Fire Ants and Leaf-Cutting Ants

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17
The Trail Pheromone Complex of *Solenopsis invicta* and *Solenopsis richteri*

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Research on the trail pheromone complex of Solenopsis invicta Buren and S. richteri Forel began over 25 years ago, when Wilson (1959) reported on its source and possible chemical nature. Since then, we have found that the trail pheromone system is part of a highly integrated symphony of pheromone and context related interactions, whose behavior releaser effects are not wholly independent. As will be discussed later, the trail pheromone can be divided into several sub-categories, although again we must not lose sight of the whole. With this thought in mind, I will present a brief background discussion on fire ant recognition and orientation mechanisms, followed by a review of the status of research on the trail pheromone.

TERRITORIALITY AND RECOGNITION

Solenopsis species are territorial by nature and tend to monopolize a food source and foraging area due to their aggressive behavior and ability to utilize trail pheromones that recruit and orient workers to specific locations (Wilson 1962). Fire ant workers, given a choice between soil from their own nest, soil from another colony's nest, or unnested soil, invariably choose to move into soil from their home nest (Hubbard 1974). To maintain a territory, worker ants must be able to distinguish nestmates from non-nestmates on at least two levels: (1) interspecific; i.e., related species such as S. invicta and S. geminata display aggressive behavior toward each other both in and out of the context of their home colony, and (2) intraspecific nestmate recognition; i.e., workers from one colony discriminate workers from another colony of the same species and defend their nest and territory. Intraspecific recognition is subtle compared to interspecies recognition, as we have observed that non-nestmate conspecifics out of their home territory context do not fight.

Conspecifics reared under identical laboratory conditions are not as aggressive toward each other as they are toward individuals from field colonies (Obin and Vander Meer, unpublished results). This suggests that context and overlying environmental odors play important roles in Solenopsis nestmate recognition (see Jaffe, Chapter 18).

Qualitatively, pheromone components are identical, but they vary quantitatively, as evidenced by significant overall differences in cuticular hydrocarbon patterns of several S. invicta colonies (Vander Meer, unpublished). An argument can be made that the natural variation of all chemicals, environmental and innate, form a constantly changing "gestalt" or nest odor that is integrated over time and iteratively learned by workers throughout the continuum of a colony.

ORIENTATION MECHANISMS

Ants have evolved a wide variety of orientation mechanisms. Although trail pheromone systems receive the most notoriety, they represent only one possible mechanism and even when utilized may not always be the dominant stimulus. The carpenter ant, Camponotus pennsylvanicus produces trail pheromones from its hindgut; but when a strong direct light is available, worker attention to trails diminishes and the ants eventually use light as their main orientation cue to reach food (Hartwick et al. 1977). The army ant, Neivamyrmex nigrescens, utilizes a combination of tactile and chemical stimuli to follow a trail (Topoff and Lawson 1979; Topoff and Mirenda 1978). Pheidole militicida uses a combination of trail pheromones (originating in the poison gland) and visual cues (Holldobler and Moglich 1980). The wood ant, Formica rufa, imprints visual cues that are maintained into the next spring by overwintering workers (Rosengren 1977).

All of the above examples represent after-the-fact orientation mechanisms where visual and/or tactile cues have already been imprinted or a chemical trail has already been laid down providing the orientation mechanism. This leaves the question of how ants initially orientate to a trail. There are several systems described in the literature. The desert ant, Cataglyphis bicolor, uses sun compass orientation to find its way back to its nest (Wehner and Lanfranconi 1981). F. pratensis workers use visual cues determined from the asymmetry of their surroundings to locate their home after finding a food source (Kaul 1983). The arboreal ant, Paltothyreus tarsatus, keeps track of its location by orientating to forest canopy patterns (Holldobler 1980). From these studies, it is evident that there are a number of orientation cues that can be used, singly or in combination.

