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Effect of potassium silicate on epidemic components of powdery mildew on melon

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The effects of silicon (Si) supplied in the form of potassium silicate (PS) were evaluated on epidemic components of powdery mildew of melon under greenhouse conditions. The PS was applied to the roots or to leaves. In the first experiment, epidemic components were evaluated after inoculation with *Podosphaera xanthii*. In the second experiment, the disease progress rate was evaluated on plants subjected to natural infection. The area under the disease progress curve was reduced by 65% and 73% in the foliar and root treatments, respectively, compared to control plants, as a consequence of reductions in infection efficiency, colony expansion rate, colony area, conidial production and disease progress rate. However, root application of PS was more effective than foliar application in reducing most of the epidemic components, except for infection efficiency. This can be explained by the high Si concentration in leaf tissues with root application, in contrast to the foliar treatment where Si was only deposited on the external leaf surfaces. The effects of PS reported in this study demonstrated that powdery mildew of melon can be controlled, and that the best results can be achieved when PS is supplied to the roots.

Keywords: *Cucumis melo*, epidemiology, plant protection, *Podosphaera xanthii*, silicon

Introduction

Melon (*Cucumis melo*) is the main fresh fruit export of Brazil. The cultivated area increased by 47% between 2000 and 2007, reaching more than 21 500 ha in 2007, and resulting in an estimated 500 000 metric tons of fruits produced (Agrianual, 2010). This increase has occurred mainly in the north-eastern growing region because of the favourable climate that allows melon plants to be grown throughout the year. A serious threat and a limiting factor to this crop in this region is the occurrence of powdery mildew caused by *Podosphaera* (sect. *Sphaerotheca*) *xanthii*, the most common and widespread disease of cucurbits (Pérez-Garcia *et al.*, 2009). The pathogen is easily recognized by the abundant production of mycelia, conidiophores and conidia on the surface of leaves, petioles and stems (Zitter *et al.*, 1996).

Powdery mildew reduces the photosynthetic rate of melons, causes yellowing and premature death of leaves and, in severe infections, may kill the plant. Although the fungus does not infect the fruits, the reduction in leaf area and changes in the carbohydrate contents of infected leaves can reduce the number and size of fruits and alter their flavour, pulp thickness and amounts of total soluble solids and sugars (Zitter *et al.*, 1996; Abood & Lösel, 2003; Santos *et al.*, 2005; Queiroga *et al.*, 2008).

Podosphaera xanthii can be controlled through genetic resistance by using race-specific genes (Kuzuya *et al.*, 2006). However, this resistance is not durable because of rapid genetic changes within the pathogen population (Hosoya *et al.*, 2000). For this reason, chemicals are also used as a control method. However, there are several reports on the selection of isolates either resistant or insensitive to different groups of fungicides (McGrath, 2001). Thus, the development of additional control measures with low environmental impact represents an important research topic for this disease.

Despite not being considered as an essential nutrient for most plants to complete their life cycles, it has been widely reported that silicon (Si) reduces the effects of biotic and abiotic stresses (Epstein, 2009). Although the physiological and molecular mechanisms underlying this phenomenon are still poorly understood, accumulation of Si in plant tissues is known to be a necessary condition for these observable effects (Datnoff *et al.*, 2007; Dallagnol *et al.*, 2009). It has been demonstrated that Si reduces several diseases caused by necrotrophic, hemibiotrophic and biotrophic pathogens in both monocotyledons and dicotyledons (Datnoff *et al.*, 2007). In rice, which is a model plant for studies with Si, application of this element reduced the severity of blast, brown spot and

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sheath blight by affecting epidemic components (Datnoff *et al.*, 2007; Dallagnol *et al.*, 2009). In addition, control of biotrophic pathogens by Si has been reported for powdery mildew in oat, wheat, barley, rye, *Arabidopsis*, cucumber, pumpkin and strawberry (Menzies *et al.*, 1991a, 1991b; Bélanger *et al.*, 2003; Ghanmi *et al.*, 2004; Datnoff *et al.*, 2007). In cucumber, for example, Si reduced the severity of *P. xanthii* infections by reducing the number of colonies and their size, the germination frequency of conidia, and the number of haustoria per colony (Menzies *et al.*, 1991a, 1991b).

The reports by Menzies *et al.* (1991a, 1991b) suggested that Si can also be used to control powdery mildew in melon. Thus, the present study investigated the effects of foliar and root applications of potassium silicate on various epidemic components and on disease progress in melon plants grown under greenhouse conditions.

