Local and Systemic Protection Against *Phytophthora infestans* Induced in Potato and Tomato Plants by Jasmonic Acid and Jasmonic Methyl Ester

Yigal Cohen, Ulrich Gisi, and Thierry Niderman

First author: Department of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel; and second and third authors: Sandoz-Agro Research, Witterswil, CH-4108, Switzerland.

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ABSTRACT

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Jasmonic acid (JA) and jasmonic methyl ester (JME) are plant lipid derivatives that have been postulated to play a role in plant wound and pathogen responses. Here we report that JA and JME applied as foliar sprays to potato (cv. Bintje or Alpha) or tomato (cv. Baby) plants protected them against a challenge infection with *Phytophthora infestans*. Local protection against *P. infestans* isolates S49 or MR1 was evident in plants treated with as low as 62.5 μg (~0.3 μmol)/ml, whereas systemic protection was evident in plants treated with jasmonates at 1000 μg (~4.8 μmol)/ml. Jasmonates at 4.8 μmol/ml did not inhibit fungal mycelial growth in agar cultures. JA and JME stimulated direct germination of sporangia in vitro and on planta. SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) analysis of acid-soluble proteins of treated tomato leaves revealed enhanced levels of two proteinase inhibitors and ~24- and 29- to 30-kDa proteins. Jasmonates did not induce phytoalexin synthesis in treated potato or tomato plants. Systemin did not protect either potato or tomato from the blight fungus. We speculate that metabolites induced by jasmonates are responsible for the protection against *P. infestans*.

Additional keywords: induced resistance.

Jasmonic acid (JA) [3-oxo-2-(2′-*cis*-pentenyl)-cyclopentane-1-acetate) and jasmonic methyl ester (JME) are naturally occurring growth regulators widely distributed in plants (23,29). JME was first isolated as an odiferous constituent of the essential oil of *Jasminum grandiflorum* L. (9). JA and 7-*iso*-JA are synthesized in plants from linolenic acid in lipoxygenase-dependent pathways (1,31). JA and JME inhibit plant growth and promote the senescence of detached leaves (literature [1]). JA inhibits pollen germination of *Camellia* (36,37) and seed germination of *Brassica napus* L. and *Linum usitatissimum* L. (34) and causes loss of chlorophyll in 27 plant species (17). It induces the accumulation of vegetable storage proteins in soybean leaves and suspension cultures (1,19,28) as well as in *B. napus* (34). Weidhase et al (32) showed that JME induces a typical class of proteins (jamonate-induced proteins) in leaf cells of 26 plant species. The structures of JA and JME are similar to mammalian eicosanoids, which are potent modulators of inflammatory responses (1).

Creelman et al (8) showed that wounded stems of soybean rapidly accumulate JA and JME. Addition of JME to soybean

suspension culture increased mRNA levels of three wound-responsive genes (chalcone synthase, vegetable-storage proteins, and proline-rich [PR] cell wall protein). Yeast elicitor caused a rapid transient accumulation of JA and JME by plant cell suspension culture (15). Addition of JME increased transcription of phenylalamine ammonia lyase and the accumulation of secondary metabolites.

Ryan and collaborators have shown that wounding by chewing insects and other mechanical damage results in the localized and systemic accumulation of high levels of proteinase-inhibitor (PI) proteins in leaves of several plant families (literature [3,11]). These newly synthesized PI proteins are part of the array of defensive chemicals that can protect tobacco against insect pests (18,20) and tomato against bacteria (25). Ryan and coworkers recently reported that atmospheric JME and JA strongly induced the accumulation of PI proteins in leaves of tomato, tobacco, and alfalfa (11,12). They also showed that an 18-amino acid polypeptide named systemin that occurs in minute amounts in tomato plants induces, in femtomole concentrations, increases in PI proteins in tomato plants (26). In their latest report, Farmer and Ryan (13) demonstrated that three of the octadecanoic precursors of JA, i.e., linolenic acid, 13(*S*)-hydroperoxylinolenic acid, and 12-oxo-phytodienoic acid induce accumulation of PI proteins in tomato plants after foliar application. They proposed a model in which wounding by herbivores would release systemin, which interacts with the plant plasma membrane receptors, leading to the activation of lipase and the release of linolenic acid into the cytoplasm. Linolenic acid would be converted to JA, which is postulated to interact with a receptor to activate PI gene expression (13). Wildon et al showed (33) that the activation of proteinase inhibitors in tomato plants by wounding takes place even when translocation in the phloem is completely inhibited and suggested that an electrical rather than a chemical signal is the underlying mechanism of the systemic wound response in tomato.

We were interested in learning if JA, JME, or systemin may induce local or systemic protection of a plant against fungal attack. For this purpose, we selected the tomato and potato-late blight system. Plants were treated with jasmonates or systemin, and their response to the late blight fungus was measured. We also studied the direct effect jasmonates have on *Phytophthora infestans* (Mont.) de Bary in vitro.

MATERIALS AND METHODS

Plants and pathogen. Two cultivars of potato (*Solanum tuberosum* L.), Bintje and Alpha, were used. Plants were grown from tubers in 1-L pots in the greenhouse (18–24 C) in sandy loam/peat/vermiculite (1:1:1 v/v) and were used when they had developed nine to 11 compound leaves. Tomato (*Lycopersicon esculentum* Mill.) cultivar Baby also was used. Plants were grown from seed in 0.5-L pots filled with the soil mixture mentioned above in the greenhouse (19–28 C) and were used when they had developed five to six leaves. Isolates S49 (metalaxyl-sensitive, Sandoz collection) and MR1 (metalaxyl-resistant, our collection [21]) of *P. infestans* were used for inoculation. The fungus was grown on potato tuber slices (S49 on Bintje, MR1 on Alpha) at 13–15 C in the dark. Freshly produced sporangia (about 1 wk after inoculation) were harvested in glass-distilled water (4 C) and used for challenge inoculations.

Chemicals and application. JME was a gift from Firmenich, Geneva. JA was supplied by Sandoz-Agro Research, Witterswil, Switzerland. Systemin was a gift from C. A. Ryan (Washington State University, Pullman).

