

## Response of *Phytophthora parasitica* var. *nicotianae* to Metalaxyl Exposure

H. D. SHEW, Assistant Professor, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616

### ABSTRACT

Shew, H. D. 1985. Response of *Phytophthora parasitica* var. *nicotianae* to metalaxyl exposure. *Plant Disease* 69:559-562.

Isolates of *Phytophthora parasitica* var. *nicotianae* were collected from seven flue-cured tobacco fields before initial metalaxyl use and after one, two, or three consecutive years of use for control of tobacco black shank. Eight hundred seventy-seven isolates were screened for inhibition of mycelial growth on 5% V-8 juice agar amended with 0.1, 1, 10, or 100  $\mu\text{g}$  of metalaxyl per milliliter of medium. Inhibition increased as fungicide concentration increased; however, at low fungicide concentrations, the range in inhibition was less for isolates collected after exposure and the percentage of isolates inhibited at the 0.1- and 1- $\mu\text{g}$  a.i./ml concentrations of the fungicide decreased after 1-3 yr of exposure. The change in sensitivity resulted in mean ED<sub>50</sub> values of 0.4, 0.3, 0.7, and 1.2  $\mu\text{g}$ /ml for isolates exposed to metalaxyl for 0, 1, 2, or 3 yr, respectively. An in vivo assay that measured mean inhibition of root infection on a susceptible tobacco cultivar gave results similar to those obtained in the in vitro test for inhibition of mycelial growth. Continuous use of metalaxyl in soil enhanced selection of *P. parasitica* var. *nicotianae* populations less sensitive to metalaxyl, and thus periodic removal of this selection pressure through crop or fungicide rotation should be considered.

Metalaxyl (Ridomil) is currently used for control of many *Phytophthora* root rots. The rapid development of resistance to metalaxyl by some members of the Peronosporales (2,4,9,10), however, has caused concern for the continued effectiveness of this fungicide. Bruin and Edgington (2) reported development of resistance by *Phytophthora capsici* isolates after repeated transfers on agar medium amended with sublethal concentrations of metalaxyl. They reasoned that low concentrations of the fungicide also would exist in soil and concluded that development of resistance would be likely in soil continuously treated with the fungicide. Sanders (9) recently reported loss of efficacy and resistance of *Pythium aphanadermatum* to metalaxyl on turfgrass.

The in vitro sensitivity of several soilborne *Phytophthora* spp. to metalaxyl has been investigated (1,3,5,6,12,13). Isolates of the various species were

collected before initial metalaxyl exposure and screened for their responses to different fungicide concentrations. A wide range in fungicide sensitivity was shown by isolates within and among species, but none of the isolates showed a high level of resistance to metalaxyl. These studies provided information on the natural variation in sensitivity to metalaxyl within a given *Phytophthora* sp. and should serve as a base for determining shifts in sensitivity after exposure to the fungicide. At present, however, research is lacking on the effects of repeated metalaxyl exposure under field conditions on soilborne *Phytophthora* spp.

Black shank, caused by *Phytophthora parasitica* Dast. var. *nicotianae* (Breda de Haan) Tucker, is an economically important disease of flue-cured and burley tobacco (8). Disease losses are minimized best by a combination of practices including crop rotation, host resistance, and chemical application. Black shank is most severe in fields where crop rotation is not practiced. The objective of this study was to determine the effects of repeated use of metalaxyl at recommended field rates on the sensitivity of populations of *P. parasitica* var. *nicotianae* in fields continuously planted to flue-cured tobacco.

