

DEVELOPMENTAL STAGE OF HERBIVORE *Pseudaletia separata* AFFECTS PRODUCTION OF HERBIVORE-INDUCED SYNOMONE BY CORN PLANTS

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Abstract—The female parasitic wasp *Cotesia kariyai* discriminated between the volatiles of corn leaves infested by younger host larvae *Pseudaletia separata* (first to fourth instar) and uninfested leaves in a Y-tube olfactometer; the wasps were attracted to the infested leaves. In contrast, when corn plants were infested by the later stages (fifth and sixth instar) of the armyworm, the wasps did not distinguish between infested corn leaves and uninfested corn leaves in the olfactometer. Mechanically damaged leaves were no more attractive than undamaged leaves, and host larvae or their feces were not attractive to the parasitoid. Through chemical analysis, the herbivore-induced plant volatiles were identified in the headspace of infested corn leaves. The herbivore-induced volatiles (HIVs) constituted a larger proportion of the headspace of corn leaves infested by early instar armyworms than of corn leaves infested by late instar armyworms. Application of third-instar larval regurgitant onto artificially damaged sites of leaves resulted in emission of parasitoid attractants from the leaf, whereas leaves treated with sixth-instar regurgitant did not. The function of this herbivore-stage related specificity of herbivore-induced synomones is discussed in a tritrophic context.

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Key Words—*Pseudaletia separata*, *Cotesia kariyai*, *Apanteles*, herbivore-induced synomone, Y-tube olfactometer, leaf volatiles, terpenoids, indole, regurgitant, herbivore developmental stage specificity.

INTRODUCTION

Plants defend themselves against herbivores in various ways. These may comprise plant characteristics that affect herbivore performance directly or indirectly through natural enemies of the herbivores (Price et al., 1980; Price, 1981; Rhoades and Cates, 1976; Rhoades, 1985; Dicke and Sabelis, 1988a). These defense mechanisms may function constitutively (Rhoades and Cates, 1976; Price, 1981) or they may be induced by herbivory (Tallamy and Raupp, 1991; Dicke et al., 1990b; Dicke and Sabelis, 1988a; Takabayashi et al., 1994; Turlings et al., 1990).

In this study, we have investigated induced indirect defense of plants, involving the attraction of natural enemies to plants that are infested by herbivores. Plants that are infested by herbivores may emit so-called "herbivore-induced synomones" (Dicke and Sabelis, 1988b; Vet and Dicke, 1992). These are chemicals that are produced by the plant in response to damage done by specific herbivores. The chemicals are not emitted, or are only emitted in trace amounts, upon mechanical damage. Natural enemies of the herbivore use these chemicals in long-/short-range herbivore location (Dicke et al., 1990a,b; Sato, 1979; Turlings et al., 1990). Herbivore-induced synomones may be very specific stimuli: they may be specific for the herbivore species, the plant species, and/or the plant cultivar (Dicke et al., 1990b; Takabayashi and Dicke, 1993; Takabayashi et al., 1994; Turlings et al., 1993; Dicke, 1994). The value of this specificity has been discussed by Vet and Dicke (1992), who hypothesized that natural enemies of herbivores are faced with a reliability-detectability problem during foraging for their herbivore victims. Contact kairomones left by herbivores are the most reliable in indicating herbivore presence and identity, but they are difficult to detect from a distance (high reliability and low detectability). On the other hand, constitutively emitted plant volatile synomones are more detectable at a distance but less reliable in indicating herbivore presence and identity (low reliability and high detectability). Herbivore-induced synomones may combine the high detectability of plant volatiles with a high reliability in indicating herbivore presence. For plants, the specificity of the herbivore-induced synomones may be an important characteristic because it could ensure that the right natural enemy species is attracted.

Moreover, many parasitoid species are more specific and can attack only a limited range of host developmental stages within a species (Waage and Great-

head, 1986). If parasitized herbivores inflict less total damage to a plant than unparasitized herbivores, attraction of the natural enemies during an early phase of herbivore infestation would be beneficial. Yet, if quantities of herbivore-induced synomones are correlated to the extent of herbivory, the natural-enemy attracting stimuli would be expected to be most abundant during the later phase of herbivory. Thus, we were primarily interested in the question of whether the emission of herbivore-induced synomones is specific for the herbivore instars inflicting the damage. In this paper, we describe the effect of herbivore instars that damage plants on the attraction of natural enemies. This is done for a tritrophic system consisting of corn plants, the armyworm *Pseudaletia separata* Walker (Lepidoptera: Noctuidae) and the gregarious parasitic wasp *Cotesia* (= *Apanteles*) *kariyai* Watanabe (Hymenoptera: Braconidae).