JA and JME usually were used as acetone sprays (Table 1). In some experiments, sonicates in distilled water or in 15 mM phosphate buffer (pH 6.5) or emulsions in N-25 (Sandoz emulgator) were used. The compounds were sprayed, using a fine glass atomizer, onto the upper (adaxial) leaf surfaces of the plants unless stated otherwise. In some experiments, JA and JME were applied to the cut end of petioles of detached leaves. Control plants were treated with either acetone, water, buffer, or N-25 in water. Plants were incubated at 20 C (16 h of light per day, 120–240 $\mu E\ m^{-2}\ s^{-1}$) and taken for inoculation 5 days after treatment, unless stated otherwise.

Systemin was dissolved in water and applied by petiole feeding. Detached leaves (with three or five leaflets) were placed with their cut petiole end in 0.5-ml solutions of systemin of various concentrations at 20 C (140 $\mu E\ m^{-2}\ s^{-1}$). Leaves were removed from solutions after they took in ~ 350 μl (about 4 h) or all the solution (24 h), were placed on wet filter paper in petri dishes, and were inoculated with *P. infestans* (MR1) 1 or 2 days later.

Inoculation and disease assessment. Tomato and potato were inoculated by spraying the upper surfaces of leaves (in some experiments, the lower surfaces) with 3 or 10 ml of sporangial suspension of *P. infestans*, respectively. With isolate S49, inoculum contained (unless stated otherwise) about 4,000 and 7,500 sporangia/ml for tomato and potato, respectively. With isolate MR1, inoculum contained (unless stated otherwise) about 2,500 and 5,000 sporangia/ml for tomato and potato, respectively. Inoculated plants were placed in a moist chamber at 18 C in the dark for 20 h and were returned to the growth chamber at 20 C. Late blight development was recorded 5 days after inoculation by counting the number and measuring the size of lesions by the method described previously (6). In some experiments, disease severity was estimated visually with a 0–4 scale, as described earlier (6). Systemin-treated detached leaves were inoculated either by placing droplets containing 20 sporangia each or by whole spraying with 2,000 sporangia/ml. Leaf area covered with blight lesions was recorded 4 days after challenge. Water-treated leaves served as controls. Percent protection against late blight was calculated as percent protection = $100(1 - x/y)$ when x and y are disease values in treated and control-challenged plants, respectively.

Fungal germination tests. Freshly produced sporangia of *P. infestans* were suspended (2×10^4 sporangia/ml) in double-distilled water (4 C). Droplets (10 μl) were applied to depressions in glass slides coated with 10 μl of JME or JA in acetone (acetone evaporated before fungus was applied, [Table 1]). Slides were placed on moist filter paper in petri dishes and incubated at 15 C (isolate S49) or 13 C (isolate MR1). Percentage of empty sporangia and of sporangia germinating directly with a germ tube(s) and the number of germinating cystospores per microscope

TABLE 1. Details of the experiments conducted with jasmonates (in acetone) in vivo and in vitro against *Phytophthora infestans*

Treated unit	Goal	Unit area (cm^2)	Unit of fresh wt. (g)	Jasmonates applied			
				Vol./ml	μg/ml	μmol per unit	μmol per gfw
6-leaf tomato	Local protection	300	5	1	62.5–2,500	0.3–12	0.06–2.40
9-leaf potato	Local protection	400	22	1.5	62.5–1,000	0.45–7	0.02–0.32
9-leaf potato	Systemic protection	400	22	1	1,000–2,000	4.8–9.5	0.22–0.43
Agar plates	Growth inhibition	18.1	10	0.1	31–2,000	0.015–9.5	0.002–0.95
Glass depressions	Germination inhibition	0.2	0.2	0.01	31–2,000	0.0015–0.095	0.008–0.48
2-leaf tomato[a]	Protinase-prohibitor protein accumulation	15	0.2			0.001–0.1	0.005–0.5

[a] Details from Farmer and Ryan (13) are presented for comparison.

field (1.77 mm^2) were recorded 20 h later. Germination tests also were conducted in planta by applying 10-μl sporangial droplets containing 100 sporangia each to detached treated leaves and staining with Calcofluor (American Cyanamid Co., Chemicals Group, Wayne, NJ) with the UV-microscope method described previously (7).

Fungal growth in vitro. The effect of JME and JA on fungal growth in vitro was tested with *P. infestans* S49 and MR1 in liquid as well as in solid media. For liquid cultures, the compounds were dissolved in 60% ethanol, and 30 μl was applied to 3 ml of rye seed liquid medium (water extract from 60 g of rye seed, 20 g of D-glucose, and 2 g of yeast extract in 1 L of water) in 5-cm petri dishes. A 5-mm agar plug containing the fungal mycelium was inoculated into each petri dish (five dishes per concentration) before the medium (3 ml) was added. Plates were incubated at 18 C in the dark, and fungal dry weight (20 h at 60 C) was determined after 11 days. The dry weight of an agar plug was subtracted from the colony weight. For solid-medium cultures, 100 μl of acetone solutions was evenly distributed on the surface of the agar medium in 4.8-cm petri dishes (Table 1). After acetone has evaporated (20 min), plates were inoculated with a 5-mm agar plug containing the fungus. Plates were incubated at 18 C in the dark. Colony diameter was measured after 1 wk.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Five-leaf tomato plants (cv. Baby) were sprayed with acetone solutions of JA or JME (100 or 1,000 μg/ml, respectively), placed in a 20 C growth chamber, and analyzed on 12.5% homogeneous polyacrylamide gels for acid-soluble (pH 2.8) proteins at 6 days, according to Christ and Mösinger (4). Acetone-sprayed plants served as controls.

Chlorophyll and carotenoid determination. Potato plants (cv. Alpha) were sprayed with acetone solutions of jasmonates; leaf samples were taken 3 days later and extracted with 80% acetone. Chlorophylls and total carotenoids were determined spectroscopically according to Lichtenthaler (22).

