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# Identification of DNA Markers Linked to Metalaxyl Insensitivity Loci in *Phytophthora infestans*

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**A total of 24 isolates of *Phytophthora infestans* were tested and analyzed for their resistance to metalaxyl fungicides. Sensitivity to metalaxyl was determined by growing isolates on 20% V8 medium amended with 0, 5, and 100 μg/ml metalaxyl. Four isolates among the 24 tested were resistant to metalaxyl. Eleven isolates were intermediate and nine isolates were sensitive. Amplified fragment length polymorphism (AFLP) assay was used to identify the amplification products of resistant isolates. As a result, selected fragments were cloned, sequences and primer pairs were developed which linked to metalaxyl insensitivity in *P. infestans* using competitive PCR.**

**KEYWORDS: AFLP, Fungicide resistant, *Phytophthora infestnas***

Late blight has reemerged over recent years as a significant threat to potato and tomato production worldwide (Fry and Goodwin, 1997). This can be largely attributed to genetic changes in the pathogen, *Phytophthora infestans* (Mont.) de Bary. The development of resistance to fungicides is a major problem in the management of plant diseases (Russell, 1995). Phenylamide fungicides such as metalaxyl, when they were first introduced, displayed excellent performance as systemic fungicides with protective, curative, and long-lasting activity against many important plant pathogenic fungi within the Peronosporales (Schwinn and Staub, 1995). However, strains insensitive to phenylamides have since appeared in many important crop systems (Gisi and Cohen, 1996). For example, not long after the introduction of metalaxyl to the European market in the 1970s, insensitive strains of the late blight pathogen *P. infestans* were detected (Davidse *et al.*, 1981; Dowley and O'Sullivan, 1981) and, by the 1990s, insensitivity was reported in North America and other regions (Deahl *et al.*, 1993; Goodwin *et al.*, 1996). Metalaxyl insensitivity has also developed in other *Phytophthora* species (Bhat *et al.*, 1993; Goodwin and McGrath, 1995; Hunger *et al.*, 1982), downy mildews (Crute, 1987), and related pathogens (Mathur *et al.*, 1995; Morton and Urech, 1988; Sanders, 1984), although phenylamides are still effective in many situations. While the occurrence of resistant strains has been well documented, much remains to be learned concerning the genetic and molecular basis of resistance and the development of insensitive genotypes. Insensitive strains of *P. infestans* from different parts of the world are genetically diverse based on their isozyme and DNA fingerprints (Forbes *et al.*, 1998; Goodwin *et al.*, 1998), and it follows that insensitivity likely evolved on multiple occasions. However, it is not known whether a change at the same locus is always responsible. By analogy with other systems, insensitivity might result from a variety of mechanisms including inactivation, altered transport, or altered metabolism of the fungicide, or a change at the phenylamide target (Steffens *et al.*, 1996). Therefore, several different loci could contribute to insensitivity. The precise cellular target of the phenylamides remains to be elucidated, but biochemical studies indicate an effect on the synthesis of ribosomal RNA (Davidse, 1995; Fisher and Hayes, 1984). Understanding the basis of insensitivity would help illuminate the target and metabolism of this important class of fungicides.

The objective of this study was to characterize the genetic basis of metalaxyl insensitivity in *P. infestans*, and to lay the groundwork for future molecular studies of the trait molecular markers linked to metalaxyl insensitivity loci were studies.

## Materials and Methods

***Phytophthora infestans* strains and culture conditions.** Isolates of *P. infestans* used in this study are described in Table 1. These were collected from various places in Korea. Mycelia from the surface of the slice were picked and aseptically transferred to V8 medium. The plates were incubated at 17~20°C and, after seven days, sporangia were transferred to 20% V8 medium for subsequent studies. Mycelium for isolation of DNA was obtained by growing *P. infestans* in liquid V8 medium for five to ten days.