Cotesia kariyai is one of the dominant parasitoid species of the common armyworm *P. separata* (Sato, 1988; Hirai, 1984). The wasps search for and attack host larvae actively during the day, while nocturnal host larvae hide in the ground or in the sheath of the host plant (Sato et al., 1983; Kanda, 1988). An intriguing question then is how the diurnal parasitoids find the nocturnal host larvae (Sato et al., 1983). It has been demonstrated that feces, feeding traces, and exuviae of the armyworm on an infested corn plant elicit strong behavioral responses in the parasitoid females upon contact with their antennae: the wasps are arrested and show area-restricted search patterns (Takabayashi et al., 1985; Takabayashi and Takahashi, 1989). This behavior increases the chances of finding a host that hides in the sheath of the infested corn plant or in the ground where host feces are scattered. The kairomones that mediate these behavioral responses were identified to be 2,5-dialkyltetrahydrofuran homologs that were present in host feces, feeding traces, and exuviae (Takabayashi and Takahashi, 1986a,b). Upon antennal contact with a *P. separata* larva, the wasp immediately inserts her ovipositor and lays a clutch of eggs.

The contact kairomones are highly reliable cues of host presence, but the question as to how the parasitoids locate the infested plants from a distance remains unanswered. *Cotesia kariyai* females are known to respond to volatiles from uninfested corn plants (Takabayashi et al., 1991a), but so far it is not known whether they are able to discriminate between uninfested plants and *P. separata*-infested plants. Other *Cotesia* species are known to distinguish uninfested plants from plants infested by their hosts on the basis of volatile infochemicals (Sato, 1979; Turlings et al., 1990; Kaiser and Cardé, 1992; Steinberg et al., 1992, 1993). Here we show that *C. kariyai* also has this ability. In addition we show that in *Cotesia kariyai* this discrimination on the basis of herbivore-induced synomones is affected by the stage of the herbivores infesting the plant.

METHODS AND MATERIALS

Insects

Pseudaletia separata has six larval instars. It takes about 15 days for the newly hatched larva to reach the pupal stage at 25°C, 17L:7D, and 50–70% relative humidity, and about three days at 25°C, 17L:7D for the adult female to initiate oviposition. The newly emerged adult female has a tendency to make a long-distance flight before the first oviposition. Females lay a cluster of ca. 100–300 eggs in one oviposition site.

Pseudaletia separata was introduced to the laboratory from a culture reared at the National Institute of Sericultural and Entomological Science in Tsukuba, Ibaraki, Japan in 1990. The insects were reared on corn leaves or artificial diet (INSECTA-LF: Nihon Nousan Kogyo, Ltd.) under conditions of 25 ± 3°C, 17L:7D, 50–70% relative humidity.

Cotesia kariyai is one of the dominant monophagous parasitoids of the common armyworm *Pseudaletia separata* in Japan (Hirai, 1984). The wasp is a gregarious endoparasitoid of second-instar to second-day sixth-instar larvae at 25°C, 17L:7D (Sato and Tanaka, 1984). The wasp cannot attack first-instar larva, probably because their small size hampers ovipositor insertion. When the wasp oviposits in a late sixth-instar host, the host pupates and the wasp offspring die.

Cotesia kariyai was introduced to the laboratory from a stock culture reared at the National Institute of Sericultural and Entomological Science in 1990. To maintain the wasp culture, fourth- or fifth-instar *P. separata* larvae were offered to female wasps for oviposition. It took about 10 days for the next generation of the wasp to egress at 25°C, 17L:7D. Egressed wasp larvae immediately made cocoons. The cluster of cocoons was collected and placed in a test tube (22 mm diam. × 200 mm long) with a small piece of paper towel moistened with 50% aqueous honey solution as food. Mating generally occurs right after emergence of the wasps. Adult wasps were stored in the dark at 10°C until used (not longer than five days). Naive *C. kariyai* were used in this study.

Corn (*Zea mays* L. vc. Royal Dent) was grown in a greenhouse (25 ± 5°C, 16L:8D). Seedlings of ca. 30–50 cm height were used for the experiments.

