Effect of leaf age on flight response of a parasitic wasp Cotesia kariyai (Hymenoptera: Braconidae) to a plant-herbivore complex

Maolin Hou,1,2 Junji Takabayashi3,4 and Yooichi Kainoh2,*

1 Key Laboratory of Biocontrol, Chinese Academy of Agricultural Sciences; Beijing 100081, P. R. China
2 Institute of Agriculture and Forestry, University of Tsukuba; Ibaraki 305–8572, Japan
3 Center for Ecological Research, Kyoto University; Kyoto 606–8502, Japan
4 CREST of JST

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Abstract
Effect of leaf age on the flight response of a parasitic wasp, Cotesia kariyai, to a plant-herbivore complex was studied in a wind tunnel. Both young and old leaves infested with host larvae (common armyworms: Mythimna separata) attracted more wasps than their respective uninfested leaves. Preference of the wasps to uninfested young leaves vs. uninfested old leaves was not significantly different in choice experiments. However, when young infested leaves were compared against old infested leaves in choice experiments, the wasps showed a significant preference for the former. In the single stand experiments, % landing response to young infested leaves was not significantly different from the old infested leaves. The fact that attractiveness of infested corn leaves to wasps differs according to leaf age suggests differential allocation of induced indirect defense in the corn plant.

Key words: Mythimna separata; Cotesia kariyai; host-plant complex; leaf age; flight response

INTRODUCTION

It is now widely accepted that plants emit a blend of volatiles in response to herbivory (Takabayashi and Dicke, 1996; Dicke and van Loon, 2000). Such volatiles are called “herbivore-induced plant volatiles (HIPV).” One well-known ecological function of HIPV is to attract carnivorous natural enemies of the inflicting herbivores. Biotic (plant species, cultivars, developmental stage, herbivore species, age, etc.; Takabayashi and Dicke, 1996; Dicke and van Loon, 2000; Shiojiri et al., 2002) and abiotic (light, temperature, humidity, nutrient etc.; Takabayashi et al., 1994; Schmelz et al., 2001, 2003; Gouinguene and Turlings, 2002; Shiojiri et al., 2002) factors are known to alter the release of HIPV in many plants. Such alterations sometimes positively or negatively affect the attractiveness of plants for specialist carnivores.

When the production of HIPV that attracts carnivorous natural enemies of herbivores increases plant fitness, such production is considered to be plant induced indirect defense against herbivores. In such a case, plants have been selected to emit HIPV in the most effective way. Leaf age is one important factor when defending against herbivores, because upper (young) leaves are more important in terms of photosynthesis and competition with other plants in the same habitat than lower (old) leaves. Thus, herbivorous damage in young leaves is more serious than in old leaves. However, little is known about the effects of leaf age on the induced indirect defense except for reports by Takabayashi et al. (1995) and Coleman et al. (1997). Takabayashi et al. (1994) compared the attractiveness of leaf age of cucumber plants infested by two-spotted spider mites, Tetranychus urticae. Young infested leaves attracted the predatory mites Phytoseiulus persimilis, while old infested leaves did not attract the mites. Coleman et al. (1997) also reported a similar phenomenon in a tritrophic system of Brassica oleracea, Pieris brassicae larvae and their parasitoids Cotesia glomerata.

In a tritrophic system consisting of corn plants and the common armyworms Mythimna separata and Cotesia kariyai (a specialist parasitoid of
M. separata larva), infested corn plants attracted wasps only when the inflicting larvae were in the early instars (Takabayashi et al., 1995). Parasitization of young larvae resulted in a decrease in the damage caused by larvae, while parasitization of old larvae did not affect the amount of damage caused. It appears that corn plants attract C. kariyai only when the recruitment is adaptive for the plants. Also, upper young corn leaves were more susceptible than lower old leaves to the larvae (Sato et al., 1983). This may suggest that damaged upper young leaves could be more defensive by attracting C. kariyai females than lower old leaves. Our past studies were carried out using corn seedlings and thus the effects of leaf age have not yet been tested in this tritrophic system. Therefore, we examine the influence of leaf age on the flight response of C. kariyai to a complex of corn plants and M. separata larvae. The allocation of induced indirect defense using HIPV in plants is discussed.

MATERIALS AND METHODS

Plants and insects. In 2001, Mythimna separata was introduced to the laboratory in the University of Tsukuba, from a culture reared at the Center for Ecological Research, Kyoto University. The insects have been reared on artificial diet (Silkmate 2(S), Nosan Corporation) in the laboratory under conditions of 25±1°C, 16L:8D photoregime, 50–70% RH.

