Dispersal and Host Finding Behaviour of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae)

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Contents

1. Introduction

The larger grain borer, *Prostephanus truncatus* (Horn) (Col.: Bostrichidae) has long been known as a sporadic pest of stored maize (*Zea mays* L., Gramineae) in Central America and Mexico (Giles & Leon, 1974). In the late 1970s and early 1980s, the pest was accidentally introduced to East and West Africa (Dunstan & Magazini, 1981; Krall, 1984), presumably with imported Mexican maize (Nissen *et al.*, 1991), and initial attempts to inhibit its establishment and spread, by eradication campaigns in the outbreak areas, failed (Krall, 1984; Mallya, 1992). In the following years, the pest spread rapidly and to date its occurrence has been confirmed for 13 African countries (Fig. 1.1; Hodges, 1994; Adda *et al.*, 1996; Sumani & Ngolwe, 1996). In some of the outbreak countries, it has become the most serious pest of farm-stored maize and dried cassava (*Manihot esculenta* Crantz, Euphorbiaceae) (e.g., Hodges *et al.*, 1985; McFarlane, 1988).

Control of the pest could easily be achieved by fumigating the maize in air-tight bulk stores with phosphine (Hodges, 1986) or methyl bromide (Detmers, 1993), and keeping the commodities in insect-tight stores afterwards. As, however, in the affected countries, the greater part of the commodities is stored on farm and in small quantities, the methods were not considered suitable for extensive application. Binary insecticides, with a pyrethroid component to control *P. truncatus* (Giga & Canhao, 1991) and with an organophosphate against *Sitophilus zeamais* Motsch. (Col.: Curculionidae) (Dick, 1988), the most important cosmopolitan pest of stored cereals (Haines, 1991), were found to control both pests successfully, on maize grains as well as on maize cobs (Golob & Hanks, 1990; von Berg & Biliwa, 1990). However, socio-economic constraints prevented a widespread adoption of this approach, especially in West African countries (Markham *et al.*, 1994a; 1994b). Agbaka (1996) reported in a recent study conducted in south-western and central Benin that only a small proportion of the farmers used the recommended binary products for the protection of their maize stores, and noticed that misuse of broad spectrum insecticides (which were actually destined for the control of cotton pests) was prevalent. In addition, synthetic pyrethroids have already been observed to induce insecticide resistance in laboratory populations of *P. truncatus* (Golob *et al.*, 1990).

As an exotic pest which was reported to be only a minor or sporadic problem in its area of origin (Giles & Leon, 1974; Hoppe, 1986), *P. truncatus* was regarded as a prime candidate for classical biological control. *Teretriosoma nigrescens* Lewis

