Plant Pathology (2007) **56**, 836–842 Doi: 10.1111/j.1365-3059.2007.01625.x

Sensitivity of *Phytophthora infestans* to mandipropamid and the effect of enforced selection pressure in the field

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Sensitivity to the new carboxylic acid amide (CAA) fungicide mandipropamid (MPD) in *Phytophthora infestans* was measured for isolates collected between 1989 and 2002 in Israel prior to the commercial use of MPD (baseline sensitivity, 44 isolates), and from MPD-treated (25 isolates) and untreated fields (215 isolates) in nine European countries and Israel between 2001 and 2005. All isolates were sensitive to MPD, with EC_{50} values ranging between 0·02 and 2·98 μg mL^{-1}. Plastic-tunnel (UK), shade-house (Israel) and field experiments (Israel) conducted during 2001–05 showed that enforced selection pressure, applied preventively or curatively, imposed by repeated sublethal (5 μg mL^{-1}) or excessive (500–1000 μg mL^{-1}) doses of MPD on mixed isolates of *P. infestans* produced no isolates resistant to the compound. The results of this study indicate that the probability of a buildup of resistant sub-populations of *P. infestans* to mandipropamid in the field is low.

Keywords: CAA fungicides, chemical control, fungicide resistant populations, late blight, oomycetes, potato

Introduction

Resistance to a fungicide in target pathogens would normally reduce its effective and long-term performance in the field. The probability of development of resistant sub-populations depends largely on the mode of action of a fungicide. Compounds with a site-specific mode of action are more vulnerable than multi-site compounds (Levy *et al*., 1983). For example, resistance against metalaxyl, an rRNA polymerase inhibitor, developed in *Pseudoperonospora cubensis* 2 years after it was first introduced commercially for the control of downy mildew in cucumber (Reuveni *et al*., 1980) and soon afterwards in *Phytophthora infestans* in potato (Gisi & Cohen, 1996). The probability of development of resistant sub-populations has a strong influence on resistance management strategies. High-risk fungicides would normally be marketed in a mixture with a multi-site fungicide to reduce the buildup of resistant sub-populations (Levy *et al*., 1991), whereas low-risk fungicides may be marketed as solo products. Experimental field trials and sensitivity monitoring provide an indication of the risk of resistance evolution against a new fungicide and whether the risk is low, medium or high.

Mandipropamid is a new fungicide effective against foliar oomycete pathogens, but it does not control *Pythium* spp. (Huggenberger *et al*., 2005). Together with dimethomorph, flumorph, iprovalicarb and benthiavalicarb, it was classified into one group of carboxylic acid amide (CAA) fungicides, mainly because field isolates of *Plasmopara viticola*, the causal agent of grape downy mildew, showed cross-resistance to all members of the group (FRAC CAA working group report 2005, www.frac.info; Gisi *et al*., 2007).

The mode of action of CAA fungicides is not known. Morphological studies (Albert *et al*., 1991; Cohen *et al*., 1995; Jende *et al*., 1999, 2002; Matheron & Porchas, 2000; Mehl & Buchenauer, 2001; Reuveni, 2003) indicated that dimethomorph, iprovalicarb and benthiavalicarb, as well as the experimental CAA XR-539 (Young *et al*., 2005), inhibit cell wall biosynthesis/assembly. This was attributed to the facts that they inhibit the encystment of zoospores (of various *Phytophthora* species and *P. viticola*) and cause their lysis, inhibit the regeneration of protoplasts of *P. infestans*, and alter the staining pattern of cell walls with fluorochromes. Biochemical studies with the mandelamide compound SX 623509 in mycelium of *P. infestans* suggested alterations in phospholipid biosynthesis, with inhibition of phosphatidylcholine (lecithin) biosynthesis as a main target (Griffiths *et al*., 2003). The suggested inhibited enzyme was phosphocholinetransferase (Griffiths *et al*., 2003), the last enzyme in the Kennedy pathway.