Fire ants are very efficient foragers and search an area by

walking in a looping pattern until they find a food source (Wilson 1962). Then they lay a chemical trail directly back to their nest. Since they do not retrace their looping search path, they must utilize a different homing mechanism while laying down the initial chemical trail. Marak and Wolken (1965) found that trail-following S. invicta workers reversed direction if the light source was reversed. More recently, we investigated the effects of light on initial trail formation and showed conclusively that a light source is the dominant visual cue (not in the sun compass sense) for successful trail formation. If the light source is continuously rotated, the ants are unable to form a trail. However, once the chemical trail is established, rotation of the light has no effect on trail activity (Vander Meer and Lofgren, unpublished results). In addition to light, fire ants have been reported to exhibit context-specific positive geotaxis, the context being those workers involved as sanitary engineers (Howard and Tschinkel 1976). Stratten and Coleman (1973) found that fire ants were capable of learning the position of a food source by using distal-visual cues. So, all in all, the more we learn about fire ants, the more sophisticated and versatile their behavior appears.

The above review illustrates that multiple "correct" answers to broad behavioral questions are the rule rather than the exception when studying fire ants or any other social insect. This is further illustrated by the following detailed discussion of the trail pheromone complex.

TRAIL PHEROMONE

Wilson (1959) established that the Dufour's gland is the source of the fire ant trail pheromone. This gland is located at the base of the sting apparatus, and its contents are emitted through the stinger. A foraging worker coming back from a food source lays a chemical trail by periodically touching its stinger to the substrate on which it is walking. Other workers are recruited to the trail and, depending on the nature of the food source, reinforce the trail with pheromone. Soon a line of ants following the trail is evident. This is in fact the end product of the trail pheromone response. An understanding of the fire ant trail pheromone system, however, is dependent on a knowledge of its chemistry and the behavioral hierarchy of the trail pheromone responses. For the ants to exploit a new food source, the trail pheromone must first attract or recruit workers to the trail. One might expect that orientation along the trail would be an automatic next step, but remarkably this is not the case. It is evident now that, in S. invicta, the Dufour's gland contents contain an orientation primer pheromone that is required to release the orientation response. Thus, successful foraging for S. invicta involves a hierarchy of behavior starting with recruitment, followed

by orientation priming, followed by orientation. I will discuss our work with S. invicta, followed by a discussion of the less complicated S. richteri system, and then present an explanation of some of these results based on fire ant trail pheromone chemistry.

Orientation Sub-category

Over the many years since the discovery of S. invicta's trail pheromone (Wilson 1959), a very reliable trail pheromone bioassay has been developed that utilizes a natural worker trail leading up a ramp and over a platform to a food source. A section of the platform can be removed and replaced with a section treated with the test material (Barlin et al. 1976; Jouvenaz et al. 1978). This bioassay was used to isolate and identify two alpha-farnesene and two homofarnesene components (Fig. 1) of the trail pheromone (Vander Meer et al. 1981; Alvarez and Vander Meer 1983). Z,E-alpha-farnesene was quantitatively the major component (ca 6 ng/worker) and elicited the most sensitive response in trail orientation bioassays (0.4 pg/cm). Z,E-alpha-farnesene and an equivalent amount of Dufour's gland extract had identical activities in an orientation bioassay, which indicated that Z,E-alpha-farnesene was solely responsible for orientation activity. Williams et al. (1981a) used the same type of bioassay to isolate and identify Z,Z,Z-allofarnesene (Fig. 1) as the trail pheromone of S. invicta. However, in our laboratory we found the following: (1) the major active component in Dufour's gland extracts is not thermally labile, whereas Z,Z,Z-allofarnesene is thermally labile; (2) Z,Z,Z-allofarnesene synthesized by the method of Williams et al. (1981b) had a different retention time than the major trail pheromone component identified from Dufour's gland extracts as Z,E-alpha-farnesene; and (3) Z,Z,Z-allofarnesene gave positive results only at high concentrations compared to Dufour's gland extracts and synthetic Z,E-alpha-farnesene. Therefore, the rest of this discussion will be based on Z,E-alpha-farnesene as the major and most active trail pheromone component.

