THE ATTRACTION OF *DROSOPHILA BUSCKII* BY SUBSTRATE AND AGGREGATION PHEROMONE UNDER LABORATORY CONDITIONS

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Keywords: *Drosophila busckii*, aggregation pheromones, chicory, *Erwinia carotovora* subsp. *carotovora*, pest management.

Summary

Drosophila use substrate odours and pheromones to localise oviposition places. *D. busckii* oviposits normally on rotten plant substrates, in which bacteria and yeasts grow. *Drosophila* species can be a vector for these bacteria and yeasts and infect new substrates with these microorganisms (Fogleman and Foster, 1988). Boers (1997) showed that *D. busckii* can be a vector of the bacteria *Erwinia carotovora* subsp. *carotovora* (here referred to as Ecc). Ecc induces softrot in for example chicory. *Drosophila busckii* is therefore considered a pest species in commercial chicory growth, and growers use permetrin against them. A good monitoring system is necessary to avoid useless spraying of the pesticide.

For the development of a monitoring trap, the selection of an attractive substrate is needed. Our experiments showed that the flies were significantly more attracted to thawed chicory than to thawed cauliflower. With the addition of pheromones and Ecc, the substrate became even more attractive. There is a minimum amount of pheromones and time for the bacteria needed to make the substrate attractive. The tested substrates were significantly more attractive to female than to male flies.

INTRODUCTION

In many insects that use patchy, temporary resources like fruits and fungi, the larvae and the adults aggregate at breeding places. These aggregations are important for the structure of those insect communities. The aggregations are often formed by chemical communication among conspecifics, *i.e.* by aggregation pheromones. *Drosophila* also form aggregations on temporary resources. In these rotting substrates, bacteria and yeasts are present, which can be transferred to healthy material by *Drosophila*. *Drosophila busckii* is an important vector of the bacteria *Erwinia carotovora* subsp. *carotovora* (Ecc) (Boers, 1997). Ecc is a member of the group of bacteria that induce soft rot in several vegetables, for example chicory. The bacteria are not host specific and they can only enter tissue at damaged sites. Ecc can become a serious problem during cultivation of chicory and also after harvest of the crop.

Drosophila busckii has established in the Netherlands and is an extreme generalist on many different rotting materials. Just as other *Drosophila* species, *D. busckii* uses odours of food substrates and of conspecifics (the aggregation pheromones) to localise possible feeding and/or oviposition sites. The aggregation pheromones attract both sexes equally in an olfactometer (Schaner *et al.*, 1989) and they even attract individuals that are not yet sexually active. The two major components of the aggregation pheromone of *D. busckii* have been identified as (S)-2-pentadecyl acetate and 2-pentadecanone (Schaner *et al.*, 1989). *D. busckii* is also attracted to racemic 2-pentadecyl acetate.

The commercial growing of chicory can be distinguished in two distinct phases, the root production stage and the forcing stage, in which the commercial product (the shoot) is grown from the roots. The roots of the chicory plants are harvested from a field in autumn and the major part of the leaves is removed. The roots are stored at 4°C and

after this cold period they are transferred to the growing cell (air temperature: 19°C and water temperature 22°C) where the forcing takes 3-4 weeks time. Although the roots are cleaned before transfer to the growing cell, eggs and pupae of *D. busckii* can easily be transported with the roots into the growing cell. These eggs and pupae develop into adults that can cause problems in the forcing stage of the chicory. The flies can become a pest by transferring Ecc from infected to healthy chicory shoots. The growers can decide to use permethrin to control the pest. This pesticide is now applied preventive once per two weeks. However, an environmentally more acceptable alternative would be to restrict the use of the insecticide to the times when *D. busckii* are present in the forcing cell. To enable such policy, a good monitoring system is necessary.

In this study different substrates were tested, to select an attractive substrate for *D. busckii* that can be used in a trap for monitoring or even for control of *D. busckii*.

MATERIAL AND METHODS

Drosophila busckii

A population of *D. busckii* (79 individuals) was collected from different organic waste bins near a student complex and with those individuals a culture was initiated. Later two populations were started, one that was reared on thawed cauliflower and the other on thawed chicory. These two populations were reared in two different climate rooms, both with a 16:8 L:D regime, at 20.0°C ±0.5; humidity was not controlled.

