

## RELEASER AND PRIMER PHEROMONES IN COLLEMBOLA

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**Abstract**—In Collembola, pheromones appear to be present in the faecal pellets. Pheromone release after cessation of faeces production points to the digestive tract as a possible site of biosynthesis.

During the pre-moulting periods Collembola do not react to pheromones, possibly due to their low activity at that time, whereas the production of the pheromones continues.

Starvation periods of up to 14 days diminish pheromone release but do not cause complete cessation. Production per animal seems to decrease at increasing densities.

The effect of pheromones on the reproductive efficiency of Collembola is discussed in the context of their physiological and behavioural ecology.

*Key Word Index:* Pheromone synthesis, Collembola, faecal pellets, starvation, instar, dose–response relationship, reproductive efficiency

### INTRODUCTION

Odours play an important role in communication among animals. These olfactory cues, which are considered as exocrine semiochemicals, function in the air (e.g. chemo-orientation in Lepidoptera; Bartell, 1977), in the water (e.g. alarm pheromones in fish; Pfeiffer, 1974), and on the ground (e.g. trail pheromones in termites; Kaib *et al.*, 1982). Also in the soil ecosystem, chemical communication has been demonstrated, viz. in the Collembola, a group of major significance (Verhoef *et al.*, 1977; Mertens *et al.*, 1979; Leinaas, 1983).

The work concerns species-specific chemicals: releaser pheromones, which elicit an aggregative behaviour in both males and females of the three epedaphic living species *Orchesella cincta* (L.), *Orchesella villosa* (Geof.) and *Tomocerus minor* (Lubbock) (Verhoef *et al.*, 1977). Pheromones are produced by males as well as females as was confirmed for the euedaphic living *Onychiurus armatus* (Tullb.) (Joose and Koelman, 1979).

The pheromones are weakly volatile in the three above-mentioned entomobryids (Verhoef *et al.*, 1977) and in the isotomid *Folsomia candida* (Willem) (Leonard and Bradbury, 1984). The pheromones of *Hypogastrura viatica* (Tullbg.) and *O. cincta* can be transported by water (Mertens and Bourgoignie, 1977; Mertens *et al.*, 1979). The antennae play an important role in the perception of pheromones (Verhoef *et al.*, 1977). According to Altner and Thies (1978) the antennae may even produce pheromones (: antennal eversible glands in *Hypogastrura socialis* (Uzel)).

It has been suggested that in Collembola the pheromones enhance reproductive efficiency (Verhoef *et al.*, 1977). Mating in Collembola is indirect; spermatophores are deposited by males and subsequently taken up by females (Schaller, 1971). Thus, aggrega-

tion of both sexes may increase the encounter frequency of females and spermatophores.

It has also been suggested that once the Collembola are aggregated, primer pheromones may elicit reproductive synchrony (Verhoef *et al.*, 1977). This hypothesis is supported by the observation that in semi-natural conditions aggregations of *O. cincta* are to a large extent in similar stages of their moulting and reproduction cycles (Joose and Verhoef, 1974). Synchronization of moulting has been described by Leinaas (1983) for *Hypogastrura lapponica* (Axelson) and *H. socialis*. He suggests that the synchronization is controlled by chemical communication through pheromones. Whether reproduction is synchronized has not yet been studied. There are, however, clues that pheromones may stimulate the spermatophore-depositing behaviour (in *Sinella curviseta* Brook; Waldorf, 1974).

Pheromones in Collembola have also a kairomone-action: predators such as the carabids *Notiophilus biguttatus* F. and *Loricera pilicornis* (Fabricius) react to places conditioned by Collembola (Ernsting, 1978; Bauer, 1982). Thus, considering the possible effects of pheromones on the population numbers of Collembola, they may have a positive as well as a negative influence.

The above literature on the substances produced by individual Collembola and received by conspecifics in which they release a specific reaction as a definite behaviour (aggregation) or a developmental process (synchronization of moulting, spermatophore production) suggests that the substances are pheromones in accordance with the definition of pheromone by Karlson and Luscher (1959).

In the present study various aspects of the pheromones are examined in two epedaphic, coexisting species (*Orchesella cincta* and *Tomocerus minor*). These are the production site, the course of production and perception during an instar, the

dose-response relationships between pheromone concentration and locomotor activity, and the effects on reproductive efficiency.

