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Diversity in the effect of an extract from *Fallopia sachalinensis* on isolates of cucurbit powdery mildews grown on melon

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Abstract: Powdery mildew caused by *Podosphaera xanthii* and *Golovinomyces cichoracearum* is one of the principal diseases on cucurbit crops in temperate climates. In order to control this disease, various biological methods, including induced resistance by the use of extracts from *Fallopia sachalinensis*, have been identified. This study was conducted to characterise the diversity of susceptibility to this plant extract among 52 isolates of *P. xanthii* and 5 isolates of *G. cichoracearum* collected from various cucurbit species in different production areas. To this end, disks excised from melon leaves were soaked in a preparation of *F. sachalinensis* extract (1% w/v) or in a control solution, inoculated with fresh conidia of powdery mildew 24 hours after treatment and placed in a growth chamber. Ten days after inoculation, symptoms were rated in classes based on a visual estimation of the leaf area infested by powdery mildew. Additionally, spore production on the leaf disks was assessed. The plant extract significantly decreased the severity of disease for all the powdery mildew isolates tested. On average, spore production was only ca. 20% in presence of the plant extract relative to the untreated control. However, the extent of the reduction in spore production varied widely among isolates.

Key words: Induced resistance, *Fallopia sachalinensis* extract, *Podosphaera xanthii*, *Golovinomyces cichoracearum*

Introduction

Powdery mildew is one of the principal diseases on cucurbits in temperate and sub-tropical climate conditions. The ascomycetes *Podosphaera xanthii* (syn. *Sphaerotheca fuliginea*) and *Golovinomyces cichoracearum* (syn. *Erysiphe cichoracearum*) are reported to be the two main causal agents of the disease on melon. Development of melon cultivars resistant to the disease has been complicated by the presence of different races of both fungi (Pitrat *et al.*, 1998). Therefore, fungicides have been widely used but the low number of registered molecules and the possible emergence of resistant strains of the pathogens complicate their use (Hollomon & Wheeler, 2002). Various biological methods, mainly based on the utilization of antagonistic microorganisms or plant extracts, have been studied (Bélanger & Labbé, 2002) and a plant extract from the giant knotweed *Fallopia* (syn. *Reynoutria*) *sachalinensis* has shown high efficacy to control powdery mildews on various crops including cucumber (Konstantinidou-Doltsinis & Schmitt, 1998). This product is commercialized as Milsana (Germany) and Regalis (USA) as a plant strengthener or as a biopesticide, respectively. The mode of action of this plant extract is associated with induced resistance. In cucumber, accumulation of phenolic compounds showing antifungal activity, and increased activity of enzymes involved in the production of phenolic compounds, have been observed following treatments with this plant extract (Daayf *et al.*, 1997, Fofana *et al.*, 2002).

To our knowledge, the diversity of the efficacy of resistance-inducing products against plant pathogen populations has not been studied. However, as for fungicides, knowledge on

the baseline sensitivity of plant pathogens to resistance-inducing compounds appears to be necessary to determine the risk of possible adaptation of pathogen populations in response to selection pressure. A study was then carried out to obtain the initial data regarding the range of sensitivity of cucurbit powdery mildews to the *F. sachalinensis* extract, and to detect any differences in sensitivity between isolates that might lead to development of resistance.

Material and methods

Collection of fungal isolates and inoculum production

Fifty two isolates of *P. xanthii* were sampled from cucurbit crops between 1989 and 1996 in different locations and were conserved in liquid nitrogen as previously described (Bardin *et al.*, 2007). Five isolates of *G. cichoracearum* were collected from cucurbit crops in France between 1989 and 1996 and maintained by sub-culturing on surface sterilized cotyledons of *Lagenaria leucantha* on an agar medium. These isolates were representative of the different races defined by Pitrat *et al.* (1998). All isolates were single-spored.

Inoculum was produced on cotyledons incubated in a growth chamber (21°C, 12 hours light) for 14 days as described by Bardin *et al.* (2007).

Preparation of the extract of F. sachalinensis

The extract from *F. sachalinensis* was prepared from dried and ground plant powder. One gram of powder was mixed with 4ml acetone. After 10 minutes, 100ml of a water solution containing 0.0125% of Tween 20, heated beforehand to 50°C, were added and the mixture was allowed to sit for 1 hour, while being occasionally stirred. The mixture was then filtered through filter paper and the water extract (1% w/v) was used fresh.

Bioassays using detached melon leaves

Leaf disks (1.5cm diameter) were excised from the first and second fully developed leaves of four-week old melons cv. 'Védrantais'. They were soaked (adaxial side up) for 1 minute in a 1% (vv) solution of the plant extract or in two types of controls (water or a solution containing 0.0125% vv Tween 20 and 4% vv acetone). Leaf disks were then blotted on filter paper, placed (adaxial side up) in Petri plates on 1% water agar amended with 30ml/l benzimidazole, and kept for 24 hours in a growth chamber (21°C, 12 hours of light). Inoculation was performed by blowing dry conidia from the surface of a mildewed cotyledon into a settling tower. Inoculum concentration was checked by placing a haemocytometer next to the leaf disks at the bottom of the tower and assessed under a microscope (x100). Inoculum density was comprised between 141 and 843 spores/leaf disk according to the isolate. The leaf disks were then incubated in a growth chamber (21°C, 12 hours of light). For each strain, ten disks were inoculated per type of treatment and three repetitions were carried out.

Disease assessment and data analysis

Ten days after inoculation, the symptoms were rated individually for each leaf disk on a scale from 0 (no detectable fungal growth) to 9 (entire disk covered with heavy sporulation). Then, spore production on the disks was quantified. Disks were immersed in a solution containing 0.02% Tween 80 and vortexed to detach the spores. Spore concentration was assessed under the microscope with the help of a haemocytometer and results were expressed as numbers of spores produced per leaf disk, 10 days after inoculation.