### MATERIALS AND METHODS

**Collection of fungal isolates.** Eight hundred seventy-seven isolates of *P. parasitica* var. *nicotianae* were collected over a 4-yr period beginning in 1980 from flue-cured and burley tobacco. Three hundred seventeen of the isolates were

obtained from stock cultures (collected before metalaxyl use) or isolated from six flue-cured tobacco fields in eastern North Carolina before the initial use of metalaxyl for black shank control (12). These 317 isolates were designated year 0. The remaining 560 isolates, isolated from the six previously mentioned fields and one additional tobacco field in eastern North Carolina, also included a group of isolates collected from miscellaneous sources. The 560 isolates were collected after 1, 2, 3, or 4 yr (20 isolates from field 7 were collected after year 4) of continuous exposure to metalaxyl in the field. All fields received one preplant application of metalaxyl per year at recommended field rates for black shank control (1.1, 2.2, or 3.3 kg/ha). All isolates that were not originally obtained from stock cultures (year 0) were isolated from fungal colonies on soil assay plates (11) (usually single chlamydospore origin) or from mycelial growth from infected tobacco roots on a selective agar medium (7,11). All isolates were maintained on 5% clarified V-8 juice agar slants under oil at 22-25 C.

**In vitro screening procedure.** The effect of metalaxyl on mycelial growth in vitro was determined on 5% V-8 juice agar. The 2EC formulation of metalaxyl (25.11% a.i.) was used to establish fungicide concentrations of 0.1, 1, 10, and 100  $\mu\text{g}$  a.i./ml of medium. Metalaxyl was added to cooled (45-50 C) agar medium, and 17 ml of medium was poured per 9-cm-diameter petri plate. A 6-mm-diameter agar disk was cut from the margin of each fungal isolate growing on 5% V-8 agar and transferred to the center of each agar plate. Mycelial growth along the longest radius was measured to the nearest millimeter after 7 days of incubation in the dark at 22-25 C. There were at least three replicates per isolate at each fungicide concentration. Data were analyzed by analysis of variance.

**In vivo screening procedure.** Inoculum of *P. parasitica* var. *nicotianae* used to infest soil was produced in soil on Hicks tobacco seedlings as described previously (11). Inoculum of each fungus isolate was produced separately. Inoculum density for each isolate in the infested soil was determined on the selective agar medium (7,11) before diluting the infested soil with a 1:1 mixture of steamed (80 C for 60 min) sandy loam soil and coarse sand to

Paper 9447 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh.

Use of trade names does not imply endorsement by the North Carolina Agricultural Research Service of the products named or criticism of similar ones not mentioned.

Accepted for publication 17 November 1984 (submitted for electronic processing).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

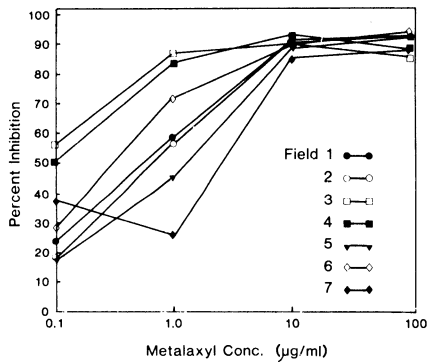


Fig. 1. Mean inhibition of growth of *Phytophthora parasitica* var. *nicotianae* isolates from seven flue-cured tobacco fields on metalaxyl-amended 5% V-8 juice agar.

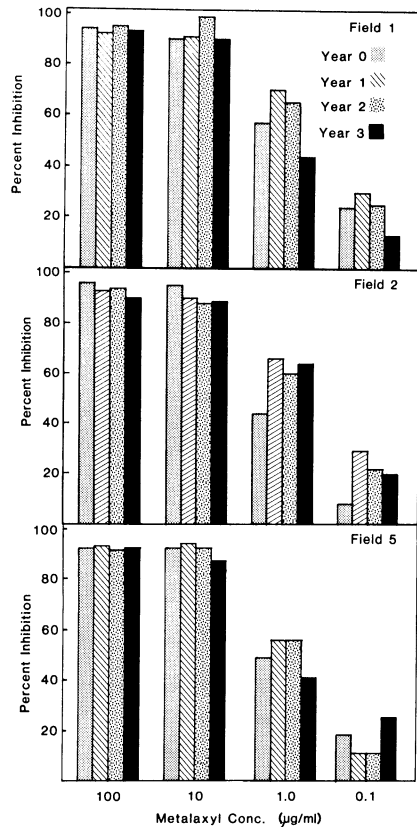


Fig. 2. Influence of numbers of metalaxyl applications on the inhibition of growth of *Phytophthora parasitica* var. *nicotianae* isolates from three flue-cured tobacco fields.