Whilst resistance to CAAs in *P. viticola* was detected in 1994, shortly after the introduction of dimethomorph in France (G. Albert, personal communication, 1996; H. Steva, personal communication, 2000; Gisi *et al*., 2007), no

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Accepted 23 January 2007

resistance has been reported in field isolates of *P. infestans*, even though dimethomorph has been in use for more than 15 years. One reason for this might be that putative resistant isolates of *P. infestans* have lower fitness: in agar cultures, artificially produced resistant mutants of *P. infestans* lost their resistance, possibly as a result of being ousted by sensitive isolates having greater fitness (Bagirova *et al*., 2001). Another reason for the difference in resistance evolution between the two pathogens might be related to the fact that oospores of *P. viticola* initiate epidemics in the spring, whereas *P. infestans* does not necessarily do so. In *P. viticola*, resistance to CAAs in field isolates was found to be controlled by recessive genes (Gisi *et al*., 2007). Recessive genes for resistance would need several sexual recombinations to be fixed in the population and expressed phenotypically, which in *P. infestans* may not happen frequently enough for establishment in the field.

The lack of resistant field isolates in *P. infestans* encouraged several researchers to produce artificial mutants resistant to CAAs by applying mainly UV irradiation to mycelia *in vitro* (Chabane *et al*., 1993; Stein & Kirk, 2004; Yuan *et al*., 2006) or to sporangia (Rubin *et al*., 2006; Rubin & Cohen, 2006). Bagirova *et al.* (2001) applied NMU (*N*-nitrosomethylurea) to cystospores. Stein & Kirk (2004) reported that dimethomorph-resistant mutants had reduced growth rates on non-amended medium and reduced frequency of infection of leaf discs and whole tubers. They concluded that the likelihood of resistance to dimethomorph appearing in nature is negligible. Similar results were generated by Bagirova *et al.* (2001) for dimethomorph and by Yuan *et al.* (2006) for flumorph. The latter authors concluded that the risk of resistance development was much lower for flumorph than for metalaxyl. Rubin *et al.* (2006) and Rubin & Cohen (2006) showed that mutants with stable resistance to mefenoxam were readily obtained by exposing sporangia to UV light, whereas mutants with resistance to dimethomorph lost resistance after repeated inoculations of dimethomorph-treated tomato leaves.

In addition to mutagenesis, enforced selection is another tool in estimating resistance risk for a new fungicide. The aim of this study was to estimate the likelihood of *P. infestans* developing resistance to the new CAA fungicide mandipropamid by imposing enforced selection pressure on naturally or artificially inoculated crops in the field, with repeated applications of either sublethal or excessive doses of MPD. This type of information is of critical importance, not only with regard to formulating appropriate resistance management strategies to be employed commercially, but also for studying the biochemical mode of action of MPD.

Materials and methods

Fungicide

Mandipropamid (MPD), code number NOA 446510, molecular weight 412, formulated as 250SC (soluble concentrate) was supplied by Syngenta. The product was suspended in water to the concentration required. All concentrations are represented in units of active ingredient.

Origin of field isolates

Sensitivity to MPD was measured for 67 Israeli field isolates of *P. infestans* collected from commercial, MPD-untreated fields between 1989 and 2002 (44 isolates), and between 2003 and 2005 (23 isolates). Isolates originating from potato or tomato crops were maintained until use on potato tuber slices or detached tomato leaves at 12°C as described previously (Cohen, 2002). Sensitivity to MPD was also determined for the reference Swiss isolate (#96, A1 mating type, sensitive to metalaxyl) from the Syngenta collection.

Sensitivity to MPD was determined for 192 field isolates of *P. infestans* collected between 2001 and 2005 from 73 trial sites in nine European countries (Switzerland, Germany, Denmark, Spain, France, the Netherlands, Poland, Russia and the UK). For the 122 of these isolates which were collected during 2004 and 2005, sensitivity to both MPD and mefenoxam (metalaxyl-M) was determined. Of these 122 isolates, 97 were collected from untreated plots and 25 from MPD-treated plots. In most trial sites, mandipropamid was applied at the recommended n-rate of 150 g a.i. ha^{-1} up to eight times per season, thus imposing a strong selection pressure on the pathogen population. Infected leaf samples (bulk isolates) were collected at different time intervals during the season and the pathogen was tested for sensitivity to MPD using the potato leaf disc spray assay described below.

