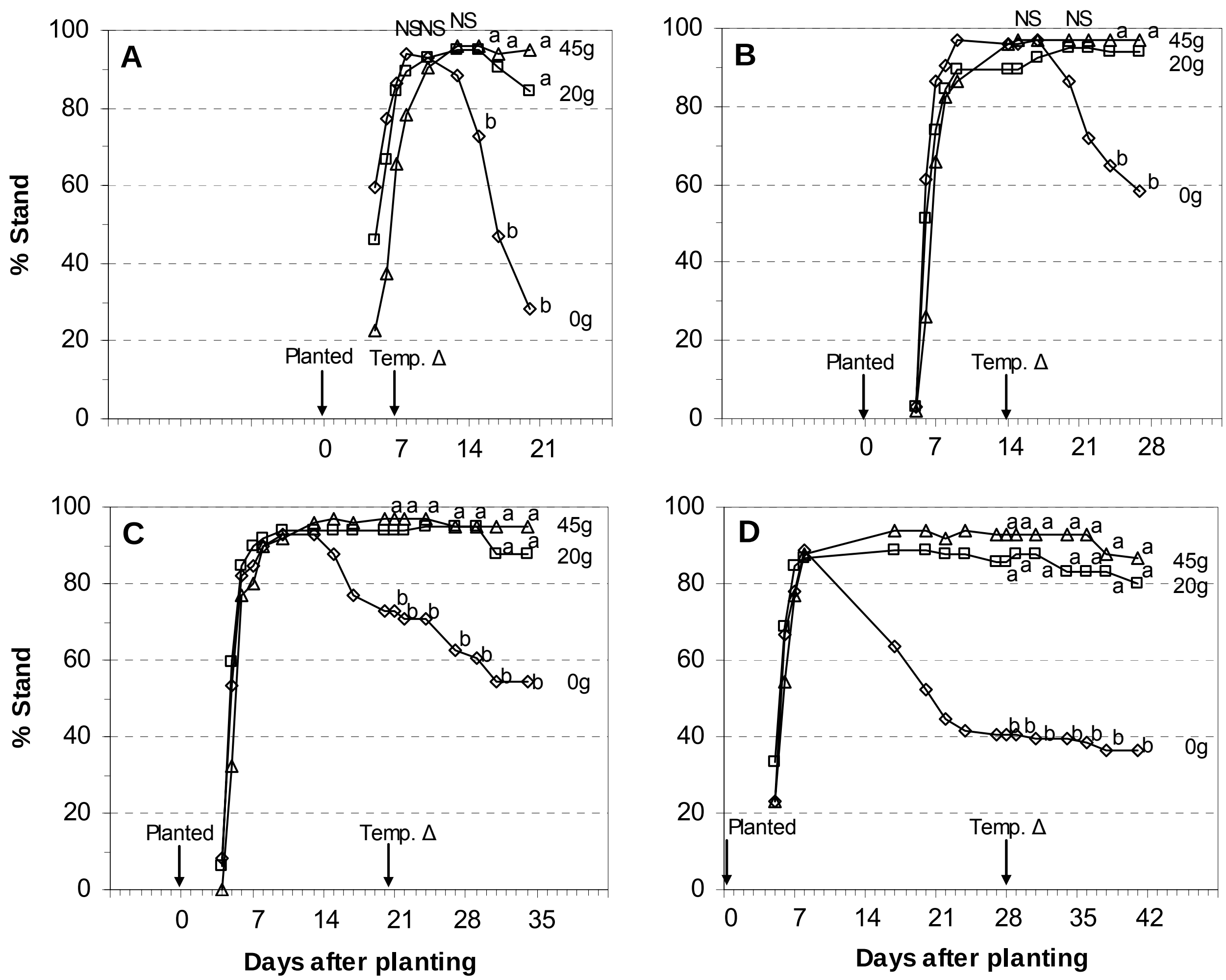


**Figure 2.** Percent stand of sugarbeet Beta 1305R when seeds were treated with 0, 20 g (minimum build-up pellet), or 45 g (standard pellet) of Tachigaren (70 WP) per unit (100,000 seed). All seed also was treated with standard rates of the fungicides Apron + Thiram to control seed rot caused by species of *Pythium* and *Rhizoctonia solani*. Seed was sown in soil collected from a field near Hillsboro, North Dakota that was naturally infested with moderate levels of inoculum of *Aphanomyces cochlioides*. Plants were grown at 64 $^{0}$F and then temperatures were increased to 72 – 78 $^{0}$F (Δ= temperature change) for 4 days to initiate infection by *A. cochlioides* at **A.**) 1 week, **B.)** 2 weeks, **C.)** 3 weeks, and **D.)** 4 weeks after planting. Then temperatures were reduced to 72 – 74 $^{0}$F and soil was kept wet for 10 days to increase disease development. The trial ended 2 weeks after environmental conditions changed to

favor infection by *A. cochlioides*. Each data point is an average of six replicates. For each day after inoculation, points marked with a different letter are significantly different ($P = 0.05$); NS = not significantly different.

