Three metalaxyl sensitivity levels in Australian isolates of *Pseudoperonospora cubensis* (Berk. et Curt.) Rost.

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Summary. Isolates of *Pseudoperonospora cubensis* from the Murrumbidgee Irrigation Area (MIA), New South Wales, and the Burdekin district, Queensland, were compared for metalaxyl sensitivity with a known sensitive isolate. Several different fungicide application techniques were used (foliar sprays, soil drenches, floating leaf discs), but the relativity of metalaxyl sensitivity between isolates was maintained. Isolates from the Burdekin district were highly resistant and came from fields where control of downy mildew was poor. Isolates from the MIA showed intermediate sensitivity but there was no apparent loss of field control. In a floating disc experiment the EC50 values of sensitive, intermediate, and resistant isolates were in the ranges >0.01–<0.1, >1–<10, and >100 μg metalaxyl/mL, respectively.

Introduction

Cucurbit downy mildew [*Pseudoperonospora cubensis* (Berk. et Curt.) Rost.] is a common disease of cucurbits in Australia. The most susceptible crop species are rockmelon and honeydew melon (*Cucumis melo* L. var. *reticulatus* and *C. melo* L. var. *inodorus*), cucumber (*Cucumis sativa* L.), and zucchini (*Cucurbita pepo* L. var. *melopepo*). Control of the disease in these field-grown crops often demands the use of fungicides such as dithiocarbamates or dithiocarbamate–phenylamide mixtures. Phenylamide-containing fungicides, in particular, have been very effective since registration in 1980.

Isolates of *P. cubensis* with resistance to the phenylamide fungicide metalaxyl were first reported by Reuveni *et al.* (1980) in glasshouse-grown cucumbers in Israel and are known to occur in Greece (Georgopoulos and Grigoriu 1981), Italy (D'Ercole and Nipoti 1985), the USA (Moss 1987), and Russia (Grin'ko 1992). In Australia, a few isolates have been tested, but until 1993, all were phenylamide sensitive. In March 1993, several isolates of the fungus were collected from the Murrumbidgee Irrigation Area (MIA), New South Wales, where field control with phenylamide fungicides appeared satisfactory. Further isolates were later obtained from the Burdekin district near Ayr, Queensland, from crops in which disease control was poor. In fungicide sensitivity tests, 3 metalaxyl sensitivity types (sensitive, intermediate, resistant) were present. This paper presents the results of the several different techniques used to compare isolates that show relative sensitivity as a constant character.

Materials and methods

Isolates

Isolate 1550 was collected in the Burdekin district, near Clare, in September 1986 ex. pumpkin (*Cucurbita maxima* Duchesne). Tests showed it to be metalaxyl sensitive, and it was stored in liquid nitrogen as a reference isolate using the method of Bromfield and Schmitt (1967). It was recovered from storage for use in these experiments in May 1993.

Ten isolates (3914-1 to 3914-10) were collected ex. rockmelon and honeydew melon from 10 farms in the MIA near Griffith in March 1993. Downy mildew was severe in unsprayed areas but of minor significance in fields sprayed with phenylamide-based fungicides. Three isolates (4031, 4044, 4045) came from rockmelon and honeydew crops near Ayr in November 1993. Field control of downy mildew with fungicides was poor. Isolate 4085 was collected near Gympie, Queensland, in December 1993 ex. a self-sown cucumber plant.

Maintenance of isolates was by weekly transfer to detached cucumber cv. Crystal Salad cotyledons or leaves on water agar in Petri dishes. Droplets of sporangial suspension prepared by shaking sporulating leaf segments in distilled water were placed on the inverted leaf surfaces. Residual water was removed with sterile blotting paper after 2 days, as it interfered with sporulation if allowed to remain. Cultures were kept on a laboratory bench in diffuse light at 22–24°C. If large quantities of inoculum were required, young cucumber plants were spray-inoculated and incubated in a moist chamber (22°C) overnight. Sporulation was induced 7–10 days later by reincubating.

