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A foliar spray of micronutrient solutions induces local and systemic protection against powdery mildew (*Sphaerotheca fuliginia*) in cucumber plants

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Abstract

A single spray of solutions of 0.005M H_3BO_3, 0.0025M $CuSO_4$, and 0.0025 $MnCl_2$, on the upper surface of the first true leaf of cucumber plants 2 h before inoculation with a conidial suspension of *Sphaerotheca fuliginea*, induced systemic protection against powdery mildew in leaves 2 and 3 without causing any damage on the induced leaf (first leaf). A similar level of systemic protection was observed when plants were induced by micronutrients, 2, 24 and 72 h before challenge with *S. fuliginea*. The level of protection induced by various concentrations varied from solution to solution. In general, the systemic protection induced by K_2HPO_4 was similar to that by the microelements. Spraying of a 1:1 mixture of phosphate and micronutrient solutions did not improve the systemic protection over that obtained with each of the solutions alone. Increasing the inoculum concentration of *S. fuliginea* increased the number of powdery mildew colonies produced on both induced and non-induced plants and has relatively affected the systemic protection on induced plants. A single foliar spray of micronutrient solutions, as a prophylactic treatment, on the upper surface of all the leaves of 3-leaf stage cucumber plants significantly inhibited powdery mildew development. A single spray of $MnCl_2$ on leaf 1 elevated peroxidase activity in the soluble fraction and caused an enhancement of β-1,3-glucanase content in the ionically bound fractions of leaf 2 of non-inoculated plants. Forty-eight hours after inoculation, the level of both fractions of the enzymes increased in non-treated plants and decreased (β-1,3-glucanase) or remained unchanged (peroxidase) in treated (induced) plants as compared to non-treated plants. The possible mechanism for this protection, and the use of microelements and phosphate solutions as inducers for systemic protection and as agents for disease control are discussed.

Introduction

Cucumber powdery mildew, caused by *Sphaerotheca fuliginea*, is a major disease, attacking both field- and greenhouse-grown cucumber plants. Increasing pesticide levels on food crops have stimulated the demand for alternative, environmentally friendly methods for disease control. Attempting to meet this goal, we previously reported that a foliar spray of NPK fertilizers can enhance growth of cucumber and maize plants and induce systemic resistance against powdery mildew in cucumbers (Reuveni, M. et al., 1993; Reuveni, M. et al., 1995a, b) and against northern leaf blight and common rust in maize (Reuveni, R. et al., 1994a). This induced protection was evident regardless of the leaf position or the rate of NPK accumulation in the upper protected maize leaves (Reuveni, R. et al., 1996). It remains unclear, however, whether the mechanism of this protection is associated with stress induced by the salts (Mucharromah and Kuc', 1991; Ye et al., 1995) or is related to an improved nutrition status or increased photosynthesis, as reported by Murray and Walters (1992) in uninfected leaves of rusted broad bean.

Activation of genes for defence in plants can be induced systemically by signalling molecules produced at the sites of the inducer-agent and transported by diffusion, or through the vascular system of the host plant (Ye et al., 1995). Salicylic acid has been

suggested as an endogenous signalling molecule that mediates systemic acquired resistance (SAR) in several host pathogen systems (Yalpani et al., 1991) while Ca^{++} ions play an important role in the induced production of salicylic acid and chitinase, one of the pathogenesis-related proteins (Schneider-Muller et al., 1994).

