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CHEMICAL CONTROL OF RHODODENDRON TWIG BLIGHT CAUSED BY *PHYTOPHTHORA RAMORUM*

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Abstract: Biological activity of 6 fungicides in the inhibition of *Phytophthora ramorum* sporulation and development of blight on rhododendron leaves and stems were evaluated. All tested compounds at dose 8 μg of a.i./cm^3 already inhibited zoosporangia formation at least in 73%. On leaf petioles and leaf disks, taken from rhododendron one week after treatment with fungicides, formation of chlamydospores was especially suppressed by fenamidone + phosetyl Al and oxadixyl + mancozeb whereas development of spores was not inhibited by cymoxanil + famoxate. All tested compounds significantly inhibited the development and spread of twig blight on rhododendron. However, furalaxyl, applied as spraying of plants 48 hrs before or after inoculation of leaves and stems by *P. ramorum* was the most effective.

Key words: fungicide, sporulation, leaf, stem, necrosis, inhibition

INTRODUCTION

Phytophthora ramorum Werres, de Cock & Man in't Veld is a serious pathogen of Californian oak species and ericaceous plants, mainly rhododendron. Disease symptoms are especially observed on the top of twigs as browning of stems and leaf blades. The symptoms are often observed on other parts of stems as well. Infected leaves can be easily dropped down and become important providers of inoculum that may be spread with water and wind. In moist conditions and temperature above 10°C zoosporangia and chlamydospores are formed on lesions on leaf blades and stems resulting in secondary inoculum. Zoospores released from zoosporangia are disseminated during irrigation or rain and wind on other rhododendrons or other potential host plants. Growers prune out infected shoots to control the disease. Biological and chemical control, however, are necessary to prevent infection. Orlikowski (2003) found that grapefruit extract, applied for plant spraying, significantly inhibited the spread of *P. ramorum* on infected leaves and stems of rhododendron. In studies of Garbelotto and Rizzo (2001) and Garbelotto et al. (2002)

phosetyl – Al, metalaxyl, copper sulfate, copper hydroxide and phosphonate reduced lesion development on *Quercus agrifolia*.

The objective of this study was to evaluate the direct activity of 6 fungicides in inhibiting sporulation and ability of these compounds to control of twig blight of rhododendron caused by *P. ramorum*.

MATERIALS AND METHODS

Fungicides. Chemical compounds listed in table 1 were used *in vitro* and in greenhouse trials. Concentrations of tested compounds are given in tables 2–5. Plants were sprayed to runoff 48 hrs before or 48 hrs after inoculation of rhododendron shoots with *P. ramorum*. Control plants were sprayed with water.

Laboratory trials. Efficacy of 6 tested compounds was determined in the control of zoosporangia production of *P. ramorum*, isolate RH 122. Soil extract amended with 6 different compounds (Tab. 1) at concentration from 0 (control) to 200 $\mu g/cm^3$ was seeded with 5 mm diam. disks (4 per 90 mm Petri dish) taken from the edge of 10-day-old colony grown on oatmeal agar at 20°C. After 3 and 6-day-incubation at 20°C in the dark number of zoosporangia were counted.

Greenhouse trials. Plants of two-year old hybrid rhododendron cv. Nova Zembla, each with 4–5 shoots with young leaves and stems, grown in 3 dm^3 pots were used. Plants were transferred on greenhouse bench and grown at temperature 15°–21°C under the plastic tunnel to increase air humidity above 92%. Leaf blades and top parts of stems were inoculated with 3 mm diam. disks of *P. ramorum,* isolate RH 122**,** taken from 10-day-old V8 culture grown at 20°C in the dark. Length and diameter of necrosis on stems and leaves were measured 7 and 14 days after inoculation.

Seven days after treatment of plants with fungicides, 16 leaves with necrotic tissues were taken from each combination. Infected leaf petioles or leaf blade disks were cut on 3 mm diam. plugs and transferred into soil extract in 90-mm Petri dishes. After 3 day-incubation period, number of formed zoosporangia and chlamydospores were counted on the edge of each disk.

Experimental design was completely randomised with 4 replications and 4 Petri dishes (laboratory trials) and 4 plants in each rep. Each Petri dish contained 4 disks of mycelium or leaf plugs. In greenhouse trials at least 4 stems and 15 leaves on each plant were inoculated. Trials were repeated twice.

Table 1. Fungicides used for control of *Phytophthora ramorum*

Commercial name	Active ingredients
Aliette 80 WP	80% phosetyl Al
Fongarid 25 WP	25% furalaxyl
Mildex 711,9 WG	44 g fenamidone + 667 g phosetyl Al/dm^3
Previcur Energy 840 SL	530 propomocarb + 310 g phosetyl Al/dm^3
Sandofan Manco 64 WP	8% oxadixyl + 56% mancozeb
Tanos 50 WG	25% cymoxamil + 25% famoxate

RESULTS AND DISCUSSION

Laboratory trials. All compounds applied at 8 μg of a.i./cm^3 inhibited formation of zoosporangia at least in 73%. At this concentration fenamidone with phosetyl Al and furalaxyl were the most effective (Tab. 2). Increase of concentration to 200 μg/cm^3 resulted in completely or almost completely inhibition of zoosporangia formation (Tab. 2).