Materials and methods

Plant material and growth

The same melon hybrid (cv. Jangada) and growing conditions were employed in both experiments described below. Seeds were sown in 8-L plastic pots containing 2 kg Plantmax substrate (Eucatex), the physicochemical characteristics of which were as follows: 335·5 g kg^{-1} of ash, pH $CaCl_2$ (0·01 M) = 5·3; P (resin) = 315 mg dm^{-3}; S = 494 mg dm^{-3}; K (resin) = 16·8 mmol dm^{-3}; Al^{3+}, Ca^{2+}, Mg^{2+} and H + Al = 0·0, 150, 125 and 52 mmol dm^{-3}, respectively; base saturation = 85%; organic matter = 265 g dm^{-3}; B, Cu, Fe, Mn and Zn = 1·2, 0·9, 160, 7·4 and 2·0 mg dm^{-3}, respectively; and density = 0·37 kg dm^{-3}. The concentration of available Si (extracted with 0·01 M $CaCl_2$) was 25·2 mg dm^{-3}. Plants were kept in a greenhouse during the experiments and after the emergence of the cotyledons they were fertilized weekly with 50 mL nutrition solution composed of 40 mM KNO_3, 10 mM $NH_4H_2PO_4$, 10 mM $MgSO_4{\cdot}7H_2O$, 15 mM $Ca(NO_3)_2{\cdot}4H_2O$, 2·4 mM $ZnSO_4{\cdot}7H_2O$, 3 mM H_3BO_3, 10 mM K_2SO_4, 3·3 mM CH_4N_2O and 7·5 mM $NH_4H_2SO_4$. The photon flux density on sunny days inside the greenhouse (at noon) quantified with a Li 250 Light meter (Li-Cor) was approximately 800 μmol photons m^{-2} s^{-1}. The daily mean temperatures ranged between 20 and 28°C.

Effects of Si on epidemic components

The effects of Si applied as potassium silicate (PS) on epidemic components were evaluated in two trials conducted in September 2008 and April 2009. The source of PS was the commercial product Sili-K (Unaprosil), which is composed of 15% K_2O (210 g L^{-1}) and 12·2% soluble Si (171 g L^{-1}). The product was applied by spraying all leaves (foliar treatment) or through the irrigation water (root treatment). The control treatment consisted of plants sprayed with water only. The three treatments were arranged in a completely randomized design with eight replications, each consisting of a single pot containing two plants.

For foliar application, PS was applied at 30 mL L^{-1} (pH 10·2), 24 h before inoculation, as a fine mist on the upper surface of the leaves until runoff using a 0417·02·00 model handheld manual sprayer (Guarany). For the root treatment, pots were irrigated daily with 250 mL PS solution diluted in deionized water at a final Si concentration of 2 mM (0·34 mL L^{-1} of PS) with the pH adjusted between 5·5 and 6·5 using NaOH (1 M) or HCl (1 M) as necessary. In order to normalize the amount of potassium supplied to plants in the root treatment, plants from the foliar and control treatments were irrigated daily with 250 mL 1·53 mM KCl (Sigma-Aldrich) solution with the pH ranging from 5·5 to 6·5 and adjusted as described above.

An isolate of *P. xanthii* was obtained from a commercial melon crop and multiplied successively on melon cv. Jangada. Conidial suspension was prepared according to Cohen (1993) with some modifications. Conidia were blown off infected leaves 10 days after inoculation using a hair dryer and the newly produced conidia were harvested 24 h later in water containing 0·002% Tween. The suspension was homogenized and the concentration adjusted to 2×10^4 conidia mL^{-1}. This suspension was sprayed onto melon leaves as a fine mist using a 0417·02·00 model handheld manual sprayer (Guarany).

