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Sensitivities of *Phytophthora infestans* to Metalaxyl, Cymoxanil, and Dimethomorph

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Abstract

The isolates of *Phytophthora infestans* on tomato in the Guangxi Zhuang Autonomous Region, China, were determined for the sensitivities to metalaxyl, cymoxanil and dimethomorph to give the basic information for integrating disease management. Sensitivities were tested by measuring the radial growth on agar medium amended with fungicide, compared with the floating-leaf-disk method. 239 isolates were collected from eight tomato growing areas during 2000-2006. The testing results indicated that the frequencies of sensitive, intermediate, and resistant isolates to metalaxyl were 42.26, 35.98, and 23.53%, respectively. Variations in sensitivities amongst isolates from different areas or different years were very high for metalaxyl. All isolates from Tianlin and Wuxuan were sensitive to metalaxyl, but the metalaxyl-resistant isolates predominated in Tianyang, with the frequency of 51.35%. The EC_{50} values of certain isolates from Tianyang were higher than 500 μg mL^{-1} and their resistance levels were over 100000 folds. Cymoxanil has been used for nearly 10 years in Guangxi, and dimethomorph has been used for 5-6 years. However, there was no decrease in sensitivity of *P. infestans* populations and the sensitivities of the pathogen were nearly normally distributed. Hence, their mean EC_{50} value [cymoxanil (0.1647 ± 0.0255) μg mL^{-1}, dimethomorph (0.0970 ± 0.0052) μg mL^{-1}] could be used as the baseline sensitivities for monitoring the field resistance development. The comparison with the floating-leaf-disk method indicates that both the techniques provided equivalent results. These studies suggested that metalaxyl can be continuously applied in Tianlin, Wuxuan, and Nanning due to the resistant isolates that have not been found, while for those areas with resistant isolate, the use of metalaxyl should be reduced or alternated, and cymoxanil or dimethomorph was recommended for controlling late blight disease of tomato.

Key words: *Phytophthora infestans*, metalaxyl, cymoxanil, dimethomorph, sensitivity

INTRODUCTION

Late blight caused by *Phytophthora infestans* is one of the most important diseases on potato and tomato (Tian *et al*. 2000; Wang *et al*. 2002; Yang *et al*. 2003; Bi *et al*. 2003). Because of the lack of resistant cultivar, chemical control is still the main measure for the diseases management and the fungicides metalaxyl, cymoxanil, and dimethomorph are widely used. Metalaxyl is one of the phenylamide fungicides, highly active against oomycete fungi. The chemical had provided effective control to the late blight. But after years of use, resistance started to become a serious concern. In 1980, isolates of *P. infestans* resistant to phenylamide fungicide were detected in the potato growing regions in Ireland, the Netherlands and Switzerland. The resistant isolates could pass winter inner the potato tubers, causing severe

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epidemics. After that, reports from some countries, such as Finland, Israel, Denmark, Norway, the United States, Canada, and Mexico, had documented the resistance (Kadish and Cohen 1988; Shattock 1988; Deahl *et al*. 1993; Sujkowski *et al*. 1995; Goodwin *et al*. 1996; Kato *et al*. 1997; Hermansen *et al*. 2000; Wang *et al*. 2002; Yang *et al*. 2003; Bi *et al*. 2003). Since it could cause the declining ability of fungicide to control disease and the increasing of disease incidence and severity, followed by the increasing use of fungicide and the increasing environment pollution, resistance had become an increasingly serious concern worldwide in the recent 20 years. Fungicide resistance of many plant pathogens had been studied in many countries. In developed countries like in America and Europe, not only was the resistance development monitored for the released fungicides, but also the resistance rise of new fungicides were assayed before its commercialization and the results of the assay were used to direct the management decisions regarding spray programs (Li *et al*. 2002). Plant pathogen resistant to fungicide was studied in China in recent years, and the reports about resistance to metalaxyl occurring in *P. infestans* causing potato late blight had been documented increasingly (Wang *et al*. 2002; Yang *et al*. 2003; Bi *et al*. 2003; Ryu *et al*. 2003; Chen *et al*. 2004; Yan *et al*. 2005a, b). However, only a few reports about metalaxyl resistance occurring in *P. infestans* causing the same disease on tomato were documented (Wang *et al*. 2002; Yang *et al*. 2003; Bi *et al*. 2003; Chen *et al*. 2004; Cao *et al*. 2006).

Tomato was one of the main crops in Guangxi, China, especially in the vegetable growing areas, playing an important role in the economy. The outbreak of late blight was frequent, causing severe epidemics in the winter-spring season if rain was abundant. Metalaxyl was widely used to control this disease. But the controlling often got little metalaxyl effectiveness in the fields and the yields of tomato decreased, or even was completely lost (Zhu *et al*. 2002). It was emergent to know if the pathogen had occurred resistance to metalaxyl in these tomato growing areas. But no study about this was reported. Cymoxanil was used in Guangxi in the late 1990s and dimethomorph was used in the early 2000s. To avoid or delay the appearance of resistance, sensitivities to both the fungicides should be tested and the baseline sensitivities for monitoring the field resistance development should be built.

In this article, isolates of *Phytophthora infestans* were collected from the tomato growing areas in Guangxi and their sensitivities to metalaxyl, cymoxanil, and dimethomorph were tested. The geographical distribution of resistance and the resistant levels were analyzed. This could give the basic information for integrating disease management and avoid or delay the appearance of resistance.