One additional possible mechanism of systemic protection, which is induced by ions or salts, involves alteration of the timing and intensity with which several defence-associated mechanisms are expressed, as reported for Si fertilization in cucumber (Abood et al., 1991; Cherif et al., 1994; Kent, 1941). Trace elements may play an important role in protection by affecting plant susceptibility to fungal or bacterial phytopathogens (Graham, 1983). They may also affect the predisposition of plants to viral diseases, which have been reported to increase or decrease the resistance of asparagus bean to tobacco necrotic virus (TNV) (Pennazio and Roggero, 1988). However, the induction of systemic resistance to foliar pathogens by a foliar spray of trace element solutions has not been extensively investigated. The most important feature of these elements, in this regard, is their variable valency, which allows them to be involved in oxidation changes involving one electron, and, therefore, to act as cofactors of metalo-protein enzymes such as peroxidase, for which Mn ions serve as an inducing agent (Fowler and Morgan, 1972; Reuveni, R. and Perl, 1979). Biochemical changes associated with induced systemic resistance, including enhancement of levels of β-1,3-glucanase and chitinase, and activity of peroxidase in immunized cucumber plants as a result of phosphate treatment, have already been thoroughly investigated (Gottstein and Kuc', 1989; Irving and Kuc', 1990).

The present paper provides further evidence on the effectiveness of micronutrient solutions in inducing systemic protection against powdery mildew in cucumber plants. In addition, the levels of β-1,3-glucanase and the activity of peroxidase were investigated, to evaluate their potential role in a mechanism for inducing systemic protection against powdery mildew in the upper leaves of plants, by foliar spray of a micronutrient solution.

Materials and methods

Cucumber plants (*Cucumis sativus* L. 'Delilla') were grown in a greenhouse in 10 cm-diameter plastic pots containing peat, soil and vermiculite (1:1:1, v/v). Twice a week, plants were watered to saturation with a 0.1% 20-20-20 (N-P-K) fertilizer solution.

Induction of systemic protection. Plants with the first true leaf expanded and the second true leaf approximately two-thirds expanded were used in all experiments (unless stated otherwise). Aqueous solutions various concentrations ranging from 0.0025 M to 0.02 M of micronutrients were freshly prepared in distilled water and used for induction. These concentrations were found to be effective to induce systemic protection against powdery mildew. The upper surface of leaf 1 (unless stated otherwise) was sprayed with 1–1.5 ml of the solution or water. To avoid contamination, the other parts of the plant were covered. The treated plants were grown under greenhouse conditions until challenged with a conidial suspension of *S. fuliginea* and transferred to a growth chamber.

In experiments, to determine the 'duration' and the speed of induction of systemic resistance in the greenhouse, solutions were sprayed on the first fully expanded true leaf at intervals of various numbers of days, and the plants were challenged on the same day after the first treatment.

Pathogen and inoculation. An isolate of *S. fuliginea* obtained from plants in a field was maintained on cucumber plants grown in a growth chamber. Inoculum was obtained from freshly sporulating infected leaves 9–12 days after inoculation. Conidia were gently brushed into a small quantity of distilled water containing two drops of Tween-20, and counted with the aid of a hemocytometer, to obtain a suspension containing a known number of conidia per millilitre. In experiments, to determine the effect of inoculum concentration on induced systemic resistance, conidial suspensions of various concentrations were prepared and used to inoculate the plants which had been induced by microelements. For inoculation the upper surfaces of all the upper leaves above leaf 1 (unless otherwise stated) of each plant were uniformly sprayed with a conidial suspension delivered from a hand sprayer. After inoculation, plants were incubated in a dew chamber at 20 °C for 24 h in darkness. Plants were then kept in a growth chamber (24 °C, 120 Ein.m-2.s-1, 16 h of light per day) for disease development. The non-inoculated control plants were kept under similar conditions in a separate growth chamber, in order to avoid contamination.

Assessment of induced systemic protection. Induced systemic resistance was determined by counting the number of colonies of powdery mildew produced on each of the upper leaves. Data obtained on various numbers of days after inoculation, and which usually included maximum lesion development, are presented. At least six to eight plants were used for each treatment and each experiment was conducted at least three times. ANOVA was performed to analyze the data, and the significance of differences among treatments was determined according to Duncan's Multiple Range Test.

