## EVALUATION OF NOVEL STRATEGIES FOR REDUCTION OF *APHANOMYCES COCHLIOIDES* ON SUGARBEET BY GREEN MANURE CROPS AND SOIL SOLARIZATION

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Aphanomyces cochlioides (= A. cochlioides) is a soilborne "water mold" that causes seedling stand loss and chronic root rot of older sugarbeet plants when soil is warm and wet. Unusually wet summers in the last 10 years have favored increases in the prevalence and severity of Aphanomyces diseases on sugarbeet. In 1999, about 51% of acres planted to sugarbeet in Minnesota and North Dakota were estimated as infested with A. cochlioides. This pathogen produces thick-walled oospores in infected roots, which survive in soil for years, even when a sugarbeet crop is not grown. Little is known about factors affecting survival of oospores, but a visual technique was recently described to distinguish viable (living) from dead oospores (2, 3).

Current control measures for Aphanomyces damping-off and root rot include early planting (to avoid warm, wet soils favorable for infection), seed treatment with the fungicide Tachigaren (hymexazol), planting partially resistant varieties, water management (installing tiles or ditches to improve soil drainage, cultivating to dry soil), and weed control (*A. cochlioides* infects several common weed species, e.g., pigweed, lamb's-quarters, kochia). When fields have high potential for disease, producers are advised to avoid planting sugarbeet because if the season is wet and warm, control options are inadequate and do not result in an economic return.

Since disease control options are limited in effectiveness, other novel strategies are being explored. Green manure crops are reported to suppress several soilborne pathogens and pests on many crops (5). Examples of disease suppression by green manure crops include: sorghum sudan grass for Verticillium wilt on potato, buckwheat for scab on potato, oilseed radish for the sugarbeet cyst nematode, and oat for Aphanomyces root rot on pea and sugarbeet (8). Soil solarization is done by covering wet soil with clear polyethylene plastic to capture solar energy and increase soil temperatures, ideally to 97-122 °F in the upper 12 inches. High soil temperatures can "pasteurize" soil and rid it of certain soilborne fungi, weed seeds, and other pests (5). Equipment is available for large-scale solarization and it has been applied most effectively in geographic areas with intense solar radiation to control a wide range of soilborne plant pathogens on economic crops. Solarization also can be effective in temperate regions when combined with green manure crops, reduced dosages of chemicals, or biological control organisms (5). For instance, Fusarium wilt of cabbage was most reduced when plots with cruciferous residues were covered with plastic tarp and solarized compared to either treatment alone (7).

#### **OBJECTIVES**

The purpose of this research was to determine the effect of several green manure crops and soil solarization for 1) suppression of Aphanomyces root rot on sugarbeet in infested fields and 2) survival of oospores of *A. cochlioides*. This report provides results for two field locations; one was established in 2001 and sown to sugarbeet in 2002 and the repeat trial was initiated in 2002 and will be planted to sugarbeet in 2003.

## MATERIALS AND METHODS

**Precrop and solarization treatments.** The first trial was initiated on May 15, 2001 in the Aphanomyces Nursery at the University of Minnesota, Northwest Research and Outreach Center (NWROC), Crookston and the second was established on May 24, 2002 in a growers' field near Crookston that was naturally infested with *A. cochlioides*. In 2001, seed of green manure crops were sown by hand and raked into soil. Crops included buckwheat var. Koto, oilseed radish var. Colonel and sorghum sudan grass var. Green Grace Supreme sown at the equivalent of 45, 18, and 13.5 lb/A, respectively. In 2002, the same crops were drill-seeded (except for oilseed radish, which was sown by hand) and two were added: oat var. Dane and wheat var. 2375 (each sown at 2 bu/A). Oat has been shown to suppress Aphanomyces on sugarbeet and wheat commonly is grown the season before sugarbeet. The control was fallow soil. Each plot measured 20 x 30 ft in 2001 but plots were increased to 40 x 30 ft in 2002. Treatments were arranged in a randomized block design with six replicates in 2001 and four in 2002.

At planting, soil cores (6, 2.5-inch diameter) were collected to a depth of 6 inches and combined per plot. Soil samples were evaluated by a sugarbeet seedling assay in the greenhouse and Aphanomyces soil index values were determined (9). Values ranged from 0 to 100; 0 = healthy and 100 = all sugarbeet seedlings dead.