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**Table 1.** Fungal isolates used in this study

| Line number | Isolates name | Host | Source |
|---|---|---|---|
| 1 | 20B02 | *Solanum tuberosum* L. | Buyeo |
| 2 | KNU(Z)10 | *S. tuberosum* L. | Kangnung |
| 3 | KNU(Z)11 | *S. tuberosum* L. | Kangnung |
| 4 | SC2-3 | *S. tuberosum* L. | Sacheon |
| 5 | WS2-1 | *S. tuberosum* L. | Wangsan |
| 6 | WS2-5 | *S. tuberosum* L. | Wangsan |
| 7 | WS2-10 | *S. tuberosum* L. | Wangsan |
| 8 | WS4-3 | *S. tuberosum* L. | Wangsan |
| 9 | WS4-15 | *S. tuberosum* L. | Wangsan |
| 10 | WS6-14 | *S. tuberosum* L. | Wangsan |
| 11 | WS8-10 | *S. tuberosum* L. | Wangsan |
| 12 | WS9-1 | *S. tuberosum* L. | Wangsan |
| 13 | SC2-2 | *S. tuberosum* L. | Sacheon |
| 14 | SC1-6 | *S. tuberosum* L. | Sacheon |
| 15 | SC1-3 | *S. tuberosum* L. | Sacheon |
| 16 | KJ-2 | *S. tuberosum* L. | Kimje |
| 17 | WS2-3 | *S. tuberosum* L. | Wangsan |
| 18 | NW-7 | *S. tuberosum* L. | Namwon |
| 19 | KR-3 | *S. tuberosum* L. | Unknown |
| 20 | YY-11 | *S. tuberosum* L. | Yangyang |
| 21 | YY-12 | *S. tuberosum* L. | Yangyang |
| 22 | YY-15 | *S. tuberosum* L. | Yangyang |
| 23 | YY-19 | *S. tuberosum* L. | Yangyang |
| 24 | YY-27 | *S. tuberosum* L. | Yangyang |

**Metalaxyl test.** Sensitivity to metalaxyl was determined for the 24 isolates using 20% V8 medium supplemented with metalaxyl at concentrations of 0, 5 and 100 $\mu g/ml$. The test was replicated 3 times. Metalaxyl was prepared as a 100 mg/*ml* stock solution in pure dimethylsulfoxide (DMSO) and appropriate volumes were added to molten (40°C) agar after autoclaving to bring it to the desired concentrations. Agar plugs approximately 9 mm in diameter were cut from the edges of 10~14 day old colony and placed at the center of each metalaxyl amended plate. After incubation for 10 days at 17~20°C, diameters of each colony were measured. Estimation of the growth rate relative to the control (0 $\mu g/ml$ metalaxyl) were also calculated. Subsequently, isolates were separated into groups depending on their sensitivity to metalaxyl following the procedures used at the International Potato Center (Forbes, 1997). Sensitive isolates were those with growth <40% of the control at 5 and 100 $\mu g/ml$; intermediate isolates had a growth ≥40% of the control at 5 $\mu g/ml$ but less than 40% of the control at 100 $\mu g/ml$; resistant isolates had growth ≥40% of the control at 100 $\mu g/ml$.

**AFLP analysis.** AFLP DNA fingerprinting was performed essentially as described by Vos *et al.* (1995). Genomic DNA (1 $\mu g$) was digested with the restriction endonucleases *Eco*RI and *Mse*I. Doble-stranded adapters were then ligated to the ends of the restriction fragments followed by ethanol precipitation and resuspension in 40 $\mu l$ of distilled water. Pre-amplification PCR was performed using standard adapter primers containing no selective nucleotides, followed by selective amplification using similar primers with two or three selective bases.

