Effects of Phenylamide Pesticides on the GSH-Conjugation System of *Phytophthora* spp. Fungi*

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Phytophthora cactorum, Ph. cambivora, Ph. cinnamomi, Ph. citricola, Ph. cryptogea, Ph. drechsleri, Ph. infestans, Ph. megasperma, Ph. parasitica, and *Ph. syringae* contain GSH-conjugation systems as indicated by the presence of active GST enzyme in addition to GSH. Basal levels of both GSH and GST in the thalli of *Phytophthora* strains studied did not correlate with either fungal sensitivity to phenylamides (acetochlor, butachlor, metolachlor, dimethachlor, propachlor, ofurace, CGA-29212, metalaxyl, RE-26745, benalaxyl, furalaxyl, LAB-149202) or their acquired resistance to metalaxyl. Thalli of *Phytophthora* strains from axenic cultures exposed to sublethal concentrations of the above pesticides contained significantly higher levels of both GSH and GST than the untreated controls. This response was independent of the sensitivity and tolerance of the strains to phenylamides.

When the responses of *Phytophthora* strains to phenylamide chemicals were compared by means of principal component analysis, four independent components were detected accounting for 88% of the total variation. Biological properties (basal and induced levels of GST and GSH, growth intensity, degree of acquired resistance to metalaxyl, sensitivity to propiconazole and to *cis*- and *trans*-tridemorph) of the strains contributed differently to this variation. It was concluded that, in contrast to plants, sensitivity or tolerance of *Phytophthora* species to phenylamide pesticides is not regulated by the efficiency of the GSH-conjugation system. In addition, our data clearly indicate that the acetanilide pesticides have multiple sites of action in the *Phytophthora* genus.

Introduction

The glutathione (γ-L-glutamyl-L-cysteinyl-glycine, GSH) conjugation system is an important pathway detoxyfying electrophilic compounds and contributes to the tolerance of living cells to xenobiotics [1–3].The key role of GSH-conjugation system in the metabolism of chloroacetanilide herbicides as related to the sensitivity or the resistance of weeds to them has been demonstrated [3–9]. Phenylamide fungicides are structurally related to chloroacetanilide herbicides (Table I) and they are highly selective against *Phytophthora* species and other taxons of Peronosporales [10, 11]. *Phytophthora* species are distinct form other fungi by phylogeny, cytology, physiology and genetics [12–15], and can be included into the plant kingdom [16], and considered as related to algae [17].

Since *Phytophthoras* [18–21] and related species [22–24] show differential sensitivity (occasionally tolerance) to phenylamide fungicides, this investigation was undertaken to determine, whether their GSH-conjugation system mediates their response to phenylamides. Some preliminary results have recently been published [25].

Materials and Methods

Fungi

Phytophthora spp. fungi were taken from an earlier study [21] and were maintained on green pea agar (GPA) slants at 19 ± 1 °C. A piece of mycelial mat was transferred into 90 mm Petri dishes containing the same medium to maintain colonies for further inoculation.

Chemicals

Glutathione, CDNB, DTNB (Fluka AG, Switzerland), cycloheximide (Calbiochem, Switzerland) and Coomassie Brilliant Blue G-250 (Serva, F.R.G.) were commercial samples. Twelve acetan-

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Table I. List of chemicals tested.

No.	Common name	Chemical name	Trade name	Pesticidal activity
I	Metalaxyl[a]	Methyl-N-(2-methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate	Ridomil 25 wp	F
II	CGA 22219[b]	Methyl-N-(2-chloroacetyl)-N-(2,6-xylyl)-DL-alaninate	experimental	F
III	RE 26745[b]	2-Methoxy-N-2,6-xylyl-acetamido-τ-butyrolactone	experimental	F
IV	Ofurace[b]	(±)-α-2-Chloro-N-2,6-xylyl-acetamido-τ-butyrolactone	Milfuram 50 wp	F
V	Benalaxyl[a]	Methyl-N-phenylacetyl-N-(2,6-xylyl)-DL-alaninate	Galben 25 wp	F
VI	Furalaxyl[a]	Methyl-N-(2-furoyl)-N-(2,6-xylyl)-DL-alaninate	Fongarid 25 wp	F
VII	LAB 149202 F[c]	Methyl-N-isoxazol-5-yl-N-(2,6-xylyl)-DL-alaninate	experimental	F
VIII	Cyprofuram[b]	(±)-α-(N-(3-Chlorophenyl)cyclopropanecarboxamido)-τ-butyrolactone	Vinicur 50 wp	F
IX	Acetochlor[a]	2-Chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)-acetamide	Harness 96 ec	H
X	Butachlor[a]	N-Butoxymethyl-2-chloro-2′,6′-diethylacetanilide	Machete 60 ec	H
XI	Dimethachlor[a]	2-Chloro-N-(2-methoxyethyl)acet-2′,6′-xylidine	Teridox 50 ec	H
XII	Metolachlor[a]	2-Chloro-N-(2-methoxy-1-methylethyl)-N-(2-ethyl-6-methyl-phenyl)acetamide	Dual 72 ec	H
XIII	Propachlor[a]	2-Chloro-N-isopropylacetanilide	Ramrod 65 wp	H

F, fungicide; H, herbicide.
[a] Active substance extracted from the corresponding commercial product.
[b] Synthesized in the Institute for Plant Protection, Kleinmachnow (Germany).
[c] Supplied by the manufacturer.