On July 11, 2001 (8 weeks after planting), all green manure crops were mowed and the residue was disked and rototilled into soil to a 3- to 4-inch depth (soil was too dry and compacted to incorporate residue deeper) Amounts of buckwheat, oilseed radish, and sorghum sudan grass incorporated into plots averaged 8, 17, and 6 tons fresh weight/A, respectively. In 2002, green manure crops were mowed

and incorporated on July16 (7½ weeks after planting) and amounts of buckwheat, oilseed radish, sorghum sudan grass, oat, and wheat averaged 6, 17, 13.5, 12, and 10 tons fresh weight/A, respectively. Fallow control plots also were disked and rototilled. Each main plot (green manure crops and fallow) then was split into two subplots (one for solarization and the other not solarized). Soil samples were collected in each subplot and later indexed for Aphanomyces root rot in the greenhouse, as previously described.

Thermocouples were buried at 3, 6, and 9 inches in subplots of one replicate and soil temperatures were monitored and recorded on a Watchdog (Spectrum Technologies, Plainfield, IL) data logger every 15 minutes during the weeks of solarization. In 2001, the trial was irrigated (1.2 inches) and then plots designated for solarization were covered with a clear, horticultural grade polyethylene plastic (3 mil thick) on July 13. Edges of tarps were manually buried in furrows along borders of solarized subplots. Plots were similarly covered with plastic on July17, 2002 but were not irrigated due to lack of facilities. In 2002, plots had received about 6.6 inches of rainfall in June and 0.2 inches in July before solarization. Precipitation data were recorded during solarization in 2002; after tarps were applied, another 0.5 inches of rainfall occurred in July and 6.8 inches occurred in August.

**Oospore survival.** Oospores of *A. cochlioides* also were buried in soil to observe the effect of green manure crops, with and without soil solarization, on oospore survival. To set up this experiment, oospores were produced in the laboratory by placing excised, 0.75-inch segments of 2-week-old sugarbeet hypocotyls (portion of the seedling between point of seed attachment and cotyledonary leaves) in sterile water. Zoospores of *A. cochlioides* then were added and tissues were incubated in the dark at  $68 \pm 5$  °F for 7 weeks. Several hypocotyls then were macerated and the contents were microscopically examined to determine total number of oospores. In 2001 and 2002 there were an average of 8,000 and 15,000 oospores/hypocotyl, respectively. One hypocotyl segment was placed in the bottom of a bag (1 x 1 inch) of a nylon monofilament mesh fabric (less than  $10\mu$  pores), which was closed with string and placed in a pan of water to prevent drying until buried in soil. All hypotocotyls had been microscopically examined to ensure they contained oospores. Bags with oospores were buried at depths of 3, 6, and 9 inches in the green manure crop and fallow subplots designated to be solarized or not solarized (four replicates/treatment). Two bags were buried per depth, one for retrieval immediately after solarization and another for removal 4 weeks later (effects of solarization on survival structures, such as oospores, can be delayed).

Tarps were removed from subplots after solarization for 9 and 7 weeks in 2001 and 2002, respectively. One set of oospores then was removed from the three depths of each subplot and nearly 4 weeks later, the second set was removed. Retrieved bags were placed in plastic bags, moistened with water, and stored in a refrigerator until examined. Each bag was carefully opened along the outside seams. Hypocotyls were removed and microscopically inspected to determine the amount of tissue and number of oospores present. Relative amounts of hypocotyl tissue were assessed on a 0-5 scale: 0 = no tissue present, 1 = 1-20% of original tissue present (or only vascular tissue remaining), 2 = 21-40%, 3 = 41-60%, 4 = 61-80%, and 5 = 81-100% of original tissue intact. Relative numbers of oospores also were assessed on a 0-4 scale: 0 = none observed, 1 = 100 or fewer, 2 = more than 100 to 1,000, 3 = more than 10,000 to 10,000, and 4 = more than 10,000. Hypocotyl tissue was transferred to a microscope slide and oospores were examined at 400X magnification to assess if they were viable (alive) or dead (2). A minimum of 100 oospores were evaluated per sample, if available. When hypocotyls were severely deteriorated, interiors of mesh bags were microscopically scanned for oospores; if present, they were removed with two-sided transparent cellophane and assessed for viability.

In 2002, another method was used to quantify number of total oospores (living and dead) and number of living oospores. After condition of each hypocotyl had been assessed, as described above, it was placed in a 2 ml Wheaton tissue grinder; 1 ml of distilled water was added; and the plunger was depressed fifteen times. Contents were transferred to a 1.5 ml tube and centrifuged 10 minutes at 10,000 rpm. A 750  $\mu$ l aliquot of supernatant (not containing oospores) was decanted and the remaining 250  $\mu$ l (containing oospores and macerated hypocotyl tissue) was vortex-mixed for several seconds. Subsamples were placed in a Speirs-Levy eosinophil counting chamber and oospores were microscopically examined and counted.