The sequence of the adapter fitting the *Eco*RI site was as follow;

5'-CTCGTAGACTGCGTACC
CTGACGCATGGTTAA-5

The sequence of the adapter fitting the *Mse*I site was as follow;

5'-GACGATGAGTCCTGAG
TACTCAGGACTCAT-5

Pre-amplification PCR started with a cycle of 30s at 94°C, 1 min at 60°C, and 1 min at 72°C and was followed by 20 cycles. After the pre-amplification, the reaction mixture was diluted to 200 $\mu l$ with distilled water. For the selective amplification of a limited number of DNA restriction fragment, the secondary template DNA was amplified with primers containing two or three selective 3' nucleotides (E+2 or E+3 and M+3 primers). Sixty six primer combinations were used. In the E+2 or E+3 primers, the core sequence E was 5'-GACTGCGTACCAATTC. In the M+3 primers, the core sequence M was 5'-GATGAGTCCTGAGTAA. The following primer combinations were used: E+AT/M+CAG, E+AT/M+CAC, E+AT/M+CTA, E+AT/M+CTT, E+AT/M+CAA, E+AT/M+CTC, E+AC/M+CAG, E+AC/M+CAC, E+AC/M+CTA, E+AC/M+CTT, E+AC/M+CAA, E+AC/M+CTC, E+TA/M+CAG, E+TA/M+CAC, E+TA/M+CTA, E+TA/M+CTT, E+TA/M+CAA, E+TA/M+CTC, E+TG/M+CAG, E+TG/M+CAC, E+TG/M+CTA, E+TG/M+CTT, E+TG/M+CAA, E+TG/M+CTC, E+AG/M+CAG, E+AG/M+CAC, E+AG/M+CTA, E+AG/M+CTT, E+AG/M+CAA, E+AG/M+CTC, E+TC/M+CAG, E+TC/M+CAC, E+TC/M+CTA, E+TC/M+CTT, E+TC/M+CAA, E+TC/M+CTC, E+AA/M+CAG, E+AA/M+CAC, E+AA/M+CTA, E+AA/M+CTT, E+AA/M+CAA, E+AA/M+CTC, E+ACT/M+CAG, E+ACT/M+CAC, E+ACT/M+CTA, E+ACT/M+CTT, E+ACT/M+CAA, E+ACT/M+CTC, E+AGC/M+CAG, E+AGC/M+CAC, E+AGC/M+CTA, E+AGC/M+CTT, E+AGC/M+CAA, E+AGC/M+CTC, E+ACC/M+CAG, E+ACC/M+CAC, E+ACC/M+CTA, E+ACC/M+CTT, E+ACC/M+CAA, E+ACC/M+CTC, E+ATC/M+CAG, E+ATC/M+CAC, E+ATC/M+CTA, E+ATC/M+CTT, E+ATC/M+CAA, and E+ATC/M+CTC. For the selective amplification, the following PCR profile was used; the first cycle with 30s at 94°C, 30s at 65°C, and 1 min at 72°C followed by 11 cycles with a stepwise lowering of annealing temperature by 1°C in each cycles and 23 cycles with an annealing temperature of 56°C. Amplification products were separated using standard 6% denatured polyacylamide gel-electrophoresis and detected using autoradio-

graphic procedures.

**Cloning and sequencing of the metalaxyl-resistant AFLP markers.** The fungicide resistance-associated band, generated by AFLP with the E+3 and M+3 primer combination from the metalaxyl resistance isolates of *P. infestans*, was recovered from agarose gels, by selective amplification of AFLP as described above. Selective amplification products were cloned into pGEM-T® Easy (Promega, USA) and transformed into electro-competent *E. coli* (Strain JM109) according to the suppliers instructions (Promega, USA). Selected clones were sequenced using the Dye Terminator Sequencing (Bionex, Korea). Primer3 was used for the selection of primers for use in PCR (Lincoln *et al.*, 1991). As a result, two primer pairs R1F/R1R and R2F/R2R were generated for the detection of metalaxyl resistant isolates.

