### SUGARBEET SEEDLING AGE AND SUSCEPTIBILITY TO APHANOMYCES COCHLIOIDES

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Aphanomyces cochlioides (= A. cochlioides) is a soilborne "water mold" that causes damping-off of sugarbeet seedlings and root rot throughout the growing season. Development of disease depends on susceptibility of the sugarbeet variety, population of A. cochlioides in soil, and environmental conditions during the growing season. The pathogen produces motile zoospores that swim short distances through water in soil to infect sugarbeet roots. Infection and disease development are favored in warm, wet soils that allow production and motility of zoospores.

Many sugarbeet varieties are susceptible to *A. cochlioides*. In recent years, several varieties with partial resistance to *A. cochlioides* have been commercially available, but none are immune. If the pathogen is present in a field, timing and severity of disease are primarily determined by inoculum densities and occurrences of wet, warm soil conditions. Infections occur when warm weather follows significant rainfall any time from seedling emergence until harvest. Observation of field samples and past research suggest that seedlings of partially resistant and susceptible varieties are both highly susceptible to damping-off and root rot within 1 month after planting. Once beyond the seedling stage, plant resistance becomes expressed in partially resistant varieties.

# **OBJECTIVE**

The objective of this study was to determine susceptibility of seedlings of a susceptible and partially resistant sugarbeet variety inoculated with *A. cochlioides* at various ages.

## MATERIALS AND METHODS

Sugarbeet seed of a susceptible variety (ACH 261) and a partially resistant variety (ACH 205) were sown at a  $\frac{3}{4}$ -inch depth in 4.5-inch square pots (13 seeds per pot) containing a commercial soil mix (Sunshine LG3). Stand was thinned to 10 seedlings per pot 1 week after planting. Planting was staggered over time so seedlings of four plant ages (1, 2, 3, and 4 weeks after planting, hereafter referred to as 1-, 2-, 3-, and 4-week-old seedlings) could be inoculated with zoospores of *A. cochlioides* on the same day. Plants were grown in a controlled environment chamber at  $69 \pm 2^{0}$ F with a 16-hour day length prior to inoculation. Plants were inoculated with 0, 100,000, or 200,000 zoospores in 50 ml deionized water per pot in the first experiment and with 0, 50,000, or 100,000 zoospores in 50 ml deionized water per pot in the second experiment. After inoculation, temperatures in the controlled environment chamber were raised to  $77 \pm 2^{0}$ F and soil was watered regularly to favor infection and disease development. Both experiments were arranged in a randomized block design with 10 replicates per treatment.

Plant stand was counted on the day of inoculation and at least twice weekly for the following 2 weeks. Samples of dying seedlings were removed and assayed to confirm infection by A. cochlioides. Hypocotyls were excised, surface-treated for 15 seconds in 0.5% sodium hypochlorite, rinsed twice in sterile deionized water (SDW), and placed in Petri dishes containing SDW. Hypocotyls were microscopically examined after 24-48 hr for A. cochlioides. Two weeks after inoculation, remaining plants were removed from soil, washed, rated for disease, and a root rot index (0-100 scale, 0 = all plants healthy, 100 = all plants dead) was calculated.

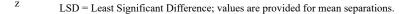
Plant stand after inoculation and root rot index values were subjected to analysis of variance (ANOVA) and when significant ( $P \le 0.05$ ), means were separated by Least Significant Difference. Non-inoculated controls showed no indication of disease and thus, were eliminated from the ANOVA to make more sensitive comparisons between varieties and among plant ages.

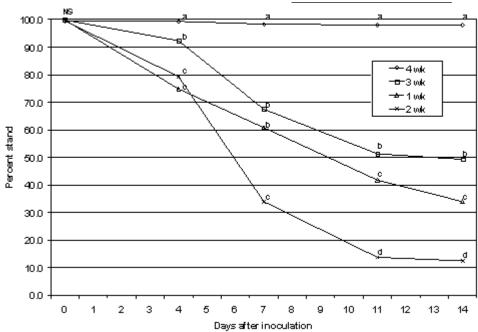
Table 1. Effect of concentration of zoospore inoculum of Aphanomyces cochlioides on percent final stand and rot root of sugarbeet seedlings 2 weeks after inoculation.

Inoculum concentration (zoospores/pot)	Percent final stand <sup>y</sup>	Root rot index <sup>y</sup>
Non-inoculated control	99	2

50,000	47	74
100,000	41	76
$LSD (P = 0.05)^{z}$	4.0	2.2

Each value based on 800 plants (80 pots) averaged across seedlings inoculated when 1-, 2-, 3-, or 4-week old of two varieties (ACH 261 [susceptible] and ACH 205 [partially resistant] to A. cochlioides).



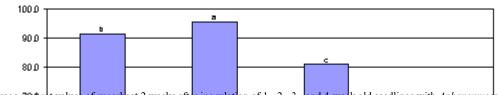


**Fig. 1.** Percent stand of sugarbeet 2 weeks following inoculation with zoospores of *Aphanomyces cochlioides* when plants were 1-, 2-, 3-, and 4-weeks old. Each data point is based on 400 plants (in 40, 4.5-inch diameter pots) averaged across inoculum concentrations (50,000 and 100,000 zoospores/pot) and two varieties (ACH 261 [susceptible] and ACH 205 [partially resistant] to *A. cochlioides*). For each time of data collection, values followed by the same letter are not statistically different (*P* = 0.05).