Uniform pesticide preparations were made of chromatographically pure substances by dissolving them (50 mg) in aliquots (100 µl) of cyclohexanone + t-butanol (1 : 1, v/v) containing 51 (w/v) detergent composition (2 part of Tween 20, 1 part of Tween 40, 1 part of Tween 80, 1 part Emulsogen I-50).

ilides (Table I), tridemorph diastereomers (BASF, F.R.G.) and propiconazole (Ciba-Geigy, Switzerland) were used throughout this study. Suspensions/solutions of pesticides were prepared from chromatographically pure active substances by using appropriate solvents (for details see note of Table I).

Toxicity measurements

Active substances in different concentrations were incorporated into GPA medium and poured (15 ml) into 90 mm glass Petri dishes. The plates were inoculated with 5 mm agar discs cut from the edges of 6-day-old colonies, and the inoculated plates were incubated at 19 ± 1 °C. Three plates of each treatment were used to estimate the variance; colony diameters were measured to the nearest mm. The sensitivity of each *Phytophthora* strain to pesticides was characterized by ED_{50} values expressed as micromol/liter (*i.e.*, the pesticide concentration in the medium causing 50% reduction in radial growth) calculated by means of a curve fitting method based on a log/logistic function [26]. The survival of the thalli to the pesticide exposure was evaluated by transferring the treated inocula to a medium free of fungicides.

GST assay

Phytophthora strains were grown in glass Petri dishes on the surface of GPA medium covered with cellophane layers to have well aerated cultures and free of the medium thalli. Cytosol was extracted (the homogenized mass of thalli in TRIS·HCl buffer pH 8.3 was centrifuged at 12,000 rpm, 10 min), then the GST activity was measured in the supernatant according to a spectrophotometric method [4] using CDNB and GSH as substrates.

GSH assay

Measurements of the GSH levels in the thalli of *Phytophthora* strains were carried out in supernatants obtained as above using DTNB as reagent for sprectrophotometric analysis following previously published procedures [5].

Protein estimation

Protein content in the extracts was measured by Coomassie Blue dye binding method [27].

Data analysis

Experiments were repeated at least four times. Significant differences were tested either by F- or

t-distributions according to Clark [28]. The growth response data were analyzed using multiple and Spearman's rank correlation analyses, and principal component analysis (PCA) [29]. Relationships between sensitivity of strains to pesticides and their biological properties (basal and induced levels of GST and GSH, growth intensity, degree of acquired resistance to metalaxyl, sensitivity to other pesticides) were analyzed by principal component regression analysis (PCRA). The results of PCA and PCRA were interpreted as described by Sváb [29] and Oros [21].

Results and Discussion

Response of Phytophthora spp. to acetanilide fungicides and herbicides

The sensitivity of *Phytophthora* species to fungicides (Table II) and herbicides (Table III) is quite

Table II. Efficacy (ED_{50}, μM) of acetanilide fungicides against *Phytophthora* species.