The correlation between disease symptoms and spore production was performed using Spearman rank correlation test. Other statistical analyses consisted of one- or two-way

analysis of variance (ANOVA, $\alpha = 0.05$), followed when appropriate by a comparison of means using the test of Newman and Keuls.

Results and discussion

Estimation of the aggressiveness of isolates

All powdery mildew isolates grew and sporulated on the control leaf disks. Spore production by the 52 isolates of *P. xanthii* ranged from $2.4x10^5$ to $1.0x10^6$ spores/leaf disk. For *G. cichoracearum* spore production by the 5 isolates ranged from $1.4x10^5$ to $1.2x10^6$ spores/leaf disk. The disease severity index (on a scale from 0 to 9) was significantly correlated with the number of spores present on the leaf disks ($R = 0.88$ and $R = 0.93$, $P < 0.0001$ for *P. xanthii* and *G. cichoracearum*, respectively). Significant differences in disease severity and sporulation were detected among isolates of both species (ANOVA, $p < 0.001$).

Protective effect of the* F. sachalinensis *extract

The plant extract significantly decreased both disease severity and the sporulation level for all the isolates tested, suggesting that induced resistance by this product was highly effective against all tested powdery mildew fungi populations. On average for *P. xanthii* and *G. cichoracearum*, spore production on the leaf disks in presence of the plant extract was only approximately 20% and 17%, respectively, as compared to the untreated control (ANOVA, $P < 0.001$, Table 1).

Table 1. Effect of an extract of *Fallopia sachalinensis* on the development of powdery mildew on leaf disks of melon. Average based on the analysis of 52 isolates of *P. xanthi* and 5 isolates of *G. cichoracearum*.

	Podosphaera xanthii				*Golovinomyces cichoracearum*			
	Spores / leaf disc (X1000)		Scale (0-9)		Spores / leaf disc (X1000)		Scale (0-9)	
Water control	673	a[y]	7.32	a	824	a	7.87	a
WTA control [x]	734	a	7.08	b	not done		7.50	a
Plant extract	141	b	2.28	c	138	b	1.87	b

x WTA control: Water Tween Acetone control.

y Numbers in column followed by the same letters are not significantly different ($p < 0.05$) based on Newman-Keuls tests for multiple mean comparisons.

However, the extent of reduction in spore production varied widely among isolates and significant differences in sensitivity to the plant extract-induced resistance in melons were detected (ANOVA, $P < 0.001$, Figure 1). A baseline sensitivity of powdery mildew populations (*P. xanthii*) to the direct effect of an extract from rhubarb has been established (Yang *et al.*, 2008), but to our knowledge, this is the first study characterizing the baseline

sensitivity to a plant-resistance inducing compound. The diversity of sensitivity was relatively high because the range factor (maximal sporulation value/minimal sporulation value of isolates) was close to 13 for *P. xanthii* and 10 for *G. cichoracearum*. This suggests that these fungi may have a potential to sporulate on *F. sacchalinensis* extract induced plants and continuous use of this plant extract on a commercial scale may lead to the selection of resistant isolates. However, germination of *P. xanthii* spores produced on *F. sachalinensis* extract treated cucumbers *in vitro* was only 50% compared to spores from untreated plants (Schmitt, personal communication). To finally elucidate the potential risk of resistance development in *P. xanthii* and *G. cichoracearum* successive generations of these fungi under selection pressure with the plant extract should be realized.

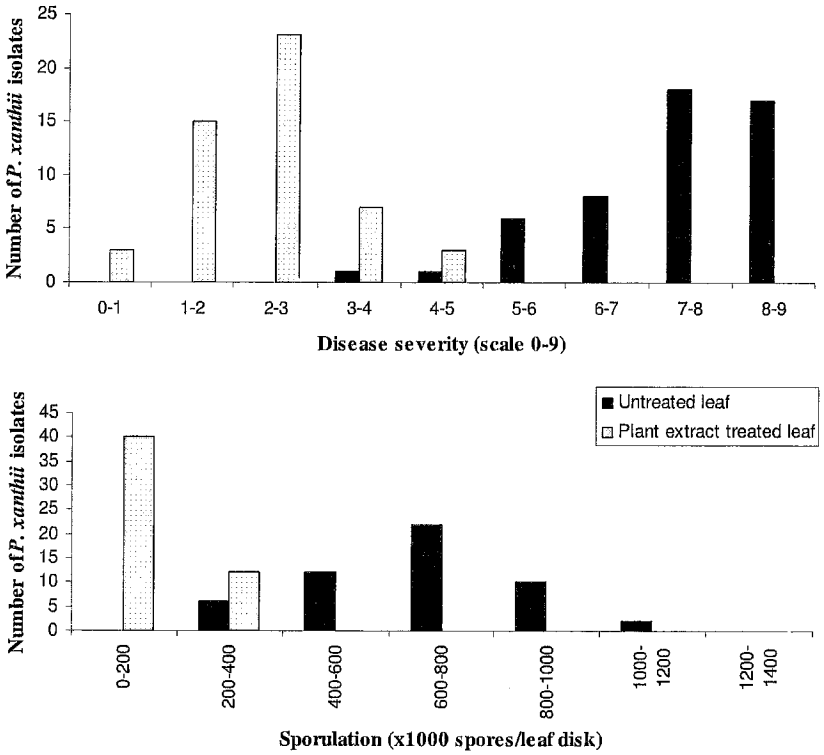

Figure 1. Effect of *Fallopia sachalinensis* plant extract on the development and sporulation of *Podosphaera xanthii* isolates on leaf disks of melon

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