Evidence for Resistance to Metalaxyl in Isolates of *Peronospora hyoscyami*

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ABSTRACT

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Isolates of the tobacco blue mold fungus (*Peronospora hyoscyami*) were obtained between 23 July and 8 August 1980 from areas of western North Carolina that grow Burley tobacco. Only fields treated with metalaxyl (4.7 L/ha, preplant soil incorporation) were sampled. Mass conidial suspensions from nine plants per field were obtained by swirling exised lesions in sterile distilled water. Potted tobacco plants in the four-leaf stage were treated with metalaxyl at 0–200 μg (a.i.)/ml and were inoculated 24 hr later with 2 ml of a suspension of 2,000 conidia per milliliter. Three of the 14 isolates screened were able to form lesions and sporulate on plants treated with ≤100 μg/ml of metalaxyl; the conidia produced on these plants were used successfully to infect other metalaxyl-treated tobacco plants. No symptoms or signs of infection were visible on plants treated with as little as 25 μg (a.i.)/ml and inoculated with the other 11 isolates. These data suggest that metalaxyl-resistant isolates of *P. hyoscyami* may exist widely in nature and that continued use of the fungicide should be carefully evaluated and monitored.

Production of tobacco (*Nicotiana tabacum* L.) in the United States and Canada was severely affected in 1979 and 1980 by epidemics of blue mold along the Eastern Coast, caused by *Peronospora hyoscyami* de Bary (syn. *P. tabacina* Adam) (7). Although the 1980 growing season was warmer, drier, and sunnier than the 1979 season and generally paralleled the 30-yr synoptic average, the 1980 epidemic progressed rapidly from south to north, beginning in Cuba and Jamaica (about 1 January) and was last reported in Ontario, Canada, on 5 August (6).

The fungicide metalaxyl (Ridomil, CGA 48988) was widely used by tobacco growers in 1980 and provided highly effective control of blue mold. Metalaxyl has also been effective against numerous other phycomycetous pathogens. Several investigators (1,5,9) have noted in vitro and in vivo efficacy of metalaxyl against *Phytophthora cinnamomi* Rands, *P. infestans* (Mont.) de Bary, and *P. nicotianae* Breda de Haan var. *nicotianae* Waterhouse. Bruck et al (2) reported lack of in vitro efficacy against *P. infestans* (races 1, 2, 3, 4). Resistance to metalaxyl has been reported in *Pseudoperonospora cubensis* (Berk & Curt.) Rostow (8) and *Phytophthora infestans* (Ciba-Geigy Corp., *personal communication*). A preliminary report of resistance to metalaxyl in *Peronospora hyoscyami* has been presented (3).

The purpose of our study was to assay isolates of *P. hyoscyami* from the natural population in North Carolina in 1980 for resistance to metalaxyl.

MATERIALS AND METHODS

Field blue mold first appeared in North Carolina in Columbus County on 1 May 1980. During the next 8 wk, all 72 tobacco-producing counties in North Carolina had active blue mold occurrences. The disease was first reported from the Burley-producing (mountain) region of the state in plant beds on 23 May and in field tobacco on 6 June. During the growing season, growers and extension agents observed actively sporulating blue mold lesions in fields reportedly treated with metalaxyl according to label recommendations (4.7 L/ha, preplant soil incorporation). Two growers from Haywood County in western North Carolina reported active blue mold in their fields after preplant treatments and an additional foliar spray (2.3 L/ha).

We began our survey on 23 July and continued sampling until 8 August. A survey crew was dispatched to collect isolates associated with reported metalaxyl "failures" based on the following criteria: 1) The extension agent confirmed active sporulating blue mold lesions in three or more metalaxyl-treated fields within the county. 2) The agent was assured that metalaxyl had been incorporated, according to label instructions, in the fields before planting. 3) The grower(s) would provide pertinent data during a personal interview and would permit destructive sampling within the field.