Sensitivity testing of *Phytophthora infestans* isolates

All tests were carried out *in vivo* using one of the following three methods:

Method (i) – European isolates

Leaf discs (12 mm in diameter) were taken from middle leaves of 10- to 11-leaf potato cv. Bintje plants, each placed, lower surface uppermost, on 1 mL of solid 0·2% water agar in 24-well titre plates and sprayed with MPD at 0, 0·001, 0·01, 0·1, 1 or 10 μg mL^{-1}. After 1 h the discs were each drop-inoculated with a 20-μL sporangial suspension (20 000 sporangia mL^{-1}) of the test isolate (one drop per disc). The sensitivity test for mefenoxam was performed by floating the leaf discs on fungicide solution instead of spray application; inoculation was as for MPD. Plates were incubated at 20°C for a week, after which the percentage of the leaf disc area showing sporulation of the pathogen was visually estimated. Percentage inhibition of sporulation at each fungicide concentration was calculated relative to the untreated control (no fungicide) and EC_{50} values were calculated by using AGSTAT analysis (Syngenta internal software).

Method (ii) – Israeli field isolates

Tomato leaf discs (12 mm in diameter) were taken from middle leaves of 10- to 11-leaf cv. Baby plants. Discs were each placed (lower side upward) on 1 mL suspension of MPD (0, 0·01, 0·1, 1, 10 or 100 μg mL^{-1}) in 24-well titre plates. Sporangia of 44 different isolates were collected from sporulating potato tuber slices or detached leaves of tomato, their concentration adjusted to 5000 sporangia mL^{-1}, and one 10-μL droplet inoculated onto each leaf disc. Plates were incubated at 18°C in the dark for 20 h and then at 20°C (12 h light per day). At 7 days post-inoculation (d.p.i.), leaf discs were collected from each treatment and the number of sporangia produced counted with the aid of a haemocytometer. Percentage inhibition of sporangial production by each fungicide concentration was calculated relative to the untreated control (no fungicide) and EC_{50} values were calculated by AGSTAT analysis.

The sensitivity of another 23 isolates to MPD was tested on detached leaves of tomato. For these isolates, mating type was also determined. MPD at 0·001–100 μg mL^{-1} (×10 dilution steps) was sprayed onto the lower leaf surface of detached tomato leaves in Nunk dishes (20 × 20 × 3 cm). Untreated leaves served as controls. Sporangial suspensions (5000 mL^{-1}) of the various isolates were each inoculated onto the leaves by spraying (10 mL per dish). Dishes were incubated as described above and the percentage of sporulating leaf area was visually estimated 6 d.p.i. Disease control data were subjected to AGSTAT analysis for calculation of EC_{50} values.

Method (iii) – Putative resistant mutants in enforced selection experiments

In the Israeli experiments, individual leaflets carrying late blight lesions (normally one lesion per leaflet) were periodically collected from the enforced selection trials in shade houses or the field. Healthy tomato leaves were placed, lower surface uppermost, on wet filter paper in Nunk dishes, and six leaflets per treatment were used for inoculation. Leaflets were each inoculated with ten 20-μL droplets of a mixture (1:1) composed of sporangial suspension of the tested isolate and MPD (0·01, 0·1, 1, 10 or 100 μg mL^{-1}). Dishes were incubated as above and the proportions of sporulating leaf area in each leaflet determined at 7–10 d.p.i. Disease control data were subjected to AGSTAT analysis for calculation of EC_{50} values. Testing of putative mutants in the UK experiment is described below.