#### MATERIALS AND METHODS

The two species, *O. cincta* and *T. minor*, were collected in a mixed birch-pine forest near Hilversum and in a pine stand near Dronten, the Netherlands.

##### Localization of the pheromones

Because, in insects, pheromones have been shown to be present on the cuticle (Howard and Blomquist, 1982), and in the gut and the faeces (Byers, 1983), in the present study the attractiveness of the cuticle (as exuviae) and of faeces were examined and compared with that of the whole animal. Furthermore, to study the role of the food, the attractiveness of starved animals was measured.

Production and/or perception of pheromones was established with two types of tests: (a) Preference tests were carried out in preference boxes (see Verhoef *et al.*, 1977). In these boxes test animals can choose between nine sectors: One conditioned by either whole animals (100) or cast cuticles (= exuviae) (50) or faeces (about 10 mg dry weight) and eight unconditioned control sectors. Conditioning took place during 20 h at 20°C. For *T. minor* individual animals were tested. Each experiment consisted on 8 × 1 test animals. Previous experiments have shown that for *O. cincta* small groups of test animals (5) have to be used (see Verhoef *et al.*, 1977). The statistical analysis is given in Verhoef *et al.* (1977) and Morse (1979). (b) As the preference reaction of the animals can be analyzed as a decrease in locomotor activity at conditioned places (the so-called arrestant action), the effect of pheromones on this parameter was also used as a bioassay. The design of the apparatus is given in Fig. 1.

The activity of the test animals was studied under red light by scoring during 1 s observation periods every minute, whether individual animals were active or not. The total procedure lasted 50 min per animal. The activity was expressed as percentage of maximally possible activity.

Conditioning took place with fed animals (5) or with animals starved for 1, 2, 5 or 7 days at 20°C (4 × 5). These five conditioning procedures (each in triplicate) were distributed randomly over the 20 holes, together with 5 control tubes (not conditioned). The experiment was repeated eight times. Conditioning times was 20 h at 20°C.

These experiments on the effects of starvation on pheromone production were followed by preference tests, as described under (a). Conditioning took place with 30 animals starved for 7 days and 30 animals starved for 14 days. Each experiment was repeated six times.

##### Course of production and perception during an instar

Preference tests were carried out as described above.

The conditioning animals were synchronized by starving them for 1 week (at 20°C) and by feeding them subsequently at the same moment. From one-hundred synchronized animals, thirty specimens were taken at three times during the instar to condition the sector. These were: (1) directly after the moult, (2) two days after the moult (= the feeding period), (3) four days after the moult (= just before the next moult). On each of the three test-days, out of fifty unsynchronized, individually kept test animals, 5 animals of each of the following time points were selected. These were: (1) directly after the moult, (2) one day after the moult (= the feeding period), (3) three days after the moult (= the end of the feeding period), (4) four days after the moult (= just before the next moult).

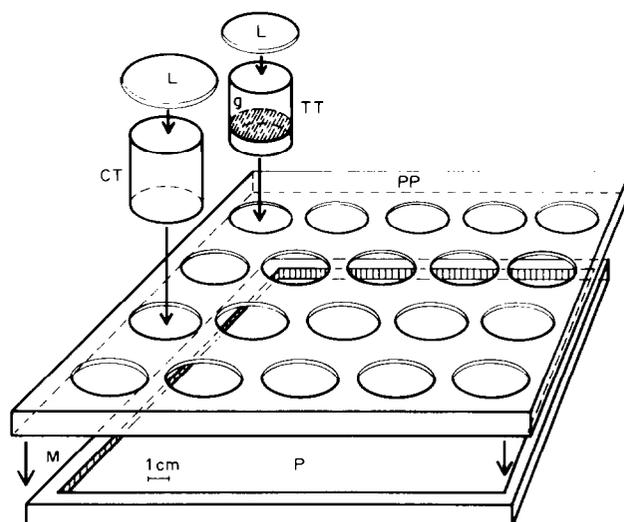


Fig. 1. Apparatus to measure the effect of the pheromones on locomotor activity. M = brass mould filled with plaster (P); PP = perspex plate with 20 holes which covers mould; CT = glass conditioning tube with lid (L). After conditioning, the 20 conditioning tubes are replaced by 20 glass testing tubes (TT), with lid (L). Test animals are situated on gauze floors (g), 6 mm above plaster floor (P).