Before collecting samples in the N.C. mountains on 23 July, 29 July, and 8 August, the survey crew and the county agent gathered information on cultivars and fungicides used, first occurrence of blue mold, and cultural practices. Nine plants with profusely sporulating lesions were selected to represent affected areas of the field. Leaves from three stalk positions of each plant were collected, placed in plastic bags, and transported in an ice chest.

Coincident with the collection of field isolates, four four-leaf stage tobacco plants (Bergerac C or Ky 14 cultivars) at the university were sprayed abaxially and adaxially to run-off with metalaxyl at 0, 25, 50, 100, and 200 μg (a.i.)/ml. Approximately 5 ml of solution was needed to cover each plant.

The *P. hyoscyami* isolates arrived at the laboratory within 24 hr after their collection. Conidial slurries representing a mass conidial isolate from each field were made by excising sporulating tissue from all leaves from a given field and agitating the lesions in 6 ml of distilled water for 20 sec on a Vortex Genie mixer

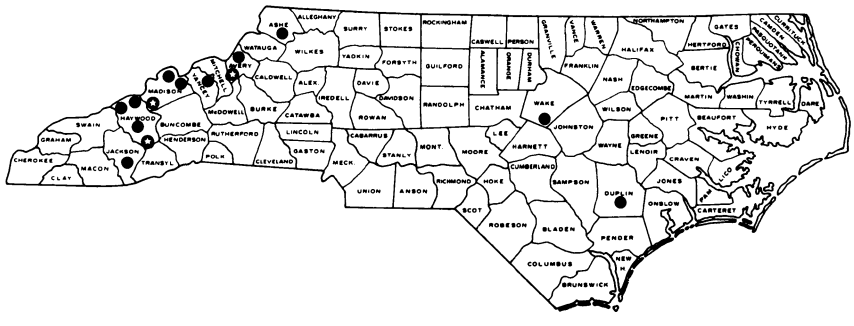

Fig. 1. Collection sites of *Peronospora hyoscyami* in North Carolina. Solid black circles represent sites of metalaxyl-sensitive isolates; stars in black circles indicate sites of metalaxyl-resistant isolates.

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(Scientific Industries, Inc., Bohemia, NY 11716). An aliquot (1 ml) of the concentrated suspension ($3–5 \times 10^5$/ml) was dispensed onto three plates of water agar to determine in vitro germinability.

The remainder of the suspension was diluted to 2,000 conidia per milliliter with distilled water, and 2 ml of the dilute suspension was sprayed with a DeVilbiss atomizer onto the tobacco plants 24 hr after their treatment with metalaxyl. Inoculated plants were placed in a growth chamber (100% RH, 18 C) with constant fluorescent illumination for 7–10 days until chlorotic lesions appeared. After lesions developed, each plant was placed in a plastic bag and then covered with a brown paper bag. The bagged plants were moved to an air-conditioned growth chamber (18 C), and lesions with profuse sporulation developed on some plants within 48 hr. All treatments were replicated four times (four plants per treatment). The experiment was repeated at least three times, using the above procedure and subculturing the inoculum collected from the previous treatment.

RESULTS

Fourteen isolates from eight counties, including two from flue-cured tobacco-growing areas, were investigated (Fig. 1). Conidia from all the isolates screened showed high initial germinability (45–97%) within 24 hr of seeding on water agar plates (Table 1). Eleven of 14 isolates initiated no lesions even at the lowest metalaxyl concentration (25 μg [a.i.]/ml), but three isolates (Avery 2, Madison 2, and Haywood 4) caused lesions and sporulated on plants treated with all metalaxyl concentrations ≤100 μg (a.i.)/ml (Table 1). Furthermore, conidia transferred from these plants to similarly treated plants also induced sporulating lesions. No lesions formed on any of the plants treated with metalaxyl at 200 μg/ml. There was no obvious difference between the two tobacco cultivars used in the bioassays in either metalaxyl or lesion response. Resistance of the three isolates to metalaxyl was confirmed by identical results of the procedure repeated at least three times.