MATERIALS AND METHODS

Samples collection and isolation

Tomato leaves, stems, and fruits naturally infected by *P. infestans* were collected from 8 tomato production areas in Guangxi during the winter and spring of 2000-2006, with one sample kept in one plastic bag. Isolation was made according to the methods described by Tian *et al*. (2000) and Zhu *et al*. (2003). After the samples were kept in a moist condition for 1-3 days, mildew on the lesion was picked off using a sterile needle, and then put on rye agar medium amended with rifampicin 20 µg mL^{-1}, ampicillin 200 µg mL^{-1}, and nystatin 100 µg mL^{-1}, and incubated at 18°C in darkness for 3-7 days. After 1 or 2 transfers of hyphal tips on media containing antibiotics, 239 pure isolates were obtained. Amongst them, 11 isolates from Tianyang County and 10 isolates from Wuming County were collected in 2000, 13 isolates from Tianyang County were collected in 2003, and the other 205 isolates were collected from 2005 to 2006. Pure isolates were incubated on rye agar medium at 18°C in darkness for 8-10 days and then were determined for the sensitivities to fungicides. They were also maintained on autoclaced rye grain medium, which was prepared according to the method described by Wang (2003).

Fungicides and preparation of agar medium amended with fungicides

Metalaxyl was provided by Jiangshu Baoling Chemical Corporation, China, as the 95.4% technical grade, cymoxanil was provided by Dupont de Nemours and Company (Shanghai, China) as the 95% technical grade, and dimethomorph was obtained from a fungicide market and its commercial formulation was 50% (BASF Co.). Metalaxyl and cymoxanil were first dissolved in acetone, and then were made into a set of stock solution with sterile distilled water. Each stock solution, including the control (zero fungicide), contained the same amount of acetone. Dimethomorph was made into a set of stock solutions with sterile distilled water. Test medium was constructed by adding 1 mL of stock solution to 100 mL of molten, cooled rye agar medium (50°C) and then dispensing the mingled solution into the petri plates (9 cm diameter).

Preparation of tomato plant

Tomato susceptible *cv*. Ts19 was grown in the greenhouse and plants with 12-15 leaves were used.

Resistance test

Fungicide sensitivities were tested by measuring radial growth on agar medium amended with fungicides (*in vitro*), compared with the floating-leaf-disk method (*in vivo*).

In *in vitro* assessment, fungicide sensitivity was determined by comparing the radial growth of each isolate on rye agar containing fungicide with the growth of the same isolate on medium without the fungicide. Before the formal test was taken, preparatory test was done. Mycelial plugs (5 mm diameter) of each isolate were transferred to rye agar medium plates amended with fungicides in a concentration of 0.1, 1.0, 10, or 100 μg mL^{-1} and incubated at 18°C for 7 days. According to the growth of each isolate in the preparatory test, a set of different concentrations of fungicides for formal test was set. There were three groups concentrations for metalaxyl: The final concentrations of metalaxyl in the media were 0, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, and 1.0 μg mL^{-1} for the very sensitive isolates, while those for intermediate and resistant isolates were 0, 0.01, 0.1, 1.0, 10, 100, 500, 1 000 μg mL^{-1} and 0, 50, 100, 200, 400, 800, 1 200, 1 600 μg mL^{-1}, respectively. For cymoxanil, the final concentrations in the media were 0, 0.005, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, and 10 μg mL^{-1}, and for dimethomorph were 0, 0.01, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.2 μg mL^{-1}. In the formal test, mycelial plugs (5 mm diameter) were cut from the margin of actively growing colonies of each isolate and placed with the mycelium in contact with the test medium in the middle of the plates. The plates were incubated at 18°C in darkness for 8-15 days with three replicates per concentration for each isolate tested. Colony diameters were measured in two perpendicular directions on all plates when the control (colony growing in the absence of fungicide) had a diameter of at least 50 mm. The measurements were averaged and corrected for the size of the agar plug and regression equation, coefficient (r) and EC_{50} values were calculated. According to the method described by Wang *et al*. (2002), each isolate was classified as resistant (EC_{50} value > 10 μg mL^{-1}), intermediate [0.01 μg mL^{-1} ≤ EC_{50} value ≤ 10 μg mL^{-1} (metalaxyl), 0.1 μg mL^{-1} ≤ EC_{50} value ≤ 10 μg mL^{-1} (cymoxanil, dimethomorph)] or sensitive [EC_{50} value < 0.01 (metalaxyl), EC_{50} value < 0.1 μg mL^{-1} (cymoxanil, dimethomorph)].

In *in vivo* assessment (floating leaf-disk method), 60 isolates with typical different sensitivity to metalaxyl, cymoxanil and dimethomorph *in vitro* were selected for sensitivity test using the floating leaf-disk method. The inoculative suspension was prepared according to the method described by Yang *et al*. (2003): Cultures of *P. infestans* were grown on rye agar plates at 18°C in darkness for 7-12 days. Sporangia were harvested from the plates by washing with sterile water and rubbing the colony surface. The concentration of the sporangial suspension was adjusted to 4×10^4 sporangia per milliliter with a hemacytometer, and then the suspension was incubated at 4°C for 2.5 h for zoospore release. Leaf disks (15 mm diameter) were cut with a cork borer from fully expanded leaflets of *cv*. TS19 grown in the greenhouse, put in 0.5% sodium hypochlorite solution for 1 min and then washed 3 times with sterile distilled water. Disks with dry surface were floated abaxial side up on solutions of 0.01, 0.1, 1.0, 10.0, 100.0, and 1 000.0 μg mL^{-1} of metalaxyl, cymoxanil or dimethomorph (15 mL), 5 disks per petri dish (60 mm diameter). Disks floated on distilled water without fungicides were served as controls. After floating for 2 h, each disk was inoculated with 20 μL of a suspension containing 4×10^4 sporangia per milliliter applied with a micropipette. The closed plates were kept at 18°C with 16 h light per day. After 5-6 days of incubation, the leaf disks were observed using a stereo microscope to estimate growth and sporulation. The area of each leaf disk producing sporulating mycelium was estimated.

The isolates were rated as resistant if they sporulated on leaf disks in the fungicide concentrations of 100 μg mL^{-1}. Those sporulating in the fungicide concentrations of 1 or 10 μg mL^{-1} were rated intermediate, and those sporulating only in water or 0.1 μg mL^{-1} were rated sensitive (Hermansen *et al*. 2000).