### Dose-response relationship between pheromone concentration and locomotor activity

The locomotor activity in these experiments was measured using the apparatus shown in Fig. 1. For *O. cincta* the different pheromone concentrations were achieved by conditioning (20 h, 20°C) with 0, 5, 10 or 25 animals, each tested five times. The entire experiment was repeated five times. In the case of *T. minor* 0, 1, 5 or 20 animals were used, each tested five times. The experiment was repeated eight times.

### Primer effect of the pheromones

The effect of the pheromones on reproduction was measured by establishing the time it took a sexually mature pair of animals (1♂ + 1♀) to produce a batch of eggs. Conditioning (48 h, 20°C) took place in boxes (dia 4.7 cm) with a moist plaster floor with 0, 5, 10, 20 or 40 animals. Each experiment was repeated five times. In each box a test pair was placed and by daily observation the number of days at which 100% of the boxes of a certain pheromone concentration contained a batch of eggs, was established.

## RESULTS

### Localization of the pheromones

The results (Table 1) exclude the exuviae as a possible site of the pheromones. Faecal pellets elicit a positive reaction. However, the effect seems weaker compared with conditioning by whole animals. The effects of starvation on pheromone production in *T. minor* are shown in Fig. 2. Conditioning with fed animals (0 days of starvation) causes a decrease in locomotor activity compared with the control situation. This shows the arrestant action of the pheromones. After 24 h of starvation at 20°C faecal production ceases. Still the test animals show low activity which indicates that pheromonal action of the conditioning animals continues.

After 7 days of starvation pheromone release has decreased. By then the locomotor activity of the test animals is comparable to the control value. In the supplementary preference tests on the production of pheromones after 7 days and 14 days of starvation, the conditioned sector was favoured ( $P < 0.001$ ). This means that although the pheromone release has diminished, it is not reduced to zero.

### Course of production and perception during an instar

Pheromones are produced throughout the instar (Table 2). There is no difference between the production of feeding animals (M + 2) and that of

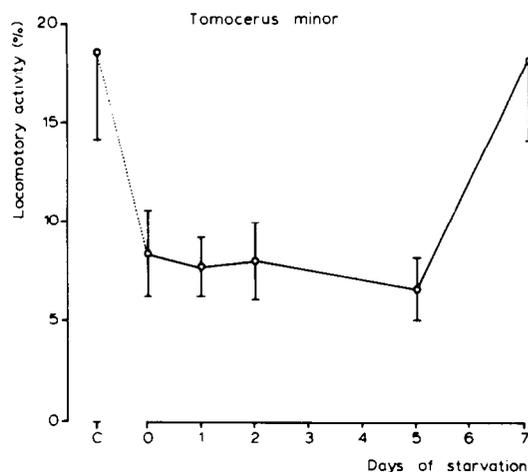


Fig. 2. The effect of the duration of starvation on locomotor activity in *T. minor*. Locomotor activity = percentage of maximally possible activity. C = control (: not conditioned).

non-feeding animals (M and M + 4). However, perception seems to depend on the physiological stage of the animals: during the pre-moulting period (M + 4) the reaction of the animals decreases. This might be caused by a decrease in the sensibility for chemical stimuli during this period. However, the general decrease in locomotor activity which characterizes this period makes it difficult to make a definite statement on perception of the stimuli.

### Dose-response relationship between pheromone concentration and locomotor activity

In both *O. cincta* and *T. minor* there is a clear relationship between pheromone concentration and locomotor activity (Figs 3 and 4).

Control *O. cincta* are much more active than *T. minor* (63.8 vs 12.3%). The arrestant effect of the pheromones is manifest more in *O. cincta* than in *T. minor*: the amount of pheromone produced by the same number of animals decreases the activity of *O. cincta* more than that of *T. minor* (e.g. 20 animals decrease the activity with 75 and 53%, respectively). The curvilinear character of the relationships given in Figs 3 and 4 might indicate a decreasing production per animal at increasing densities for both species (see Discussion).

