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Restriction of potato and tomato late blight development by sub-phytotoxic concentrations of boron

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Boron is a microelement required for normal growth and development of plants but its positive effect is restricted to a narrow range of concentrations. The gradual increase in use of recycled water, which contains high concentrations of boron for irrigation, has already raised the level of boron in soils and plants in southern Israel. This research was conducted to examine the direct effects of sub-phytotoxic boron concentrations on potato late blight epidemics and to explore the mode of action of boron against *Phytophthora infestans*. When boron was applied alone to field grown potato plants it did not affect the epidemic. However, together with a reduced rate of the fungicide Melody Duo (propineb + iprovalicarb), boron improved late blight suppression compared to plants treated with the fungicide alone. The ED_{50} of boron against *P. infestans* ($256{\cdot}4$ mg L^{-1}) was about 6400 times higher than the ED_{50} value of the fungicide chlorothalonil ($0{\cdot}04$ mg L^{-1}), indicating that boron does not have a direct fungicidal activity that would explain the level of protection seen in the field. In greenhouse experiments conducted with potted tomato plants, boron decreased late blight severity in both treated leaves and distant leaves not treated with boron. The results suggest that boron is active locally but also may induce systemic acquired resistance against *P. infestans*.

Keywords: iprovalicarb, *Phytophthora infestans*, propineb, *Solanum lycopersicum*, *Solanum tuberosum*, systemic acquired resistance

Introduction

Boron is a microelement essential for plant development and metabolism. It is involved in plant cell wall stability (Blevins & Lukaszewski, 1998), sugar transport (Brown & Hu, 1996), cell membrane function (Blevins & Lukaszewski, 1998), and gene expression (Camacho-Cristobal *et al.*, 2008). However, boron's positive influence is restricted to a narrow range of concentrations in soil solution. Boron deficiency may limit leaf and root development and reduce plant growth (Blevins & Lukaszewski, 1998). On the other hand, excess concentrations are phytotoxic and may cause chlorotic and necrotic lesions on leaves. Accumulation of this element in plant tissues in high concentrations may lead to leaf death and even whole-plant mortality (Nable *et al.*, 1997). In species in which boron is phloem mobile (e.g. apple, almond and nectarine) the symptoms of toxicity are flower and fruit disorders, bark necrosis, which appears to be due to death of cambial tissues, and stem die back (Brown & Hu, 1996). The extent of boron toxicity symptoms is a function of boron accumulation in the plant organs, which in turn depends on the boron concentration in soil solution, length of exposure, transpiration rate, and plant genotype (Nable *et al.*, 1997).

In Israel, 91% of all municipal sewage is treated, 73% of which is used for irrigation (Tal, 2006). Fresh and recycled water differ in soluble organic matter and minerals such as nitrogen, phosphorous, potassium, sodium and chloride. However, one of the main differences between these two sources of irrigation water is the concentration of boron. Whereas concentrations in the range of $0{\cdot}08$–$0{\cdot}20$ mg L^{-1} are common in fresh water, the boron concentration in recycled water ranges from $0{\cdot}46$–$1{\cdot}74$ mg L^{-1} (Tarchitzky & Chen, 2004). An increase of boron has already been documented in Israeli soils irrigated with recycled water, especially in arid and semi arid zones (Tarchitzky *et al.*, 2006). Tarchitzky *et al.* (2006) have shown that the amount of boron applied to the soil with the Tel Aviv metropolitan recycled water is around 2 kg ha^{-1} per year, which is 4–6 times higher than the amount of boron applied with fresh water, and consequently the boron concentration in the soil solution of fields irrigated with effluent was higher than that of fields irrigated with fresh water. Boron is absorbed by plants directly from the soil solution as boric acid ($B(OH)_3$), and

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there is a strong linear correlation between boron concentration in the soil solution and in the plant tissues (Yermiyahu *et al.*, 2001). Since the severity of plant diseases is often influenced by the host's physiological and nutritional state (Marschner, 1995; Dordas, 2008), it is crucial to study and understand the potential influence of boron and its mode of action on plant diseases.