No.	*Phytophthora* species	Fungicides							
		Metalaxyl **I**	CGA 22219 **II**	RE 26745 **III**	Ofurace **IV**	Benalaxyl **V**	Furalaxyl **VI**	LAB 149202 **VII**	Cyprofur **VIII**
1	*cactorum* w	0.590 f g	0.132 d	0. 728 g	2.80 i	5.53 j	0.564 f g	0.827 g	17.8 k l
2	*cactorum*	6.91 j	59.02 n o	0.639 g	2.47 h i	15.1 k l	1.95 i h	0.573 f	18.7 k l
3	*cryptogea*	0.0251 b	0.290 e f	1.13 g h	3.27 i	5.44 j	0.199 e	0.364 f	7.15 j k
4	*cambivora* w	15.7 k l	0.769 g	1.03 g	3.97 i	0.768 g	0.163 e	0.265 f e	10.2 j k
5	*cambivora* r	37.9 m n	2.0526 h	0.578 f g	1.65 h i	0.605 g	13.6 k l	0.173 de	5.08 i j
6	*citricola*	0.0430 b c	0.250 e	5.52 j	21.4 l	18.6 k l	0.697 g	1.125 g	114.4 o p r
7	*cinnamomi* w	2.51 i	2.27 h	1.39 h	25.3 l	30.7 m n	1.427 h i	0.232 e	18.4 k l
8	*cinnamomi* r	37.9 m n	0.0519 b c	1.39 h	71.7 n o	97.2 o	1.26 h	0.164 e d	36.8 m n
9	*megasperma*	2.03 h g	0.0706 c d	0.459 f g	1.74 i h	2.86 i	0.465 f g	0.103 d	1.72 h i
10	*parasitica* w	0.856 g	0.252 e	2.45 i	50.6 n o	16.9 k l	0.697 g	0.529 f	35.4 m
11	*parasitica* r	2.83 i j	6.95 j	1.42 h	13.1 k l	20.9 l m	1.05 g	0.323 e f	24.3 l m
12	*parasitica* rev	0.244 e	0.0921 c d	1.59 h	61. 2 o	5.53 j	0.365 f	0.463 f g	26.8 l m
13	*syringae*	0.0752 c d	0.0570 b c	0.0630 c	0.103 d	0.705 g	0.0631 c	0.0496 b c	1.22 h
14	*dreschleri*	65.8 o	26.31 m	51.6 n o	1.18 g	149.4 p r	11.8 k l	12.04 k l	96.7 o p
15	*infestans*	0.004 a	0.004 a	0.098 c d	0.027 b	n.d.	n.d.	n.d.	n.d.

Values labeled by the same letter are not significantly different at P = 5% level. Abbreviations: w, wild type; r, resistant to metalaxyl; rev, revertant of metalaxyl-resistant mutant; n.d., not determined.

Table III. Fungitoxic efficacy (ED_{50}, μM) of acetanilide herbicides to *Phytophthora* species.

No.	*Phytophthora* species	Herbicides				
		Acetochlor **IX**	Butachlor **X**	Metolachlor **XI**	Dimethachlor **XII**	Propachlor **XIII**
1	*cactorum* w	78.6 j	5022.9 s r	54.5 i j	195.5 l m	56.8 i j
2	*cactorum* r	180.8 l m	2.67 e	84.2 i j k	165.4 k l m	63.5 i j
3	*cryptogea*	2.55 e	15.5 f g	42.5 h i	1.27 c d	54.6 i j
4	*cambivora* w	13.6 f	899.6 n o	35.2 h	243.9 m	77.6 j k
5	*cambivora* r	152.9 k l m	1068.2 o p	67.5 i j	135.1 k l m	68.5 i j
6	*citricola*	0.815 b c	0.456 a b	74.3 j	10.8 f	2.14 d e
7	*cinnamomi* w	205.4 l m	1358.3 o p r	195.2 l m	3048.9 r	237.8 m
8	*cinnamomi* r	231.1 l m	1399.2 o p r	247.4 l m	2529.1 p r	237.7 m
9	*megasperma*	59.5 i j	33.9 h i	42.5 h i j	22.2 g h	18.9 f g
10	*parasitica* w	23.6 g h	3280.2 r s	39.8 h i	112.6 k l	28.7 g h
11	*parasitica* r	34.9 h i	14.7 f	26.1 g h	100.5 j k l	0.21 a
12	*parasitica* rev	38.3 h i	1137.5 o p	2.01 d e	17.4 g f	23.8 g h
13	*syringae*	69.5 i j	88.2 j k	2.15 d e	77.4 j	25.6 g h
14	*dreschleri*	11.0 f	6485.5 s	727.8 n	>8000	163.6 k l m

Values labeled by the same letter are not significantly different at P = 5% level. Abbreviations: w, wild type; r, resistant to metalaxyl; rev, revertant of resistant to metalaxyl mutant.

variable. For example, *P. syringae* (the most sensitive species) is about 60 times more sensitive than *P. drechsleri* (the most tolerant one). Sensitivities of the strains to the fungicidal and to the herbicidal acetanilides correlated only for strains with acquired resistance to metalaxyl (Fig. 1 A). This is in contrast to the variations in growth responses to these compounds where significant correlation was observed for all strains (Fig. 1 B), *i.e.* the strain responding selectively to the first group of compounds responded by the same manner to the second one as well. The strains with acquired resistance to metalaxyl (**I**) were less sensitive to benalaxyl (**V**), furalaxyl (**VI**) and acetochlor (**IX**), and more sensitive to dimethachlor (**XI**) and LAB 149202 F (**VII**) than their parental strains. The effect of the presence of the gene of acquired resistance to metalaxyl on the response of the given strains to the other compounds was strain-dependent (Tables II and III), and it was not possible to predict the nature (positive or the negative) of the cross resistance to various compounds even for the closest structural analogues (**II** and **III**) of metalaxyl.