**Sugarbeet following precrops and soil solarization.** On May 22, 2002, non-solarized plots established in 2001 were fertilized with nitrogen (100 lb/A) to equal nitrogen in solarized plots. Sugarbeet seed of variety Beta 2088 was sown at a 1.25-inch spacing in 30 foot rows, 22 inches apart; there were six rows of sugarbeet in each subplot established in 2001. Counter was applied in a 7-inch band at 1.8 lb active ingredient/A at planting. Microrates of herbicides were applied on June 18 (0.5 pint Betamix, 1/8 oz Upbeet, 60 ml Select, 40 ml Stinger, 1½ pint Scoil), June 22 (0.5 pint Betanex, 1/8 oz Upbeet, 60 ml Select, 40 ml Stinger, 1½ pint Scoil), and June 27 (0.5 pint Betanex, 1/8 oz Upbeet, 5.3 oz Poast, 40 ml Stinger, 1½ Scoil [product/A]). Plots also were cultivated on July 1; thinned on July 2; and irrigated on May 29, June 3 and 7, and July 3 at 253, 181, 181, and 217 gallons/two middle rows of each subplot, respectively. Rainfall was abundant after early July (3.7 inches), followed by 9.2 inches in August and further irrigation was not necessary. Fungicides for controlling Cercospora leaf spot were applied on August 2 and 20 and September 4 with 5 oz Supertin, 13 oz Eminent, and 5 oz Supertin (product/A), respectively.

healthy, 7 = root completely rotted and foliage dead). Ten of these roots were analyzed for yield and sucrose quality by the American Crystal Sugar Company Quality Laboratory, East Grand Forks, MN.

**Data analysis**. Data for relative amounts of sugarbeet hypocotyl tissue, relative number of total oospores, percent living oospores, number of total oospores (living and dead), and number of living oospores were subjected to appropriate transformations (if needed) and Analysis of Variance. If significant ( $P \le 0.05$ ), means were separated by Least Significant Difference (LSD). Correlations were calculated for relative amounts of hypocotyl tissue and total oospores (living and dead) and for relative amounts of hypocotyl tissue and total oospores (living and dead) and for relative amounts of hypocotyl tissue and total oospores (living and dead) and for relative amounts of hypocotyl tissue and living oospores.

# RESULTS

**Greenhouse assay for Aphanomyces soil index values.** Before green manure crops were sown in the Aphanomyces Nursery in 2001 and in the *A. cochlioides*-infested field near Crookston in 2002, average soil index values were 97 and 99, respectively (<u>Table 1</u>). After incorporation of green manure crops (and before solarization), soil index values were reduced by all precrop treatments in both seasons by varying amounts (<u>Table 1</u>). Buckwheat resulted in the greatest reduction of root rot in 2001 and among the least change in 2002; oat resulted in the greatest reduction in root rot in 2002; and sorghum sudan grass resulted in the least reduction in both seasons. Soil index values for fallow plots remained virtually the same during this period in both seasons.

Soil temperatures attained by solarization. Maximum soil temperatures recorded in green manure crop subplots (illustrated for oilseed radish) were similar to the fallow control (solarized or not solarized) in both seasons (Table 2). At the 3-inch depth, solarization resulted in maximum soil temperatures between 102–110 °F, and generally were nearly 20 °F higher than temperatures recorded in non-solarized soils. A less dramatic temperature differential between solarized and non-solarized soils occurred with increasing soil depths. At 9 inches, maximum soil temperatures were at least 10 °F higher in solarized (90-93 °F) than in non-solarized (76 - 81 °F) soils. The highest ambient temperature recorded during the solarization periods was 96 °F on August 5, 2001 and 90 <sup>0</sup>F on August 2, 2002.

**Oospore survival.** Examination of sugarbeet hypocotyls and oospores removed from soil immediately after solarization in both seasons and a second sampling 4 weeks later in 2001 (samples are not completed for 2002) revealed various stages of tissue decomposition in both seasons. Some hypocotyls were fairly intact and the cortex surrounding vascular tissue contained abundant oospores. In other cases, the cortex was severely decomposed and

Table 1.Aphanomyces soil index values determined in the greenhouse for field soil collected when green manure crops were sown<br/>(May 15, 2001; May 24, 2002) and after they were incorporated (July 11, 2001; July 16, 2002) the day before solarization<br/>began; the control was fallow soil. In 2001, plots were located in the Aphanomyces Nursery at the University of Minnesota,<br/>Northwest Research and Outreach Center, Crookston and in 2002, were in a growers' field near Crookston that was naturally<br/>infested with Aphanomyces cochlioides.

