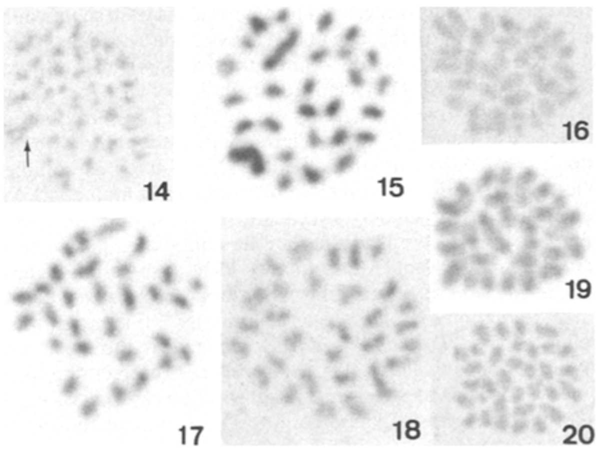

Figures 14–20. Chromosomes of *Cryptocephalus*. Spermatogonial metaphases of *C. primarius* with 2n=40, the X-chromosome arrowed (14), *C. vittula* 2n=28 (15), *C. crassus* 2n=32 (16), *C. ocellatus* (17) and *C. ochroleucus* (18) with 2n=30, *C. sexmaculatus* 2n=28 (19), and *C. fulvus* 2n=32 (20). Note the large autosome pair of *C. vittula* and *C. sexmaculatus*. Figures 14, 16 and 20 ×2000, others ×2500.

Xy_p or the Xy_r is the most primitive sex-determining system of the subfamily. Anyhow the Cryptocephalinae differ strikingly in this respect with regard to their most closely related subfamilies, such as the Megalopodinae and Clytrinae, in which unpaired sex chromosomes, X+y formula, seem to be the rule in the few investigated species of the former[15] and in several species of the latter[14,16]. In this sense, the Cryptocephalinae have kept more archaic sex-determining systems than those of Megalopodinae and Clytrinae, in spite of being more advanced on morphological grounds. Therefore, other chromosomal characteristics like the low number, 2n=20 in Megalopodinae[15] and 2n=20–24 in most Clytrinae[14,16], are probably better karyological hints of primitivism than the sex-determining systems.

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Identification of a new predaceous stink bug pheromone and its attractiveness to the eastern yellowjacket[1]

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Summary. Males of *Podisus fretus* (Hemiptera: Pentatomidae) release a long-range attractant pheromone containing linalool (49.0%), (*E*)-2-hexenal (34.5%), benzyl alcohol (12.0%), nerolidol (2.0%), α-terpineol (1.1%), and traces of several other compounds. The eastern yellowjacket, *Vespula maculifrons* (Hymenoptera: Vespidae), is attracted to artificial pheromones for *P. fretus* and for the sympatric species, *Podisus maculiventris*.

Key words. Pheromone; kairomone; semiochemical; attractant; *Vespula maculifrons; Podisus fretus;* Hemiptera; honeybee.

Foraging workers and queens of the eastern yellowjacket, *Vespula maculifrons* (Hymenoptera: Vespidae), are attracted to the pheromone of the predaceous spined soldier bug, *Podisus maculiventris* (Hemiptera: Pentatomidae)[2]. Individual components of the bug's pheromone (table 1) were unattractive to this wasp in 1983 field tests, but 1:1 mixtures of either (*E*)-2-hexenal and α-terpineol or (*E*)-2-hexenal and linalool were as attractive to yellowjacket workers as the entire pheromone in 1982 and 1983 tests[2]. Linalool constitutes less than 1% of the spined soldier bug pheromone (table 1)[3], suggesting that *V. maculifrons* is not specifically attracted to the pheromone of *P. maculiventris*[2]. However, we now report the discovery of a second *Podisus* species in our study area, provisionally identified as *P. fretus,* whose major pheromone components are (*E*)-2-hexenal and linalool. In addition, we have collected yellowjackets, honeybees, and *P. fretus* adults in pheromone-baited traps deployed in coniferous and deciduous forests for an entire season to more closely examine the chemical relationship between *Podisus* pheromones and yellowjackets.