Phytoalexin extraction. Extraction was performed according to Hammerschmidt and Kuc (16). Tomato and potato plants were extracted 3 days after having been sprayed with JME or JA in acetone. Leaf tissue was extracted by shaking in 40% ethanol for 4 h. Ethanol was evaporated at 38 C under vacuum, and the water fraction was partitioned three times against chloroform. The chloroform was allowed to evaporate, and residue was taken in chloroform, spotted on silica gel plates, and run in chloroform/methanol (95:5). Plates were examined under UV light (254 and 366 nm) and sprayed with vanillin/sulphuric acid in methanol to detect the presence of new compounds (16). Phytuberin and capsidiol were cospotted for reference purposes. Phytuberin was isolated from potato tuber slices (cv. Alpha) inoculated with *P. infestans* S49 (incompatible), and capsidiol was isolated from tobacco plants (cv. Ky16) infected with blue mold (*Peronospora tabacina* D.B. Adam, compatible).

Recovery of jasmonates. Plants were sprayed with acetone solutions (1,000 μg/ml) of JA or JME and placed in a growth chamber at 20 C (12 h of light per day, 130 $\mu E\ m^{-2}\ s^{-1}$) in either the open air (potato) or inside sealed transparent plastic boxes (tomato). Control plants were sprayed with acetone and kept in another similar chamber. Four plants were dissected above soil level at 0, 1, 2, 5, and 7 days after spraying, and individual leaflets were dipped for 10 s in acetone to extract jasmonates from the leaf surface. The acetone was evaporated, and the residue was dissolved in chloroform. Leaves were placed in 40% ethanol and shaken for 4 h, as described above, to extract jasmonates from inside the tissues. Extracts were spotted on thin-layer chromatography (TLC) plates, run in 95:5 chloroform/methanol, sprayed with vanillin/sulfuric acid in methanol (Y. Cohen, *unpublished data*), and heated at 110 C for 10 min. JA and JME were spotted on the plates for reference purposes. The detection limit in TLC was 0.62 μg (=2.95 nmol for JA and 2.75 nmol for JME).

Statistical analyses. Experiments were performed at least twice with six replicate plants per treatment. Results given below were taken from representative experiments. Control plants were kept in a separate growth chamber to avoid vapor effects of JME. Duncan's multiple range test ($\alpha = 0.05$) was used for mean separation.

RESULTS

Toxicity and persistence. JA or JME at >1,000 μg/ml produced a slight chlorosis in potato and tomato plants at 2–3 days after spraying, regardless of the formulation used. Chlorosis intensified at 2,500 μg/ml and was accompanied by a slight growth retardation at concentrations >5,000 μg/ml. Acetone-sprayed plants

TABLE 2. Effect of jasmonic acid (JA) and jasmonic methyl ester (JME) applied as a spray to potato plants (cv. Bintje) on late blight development (*Phytophthora infestans* isolate S49) related to the time interval between spray treatment and challenge inoculation[a]

Interval	Spray treatment and conc. (μg/ml)	Number of lesions/plant	Lesion size (mm)	Blighted area (mm^2 or %)	Protection (%)
2 h	None	48 ± 17	4 ± 0.5	603	...
	N-25, 20	51 ± 14	4 ± 1	640	6
	N-25, 200	59 ± 22	4 ± 1	741	−23
	JA, 100	7 ± 7	3 ± 1	49	92
	JA, 1,000	0	...	...	100
	JME, 100	37 ± 14	4 ± 0	465	23
	JME, 1,000	6 ± 1	3.5 ± 0.5	58	90
5 days	None	28 ± 4	4.2 ± 0.5	388	...
	JA, 100	10 ± 5	3 ± 1	71	82
	JA, 1,000	3 ± 2	2.2 ± 1.4	11	97
	JME, 100	nt			
	JME, 1,000	nt			
8 days	None	121 ± 17	6.7 ± 0.9	4,300	...
	JA, 100	79 ± 28	4.7 ± 1.5	1,400	67
	JA, 1,000	52 ± 13	4.9 ± 1.6	980	77
	JME, 100	83 ± 15	4.4 ± 0.8	1,260	71
	JME, 1,000	118 ± 7	5.9 ± 1.1	3,270	24
11 days	None	...	...	64 ± 10	...
	JA, 100	...	...	69 ± 10	−8
	JA, 1,000	...	...	44 ± 6	31
	JME, 100	...	...	63 ± 9	2
	JME, 1,000	...	...	53 ± 13	17

[a]Jasmonate solutions contained N-25 at 20 or 200 μg/ml. Challenge was carried out with 7,500 sporangia/ml, except at day 8 when 10,000 sporangia/ml were used. Figures in day 11 are the percentage of blighted leaf area. Disease readings were taken 5 days post-inoculation. nt = not tested. ± = standard deviation of the mean ($n = 6$).

contained chlorophyll a, chlorophyll b, and total carotenoids at 1.8, 4.9, and 3.3 mg/g of fresh weight, respectively. JA and JME at 100 μg/ml as well as JA at 1,000 μg/ml did not significantly affect these contents. However, JME at 1,000 μg/ml reduced the content of chlorophyll a by 18%, chlorophyll b by 19%, and total carotenoids by 20%. Repeated experiments revealed that JA persisted considerably longer compared to JME on surfaces and inside leaf tissues of either potato or tomato, regardless of whether plants were in plastic boxes or in the open air. Thus, JA was recovered from leaf surfaces (10-s dip in acetone) or from inside leaf tissue (4 h of shaking in 40% ethanol) for as long as 7 days (longest period tested) after application (1,000 μg/ml) to the plants as an acetone spray. In contrast, JME could be recovered from plants extracted immediately after spraying (1,000 μg/ml) but not from plants extracted 1–7 days after spraying. The endogenous level of jasmonates in nontreated plants was <0.1 μmol/g of fresh weight.

Local protection of potato plants. Both JA and JME applied as sprays to the adaxial (upper) leaf surfaces of Bintje plants protected against a challenge inoculation with isolate S49 applied to the adaxial leaf surfaces (Table 2). The degree of protection depended on jasmonate concentration and the time interval between spraying and challenge. When the interval period was 2 h, percent protection (relative to nontreated challenged plants) was 92 and 100% for JA at 100 and 1,000 μg/ml, respectively (Table 2). When the interval period was 5 days, JA kept its high protective activity. Lengthening the interval period to 8 days (Table 2) reduced percent protection to 67 and 77% with JA and to 71 and 24% with JME at 100 and 1,000 μg/ml, respectively (Table 2). Lengthening the interval period to 11 days reduced percent protection to 31 and 17% with JA and JME at 1,000 μg/ml, respectively.