S. richteri's Dufour's gland profile is dominated by a single peak composed of two isomeric tricyclic homosesquiterpenes designated C-1/C-2. These two compounds are as active in the orientation bioassay as an equivalent amount of Dufour's gland extract. Therefore, they elicit 100% of the orientation response. C-1 and C-2 are found in S. invicta Dufour's glands at only about 75 pg/worker compared with 4000 pg/worker in S. richteri Dufour's glands. No alpha-farnesenes or homofarnesenes have been found in S. richteri's Dufour's gland extracts. Barlin et al. (1976) and Jouvenaz et al. (1978) used the ramp/platform food trail bioassay to show that the two imported fire ant species are capable of orienting to each other's Dufour's gland extracts. We now know that this bioassay

specifically measures the orientation sub-category of the trail pheromone, and we can provide an explanation of these results based on trail pheromone chemistry. No species specificity was observed in this bioassay because S. invicta is moderately sensitive to C-1/C-2 (>4 pg); and at the bioassay concentration of 0.01 worker equivalents (WE)/cm (Barlin et al. 1976), there is >10 times enough C-1/C-2 in S. richteri's Dufour's gland extract to elicit a response from S. invicta. S. richteri is not sensitive to Z,E-alpha-farnesene (>60 pg/cm) and would elicit a marginal response to 0.01 WE/cm (equivalent to 60 pg/cm Z,E-alpha-farnesene) of S. invicta's Dufour's gland. However, the amount of C-1/C-2 in S. invicta's Dufour's gland is about 10X that needed (>50 fg/cm) to elicit an orientation response from highly sensitive S. richteri.