The following epidemic components were evaluated on the third, fourth and fifth leaf of each plant: latent period (LP), infection efficiency (IE), rate of colony expansion (r_c), colony area (CA), conidial production per colonized area, and disease severity. The LP (h) was assessed by examining the leaves every 12 h after inoculation and was defined as the interval between inoculation and visualization of the first sporulating colonies. The IE was expressed as the ratio between the number of colonies per cm^2 of leaf and the estimated number of conidia deposited per cm^2 after inoculation. To achieve this, coverslips (2 × 2 cm) were placed randomly on the upper surface of three leaves per replicate before inoculation and the number of conidia deposited on each coverslip was counted just after inoculation using an E200 light microscope (Nikon). The number of colonies per leaf area (cm^2) was counted 7 days after inoculation at three distinct regions of the leaves chosen at random. The r_c was the angular coefficient obtained by the linear regression of colony diameter as a function of time. Mean colony area was calculated from the measurement, with a translucent ruler, of five colonies per leaf, picked at random 10 days after inoculation (i.e. when colonies started to coalesce on the control plants). The production of conidia per colonized area was estimated based on three samples of 2 cm^2 leaf tissue taken from three leaves of four replicates from each treatment 13 days after inoculation. Twenty-four hours before sampling, old conidia were dislodged as described above and a Citoval 2 stereomicroscope (Carl Zeiss) was used to ensure that all old conidia were removed from the leaf surface. Images of

the leaf samples were captured by a Power Shot A430 digital camera and the colonized area was assessed using the software QUANT (Liberato, 2003). Conidia were sampled by rinsing the leaf samples in 5 mL water with 0·002% Tween and were counted using a haemocytometer. The number of conidia per colonized area was expressed as the ratio between the total number of conidia and the colonized area (mm^2) of each sample. Disease severity was evaluated on individual leaves every 48 h from 8 to 20 days after inoculation as the percentage leaf area covered by powdery mildew (McGrath, 1996) and data were used to calculate the area under the disease progress curve (AUDPC) for each leaf of each plant according to Shaner & Finney (1977).

Analyses of Si and K in leaf tissues

Leaf samples taken from plants from the trials described above were analysed for Si content by both colorimetric and X-ray analyses. In the first case, leaf samples were collected from each replicate at the end of the trials, rinsed with deionized water, dried for 72 h at 65°C, and ground to a fine powder using a mortar and pestle. The silicon concentration was determined by colorimetric analysis of 0·1 g alkali-digested tissue (Korndörfer *et al*., 2004). The potassium concentration was also determined in a flame photometer using 0·2 g dry tissue submitted to nitric-perchloric acid digestion (Zasoski & Burau, 1977).

The X-ray analysis was carried out in order to compare the external and internal leaf contents of Si and K. For this, four leaf discs (1 cm^2) were sampled randomly from each plant at the end of the trials. Two discs were rinsed with deionized water and dehydrated for 120 h in silica gel, while the other two were dehydrated in the same way without rinsing. Afterwards, the discs were mounted on aluminium stubs and covered with carbon (MED 010, Balzers Union). The samples were examined under a scanning electron microscope (Zeiss DSM 940A) coupled with an energy dispersive X-ray spectroscopy device (EDS, Oxford Instrument Link ISIS) operating at 15 kV with a working distance of 10 mm. The elements Si and K were identified in the EDS spectrum by their expected α line energy peaks of 1·739 and 3·312 keV, respectively.

Effects of Si on disease progress rate

A second experiment was carried out in March–April 2009 and repeated in September–October 2009 to evaluate the effect of PS on the progress of powdery mildew. The treatments were the same as described previously, except that PS was sprayed on leaves (foliar treatment) weekly starting with the appearance of the first leaf. The inoculum source for natural infection consisted of melon plants infected with *P. xanthii* randomly distributed around the experimental area. The three treatments were arranged in a completely random design with five replications. The experimental unit consisted of one plant per pot.

Disease severity was evaluated every 4–5 days on every leaf after visualization of the first symptoms as described above. Disease severity was analysed separately in the lower, middle and upper portions of the plants because of differences in leaf phenology. Disease progress curves for the three portions were obtained by plotting disease severity as a function of time.

Data analyses

Homogeneity of variances for all variables was tested using Cochran's test (Gomez & Gomez, 1994). Analyses of variance (ANOVA) and comparisons of treatments means by Tukey's test ($P = 0{\cdot}05$) were done using SAS (version 8·0; SAS Institute, Inc., 1989). The disease progress rate (r) was determined using the software STATISTICA version 7 (Statsoft) and the logistic model $Y = 1/1 + \exp(-(\beta + r^*t))$, where Y is the disease severity expressed as a proportion, β is the constant of integration, r is the disease progress rate and t is the time in days (Campbell & Madden, 1990). Progress rates were compared among treatments by a t-test using the formula $t_{cal\ (5\%)} = ((P_1 - P_2)/\sqrt{\sigma_{p1}{}^2 + \sigma_{p2}{}^2})$, where P is the value of the variable and σ is the standard deviation (Campbell & Madden, 1990).