Applying JA as a spray to the abaxial (lower) leaf surface protected the adaxial (upper) leaf surfaces of Bintje from isolate S49 applied 5 days later (Table 3). However, the degree of protection was lower (85% at 1,000 μg/ml) compared to that achieved with a similar spray applied to the upper leaf surfaces (Table 2).

Other experiments were conducted to evaluate the local effect of jasmonates dissolved in acetone on the development of isolate MR1 on Bintje (Fig. 1) and Alpha (Fig. 2) potatoes. Acetone-treated plants were 83% blighted in 5 days, whereas plants treated with either JA or JME (62.5–1,000 μg/ml) were significantly protected up to 45–67%, with no clear dose-response effect and no significant difference between JA and JME at all concentrations used (Fig. 1). In the Alpha experiment, acetone-treated plants were 88% blighted, and disease was significantly ($P < 0.05$) suppressed by JA or JME at 62.5–1,000 μg/ml (Fig. 2). At all concentrations used, JA was a significantly better protector compared to JME (Fig. 2).

Systemic protection of potato plants. Two experiments were conducted to evaluate the systemic protection by jasmonates in cv. Bintje. In the first, the three lower leaves of nine-leaf plants were sprayed with a sonicate of JME in water at 2,000 μg/ml. In the second, the five lower leaves of 11-leaf plants were sprayed with JA (emulsified in N-25) at 1,000 μg/ml. Plants were chal-

TABLE 3. Development of late blight on potato plants (cv. Bintje) treated on lower leaf surfaces with jasmonic acid (JA) and challenged on upper leaf surfaces with *Phytophthora infestans* (isolate S49) 5 days after spraying ($n = 6$)

Spray	Conc. (μg/ml)	Number of lesions/plant[a]	Lesion size (mm)	Blighted area/plant (mm^2)	Protection (%)
None	...	42 ± 14	4.8 ± 0.3	760	...
JA + N-25	1,000 + 200	10 ± 7	4.3 ± 0.9	113	85
	2,500 + 500[b]	16 ± 11	4.3 ± 1	232	70
	5,000 + 1,000[b]	10 ± 5	3.8 ± 1	113	85
	10,000 + 2,000[c]	12 ± 7	4.4 ± 0.8	182	76

[a] Disease was recorded 5 days post-inoculation.
[b] Slight chlorosis.
[c] Chlorosis and some stunting.

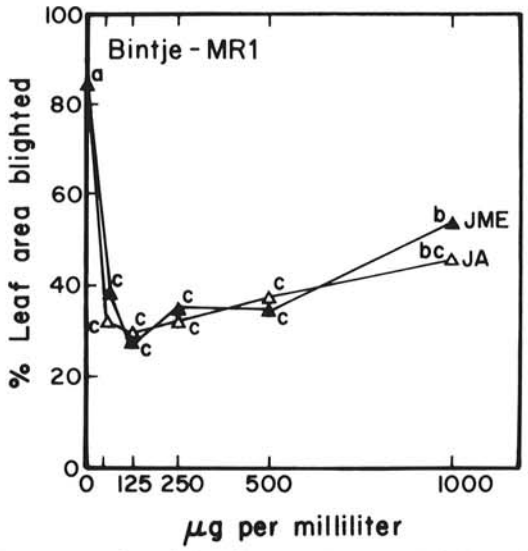

Fig. 1. Local protection of 12-leaf potato plants (cv. Bintje) against late blight (*Phytophthora infestans* isolate MR1) by jasmonic acid (JA) or jasmonic methyl ester (JME). Jasmonates were dissolved in acetone and applied to adaxial leaf surfaces at the indicated concentrations. Plants were challenged on adaxial leaf surfaces with 1,200 sporangia/ml 2 days after treatment, and disease was recorded 5 days post-inoculation. Values followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

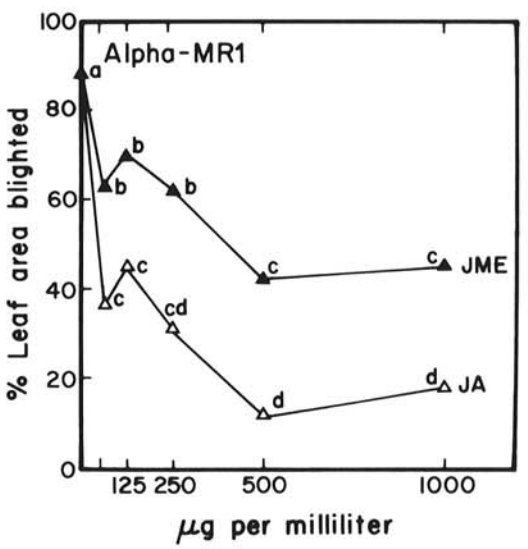

Fig. 2. Local protection of potato plants (cv. Alpha) against late blight (*Phytophthora infestans* isolate MR1) by jasmonic acid (JA) or jasmonic methyl ester (JME). Jasmonates were dissolved in acetone and applied to adaxial leaf surfaces at the indicated concentrations. Plants were challenged on adaxial leaf surfaces with 5,000 sporangia/ml 2 days after treatment, and disease was recorded 5 days post-inoculation. Values followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

TABLE 4. Systemic protection of upper leaves of potato plants (cv. Alpha) against *Phytophthora infestans* (MR1) induced by jasmonic acid (JA) or jasmonic methyl ester (JME) applied to the lower leaves[a]