**Dot-hybridization.** Standard molecular biological method was used unless otherwise stated. The AFLP DNA fragment was labelled using DIG-11-dUTP (digoxigenein-3-O-methylcarbornyl-(-amino-caproyl-5-(-3-aminoallyl)-uridine-5'-triphosphate) according to the menufactuers' instructions (Specific PCR Reaction, Boehringer-Mannheim, Germany). A total of 100 $\mu l$ of the PCR labeling reaction mixture containing 10 ng of template DNA, 0.5 $\mu$M of the each primers (PUC/M13 forward and PUC/M13 reverse), 1×PCR buffer, 5 units of Taq polymerase (Quantum, USA), 0.1 mM of dGTP, dCTP and dATP, 0.09 mM dTTP and 1 mM dUTP (Boehringer-Mannheim, Germany) was prepared and reacted in a program of 90s at 94°C, 90s at 50°C, 2 min at 72°C for 24 cycles and 4 min at 72°C. PCR products amplified by designed primer set (R1F/R1R and R2F/R2R) were electrophoretically separated on 0.8% agarose gel. They were transferred onto Hybond N membranes (Quantum, USA) using a vacuum transfer system. Identification of homologous sequences of DNA, using a labelled probe, was possible with the DIG-detection chemiluminescent method (Boehringer-Mannheim, Germany).

## Results and Discussion

**Metalaxyl resistance test.** The addition of metalaxyl to culture medium significantly reduced growth rates in most of the isolates tested (Table 2). However, among the twenty-four tesed isolates, four *P. infestans* isolates were considered to be resistant to metalaxyl.

Their growth rates at 100 $\mu g/ml$ ranged from 57.6 to 83.3% (20B02, KNU(Z)10, KNU(Z)11 and SC2-3). Overall, eleven isolates tested were intermediate resistant to metalaxyl (WS2-1, WS2-5, WS2-10, WS4-3, WS4-15, WS6-14, WS8-10, WS9-1, SC2-2, SC1-6 and SC1-3), nine showed sensitivity (KJ-2, WS2-3, NW-7, KR-3, YY-11, YY-12, YY-15, YY-19 and YY-27; Table 2).

**Table 2.** Mean colony diameter (mm) after 10 days of incubation and percent (%) mycelial growth rates relative control of *P. infestans* isolates at two metalaxyl concentration

| Isolates | Control colony diameter in mm (0 $\mu g/ml$) | % growth rates | | Resistance rate[a] |
|---|---|---|---|---|
| | | (5 $\mu g/ml$) | (100 $\mu g/ml$) | |
| 20B02 | 78.0 | 94.9 | 83.3 | R |
| KNU(Z)10 | 81.0 | 91.3 | 67.0 | R |
| KNU(Z)11 | 83.0 | 85.9 | 64.7 | R |
| SC2-3 | 74.0 | 92.3 | 57.6 | R |
| WS2-1 | 68.8 | 41.9 | 31.9 | IR |
| WS2-5 | 67.0 | 44.3 | 35.1 | IR |
| WS2-10 | 67.0 | 43.9 | 32.4 | IR |
| WS4-3 | 66.8 | 43.3 | 35.3 | IR |
| WS4-15 | 69.9 | 44.6 | 35.6 | IR |
| WS6-14 | 60.6 | 42.4 | 32.3 | IR |
| WS8-10 | 63.7 | 43.4 | 28.0 | IR |
| WS9-1 | 63.3 | 59.4 | 28.9 | IR |
| SC2-2 | 72.0 | 52.1 | 39.2 | IR |
| SC1-6 | 69.0 | 49.8 | 32.5 | IR |
| SC1-3 | 71.4 | 41.2 | 30.2 | IR |
| KJ-2 | 66.5 | 23.8 | 14.3 | S |
| WS2-3 | 71.2 | 26.3 | 13.9 | S |
| NW-7 | 85.0 | 36.5 | 12.9 | S |
| KR-3 | 85.0 | 37.6 | 15.2 | S |
| YY-11 | 85.0 | 35.3 | 15.3 | S |
| YY-12 | 85.0 | 31.8 | 15.2 | S |
| YY-15 | 65.0 | 0 | 0 | S |
| YY-19 | 73.0 | 24.2 | 11.9 | S |
| YY-27 | 81.0 | 37.4 | 15.7 | S |

[a]R: Resistance; IR: Intermediate resistance; S: Sensitive.