Inhibition of powdery mildew by micronutrient solutions. The inhibitory effect of micronutrient solutions on the development of powdery mildew on cucumber leaves was determined following a single foliar spray of each solution. Fresh solutions of 0.0025 M $CuSO_4$, 0.005 M $MnCl_2$, 0.005 M H_3BO_3, 0.05 M K_2HPO_4 and 0.01% Pyrifenox (Dorado 480 EC, a systemic fungicide belonging to the sterol biosynthesis inhibitors) were prepared and sprayed on the upper surface of each of four-leaf-old cucumber plants as prophylactic treatment. Control plants were sprayed with water. Plants were kept in the greenhouse and three days later were inoculated with 3×104 conidia of *S. fuliginea* per ml as described above. Eight plants were used for each treatment and the experiment was conducted twice.

Determination of peroxidase activity and β-1,3-glucanase content. Plants with two leaves were used for this experiment. For induction, a fresh aqueous solution of 0.005 M of $MnCl_2$ was sprayed on the upper surface of leaf 1. As controls, non-induced plants were sprayed with water. Two hours after induction, the second leaf of each of the five plants in each treatment was challenged (inoculated) with *S. fuliginea* (3×104 conidia per ml), as described above. Control plants were kept under similar conditions in a separate growth chamber, in order to avoid contamination. The second leaf (leaf no. 2) of each of five replicate plants from each treatment was removed 48 h after inoculation and used for analysis. As a control, leaf number 2 was removed from untreated control plants at the beginning of the experiment (time zero). A 0.5-g sample from each leaf was homogenized with 1 ml cold 0.05 M sodium acetate buffer (pH 5.0) with a mortar and pestle. The homogenate was centrifuged at 14,000g for 20 min at 4–6 °C, and the supernatant was used to determine enzyme activity.

Extraction of ionically bound proteins was performed by rehomogenizing the pellets from the above extraction with the same buffer, containing 1 M NaCl. Samples were incubated at 4–5 °C for 24 h and centrifuged as described above. Peroxidase activities of both soluble and ionically bound supernatant samples were determined. A 20-μl sample of the supernatant was added to 3 ml of the assay mixture, which consisted of a solution of 0.1 M sodium phosphate buffer (pH 6.0), 1 mM H_2O_2 and 0.1 mM *O*-methoxy-phenol (guaiacol). The increased absorbance density at 470 nm was recorded with a spectrophotometer and the enzyme activity was expressed as the change in absorbance per minute per gram fresh weight.

β-1,3-glucanase content was determined by the laminarin dinitrosalicylate method previously described by Abeles and Forrence (1970), with the following modification: 25 μl of the enzyme extract were added to 75 μl of 2% laminarin and incubated at 40 °C for 60 min. The reaction was halted by adding 400 μl of dinitrosalicylic reagent, heated for 10 min in a boiling-water bath, cooled and diluted with 2.5 ml of water. Samples were vortexed and the absorbance at 500 nm was determined.

Results

Effect of a foliar spray of micronutrient solutions on induced systemic protection against powdery mildew. Spraying of micronutrient solutions of various concentrations on the upper surface of leaf 1 induced a systemic protection on leaves 2 and 3 without causing any stress signs or damage. Based on comparison with the number of powdery mildew colonies produced on plants treated with water at 15 days after inoculation, a single spray of B, Mn and Cu effectively induced systemic protection against powdery mildew (Table 1). Increasing the concentration of each trace element solution had little or no significant effect (Table 1). The systemic protection on leaves 2 and 3 was evident up to 21 days after inoculation with *S. fuliginea* (Figure 1, Table 2), and was expressed by reductions ranging from 34 to 68%, and 40 to 67% in the number of powdery mildew colonies, as rated 14 and 21 days after inoculation, respectively, in comparison with plants treated with water (Figure 1).