|                        |                           | Soil index value <sup>z</sup> |        |                           |                               |        |  |  |  |  |
|------------------------|---------------------------|-------------------------------|--------|---------------------------|-------------------------------|--------|--|--|--|--|
|                        | 2001                      |                               |        |                           | 2002                          |        |  |  |  |  |
| Soil Treatment         | Before green<br>crop sown | After green crop incorporated | Change | Before green<br>crop sown | After green crop incorporated | Change |  |  |  |  |
|                        |                           |                               |        |                           |                               |        |  |  |  |  |
| Buckwheat              | 96                        | 63                            | -33    | 99                        | 90                            | -9     |  |  |  |  |
| Oat                    | -                         | -                             | -      | 99                        | 79                            | -20    |  |  |  |  |
| Oilseed radish         | 98                        | 75                            | -22    | 100                       | 86                            | -14    |  |  |  |  |
| Sorghum sudan grass    | 96                        | 78                            | -18    | 99                        | 90                            | -9     |  |  |  |  |
| Wheat                  | -                         | -                             | -      | 99                        | 83                            | -16    |  |  |  |  |
| Fallow soil<br>Average | <u>98</u><br>97           | 97                            | 1      | $\frac{100}{99}$          | 96                            | -4     |  |  |  |  |

z

Each value based on planting 25 sugarbeet seed of variety ACH 261/pot (four pots/soil treatment/replicate). Six soil cores had been collected to a 6-inch depth per treatment and combined. Four weeks after planting, index values were determined on a 0-100 scale where 0=plant healthy, 100=all plants dead or severely rotted; - = precrop not grown. Each value is based on six replicates in 2001 and four replicates in 2002.

Table 2.Maximum soil temperatures recorded in fallow and green manure precrop plots (illustrated for oilseed radish) that were<br/>solarized (soil covered with clear, polyethylene plastic) or not solarized from July 13 to September 13, 2001 in the<br/>Aphanomyces Nursery at the University of Minnesota, Northwest Research and Outreach Center, Crookston and from July<br/>17 to September 4, 2002 in a growers' field near Crookston that was naturally infested with Aphanomyces cochlioides.

|                | Maximum soil temperature (°F)/depth (inches) <sup>z</sup> |   |   |      |   |   |   |  |
|----------------|---|---|---|------|---|---|---|--|
|                | 2001  |   |   | 2002 |   |   |   |  |
| Soil treatment | 3   | 6 | 9 |      | 3 | 6 | 9 |  |

| Solarized      |     |     |    |     |    |    |
|----------------|-----|-----|----|-----|----|----|
| Oilseed radish | 109 | 97  | 93 | 102 | 99 | 90 |
| Fallow         | 106 | 100 | 91 | 110 | 96 | 90 |
|                |     |     |    |     |    |    |
| Non-solarized  |     |     |    |     |    |    |
| Oilseed radish | 90  | 86  | 81 | 91  | 83 | 76 |
| Fallow         | 88  | 84  | 81 | 91  | 86 | 80 |

Data loggers were buried at 3-, 6-, and 9-inch depths in one replicate and temperatures were monitored and recorded every 15 minutes during the term of soil solarization.

only vascular tissue, which contained a few oospores, remained (10). Occasionally, no hypocotyl tissue and no oospores (or only a few oospores) were attached to the interior of the mesh bag.

In 2001, the relative amount of sugarbeet hypocotyl tissue in mesh bags retrieved after solarization and 4 weeks later averaged 2.1 for both sampling times (data not shown). In 2002, the relative amount of hypocotyl tissue after solarization averaged 2.6 (data not shown). A rating of 2 = 21 to 40% of original tissue buried in soil was intact and 3 = 41 - 60% was intact. Green manure crops and the fallow control, solarized and non-solarized treatments, and depth of burial in soil did not have a significant effect on amount of hypocotyl tissue remaining at either sampling date in 2001 and 2002 (data not shown).