Metalaxyl sensitivity tests

Since *P. cubensis* is an obligate parasite, experiments to compare fungicide sensitivity of isolates must be completed on plant tissue. Such tests are subject to more environmental variables than those in which fungicides can be incorporated into synthetic media. For this reason, tests were repeated using different techniques to ensure that the relative response of

isolates to changes in fungicide dosage was constant. In all experiments cucumber cv. Crystal Salad was the source of plants or leaf discs, and Ridomil WP25 was the source of metalaxyl.

Experiment 1. Comparison of 10 isolates from the MIA. Cucumber plants were grown in a glasshouse and sprayed at weekly intervals with metalaxyl at concentrations of 0, 10, or 100 μg a.i./mL. Concurrently, the 10 isolates from the MIA (3914-1 to 3914-10) were maintained to provide inoculum. Leaf discs (12 mm diameter) were cut from the youngest fully expanded leaves at 24 h after a metalaxyl application. Such leaves had received 2 fungicide applications. Leaf discs were placed inverted on water agar in Petri dishes and inoculated with 10-μL droplets (1×10^4 sporangia/mL) of each of the isolates (1 droplet/disc). There were 3 replicate discs per treatment. After 2 days, inoculum droplets were removed with blotting paper. Five days later, discs were examined and rated on a 0–3 scale: 0, no symptoms; 1 chlorotic spot with either no sporulation or very few sporangiophores; 2 sporulation moderate; 3, sporulation intense.

Experiment 2. Comparison of isolate 3914-1 with 1550. Cucumber plants were grown, 1 plant/10-cm-diameter pot, until the second true leaf was almost fully expanded. Eight replicate plants were then thoroughly sprayed with metalaxyl at concentrations of 0, 1, 10, and 100 μg/mL. Four plants from each treatment were inoculated 24 h later with sporangial suspensions (2×10^4/mL) of isolate 3914–1 or 1550 applied to all leaf surfaces by a Preval atomiser. Plants were enclosed in moist plastic bags overnight and again a week later to induce sporulation. Two leaves from each plant were rated on a 0–3 scale (% leaf area affected): 0, no symptoms; 1, <10%; 2, 10–50%; 3, >50%.

Experiment 3. Comparison of isolates 3914-1, 1550, 4031. Glasshouse-grown cucumber plants were raised, 1 plant/10-cm-diameter pot in U.C. mix (1:1 sand:peat) until 2 true leaves had emerged. Nine plants were thoroughly sprayed with each of the 6 concentrations of metalaxyl (0.25, 1, 2.5, 10, 25, 100 μg/mL). Six plants were treated with each of the 3 concentrations of metalaxyl drench (2.5, 25, 250 μg/mL) applied at 20 mL/pot. Nine plants were left as untreated controls. After 4 h, when spray deposits were dry, sporangial suspensions (1×10^4/mL) of isolates 3914-1, 1550, and 4031 were used to inoculate 3 plants in each spray treatment and 2 plants in each drench treatment. Inoculum was atomised over all leaf surfaces. Plants were enclosed overnight in moist plastic bags and kept at 15–20°C. One week later, plants were again enclosed overnight and rated for disease severity. Two leaves/plant were rated on a 0–6 scale (% leaf area affected): 0, no symptoms; 1, 1–3 chlorotic spots (<1%); 2, 1–10%; 3, >10–25%; 4, >25–50%; 5, >50–75%; 6, >75%.

Experiment 4. Comparison of isolates 3914-1, 4085, 4044, 4045, 1550. Leaf discs (12 mm diameter) were cut from cucumber cotyledons. Metalaxyl solutions (0, 0.001, 0.01, 0.1, 1, 10, 100 μg/mL) were prepared using sterile distilled water, and each solution was allocated to a 24-well culture dish (2 mL/well). Leaf discs (1/well) were floated inverted on the solutions. Sporangial suspensions (1×10^4/mL) of the test isolates were prepared and a single 10-μL droplet was placed in the centre of each disc. Each treatment was replicated 4 times. The inoculum droplet was removed with blotting paper after 2 days. Discs were incubated at 20°C for 12 days, then rated on a 0–3 scale for sporulation intensity: 0, no symptoms; 1, reaction spot with no sporulation; 2, sporulation low–moderate covering <50% of disc surface; 3, sporulation covering >50% of disc.

