

SPECIFICITY OF PHEROMONAL AND KAIROMONAL  
RESPONSE OF THE ISRAELI PINE BAST SCALE  
*Matsucoccus josephi* AND ITS PREDATOR  
*Elatophilus hebraicus*

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**Abstract**—It has been demonstrated previously that the sex pheromone of the Israeli pine bast scale, *Matsucoccus josephi*, (2E,5R,6E,8E)-5,7-dimethyl-2,6,8-decatrien-4-one (**1**) is also a potent kairomone of the scale insect's predator *Elatophilus hebraicus*. Surprisingly, the sex pheromones of *M. feytaudi* (**2**) and *M. matsumurae* (**3**) also attract *E. hebraicus*. These results have prompted us to prepare a series of analogs of **1** with variations in the two moieties attached to the C=O group (**4**–**9**) in order to probe the structure–activity relationship of the pheromonal/kairomonal response of *M. josephi* and *E. hebraicus*. The most selective and active pheromone analog is **8**, attracting only *M. josephi* males and the most selective and active kairomone analog is the *M. feytaudi* pheromone **2**, attracting only adults of *E. hebraicus*. A dose–response field test of these analogs and the chiral and racemic *M. josephi* pheromone **1** indicates that the specificity is maintained at a broad range between 25 and 400 µg corresponding to **1**. Analog **5**, which is neither a parapheromone nor a kairomone, and analog **8**, which is only a parapheromone, are not inhibitory to *M. josephi* or to *E. hebraicus*. Our study indicates that alterations in the diene side chain of **1**, common to all three *Matsucoccus* pheromones, strongly reduce the kairomonal activity while structural changes in the second side chain significantly reduce the pheromonal activity. The discovery of selective analogs of **1** has practical implications and enables specific monitoring of *M. josephi* or *E. hebraicus*. Particularly important is the possibility to mass-trap males of *M. josephi* without reducing the population of *E. hebraicus*.

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**Key Words**—*Matsucoccus josephi*, Homoptera, Matsucoccidae, *Elatophilus hebraicus*, Hemiptera, Anthocoridae, sex pheromone, kairomone, analog.

#### INTRODUCTION

The sex pheromone of *Matsucoccus josephi* Bodenheimer and Harpaz has been isolated and identified as (2*E*,5*R*,6*E*,8*E*)-5,7-dimethyl-2,6,8-decatrien-4-one (Figure 1, **1**) (Dunkelblum et al., 1993, 1995). Chiral capillary analysis and gas chromatographic electroantennographic detection (GC-EAD) was applied directly for the determination of the absolute configuration of the pheromone (Dunkelblum et al., 1995). Structural comparisons of the *M. josephi* sex pheromone has revealed similarities with those of the allopatric *Matsucoccus* spp.: *M. feytaudi* Ducasse (**2**) (Einhorn et al., 1990) and *M. matsumurae* Kuwana (**3**) (Lanier et al., 1989). All three pheromones have the same chiral ketodiene moiety (bold), with the same absolute configuration, but differ in the remainder of the molecule (Figure 1).

*Elatophilus* spp. occur in pine forests and are closely associated with pine bast scales, *Matsucoccus* spp. (Mendel et al., 1991, 1994). Generally, *Elatophilus* spp. are monophagous (Mendel et al., 1991); however, there is evidence that a single *Elatophilus* species may prey on different pine bast scales (Covasi et al., 1991). Recently we have shown that *E. hebraicus* Pericart, the principal predator of *M. josephi*, is strongly attracted to the sex pheromone of its prey (**1**) and to the pheromones of *M. feytaudi* (**2**) and *M. matsumurae* (**3**) despite the fact that these two *Matsucoccus* spp. do not occur in the natural range of *E. hebraicus* (Dunkelblum et al., 1996).

The objective of this study was to determine the specificity of the pher-

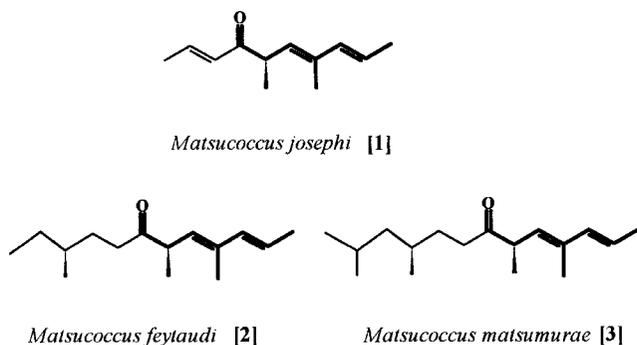


FIG. 1. The structure of the known sex pheromones of *Matsucoccus* spp. The keto-diene moiety, common to all three pheromones, is marked in bold.

omonal and kairomonal response of *M. josephi* and *E. hebraicus*, in the forest, to specially designed analogs of the *Matsucoccus* sex pheromone.