To date, there are few data available on boron's effects on plant disease in general (Kataria & Sundar, 1985; Dixon, 1996), and on diseases caused by foliar pathogens, in particular (Simoglou & Dordas, 2006; Dordas, 2008). In a few reports, the effects of high, phytotoxic concentrations are discussed. For example, Smilanick & Sorenson (2001) studied the effect of boron on stored citrus against *Penicillium digitatum*. Similarly, Rolshausen & Gubler (2005) showed that addition of boric acid significantly reduced the severity of eutypa dieback caused by *Eutypa lata* in grapevines. These studies addressed the direct effects of boron on disease development and did not elaborate on the mechanisms of disease suppression.

Potatoes are grown in Israel (30°E, 31°N) in two main regions: the northern Negev (*ca.* 11 000 ha) and the coastal plain (*ca.* 3000 ha). The climate in Israel is semi arid with mild winters and hot, rainless summers. There are two main seasons for potato production, autumn and spring. For the autumn crop, potatoes are planted in late August to early September and harvested during December and January. For the spring crop, potatoes are planted in late January to February and harvested during late May–July (Shtienberg *et al.*, 1996). All potato fields are irrigated (predominately by overhead sprinklers) but the source of the water used for irrigation varies among the different production areas. Fresh water is used on the coastal plain, and recycled water is used in the northern Negev (Tal, 2006; Tarchitzky *et al.*, 2006). The oomycete *Phytophthora infestans*, the causal agent of late blight, is the most destructive foliar pathogen of potatoes in Israel (Shtienberg & Yaniv, 1999). Nonetheless, observations made in potato fields over the years suggested that the occurrence and intensity of late blight and the resultant losses differ among the two regions. Whereas severe epidemics occasionally develop on the coastal plain, epidemics in the northern Negev are usually moderate. Preliminary simulation data obtained from the Cornell LATEBLIGHT simulator (Bruhn & Fry, 1981; Andrade-Piedra *et al.*, 2005) accurately predicted the late blight epidemic which developed in the coastal plain (Frenkel *et al.*, 2003), but over-estimated the epidemics which developed in the northern Negev, implying that climatic parameters and host resistance are probably not the main reasons for the differences in the epidemics (Frenkel *et al.*, 2003). Several factors (and their interactions) may create these differences, among which are microclimatic conditions, differences in production practices (Shtienberg & Yaniv, 1999; Grünwald *et al.*, 2000), and variation in the aggressiveness of the prevailing *P. infestans* isolates (Andrivon *et al.*, 2007). However, as the different sources of the irrigation water and their different boron concentrations coincide with the variable intensity of late blight epidemics in the different regions, it appeared possible that boron may also play a role in determining late blight intensity.

It is hypothesized that boron reduces severity of late blight caused by *P. infestans* and that its mode of action is not limited to direct fungicidal activity. The specific objectives were: (i) to examine the direct effects of boron on late blight epidemics; and (ii) to investigate the mode of action of boron against *P. infestans*.

Material and methods

Suppression of late blight by foliar application of boron

Field experiments were conducted to determine if applying boron to foliage affected the intensity of late blight epidemics. The experiments were carried out in spring 2000 and 2001 in Sde Elyahu, located in the coastal plain region. Potato tubers were planted on 1 February 2000 (cv. Nicola) and 15 January 2001 (cv. Mondial). Both cultivars are highly susceptible to *P. infestans*. The crops were cultivated and maintained as recommended to potato growers in that region. The fields were irrigated with fresh water and were not sprayed with fungicide, unless otherwise stated.

The experiments were laid out in a complete randomized block design with four replicates per treatment. The size of each experimental plot was 3 × 7 m (in 2000) and 4 × 7 m (in 2001). The experiments comprised five treatments, as follows: Treatment 1: untreated control; 2, boron (boric acid, Merck) at a rate of 700 mg L^{-1}; 3, Melody Duo (propineb + iprovalicarb, 61·25 + 5·5% a.i., WP, Bayer AG) at a full rate of 3·5 kg ha^{-1}; 4, Melody Duo at a reduced rate of 2 kg ha^{-1}; and 5, boron at a rate of 700 mg L^{-1} plus Melody Duo at a rate of 2 kg ha^{-1}. As potato fields in that area are subjected to occasional severe late blight epidemics and because it was anticipated that boron, if effective against *P. infestans*, will not be as effective as a chemical fungicide, there was concern that epidemics would develop so fast that it would not be possible to elucidate the effects of boron. Therefore, both experiments included a treatment where Melody Duo at a reduced rate (2 kg ha^{-1}) was added to boron at the rate given above.