Results

Experiment 1

All 10 isolates from the MIA sporulated on leaves sprayed with 0 and 10 μg metalaxyl/mL, with mean (± s.e.) disease ratings 2.9 ± 0.5 and 2.6 ± 0.5, respectively. Five isolates produced low disease severity (0.3 ± 0.6) on leaves sprayed with 100 μg/mL. There were no significant differences between isolates in any of the 3 treatments.

Experiment 2

Both isolates caused severe damage on unsprayed plants but the standard sensitive isolate 1550 caused only slight damage on plants sprayed with 1 μg metalaxyl/mL and no symptoms on plants sprayed with 10 and 100 μg/mL (Table 1). Isolate 3914-1, in comparison, caused severe damage on plants treated with 1 μg metalaxyl/mL. There was a small but significant reduction in severity at 10 μg/mL and only trace symptoms at 100 μg/mL.

Experiment 3

The 3 isolates (1550, 3914-1, 4031) severely affected untreated control plants but showed consistently different sensitivities to metalaxyl in the spray and drench treatments (Table 2). With the spray treatments, the severity of disease caused by isolate 1550 was reduced from rating 6 to 3 at 0.25 μg metalaxyl/mL. A similar reduction occurred with isolate 3914-1 at 25 μg/mL, but there was no reduction in disease severity with isolate 4031 at any concentration of metalaxyl. Following drench applications of metalaxyl, isolate 1550 was greatly inhibited by the 25 μg/mL treatment, and isolate 3914-1 by 250 μg/mL. Isolate 4031 was unaffected by the 250 μg/mL drench treatment, even though phenylamide toxicity symptoms were apparent.

Experiment 4

Two isolates (1550, 4085) were sensitive, with no infection occurring on leaf discs floating on a 0.1 μg/mL solution of metalaxyl (Table 3). Isolate 3914-1 showed

Table 1. Experiment 2. Comparative severity of two isolates of *Pseudoperonospora cubensis* on cucumber plants sprayed with a range of metalaxyl concentrations (values are means of eight leaves)

Disease severity (% leaf area affected): 0, no symptoms; 1, <10%; 2, >10–50%; 3, >50%

Metalaxyl conc. (μg/mL)	Isolate 1550	Isolate 3914-1
0	3.0	3.0
1	0.9	3.0
10	0	2.1
100	0	0.1
l.s.d. (*P = 0.05*)	0.8	0.3

Table 2. Experiment 3. Disease severity caused by three isolates of *Pseudoperonospora cubensis* on cucumber plants treated with foliar or drench applications of metalaxyl (values are means of six leaves for spray treatments and four leaves for drench treatments)

Disease severity (% leaf area affected): 0, no symptoms; 1, 1–3 chlorotic spots/leaf, <1%; 2, 1–10%; 3, >10–25%; 4, >25–50%; 5, >50–75%; 6, >75%

Treatment	Isolate: 1550	3914-1	4031
Untreated	6	6	6
Metalaxyl spray (μg/mL)			
0.25	3	6	6
1.0	1	6	6
2.5	0.3	6	6
10	0	6	6
25	0	2.3	6
100	0	0	6
Metalaxyl drench (μg/mL in 20 mL/pot)			
2.5	5	6	6
25	0.5	6	6
250	0	0.5	6

Table 3. Experiment 4. Sporulation of five isolates of *Pseudoperonospora cubensis* on cucumber leaf discs floating on metalaxyl solutions

Number of infected leaf discs is indicated in parentheses (maximum possible, four)

