Local and Systemic Control of *Phytophthora infestans* in Tomato Plants by DL-3-Amino-n-Butanoic Acids

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ABSTRACT

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Tomato plants (six- to seven-leaf stage) sprayed with the nonprotein amino acid DL-3-amino-n-butanoic acid (DL-β-amino-n-butanoic acid) were protected against a challenge infection with *Phytophthora infestans*. A single foliar spray (19.4 mM, 2,000 ppm) applied either before or after inoculation provided more than 95% control of the disease compared with unsprayed challenged plants. The concentration of the racemate required to achieve 50% control of the disease was 175 ppm (1.7 mM). DL-2-Amino-n-butanoic acid was half as effective compared with the DL-3-amino-n-butanoic acid, whereas the 4-amino-n-butanoic acid was ineffective against the blight. DL-3-Amino-n-butanoic acid protected against seven isolates of *P. infestans* in seven cultivars of tomato, which carry various levels of susceptibility to the blight pathogen. It produced minor phytotoxic symptoms in tomato leaves. It had no effect on sporangial germination in water or on leaf surfaces of tomato nor on mycelial growth in rye seed liquid medium.

Additional keywords: aminobutyrates, induced resistance, late blight, *Lycopersicon esculentum,* plant defense.

Nonprotein amino and imino acids are widespread secondary plant metabolites with extremely diversified chemical and physiological properties. Some members of this group are biologically toxic. About 240 nonprotein amino acids are known to occur in plants, mainly stored in seeds of Leguminaceae to provide nitrogen to the germinating plant (9).

4-Amino-n-butanoic acid (γ-amino-n-butanoic acid, piperidinic acid, GABA) is widely distributed in higher plants (10). It has been extensively studied as a strong inhibitory neurotransmitter in humans and animals (9,10). It is produced from glutamate by α-glutamic acid decarboxylase. DL-2-Amino-n-butanoic acid

(α-amino-n-butanoic acid) was reported in *Pisum* (9), and a single report (4) showed the occurrence of DL-3-amino-n-butanoic acid (β-amino-n-butanoic acid) in root exudates of tomato plants.

Little information is available in the literature on the effects of exogenously applied amino acids on plant disease. The increase in 4-aminobutanoic acid content and glutamate decarboxylase activity have been observed in many plants under a variety of environmental stress conditions (10). Papavizas (8) showed that DL-3-amino-n-butanoic acid and DL-threo-3-methylaspartic acid controlled Aphanomyces root rot in pea plants. Asselin et al (1) and Lotan and Fluhr (7) showed that amino-n-butanoic acids induce pathogenesis-related proteins in tobacco.

Lotan and Fluhr also reported (7) that DL-2-amino-n-butanoic acid induced the production of the phytoalexin capsidiol in tobacco. It was, therefore, tempting to look for the effects of various amino acids on fungal and disease development in the tomato-late blight pathosystem with the hope of identifying molecules capable of protecting plants against disease. It became apparent that while protein amino acids had no such activity, the nonprotein amino acid DL-3-amino-n-butanoic acid was strongly inhibitory to the development of late blight in tomato. As shown in this study, this compound lacks activity against the late blight fungus in vitro, which makes it a possible inducer of plant defense.

MATERIALS AND METHODS

Plants. Most of the experiments were done with the tomato (*Lycopersicon esculentum* Mill.) F_1 hybrids Baby and Florida Basket. Some experiments were conducted with the F_1 hybrids Rheinland Rhum, Berner Rose, Mignon, Montfavet, and Ricello (seeds supplied by Mauser, Dubendorf, Switzerland).

Plants were grown in the greenhouse (15–28 C) in pots (12 cm in diameter) filled with sandy loam, peat, and perlite mixed in equal volumes. Plants were fertilized twice a week with 0.5% N-P-K (20:20:20). Plants were used for experimentation when they had six or seven compound leaves (unless otherwise stated).