DISCUSSION

Three of 14 isolates of *P. hyoscyami* exhibited resistance to metalaxyl on plants treated at the rate of 100 μg (a.i.)/ml in the greenhouse. Sporulation was the indication that infection had occurred. Therefore, some colonization without sporulation may have occurred with sensitive isolates at metalaxyl concentrations above the control (0.0 μg [a.i.]/ml) level and also with the three metalaxyl-resistant isolates above the 100 μg (a.i.)/ml rate.

After each experiment, actively sporulating lesions from each isolate were stored by the methods of Cohen and Kuć (4). However, attempts to recover viable isolates from the leaf tissue 2 mo after freezing were unsuccessful.

The various developmental stages of *P. hyoscyami* apparently did not respond to metalaxyl in the manner described for *Phytophthora infestans* (2). The three metalaxyl-resistant isolates showed no suppression of infection, lesion development, sporulation, or conidia viability even on plants treated at the 100 μg (a.i.)/ml concentration. Resistance to metalaxyl was not significantly ($P < 0.05$) correlated with conidia viability of the isolates on water agar. Although the results suggest that the resistance of *P. hyoscyami* to metalaxyl may be stable, we were unable to "train" the three metalaxyl-resistant isolates to complete their life cycles on plants treated with 200 μg/ml.

The survey to collect isolates of *P. hyoscyami* potentially resistant to metalaxyl ended on 8 August because hot, dry weather resulted in cessation of sporulation. The epidemic subsided shortly after this, even in areas with moderate to heavy infection; hence, the full potential impact of metalaxyl-resistant isolates of *P. hyoscyami* was probably averted or delayed and could not be assessed further from the epidemiological data available. The relevance of our data to regional or continental spread and local multiplication is difficult to assess. The ability of conidia or oospores to overwinter in the tobacco-producing areas of the United States and Canada has not been adequately determined. Long-distance transport of conidia on wind currents from the Caribbean has been postulated but not proved. Therefore, the source of inoculum and the influence of selection for metalaxyl-resistance in *P. hyoscyami* in the United States is uncertain.

These data do not explain either a resistance mechanism or the possibility of naturally occurring metalaxyl-tolerant strains. Nevertheless, the parasitic fitness of such a naturally occurring strain could forewarn of serious consequences from future selection pressure. Metalaxyl provides a valuable tool to tobacco growers, but its deployment must be carefully considered. Combination of metalaxyl with protectant fungicides should be considered as one method to delay the potential increase of metalaxyl-resistant strains of *P. hyoscyami*.

Table 1. Effect of metalaxyl treatment of tobacco on the response of 14 *Peronospora hyoscyami* isolates

Origin of isolate[a]	Percent germination[b]	Sporulation[c] on plants treated with metalaxyl (μg [a.i.]/ml)				
		0	25	50	100	200
Duplin 1	85	+	−	−	−	−
Wake 1	71	+	−	−	−	−
Ashe 1	45	+	−	−	−	−
Avery 1	88	+	−	−	−	−
Avery 2*	79	+	+	+	+	−
Yancy 1	53	+	−	−	−	−
Madison 1	95	+	−	−	−	−
Madison 2*	77	+	+	+	+	−
Madison 3	47	+	−	−	−	−
Haywood 1	81	+	−	−	−	−
Haywood 2	55	+	−	−	−	−
Haywood 3	69	+	−	−	−	−
Haywood 4*	84	+	+	+	+	−
Jackson 1	66	+	−	−	−	−

[a] Isolate names indicate the North Carolina county of origin; asterisks indicate where isolates occurred with observed resistance to metalaxyl.
[b] Germination of 1-ml mass conidial suspension (2,000 conidia per milliliter) on water agar in petri plates; percent germination was determined 24 hr after seeding conidia to the plates.
[c] + = sporulating lesions, − = no lesions or sporulation.

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