Experiment	Compound applied and conc. (μg/ml)	Disease record on upper leaves: Days 3 or 4[b] Lesions per plant	Lesion size (mm)	Area blighted (mm^2)	Protection (%)	Day 5 Blighted leaf area (%)	Protection (%)
1	None	90 ± 29	5 ± 0	1,767	...	71 ± 12	...
	JA, 1,000	67 ± 24	5 ± 1	1,316	26	33 ± 7	54
	JA, 2,000	44 ± 3	3.2 ± 0.2	354	80	18 ± 3	75
	JME, 1,000	60 ± 33	3.3 ± 0.5	513	71	28 ± 13	61
	JME, 2,000	53 ± 24	4 ± 1	660	63	19 ± 7	73
2	Acetone	157 ± 29	...	...	...	98 ± 5	...
	JA, 1,000	23 ± 5	...	...	85	20 ± 3	80
	JA, 2,000	16 ± 3	...	...	90	23 ± 2	77
	JME, 1,000	15 ± 5	...	...	90	15 ± 8	85
	JME, 2,000	11 ± 5	...	...	93	13 ± 3	77

[a]The three lower leaves of 11-leaf plants were treated as indicated and challenged 5 (experiment 1) or 6 (experiment 2) days later with 1,000 sporangia/ml. Disease recordings were made on leaves four through 13 at 3–5 days post-inoculation. ($n = 6$).
[b]On day four during experiment 1 and on day three during experiment 2.

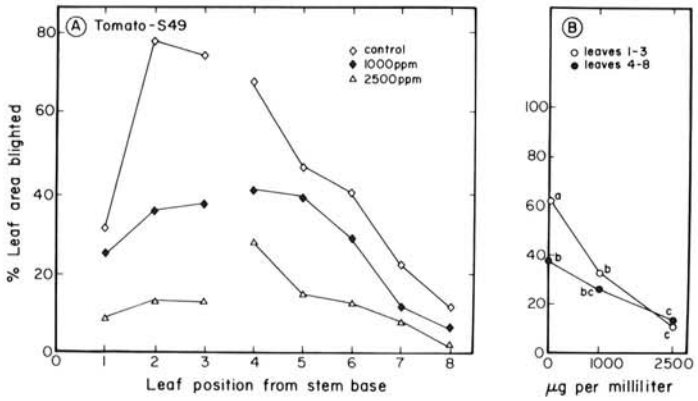

Fig. 3. Local and systemic protection of tomato plants (cv. Baby) against late blight (*Phytophthora infestans* isolate S49) by jasmonic acid. The compound was sonicated in water and applied to the three lower leaves of six-leaf plants. Plants were challenged with 3,750 sporangia/ml 5 days after treatment, and disease was recorded 4 days post-inoculation. Data for leaves one through three represent local protection, and for leaves four through eight, they represent systemic protection. **A,** Mean disease versus leaf age. **B,** Mean disease versus concentration. Values followed by the same letter in **B** are not significantly different at the 5% level (Duncan's multiple range test).

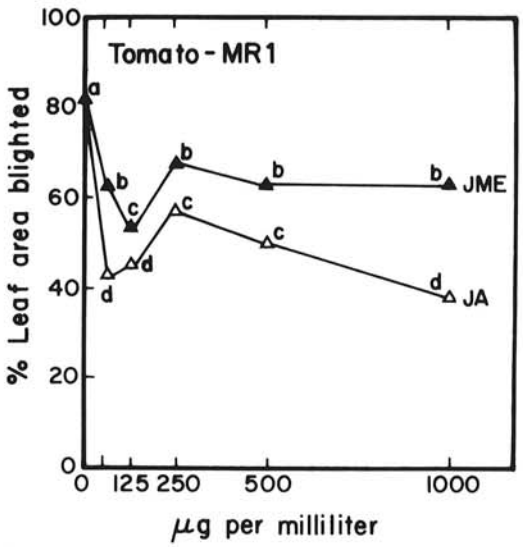

Fig. 4. Local protection of tomato plants (cv. Baby) against late blight (*Phytophthora infestans* isolate MR1) by jasmonic acid (JA) or jasmonic methyl ester (JME). Compounds were dissolved in acetone and applied to adaxial leaf surfaces at indicated concentrations. Plants were challenged with 2,000 sporangia/ml on adaxial leaf surfaces, and disease was recorded 4 days post-inoculation. Values followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

lenged 5 days after spraying. The upper nontreated challenged leaves were protected up to 58 and 65% in the first and second experiments, respectively, compared with challenged plants whose lower leaves were treated with water or N-25.

Two other experiments were conducted to evaluate the systemic protection induced in cv. Alpha. Results (Table 4) show that both compounds significantly protected the nontreated foliage from the late blight fungus. Percent protection at 5 days post-inoculation ranged between 54 and 73% in the first experiment and between 77 and 85% in the second.

Detached leaves of Alpha fed through the cut end of the petiole with JA or JME (in N-25 or in buffer) at 100 or 1,000 μg/ml were not protected from isolate MR1. Both compounds induced chlorosis in laminae and some necrosis in rachis at 1,000 μg/ml. Detached shoots of potato (Alpha) placed in buffer sonicates of JA or JME (up to 1,000 μg/ml) were not protected against isolate MR1.

Local and systemic protection of tomato plants. Six-leaf tomato plants were treated with sonicates of JA on the lower three leaves. Plants responded with yellowing of top leaves 2–3 days after spraying with JA at ≥1,000 μg/ml. Some necrosis was seen in leaves treated with the compound at 5,000 μg/ml. Data on the local and systemic protection of these tomato plants against isolate S49 are given in Figure 3A and B. Percentage of local and systemic protection increased with increasing dosage of JA up to 2,500 μg/ml (Fig. 3A and B). At this concentration, local and systemic protection reached a level of 82 and 66%, respectively. In another experiment four-leaf tomato plants were sprayed with acetone solutions of JA or JME (62.5–1,000 μg/ml) on adaxial leaf surfaces and were inoculated with isolate MR1 5 days after spraying. Acetone-treated plants did not develop any toxic symptoms. JA and JME at 1,000 μg/ml produced chlorosis in upper leaves 2–3 days after spraying. At 4 days after inoculation, control (acetone-sprayed) challenged plants were 81% blighted (Fig. 4). Plants treated with JME were only slightly protected, except at 125 μg/ml (34%), whereas plants treated with JA exhibited between 29 (250 μg/ml) and 54% (1,000 μg/ml) protection (Fig. 4). JA provided significantly better protection compared to JME at all concentrations used (Fig. 4).