Methods and materials. On 8 April 1984 a large dark brown female pentatomid was captured on the outside of a trap baited with *P. maculiventris* artificial pheromone. The female laid eggs, the resulting larvae were reared on *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae[4], and the first adults emerged 37 days after oviposition. The large dorsal abdominal pheromone glands were dissected from 1-week-old males[4], and the methylene chloride extracts were analyzed by gas chromatography/mass spectrometry (GC/MS) (Finnigan 4510) using a 30-m fused silica bonded methyl silicone capillary column (0.25-mm ID; 0.1-μm phase film; DB-1™; J & W Scientific, Rancho Cordova, CA), temperature programmed from 45°C (isothermal for 2 min) to 240°C at 15°/min. Compounds were identified by comparison of their electron impact mass spectrum (MS) to the published MS and/or the MS of authentic standards (nerolidol and (*E*)-2-hexenal from Bedoukian Research Inc, Danbury, CN; *cis*- and *trans*-piperitol from PCR Research Chemical Inc., Gainesville, FL; the remaining compounds from Aldrich Chemicals, Milwaukee, WI). All compound identifications were confirmed by comparison of the GC retention of the natural product to that of the standard using a Varian 3700 GC with a 15-m DB-1 capillary column, under isothermal conditions. Percentages of compounds in gland extracts were determined by GC analysis using a Shimadzu C-R3A peak area integrator.

Artificial pheromone blends for *P. fretus* and *P. maculiventris* were field tested during 1984 and 1985 in coniferous and deciduous forest tracts surrounding a 9.7-ha pasture at the Beltsville

Table 1. Compounds identified from the dorsal abdominal pheromone glands of *Podisus fretus* and *P. maculiventris* males (numbers are percentages based on GC peak areas)

Compound	*P. fretus*[a]	*P. maculiventris*[b]
(*E*)-2-Hexenal	34.52	45.07
(*E*)-2-Hexenol	0.51	–
(*E*)-2-Octenol	0.16	–
Benzyl alcohol	11.99	6.41
Benzaldehyde	0.34	–
α-Terpineol	1.14	45.10
Linalool	48.96	0.83
Terpinen-4-ol	0.12	0.93
trans-Piperitol	0.30	1.59
cis-Piperitol	–	0.07
Nerolidol	1.96	–

[a] Weighted-averages of 8 individually analyzed males; identified volatiles accounted for 98.33% of the total volatiles. [b] Composition previously reported[3,4].