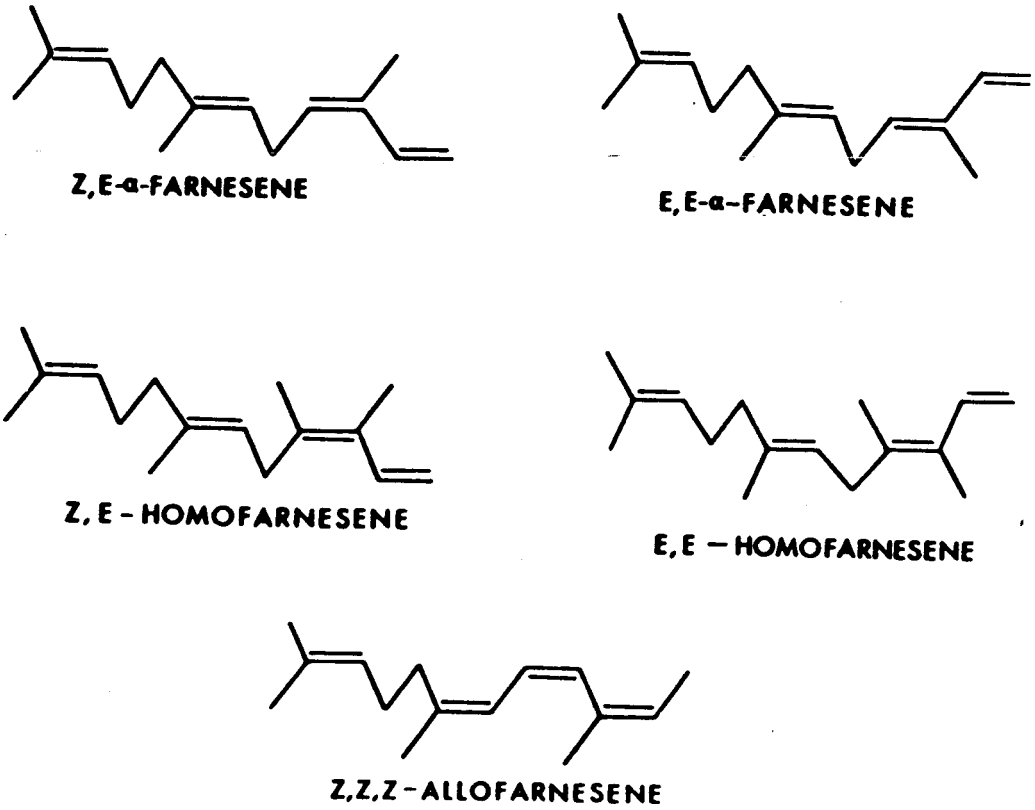

FIGURE 1. Structures of S. invicta trail pheromones isolated using a trail orientation bioassay (Vander Meer et al. 1981; Alvarez and Vander Meer 1983; and Williams et al. 1981a, b).

Recruitment Sub-category

Two bioassays were used to measure the recruitment sub-category. One was based on the response of workers to a point source of Dufour's gland extract. In this test workers are not only attracted to the spot, but they also aggregate and bite at the

substrate. The bioassay was structured so that multiple samples and controls could be run simultaneously. It was quantified by counting the number of ants responding at 5-minute intervals for a total time of 30 minutes. Unexpectedly, neither Z,E-alpha-farnesene nor the other compounds isolated using the orientation bioassay elicited recruitment activity when presented to S. invicta workers as a point source. However, we determined that 85% of Dufour's gland recruitment activity could be obtained with a mixture of Z,E-alpha-farnesene and the two isomeric tricyclic homofarnesenes, C1/C2. These compounds occur at only 75 pg of C-1/C-2 per S. invicta worker and have a profound behavioral effect in combination with the major orientation pheromone. However, the fact that we did not obtain a 100% response indicates that there are still some components missing. A similar situation exists with S. richteri, where C-1/C-2 gave 85% of Dufour's gland activity in the point source bioassay (Vander Meer et al. 1985).

Cross species tests using the point source bioassay showed clear species specificity (Vander Meer et al. 1985). This can be rationalized chemically by the fact that S. invicta requires a combination of C-1/C-2 and Z,E-alpha-farnesene. That combination is not present in S. richteri Dufour's gland extracts. S. richteri, however, requires C-1/C-2, which is over 50 times less concentrated in S. invicta Dufour's glands. Concentration/activity studies have shown that S. richteri workers do not respond in the point source bioassay to the quantities of C-1/C-2 found in S. invicta Dufour's glands (Vander Meer et al. 1985).

The second recruitment bioassay measured the attraction of workers to volatile components of Dufour's gland extracts using a Y-tube olfactometer. S. invicta responded poorly to Z,E-alpha-farnesene or C-1/C-2 alone when compared to Dufour's gland extracts; however, their response to a combination of the compounds was statistically indistinguishable from those of an equivalent concentration of Dufour's gland extract. Similarly, the response of S. richteri workers in the olfactometer to C-1/C-2 and an equivalent amount of S. richteri Dufour's glands was identical (Vander Meer, unpublished data). Olfactometer species-specificity tests comparing the responses of S. richteri and S. invicta to each others Dufour's gland extracts did not show clear differentiation. Both species showed significant attraction to each others trail pheromones, although S. invicta was most capable of responding both to its own and S. richteri's Dufour's gland extract.

Orientation Primer Sub-category

The hypothesis for an orientation primer pheromone originated with the observation that the orientation pheromone, Z,E-alpha-farnesene, did not induce orientation in randomly foraging workers.

A positive response was obtained only when test workers were already following a trail. Therefore, a bioassay was devised that measured the response of non-trailing ants to streaked test samples. We found that the addition of an equivalent amount of Dufour's gland extract at the beginning of a Z,E-alpha-farnesene trail increased the orientation activity of Z,E-alpha-farnesene greater than 4X (Vander Meer et al. 1984). In contrast, the recruitment mixture Z,E-alpha-farnesene and C-1/C-2 had no orientation priming effects. Based on the orientation and orientation primer bioassay results, we concluded that the components required to initiate orientation are not themselves required for orientation. These statements define a primer pheromone (Nordlund 1981).