RESULTS

Sensitivity to fungicides *in vitro*

Response to metalaxyl 239 isolates were tested during 2000 to 2006. The testing results (Tables 1 and 2) indicated that the frequencies of metalaxyl sensitive (MS), metalaxyl intermediate (MI), and metalaxyl resistant (MR) isolates were 42.26% (101/239), 35.98% (86/239), and 23.53% (52/239), respectively. Differences in the frequency of sensitivity were evident between isolates from different areas. All isolates from Tianlin and Wuxuan were sensitive to metalaxyl. In these areas, tomato was widely planted only in recent years and metalaxyl was seldom used. The vast majority of isolates (65.71%) were sensitive to metalaxyl in Nanning, where tomato has been widely planted for decades, but there were very few late blight and very few metalaxyl were used. However, in Tianyang, the most important tomato production area in Guangxi, tomato has been widely planted for decades, late blight occurred very frequently and metalaxyl was used intensively, metalaxyl resistant isolates were predominant, with the frequency of 51.35%. The EC_{50} values of some isolates from this area were higher than 500 μg mL^{-1} and their resistance levels were over 100000 folds. The vast majority of isolates (87.88%) were metalaxyl intermediate in Wuming, where tomato was widely planted a few years later than in Tianyang, but late blight occurred very frequently during the tomato growing season and metalaxyl was used intensively.

Response to cymoxanil The testing results (Tables 3 and 4) indicated that the frequencies of cymoxanil sensitive (CS) and cymoxanil intermediate (CI) were 37.24% (89/239) and 62.76% (150/239). No cymoxanil resistant isolate was found. There was no evident difference in the frequency of sensitivity between isolates from different areas. From the cymoxanil sensitivity distribution (Fig.1), it was observed that the sensitivities of *P. infestans* were unimodal, nearly normally distributed, and there was no decrease in sensitivity of the pathogen populations had been detected. 239 isolates had EC_{50} values in the range of 0.0429 to 2.9818 μg mL^{-1}. The vast majority of isolates

Table 1 Metalaxyl sensitivity *in vitro* amongst isolates of *Phytophthora infestans* from different areas in Guangxi during 2000-2006

Area	No. of isolate	No. of MS	Frequency of MS (%)	No. of MI	Frequency of MI (%)	No. of MR	Frequency of MR (%)
Tianyang	74	27	36.49	9	12.16	38	51.35
Tiandong	27	6	22.22	18	66.67	3	11.11
Tianlin	28	28	100.00	0	0.00	0	0.00
Nanning	35	23	65.71	12	34.29	0	0.00
Wuming	33	2	6.06	29	87.88	2	6.06
Liuzhou	19	4	21.05	10	52.63	5	26.32
Wuxuan	6	6	100.00	0	0.00	0	0.00
Guilin	17	5	29.41	8	47.06	4	23.53
Total	239	101	42.26	86	35.98	52	21.76

MS, metalaxyl sensitive; MI, metalaxyl intermediate; MR, metalaxyl resistant. The same as in Table 2.

Table 2 Metalaxyl sensitivity level of some isolates of *Phytophthora infestans*

Isolate	Area	Regression equation	Coefficient (*r*)	EC_{50} value (μg mL^{-1})	Sensitivity type
TZ04	Tianyang	$Y = -2.9122 + 3.0505x$	0.9937	392.4502	MR
TZ25	Tianyang	$Y = -3.7256 + 3.1911x$	0.9942	542.3859	MR
TZ42	Tianyang	$Y = -1.1070 + 2.5174x$	0.9925	266.6521	MR
TZ47	Tianyang	$Y = -1.5213 + 2.6406x$	0.9944	294.8435	MR
BY03	Tianyang	$Y = -2.7644 + 2.8844x$	0.9915	491.8805	MR
ET17	Tianyang	$Y = -6.1929 + 4.0702x$	0.9903	562.3155	MR
TD04	Tiandong	$Y = -1.3683 + 2.4370x$	0.9978	410.3709	MR
NN18	Nanning	$Y = 7.4100 + 1.2352x$	0.9937	0.0112	MI
SQ18	Wuming	$Y = 5.1060 + 0.3775x$	0.9965	0.5239	MI
HM16	Wuming	$Y = 5.4723 + 0.7369x$	0.9930	0.2286	MI
TP02	Wuming	$Y = 4.8808 + 0.6097x$	0.9832	13.7055	MR
LZ11	Liuzhou	$Y = 6.1830 + 0.5255x$	0.9861	0.0056	MS

(238/239) had EC_{50} values ≤ 1.0000 μg mL^{-1}, and only one isolate (TD04) from Tiandong had an EC_{50} value (2.9818 μg mL^{-1}) > 1.0000 μg mL^{-1}. Hence, the mean EC_{50} value [(0.1647 ± 0.0255) μg mL^{-1}] could be used as the baseline sensitivities for monitoring the field resistance development of *P. infestans*.

Response to dimethomorph The testing results (Tables 5 and 6) indicated that the frequencies of dimethomorph sensitive (DS) and dimethomorph intermediate (DI) were 54.81% (131/239) and 45.19% (108/239). No dimethomorph resistant isolate was found. There was no evident difference in the frequency of sensitivity between isolates from different areas. From the dimethomorph sensitivity distribution (Fig.2), the sensitivities of *P. infestans* were unimodal, nearly normally distributed, no decrease in sensitivity of the pathogen populations had been detected. 239 isolates had EC_{50} values in the range of 0.0310 to 0.2020 μg mL^{-1}. The highest EC_{50} value was 6.5 folds of the lowest. Hence, the mean EC_{50} value [(0.0970 ± 0.0052) μg mL^{-1}] could be used as the baseline sensitivities for monitoring the field resistance development of *P. infestans*.