Fungus. Most of the experiments were done with the isolate MR1 of *Phytophthora infestans* (Mont.) de Bary collected at Gevuloth, Israel, in 1984 (5). Some experiments were conducted with the Israeli isolates MS1, M52, MS3, MR2, and MR3 (5). All isolates (except MS1) carry the virulence factors 1, 3, 4, 7, 8, and 10 and belong to the A2 mating type. MS1 belongs to the A1 mating type and carries only the virulence factors 1, 3, 4, and 7 (5). Some experiments included the Swiss isolate S49 (Sandoz, Basel, Switzerland), which belongs to the A1 mating type. The virulence factors for this isolate are not known. The fungus was grown on potato tuber slices (cultivar Alpha, except for isolate S49, which was grown on Bintje potato slices) at 15 C in the dark. Fresh sporangia were harvested at 6 days after the slices were inoculated into double distilled water (4 C), and the concentration was adjusted to 10,000 sporangia per milliliter (unless stated otherwise).

Treatment and inoculation of intact plants. Most of the experiments were done with three isomers of amino-n-butanoic acid: DL-2-amino-n-butanoic acid, DL-3-amino-n-butanoic acid, and 4-amino-n-butanoic acid. Some experiments were done with the *iso* derivatives of DL-2- and DL-3-aminobutanoic acid. Other compounds tested (mostly amino acids) are listed in Results. The compounds were each dissolved in water (up to 2,000 ppm) and sprayed on either the abaxial or adaxial leaf surfaces of tomato plants with a fine glass atomizer (about 3 ml per plant). Usually, six plants were used for each treatment concentration. The plants were left on the bench until droplets dried (usually 2–3 h) and then placed in a growth cabinet calibrated to 22 C and 14 h of light (120 $\mu E \cdot m^{-2} \cdot s^{-1}$) per day until they were inoculated. The systemic protection of upper leaves was tested in plants sprayed only on the three lower leaves. In some experiments, 3-amino-n-butanoic acid was also applied curatively 24 h postinoculation.

The challenge inoculation (isolate MR1, unless stated otherwise) with *P. infestans* was conducted at various time intervals after spray, from 30 min to 12 days, by spraying a sporangial suspension onto the adaxial leaf surfaces (about 5 ml per plant) with a glass atomizer. In one experiment, inoculum droplets (10 μl containing about 100 sporangia) were placed on the leaf surfaces, two droplets per leaflet, one from each side on the main vein.

Inoculated plants were kept at 100% RH in the dark for 24 h at 18 C and then returned to a growth cabinet maintained at 20 C with 12 h of light (120 $\mu E \cdot m^{-2} \cdot s^{-1}$) per day.

Feeding and inoculation of detached leaves. Leaf 4 (from the stem base) with five leaflets was excised from plants (seven-leaf stage). The cut petiole end of each leaf was placed in 1 ml of aqueous solution of the test compound in 2-ml vials at 20 C (120 $\mu E \cdot m^{-2} \cdot s^{-1}$, 70% RH) until the solution was taken up by the leaf (usually 2–3 h). The leaves (three per treatment) were then removed from the vials and placed in 20- × 20-cm plastic plates (Nunk) on wet filter paper with their adaxial surfaces uppermost. Each leaflet was then inoculated with two droplets of the sporangial suspension (10 μl, MR1, 2,500 sporangia per milliliter), one droplet on each side of the midvein. Plates were incubated for 20 h at 13–15 C in the dark and then transferred to the growth chamber (20 C) to allow for disease development. Seven days later, lesion diameter was recorded and percentage protection calculated relative to the water-treated leaves.

Estimation of disease development. Disease development was monitored 4 days (unless stated otherwise) after challenge inoculation by visually estimating the proportion of a leaf area occupied by blight lesions on a 0–4 scale, in which 0.1 = a few circular 1- to 2-mm lesions; 0.5 = up to ~5% of the leaf area blighted; 0.75 = >5 but <10%; 1 = >10 but <25%; 2 = >25 but <50%; 3 = >50 but <75%; 3.5 = about 76–85%; 3.75 = 86–95%; and 4 = 96–100%. Mean disease severity for a plant was calculated as the mean of disease severity values of all the leaves of that plant. In one series of experiments, the area occupied by blight lesions was measured by tracing it on transparent paper and then weighing the paper. Percentage protection against the disease was calculated as control (%) = 100 $(1 - x/y)$, in which x and y are disease severity values in treated, challenged and control (untreated, challenged) plants, respectively. In most experiments, six plants were used per treatment. Experiments were repeated at least twice.

