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Role of induced resistance in interactions of *Epilachna vigintioctopunctata* with host and non-host plant species

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Abstract

The process of host/non-host determination was dissected in interactions of *Epilachna vigintioctopunctata*, a specialist herbivore of solanaceous plants, with various plant species. On host plants (tomato and egg plant) the ladybird beetle started feeding within 5 min. On red pepper, another solanaceous plant, it also started feeding within 5 min, but did not continue the feeding as vigorously as on tomato or eggplant. This result suggests that the ladybird beetle recognizes red pepper as a host plant but does not overcome its constitutive resistance. On Chinese cabbage, the ladybird beetle did not start feeding as quickly as on the host plants, but once started, it continued feeding as vigorously as on the host plants. This result suggests that the ladybird beetle does not recognize Chinese cabbage as a host plant but overcomes its constitutive resistance. Subsequently, the effect of induced resistance in a host (tomato) and non-hosts (Chinese cabbage and *Arabidopsis*) was evaluated. The treatment with methyl jasmonate (MeJA) showed no effects in tomato but decreased the damaged area in Chinese cabbage and *Arabidopsis*. A feeding test with *Arabidopsis* mutants supported the idea that induced resistance via the jasmonic acid (JA) pathway is effective against the ladybird beetle on the cruciferous plants. We suggest that a specialist herbivore has to overcome not only constitutive resistance but also induced resistance to utilize the non-host plant as a host, and that induced resistance is one of the factors that determine host specificity of the specialist.

Keywords: *Epilachna vigintioctopunctata*; Solanaceous plant; Host-specificity; Induced resistance

1. Introduction

In addition to constitutive resistance mechanisms, plants have evolved inducible mechanisms against attack by herbivores and pathogens [1]. Induced resistance against subsequent attack is acquired following inoculation with pathogens [2], non-pathogenic root-colonizing bacteria [3] or feeding by herbivores [4]. One type of induced resistance is characterized by the accumulation of salicylic acid (SA). Exogenous treatments with SA itself, 2,6-dichloronicotinic acid (INA), or the benzothiadiazole derivative BTH activate the SA-dependent pathway, resulting in the induction of resistance against some pathogens. For example, SA-treated soybean cells responded to an avirulent strain of *Pseudomonas syringae* with more rapid expression of hypersensitive response and H_2O_2 generation [5]. The treatment with BTH induced systemic resistance to pathogens in tobacco [6], wheat [7] and *Arabidopsis* [8].

The jasmonic acid (JA)-dependent pathway was also well documented as a signaling pathway to induced resistance [9]. JA is synthesized via octadecanoid pathway from linolenic acid [10] and increased by several stimuli including

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mechanical wounding and water deficit [11], herbivores [12], pathogens [13], microbial cell wall elicitors [14] and plant signaling peptide systemin [15]. The increased JA activates a set of defense genes that are different from SA-inducible genes [16]. Among the set of genes, proteinase inhibitor I and II genes in tomato have been well characterized. These inhibitors interact with proteinases in herbivore gut and affect proteolysis [17]. Actually, an octadecanoid pathway-deficient mutant of tomato lost the ability to induce the proteinase inhibitors, and was concomitantly compromised in the resistance to *Manduca sexta* [18]. The critical role of the JA-dependent pathway in insect resistance was also demonstrated in *Arabidopsis*. The *fad* mutant, that is deficient in the jasmonate precursor linolenic acid, was severely attacked by larvae of *Bradysia impatiens* [19]. Interestingly, Stotz et al. [20] found that ethylene signaling reduced resistance of *Arabidopsis* against Egyptian cotton worm (*Spodoptera littoralis*) but not diamondback moth (*Plutella xylostella*). These results suggested that individual herbivore evolved a specific strategy for the adaptation to the induced resistance in its host plant.

The vast majority of phytophagous insects exhibit a high degree of host plant specificity [21]. Mechanisms of the host-specificity have been intensively studied from the viewpoint of recognition of stimulatory and deterrent compounds [22]. For example, glycosinolates and indioside D were identified as stimulatory compounds for *Pieris rapae* and *M. sexta*, respectively. They were involved in the host recognition after hatched larvae had experienced their host [23,24]. A saponin, identified as a deterrent compound to *P. xylostella*, was correlated with the resistance of *Barbarea vulgaris* to the diamondback moth [25]. These reports are interpreted as suggesting that constitutive compounds play important roles in the recognition of host/non-host plants by phytophagous insects. However, there have been few reports on the relationship between induced resistance and host ranges.