Bioassay

A Y-tube olfactometer was used to study the response of the wasps toward leaf volatiles (for details of the olfactometer setup, see Takabayashi et al., 1991b). The number of wasps that walked into the sample cage and the control cage was counted. Sign-tests determined whether the results differed significantly from a 50:50 distribution of the wasps (null hypothesis).

Olfactory Response of C. Kariyai to Corn Leaf Volatiles

The attraction of corn leaves infested by *P. separata* larva(e) of different developmental stages was tested. Infested leaves vs. uninfested leaves were compared in the Y-tube olfactometer.

Corn Plants Infested by Young Armyworms. Five intact corn plants (ca. 30 cm height) in a clear plastic cage (50 × 50 × 50 cm) were offered to five second-instar larvae in the climate room (25 ± 3°C, 17:7D, 50–70% relative humidity). Five days later, the armyworms were third instar. The host larvae and feces were then carefully removed from the plants with a brush.

Corn Plants Infested by Old Armyworms. Five intact corn plants (ca. 30 cm height) in a clear plastic cage (50 × 50 × 50 cm) were offered to five fourth-instar larvae in a climate room (25 ± 3°C, 17L:7D, 50–70% relative humidity). Five days later, the armyworms were fifth- to sixth-instar. The larvae and feces were then carefully removed from the infested plant with a brush.

Artificially Damaged Corn Leaves. Ten leaves of an uninfested corn plant (ca. 30 cm) were damaged slightly with sandpaper (ca. 30% of each leaf area) to imitate the young larva's infestation and with scissors (ca. 30% of each leaf area) to imitate the old larva's infestation. One hour later, the leaves were used for the bioassay.

Corn Plants Infested by Armyworms of Each Instar. Infested corn plants were further classified according to the instar of the armyworm that fed on them. Three corn plants were offered to armyworms of each instar during 2–6 hr, at 25 ± 3°C, 50–70% relative humidity. The number of armyworms offered and the infested area of each infested leaf are listed in Table 1.

Imitation of Caterpillar Damage on Corn Leaves. To imitate infestation by armyworm caterpillars, the corn leaf surface (ca. 30%) was damaged artificially with sand paper and then the regurgitant (5 larvae equivalent) of third-instar larvae or sixth-instar larvae was applied to the damaged part of the corn leaf.

TABLE 1. CORN LEAF SAMPLES USED FOR BIOASSAY AND CHEMICAL ANALYSIS

Infested by	Damaged leaf area (%)	Armyworms (N)	Bioassay ^a	Chemical analysis ^a
First instar	< 10	150	+	+
Second instar	< 10	100	+	+
Third instar	ca. 10	40	+	+
Fourth instar	ca. 40	10	+	–
Fifth instar	ca. 50	5	+	–
Sixth instar	ca. 50	3	+	+

^a + = tested, – = not tested.

To collect the one equivalent regurgitant, the host was gently squeezed and the regurgitant was rapidly collected with 5- μ l glass capillary tubes. Four leaves were treated. Six hours after treatment, the attraction of the treated leaves vs. undamaged leaves was compared in an olfactometer.

Chemical Analysis of Corn Leaf Volatiles

Infested corn leaves (as listed in Table 1), artificially damaged corn leaves, and uninfested corn leaves were used for chemical analysis. The headspace volatiles of each sample (50–100 g plant material in a 2-liter glass bottle) were collected on Tenax-TA for 30–60 min. The methods used to collect the volatiles of corn leaves were the same as previously reported (Takabayashi et al., 1991b).

The trapped headspace volatiles were subsequently analyzed with a gas chromatograph–mass spectrometer (GC-MS: VG MM7070F) of which the gas chromatograph was equipped with a thermal desorption cold trap injector (TCT, Chrompack). The GC conditions were: a Supelcowax 10 capillary column (60 m, 0.25 mm ID, 0.25- μ m film thickness) and the oven temperature programmed from 40°C (4 min hold) to 100°C at a rate of 2°C/min, and then to 270°C at a rate of 6°C/min. The TCT conditions were: thermodesorption at 250°C during 10 min, while at the same time the cold trap was maintained at a temperature of –100°C. The analysis started by fast heating of the cold trap to 220°C. The mass spectrometer was working in the 70 eV electron impact (EI) ionization mode and scanning from 300 to 24 amu at a rate of 1 decade/sec.

The chemical structure of each compound was elucidated by comparison of the mass spectra with those of authentic chemical samples or by comparison with data in the mass spectra library of the Department of Organic Chemistry, Wageningen Agricultural University.