Vegetables

The cauliflower and the chicory, used for the experiments and for rearing, were bought at the local fresh market. During the season different cultivars were used but that did not seem to influence the experiments. The vegetables were stored in a refrigerator (4°C) or were frozen (-8°C) after purchase, depending on experimental treatment (see below). Freezing of the vegetables resulted in a soft substrate with a structure that is similar to a rotting substrate.

Erwinia carotovora subsp. carotovora (Ecc)

The bacteria used in the experiments were obtained from the Laboratory of Phytopathology (Wageningen University). The bacteria were cultured on nutrient agar (Schaad, 1988; Klement *et al.*, 1990) and stored in a climate chamber at 25.5°C±0.5. To infect the chicory, a colony of bacteria was first put on a new agar plate and the new colony was deluded one day later in IM PBS. The chicory was then infected by puncturing holes in it with a wooden toothpick, which was dipped in the IM PBS solution with the bacteria (10^8 bacteria per ml).

Pheromones

The aggregation pheromone of *D. busckii* is a mixture of components. The two most important components, 2-pentadecyl acetate (purchased of IPO Pherobank) and 2-pentadecanone (of Fluca Chimika), (Schaner *et al.*, 1989) were dissolved in n-hexane (pro-analysi) and stored at -20°C in a Chrompack screw cap vial (1ml) with Teflon screw cap. The amounts of pheromone used were based on Schaner *et al.* (1989), namely 120 ng 2-pentadecyl acetate and 50 ng 2-pentadecanone as one fly equivalent.

Flight cage

To test the relative attractiveness of two or more substrates, a flight cage set-up was designed. The flight cage was 29x38x55cm and the sidewalls were constructed with insect gauze; the bottom and a part of the ceiling were of plastic. In each cage about 200-400 flies of mixed sexes (sex ratio 1:1) and different ages were released. In these cages, the different substrates (6-7 g) were offered in plastic vials (3.5 cm high and a diameter of 1.1 cm of Greiner labortechnik©). Three or 4 vials per substrate type were offered

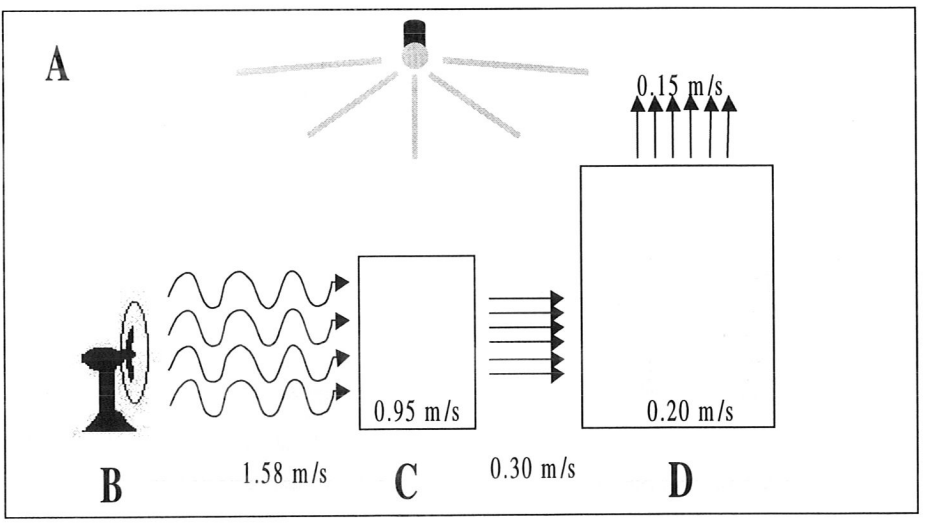
simultaneously to avoid strong location effects. For each replication new substrates were used. The vials were partly covered with parafilm leaving an opening (0.5 cm) through which the flies could enter. The vials were closed and removed from the cage after 5 or 10 minutes. The trapped flies were counted and sexed.
The experiments were executed in a fumehood. A small fan was used to create an airflow in the experimental cage. Between the experimental cage and the fan, a second insect-gauze cage was placed to reduce windspeed and make the airflow more laminar (see figure 1). Thirty minutes before the experiment started the fan and lights were turned on to allow the flies to acclimatise. Water was available for the flies in the experimental cage. The flies that were not trapped during a trial can be used as non-experienced individuals for the next trial (Bartelt and Jackson, 1984). The time between two trials with the same cage was at least 15 minutes. At most 4 trails per day were carried out per cage. Room temperature was kept at approximately 21 °C with an air-conditioner; humidity was not controlled.

