Forage legumes / Legumineuses fourrageres

CROP: Alfalfa

LOCATION: South Western Ontario

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TITLE: SURVEY OF *APHANOMYCES* SP. IN ALFALFA FIELDS IN SOUTH WESTERN ONTARIO

METHODS: Eighty three established alfalfa fields scattered in 18 counties across south western Ontario were visited during mid May and early July, 1992. The alfalfa stands ranged from 1 to 8 years old and were on different types of soil. Soil samples were taken from five random sites in each field, and **3-5** L of soil was collected from most of the fields. The samples were stored at -10°C in a cold room for **3-5** weeks before processing.

After being taken out from storage, the soil samples were saturated with distilled water, incubated for 10 days at 20°C in a growth room, and potted. Four pots (10-cm diameters) of soil were prepared for each field, placed in an aluminum-foil tray, and watered. Sixty alfalfa seeds of cultivar Saranac were sown in each pot, and kept at 22°C for 3 days. Roughly at the stage of early seedling emergence, the soils were flooded for 7 consecutive days.

Alfalfa seedlings were removed from the soil after flooding. Roots and hypocotyl with water-soaked discoloration were surface-disinfested with a 0.6% NaOCl solution, placed on metalaxyl-benomyl-vancomycin agar, a semiselective medium for *Aphanomyces* sp., and incubated at 20°C for 4-7 days. Colonies with mycelium typical of *Aphanomyces* spp. were transferred to cornmeal agar and incubated at 20°C for further identification. After surface disinfestation, some diseased seedlings were placed in 9-cm petri dishes containing 10 mL of distilled water. Growth of fungi from the seedlings was examined under a microscope.

To confirm the pathogenicity of the isolates of *Aphanomyces* sp., an inoculation test was conducted in a controlled environment. Inoculum of *Aphanomyces* sp. was produced through procedure modified from previously reported methods. Briefly, mycelium grown on potato dextrose agar was transferred to a broth containing 2% peptone and 0.5% glucose, and cultured with shaking for 7 days at 22°C. The contents were then broken into small pieces with a food blender. Twenty-five alfalfa seeds of cultivar Apollo II were planted in microwave-sterilized soil in a 10-cm pot. The seedlings in each pot were inoculated with 10 mL of the inoculum using a syringe **4** days after planting, and treated pots were placed in aluminum-foil trays. Two hours later, water was added to the trays to a level 5 cm from the top of the pots and was maintained there for 7 consecutive days. Seedlings were then removed from the soil and examined for the presence of the pathogen using the procedure described above.

In addition, diseased alfalfa plants from eight new spring seedings with extensive root rot damage were surface disinfested using the method described above, and incubated on the semiselective medium and water agar **(0.5%)** for identification of the causal agent.

RESULTS AND DISCUSSION: *Aphanomyces* sp. was found in 6 of **83** alfalfa fields examined, and the infested fields occurred in five counties (Figure 1). Alfalfa had been grown in the infested fields for 2 to 4 years, and soils in the fields were generally clay-loam. One of the fields traditionally had root-rot problems, but no typical root-rot symptoms were observed at the time of field visit.

Other fungi isolated most frequently from the soil samples using the semiselective medium were *Fusarium* spp. and *Rhizopus* spp. However, pathogenicity of the two genera on alfalfa seedlings was not observed in an inoculation test in a controlled environment.

Phytophthora megasperma f.sp. *medicaginis* was often isolated from diseased plants collected from new alfalfa spring seedings with serious root rot damage, and most of those stands were cultivars susceptible to moderately resistant to the pathogen. But in a few cases, *Phytophfhora* root rot occurred

widespread and severely in resistant varieties. *Aphanomyces* sp. was not observed in those diseased plants obtained from fields.

The survey covered a large area in south-western Ontario to investigate distribution of *Aphanomyces* sp. in alfalfa fields. The results indicated that the pathogen was isolated from only 7% of the fields sampled, which was significantly lower than figures released from a previous study around the London, Ontario area.

Aphanomyces is a water mold which requires saturated soil conditions for infection. The crop season of 1992 was exceptionally wet and cool in south-western Ontario, but damage to new alfalfa seeding caused by the pathogen was not observed in our field trips. In controlled environments, isolates of *Aphanomyces* sp. recovered from the area caused severe damage to alfalfa seedlings when the plants were flooded for 7 days after inoculation. The results may suggest that *Aphanomyces* **sp.** is potentially a serious pathogen of alfalfa in Ontario. However, more field observation is needed to understand the real impact of the disease on alfalfa production.