The phytophagous ladybird beetle, *Epilachna vigintioctopunctata*, widely inhabits from tropical to temperate regions throughout Asia and Oceania. The ladybird beetle is an oligophagous specialist herbivore of solanaceous plants [26], but mechanisms of the determination of its host range are still unclear. In this study, we examined three possible factors that may be involved in the determination of the host-specificity of *E. vigintioctopunctata*, i.e., host recognition, effects of constitutive resistance and induced resistance. Our results suggested that a specialist herbivore has to overcome not only constitutive resistance but also induced resistance to utilize a plant species as a host.

2. Materials and methods

2.1. Insects and plants

E. vigintioctopunctata was collected from a tomato field at Kobe University and kept in a climate room (25 °C; 50–70% relative humidity; 16L:8D) with tomato leaves as a diet.

Test plants were tomato (*Lycopersicon esculentum*) cv. Redpear, Chinese cabbage (*Brassica campestris*) cv. Muso, Cucumber (*Cucumis sativus*) cv. Hokushin, and Komatsuna (*B. campestris*) obtained from Takii corporation, Japan, red pepper (*Capsicum annuum*) cv. Takanotsume and Pea (*Pisum sativum*) cv. Hyogo-kinusaya obtained from Sakata seed corporation, Japan, eggplant (*Solanum melongena*) cv. Shiki, Japanese radish (*Raphanus sativus*) cv. Natsudaikon, and pumpkin (*Cucurbita moschata*) cv. Azuma-ebisu obtained from Tohoku corporation, Japan, and kidney bean (*Phaseolus vulgaris*) cv. Nagauzurasaito obtained from Takayama seed corporation, Japan. Seeds of these test plants were sown in Sakata supermix soil (Sakata Co., Yokohama, Japan) in plastic pots (10.5 cm in diameter) (one seed/pot) and grown in a controlled-environment room with a 12 h photoperiod of fluorescent lighting (150 $\mu mol\ s^{-1}\ m^{-2}$) at 22–25 °C.

Arabidopsis thaliana ecotype Columbia and mutants derived from Columbia were also employed. The *fad7-2* and *fad7-1/8-1* mutants [27], which are deficient in fatty acid desaturation gene(s) and the wild type Columbia, were obtained from *Arabidopsis* Stock Center (Nottingham, UK). The SA deficient *eds16-1* mutant [28] and SA-non-accumulating Col-*nahG* plant were provided by M.C. Wildermuth and F.M. Ausubel (Massachusetts General Hospital). The JA-insensitive *jar1-1* mutant [29] was provided by P.E. Staswick (University of Nebraska). Seeds of these lines were sown in Kakiuchi professional soil (Kakiuchi Co., Tokyo, Japan) in plastic pots (8 cm in diameter) and grown in the controlled-environment room with a 12 h photoperiod of fluorescent lighting (150 $\mu mol\ s^{-1}\ m^{-2}$) at 22 °C. Ten days after sowing, the seedlings were thinned out to three seedlings/pot.

2.2. Estimation of host recognition by the ladybird beetle

A pot with 4–5-week-old seedling(s) was placed in a cylindrical plastic case. Three 4-day-old adults, that had been starved for 24 h beforehand, were released to the case and maintained in a growth chamber at 25 °C under 16 h photoperiod of fluorescent lighting (150 $\mu mol\ s^{-1}\ m^{-2}$). Ten pots were employed for each plant species, and the number of seedlings with feeding marks was recorded at 5, 15, 30 min and 1, 3, 6, 12, 24 h for the first day and every 12 h thereafter up to 168 h after the release. This experiment was replicated twice. Therefore, the total number of seedlings employed was 60 (3 seedlings/pot × 10 pot/replicate × 2 replicates) for *Arabidopsis* and 20 (one seedling/pot × 10 pot/replicate × 2 replicates) for the other plant species. The rate of plants with no feeding marks was calculated as survival rate by Kaplan–Meier method and statistically analyzed by the log–rank test [30].