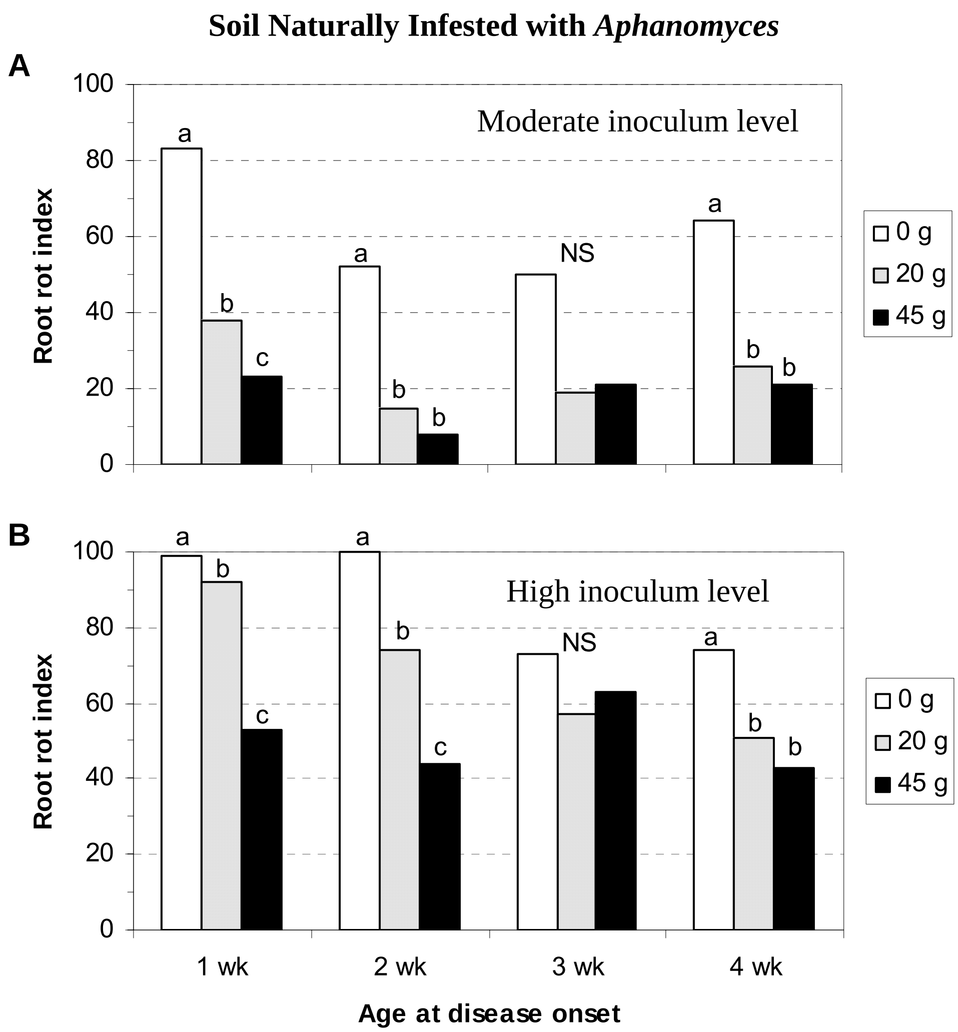


**Figure 3.** Root rot index values of sugarbeet seedlings of Beta 1305R from seed treated with 0, 20 g (minimum build-up pellet), or 45 g (standard pellet) of Tachigaren (70 WP) per unit (100,000 seed). All seed also was treated with standard rates of the fungicides Apron + Thiram to control seed rot caused by species of *Pythium* and *Rhizoctonia solani*. Seed was sown in soil collected from two fields naturally infested with *Aphanomyces cochlioides* with **A.)** moderate levels of inoculum (Hillsboro, North Dakota) and **B.)** high levels of inoculum (Breckenridge, Minnesota). Plants were grown at 64 $^{0}$F and then temperatures were increased to 72 – 78 $^{0}$F for 4 days to initiate infection by *A. cochlioides* at 1, 2, 3, or 4 weeks after planting. Then temperatures were reduced to 72 – 74 $^{0}$F and soil was kept wet for 10 days to increase disease development. The trial ended 2 weeks after environmental conditions were changed to favor infection by *A. cochlioides*. The RRI values are on a 0 to 100 scale, where 0 = no disease and 100 = all plants dead and roots severely rotted. Each data

point is an average of six replicates. For each plant age at disease onset, bars with a different letter are significantly different ($P = 0.05$); NS = not significantly different.

When disease-onset conditions occurred 1 week after planting, RRI values were significantly lower for plants from seed treated with 45 g of Tachigaren compared to 20 g, and both rates resulted in less root rot than the untreated control. Plants with disease onset at 2 and 4 weeks after planting resulted in statistically lower and equal RRI values for both rates of Tachigaren seed treatment compared to the untreated control. When plants were exposed to favorable disease conditions 3 weeks after planting, RRI values were variable and not significantly different among seed treatments, although they tended to be equally lower for both rates of Tachigaren compared to untreated seed.