Foliar applications of boron and Melody Duo were initiated 22 or 48 days after planting (in both experiments) and before disease onset. Subsequent sprays were applied at 5–8 day intervals until the end of the season; 11 sprays were applied in the 2000 experiment and 10 in 2001. Sprays were applied with a motorized back-pack sprayer (Echo) equipped with cone jet ×6 nozzles at a pressure of 5 atm, in a volume of 200 L ha^{-1}.

Experimental plots were inspected visually for late blight symptoms, starting soon after emergence and continuing at 3–7 day intervals until the end of the experiments. Two specific field workers assessed the disease severity (as a percentage) for each plot and the disease severity scores were averaged between assessors. The season-long disease assessments were used to calculate the area under the disease progress curve (AUDPC, Shaner & Finney, 1977). The period used for calculating the AUDPC values lasted from the date of disease onset until the end of the experiments. AUDPC values recorded in treated plots (A_t) and in untreated control plots (A_u) were used to calculate control efficacy (CE, %) achieved by each of the treatments: $CE = (1-A_t/A_u) \times 100$. By the end of the experiments (125 and 120 days after planting in 2000 and 2001, respectively) tubers were manually harvested from an area of 5 m^2 located in the centre of each experimental plot. The tubers were weighed and the yield per hectare was calculated. Data from the replicated plots were statistically analyzed using JMP-IN version 5 (SAS Institute Inc.). When the F statistic of ANOVA was significant at $P \leq 0{\cdot}05$, the least significant difference (LSD) was calculated according to the HSD test.

Potato leaves, sampled from the field experiments, were used to determine the boron concentration in the foliage. Ten diagnostic leaves were randomly sampled from each experimental plot of treatments 4 (Melody Duo 2·0 kg ha^{-1}) and 5 (700 mg L^{-1} boron plus Melody Duo 2·0 kg ha^{-1}). The leaves were washed with distilled water, dried on blotting paper and then in an oven at 65°C, and ground. A sample of 0·25 g was then placed in an oven at 550°C for 4 h. Five millilitres of 1M HCl were added to the cooled ash and the solution was filtered after 15 min. A 3 mL aliquot was taken for boron analysis using the azomethine-H procedure (Gupta & Stewart, 1975).

Mode of action of boron against *P. infestans*

Phytophthora infestans isolate #137 (isolated from tomato and provided by Professor Y. Cohen, Department of Biology, Bar Ilan University, Israel) was used in all artificially-inoculated experiments of this study. The isolate was maintained on potato tubers (cv. Alpha); tubers were cut into thin slices, washed with sterile double distilled water and dried. Hyphae and sporangia were transferred from 10-day-old sporulating slices using a paint brush. Newly inoculated slices were incubated in Petri dishes at 15°C in the dark for 7–10 days. To maintain isolate aggressiveness, tomato plants (cvs 144 or 189; both highly susceptible to *P. infestans*) were inoculated and the pathogen was re-isolated and used for inoculating potato tuber slices.

The fungicidal effect of boron on *P. infestans* was determined on detached potato leaves (cv. Mondial). During March 2001 healthy leaves (the fourth mature leaf from the top) were collected from untreated plots of the Nir Elyahu experiment. The leaves were washed in distilled water, dried and placed horizontally on plastic net in a plastic container (15 × 30 × 33 cm). Leaf petioles were imbedded into wet floral foams and water was added to the containers to maintain leaf viability. Sporangia suspensions were mixed with increasing concentrations of boric acid ranging from 1 to 1000 mg boron L^{-1} or with the fungicide chlorothalonil (Bravo, 50% a.i., Diamond Shemrock Corp.) at concentrations ranging from 0·01 to 100 mg a.i. L^{-1}. For each leaf, six leaflets were inoculated by placing a 20 μL drop containing 200 *P. infestans* sporangia (1×10^4 sporangia mL^{-1}) mixed with boron or with a fungicide in the centre of each leaflet. Water agar (0·1%) was added to the sporangia suspension and leaves inoculated with this suspension (without boron or chlorothalonil) or with distilled water as controls. The containers were covered with plastic bags to maintain high relative humidity and placed in a growth chamber at 15°C and 12 h photoperiod, for 120 h. After 5 days of incubation, areas of the resultant lesions were measured and the control efficacy values calculated. ED_{50} values were estimated from the dose-response curves after transforming the boron/fungicide concentrations to log units and the control efficacy values to probit values. The experiment was laid out in a randomized block design with four replicates per treatment and was repeated once.