RESULTS

Effect of Armyworm Infestation on Response of Parasitoid to Corn Leaf Volatiles. Naive wasps preferred corn leaves infested by young larvae to uninfested leaves in the olfactometer (Figure 1). However, the wasps did not respond to larvae or their feces, isolated from corn leaves infested by young larvae when compared with clean air in the olfactometer (Figure 1).

The naive wasps did not prefer the odor of corn leaves infested by late-instar armyworms over that of uninfested leaves (Figure 1). The wasps also did not respond to isolated larvae or their feces when compared with clean air in the olfactometer (Figure 1).

The wasps showed no preference for leaves artificially damaged with sandpaper over undamaged leaves, or leaves artificially damaged with scissors over undamaged leaves (Figure 1).

Effect of Developmental Stage of Armyworm on Attractiveness of Infested Plant to Wasps. Corn leaves with ca. 10% (area) infestation by first-, second-, or third-instar host larvae attracted wasps more than uninfested leaves (Figure 2). Leaves with ca. 40% (area) infestation by fourth-instar larvae were also preferred by the wasp (Figure 2). However, when 50% of the area of corn leaves was infested by fifth- or sixth-instar larvae, they were not preferred by the wasp (Figure 2).

The six experiments in Figure 2 were also compared using the *G* test (Sokal and Rohlf, 1981). The attraction of leaves infested by first instars, second instars, third instars, and fourth instars were not significantly different. The attraction

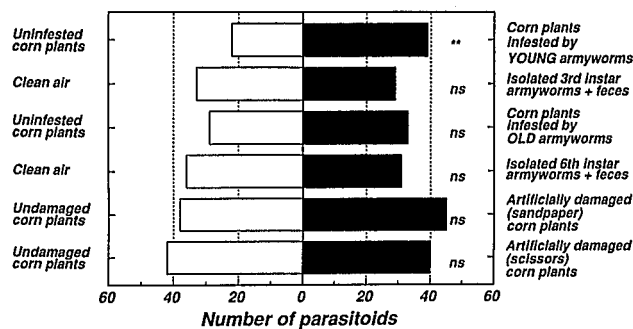


FIG. 1. Olfactory response of *Cotesia kariyai* to infested/uninfested corn plants, and armyworms plus feces. ** 0.01 > *P* > 0.001; ns: not significantly different by sign test.

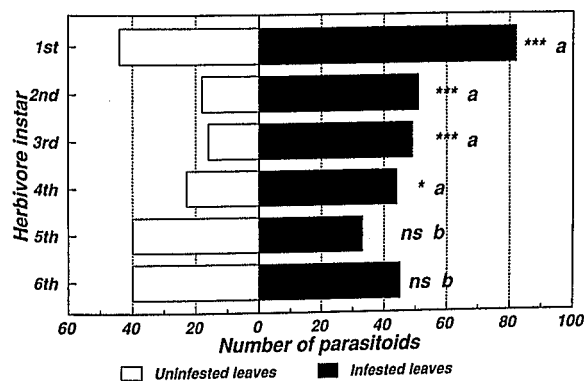


FIG. 2. Olfactory response of *Cotesia kariyai* to corn leaves infested by first- to sixth-instar armyworms separately vs. uninfested leaves. * 0.05 > *P* > 0.01; *** 0.001 > *P*; ns: not significantly different by sign test. Columns that are followed by the same letter do not differ significantly from each other by *G* test (*P* < 0.05).