Enforced selection experiments in plastic tunnel, shade house and open field

A total of four experiments were performed to evaluate whether continuous exposure over an extended period of time to sublethal concentrations of MPD, or the repeated use of excessive doses, would select resistant isolates from mixed populations. Both approaches can be regarded as worst-case situations of product use potentially selecting fungicide resistance. A plastic-tunnel experiment (A) was performed in 2001 at the Syngenta Research Centre, Jealotts Hill, UK (Table 1) using the method described previously for phenylamide fungicides (Kadish & Cohen, 1989). Potato plants (cv. Bintje) were grown in a 6- × 10-m plastic tunnel in three rows. The middle row was left untreated, whereas the two outer rows were sprayed (with the aid of a hand-held manual sprayer) 11 times at weekly intervals with a sublethal concentration (5 μg mL^{-1}) of MPD. A mixture of 20 UK field isolates of *P. infestans* was inoculated onto all potato rows. Sporulating leaflets were collected every week from treated and untreated plants and the sensitivity to MPD of the sporangia they harboured was determined using method (i).

Three experiments (B, C and D) were conducted at the Bar-Ilan University Farm, Israel, during 2002–05 (Table 1), in which selection pressure was imposed by applying excessive doses of MPD, and the appearance of putative resistant mutants was monitored. One experiment (D) was carried out in the open field and two in shade houses. Potato crops were grown in 300-m^2 (6 × 50 m) shade houses (covered with 50-mesh antivirus, white plastic nets). Three rows of potato cv. Alpha plants, each 40 m long, were grown in each shade house. The middle row was left untreated (CK, control), one row was sprayed with 2 L of MPD at 500 μg mL^{-1} (corresponding to 300 g a.i. ha^{-1} = twice the normal field rate), the other with 1000 μg mL^{-1} (600 g a.i. ha^{-1} = four times the normal field rate). Sprays were applied at 7- to 9-day intervals (3–4 sprays per season) with the aid of a motorized back-pack sprayer. To avoid drift of spray mist, the other two rows were covered with a plastic sheet during fungicide application. A few hours after the first spray (preventive application) all three rows were spray-inoculated (1 L per row, manual back-pack sprayer) with a sporangial suspension (1000 sporangia mL^{-1}) composed of an artificial mixture of *P. infestans* isolates (Table 1). In 2002, a mixture of 65 field isolates was used (Expt. B); in

Table 1 Experiments designed to enforce buildup of resistance to mandipropamid in *Phytophthora infestans*

Experiment	Year	Crop (cultivar)	Place	Country	Timing of first spray	Number of sprays	Concentration (μg mL^{-1})[a]	Inoculation	Number of isolates
A	2001 May–June	Potato (Bintje)	Plastic tunnel	UK	Preventive	11	5	Artificial	20
B	2002 October	Potato (Alpha)	Shade house	Israel	Preventive	3	1000 (4 × *n*)	Artificial	65
C	2004 December	Potato (Alpha)	Shade house	Israel	Preventive	4	500 (2 × *n*), 1000 (4 × *n*)	Artificial	165
D	2005 March	Potato (Alpha)	Field	Israel	Curative	3	500 (2 × *n*), 1000 (4 × *n*)	Natural	N/A

[a]Values in parentheses show relationship to normal (*n*) field rates.

2004, a mixture of 165 isolates, composed of 75 field isolates and 90 F_1 isolates produced from them were used (Expt. C) (Irzhansky & Cohen, 2006; Rubin & Cohen, 2006). Isolates had diverse characteristics including various levels of sensitivity to metalaxyl, A1 and A2 mating types, simple and complex races, and several SSR and AFLP genotypes (Klarfeld *et al*., 2006; Rubin *et al*., 2006).

In the open-field experiment (Expt. D), 18 plots (12 m^2 each) of potato cv. Alpha were allowed to become naturally infected with late blight. When about 5% of the leaf area was infected, six plots (randomly selected) were treated with 2 L of MPD at 500 μg mL^{-1} (curative application, four treatments), six plots with 1000 μg mL^{-1}, and six plots were left untreated.

In all experiments, individual infected leaflets (with single lesions) were periodically collected from each row and allowed to sporulate under moist conditions. The sporangia produced were assayed for sensitivity to MPD as described in method (iii). In the open-field experiment, sporangia were washed with water over a Millipore membrane before use.