Table 2. Inhibition (in %) of zoosporangia formation of *Phytophthora ramorum* in soil extract amended with different chemicals after 6-day-incubation

Active ingredients	μg of a.i./cm^3		
	8	40	200
Cymoxanil + famoxate	75 a	83 b	98 ab
Fenamidone + phosetyl Al	95 c	100 c	100 ab
Furalaxyl	90 c	98 c	100 ab
Oxadixyl + mancozeb	84 b	87 bc	99 ab
Phosetyl Al	73 a	75 a	92 a
Propamocarb + phosetyl Al	77 ab	84 b	90 a

Means followed by the same letter do not differ with 5% of significance (Duncan's multiple range test)

Greenhouse trials. One fold fungicide application of spraying of shoots resulted in the suppression of twig blight development (Tabs. 3, 4). Efficacy of fungicides in protection of rhododendron from infection by *P. ramorum* varied greatly. Furalaxyl and oxadixyl + mancozeb applied 48 hrs before or after inoculation of leaf blades with *P. ramorum* were the most effective in the inhibition of spot development (Tab. 3). Fenamidone + phosetyl Al and phosetyl Al alone inhibited necrosis spread at least in 50%. Application of cymoxanil + famoxate 48 hrs prior inoculation was also satisfactory. Spots developed quickly during the first weak but after the next 7 days increase of spot diam. was strongly limited. Propamocarb + pho-

Table 3. Effectiveness of some chemicals in the control of *Phytophthora ramorum* on rhododendron leaves
Inoculation: 2003.05.05

Treatment	μg/ of a.i./cm^3	Diameter of necrotic spots (in mm) on inoculated leaf blades			
		48 hr before spraying		48 hr after spraying	
		a	b	a	b
Control	–	17.7 e	36.5 d	19.0 d	36.4 d
Cymoxanil + famoxate	125 + 125	13.2 d	14.5 b	5.8 ab	18.9 bc
Furalaxyl	250	0 a	4.5 a	3.4 a	7.5 a
Fenamidone + phosetyl Al	44,4 + 667	5.5 b	12.1 b	7.6 bc	15.1 b
Oxadixyl + mancozeb	160 + 1120	3.8 b	10.1 ab	3.6 a	4.5 a
Phosetyl Al	1600	9.0 c	14.0 b	4.3 a	17.6 b
Propamocarb + phosetyl Al	1060 + 620	14.9 de	21.3 c	9.9 c	24.9 c

Explanation – see table 2
a – 7 days after inoculation
b – 14 days after inoculation

Table 4. Effectiveness of some chemicals in the control of *Phytophthora ramorum* on rhododendron stems
Inoculation: 2003.05.05

Treatment	μg/ of a.i./cm^3	Length of necrosis (in mm) on inoculated stem parts			
		48 hr before spraying		48 hr after spraying	
		a	b	a	b
Control	–	24.3 f	44.9 e	27.0 d	43.3 d
Cymoxanil + famoxate	125 + 125	16.8 e	41.3 de	25.3 d	18.3 b
Furalaxyl	125	0 a	7.5 a	6.1 a	5.0 a
Fenamidone + phosetyl Al	44.4 + 667	20.1 d	26.1 b	12.0 b	33.0 c
Oxadixyl + mancozeb	160 + 1120	8.1 b	6.4 a	4.8 a	16.9 b
Phosetyl Al	1600	8.4 b	30.8 bc	18.3 c	21.6 b
Propamocarb + phosetyl Al	1060 + 620	12.4 c	35.6 cd	16.6 bc	23.0 b

Explanation – see table 2
a – 7 days after inoculation
b – 14 days after inoculation

setyl Al, applied as plant spraying, suppressed the disease spread only at 42% and 32% when applied 48 hrs before or after leaf infection, respectively (Tab. 3).

All tested compounds inhibited the spread of necrosis on rhododendron stems (Tab. 4). Furalaxyl, however, applied 48 hrs before or after inoculation was the most effective. Cymoxanil + famoxate, propamocarb + phosetyl Al and phosetyl Al used as spray 48 hrs after stem inoculation only slightly inhibited stem blight development. Application of these compounds before inoculation of plants was much more effective (Tab. 4).