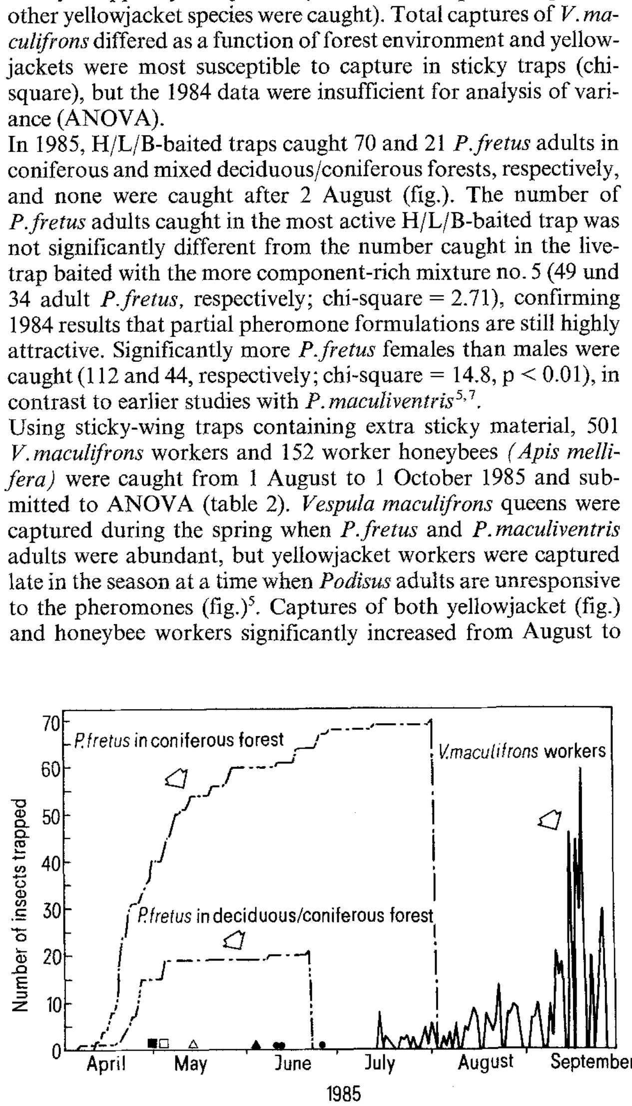
Agricultural Research Center. Maturing scrub pine and loblolly pine dominate the eastern borderland of the pasture, whereas the western borderland is a mixed deciduous forest with oaks, sweet gum, tulip tree, and red maple being common, and occasional mature pines. The northern borderland ranged from the successional coniferous forest on the east to the climax deciduous forest on the west. Beginning 31 May 1984, two *P. maculiventris* pheromone blends were tested, one containing all the components and one with all components except linalool, each made with 2 volumes of racemic α-terpineol; and five *P. fretus* pheromone blends were tested containing: 1) all components, 2) the three major components, 3) the four major components, 4) all components but with 2 volumes of racemic linalool, and 5) all components except α-terpineol and with 2 volumes of racemic linalool (proportions as in table 1). These blends were mixed with plasticized polyvinyl chloride (PVC) (20% W/W) and a 350-mg piece was used as bait every 3 days in one set of sticky-wing traps (eight traps including a control) and in two sets of traps designed to capture insects alive (live-traps) deployed as previously described[5] in the forest tracts around the pasture. Additionally in 1984, six sticky-wing traps were deployed along the northern borderland of the pasture: two traps were baited every 1–3 days with 25 µl of a 1:1 (V/V) mixture of (*E*)-2-hexenal/linalool (H/L), two traps were similarly baited with (*E*)-2-hexenal/α-terpineol (H/T), and two traps were unbaited controls. From 26 March through 1 October 1985 a PVC-formulation[5] consisting of (*E*)-2-hexenal/linalool/benzyl alcohol (H/L/B; 1:2:0.18, V/V/V) was used to bait a live-trap and a sticky-wing trap along the eastern and northern borderlands. One live-trap was baited with mixture no. 5 used in 1984 and deployed in an adjacent coniferous forest area for comparison to H/L/B-baited traps. These traps were rebaited every 3 days and monitored daily. Finally, during the same period in 1985, six sticky-wing traps fortified with extra sticky material (Tack Trap™, Animal Repellents Inc., Griffin, GA) were deployed in a coniferous forest tract behind the Insect Physiology Laboratory and the contiguous tract of deciduous forest forming the western border of the pasture; one H/L- and one H/T-baited trap, rebaited every 1–3 days with 20 µl of neat material (1:1, V/V), were deployed in each forest tract, plus unbaited control traps.

Results. The new *Podisus* species captured in our study area has characters of both *Podisus fretus* and *P. serieventris*[6], and has been provisionally identified as *P. fretus* (J. E. McPherson and T. J. Henry, personal communication).

