The Effects of Surface-Applied Jasmonic and Salicylic Acids on Caterpillar Growth and Damage to Tomato Plants1

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ABSTRACT. We tested the role of salicylic acid (SA) and jasmonic acid (JA) in altering the tomato plant’s defense against herbivory by tobacco hornworm. Treatments of SA or JA were topically applied to tomato plants, hornworm consumption was allowed to proceed for 12 days, and harvest analyses were performed. Measurements taken included a subjective plant rating (1-10 score), plant dry mass, caterpillar mass, and the number of times the caterpillars fell off the plant. Results showed significant effects of exogenously applied SA and JA on the defense of tomato plants against insect herbivory. Plants treated with SA had little resistance to the feeding caterpillars and the plant lost more biomass to them. JA, in contrast, apparently increased the defensive mechanisms of the plant, resulting in lower caterpillar growth and increased caterpillar detachment from plants. The data are consistent with a model where JA, endogenous or exogenously applied, is necessary for defense against insect herbivory and SA disrupts JA biosynthesis and/or pool accumulation.

INTRODUCTION

Jasmonic acid (JA) is an endogenous plant growth regulator widely distributed in higher plants (Meyer and others 1984; Taito 1990). In response to injury, a plant may produce JA, which induces the expression of defensive compounds such as insect proteinase inhibitors. JA may also be systemically distributed throughout the plant and create volatile gases, which in turn may induce neighboring plants to increase their defense allocations as well as attract parasitic wasps to attack the infesting herbivores (Creelman and Mullet 1995; McConn and others 1997; Thaler and others 1996; Turris and others 1995). The synthesis of jasmonic acid takes place via the octadecanoid pathway (Fig. 1). The precursor of jasmonic acid is linolenic acid. Linolenic acid is converted to hydroxylinolenic acid by lipoxygenase. After reactions catalyzed by allene oxide synthase (AOS) and allene oxide cyclase, phytodienoic acid is formed and through oxidation, jasmonic acid is formed (Creelman and Mullet 1997; Pan and others 1998). The jasmonic acid then facilitates the induction of plant defensive genes.

Salicylates, when synthesized or applied to plants, inhibit AOS activity, which in turn inhibits the production of jasmonic acid and proteinase inhibitors (Fig. 1) (Raskin 1992; Doares and others 1995; Pan and others 1998). Consequently, a plant given salicylates will become less able to defend itself against insect attack. In contrast, elevated SA in plants has often been associated with increased pathogen resistance (Yang and others 1997). However, the relationship between pathogen and insect resistance is still under debate (Apryanto and Potter 1990; Hatcher 1995). Some studies show mutual antagonism of JA vs. SA pathways, with consequent increase in pathogen resistance but decrease in insect resistance with the exogenous application of SA, while others have shown cross protection for both insect and pathogen resistance (for example, Inbar and others 1998; Thaler and others 1999). The plant response apparently depends on the plant-challenger system and the type and strength of the elicitor.

Several investigations have centered on the defense of the tomato (Lycopersicon esculentum) and their insect pests. Howe and others (1996) found that a tomato mutant that was deficient in the capacity to induce defense genes, via the octadecanoid pathway, was much more susceptible to damage by the tobacco hornworm (Manduca sexta). Thaler and others (1996) found that exogenously applied JA increased defense against beet armyworm (Spodoptera exigua), and showed that field-applied JA enhances the production of chemical defenses in tomato. Thaler (1999) also showed that JA, when exogenously applied to tomato, not only induced additional plant resistance to beet armyworm damage, but also doubled the incidence of parasitism of the endoparasitic wasp Hypoaspis exigua on the armyworm. These results may indicate a potential use of JA in inhibiting agricultural pests.

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In this experiment, we investigate the tomato-hornworm system, a well-studied and economically important crop-insect pest system. We test the effect of surface-applied salicylic acid (SA) and jasmonic acid (JA) on tomato plants, in conjunction with the damage induced by herbivory with the tobacco hornworm.