2.3. Estimation of induced resistance in tomato, Chinese cabbage and Arabidopsis

Chemicals used for activation of induced resistance were benzothiadiazole (BTH) (a gift from Novartis Crop. Protection, AG, an activator of the SA-dependent pathway) and methyl jasmonate (MeJA) (Wako Chem. Ltd., Japan, an activator of the JA-dependent pathway). A pot with a 3–4-week-old tomato seedling, a 3–4-week-old Chinese cabbage seedling, or three 4–5-week-old *Arabidopsis* seedlings was placed in the cylindrical plastic case, and sprayed with 0.5 mM BTH, 0.2 mM MeJA, or water. The case was capped with a plastic lid to keep the humidity and maintained in a growth chamber with a 16 h photoperiod of fluorescent lighting (150 μmol s^{-1} m^{-2}) at 25 °C for 24 h. After the incubation, the plastic lid was replaced with a net lid, and the treated seedling was subjected to the feeding test. In tomato, a fourth-instar larva, or a 4-day-old adult was released to a case with a single seedling and incubated further in the growth chamber. Twenty-four hours after release, the consumed area on the leaves was copied to a transparent sheet by tracing it, and analyzed by the software, scion image (Scion corporation, USA). In Chinese cabbage, three fourth-instar larvae, or 4-day-old adults were released to a case with a single seedling, and the consumed area was estimated at 24 h after release as described above. In *Arabidopsis*, three 4-day-old adults were released to a case with three seedlings. Seven days after release, the damage of each leaf was rated using 11 progressive grades from 0 (no damage) to 10 (complete consumption). The feeding index (FI) was calculated as follow: FI = [(sum of the grade scores)/(number of observed leaves × 10)] × 100. In the experiment with the mutants of Columbia, 4–5-week-old seedlings were directly subjected to the feeding test without chemical treatment. These experiments were repeated ten times for adult insects and five times for larvae.

2.4. Detection of gene expression by RT-PCR

A leaf of 3–4-week-old tomato or Chinese cabbage was detached, placed in a plastic plate (14 cm × 10 cm × 1.5 cm), and treated with 0.2 mM MeJA or water. The plastic plate with the treated leaf was covered with a plastic lid and placed in the dark at 25 °C for 12 h. Two 4-day-old adults were released to a plate, which was then incubated for 2 h at 25 °C under fluorescent lighting (150 μmol s^{-1} m^{-2}). After removing the beetles, the attacked leaf was further incubated for 3 or 6 h under the same condition. In the control, the leaf treated with MeJA or water was incubated without insects for 5 h (2+3) or 8 h(2+6).

Total RNA was isolated according to Perry and Francki [31]. First-stranded cDNA was synthesized using oligo (dT) primer and reverse-transcriptase ReverTra Ace (TOYOBO, Japan). In tomato, the cDNA was subjected to PCR amplification of *PINII* (the proteinase inhibitor II gene; GenBank Accession No. AB110700), using a primer pair 5′-TGTTGATGCCAAGGCTTGTA-3′/5′-AGCAACCCTTGTACCCTGTG-3′. The expression of *LHA2* (the plasma membrane H^+-ATPase gene; GenBank Accession No. AF179442) was also monitored as a control using a primer pair 5′-GCCAAAGGTGTTGACGCAGATAC-3′/5′-CACTCCAAGATTCAAAGCCCTCCT-3′. In Chinese cabbage, the expression of *CYP* (a cytochrome P450 gene, *CYP79B1*, GenBank Accession No. AF528173) and *TUB* (a putative tubulin gene, GenBank Accession No. D78496) were monitored using primer pairs 5′-TCGCGAGACTTCTTCAAGGT-3′/5′-AAACCGACCAAACTCTCTGG-3′ and 5′-CTCGATGGCCTCAACCTTTA-3′/5′-ATGTTGCTCTCGGCTTCTGT-3′, respectively. The PCR reaction was performed in a 50 μl reaction mixture containing 1U rTaq polymerase, 1 pM each primer, 0.2 mM dNTP mix, 2.5 mM $MgCl_2$ and 10 μl 10× buffer (100 mM KCl containing 1% Triton X-100) with one denaturation cycle of 1 min at 94 °C and 30 cycles of 1 min at 94 °C, 50 s at 60 °C, and 40 s at 74 °C. The PCR product (10 μl) was fractionated by electrophoresis on 2% agarose gel and stained with ethidium bromide. This experiment was repeated three times.

3. Results

3.1. Feeding behaviors of the ladybird beetle on various plant species

Four-day-old adults of *E. vigintioctopunctata* were released to various plant species, and the number of attacked seedlings was recorded from 5 min to 7 days after the release. Significant difference was detected by the log–rank test between five groups ($P < 0.05$): group 1 (tomato, eggplant and red pepper), group 2 (Chinese cabbage), group 3 (*Arabidopsis*, Komatsuna, Japanese radish and kidney bean), group 4 (pea and cucumber) and group 5 (pumpkin).

The beetles released to the host plants, i.e., tomato and eggplant, immediately brought their mouthpart into contact with the leaf surface. They attacked the host plants within 5–15 min, and produced feeding marks on almost all seedlings by 30 min (Fig. 1A). They continued feeding vigorously, and caused extensive damage to the seedlings by 24 h (Fig. 2A). On red pepper the beetles also started feeding within 5–15 min (Fig. 1A), but did not continue the feeding as vigorously as on tomato or eggplant. The feeding marks looked sporadic dots even 24 h after release (Fig. 2B).