**AFLP analysis.** AFLP analysis was performed on twenty-four isolates of *P. infestans* using sixty six primer combinations to identify amplification products of resistant isolates. Two primer combinations produced amplification profiles in which at least one fragment was common to resistant isolates of *P. infestans* and not shared by any isolates (primer combination: M+CAA/E+ACT and M+CTA/E+ACC) which were selected for further study (Fig. 1). Primer combination M+CTA/E+ACC strongly amplified a fragment of approximately 220 bp from DNA of resistant isolates, and the other combination amplified a fragment of approximately 180 bp. These fragments were named R1 and R2, respectively. The two selected fragments were isolated, cloned into pGEM-T® Easy (Promega, USA) and transformed into *E. coli* strain JM109 as described above.

**Sequencing of the AFLP markers.** The AFLP fragments R1 and R2 were amplified, cloned and sequenced (Fig. 2). The data obtained showed the presence of the initial AFLP primers used at both ends of the insert DNA. The specific primer pairs, R1F(5'-GCTACATGTGAG-

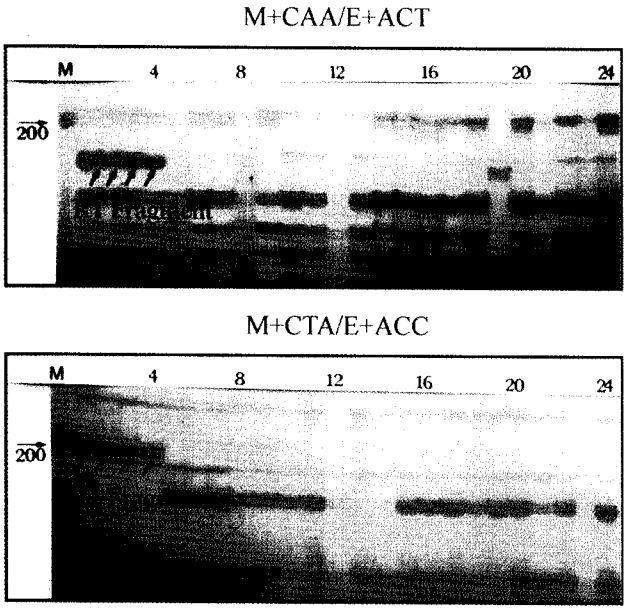


**Fig. 1.** Amplification fragment length polymorphism profile. DNA fingerprints generated by primer combination M+CTA/E+ACC and M+CAA/E+ATC. The marker fragments are indicated by arrows. List of isolates in this study was described in Table 1.

R1 Fragment

```
1   GACTGCGTAC  CAATTCACTA  CGTGTATCAA  ACGTGCTACA  TGTGAGGAAA
51  ATCCTTCTGG  AGACGGCAAA  GCCGGCAAAT  TGTCTATGAT  CGATCTTGCG
101 TCTGAGGTCT  GTCAAGGAGA  GTTGAACGAC  CAGGTGGTCC  ACGCGTGCTC
151 AACTGATTAC  GGTGCCTTAC  CTTGTTACTC  AGGACTCATC
```

R2 Fragment

```
1   GACTGCGTAC  CAATTCACCA  ACAGGGACAT  GGCATGAGAC  AGAGCGGAAT
51  TTCGCATCCA  CTTGAGCTGT  AGCCGTATCC  ATCGGGATCG  TAGTCGGCGA
101 GCCAAATCGG  AACCGTGTGG  TGAAGTCGTC  GCTGAAAAAG  ATTCTAAGGT
151 GAAATACTGT  AGCTCAATCT  GGGTGTGTTA  GTTACTCAGG  ACTCATCAAT
```

**Fig. 2.** Sequence of the marker fragment R1 and R2. The position of the primer M+3 and E+3 are underlined and the specific primer R1F, R1R, R2F, and R2R are shaded.

GAAA ATCC-3'), R1R(5'-GTAAGGCACGTAATCAGTT-3'), R2F(5'-AACAGGGACATGGCATGA GA-3'), and R2R (5'-ACACACCCAGATTGAGCTAC-3') were designed from the sequence of the selected clone (R1 and R2 clone), and used to amplify DNA from resistant isolates of *P. infestans* (Fig. 2).