TABLE 2. RELATIVE AMOUNTS OF COMPOUNDS FOUND IN HEADSPACE OF UNINFESTED, ARTIFICIALLY DAMAGED, AND INFESTED LEAVES^a

Compounds	Host instar				
	UD ^b	ADL ^c	1st/ 2nd ^d	3rd	6th
Aldehydes					
Pentanal		0.5			
2-Pentenal		0.7			1.0
Hexanal	8.6	5.0			9.2
2-Hexenals (E/Z)		4.9	4.4	3.2	16.6
Heptanal	5.6	0.4			
Alcohols					
(Z)-3-Hexen-1-ol	8.4	23.5	2.0	1.5	8.4
1-Pentanol	tr ^e	0.2	0.1		
1-Hexanol		0.7			
1-Octanol	3.1		0.4		
Ketones					
4-Methyl-2-pentanone	3.8	1.1			
3-pentanone		1.0	0.5	0.5	4.1
1-Pentene-3-one		1.5	0.2	0.6	5.0
6-Methyl-5-heptene-2-one		0.8			
2-Nonanone	3.0	0.3			
1-Octanone	0.7				
Acetates					
1-Butyl acetate	3.8	0.4	0.1		
(Z)-3-Hexen-1-yl acetate	31.8	38.3	5.5	5.4	19.7
1-Hexyl acetate		1.1	0.2	0.1	2.1
Terpenoids					
α -Pinene	5.3	15.7			3.2
β -Pinene	3.3	0.6			
Myrcene	4.2	0.2	0.9	1.2	1.8
Limonene	9.8	1.6	0.1	0.2	4.9
Linalool	3.0	0.6	3.5	16.0	8.3
Others					
Methyl salicylate	3.4	0.4			0.3
Total (%)	97.8	99.5	17.9	28.7	84.6
Total peak area/g leaf	218.3	850.5	184.5	203.9	430.3

^aNumber of replications: 2.^bUD: undamaged leaves.^cADL: artificially damaged leaves.^dData of leaves infested by first and second instars were combined.^etr: less than 0.1%

TABLE 3. RELATIVE AMOUNTS OF COMPOUNDS FOUND IN HEADSPACE OF INFESTED LEAVES BUT NOT (OR FOUND IN TRACE AMOUNTS) IN UNINFESTED LEAF HEADSPACE^a

Compounds	Host instar				
	UD	ADL	1st/ 2nd	3rd	6th
Aldehydes					
2-Methyl-2-propenal				0.1	
3-Methylbutanal				tr	
2-Pentenal				0.1	
Alcohol					
(<i>E</i>)-2-Hexen-1-ol				0.1	
2-Methyl-1-propanol			0.4	0.5	1.2
Ketones					
3-Hydroxy-2-butanone			3.5		7.4
Acetates					
1-Pentene-3-ol acetate				0.4	
Isopentyl acetate			0.1	0.2	
2-Methylbutyl acetate			0.6	0.2	
(<i>E</i>)-3-Hexen-1-yl acetate			0.4		
2-Hexen-1-yl acetate				0.9	
Benzyl acetate			0.1		
Geranyl acetate			0.3	1.4	
2-Phenylethyl acetate			1.3		
Terpenoids					
(3 <i>E</i>)-4,8-Dimethyl-1,3,7-nonatriene	2.1	0.4	12.3	22.6	3.4
(3 <i>Z</i>)-4,8-Dimethyl-1,3,7-nonatriene			1.0	0.9	
α -Copaene			1.1	0.4	
Linalool oxide				0.3	
(<i>E</i>)- α -Bergamotene			12.0	7.6	
β -Caryophyllene			1.3		
(<i>E</i>)- β -Farnesene			32.3	18.2	
β -Sesquiphellandrene			0.5		
(3 <i>E</i> , 7 <i>E</i>)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene			1.3	1.0	
unknown terpenoid			0.4	0.9	
Nitriles					
2-Methylpropanenitrile			0.3	0.2	
2-Methylbutanenitrile			0.2	tr	
Oximes					
Butanal <i>O</i> -methyloxime			4.4		
2-Methylbutanal <i>O</i> -methyloxime				0.2	
2-Methylpropanal <i>O</i> -methyloxime			1.2	1.9	
Other					
Indole			7.0	13.0	3.2
Total (%)	2.1	0.4	82.0	71.1	15.2
Total peak area/g leaf	2.6	34.2	846.2	928.8	77.3

^aSee footnotes to Table 2.

of leaves infested by fifth instars and sixth instars was also not significantly different.

Mechanism of Production of Parasitoid Attractants. Leaves treated with third-instar regurgitant on a mechanical wound (five larvae equivalent per leaf) attracted wasps more than untreated leaves (Figure 3). In contrast, leaves treated with sixth-instar regurgitant (five larvae equivalent per leaf) did not attract wasps more than the untreated leaves (Figure 3). A 20-larvae equivalent of regurgitant of third- or sixth-instar larvae impregnated into a filter paper (2 × 2 cm) did not attract wasps (Figure 3).

Chemical Analysis. The main volatile compounds from uninfested corn leaves were hexanal, (*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, and limonene (Table 2). There were many compounds that were found in the volatiles of leaves infested by armyworms that were not detected or detected in only trace amounts in uninfested leaf volatiles (Table 3).

