Resistance of *Phytophthora capsici* to metalaxyl in plastic-house capsicum crops in southern Italy*

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In Calabria (southern Italy), control of crown and root rot of capsicum caused by *Phytophthora capsici* has relied primarily on soil drenches of metalaxyl. However, severe outbreaks occur every year in glasshouse crops, in which the practice of using plastic mulch and furrow irrigation favours the disease. Single-hypha isolates of *P. capsici* collected in Calabria in 1992/1998 were tested *in vitro* for their level of sensitivity to metalaxyl. Isolates of other species of *Phytophthora* were used as reference. Fungicide sensitivity was determined by plating mycelial plugs onto potato dextrose agar amended with metalaxyl, at final concentrations ranging from 0.1 to 1000 $\mu g\,mL^{-1}$ a.s. Inhibition of radial growth (%) was determined when colonies on unamended medium had covered approximately two-thirds of the plate. The ED_{50} values for inhibition of mycelial growth of *P. capsici* isolates ranged from 1.41 to 44.6 $\mu g\,mL^{-1}$ a.s. More than 80% of the *P. capsici* isolates from commercial plastic-house crops in Calabria showed a moderate level of resistance as they were inhibited less than 60% at 5 $\mu g\,mL^{-1}$ but more than 60% at 100 $\mu g\,mL^{-1}$.

Introduction

Phytophthora blight caused by *Phytophthora capsici* is the most important disease of capsicum in the coastal provinces of Catanzaro and Vibo Valentia, in Calabria (southern Italy), and is becoming a limiting factor for plastic-house capsicum crops in this area where an old local selection Riggitano, very susceptible to root and crown rot caused by *P. capsici*, is the prevalent cultivar. In addition, the use of plastic mulch and furrow irrigation greatly increases the incidence of this disease. Although soil and space solarization have proved effective in reducing both the inoculum of *P. capsici* in the soil and the incidence of phytophthora blight in experimental plots (Polizzi *et al.*, 1994), severe outbreaks often occur in commercial crops in both solarized and methyl bromide-sterilized soils despite repeated applications of metalaxyl, as seed and root treatments of seedlings before transplanting or as a soil drench.

The objective of the present study was to verify the hypothesis that failure of metalaxyl to control phytophthora blight in Calabria is due to development of resistance in populations of *P. capsici* as a consequence of repeated applications of this fungicide. Whereas naturally occurring resistance to metalaxyl has been observed for various downy mildews, few cases have been documented for soil-borne *Phytophthora* species. Only recently, insensitivity to both mefenoxam (the active enantiomer contained in the racemic fungicide metalaxyl) and metalaxyl has been reported in field isolates of *P. capsici* from capsicum, cucurbit and aubergine crops in the USA (Parra & Ristaino, 1998; Mathis *et al.*, 1999; Lamour & Hausbeck, 1999).

Materials and methods

P. capsici was isolated from infected tissues and from rhizosphere soil of capsicum on selective medium (Masago *et al.*, 1977). Sixty-two single-hypha isolates of *P. capsici* were obtained from plastic-house capsicum crops of cv. Riggitano in the Lamezia Terme plain, one from a plastic-house capsicum crop in the Gioia Tauro plane and four from kitchen-garden crops in the Vibo Valentia area (Table 1). The isolates were paired on cleared V8-agar with reference isolates to determine their mating type. Reference isolates of other *Phytophthora* species were also used (Table 2). The isolates of *Phytophthora nicotianae* from *Hibiscus rosa-sinensis* were from a glasshouse, where metalaxyl had repeatedly been applied as a soil drench, while the isolate from sour orange was from a citrus orchard where metalaxyl had never been used and the isolate from *Myrtus communis* was from a nursery treated with metalaxyl only recently.

Wettable powder formulation of metalaxyl containing 35% a.s. was used in all tests. Sensitivity was determined by

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Table 1 *In vitro* responses to metalaxyl of isolates of *Phytophthora capsici** from capsicum crops