Table 3. Survival of oospores of *Aphanomyces cochlioides* within sugarbeet hypocotyls buried in field trials conducted in 2001 and 2002. Oospores were buried at three depths within 1 day after various green manure crops had been incorporated into soil and then plots were solarized (covered with a clear polyethylene tarp on July 13 - September 13, 2001 [9 weeks] and July 17 - September 4, 2002 [7 weeks]); controls included non-solarized plots of each green manure precrop and fallow plots (solarized and non-solarized). Hypocotyls were retrieved and oospores within this tissue were microscopically assessed for viability immediately after solarization and 4 weeks later in 2001 and immediately after solarization in 2002. The 2001 trial was established in the Aphanomyces Nursery at the University of Minnesota, Northwest Research and Outreach Center, Crookston and the 2002 trial was in a growers' field near Crookston that was naturally infested with *A. cochlioides*.

|                                 | 2001 - % ] | Living oospores <sup>u</sup> | 2002 – Number of oo     | spores at 7 weeks <sup>u.v</sup> |  |
|---------------------------------|------------|------------------------------|-------------------------|----------------------------------|--|
| Treatment                       | 9 weeks    | 13 weeks                     | Total (living and dead) | Living                           |  |
|                                 |            |                              |                         |                                  |  |
| <u>Precrop<sup>w</sup></u>      |            |                              |                         |                                  |  |
| Sorghum sudan grass             | 52         | 41                           | 7,430                   | 1,770                            |  |
| Oilseed radish                  | 48         | 31                           | 5,950                   | 1,790                            |  |
| Buckwheat                       | 32         | 36                           | 8,040                   | 1,470                            |  |
| Oat                             | -          | -                            | 7,040                   | 1,660                            |  |
| Wheat                           | -          | -                            | 7,580                   | 1,900                            |  |
| Fallow                          | <u>33</u>  | <u>35</u>                    | <u>6,280</u>            | 1,460                            |  |
| Mean                            | 41         | 36                           | 7,050                   | 1,675                            |  |
| LSD $(P < 0.05)^{z}$            | NS         | NS                           | NS                      | NS                               |  |
| , <b></b>                       |            |                              |                         |                                  |  |
| Soil treatment <sup>x</sup>     |            |                              |                         |                                  |  |
| Solarized                       | 35         | 43                           | 7,180                   | 2,230                            |  |
| Non-solarized                   | 47         | 28                           | 6,920                   | 1,117                            |  |
| Mean                            | 41         | 36                           | 7,050                   | 1,675                            |  |
| LSD $(P \le 0.05)^{Z}$          | NS         | 13                           | NS                      | 809                              |  |
|                                 |            |                              |                         |                                  |  |
| Depth (inches) <sup>y</sup>     |            |                              |                         |                                  |  |
| 3                               | 38         | 25                           | 6,670                   | 1,770                            |  |
| 6                               | 34         | 35                           | 6,920                   | 1,420                            |  |
| 9                               | 52         | 48                           | 7,750                   | 1,840                            |  |
| Mean                            | 41         | 36                           | 7,050                   | 1,675                            |  |
| LSD $(P \le 0.05)^{\mathbb{Z}}$ | NS         | 16                           | NS                      | NS                               |  |

u

v

Z

Each value for percent living oospores and number of total oospores (living and dead) is based on observing and counting at least 1,800 oospores per precrop in 2001 and 2002; 3,600 per soil treatment in 2001 and 5,400 in 2002; and 2,400 per depth in 2001 and 3,600 in 2002. These values reflect oospores that were observable after they were retrieved from soil; many oospores died as sugarbeet hypocotyls decomposed in soil and therefore, could not be counted. Number of living oospores per 0.75-inch hypocotyl before burial in soil in 2001 and 2002 averaged 8,000 and 15,000, respectively.

- W Each value averaged across soil treatment and depths; = cover crop not sown. In 2001, precrops were sown on May 15 and incorporated on July 11 and in 2002, they were sown on May 24 and incorporated on July 16.
- X Each value averaged across precrop treatment and depth.
- y Each value averaged across precrop treatment and soil treatment.
- <sup>Z</sup> LSD = Least Significant Difference; if significant, LSD value provided for mean separations; NS = not significant.

Immediately after solarization in 2001, the <u>relative number</u> of total oospores (living and dead) remaining in buried sugarbeet hypocotyls averaged 2.2 and when the second set of oospores was removed 4 weeks later, averaged 2.1 (data not shown; a rating of 2 = 100 - 1,000 oospores). After solarization was completed in 2002, the relative number of total oospores in buried sugarbeet hypocotyls averaged 2.7. Green manure crop and fallow treatments, solarized and non-solarized treatments, and depth of burial did not significantly affect relative number of total oospores (living and dead) in hypocotyls in either year (data not shown).