Sporangial germination and mycelial growth in vitro. Fresh sporangial suspensions of isolates S49 and MR1 were mixed with amino-n-butanoic acids of various concentrations and applied (20 μl) to depression glass slides in triplicates. The slides were incubated at 15 C in the dark for 20 h. Germinating sporangia and cystospores were counted under the microscope. The mycelial growth of these isolates was examined on liquid rye seed-extract medium (60 g of rye seed, 20 g of sucrose, and 2 g of yeast extract per liter of water) amended with autoclaved amino-n-butanoic acids of various concentrations. There were three petri dishes per treatment concentration. Autoclaving had no effect on the activity of the 2- and 3-isomers tested on tomato plants (see Results). The dry weights of mycelial mats were determined 2 wks after the plates were inoculated. Sporangial germination on planta was tested with Calcofluor staining and epifluorescence as described (3); one disk (1 cm in diameter) was taken from each leaf of three treated and three untreated plants.

RESULTS

Phytotoxicity. The pH values of the water solutions (2,000 ppm) were 6.82, 6.77, and 6.73 for DL-2-amino-n-butanoic acid, DL-3-amino-n-butanoic acid, and 4-amino-n-butanoic acid, respectively. Of the seven F_1 hybrids tested, Mignon was the most sensitive to a foliar spray with DL-3-amino-n-butanoic acid. Plants sprayed with 2,000 ppm developed necrotic areas on the lower leaves within 2 days after spray. On the other hand, all six of the other hybrids exhibited minor sensitivities to this compound applied at concentrations of up to 2,000 ppm. Thus, Florida Basket showed no visible symptoms, while Baby produced about 10 necrotic microlesions (0.5 mm in diameter) per leaflet on lower leaves and occasional chlorotic microlesions on upper leaves. In no instance was growth of the plants inhibited, nor were any symptoms produced at concentrations of 1,000 ppm or less. DL-

2-Amino-n-butanoic acid and 4-amino-n-butanoic acid produced no symptoms on Florida Basket or on Baby (other cultivars were not tested) after sprays with concentrations of 2,000 ppm or less. Comparative tests with tobacco (Ky14 and Ky16) showed much higher susceptibilities to DL-2- and DL-3-amino-n-butanoic acids but not to 4-amino-n-butanoic acid; plants sprayed with 200 ppm produced means of 250 and 150 necrotic microlesions (1–1.5 mm in diameter) with DL-2- and DL-3-amino-n-butanoic acid, respectively, at 48 h after spray.

Effect of amino-n-butanoic acids on sporangial germination and mycelial growth. Sporangial germination in vitro of isolates MR1, MR3, or S49 was unaffected by either the DL-2-, DL-3-, or the 4-isomer of amino-n-butanoic acid up to a concentration of 1,000 ppm. The number of germinating cystospores and the length of germ tubes were similar to those observed in water. None of the amino-n-butanoic acids adversely affected growth of *P. infestans* (isolates MS3 and S49) in liquid cultures when amended at final concentrations of up to 1,000 ppm. Calcofluor staining of inoculated leaf disks (20 h) revealed abundant cystospore germination on the surfaces of control as well as on DL-3-amino-n-butanoic acid-treated (2,000 ppm, 5 days after spray) leaves.

Disease control with amino-n-butanoic acids. Pooled data from several experiments made with seven cultivars of tomato revealed that DL-3-amino-n-butanoic acid provided a significantly higher level of control (84%) of late blight compared with DL-2-amino-n-butanoic acid (36%) and 4-amino-n-butanoic acid (15%) (all applied at 2,000 ppm). Three other related amino-n-butanoic acids, L-2-amino-n-butanoic acid, DL-2-amino*iso*butanoic acid, and DL-3-amino*iso*butanoic acid, provided no control of the blight in tomato plants. Detailed experiments, therefore, were conducted with the DL-3-amino-n-butanoic acid.

