Methyl Jasmonate Induces Papain Inhibitor(s) in Tomato Leaves

Caroline J. Bolter

Agriculture Canada Research Centre, 1391 Sandford Street, London, Ontario, N5V 4T3, Canada

Leaves of 18- to 24-d-old tomato (Lycopersicon esculentum) plants exposed to gaseous methyl jasmonate (MJ) for 24 h at 30°C in continuous light contained high levels of soluble protein that inhibited papain. Chromatographic analysis demonstrated that the active protein had a molecular mass of 80 to 90 kD. Induction of papain inhibitor was directly related to the concentration of airborne MJ up to a maximum of 0.1 μL MJ per treatment and depended on the duration of exposure up to 18 h. Inhibitor activity in plants treated for less than 18 h increased with time after treatment. Levels remained constant for up to 4 d after treatment, after which time activity decreased. The youngest leaf, leaf 5, consistently lost activity at a faster rate than older, lower leaves. Inhibitor concentration in all leaves was reduced to minimum levels by 11 d after MJ treatment, but did not return to control levels. Treatment with MJ in the dark did induce inhibitor activity, but at a significantly lower rate. Polyclonal antibodies raised to purified potato tuber skin cysteine proteinase inhibitors (CPI) cross-reacted with the tomato inhibitor, suggesting that the tomato papain inhibitor and the potato CPI are closely related. No papain inhibitor activity was observed in extracts from wounded tomato leaves, nor was there any immunoreactivity with antibodies raised to potato tuber skin CPI.

Physical damage to the leaves of plants from a number of families resulted in the synthesis of PI at the wound site as well as in distal unwounded leaves (Green and Ryan, 1972b; Walker-Simmons and Ryan, 1977; Brown and Ryan, 1984). It was proposed that induction of PI is part of a plant’s defense system against herbivory (Ryan, 1973), for example, by inhibiting insect digestive enzymes and the extracellular proteinases produced by some pathogens (Mosolov et al., 1976). PIs, induced in tomatoes (Lycopersicon esculentum) after beet armyworm herbivory, were thought to be the major factor responsible for reducing the suitability and desirability of the wounded leaves (Broadway et al., 1986). In addition, there was a significant reduction in both growth and development when larvae of two noctuid species were provided with artificial diets containing soybean trypsin inhibitor or potato PI II (Broadway and Duffey, 1986).

A number of endogenous factors have been identified that induce PIs, including systemin, an 18-amino acid polypeptide derived from plants (Pearce et al., 1991), plant growth regulators such as auxin and ABA (Peña-Cortés et al., 1989), and oligosaccharides from the damaged cell wall (Bishop et al., 1981). Recently, Farmer and Ryan (1990) observed that JA and MJ, secondary products of lipoxygenase-catalyzed oxidation of linolenic acid, were also potent inducers of PI in tomato, tobacco, and alfalfa leaves when applied exogenously. These observations, combined with the finding that octadecanoid precursors of JA, particularly linolenic acid, induced PIs (Farmer and Ryan, 1992), suggested that jasmonates could be a component in the pathway for PI gene expression after wounding (Farmer et al., 1992). Growth-regulating properties of JA were first reported in 1981, and since then JA and MJ have been detected in over 30 plant species (Meyer et al., 1984; Berg and Ewing, 1991), indicating that this pathway might be ubiquitous. JA and MJ are structurally similar to and have many physical properties in common with ABA (Anderson, 1989), which also induces PIs (Hildmann et al., 1992).

Despite this focus on PI I and II, it is clear that the induction of Ser proteinase inhibitors may not be an effective defense mechanism against all insect species (Wolfson and Murdoch, 1990a). Benz (1978) demonstrated that although wounding potato leaves led to a doubling of Ser proteinase inhibitor activity, the development of Colorado potato beetles provided with previously injured plants was not significantly different from those reared on intact plants. It was shown that Ser proteinases were not important digestive enzymes in many Coleoptera and that the Kunitz trypsin inhibitor had no effect on the proteinase activity in homogenates of larval bruchid beetles (Kitch and Murdoch, 1986) or cowpea weevils (Gatehouse et al., 1985). Murdock et al. (1987) demonstrated that Cys (or thiol) proteinases, not Ser proteinases, were responsible for most of the proteolytic activity in the digestive tract of many Coleoptera. Indeed, the rice CPI, oryzacystatin, significantly inhibits the digestive proteinases of two species of stored grain beetles (Liang et al., 1991). In addition, it was shown that the specific CPI, E-64 (t-trans-epoxysuccinyl-leucylamido-[4-guanidino]-butane), supplied in the insect diet, suppressed the growth and development of larval cowpea weevils (Murdock et al., 1988), common bean weevils (Hines et al., 1990), and Colorado potato beetles (Wolfson and Murdoch, 1987). Growth suppression in the Colorado potato beetle is not surprising since it is known that the Cys proteinases cathespin B and H are two of the most important proteinases in the gut of this insect (Thie and Houseman, 1990).