Choice experiments

Several choice experiments were performed to select the most attractive substrate. A first experiment was conducted to determine whether the substrate on which the flies had been reared influenced their choice.

When in an experiment pheromones were added to a substrate, the control treatment consisted of adding the solvent (hexane) to an identically treated substrate. When the pheromones or hexane were tested for their activity without substrate, they were applied on filter paper. In experiments with thawed chicory or cauliflower, the substrate was at room temperature at the moment of the experiment.

CHICORY VS CAULIFLOWER (BOTH THAWED): Two different *D. busckii* populations were tested in separate cages: One was reared on cauliflower and the other was reared on chicory. The flies had five minutes to make a choice. The flies had been starved for at least 12 hours before the experiments started. Number of replications: 28.



A: fumehood with light source B: fan C: cage D: experimental cage

***Figure 1**: Experimental set-up of the flight cage.*

THAWED CHICORY AND PHEROMONES: The attractiveness of pheromones (5 fly equivalents) was tested both in combination with thawed chicory and without chicory. The controls consist of thawed chicory with hexane and hexane only. All four treatments were offered simultaneously. The flies had five minutes to make a choice. The flies had been starved for 12 hours before the experiment started. Number of replications: 32.

FRESH CHICORY AND PHEROMONES: The first experiments were done with either 5 or 25 fly equivalents and an experimental time of 5 minutes. When it became clear that *D. busckii* had no preference it was decided not to starve the flies, this in contrast to previous work (Bartelt *et al.*, 1989, Schaner *et al.*, 1989), to use 25 fly equivalents of pheromone and also prolong the experimental time to 10 minutes. Number of replications: 22.

ECC CHICORY AND PHEROMONES: Fresh chicory was infected with Ecc, 60 hours before the experiment, and 25 fly equivalents of pheromone were added. The *D. busckii* were not starved and they had 10 minutes to choose between the Ecc chicory with pheromones and the Ecc chicory with hexane (control). Number of replications: 16.

ECC CHICORY VS THAWED CHICORY (BOTH PHEROMONES): Fresh chicory was infected with Ecc bacteria, 60 hours before the experiment. 25 fly equivalents of pheromone were added to both the Ecc infected substrate and thawed chicory. The flies were not starved and they had 10 minutes to choose. Number of replications: 15.

Statistics

The data of the replicas were pooled. The data of the two choice tests between different substrates and the total number each sex were analysed with a χ^2-test with one degree of freedom and α=0,05. The data of the four choice test were analysed with the Kruskal-Wallis test with α=0,05 and a multiple comparison between treatments. It was assumed that the flies reacted independently of each other.

RESULTS

CHICORY VS CAULIFLOWER: The two fly populations, both the one reared on chicory and the one reared on cauliflower had a clear preference for the previously frozen chicory. (Chicory-reared flies χ^2= 312.4 and cauliflower-reared flies χ^2= 300.6, for both populations p<< 0.001) (Fig. 2). There is no effect of the flies' rearing substrate on choice.

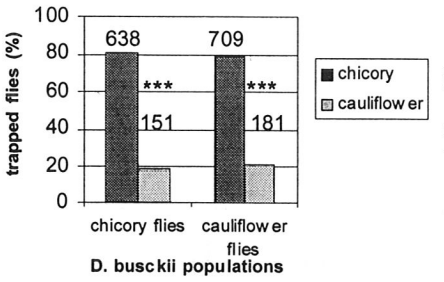


***Figure 2**: The percentage and the real numbers of the trapped flies with the chicory and cauliflower substrates for the two* D. busckii *populations. ***: p<< 0.001*

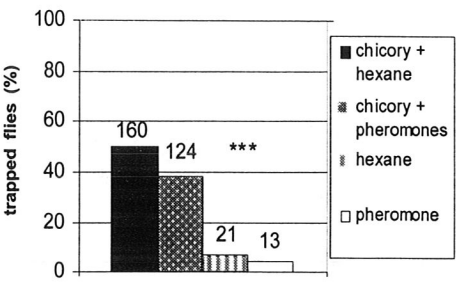


***Figure 3**: The percentage and the real numbers of the trapped flies with the different substrates ***: p<< 0.001*

THAWED CHICORY AND PHEROMONES: The results are shown in figure 3. The combination of substrate with hexane or pheromones was more attractive to the flies than hexane only or pheromones only. There was no difference in attraction between substrate with pheromones and substrate with hexane.