Sporulation rating (mean of four discs): 0, no symptoms; 1, water-soaked lesion, no sporulation; 2, sporulation covering <50% of leaf disc; 3, sporulation covering >50% of leaf disc

Metalaxyl conc. (μg/mL)	Isolate 1550	4085	3914-1	4044	4045
0	3(4)	3(4)	2.7(4)	3(4)	3(4)
0.001	3(4)	3(4)	3(4)	3(4)	3(4)
0.01	2(4)	3(4)	3(4)	3(4)	3(4)
0.1	0(0)	0(0)	3(4)	2.7(4)	3(4)
1	0(0)	0(0)	3(4)	3(4)	3(4)
10	0(0)	0(0)	0(0)	2(3)	3(4)
100	0(0)	0(0)	0(0)	2(3)	1.7(3)

intermediate sensitivity and sporulated on discs floating on 1 μg/mL but not 10 μg/mL. Two isolates from the Burdekin district sporulated on discs floating on 100 μg/mL.

Discussion

In the experiments described, there were 3 levels of sensitivity to metalaxyl in isolates of *P. cubensis*. Isolates such as 1550 and 4085 were similar to the sensitive isolates of Reuveni *et al.* (1980), while isolates from the Burdekin district (4031, 4044, 4045) were similar to their resistant isolates. Isolates from the MIA, typified by 3914-1, had an intermediate sensitivity to metalaxyl not previously observed in this fungus. Strains with intermediate metalaxyl sensitivity have been recognised in field collections of other oomycetous plant pathogens including *Plasmopara viticola* (Herzog and Schüepp 1985), *Phytophthora infestans* (Pappas 1985), and *Bremia lactucae* (Crute 1992). Comparing the results of Pappas (1985) for *P. infestans* with our results in Table 3, there is a close similarity in the 3 sensitivity types in these 2 species using the floating disc technique. In both, resistant isolates grew on discs floating on 100 μg metalaxyl/mL, intermediate isolates grew on 1 μg/mL but not 10 μg/mL, while sensitive isolates were inhibited by 0.1 μg/mL. Isolates of *P. cubensis* with intermediate sensitivity in the MIA were not associated with loss of disease control, although Pappas (1985) reported that metalaxyl dosages needed to be increased to control intermediate isolates of *P. infestans* in the field.

Inheritance studies with *P. infestans* (Shattock 1988) and *Bremia lactucae* (Crute *et al.* 1987) showed that intermediate resistance is controlled by a single locus exhibiting incomplete dominance. Therefore, sensitive and resistant isolates are homozygous while intermediate isolates are heterozygous. Intermediate sensitivity is probably the first step in the selection of highly resistant strains. Supporting field evidence is the presence of reduced sensitive (intermediate) isolates of *P. viticola* at Wädenswil, Switzerland, in 1979 (Herzog and Schüepp 1985) before resistant isolates were first detected in 1981 (Clerjeau and Simone 1982). In Greece, intermediate isolates of *P. infestans* on tomato were collected by Pappas (1985) in 1983, but resistant isolates were not detected until a year later at the same site. It seems probable that the reduced sensitivity of isolates of *P. cubensis* from the MIA represents a transitory stage, and later collections will show the presence of resistant types. Whether intermediate isolates will still be found once resistance occurs is questionable, since such isolates have not been detected elsewhere. In both *P. infestans* and *B. lactucae,* intermediate types continue to be found in field collections (Crute 1992; Deahl and De Muth 1993), possibly a result of mating between homozygous sensitive and resistant strains (Crute 1992). Sexual reproduction in *P. cubensis* is erratic (Cohen 1981) and is known only in Russia, China, and Japan (Palti 1975); hence, intermediate isolates are unlikely to be continually generated by this mechanism.

The presence of fungicide-resistant strains of *P. cubensis* will make downy mildew control in Australian cucurbit crops more difficult. Experience in Israel (Samoucha and Cohen 1984) showed that crop protection with protectant fungicides such as mancozeb may be inadequate and there is a need for the commercial development of effective alternative fungicides such as dimethomorph (Golyshin *et al.* 1992).