**Specific PCR and Dot-hybridization.** The two primer pairs, R1F/R1R and R2F/R2R, were used to amplify specific fragment from resistant isolates. PCR primers were designed from the sequence of the cloned AFLP markers. These primer combinations amplified the DNA of four

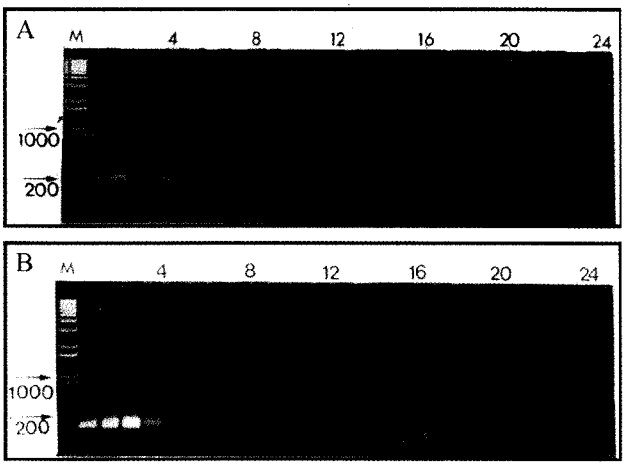


**Fig. 3.** Amplification of DNA from *P. infestans* using primer pair A. R1F/R1R and B. R2F/R2R. Lanes 1~24: DNA from isolates 20B02, KNU(Z)10, KNU(Z)11, SC2-3, WS2-5, WS2-10, WS4-3, WS4-15, WS6-14, WS8-10, WS9-1, SC2-2, SC1-6, SC1-3, KJ-2, WS2-3, NW-7, KR-3, YY-11, YY-12, YY-15, YY-19, and YY-27.

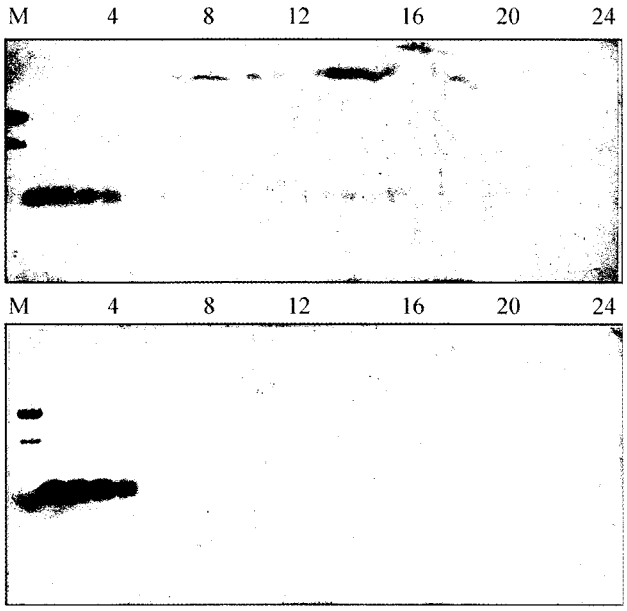


**Fig. 4.** Dot-hybridization profiles using the cloned R1 fragment (upper figure) and R2 fragment (lower figure) as a probe.

resistance isolates of *P. infestans* and none of the other intermediate and sensitive isolates (Fig. 3). Southern blotting of the specific PCR gel, and hybridization with labeled insert DNA from pR1 and pR2, showed that the PCR products were homologous to the original metalaxyl resistance AFLP marker (Fig. 4). No signals were observed in lanes of other intermediate and sensitive isolates.

In this study, the basis of insensitivity to metalaxyl in twenty four isolates of *P. infestans* was examined. Result