The herbivore-induced plant chemicals in the headspace of corn leaves infested by first- to second- and third-instar larvae were similar, and their combined amounts comprised ca. 70% of the total headspace volatiles emitted (Table 3). The major herbivore-induced compounds were terpenoids, indole, and oximes. (*3E*)-4,8-Dimethyl-1,3,7-nonatriene, (*E*)- α -bergamotene and (*E*)- β -farnesene were found as major compounds in the headspace of leaves infested by first to second instars and leaves infested by third instars. However, when corn leaves were infested by sixth-instar larvae, few compounds were found as herbivore-induced plant chemicals [indole, 3-hydroxy-2-butanone, (*3E*)-4,8-

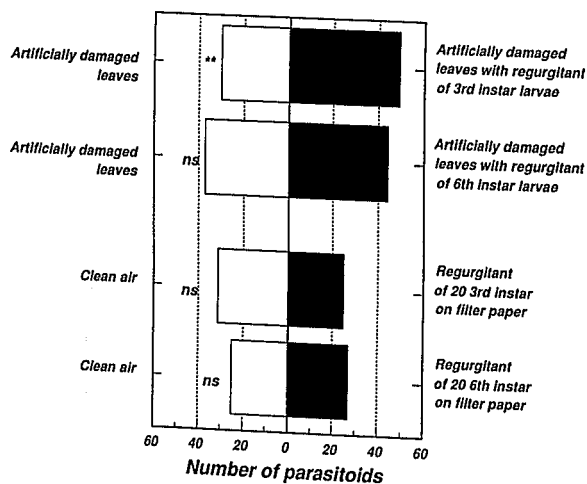


FIG. 3. Olfactory response of *Cotesia kariyai* to artificially damaged corn leaves treated with regurgitant of third-instar and sixth-instar armyworms. ** $0.01 > P > 0.001$; ns: not significantly different by sign test.

dimethyl-1,3,7-nonatriene and 2-methyl-1-propanol] and their total relative amount was only 15.2% (Table 3). The total peak area (arbitrary units) of sixth-instar armyworm-induced plant volatiles per gram leaf (77.3) was smaller than that of first-/second-instar, and third-instar armyworm-induced plant volatiles (846.2 and 928.8, respectively) (Table 3). Thus, there are qualitative and quantitative differences in the production of herbivore-induced plant volatiles depending on the developmental stage of the herbivores.

DISCUSSION

Cotesia kariyai is attracted by uninfested corn leaves, mediated by green leaf volatiles such as (*Z*)-3-hexenol (Takabayashi et al., 1991a). Artificially damaged leaves are not more attractive than undamaged corn leaves, while infochemicals from corn leaves infested by young armyworms were highly preferred compared to uninfested corn leaves. Neither the caterpillars themselves nor their feces are attractive to the parasitoids. The infochemicals that are emitted from host-infested leaves are not just the result of mechanical damage, but are specific for herbivore infestation. Similar phenomena are observed in other tritrophic systems (Turlings et al., 1990, 1991; Steinberg et al., 1992, 1993; Mattiacci et al., 1994). Moreover, the composition of the infochemicals emitted from armyworm-infested corn leaves is dependent on the herbivore developmental stage that feeds on the plant.

Damage by younger instars (first through third) attracts the parasitoids, while damage by older instars (fourth through sixth) does not. This difference was studied more precisely with corn leaves infested by each instar larvae separately. This revealed that corn plants infested by first-, second-, third-, or fourth-instar armyworms attract the wasp, whereas corn plants infested by fifth or sixth instars do not.

Chemical analysis supports our behavioral data. Corn leaves infested by younger instar larvae produce large amounts of herbivore-induced volatiles (70–80% of total volatile emission). However, corn leaves infested by sixth-instar larvae produce few herbivore-induced volatiles and their combined amount comprised only ca. 15% of the total volatile emission (Table 3). This may explain the attraction of leaves infested by young armyworms and the nonattraction of leaves infested by old armyworms.

Application of regurgitant of third-instar larvae onto artificially damaged leaves resulted in the attraction of parasitoids, whereas application of regurgitant of sixth instar leaves did not. It is likely that this effect was caused by buccal secretion or saliva of the host larvae, as has been found for other tritrophic systems (Sato, 1979; Turlings et al., 1990, 1991; Mattiacci et al., 1994). Furthermore, our data suggest that the buccal secretion or saliva of third instars and

those of sixth instar differ in composition and, thus, affect the plant differently with regard to the production of the wasp attractants.

