Diagnostic Tip of the Month

Diagnostic Tip of the Month: Testing for Races of *Aphanomyces euteiches* in Alfalfa Soils

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The following describes an assay that we use at the Plant Disease Diagnostics Clinic (PDDC) in an effort to determine which (if any) races of *Aphanomyces euteiches* are present in a soil samples collected from alfalfa production areas. Currently, two races of *Aphanomyces euteiches* (designated race 1 and race 2) have been documented on alfalfa. This assay also provides information on the presence of *Phytophthora megasperma* f. sp. *medicaginis*. Our assay is a modification of an assay developed by Craig Grau, Department of Plant Pathology, University of Wisconsin-Madison.

Fig. 1: Starting dates of *Aphanomyces* race surveys are staggered so that assays at a variety of stages are always in progress at the PDDC. Assays are conducted on light carts at ambient temperature (~24 C).

This assay requires approximately 1.5 gal. of soil collected from an area (henceforth referred to as a "field") that is a maximum of 5 acres in size. Optimally, approximately one cup of soil should be collected from each of 25 sites within a "field", yielding a total, bulked sample that is between one and two gallons. Sites within a field should be selected as randomly as possible, and soil should be collected from the upper 6 in. of the soil profile.

Once a soil sample arrives at the PDDC, we sieve the soil through hardware cloth (with ¼ in. × ¼ in. openings) to yield soil of a more uniform consistency. Sieving also removes rocks, plant debris and large, hard soil clumps. If a soil is overly wet upon arrival, we air dry it before sieving. If a soil is overly dry, we often must use a hammer to break up larger clumps before sieving is possible. We autoclave sieves between samples to avoid cross-contamination. Once sieved, the soil is mixed thoroughly and then used to completely fill twelve 3 in. × 3 in. × 2¼ in. cell pack inserts with drainage holes (we use D1801 inserts which come in sheets of 18 cells and are available through Carlin Horticultural Supplies, www.carlinsales.com). Filled cells are placed in a 10 in. X 20 in. plastic tray without drainage holes (also available through Carlin Horticultural Supplies).

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Four varieties/breeding lines of alfalfa are used for the assay: Vernal, Dart, WAPH-1 and WAPH-5. Vernal is susceptible to race 1 and race 2 of *A. euteiches* as well as *P. megasperma* f. sp. *medicaginis.* Dart is susceptible to race 1 and race 2 of *A. euteiches* and has resistance to *P. megasperma* f. sp. *medicaginis.* WAPH-1 is susceptible to race 2 of *A. euteiches* and has resistance to *P. megasperma* f. sp. *medicaginis* and race 1 of *A. euteiches*. WAPH-5 has resistance to race 1 and race 2 of *A. euteiches* and to *P. megasperma* f. sp. *medicaginis.* Vernal and Dart are commercially available alfalfa varieties; WAPH-1 and WAPH-5 are breeding lines developed by Craig Grau, Department of Plant Pathology, University of Wisconsin-Madison. We plant 90 scarified seeds of a given variety/breeding line in each cell, and use three replications of each variety/breeding line per assay. Seeds are distributed as evenly as possible on the surface of the soil in each cell, and subsequently the seeds are agitated slightly so that they become covered with a very thin layer (~1/8 in.) of soil. After planting, the 12 resulting cells are arranged in a completely randomized design within the plastic tray and each cell is labeled with a plastic pot label numbered 1 through 12 (each number corresponding to a particular variety/breeding line). This numbering system allows us to blindly evaluate the plants in each cell at the end of the assay.

The assay can be performed in a growth chamber (we have used 24 C day/night or 24 C day/22 C night with a photoperiod of 12 hours), or in a greenhouse at ambient temperatures (~24 C) and light. We currently perform the assay in our clinic under grow lights (~24 C with a photoperiod of 12 hours). After planting, we initially lightly moisten the soil and keep it lightly moistened (watering from below) to promote seed germination. After ten days, we count and record the number of seed that have germinated in each cell. After the germination count is complete, we fill the plastic trays to the top with water, thus saturating the soil within the cells, and maintain this water level for two weeks.

Fig. 2: At the end of an assay, differences in plant growth are typically visible. Substantial stunting and plant mortality can occur in some varieties/breeding lines.

After two weeks, we stop adding water and allow seedlings to incubate one additional week, only adding water if the trays are completely devoid of water.

