Metalaxyl resistance in Sunflower Downy Mildew and Control through Genetics and Alternative Fungicides.

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Abstract

Sunflower downy mildew (*Plasmopara halstedii*) isolates from the north-central area of the United States (North Dakota, South Dakota, and Minnesota) were evaluated in 1998 and 1999 for tolerance to APRON (metalaxyl). In 1998, 95% of the isolates were tolerant, and 80% of the 1999 isolates were tolerant from 10-100% infection on APRON-treated seed. Single-plant and single-spore isolates consistently produced 95-100% infection on APRON-treated seed, suggesting that isolates producing intermediate levels of infection on APRON-treated seed are thus mixtures of both metalaxyl-resistant and sensitive strains. Metalaxyl resistant isolates produced equivalent infection on seed treated at the U.S. rate (61,2 g ai/100 kg), the European rate (210 g ai) and seed treated at 630 g a.i/100 kg. Metalaxyl resistance was not correlated with any physiological race, with the predominant races being race 4 (730) and race 5 (770). Sunflower genotypes with multi-race immunity, such as USDA line HA-335 and commercial hybrids Bilto and Maya, were equally resistant against both metalaxyl-resistant and sensitive mildew strains. The addition of cofungicides, such as mancozeb, to metalaxyl gave only partial control of metalaxyl resistant strains, equivalent to control with the cofungicide alone. Of alternative fungicides tested, the most effective were azoxystrobin and RP 407213. In order to forestall fungicide resistance, a coordinated effort between public researchers, seed and chemical companies in the U.S. will be made to formulate and utilize a 2- or 3-way fungicide mixture, which may include azoxystrobin, metalaxyl, and a cofungicide such as mancozeb.

MATERIALS and METHODS

APRON-tolerance tests. Sunflower leaves with downy mildew sporulation were collected from fields and the spores were either increased by inoculating onto sunflower seedlings, or used immediately The APRON-tolerance test consisted of planting non-treated and APRON-treated seed (U.S. rate of 625 ppm ai) of a commercial oilseed hybrid in small flats containing sand/perlite. On the 3rd and 4th day after planting, the flats were drenched with a 20,000 spore/ml suspension . Initially, three replications of 40 seeds/flat of APRON treated seed were used, with one flat of 40 seeds of non-treated seeds as a viability check. In later tests, two replications of non-treated and APRON-treated seed were used. The flats were grown in the greenhouse for 11 days following inoculation, and then transferred overnight to a chamber maintained at 65 F and 100% RH, and evaluated the next day. Those mildew isolates producing infection on both non-treated and APRON-treated seedlings were categorized as APRON-resistant, while those displaying infection only on untreated seed were labeled as APRON-sensitive. Those samples producing <100% infection on untreated seed were repeated. Race identifications were made on a portion of the isolates to determine if APRON-tolerance was associated with a particular physiological race.

Mildew survey. A survey was conducted in 1998 to determine the incidence (% of fields with downy mildew) and the severity (% systemically infected plants within a field) of downy mildew, and thus to gauge the economic impact of the disease. Within a field, five strips of 40 plants were examined, and the number of plants showing systemic downy mildew infection was recorded.

Fungicide tests. Candidate fungicides were coated onto 100 g quantities of a commercial hybrid (Cargill 270), using 3 ml of fungicide solution and hand mixing the seed in a 2 l Erlenmeyer flask. Seeds were planted in flats containing sand/perlite and drenched as described above with spores of an APRON-resistant mildew isolate. All tests were repeated twice, with four replications of 40 seeds per treatment, for a total of at least 320 seedlings evaluated. All fungicides were tested at 3 to 5 rates (75, 150, 300, 450 and 600 ppm), unless prior information suggested higher dosages. Any degree of sporulation on the cotyledons or true leaves was interpreted as infection (lack of control by the fungicide).

The fungicides evaluated included (1) older, non-systemic compounds such as mancozeb, thiram, TCMTB, etridiazole, streptomycin, chlorothalonil, and chloroneb; (2) newer, currently registered products with documented activity against other Oomycetes, including cymoxanil, propamocarb, fosetyl-Al, azoxystrobin, dimethomorph, kresoxim methyl, hymexanol, and fluazinam; and (3) experimental compounds with reported activity against Oomycetes, including ohm & Haas 7281 (zoxamide), Rhone Poulenc 407213 (fenamidone), DuPont JE874 (famoxadone), bayer IFK 916, TomenAgro SZX-722 (iprovalicarb), BASF 500, and Gustafson 317. Azoxystrobin tolerance was tested by soil drenching sunflower seed treated at rates of 75, 150, 300, 450 and 600 ppm ai. Twelve downy mildew isolates (10 from the U.S. and 2 from Spain) were used and the seedlings scored for systemic infection as described above.