In both experiments, the level of protection induced on the upper leaves of plants by micronutrient solutions was similar to that induced by phosphate salts (Figure 1, Tables 1 and 2). However, use of a 1:1 mixture of

Table 1. Induction of systemic resistance against powdery mildew in cucumber plants by phosphate salts and micronutrients

Treatment[1]	Concentration	Number of colonies/plant
Control	–	70.5 a[2]
K_2HPO_4	0.05 M	29.8 bc
$MnCl_2$	0.01 M	36.3 b
	0.005 M	23.2 bc
	0.0025 M	25.3 bc
H_3BO_3	0.02 M	21.8 bc
	0.01 M	19.2 c
	0.005 M	21.2 bc
$CuSO_4$	0.01M	22.5 bc
	0.005 M	33.8 b
	0.0025 M	34.8 b

[1] A solution of salts was sprayed on leaf 1, 2 h before inoculation of leaves 2 and 3 with *S. fuliginea*.
[2] Data represents the means of the number of colonies on leaves 2 and 3 of six plants per treatment, 15 days after inoculation with powdery mildew. Means within column followed by different letters are significantly different ($P < 0.05$) according to Duncan's Multiple Range Test.

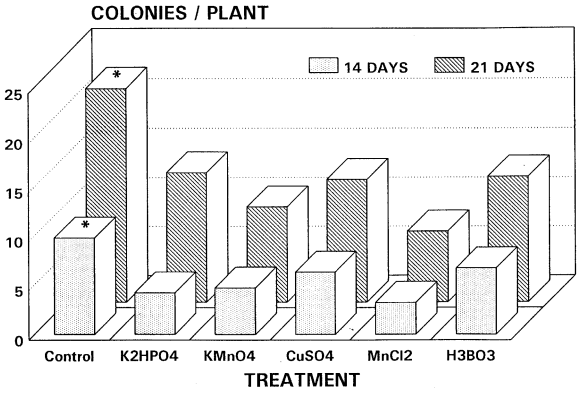

Figure 1. The effect of a foliar spray of phosphate and micronutrient solutions on induced systemic protection against powdery mildew in cucumber plants. Approximately 11.5 ml of solution (concentrations as in Table 2) was sprayed 2 h before inoculation on the upper surface of the first true leaf (leaf 1). As a control treatment, water was sprayed on leaf 1. Plants were challenged on leaves 2 and 3 with a conidial suspension of *S. fuliginea* (2×104 spores/ml). The data represents the means of six plants per treatment and the experiment was conducted three times. Bars with an asterisk indicate a significant difference ($P < 0.05$) from all other treatments according to Duncan's Multiple Range Test.

phosphate (0.05 M K_2HPO_4) and micronutrient solution (0.01 M H_3BO_3) did not enhance the systemic protection on the upper leaves in comparison to induction with either solution alone (Table 3). Attempts to induce systemic protection against powdery mildew by means of other micronutrients such as Mo, Mg and Zn were unsuccessful, as compared to the phosphate treatment (Table 4).

In order to determine the 'duration' of trace elements in the induction of local and systemic protection, solutions of K_2HPO_4, $MnCl_2$ or H_3BO_3 were sprayed on leaf 1 at 72 h (3 days), 24 h (1 day) and 2 h (0 day) before inoculation with a suspension of 20 000 conidia per ml. Powdery mildew colonies were observed initially on the 6th day after inoculation on non-induced

Table 2. Induction of systemic resistance against powdery mildew in cucumber plants by phosphate salts and micronutrients

	Number of colonies on leaves 2 and 3 on given days after inoculation		
Treatment[1]	15	18	21
Control	30.8 a[2]	45.2 a	56.8 a
K_2HPO_4 0.05 M1	17.7 bc	28.5 b	37.7 bc
KH_2PO_4+KOH 0.05 M	16.7 bc	26.2 b	31.5 bc
$KMnO_4$ 0.01 M	13.2 bc	23.8 b	33.3 bc
$CuSO_4$ 0.01 M	20.5 b	29.3 b	38.3 b
$MnCl_2$ 0.01 M	7.5 c	16.3 b	17.8 c
H_3BO_3 0.02 M	11.3 bc	22.0 b	25.2 bc

[1] Approximately 1–1.5 ml of each salt solution was sprayed on the upper surface of the first true leaf (leaf 1). As a control treatment, water was sprayed on leaf 1. Two hours after induction plants were challenged on leaves 2 and 3 with a conidial suspension of *S. fuliginea* (2×104 spores per ml).
[2] The data represents the means of six plants per treatment/experiment and the experiment was conducted three times.