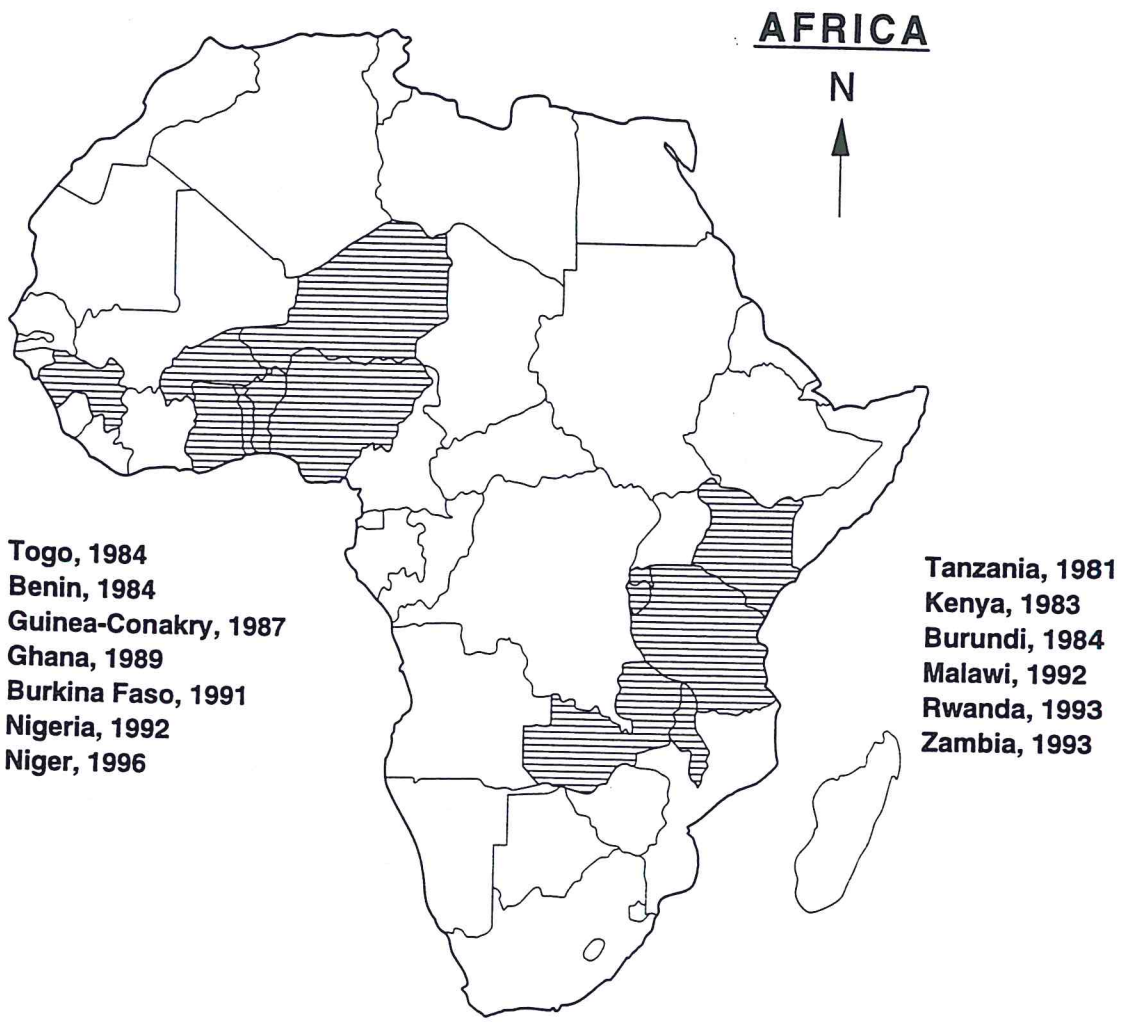


Fig. 1.1. Current distribution of *P. truncatus* in Africa.

(Col.: Histeridae) was identified as a specialized predator of *P. truncatus* in Central America and Mexico (e.g., Rees *et al.*, 1990; Boeye *et al.*, 1992), and was subsequently introduced to Togo (Biliwa *et al.*, 1992), Benin (Anonymous, 1992), Kenya (Giles *et al.*, 1995) and Ghana (Compton & Ofosu, 1994). Recent studies from south-western Benin (C. Borgemeister, pers. comm.) and from Kenya (Giles *et al.*, 1995) indicated that the introduced predator substantially reduced populations of *P. truncatus* in storage systems as well as the beetle's occurrence in non-farmland. However, it is anticipated that *T. nigrescens* alone may not solve the pest problem in Africa completely, in particular since studies conducted in Honduras and Mexico revealed that, even with *T. nigrescens* present, damage levels in maize stores may increase well over economically acceptable thresholds (Hoppe, 1986; Markham *et al.*, 1994a).

The present study was conducted within the framework of a project focussed on the development and the implementation of integrated pest management (IPM) strategies against *P. truncatus* and other storage pests. In spite of 15 years of intensive research, and while the pest's population dynamics in storage systems have been thoroughly investigated (Markham *et al.*, 1991), many questions, especially concerning the pest's behaviour and infestation strategy, are still unanswered (Hodges, 1994; Markham *et al.*, 1994a). Adaptive dispersal and location of suitable new hosts determine the survival, the colonization and the subsequent development of insect populations. A sound understanding of factors influencing these behavioural mechanisms is therefore essential to comprehend an insect's biology and ecology, and is fundamental to develop and implement IPM strategies successfully (McNeil, 1991; Markham *et al.*, 1994a).