Fungicide sensitivity variation in different years
The fungicide sensitivities between isolates collected in 2000-2006 from Tianyang and Wuming were compared. With the years passing, the metalaxyl sensitivities decreased; both the frequencies of metalaxyl resistant (MR) isolates and the mean EC_{50} values of the pathogen populations increased. However, there was no evident difference in the sensitivities to cymoxanil and dimethomorph (Tables 7-9).

Table 3 Cymoxanil sensitivity *in vitro* amongst isolates of *Phytophthora infestans* from different areas in Guangxi during 2000-2006

Area	No. of isolate	No. of CS	Frequency of CS (%)	No. of CI	Frequency of CI (%)
Tianyang	74	28	37.84	46	62.16
Tiandong	27	9	33.33	18	66.67
Tianlin	28	12	42.86	16	57.14
Nanning	35	13	37.14	22	62.86
Wuming	33	12	36.36	21	63.64
Liuzhou	19	7	36.84	12	63.16
Wuxuan	6	2	33.33	3	66.67
Guilin	17	6	35.29	11	64.71
Total	239	89	37.24	150	62.76

CS, Cymoxanil sensitive; CI, cymoxanil intermediate. The same as in Table 4.

Table 4 Cymoxanil sensitivity level of some isolates of *Phytophthora infestans*

Isolate	Area	Regression equation	Coefficient (*r*)	EC_{50} value (μg mL^{-1})	Sensitivity type
TZ25	Tianyang	$Y = 6.0158 + 1.2121x$	0.9841	0.1452	CI
TZ42	Tianyang	$Y = 5.4441 + 1.3284x$	0.9965	0.4631	CI
TZ47	Tianyang	$Y = 5.6605 + 0.6284x$	0.9926	0.0889	CS
BY03	Tianyang	$Y = 6.0295 + 1.0197x$	0.9913	0.0978	CS
TL09	Tianlin	$Y = 5.9796 + 0.9282x$	0.9955	0.0880	CS
TD04	Tiandong	$Y = 4.5107 + 1.0309x$	0.9857	2.9818	CI
SQ18	Wuming	$Y = 6.3084 + 1.8060x$	0.9942	0.1886	CI
SQ15	Wuming	$Y = 5.9531 + 2.1651x$	0.9924	0.3629	CI

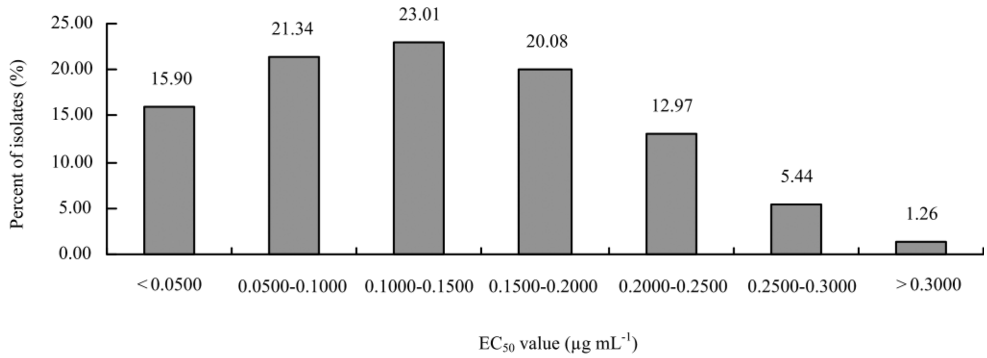

Fig. 1 Cymoxanil sensitivity distribution of the isolates of *Phytophthora infestans*.

Sensitivity to fungicides *in vivo*

The testing results *in vivo* (Tables 10-12) showed, amongst 60 isolates selected for sensitivity test using the floating leaf-disk method, 56 isolates had the same sensitivities as *in vitro*. The isolates which were resistant *in vitro* could sporulate on leaf disks in the fungicide concentrations of 100 µg mL^{-1}, while the isolates which were intermediate *in vitro* could sporulate in the fungicide concentrations of 1 or 10 µg mL^{-1}, and the isolates which were sensitive *in vitro* could sporulate only in water or 0.1 µg mL^{-1}. For example, TD04, which had the EC_{50} values *in vitro* for metalaxyl, cymoxanil, and dimethomorph of 410.3709, 2.9818, and 0.1136 µg mL^{-1}, respectively, could produce a considerable number of sporulations even in the metalaxyl concentrations of 1 000 µg mL^{-1}. It could also produce an intermediate number of sporulations in the cymoxanil concentrations of 1.0 µg mL^{-1} and a few sporulations in the dimethomorph concentrations of 1.0 µg mL^{-1}, but could not make any lesions at the leaf disks floating in the cymoxanil or dimethomorph concentrations of 10 µg mL^{-1}. However, there were 4 isolates (ET05, SQ19, TT02, and TZ42), which had different responses *in vivo*. For example, TZ42, which had the EC_{50} values *in vitro* for metalaxyl, cymoxanil and dimethomorph of 266.6521, 0.4631, and 0.1456 µg mL^{-1}, respectively, could not sporulate on leaf disks

Table 5 Dimethomorph sensitivity *in vitro* amongst isolates of *Phytophthora infestans* from different areas in Guangxi during 2000-2006

Area	No. of isolate	No. of DS	Frequency of DS (%)	No. of DI	Frequency of DI (%)
Tianyang	74	39	52.70	35	47.30
Tiandong	27	15	55.56	12	44.44
Tianlin	28	16	57.14	12	42.86
Nanning	35	20	57.14	15	42.86
Wuming	33	17	51.52	16	48.48
Liuzhou	19	10	52.63	9	47.37
Wuxuan	6	4	66.67	2	33.33
Guilin	17	10	58.82	7	41.18
Total	239	131	54.81	108	45.19

DS, dimethomorph sensitive; DI, dimethomorph intermediate. The same as in Table 6.