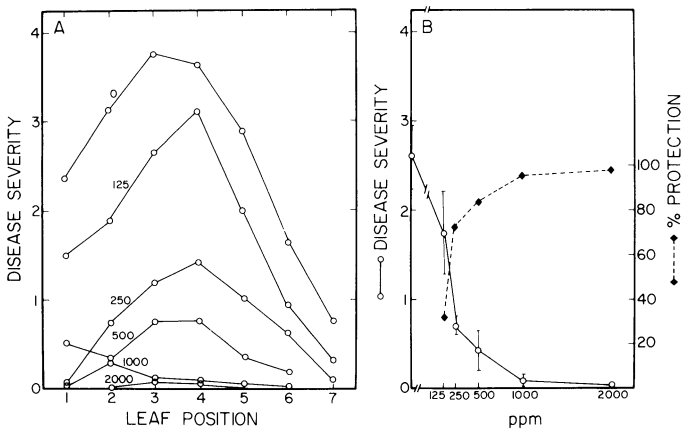

Fig. 1. Protection of tomato plants (cultivar Baby) against late blight (*Phytophthora infestans,* isolate MR1) by DL-3-amino-n-butanoic acid. Plants were sprayed with various doses (125–2,000 ppm) of the compound and challenged 1 day later. **A,** Mean disease severity on individual leaves (from six plants) estimated 4 days after challenge inoculation; **B,** mean disease severity per plant. Bars represent standard deviations of the mean.

Figure 1 exemplifies the magnitudes of disease severity and protection achieved in tomato plants (Baby) challenged 1 day after treatment with DL-3-amino-n-butanoic acid. Disease severity was dependent on leaf age (position on the stem) and concentrations of the chemical. Middle leaves were most blighted and least protected, compared with bottom or top leaves, at all concentrations tested (Fig. 1A). Mean protection, calculated for all leaves on a plant (Fig. 1B), increased with increasing concentrations of the compound. Maximal protection (91–97%) was seen in plants sprayed with 2,000 ppm. At 500 ppm, 84% protection was observed. EC_{50} value (50% effective concentration) calculated after logit transformation from Figure 1B was 175 ppm (1.7 mM).

Detailed lesion-size records were taken from the cultivar Florida Basket. Plants (seven-leaf stage) were spray challenged with *P. infestans* 1 day after being sprayed with DL-3-amino-n-butanoic acid, and lesions were measured 3 days later, before coalescence. The compound, applied at 1,000 or 2,000 ppm, drastically and significantly reduced lesion size, compared with challenged, untreated plants. Mean blighted areas per plant ($n = 6$) were 152, 8.8, and 2.6 cm^2 in plants treated with 0, 1,000, and 2,000 ppm of the compound, respectively. The area occupied by blight lesions in treated plants was largest in leaf 3 (from the stem base).

Droplet inoculation showed a large difference in lesion development between untreated plants and plants treated with 2,000 ppm of DL-3-amino-n-butanoic acid. Four days after inoculation, control plants produced lesions whose mean diameters ranged between 4 and 12 mm, whereas treated plants produced lesions 0–2 mm in diameter. Seven days after inoculation, the mean diameters of the lesions developed in control and treated plants were 6–25 and 0–5.4 mm, respectively (Table 1). Leaf 2 was least protected, probably because of a rapid translocation of the compound to upper leaves (Y. Cohen and U. Gisi, *unpublished results*).

DL-3-Amino-n-butanoic acid was effective in controlling late blight induced by seven isolates of *P. infestans.* Thus, plants (Baby) treated with 2,000 ppm of the compound and challenged 2 days later were protected, 4 days after challenge, at 88, 94, 96, 85, 96, 94, and 99% against isolates MS1, MS2, MS3, MR1, MR2, MR3, or S49, respectively.

DL-3-Amino-n-butanoic acid also had a strong curative activity. Plants treated with this compound 24 h postinoculation were highly (85–100% in various leaves) protected against *P. infestans* (data not shown). Table 2 shows that the residual activity of the compound against the blight lasted for at least 12 days. (Longer periods were not tested.) The newly developed leaves were also protected.