Based on a simple conceptual model of dispersal and host finding (Fig. 1.2), I tried to clarify some of the aspects which determine the successive behavioural steps. Individual beetles disperse from a breeding habitat, which may be a maize store or dried cassava. However, it may also be a woody plant, assuming that *P. truncatus*, which belongs to a family of wood-boring beetles (Freude *et al.*, 1969), is only facultatively associated with stored commodities. The beetle has been found to reproduce in certain woody plant species in laboratory experiments (Nang'ayo *et al.*, 1993) and in the field (Ramírez-Martínez *et al.*, 1994; R. H. Markham, pers. comm.; F. Nang'ayo, pers. comm.), and has been caught with pheromone traps in non-farmland, both in the neotropics and in Africa (Rees *et al.*, 1990; Nang'ayo *et al.*, 1993; Giles *et al.*, 1995). Strong seasonal fluctuations in the flight activity of *P. truncatus* have been observed and factors influencing the suspected underlying dispersal fluctuations have been discussed (Tigar *et al.*, 1994b; Borgemeister *et al.*, 1997); however, stimuli which actually lead to dispersal remain unknown (Hodges, 1994). Therefore, I investigated intrinsic and extrinsic factors which may influence dispersal (Chapter 3).

Having left the old habitat, the beetles have to locate an appropriate new resource for feeding and reproduction, either by random or by directional search, i.e., guided by stimuli of, or associated with, the new patch (primary attraction). Investigations on suspected cues which may determine primary host finding in *P. truncatus* are described, and their relevance discussed, in Chapter 4.

Male *P. truncatus* on a suitable food source produce an aggregation pheromone (Smith *et al.*, 1996), which attracts male and female conspecifics (secondary