Ten compounds were identified in the dorsal abdominal gland secretion of *P. fretus*, with linalool and (*E*)-2-hexenal (known as leaf aldehyde) accounting for 83% of the total volatiles (table 1). A total of 39 *P. fretus* adults, 208 *P. maculiventris* adults, and 432 *V. maculifrons* workers and queens were trapped in live-traps during 1984 using artificial pheromones prepared from commercially available standards of some or all of the compounds identified in each *Podisus* species. The pheromones were significantly species-specific for the bugs (1 *P. fretus* male was caught in a *P. maculiventris*-pheromone-baited trap) and, whereas *P. maculiventris* captures did not significantly differ between forest areas ($p > 0.05$, chi-square), *P. fretus* did not occur in purely deciduous forest. Partial *P. fretus* pheromones were more attractive (no. 3, $p < 0.05$) or as attractive (no. 2, $p > 0.05$) as complete pheromone no. 1, and complete pheromones containing 2 volumes of racemic linalool (no. 4 and 5) were more attractive ($p < 0.05$) than complete pheromone containing 1 volume of racemic linalool (no. 1) (chi-square). The latter result suggests that, as in *P. maculiventris*[5], the major monoterpenol in the natural *P. fretus* pheromone is one enantiomer with the antipode being inactive. *Vespula maculifrons* was by far the most commonly trapped yellowjacket (six workers representing three other yellowjacket species were caught). Total captures of *V. maculifrons* differed as a function of forest environment and yellowjackets were most susceptible to capture in sticky traps (chi-square), but the 1984 data were insufficient for analysis of variance (ANOVA).

In 1985, H/L/B-baited traps caught 70 and 21 *P. fretus* adults in coniferous and mixed deciduous/coniferous forests, respectively, and none were caught after 2 August (fig.). The number of *P. fretus* adults caught in the most active H/L/B-baited trap was not significantly different from the number caught in the live-trap baited with the more component-rich mixture no. 5 (49 und 34 adult *P. fretus*, respectively; chi-square = 2.71), confirming 1984 results that partial pheromone formulations are still highly attractive. Significantly more *P. fretus* females than males were caught (112 and 44, respectively; chi-square = 14.8, $p < 0.01$), in contrast to earlier studies with *P. maculiventris*[5,7].

Using sticky-wing traps containing extra sticky material, 501 *V. maculifrons* workers and 152 worker honeybees (*Apis mellifera*) were caught from 1 August to 1 October 1985 and submitted to ANOVA (table 2). *Vespula maculifrons* queens were captured during the spring when *P. fretus* and *P. maculiventris* adults were abundant, but yellowjacket workers were captured late in the season at a time when *Podisus* adults are unresponsive to the pheromones (fig.)[5]. Captures of both yellowjacket (fig.) and honeybee workers significantly increased from August to



Podisus fretus adults and eastern yellowjackets (*Vespula maculifrons*) caught in field traps during 1985. Plots of *P. fretus* captures are cumulative totals from four traps baited with (*E*)-2-hexenal/linalool/benzyl alcohol (H/L/B): one live-trap and one sticky-wing trap were deployed in a coniferous forest tract and a second set of traps was deployed in a contiguous tract of mixed deciduous/coniferous forest. The plot of *V. maculifrons* workers is from daily captures of four sticky-wing traps (fortified with extra sticky material) baited with either (*E*)-2-hexenal/α-terpineol (H/T) or (*E*)-2-hexenal/linalool (H/L) deployed in an adjacent forest area consisting of contiguous tracts of coniferous and deciduous forest. Symbols denote *V. maculifrons* queens caught in chemically-baited live-traps (open symbols) and sticky-traps (closed symbols) (square = H/L/B; triangle = H/L; circle = H/T).

Table 2. Eastern yellowjackets *(Vespula maculifrons)* and honeybees *(Apis mellifera)* caught in field traps baited with blends of (*E*)-2-hexenal with either α-terpineol (H/T) or linalool (H/L) from 1 August through 1 October 1985

Class	Treatment	Mean no. workers/trap[a] *V. maculifrons*	*A. mellifera*
Lure	H/T	15.9 a	2.1 b
	H/L	15.4 a	6.0 a
	Unbaited control	0.4 b	1.9 b
Forest	Deciduous	19.8 a	4.6 a
	Coniferous	11.6 b	2.1 b
Forest lure	Deciduous – H/T	22.0 a	0.8 bc
	Deciduous – H/L	17.5 ab	9.4 a
	Deciduous – control	0.4 d	3.6 b
	Coniferous – H/T	9.9 c	3.5 b
	Coniferous – H/L	13.3 bc	2.6 b
	Coniferous – control	0.4 d	0.3 c

[a] Means within a class followed by the same letter are not significantly different at the 5% level (Duncan's Multiple Range Test). Data were collected from six traps: one H/T-baited trap, one H/L-baited trap, and one unbaited control trap deployed in a coniferous forest tract and a second set of traps in a contiguous tract of deciduous forest. Means are for periods of 5 rebaiting days (eight periods) and were square root-transformed for analysis.