The efficacy of acetanilides was also highly variable: the fungicides are about two orders of magnitude more efficient than the herbicides (for example the experimental fungicide LAB 149202 F is about 1000 times more active than the herbicide butachlor). By means of multiple correlation analysis (Table IV) the compounds form well-separated clusters with correlations of various significance within the clusters. Seven compounds (**I, III, V, VI, VII, XI** and **XII**) form a closely related ($P = 0.1-1\%$) cluster; **VIII, IX, X** and **XIII** are related to this main cluster with lower significance ($P = 1-10\%$), while CGA 22219 (**II**) and ofurace (**IV**) are significantly distinct from the others ($P > 10\%$). By means of Student's *t*-probe (Table IV) the compounds form two clusters with significantly different intrageneric broad spectrum; the first one comprises three compounds of herbicidal activity (**IX, XI** and **XIII**), and the second one all the others. Thus, the qualitative differences between herbicidal and fungicidal acetanilides are clearly evident in our assay system. Interestingly, in quantitative evaluations the clustering of the compounds is different.

Influence of acetanilides on GSH levels and GST activity of Phytophthora spp.

The GST enzyme in *Phytophthora* species has an optimum at pH 6.5–8.5. Both content of GSH

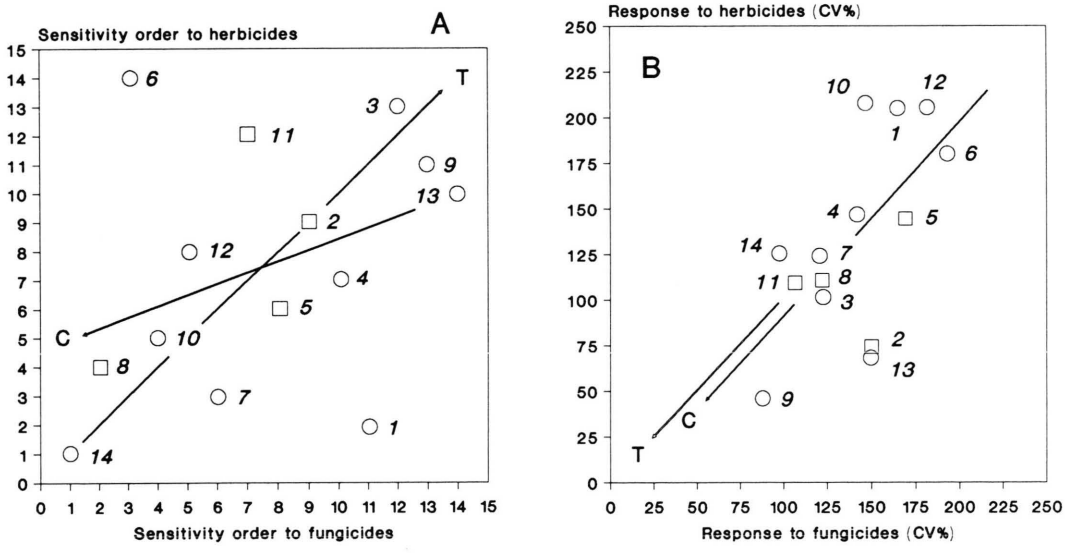

Fig. 1. Comparing sensitivity (A) and selectivity of the response (B) of *Phytophthora* strains to fungicides and herbicides. The lines on the rank map (A) and on the strain selectivity chart (B) represent regressions between responses of *Phytophthoras* to fungicides and herbicides: theoretical (T) and calculated (C) from the data for wild (circles) and metalaxyl-resistant (squares) strains. The numeration of the strains corresponds to that in Table II.

Table IV. Analysis of variation (matrix A) and multiple correlation (matrix B) of efficacy data of acetanilide pesticides against *Phytophthora* species.