Results

Effects of Si on epidemic components

Data from the two trials were analysed together since all components showed normal distributions and homogeneous variances (data not shown). Both leaf and root applications of PS significantly reduced ($P \leq 0{\cdot}05$) all components of resistance compared to the control treatment (Fig. 1), except for LP (Fig. 1a). No difference in the IE was detected between the leaf and root treatments ($P = 0{\cdot}24$), but it was 57% and 52% lower, respectively, in these treatments than in the control (Fig. 1b). However, compared to the foliar treatment, the root treatment significantly reduced ($P \leq 0{\cdot}05$) r_c by 28% (Fig. 1c), CA by 38% (Fig. 1d), conidial production per cm^2 colonized area by 54% (Fig. 1e) and AUDPC by 24% (Fig. 1f).

Analyses of Si and K in leaf tissues

Colorimetric determination of the Si concentration in leaf samples indicated that root applications increased the concentration of Si by up to 210% and 170% compared to the control and the foliar treatments, respectively (Fig. 2a). By contrast, no difference ($P = 0{\cdot}32$) was detected between the foliar and the control treatments. No significant difference was found ($P = 0{\cdot}55$) among the treatments regarding the concentration of K (Fig. 2b).

The X-ray analysis of non-rinsed leaves indicated that the energy peaks of Si were higher in the root (Fig. 3a) and leaf treatments (Fig. 3c) than the control (Fig. 3e), and that rinsing of the leaves decreased these peaks. However,

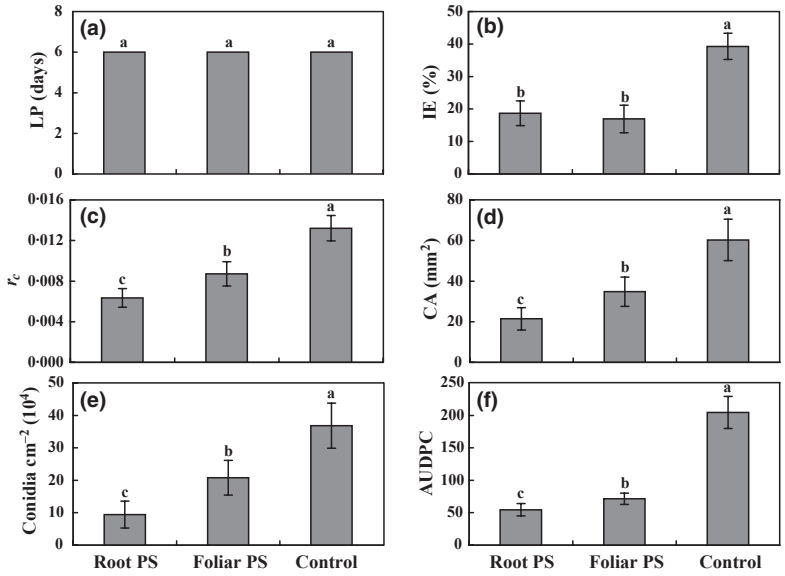

Figure 1 Latent period (LP; a), infection efficiency (IE; b), colony expansion rate (r_c; c), colony area (CA; d), number of conidia produced per leaf area (e) and area under the disease progress curve (AUDPC; f) of melon plants that received root, foliar or no applications (control) of potassium silicate (PS) and were inoculated with *Podosphaera xanthii*. Bars represent standard deviation of the mean. The data shown represent the combination of the two experiments (n = 16). Means followed by different letters in the same graph are significantly different based on Tukey's test.

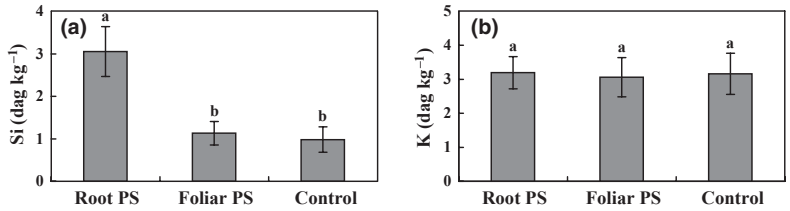

Figure 2 Concentrations of silicon (Si; a) and potassium (K; b) in leaves of melon plants that received root, foliar or no applications (control) of potassium silicate (PS). Bars represent standard deviation of the mean. The data shown represent the combination of the two experiments (n = 16). Means followed by different letters in the same graph are significantly different based on Tukey's test.

in the root treatment (Fig. 3b), this decrease was not as intense as in the foliar (Fig. 3d) and control (Fig. 3f) treatments.