The possibility that boron affects *P. infestans* by modifying the response of the host plant, rather than by directly inhibiting the pathogen, was evaluated using tomato (*Solanum lycopersicum*) plants as a host. Tomatoes rather than potatoes were used in these experiments as they are easier to grow and handle in pot experiments. Both crops are equally susceptible to the *P. infestans* isolate used. Tomato cultivars 144 or 189 (both highly susceptible to *P. infestans* isolate #137) were grown in a greenhouse at 25–30°C under natural light. Seedlings were planted in 20 cm pots filled with perlite, watered daily with 130 mL water per pot and fertilized with boron-free liquid fertilizer 0·1% 'Shefer 1' (Fertilizers and Chemical Materials Ltd) which contained 7:3:7 of $N:P_2O_5:K_2O$, and trace amounts of Fe, Mn, Zn, Cu and Mo. Plants were grown for 1 month before use.

Two sets of experiments were carried out in this part of the study. In the first, detached leaves were used and local boron effects (within treated leaves) were examined. Three leaflets located on one side of the sampled leaves were dipped for 1 min in solutions containing 0 (control), 10, 25 or 50 mg boron L^{-1}. Three leaflets on the opposite side of the leaves were dipped in sterile distilled water. The leaves were then placed in 15 mL tubes filled with double distilled water containing 0·1% fertilizer 'Shefer 1' and 10 μM $CaCl_2$. The tubes were placed in a growth chamber at 20°C and 12 h photoperiod. After 72 h, each leaflet was inoculated with a 20 μL drop containing 200 *P. infestans* sporangia (1×10^4 sporangia mL^{-1}) as described above. Control leaflets were inoculated with distilled water. The leaves were covered with two polyethylene bags to maintain moisture and incubated at 15°C for an additional 120 h. Then, the plastic bags were removed and the sizes of the resultant lesions were measured on all inoculated leaflets. Each treatment was repeated four times; the experiment was laid out in a complete randomized design and repeated once. Boron concentration was determined in dipped leaflets and in the opposite, non-dipped leaflets, using leaves that were not inoculated with the pathogen. The method used for quantifying boron concentration was described above.

In the second set of experiments, potted tomato plants were used and distant boron effects (in non-treated leaves) were examined. The fifth leaves (from the top) of 1-month-old plants were dipped in boron solution for 1 min. The concentrations used were 0 (control), 50, 100, 250, 500 and 800 mg boron L^{-1}. High boron concentrations were used in these experiments to determine whether the magnitude of the effect is related to the concentration of boron. After dipping the leaves in the solutions, the plants were returned to the greenhouse for 72 h. Then, the fifth (dipped leaves) and the fourth (non-dipped leaves) were detached and placed in 15 mL tubes filled with double distilled water containing 0·1% fertilizer 'Shefer 1' and 10 μM $CaCl_2$. Six leaflets in each leaf were inoculated with *P. infestans* sporangia and then incubated and assessed as described above. Each treatment was repeated five times; the experiment was laid out in a complete randomized design and was repeated once. Boron concentration was determined in the fifth (dipped) and fourth (non-dipped) leaves that were not inoculated. Regression analyses were used to quantify the relationships between boron concentration in the solution and the resultant late blight lesion areas.