**High Aphanomyces inoculum level.** In the Breckenridge soil, Aphanomyces damping-off occurred earlier (Figure 4) and root rot was more severe (Figure 3) than in the Hillsboro soil (Figure 2). There were significant interactions between plant age and seed treatment following onset of disease-favorable environmental conditions (increase in temperature from 64°F to 75-78°F and increases in soil moisture) for most stand data collected during the trial and for root rot index values.

When disease-favorable conditions were initiated 1 and 2 weeks after sowing (Figure 4A and 4B, respectively), Aphanomyces damping-off had not yet occurred. When disease-favorable conditions were applied 1 week after planting, seedlings from untreated and Tachigaren-treated (20g) seed started damping-off 3 to 6 days later (Figure 4A). Plants from seed treated with 45 g of Tachigaren began dying 8 days after the environment changed, but stands remained significantly higher compared to the 20 g rate and untreated seed. Final stands were significantly different among seed treatments; the highest stand was from seed treated with 45 g of Tachigaren (73%), intermediate for 20 g (14%), and lowest for untreated seed (2%). When the environmental changes occurred 2 weeks after planting, plant populations began declining 1 day later for seedlings from untreated seed and remained significantly lower compared to stands from both rates of Tachigaren seed treatment (Figure 4B). Eight days after the environment changed, plant stands started declining when seed was treated with 20 or 45 g of Tachigaren. Final stands were significantly different among seed treatments with the highest stand from the 45 g rate of Tachigaren (86%), intermediate for 20 g (39%), and lowest for untreated seed (0%).