On *Brassicaceae* the beetles also brought their mouthpart into contact with the leaf surface as quickly as on the solanaceous plants. However, they did not start feeding immediately but started wandering in the case. After wandering for 0.5–3 h, the beetles started feeding (Fig. 1B). The most prominent was the feeding behavior on Chinese cabbage; the beetles produced feeding marks on a half of seedlings within 1 h (Fig. 1B). They continued feeding on Chinese cabbage as vigorously as on tomato and eggplant, and severely damaged the Chinese cabbage seedlings within

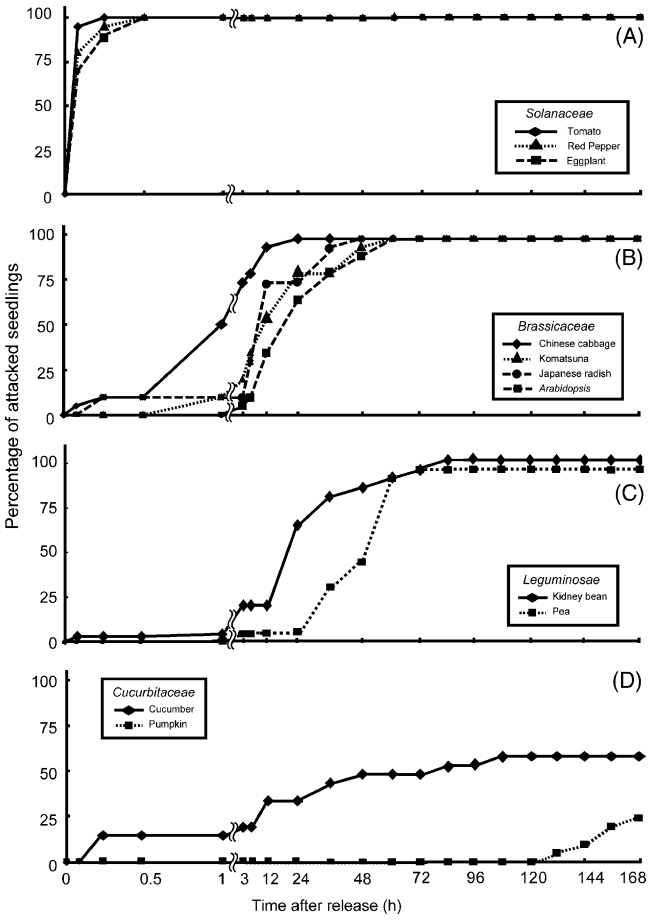

Fig. 1. Host recognition by *E. vigintioctopunctata*. After the release of 4-day-old adults, the number of seedlings with feeding marks was recorded.

24 h (Fig. 2C). On the other cultivated *Brassicaceae* plants, i.e., Komatsuna and Japanese radish, the beetles also consumed the leaves vigorously. On *Arabidopsis* the consumed area was very small at 24 h, but increased gradually up to 5–7 days (Fig. 2D).

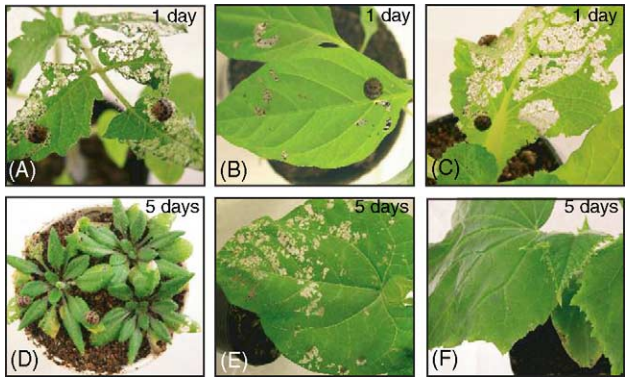

Fig. 2. Feeding marks produced by *E. vigintioctopunctata* on tomato (A), red pepper (B), Chinese cabbage (C), *Arabidopsis* (D), kidney bean (E) and cucumber (F). Three 4-day-old adults were released to a pot with one (A–C, E and F) or three (D) seedlings and incubated for 1 day (A–C) or 5 days (D–F).

On *Cucurbitaceae* the beetles showed a recognition behavior similar to that on *Brassicaceae*; they quickly brought their mouthpart into contact with the leaf surface but, instead of feeding, started wandering in the case. On cucumber, feeding marks were observed at 15 min after release in a few seedlings, but did not extend thereafter (Fig. 2F). Even after 120–168 h (5–7 days), about 40% of seedlings had not been attacked (Fig. 1D). On pumpkin feeding marks were scarcely observed until 120 h after release (Fig. 1D), and if any, only on cotyledons. It was not until 132 h that the beetles produced a few, tiny feeding marks on true leaves.