of metalaxyl test revealed that four *P. infestans* isolates were considered highly resistant to metalaxyl. Amplified fragment length polymorphism assays were used to identify amplification products characteristic of resistant isolates. DNA markers linked to insensitivity were obtained using AFLP. DNA markers were identified that flanked the major insensitivity locus. A previous study by Dr. Judelson laboratory revealed a complex pattern of inheritance for insensitivity in *P. infestans* involving both major and minor loci (Fabritius *et al.*, 1997). That major determinants (*MEX* loci) existed concurred with conclusions by other groups based on studies of various *Phytophthora* spp. (Bhat *et al.*, 1993; Chang and Ko, 1990; Shattock, 1988; Vorobeva *et al.*, 1992). The nature of the molecular changes that confer insensitivity to phenylamides currently can only be the subject of speculation. Phenylamides are reported to specifically inhibit the synthesis of ribosomal RNA, but their precise target is unknown (Davidse, 1995; Davidse *et al.*, 1983; Fisher and Hayes, 1984). RNA polymerase I is sometimes suggested as the target, but the complex nature of the eukaryotic transcriptional apparatus presents multiple potential targets (Halle and Meisterernst, 1996). Moreover, the ability of phenylamides to impair transcription in mycelial extracts, but not purified nuclei, may suggest a less direct mode of action (Davidse *et al.*, 1983). Assuming that more than one major locus may be implicated in *P. infestans*, the genetics of phenylamide insensitivity would be similar to that of carboximide and kasugamycin resistance in which multiple mechanisms have been described (Gunatillkeke *et al.*, 1976; Taga *et al.*, 1978). DNA markers linked to metalaxyl insensitivity loci have applications beyond those presented here. Such markers may be used to study the development and spread of insensitivity through populations or for assays to predict insensitivity; probes tightly linked to the gene should be more appropriate than the unlinked markers that are currently employed (Goodwin *et al.*, 1996). Also, whether homozygous resistant isolates resulted from selfing, mitotic crossing-over, or second mutations in heterozygotes might be determined with the aid of the flanking DNA markers. Such studies may indicated how resistance to new fungicides might develop.

## References


Bhat, R. G., McBlain, B. A. and Schmitthenner, A. F. 1993. The inheritance of resistance to metalaxyl and to fluorophenylaminine in matings of homothallic *Phytophthora sajae*. *Mycol. Res.* **97**: 865-870.

Chang, T. T. and Ko, W. H. 1990. Resistance to fungicides and antibiotics in *Phytophthora parasitica*: Genetic nature and use in hybrid determination. *Phytopathology* **80**: 1414-1421.

Crute, L. R. 1987. The occurrence, characteristics, distribution, genetics, and control of a metalaxyl-resistant pathotype of Bremia lactucae in the United Kingdom. *Plant Dis.* **71**: 763-767.

Davidse, L. C. 1995. Phenylamide fungicides-Biochemical action and resistance. Pages 347-354 in: Modern Selective Fungicides, Properties, Applications, Mechanisms of Action. H. Lyr, ed. Gustav Fisher Verlag, New York.

_____, Hofman, A. E. and Vwlthuis, G. C. M. 1983. Specific interference of metalaxyl with endogeneous RNA polymerase activity in isolated nuclei from *Phytophthora megasperma* f. sp. *Medicaginis*. *Exp. Mycol.* **7**: 344-361.

_____, Looijeu, D., Turkensteen, L. J. and van der Als, D. 1981. Occurrence of metalaxyl-resistant strains of potato blight in Dutch potato fields. *Neth. J. Plant Pathol.* **87**: 65-68.

Deahl, K. L., Inglas, D. A. and DeMuth, S. P. 1993. Testing for resistance to metalaxyl in *Phytophthora infestans* isolates from northwestern Washington. Am. *Potato J.* **70**: 779-795.

Dowley, L. J. and OSullivan, E. 1981. Metalaxyl-resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland. *Potato Res.* **24**: 417-421.

Fabritius, A. L., Shcttock, R. C. and Judelson, H. S. 1997. Genetic analysis of metalaxyl insensitivity loci in *Phytophthora infestans* using linked DNA markers. *Phytopathology* **87**: 1034-1040.

Fisher, D. J. and Hayes, M. D. 1984. Studies of mechanisms of metalaxyl fungitoxicity and resistance to metalaxyl. *Crop Prot.* **3**: 117-185.

Forbes, G. A. 1997. Manual for Laboratory Work on *Phytophthora infestans*. International Potato Center, Training manual. Quito, Ecuador, 33 pp.