In 2001, there were significant and positive correlations between the relative amount of hypocotyl tissue and relative number of total oospores (living and dead) in solarized and non-solarized plots at the three depths immediately after solarization (10). Number of total oospores present increased as the amount of intact hypocotyl tissue increased. After solarization in 2002, there again was a significant and positive correlation between the relative amount of hypocotyl tissue and total number of oospores (living and dead) in solarized plots at 3, 6, and 9 inches (Fig. 1A). In non-solarized plots, however, this relationship occurred only for oospores buried at the 6-inch depth (Fig. 1B). Oospores rarely survived or were not found in mesh bags containing severely decomposed sugarbeet hypocotyls, indicating that all or most had died and decomposed.

Viability assessments of oospores in both years are summarized in <u>Table 3</u>. There were no significant interactions between main treatments in either year, so data are presented only for main treatments. Of oospores present in sugarbeet hypocotyls retrieved from soil after solarization and 4 weeks later in 2001, 41% and 36% were alive, respectively. Oospores were directly quantified for samples removed after solarization in 2002. An average of 1,675 out of 7,053 present (24%) were alive (nearly 76% of oospores observed were dead). An average of 15,000 oospores were present in hypocotyls when they were buried, so overall survival after burial in soil for 7 weeks was about 11%.

For samples removed immediately after solarization in both years, percentages or numbers of living oospores observed were not significantly affected by green manure crop and fallow treatments in solarized and non-solarized plots (<u>Table 3</u>). In 2001, solarization for 9 weeks had no effect on percent living oospores but 4 weeks later, survival was significantly greater in solarized soil. In 2002, total numbers of oospores were the same in solarized and non-solarized soils, but the number of living oospores was significantly higher in solarized soil. Oospore viability was not significantly affected in either season by depth of burial after solarization was completed, but viability was significantly higher with increasing depth of burial for samples collected 4 weeks later in 2001.

After solarization in 2001, there was a significant and positive correlation between amounts of hypocotyl tissue and percent living oospores for samples collected in solarized soils at all depths and in non-solarized soils at 3 and 9 inches; in non-solarized soils at 6 inches, about 25% of oospores were viable regardless of amount of hypocotyl tissue present (data not shown). For samples collected 4 weeks later, correlations between percent living oospores and relative amounts of hypocotyl tissue in solarized and non-solarized soils were significant and positive for samples buried at 6- and 9-inch depths but not at 3 inches; at 3 inches, a low percentage of oospores (about 25%) were alive in solarized and non-solarized soil regardless of the amount of hypocotyl tissue present (10). In 2002, there was a significant and positive correlation between amount of hypocotyl tissue and the number of living oospores buried in solarized soil at 3 and 9 inches, but not at 6 inches (Fig. 2A) but there was no correlation for these factors in non-solarized soil at any depth (Fig. 2B).

**Sugarbeet following precrops and soil solarization.** There were no significant interactions between main treatments (precrop or soil solarization) for data collected on sugarbeet in 2002, so data are presented only for main treatments (<u>Table 4</u>). Green manure crops grown the previous season and the fallow control had no significant effect on sugarbeet stands at 2 and 4 weeks after planting. Stands at both dates were significantly higher in solarized than non-solarized plots although stands decreased after emergence in both treatments because of Aphanomyces damping-off. Precrop also did not affect severity of Aphanomyces root rot on sugarbeet roots rated 6 weeks after planting but disease ratings were significantly lower in plots that had been solarized compared to non-solarized plots.

At harvest, precrop treatments had no significant effect on number of marketable sugarbeet roots, Aphanomyces root rot ratings, yield, or quality. Solarization of plots the previous season resulted in a significant reduction in root rot and significant increases in tons of roots and recoverable sucrose per acre, but had no effect on number of marketable roots, percent sucrose, or pounds of sucrose per ton when compared to non-solarized plots.

18,000 Total no. oospores (living + dead) Δ Ŷ 9" (\*)\$3" 15,000 Δ **¤**6" 12,000 ∆9" 盦 9,000 6,000 ŏ 4 3,000 Ē 0 0 1 2 3 4 5

Relative amount hypocotyl tissue

Β

Α

Α

Non-solarized Soil



Fig. 1. Relationship between relative amount of **Relative** parapulative parapulative parapulation of parameters (living and dead) of Aphanomyces cochlicides (hypocotyl contained about 15,000 oospores/0.75-inch segment and were placed in nylon mesh bags) after burial in field plots at 3, 6, and 9 inches (July 17, 2002) that then were A) solarized and B) not solarized until September 4. Relative amount of hypocotyl tissue based on a 0 - 5 scale, where 0 = no tissue present and 5 = 81-100% of tissue originally buried was intact. NS = no significant relationship and \* = significant relationship,  $P \le 0.05$ .