Feeding behaviors on *Leguminosae* were different between plant species. On pea, the beetles started feeding after wandering for 24–36 h, and the number of attacked seedlings increased thereafter (Fig. 1C). However, the feeding marks did not enlarge and were still pinhead holes even after 7 days. This result indicates that pea is a complete non-host as pumpkin and cucumber. On the other hand, the behavior on kidney bean was rather similar to that on *Arabidopsis*. After wandering for 1–3 h, the beetles started feeding and produced tiny feeding marks (Fig. 1C). The consumed area was small at 24 h, but increased gradually up to 5–7 days (Fig. 2E).

3.2. Effects of chemically induced resistance on feeding on tomato, Chinese cabbage and Arabidopsis

The feeding test mentioned above showed that 4-day-old adults feed on Chinese cabbage as well as on tomato at least for a short term. Generally speaking, however, cruciferous plants are non-hosts of the ladybird beetle while solanaceous plants are hosts. To find differences between tomato and Chinese cabbage in reactions to the ladybird beetle, effects of induced resistance were examined. When tomato seedlings treated with BTH, MeJA, or water were infested with 4-day-old adults, no difference was observed in consumed areas among the three treatments (Fig. 3A). Similar results were obtained with the fourth-instar larvae (data not shown). These results indicate that the induced resistance in tomato is ineffective against the ladybird beetle. In other words, the ladybird beetle completely overcomes the induced resistance of the host plant, tomato.

On the other hand, induced resistance in Chinese cabbage showed a drastic effect on the feeding behavior of the ladybird beetle. The MeJA treatment reduced the consumed leaf area prominently, and protected the Chinese cabbage seedlings almost completely against the 4-day-old adults (Fig. 3B). Similar results were obtained with the fourth-instar larvae (data not shown). Furthermore, the MeJA treatment reduced the consumed area in *Arabidopsis* (Fig. 3C). These results suggest that induced resistance via the jasmonic acid pathway is effective against the ladybird beetle in Chinese cabbage and *Arabidopsis*. Interestingly, the BTH treatments showed no effects in

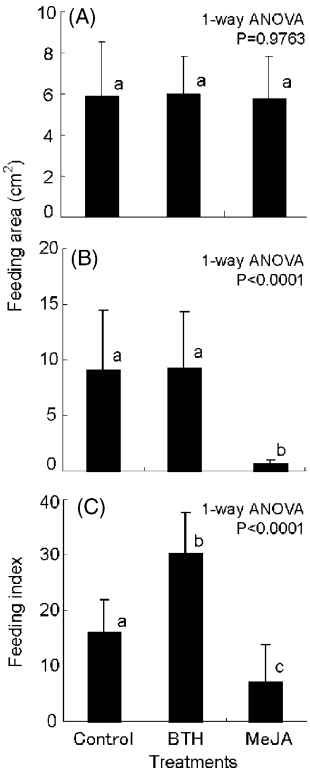

Fig. 3. Effects of induced resistance on consumed area in tomato (A), Chinese cabbage (B) and *Arabidopsis* (C) infested with 4-day-old adults of *E. vigintioctopunctata*. In tomato and Chinese cabbage, one and three insects, respectively, were released to each pot with one seedling that had been treated with BTH, MeJA and water (Cont), and the consumed area was estimated 24 h after release. In *Arabidopsis*, three insects were released to each pot with three seedlings that had been treated with those chemicals, and the consumed area was estimated by feeding indices (see the text) 7 days after release. Bars indicate standard deviations from 10 replications. Means with the same letter are not significantly different in Tukey–Kramer test ($P < 0.05$) after 1-way ANOVA.

Chinese cabbage (Fig. 3B) but showed an adverse effect in *Arabidopsis* (Fig. 3C).

3.3. Effects of the MeJA treatment and the feeding on the expression of MeJA-inducible genes in tomato and Chinese cabbage

The feeding behavior of the ladybird beetle on the MeJA-treated tomato (Fig. 3) raised a question whether the feeding or the MeJA treatment actually activated the pathway of induced resistance. To address this question, total RNA was extracted from MeJA- or water-treated leaves of tomato and Chinese cabbage with or without feeding, and subjected to RT-PCR analysis for the expression of genes involved in the JA pathway-mediated induced resistance. In tomato, the *PINII* gene encoding proteinase inhibitor II was chosen as a representative of such genes because this inhibitor is among the best-studied proteins synthesized in response to JA or MeJA [32,33]. In Chinese cabbage, an ortholog of proteinase inhibitor genes has been reported [34], but there has been no

