# VARIABILITY OF SPORE PRODUCTION AND AGGRESSIVENESS OF *APHANOMYCES COCHLIOIDES* ON SUGARBEET

Jason R. Brantner and Carol E. Windels

Research Fellow and Professor of Plant Pathology, respectively, University of Minnesota, Northwest Research and Outreach Center, Crookston

*Aphanomyces cochlioides* is a soilborne fungus that can cause damping-off on sugarbeet seedlings and root rot throughout the growing season. The amount of disease in a given field varies depending on susceptibility of the sugarbeet variety, population of *A. cochlioides* in the soil, and environmental conditions during the growing season. Infection and disease development are favored by warm, wet soil conditions.

The fungus produces two different types of spores that are important in its disease cycle. Oospores are sexual reproductive spores that are formed in infected host material (sugarbeets or many weeds including pigweed, kochia, and lambsquarters). They have thick walls and function as survival structures allowing the fungus to maintain its population for years between sugarbeet crops. Oospores are stimulated to germinate by root exudates from growing sugarbeet plants and can either infect sugarbeet roots directly, or more often, produce motile spores called zoospores. Zoospores can swim short distances in the soil moisture to infect sugarbeet roots. They are delicate structures and do not persist for extended periods of time.

There are many sugarbeet varieties with partial resistance to *A. cochlioides*. None of these varieties, however, have complete resistance, and during very wet and warm growing seasons, the partial resistance in these varieties can be overwhelmed. Sugarbeet seed companies are working on development of new varieties with increased resistance to *A. cochlioides*. Information on pathogen variability can assist breeders in selecting isolates of *A. cochlioides* for screening germplasm for resistance to Aphanomyces damping-off and root rot. It also can provide some information on the effectiveness of resistant varieties in various sugarbeet growing areas with different populations of *A. cochlioides*.

## OBJECTIVES

The objectives of this study were to evaluate isolates of *A. cochlioides* from northern North Dakota, southern Minnesota, and Texas for their ability to produce zoospores and oospores, and for their aggressiveness on a partially resistant, moderate, and susceptible sugarbeet variety.

## MATERIALS AND METHODS

Sixteen isolates of *A. cochlioides* used in this study were baited from soil collected from sugarbeet fields near Cavalier, North Dakota, Buffalo Lake, Minnesota, and Texas. Due to the time required for counting spores, tests for spore production were done utilizing a randomized block design with four replicates and time as a blocking factor.

**Zoospore production.** Cultures were grown for 2 days in peptone glucose broth. Mycelial mats were rinsed two times in a half strength mineral salts solution, and then incubated approximately 16 hours in a mineral salts solution to stimulate production of zoospores. Zoospores then were counted using a Speirs-Levy counting chamber (specialized haemocytometer). The experiment was repeated.

**Oospore production.** Cultures were grown for 4-5 weeks in oat broth. Mycelial mats were removed and blended in 100 ml sterile deionized water. Total volume was brought to 150 ml and oospores were counted using a Speirs-Levy counting chamber.

**Aggressiveness on sugarbeet.** Aggressiveness of isolates was determined by inoculating 4-week-old sugarbeet plants with zoospores. Seed of a partially resistant, moderate, and susceptible variety was sown at a 3/4-inch depth in 6-inch pots (3 seeds of 1 variety/pot) containing autoclaved soil mix (3 parts field soil:1 part sand:1 part peat moss). Pots were placed in the greenhouse at 60-75 ºF with a 16-hour photoperiod. After 2 weeks, plants were thinned to one per pot and fertilized with approximately 3 grams of osmocote 14-14-14 slow release fertilizer.

Plants were inoculated 4 weeks after planting. Zoospores were prepared and counted as described above. Soil was removed from the root to about 1 inch below the soil surface. Each plant was inoculated with 200,000 zoospores in 50 ml sterile deionized water, dispensed around the root, and then soil was returned to the root. Pots were arranged in a randomized block design with four replicates in a growth room at 83 ºF daytime and 77 ºF nighttime with a 16-hour photoperiod. Soil was watered to maintain high soil moisture. Four weeks after inoculation, roots were removed, washed, weighed, and rated for Aphanomyces root rot on a 0 to 7 scale (0 = root clean and healthy, 7 = root completely rotted and foliage dead).

## RESULTS AND DISCUSSION

Zoospore production varied significantly among isolates within and between sampling locations (Figure 1A). Production ranged from a low of 125 zoospores/ml for isolate Tx24 (from Texas) to a high of 194,000 zoospores/ml for isolate C2 (from Cavalier, ND). Zoospore production was highly variable over time (data not shown), but isolates that produced high numbers of zoospores were consistently better producers than isolates that produced low numbers of zoospores.