No.	Chemicals (matrix A)	Chemicals (matrix B) I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
I	Metalaxyl	–	0.24	0.74	−0.02	0.81	0.81	0.73	0.36	0.19	0.53	0.83	0.78	0.55
II	CGA 22219	0.70	–	0.32	−0.28	0.27	0.24	0.35	0.14	0.25	0.07	0.33	0.26	0.08
III	RE 26745	1.14	0.40	–	−0.17	0.82	0.59	0.99	0.64	−0.28	0.68	0.93	0.89	0.30
IV	Ofurace	0.76	1.55	2.11	–	0.21	−0.27	−0.19	0.17	0.25	0.02	−0.03	−0.03	0.29
V	Benalaxyl	1.06	1.49	1.72	0.61	–	0.45	0.81	0.59	0.14	0.59	0.94	0.92	0.65
VI	Furalaxyl	1.67	0.98	0.79	2.73	1.96	–	0.59	0.23	−0.13	0.43	0.59	0.54	0.25
VII	LAB 149202 F	1.92	1.28	1.26	2.99	2.07	0.85	–	0.62	−0.27	0.72	0.92	0.88	0.29
VIII	Cyprofuram	1.55	2.11	2.45	1.01	0.25	2.79	2.93	–	0.29	0.37	0.58	0.51	0.09
IX	Acetochlor	2.92	3.18	3.31	2.65	2.08	3.44	3.50	2.05	–	−0.12	0.06	0.09	0.65
X	Butachlor	2.60	2.61	2.62	2.59	2.58	2.62	2.62	2.57	2.49	–	0.68	0.67	0.33
XI	Dimethachlor	1.99	2.09	2.14	1.88	1.69	2.18	2.21	1.65	0.69	2.41	–	0.98	0.68
XII	Metolachlor	1.67	1.68	1.69	1.67	1.65	1.69	1.69	1.65	1.57	0.52	1.50	–	0.61
XIII	Propachlor	2.80	3.05	3.18	2.54	1.98	3.31	3.37	1.95	0.06	2.49	0.72	1.57	–

The matrix A contains Student's *t*-values, related to differences in variations of efficacy (Ed_{50}-values) of acetanilides against 14 *Phytophthora* species (FG = 12; $t_{P=10\%} = 1.82$; $t_{P=5\%} = 2.28$; $t_{P=2\%} = 2.764$; $t_{P=1\%} = 3.20$; $t_{P=0.1\%} = 4.59$). The matrix B contains Bravais' correlation coefficient (r) related to similarity in efficacy (ED_{50}-values) of acetanilides against 14 *Phytophthora* species (FG = 13; $r_{P=10\%} = 0.44$; $r_{P=5\%} = 0.51$; $r_{P=2\%} = 0.59$; $r_{P=1\%} = 0.64$; $r_{P=0.1\%} = 0.76$).

and activity of GST (determinants of the efficiency of the GSH conjugation system) in the thalli of various strains show great variations (Table V). The levels of GSH and GST are in good correlation ($P < 1\%$). There is no correlation, however, between the levels of GSH or GST and the other biological characteristics of the strains included into the Table V.

Table V. Biological properties and sensitivity of *Phytophthora* species to selected fungicides of different mode of action.

No.	*Phytophthora* species[a]	Biological properties Growth [mm/day]	Mutation rate [$\times 10^{-6}$ %]	Levels[b] of GSH B	GSH I	GST B	GST I	Growth inhibition[c] [%] by Tridemorph *cis-*	Tridemorph *trans-*	Propiconazole
1	*cactorum* w	14.3 a	2.4	243 a	214 a	0.30 a	0.52 a	63.5	40.0	37.7
2	*cactorum* r	11.0 b	–	558 b	995 h	0.71 b	1.40 d	72.4	21.1	24.2
3	*cryptogea*	14.5 a	0.02	679 c	968 h	1.01 c	1.97 g	62.2	55.5	27.3
4	*cambivora* w	10.8 b	0.3	315 d	471 e	0.75 b	0.66 b e	72.6	40.6	34.3
5	*cambivora* r	10.0 c	–	484 e	412 i	0.68 b	0.60 e	68.4	23.0	38.4
6	*citricola*	13.3	0.14	752 c j	800 j	1.36 d g	1.29 g	96.0	58.3	34.8
7	*cinnamomi* w	8.8 e	0.4	456 e	437 i e	0.57 e	0.47 e	85.7	8.9	32.3
8	*cinnamomi* r	7.3 f	–	121 f	119 f	0.36 a	0.37 a	83.1	16.4	25.8
9	*megasperma*	7.0 f	0.15	243 a	214 a	0.08 f	0.08 f	65.6	45.3	37.8
10	*parasitica* w	7.8	0.5	243 a	216 a	0.46 a e	0.50 e	80.9	17.5	21.9
11	*parasitica* r	8.8 e	–	273 a d	245 a	0.38 a	0.55 e	92.1	19.7	23.5
12	*parasitica* rev	10.0 c	–	n.d.	n.d.	n.d.	n.d.	98.7	27.3	28.6
13	*syringae*	3.0 g	0.02	n.d.	n.d.	0.48 e	1.19 g	82.2	58.6	16.7
14	*dreschleri*	12.4	–	83 g f	97 f	0.14 f	0.16 f	n.d.	n.d.	n.d.
15	*infestans*	4.4 g	0.5	73 g	239 a	0.30 a	0.52 e	87.8	43.9	36.6

Values of a parameter labeled by the same letter are not significantly different at P = 5% level.

[a] w, wild type; r, resistant to metalaxyl; rev, revertant of metalaxyl-resistant mutant.