Results

Baseline sensitivity to MPD of Israeli isolates

The EC_{50} values for 44 isolates of *P. infestans* collected between 1989 and 2002 from fields which had never been treated with MPD are shown in Fig. 1a; they ranged between 0·007 and 1·17 μg mL^{-1}, with a mean ± SD of 0·29 ± 0·32 μg mL^{-1}. The EC_{50} value of the reference isolate #96 was 0·26 μg mL^{-1}. Amongst these isolates, 22, 10 and 7 were sensitive, intermediately-resistant, and resistant to metalaxyl, respectively (data not shown, five isolates not determined). Sensitivity to MPD was not associated with the level of sensitivity to metalaxyl.

Sensitivity to MPD of Israeli and European isolates

The sensitivity to MPD of 12 A1- and 11 A2-mating-type isolates collected in Israel between 2003 and 2005 was measured on detached leaves of tomato plants. The EC_{50} values for the A1 isolates ranged between 0·009 and 0·48 μg mL^{-1}, with a mean ± SD of 0·126 ± 0·135 μg mL^{-1}. Values for the A2 isolates ranged between 0·009 and 0·30 μg mL^{-1}, with a mean of 0·077 ± 0·106 μg mL^{-1}. There was no significant difference (Fisher's LSD test) between the two mating-type groups in the effectiveness of MPD, measured as the EC_{50} values.

The EC_{50} value of the reference isolate #96 used for sensitivity tests to MPD on potato leaf discs ranged between 0·76 and 0·89 μg mL^{-1}. All of the isolates sampled from field trial stations across Europe during 2001, 2002 and 2003 (11, 29 and 30, respectively) were sensitive to mandipropamid (data not shown).

The sensitivity data of 122 isolates collected during 2004 (75 isolates) and 2005 (47 isolates), from untreated (97 isolates) and MPD-treated (25 isolates) fields across Europe, are presented (combined) in Fig. 1b. The EC_{50} values for the 2004 isolates ranged between 0·025 and 1·84 μg mL^{-1}, with a mean of 0·609 ± 0·380 μg mL^{-1}, and