Oospore production also varied significantly among isolates within and between sampling locations (Figure 1B). Production ranged from a low of 1,000 oospores/ml for isolates B13 (from Buffalo Lake, MN) and Tx1 (from Texas) to a high of 42,000 oospores/ml for isolate C2 (from Cavalier, ND). In general, isolates from Cavalier, ND produced the most oospores, followed by isolates from Buffalo Lake, MN, while isolates from Texas produced relatively low numbers of oospores. This greater production of oospores in North Dakota and Minnesota may be an adaptation for survival over winter.

Six isolates produced enough zoospores to be tested for aggressiveness. These isolates varied in aggressiveness (Figure 2), with root rot ratings across varieties ranging from 1.9 for isolate B13 (from Buffalo Lake, MN) to 4.5 for isolate C2 (from Cavalier, ND). The partially resistant variety had a root rot rating of 2.0, which was significantly lower than ratings for the moderate (2.5) and susceptible variety (2.9) (data not shown). In addition, root weights (expressed as percent of non-inoculated control) were significantly higher for the partially resistant (58%) and moderate variety (53%) than for the susceptible variety (39%) (data not shown). There was no significant interaction between isolate and variety. This is very important because it provides some evidence that commercial varieties with partial resistance to *Aphanomyces* will respond similarly to populations of *A. cochlioides* when planted in southern Minnesota and North Dakota (no isolates from Texas were in the aggressiveness test). Isolates that are both good spore producers and highly aggressive should be chosen for screening procedures that utilize inoculations with spores.

## SUMMARY

*Aphanomyces cochlioides* isolates from northern North Dakota, southern Minnesota, and Texas varied significantly in zoospore and oospore production and in aggressiveness on sugarbeet.

Differences were found in amount of disease for partially resistant, moderate, and susceptible varieties, but response to isolates from both locations was similar.