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After this 31 day incubation period (10 days for seed germination, 14 days with saturated soil conditions, 7 days with reduced watering), we evaluate each individual plant in each cell for root/hypocotyl discoloration using the following numerical scale:

1. = No necrosis of roots and hypocotyls.
2. = Slight necrosis of roots and hypocotyls.
3. = Necrosis of roots and lower hypocotyl, slight chlorosis of cotyledons, and moderate stunting of stem(s).
4. = Extensive necrosis of roots, hypocotyls and cotyledons, and severe stunting of stem(s).
5. = Dead seedling.

Scores from all plants in a given cell are averaged and the 12 resulting data points (three replications of four varieties/ breeding lines) are analyzed using analysis of variance (ANOVA). If the ANOVA indicates statistically significant differences in the varieties/ breeding lines (we recommend using $\alpha = 0.05$ or $\alpha = 0.10$), we follow the ANOVA with comparisons of the variety/ breeding line means using least significant difference (LSD) analysis.

We initially compare mean values for Vernal and Dart. If Dart has a statistically significantly lower mean than Vernal, we conclude that *P. megasperma* f. sp. *medicaginis* is present in the soil. In this situation, we subsequently compare the mean value for Dart with the mean values for WAPH-1 and WAPH-5 to determine if *A. euteiches* race 1 and/or race 2 are present (see below). If the means for Vernal and Dart are not statistically significantly different, we conclude that *P. megasperma* f. sp. *medicaginis.* is not present in the soil (at least not at a detectable level), and the mean value for Vernal is subsequently used for further comparisons with the mean values for WAPH-1 and WAPH-5 to determine if *A. euteiches* race 1 and race 2 are present.

If mean values for WAPH-1 and WAPH-5 are not statistically significantly different, and the mean value for WAPH-1 is statistically significantly lower than the mean value for Vernal or Dart (see above to determine which mean to use as a standard), we conclude that race 1 of *A. euteiches* is present and race 2 of *A. euteiches* is not (at least not at a detectable level).

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Fig. 3: At the end of an assay, individual plants are rated on a scale of 1 (healthy) to 5 (dead). Typical plants that would be rated with scores of 1 (left) to 4 (right) are shown in this photo.

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If the mean value for WAPH-5 is statistically significantly lower than the mean value for WAPH-1, and mean values for both WAPH-1 and WAPH-5 are statistically significantly lower than the mean value for Vernal or Dart (see above to determine which mean value to use as a standard), then we conclude that both race 1 and race 2 of *A. euteiches* are present in the soil. If the mean value for WAPH-5 is statistically significantly lower than the mean value for WAPH-1, and the mean value for WAPH-1 is not statistically significantly lower than the mean value for Vernal or Dart (see above to determine which mean to use as a standard), then we conclude that race 2 of *A. euteiches* is present in the soil and that race 1 of *Aphanomyces* is not (at least not at a detectable level).

Often, in the initial ANOVA, we do not see statistically significant differences between the mean values of any of the varieties/breeding lines. If the mean values for all of the varieties/breeding lines are (approximately) less than 3 (i.e., in general, plants of all varieties look relatively healthy), we then tend to conclude that there is no evidence of either race of *A. euteiches* or *P. megasperma* f. sp. *medicaginis* in the soil. If the mean values for all of the varieties/breeding lines are (approximately) greater than 3 (i.e., in general, plants of all varieties look relatively unhealthy), then we begin to be concerned about whether there might be other (currently undocumented) races of *A. euteiches,* other pathogens or even other abiotic factors in the soil that may be a problem. We often advocate additional follow-up testing in these situations. Note that healthy/not healthy cutoff value of 3 is somewhat arbitrary and is based on our experience with the test.

Once the assay is complete, we supply a written report to our clients, including the mean values for each of the alfalfa varieties/breeding lines tested and our interpretation of the results. Because this test is relatively labor intensive, our current charge is $100 per soil sample.

As you can see, this assay is a combination of both science and art. While the assay itself is relatively straightforward to conduct, interpretation of results can become quite complex and may not always be clear-cut. If you are interested in performing this assay and would like advice (or just a sympathetic ear), feel free to contact me at bdh@plantpath.wisc.edu or (608) 262-2863.

National Database

National Database Subcommittee Update

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The National Database Subcommittee met on January 16, 2008 to continue our work on reviewing the massive EPA Pest and Host lists and revising guidelines for uploading documents that will clarify how sample diagnoses should be transmitted to the National Repository at Purdue University. During this meeting a number of issues were addressed. Please refer to the national database subcommittee web page of the NPDN web site for complete minutes of this meeting (login and password required).

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