### Primer effect of the pheromones

In Fig. 5 the relationship between pheromone concentration and reproductive efficiency is given. Reproductive efficiency is expressed as the reciprocal

Table 1. Preference tests for *O. cincta* and *T. minor* in boxes with one sector conditioned by either whole animals†, or faeces or exuviae

Tested species	Conditioning	(n)	( $P_b$ )
<i>O. cincta</i>	<i>O. cincta</i> †	5	0.0115*
<i>O. cincta</i>	Faeces of <i>O. cincta</i>	3	0.0329*
<i>O. cincta</i>	Exuviae of <i>O. cincta</i>	6	0.0535 NS
<i>T. minor</i>	<i>T. minor</i> †	6	0.00002***
<i>T. minor</i>	Faeces of <i>T. minor</i>	5	0.01084*
<i>T. minor</i>	Exuviae of <i>T. minor</i>	6	0.49327 NS

† $P_b = P$  determined by the binomial test; NS = not significant;

\* =  $0.01 < P < 0.05$ ; \*\*\* =  $P < 0.001$ .

Table 2. Tests on stage-dependent production and perception in *O. cincta*

Stage of tested animals	Stage of conditioning animals		
	M	M + 2	M + 4
M	0.0014**	0.0014**	0.0014**
M + 1	0.0014**	0.0329*	0.0014**
M + 2	0.0014**	0.0014**	0.0014**
M + 4	0.0329*	0.2634 NS	0.2634 NS

M = Directly after the moult; M + 1, M + 2, M + 4 = 1, 2, 4 days after the moult. \* = 0.01 < P < 0.05, \*\* = 0.01 < P < 0.001. In the table P determined by the binomial test is given.

of the number of days it took all pairs of a pheromone concentration to produce a batch of eggs.

The reproductive efficiency for control *O. cincta* and *T. minor* is similar. The effect of an increasing pheromone concentration is greater for *O. cincta* than

for *T. minor*. The pheromone concentration produced by 40 animals causes an increase of the reproductive efficiency by factors of 6.6 and 2.3, respectively.

Expressed in number of days this means that maximally it takes about 3 weeks before control *O. cincta* and *T. minor* produce a batch of eggs. There are control pairs which produce eggs already within 3 days. At high pheromone concentration all *O. cincta* oviposit within 3 days. *T. minor* produces the eggs within 1 week. The increase of reproductive efficiency might be caused by a synchronizing effect of the pheromones.

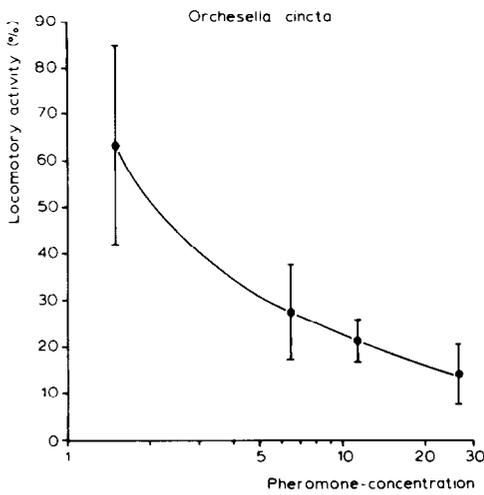


Fig. 3. The dose-response relationship between pheromone-concentration and locomotor activity in *O. cincta*. Pheromone-concentration = number of conditioning animals.

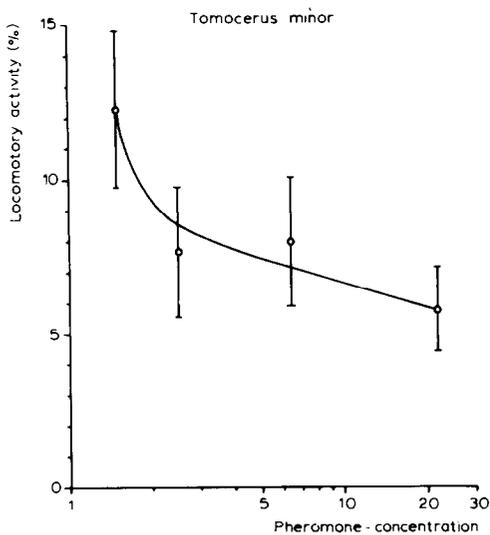


Fig. 4. The dose-response relationship between pheromone-concentration and locomotor activity in *T. minor*. Pheromone-concentration = number of conditioning animals.

DISCUSSION

The results show that the faecal pellets of *Collembola* contain pheromones. The fact that pheromone release continues after cessation of faecal production indicates that the sites of biosynthesis are located in or near the digestive tract.