[b] Basal (B) and induced (I) levels of GSH (μg/g f.w.) and GST (μmol/min g f.w.) after 24 h exposure to 1 mg/l metalaxyl in the thalli of *Phytophthora* strains (n.d., not detected).

[c] Inhibition of longitudinal growth ($LSD_{P=5\%} = 6.7$) by tridemorphs (50 mg/l) and propiconazole (25 mg/l).

Levels of GSH and GST are significantly increased in the thalli of *P. cryptogea, P. infestans* and *P. syringae* after exposure to acetanilides (Table VI) of widely different fungicidal potency. The induced levels of GSH and GST by metalaxyl in the strains investigated are in good correlation (Fig. 2), and the extent of induction does not seem to be related to the sensitivity of the strains to metalaxyl. The increase of the GST activity due to metalaxyl exposure is completely suppressed by the protein synthesis inhibitor, cycloheximide. The extent of the induction of GST by metalaxyl and acetochlor depends on the concentration of the inducers, and takes place at levels that are completely inhibitory for longitudinal growth of hyphae (Fig. 3). At the doses of acetanilide pesticides that stop the longitudinal growth of hyphae, mass accumulation of the thalli continues. When the inocula are transferred from this "lethal" acetanilide treatment to an untreated agar plate the thalli resume growth, showing a typical fungistatic activity of the acetanilides. Ofurace and acetochlor, however, are different: they exhibit a real lethal effect. The extent of the induction of GST correlates with pesticide inhibition of mass accumulation of the thalli (Fig. 4).

Table VI. Effects of phenylamide fungicides (1 mg/l, 24 h exposure) on the activity of GST (μmol/min g f.w.) in thalli of *Phytophthora* species.

No.	Treatments	Species *cryptogea*	*infestans*	*syringae*
	Untreated	1.01 a	0.30 c	0.48 d e
I	Metalaxyl	1.97	0.52 d f	1.19 b
II	CGA 22219	1.17 b	0.28 c	0.59 f
III	RE 26745	0.95 a	0.32 c	1.11 b
IV	Ofurace	1.63	0.41 e	0.66 f

Values labeled by the same letter are not significantly different at P = 5% level.

The efficacies (ED_{50} values) of acetanilides against various *Phytophthora* strains were subjected as variates to principal component analysis (PCA) (Table VII). The first (i) principal component (PC) accounted for more than half of the total variation indicating that the effects of most of the compounds positively correlate with each other. This PC of the correlation matrix for acetanilides appears to relate to the efficacy of these compounds, and with a low significance to the basal level of GSH in the thalli. The second PC (ii) accounted for less variation (16%) explaining strain sensitivity to ofurace (**VI**), acetochlor (**IX**) and propachlor (**XIII**), and correlates with the sen-

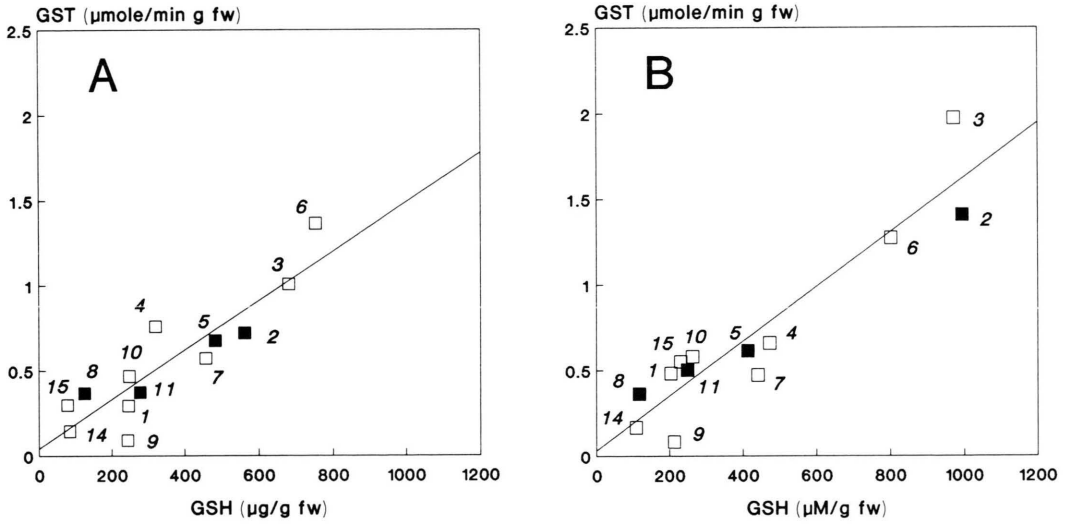

Fig. 2. Correlations between basal (A) and induced by metalaxyl (B) levels of GSH and GST in *Phytophthora* strains. GSH and GST were induced by 24 h exposure to 1 mg/l metalaxyl. The numeration of wild and metalaxyl-resistant strains (open and filled symbols, respectively) corresponds to that in Table II.