establish a final inoculum density of two propagules per gram of soil. Metalaxyl was mixed immediately into the infested soil at concentrations of 0, 0.1, 0.5, 1, and 5 µg a.i./g dry weight of soil. The soil was placed in 10-cm-diameter pots and a 1-mo-old Hicks tobacco seedling was transplanted into each pot after about 24 hr. There were five seedlings per fungicide concentration and the test was repeated twice. Percent plant infection was determined 3 wk after transplanting by carefully removing the seedlings from the soil, surface-sterilizing the root system in 0.5% NaClO for 20 sec, and plating the entire root system of each plant on the selective medium. Plates were checked

daily for growth of *P. parasitica* var. *nicotianae* from the roots. Root rot severity was rated on a scale of 1-5, where 1 = healthy, 2 = <5% root rot, 3 = 6-50% root rot, 4 = >50% root rot and/or crown rot, and 5 = dead plant.

## RESULTS

**In vitro screening.** Mean growth inhibition increased with increasing metalaxyl concentration for isolates collected after 0, 1, 2, and 3 yr of fungicide exposure (Table 1). In relation to year 0 isolates, mean inhibition increased for year 1 isolates but decreased for year 2 and 3 isolates, resulting in ED<sub>50</sub> values of about 0.4, 0.3, 0.7, and 1.2 µg/ml for year 0, 1, 2, and 3 isolates, respectively (Table 1).

Isolates from all fields and all years were extremely variable in their responses to the fungicide in vitro (Table 1). At 10 and 100 µg/ml, the range in response of year 1, 2, and 3 isolates was similar to that of year 0 isolates. However, at 0.1 and 1 µg/ml, isolates were less sensitive after metalaxyl exposure (Table 1). For example, at 1 µg/ml the minimum inhibition for a year 0 isolate was 0% (growth same as control) and the maximum inhibition 100% of the control. After the third year of fungicide exposure, the range in response was +34% (growth greater than control) to a maximum of 80% inhibition. A similar shift in response was observed at the 0.1-µg/ml concentration (Table 1).

Mean inhibition of *P. parasitica* var. *nicotianae* isolates collected over the 4-yr period varied with field of origin (Fig. 1). Mean ED<sub>50</sub> values ranged from <0.1 µg/ml for isolates from fields 3 and 4 to >2 µg/ml for isolates from fields 5 and 7. Isolates from field 7 responded to metalaxyl differently than isolates from other fields, with mean inhibition of growth greater at 0.1 than at 1 µg/ml (Fig. 1).

Response of *P. parasitica* var. *nicotianae* populations to repeated metalaxyl exposure also varied with field. In general, pathogen populations were more sensitive to metalaxyl after the first year of exposure but became less sensitive after 2 or 3 yr of exposure (Table 1, Fig. 2). For example, in field 1, percent inhibition at 1 µg/ml was 57, 70, 65, and 44% after 0, 1, 2, and 3 yr of exposure, respectively (Fig. 2). In contrast, isolates from field 2 remained more sensitive than year 0 isolates even after 2 and 3 yr of exposure (Fig. 2).

Frequency distribution plots also demonstrated changes in response to metalaxyl after repeated exposure (Figs. 3 and 4). For example, in field 7, the percentage of total isolates inhibited less than 10% at 1 µg/ml increased from 0 to 75% between years 1 and 4 (year 0 and 2 isolates not obtained) (Fig. 3). In field 1, the percentage of total isolates inhibited less than 10% at 0.1 µg/ml was 11, 3, 0, and 35% after 0, 1, 2, and 3 yr of