Isolate	Year	Location†	Origin	ED_{50}	ED_{95}
				$\mu g\ mL^{-1}$	
1/1	1992	LT	Collar	5.93	142
1/2	1992	LT	Collar	8.28	232
2/1	1992	LT	Collar	6.07	136
2/2	1992	LT	Collar	10.20	295
275	1992	LT	Collar	7.40	154.9
2/7	1992	LT	Collar	10.50	288.4
2/8	1992	LT	Collar	9.80	331
2/9	1992	LT	Collar	6.30	155
2/10	1992	LT	Collar	6.50	141.2
2/11	1992	LT	Collar	4.68	123
3/2	1992	LT	Collar	7.10	186
3/3	1992	LT	Collar	6.20	120
3/4	1992	LT	Collar	7.41	155
3/5	1992	LT	Collar	6.80	199
3/6	1992	LT	Collar	8.51	209
3/7	1992	LT	Collar	6.92	182
7/2	1992	LT	Collar	10.0	214
7/4	1992	LT	Collar	6.31	123
8/1	1992	LT	Collar	6.02	144
8/2	1992	LT	Collar	8.32	174
8/3	1992	LT	Collar	7.58	257
8/4	1992	LT	Collar	6.06	130.2
8/5	1992	LT	Collar	6.82	185
A1	1993	LT	Soil	4.20	135.2
A2	1993	LT	Soil	5.13	117.8
A3	1993	LT	soil	4.87	165.9
A4	1993	LT	Soil	3.89	151
A5	1993	LT	Soil	3.71	131.8
A6	1993	LT	Soil	4.02	151
A7	1993	LT	Soil	3.89	158.5
A8	1993	LT	Soil	4.07	115
A11	1993	LT	Soil	3.89	59
C1	1993	LT	Soil	4.47	60.2
C2	1993	LT	Soil	4.80	77.6
C3	1993	LT	Soil	3.63	45.7
C4	1993	LT	Soil	2.51	41.7
C5	1993	LT	Soil	4.70	95.5
C6	1993	LT	Soil	3.47	54.9
C7	1993	LT	Soil	3.16	44.7
C8	1993	LT	Soil	3.55	50
C10	1993	LT	Soil	3.50	72
C11	1993	LT	Soil	3.80	46.3
C12	1993	LT	Soil	3.77	53.2
D3	1993	LT	Soil	2.10	45.7
E1	1993	LT	Soil	1.90	47.9
P1	1993	LT	Root	8.50	148
P2	1993	LT	Root	4.70	77.6
P3	1993	LT	Root	4.10	83
P4	1993	LT	Root	10.20	537
P5	1993	LT	Root	4.60	83.2
P6	1993	LT	Root	4.57	85.1
P7	1993	LT	Root	6.02	148
P8	1993	LT	Root	3.89	61.6
P9	1993	LT	Root	4.90	72.4
P10	1993	LT	Root	4.85	84.0
P11	1993	LT	Root	6.01	86.4
P12	1993	LT	Root	4.83	85.1
P13	1993	LT	Root	6.03	86.3
AI/1	1996	VV	Collar	2.95	66.0
BI/3	1996	VV	Collar	1.41	46.8
CI/1	1996	VV	Collar	2.69	53.7
DI/3	1996	VV	Collar	2.45	48.2
R1	1997	GT	Collar	44.66	> 1000
LT	1998	LT	Collar	8.70	281.8
PC	1998	LT	Collar	8.91	210
CB	1998	LT	Collar	9.54	320.2
A8	1998	LT	Collar	5.01	130.1

ED_{50} and ED_{95} values for inhibition of radial growth of the mycelium.

*All isolates were A2 mating type.

†LT, Lamezia Terme; GT, Gioia Tauro; VV, Vibo Valenzia.

Table 2 *In vitro* responses to metalaxyl of isolates of various species of *Phytophthora* used as a reference

Isolates	Mating type	Host	Location	ED50 $\mu g\,mL^{-1}$	ED95 $\mu g\,mL^{-1}$
P. cinnamomi 8A3R	A2	*Persea americana*	Sicilia (IT)	9.54	> 1000
P.cinnamomi IMI 70473*	A2	*Erica* sp.	UK	2.51	302
P. capsici CH2	A1	*Convolvulus cneorum*	Toscana (IT)	70.79	> 1000
P. citrophthora CH1	–	*Convolvulus cneorum*	Sicilia (IT)	8.91	> 1000
P. nicotianae C301	A2	*Myrtus communis*	Sardegna (IT)	7.94	141.2
P. nicotianae A	A1	*Hibiscus rosa-sinensis*	Calabria (IT)	6.10	420
P. nicotianae B	A2	*Hibiscus rosa-sinensis*	Calabria (IT)	2.50	405
P. nicotianae RT	A1	*Citrus aurantium*	Sicilia (IT)	6.40	350

*From the culture collection at the International Mycological Institute, Egham (GB).

plating 6-mm-diameter mycelial disks onto potato dextrose agar (PDA) amended with metalaxyl. Fungicide was prepared as a concentrated stock solution in distilled water and added to the medium after autoclaving at final concentrations of 0.1, 0.5, 1, 5, 10, 50, 100, 200 and 1000 μg a.s. mL^{-1}. The plates were incubated in the dark at 25 °C. Four replicate Petri dishes were used per treatment and each test was performed twice. The percent of inhibition of radial growth compared with the growth on unamended media was determined at 6 days, when colonies on unamended medium had covered approximately two-thirds of the plate. The ED_{50} and ED_{95} values were calculated from the linear equations relating fungicide concentrations to percent inhibition.