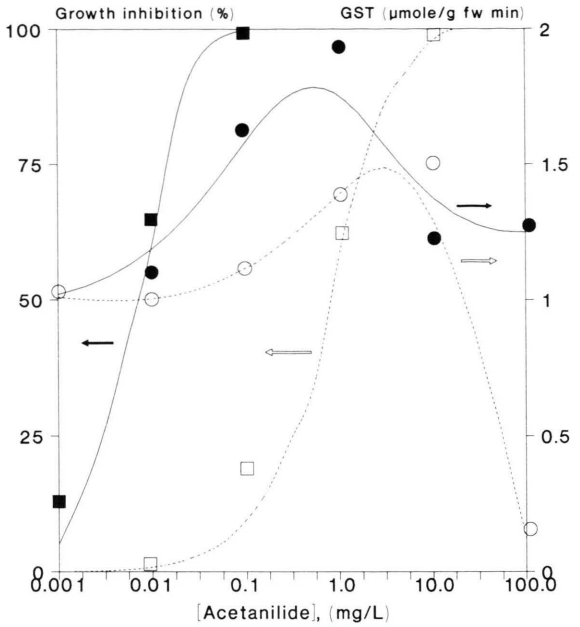

Fig. 3. Growth inhibitory (squares) and GST inducing activities (circles) of acetanilides on *Phytophthora cryptogea*. Continuous and dashed lines correspond to responses to acetochlor (**IX**) and metalaxyl (**I**) (open and filled symbols), respectively.

sitivity of the strans to *trans*-tridemorph (Fig. 5). The third PC (iii) accounted for a variation (11%) somewhat more than a single variable (7.7%) and relates to responses to CGA 22219 (**II**), ofurace (**IV**) and cyprofuram (**VIII**), and correlates with the sensitivity of the strains to *cis*-tridemorph. The fourth PC (iv) influenced solely by CGA 22219 (**II**) accounted for a variation (6.96%) at the limit of the conventionally acceptable significance [29] and correlates with the sensitivity of the strains to propiconazole (Fig. 5). PCRA results for the other parameters in Table V (growth rate, mutation rate, GST levels) are not shown because they correlate with PCs i–iv poorly.

On the basis of the results of the PCA we have attempted to form clusters of the species and of the compounds. The results show that clones of a species may belong to different clusters, indicating that the properties responsible for separation are not species-specific. High variability of the sensitivity of *Phytophthoras* to acetanilides seems to be due to their heterokaryotic and coenocytic vegetative body (thallus), in addition to the diploidic cell nuclei [14]. These make possible the presence of several alleles of a gene (a heterozygotic status),

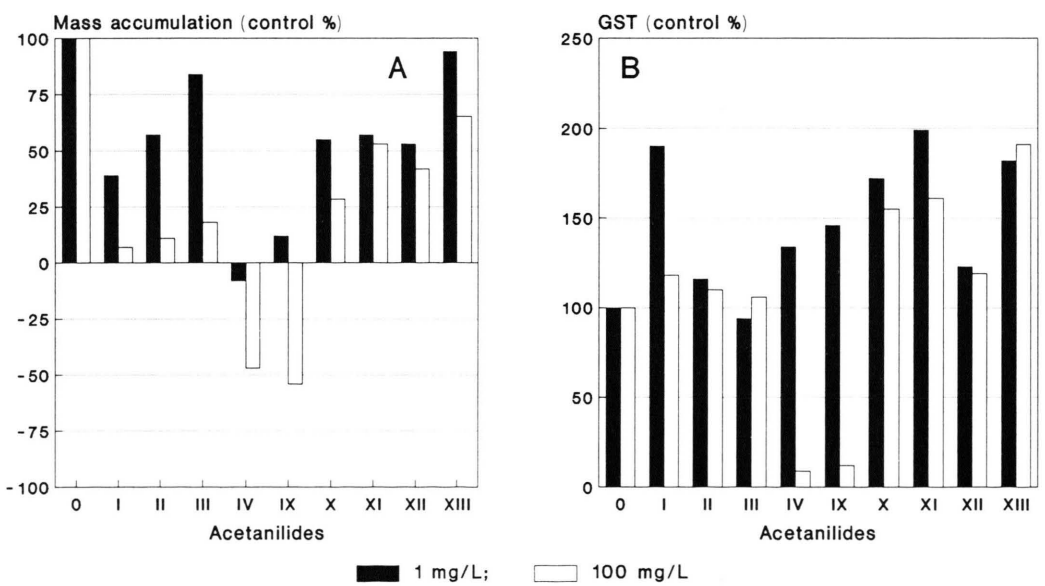

Fig. 4. Mass accumulation (A) and GST activity (B) of *Phytophthora cryptogea* thalli exposed to acetanilides. Exposure time 24 h.