C. kariyai is a gregarious endoparasitoid of 2nd to early 6th instars of M. separata caterpillars. In 2001, the wasp was introduced to the laboratory from a stock culture of the Center for Ecological Research, Kyoto University. To maintain the wasp culture, 3rd to 4th instar larvae of M. separata were offered to female wasps for oviposition. Soon after emergence from the host, the wasp larvae spin cocoons. Clusters of cocoons were placed in a plastic cage (15×17×24 cm) for emergence and wet cotton and honey were provided as water and food for the wasps emerging from the cocoons. Mating occurred immediately after emergence of the wasps. Adult wasps were stored in the laboratory under conditions of 15±2°C, 50–70% RH and 16L:8D until used in the experiments. Wasps were used in experiments within a maximum of 7 d. Oviposition-inexperienced females were used to test innate responses.

Potted corn plants (Zea mays L. cv. Nasuhomare) (one plant per pot) were grown in a growth chamber (25±3°C, natural light condition). Six- to 8-week-old plants, ca. 100–140 cm in height, were used for the experiments.

Flight responses of parasitoids to corn plants. The flight responses of female C. kariyai to corn leaves were observed in a wind tunnel (50×50×150 cm; 20–30 cm/s wind velocity; 25±1°C, 50±10% RH and ca. 2,200 lx) (see Fukushima et al., 2001 for details of the wind tunnel). As an odor source, a piece of leaf (hereafter called a leaflet) vertically standing on a small vial (10 ml) with moist cotton wool was placed upwind of the tunnel. In the preference test, two leaflets were placed 12 cm apart from each other upwind of the tunnel. The position of the two leaflets was exchanged every 7–10 bioassays.

C. kariyai females were individually confined in a plastic vial (3 ml) prior to the experiments. A vial was placed on a platform (23 cm high) set 15 cm downwind of the leaf. Then we carefully opened the vial and observed the behavior of the wasps for a maximum of 5 min. Those that did not fly from the vial within 5 min were discarded. The first landing by each wasp on one of the leaflets was recorded as a choice. A wasp landing on a leaflet was immediately removed from the wind tunnel with an insect aspirator. If the wasp did not land on either of the two leaflets within 5 min, it was considered a no-choice result. The odor sources were replaced by new ones every 20–30 trials.

The odor sources were prepared as follows. Ten 2nd-instar M. separata larvae were introduced to either the second or fifth corn leaf (Fig. 1, hereafter called young leaf and old leaf, respectively) for 14 h (3L:8D-3L in 16L:8D light condition, 25±3°C, 40–60% RH). When the feeding area was approximately 40% of the total leaf area, the larvae on the leaf were removed, their associated products such as feces were carefully removed with a fine brush and the leaf was washed with tap water. During the observations of flight responses, the same number of M. separata larvae (one or two) were placed on the infested leaves to create a host-plant complex. Because the height of the wind tunnel was 50 cm, the tips of both the second and the fifth leaf were cut at ca. 40 cm. After this cutting, the damaged areas of the young leaflet and the old leaflet were confirmed to be close. The leaflet was
set vertically in a small plastic vial filled with moist cotton wool. The following comparisons were tested: 1) the damaged young (second) leaflet vs. the undamaged young leaflet, 2) the damaged old (fifth) leaflet vs. the undamaged old leaflet, and 3) the damaged young leaflet vs. the damaged old leaflet. Further, single stand experiments were separately tested to observe responses of the wasps to infested young or old leaflets. If the wasp did not land on the leaflet within 5 min, it was considered a no-response result.

Significance of preferences in dual-choice tests was analyzed using a binominal test under the null hypothesis that wasps had a 1 : 1 distribution over the two groups of plants. The number of wasps that did not make a choice was ignored. The results of the single stand flight experiments were compared with a $\chi^2$ test.

**RESULTS AND DISCUSSION**

At first, we tested whether both a young leaflet and an old leaflet became more attractive when infested by the host larvae. Compared to the young uninfested leaflet, the wasps showed significant flight preference for the young infested leaflet (Fig. 2A, binominal test; $p=0.0041$). Likewise, the wasps showed significant flight preference for the old infested leaflet over the old uninfested leaflet (Fig. 2B, binominal test; $p=0.0118$). Takabayashi et al. (1995) reported that young corn plants (ca. 30 cm high) (cv. Royal dent) infested by the host larvae of early instars (second to fourth instar) attracted *C. kariyai*. The present data showed that even corn plants (cv. Nasuhomare) of 100 to 140 cm in height attracted the wasps irrespective of their leaf age, when infested by early instars of *M. separata* larvae.