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References

Bromfield, K. R., and Schmitt, C. G. (1967). Cryogenic storage of conidia of *Peronospora tabacina*. *Phytopathology* **57**, 1133.

Clerjeau, M., and Simone, J. (1982). Apparition en France de souche de mildiou (*Plasmopara viticola*) résistantes aux fongicides de la famille des anilides (métalaxyl, milfurame). *Le Progrès Agricole et Viticole* **99**, 59–61.

Cohen, Y. (1981). Downy mildew of cucurbits. *In* 'The Downy Mildews'. (Ed. D. M. Spencer.) pp. 341–54. (Academic Press Inc.: New York.)

Crute, I. R. (1992). Downy mildew of lettuce. *In* 'Plant Diseases of International Importance. Vol II. Diseases of Vegetables and Oil Seed Crops'. (Eds H. S. Chaube, U. S. Singh, A. N. Mukhopadhyay and J. Kumar.) pp. 165–85. (Prentice-Hall Inc.: Englewood Cliffs, NJ, USA.)

Crute, I. R., Norwood, J. M., and Gordon, P. L. (1987). The occurrence, characteristics and distribution in the United Kingdom of resistance to phenylamide fungicides in *Bremia lactucae* (lettuce downy mildew). *Plant Pathology* **36**, 297–315.

Deahl, K. L., and De Muth, S. P. (1993). First report of resistance of *Phytophthora infestans* to metalaxyl in eastern Washington and south-western British Columbia. *Plant Disease* **77**, 429.

D'Ercole, N., and Nipoti, P. (1985). Downy mildew of melon due to *Pseudoperonospora cubensis* (Berk. et Curt.) Rostow. *Informatore Fitopathologico* **35**, 61–3. [Abstract in *Review of Plant Pathology* (1985) **64**, 460. Abstract no. 4574.]

Georgopoulos, S. G., and Grigoriu, A. C. (1981). Metalaxyl-resistant strains of *Pseudoperonospora cubensis* in cucumber greenhouses of southern Greece. *Plant Disease* **65**, 729–31.

Golyshin, N. M., Maslova, A. A., and Goncharova, T. F. (1992). A new systemic fungicide against peronosporosis. *Zashchita Rastenii (Moskva)* No. 12, 13. [Abstract in *Review of Plant Pathology* (1993) **72**, 440. Abstract no. 3804.]

Grin'ko, N. N. (1992). Tolerance of the causal agent of downy mildew of cucumber. *Zashchita Rastenii (Moskva)* No. 12, 14. [Abstract in *Review of Plant Pathology* (1993) **72**, 440. Abstract no. 3805.]

Herzog, J., and Schüepp, H. (1985). Three types of sensitivity to metalaxyl in *Plasmospora viticola*. *Phytopathologische Zeitschrift* **114**, 90–3.

Moss, M. A. (1987). Resistance to metalaxyl in the *Pseudoperonospora cubensis* population causing downy mildew of cucumber in South Florida. *Plant Disease* **71**, 1045.

Palti, J. (1975) Pseudoperonospora cubensis. CMI descriptions of Pathogenic Fungi and Bacteria No. 457.

Pappas, A. C. (1985). Metalaxyl resistance in *Phytophthora infestans* on greenhouse tomatoes in Greece. *Plant Pathology* **34**, 293–6.

Reuveni, M., Eyal, H., and Cohen, Y. (1980). Development of resistance to metalaxyl in *Pseudoperonospora cubensis*. *Plant Disease* **64**, 1108–9.

Samoucha, Y., and Cohen, Y. (1984). Differential sensitivity to mancozeb of metalaxyl-sensitive and metalaxyl-resistant isolates of *Pseudoperonospora cubensis*. *Phytopathology* **74**, 1437–9.

Shattock, R.C. (1988). Studies on the inheritance of resistance to metalaxyl in *Phytophthora infestans*. *Plant Pathology,* **37**, 4–11.

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