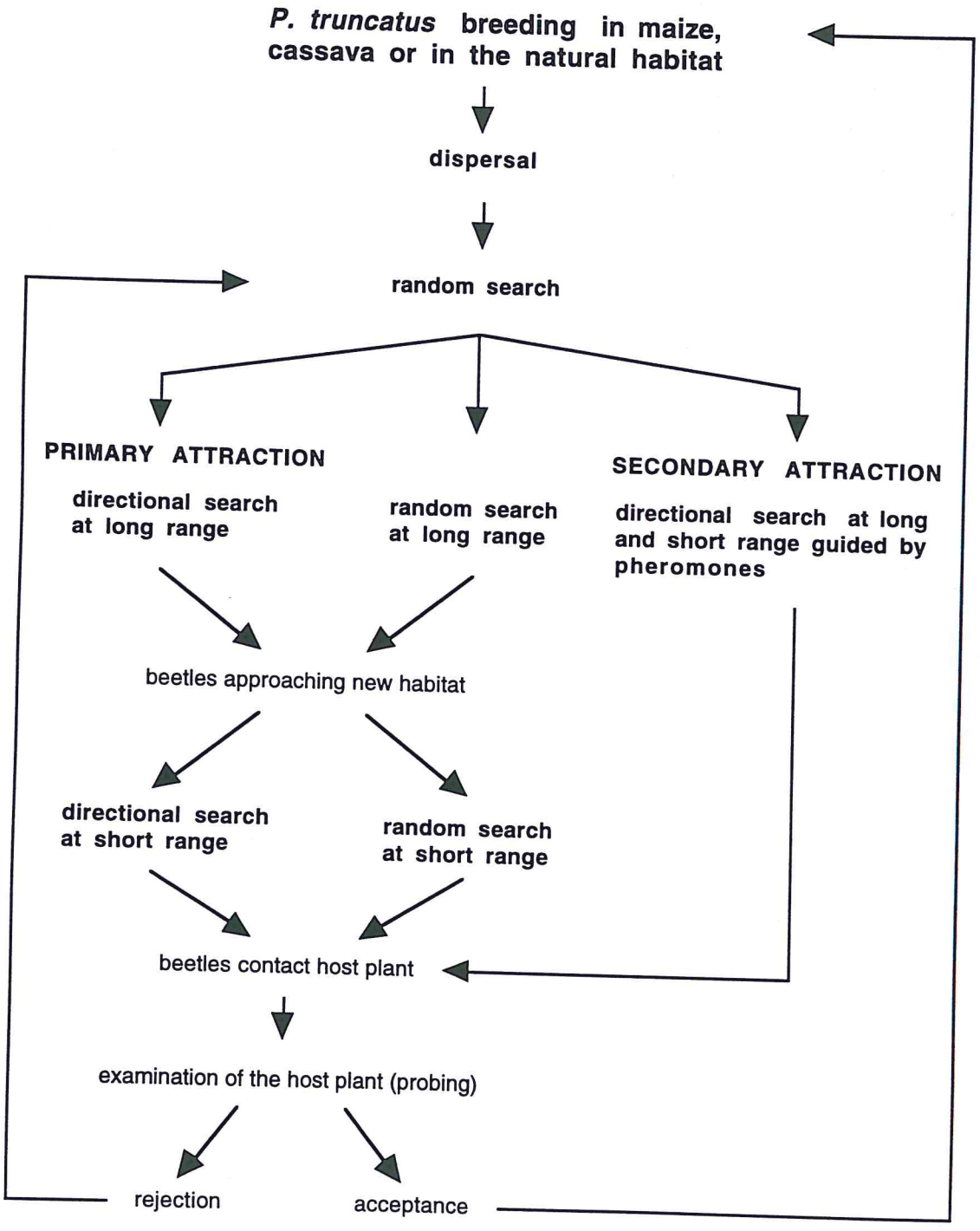


Fig. 1.2. Basic concept for the investigations of dispersal and host finding of *P. truncatus*.

attraction). The two main components of this pheromone, trivially named Trunc-call 1 (or T1) and Trunc-call 2 (or T2) have been identified (Cork *et al.*, 1991), and the synthetic analogs are commercially available, mainly used for monitoring purposes (e.g., Richter & Biliwa, 1991; Tigar *et al.*, 1994b). Conditions of pheromone production and behavioural as well as physiological responses to the pheromone were studied, and the role of small initial store infestations, possibly initiated by individual males, discussed (Chapter 5). Since *T. nigrescens* responds to its prey's aggregation pheromone (Boeye *et al.*, 1992), the predator was included in part of the investigations.

Extensive definitions of biological terms are avoided within the main text, but can be found in the appended glossary (Chapter 7).

Unless stated otherwise, experiments were conducted at the Biological Control Center for Africa of the International Institute of Tropical Agriculture (IITA) in Abomey-Calavi (near Cotonou), Republic of Benin, West Africa.

2. General materials and methods

2.1. Maize variety

Maize cobs, grains and maize flour used in any of the experiments were of the improved IITA maize variety TZSR-W (Meikle *et al.*, 1997a).

2.2. Insect rearing

Initial laboratory cultures of *P. truncatus* were set up with individuals collected in the Mono District of south-western Benin in 1992. Freshly collected beetles, originating from the same region, were routinely added to the cultures, every four to six months. The insects in these basic cultures were reared on 350 g maize grains in 1000 ml glass jars covered with brass gauze at 30 ± 1 °C and 75 ± 5% r.h. under a L12:D12 photoperiod. The maize was infested with 200 unsexed adult *P. truncatus*, all of which were removed after two weeks to obtain a synchronized F1. The progeny of these basic cultures was used in many of the experiments described in this thesis.

2.3. Sex determination

In this study, the sex of live and dead *P. truncatus* adults was determined by carefully squeezing the abdomen of the beetles, which extrudes the genitals, since external sex determination based on clypeal tubercle differences (Shires & McCarthy, 1976) reaches only an accuracy level of about 90%. When genital examination was used to sex live beetles, care was taken not to injure the insects, and only obviously non-injured individuals were selected. To verify if this sex determination method caused internal injuries which were externally invisible, the mortality and reproduction of insects sexed by this method were investigated.

Potential effects on mortality

Maize grains (170 g in 250 ml glass jars) were infested with 100 obviously non-injured *P. truncatus* whose sex had been determined with the method described above, and maintained at 30 ± 1 °C and 75 ± 5% r.h. under a L12:D12 photoperiod.