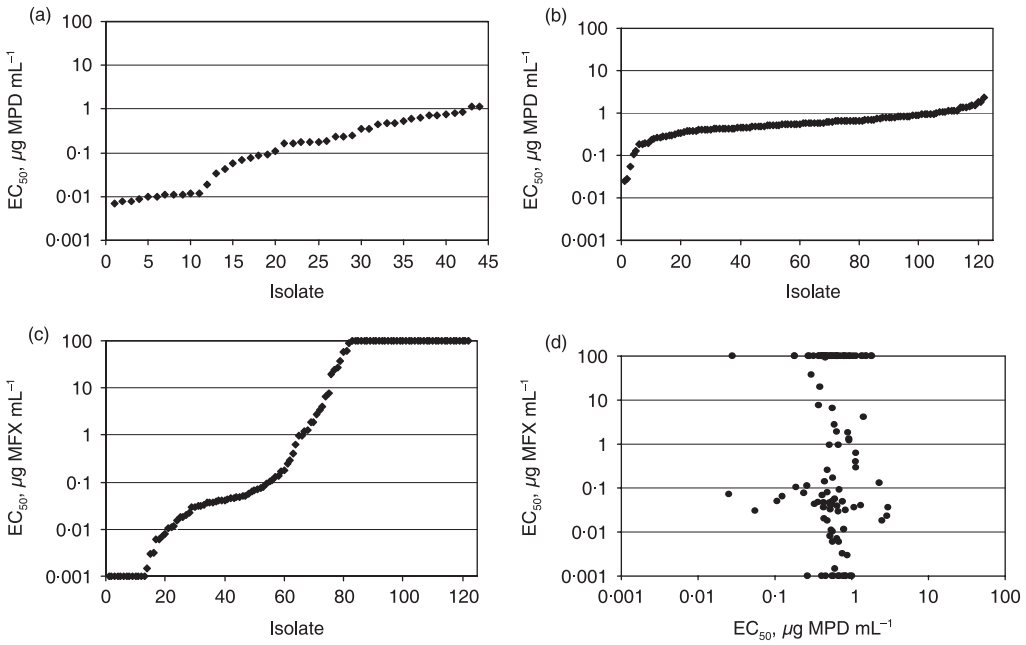

Figure 1 Sensitivity (EC_{50}) of Israeli and European field isolates of *Phytophthora infestans* to mandipropamid (MPD) and mefenoxam (MFX). (a) Baseline sensitivity to MPD of 44 isolates collected from potato or tomato fields in Israel during 1989–2002 (tomato leaf disc float assay). (b) Sensitivity to MPD of 122 isolates collected from 36 trial sites in Europe during 2004–05 (potato leaf disc spray assay). (c) Sensitivity to MFX of the same 122 European isolates (potato leaf disc float assay). (d) Comparison of sensitivities to MPD and MFX of the same 122 European isolates.

for the 2005 isolates between 0·180 and 2·98 (mean 0·784 ± 0·560) μg mL^{-1}. Kruskal-Wallis one-way analysis of variance on ranks revealed no significant difference in EC_{50} values between the two groups of isolates (collected during 2004 or 2005, $P = 0{\cdot}05$). The difference in sensitivity to MPD between the most and least sensitive isolate was 150-fold. The 97 isolates collected from untreated plots showed a mean EC_{50} value of 0·696 ± 0·477 μg mL^{-1}, compared with 0·557 ± 0·383 μg mL^{-1} for the 25 isolates collected from MPD-treated plots. No significant difference in sensitivity to MPD was found between the two groups of isolates (collected from MPD-treated or untreated fields, $P = 0{\cdot}131$).

Sensitivity of these 122 isolates to mefenoxam (metalaxyl-M) is shown in Fig. 1c. The reference isolate #96 had an EC_{50} of 0·015 μg mL^{-1}. Isolates 1–21 had EC_{50} values <0·01 μg mL^{-1} (considered highly sensitive; Rubin & Cohen, 2006), isolates 22–52 had EC_{50} 0·011–0·1 μg mL^{-1} (considered sensitive), isolates 53–75 had EC_{50} 0·11–10 μg mL^{-1} (considered intermediately resistant) and isolates 76–122 had $EC_{50} > 10$ μg mL^{-1} (considered resistant). Since the genotype of intermediately resistant isolates is not well defined, the borderline between sensitive and intermediately resistant isolates can be drawn at any EC_{50} between 0·11 and 1·0 μg mL^{-1}. Unlike the continuous sensitivity distribution to MPD (Fig. 1b), the sensitivity distribution to mefenoxam was bimodal, clearly separating sensitive and resistant isolates (Fig. 1c). The overall range of sensitivity to mefenoxam was broad (a factor of > 100 000). Sensitivity to MPD was not correlated with sensitivity/resistance to mefenoxam ($r^2 = 0{\cdot}0012$, Fig. 1d), suggesting that no cross-resistance occurs between the two fungicides.

Enforced selection experiments in the field

At the end of the plastic-tunnel experiment carried out in the UK (Expt. A), the percentage infected leaf area reached 55% and 6·6% in untreated and treated plants, respectively (Fig. 2a). The EC_{50} value of MPD of the founding population was 0·05 μg mL^{-1}. The EC_{50} values of a total of 33 isolates retrieved from the treated plants and 33 isolates retrieved from the untreated plants throughout the experiment were not significantly different from each other, nor from the value of the founding population, indicating that no resistance had built up.

