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ROLE OF VOLATILE INFOCHEMICALS EMITTED BY FECES OF LARVAE IN HOST-SEARCHING BEHAVIOR OF PARASITOID *Cotesia rubecula* (HYMENOPTERA: BRACONIDAE): A BEHAVIORAL AND CHEMICAL STUDY

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Abstract—The role of volatile infochemicals emitted by feces of larvae in the host-searching behavior of the parasitoid *Cotesia rubecula* was evaluated during single- and dual-choice tests inside a wind tunnel. The following treatments were tested: feces produced by second and fourth instars of *Pieris rapae* (preferred host), second instars of *P. brassicae* (inferior host), second instars of *P. napi* (nonhost), and wet feces of second instars of *P. rapae*. During a single-choice situation females of *C. rubecula* oriented to all types of feces tested. When a preference was to be made, *C. rubecula* preferred feces of second instars of *P. rapae* over that of fourth, feces of *P. rapae* over that of *P. brassicae*, feces of *P. napi* over that of *P. brassicae*, and wet over normal host feces. No preference was exhibited between feces of second instars of *P. napi* and that of second instars of *P. rapae*. The relative importance of infochemicals from host feces versus plant damage caused by host larvae to the searching behavior of *C. rubecula* was also evaluated. Plant damage was more important to the searching females than host feces when feces were present in specific concentrations in relation to damage. The volatiles released by normal and wet feces of second instars of *P. rapae*, wet feces of fourth instars of *P. rapae*, and normal and wet feces of *P. brassicae* were collected and identified. Overall, 85 chemical compounds were recorded belonging to the following chemical groups: alcohols, ketones, aldehydes, esters, isothio-

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cyanates, sulfides, nitriles, furanoids, terpenoids and pyridines. The blend of chemicals emitted by feces of different instars of *P. rapae* and different species of *Pieris* exhibited an instar and species specificity in both quantity and quality. Wetting of normal feces increased the amount of volatile chemicals released, and it was also responsible for the appearance of new compounds. The role of feces of larvae in the host-seeking behavior of *C. rubecula* is discussed.

Key Words—Volatile infochemicals, kairomone, host feces, parasitoid, *Cotesia rubecula*, host-searching behavior, *Brassica oleracea gemmifera*, *Pieris rapae*, *Pieris brassicae*, *Pieris napi*, Lepidoptera.

INTRODUCTION

During their search for hosts, parasitoids attacking larvae of Lepidoptera are known to utilize volatile chemicals emitted by plants infested by their hosts. Some of the chemicals emitted by host-infested plants may be released by the plant itself and others may originate from activities related to the biology of larvae, e.g., feces, silk, etc. (for an overview see Vinson, 1976, 1984; Vet and Dicke, 1992). Some of the volatiles emitted by the host plant are produced independently of the presence of larvae, and others may appear only during or after feeding of larvae has occurred (Whitman and Eller, 1990; Turlings et al., 1990, 1991, Turlings and Tumlinson, 1992; Agelopoulos and Keller, 1994c; Blaakmeer et al., 1994; Mattiacci et al., 1994; Potting et al. 1995, Takabayashi et al., 1995). Plant compounds induced by larval feeding are extensively used by female parasitoids during location of host-infested plants (Loke et al., 1983; Nadel and van Alphen, 1987; Steinberg et al., 1993; Turlings et al., 1990; Agelopoulos and Keller, 1994b; Geervliet et al., 1994, Takabayashi et al., 1995). Volatiles emitted by feces of larvae may also be involved in orientation to host-infested plants since many species of parasitoids are known to be attracted to feces of their host larvae (Lecomte and Thibout, 1986, 1993; Eller et al., 1988a,b; Elzen et al., 1987; Ding et al., 1989; Ramachandran et al., 1991; Turlings et al., 1991; Cortesero et al., 1993; Steinberg et al., 1993; Agelopoulos and Keller, 1994a; Geervliet et al., 1994). The aim of this study was to investigate the role of host feces in orientation to host-infested plants by the parasitoid *Cotesia rubecula*.

Cotesia rubecula Marshall (Hymenoptera: Braconidae) is a larval endoparasitoid of *Pieris rapae*. *Pieris rapae* is the preferred host of *C. rubecula*, and all host stages can be attacked. Other host species have also been reported (Shenefelt, 1972). For example, it is known that females of a Dutch population of *C. rubecula* develop most successfully in *P. rapae* although they do oviposit and develop less successfully in *Pieris brassicae* as well (Geervliet and Brodeur, 1992). Although the parasitoid accepts *Pieris napi* as a host, the parasitoid cannot successfully develop in this species (Geervliet and Brodeur, 1992). Dur-

ing their search for hosts, females of *C. rubecula* are known to be attracted from a distance to host-infested plants (Nealis, 1986, 1990; Keller, 1990), and their searching behavior is influenced by experience (Kaiser and Cardé, 1992; Geervliet et al., 1993). Detailed studies on the role and identity of plant volatiles involved in long-distance orientation to host-infested plants have been conducted for two different populations of *C. rubecula*: an Australian population (Agelopoulos and Keller, 1994a–c) and a Dutch one (Geervliet et al., 1993, 1994, 1995; Blaakmeer et al., 1994). For both populations the same holds true: females of *C. rubecula* rely mainly on plant volatiles related to damage to locate host-infested plants. They are able to distinguish from a distance between plants damaged by larvae of Lepidoptera and those damaged by mechanical means, but they are not able to discriminate between host plants damaged by larvae of different species of Lepidoptera. However, in a more complex situation where plants infested by *P. rapae* are simultaneously present with plants infested by *P. brassicae*, females of *C. rubecula* preferably land on plants infested by *P. rapae* (Wiskerke and Vet, 1994). Since the blends of volatiles released by cabbage plants damaged by different Lepidoptera is not species specific (Blaakmeer et al. 1994), the question still remains as to which component of the plant-herbivore complex provides species-specific information to the searching females. Studies on other sources of volatiles related to infestation that may be involved in orientation to host-infested plants have revealed that females of *C. rubecula* are not attracted to host larvae (Kaiser and Cardé, 1992; Agelopoulos and Keller, 1994a), but they are attracted to feces of the host (Agelopoulos and Keller, 1994a; Geervliet et al., 1994). In the chemical study by Agelopoulos and Keller (1994c), there is evidence of species specificity related to volatiles emitted by feces of larvae, but the question still remains as to whether feces are the component that provides species-specific information to the searching females.

In this study we explored further the role of feces in attracting *C. rubecula* to its host. By conducting single- and dual-choice tests and by using feces of different host instars and feces of three closely related species of *Pieris*, we tested whether the information released by feces and utilized by *C. rubecula* was: (1) host-instar specific, (2) species specific, (3) dose dependent, and (4) affected by feces moisture. The relative importance of volatiles released by feces, compared to those released by plant damage was also evaluated. Finally, we sought to identify the volatiles released by feces of different instars of *P. rapae*, feces of *P. rapae* with different moisture levels, and feces of *P. brassicae*.