Table VII. Relative weights of common properties of acetanilides as principal component variables in determining the longitudinal growth of *Phytophthora* species.

No.	Variables (compounds)	Principal components[a] i	ii	iii	iv
I	Metalaxyl	0.8674*	0.1599	−0.1652	−0.2340
II	CGA 22219	0.3339	−0.0033	−0.5971*	0.6867*
III	RE 26745	0.9405*	−0.3123	0.0225	0.0319
IV	Ofurace	−0.0295	0.5247+	0.7267*	0.1359
V	Benalaxyl	0.9271*	0.1912	0.1900	0.1215
VI	Furalaxyl	0.6672*	−0.0397	−0.4322	−0.3750
VII	LAB 149202F	0.9362*	−0.3255	−0.0081	0.0386
VIII	Cyprofuram	0.5998*	−0.3015	0.4332+	0.3636
IX	Acetochlor	0.0305	0.9120*	−0.3201	0.0825
X	Butachlor	0.7249*	−0.1302	0.1720	−0.2395
XI	Metolachlor	0.9891*	0.0403	0.0110	0.0412
XII	Dimethachlor	0.9604*	0.1134	0.0518	−0.0013
XIII	Propachlor	0.5618*	0.7511*	0.0487	−0.0666
Eigenvalues		7.0332	2.008	1.4602	0.9051
Percentage variation		54.10	15.85	11.23	6.96
Cumulative percentage		54.10	69.95	81.19	88.15

[a] The principal components are shown in the order of the amount of variation they represent. Eigenvalues having percentage variation less than 5% were omitted. The principal component loadings marked with symbols * and +, are affected significantly by the given variable ($P < 5\%$ and $P = 5-10\%$, respectively).

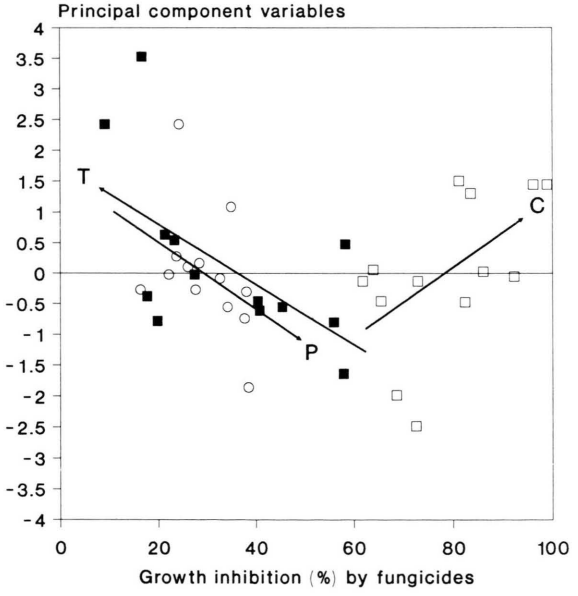

Fig. 5. Relationships between principal component variables from acetanilide efficacy data and sensitivity of *Phytophthoras* to fungicides of different modes of action. Regression plots for principal component variables **II** (filled squares), **III** (open squares) and **IV** (circles) *vs.* fungus response to *trans*-tridemorph, *cis*-tridemorph and propiconazole (lines T, C and P, respectively).

phenotypic expression of which may be related to the differences in their response to acetanilides. Clustering of the compounds can not be explained by a single chemical or physical property. These findings suggest that acetanilide pesticides have multiple sites of action on *Phytophthoras*, and their overall effect is a composite result of influences at different target sites. The presence or absence of a target site is determined by species characteristics as well as by environmental factors which can influence the phenotypical expression of the genotype. This may explain the unexpectedly large differences between effects of chemically closely similar compounds, as well as the similarities between the effects of compounds that may or may not conjugate with GSH.

Conclusion

Our data demonstrate that *Phytophthora* species can be characterized by widely different GSH-conjugation potency, which may be influenced by acetanilides. The alkylating ability of a compound (presence or absence of a 2-chloroacetyl group in the molecule) in this respect does not seem to be es-

sential. The poor correlation between the growth response of the fungi to acetanilides and their GSH or GST levels indicates that the activity of the GSH-conjugation system in *Phytophthoras* is no decisive in the regulation of their sensitivity to these chemicals. Based on our data we assume that acetanilide pesticides have multiple sites of action on the *Phytophthora* genus. For more accurate conclusions it is necessary to increase the number of species and compounds involved in the study. The results presented in this paper underline the importance of improving our knowledge on the biology and physiology of Oomycota microorganisms which, in our opinion, is a prerequisite of the development of new antioomycete compounds with high potency.

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