Induced resistance to the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae) in three soybean cultivars

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Abstract

We investigated induced resistance to the common cutworm, *Spodoptera litura*, in three soybean cultivars, ‘Bay’, ‘Fukuyutaka’, and ‘Himeshirazu’. Levels of induced resistance caused by *S. litura* herbivory varied among the cultivars. High induced resistance was found in ‘Bay’, with less food consumption by larvae (78.2%), less larval weight gain (67.3%), longer larval duration (114.2%), and lighter pupal weight (79.7%) than on uninjured plants (100%). The same trends were observed in larvae feeding on leaves of previously damaged plants of ‘Fukuyutaka’, which is susceptible to *S. litura*, but only larval duration was significantly prolonged (107.3%). No apparent effect of previous herbivory on plants was found in ‘Himeshirazu’, which is constitutively resistant to *S. litura*. Prior mechanical injury to leaves of ‘Bay’ also decreased subsequent food consumption by larvae (82.9%), but had little effect on larval growth.

Key words: Induced resistance; soybean; Bay; *Spodoptera litura*

INTRODUCTION

The common cutworm, *Spodoptera litura* (Fabricius), is a polyphagous insect and one of the most serious pests of soybean in southwestern Japan. Soybean resistance to *S. litura* has been studied, and some cultivars, e.g. PI229358 (‘Sodendaizu’) and ‘Himeshirazu’, showed high resistance (Hara and Ohba, 1981; Komatsu et al., 2004). Soybean breeding lines developed from resistant cultivars suffered lower damage by a few soybean pests, including *S. litura*, in the field (Endo et al., 2002; Wada et al., 2006). Thus, the use of soybean resistance to insects offers an important tool in integrated pest management in Japan.

We found that the soybean cultivar ‘Bay’ showed a high level of antibiotic to *S. litura* when larvae were reared on whole plants (Endo et al., 2005). Larvae reared on ‘Bay’ had significantly longer larval duration, lower pupal weight, and lower pupation rate than larvae reared on ‘Fukuyutaka’, which is susceptible (Endo et al., 2005); however, we could not confirm such strong antibiotic when larvae were reared on isolated ‘Bay’ leaves (N. Endo, unpublished data). This suggests that antibiotic effects may be triggered by stress caused by larvae on plants, such as larval herbivory (induced resistance).

Many studies have demonstrated that previously damaged soybean plants are less suitable for herbivorous insects (Kogan and Fischer, 1991). Insect herbivory induces both antixenotic (Chiang et al., 1987) and antibiotic effects (Lin and Kogan, 1990) on the Mexican bean beetle, *Epilachna varivestis*. The soybean looper, *Pseudoplusia includens*, reared on leaves from previously injured plants, had an 8.5% longer larval development time and a 10.4% lower pupal weight than loopers reared on leaves from uninjured plants (Lin and Kogan, 1990). Mechanical injury to soybean leaves also increased the level of resistance to *E. varivestis* (Lin et al., 1990) and *P. includens* (Reynolds and Smith, 1985). Therefore, we aimed in this study to determine whether *S. litura* feeding on soybean leaves really induces resistance and to determine any effect of mechanical injury on the expression of this induced resistance.

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MATERIALS AND METHODS

Plants. We used three soybean cultivars, ‘Bay’, ‘Fukuyutaka’, and ‘Himeshirazu’. We used the same cultivars in our previous study (Endo et al., 2005), which investigated the resistance of ‘Bay’. ‘Bay’ was bred in the United States (Buss et al., 1979) and has been used as experimental material at Saga University. ‘Fukuyutaka’ is a commercial cultivar grown in southwestern Japan, and is susceptible to *S. litura*. ‘Himeshirazu’ is resistant to *S. litura* and shows a strong antibiotic effect against *S. litura* (Komatsu et al., 2004).

Soybean seeds were sown in Wagner pots (1/5,000 a) filled with field soil on 31 May (‘Bay’, ‘Fukuyutaka’, and ‘Himeshirazu’) and on 1 July 2005 (‘Bay’ only). The plants were kept in a wire-meshed greenhouse to exclude insects, where the plants grew in near-natural conditions except being shielded from rain. About two months later, the fully developed plants were used for bioassay. The developmental stages of the plants were defined according to Fehr et al. (1971).

Insects. Eggs of *S. litura* were supplied by Sumitomo Chemical Co., Ltd., and the larvae were kept in the laboratory (24±1°C under 16L–8D photoregime). Larvae were reared on an artificial diet (Insecta LFS; Nihon-Nosan Kogyo Co., Yokohama, Japan) until the fifth instar. Before molting to the sixth (final) instar, each larva was transferred to a plastic cup. About 16 h later, larvae that had finished molting were used for bioassay.