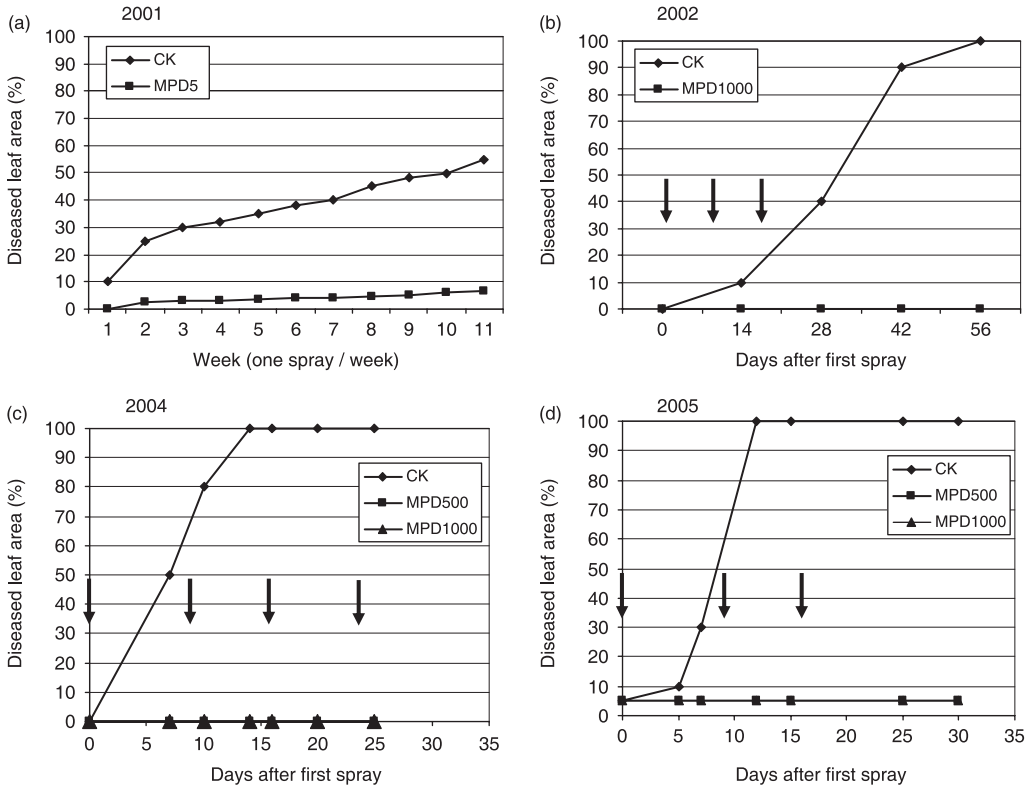

Figure 2 Efficacy of mandipropamid (MPD) in controlling late blight in potato in plastic-tunnel, shade-house and field trials. (a) Plastic-tunnel experiment (A) with potato cv. Bintje in May–June 2001; 11 sprays (5 μg mL^{-1}) applied (once a week); inoculation with a mixture of 20 isolates after the first spray. (b) Shade-house experiment (B) with potato cv. Alpha in October 2002; three sprays (500 or 1000 μg mL^{-1}) applied; inoculation with a mixture of 65 isolates after the first spray. (c) Shade-house experiment (C) with potato cv. Alpha in December 2004; four sprays (500 or 1000 μg mL^{-1}) applied; inoculation with a mixture of 165 isolates after the first spray. (d) Field experiment (D) with potato cv. Alpha in March 2005; natural late blight disease; three sprays (500 or 1000 μg mL^{-1}) applied, first one when blight-affected leaf area ~5 %. CK = untreated control.

In the three experiments carried out in Israel, disease in the untreated control rows developed rapidly because of the favourable environmental conditions, but was strongly suppressed in the MPD-treated rows (Fig. 2b–d). Thirty infected leaflets were collected from the untreated control plants in each experiment during the season and 2–30 infected leaflets (depending on disease level) from the treated plants. A total of 227 isolates were collected from the three experiments (B-D). They all were sensitive to MPD regardless of whether they had been collected from treated or untreated plants. They were all sensitive and completely inhibited at a concentration of 0·05 μg MPD mL^{-1}.

Discussion

In this work, two important factors that can be used for assessing the potential resistance risk for mandipropamid in *P. infestans* were investigated: (i) the baseline sensitivity and sensitivity of treated and untreated isolates collected from field populations in Europe and Israel, and (ii) the reaction of populations to enforced selection in the field. Experiments were carried out to examine whether the continuous exposure of *P. infestans* populations on potato crops to sublethal or repeated exposure to excessive doses of MPD could encourage the selection of resistant sub-populations.