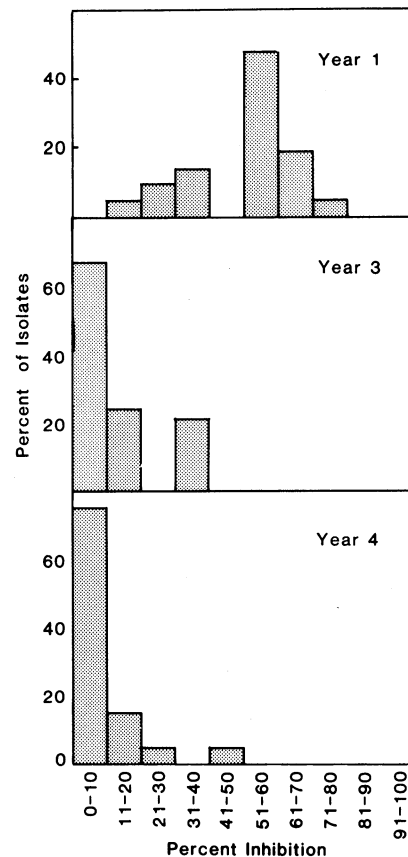


Fig. 3. Frequency distribution of growth response among *Phytophthora parasitica* var. *nicotianae* isolates from field 7 to metalaxyl at 1 µg/ml in 5% V-8 juice agar.

Table 1. Influence of metalaxyl exposure on mean inhibition of *Phytophthora parasitica* var. *nicotianae* isolates in vitro

Years of exposure	No. of isolates	Growth inhibition (%) <sup>a</sup> at indicated metalaxyl conc. (µg/ml)				ED <sub>50</sub> (µg/ml)
		0.1	1	10	100	
0	317	31 (+34-97) <sup>b</sup>	63 (0-100)	91 (35-100)	91 (35-100)	0.4
1	271	34 (+15-94)	71 (19-98)	90 (64-100)	90 (61-100)	0.3
2	124	20 (+69-54)	60 (3-90)	90 (72-98)	93 (79-100)	0.7
3	145	22 (+36-58)	42 (+34-80)	88 (60-98)	92 (59-100)	1.2

<sup>a</sup> Mean inhibition compared with growth on fungicide-free 5% V-8 juice agar after 7 days in the dark at 22-25 C.

<sup>b</sup> Values in parentheses indicate the range in percent growth inhibition for all isolates collected during a given year. Plus values indicate growth was greater on metalaxyl-incorporated agar than on fungicide-free agar for some isolates.

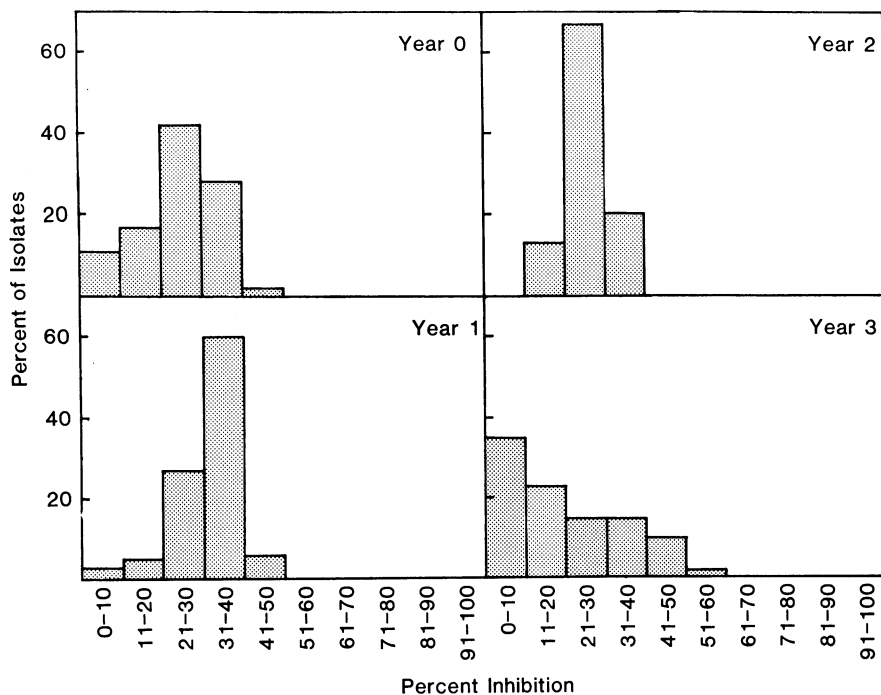


Fig. 4. Frequency distribution of growth response among *Phytophthora parasitica* var. *nicotianae* isolates from field 1 to metalaxyl at 0.1 µg/ml in 5% V-8 juice agar.

exposure, respectively (Fig. 4).