Table 3. Induction of systemic resistance against powdery mildew in cucumber plants by phosphate salts and micronutrients

	Number of colonies on leaves 2 and 3 on given days after inoculation	
Treatment[1]	11	16
Control	22.0 a[2]	37.7 a
K_2HPO_4 0.05 M1	12.8 b	18.2 b
H_3B0_3 0.01 M	11.3 b	17.3 b
K_2HPO_4 0.05 M1 + H_3B0_3 0.01 M	14.0 b	21.8 b

[1] A solution of salts was sprayed on leaf 1, 2 h before inoculation of leaves 2 and 3 with *S. fuliginea*.
[2] Data represents the means of six plants per treatment. Means within column followed by different letters are significantly different ($P < 0.05$) according to Duncan's Multiple Range Test.

Table 4. Effect of a foliar spray of phosphate and micronutrient solutions on induced systemic protection against powdery mildew on cucumber plants

Treatment[1]	Number of colonies/leaves 2 and 3
Control	16.3 a[2]
K_2HPO_4 0.05 M	3.0 b
Na_2MoO_4 0.01 M	10.2 a
$MgSO_4$ 0.02 M	15.9 a
$ZnSO_4$ 0.2%	18.0 a

[1] A solution of salts was sprayed on leaf 1, 2 h before inoculation of leaves 2 and 3 with *S. fuliginea*.
[2] Mean numbers of powdery mildew colonies produced in leaves 2 and 3 of each of six plants per treatment. The experiment was conducted three times and numbers are means of these experiments.

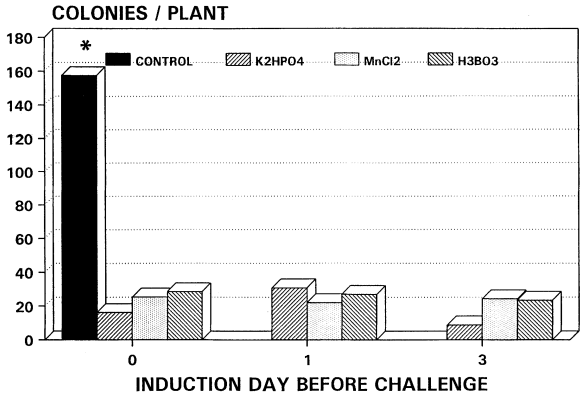

Figure 2. The effect of the number of days between induction and inoculation on induced systemic protection against powdery mildew in cucumber plants by phosphate and micronutrient solutions. Approximately 1–1.5 ml of solution (concentrations as in Table 2) was sprayed on the upper surface of the first true leaf (leaf 1), on the given number of days before inoculation. As a control treatment, water was sprayed on leaf 1. Plants were challenged on leaves 2 and 3 with a conidial suspension of *S. fuliginea* (3×104 conidia/ml). The data represents the means of eight plants per treatment and the experiment was conducted three times. An asterisk on control treatment indicates significance ($P < 0.05$) according to Duncan's Multiple Range Test.

control plants, whereas no powdery mildew symptoms were seen at this stage on any induced plants. On the 10th day after inoculation, the number of colonies per plant – i.e., on leaves 2, 3 and 4 increased remarkably on the control plants, while many fewer colonies developed on the induced plants (Figure 2). Inductions applied 2, 24 or 72 h before inoculation did not differ significantly in their resulting systemic protection on upper leaves. Analysis of variance performed for each leaf indicated a significant ($P < 0.05$) effect of K_2HPO_4, $MnCl_2$ and H_3BO_3 on the number of colonies produced on leaves 1, 2, 3 and 4. In all cases, the number of powdery mildew colonies was much greater on leaves of non-induced control plants than on leaves of induced plants (Table 5). In general, the highest level of induced systemic resistance against powdery mildew was observed on leaf 2. K_2HPO_4 and trace elements also induced a significant ($P < 0.05$) local protection on the first (induced) leaf, and inhibited disease development (Table 5).