Solarized Soil

Solarized Soil



Non-solarized Soil



Fig. 2. Relationship between relative amount of sugarbeet hyperbyl tissue and number of <u>living oospores of Aphanomyces. coch</u> ioides (hypocotyl contained about 15,000 oospores/0.75-inch segment) after burial infield plots at 3, 6, and 9 inches (July 47, 2002) that then were A) solarized and B) not solarized until September 4. Relative amount of hypocotyl tissue based on a 0-5 scale, where 0 = no tissue present and  $5 = 81 \pm 100\%$  of tissue originally buried was intact. NS = no significant relationship and \* = significant relationship,  $P \le 0.05$ . 4 5

Table 4.Sugarbeet seedling emergence application of the processing of the processing emergence application of the processing emergence applic

|  | No. plants/60 ft row<br>(WAP) <sup>v</sup> |     | Root rot<br>rating   | No. marketable               | Root rot <sup>x,y</sup> | Yield              |      | Su   | crose <sup>x</sup> |
|--|--|-----|----------------------|------------------------------|-------------------------|--------------------|------|------|--------------------|
|  | 2  | 4   | (6 WAP) <sup>w</sup> | roots/60 ft row <sup>x</sup> | rating                  | (T/A) <sup>x</sup> | %    | lb/T | lb recoverable/A   |
|  |  |     |                      |                              |                         |                    |      |      |                    |
| Precrop  |  |     |                      |                              |                         |                    |      |      |                    |
| Sorghum sudan<br>grass   | 421  | 372 | 1.28                 | 70                           | 2.6                     | 22.1               | 15.5 | 279  | 6,167              |
| Buckwheat  | 415  | 370 | 1.25                 | 70                           | 2.5                     | 22.5               | 15.8 | 286  | 6,421              |
| Oilseed radish   | 424  | 375 | 1.23                 | 67                           | 2.5                     | 21.6               | 15.8 | 288  | 6,202              |
| Fallow   | 432  | 372 | 1.33                 | 68                           | 2.7                     | 21.7               | 15.4 | 276  | 5,984              |
| $\frac{\text{LSD} (P \le 0.05)^{\text{z}}}{(P \le 0.05)^{\text{z}}}$ | NS   | NS  | NS                   | NS                           | NS                      | NS                 | NS   | NS   | NS                 |
|  |  |     |                      |                              |                         |                    |      |      |                    |

| Soil treatment |     |     |      |    |     |      |      |     |       |
|----------------|-----|-----|------|----|-----|------|------|-----|-------|
| Solarized      | 477 | 427 | 1.15 | 70 | 2.4 | 23.2 | 15.6 | 281 | 6,497 |
| Non-solarized  | 369 | 318 | 1.40 | 68 | 2.7 | 20.8 | 15.7 | 284 | 5,890 |
| LSD ( $P \leq$ | 14  | 16  | 0.23 | NS | 0.2 | 1.1  | NS   | NS  | 306   |
| $(0.05)^{Z}$   |     |     |      |    |     |      |      |     |       |

V Seeds planted at a 1.25-inch spacing on May 24, 2002. For each precrop, values are averaged across solarized and non-solarized plots (six replicates); for each soil treatment, values are averaged across all precrops (six replicates).

- W Root rot rating 6 WAP based on a 0 4 scale, 0 = root healthy, 4 = more than 75% of root surface scarred and/or no root tip. For each precrop (40 roots per replicate), values are averaged across solarized and non-solarized plots and for each soil treatment, values are averaged across all precrops (three replicates).
- X For each precrop, values are averaged across solarized and non-solarized plots and for each soil treatment, values are averaged across precrops (five replicates).
- Y Root rot rating based on a 0-7 scale, 0 = root healthy; 7 = root completely rotted and foliage dead.
- LSD = Least Significant Difference; if significant, LSD value provided for mean separations; NS = not significant.

#### DISCUSSION

Solarization of field plots in 2001 improved sugarbeet growth and yield and reduced Aphanomyces root rot in 2002 compared to soil that had not been solarized. Katan (5) has reported that increased growth responses beyond pest control often occur in solarized soils. Several mechanisms may be involved including chemical (where there is a release of mineral nutrients or growth factors and nullification of toxic products) or biological (elimination of minor pathogens and stimulation of beneficial microorganisms), or both. Halloin (4) solarized fields without a history of soilborne diseases in Michigan and also reported an increase in sugarbeet yield the subsequent year, which he attributed to increased numbers and longevity of feeder roots.