METHODS AND MATERIALS

Plants and Insects. Brussels sprouts plants, *Brassica oleracea gemmifera* cv. Titurel, were grown under greenhouse conditions (20–30°C, 50–80% rel-

ative humidity, 16L:8D). *Pieris rapae* and *P. brassicae* (Lepidoptera: Pieridae) were reared on Brussels sprouts plants inside a climate-controlled room (20–22°C, 50–70% relative humidity, 16L:8D), and *P. napi* was reared on Brussels sprouts plants under greenhouse conditions (20–30°C, 50–80% relative humidity, 16L:8D). The parasitoid *C. rubecula* was reared on *P. rapae* at 20–22°C, 50–70% relative humidity, 16L:D8. Upon emergence, mated females of *C. rubecula* were released into a gauze cage with honey and water. Two- to 3-day-old "experienced" females were used for the bioassays. Experience of females was obtained by introducing inside the cage a leaf of Brussels sprouts bearing feeding damage and feces of the host. The leaf remained there for 22 hr and was removed 4 hr prior to testing.

Bioassays. All tests were performed inside a wind tunnel under the following conditions: 25°C, 45–60% relative humidity, and wind speed 50 cm/sec (description of wind tunnel by Geervliet et al., 1994). One female, held in a vial that was placed in vertical position, was introduced into the wind tunnel 1 m away from the stimulus. The observation started when the plug of cotton wool restricting the insect inside the vial was removed. A maximum of six tries was given to insects that failed during earlier attempts. "Attraction to stimulus" was defined as a complete flight of the insect inside the odor plume and successful location (landing) of the stimulus. Insects that flew half way to the stimulus were given a second try. To reduce the effect of day-to-day variation in response, the same test was conducted on at least three different days in different weeks. Feces were collected by placing Petri dishes under infested leaves for a period of 24 hr (25°C, 50–60% relative humidity).

Single-Choice Tests. The response of females of *C. rubecula* to a single stimulus was observed for the following: feces of second instars of *P. rapae*, feces of fourth instars of *P. rapae*, feces of second instars of *P. brassicae*, and feces of second instars of *P. napi*. For each bioassay, 65 mg of feces was used and placed on a flat surface at the upwind end of the wind tunnel. Five, 10, and 20 mg of feces of late second instars of *P. rapae* were used to record dose-dependent responses. The three different doses were tested on the same day.

Dual-Choice Tests. The landing preference of *C. rubecula* was tested for the following: (1) feces of late second instars of *P. rapae* vs. feces of early fourth instars of *P. rapae* (second vs. fourth *P. rapae*), (2) feces of late second instars of *P. rapae* vs. wet feces of late second instars of *P. rapae* (second *P. rapae* vs. wet second *P. rapae*), (3) feces of late second instars of *P. rapae* vs. feces of late second instars of *P. brassicae* (*P. rapae* vs. *P. brassicae*), (4) feces of late second instars of *P. rapae* vs. feces of late second instars of *P. napi* (*P. rapae* vs. *P. napi*), and (5) feces of late second instars of *P. brassicae* vs. feces of late second instars of *P. napi* (*P. brassicae* vs. *P. napi*). "Wet" feces is defined as feces wetted prior to testing with three drops of water. "Normal" feces is rather dry. In each case, 65 mg of feces was used.

The two stimuli were placed on separate flat surfaces located 5 cm apart and 1 m away from the release point of insects. To avoid bias caused by the left or right position of stimuli inside the wind tunnel, the stimuli were alternated after every fifth observation. To evaluate the relative significance of feces over plant damage in leading *C. rubecula* to its host, the following were tested: (1) a leaf carrying feeding damage of 10 instars of *P. rapae* vs. a leaf carrying feeding damage of 10 instars of *P. rapae* plus 10 mg of feces of *P. rapae* (D_{10} vs. $D_{10}F_{10\,mg}$), (2) a leaf carrying feeding damage of 10 instars of *P. rapae* vs. a leaf carrying feeding damage of 10 instars of *P. rapae* plus 65 mg of feces of *P. rapae* (D_{10} vs. $D_{10}F_{65\,mg}$), (3) a leaf carrying feeding damage caused by 20 instars of *P. rapae* vs. a leaf carrying feeding damage of 10 instars of *P. rapae* plus 65 mg of feces of *P. rapae* (D_{20} vs. $D_{10}F_{65\,mg}$). For all tests, damage and feces were produced by late second instars of *P. rapae*. Damage on the leaves was produced over a period of 24 hr. Prior to testing, larvae and larval by-products were removed. In the cases where leaf and feces were used together, the feces were located on a flat surface next to the leaf. To avoid bias caused by left or right position of stimuli inside the wind tunnel, the stimuli were alternated after every fifth observation. Production of feces by second and fourth instars of *P. rapae* was accomplished by placing 20 late second instars and 20 early fourth instars of *P. rapae* individually on single leaves held in vials with water where they were left to feed for 24 hr. The feces produced by each larva were collected and weighed.

Statistics. The chi-square test for goodness-of-fit was used to determine preference for one of the stimuli tested during dual choice situation (distribution of expected values 50:50).

Collection of Volatiles from Larval Feces and Chemical Analysis. Feces used for identification of volatile components were collected by placing Petri dishes under infested leaves for a period of 24 hr (25°C, 50–60% relative humidity). Volatiles from feces were collected by placing feces in a glass flask (50 ml), which was closed with a glass top having a ground glass connection and an inlet and outlet glass tube. Purified air was passed through the inlet tube with an air flow of 90 ml/min. Volatile chemicals emitted by feces were transported with the aid of airflow to the outlet tube, where they were trapped on 90 mg of Tenax-TA. The Tenax-TA was placed inside a glass tube connected immediately to the outlet tube (Dicke et al., 1990). Thermodesorption was the method used to release the volatiles trapped on Tenax-TA (description of method by Dicke et al., 1990). The samples were analyzed by using a gas chromatograph–mass spectrometer (Finnigan MAT 95). The GC column used was Supelcowax 10 (60 m × 0.25 mm ID; 0.25-μm film thickness), and the temperature conditions were 3 min at 40°C, increased by 3°C/min to 140°C, and after that increased by 6°C/min to 270°C. The mass spectrometer was operated in the 70-eV electron impact ionization mode. Control samples (clean air) were also

analyzed to ascertain which chemicals were specific to feces. Identification of compounds was based on the commercial spectra libraries NIST and WILEY and on our own collection of spectra of natural compounds. Volatiles released by 250 mg of feces produced by late second instars of *P. rapae* (samples named as normal) were collected for a period of 2.5 hr. At the end of that collection period, the same feces were wetted with nine drops of distilled water and volatiles were collected for a further 2.5 hr (samples named as wet). The same procedure was followed for 250 mg of feces produced by late second instars of *P. brassicae*. Volatiles were also collected from 250 mg of feces produced by early fourth instars of *P. rapae*. The samples for that particular treatment were all wetted with nine drops of distilled water. Three samples were collected for each treatment.