Results

Suppression of late blight by foliar application of boron

The suppression of late blight by applying boron to foliage was examined in spring 2000 and 2001 in fields irrigated with fresh water. The first disease symptoms were observed in untreated plots 64 and 54 days after planting in the 2000 and 2001 experiments, respectively. Within less than 14 days, disease severity in these plots reached 80% and final disease severity scores were >95% (Fig. 1c,d; Table 1). Application of Melody Duo at the recommended rate significantly suppressed disease in both experiments (CE = 93·3 and 91·1%, respectively) and increased yield (by 48·7 and 25 t ha^{-1}, respectively, Table 1). As expected, under such severe epidemic pressure, foliar application of boron did not affect disease development and did not increase yields as compared with untreated plots, in both experiments. The reduced rate of Melody Duo was only partially effective against late blight, but it enabled the detection of the effect of boron. Addition of boron to the reduced rate treatments improved disease suppression in both experiments. The contribution of boron was significant in 2000 but not in 2001 (Fig. 1c,d; Table 1). Boron concentration in leaves sampled from plots where it was not applied ranged from 21 to 44 mg kg^{-1} in both experiments. In leaves sampled from boron-treated plots, boron concentrations fluctuated markedly over time, ranging from 36 to 132 mg kg^{-1}. Despite this fluctuation, boron concentrations in boron treated leaves were higher than in control leaves (Melody Duo 2·0 kg ha^{-1}) in six out of seven samples in 2000 and in all four samples in 2001 (Fig. 1a,b). Phytotoxicity was not observed in the foliage of the boron-treated plots in either of the experiments.

Mode of boron action against *P. infestans*

Suppression of late blight epidemics by boron may have resulted from direct inhibition of the pathogen, *P. infestans*. *In vitro* experiments revealed that boron is a relatively weak fungicide. The ED_{50} concentration of boron was 256·4 mg L^{-1}, about 6400-fold higher than the measured ED_{50} for the fungicide chlorothalonil (0·04 mg L^{-1}).

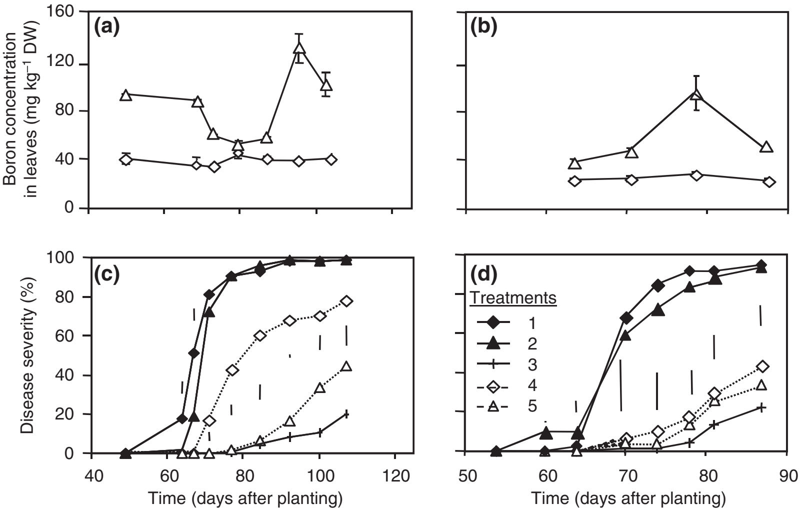

Figure 1 Effects of foliar application of boron and the propineb + iprovalicarb fungicide Melody Duo on potato late blight in experiments conducted in spring 2000 (a, c) and spring 2001 (b, d). Treatments: 1, untreated control; 2, boron (700 mg L^{-1}); 3, Melody Duo (3·5 kg ha^{-1}); 4, Melody Duo (2·0 kg ha^{-1}); 5, Melody Duo (2·0 kg ha^{-1}) + boron (700 mg L^{-1}). (a, b) Boron concentration in diagnostic leaves; vertical bars indicate the standard error. (c, d) Disease progress curves; vertical bars indicate the least significant difference (LSD) at $P = 0·05$.

Table 1 Effects of foliar application of boron and the propineb + iprovalicarb fungicide Melody Duo (MD) on late blight development caused by *Phytophthora infestans* and on potato yield in field experiments conducted in spring 2000 and 2001

Treatment	2000 Final disease severity (%)	 AUDPC (%×day)[a]	 Yield (t ha^{-1})	2001 Final disease severity (%)	 AUDPC (%×day)	 Yield (t ha^{-1})
Untreated control	99·0 a[b]	3801 a	21·6 a	97·2 a	1758 a	34·0 a
Boron (700 mg L^{-1})	98·7 a	3625 a	20·7 a	96·2 a	1659 a	38·0 a
MD (3·5 kg ha^{-1})	20·0 d	253 c	70·3 c	22·0 c	156 b	59·0 b
MD (2·0 kg ha^{-1})	77·5 b	2146 b	40·8 b	45·0 b	411 b	63·0 b
MD (2·0 kg ha^{-1}) + boron (700 mg L^{-1})	45·0 c	608 c	70·3 c	35·0 bc	311 b	55·0 b

[a] AUDPC = area under the disease progress curve. The curves are presented in Fig. 1.