Root examinations. A series of field trials with Quadris-treated seed in 1999, both in the U.S. and in France, were considered failures because of the high incidence of downy mildew on seed treated at rates which gave nearly complete control in greenhouse trials. To investigate what may have happened, a root examination step was added to the fungicide evaluations.

This entailed growing the apparently healthy seedlings an additional week (or 18-21 days post inoculation), digging up the seedlings, and rinsing off the sand. The plants were cut off at the hypocotyl and the roots placed in wet blotter paper lined plastic boxes and held at 16 C for 48 hours. The roots were then examined under a dissecting microscope at 25-50 X.

RESULTS

<u>APRON-tolerance tests</u>. A total of 102 downy mildew samples were collected in 1998 from fields in North Dakota, South Dakota, Minnesota (U.S.) and Manitoba (Canada) with 98 fields or 96% showing some degree of APRON tolerance. The «APRON-resistant» isolates produced from 1 to 100% infection on APRON treated seed, which suggests that the bulk field collections were in many cases mixtures of both APRON-sensitive and APRON-resistant strains. Only 34 DM isolates (or 33% of the fields sampled) produced 90-100% infection on APRON treated seeds, and thus these 34 fields would be classified as having the APRON-resistant strain predominantly. Conversely, 63% of the DM isolates produced from 1 to 89% infection on APRON-treated seeds, and these fields obviously contained a range of mixtures of APRON-resistant and APRON-sensitive strains. The only DM isolates showing no APRON resistance were collected from breeding nurseries in which there was minimal or no history of APRON usage. Downy mildew samples from 1999 were collected only from non-treated seeds, in an attempt to get a non-biased estimate of the proportion of APRON-tolerant isolates. Of the 60 mildew isolates collected in 1999, 85% of them were APRON-tolerant. Isolates showing <100% infection on APRON-treated seed were retested, using spores collected from APRON-treated seed. These repeated inoculations always resulted in 90-100% infection on APRON-treated seed, implying that the original spore collection was a mixture of APRON-resistant and APRON-susceptible individuals. Repeated single-spore isolations from APRON-resistant isolates always produced APRON-resistant subcultures which gave 90-100% infection on APRON-treated seed, which suggests that APRON resistance is not a quantitative response of *Plasmopara halstedii*.

<u>Race determinations and genetic resistance</u>. Sixty APRON-resistant isolates were identified to race, with 48% typed as race 4 (730) and 32% as race 5 (770). Thus, APRON-resistance was not linked with a specific physiological race, nor was APRON resistance associated with new races. None of the APRON-resistant isolates infected USDA lines HA-335 or RHA-340, which continue to display immunity to all races of downy mildew examined to date. Similarly, European hybrids known to have multi-race mildew resistance (BILTO, MAYA) were also uninfected by the APRON-resistant strains.

<u>Downy mildew survey</u>. A total of 169 sunflower fields were inspected in North Dakota, South Dakota and Minnesota in 1998. The incidence of systemic downy mildew infection in the three state area was 37% (63 of 169 fields). Overall severity (% plants showing systemic downy mildew/total number of plants examined) was 0.9% for the tri-state area. A majority of the fields (63%) were free from downy mildew. Only 2% of the 169 fields examined had >5% systemically infected plants. Yield losses would not be expected in fields showing <5% infection. Since the survey was done in August, the survey undoubtedly underestimated severity, as many infected seedlings would have died early in the summer.

Fungicide tests. A series of tests were conducted to determine which fungicide, alone and in combination, and at what rates, would control the new APRON-resistant mildew strains. Initial tests compared metalaxyl (marketed as ALLEGIANCE by Gustafson) and mefonoxim (marketed as APRON-XL by Novartis), both at the US rate of 1 oz ai/cwt (612 ppm) and the export rate of 3.4 oz ai/cwt (2125 ppm). When inoculated with an APRON-resistant strain, seed treated at the US rate of ALLEGIANCE and APRON-XL had 92 and 97% infection, respectively, and 87 and 92% at the export rate. The fungicide oxadixyl (marketed as ANCHOR by Novartis), which is also registered for use in the US on sunflower, similarly did not control downy mildew, as would be expected because oxadixyl is an analogue of metalaxyl.