Results

All isolates of *P. capsici* from Calabria were A_2 mating type. Sensitivity to metalaxyl varied greatly among these isolates. The ED_{50} values for inhibition of mycelial growth, in fact, ranged from 1.41 to 44.66 $\mu g\,mL^{-1}$ (Table 1). Isolates of *P. capsici* obtained from kitchen gardens were significantly more sensitive to metalaxyl than isolates from commercial plastic-house crops, suggesting a possible selective effect of treatments with this fungicide. A significant difference in sensitivity to metalaxyl was also observed between isolates from soil and those from collar and roots in plastic-house crops.

The mean ED_{50} value (± SE) for soil isolates from the Lamezia Terme area in 1993 was $3.77 \pm 0.24\,\mu g\,mL^{-1}$, whereas the corresponding value for isolates from roots was $5.63 \pm 0.50\,\mu g\,mL^{-1}$. The ED_{50} values ranged from 1.9 to 5.13 $\mu g\,mL^{-1}$ for isolates from soil, from 3.89 to 10.2 $\mu g\,mL^{-1}$ for isolates from roots, and from 4.68 to 44.66 $\mu g\,mL^{-1}$ for isolates from collar. More than 70% of isolates obtained from infected plant tissues showed an ED_{50} value higher than 6 $\mu g\,mL^{-1}$. The values for collar isolates from plastic-house crops were $7.37 \pm 0.32\,\mu g\,mL^{-1}$ whereas the corresponding value for isolates from kitchen gardens were significantly lower ($2.37 \pm 0.33\,\mu g\,mL^{-1}$). The least sensitive isolate ($ED_{50} = 44.66\,\mu g\,mL^{-1}$) was obtained from a plastic-house crop in the Gioia Tauro area, in 1997. The isolates from plastic-house crops were inhibited 1.3–11.8% at 0.1 $\mu g\,mL^{-1}$, 5.3–19.5% at 0.5 $\mu g\,mL^{-1}$, and 6.7–27.3% at 1 $\mu g\,mL^{-1}$ of metalaxyl.

According to the criterion recently used by Parra & Ristaino (1998) in a screening of *P. capsici* populations for sensitivity to metalaxyl, based on the response of mycelial growth to different dosages of fungicide, 26% of the *P. capsici* isolates from Calabria should be considered sensitive, as they were inhibited more than 60% at 5 $\mu g\,mL^{-1}$, whereas all the others showed a moderate level of resistance as they were inhibited less than 60% at 5 $\mu g\,mL^{-1}$ but more than 60% at 100 $\mu g\,mL^{-1}$. No isolate appeared highly resistant. In fact, at 100 $\mu g\,mL^{-1}$, all isolates were 80–100% inhibited and only one isolate, from a plastic-house crop in the area of Gioia Tauro, showed an inhibition level of 65%, which is very close to the critical value (60%) used to classify an isolate as insensitive. Most isolates from plastic-house soil were sensitive whereas all isolates from plants in plastic houses were moderately resistant. Conversely, the four isolates from plants grown in kitchen gardens were sensitive.

Discussion

The isolates of *P. capsici* from plastic-house capsicum crops tested in this study proved to be less sensitive to metalaxyl than isolates of the same species originating from other countries, tested by Coffey & Bower (1984). The ED_{50} values for inhibition of mycelial growth for isolates of *P. capsici* from Calabria were 10–190 times higher than the ED_{50} of 0.1 $\mu g\,mL^{-1}$ determined about 15 years ago for a field isolate of *P. capsici* from northern Italy (Romano & Garibaldi, 1984). The results of this study are consistent with those of other authors (Erwin & Ribeiro, 1996) who showed that considerable inter- and intraspecific variation in sensitivity to metalaxyl, determined as *in vitro* inhibition of mycelial growth, occurs naturally in field populations of soil-borne species of *Phytophthora*.

There is evidence to suggest that the level of sensitivity to metalaxyl in field populations of *P. capsici* from Calabria is

correlated with the use of this fungicide to control phytophthora blight. In fact, isolates from commercial capsicum crops grown under plastic houses were significantly less sensitive to this fungicide than isolates from kitchen gardens. In addition, isolates obtained directly from soil in plastic-house crops showed a higher sensitivity to metalaxyl than isolates obtained from infected plant tissue. The most likely explanation is that metalaxyl treatments result in a reduced selective pressure in the soil, because they affect directly only a small portion of the total population of *P. capsici*. Moreover, repeated treatments with this fungicide in the soil may induce its degradation by a rapid increase of a microbial flora capable of metabolizing it (Bailey & Coffey, 1985). However, it cannot be ruled out that strains of *P. capsici* resistant to metalaxyl are more fit; that is, they have a greater ability to infect, sporulate and colonize host tissue than the sensitive strains. In Israel, this last hypothesis has been demonstrated for populations of *P. infestans* resistant to metalaxyl (Kadish & Cohen, 1989).