[b] Within each column, values followed by the same letters do not differ significantly determined by the HSD test (at $P = 0{\cdot}05$).

The possibility that boron affects *P. infestans* by modifying host response to the pathogen was examined in two sets of experiments carried out with tomatoes. In the first, detached tomato leaves were used. Boron concentration in non-dipped leaflets was not altered (Fig. 2). Nevertheless, as the boron concentration in the solution increased the size of late blight lesions, in both dipped and in the non-dipped leaflets, decreased (Fig. 3). In the second set of experiments potted tomato plants were used. Boron concentration in the dipped, fifth leaves, was linearly related to the concentration of the element in the solution but boron concentration in the fourth, non-dipped, leaves was not altered (Fig. 4). Although phytotoxic effects were not observed on dipped leaves until the termination of the experiments, the texture of the leaves that were dipped in 800 mg boron L^{-1} was altered and the leaves became flimsy. Increasing the boron concentration in the solution up to 100 mg L^{-1} resulted in a gradual decrease in lesion size in the fourth, non-treated, leaves (Fig. 5). Boron concentration in the fifth leaves, dipped in 100 mg boron L^{-1} was 206·1 mg kg^{-1} DW (Fig. 4). Increasing the boron concentration in the solution further did not have any additional effect on the size of late blight lesions in non-dipped leaves (Fig. 5).

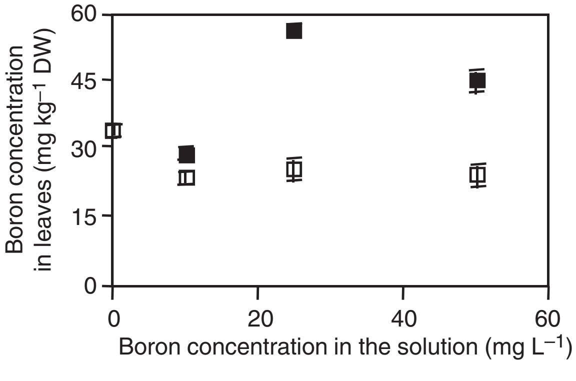

Figure 2 The relationship between boron concentration in the solution and its concentration in leaflets of tomato plants that were dipped (■) or not dipped (□) in the solution for 1 min. Vertical bars indicate the standard error.

Discussion

Boron is often found in high concentrations in association with agriculture in arid and semi arid regions where saline soils and saline irrigation water can be prevalent. Wastewater effluents used for irrigation are also sources of excess boron in agricultural systems (Tsadilis, 1997). There is a steady increase in irrigation with recycled water in many semi arid countries including south-western USA (Takano & Levine, 1996), Australia, Greece (Tsadilis, 1997) and Israel (Tarchitzky & Chen, 2004). In the Negev desert of Israel about 72% of the agricultural production areas already depend on such water. Although biological treatment and soil filtration greatly improve the quality of the recycled water, concentrations of several organic and inorganic compounds differ significantly between fresh and treated water, with boron constituting one of the most apparent differences (Tarchitzky *et al.*, 2006). Boron can be removed from effluent as a part of tertiary treatment processes and from soils (Nable *et al.*, 1997; Yilmaz *et al.*, 2005). These options, while effective, are particularly expensive. As a result, boron has become a 'fixed environmental parameter' and therefore needs to be furthered explored and considered while practicing agriculture in these areas.