When disease-favorable environmental conditions increased 3 and 4 weeks after planting (Figure 4C and 4D, respectively), Aphanomyces damping-off was already occurring. When environmental changes were applied 3 weeks after planting, damping-off had started 13 days after planting (8 days before the environment change) for plants from seed treated with 45 g of Tachigaren or that were untreated (Figure 4C). Stand losses were not statistically different among seed treatments throughout the trial, but were greatest for seedlings from untreated seed, intermediate for 45 g Tachigaren, and least for the 20 g rate. Final stands averaged 46, 65, and 53% from seeds treated with 0, 20, or 45 g of Tachigaren, respectively. When environmental conditions were increased to favor disease 4 weeks after planting, seedlings from untreated seed had already been dying between 8 and 21 days after planting before stabilizing for the remainder of the trial (Figure 4D). Plants from seed treated with 20 and 45 g of Tachigaren also declined in stand at 7 to 10 days before environmental conditions were changed but final stands were statistically equal and higher compared to untreated seed. Final stands averaged 27, 61, and 72% for 0, 20, and 45 g of Tachigaren seed treatment, respectively.

There were significant interactions between plant age at onset of disease-favorable environmental conditions and seed treatment for RRI values. Overall, as plants were increasingly older at onset of environmental conditions favorable for infection by *A. cochlioides*, RRI values of plants from seed that was untreated or amended with 20 g of Tachigaren tended to decrease, while RRI values of plants from seed treated with 45 g of Tachigaren remained consistently low (Figure 3B). When environmental conditions changed 1 or 2 weeks after planting, RRI values of seedlings were statistically different among seed treatments. Disease ratings were lowest for plants from seed treated with 45 g of Tachigaren, intermediate for the 20 g rate, and highest with untreated seed. When disease-favorable environmental conditions were established 3 weeks after planting, there were no significant differences in RRI values of seedlings from any seed treatment. Changing environmental conditions to favor *A. cochlioides* 4 weeks after planting resulted in significantly equal and lower RRI values for plants from seed treated with 20 and 45 g of Tachigaren compared to untreated seed.