#### METHODS AND MATERIALS

**Chemicals.** Most of the tested pheromones and analogs were chiral. The new analogs (Figure 2, **6–9**) were prepared especially for this study and their quantity was very limited. Two analogs (**4** and **5**) were available only as racemic mixtures. We know from previous field work, however, that racemic pheromones could be used effectively since the unnatural stereoisomers do not affect the pheromonal and kairomonal activity (Dunkelblum et al., 1995, 1996; Jactel et al., 1994; Mendel et al., 1995). We assume that this is also the case with the analogs.

The racemic *M. josephi* pheromone (*2E,6E/Z,8E*)-5,7-dimethyl-2,6,8-decatrien-4-one (**1A** + **1B**) was available as mixture (56% *E* + 44% *Z*) from previous work (Zegelman et al., 1993). The chiral *M. josephi* pheromone (*2E,5R,6E,8E*)-5,7-dimethyl-2,6,8-decatrien-4-one (**1**) was prepared by Mori and Amaike (1994). The racemic analogs, (*6E/Z,8E*)-5,7-dimethyl-6,8-decadien-4-one (52% *E* + 48% *Z*, **4A** + **4B**) and (*6E/Z,8E*)-2,4,6-trimethyl-1,6,8-nonatrien-3-one (60% *E* + 40% *Z*, **5A** + **5B**) were prepared by the same procedure as

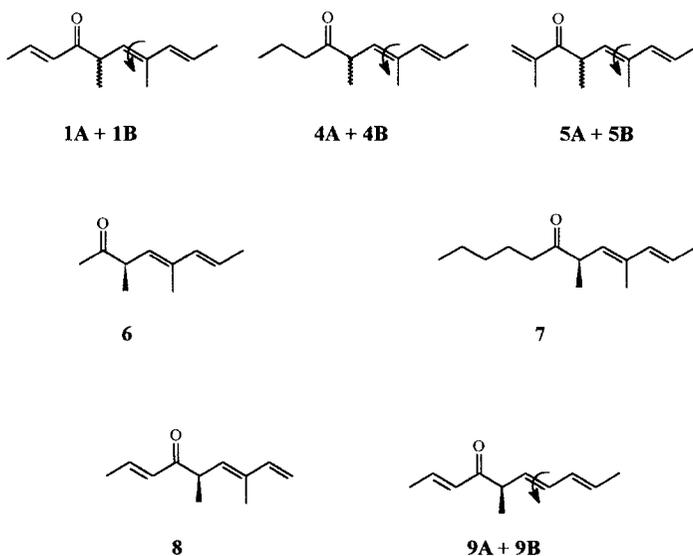


FIG. 2. The structure of analogs of the *Matsucoccus josephi* sex pheromone (**1**).

the racemic *E/Z* mixture of the *M. josephi* pheromone (Zegelman et al., 1993), starting from butyraldehyde and methacrylaldehyde, respectively (the abbreviated form of **4** and **5** will be used for the racemic analogs **4A** + **4B** and **5A** + **5B** throughout the text).

The chiral analogs (3*R*,4*E*,6*E*)-3,5-dimethyl-4,6-octadien-2-one (**6**), (2*E*,4*E*,6*R*)-4,6-dimethyl-2,4-pentadecadien-7-one (**7**), (2*E*,5*R*,6*E*)-5,7-dimethyl-2,6,8-nonatrien-4-one (**8**) and a mixture (55% *E* + 45% *Z*) of (2*E*,5*R*,6*E*,8*E*)-5-methyl-2,6,8-decatrien-4-one (**9A**) and (2*E*,5*R*,6*Z*,8*E*)-5-methyl-2,6,8-decatrien-4-one (**9B**) (in text designated as **9**) were prepared in the Mori laboratory, and the details of the synthesis will be published separately. The structures of the racemic *E/Z* *M. josephi* pheromone and analogs are presented in Figure 2.

All compounds were analyzed and their *E/Z* ratios determined on a capillary DB-5 column (30 m × 0.25 mm, J & W, Folsom, California). The column was kept at 50°C for 2 min, then programmed to 145°C at 10°C/min. After 8 min it was programmed to 200°C at the same rate. The column was operated in the splitless mode, the purge valve was opened 1 min after injection, and the carrier gas (helium) pressure was maintained at 1 atmosphere. The *Z* isomers always eluted first.

