Histological and Biochemical Changes in the Tissue of Pumpkin Fruit Injured by Lygus disponsi LINNAVUORI (Hemiptera: Miridae)¹

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When Lygus disponsi LINNAVUORI fed on pumpkin fruits, their styles passed through intercellularly to reach the vascular system, where a feeding cavity often formed. The cells surrounding the stylet track and feeding cavity went on swelling till 6 days after the injury and turned so that they were arranged regularly along the stylet track, the feeding cavity and the epithelium. Starch granules disappeared from or decreased in number in the enlarged cells.

Polyphenoloxidase activity rose just after the infestation period, but soon dropped and remained at the control level from the 3rd day on. Acid phosphatase activity was the highest at the 6th day after the infestation period. IAA-oxidase activity rose markedly as a result of the injury, but decreased very quickly. The mechanism of tubercle formation is discussed.

INTRODUCTION

The bug Lygus disponsi LINNAVUORI (Hemiptera: Miridae) is common in Hokkaido and has two or three generations a year. The adults and nymphs feed mainly on reproductive organs of wild cruciferous and fabaceous plants, but they sometimes invade fields of various crops and injure them (HORI, 1967; HORI and HANADA, 1970; HORI and KURAMOCHI, 1984). TORIKURA (1985) found the bugs L. disponsi attacking pumpkin fruits and producing tubercles at the feeding points (Fig. 1). Heteropterous insects have seldom been known to cause swellings on plants by feeding, though they cause different types of malformations on various plants and plant parts; in only a few cases have insects produced swelling at the feeding points (cf. CARTER, 1962; MILES, personal communication).

In the present study, histological and biochemical changes in the injured parts of pumpkin fruits were investigated to learn the mechanism of the swelling formation.

MATERIALS AND METHODS

Insects used. Adult bugs collected from the field in late May, 1985, were reared in petri dishes provided with cruciferous plants as food and an ovipositional object. Eggs

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laid in the plant tissues were removed to other petri dishes and kept till hatching at room temperature. Nymphs were reared in cages provided with food plants (cruciferous plants) under the same conditions until they reached adult stage. First generation adults were used for experiments.

**Experimental plants.** Seedlings of pumpkin plant (purchased from the Memuro Agriculture Co-operative Association were transplanted in 3 m wide rows and 0.5 m apart in the experimental field of Obihiro University of Agriculture and Veterinary Medicine. In 1985 the fruiting time was late June. Fruits 10 cm in diameter and still growing were used for experiments.

**Artificial infestation and plant tissues used for investigations.** A pair of plastic cups (5 cm in top diameter, 4 cm in bottom diameter and 5.6 cm in height) from which the bottom had been removed and replaced with a sheet of net cloth was set on each of 24 pumpkin fruits as seen in Fig. 2. A thin sheet of artificial sponge ring was inserted between the cup and the fruit to fill any possible space there, and then the cup was fixed by sticky tape. Ten adult bugs were caged in one of the cups (feeding cup) for 4 days; the other was used for control (control cup). When a bug in the feeding cup died, it was replaced by a new one. Fruit tissue for histological observation and chemical analysis was cut out of injured and control parts immediately, 3, 6, 9, 12, 15, 18 and 21 days after the infestation period. The observation and analysis were repeated three times.

**Histological observations.** As soon as the tissues were cut from the pumpkin fruits, they were fixed with FAA fixing fluid (100 ml of ethyl alcohol, 6.5 ml of formaline, and 2.5 ml of acetic acid) for 48 hr. Following the general procedure of preparation, they were cut into sections 10 nm thick and stained with acid fennelum and safranin. Carbohydrates were detected with periodic acid-Schiff reaction (cf. Sano, 1965).

**Chemical analyses.** Injured and uninjured (control) tissues were cut out in about 6 mm thicknesses from the surface of the pumpkin fruits. They were extracted with 10 ml distilled water per gram of fresh weight in a mortar by pestling with quartz sand. After refrigerated centrifugation at 26,000 g for 20 min, the supernatants were used for various assays and analyses, mainly following the methods described by Hori (1973) with some modification. The test solutions were diluted 10-fold for determination of peroxidase activity and phenol compound, and 40-fold for acid phosphatase activity, but they were not diluted for polyphenoloxidase and IAA-oxidase assays. Polyphenoloxidase assay was performed by following a modified Ponting-Joslyn method (1948):

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**Fig. 1.** Tubercles formed on a pumpkin fruit as a result of injury by _L. disparis_. Arrow shows a large swelling.

**Fig. 2.** A feeding cup with 10 bugs and a control cup without bugs set on a pumpkin fruit.
Reaction mixture consisted of 1.0 ml phosphate buffer (pH 7.0, 0.07 M), 1.0 ml catechol (1.6%), 1.0 ml saturated sulfuric acid and 1.0 ml enzyme solution. The reaction was initiated by adding the enzyme solution to the mixture of the first three ingredients in a glass cell. Absorbancy at 350 nm was then recorded 30 sec after the beginning of the reaction. For IAA-oxidase assay, phosphate buffer of pH 5.0 and 0.05 M used in the previous investigation (Hori, 1973) was replaced by that of pH 6.0 and 0.067 M.