Influence of previous leaf damage on food consumption by larvae and on larval development. Experiment 1 was conducted to examine the effect of *S. litura* herbivory on the induction of resistance in the three cultivars. The experiment was carried out from 26 July with soybean plants sown on 31 May. The plant stages were R3–R4 (‘Bay’), R2 (‘Fukuyutaka’), and V14–V15 (‘Himeshirazu’). Ten plants (1/pot) of each cultivar were divided into two groups of five plants each (treatment and control). We released 15 sixth-instar larvae on the leaves on the lower half of each treatment plant for 12 h; these caused approximately 20–30% defoliation. The control plants (uninjured) received no larvae. Five days after the initial injury, the intact upper leaves from injured and uninjured plants were used in the experiment. Leaves were taken from each plant and placed in a Petri dish (2 cm×9 cm) with moist filter paper on the bottom. A newly molted sixth-instar *S. litura* larva (mean±SE, 195.9±1.8 mg) was introduced into each dish. The dish was kept in an incubator at 24±1°C under a 16L–8D photoregime. We weighed each larva and the leaves at the beginning of the test and 24 h later to calculate food consumption by the larva and larval weight gain. Leaves were exchanged with fresh ones from the same plant every day until pupation. We recorded the duration of the sixth instar (including prepupa), pupal weight (on the 2nd day after pupation), and survivorship until adult emergence. To measure the duration of the sixth instar, we checked pupae every 12 h. We calculated an index (efficiency of conversion of ingested food, ECI) to evaluate the efficiency of food digestion by the larvae: ECI=100×WG/FC, where WG is larval weight gain (mg) and FC is food consumption (mg) by the larva during the first 24 h.

Experiment 2 was conducted to evaluate the effect of mechanical injury. The experiment was carried out from 23 August with ‘Bay’, which was sown on 1 July. Fifteen plants (R3 stage) were divided into three treatment groups of five plants each. In one group, we used a hole punch to punch many holes (6 mm in diameter) in leaves on the lower half of the plants to provide mechanical injury (ca. 25% defoliation). The other two treatments were the same as those in experiment 1. Three days after the initial injury, we used the upper leaves of the plants in each treatment for bioassays with sixth-instar *S. litura* larvae (mean±SE, 208.4±1.8 mg). The other procedures were the same as those in experiment 1.

Statistical analysis. In experiment 1, the effects of larval herbivory on food consumption by larvae and on larval development were analyzed by *t*-test. Larval survival rates were compared by Fisher’s exact test. In experiment 2, the effects of mechanical injury and larval herbivory on consumption by larvae and on larval development were analyzed by one-way ANOVA, followed by the Tukey–Kramer test, using JMP Ver. 5.0 (SAS Institute Inc., Cary, NC). Differences in mortality rates among treatments were analyzed by multiple comparisons of proportions (Zar, 1996).
RESULTS

Food consumption and subsequent larval growth were compared between larvae feeding on previously injured plants and on uninjured plants (Table 1). Larvae reared on the leaves of previously injured ‘Bay’ showed significantly (p<0.01) less food consumption (78.2%) and less weight gain (67.3%) than those reared on uninjured control plants (100%) (Table 1). The same trend was observed in ‘Fukuyutaka’, but no significant difference was found between treatments in either food consumption or weight gain (p>0.05, Table 1). The duration of the sixth instar of *S. litura* reared on leaves of previously damaged plants was longer on ‘Bay’ (114.2%, p<0.001) and ‘Fukuyutaka’ (107.3%, p<0.01) than on the control (100%) (Table 2). Weights of pupae reared on leaves of previously injured plants were less on ‘Bay’ (79.7%, p<0.01) and ‘Fukuyutaka’ (92.7%, p>0.05) than on leaves of uninjured plants (100%) (Table 2). On ‘Himeshirazu’, food consumption by larvae, larval weight gain, and ECI of larvae fed on leaves of both treatments were much lower than those on other cultivars (Table 1), and larval injury to the plants had no significant effect on larval development (Table 2).