Abbreviations: CPI, cysteine proteinase inhibitor; HSS, high-speed supernatant of 10X dilute leaf extract; JA, jasmonic acid; MJ, methyl jasmonate; PI, proteinase inhibitor; PI I, proteinase inhibitor I; PI II, proteinase inhibitor II.
This study was conducted to investigate the induction of papain inhibitor in tomato leaves after MJ treatment.

**MATERIALS AND METHODS**

**Materials**

MJ was purchased from Bedoukian Industries (Danbury, CT). All other chemicals were purchased from Sigma.

**Plant Growth**

Young tomato (*Lycopersicon esculentum* var Bonny Best) plants were utilized 18 to 24 d after planting. At this time they were 10 to 12 cm high and had four, well-developed leaves and a small apical leaf. Seeds were germinated and grown in the greenhouse maintained at 25 ± 6°C, with a 16-h light/8-h dark photoperiod using a 400-W high-pressure sodium (GE Lucalo) lamp to supplement daylight. Plants were fertilized weekly with a 1-g L⁻¹ solution of 20:20:20 (N:P:K) (Plant Products Ltd., Bramalea, Ontario, Canada).

**Treatment of Plants with MJ**

Tomato plants, 18 to 24 d old, were exposed to MJ vapors in a 12.6-L air-tight glass chamber with 1 × 2 cm pieces of Whatman No. 1 filter paper to which 10 μL of MJ oil diluted (10× to 10³×) in 95% ethanol had been applied. The MJ source was placed at least 4 cm away from the closest leaf. Control plants were exposed to 95% ethanol. The chambers were fertilized weekly with a 1-g L⁻¹ solution of 20:20:20 (N:P:K) (Plant Products Ltd., Bramalea, Ontario, Canada).

**Treatments of Plants by Wounding**

Tomato leaves were wounded according to the method of Green and Ryan (1972a) by crushing the leaves between a small circular wooden dowel (0.8 cm diameter) and a rat-tail file. Each of the five leaflets comprising leaf 2 (with counting starting from the trifoliate leaf as leaf 1) was damaged once across the main vein and a second time 20 h later. The wounded leaf and leaf 3 were assayed for papain and trypsin inhibitor activity 24 h after the first wound (Hildmann et al., 1992). Plants were incubated in a growth chamber under constant illumination (400 μmol m⁻² s⁻¹) at 30°C for 24 h unless otherwise specified. After MJ treatment the plants were removed from the chambers and incubated in a growth chamber with the same illumination under 16-h day at 28°C with a night temperature of 23°C and constant 80% RH. In other experiments tomato plants were exposed to MJ in constant dark at 30°C and then incubated in the dark at the same temperature.