In these field experiments boron was applied directly to the foliage by spraying and not as an additive to the irrigation water, since the aim was to increase its concentration in the foliage throughout the experiments. In general, boron levels in plants sprayed with boron was significantly higher, but its concentration fluctuated markedly over time (Fig. 1a,b). Boron is not a fungicide and its effect on late blight development could not be detected under conditions highly conducive to *P. infestans* development. Nevertheless, as an additive, boron improved the efficacy of a reduced dose of Melody-Duo. The effect was significant in 2000 (higher yield, lower AUDPC and final disease severity) but not in 2001 (Fig. 1, Table 1), However, some influences were detected in 2001 as final disease severity in plots treated with a reduced rate of Melody-Duo differed significantly from that observed in plots treated with the full rate of the fungicide. Adding boron to the reduced rate treatment improved disease

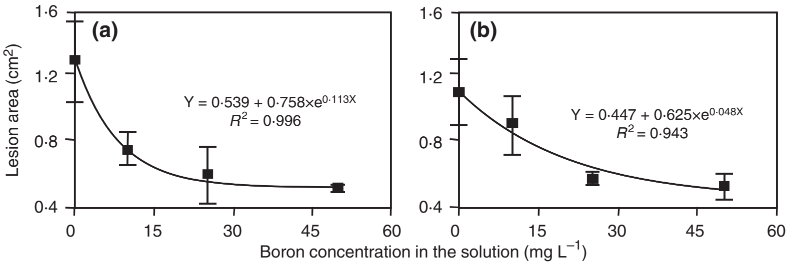

Figure 3 Local and systemic acquired resistance induced by boron against *Phytophthora infestans* in detached tomato leaves. Leaflets on one side of the leaves were dipped in boron solutions at various concentrations for 1 min; after 72 h, the dipped (a) and the opposite, non-dipped (b), leaflets were inoculated. Lesion area was measured 96 h later. Vertical bars indicate the standard error.

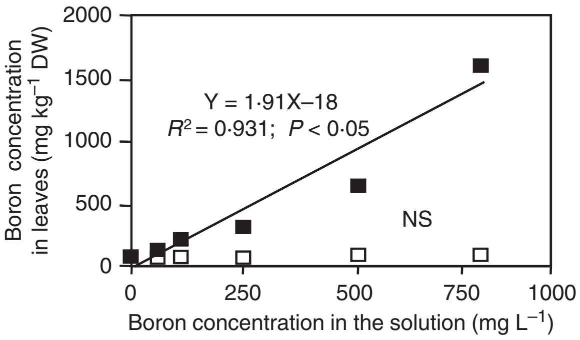

Figure 4 Relationship between the boron concentration in solution and its concentration in leaves of tomato plants that were dipped in the solution for 1 min (fifth leaf from the top, ■) and with leaves of the same plants that were not dipped in the solution (fourth leaf from the top, □).

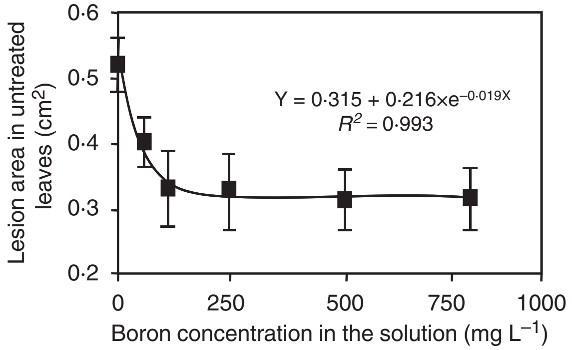

Figure 5 Systemic acquired resistance induced by boron against *Phytophora infestans* in potted tomato plants. The fifth leaf from the top of each plant was dipped in boron solutions at various concentrations for 1 min. After 72 h, non-dipped leaves (fourth leaves from the top) were detached and inoculated. Lesion area was measured 96 h later. Vertical bars indicate the standard error.

suppression and differences between this treatment and the full-rate fungicide treatment were insignificant (Fig. 1d, Table 1). These results, together with the controlled experiment studies on tomato, lead to the conclusion that boron, in sub-phytotoxic concentrations, restricts late blight development. Very few reports have addressed the individual effect of boron on plant pathogens and its interaction with other control measures. Kataria & Sunder (1985) reported that combining boron with fungicides improved the suppression of *Rhizoctonia solani* in cowpea (as compared to using the fungicides alone); Rolshausen & Gubler (2005) and Duffy *et al.* (1997) combined boron and biocontrol agents against eutypa die back in grapes and take-all in wheat, respectively. Interestingly, Ruiz *et al.* (1999) reported that combining the fungicide carbendazim with boron may influence antimicrobial compounds in tobacco tissues. Carbendazim increased the production of phenylalanine lyase in the plant tissue while boron increased the production of polyphenol oxidase (PPO). PPO uses phenolic compounds synthesized by phenylalanine ammonia lyase (PAL) to form quinonic compounds which are toxic against a wide range of microbial pathogens (Ruiz *et al.*, 1999).