The effect of the interaction between the concentration of the challenge inoculum and leaf age on the protection induced by

TABLE 1. Effect of DL-3-amino-n-butanoic acid (2,000 ppm) on lesion development of *Phytophthora infestans* (isolate MR1) on tomato plants (cultivar Baby)[a]

Leaf position from stem base	Mean lesion diameter and percentage protection					
	4 days after challenge			7 days after challenge		
	Control (mm)	DL-3-Amino-n-butanoic acid-treated (mm)	Percentage protection	Control (mm)	DL-3-Amino-n-butanoic acid-treated (mm)	Percentage protection
1	12	0.3	97	20	2.7	86
2	12	2.0	83	20	5.4	73
3	10	0.7	93	25	1.1	96
4	7	0.3	96	22	0.3	99
5	6	0	100	14	0	100
6	5	0	100	9	0	100
7	4	0	100	6	0	100

[a] Plants were sprayed with DL-3-amino-n-butanoic acid and challenged 4 days later by placing two 10-µl droplets containing about 100 sporangia per droplet on each leaflet. Lesion diameters were measured 4 and 7 days after challenge. Figures for control (untreated, challenged) plants were rounded to 1 mm, whereas those for plants treated with DL-3-amino-n-butanoic acid were rounded to 0.1 mm. Standard deviations of the means ($n = 24$) did not exceed 20% of the values presented. Values for treated plants were significantly ($P < 0.05$) different from the corresponding values for control plants (Student's t test).

DL-3-amino-n-butanoic acid was investigated in the cultivar Florida Basket. Plants were either not treated or treated with a 2,000-ppm solution of DL-3-amino-n-butanoic acid and were challenged 48 h later with sporangial suspensions containing 0.5, 1, or 1.7 × 10^4 sporangia per milliliter. Disease records taken 4 days after challenge inoculation are presented in Figure 2. Disease severity in challenged, untreated plants increased, as expected, with increasing inoculum concentrations. Lower and middle leaves were more severely blighted compared with upper leaves at all inoculum concentrations. Treated plants were protected against the blight, more so when challenged with 0.5 than with 1 or 1.7 × 10^4 sporangia per milliliter. Leaves 4–8 were 75–100% protected, regardless of the inoculum load they received (Fig. 2).

The dynamics of disease development in control untreated, challenged and treated, challenged plants were followed in eight-leaf plants (Baby) sprayed with 500, 1,000, and 2,000 ppm of DL-3-amino-n-butanoic acid. Three days after challenge, control plants were about 50% blighted, and plants treated with 500 and 1,000 or 2,000 ppm were 93 and 100% protected, respectively.

TABLE 2. Residual activity of DL-3-amino-n-butanoic acid (2,000 ppm) against late blight in tomato plants (cultivar Baby) at 12 days after spray[a]

Leaf position from stem base	Disease severity ± SD (0–4 scale)[b]		
	Control	DL-3-amino-n-butanoic acid-treated	Percentage protection
1	1.4 ± 0.9	0.04 ± 0.05	97
2	3.1 ± 1.0	0.07 ± 0.05	98
3	3.3 ± 0.5	0.45 ± 0.30	86
4	3.5 ± 0.6	0.43 ± 0.15	88
5	3.4 ± 0.5	0.96 ± 0.42	72
6	3.1 ± 0.5	0.60 ± 0.50	81
7	2.3 ± 0.9	0.37 ± 0.30	84
8	1.4 ± 0.6	0.05 ± 0.05	96
9	0.8 ± 0.5	0	100
10	0.3 ± 0.3	0	100
11	0.1 ± 0	0	100
12	0.1 ± 0	0	100
Mean per plant[c]	1.88 ± 0.45	0.25 ± 0.06	87

[a] Six-leaf plants were sprayed with DL-3-amino-n-butanoic acid and challenged 12 days later with *Phytophthora infestans* (isolate MR1). Disease was estimated 4 days after challenge.

[b] Calculated from means of individual leaves ($n = 6$). 0.1 = a few circular 1–2-mm lesions; 0.5 = up to ~5% of the leaf area blighted; 0.75 = >5 but <10%; 1 = >10 but <25%; 2 = >25 but <50%; 3 = >50 but <75%; 3.5 = about 76–85%; 3.75 = 86–95%; and 4 = 96–100%.