Effect of concentration of S. fuliginea *inoculum on induced systemic protection*. The first leaf of two-leaf-old plants was sprayed either with a solution of $MnCl_2$ or H_3BO_3 (induced), or with water (control). Two hours later, plants were inoculated with freshly prepared suspensions of 1, 3 and 16×104 conidia per ml. The number of powdery mildew colonies produced per plant was counted 12 days after inoculation. Overall, factorial analysis of variance indicated that spraying with trace elements induced a significant systemic protection as compared to water-sprayed control plants. Increasing the inoculum concentrations from 1 to 16×104 conidia per ml, significantly increased the number of colonies produced on both induced and non-induced plants, and relatively affected the systemic protection (Table 6). This relative protection is expressed as the high level of reduction in the powdery mildew colonies as affected by both salts under the highest concentration of the challenge inoculation.

Inhibition of powdery mildew development by prophylactic treatment with micronutrients and phosphate solutions on cucumber plants. Foliar sprays of phosphate, trace elements and the systemic fungicide pyrifenox solutions on the upper surfaces of all leaves of each tested plant, 3 days before inoculation with *S. fuliginea* (3×104 conidia per ml) inhibited powdery mildew development on these plants (Figure 3). The total number of colonies produced on phosphate- and micronutrient-treated plants was reduced by 95% and 81–97%, respectively, as compared to water-treated control plants. Treatment with pyrifenox, however, was less effective and caused a reduction of 67% in the total number of colonies (Figure 3).

Peroxidase activity and levels of β-1,3-glucanase contents in cucumber induced leaves as affected by foliar

Table 5. Induction of systemic resistance against powdery mildew in cucumber plants by phosphate salts and micronutrients

Treatment[1]	Days after inoculation	Number of colonies on leaf number			
		1	2	3	4
Control	0	56.3 a[2]	56.8 a	38.7 a	5.7 a
K_2HPO_4 0.05 M1	0	0.0 b	1.5 c	6.5 b	0.8 b
$MnCl_2$ 0.005 M	0	3.5 b	10.7 bc	10.2 b	0.0 b
H_3BO_3 0.01 M	0	3.8 b	12.2 bc	7.5 b	0.0 b
K_2HPO_4 0.05 M	1	0.0 b	12.3 bc	14.5 b	4.0 ab
$MnCl_2$ 0.005 M	1	3.7 b	13.2 b	5.2 b	0.0 b
H_3BO_3 0.01 M	1	4.5 b	14.0 b	8.3 b	0.2 b
K_2HPO_4 0.05 M	3	0.2 b	7.8 bc	8.0 b	0.2 b
$MnCl_2$ 0.005 M	3	2.5 b	12.0 bc	10.0 b	0.8 b
H_3BO_3 0.01 M	3	4.0 b	12.7 b	10.2 b	1.7 ab

[1] A solution of salts was sprayed on leaf 1, 2 h (day 0), 1 day and 3 days before inoculation of leaves 2 and 3 with *S. fuliginea.*
[2] Data represents the means of six plants per treatment at 10 days after inoculation with *S. fuliginea*. Means within a column followed by different letters are significantly different ($P < 0.05$) according to Duncan's Multiple Range Test.

Table 6. Effect of concentration of challenge inoculation on induction of systemic resistance against powdery mildew in cucumber plants by micronutrients

Treatment[1]	Number of colonies/plant at various concentrations of *S. fuliginea* (conidia/ml)			
	1 × 104	3 × 104	16 × 104	Means
Control	41.8	53.7	151.0	82.2 a[2]
$MnCl_2$ 0.005 M	31.8	47.2	91.5	56.8 b
H_3B0_3 0.01 M	6.5	46.3	91.5	48.1 b
Means	26.7 C[3]	49.1 B	111.3 A	

[1] Solutions were sprayed on leaf 1, 2 h before inoculation with *S. fuliginea*. Data represents the means of six plants per treatment.
[2] Mean numbers within column (induction treatment) followed by different lower case letters are significantly different ($P < 0.05$) according to Duncan's Multiple Range Test.
[3] Mean numbers in row (inoculum concentration) followed by different upper case letters are significantly different ($P < 0.05$) according to the same test.