Isolates of *P. infestans* collected in Israel (where MPD has never been used) during 1989–2006 from potato or tomato crops were all sensitive to MPD. This suggests that resistance to MPD did not occur in this country. A total of 192 isolates of *P. infestans* were collected between 2001 and 2005 in nine European countries from 73 potato field trials. All isolates showed no difference in sensitivity to MPD in comparison with a sensitive reference isolate. Also, no difference in sensitivity was found between isolates collected from treated and untreated plots. This indicates that repeated applications of MPD, often in the same location, did not select resistance to MPD. In addition, no resistant isolates were detected against other CAA fungicides such as dimethomorph or iprovalicarb over the last decade (CAA FRAC working group www.frac.info).

MPD was applied in excessive doses in three experiments in Israel to explore whether such heavy selection pressure could result in the appearance of resistant isolates. In two experiments, a large number of diverse field isolates (and their sexual progeny) were used to artificially inoculate the host crops, while in the third experiment a natural infection occurred. In the artificially inoculated crops, MPD was applied preventatively, and in the naturally infected crop as a curative treatment. In all experiments, MPD provided excellent control of late blight and no resistant isolates of *P. infestans* were recovered from either the treated or the untreated control plants. Similar exposure to metalaxyl resulted in the appearance of resistant isolates of *P. cubensis* in greenhouse-grown cucumber within 2 years (Reuveni *et al*., 1980) and of *P. infestans* in potato within 4 years after introduction of the product (Gisi & Cohen, 1996), suggesting that for the control of *P. infestans* MPD is a low-risk fungicide compared with metalaxyl. The results obtained in this study show that the probability of *P. infestans* developing sub-populations resistant to mandipropamid in the field as a result of selection pressure is low. Also, many years of commercial use of other CAAs (especially dimethomorph and iprovalicarb) did not select CAA-resistant isolates. Therefore, the resistance risk in *P. infestans* for the entire class of CAA fungicides can be considered low.

With the exception of the phenylamide fungicides (e.g. mefenoxam), none of the existing fungicide classes used to control *P. infestans*, such as cyanoacetamide-oximes (cymoxanil, launched in 1976), carbamates (propamocarb, 1978), dinitroanilines (fluazinam, 1988), QoIs (e.g. azoxystrobin, 1992; and famoxadone, 1996), QiIs (cyazofamide, 1998) and benzamides (zoxamide,1998) have selected resistance in field populations (Kuck & Russell, 2006). However, in the same period of time, resistance evolved in *P. viticola* to several of these fungicide classes, including phenylamides, cymoxanil, CAAs and QoIs (the other classes are not used for *P. viticola* control) (Gisi, 2002; Gisi *et al*., 2007). Therefore, FRAC has proposed to down-grade the resistance risk for *P. infestans* from high to medium (except for phenylamides) (Pathogen Risk List, www.frac.info).

The repeated absence of MPD-resistant isolates in the enforced selection experiments and in all the field trials across Europe described here during the last five years may result from one or more of the following reasons: (i) multiple targets or multiple loci are associated with the activity of MPD; additive mutations in one or several genes are involved in resistance and many selection cycles are required for achieving stable resistance; (ii) inheritance of all or some of the resistant loci is recessive (as in *P. viticola*, Gisi *et al*., 2007), thus requiring several sexual recombination cycles to be expressed phenotypically; and/or (iii) resistance in *P. infestans*, unlike that in *P. viticola*, might be lethal or associated with severe penalties, such as reduced growth and fitness, thus avoiding competition with wild-type populations.

Whilst wild-type field isolates failed to attack MPD-treated crops, artificially produced mutants of *P. infestans* may do so. Current experiments are aimed at elucidating such a possibility. Although this study suggests that isolates resistant to CAA fungicides may not appear in field populations, suitable anti-resistance precautions should be taken, including continuous sensitivity monitoring, preventive use of the products, limitation of the number of applications per season and use of CAA products within a spray programme with fungicides of other chemical classes (see CAA FRAC recommendations, www.frac.info).

Acknowledgements

We are grateful to Dominique Edel of Syngenta Crop Protection, Stein, Switzerland, for providing the sensitivity data of the European isolates, and to Eli Brinder of Bar-Ilan University for conducting the field experiments.

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