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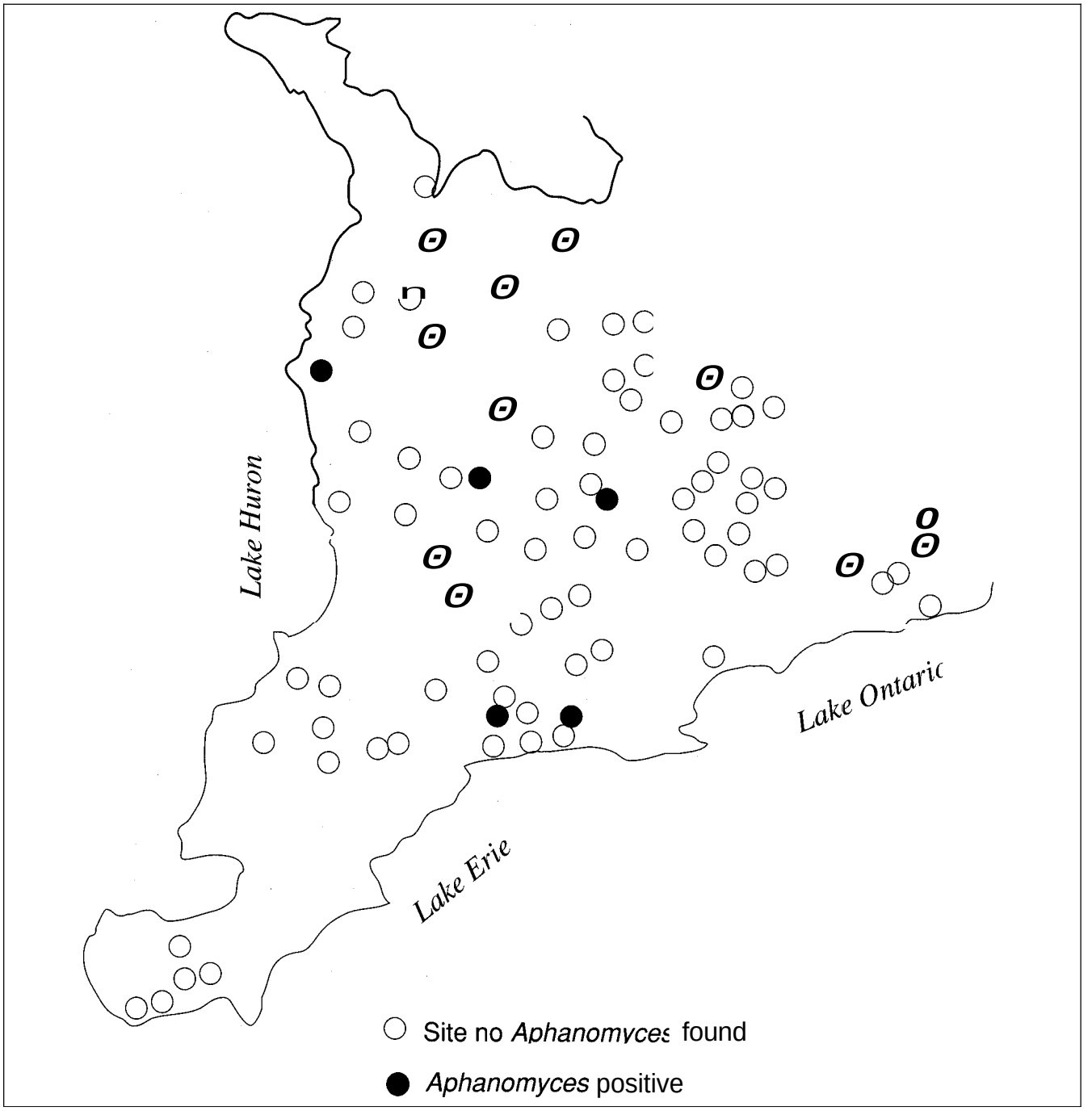
REFERENCES

1. Beghdadi, A., C. Richard, and D. Dostaler. **1992.** L`*Aphanomyces eufeiches* des luzernieres du Quebec: isolement, morphologie et variabilite de la croissance et du pouvoir pathogene. Can. J. Bot. 70:1903-1911.
2. Delwiche, P.A., C.R. Grau, E.B. Holub and J.B Perry. **1987.** Characterization of *Aphanomyces eufeiches* isolates recovered from alfalfa in Wisconsin. Plant Dis. 71:155-161.
3. McKeen, W.E. and J.A. Traquair. **1980.** *Aphanomyces* sp., an alfalfa pathogen in Ontario. Can. J. Plant Path. 2:42-44.



'igure 1. Survey of *Aphanomyces* **sp.** in South Western Ontario (1992)