The results of experiment 2 show that mechanical injury of ‘Bay’ leaves significantly decreased food consumption by larvae in the first 24 h (82.9%, p<0.05) (Table 3); however, the duration of the sixth instar and pupal weight were not affected (Table 4). On the other hand, food consumption by larvae feeding on leaves after larval herbivory declined to 88.3%, but the difference was not significant (Table 3). Antibiotic effects (prolonged larval duration and reduced pupal weight), however, were again observed in larval herbivory treatments (Table 4). Larvae feeding on leaves from previous herbivory plants showed increased mortality, with only 10% attaining adult eclosion, while 40% of larvae on mechanically injured plants

### Table 1. Effects of previous herbivory on three soybean cultivars on food consumption by and development of *Spodoptera litura* larvae (mean±SE) (Experiment 1)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Treatment</th>
<th>Food consumed (mg)</th>
<th>Weight gain (mg)</th>
<th>ECI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bay</td>
<td>Uninjured</td>
<td>139.2±7.3</td>
<td>67.2±10.4</td>
<td>48.4±2.8</td>
</tr>
<tr>
<td></td>
<td>Larval herbivory</td>
<td>108.8±8.2</td>
<td>45.2±7.5</td>
<td>42.1±2.0</td>
</tr>
<tr>
<td>Fukuyutaka</td>
<td>Uninjured</td>
<td>150.4±11.9</td>
<td>74.2±14.9</td>
<td>48.8±2.6</td>
</tr>
<tr>
<td></td>
<td>Larval herbivory</td>
<td>129.8±8.7</td>
<td>58.9±8.9</td>
<td>46.1±2.5</td>
</tr>
<tr>
<td>Himeshirazu</td>
<td>Uninjured</td>
<td>87.3±10.1</td>
<td>21.8±10.0</td>
<td>26.7±3.6</td>
</tr>
<tr>
<td></td>
<td>Larval herbivory</td>
<td>89.3±13.8</td>
<td>24.0±12.9</td>
<td>27.0±3.9</td>
</tr>
</tbody>
</table>

Food consumption by larvae and larval development were examined during 24 h with sixth instar larvae (20 replicates). Significant difference is represented by asterisks (t-test: **p<0.01).

a ECI is efficiency of conversion of ingested food: (weight gain/food consumed)×100.

### Table 2. Effects of previous herbivory on three soybean cultivars on development (mean±SE) and survivorship of *Spodoptera litura* larvae (Experiment 1)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Treatment</th>
<th>Duration of sixth instar (day)</th>
<th>Pupal weight (mg)</th>
<th>Pupation (%)</th>
<th>Eclosion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bay</td>
<td>Uninjured</td>
<td>7.16±0.08</td>
<td>184.2±7.0</td>
<td>95.0</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td>Larval herbivory</td>
<td>8.18±0.17</td>
<td>146.8±5.8</td>
<td>95.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Fukuyutaka</td>
<td>Uninjured</td>
<td>6.75±0.12</td>
<td>210.1±6.2</td>
<td>100.0</td>
<td>75.0</td>
</tr>
<tr>
<td></td>
<td>Larval herbivory</td>
<td>7.24±0.11</td>
<td>194.7±5.0</td>
<td>95.0</td>
<td>55.0</td>
</tr>
<tr>
<td>Himeshirazu</td>
<td>Uninjured</td>
<td>8.75±0.75</td>
<td>145.5±19.5</td>
<td>10.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Larval herbivory</td>
<td>7.75±0.75</td>
<td>146.5±11.5</td>
<td>10.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Development and survivorship of the larvae were examined from the beginning of the sixth instar to adult eclosion (20 replicates). Significant difference is represented by asterisks (*p<0.05, **p<0.01, ***p<0.001) according to t-test or Fisher’s exact test (percentage data).
and 70% on uninjured plants completed development to adults (Table 4).

DISCUSSION

Insect-induced resistance in soybeans to various species has been reported, including some lepidopteran species such as *P. includens* (Lin and Kogan, 1990) and the corn earworm *Helicoverpa zea* (Bi et al., 1994). We have shown for the first time that previous herbivory by *S. litura* larvae on soybean plants induces antibiotic effects on subsequent larval feeding and growth. In addition, damage to leaves was confined to the lower part of the plants, but we found antibiosis by intact leaves on the upper part of plants; thus, the induction of resistance is systemic.

Underwood et al. (2000) showed that the extent of resistance induced by *E. varivestis* varied among soybean genotypes. Our results also demonstrate that antibiotic effects caused by *S. litura* herbivory vary among cultivars: a high level of resistance was induced in ‘Bay’, a low level was induced in ‘Fukuyutaka’, but no apparent effect of herbivory was found in ‘Himeshirazu’.