Table 6 Dimethomorph sensitivity level of some isolates of *Phytophthora infestans*

Isolate	Area	Regression equation	Coefficient (*r*)	EC_{50} value (µg mL^{-1})	Sensitivity type
TZ25	Tianyang	$Y = 7.0989 + 1.9064x$	0.9976	0.0792	DS
TZ42	Tianyang	$Y = 8.2926 + 3.9338x$	0.9902	0.1456	DI
BY03	Tianyang	$Y = 7.9052 + 3.3013x$	0.9912	0.1318	DI
TL09	Tianlin	$Y = 8.3837 + 3.3001x$	0.9892	0.0943	DS
TD04	Tiandong	$Y = 6.4573 + 1.5430x$	0.9927	0.1136	DI
NN18	Nanning	$Y = 7.2906 + 1.5180x$	0.9885	0.0310	DS
SQ18	Wuming	$Y = 7.0427 + 2.2684x$	0.9922	0.1258	DI
SQ15	Wuming	$Y = 6.7695 + 2.5474x$	0.9912	0.2020	DI
GL07	Guilin	$Y = 9.0479 + 3.1574x$	0.9811	0.0522	DS

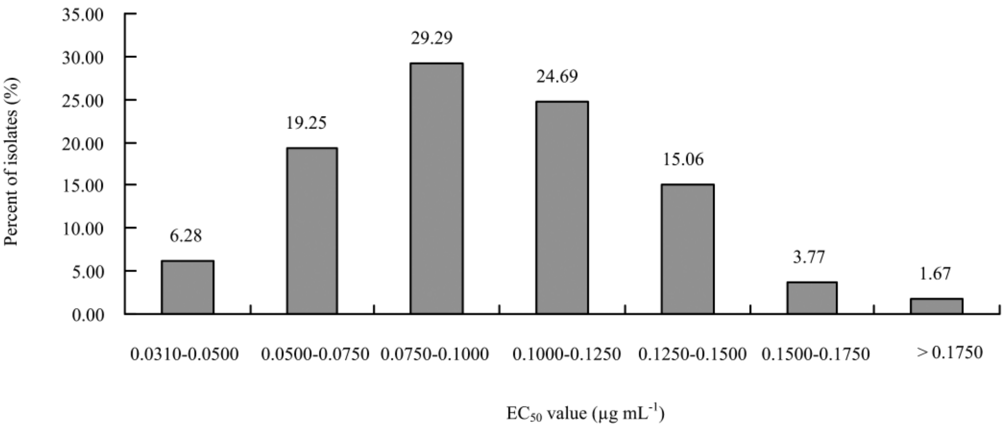

Fig. 2 Dimethomorph sensitivity distribution of isolates of *Phytophthora infestans*.

in the metalaxyl concentrations of 100 μg mL^{-1}, the cymoxanil or dimethomorph concentrations of 1.0 μg mL^{-1}. It could only make a small brown lesion of hypersensitive necrotic reaction at the inoculating site.

Cross-resistance

The sensitivities of 239 isolates to metalaxyl, cymoxanil and dimethomorph were compared. Cymoxanil and dimethomorph gave equal effect on MS, MI, and MR isolates and the CS isolates were not necessarily sensitive to dimethomorph. This indicated that there was no cross-resistance amongst metalaxyl, cymoxanil and dimethomorph in field populations of *P. infestans*.

DISCUSSION

Fungicides have to be sprayed more frequently, concomitant with the increasing concentration after fungicide resistance has occurred in plant pathogen, adding disease controlling costs, and causing environment hazards. To avoid or delay the appearance of resistance, sensitivities to fungicides in different areas should be monitored and taken into account prior to making late blight management decisions. The isolates of *P. infestans* collected from eight tomato grown areas in Guangxi were determined for the sensitivities to metalaxyl, cymoxanil, and dimethomorph in this study. The result indicated differences in the frequency and levels of metalaxyl sensitivity were evident amongst isolates from different areas, depending on the tomato planted years, the frequency of late blight outbreak, and the application of fungicide. In Tianyang, the most important tomato growing area in Guangxi, tomato had been planted for decays and the outbreak of late blight was frequent and severe. Late blight could be found even in dry years. In these fields, metalaxyl had been sprayed regularly, about 10 times or more during a tomato growing season. As a result, the resistance to metalaxyl is very common. The fungicide was sprayed

Table 7 Metalaxyl sensitivity *in vitro* amongst the isolates of *Phytophthora infestans* collected in different years from the same area

Area	Year	No. of strain	Frequency of sensitive strain (%)	Frequency of intermediate strain (%)	Frequency of resistant strain (%)	EC_{50} value (μg mL^{-1})	
						Range	Mean
Tianyang	2000	11	54.55	27.27	18.18	0.0007-357.8102	41.3081
Tianyang	2003	13	38.46	30.77	30.77	0.0052-392.4502	68.8422
Tianyang	2005	23	39.13	4.35	56.52	0.0036-542.3859	93.4088
Tianyang	2006	27	25.93	3.70	70.37	0.0078-562.3155	121.5131
Wuming	2000	10	20.00	80.00	0.00	0.0027-0.2286	0.0652
Wuming	2005	10	0.00	100.00	0.00	0.0360-0.7729	0.2571
Wuming	2006	13	0.00	84.62	15.38	0.0536-14.1758	2.8793

Table 8 Cymoxanil sensitivity *in vitro* amongst the isolates of *Phytophthora infestans* collected in different years from the same area

Area	Year	No. of strain	Frequency of sensitive strain (%)	Frequency of intermediate strain (%)	EC_{50} value (μg mL^{-1})	
					Range	Mean
Tianyang	2000	11	36.36	63.64	0.0429-0.5671	0.1825
Tianyang	2003	13	38.46	61.54	0.0543-0.4563	0.2142
Tianyang	2005	23	34.78	65.22	0.0697-0.6042	0.1907
Tianyang	2006	27	40.74	59.26	0.0510-0.4631	0.1738
Wuming	2000	10	40.00	60.00	0.0660-0.4132	0.1965
Wuming	2005	10	30.00	70.00	0.0771-0.4527	0.1792
Wuming	2006	13	38.46	61.54	0.0880-0.3629	0.1814