RESULTS

Bioassays. During a single-choice situation, females of *C. rubecula* oriented to and landed on all types of feces tested irrespective of instar or species that had produced them (Table 1). *C. rubecula* was able to locate the source of volatiles even when very small amounts (e.g., 5 mg of feces) of host feces were used (Table 2). A higher degree of attraction was observed for 20 mg of feces over that of 5 and 10 mg. However, the attraction of females to the different doses tested was not significantly different (contingency table, $P = 0.07$). *C. rubecula* preferred feces of *P. rapae* over that of *P. brassicae*, but it did not show preference for feces of *P. rapae* when tested against that of *P. napi*. Feces of *P. napi* were also preferred over that of *P. brassicae* (Table 3). Preference was also exhibited for feces of second instars over feces of fourth instars of *P. rapae* (Table 3). The preference of females for leaves damaged by the host over leaves damaged by the host that also carried feces of the host depended on the amount of feces present on the leaves (Table 4). When leaves carrying

TABLE 1. ORIENTATION OF FEMALES OF *C. rubecula* TO 65 mg OF FECES PRODUCED BY LARVAE OF DIFFERENT INSTARS AND DIFFERENT SPECIES OF *Pieris* ($N = 30$) IN SINGLE-CHOICE EXPERIMENTS

Source of feces	Attraction	No attraction
Second instars of *P. rapae*	24	6
Fourth instars of *P. rapae*	29	1
Second instars of *P. brassicae*	23	7
Second instars of *P. napi*	18	12

TABLE 2. DOSE-RELATED RESPONSES OF FEMALES OF *C. rubecula* TO FECES OF SECOND INSTARS OF *P. rapae* ($N = 30$)

Amount of feces (mg)	Attraction	No attraction
20	25 (83%)	5
10	19 (63%)	11
5	17 (56%)	13

damage caused by 10 second instars of *P. rapae* were tested against leaves carrying the same amount of damage plus 10 mg of feces of *P. rapae*, the females did not show preference for any of the two stimuli. When the 10 mg of feces was replaced with 65 mg, the females showed a preference for the latter. Leaves bearing damage caused by 20 second instars of *P. rapae* were preferred over leaves carrying feeding damage of 10 second instars of the host plus 65 mg of host feces.

A 10-fold increase in production of feces was observed from second (0.62 ± 0.03 m/larva/24 h, mean ± SEM) to fourth instars of *P. rapae* (5.87 ± 0.17 m/larva/24 h).

TABLE 3. PREFERENCE OF *C. rubecula* FOR FECES OF DIFFERENT SPECIES OF *Pieris*, DIFFERENT INSTARS OF *P. rapae*, AND NORMAL VERSUS WET FECES OF *P. rapae*[a]

Choice test	Attraction		No attraction
Second vs. fourth *P. rapae* ($N = 66$)	2nd	4th	
	29	12*	25
Second *P. rapae* vs. wet second *P. rapae* ($N = 34$)	*frass*	*wet frass*	
	5	22*	7
Second *P. rapae* vs. second *P. brassicae* ($N = 40$)	*P. rapae*	*P. brassicae*	
	23	8*	9
Second *P. rapae* vs. second *P. napi* ($N = 75$)	*P. rapae*	*P. napi*	
	15	17 NS	43
Second *P. brassicae* vs. second *P. napi* ($N = 49$)	*P. brassicae*	*P. napi*	
	8	22*	19

[a]NS = not significantly different, *significantly different $P \leq 0.05$.

TABLE 4. PREFERENCE OF FEMALES OF *C. rubecula* TO PLANT DAMAGE CAUSED BY HOST LARVAE OVER HOST FECES

Choice test	Attraction		No attraction
D_{10} vs. $D_{10} + F_{10mg}$ ($N = 46$)	D_{10}	$D_{10} + F_{10mg}$	
	19	16 NS	11
D_{10} vs. $D_{10} + F_{65mg}$ ($N = 32$)	D_{10}	$D_{10} + F_{65mg}$	
	9	22*	1
D_{20} vs. $D_{10} + F_{65mg}$ ($N = 35$)	D_{20}	$D_{10} + F_{65mg}$	
	26	7*	2

[a] D_{10}, D_{20} = plant damage caused by 10 and 20 second instars of *P. rapae* respectively, F = feces, D + F = damage and feces together, NS = not significantly different, *significantly different $P \leq 0.05$).

Chemical Analysis. Overall, 85 volatile chemical compounds were identified from the various treatments tested belonging to the following chemical groups: alcohols, ketones, aldehydes, esters, isothiocyanates, sulfides, nitriles, furanoids, terpenoids, and pyridines (Table 5). The blends of volatiles released by feces of larvae of different species of *Pieris*, different instars of *P. rapae*, and by feces affected by moisture are different in both quantity and quality (Table 5; Figure 1). The average amount of the identified compounds is represented in Table 5 by units of peak area. Depending on the chemical nature of the compound, one unit of peak area is equal to 0.3–0.5 ng. Wet feces of second instars of *P. rapae* and *P. brassicae* emitted the highest amounts of volatiles (total peak area, Table 5). Wet feces of fourth instars of *P. rapae* emitted much less and normal feces of second instars of *P. rapae* and *P. brassicae* emitted the lowest amount of chemicals.

Most of the compounds detected were present in all treatments tested, but some compounds or classes of compounds were present in one treatment only (Table 6). For example, nitriles were solely, and pyridines mainly, present in feces of fourth instars of *P. rapae*. Feces of *P. brassicae* emitted more aldehydes, alcohols, and ketones in comparison with that of *P. rapae*, and feces of second instars of *P. rapae* exhibited the least qualitative specificity but showed a quantitative specificity for a number of compounds. For example, compounds such as 2-methyl-1-propanol, 1-penten-3-ol, 1-penten-3-one, 3-methylbutanal, pentanal, and β-cyclocitral appeared in high concentration in that treatment (Table 5, Figure 2).

TABLE 5. VOLATILE COMPOUNDS EMITTED BY FECES OF SECOND AND FOURTH INSTARS OF *P. rapae* AND SECOND INSTARS *P. brassicae*[a]