#### RESULTS

Results from both trials were similar, so data from the second trial are presented. In the non-inoculated control, final stand was 99%, the root rot index was only 2 (<u>Table 1</u>), and *A. cochlioides* was not isolated. Final stands for plants inoculated with 50,000 or 100,000 zoospores per pot (averaged across varieties and plant ages at time of inoculation) were very similar but statistically higher for lower inoculum concentration whereas, root rot indices for both inoculum concentrations were statistically the same (<u>Table 1</u>). Therefore, data were combined for zoospore concentrations in subsequent analyses of data.

There were no interactions between variety and plant age at time of inoculation, so these main effects will be presented separately. During the 2 weeks after inoculation, there were significant differences in stand, depending upon age of plants at time of inoculation. At only 4 days after inoculation (Fig. 1), plant stands (averaged across



Average Rule ret values of sugarbeet 2 weeks after inoculation of 1-.2-,3-, and 4-week old seedlings with Aphanomyce's cochlioides. Each bar in the graph is based on 400 plants (in 40, 4.5-inch diameter pots) averaged across inoculum concentrations (50,000 and 100,000 zoospores/pot) and two varieties (ACH 261 [susceptible] and ACH 205 [partially resistant] to A. cochlioides). Bars noted by the same letter are not statistically different (P = 0.05).

inoculum concentration and varieties) were already declining and damping-off was greatest and equally significant (P = 0.05) for plants 1 or 2 weeks old at inoculation; intermediate for plants 3 weeks old at inoculation; and there was no stand loss for plants 4 weeks old at time of inoculation. Stands continued to decline and by 2 weeks after inoculation, there were significant differences in final stand among the four plant ages, which averaged 12, 34, 50, and 98% when inoculated at 2, 1, 3, and 4 weeks after planting, respectively.

Root rot index values (averaged across inoculum concentrations and varieties) were significantly different (P = 0.05) for each plant age at inoculation (Fig. 2). Seedlings inoculated at 2 weeks after planting had the highest root rot index, followed in descending order by plants that were inoculated when 1, 3, or 4 weeks old (Fig. 2).

#### Plantage at inoculation

Both varieties lost considerable stand during the 2 weeks following inoculation but the partially resistant variety had slightly higher stands than the susceptible variety (Fig. 3, data are averaged across inoculum concentrations and plant ages at time of inoculation). There were no significant differences in stand between varieties, except at 4 days after inoculation, when the partially resistant variety had a statistically higher (P = 0.05) stand compared to the susceptible variety. Root rot index values were statistically lower (P = 0.05) for the partially resistant variety (73) than the susceptible variety (77) (data not shown).

#### DISCUSSION

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Susceptibility of seedlings to *A. cochlioides* is dependent on plant age when infection occurs and is not significantly affected by partial resistance or susceptibility of varieties at this early stage of plant development. Plants that were 1 or 2 weeks old when inoculated were extremely susceptible. These results illustrate the potential for stand losses in fields when warm, wet conditions occur shortly after emergence. Even if the duration of conditions favorable for disease is short, appreciable stand loss can occur. Three-week-old plants also were fairly susceptible. Plants that were 4 weeks old when inoculated were quite resistant to disease and did not lose much stand during the 2 weeks after inoculation (Fig. 1). It would take more inoculum and longer duration of disease-favorable conditions to cause stand loss when 4-week old plants become infected compared to younger seedlings.

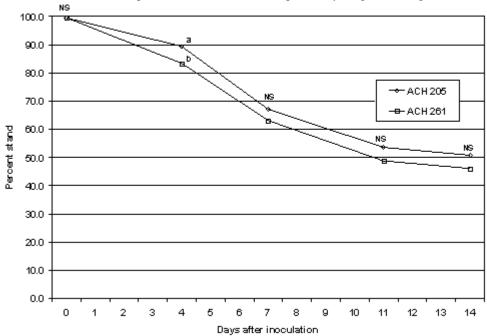


Fig. 3. Percent stand of a sugarbeet variety that is susceptible (ACH 261) and partially resistant (ACH 205) to *Aphanomyces cochlioides* during 2 weeks after inoculation. Each data point is based on 800 plants (in 80, 4.5-inch diameter pots) averaged across inoculum concentrations (50,000 and 100,000 zoospores/pot and plant ages at time of inoculation (1-, 2-, 3-, and 4-weeks old). For each time of data collection, values followed by the same letter are not statistically different (*P* = 0.05).

Differences in stand between the susceptible and partially resistant varieties were statistically significant (P = 0.05) at 4 days after inoculation (83 and 89, respectively) and for root rot indices at 2 weeks after inoculation (77 and 73, respectively). This indicates that subtle differences between varieties can be detected at the seedling stage if there is enough replication. These differences, however, were small and may not be biologically meaningful. Root rot indices of 77 and 73 (averaged across plant ages) are high and indicate both varieties were susceptible.

This data strongly suggests that protecting seedlings from infection by *A. cochlioides* for the first 4 weeks after planting is critical in fields with a history of Aphanomyces. Seed treatment with Tachigaren reduces stand loss and damping-off caused by *A. cochlioides* and the fungicide remains active for about 3 to 4 weeks after planting. When environmental conditions are dry and/or cool during the first few weeks after emergence, *A. cochlioides* will cause little if any damage, but if warm, wet conditions occur, stand loss can be rapid and severe on young, unprotected seedlings. When plants are 4 weeks old, some resistance to *A. cochlioides* becomes measurably active in sugarbeet varieties, provided plants are not overwhelmed by high inoculum densities and exceedingly favorable conditions for infection and disease development. As plants mature, resistance becomes actively expressed in partially resistant, but not susceptible, varieties (2000 Sugarbeet Research and Extension Reports 31:243-246).

### **ACKNOWLEDGEMENTS**

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