Table 9 Dimethomorph sensitivity *in vitro* amongst the isolates of *Phytophthora infestans* collected in different years from the same area

Area	Year	No. of strain	Frequency of sensitive strain (%)	Frequency of intermediate strain (%)	EC_{50} value (μg mL^{-1})	
					Range	Mean
Tianyang	2000	11	45.45	54.55	0.0418-0.1421	0.1189
Tianyang	2003	13	53.85	46.15	0.0516-0.1256	0.1142
Tianyang	2005	23	56.52	43.48	0.0479-0.1318	0.1201
Tianyang	2006	27	51.85	48.15	0.0318-0.1456	0.1135
Wuming	2000	10	50.00	50.00	0.0447-0.1423	0.1195
Wuming	2005	10	60.00	40.00	0.0602-0.1629	0.1165
Wuming	2006	13	46.15	53.85	0.0523-0.2020	0.1214

Table 10 Metalaxyl sensitivity *in vivo* amongst 60 isolates of *Phytophthora infestans*

Isolate	Area	Response *in vitro*	Sporulation at different concentration *in vivo* (μg mL^{-1})						
			CK	0.01	0.1	1.0	10.0	100.0	1 000.0
TL01	Tianlin	MS	++	-	-	-	-	-	-
TL09	Tianlin	MS	+++	+	-	-	-	-	-
TL14	Tianlin	MS	++	-	-	-	-	-	-
TZ06	Tianyang	MS	+++	+	+	-	-	-	-
TZ11	Tianyang	MS	++	-	-	-	-	-	-
TT26	Tianyang	MS	+++	+	+	-	-	-	-
BY01	Tianyang	MS	+++	+	+	-	-	-	-
ET07	Tianyang	MS	+++	+	-	-	-	-	-
NM10	Tianyang	MS	+++	+	+	-	-	-	-
TD01	Tiandong	MS	+++	+	-	-	-	-	-
TD07	Tiandong	MS	+++	+	-	-	-	-	-
HM02	Wuming	MS	+++	+	-	-	-	-	-
SQ09	Wuming	MS	+++	+	+	-	-	-	-
NN05	Nanning	MS	+++	+	-	-	-	-	-
NN25	Nanning	MS	+++	+	-	-	-	-	-
LZ02	Liuzhou	MS	+++	+	+	-	-	-	-
LZ11	Liuzhou	MS	+++	+	+	-	-	-	-
GL06	Guilin	MS	+++	+	+	-	-	-	-
GL17	Guilin	MS	+++	+	-	-	-	-	-
WX05	Wuxuan	MS	+++	+	-	-	-	-	-
ET05	Tianyang	MI	++	+	+	-	-	-	-
TZ13	Tianyang	MI	+++	+++	++	++	+	-	-
TZ22	Tianyang	MI	+++	+++	++	++	+	-	-
TZ26	Tianyang	MI	+++	+++	+++	++	+	-	-
TD05	Tiandong	MI	+++	++	+	+	-	-	-
TD08	Tiandong	MI	+++	+++	++	+	+	-	-
LF02	Tiandong	MI	+++	+++	++	+	-	-	-
LF09	Tiandong	MI	+++	+++	++	++	+	-	-
HM07	Wuming	MI	+++	++	+	+	-	-	-
SQ03	Wuming	MI	+++	+++	+++	++	+	-	-
SQ14	Wuming	MI	+++	+++	+++	++	+	-	-
SQ18	Wuming	MI	+++	+++	++	+	+	-	-
SQ19	Wuming	MI	++	+	+	-	-	-	-
TP05	Wuming	MI	+++	+++	+++	++	+	-	-
NN04	Nanning	MI	+++	++	+	+	-	-	-
NN18	Nanning	MI	+++	++	+	+	-	-	-
LZ03	Liuzhou	MI	+++	+++	++	+	+	-	-
LZ19	Liuzhou	MI	+++	+++	++	+	+	-	-
GL01	Guilin	MI	+++	++	+	+	+	-	-
GL05	Guilin	MI	+++	+	+	+	-	-	-
BY03	Tianyang	MR	+++	+++	+++	+++	+++	+++	++
ET03	Tianyang	MR	+++	+++	+++	+++	+++	++	+
ET04	Tianyang	MR	+++	+++	+++	+++	+++	++	+
ET17	Tianyang	MR	+++	+++	+++	+++	+++	+++	++
NM02	Tianyang	MR	+++	+++	+++	+++	+++	++	+
NM06	Tianyang	MR	+++	+++	+++	+++	+++	++	+
TT02	Tianyang	MR	+++	+++	++	+	+	-	-
TT27	Tianyang	MR	+++	+++	+++	+++	++	+	+
TZ04	Tianyang	MR	+++	+++	+++	+++	+++	+++	++
TZ25	Tianyang	MR	+++	+++	+++	+++	+++	+++	++
TZ42	Tianyang	MR	+++	++	+	+	+	-	-
TZ47	Tianyang	MR	+++	+++	+++	+++	+++	++	+
TD04	Tiandong	MR	+++	+++	+++	+++	+++	+++	++
TD14	Tiandong	MR	+++	+++	+++	+++	++	++	+
LF06	Tiandong	MR	+++	+++	+++	++	++	+	-
SQ25	Wuming	MR	+++	+++	+++	++	++	+	-
TP02	Wuming	MR	+++	+++	+++	++	+	+	-
LZ10	Liuzhou	MR	+++	+++	+++	+++	++	+	+
LZ12	Liuzhou	MR	+++	+++	+++	++	++	+	-
GL03	Guilin	MR	+++	+++	+++	++	+	+	-

+++, the leaf-disk surfaces covered with a large number of sporulations; ++, the leaf-disk surfaces covered with an intermediate number of sporulations; +, the leaf-disk surfaces covered with a few sporulations; -, no sporulation on the leaf-disk surfaces. The same as below.

but the controlling often got little metalaxyl effectiveness in the fields and the yields of tomato decreased, or even was completely lost. However, in Tianlin and Wuxuan, where only in recently years was tomato planted in winter-spring, the optimum season for the occurring of late blight, this disease occurs sometimes, but fungicide was used very seldom and all isolates tested were metalaxyl sensitive.