	Compounds	*P. rapae*			*P. brassicae*	
		Second instars, normal ($N = 3$)	Second instars, wet ($N = 3$)	Fourth instars, wet ($N = 3$)	Second instars, normal ($N = 3$)	Second instars, wet ($N = 3$)
Alcohols						
1.	methanethiol		$2.0 \pm 2.0^{++}$	$0.2 \pm 0.2^{+}$		$0.8 \pm 0.8^{+}$
2.	methanol					$44 \pm 23^{++}$
3.	2-propanol*					$0.5 \pm 0.3^{++}$
4.	ethanol*				$0.6 \pm 0.3^{++}$	$1.4 \pm 0.3^{++}$
5.	2-methyl-1-propanol*	$9.3 \pm 5.6^{+++}$	$54 \pm 45^{+++}$	$14 \pm 10^{+++}$	$1.6 \pm 0.7^{+++}$	$21 \pm 13^{+++}$
6.	3-pentanol*			$9.7 \pm 7.8^{++}$		$0.7 \pm 0.7^{+}$
7.	1-penten-3-ol*	$5.0 \pm 2.1^{+++}$	$141 \pm 17^{+++}$	$8.5 \pm 3.9^{+++}$	$3.1 \pm 2.8^{+++}$	$83 \pm 43^{+++}$
8.	3-methyl-2-pentanol			$0.9 \pm 0.5^{++}$		
9.	3-methyl-1-butanol*			$59 \pm 49^{+++}$		$0.2 \pm 0.2^{+}$
10.	2-methyl-1-butanol			$0.9 \pm 0.5^{++}$		
11.	1-pentanol*	$1.3 \pm 0.3^{+++}$	$30.7 \pm 6.3^{+++}$	$23 \pm 14^{+++}$	$0.83 \pm 0.60^{++}$	$16.7 \pm 8.6^{++}$
12.	(*E*)-2-penten-1-ol		$5.7 \pm 0.7^{+++}$	$8.0 \pm 8.0^{+}$		$4.3 \pm 2.3^{++}$
13.	(*Z*)-2-penten-1-ol*	$2.5 \pm 1.8^{+++}$	$48.0 \pm 3.6^{+++}$	$7.0 \pm 6.0^{+++}$	$3.0 \pm 2.5^{++}$	$40 \pm 20^{+++}$
14.	1-hexanol*		$4.7 \pm 1.5^{+++}$	$6.0 \pm 1.5^{+++}$		$3.2 \pm 1.5^{+++}$
15.	(*Z*)-3-hexen-1-ol*	$8.3 \pm 2.6^{+++}$	$137 \pm 50^{+++}$	$95 \pm 48^{+++}$	$7.3 \pm 7.3^{+}$	$110 \pm 83^{+++}$
16.	(*E*)-2-hexen-1-ol*			$4.5 \pm 4.2^{++}$		
17.	1-octen-3-ol*		$8.0 \pm 2.1^{+++}$	$1.1 \pm 0.5^{+++}$		$5.0 \pm 2.6^{++}$
18.	1-heptanol		$1.7 \pm 0.3^{+++}$	$1.2 \pm 0.4^{+++}$		
19.	2-methylthioethanol			$6.4 \pm 5.8^{+++}$		
20.	1-octanol	$2.3 \pm 1.3^{+++}$	$5.7 \pm 1.2^{+++}$	$2.0 \pm 0.6^{+++}$	$1.6 \pm 0.5^{+++}$	$3.4 \pm 1.6^{+++}$

TABLE 5. Continued

	Compounds	*P. rapae*			*P. brassicae*	
		Second instars, normal ($N = 3$)	Second instars, wet ($N = 3$)	Fourth instars, wet ($N = 3$)	Second instars, normal ($N = 3$)	Second instars, wet ($N = 3$)
Ketones						
21.	2-butanone				2.3 ± 1.9^{++}	36 ± 35^{++}
22.	3-methyl-2-butanone					0.8 ± 0.4^{++}
23.	2-pentanone	1.6 ± 0.4^{+++}	17.3 ± 5.9^{+++}	0.6 ± 0.3^{++}	2.5 ± 1.0^{+++}	31 ± 27^{+++}
24.	2,3-butanedione	0.8 ± 0.2^{+++}	36.5 ± 5.3^{+++}	19.0 ± 7.1^{+++}	0.8 ± 0.5^{++}	35 ± 18^{+++}
25.	3-methyl-2-pentanone*		7.1 ± 2.9^{+++}	2.5 ± 1.8^{++}		2.3 ± 1.2^{++}
26.	1-penten-3-one*		53.7 ± 2.7^{+++}	2.3 ± 1.4^{+++}	0.3 ± 0.3^{+}	33 ± 16^{+++}
27.	3-hexanone					1.5 ± 0.8^{++}
28.	2,3-pentanedione*		11.7 ± 0.9^{+++}	1.8 ± 1.1^{+++}		5.4 ± 2.9^{++}
29.	3-penten-2-one* *isomer*		9.3 ± 1.8^{+++}			3.8 ± 1.9^{+++}
30.	2-heptanone		3.7 ± 2.7^{+++}	0.5 ± 0.3^{++}		0.9 ± 0.8^{++}
31.	6-methyl-2-heptanone					2.3 ± 1.5^{++}
32.	2,4-pentanedione		10.0 ± 10.0^{++}	8.3 ± 8.3^{+}		
33.	3-hydroxy-2-butanone			29 ± 15^{+++}	4.7 ± 4.7^{+}	3.3 ± 3.3^{+}
34.	2,2,6-trimethylcyclohexanone		8.3 ± 0.7^{+++}			4.7 ± 2.3^{++}
35.	6-methyl-5-hepten-2-one	1.0 ± 0.5^{+++}	14.0 ± 2.0^{+++}	1.2 ± 0.4^{+++}	1.4 ± 0.8^{+++}	11.1 ± 5.6^{+++}
36.	4-hydroxy-4-methyl-2-pentanone	1.7 ± 1.7^{+}	0.3 ± 0.2^{++}	0.7 ± 0.6^{++}		
37.	2,5 hexanedione	1.8 ± 1.6^{++}	2.3 ± 1.2^{++}	1.3 ± 1.3^{+}		
38.	3-nonen-2-one *isomer*					1.2 ± 0.6^{++}
39.	3,5-dimethyl-2-cyclohexen-1-one		8.0 ± 6.6^{++}			
40.	3,3,5-trimethyl-2-cyclohexen-1-one		11 ± 0^{+++}	1.0 ± 0.6^{+++}		
41.	3,5 octadien-2-one		8.7 ± 0.3^{+++}		0.5 ± 0.5^{+}	3.7 ± 1.9^{+}
42.	dihydrofuranone					3.0 ± 1.7^{++}
Aldehydes						
43.	acetaldehyde					9.7 ± 7.8^{++}
44.	propanal		2.2 ± 1.0^{+++}	0.10 ± 0.05^{++}		7.7 ± 6.7^{++}
45.	2-methylpropanal		6.9 ± 2.5^{+++}	3.2 ± 1.4^{+++}		7.3 ± 5.9^{++}