RESULTS AND DISCUSSION

Histological and histochemical observations

The stylet track stained red-purple with acid femalaun-safranin passed intercellularly toward the vascular system of pumpkin fruit, where a feeding cavity was often formed (Figs. 3a and b). Cells surrounding the track and cavity were enlarged. Starch granules disappeared or decreased in number in the enlarged cells and centered in the feeding cavity, and the stylet track adjoined the cavity (compare Figs. 3b and 4d). This may suggest that these granules were consumed as energy for cell hypertrophy and/or brought out from broken enlarged cells to the feeding cavity as a food source for...
Table 1. The number of cells contained in a defined area of pumpkin tissues injured by *L. dispensi* and uninjured control tissues

<table>
<thead>
<tr>
<th>Days after infestation</th>
<th>Sample no.</th>
<th>Number of cells</th>
<th>At 0.06 mm depth</th>
<th>Relative value</th>
<th>At 0.83 mm depth</th>
<th>Relative value</th>
<th>Mean relative value</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Injury</td>
<td>Control</td>
<td></td>
<td>Injury</td>
<td>Control</td>
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<tr>
<td>0</td>
<td>(1)</td>
<td>16.07</td>
<td>16.11</td>
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<td>38.33</td>
<td>44.11</td>
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<td></td>
<td>(2)</td>
<td>17.67</td>
<td>18.67</td>
<td>94.64</td>
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<td>53.00</td>
<td>95.38</td>
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<td>8.00</td>
<td>13.11</td>
<td>61.02**</td>
<td>45.33</td>
<td>51.89</td>
<td>87.38</td>
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<td>11.56</td>
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<td>88.12</td>
<td>34.34</td>
<td>45.44</td>
<td>75.58**</td>
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<td>8.22</td>
<td>13.33</td>
<td>61.67**</td>
<td>33.44</td>
<td>41.78</td>
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<td>105.68</td>
</tr>
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</table>

* (1) and (2) show samples from different pumpkin fruits.

* Values are expressed as the average of 9 determinations.

* Significant different from control at *p*<0.05 (*t*-test).

** Significantly different from control at *p*<0.01 (*t*-test).

the bugs. Starch concentrations were known to be low or absent in the neighboring gall tissues of the larval chamber of *Urophora* sp. (BRONNER, 1977; Lalonde and Shorthouse, 1984).

Table 1 shows a comparison of the number of cells contained in 2.5×10⁻⁸ mm² or 4.4×10⁻² mm² of injured and uninjured tissues. Just after the infestation period, the cell group surrounding the stylet track swelled (Table 1 and compare Fig. 3 c with 3 d). Thereafter, the cells continued swelling till the 6th day after the infestation period (Table 1 and compare Fig. 4 a with 4 b). At the 9th day (compare Fig. 4 c with 4 d), the 12th day, the 15th day and the 18th day (compare Fig. 4 e and with 4 f), the size and number of cells in the injured parts were not greatly different from those in the control parts. On the other hand, the injury changed the cell arrangement in the tissue, which was random in the control, so that it stood in a row toward the stylet track, the feeding cavity and even the epithelium of pumpkin fruit (compare figures of the injured and control parts).

Chemical analyses.

Enzyme activity and the amount of phenol compound per gram of dry weight in the injured tissue were expressed as the values relative to those in the control tissue (=100). Absorbance values per gram of dry weight of tissue in the control part just following infestation were: 1.61 for PPO, 258.39 for AP, 3.91 for phenol, 4.79 for PO and 1.14 for IAA-oxidase.

Polyphenoloxidase (PPO) activity in the tissue rose just after infestation (though not significant statistically), but soon decreased and remained at the level of the control from 3 to 21 days later (Fig. 5); this was quite different from the case of sugar beet leaf tissues injured by *L. dispensi*, where a high level of PPO-activity remained in the
injured tissues 18 days after the infestation (Hori, 1973). The amount of phenol compounds in the fruit tissue tended to increase as a result of the injury, but soon returned to the original level (Fig. 5). Not much quinone may be produced in the bug-injured part of a pumpkin fruit, because raised PPO and phenol level returned immediately to their original levels. Thus no necrosis was caused and histological observation showed that only swelling was formed in the injured part.

Acid phosphatase (AP) activity in the tissue increased rapidly from just after the infestation period to 6 days later (though not statistically significant), then decreased quickly till the 9th day and returned to the control level on the 15th day (Fig. 5).
Peroxidase (PO) activity was very high immediately after the infestation period (8-fold of control) \((p<0.05)\), but dropped rapidly to a level just double the control 3 days later and this level was kept for 21 days (Fig. 6). The change of IAA-oxidase activity in the injured part with the lapse of time after infestation was very similar to that of PO activity (Fig. 6), indicating that IAA-oxidase activity in the injured part came mainly from the function of PO. The increase of IAA-oxidase in the injured tissue just after the attack is suggestive of the increase of IAA content itself in the tissue. Hori (1974) demonstrated the existence of IAA-synergist (IAA-oxidase inhibiting substance, etc.) in the salivary gland of L. dispersi and stressed the importance of IAA-synergist in insect saliva in relation to the leaf-malformation of sugar beet formed by the injury of L. dispersi and the mechanism of gall-formation. Such an IAA-oxidase inhibiting substance (IOIS) may penetrate the surrounding tissues from the feeding points via the saliva injected into those tissues and may inhibit IAA-oxidase activity there. The quick decrease of IAA-oxidase within 3 days after infestation might be due to the spread of IOIS from the injured part. This, in turn, could stimulate the rise of IAA activity on the 3rd and the 6th day, when the cells surrounding the injured point swell (Fig. 4a), and acid phosphatase activity necessary for energy metabolism in relation to cell hypertrophy rises (Fig. 5). A low level of IAA-oxidase activity from the 9th day might be the result of the decrease of IAA itself. Analyses of IAA amount to verify this hypothesis will be performed in the near future.

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Pumpkin Injured by *Lygus* Bug

REFERENCES