*Field Bioassay.* Five-replicate tests were set up in randomized complete blocks in the Hanita forest (Northwestern Galilee) and Yatir forest (Southern Judean Hills) in stands of Aleppo pine (*Pinus halepensis* Mill.) and brutia pine (*Pinus brutia* Ten). Triangular sticky traps, baited with rubber septa impregnated with synthetic pheromone or analog in 200 μl hexane were used. Control traps were baited with dispensers impregnated only with hexane. Traps were suspended at 1.8 m above ground at least 25 m apart. The trapped scale males and the predatory bug, both males and females, were counted under a stereomicroscope.

The first comprehensive field test was conducted in August 1997 and included all available analogs; it was repeated, at a higher flight activity of the predator, in July 1998. The tests were conducted in two forests and the activity of the analogs (at one dosage) was compared with that of the chiral *M. josephi* pheromone (**1**) and the racemic *E/Z* mixture (**1A** + **1B**) containing approximately 25% of **1**. The second experiment, conducted in May 1998, tested the dose–response of the chiral *M. josephi* pheromone (**1**), the racemic *E/Z* mixture (**1A** + **1B**) containing approximately 25% of **1**, the chiral analog (**8**) and the *M. feytaudi* pheromone (**2**) in the range of 25–400 μg corresponding to **1**. The dosages of the *M. feytaudi* pheromone (**2**) and analog (**7**) were adjusted according to their lower volatility having been previously measured for the relative volatilities of the different *Matsucoccus* pheromones (Dunkelblum et al., 1996). Analog **7** has the same molecular weight as the *M. matsumurae* pheromone and has therefore a similar volatility. The last field experiment, conducted in April–May 1999, tested the possibility that analog **5** is an inhibitor to *M. josephi* and to *E.*

*hebraicus* or analog **8** is an inhibitor to *E. hebraicus*. The attractancy of **1**, **5**, and **8** was compared with that of mixtures of **1 + 5** and **1 + 8**.

*Statistical Analysis.* Trap catch data were transformed to  $\sqrt{x} + 0.5$  and then subjected to analysis of variance, followed by the Student-Neuman-Keuls multiple range test (at  $P < 0.05$ ), to determine significance between means.

## RESULTS

The activity of analogs **4–9** was compared with that of the chiral and racemic *M. josephi* pheromone (**1**) in several field tests during 1997, 1998, and 1999. Preliminary results indicated that some of the analogs exhibit relatively high activity with significant specificity, either as parapheromones or as kairomones. The comprehensive field tests conducted in 1997 and 1998 compared the pheromonal and kairomonal activity of all analogs with that of the chiral *M. josephi* pheromone (**1**). The results (Table 1) indicate that analog **8** is an active parapheromone, analogs **4** and **9** have low activity, and the rest are inactive. Analog **4** and **7** are the only ones with considerable kairomonal activity, but much less than that of the pheromone **1**. Comparison of the activity of the *M. josephi* pheromone (**1**) with that of the racemic *E/Z* pheromone (**1A + 1B**), as well as with those of the chiral analog (**8**) and the chiral *M. feytaudi*

TABLE 1. CATCH OF MALE *Matsucoccus josephi* AND ADULT *Elatophilus hebraicus* (MEAN/TRAP/DAY)<sup>a</sup> IN TRAPS BAITED WITH *Matsucoccus josephi* PHEROMONE AND ANALOGS

Pheromone/analog	Test 1, Aug 14–26, 1997 (12 days), Hanita		Test 3, Jul 14–22, 1998 (8 days), Yatir	
	<i>Matsucoccus josephi</i>	<i>Elatophilus hebraicus</i>	<i>Matsucoccus josephi</i>	<i>Elatophilus hebraicus</i>
<b>1</b> Chiral pheromone (50 µg)	53.3a	2.8a	56.0a	26.2a
<b>4A + 4B</b> Racemic <i>E/Z</i> analog (200 µg) (~25% <i>E-R</i> )	2.3c	1.1b	8.0c	10.3b
<b>5A + 5B</b> Racemic <i>E/Z</i> analog (200 µg) (~25% <i>E-R</i> )	0.0d	0.0c	0.1d	0.4c
<b>6</b> Chiral analog (50 µg)	0.1d	0.0c	0.8d	1.1c
<b>7</b> Chiral analog (600 µg)	0.2d	0.4c	0.8d	10.6b
<b>8</b> Chiral analog (50 µg)	11.7b	0.0c	34.4b	0.5c
<b>9A + 9B</b> Chiral <i>E/Z</i> analog (100 µg) (~50% <i>E-R</i> )	1.4c	0.1c	8.4c	0.3c
Control	0.0d	0.0c	0.8d	0.5c

<sup>a</sup>Means followed by the same letter in columns are not significantly different at  $P > 0.05$ .