Solarization tended to result in greater survival of *A. cochlioides* oospores than in non-solarized soil. Soil solarization typically hastens decline of survival propagules of some soilborne fungi and other pests by generating high temperatures that directly kill propagules or weaken them so they are vulnerable to parasitism by other soilborne organisms. Some climates do not result in sufficiently high temperatures during solarization to kill pathogens, or certain pathogen propagules may be resistant to heat. There are no reports in the literature documenting the effects of solarization on survival of *Aphanomyces* species (5). In our study, soil temperatures reached under polyethylene tarps may have been inadequate, or did not prevail for a sufficient length of time, to effectively reduce numbers of oospores. Dyer has shown that 90% of oospores of *A. cochlioides* die when exposed to 104 °F for 72 hours or to 122 °F for 4 hours. Tropical climates can attain temperatures of 120 °F at a 9-inch depth in tarped soils, but our study reached temperatures slightly over 105 °F at 3 inches. Perhaps solarization increased soil fungistasis so oospores did not germinate (through altered microbial populations or induced dormancy) or certain soil factors preconditioned oospores so they were less vulnerable to dying. Dyer found that oospores of *A. cochlioides* exposed to low humidity (desiccation) were less likely to die when exposed to typically lethal temperatures (*unpublished*).

It is unknown if oospores of *A. cochlioides* in infested fields, and those buried in mesh bags in soil, respond similarly to green manure crops, solarization, and depth of burial. Green manure crops reduced Aphanomyces root rot index values immediately after soil-incorporation compared to fallow soil but they did not directly affect viability of buried oospores compared to fallow soil, nor did they benefit the subsequent sugarbeet crop in 2002 (in combination with solarization or alone). Perhaps disease suppression by green manure crops is short-term and does not carry over the winter to benefit a subsequent sugarbeet crop. Previous greenhouse trials have shown that a green oat precrop consistently reduces Aphanomyces damping-off on sugarbeet but in the field, may not perform consistently. Other reports indicate that growing a green oat precrop in producers' fields reduces Aphanomyces root rot of peas caused by *A. euteiches* (8).

Considerable decomposition of sugarbeet hypocotyls buried in soil occurred from mid July through early September throughout the upper 9 inches of soil in solarized and non-solarized plots. The top 12 inches of soil is most highly populated by saprophytic microorganisms and soilborne fungal pathogens. Soil temperatures at these depths also are favorable for microbial activity and decomposition of hypocotyl tissue and oospores. In 2002, depth of burial of oospores in soil did not affect survival but in 2001, survival was significantly higher at 3- than at 9-inch depths. Such discrepancies may be caused by varying soil environmental factors (biological, physical, chemical).

A direct relationship between the amount of *A. cochlioides*-infested sugarbeet hypocotyl tissue persisting in soil and numbers of oospores in the hypocotyl illustrates the dependency of oospores on previously infected plant tissue for survival. Fresh organic matter

introduced into soil is vulnerable to immediate microbial decomposition, which accounts for rapid loss of tissue (and hence, loss of oospores embedded in hypocotyls) after burial in soil. Hypocotyl tissue also is immature and delicate and decomposes readily. Oospores of *A. cochlioides* may survive longer in diseased, mature sugarbeet roots that have secondary thickenings and decompose slowly in soil.

Observation of the rapid decline in oospore survival where *A. cochlioides*-infected hypocotyls decomposed in soil has not been previously reported, although Boosalis and Scharen (1) found oospores of *A. euteiches* associated with diseased plant debris extracted from soil. We found that maceration of individual hypocotyls to quantify oospores revealed that only 11% survived after burial for 2 months, regardless of green manure crop or soil solarization. *A. cochlioides* oospores average  $21 \pm 5 \mu$  in diameter and would be too large to pass through 10 $\mu$  pores of the nylon mesh bags. Based on a most probable number assay, Pfender and Hagedorn (6) reported a substantial loss of inoculum of *A. euteiches* of nearly 50% within 1 year after growing peas. Survival of only a few oospores in a small volume of soil, however, can be deceptive. A single oospore of *A. cochlioides* produces 100 to 200 zoospores of primary inoculum, so a small number of surviving oospores could result in considerable disease under warm, wet conditions.

To date, application of soil solarization to reduce Aphanomyces diseases (or increase sugarbeet growth and yield) in our region appears to be inadequate and impractical for growers' fields. A second year of evaluating the effects of green manure precrops will further define if there is any benefit in this practice. To date, the most significant result of this study is the observation that oospores of A. *cochlioides* die as previously colonized sugarbeet debris decomposes in soil. That is, oospore survival is dependent upon condition of host debris.

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