**Measurement of Papain Inhibitor Activity**

Inhibitor activity was measured by observing its effect on the endopeptidase activity of papain using the method of Arnon (1970). This method follows the breakdown of casein by papain spectrophotometrically. In the absence of inhibitor, papain activity is unaffected by the partially purified plant extract, and release of TCA-soluble peptides from the substrate is high. When papain inhibitors have been induced in the leaf tissue, their presence in leaf extracts inhibit papain, leading to a decrease in the TCA-soluble peptides. Plant extract was produced by squeezing the juice from leaf 3 using an ice-cold garlic press. Leaf extract was diluted 10× with 50 mM Tris buffer, pH 8, containing 0.15 M NaCl and 2 mM sodium EDTA and centrifuged for 30 min at 350,000g using a Beckman TL-100 ultracentrifuge. The HSS was further diluted 10×. Papain (0.25 μM) was incubated with the diluted HSS (20 μL unless otherwise stated) in the presence of 5 mM Cys for 10 min at 37°C in a total volume of 300 μL using the same buffer. After incubation the assay medium was cooled to 4°C before addition of 100 μL of 1% ice-cold casein. The reaction medium was then incubated at 37°C for 10 min and rapidly cooled to 4°C while 700 μL of 8% TCA was added to stop the proteolytic action of papain. The tubes were centrifuged at 15,000 rpm in an Eppendorf 5415C for 5 min and the absorbance of the supernatant was measured at 280 nm. The concentration of papain used in the experiment was determined using an activity versus concentration curve. A papain concentration with activity in the linear portion of the curve was used, where Aₜₐᵢᵢ after subtraction of background levels varied between 0.15 and 0.2. Some experiments were also performed using crude plant extract. In addition, inhibitor activity was investigated using 0.2 μL KHP0₄ buffer, pH 6, containing 2 mM EDTA. Controls with and without papain were performed to ensure that the HSS (10× diluted) and the crude plant extract (100× diluted) had no direct effect on casein.

**Measurement of Trypsin Inhibitor Activity**

A modification of the method used by Kunitz (1947) similar to that described above for the papain inhibitor was used. Leaf extract diluted with 4.6 mM Tris buffer, pH 8, containing 1.15 mM CaCl₂ was centrifuged at 350,000g, and diluted HSS (40×) was incubated with 0.16 μM trypsin for 10 min at 37°C. After cooling on ice, 100 μL of 1% casein was added. The reaction mixture was incubated as above, the proteolytic action of trypsin was stopped using TCA, and the concentration of TCA-soluble peptides was measured spectrophotometrically at 280 nm. A trypsin concentration that fell on the linear part of an activity versus concentration curve was used.

**Molecular Mass of the CPI**

The HPLC apparatus used in this procedure was a Waters system consisting of two model 510 pumps, a refrigerated WISP 710B auto-injector, a model 441 absorbance detector (280 nm), and an 840 system controller. Plant leaf extract was diluted 10-fold using a 50 mM KHP0₄ buffer, pH 6, containing 0.15 mM NaCl and 2 mM sodium EDTA, and centrifuged at 350,000g for 30 min (Beckman TL-100 ultracentrifuge). The supernatant (150 μL) was injected into a Waters' Protein Pak 300sw molecular exclusion column (7.5 mm (i.d.) × 30 cm), that had been equilibrated and eluted with the KHP0₄ buffer described above at a flow rate of 0.25 mL/ min. Fractions of 250 μL were collected and maintained on ice. Papain inhibitor activity was measured using 60 μL of each fraction. Various protein standards of known molecular mass were run through the column to ascertain the approximate molecular mass for the papain inhibitor.
Immunoblotting

Gel electrophoresis was carried out using a 10% SDS-polyacrylamide gel and run for 60 min at 200 V. Pure potato CPI extracted from potato tuber skin crystals according to the method of Rodis and Hoff (1984) was loaded in lane 1 (0.5 µg protein/lane). Crude extract from leaves of MJ-treated tomato plants was loaded in lane 2 (10 µg protein/lane) and extract from control plants not exposed to MJ was loaded in lane 3. The gel was rinsed in transfer buffer and transferred to nitrocellulose electrophoretically according to the method of Towbin et al. (1979). Polyclonal antibodies raised in rabbits to nitrocellulose electrophoretically according to the method of Towbin et al. (1979) were used to reveal the position of immunoreactive material. Bands were visualized using alkaline phosphatase conjugated to anti-rabbit antibodies followed by Sigma Fast Substrate Tablets. Color development, which indicated immunoreactivity with potato tuber skin CPI, was stopped with PBS-EDTA. Crude extracts from leaves 1, 2, and 3 of plants wounded as described above were also tested for immunoreactivity to potato CPI.

Protein Assay

Proteins were measured using the technique described by Bradford (1976) using γ-globulin as a standard.

RESULTS

The experiments performed here were undertaken to explore papain inhibitor induction prior to investigating its potential effects on the Cys proteinases found in the gut of certain insects, such as the Colorado potato beetle. The method used to measure papain activity utilizes a buffer of pH 8 (Arnon, 1970). However, since many Coleoptera, including Colorado potato beetle, have a mildly acidic midgut (pH 5-6), a comparison was made between papain and inhibitor activities at pH 8 and pH 6. It was observed using Student's t test, that the inhibitory activity of the HSS was not significantly different at the two pH levels. In addition, the rate of papain proteolytic activity, using casein as a substrate, was significantly faster at pH 8 than at pH 6, which allowed a greater degree of sensitivity. For this reason experiments were carried out at pH 8.