TABLE 2. CATCH OF MALE *Matsucoccus josephi* (MEAN/TRAP/DAY)<sup>a</sup> IN TRAPS BAITED WITH DIFFERENT DOSES OF *Matsucoccus* PHEROMONES AND ANALOGS IN YATIR FOREST, MAY 20–JUNE 1, 1998 (12 DAYS)

Pheromone/analog	Concentration ( $\mu\text{g}$ )				
	25	50	100	200	400
<b>1</b> Chiral pheromone ( <i>M.j.</i> )	48.4b;x	57.8b;x	69.3ab;x	94.0a;x	78.8ab;x
<b>8</b> Chiral analog	15.7c;y	26.3b;y	36.7a;y	29.7b;y	35.4a;y
	100	200	400	800	1600
<b>1A + 1B</b> Racemic <i>E/Z</i> pheromone (~25% <i>E-R</i> ) ( <i>M.j.</i> )	33.2d;x	42.9bc;x	66.3ab;x	63.2ab;x	95.4a;x
	110	220	440	880	1760
<b>2</b> Chiral pheromone ( <i>M.f.</i> )	0.2a;z	0.1a;z	0.1a;z	0.3a;z	0.7a;z
Control	0.3z				

<sup>a</sup>Means followed by the same letter in rows (a–d) and columns (x–z) are not significantly different at  $P > 0.05$ .

pheromone (**2**), confirm the selectivity of **2** and **8** at a wide range of loadings (Tables 2 and 3). Evaluation of the possible inhibitory effects by two representative analogs **5** and **8** indicate clearly that neither analog is an inhibitor (Table 4).

TABLE 3. CATCH OF ADULT *Elatophilus hebraicus* (MEAN/TRAP/DAY)<sup>a</sup> IN TRAPS BAITED WITH DIFFERENT DOSES OF *Matsucoccus* PHEROMONES AND ANALOGS IN YATIR FOREST, MAY 20–JUNE 1, 1998 (12 DAYS)

Pheromone/analog	Concentration ( $\mu\text{g}$ )				
	25	50	100	200	400
<b>1</b> Chiral pheromone ( <i>M.j.</i> )	21.4c;x	46.3b;x	42.3b;x	73.0a;x	41.8b;x
<b>8</b> Chiral analog	0.8b;y	0.9ab;z	1.0ab;z	0.9ab;z	1.1a;y
	100	200	400	800	1600
<b>1A + 1B</b> Racemic <i>E/Z</i> pheromone (~25% <i>E-R</i> ) ( <i>M.j.</i> )	13.0c;x	25.6b;xy	30.3ab;xy	33.4ab;y	44.1a;x
	110	220	440	880	1760
<b>2</b> Chiral pheromone ( <i>M.f.</i> )	10.3b;x	13.3b;y	20.6b;y	20.8b;y	30.0a;x
Control	0.3y				

<sup>a</sup>Means followed by the same letter in rows (a–d) and columns (x–z) are not significantly different at  $P > 0.05$ .

TABLE 4. CATCH OF MALE *Matsucoccus josephi* AND ADULT *Elatophilus hebraicus* (MEAN/TRAP/DAY)<sup>a</sup> IN TRAPS BAITED WITH *Matsucoccus josephi* PHEROMONE, ANALOGS, AND MIXTURES IN YATIR FOREST

Pheromone/analog or pheromone + analog	First period Apr 19–26, 1999 (7 days)		Second period Apr 26–May 6, 1999 (10 days)	
	<i>Matsucoccus josephi</i>	<i>Elatophilus hebraicus</i>	<i>Matsucoccus josephi</i>	<i>Elatophilus hebraicus</i>
<b>1</b> Chiral pheromone (50 µg)	59.9a	51.5a	29.5a	142.7a
<b>5A + 5B</b> Racemic <i>E/Z</i> analog (200 µg) (~25% <i>E-R</i> )	1.2c	3.7b	0.2c	7.9b
<b>8</b> Chiral analog (50 µg)	16.2b	3.2b	6.5b	7.9b
<b>1</b> (50 µg) + <b>5A + 5B</b> (200 µg) (~25% <i>E-R</i> )	54.0a	67.7a	35.4a	181.0a
<b>1</b> (50 µg) + <b>8</b> (50 µg)	67.2a	46.2a	29.2a	124.8a
Control	1.0c	3.4b	0.3c	7.3b

<sup>a</sup>Means followed by the same letter in columns are not significantly different at  $P > 0.05$ .