The absence of attractiveness of the exuviae (i.e. cast cuticles) indicates that intact cuticles are probably not a pheromone site either. However, it is possible that the lack of attractiveness of the cast cuticles is due to disrupted pheromone production by

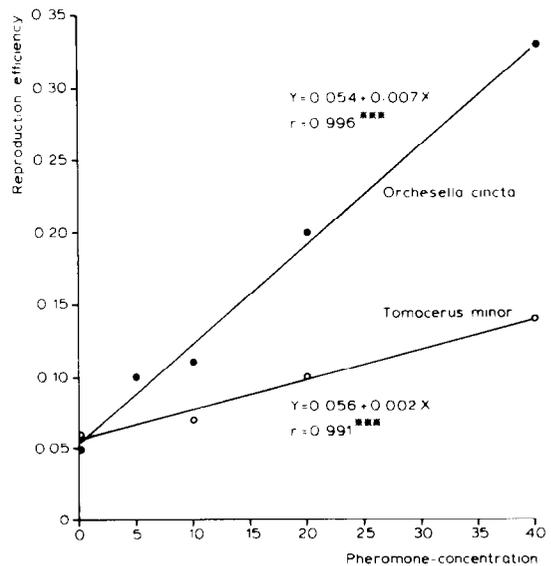


Fig. 5. The relationship between pheromone-concentration and reproduction efficiency in *O. cincta* and *T. minor*. Pheromone-concentration = number of conditioning animals. Reproduction efficiency = reciprocal of the number of days it took all pairs of animals of a pheromone-concentration to produce a batch of eggs.

the epidermal cells after ecdysis. The weakly volatile character of the pheromones could yet guarantee pheromonal action of the exuviae during a relatively long time.

It is remarkable that during the pre-moulting period when, besides the cuticle, the digestive tract also is renewed, production, or at least release of pheromones continues. In the cockroach *Blaberus craniifer* Burmeister there is no pheromone production during the pre-moulting period. However, animals in that period do not react to pheromones (Brossut *et al.*, 1974), which is in agreement with our results on Collembola.

Long-term food deprivation (more than 5 days) reduces pheromone production in *Tomocerus minor*. However, there is no total cessation of release, at least not after 14 days of starvation. This agrees with data on different cockroaches (Brossut *et al.*, 1974) and for ixodid ticks (Hajkova and Leahy, 1982).

In *Orchesella cincta* too, pheromone release has been established after 7 days of starvation (unpublished data). In these conditions this species appears to enter a state of quiescence (Testerink, 1982), reflected by a lowered metabolic rate and transpiration rate (Verhoef and Li, 1983). Even in this state pheromone release seems to be relevant.

The obtained curvilinearity of the dose-response relationship curves might indicate a decreasing production per animal at increasing densities. This has also been found in spruce budworm moths (Morse *et al.*, 1982). The lower release may reflect binding of pheromone onto the bodies of the other animals, reduction in production due to physical interaction of the animals or possible modulation of pheromone release rates by the animals when the external concentration of pheromone is high (Morse *et al.*, 1982).

Comparison of the preference-reactions shows that *T. minor* reacts more positively than *O. cincta*. This might be caused by the fact that in the case of *O. cincta* the tests were performed on groups of animals. A group itself may also function as a pheromone source.

The effect on the locomotor activity and reproductive efficiency is more evident for *O. cincta* than for *T. minor*. It is interesting to consider this in relation to their habitat selection. Although *O. cincta* and *T. minor* often coexist in forest soils, there are clear differences in their micro-distribution. *O. cincta* is found more superficially, whereas *T. minor* prefers the deeper litter layers (Verhoef, unpublished). This difference is also reflected in their physiological and behavioural ecology: *O. cincta* has a higher resistance against desiccation (Verhoef and Witteveen, 1980; Verhoef *et al.*, 1983), a higher metabolic rate (Testerink, 1982) and a higher locomotor activity (Ernsting and Jansen, 1978) than *T. minor*. The latter species is restricted to a life in rather permanent aggregations in moist places (Verhoef and Van Selm, 1983). *O. cincta* has a more dispersed distribution (Verhoef and Nagelkerke, 1977) and can be found high up in trees.

The fact that the pheromonal action in creating aggregations of reproductively synchronous animals is stronger in the more mobile, dispersed species, may lead to the hypothesis, that the progressive adaptation to terrestrial life within the group of Collembola (see Verhoef and Nagelkerke, 1977) is attended by an

increasing importance of both releaser and primer pheromones.

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