Feeding detached leaves of tomato with JA or JME gave no protection against isolate MR1 up to a concentration of 2,000 μg/ml. Chlorosis in laminae and vein necrosis developed at ≥1,000 μg/ml.

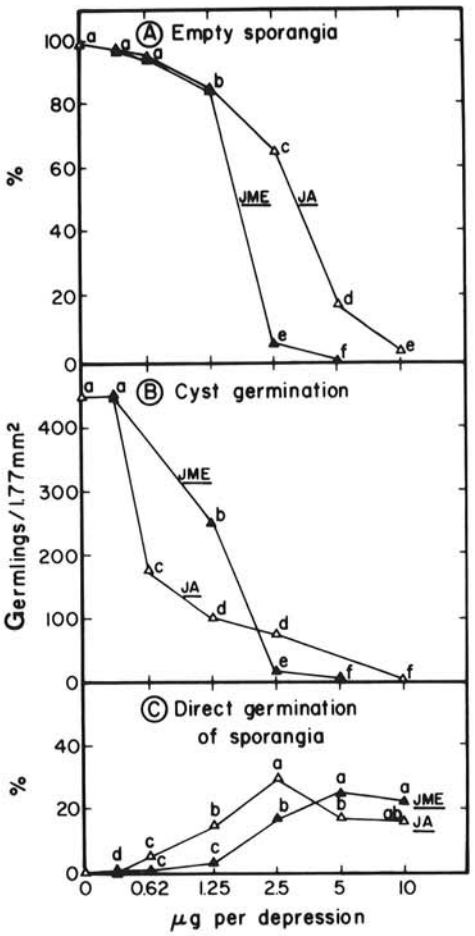

Fig. 5. Effect of jasmonates on germination of *Phytophthora infestans* isolate MR1 at 13–15 C. Ten microliters of acetone solutions of jasmonic acid (JA) or jasmonic methyl ester (JME) was applied to a depression in glass slides, and 20 μl of sporangial suspension was added after acetone was evaporated. Germination was recorded after 20 h. **A,** Percentage of sporangia that discharged zoospores. **B,** Number of germinating cystospores per microscope field. **C,** Percentage of sporangia-producing germ tubes. Values followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

Sporangial germination of *P. infestans*. Germination of isolate MR1 was tested in depressions in glass slides previously coated with increasing dosages of JA and JME. Although JA and JME did not dissolve in water, they did affect sporangial germination. JA and JME inhibited discharge of zoospores from sporangia in a dose-dependent manner (Fig. 5A). Complete inhibition was seen with JME at 5 μg per depression and 95% inhibition with JA at 10 μg per depression. Only some of the zoospores that discharged from the sporangia encysted and germinated in the presence of JA or JME (Fig. 5B). Complete inhibition of cyst germination was seen with JME and JA at 5 and 10 μg per depression, respectively. Interestingly, JA and JME stimulated direct germination of sporangia. Although no sporangia produced germ tube(s) in water (at 13–15 C), as many as 30 and 25% did so in the presence of JA and JME, respectively (Fig. 5C). Germination was manifested by the production of 3–4 germ tubes (20–50 μm long) from the distal end of the sporangium.

Similar experiments were conducted in planta. Potato plants (Alpha) were sprayed with acetone solutions of JA or JME. Leaflets were detached 1 h and 1, 2, 3, 4, and 7 days after spraying, were placed in moistened petri dishes, and were inoculated with sporangia of isolate MR1. Plates were incubated at 18 C in the dark for 20 h. UV-microscope observations after Calcofluor staining revealed that the fungus germinated on control (acetone-treated) leaves by cystospores, with no direct germination of sporangia observed. JA prevented zoospore release and stimulated direct germination at all sampling dates, except day 7 (Table 5). JME stimulated direct germination at 1 h and at 1 day, but, gradually, at 2 and 3 days, allowed for mixed, direct, and indirect germination (data not shown). At 4 days, JME mainly allowed cystospore germination (Table 5). At 7 days, both compounds only allowed cystospore germination (Table 5).

Mycelial growth of *P. infestans* in vitro. When added to rye seed-extract liquid medium, both JA and JME (dissolved in 60% ethanol) were inhibitory to mycelial growth of *P. infestans*. ED_{90} values for isolate S49 were 252 ($R^2 = 0.84$) and 317 μg/ml ($R^2 = 0.91$) for JME and JA, respectively, and for isolate MR1, values were 798 ($R^2 = 0.84$) and 670 μg/ml ($R^2 = 0.92$) for JME and JA, respectively. On agar plates, JME was not significantly inhibitory to either isolate at 200 μg per dish (highest concentration tested). Growth was stimulated by 10% at lower doses. JA caused 48 and 74% inhibition of S49 and MR1, respectively, at 200 μg per dish (Fig. 6).

Effect of systemin on late blight development. Systemin fed to detached leaves of both potato cultivars and the tomato cultivar

TABLE 5. Mode and frequency of sporangial germination of *Phytophthora infestans* MR1 on leaflets of potato (cv. Alpha) pretreated with acetone solutions of jasmonic acid (JA) or jasmonic methyl ester (JME) at 18 C (Calcofluor staining)[a]

Compound and conc. (μg/ml)	Abundance[b]					
	Germinating cytospores			Germinating sporangia		
	1 h	4 days	7 days	1 h	4 days	7 days
None	+++	+++	+++	−	−	−
Acetone	+++	+++	+++	−	−	−
JA						
2,000	−	−	+++	±	+++	−
1,000	−	−	+++	++	+++	−
500	−	−	+++	++	++	−
250	−	−	+++	++	++	−
125	−	−	+++	++	++	−
62.5	−	−	+++	++++	++	−
JME						
2,000	−	+++	+++	+	+	−
1,000	−	++	+++	+++	±	−
500	−	++	+++	+++	±	−
250	−	++	+++	++	±	−
125	−	++	+++	++	±	−
62.5	+	++	+++	++	±	−

[a]Leaflets (five per treatment) were detached for the assay 1 h or 4 or 7 days after application of jasmonates, and each was inoculated with a 10-μl droplet containing 100 sporangia. Germination was recorded 20 h after inoculation with the aid of a UV, epifluorescent microscope.