The energy peak of K in non-rinsed leaves was lower in the root (Fig. 3a) than the foliar (Fig. 3c) and control treatments (Fig. 3e). For rinsed leaves, the energy peaks of the root and leaf treatments (Fig. 3b, d, respectively) were lower than that of the control (Fig. 3f).

Effect of Si on disease progress rate

The high R^2 values showed that the data were a good fit to the logistic model (Table 1). Compared to the control, the application of PS reduced the disease progress rate (r) irrespective of its form of application or leaf position (Table 1). However, root application was significantly more effective in reducing r than foliar application, the exception being for the middle leaves of the plant in the second trial (Table 1).

The lower r values in response to PS applications were reflected in the disease progress curves (Fig. 4a–d). In both treatments with PS, the disease progress was reduced on both the lower (Fig. 4a,b) and middle (Fig. 4c,d) leaves of the plants. Disease progress curves were not plotted for the upper leaves because of the small number of data points resulting from the duration of the trials. However, in the last evaluation of both trials, disease severity was lower for the PS treatments than for the controls (Fig. 4e,f). Additionally, application of PS through the roots was significantly more effective in reducing severity levels in these leaves than were foliar applications.

Discussion

The concentration of Si in plant tissues varies greatly among species, ranging from <0·1 dag kg^{-1} to more than 10 dag kg^{-1} dry weight (Datnoff *et al.*, 2007). Based on this, plants are classified as accumulators, intermediate or

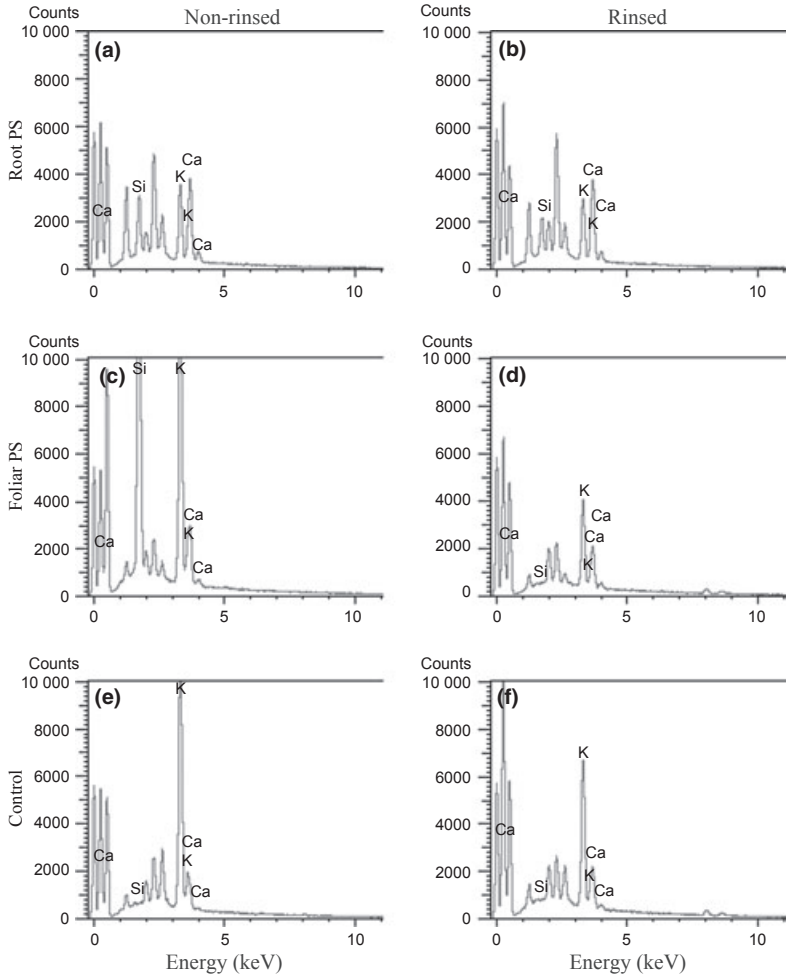

Figure 3 X-ray microanalysis of non-rinsed (a, c and e) and rinsed (b, d and f) leaves of melon plants that received root (a and b), foliar (c and d) or no applications (control; e and f) of potassium silicate (PS).