The risk of fungicide resistance can be high, intermediate, or low, depending on both the fungicide and the pathogen. Although cymoxanil has been used nearly for 10 years in Guangxi, and dimethomorph has been used for 5-6 years, no resistant isolate was detected. This indicated that the risks of cymoxanil and dimethomorph resistance are relatively low. The results were similar to those of Wang *et al*. (2002) and Yan *et al*. (2005b). However, one isolate (TD04) was detected with the EC_{50} value of 2.9818 μg mL^{-1} to cymoxanil and could sporulate on leaf disks in the cymoxanil concentration of 1.0 μg mL^{-1}.

Variation in response to metalaxyl by fungi growing saprophytically or within plant tissues, as well as variation in host cultivar metabolism and disease resistance properties may account for some of the differences between *in vitro* and *in vivo* testing for metalaxyl resistance (Deahl *et al*. 1993). To reduce these differences, TS19, a cultivar containing no resistance genes to *P. infestans*, was used for *in vivo* testing in this study. The leaf disks were pretreated with sodium hypochlorite and sterile distilled water, and the testing materials, such as petri dish, distilled water, etc., were sterile. Amongst 60 isolates tested *in vivo*, 56 isolates had the sensitivities correlated with those *in vitro*, showing similar results to those of Matuszak *et al*. (1994). Matuszak had compared *in vitro* and *in vivo* testing for 20 isolates and the relationship between the methods was r^2 for 0.94 at 5 μg mL^{-1} of metalaxyl, r^2 for 0.98 at 100 μg mL^{-1} of metalaxyl.

CONCLUSION

The studies suggested that metalaxyl can be continuously applied in Tianlin, Wuxuan, and Nanning, due to the resistant isolates not being found in these areas. But in the other areas, the metalaxyl resistance has developed. Especially in Tianyang, the tomato main growing area,

Table 11 Cymoxanil sensitivity *in vivo* amongst 60 isolates of *Phytophthora infestans*

Isolate	Area	Response *in vitro*	Sporulation at different concentration *in vivo* (μg mL⁻¹)					
			CK	0.01	0.1	1.0	10.0	100.0
TL01	Tianlin	CS	++	+	+	-	-	-
TL09	Tianlin	CS	+++	+++	++	-	-	-
TT26	Tianyang	CS	+++	+++	++	-	-	-
TT27	Tianyang	CS	+++	+++	++	-	-	-
TZ26	Tianyang	CS	+++	++	+	-	-	-
TZ47	Tianyang	CS	+++	+++	++	-	-	-
BY03	Tianyang	CS	+++	+++	++	-	-	-
ET17	Tianyang	CS	+++	+++	++	-	-	-
LF06	Tiandong	CS	+++	+++	++	-	-	-
TD05	Tiandong	CS	+++	++	+	-	-	-
TD07	Tiandong	CS	+++	+++	++	-	-	-
SQ03	Wuming	CS	+++	++	+	-	-	-
SQ19	Wuming	CS	++	+	-	-	-	-
TP02	Wuming	CS	+++	+++	++	-	-	-
NN18	Nanning	CS	+++	++	+	-	-	-
NN25	Nanning	CS	+++	+++	++	-	-	-
LZ03	Liuzhou	CS	+++	++	+	-	-	-
LZ11	Liuzhou	CS	+++	++	+	-	-	-
LZ12	Liuzhou	CS	+++	+++	++	-	-	-
GL03	Guilin	CS	+++	+++	++	-	-	-
GL05	Guilin	CS	+++	+++	++	-	-	-
TL14	Tianlin	CI	++	++	++	+	-	-
BY01	Tianyang	CI	+++	+++	++	+	-	-
ET03	Tianyang	CI	+++	+++	++	+	-	-
ET04	Tianyang	CI	+++	+++	++	+	-	-
ET05	Tianyang	CI	++	++	+	-	-	-
ET07	Tianyang	CI	+++	+++	++	+	-	-
NM02	Tianyang	CI	+++	+++	++	+	-	-
NM06	Tianyang	CI	+++	+++	++	+	-	-
NM10	Tianyang	CI	+++	+++	++	+	-	-
TT02	Tianyang	CI	+++	+	+	-	-	-
TZ04	Tianyang	CI	+++	+++	++	+	-	-
TZ06	Tianyang	CI	+++	+++	++	+	-	-
TZ11	Tianyang	CI	++	++	++	+	-	-
TZ13	Tianyang	CI	+++	+++	++	+	-	-
TZ22	Tianyang	CI	+++	+++	++	+	-	-
TZ25	Tianyang	CI	+++	+++	++	+	-	-
TZ42	Tianyang	CI	+++	++	+	-	-	-
LF02	Tiandong	CI	+++	+++	++	+	-	-
LF09	Tiandong	CI	+++	+++	++	+	-	-
TD01	Tiandong	CI	+++	++	++	+	-	-
TD04	Tiandong	CI	+++	+++	+++	++	-	-
TD08	Tiandong	CI	+++	+++	++	+	-	-
TD14	Tiandong	CI	+++	+++	++	+	-	-
HM02	Wuming	CI	+++	+++	++	+	-	-
HM07	Wuming	CI	+++	+++	++	+	-	-
SQ09	Wuming	CI	+++	+++	++	+	-	-
SQ14	Wuming	CI	+++	+++	++	+	-	-
SQ18	Wuming	CI	+++	+++	++	+	-	-
SQ25	Wuming	CI	+++	+++	++	+	-	-
TP05	Wuming	CI	+++	+++	++	+	-	-
NN04	Nanning	CI	+++	+++	++	+	-	-
NN05	Nanning	CI	+++	+++	++	+	-	-
LZ02	Liuzhou	CI	+++	+++	++	+	-	-
LZ10	Liuzhou	CI	+++	+++	++	+	-	-
LZ19	Liuzhou	CI	+++	+++	++	+	-	-
GL01	Guilin	CI	+++	+++	++	+	-	-
GL06	Guilin	CI	+++	+++	++	+	-	-
GL17	Guilin	CI	+++	+++	++	+	-	-
WX05	Wuxuan	CI	+++	++	++	+	-	-