The orientation primer pheromone alters a worker's physiological state such that it maximizes the behavioral releaser effects of the trail orientation pheromone. There are precedents for this concept in the literature. Ants have evolved a wide variety of mechanisms to recruit and orientate workers to food sources, ranging from primitive tandem calling in Leptothorax acervorum (Moglich et al. 1974) to the complex multiple recruitment systems of the African weaver ant, Oecophylla longinoda (Holldobler and Wilson 1978). A successful L. acervorum forager attracts a nestmate with chemicals and then physically guides them to the food source; consequently, trail orientation pheromones are not utilized. This recruitment mechanism may represent one of the first evolutionary steps toward trail pheromones and mass communication in myrmecine ants (Holldobler 1978). In other ant species, foraging workers lay down chemical trails that have orientation effects but do not release recruitment and orientation behavior. In these examples, motor displays and mechanical signals are important to induce or stimulate actual trail following (Holldobler 1978). For instance, F. fusca scouts must perform a waggle display to excite nestmates into following their trail pheromone (Moglich and Holldobler 1975). The orientation primer pheromone is the chemical analog to the physical waggle display.

Further up the evolutionary ladder, recruitment strategies involve trail pheromones that elicit recruitment, priming or modulation, and/or orientation. S. invicta falls into this category because a single glandular source is responsible for all mass-foraging activities. The orientation primer pheromone has not been chemically defined; however, species-specificity tests showed that S. invicta was primed by its own Dufour's gland extract. Surprisingly, their response to the S. richteri Dufour's gland extract increased by a factor of two. In contrast, S. richteri only responded to its own Dufour's gland extract, ignoring the material from S. invicta (Vander Meer et al. 1985). This is an example of one-way species specificity. At the present time, we do not have enough information to explain these results in terms of Dufour's gland chemistry.

SUMMARY

When species specificity bioassay results are collated, we find an almost complete range of responses (Table 1). There is (1) total lack of specificity in the orientation bioassay, (2) total specificity with the recruitment or point-source bioassay, (3) one-sided specificity using the orientation primer bioassay, and (4) ambiguous results using the olfactometer. Although this appears to be about as confusing a situation as possible, we are developing an understanding of these results based on our knowledge of the chemistry of S. invicta's and S. richteri's Dufour's gland contents. Besides the alpha-farnesenes and homofarnesenes, n-heptadecane and n-nonadecane had been reported previously in S. invicta's Dufour's gland extract but had no associated behavioral releaser effects (Barlin et al. 1976). Also present in Dufour's gland extracts (but inactive) are the five normal methyl and dimethyl branched hydrocarbons that are ubiquitous to S. invicta and are characteristic of the species (Nelson et al. 1980; Vander Meer and Wojcik 1982). Preliminary experiments indicate that these compounds are important in synergizing orientation primer effects (Vander Meer, unpublished results).

Mass-foraging in both species is moderated by chemicals produced by a single gland, and there are at least three broad behavioral categories (recruitment, orientation primer, and orientation) released by these chemicals. The simplest situation is that a single chemical or group of chemicals is responsible for all three trail pheromone sub-categories. The most complicated is if a different chemical or set of chemicals is responsible for each sub-category. S. invicta comes close to the latter situation except Z,E-alpha-farnesene is a common component for all elements of the trail pheromone. In contrast, S. richteri utilizes a single mixture of components (C-1/C-2) to release 100% of the orientation and recruitment sub-categories, and these same components release 85% of the orientation priming activity. Future research will further define trail pheromone sub-categories and their interrelationships, as well as their potential as an adjunct to control methods.

TABLE 1. Species specificity of S. invicta and S. richteri to four sub-categories of their respective trail pheromones.

Sub-category	Species	
	S. invicta	S. richteri
Recruitment (olfactometer)	±	±
Recruitment (point source)	+	+
Orientation primer	-	+
Orientation	-	-

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