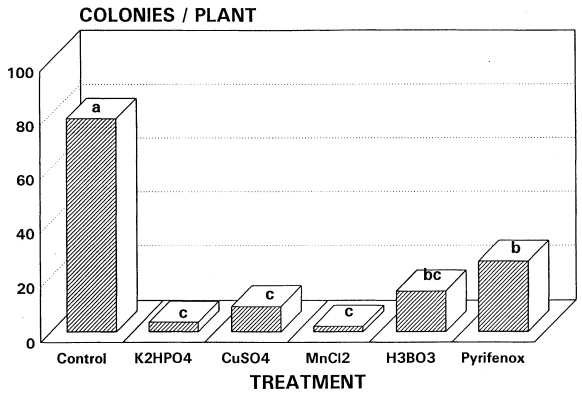

Figure 3. The inhibitory effect of micronutrients, phosphates, and a systemic fungicide solution against powdery mildew in cucumber plants. Solutions (concentrations as in Table 2) were sprayed on the upper surface of each of the four-leaf-old plants. Three days later, plants were inoculated with a conidial suspension of *S. fuliginea* (3 × 104 conidia/ml) as described in Materials and methods. The experiment was conducted three times. Numbers are mean numbers of colonies produced on leaves of six plants per treatment/experiment as recorded 9 days after inoculation. Different letters within columns indicate significant differences ($P < 0.05$) according to Duncan's Multiple Range Test.

spray of $MnCl_2$. Increased activities of both soluble and ionically bound fractions of peroxidase were detected in leaf 2 of cucumber plants 48 h after inoculation with *S. fuliginea* (Table 7). Foliar spray of $MnCl_2$ increased peroxidase activity in non-inoculated control plants and decreased the activity of the soluble fraction in the $MnCl_2$-treated and inoculated but protected plants. Inoculation with the pathogen caused an increase of β-1,3-glucanase content in both soluble and ionically bound fractions (Table 8). Foliar spray of $MnCl_2$ caused an increase in the β-1,3-glucanase content mainly in the ionically bound fraction of the non-inoculated control plants. Lower levels of the enzyme were detected in the protected plants which were induced by $MnCl_2$ and challenged by the pathogen.

Table 7. Changes in peroxidase activity in upper leaf (Leaf 2) of cucumber plants induced by $MnCl_2$ on leaf 1

Treatment[1]	Peroxidase activity (× 102)		
	Soluble	Ionically bound	Total
Non-treated control (0 h)	102.0±27.3	19.3±1.3	121.3±2.6
48h after inoculation			
Non-treated control	104.2±12.9	18.8±2.9	123.0±11.2
$MnCl_2$ control	196.8±33.6	19.7±3.4	216.5±38.6
Non-treated inoculated	152.3±28.9	29.4±9.4	181.6±37.8
$MnCl_2$ inoculated	111.7±40.1	32.4±24.6	144.2±64.8

[1] A fresh solution per g of 0.005 M $MnCl_2$ was sprayed on the upper surface of leaf 1 (induced). As a control, water was sprayed on leaf 1. Leaf samples at zero time were obtained just before induction and those at 48 h after induction and also of those after induction + inoculation as described in Materials and methods.
[2] Peroxidase activity is expressed as change in absorbance per min per g fresh weight, at 470 nm. Numbers are means ± standard error of five samples per treatment.