46.	2-methylbutanal		39.7 ± 6.3^{+++}	26 ± 13^{+++}	0.4 ± 0.2^{++}	38 ± 12^{+++}
47.	3-methylbutanal		105.7 ± 8.5^{++}	80 ± 27^{+++}	0.4 ± 0.2^{++}	58 ± 28^{+++}
48.	pentanal	0.8 ± 0.2^{+++}	36.5 ± 5.3^{+++}	3.4 ± 1.2^{+++}	1.3 ± 0.7^{+++}	11.9 ± 5.5^{+++}
49.	2-butenal		8.3 ± 1.9^{+++}			4.3 ± 2.2^{++}
50.	(*Z*)-2-pentenal*		3.7 ± 0.9^{+++}			4.3 ± 2.3^{++}
51.	(*E*)-2-pentenal*		57.0 ± 7.6^{+++}	1.4 ± 0.6^{+++}	0.2 ± 0.2^{+}	40 ± 22^{+++}
52.	heptanal	0.7 ± 0.3^{+++}	6.3 ± 0.9^{+++}	0.90 ± 0.05^{+++}	1.0 ± 0.2^{+++}	8.8 ± 5.5^{+++}
53.	(*Z*)-2-hexenal*		1.3 ± 1.3^{+}			6.0 ± 6.0^{+}
54.	(*E*)-2-hexenal*	2.3 ± 1.5^{++}	174 ± 20^{+++}	20 ± 12^{+++}	7.0 ± 6.5^{++}	191 ± 114^{+++}
55.	4-heptenal *isomer*					3.3 ± 1.7^{++}
56.	methylthioacetaldehyde	1.0 ± 1.0^{++}	29.3 ± 8.3^{+++}	4.9 ± 4.1^{++}	1.8 ± 0.7^{+++}	94 ± 60^{+++}
57.	octanal*	2.3 ± 0.3^{+++}	5.6 ± 1.9^{+++}	6.7 ± 6.2^{++}	3.3 ± 1.9^{+++}	6.8 ± 3.2^{+++}
58.	(*E*)-2-heptenal		7.3 ± 2.4^{+++}			4.3 ± 2.2^{++}
59.	2-octenal *isomer*					2.3 ± 1.2^{++}
60.	2,4-heptadienal *isomer*		34.0 ± 2.5^{+++}	2.0 ± 2.0^{+}	0.3 ± 0.3^{+}	22 ± 10^{+++}
61.	2,4-heptadienal *isomer*		45.7 ± 7.2^{+++}	5.8 ± 5.1^{++}		27 ± 15^{++}
62.	2-nonenal *isomer*				0.6 ± 0.6^{+}	1.8 ± 1.8^{+}
63.	phenylacetaldehyde					2.3 ± 0.9^{+++}
Esters						
64.	(Z)3-hexen-1-ol isovalerate*		1.2 ± 0.2^{+++}			
Isothiocyanates (ITC)						
65.	methyl ITC*	1.0 ± 0.5^{+++}	15.6 ± 6.7^{+++}	5.3 ± 2.3^{+++}	0.6 ± 0.2^{+++}	32 ± 18^{+++}
66.	but-3-en-1-yl ITC			2.7 ± 1.3^{+++}		
67.	allyl ITC		3.3 ± 3.3^{+}	12 ± 10^{+++}		1.6 ± 1.2^{++}
Sulfides						
68.	dimethyl disulfide*	5.0 ± 0.6^{+++}	303 ± 84^{+++}	113 ± 45^{+++}	3.9 ± 1.3^{+++}	267 ± 194^{+++}
69.	methylvinyldisulfide		2.7 ± 0.9^{+++}			9.3 ± 4.8^{++}
70.	dimethyl trisulfide*	8.0 ± 3.6^{+++}	745 ± 327^{+++}	57 ± 25^{+++}	5.8 ± 2.6^{+++}	831 ± 588^{+++}
71.	methyl(methylthio)methyl disulfide		11.3 ± 5.8^{+++}	0.2 ± 0.2^{+}		15.7 ± 12.3^{+++}
72.	2,4-dithiopentane			0.7 ± 0.3^{+++}		
Nitriles						
73.	2,4-pentadienenitrile *isomer*			4.1 ± 2.5^{+++}		
74.	2,4-pentadienenitrile *isomer*			7.3 ± 3.8^{+++}		
75.	4-methylthiobutanenitrile			9.3 ± 5.3^{+++}		

TABLE 5. Continued

Compounds		*P. rapae*			*P. brassicae*	
		Second instars, normal (N = 3)	Second instars, wet (N = 3)	Fourth instars, wet (N = 3)	Second instars, normal (N = 3)	Second instars, wet (N = 3)
Furanoids						
76.	2,4-dimethylfuran		1.3 ± 0.7''			
77.	2-ethylfuran*					0.5 ± 0.3''
Terpenoids						
78.	linalool		1.2 ± 0.4'''			0.9 ± 0.7''
79.	safranal		4.0 ± 0.6'''			
80.	β-cyclocitral	2.1 ± 0.5'''	114.0 ± 9.2'''	10.1 ± 6.6'''	3.4 ± 2.4'''	65 ± 34'''
Pyridines						
81.	2,4-dimethylpyridine			4.3 ± 2.3''		
82.	2,4,6-trimethylpyridine	0.2 ± 0.2'	13.7 ± 7.8'''	29 ± 24'''	0.3 ± 0.3'	2.1 ± 1.5''
83.	2-ethyl-4,6-dimethylpyridine			0.7 ± 0.6''		
Unknown						
84.	Unknown 1		14.3 ± 1.5'''	1.2 ± 0.9''	0.3 ± 0.3'	9.1 ± 4.5'''
85.	Unknown 2	2.5 ± 1.3'''	196 ± 18'''	14.7 ± 9.8'''	2.4 ± 1.1'''	125 ± 70'''
Total peak area (mean ± SE)		62 ± 9	2633 ± 917	746 ± 483	64 ± 56	2515 ± 1949

[a]The number after each compound represents the average peak area (±SE) in samples of the same treatment. Depending on the chemical nature of compounds, one unit of peak area is approximately equal to 0.3–0.5 ng [N = number of samples analysed in each treatment; + = presence of compound in one sample, ++ = present in 2 samples; +++ = present in all 3 samples; * = presence of compound in the volatile blend released by Brussels sprouts infested by *P. brassicae* (Mattiacci et al., 1994), *isomer* = the identity of the geometric isomer of the compound is unknown].