_____, Goodwin, S. B., Drenth, A., Oyarzun, P., Ordonez, M. E. and Fry, W. E. 1998. A global marker database for *Phytophthora infestans*. *Plant Dis.* **82**: 811-818.

Fry, W. E. and Goodwin, S. B. 1997. Resurgence of the Irish potato famine fungus. *Bioscience* **47**: 363-371.

Gisi, U. and Cohen, Y. 1996. Resistance to phenylamide fungicides: A case study with *Phytophthora infestans* involving mating type and race structure. *Annu. Rev. Phytophathol.* **34**: 549-572.

Goodwin, S. B. and McGrath, M. T. 1995. Insensitivity to metalaxyl among isolates of *Phytophthora erythroseptica* causing pink rot of potato in New York. *Plant Dis.* **79**: 967.

_____, Smart, C. D., Sandrock, R. W., Deahl, K. L., Punja, Z. K. and Fry, W. E. 1998. Genetic change within populations of *Phytophthora infestans* in the United States and Canada during 1994 to 1996: Role of migration and recombination. *Phytopathology* **88**: 939-949.

_____, Sujkowski, L. S. and Fry, W. E. 1996. Widespread distribution and probable origin of resistance to metalaxyl in clonal genotypes of *Phytophthora infestans* in the United States and western Canada. *Phytopathology* **86**: 793-800.

Gunatillkeke, I. A. U. N., Arst, H. N. and Scazzochio, C. 1976. Three genes determine the probable the carboxin sensitivity of mitochondrial succinate oxidation in *Aspergillus nidulans*. *Genet. Res.* **26**: 297-305.

Halle, J. P. and Meisterenst, M. 1996. Gene expression: Increasing evidence for a transcriptosome. Trends Genet. **12**: 161-163.

Hunger, R. M., Horner, P. B. and Hansen, E. M. 1982. Tolerance of *Phytophthora megasperma* isolates to metalaxyl. *Plant Dis.* **66**: 645-649.

Lincoln, S. E., Daly, M. J. and Lander, E. S. 1991. Primer. The Whitchcad Institute for Biomedical Research.

Mathur, S., Wu, C., Liu, J. Q. and Rimmer, S. R. 1995. Inheritance of virulence and fungicide resistance in Albugo candida.

*Can. J. Plant Pathol.* **17**: 360.

Morton, H. V. and Urech, P. A. 1988. History of the development of resistance to phenylamide fungicides. Pages 59-62 in: Fungicide Resistance in North America. C. J. Delp, ed. The American Phytopathological Society, St. Paul, MN.

Russell, P. E. 1995. Fungicide resistance: Occurrence and management. *J. Agric. Sci.* **124**: 317-323.

Sander, P. L. 1984. Failure of metalaxyl to control Pythium blight on turfgrass in Pennsylvania. *Plant Dis.* **68**: 776-777.

Schwinn, F. and Staub, T. 1995. Oomycetes fungicides. Pages 323-346 in: Modern Selective Fungicides, Properties, Applications, Mechanisms of Action. H. Lyr, ed. Gustav Fischer Verlag, New York.

Shattock, R. C. 1988. Studies on the inheritance of resistance to metalaxyl in *Phytophthora infestans. Plant Pathol.* **37**: 4-11.

Steffens, J. J., Pell, E. J. and Tien, M. 1996. Mechanisms of fungicide resistance in phytopathogenic fungi. *Curr. Opin. Biotechnol.* **7**: 348-355.

Taga, M., Nakagawa, H., Tsuda, M. and Ueyama, A. 1978. Ascospore analysis of kasugamycin resistance in *Phytophthora infestans* to acylalanine fungicides. *Plant Dis. Rep.* **63**: 385-389.

Vorob'eva Yu, V., Shemyakina, V. P. and Kvasnyuk, N. Y. 1992. The inheritance of ridomyl resistance by sexual reproduction of *Phytophthora infestans. Genetika* **28**: 99-106.

Vos, P., Hoders, R., bleeker, M., reijans, M., Lee, V. T., Hornes, M., Fritjers, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP: A new concept for DNA fingerprinting. *Nucl. Acids Res.* **23**: 4407-4414.