[b]−: no germination occurred; ±: erratic occurrence of germinating spores in various samples; +, ++, +++: about 5–10, 10–50, and 50–100 germinating cytospores and about 1–5, 5–10, and 10–30 directly germinating sporangia per site inoculated, respectively.

at various concentrations (0.03–1,000 μg/ml) had no significant effect on the development of late blight caused by isolate MR1. A slight and erratic reduction in blight development was observed in some treated potato leaves but not in tomato leaves, which were all blighted, as were the water-treated leaves.

SDS-PAGE. Figure 7 presents Coomasie blue-stained gels of acid-soluble proteins extracted from tomato leaves sprayed with jasmonates. At 6 days, at least four new protein bands were observed: two bands below the 14-kDa marker, probably proteinase inhibitors I and II (12,13), a 24- and a 29- to 30-kDa band.

Phytoalexins. Phytoalexins were extracted from potato (Alpha) and tomato plants 3 days after they were sprayed with acetone solutions of JA or JME (20–2,000 μg/ml). Acetone-treated plants served as controls. TLC analyses recovered no phytoalexins in either tomato or potato plants as a result of JA or JME treatment. However, vanillin-H_2SO_4 staining of the plates showed the presence of parental JA in extracts of JA-treated plants (at 200–2,000 μg/ml) and minute parental JME in extracts of JME-treated plants (at 2,000 μg/ml). Authentic JA and JME were stained brown with R_f's of 0.25 and 0.75, respectively. Phytuberin stained purple-red with R_f 0.73, and capsidiol stained gray with R_f 0.23.

DISCUSSION

Recently published work (8,11–14,26,27,29–31) indicates that jasmonates may induce disease resistance-related responses in a wide variety of plants. We have investigated the effects of jasmonates on the pathogen *P. infestans*, on the disease in tomato and potato, as well as on putative resistance responses of tomato and potato. In potato and tomato, jasmonates induced visible chlorosis (especially pronounced in tomato plant apices), slight growth retardation, loss of chlorophylls and carotenoids, and enhanced levels of acid-soluble proteins. In *P. infestans*, jasmonates affected the mode of sporangial germination, shifting it from indirect germination by zoospores to direct germination with germ tubes, and inhibited mycelial growth at relatively high dosages. Jasmonates also significantly reduced late blight development in both potato and tomato plants. Lesion number and size and leaf area blighted were reduced in jasmonate-treated plants, compared to control (solvent-treated) plants with concentrations that produced no damage to the plant.

Local and systemic protection of potato was seen in plants treated with as little as 0.45 and 4.8 μmol per plant, respectively, which seems much higher compared to the results of Farmer and Ryan (13) who used 0.001–0.1 μmol per tomato plant. However, when calculated relative to the plant's weight (Table 1), we used 0.02 and 0.22 μmol/g of fresh weight, whereas Farmer and Ryan (13), due to the much smaller plants they used, applied 0.005–0.5 μmol/g of fresh weight. Our concentrations are, therefore, 0.64 to four times larger compared to those of Farmer and Ryan (13), and fit well with physiological responses reported for jasmonates.

A basic question arising from our data is do jasmonates protect potato and tomato plants because of a direct fungitoxic effect on *P. infestans* or do they protect against the late blight disease because of induced resistance mechanisms operating in the treated plants. The data showing that JA and JME inhibit cystospore germination and mycelial growth in vitro seem to support the case of direct inhibition. However, a close look at these and the many other data favors induced resistance. First, jasmonates did not inhibit late blight development in tomato or potato leaves when fed through their cut petiole ends with up to 9.5 μmol per leaf (although phytotoxic symptoms were produced). Second, JME did not inhibit fungal growth on agar plates at 9.5 μmol per plate (the highest concentration tested), whereas it protected against disease at a concentration as low as 9.3 μmol per plant. Third, the kinetics of sporangial germination on leaf surfaces as a function of time lapsed between jasmonate application and inoculation showed that direct sporangial germination occurred with the highest concentration tested; with time, it gradually shifted back to the normal mode of indirect germination. Fourth, recovery tests we did with jasmonate-treated plants failed to detect JME at ≥1 day after spraying, although we were successful in detecting JA for as long as 7 days after applying spray. The fact that JME protected against late blight at ≥1 day after applying spray supports the idea of a mechanism of induced resistance. Fifth, jasmonates applied to bottom leaves of potato and tomato systemically protected the upper leaves from the blight. They also showed a translaminar protective activity. Therefore, we assume that the protection observed when challenge inoculation was done shortly after jasmonate application may only partially result from a direct effect on the fungus, whereas the protection achieved later (2–8 days after application) is probably a result of induced resistance.

Only a single report is available in the literature on the effect of exogenously applied JA on plant disease development. Schweizer et al (27) showed that JA protected barley from *Erysiphe graminis* f. sp. *hordei* by preventing appressorial differentiation of the fungus. They found that JA induced the accumulation

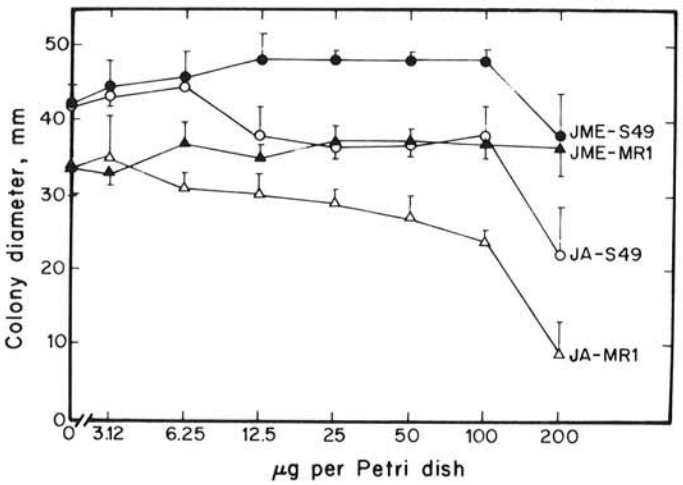

Fig. 6. Growth of *Phytophthora infestans* on agar medium amended with jasmonates (jasmonic acid [JA] or jasmonic methyl ester [JME]). Petri dishes 48 mm in diameter with 10 ml of rye seed-extract agar medium were amended with 100-μl acetone solutions of jasmonates. When the acetone evaporated, plates were inoculated in the center with the fungus and were incubated at 20 C in the dark. Colony diameter was measured 7 days after inoculation. Bars represent the standard error. ($n = 6$).