FRESH CHICORY AND PHEROMONES: The fresh chicory with pheromone attracted slightly more *D. busckii* than fresh chicory with hexane (control) (Table 1).

ECC INFECTED CHICORY AND PHEROMONES: Chicory that had been infected with the Ecc bacteria for 60 hours with pheromones attracted more flies than the control substrate (Table 1).

ECC INFECTED CHICORY VS THAWED CHICORY (BOTH WITH PHEROMONES): Chicory that had been infected with Ecc bacteria and to which pheromone had been added was more attractive to the flies than the thawed chicory with pheromone (Table 1).

***Table 1**: The numbers of trapped flies in the test and control substrate, the χ^2-value, the number of males and females attracted by the substrate and the levels of significance.*

Experiment	# trapped flies		χ^2 - value	p	# males trapped	# females trapped	p
	test	con					
Fresh chicory + pheromone	63	42	4.2	0.04	25	72	<<0.001
Ecc chicory + pheromone	302	166	39.5	<<0.001			
Ecc chicory + pheromone vs thawed chicory + pheromone	479	111	229.5	<<0.001			
Thawed chicory + pheromone					68	135	<<0.001

DISCUSSION

Choice experiments

Thawed chicory was more attractive than thawed cauliflower to two *D. busckii* populations, one reared on chicory and one reared on cauliflower. This shows that the substrate on which the flies had been reared did not influence their choice. In nature the flexibility of substrate means that the *D. busckii* can utilise any suitable substrate that becomes available. The fruit flies can switch to chicory plants when the plants are available in the field.

Pheromone in combination with hexane did not attract significant numbers of fruit flies. When the pheromone (at least 25 fly equivalents) was added to a substrate, however, the substrate became more attractive to the fruit flies. The odours of the substrates and the pheromone seem to work synergistically. Bartelt *et al.* (1986,1989) and Schaner *et al.* (1987) also reported this for other *Drosophila* species and other substrates. Thawed and fresh chicory, both with a low amount of pheromone did not attract more flies than the control substrate. However, when more pheromone was added, the experimental time was enlarged and the flies were not starved, then the discrimination between the substrates became stronger.

The odours of the substrate seem essential whereas the odours of the pheromone are supplementary for the choice of trapping vial. When Ecc chicory was tested against thawed chicory, both with the same amount of pheromones, the flies chose significantly more often for the Ecc chicory. This suggests that microbial growth is needed to make a substrate attractive for feeding or ovipositing.

The sex ratio of the trapped male and female flies was unequal. The female flies were attracted in larger numbers. Schaner *et al.* (1989) found for another *Drosophila* species an equal attraction of both sexes by aggregation pheromones. In contrast to the

experiments of Schaner *et al.*, in our experiments the pheromones were mostly offered to the flies in combination with substrate. This biased sex ratio, together with the fact that the flies were not starved and had had the opportunity to mate, might suggest that pheromones are most important for the flies when searching for oviposition sites.

The starvation of flies had a negative influence on the discrimination ability of the flies in our experiments. Possibly, the starvation lowered the energy level of the flies to such a level that that the selectivity of the flies was reduced

Chicory with pheromones, which was infected for 12 hours with Ecc was not more attractive to the flies than the control substrate (results not shown in this paper). However when the infection time of Ecc was prolonged to 60 hours the substrate with pheromones became highly attractive to the flies.

According to our results, the trap for monitoring the presence of *D. busckii* should contain chicory, which was infected for at least 60 hours with Ecc, together with pheromones. Preliminary results of experiments in growing cells do indeed show that traps with this substrate catch *D. busckii*.

ACKNOWLEDGEMENTS

We would like to thank Gitte Schober of the Laboratory of Phytopathology (Wageningen University) for her help and her helpful comments. We would also like to thank the PAV in Lelystad and especially Gijs van Kruistum for providing facilities to conduct experiments in the field and a chicory growth chamber.

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