**Soil Naturally Infested with *Aphanomyces*: High Inoculum Level**

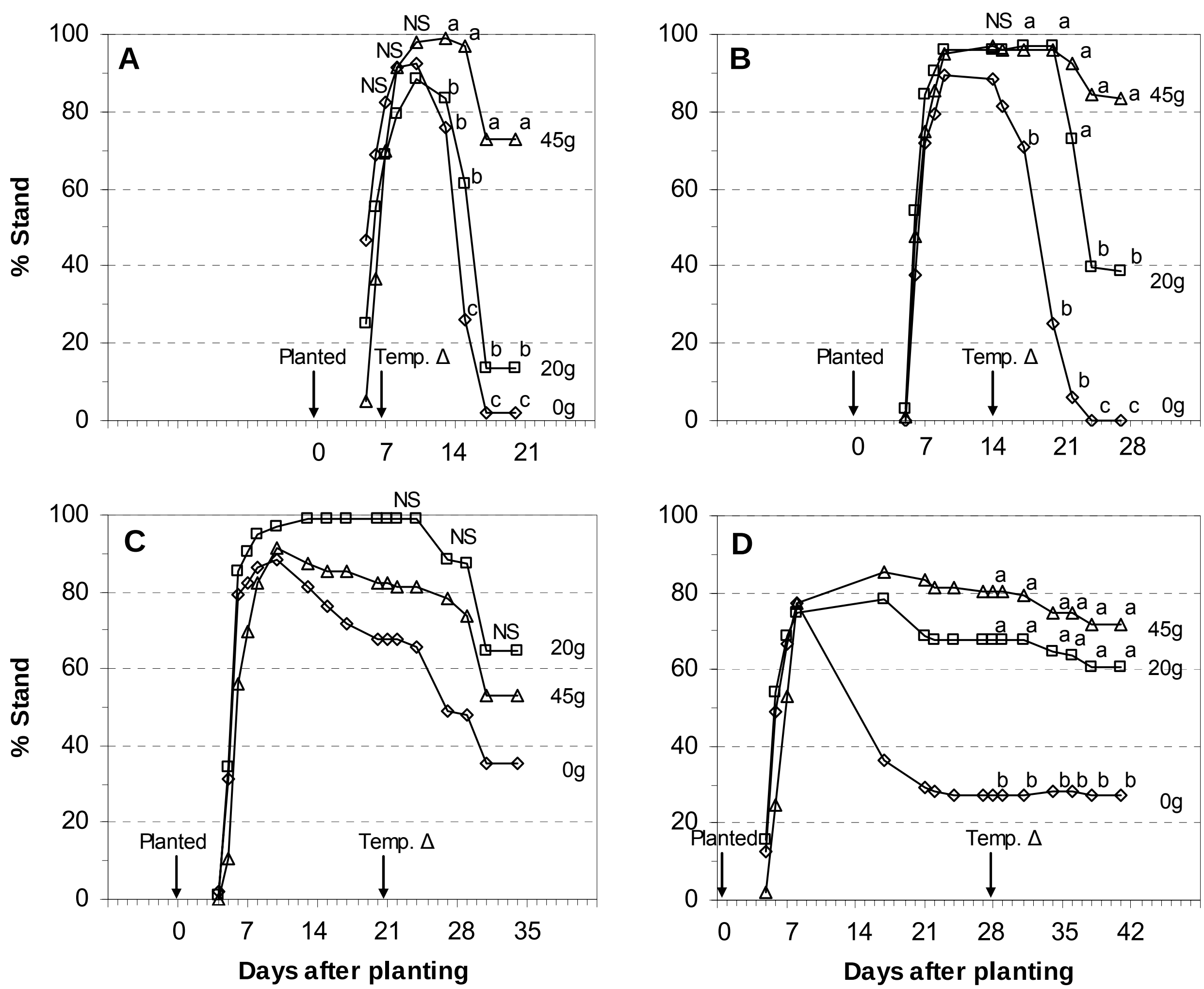

**Figure 4.** Percent stand of sugarbeet Beta 1305R when seeds were treated with 0, 20 g (minimum build-up pellet), or 45 g (standard pellet) of Tachigaren (70 WP) per unit (100,000 seed). All seed also was treated with standard rates of the fungicides Apron + Thiram to control seed rot caused by species of *Pythium* and *Rhizoctonia solani*. Seed was sown in soil collected from a field near Breckenridge, Minnesota that was naturally infested with high levels of inoculum of *Aphanomyces cochlioides*. Plants were grown at 64 $^{0}$F and then temperatures were increased to 72 – 78 $^{0}$F (Δ= temperature change) for 4 days to initiate infection by *A. cochlioides* at **A.**) 1 week, **B.)** 2 weeks, **C.)** 3 weeks, and **D.)** 4 weeks after planting. Then, temperatures were reduced to 72 – 74 $^{0}$F and soil was kept wet for 10 days to increase disease development. The trial ended 2 weeks after environmental conditions were changed to favor infection by *A. cochlioides*. Each data point is an average of six replicates. For each day after inoculation, points marked with a different letter are significantly different ($P$ = 0.05); NS = not significantly different.

## DISCUSSION

Seed treatment with Tachigaren provided significant benefit compared to untreated seed over a wide range of seedling ages at onset of infection by *A. cochlioides*. Differences in efficacy of Tachigaren occurred depending on rate, level of pathogen inoculum, and age of plants at onset of disease.