excluder species (Takahashi *et al.*, 1990). Melon has been classified as an intermediate-type plant (Takahashi *et al.*, 1990; Voogt & Sonneveld, 2001), which was also confirmed in this study since plants that received root applications of PS accumulated up to 3 dag Si kg^{-1} in the shoot dry weight, according to the colorimetric analyses. Furthermore, as shown for rice (Rezende *et al.*, 2009), X-ray analyses indicated that Si accumulated in leaves only when Si was root-applied, since foliar-applied Si could be easily removed by rinsing.

Despite the differences of Si distribution related to the way in which PS was applied to the plants, both root and foliar applications affected all the epidemic components when compared to the non-Si control treatment, except for the LP. However, the effects of PS application to roots were more pronounced since it resulted in greater reductions of r_c, CA and conidial production than foliar application. The effects of both forms of PS application on the epidemic components correlated with lower values of AUDPC and the effect on this variable was greater when PS was supplied to the roots. The differences in the rate of colony expansion (r_c) probably impacted AUDPC more than conidial production per colonized area because of the short period during which the evaluations were made (20 days), which reflected the effects of only two extra disease cycles assuming a latent period of 6 days. Moreover, r_c was previously shown to have an impact on the final size of the colony and has a substantial effect on the shape of the disease progress curve and, consequently, on AUDPC (Berger *et al.*, 1997). However, under natural epidemic conditions, greater effects on AUDPC are expected from reductions in conidial production as the number of disease cycles is increased. In addition, it is expected that lowering the amount of conidia may increase the effectiveness of chemical control by reducing the population size, thus reducing the risks of fungicide resistance (McGrath, 2001).

The better results of root treatment with PS compared to foliar treatment may be related to differences in the distribution of Si, as discussed above. It is known that, when applied to leaves, PS deposits on the external surface of the leaf and acts both as a physical and as a chemical

Table 1 Disease progress (*r*) and coefficients of determination (R^2) estimated by the logistic model for powdery mildew (caused by *Podosphaera xanthii*) disease progress on lower and middle leaves of melon plants amended with potassium silicate (PS)

	Lower leaves		Middle leaves	
Treatment	*r*	R^2	*r*	R^2
Trial 1				
Root PS	0·089 c	0·97	0·156 c	0·97
Foliar PS	0·130 b	0·96	0·209 b	0·97
Control PS	0·253 a	0·99	0·336 a	0·98
Trial 2				
Root PS	0·106 c	0·97	0·183 b	0·98
Foliar PS	0·166 b	0·96	0·178 b	0·97
Control	0·323 a	0·99	0·334 a	0·99

Root PS = PS supplied via irrigation; Foliar PS = PS applied by foliar spray; Control = non-amended plants. Means within columns followed by different letters are significantly different based on *t*-test.

barrier as the result of an increase in both pH and osmotic potential after water evaporation (Liang *et al*., 2005; Rodrigues *et al*., 2010). The observation that the lesions which formed in leaf patches not covered by PS were similar in size to those seen with the control treatment agrees with this hypothesis. In contrast, when applied to roots, Si accumulated in the leaf tissues, where it is also reported to form a physical barrier, but in this case as a result of distinct mechanisms operating at the cellular level, i.e. stimulation of cell wall reinforcement and polymerization underneath the cuticle, increasing its rigidity, as reported in rice and strawberry (Kanto *et al*., 2004; Hayasaka *et al*., 2008). In addition to these physical effects, studies involving powdery mildew in cucumber, *Arabidopsis*, wheat and strawberry reported an anticipation in the expression of enzymes associated with the plant's defence system (priming effect) and in the production and accumulation of phenolic compounds and phytoalexins at infection sites (Bélanger *et al*., 2003; Ghanmi *et al*., 2004; Liang *et al*., 2005; Kanto *et al*., 2006; Datnoff *et al*., 2007). Thus, the greater control of powdery mildew achieved in the plants which received the root treatment could result from the combination of these cellular and priming effects.