Table 12 Dimethomorph sensitivity *in vivo* amongst 60 isolates of *Phytophthora infestans*

Isolate	Area	Response *in vitro*	Sporulation at different concentration *in vivo* (μg mL⁻¹)					
			CK	0.01	0.1	1.0	10.0	100.0
TL01	Tianlin	DS	++	++	+	-	-	-
TL09	Tianlin	DS	+++	+++	+++	-	-	-
TL14	Tianlin	DS	++	++	+	-	-	-
ET03	Tianyang	DS	+++	+++	+++	-	-	-
ET04	Tianyang	DS	+++	+++	+++	-	-	-
ET05	Tianyang	DS	++	++	+	-	-	-
NM02	Tianyang	DS	+++	+++	+++	-	-	-
NM10	Tianyang	DS	+++	+++	+	-	-	-
TT02	Tianyang	DS	+++	+++	++	-	-	-
TT26	Tianyang	DS	+++	+++	+	-	-	-
TZ04	Tianyang	DS	+++	+++	+++	-	-	-
TZ11	Tianyang	DS	++	++	+	-	-	-
TZ22	Tianyang	DS	+++	+++	++	-	-	-
TZ25	Tianyang	DS	+++	+++	++	-	-	-
TZ26	Tianyang	DS	+++	+++	+++	-	-	-
LF02	Tiandong	DS	+++	+++	++	-	-	-
TD08	Tiandong	DS	+++	+++	++	-	-	-
TD14	Tiandong	DS	+++	+++	+++	-	-	-
SQ03	Wuming	DS	+++	+++	+++	-	-	-
SQ09	Wuming	DS	+++	+++	+	-	-	-
SQ19	Wuming	DS	++	++	+	-	-	-
SQ25	Wuming	DS	+++	+++	+++	-	-	-
NN04	Nanning	DS	+++	+++	+	-	-	-
NN05	Nanning	DS	+++	+++	+	-	-	-
NN18	Nanning	DS	+++	+++	+	-	-	-
LZ10	Liuzhou	DS	+++	+++	+	-	-	-
LZ11	Liuzhou	DS	+++	+++	+	-	-	-
LZ12	Liuzhou	DS	+++	+++	+++	-	-	-
GL01	Guilin	DS	+++	+++	+	-	-	-
GL03	Guilin	DS	+++	+++	+++	-	-	-
WX05	Wuxuan	DS	+++	+++	+	-	-	-
BY01	Tianyang	DI	+++	+++	++	+	-	-
BY03	Tianyang	DI	+++	+++	+++	+	-	-
ET07	Tianyang	DI	+++	+++	+++	+	-	-
ET17	Tianyang	DI	+++	+++	+++	+	-	-
NM06	Tianyang	DI	+++	+++	+++	+	-	-
TT27	Tianyang	DI	+++	+++	+++	+	-	-
TZ06	Tianyang	DI	+++	+++	+++	+	-	-
TZ13	Tianyang	DI	+++	+++	++	+	-	-
TZ42	Tianyang	DI	+++	++	+	-	-	-
TZ47	Tianyang	DI	+++	+++	+++	+	-	-
LF06	Tiandong	DI	+++	+++	++	+	-	-
LF09	Tiandong	DI	+++	+++	+++	+	-	-
TD01	Tiandong	DI	+++	+++	+++	+	-	-
TD04	Tiandong	DI	+++	+++	+++	+	-	-
TD05	Tiandong	DI	+++	+++	+++	+	-	-
TD07	Tiandong	DI	+++	+++	++	+	-	-
HM07	Wuming	DI	+++	+++	+++	+	-	-
HM02	Wuming	DI	+++	+++	++	+	-	-
SQ14	Wuming	DI	+++	+++	+++	+	-	-
SQ18	Wuming	DI	+++	+++	+++	+	-	-
TP05	Wuming	DI	+++	+++	+++	+	-	-
TP02	Wuming	DI	+++	+++	+++	+	-	-
NN25	Nanning	DI	+++	+++	+++	+	-	-
LZ02	Liuzhou	DI	+++	+++	+++	+	-	-
LZ03	Liuzhou	DI	+++	+++	++	+	-	-
LZ19	Liuzhou	DI	+++	+++	+++	+	-	-
GL05	Guilin	DI	+++	+++	+++	+	-	-
GL06	Guilin	DI	+++	+++	+++	+	-	-
GL17	Guilin	DI	+++	+++	+++	+	-	-

metalaxyl resistant isolates were predominant, with the frequency of 51.35%. The EC_{50} values of some isolates from this area were higher than 500 μg mL^{-1} and their resistance levels were over 100000 folds. In these areas, the use of metalaxyl should be reduced or alternated. Cymoxanil has been used for nearly 10 years in Guangxi, and dimethomorph has been used for 5-6 years. However, there was no decrease in the sensitivity of *P. infestans* populations, and the sensitivities of the pathogen were nearly normally distributed. Hence, they could be recommended for controlling late blight disease of tomato and their mean EC_{50} value [cymoxanil (0.1647±0.0255) μg mL^{-1}, dimethomorph (0.0970±0.0052) μg mL^{-1}) could be used as the baseline sensitivities for monitoring the field resistance development. The comparison with the floating-leaf-disk method indicated both *in vitro* and *in vivo* techniques provided equivalent results.

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