**METHODS**

**Plant Growth**

Heirloom tomato seedlings (*Lycopersicon esculentum* var. *Mill., cv. Moskovich) were germinated under fluoresecen lights (17:7h; L:D, 21°C) in seed starters and transplanted as 16-day old seedlings into 10 cm pots and placed in a greenhouse (sodium lights; 17:7h; L:D, 24-29°C photophase and 16-18°C scotophase). Plants were allowed to grow for 12 days following transplantation before chemical or caterpillar treatments were applied. Plants were randomly placed in rows according to each treatment. Each row of six replicate plants was separated from other rows (treatments) by at least 60 cm. This spacing was necessary to reduce possible effects of spreading volatile gases or caterpillars among the various treatments. A total of 24 plants were given different treatments, with six replicates for each of the four treatments. Plant treatments included spraying every two days over a 12-day period until the experiment was terminated.

**Phytohormone Application**

Solutions of 0.01% JA (methyl jasmonate, IUPAC name: (-)-(-)-2β,3-0xy-2-(cis-2-pentenyl)-cyclopentanone) and 0.05% SA (methyl salicylate, IUPAC name: 2-hydroxybenzoic acid methyl ester; obtained from R.A. Creelman) were added to allow equal distribution of the solutions on the plant leaf surface and to allow the plant to absorb the solution more readily (Creelman, personal communication; Browse, personal communication). First, stock solutions of SA (0.10%) and JA (0.10%) were prepared with ethanol, as was a control with neither SA nor JA. Ten ml of each stock solution was then added to 990 ml of deionized water and six drops of Tween 20 (detergent, obtained from R.A. Creelman) were added to allow equal distribution of liquid on the plant leaf surface and to allow the plant to absorb the solution more readily (Creelman, personal communication; Browse, personal communication).

The plants (3%) were sprayed with hand spray bottles twice a day—once prior to the application of the caterpillars. Each plant was sprayed with a fine mist of 2.25 ml of SA, JA, control, or both SA and JA solutions. The solutions had dried prior to initial application of the caterpillars. Plants were then sprayed every two days over a 12-day period until the experiment was terminated.

**Caterpillar Treatment**

Tobacco hornworm (*Manduca sexta*) eggs were obtained from the North Carolina State University Insectary. Eggs were placed in a 25 × 25 cm plastic container with artificial diet at room temperature and in the natural light regime for January in Ohio. The artificial diet was obtained also from the NCSU Insectary and consisted of a mixture of wheat germ, casein, sucrose, torula yeast, Wesson salt mixture, sorbic acid, cholesterol, methyl paraben, streptomycin sulphate, agar, vitamin mixture (USB no. 23430, ascorbic acid, and formalin). Eggs hatched in 4-6 days. Four 1- to 2-day old larvae were placed on each caterpillar-treatment plant and allowed to consume foliage for 12 days. Caterpillars were counted daily on each treatment plant; frequently some caterpillars had detached from the plant to the soil surface. The plants were terminated and replaced.

**Analysis Of Treatment Effects**

The plants were subjectively scored beginning the day after the treatments were applied and continuing every two days until the day of harvest. The levels of rating were as follows:

1. No damage. Perfectly healthy
2. Slight leaf discoloration or chlorosis
3. Slight leaf wilting or curling
4. More leaf curling or wilting
5. Some leaves curled or wilted over half
6. Some leaves curled or wilted over three fourths
7. All leaves curled or wilted over half
8. All leaves curled or wilted fully
9. Dead leaf
10. Plant dead

After the 12 days, the 6-week-old plants were cut at the base, placed in a paper bag, dried for 48 hours in a drying oven at 70°C, and weighed (in bags) with a Fisher top-loading balance accurate to 0.01 g. Because the 18 control plants were harvested 4 days earlier than the rest of the experiment. Therefore, one would expect those plants to have slightly less dry weight than they would if allowed to grow as long as the caterpillar-applied plants. Those data are included in some of the analyses because of the additional information obtained. Data of score, plant weight, caterpillar weight, and number of caterpillar detachments were statistically analyzed in S-Plus software (Statistical Sciences 1993) using an analysis of variance (ANOVA) with multiple comparisons tested with the Tukey method. The P value used for declaring significant effects was 0.05.