Therefore, failure of metalaxyl in the control of phytophthora blight in plastic-house capsicum crops in Calabria could be due, at least in part, to the development of resistance in *P. capsici* populations. Although no isolate of *P. capsici* tested proved to be highly resistant to metalaxyl, a high proportion of isolates showed an intermediate level of resistance and the ED_{50} value for an isolate obtained from infected plant tissue was 44.6 $\mu g\,mL^{-1}$, which is very close to the maximum recommended rate of metalaxyl for field treatments. Recent reports from the USA confirm that the danger of development of resistance to metalaxyl in field populations of *P. capsici* is real and indicate that a shift in field populations of this pathogen to insensitivity has occurred in a relatively short (3 years) period (Parra & Ristaino, 1998). This should be a reason for the growers in Calabria to use metalaxyl more cautiously, and to combine chemical treatments with other means for managing phytophthora blight in commercial capsicum crops.

Résistance de *Phytophthora capsici* au métalaxyl dans les cultures de poivrons sous serre en plastique en Italie du sud

En Calabria (Italie du sud), la lutte contre la pourriture du collet et des racines du poivron causée par *Phytophthora capsici* repose principalement sur un traitement au métalaxyl, utilisé par inondation du sol. Cependant, des épidémies graves se développent tous les ans dans les cultures sous serre, dans lesquelles l'utilisation de paillage plastique et de l'irrigation par écoulement favorisent la maladie. Des isolats monohyphales de *P. capsici* collectés en Calabria de 1992 à 1998, ont été testés *in vitro* pour déterminer leur niveau de sensibilité au métalaxyl. Des isolats d'autres espèces de *Phytophthora* ont été utilisés comme témoins. La sensibilité au fongicide a été déterminée en plaçant de petits morceaux de mycélium dans des boîtes de Petri sur du milieu PDA additionné de métalaxyl à des concentrations comprises entre 0,1 et 1000 $\mu g\,mL^{-1}$. Le pourcentage d'inhibition de la croissance radiale a été déterminé lorsque les colonies du milieu non traité avec le fongicide avaient couvert environ deux tiers de la boîte. Les valeurs de la DE_{50} pour l'inhibition de la croissance du mycélium des isolats de *P. capsici* variaient entre 1,41 à 44,6 $\mu g\,mL^{-1}$ s.a. Plus de 80% des isolats de *P. capsici* provenant de cultures commerciales sous abris plastiques en Calabria ont montré un niveau de résistance modéré puisque l'inhibition était inférieure à 60% à la concentration de 5 $\mu g\,mL^{-1}$, mais supérieure à 60% à la concentration de 100 $\mu g\,mL^{-1}$.

Резистентность *Phytophthora capsici* к металаксилу на культурах перца под полиэтиленовой пленкой в южной Италии

В Калабрии (южная Италия) борьба с гнилью корневой шейки и корней однолетнего перца, вызываемыми *Phytophthora capsici*, основывалась прежде всего на обработке почвы металаксилом. Однако, серьезные вспышки происходят каждый год на тепличных культурах, где практика использования мульги из полимерной пленки и сточной ирригации способствовала заболеванию. Одногифные изоляты *P. capsici*, собранные в Калабрии в 1992-1998 гг., проверялись *in vitro* в отношении уровня их чувствительности к металаксилу. Изоляты других видов *Phytophthora* использовались в качестве контрольных. Чувствительность к фунгициду определялась путем помещения кусочков мицелия на агаре карофельной декстрозы с добавками металаксила в конечных концентрациях от 0,1 до 1000 мкг/мл д.в.. Процент блокирования роста в ширину определялся в тот момент, когда колонии на необработанной среде покрывали приблизительно две трети чашки. Значения $ЭД_{50}$ для блокирования роста мицелия изолятов *P. capsici* размещались в пределах от 1,41 до 44,6 мкг/мл д.в.. Более 80% изолятов *P. capsici* на товарных культурах под полиэтиленовой пленкой в Калабрии показали умеренный уровень устойчивости, поскольку они блокировались менее чем на 60% при 5 мкг/мл, но более чем на 60% при 100 мкг/мл.

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