Table 8. Changes in β-1,3-glucanase contents in upper leaf (leaf 2) of cucumber plants induced by $MnCl_2$ on leaf 1

Treatment[1]	β-1,3-Glucanase content[2]		
	Soluble	Ionically bound	Total
Non-treated control (0 h)	53.9±0.8	37.0±1.8	90.8±2.4
48h after inoculation			
Non-treated control	52.4±2.6	37.2±1.4	89.6±3.8
$MnCl_2$ control	59.5±9.0	60.6±6.5	120.1±12.2
Non-treated inoculated	64.1±5.5	51.9±2.9	116.0±8.2
$MnCl_2$ inoculated	56.6±2.0	47.4±1.7	104.0±2.2

[1] See footnote in Table 7.
[2] β-1,3-glucanase content is presented as mu/g. One milliunit (mu) will liberate one +g per min of reduced sugar (measured as glucose) from laminarin, at pH 5.5 and 40 °C. Numbers are means ± standard error of five samples per treatment.

Discussion

Trace elements may play an important role in plants by affecting susceptibility to fungal or bacterial phytopathogens (Graham, 1983). As far as viral diseases are concerned, some trace elements have been reported to increase the susceptibility of bean leaves to tobacco mosaic virus (Yarwood, 1954). Trace elements may also affect the predisposition of plants to viral diseases which have been reported to increase or decrease the resistance of asparagus bean to tobacco necrosis virus (TNV) (Pennazio and Roggero, 1988). Feeding Mn to cuttings of this plant decreased their natural resistance whereas Cu, Co and Ni increased resistance to TNV. However, the induction of systemic resistance to foliar fungal pathogens by a foliar spray of trace element solution has not been extensively investigated.

The present report clearly demonstrates that the level of protection against powdery mildew induced by B, Mn, and Cu, applied to the upper surface of the first leaf of cucumber plants varied from solution to solution (Table 1, Figure 1). More importantly, this significant level of systemic protection was similar to that achieved by the use of K_2HPO_4 or KH_2PO_4 (Reuveni, M. et al., 1993, 1995a, b). As we have previously reported, this systemic protection was observed on leaf 2 when the first true leaf was induced and did not decline on leaves 3 and 4 (Table 1), and was effctive when the first true leaf was induced 2 h before inoculation (day 0) (Figure 2). The rate of protection was not affected by a 1:1 mixture of phosphate and micronutrient solutions and this treatment did not improve the systemic protection as compared to that induced by either solution alone (Table 3).

The rate of development of powdery mildew colonies on protected plants was directly related to the concentration of *S. fuliginea* inoculum (Table 6). High inoculum pressure (16.0 × 104 conidia per ml), which was used to challenge the protected plants, did not affect the protection on the upper leaf (Table 6). Although this protection appeared to be relative and certainly not absolute, it is evident that it remained effective. More importantly, this relative protection may be expressed as a delay in colony development, which could affect the spread and progress of an epidemic in the field or greenhouse.

Although the action mechanism(s) of these chemicals in restricting disease development is still unknown, the systemic induction of peroxidase and β-1,3-glucanase suggests that they rapidly trigger the plant's general response to the induction and activation of the mechanism(s) for resistance in the upper, protected leaves (Tables 7 and 8). Unlike other host-pathogen systems the activity of peroxidase and β-1,3-glucanase were slightly depressed after challenge inoculation with *S. fuliginea*. The reason for this decrease is not currently understood.

The use of fertilizers and organic amendments in the control of plant disease has already been reported (Huber, 1981). The efficiency of salts of microelements to induce local and systemic protection against powdery mildew in cucumbers and that of NPK fertil-

izers against several diseases in a wide range of crops indicate that they may form a competitive alternative to the increasing use of fungicides against major diseases. NPK fertilizers have been found to be effective on cucumbers (Reuveni, M. et al., 1966), roses (Reuveni, R. et al., 1994b), wine grapes (Reuveni, M., and Reuveni, R., 1995a), nectarines and mangoes (Reuveni, M. and Reuveni, R., 1995b), as well as stimulating plant growth, as our data suggests for maize (Reuveni, R. et al., 1994a, c, 1996) and cucumbers (Reuveni, M. et al., 1993).

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