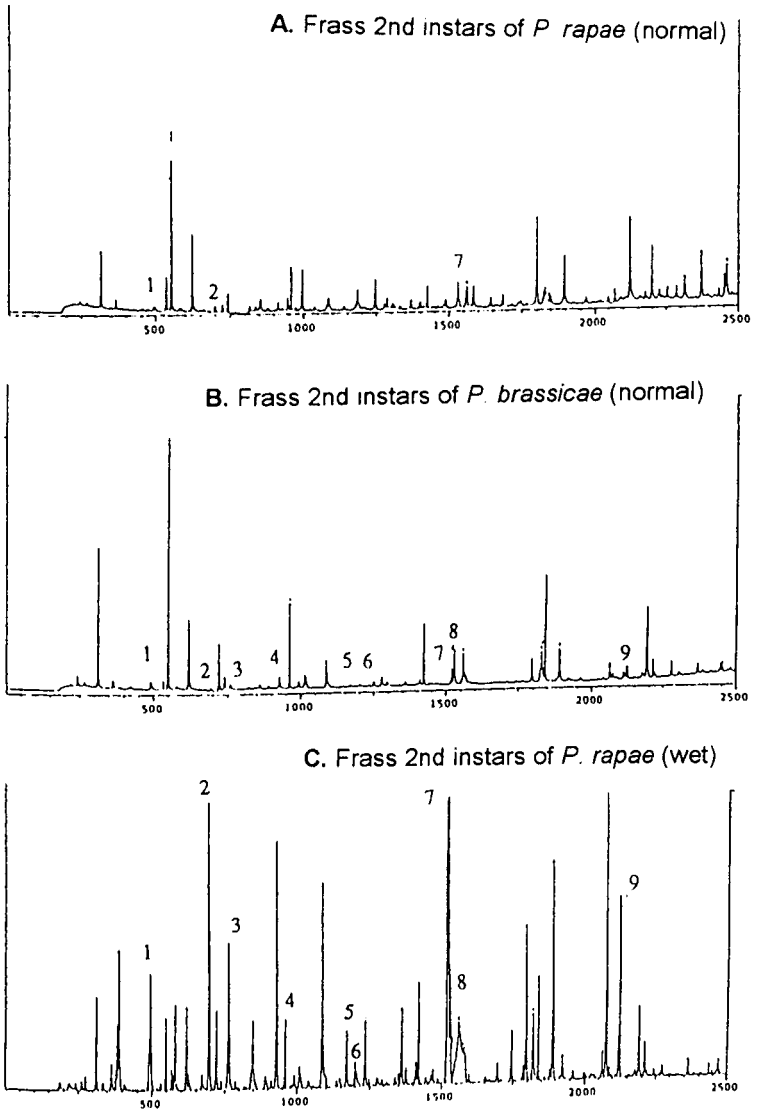

FIG. 1. Chromatograms of volatiles emitted by feces of second instars of *P. rapae* [(A) normal, (C) wet], second instars of *P. brassicae* [(B) normal, (D) wet], and fourth instars of *P. rapae* [(E) wet]. Each chromatogram represents only one of the three samples analysed in each treatment. 1 = 2-pentanone, 2 = dimethyl disulfide, 3 = 2-methyl propanol, 4 = 1-penten-3-ol, 5 = 1-pentanol, 6 = methylthioacetaldehyde, 7 = dimethyl trisulfide, 8 = (Z)-3-hexen-1-ol, 9 = β-cyclocitral.

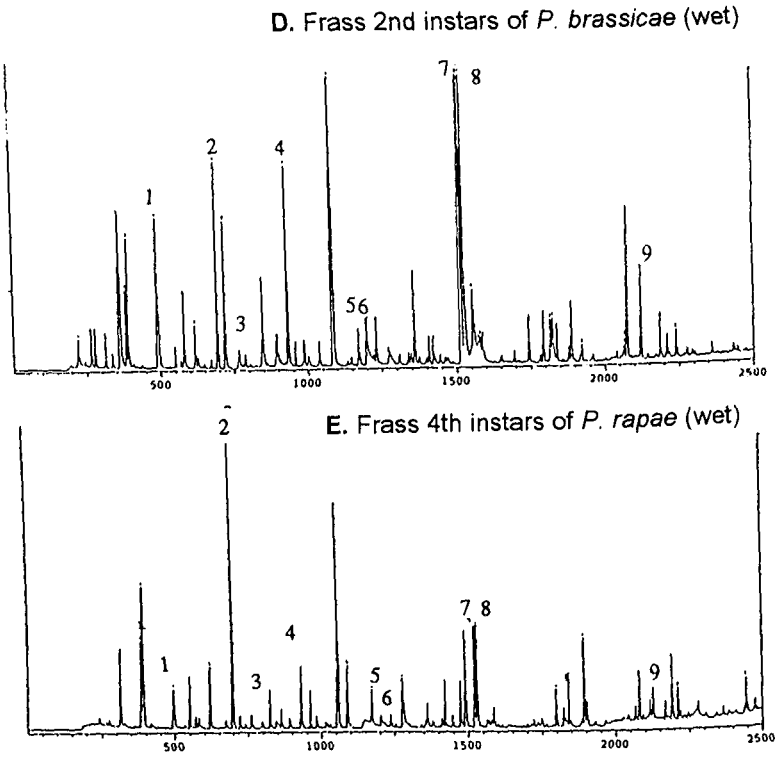

FIG. 1. Continued.

Wetting of samples not only increased the amount of compounds already present in normal samples but was also responsible for the appearance of new compounds. At present, it is not known if the appearance of new compounds is strictly related to reactions initiated by the presence of water or if those compounds were also present in normal feces but in undetectable amounts. The detection limit of the GC-MS used is approximately 0.1 ng.

Variation in the presence and concentration of some compounds between samples of the same treatment was recorded. Figure 3 shows the spectra of the unidentified compounds, unknown 1 and unknown 2.

DISCUSSION

Feces of Larvae and Their Influence on Host-Searching Behavior of Parasitoids. The host-seeking behavior of some parasitoid species is known to be influenced by feces of larvae from a distance (Lecomte and Thibout, 1986, 1993; Eller et al., 1988a,b; Elzen et al., 1987; Ding et al., 1989; Ramachandran

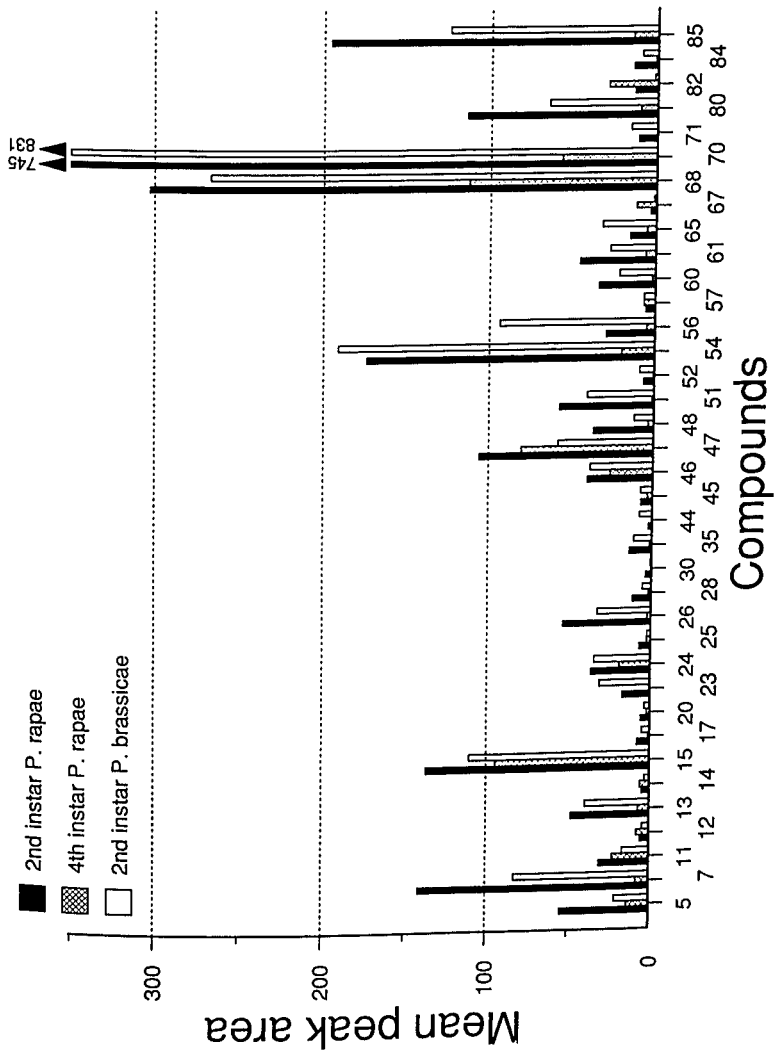

FIG. 2. Relative concentration (mean peak area) of compounds common in the treatments: second instar *P. rapae* (wet feces of second instars of *P. rapae*), fourth instar *P. rapae* (wet feces of fourth instars of *P. rapae*), second instar *P. brassicae* (wet feces of second instars of *P. brassicae*). Compound numbers are the same as in Table 5.