**In vivo screening.** Root infection in the bioassay with the susceptible tobacco Hicks decreased as metalaxyl concentration increased (Fig. 5). Percentage of plants infected was 85, 65, 43, and 9% and root rot rating was 2.2, 1.6, 1.3, and 1 at fungicide concentrations of 0.1, 0.5, 1, and 5 µg/g of soil, respectively.

## DISCUSSION

Isolates of *P. parasitica* var. *nicotianae* collected after 1, 2, 3, or 4 yr of metalaxyl exposure (one fungicide application per year) under field conditions were variable in their growth response to the fungicide in vitro. A similar response was previously observed for year 0 isolates of *P. parasitica* var. *nicotianae* (12) and for several other soilborne *Phytophthora* spp. (3,6). Isolates collected after exposure showed a similar range of sensitivity to 10 and 100 µg/ml of metalaxyl as year 0 isolates. In fact, after 1-4 yr of exposure, no isolates were recovered that showed a high level of resistance to the fungicide in vitro. In contrast, at the 0.1- and 1-µg/ml concentrations, shifts in sensitivity to the fungicide were observed after 2, 3, and 4 yr of exposure. Two types of responses to exposure were evident. First, the range in isolate sensitivity was extended, often resulting in a stimulation of mycelial growth compared with year 0 isolates (Table 1). Second, a higher percentage of the isolates from a given field were inhibited to a lesser degree at a specific fungicide concentration (Fig. 3). These responses to exposure resulted in twofold and threefold increases in ED<sub>50</sub> values for year 2 and 3 isolates, respectively.

Bruin and Edgington (2) reported development of resistance in *P. capsici*

during 12 transfers over an 8-mo period on V-8 juice agar amended with sublethal concentrations of metalaxyl. They concluded that development of resistance to acylalanine-type fungicides was very likely after continuous use because sublethal concentrations of the fungicide would exist for extended periods of time in the soil. After 4 yr of continuous use in tobacco for black shank control, resistance to metalaxyl has not been observed in North Carolina. Because soilborne fungi are infrequently in an active growth stage (mycelium), as occurred in the in vitro tests, selection pressure probably would not be as great and development of resistance would not be as common as reported for *P. capsici* (2). However, the potential of soilborne members of Peronosporales to rapidly develop resistance in vitro has been demonstrated (2,4,9,10). In this study, there was an apparent response to the selection pressure applied by repeated use of metalaxyl, resulting in reduced mean sensitivity of pathogen populations to the fungicide. Based on these observations and previous reports (2,4), the selection of strains of *P. parasitica* var. *nicotianae* highly resistant to metalaxyl may be minimized by the periodic removal of the selection pressure by either crop or fungicide rotation. Removal of metalaxyl from the growth medium reversed the adaption to metalaxyl by several *P. capsici* isolates (2).

Mean inhibition of *P. parasitica* var. *nicotianae* varied with field of origin before initial metalaxyl use (12). There was no apparent relationship between this variation and subsequent development of reduced fungicide sensitivity. In

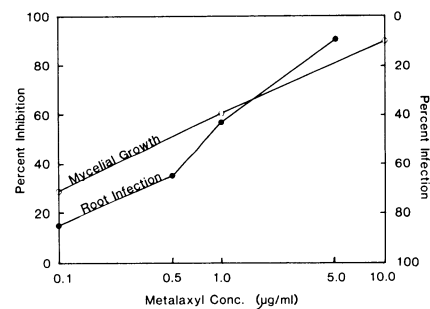


Fig. 5. Comparison of in vitro and in vivo screening procedures for determining sensitivity of *Phytophthora parasitica* var. *nicotianae* isolates to metalaxyl. Mycelial growth is the overall mean for all isolates screened and root infection is the mean infection for 19 isolates chosen at random.

addition, in most fields, sensitivity to the fungicide increased after the first year of exposure. A similar increase in sensitivity was observed after 1 yr in field microplots originally infested with a mixture of four *P. parasitica* var. *nicotianae* isolates not previously exposed to metalaxyl (H. D. Shew, unpublished).