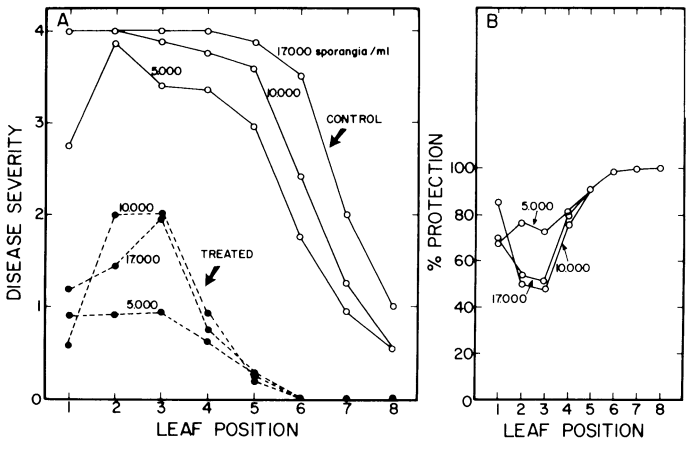

Fig. 2. Protection of tomato plants (cultivar Florida Basket) against late blight by DL-3-amino-n-butanoic acid. Plants ($n = 6$) were sprayed with 2,000 ppm of the compound and challenged 3 days later with 0.5, 1, or 1.7 × 10^4 sporangia of *Phytophthora infestans* (isolate MR1) per milliliter. **A,** Disease severity and **B,** percentage of protection on individual leaves was estimated 4 days after challenge inoculation. Mean values per plant in treated plants were significantly different ($P < 0.05$) from means of control plants at corresponding inoculum concentrations (Student's t test).

As disease progressed in control plants, the magnitude of protection decreased in induced plants. At 7 days after challenge, when 75% of the foliar area was blighted in control plants, DL-3-amino-n-butanoic acid of 500, 1,000, and 2,000 ppm provided 49, 57, and 97% protection, respectively.

Local vs. systemic induced resistance was studied in eight-leaf plants (Baby). All the leaves or only the three lower leaves were sprayed with 2,000 ppm of DL-3-amino-n-butanoic acid. Fully sprayed plants (3 ml per plant) were strongly protected (85–100% in various leaves) against the blight (middle leaves were least protected), while plants sprayed on their three lower leaves (1 ml per plant) were protected in the lower (treated, 75–95%) and upper (untreated, 60–100%) leaves, except leaf position 4 (15%). These results suggest an acropetal systemic effect of DL-3-amino-n-butanoic acid or of a metabolite it produces from the lower to the upper leaves of tomato plants. Other evidence of its systemic translocation is presented in Table 3, which shows that DL-3-amino-n-butanoic acid was highly effective against *P. infestans* when introduced into detached tomato leaves by petiole feeding. At the lowest concentration tested (250 ppm), lesion size was reduced by 77%. At ≥500 ppm, more than 90% protection was observed. In comparable experiments, DL-2-amino-n-butanoic acid and 4-amino-n-butanoic acid at 2,000 ppm provided 37 and 0% protection, respectively (data not shown). It should be noted that no phytotoxic symptoms were observed in such experiments of petiole feeding. Other experiments (data not shown) indicated that DL-3-amino-n-butanoic acid had a significant translaminar activity, regardless of whether sprayed on the abaxial or adaxial leaf surfaces.

The efficacy of DL-3-amino-n-butanoic acid (2,000 ppm) in the dark or in the light was examined in Baby. Six-leaf plants were incubated after spray at 20 C in the dark or in the light (200 $\mu E \cdot m^{-2} \cdot s^{-1}$) for 24 h and then challenged. Disease records were taken 4 days after inoculation. Mean disease values in the dark were 3.0 ± 0.3 and 0.7 ± 0.3 (77% protection) and in the light, 2.3 ± 0.2 and 0.3 ± 0.1 (87% protection) for untreated control and treated plants, respectively.