Overall, seed treatment with 45 g of Tachigaren was more consistent than 20 g in controlling damping-off and root rot of sugarbeet sown in soils naturally infested with *A. cochlioides*, regardless of plant age at onset of disease. Tachigaren is soluble in water and also decomposes in soil, so seed treated with 45 g would have a higher residual level of fungicide than seed treated with 20 g and thus, be more effective in controlling *A. cochlioides*. For instance, degradation of Tachigaren after 7 days in soil at 59, 68, 77, and 86 $^{0}$F was 3.3, 7.8, 15, and 25%, respectively (Sankyo Agro Co., Ltd., Tokyo, Japan) and decomposition continues over time (2). Performance of the 20 and 45 g rates of Tachigaren was nearly equal, however, when seed was sown in a field soil with a moderate inoculum density of *A. cochlioides.* This outcome indicates there was sufficient fungicide present from the 20 g seed treatment of Tachigaren to adequately protect seedlings. When seed was sown in soil with a history of severe *Aphanomyces*, even the 45 g rate of Tachigaren was inadequate to completely control damping-off (stand loss was as much as 47%) but overall, provided more residual protection than the 20 g rate (which typically resulted in intermediate stands compared to the untreated control).

Damping-off and root rot of seedlings also was affected by plant age at onset of disease-favorable environmental conditions. In both moderate and severely infested soils, seedlings did not die before onset of disease-favorable environmental conditions were applied 1 or 2 weeks after planting. After environmental conditions changed, however, stand losses occurred, especially in the untreated control where final stands were very low and root rot indices were very high. When disease-favorable environmental conditions were initiated 3 and 4 weeks after planting, seedlings already were dying prior to the environment change for the untreated control in the moderate disease pressure soil and for all seed treatments in the severely infested soil. This likely can be attributed to soil temperatures that were 6 °F higher than air temperature (as determined by sensors placed at ¾-inch depths) during the day because of absorption of energy from overhead lights. In the severely infested soil, seed treatment with Tachigaren did not significantly protect seedlings when disease-favorable conditions began 3 weeks after planting but did provide protection when disease favorable conditions began 4 weeks after planting. Three weeks after planting, seedlings were still very susceptible to damping-off and Tachigaren was decomposing, resulting in less disease control. By 4 weeks after planting, seedlings were starting to express resistance to *A. cochlioides* (1), so residual amounts of Tachigaren combined with plant resistance resulted in disease control.

Our controlled environment trials attempted to simulate a range of environmental conditions favorable for infection of sugarbeet seedlings by *A. cochlioides* that could occur in the field. Under favorable disease conditions, the pathogen produces infective propagules (zoospores) which infect plants but survive for only a few days (3). In the field, actual disease pressure may be greater or less than our simulation, depending on soil moisture and temperatures.

Susceptibility of seedlings to *A. cochlioides* varies with plant age and development of root tissues, but development varies tremendously under different environmental conditions in the field. Decomposition of Tachigaren seed treatment after planting also is likely to vary in field situations, depending on soil type, moisture, and temperature. Efficacy of Tachigaren seed treatment depends on several factors including breakdown in the soil, development of the seedling, population of *A. cochlioides*, and onset of conditions favorable for disease. Under these highly variable conditions, use of the 45 g rate of Tachigaren as a seed treatment results in more consistent benefits than the 20 g rate.

**SUMMARY AND RECOMMENDATIONS**

1. Tachigaren seed treatment at the 45 g rate provided more consistent protection than the 20 g rate in controlling *A. cochlioides* damping-off and root rot over a range of disease severities and plant ages at disease onset.

2. Under moderate disease pressure in naturally infested field soil, 20 and 45 g rates of Tachigaren seed treatment provided similar benefit in controlling seedling disease caused by *A. cochlioides*.

3. Under high disease pressure in fields naturally infested with *A. cochlioides*, 45 g of Tachigaren seed treatment was effective in protecting seedlings when disease onset started at 1 and 2 weeks after planting, but the 20 g rate did not provide sufficient control compare to untreated seed. Neither rate of Tachigaren provided significant protection of seedling when disease onset started 3 weeks after planting. Both rates of Tachigaren were nearly equally effective compared to untreated seed when disease-favorable conditions occurred 4 weeks after planting (when plant resistance starts to become active), but still resulted in some stand loss.

4. Protecting seedlings within a few weeks of emergence is critical since they are most susceptible to Aphanomyces damping-off and root rot during this time.

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