The benefits of PS applications were also detected in plants kept in the greenhouse and subjected to natural infection. In this case, regardless of its form of application, PS reduced the disease progress rate compared to the control treatment, resulting in lower disease severity in the lower, middle and upper leaves of the plants. In this case, however, the results were more variable between trials, perhaps because of variations in environmental conditions, but also variations in the amount and distribution of inoculum. Nevertheless, as with effects on epidemic components, supplying PS to the roots was more efficient at controlling the disease than foliar application in most comparisons.

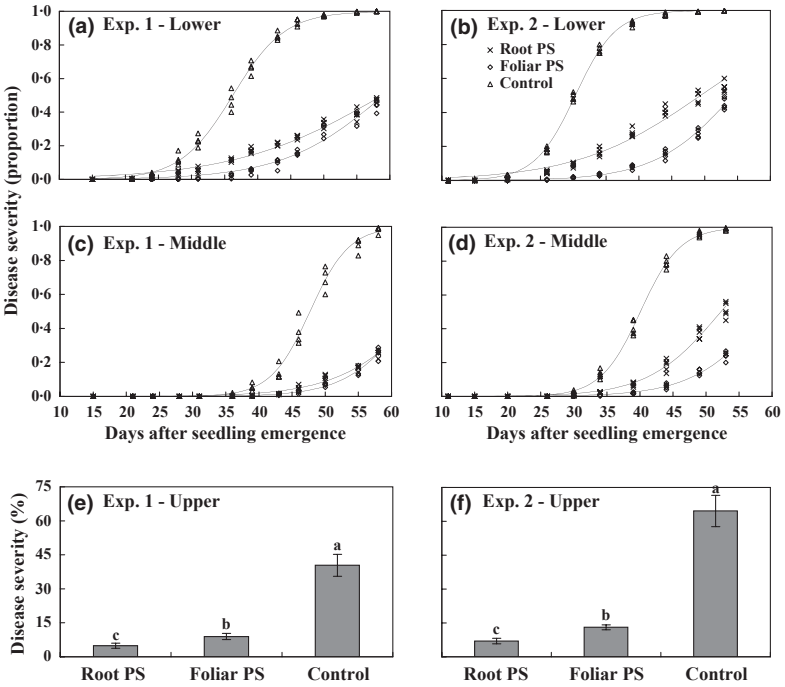

Figure 4 Disease progress curves of powdery mildew caused by *Podosphaera xanthii* in the lower (a and b) and middle parts (c and d) of melon plants, and disease severity in the last evaluation on the upper part (e and f) of plants that received root, foliar or no applications (control) of potassium silicate (PS). Values of observed disease severity (proportion) are represented by symbols, while lines represent the values of disease severity estimated by an equation adjusted by the logistical model. Means followed by different letters in graphs e and f are significantly different based on Tukey's test.

An additional effect of the reduced disease severity in plants treated with PS, regardless of its method of application, was the delay in leaf senescence in the lower and middle portions of the plant. By contrast, in plants which received the control treatment, the higher values of *r* resulted in severity values close to 100% in these leaves, resulting in their premature death. In these plants, at around 60 days after emergence, only the upper part of the plant maintained green leaves, with more than 40% and 65% of the leaf area affected by powdery mildew in the first and second trials, respectively. Premature leaf senescence would reflect negatively on the production and quality of fruit since previous studies have shown a direct correlation between the number of leaves contributing to photosynthesis and fruit quality and productivity (Hubbard *et al*., 1990; Queiroga *et al*., 2008).

The results of this study show the potential of Si to affect epidemic components and reduce powdery mildew severity in melon. The reduction in disease severity as a result of root applications of PS was similar in magnitude to that observed in cucumber and strawberry plants infected with powdery mildew and supplied with Si (Menzies *et al*., 1991b; Kanto *et al*., 2006), but the reduction in disease severity observed in the present study was not as profound as that reported for wheat, in which Si applications accounted for a reduction of nearly 90% in disease severity (Bélanger *et al*., 2003). With regard to foliar applications, reductions in disease severity were greater than those observed in cucumber and strawberry, where this form of application did not result in significant disease control (Liang *et al*., 2005; Palmer *et al*., 2006). Thus, the incorporation of PS in an integrated management programme should bring positive results, both in terms of reducing disease intensity and, consequently, the amount of fungicides used. With regard to the form of Si application, root application could be recommended both for field and protected melon crops, whereas foliar could be recommended in the latter case because of its deposition on the leaf surface. However, it could also be adopted in crops grown in the dry season with drip irrigation, as is the case in the north-eastern growing region of Brazil.

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