To test the effect of temperature on control efficacy, plants (Baby) were sprayed with water or with DL-3-amino-n-butanoic acid (2,000 ppm), kept in growth chambers at 15, 20, or 25 C for 4 days, challenged at 18 C, and returned for disease development to a growth chamber at 20 C. Four days after challenge, mean disease severity in water-treated plants was 2.1 ± 0.4, 2.3 ± 0.6, and 2.9 ± 0.1 in plants incubated at 15, 20, and 25 C, respectively, compared with 0.06 ± 0.04 (97% protection), 0.14 ± 0.05 (94% protection), and 0.46 ± 0.01 (84% protection) in treated plants incubated at 15, 20, and 25 C, respectively.

Disease control with other compounds. Other compounds, mostly protein and nonprotein amino acids, were tested for their ability to control *P. infestans* in tomato plants. Adaxial leaf surfaces of tomato plants (Baby) were sprayed with solutions (250–2,000 ppm), challenged with *P. infestans* (MR1) 2 days later, and examined for disease resistance 4 and 7 days postinoculation. L-Canavanine caused severe chlorosis in the youngest leaves at 500–2,000 ppm 2 days after spray, and fusaric acid caused necrosis

TABLE 3. Late blight development on detached tomato leaves (cultivar Baby) treated with different concentrations of DL-3-amino-n-butanoic acid by petiole feeding[a]

Concentration (ppm)	Mean lesion size (mm ± SD)[b]	Percentage protection
0	12.8 ± 1.6	...
250	3.0 ± 0.9	76.5
500	1.2 ± 0.8	90.2
1,000	0.3 ± 0.2	97.6
2,000	0.2 ± 0.1	98.4

[a] Leaf 4 from the stem base was detached from seven-leaf plants and placed with the cut end of the petiole in 1 ml of the compound. Leaves were inoculated with *Phythophora infestans* (isolate MR1, 25 sporangia per droplet per site, 30 droplets per 15 leaflets) at 2 h. Lesion sizes were recorded 7 days after inoculation.

[b] Means include zeros from unsuccessful infections.

in the youngest leaves treated with 500–2,000 ppm. Salicylic acid at 2,000 ppm caused severe necrosis in all leaves and therefore was tested with concentrations of 125–500 ppm only.

Disease records taken 4 days postinoculation showed a significant reduction (60–80%) in disease severity in plants treated with 2,000 ppm of L-homoserine, β-alanine, L-canavanine, and fusaric acid. However, 7 days postinoculation, percent control of the disease was only 20–30%. None of the following compounds gave any significant protection after 4 days: L-alanine, DL-α-aminocaprylic acid, L-aspartic acid, D-aspartic acid, L-asparagine, L-arginine-HCl, L-cysteine, cycloleucine, L-glutamic acid, L-glutamine, L-histidine-HCl, isopropylamine, L-isoleucine, L-leucine, L-lysine-HCl, L-malic acid, DL-malic acid, malonic acid, DL-norleucine, O-methyl-DL-serine, L-ornithine-HCl, L-phenylalanine, L-proline, L-serine, D-serine, DL-serine, succinic acid, L-threonine, D-threonine, DL-threo-β-methylaspartic acid, trans-4-hydroxy-L-proline, L-tryptophan, L-tyrosine, L-valine, DL-norvaline, and salicylic acid (at 125–500 ppm). At 7 days postinoculation, only plants treated with DL-3-amino-n-butanoic acid exhibited a high degree (92%) of protection.