Information on the sensitivity of soilborne *Phytophthora* spp. to metalaxyl has been obtained primarily from tests conducted in vitro (1,2,3,5,6,12,13). In this study, isolates also were screened in vitro for growth inhibition. However, since mycelial growth is only one growth stage important in root infection and colonization, in vivo tests also were conducted for comparison. The response observed in vitro was similar to the mean response in vivo. By manipulation of the initial inoculum density and the level of host resistance in the assay plant, the in vivo assay may more closely reflect the response of isolates in vitro.

## ACKNOWLEDGMENTS

This research was supported in part by grants from the Ciba-Geigy Corporation and the North Carolina Tobacco Foundation, Inc. I thank D. T. Glover and S. Bhikhai for their technical assistance, and M. Williams for photographic assistance.

## LITERATURE CITED

- Benson, D. M. 1979. Efficacy and in vitro activity of the systemic acylalanines and ethazole for control of *Phytophthora cinnamomi* root rot of azalea. *Phytopathology* 69:174-178.
- Bruin, G. C. A., and Edgington, L. V. 1981. Adaptive resistance in Peronosporales to metalaxyl. *Can. J. Plant Pathol.* 3:201-206.
- Coffey, M. D., Klure, L. J., and Bower, L. A. 1984. Variability in sensitivity to metalaxyl of isolates of *Phytophthora cinnamomi* and *Phytophthora citricola*. *Phytopathology* 74:417-422.
- Davidse, L. C. 1982. Acylalanines: Resistance in downy mildews, *Pythium*, and *Phytophthora* spp. Pages 118-127 in: *Fungicide Resistance in Crop Protection*. J. Dekker and S. G. Georgopoulos, eds. Pudoc, Wageningen, Netherlands.
- Farih, A., Tsao, P. H., and Menge, J. A. 1981. In vitro effects of metalaxyl on growth, sporulation, and germination of *Phytophthora parasitica* and *P. citrophthora*. *Plant Dis.* 65:651-654.
- Hunger, R. M., Hamm, P. B., Horner, C. E., and Hansen, E. M. 1982. Tolerance of *Phytophthora megasperma* isolates to metalaxyl. *Plant Dis.* 66:645-649.
- Kannwischer, M. E., and Mitchell, D. J. 1981.

- Relationship of numbers of spores of *Phytophthora parasitica* var. *nicotianae* to infection and mortality of tobacco. *Phytopathology* 71:69-73.
8. Lucas, G. B. 1975. Diseases of Tobacco. 3rd ed. Harold E. Parker and Sons, Fuquay-Varina, NC. 621 pp.
9. Sanders, P. L. 1984. Failure of metalaxyl to control Pythium blight on turfgrass in Pennsylvania. *Plant Dis.* 68:776-777.
10. Schwinn, F. J. 1983. New developments in chemical control of *Phytophthora*. Pages 327-334 in: *Phytophthora: Its Biology, Taxonomy, Ecology, and Pathology*. D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao, eds. American Phytopathological Society, St. Paul, MN. 392 pp.
11. Shew, H. D. 1983. Effects of soil matric potential on infection of tobacco by *Phytophthora parasitica* var. *nicotianae*. *Phytopathology* 73:1160-1163.
12. Shew, H. D. 1984. In vitro growth response of *Phytophthora parasitica* var. *nicotianae* isolates to metalaxyl. *Plant Dis.* 68:764-766.
13. Staub, T. H., and Young, T. R. 1980. Fungitoxicity of metalaxyl against *Phytophthora parasitica* var. *nicotianae*. *Phytopathology* 70:797-801.