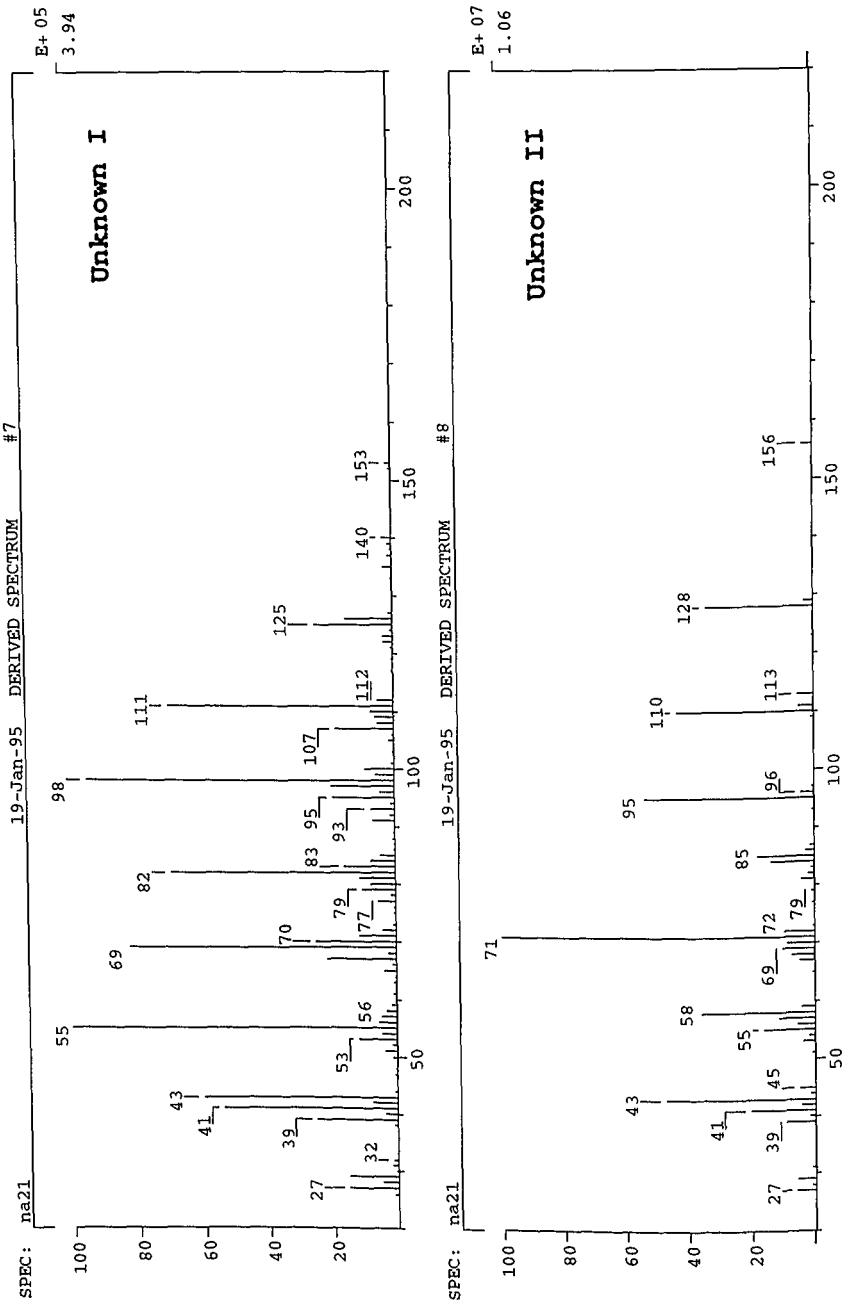

FIG. 3. Mass spectra of the unidentified compounds, unknown I and unknown II.

TABLE 6. COMPOUNDS PRESENT ONLY IN ONE OF THE FOLLOWING CLASSES: FECES OF SECOND INSTARS OF *P. rapae* (WET OR NORMAL), FECES OF FOURTH INSTARS OF *P. rapae*, AND FECES OF SECOND INSTARS OF *P. brassicae* (WET OR NORMAL)

Second instars of *P. rapae*	Fourth instars of *P. rapae*	Second instars of *P. brassicae*
(Z)-3-Hexen-1-ol isovalerate (wet)[a]	3-Methyl-2-pentanol	Methanol (wet)
2,4-Dimethylfuran (wet)	2-Methyl-1-butanol	2-Propanol (wet)
Safranal (wet)	(*E*)-2-Hexen-1-ol	Ethanol (normal, wet)[a]
	2-Methylthioethanol	2-Butanone (normal, wet)
	But-3-en-1-yl isothiocyanate	3-Methyl-2-butanone (wet)
	2,4-Dithiopentane	3-Hexanone (wet)
	2,4-Pentadienenitrile *isomer*	3-Nonen-2-one *isomer* (wet)
	2,4-Pentadienenitrile *isomer*	Dihydrofuranone (wet)
	4-Methylthiobutanenitrile	Acetaldehyde (wet)
	2,4-Dimethylpyridine	4-Heptenal *isomer* (wet)
	2-Ethyl-4,6-dimethylpyridine	2-Octenal *isomer* (normal, wet)
		2-Nonenal *isomer* (normal, wet)
		Phenylacetaldehyde (wet)
		2-Ethylfuran (wet)[a]

[a] Presence of compound in the volatile blend released by Brussels sprouts plants infested by *P. brassicae* (Mattiacci et al., 1994).

et al., 1991; Turlings et al., 1991; Cortesero et al., 1993; Steinberg et al., 1993; Agelopoulos and Keller, 1994a; Geervliet et al., 1994) and upon contact (Lewis and Jones, 1971; Sato, 1979; Nordlund and Sauls, 1981; van Leerdam et al., 1985; Nordlund and Lewis, 1985; Ding et al., 1989; Fukushima et al., 1989; Takabayashi and Takahashi, 1989; Mattiacci and Dicke, 1995). Factors related to production of feces, such as species of larvae (Lewis and Jones, 1971; Thibout et al., 1993; Agelopoulos and Keller, 1994a; present study), species of plant that larvae feed on (Nordlund and Sauls, 1981; Ramachandran et al., 1991), developmental stage of larvae (Mattiacci and Dicke, 1995; present study), and moisture content of feces (Sato 1979; van Leerdam et al., 1985; present study) are known to influence the behavior of female parasitoids from a distance and upon contact.