Of the older, non-systemic fungicides tested, only mancozeb and thiram showed partial control of downy mildew. However, when combined at rates of 1250 to 2500 ppm with newer, systemic chemicals they performed synergistically as cofungicides. Of the fungicides currently registered for use on any crop in the U.S., azoxystrobin (Quadris) displayed the highest level of control when used alone. The degree of control varied with the mildew isolate, and the range of azoxystrobin tolerance is found in Table 1. Dimethomorph, marketed in the U.S. in combination with mancozeb as RESPECT or ACROBAT, did control downy mildew, but dimethomorph alone was only partially effective. Of the experimental or non-registered compounds tested, the most effective at controlling downy mildew were fluazinam, RP 407213, RH 7281, and a BASF experimental (Table 2).

Root examination. Examination of the roots of apparently healthy seedlings revealed the presence of mildew sporulation in many instances. This prompted us to do root examinations on all seedlings lacking systemic symptoms. By growing seedlings with root colonization for several weeks, we observed that a portion of these seedlings will develop into typical systemic symptoms over the course of a month following inoculation. The amount of root colonization on seeds treated with different rates of Quadris is found in Table 2.

DISCUSSION

Metalaxyl, marketed in the U.S. for sunflower seed treatment as APRON or ALLEGIANCE, has been used extensively since 1985. Sunflower companies have had the option of combining metalaxyl with Captan, a broad spectrum fungicide with minimal activity against downy mildew, or of using oxadixyl, marketed as ANCHOR, which is in the same chemical class as metalaxyl. No other fungicides have been available for downy mildew control.

In anticipation of the possibility of sunflower downy mildew developing resistance to metalaxyl, we conducted a series of experiments in 1986 and again in 1992. All downy mildew isolates, from North America, Hungary, and Argentina, were extremely sensitive to metalaxyl. Variation in infection between mildew isolates, referred to as differences in sensitivity, was noted but none of the isolates tested produced any infection on seed treated at 100 or 600. Attempts to induce metalaxyl resistance by cycling the fungus over seed treated with low rates of metalaxyl were unsuccessful.

The high incidence of APRON-resistant mildew isolates in 1998, found uniformly across the tri-state sunflower region, suggests that APRON-resistant strains are well established in the northern Great Plains region of the U.S., and to a lesser extent in adjacent Canada. The apparent «explosion» of APRON-resistance is deceptive, and probably has been increasing slowly over the last few years at levels low enough to escape notice. In years with low rainfalls following planting, the conditions would be sub-optimal for the establishment of systemic mildew infection, but the fungus still would be able to colonize roots, but not display any aboveground symptoms. While APRON-resistance was documented in 96% of the fields examined in 1998, the varying levels of infection in our greenhouse tests suggests that most fields contain a mixture of the old, APRON-sensitive strain and the new, APRON-resistant strain.

To control the APRON-resistant strains, there are two approaches which can be used singly or in combination. First, genetic resistance to all physiological races (including all APRON resistant strains) is available, and if this resistance is incorporated into commercial hybrids, they would require no fungicide treatments. Secondly, a fungicide seed treatment could be used, which will be necessary until resistant hybrids are available. The fungicide azoxystrobin (QUADRIS) was the most effective single chemical that is currently available in the U.S. However, since it does not prevent root colonization at low rates, it would either need to be used at higher rates (i.e. 600 ppm) or combined with a cofungicide to completely prevent mildew infection. Optimistically there will be several fungicides available in a few years which have different modes of action, and thus could be combined to delay the potential of resistance developing to azoxystrobin or any other fungicide used singly.

Table 1. Tolerance of downy mildew isolates from the U.S. and Spain to varying rates of azoxystrobin (Quadris).

Isolate	**Quadris Concentration**				
	37	**75**	**150**	**300**	**450 ppm**
US-1	12	5	2.2	0.9	0.9
US-2	35	18	5	2.9	1
US-3	43	18	10	1	1
US-4	46	20	10	4.2	1
US-5	50	22	2.3	0	0
Spain-1	39	24	12	5.8	0
Mean of 12 isolates	37.8	16.7	7.1	3.5	0.9

Table 2. Control of APRON-resistant downy mildew with the most effective fungicides, alone and in combination.

Fungicide	Systemic Infection	Total Infection
Quadris @ 150 ppm	20	70
Quadris @ 300 ppm	8	35
Quadris @ 450 ppm	3	24
Quadris @ 600 ppm	0.3	14
Quadris @ 150 ppm + 2500 ppm mancozeb	11	33
Quadris @ 150 ppm + 2500 ppm fluazinam	4	6
RP 407213 @ 450 ppm	0	0.7
RP 407213 @ 600 ppm	0.4	1.0
BASF exp @ 450 ppm	0.4	8
BASF exp @ 600 ppm	0.7	2.5

* Total infection = % plants with systemic symptoms, plus % latent seedlings with root colonization.