Further, attractiveness of a young infested leaflet and an old infested leaflet were compared. Interestingly, the wasps showed significant preference to the young infested leaflet over the old infested leaflet (Fig. 3A, binominal test; $p=0.0002$). As the attractiveness of the uninfested young leaflet and the uninfested old leaflet were not significantly different (Fig. 3B, binominal test; $p=0.6875$), the difference in Fig. 3A can be explained by the different attractiveness of HIPV emitted from two types of leaflets. The percent landing response to the uninfested leaflet was very low in Fig. 3B while the percent landing response to the infested leaflet was higher than the uninfested one in Fig. 3A, which
also suggests that the wasps respond to HIPV. Single stand experiments (Table 1) showed that the attractiveness of the young infested leaflet was not significantly higher than that of the old infested leaflet ($\chi^2$ test; $p=0.100$), suggesting that the wasps' preference for a young infested leaf to an old one in choice experiments is the difference in relative attractiveness.

In a tritrophic system of corn plants, *M. separata* larvae and *C. kariyai*, the emission of *C. kariyai* attractants by corn plants infested with young larvae is considered to be adaptive for the plants (Takabayashi et al., 1995). The younger larvae show a strong tendency to conceal themselves in the cylindrical part folded by upper young leaves in the daytime (Sato et al., 1983). This observation indicates that the wasps that were attracted to the young leaves in the daytime could easily detect hosts in nearby folding leaves. Thus, the higher attractiveness of young corn leaves to old corn leaves could be a defense strategy of the corn plant to recruit *C. kariyai* to the part in which their host larvae are most likely to be. In addition, young leaves are more important than old leaves in terms of photosynthesis, growth and reproduction.

When *C. kariyai* females attack late-instar host larvae, they often receive counterattack from the larvae, but they seldom receive it from young host larvae (unpublished data). This may in part explain why the wasps choose young leaves infested by young larvae. Also, later-stage armyworms are more likely to already be parasitized by conspecific or heterospecific parasitoids (Takabayashi et al., 1995). In such a competition situation, searching for a young armyworm should be advantageous for *C. kariyai*.

An unanswered question is whether the difference between the attractiveness of young leaves and that of old leaves is due to qualitative or quantitative differences in HIPV. Takabayashi et al. (1995) compared the attractiveness of different age cucumber leaves infested with spider mites *T. urticae* and suggested that the difference in attractiveness was not due to quantitative differences in the attractants between young and old leaves, since both young and old leaves emitted equivalent amounts of compounds to attract *P. persimilis*. They speculated that the presence of the volatile compounds in the headspace of old infested leaves

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**Table 1. Flight responses of *C. kariyai* to a young infested leaflet and to an old infested leaflet when offered separately in a wind tunnel**

<table>
<thead>
<tr>
<th>Odor source</th>
<th>Number of wasps released</th>
<th>Number of wasps landing on infested leaflet (%)</th>
<th>No response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young infested leaflet</td>
<td>14</td>
<td>8 (57)</td>
<td>6 (43)</td>
</tr>
<tr>
<td>Old infested leaflet</td>
<td>29</td>
<td>9 (31)</td>
<td>20 (69)</td>
</tr>
</tbody>
</table>

* Chi-square test, $p>0.05$. 

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Fig. 3. Flight response of *C. kariyai* to young vs. old corn leaflets in a wind tunnel. A: infested leaflets ($n=65$), B: uninfested leaflets ($n=49$). ***$p<0.001$, NS: $0.05<p$ (binominal test).

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M. Hou et al.
Effect of Leaf Age on Flight Response of Parasitoid

In this study, the larvae infested young leaves and old leaves of the same plants. Under this condition, the order of attractiveness of corn leaves was: young infested leaves > old infested leaves > young uninfested leaves = old uninfested leaves. Therefore, we can hypothesize that corn plants defend themselves against *M. separata* by emitting HIPV, but they allocate the defense more to their young parts in which younger larvae are most likely to be hiding. In fact, damaged leaves released more HIPV than other undamaged leaves in the same corn plant (Takabayashi, unpublished). Clarification of the detailed patterns of relative attractiveness of young and old leaves in corn plants as well as other host food plants of *M. separata* is needed to specify the allocation of the induced indirect defense against *M. separata*.

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REFERENCES