October ($p < 0.05$), but this trend was much greater for yellowjackets (78.6% of total variance) than for honeybees (30.2% of total variance). The mean captures for H/L- and H/T-baited traps, irrespective of forest environment, were not significantly different for yellowjackets ($p > 0.95$), but the mean number of honeybees caught in H/L-baited traps was significantly greater than that for H/T-baited and control traps ($p < 0.01$). Traps in deciduous forest caught more workers of *V. maculifrons* and *A. mellifera* than traps in coniferous forest. Although eastern yellowjacket workers did not significantly prefer H/L- over H/T-baited traps in general, analysis of the interaction of forest type and lure indicated that the responses to the lures were significantly different in the two types of forest ($p < 0.05$). In deciduous forest more yellowjackets were caught in the H/T-baited trap (+4.5 yellowjackets), but in coniferous forest less yellowjackets were caught in the H/T-baited trap (–3.4 yellowjackets) (table 2). Honeybees showed a significant preference for H/L in deciduous forest, while in coniferous forest the mean H/T-baited-trap catch was greater, but not significantly so, than that of the H/L-baited trap.

Discussion. Podisus fretus reportedly prey on conifer sawfly larvae (Hymenoptera: Diprionidae)[8], which is consistent with our discovery that this species is found primarily in coniferous forests. *Podisus maculiventris*, however, is a highly polyphagous species and occurs in a variety of habitats[6]. The discovery of a pheromone attractive to a *Podisus* species possessing characters of both *P. fretus* and *P. serieventris*[6] casts doubt on the validity of these species. In any case, the species here provisionally identified as *P. fretus* is not nearly so rare as once thought[6,9], but probably not as abundant or widespread as *P. maculiventris*.

The chemical relationship between the eastern yellowjacket and the *Podisus* stink bugs is perplexing. It is possible that the attraction of *V. maculifrons* to these pheromones is merely a coincidence. Originally, we hypothesized that the combination of leaf aldehyde with linalool or α-terpineol constitutes a damaged-leaf odor that *V. maculifrons* uses as a general kairomone to find insects feeding on leaves[2]. The coexistence of *P. fretus* with *P. maculiventris* and the finding that the pheromone released by *P. fretus* males is dominated by (*E*)-2-hexenal and linalool suggests that the chemical relationship of *V. maculifrons* to the *Podisus* pheromones may be more specific than we originally thought. One of us (JRA) has, in fact, observed *V. maculifrons* workers feeding on trapped tachinid fly parasitoids of these *Podisus* spp. (unpubl. observ.), but not on the bugs themselves. However, the fact that the population of eastern yellowjacket workers is greatest toward the season's end when *P. maculiventris* and *P. fretus* are unresponsive to pheromone, argues against a specific attraction of *V. maculifrons* to *Podisus* pheromones. Linalool is a ubiquitous floral scent[10] to which honeybees can become conditioned with little tendency for habituation[11]. Therefore, honeybees searching for nectar and pollen in deciduous woods may have been attracted to H/L-baited traps due to prior conditioning. Honeybees foraging in conifers late in the season are primarily collecting pine resin, rich in α-terpineol[12], to prepare propolis for hive repair[13]. In contrast to honeybees, eastern yellowjacket workers preferred lures containing linalool in coniferous forest and lures containing α-terpineol in deciduous forest. Thus, foraging *V. maculifrons* workers are more attracted to odors unusual in the environment. This may simply be a consequence of habituation to pervasive volatiles, such as α-terpineol in coniferous forests. In honeybees, conditioning to familiar odorants facilitates exploitation of rich food sources by mass recruitment, whereas yellowjackets seem to rely more on unusual, transient scents to help them quickly locate and individually exploit casual and more varied food sources[14].

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