Figure 1 shows that a concentration of 0.1 µL of MJ (10 µL of 10X diluted MJ) or greater in a 12.6-L glass chamber gave maximal induction of papain inhibitor in 18- to 24-day-old plants. Inhibitory activity was less in plants exposed to lower concentrations of MJ. Control plants, treated in a manner identical to the experimental plants except that MJ was not present, had no detectable levels of papain inhibitor. A concentration of 1 µL of MJ was used in subsequent experiments.

No inhibitory activity was induced in leaves of plants treated for 3 h regardless of the duration of incubation after treatment (Fig. 2). However, inhibitor activity was detectable after a 6-h treatment if the plants were incubated for 12 h. A further increase was observed after a 24-h incubation. Activity was observed immediately after a 9-h treatment, and this activity increased with incubation duration. Inhibitory activity, measured immediately after treatment, increased linearly with exposure time between 9 and 18 h, when maximal levels were attained. Subsequent experiments were carried out using 24-h treatments. Inhibitory activity was significantly lower when plants were treated with MJ in continuous darkness than when they were exposed in the light.

Papain inhibitor levels were measured in individual leaves for 13 d after a 24-h treatment with MJ (Fig. 3). It was observed that inhibitor levels in tomato plants exposed to MJ at different times and incubated for more than 24 h were

Figure 1. Effect of airborne MJ on papain inhibitory activity in tomato leaves. Plants were exposed to MJ as described in "Materials and Methods." Volume of MJ (µL) is the actual volume of oil used in each experiment. Twenty microliters of HSS (10X diluted) was used at each MJ concentration. Points represent the mean of four replications.

Figure 2. Effect of duration of exposure to airborne MJ and incubation time after exposure on papain inhibitory activity in tomato leaves. Light-treated plants were exposed to MJ as described in "Materials and Methods" for 3 to 24 h in continuous illumination at 30°C. After treatment, plants were assayed immediately or incubated in a growth chamber under 16-h day at 28°C and a night temperature of 23°C for 12 or 24 h. Dark-treated tomato plants were exposed to MJ in continuous dark at 30°C for 24 h and then assayed immediately or incubated in the dark at the same temperature for 12 to 24 h. Twenty microliters of HSS (10X diluted) was used at each time point. Points represent the mean of four replications ± se.
proteases, thus, until its activity against other Cys proteinases
may have several isomers. A band representing a protein of lower mol wt was observed in lane 7 and is thought
to be a breakdown product. Extracts from leaves of control plants that had not been treated with MJ (lane 6) showed no
immunoreactivity. Immunoblotting also revealed that leaves that had been wounded with a file and dowel, as well as the
leaf above and below the wounded leaf, showed no immunoreactivity to potato tuber skin CPI (lanes 2 and 3).

**DISCUSSION**

This study was conducted to investigate the induction of CPIs, specifically papain inhibitor(s) in tomato plants after
treatment with MJ. Papain was used because it is inexpensive and easy to work with, and shows some sequence homology
with both cathepsin B and H (Barrett, 1987), the Cys proteinases found in Colorado potato beetle gut (Thie and House-
man, 1990). Initial observations showed that papain inhibition was not significantly different at pH 6 and pH 8, which
is similar to the findings of Rodis and Hoff (1984) using purified potato tuber skin CPI. It was important to establish
this because the midgut of many Coleoptera operates at pH 5 to pH 6 and this is the presumed site of action of inducible
PLs. Papain was the only protease used in these experiments, thus, until its activity against other Cys proteinases
has been investigated, the inhibitor is referred to as a papain inhibitor and not as a CPI.

Figure 3. Papain inhibitor activity in individual tomato leaves at various times after treatment with MJ. Leaf numbering was started
with the first true, trifoliate leaf as leaf 1. Plants were exposed to MJ as described in "Materials and Methods." After treatment, plants
were assayed immediately or incubated in a growth chamber under 16-h day at 28°C and a night temperature of 23°C for up to 13 d.
Twenty microliters of HSS (10X diluted) was used at each time point. Points represent the mean of three replications.