DISCUSSION

Induced resistance in a variety of host-pathogen systems is a well-documented phenomenon (6). Such resistance may be induced by biotic or abiotic agents. Induced resistance mechanisms might provide new strategies for crop protection, either through the development of transgenic plants that express molecular components of induced resistance or through the use of new chemicals that act by stimulating disease-resistance mechanisms (6,11). This paper describes a chemical effective in controlling late blight (*P. infestans*) in tomato plants. This is the nonprotein DL-3-amino-n-butanoic acid, which had no activity against *P. infestans* in vitro but which exerted a strong antifungal activity in vivo. Of the three isomers tested, only the DL-3-amino-n-butanoic acid was highly effective (>90%) against late blight in tomato. The *iso* derivatives of aminobutanoic acid were inactive, as were 31 other (mostly protein) amino acids, thus indicating a very specific action of this molecule in tomato plants that leads to protection against *P. infestans*. This compound provided not only a protective activity (for 12 days, the longest period tested) on treated and newly developed leaves on a plant but also a curative activity against the blight when applied after infection. A relatively high concentration of the amino acid was required to achieve complete or almost complete control of the disease. This may result from the fact that a DL-racemate was used. A much lower concentration of either D- or L-3-amino-n-butanoic acid would probably be required to fully control the disease. The compound had a strong translaminar activity, as could be judged from its high control efficacy when applied to the leaf surface opposite to the surface inoculated. It also had an acropetal systemic effect (basipetal movement was not tested), because it protected detached leaves fed through their petioles and upper leaves of intact tomato plants when applied to the lower leaves. The middle leaves of the hybrids tested were more susceptible to fungal attack compared with the bottom or top leaves and consequently were relatively less protected upon treatment with DL-3-amino-n-butanoic acid. We do not know the reason for the enhanced susceptibility of the middle leaves, but it is worthwhile to note that, when incubated in a cellulase plus pectinase solution, leaf disks taken from the middle leaves released their protoplasts in about half the time as those taken from bottom or top leaves (Y. Cohen, *unpublished*). DL-3-Amino-n-butanoic acid induced a higher level of resistance in plants incubated for 24 h after spray in the light compared with plants incubated in darkness, indicating a possible requirement for energy to operate the resistance mechanism (7). On the other hand, the acid performed better in plants kept at 15 C than in those kept at 20 or 25 C. The reasons for this are unknown. The mechanism by which the compound acts is not fully understood. Sporangial or cyst germination and appressoria formation of the fungus on leaf surfaces (this study) as well as penetration of the fungus into epidermal cells (A. Raviv and Y. Cohen, *unpublished data*) were similar in treated and untreated leaves. It is clear, therefore, that mycelial growth and, as a result, lesion formation are inhibited in vivo. A similar conclusion was reached by Papavizas (8) working with DL-3-amino-n-butanoic acid as a soil drench against *Aphanomyces euteiches* in peas. The lack of activity of amino-n-butanoic acids (up to 1,000 ppm) against *A. euteiches* in vitro was recently confirmed by U. Gisi (*personal communication*). Papavizas also observed a strong activity of β-methylaspartic acid against *A. euteiches* in peas that we have failed to obtain with *P. infestans* on tomato.

What causes fungal growth inhibition in vivo? One or more of the following host-mediated possibilities may be considered: DL-3-amino-n-butanoic acid is metabolized to an antifungal compound; it induces phytoalexin production; it induces synthesis of pathogenesis-related proteins. We have good reasons to believe that DL-3-amino-n-butanoic acid is not altered in tomato plants. First, isopropylamine, a possible decarboxylated product of DL-3-amino-n-butanoic acid, was found to be inactive in controlling *P. infestans* in tomato; and second, ^{14}C-DL-3-amino-n-butanoic acid was the only labeled compound we recovered from tomato plant tissues 1–6 days after foliar application of ^{14}C-DL-3-amino-n-butanoic acid (Y. Cohen and U. Gisi, *unpublished*). The phytoalexin theory also does not seem to hold, because we have failed to detect such compounds in extracts taken from tomato plants treated with DL-3-amino-n-butanoic acid. On the other hand, accumulations of pathogenesis-related proteins were found in leaves of tomato plants treated with DL-3-amino-n-butanoic acid but were not found in leaves treated with DL-2- or 4-amino-n-butanoic acid (2). This agrees with Asselin et al (1), who reported that DL-2- and DL-3-amino-n-butanoic acids induced pathogenesis-related proteins in tobacco leaf disks, and with Lotan and Fluhr (7), who showed that DL-2-amino-n-butanoic acid induced the pathogenesis-related proteins PR-1, chitinases, and β-1,3-glucanases (as well as the phytoalexin capsidiol) in tobacco plants. The fact that the β-isomer of amino-n-butanoic acid is recognized by tomato leaves to a much higher extent than are the α- or γ-isomers may indicate a specific binding to a target molecule in the host plant.

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