#### DISCUSSION

The analogs that we studied can be divided into two classes. The first one consisted of **4**, **5**, **6**, and **7** displaying alterations in the propenyl moiety characteristic of the *M. josephi* sex pheromone. The main rationale behind the design of these analogs was to evaluate the effect of saturated chains of different length on attractancy. For a valid comparison under the same conditions, analogs **4** and **5** had to be included in the test (Table 1) despite the fact that they were tested before (Dunkelblum et al., 1996). Analog **6**, with the shortest possible moiety (a methyl group), was inactive, both as pheromone and kairomone. Analog **4**, having a 3 carbon moiety, displayed a very low pheromonal activity while showing significant kairomonal attractancy. In previous field tests (Dunkelblum et al., 1996), analog **4** displayed only kairomonal activity. The branched analog (**5**) was completely inactive, both as pheromone and kairomone. Introduction of a methyl function  $\alpha$  to the ketone prevents a positive interaction with the receptors of *M. josephi* males and with the receptors of *E. hebraicus* adults, probably due to steric hindrance. Analog **7**, with a long 8 carbon moiety, exhibited only moderate kairomonal activity. The kairomonal activity of analogs **4**, **6**, and **7** depended on the length of the side chain, indicating that more than one carbon was necessary for any activity. The *M. feytaudi* and *M. matsumurae* pheromones **2** and **3**, having branched side chains, were also shown to be specific and very efficient kairomones for *E. hebraicus* (Dunkelblum et al., 1996). *M. feytaudi* **2**, which was available in larger quantity, was chosen for the present tests.

The second class of analogs consisted of **8** and **9** displaying alterations in the diene moiety, characteristic of all identified *Matsucoccus* sex pheromones. Both

act as parapheromones without any kairomonal activity. The negative influence on the pheromonal activity of the removal of the methyl group at position 7 in analog **9** (central position of the diene) was stronger than that of the removal of the terminal methyl group in analog **8**—which exhibited a higher attractancy to *M. josephi* males as compared with that of analog **9**.

The dose–response of the pheromone **1**, chiral or racemic, and of two representative analogs, **2** and **8**, was tested in order to assess the attractancy of *M. josephi* males and of *E. hebraicus* as a function of loading. Analog **8** and *M. feytaudi* pheromone **2** were chosen because they were the most selective and active parapheromone and kairomone, respectively. Analog **7** was not available in sufficient quantity for the dose–response test. A moderate and gradual increase in trap catch of both *M. josephi* males and of *E. hebraicus* as function of dose was observed (Tables 2 and 3), leveling off at a loading of 100  $\mu\text{g}$  in most cases. Both analog **8**, as parapheromone, and *M. feytaudi* pheromone **2**, as kairomone, revealed complete selectivity at the whole range of tested loadings.

All field tests showed that analog **5** is inactive, as it is neither a parapheromone nor a kairomone, and that analog **8** is a selective parapheromone with considerable activity. The question is whether these compounds are benign or inhibitory to the insects they do not attract. Equal attractancy of **1** and mixtures of **1** + **5** and **1** + **8**, rules out any inhibitory properties of both compounds (Table 4). The second period of this test was characterized by a very high flight activity of adult *E. hebraicus*, which is typical for the month of May when the adult population of the predator increases sharply almost every year (Mendel et al., 1997).

The female sex pheromone of *M. josephi* attracts conspecific males as expected, but it is also a potent kairomone, attracting both males and females of the predator, *E. hebraicus* (Dunkelblum et al., 1996). There are only very few reports on kairomonal activity of scale insect sex pheromones, particularly of parasites responding to the *Aonidiella auranti* (Maskell) and *Quadraspidiotus perniciosus* (Comstock) pheromones (see Dunkelblum, 1999, for review). The present study seems to indicate the first case of scale insect pheromone analogs displaying different specificities, as they attract either *M. josephi* males or *E. hebraicus* adults, depending on systematic structural changes. Of particular interest is the observation that alterations in the diene moiety, characteristic of all *Matsucoccus* pheromones, adversely affecting the kairomonal activity alone, while changes in the other moiety of **1** significantly reduce the pheromonal activity. This observation has practical implications, indicating the possibility of selective catch of *M. josephi* males without reducing the population of the predator *E. hebraicus*.

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