Figure 4. Immunoblot (western blots) of tomato extract using antibodies raised against purified potato tuber skin CPI. Color development
with Sigma Fast Substrate Tablets indicated immunoreactivity. Immunoblotting also revealed that leaves that had been wounded with a file and dowel, as well as the
leaf above and below the wounded leaf, showed no immunoreactivity to potato tuber skin CPI (lanes 2 and 3).
Using HPLC analysis it was observed that a proteinaceous inhibitor of papain with a molecular mass of 80 to 90 kDa was induced in tomato leaves when they were exposed to airborne MJ. When Farmer et al. (1992) treated tomato plants with airborne MJ they reported that in addition to the Ser PI's PS I (41 kDa) and PS II (23 kDa) that had been observed after wounding (Gustafson and Ryan, 1976), two other induced protein bands appeared on an SDS-polyacrylamide gel, at 64 and 87 kDa. They did not identify the two larger proteins, but they felt that they were not PIs because of their large size. However, observations made in the present study suggest that the larger of the two unidentified proteins may be a papain inhibitor. Large CPIs have been identified in the past. Rodis and Hoff (1984) extracted a single, large CPI that exists as crystals in the skin of potato tubers. It had a molecular mass of 80 kDa on a nondenaturing gel under acidic conditions (pH 4.3) and appeared to form a tetramer of 320 to 350 kDa when the extract was run under alkaline conditions (pH 8.9). In the present study it was observed that HPLC analysis carried out at pH 6 produced a much sharper peak of inhibitory activity than it did at pH 8. At pH 8 the peak was significantly broader on the high molecular mass side (data not shown), which could indicate that multimeric, active forms of papain inhibitor are also found in tomato extract exposed to alkaline conditions.

Immunoblots using polyclonal antibodies raised against potato tuber skin CPI indicated that the tomato papain inhibitor is antigenically related to the potato-derived CPI and has a similar molecular mass of 80 kDa, confirming the results of the chromatographic analysis. There appeared to be several isomers of both the potato CPI and the tomato inhibitor. The immunoblots, combined with HPLC results, demonstrated that the tomato papain inhibitor is probably a monomer. Control plants, not exposed to MJ, did not show any immunoreactivity.

Akers and Hoff (1980) reported that CPI activity could be induced in tomato leaves after they had been excised and exposed to continuous illumination at 30°C. This activity was accompanied by the formation of cubical crystals in the leaf mesophyll cells that closely resembled those observed in the tuber, and Akers and Hoff hypothesized that these crystals contained the CPI activity. Thus, it seems likely that the 87-kDa protein observed in MJ-treated tomatoes by Farmer and Ryan (1992), the papain inhibitor described in this work, the CPI induced in tomato leaves by Akers and Hoff (1980), and the CPI identified from potato tuber skin (Rodis and Hoff, 1984) are all related. It is interesting to note that although this inhibitor is constitutive in potato tuber, it is apparently not present in tomato leaves until it is induced by some form of stress, such as excision followed by constant illumination at 30°C, or MJ treatment.

Papain inhibitor was induced when plants were exposed to MJ in both light and dark conditions, although plants treated for 24 h in the dark had significantly lower levels of inhibitory activity then those exposed in the light. These levels increased only slightly after a 24-h incubation, clearly demonstrating the importance of illumination in the induction of the papain inhibitor. Variability in papain inhibitor levels measured in individual leaves during the 13 d following an exposure to MJ was observed between plants treated on different occasions, although there was a clear trend within each experiment. Variable growing conditions have been shown to result in differences in plant physiology and subsequent resistance to pathogen attack (Sinden et al., 1978), and significant differences in Ser PI induction between 16- and 21-d-old tomato plants have been observed (Wolfson and Murdock, 1990b). Thus, it was hypothesized that variability in greenhouse conditions, such as temperature, light intensity, and watering regimes, as well as plant age probably had an effect on plant physiology that was significant enough to account for the variations observed. Nevertheless, leaf 2 had consistently less papain inhibitor activity immediately after treatment than leaves 3 and 4, which increased to the same level after 24 h. Leaf 5, the youngest leaf, lost its papain activity more rapidly than the other leaves, and inhibitor concentration was reduced to minimum levels in all leaves by 11 d after treatment. These observations indicate that the high levels of inhibitor activity were only transitory, but that some activity remained in all leaves for an extended duration, which could be important in plant protection. There was no difference observed between inhibitor activity in crude leaf extracts and the corresponding HSS. Surprisingly, no correlation was observed between protein concentration in the crude homogenate and inhibitor activity (data not shown).