Herbivore-induced synomone (HIS) production by plants for specific natural enemies of herbivores can be of two classes:

1. Unconditional HIS production. This occurs when the interaction mediated by HIS is unconditionally mutualistic, i.e., adaptive to both the plant and the natural enemy.

2. Conditional HIS production. This occurs when the interaction mediated by HIS is conditionally mutualistic in the following three ways. Type 1: Adaptive to the plant and conditionally adaptive to the natural enemy. In this case, the plant should be selected to produce HIS only when it is beneficial for the natural enemy to respond to HIS. Type 2: Adaptive to the natural enemy and conditionally adaptive to the plant. In this case, the plant should be selected to produce HIS only when it is beneficial to the plant. Type 3: Conditionally adaptive to both plant and natural enemy. In this case, plant should be selected to produce HIS only when it is beneficial to both the plant and the natural enemy.

In the case of the *C. kariyai*-armyworm-corn plant interaction, the HIS production is found to be conditional. Thus, the interaction between corn plants and *C. kariyai* is conditionally mutualistic. We examine this mutualistic interaction from the viewpoint of the plant and the wasp:

From the viewpoint for the plant, the wasps can successfully parasitize the larvae during the second to early sixth instar (Sato and Tanaka, 1984). It takes 10 days at 25°C for the parasitizing wasp larvae to develop and egress from the host. *Pseudaletia separata* larvae that are parasitized by *C. kariyai* at these stages do less damage to a plant than unparasitized larvae in this period, and this effect is more pronounced when parasitism takes place in younger host instars (Tanaka et al., 1992).

When a third-instar larva of *P. separata* is parasitized by *C. kariyai*, the total amount of food consumed by this larva until pupation is ca. 36% of the total food consumed by an unparasitized larva of the same developmental stage (Tanaka et al., 1992). Thus, attracting *C. kariyai* is adaptive for the infested corn plant.

When a fifth-instar larva is parasitized by *C. kariyai*, the total food consumed by this larva until pupation is reduced to ca. 63% of the food consumed by an unparasitized larvae of the same developmental stage. The parasitized fifth-instar larva, however, has already consumed food from the first up to the fifth instar developmental stage. Therefore, the total food consumed by a larva that is parasitized at the fifth instar is 76% of the total food consumed by an unparasitized larva of the same developmental stage until pupation (data from Tanaka et al., 1992). Thus, the reduction in feeding damage is much more pronounced when the larva is parasitized in the third instar. When early sixth-

instar larvae were parasitized by *C. kariyai*, the total food consumed by the larva is almost the same as that consumed by the unparasitized host (J. Takabayashi, unpublished data, 1992). Thus, for plants, it is advantageous to attract the parasitoids when the larvae are still small, as the benefit of attracting parasitoids decreases with time. The production of the wasp attractants is likely to have costs, in terms of energy or in terms of being more detectable to other herbivores, that are not affected by the wasps (Dicke and Sabelis, 1989). Therefore, it is adaptive for an infested plant to reduce the production of attractants with decreasing benefit due to herbivore development. This is probably one of the reasons why plants stop attracting wasps when the host is older than fifth instar.

From the viewpoint of *C. kariyai*, it is clearly adaptive for the wasps to find unparasitized hosts. There are three other dominant parasitoid species for *P. separata* larva in Japan: *Cotesia ruficrus*, *Microplitis mediator*, and *Campoplex chloridae* (Sato, 1988). *Cotesia kariyai* can successfully parasitize larvae during the second through the early sixth instar (Sato and Tanaka, 1984), but later-stage armyworms are more likely to be already parasitized by conspecific or heterospecific parasitoids. *Cotesia kariyai* cannot successfully produce offspring in an armyworm that has already been parasitized more than three days earlier by a conspecific wasp (J. Takabayashi, unpublished data). Under such a competitive situation, searching for young armyworms should be advantageous for the wasps. This may also select corn plants to produce HIS when inflicting armyworms are of a younger stage.

The above findings indicate that the interaction between corn plant and *C. kariyai* is type 3—conditionally adaptive to both the plant and the natural enemy. This study provides an example for illustrating a new scheme to evaluate plant–natural enemy interactions on the basis of HIS production. The experimental design developed in this paper is applicable to other tritrophic systems in evaluating the mutualistic interactions between plants and natural enemies of herbivores through HIS.

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