**RESULTS**

**Plant Scores**

Plants with no caterpillars, regardless of chemical treatment were vigorous and healthy throughout the experiment (score of 1; Table 1). Thus, the JA and SA did
The effects of surface-applied JA and SA on tomato plant vigor and weight.

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Caterpillar added</th>
<th>No Caterpillar added Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>SA</td>
</tr>
<tr>
<td>mean plant vigor score</td>
<td>5.70 ± 0.20 a</td>
<td>4.03 ± 0.47 b</td>
</tr>
<tr>
<td>Mean plant weight, g*</td>
<td>0.63 ± 0.69 b</td>
<td>0.41 ± 0.14 a</td>
</tr>
</tbody>
</table>

*Because the plants with no caterpillars were harvested 4 days earlier, only general comparisons should be made to the caterpillar-treated plants.

1 Different letters indicate significantly different (P < 0.05) results using Tukey's multiple comparison test after the analysis of variance.

### Plant Weight

Plants treated with caterpillars had generally lower biomass compared to plants with no caterpillars (Table 1). This effect of a reduction in plant biomass via caterpillar ingestion is apparent even though the control plants that had no caterpillars applied were harvested four days earlier than the caterpillar-treated plants.

Among the plants treated with caterpillars, aboveground dry matter data showed that the SA-treated plants had significantly lower total yield compared to water-treated plants, while JA-treated plants had higher yields compared to water-treated plants and especially when compared to SA-treated plants (Table 1). JA-treated plants had twice the biomass of the SA-treated plants. When SA and JA were applied together, there was a slight, but not statistically different, increase in biomass compared to controls (0.69 ± 0.08 vs. 0.63 ± 0.09 g per plant). In general, the effects of JA vs. SA negated each other. Conceivably, exogenous JA should be able to bypass and eventually overcome the SA block on endogenous JA production, if enough exogenous JA was applied and absorbed. Perhaps this effect is beginning to be apparent in this experiment.

### Caterpillar Detachment

This metric is a measure of the total number of times caterpillars had dropped from the plant to the pot or table, cumulative over the 12 days of caterpillar consumption. Though no statistical analysis was possible on these data, the JA-treated plants had nearly twice the number of detachments compared to water-treated plants, and three times the detachments of SA-treated plants (Table 2). Those plants treated with both JA and SA had nearly the same number of detachments as compared to the JA-treated plants. These data suggest that caterpillars were making choices on preferred food, and the JA-treated plants were not preferred. Because the plants were widely spaced within the greenhouse, no evidence of crossover of caterpillars between treatments was detected.

### DISCUSSION

Evidence from plant weight, caterpillar weight, and the cumulative count of caterpillar detachments shows that the surface application of SA made tomato plants more susceptible to caterpillar damage and that JA made tomato plants less susceptible to caterpillar damage.

### Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Caterpillar weight, mg*</th>
<th>Total Detachments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>Water</td>
<td>87 ± 53 b</td>
<td>135 ± 36 c</td>
</tr>
<tr>
<td>SA</td>
<td>135 ± 36 c</td>
<td>53 ± 16 ab</td>
</tr>
<tr>
<td>JA</td>
<td>135 ± 36 c</td>
<td>53 ± 16 ab</td>
</tr>
</tbody>
</table>

*Different letters indicate significantly different (P < 0.05) results using Tukey's multiple comparisons test after the analysis of variance.
SA-treated plants had proportionately more plant tissue converted to caterpillar larvae tissue than did JA-treated plants. In both plant and caterpillar weight, SA and JA-treated plants were significantly different from the control plants. In both plant and caterpillar weight, SA and JA-treated plants were significantly different from the control plants. Thus, caterpillars being on SA-treated plants have a larger mass as they are not as inhibited by the proteinase inhibitors or other defense responses that can affect growth and reproduction of the insect.

Plants treated with JA, on the other hand, exhibit the opposite effect. JA is the end product of the octadecanoid defense pathway that leads to the activation of defense genes and production of defensive proteins. In our model, exogenous JA is absorbed and stimulates a greater production of these defensive compounds.