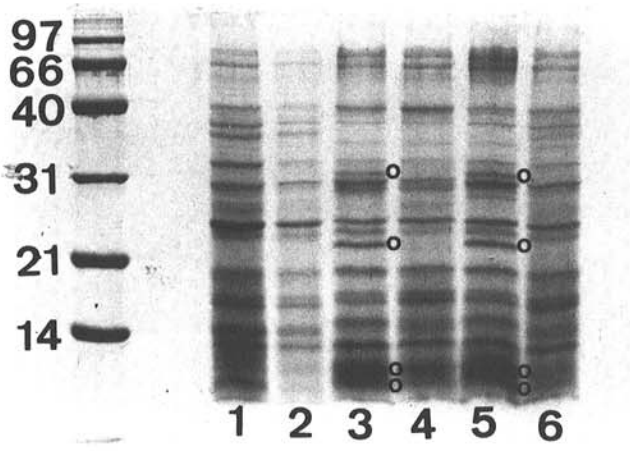

Fig. 7. Electrophoretic analysis of tomato leaf acid-soluble (pH 2.8) proteins induced by acetone sprays of jasmonic acid at 1,000 (lane 3) and 100 μg/ml (lane 4), jasmonic methyl ester at 1,000 (lane 5) and 100 μg/ml (lane 6). Lanes 1 and 2 represent nontreated plants and acetone-sprayed plants, respectively. Gel represents extracts taken 6 days after spraying. Electrophoresis of leaf proteins (10 μg) was done in 12.5% polyacrylamide gels containing sodium dodecyl sulfate and staining was done with Coomasie blue. Low molecular weight markers are on the left. Circles indicate induced proteins.

of 23-, 12-, and 10-kDa acid-soluble proteins, but these proteins were not antifungal. Neto et al (24) isolated jasmonic acid from wild rice and showed complete germination inhibition of *Pyricularia oryzae* with JA at 250 μg/ml but only 10% inhibition at 100 μg/ml. At 100 μg/ml, 30% of the germinated spores produced no appressoria.

If one accepts the hypothesis that jasmonates induce resistance in tomato and potato against late blight, a question arises as to the nature of this resistance. Jasmonates did not induce hypersensitive reaction in treated-challenged leaves nor did they protect potato tuber slices from *P. infestans* (Y. Cohen, *unpublished data*). Dipping whole tubers in sonicates of JA or JME and using acetone sprays of JA or JME applied to tubers did not protect developing potato plants from the blight (Y. Cohen, *unpublished data*). Jasmonates did not induce phytoalexins in potato or tomato foliage.

It is tempting, therefore, to assume that PI proteins that accumulate in both tomato and potato after treatment with jasmonates (11,12, and this study) have a role in the defense against late blight (3). However, there are several lines of evidence that show that this may not be the case. First, jasmonates in buffer applied to the soil surface of potted tomato plants or to cut potato stems caused slight chlorosis but induced no protection against the blight. Farmer et al (11) show that jasmonates applied similarly to the soil induced strong PI proteins to accumulate in tomato plants. Second, unsaturated fatty acids applied to lower leaves of potato protect the upper leaves from *P. infestans* (5,6). Protection increased in this order: 20:5 > 20:4 > 18:2 > 18:3 > 18:1. This order of response does not fit the findings of Farmer and Ryan (13) who showed that JA and its precursors (derivatives of 18:3) stimulate PI proteins the most. In their study, 20:4 (arachidonic acid) was totally ineffective in inducing PI proteins, whereas our data showed that 20:5 and 20:4 were most effective in inducing resistance. Also, wounding potato or tomato in our experiments (6) failed to protect against *P. infestans*, while elevating PI proteins considerably in both plants (11). Third, systemin, a signal 18-amino acid polypeptide released in tomato by wounding (26) was reported to activate PI proteins in femtomole concentrations (26). Systemin in our experiments, however, provided no protection against late blight in either potato or tomato. There is a lack of evidence concerning the sensitivity of *P. infestans* to PI proteins and the participation of *P. infestans* proteases in the process of infection. It is interesting to note that exogenously applied trypsin inhibitor (type II-S from soybean, Sigma Chemical Co., St. Louis, MO) did not affect spore germination or infection of tomato leaves with *P. infestans* up to a concentration of 100 μg/ml (Y. Cohen, *unpublished data*). In another fungal system (2), a clear correlation between pathogenicity and protease production was established. Therefore, we believe that jasmonates operate via another, as yet unknown, mechanism that protects tomato and potato plants against late blight. Plants expressing high levels of PI proteins were protected against chewing insects (18). No evidence is available, however, that such plants were protected against aphids or fungal pathogens. The recent report of Pautot et al (25) indicates that bacterial plant pathogens may belong to the group of organisms that, because of their high proteolytic activity, should be sensitive to PI proteins accumulating in their host plant.

One possibility is that the 24- and/or the 29- to 30-kDa proteins we detected in acid-soluble extracts are antifungal. This has yet to be determined. The 24-kDa protein did not react with osmotin (35) antibodies (T. Niderman, *unpublished data*), nor did we find enhanced levels of p14a, chitinases, or β-1,3 glucanases, the major pathogenesis-related proteins of tomato (4) in jasmonate-treated plants.

Another reasonable speculation on the mode of action of jasmonates in our system involves free radical formation due to lipoxygenase enhancement. Recent evidence (30) has shown that jasmonates dramatically increased lipoxygenase activity (14) and lipoxygenase-mediated lipid peroxidation and induced resistance against aphids (10).

This is a first report on the local and systemic resistance jasmonates induce in potato and tomato plants against a fungal pathogen. More studies are required to elucidate the mode of action of jasmonates against fungal pathogens in vivo.

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