The variation in percentage of attraction of females during the various tests conducted here is not uncommon for the population of *C. rubecula* kept in our laboratory. Females of that population exhibit an average attraction of 50% to host-infested plants, varying from 20% to 95%, depending on the day of testing (Geervliet et al., 1994, 1995; N.G. Agelopoulos personal observations). Variation in attraction to host-infested plants has also been observed for a Dutch population of *C. glomerata* (Steinberg et al., 1993). The reason behind the observed variation in the responses of *C. rubecula* is not known, although

decreasing barometric pressure has been shown to be one of the factors affecting the response of females of *C. glomerata* (Steinberg et al., 1993). Because of the day-to-day variation in percentage of wasps that fly upwind to the odor source, the data for the different odor sources in Table 1 (different experimental days per treatment) cannot be compared to draw conclusions on the relative attraction towards different feces. This can only be done with the data in Table 3.

Plant Origin of Chemicals in Feces. Indications of the plant origin of chemicals present in feces of larvae have been abundant in studies related to behavior of parasitoids. The differential responses of parasitoids to feces produced after feeding of host larvae on different plants (van Leerdam et al., 1985), the nonattraction of parasitoids to feces produced after feeding of larvae on artificial diet (Nordlund and Lewis, 1985; Ding et al., 1989; Ramachandran et al., 1991), and the attraction of parasitoids to feces of nonhost larvae feeding on hostplants (Lewis and Jones, 1971; Thibout et al., 1993; Agelopoulos and Keller, 1994a) are indicative of the involvement of the plant in the chemical composition of feces. Identification of volatiles released by feces of different species of larvae has revealed the plant origin of those compounds (Auger et al., 1989; Ramachandran et al., 1991; Agelopoulos and Keller, 1994c). Our chemical study further supports the plant origin of compounds emitted by feces, since most of the volatiles emitted by feces of *P. rapae* and *P. brassicae* have previously been reported in cruciferous plants (Bailey et al., 1961; Buttery et al., 1976; Wallbank and Wheatley, 1976; Cole, 1976, 1980a,b; Tollsten and Bergström, 1988; Evans and Allen-Williams, 1992; Blaakmeer et al., 1994; Mattiacci et al., 1994). The presence of some of the volatiles emitted by feces may be related to breakdown processes of plant material after digestion by larvae and the presence of others may be the end product of processes related to microorganisms inhabiting the feces (Thibout et al., 1993).

Agelopoulos and Keller (1994c) identified volatiles from "normal" frass of *P. rapae* larvae on a *capitata* variety of cabbage and recorded eight compounds, five of which are also recorded here. One of these five compounds is identified here as methylthioacetaldehyde on the basis of mass spectrum and chromatographic behavior and has the same mass spectrum as the compound labeled as methyl propyl sulfide by Agelopoulos and Keller (1994c). The spectrum has small, but significant differences from that of methyl propyl sulfide. Our analyses show that moistening of the feces greatly increases the amount of chemicals recorded.

Specificity Related to Volatiles Emitted by Feces of Different Species and Instars of Larvae. The chemical profile emitted by feces of *P. rapae* and *P. brassicae* produced after feeding of larvae on Brussels sprouts plants is species specific in both quantity and quality. Species specificity related to the blend of chemicals released by feces of larvae has also been observed for

P. rapae and *Plutella xylostella* (Lepidoptera: Yponomeutidae) (Agelopoulos and Keller, 1994c). An instar specificity, in both quality and quantity, is also exhibited in the blend of chemicals emitted by feces of second and fourth instars of *P. rapae*. The quantitative differences consist of a higher emission of chemicals in feces of second instars than of fourth instars. This may be due to higher evaporation from the feces of second instars. Pellets of feces of second instars are much smaller than those of fourth instars, and consequently the number of pellets in 250 mg of feces of second instars is greater than that in the same amount of feces of fourth instars; this increases the surface area of evaporation in feces of second instars. If higher evaporation causes the greater emission of chemicals from feces of second instars, the quantitative differences between the two will be eliminated as the amount of feces of fourth instars increases. This is possible since fourth instars produce 10 times more feces than second instars. The species and instar specificity reported in feces may be due to different digestion processes of larvae and to different microorganisms inhabiting the feces of larvae.

Effects of Volatiles Released by Feces of Larvae in Host-Searching Behavior of C. rubecula. Females of *C. rubecula* are attracted to both infested plants and feces of larvae. Many volatiles emitted by feces of *P. rapae* and *P. brassicae* are also present in the blend of chemicals emitted by Brussels sprouts plants infested by either of the two species of larvae (Blaakmeer et al., 1994; Mattiacci et al., 1994). Nevertheless, some groups of chemicals appear only in one of the two infested plants or feces. For example, esters are a predominant group of chemicals related to plant damage caused by larvae and are hardly represented in feces. The same holds true for terpenoids, which are mainly emitted by infested or uninfested Brussels sprouts plants. On the other hand, pyridines are only present in feces and are totally absent in the volatiles released by plants. The question arises as to whether the chemicals utilized by *C. rubecula* to orient to feces are chemicals common to both infested plants and feces or a combination of common and specific chemicals.

The results of our bioassays showed that during landing leaves carrying large amounts of plant damage were preferred over those carrying less damage and small amounts of feces. Although landing manifests the final preference of females, it does not convey information about the different sources of information employed from a distance or while flying before landing. Specific chemicals in feces may be utilized from a distance in discriminating between plants infested by different species of *Pieris* or instars of the host. Upon landing, plant chemicals related to plant damage may be more important, because plant damage relates to the position of the larvae while feces rarely do. Fecal pellets rarely are present on the leaf of production; in most cases they fall, contaminating the lower parts of the plant and the surrounding ground. The position of feces on or around the plant can be influenced by factors such as the orientation and

structure of leaves and plant, hygienic behavior of the larvae (Usher, 1984), factors shaking the plant (wind, storms, passing animals), the size of fecal pellet (larger pellets tend to roll more easily than smaller ones), and evaporation. When fecal pellets are produced, they are more sticky than when dry.

C. rubecula also favored wet over normal feces of its host. Moisture is one of the factors increasing the release of volatile compounds from feces, and high humidity related to climatic or microclimatic conditions (e.g., dense foliage), morning dew, or rain may increase the volatile emission of feces in favor of the parasitoid.

Although at present we have a good knowledge of the identity of volatiles that may be involved in orientation to host-infested plants, much more work has to be done to understand which of those chemicals are the most reliable indicators of host presence and to understand how females of *C. rubecula* cope with the great variability associated with the chemical information available in every moment of their search for hosts.

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