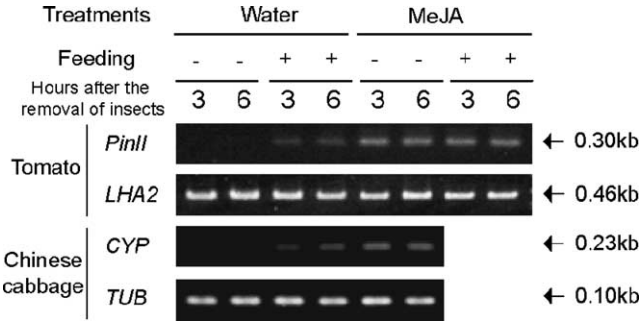

Fig. 4. Effects of the MeJA treatment and feeding by 4-day-old adults of *E. vigintioctopunctata* on the expression of the proteinase inhibitor gene (*PINII*) in tomato and the cytochrome P450 gene (*CYP*) in Chinese cabbage. The expression of these genes was detected by RT-PCR. As a control, the H^+-ATPase gene (*LHA2*) and the putative tubulin gene (*TUB*) were monitored in tomato and Chinese cabbage, respectively.

evidence showing its involvement in the induced resistance via the JA pathway. Therefore, *CYP79B1*, a cytochrome P450 (*CYP*) gene [35], was chosen as a marker for Chinese cabbage. This gene has been shown to be activated in response to MeJA [35]. An amplicon from *PINII* with the expected size (0.30-kb) was obtained from MeJA-treated tomato leaves whether the beetle was released or not (Fig. 4). This result suggests that our MeJA treatment actually activated the JA-dependent pathway in tomato. Similarly, the expression of *CYP* was detected in the MeJA-treated Chinese cabbage (Fig. 4). The effect of feeding on the *CYP* expression was difficult to evaluate in the MeJA-treated Chinese cabbage because the MeJA treatment suppressed the feeding almost completely (Fig. 3B).

The expression of *PINII* and *CYP* was also induced by the feeding alone as shown in the water-treated leaves (Fig. 4) although the level of expression appeared to be low at the early stage of infestation. The plasma membrane H^+-ATPase gene (*LHA2*) in tomato and putative tubulin gene (*TUB*) in Chinese cabbage were constitutively expressed irrespective of the presence or absence of MeJA treatments or feeding (Fig. 4).

3.4. Effects of the mutations in SA/JA pathways on feeding in Arabidopsis

In the feeding tests mentioned above, there remained a possibility that the chemicals themselves had some effects on the feeding behavior of the ladybird beetle. Therefore, we employed a transgenic line and mutants that were defective in the JA or SA pathways. When infested with 4-day-old adults of the ladybird beetle, *fad7-2*, *fad7-1/8-1* and *jar1-1*, defective in the JA pathway, were less resistant than the wild type (Fig. 5). On the contrary, *eds16-1* and *nahG*, defective in the SA pathway, showed enhanced resistance to the ladybird beetle. These results support the idea that induced resistance via the JA pathway is effective against the ladybird beetle in *Arabidopsis*.

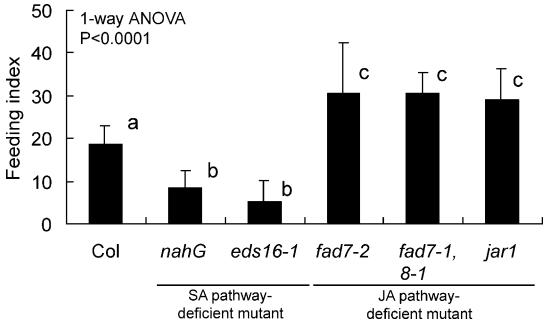

Fig. 5. Effects of the mutations in the SA and JA pathways on consumed area in *Arabidopsis thaliana* infested with 4-day-old adults of *E. vigintioctopunctata*. Three insects were released to each pot with three seedlings of ecotype Columbia (Col) or its mutants. Seven days after release, consumed area was estimated by feeding indices (see the text). Bars indicate standard deviations from 10 replications. Means with the same letter are not significantly different in Tukey–Kramer test ($P < 0.05$) after 1-way ANOVA.

4. Discussion

The ladybird beetle is known as a pest insect of solanaceous plants. Plant species reported as its host plant so far include *Lycopersicon esculentum*, *S. melongena*, *S. tuberosum*, *S. photeinocarpum* and *S. torvum* [26]. We expected that the ladybird beetle should recognize the host plants quickly after their contact to the plant surface, and therefore, that hosts and non-hosts could be distinguished by examining the time taken to start feeding. Actually, the beetles started feeding on solanaceous plants within 5–15 min (Fig. 1A), whereas they began to wander on the other plant families after their first contact to the leaf surface. These results suggest that the first step for the ladybird beetle to attack their hosts is to recognize the hosts probably via preformed compound(s) as has been documented in other phytophagous insects [21].