Analyse of the pathogen sporulation on leaves taken from rhododendron 7 days after spraying showed that fenamidone + phosetyl Al and oxadixyl + mancozeb were the most effective in the suppression of zoosporangia and chlamydospores formation (Tab. 5). Results indicated that chlamydospores were much more tolerant to tested compounds than zoosporangia. Their production was not influenced or fungicide activity was very low when cymoxanil + famoxate, phosetyl Al and propamocarb + phosetyl Al were used as plant spray (Tab. 5).

Table 5. Inhibition (in %) of zoosporangia and chlamydospores formation of *Phytophthora ramorum* on leaf disks taken from rhododendron 7 days after spraying with different compounds

Treatment	μg/ of a.i./cm^3	Zoosporangia	Chlamydospores
Cymoxanil + famoxate	125 + 125	42 a	0 a
Furalaxyl	125	50 a	29 b
Fenamidone + phosetyl Al	44.4 + 667	73 c	57 c
Oxadixyl + mancozeb	160 + 1120	57 b	44 c
Phosetyl Al	1600	38 a	9 a
Propamocarb + phosetyl Al	1060 + 620	41 a	8 a

Explanation – see table 2

The data obtained indicated on furalaxyl as the most effective compound in the *in vitro* study. The direct action of furalaxyl at 8 $\mu g/cm^3$ on *P. ramorum* resulted in the inhibition of zoosporangia formation at 90%. The systemic and curative activity of furalaxyl indicates that this compound is easily taken up by rhododendron after foliar treatment. The major route by which the compound reaches leaf blades and stems is by penetration into the green parts with subsequent acropetal transport in the transpiration stream (Staub et al. 1978). The presence of the compound in leaves protected them against the pathogen partly by inhibition of its sporulation. Antifungal activity of acylalanine fungicides is based on inhibition of RNA biosynthesis by interference with the activity of RNA polymerase- template complex (Davidse et al. 1984). In Benson (1980) trials, metalaxyl, the main acylalanine compound, protected rhododendrons against *P. heveae* infection for 56 days. Similar efficacy like with furalaxyl was obtained when oxadixyl with mancozeb were used for foliar treatment. A recent study of fungicides activity in the control of *Phytophthora cinnamomi* has shown that phosetyl Al, applied as plant spraying or drench, strongly suppressed the development of root and stem rot of Lawson cypress (Orlikowski 1996). In the present work phosetyl Al alone or in mixture with fenamidone or propamocarb almost completely inhibited *P. ramorum* zoosporangia formation *in vitro* and at least in 38% on infected rhododendron leaves. Compounds used preventively or curatively inhibited the spread of twig blight on young stems and leaves. Inhibition of the pathogen development and spread by phosphonate might be explained by selective interference with a specific biochemical process as with other systemic compounds (Ouimette and Coffey 1989). Upon application of phosetyl Al the chemical enters the plant and is metabolised to phosphoric acid, which is very toxic to *Phytophthora* spp. In studies of Afek and Sztejnberg (1989) phosetyl controlled diseases caused by *Oomycetes* in the field and in greenhouse. In authors opinion the compound acts by inducing host defence mechanisms against pathogens by increasing concentration of scoparone. Results obtained indicated that chlamydospores of *P. ramorum* were tolerant to cymoxanil + famoxate, phosetyl Al and its mixture with propamocarb. They may survive on affected rhododendrons or other host plants even when they are treated with chemicals or biocontrol agent like grapefruit extract (Orlikowski 2003). Inhibition of zoosporangia formation on leaves invaded by *P. ramorum* was also not completed. In favoured conditions they might be the source of the pathogen inoculum. In such situation repetition of plant spraying with chemicals or biocontrol agents combined with pruning of diseased shoots are necessary.

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POLISH SUMMARY

CHEMICZNA OCHRONA RÓŻANECZNIKÓW PRZED *PHYTOPHTHORA RAMORUM* – SPRAWCY ZARAZY PĘDÓW

Celem badań było określenie aktywności biologicznej 6 środków ochrony roślin w hamowaniu rozwoju *Phytophthora ramorum* oraz ochronie liści i łodyg przed patogenem. Środki dodane do wyciągu glebowego już w stężeniu 8 μg substancji aktywnej na cm^3 w co najmniej 73% hamowały tworzenie się zarodni pływkowych patogena. Na krążkach z ogonków i blaszek liściowych, pobranych z chronionych różaneczników, tworzenie się chlamydospor najsilniej hamował fenamidon z fosetylem glinu i furalaksyl, podczas gdy cymoksanil + famoksat w ogóle nie ograniczały formowania się tych zarodników. Jednorazowe opryskiwanie różaneczników furalaksylem 48 godzin przed lub po inokulacji liści i łodyg przez *P. ramorum* okazało się najskuteczniejsze w ograniczaniu rozwoju zarazy pędów. Inne środki również istotnie ograniczały rozwój nekrozy ale ich aktywność była istotnie niższa aniżeli furalaksylu.