Boron may have affected late blight by direct inhibition of the pathogen. However, boron was found to be only a very weak fungicide against *P. infestans*. The ED$_{50}$ value was 256·4 mg mL^{-1}, about 6400 times higher than the ED$_{50}$ value of the fungicide chlorothalonil. Boron concentration in recycled water ranges from 0·46 to 1·74 mg L^{-1} (Tarchitzky & Chen, 2004). In soils irrigated with recycled water, without appropriate leaching, boron accumulates over time and concentrations in the soil solution can be much higher than that found in irrigation water. Plants growing in these soils absorb the element through their vascular system and leaf concentrations may reach several hundred mg boron kg^{-1} DW. In this study, the highest rate detected in field-sprayed leaves was 132 mg boron kg^{-1} DW (Fig. 1a). In the controlled experiment studies, boron effects were apparent in leaves with concentrations of less than 60 mg boron kg^{-1} DW. At the same time, increasing boron concentration beyond 206 mg kg^{-1} DW did not influence the disease lesion size (Figs 2–5). Thus, it is likely that the observed effects of boron on late blight development did not develop from direct fungicidal inhibition of the pathogen.

The mechanisms by which boron restricted pathogen development were not studied thoroughly and are not fully understood. Boron affects late blight development in both treated and distant leaves, not directly treated with the element (Figs 3 and 5). This suggests that boron is active locally but may also induce systemic acquired resistance against *P. infestans*. Boron is still considered one of the least understood microelements of plants (Blevins & Lukaszewski, 1998; Dordas, 2008) and findings about its possible involvement in local and systemic induced resistance are scarce. However, information about boron's role in plant structure and metabolism may enable one to raise some hypotheses about its role in resistance against plant pathogens. As an example, boron promotes stability and rigidity of the cell wall structure and therefore supports the shape and strength of the plant cell (Blevins & Lukaszewski, 1998; Dordas, 2008). In addition, boron increases and enhances the deposition rate of callose (Tighe & Heath, 1982) and lignin (Lewis, 1980), both important components in papillae formation (Smart *et al.*, 1986). Boron also increases the formation of several phenolic compounds and peroxidase (Ruiz *et al.*, 1999; Camacho-Cristobal *et al.*, 2008). Preliminary experiments conducted by the authors (in collaboration with Professor Y. Kapulnik, Agriculture Research Organization, Bet Dagan, Israel) also showed that boron influences the expression of pathogenesis-related (PR) proteins. Boron induced PR-1a expression in tobacco callus cells and systemically enhanced the expression of chitinase and ETR1, a receptor for ethylene, in tomatoes. These proteins play an important role in systemic acquired resistance in plants (Neiderman *et al.*, 1995) and may provide another possible explanation for a resistance mechanism.

Although there is evidence that the element boron enhances host resistance against *P. infestans* in potatoes and tomatoes, the same cannot be concluded about the influence of recycled water on the pathogen. Indeed, simulation models suggest that late blight epidemics are less intense then expected in the southern part of Israel where irrigation with recycled water is common (Frenkel *et al.*, 2003). High boron concentrations were also detected in soils and plants irrigated with recycled water (OF, UY & DS, unpublished data). However, boron in recycled water, accumulated slower, is absorbed through the roots and may interact with other components in the water. Moreover, other elements in the water may affect the pathogen or the host resistance. In order to approach these problems, replicated experiments where some of the plots are irrigated with fresh water and others with recycled water should be carried out. In addition it needs to be emphasized that boron should not be used at this stage as a means for managing late blight. It should be noted that high boron concentrations may be phytotoxic (Ben-Gal & Shani, 2003; Yermiyahu *et al.*, 2006). In a set of additional experiments conducted in the Northern Negev (where the plants are irrigated with recycled water) it was found that application of boron to the foliage decreased the severity of early blight, caused by *Alternaria solani*, but also caused some damage in potato plants (OF, UY & DS, unpublished). Therefore, additional studies, in different environments, are required before the information presented in this study may be used for disease management.

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