The second step should be to overcome the constitutive resistance, which consists of unsuitable physical traits, preformed toxic compounds and so on [21]. The ladybird beetle continued feeding on tomato and eggplant leaves and caused destructive damage to the seedlings by 24 h, suggesting that it completely overcame the constitutive resistance of these host plants. One may argue that the second step is indistinguishable from the first step because the host range of phytophagous insects may be determined by the balance of stimulatory and deterrent compounds [22]. However, we suggest that the two steps should be conceptually discriminated with two reasons. First, the ladybird beetle started feeding on red pepper as early as on tomato and eggplant (Fig. 1A) but did not continue feeding vigorously (Fig. 2B). This result indicates that the ladybird beetle recognizes red pepper as a member of host plants but does not overcome its constitutive resistance. Red pepper may be regarded as a ‘‘resistant host’’. Second, the ladybird beetle did not start feeding on the cruciferous plants so early as on tomato and eggplant (Fig. 1B), but once started, it continued feeding vigorously. The most remarkable was Chinese cabbage, whose damage by 24 h was comparable to those of tomato (Fig. 2A and C). These results suggest that the ladybird beetle does not recognize the cruciferous plants as their hosts, but overcomes their constitutive resistance.

The third step may be to overcome the induced resistance. MeJA showed a remarkable effect on the feeding on Chinese cabbage and *Arabidopsis* (Fig. 3). It has been reported that the JA signaling pathway plays an essential role in insect resistance of *Arabidopsis* [19,36]. Our results shown here suggest that the JA signaling pathway also mediates defense reactions of the cruciferous plants against the ladybird beetle. Interestingly, the BTH treatment increased the consumed area in *Arabidopsis* (Fig. 3). Furthermore, the mutants defective in the SA signaling pathway showed enhanced resistance against the ladybird beetle (Fig. 5). Similar results have been reported in feeding tests with other insects [37,38]. Cui et al. [37] showed that *Arabidopsis* mutants compromised in the SA-mediated pathway exhibited reduced levels of feeding by *Trichoplusia ni* but that mutants with elevated levels of SA exhibited enhanced levels of *T. ni* feeding. These findings may be explained by the antagonistic cross-talk between the SA-mediated pathway and the JA-mediated pathway [39,40]. It is not clear why such cross-talk was not detected in Chinese cabbage (Fig. 3B).

By contrast, the MeJA treatment showed no effects on the feeding on tomato (Fig. 3A), indicating that the ladybird beetle have completely adapted to the induced resistance in tomato. Two possible mechanisms have been reported for adaptation; one is the suppression of the induction of resistance and the other is the acquisition of tolerance against the final deterrent compounds. Kahl et al. [41] found that ethylene induced by feeding of *M. sexta* suppressed JA-induced nicotine accumulation in *Nicotiana attenuta*. *M. sexta* may have adapted to its host through this strategy. The other mechanism for adaptation was described in *Leptinotarsa decemlineata*, *Spodoptera exigua*, *P. rapae* and *P. napi*. These insects produced insensitive proteinases to adapt to proteinase inhibitors induced in plant [42,43]. When tomato leaves were infested with the ladybird beetle, the JA-inducible *PINII* gene was activated (Fig. 4). This result suggests that the ladybird beetle may adapt to the induced resistance of tomato not via the suppression of the induction but probably via the acquisition of tolerance to final inhibitory products.

In Chinese cabbage, the ladybird beetle caused severe damage in the early stage of infestation (Fig. 2C) in spite of the activation of the P450 gene (Fig. 4). It may seem curious because MeJA, the inducer of the JA pathway, suppressed the feeding almost completely (Fig. 3B). We used the P450 gene as a marker of induced resistance in Chinese cabbage because there were no other Chinese cabbage genes that were reported to be activated by MeJA. However, the P450 gene may not be an appropriate marker for the activation of the JA pathway because it is also activated by BTH [35].

Alternatively, the JA pathway may be actually activated in the early stage of feeding, but the amount of final products with inhibitory effects may not be enough to prevent the feeding.

Induced resistance has been divided into two types; one is expressed locally at the site of primary inoculation and the other is expressed systemically in tissues remotely located from the initial treatment [1]. In the present experimental system, it is difficult to differentiate the former type of induced resistance from the constitutive resistance because it becomes effective immediately after the primary infection. On the other hand, the latter, called systemic acquired resistance (SAR), may not become effective immediately. The chemical treatment in the present study may be interpreted as strongly inducing SAR, which should have been slowly induced in distal, untreated plant parts, so that we can detect it quickly in the leaves with the primary treatment.