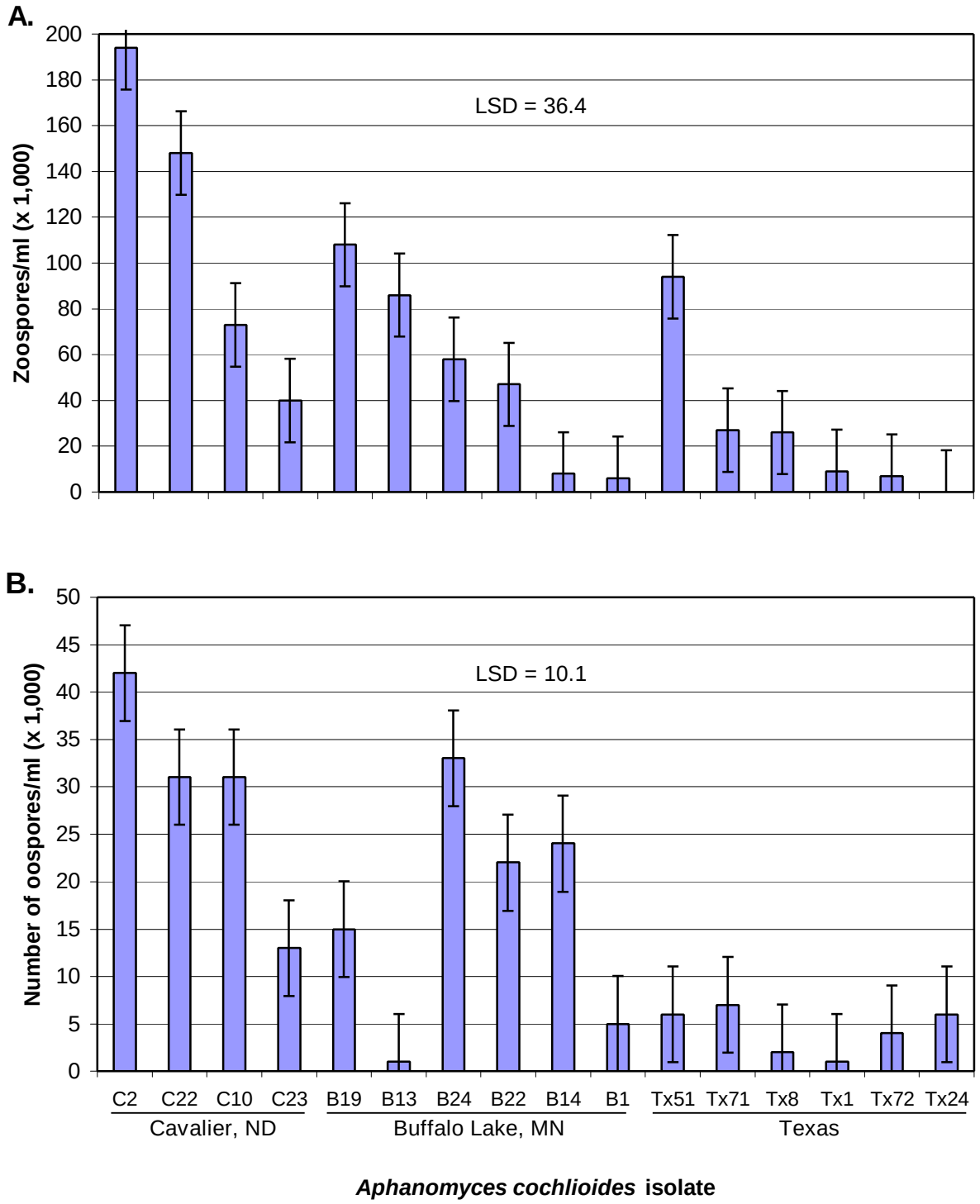


**Figure 1.** Production of **A)** zoospores and **B)** oospores of 16 isolates of *Aphanomyces cochlioides* from Cavalier, ND, Buffalo Lake, MN, and Texas. Zoospore numbers are based on two experiments, each with four replicates; oospore numbers are based on one experiment with four replicates.

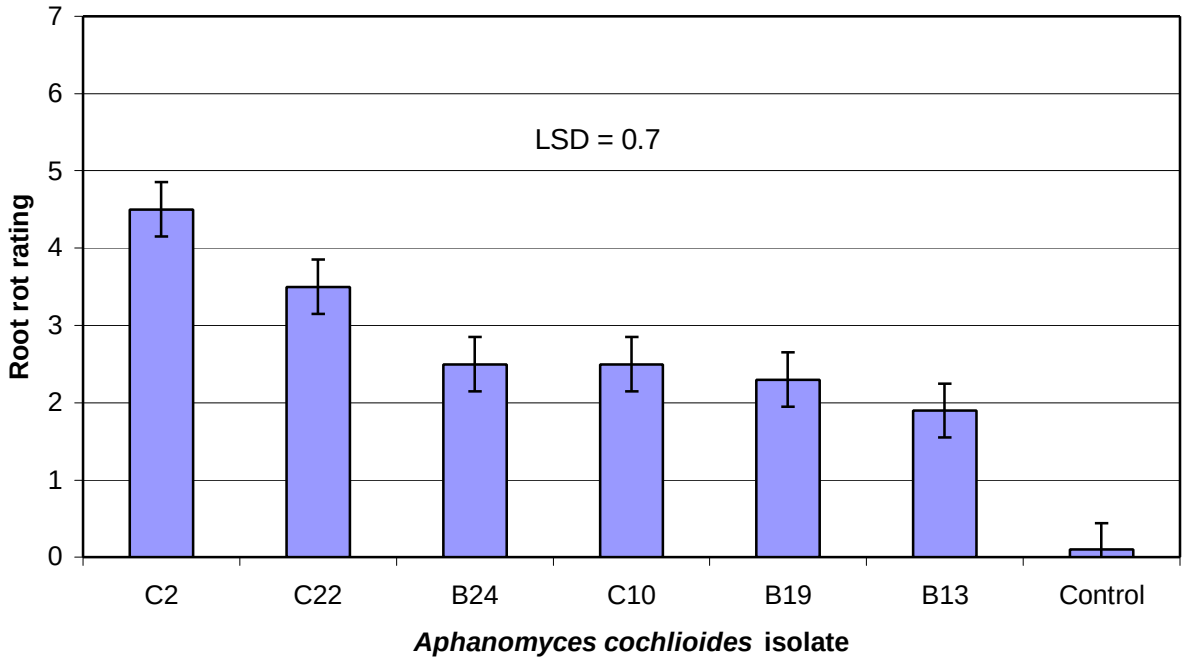


**Figure 2.** Root rot ratings (0-7 scale, 0 = healthy, 7 = root completely rotted and foliage dead) of six isolates of *Aphanomyces cochlioides* inoculated on roots of 4-week-old plants (200,000 zoospores per root) and evaluated 4 weeks later. Isolates of *A. cochlioides* were collected in Cavalier, ND (C) and Buffalo Lake, MN (B); the control was not inoculated. Each value is averaged across three sugarbeet varieties (partially resistant, moderate, and susceptible) for a total of 12 roots.

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## CONCLUSION

Varieties responded similarly with respect to disease resistance to isolates of *A. cochlioides* from northern North Dakota and southern Minnesota. Isolates used in disease resistance breeding programs should be chosen on the basis of spore production (to make screening procedures easier) and aggressiveness (to allow separation of germplasm).

## ACKNOWLEDGMENTS

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