Despite the observations that papain inhibitor is induced by certain forms of stress in tomato leaves, no inhibitory activity was measured after wounding of tomato leaves, either in wounded or unwounded leaves on the same plant. However, trypsin inhibitory activity was induced significantly, indicating that the wounding procedure had been successful. Immunoblots of extracts from wounded leaves and the leaves above and below the wound indicated that there was no immuno-cross-reactivity with potato CPI, confirming that the inhibitor had not been induced. The observations that wounding induced significant levels of trypsin inhibitor while papain inhibitory activity was undetectable suggested that there may be some fundamental differences in the regulatory control of these two inhibitors. It is interesting that with potato plants that had been wounded using the same technique as that described here, Hildmann et al. (1992) observed an increase in mRNA that translated into a peptide with homology to the rice CPI, oryzacystatin. They found that the concentration of this mRNA was the same in wounded leaves and as in those treated with MJ. However, it is possible that the mRNA levels induced in their MJ-treated plants were significantly lower than they would have been if they had exposed whole plants to airborne MJ in the light instead of incubating excised petioles in an MJ solution in the dark.

Rawlings and Barrett (1990) did not mention potato CPI when describing the evolution of proteins of the cystatin superfamily, but it seems likely that both the potato CPI previously described and the tomato papain inhibitor described in this work will require the formation of a new cystatin category before they can be included in Barrett's classification. Cystatins with disulfide bonds are classified as type 2 cystatins, whereas potato tuber skin CPI, which lacks disulfide bonds (Rodis and Hoff, 1984; Zimacheva et al., 1985), could be classified as a type 1 cystatin (Barrett, 1987). However, the potato CPI and the papain inhibitor described.
here do not fit the classical definition of type 1 cystatins, which have molecular masses of 10 to 13 kD, because they are so large. A third type of cystatin, the kininogens, has been described. These are much larger molecules containing three type 2-like domains, and it seems possible that the large potato and tomato inhibitors, like the kininogens, are similarly composed of identical replicating sequences. The potato inhibitor was found to be a multisite and polyvalent inhibitor in which there appeared to be eight proteinase binding sites, four of which could bind either papain or chymopapain, and four sites that could accommodate only chymopapain (Rodis, 1974, as reported by Richardson, 1977).

In conclusion, it has been reported here that leaves of tomato plants can be stimulated by airborne MJ to produce papain inhibitor that could play an important role in defending the plant against insects that have evolved to use Cys proteinases for digestion. Indeed, Felton et al. (1989) observed that the high sulfhydryl content of trypsin inhibitors makes them susceptible to alklylation by plant-derived quinones and that the presence of proteinase inhibitors with a low potential for alklyative inactivation may be an optimal strategy for host-plant resistance. With their lack of disulfide bonds, CPIs would seem to be good candidates.

ACKNOWLEDGMENTS

I wish to thank Dr. Alex Vardanis and Henry Bork for helping with the HPLC analysis, Dr. Steve Gleddie for raising the potato tuber skin CPI antibodies and for running the immunoblots, and Dorothy Drew and Jerry Lambert for their assistance with the preparation of the manuscript.

Received June 9, 1993; accepted August 16, 1993.
Copyright Clearance Center: 0032-0889/93/103/1347/07.

LITERATURE CITED

Kitch LW, Murdock LL (1986) Partial characterization of a major gut thiol proteinase from larvae of Callosobruchus maculatus F. Arch Insect Biochem Physiol 3: 561–575
Osadceva JH, Shukle RH, Wolfson JL (1984) Inactivation of pro-

Downloaded from on October 15, 2017 - Published by www.plantphysiol.org
Copyright © 1993 American Society of Plant Biologists. All rights reserved.
potato inhibitor of papain, chymopapain and ficin. Plant Physiol 74: 907–911


Sinden SL, Schalk JM, Stoner AK (1978) Effects of daylength and maturity of tomato plants on tomatine content and resistance to the Colorado potato beetle. J Am Soc Hortic Sci 103: 596–600