The results obtained in the present study are summarized in Fig. 6. The determination of the host range of *E. vigintioctopunctata* involves three steps, i.e., the recognition of host plants, overcoming constitutive resistance and overcoming induced resistance. Here, ''constitutive resistance'' includes the localized induced resistance. The ladybird beetle shows various degrees and types of adaptation to various plant species. In other words, plants hold the three inhibitory steps in various degrees and combinations against the feeding by the ladybird beetle. Cucumber, pumpkin and pea are recognized as non-host plants, so suffer almost no damages (Fig. 6F). Kedney bean is also recognized as a non-host, but suffer some damages probably because its constitutive resistance is imperfect (Fig. 6E). *Arabidopsis* and Chinese cabbage are not recognized as host plants, but once attacked, are damaged under our experimental condition, probably because their constitutive resistance is imperfect or ineffective (Fig. 6D and C). However, their induced resistance is effective to the ladybird beetle (Fig. 6D and C). Red pepper is recognized as a host but is not damaged severely because its constitutive resistance is effective (Fig. 2B). Tomato is severely damaged because the ladybird beetle passes through all the three steps (Fig. 2A).

To utilize Chinese cabbage as a host, the beetle would have to overcome its induced resistance (Fig. 6). This does not mean that overcoming induced resistance is the only one or final requirement for the ladybird beetle to utilize Chinese cabbage as a host. In the present study, the fitness of the ladybird beetle throughout its life cycle has not been evaluated. Also, the feeding test was performed in closed cages in the laboratory. In the field condition, the recognition of host plants could be the most important factor because, if the beetle recognizes a plant as a non-host, it would immediately leave the plant without retrying feeding. However, the factor(s) involved in the host recognition should function as a representative of background information including constitutive resistance and induced resistance. If the ladybird beetle successfully overcomes all the factors involved in the constitutive and induced resistance of, for example, Chinese cabbage, it will then find a trait of Chinese cabbage for recognizing it as a host plant.

Chemical resistance in plant has been considered as being less effective to specialist herbivores than to generalist herbivores [44,45]. This paradigm (''the specialist herbivore paradigm'' [46]) was extended to induced resistance [20,47]. Recently, however, it was reported that induced resistance was significantly effective against some specialist herbivores [48,49]. Agrawal [49] found a variation in the specificity of effects of induced resistance and suggested that

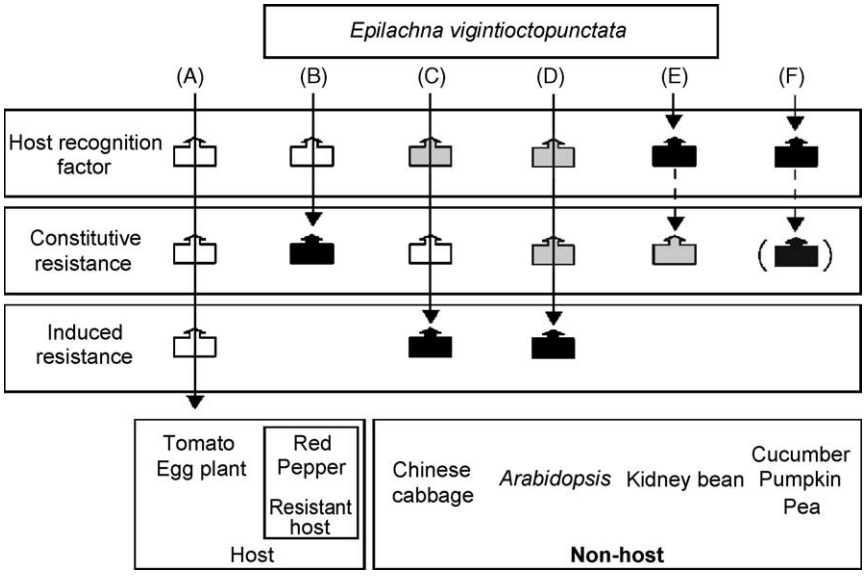

Fig. 6. Mechanism of host-specificity of *E. vigintioctopunctata*. Arrows to the top represent barriers against the ladybird beetle, which were overcome (white), partially overcome (gray), or not overcome (black).

the variation was not associated with diet specialization in the herbivores (i.e., specialists versus generalists). Our present results indicated that the fourth-instar larvae and adults of a specialist herbivore, ladybird beetle, completely adapt to induced resistance in the host plants. Further studies are needed to evaluate the specialist herbivore paradigm from the viewpoint of induced resistance.

The notable finding in the present study is the sharp contrast of the effect of induced resistance between hosts and non-hosts. Recently, Kessler et al. [50] found that the host selection of generalist herbivores is changed by the down-regulation of jasmonate pathway, suggesting that the host range is determined not only by constitutive resistance but also by induced resistance. Our data extend this idea by suggesting that induced resistance is one of the factors that determine host-specificity of the specialist.

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