This study clearly indicates the difference in resistance mechanisms between ‘Bay’ and ‘Himeshirazu’. ‘Himeshirazu’ showed strong antibiosis, irrespective of larval injury (constitutive resistance) (Table 2). ‘Bay’ showed resistance only after larval damage (induced resistance) (Table 2). Endo et al. (2005) also suggested that the mechanism of resistance to *S. litura* differs between these two cultivars. They found better larval survival and subsequent slower larval weight gain on ‘Bay’. These phenomena may be partially explained by the physiological characteristics of ‘Bay’, which switch on resistance only after larval herbivory.

Earlier studies have demonstrated that mechanical injury induces resistance to insects in soybean plants (Reynolds and Smith, 1985), but the level of induction is significantly lower than that by feeding injury (Lin et al., 1990; Srinivas et al., 2001). Our results show that mechanical injury caused less food consumption by larvae, but had little effect on larval growth, which agrees with earlier studies.

Regurgitant of herbivorous larvae may be involved in induced resistance in soybean. Lin et al. (1990) showed that inoculation of the regurgitant of *P. includens* larvae together with mechanical injury of soybean leaves induced higher resistance than mechanical injury alone, and suggested that material(s) contained in the larval regurgitate might enhance the induction of resistance. Recently, *N*-(17-hydroxylinolenoyl)-l-glutamine (volicitin), and volicitin-related compounds have been identified from the oral secretion of herbivo-

### Table 3. Effects of mechanical injury and larval herbivory in soybean cultivar ‘Bay’ on food consumption by and development of *Spodoptera litura* larvae (mean±SE) (Experiment 2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Food consumed (mg)</th>
<th>Weight gain (mg)</th>
<th>ECIa (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninjured</td>
<td>152.2±7.5 a</td>
<td>72.7±5.3 a</td>
<td>47.4±1.9</td>
</tr>
<tr>
<td>Mechanical injury</td>
<td>126.2±9.4 b</td>
<td>56.3±5.5 a</td>
<td>43.7±1.9</td>
</tr>
<tr>
<td>Larval herbivory</td>
<td>134.4±6.5 ab</td>
<td>58.4±3.0 a</td>
<td>43.8±1.5</td>
</tr>
</tbody>
</table>

Food consumption by larvae and larval development were examined during 24h with sixth instar larvae (20 replicates). Different letters indicate significant differences (*p*<0.05) by ANOVA followed by Tukey-Kramer test. ECI is efficiency of conversion of ingested food: (weight gain/food consumed)×100.

### Table 4. Effects of mechanical injury and larval herbivory in soybean cultivar ‘Bay’ on development (mean±SE) and survivorship of *Spodoptera litura* larvae (Experiment 2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of sixth instar (day)</th>
<th>Pupal weight (mg)</th>
<th>Pupation (%)</th>
<th>Eclosion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninjured</td>
<td>7.74±0.16 a</td>
<td>144.5±3.9 a</td>
<td>95.0 a</td>
<td>70.0 a</td>
</tr>
<tr>
<td>Mechanical injury</td>
<td>7.87±0.12 ab</td>
<td>135.1±5.1 ab</td>
<td>75.0 ab</td>
<td>40.0 ab</td>
</tr>
<tr>
<td>Larval herbivory</td>
<td>8.45±0.30 b</td>
<td>121.7±3.9 b</td>
<td>55.0 b</td>
<td>10.0 b</td>
</tr>
</tbody>
</table>

Development and survivorship of the larvae were examined from the beginning of the sixth instar to adult eclosion (20 replicates). Different letters indicate significant differences (*p*<0.05) by ANOVA followed by Tukey-Kramer test or by multiple comparison of proportions (Zar, 1996).
rous larvae including *S. litura* (Mori et al., 2003). These compounds elicit plant volatiles (Schmelz et al., 2003; Sawada et al., 2006) and jasmonic acid (Schmelz et al., 2003) in corn plants, which are thought to be key compounds of plant-induced resistance. Thus, even in our experiments, these compounds in the regurgitant of *S. litura* might induce a continuous systemic response for larval resistance in soybean plants.

Most studies related to induced resistance in soybean were conducted with young plants grown in a greenhouse, and thus little is known about the consequence in the field (Underwood et al., 2002). We used mature plants grown in near-field-like conditions. In addition, there is an observation that ‘Bay’ suffered less insect damage than other cultivars in the field (S. Tojo, unpublished data); therefore, we can expect practical field resistance against *S. litura* and other herbivores by ‘Bay’.

In current breeding and screening programs for soybean pest resistance, only constitutive resistance has been evaluated. The program protocols could not evaluate the dynamic resistance elicited by natural stresses, such as insect herbivory, pathogenic infection, and mechanical damage. We should focus on both constitutive and inducible levels of resistance in future breeding to fully understand the potential for plant resistance.

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