Table 2.1. Effects of sex determination by genitalia examination on mortality and reproduction. Means ± s.e.; mortality and reproduction did not differ significantly between the two treatments (mortality: Wilcoxon signed ranks test, p=0.13; reproduction: t-test, p=0.90).

	sex determination	
	genitalia examination	none or according to clypeal tubercle differences
mortality in % (after 14 days)	6.8 (± 1.0)	9.1 (± 1.1)
progeny (produced during 14 days)	33.4 (± 1.9)	33.8 (± 2.0)

Maize grains infested with 100 unsexed beetles served as controls. Ten replicates were conducted, one replicate (consisting of an unsexed and a sexed treatment) being always set up with beetles from the same basic culture jar. After 14 days, all insects were removed from the grains and the mortality determined. Mortality did not differ significantly between the two treatments (Table 2.1; Wilcoxon signed ranks test, p=0.13; Siegel & Castellan, 1988).

Potential effects on reproduction

Maize grains in small glass jars (6 cm high, 2 cm inner diameter) were infested with one male and one female adult *P. truncatus* and maintained under the same conditions as in the previous experiment. Beetles had previously been sexed either by genitalia examination, as described above, or according to clypeal tubercle differences (Shires & McCarthy, 1976). The adults were removed after 14 days and their sexes verified by dissection. Only replicates where both individuals were alive and where the second sex determination confirmed that a male and a female had been present were kept for another 35 days (n=73 for the treatment 'genitalia examination' and n=69 for the treatment 'clypeal tubercle'), and the produced adult progeny was counted. There was no significant difference between the numbers of progeny in the two treatments (Table 2.1; t-test, p=0.90; Sachs, 1991).

It can therefore be concluded that sex determination by squeezing the abdomen of the beetles, which extrudes the genitals, and selecting only non-injured beetles does not influence mortality and reproduction. Genitalia examination can therefore be regarded as an appropriate method to sex live *P. truncatus* adults accurately.

2.4. Statistical Analyses

Unless stated otherwise, statistical analyses were performed with Statview 4.01 (Abacus Concepts, 1992) on a Macintosh Powerbook 180.

3. Dispersal

3.1. Introduction

There has been much discussion on the correct use of the terms 'dispersal' and 'migration' (Kennedy, 1961; Johnson, 1969; Dingle, 1972; 1985; and references therein). Originally, 'migration' was used to describe the transfer of populations from place to place by mass flight, while 'dispersal' was defined as the scattering of populations. These definitions, however, have not been found to describe the movements of insects between habitats satisfactorily. Also the distinction between migration being an active and dispersal being a passive movement is not tenable, as, for instance, butterflies flying from flower to flower are changing their locations actively, but are surely not migrating. Migration can rather be considered to be a specialized behaviour for the displacement of the individual in space (Dingle, 1985), an adaptive change of the breeding habitat, an adaptive dispersal (Johnson, 1969). In this sense, the two terms 'migration' and 'dispersal' are used synonymously in this thesis, referring to *P. truncatus* leaving, and having left, its habitat by flight.

Dispersal is an important factor in the dynamics of most insect populations, as the resources in a given habitat are usually time-limited. When resources become scarce and insect populations more dense, only dispersing to new habitats can secure successful reproduction (Dingle, 1985). On the other hand, leaving the original habitat increases the risk of mortality and uses up energy which could otherwise be spent on reproduction. Hence, dispersal should only occur, when the probability of finding an appropriate resource is high enough to compensate for the costs of dispersal and for the loss of the advantages that could have been gained from the former location (Parker & Stuart, 1976; Ziegler, 1978). The 'decision' to disperse or not to disperse should, therefore, be determined by factors that either reflect the decreasing quality of the original patch, for instance, prolonged search for suitable oviposition sites (Roitberg *et al.*, 1982), decrease in food value (Johnson, 1969) or crowding (Shaw, 1970), and/or indicate the likelyhood of a successful search for other suitable habitats, for example, temperature and humidity changes (Borden *et al.*, 1986) or other seasonal cues (Dingle, 1972; 1985), which possibly coincide with the availability of adequate new resources.

In a first approach, I investigated therefore the effect of crowding on the dispersal of *P. truncatus* (Chapter 3.2). In addition, it was postulated that the male-produced pheromone may act as an indirect indicator for habitat suitability. The pheromone

produced by *P. truncatus* may not only attract conspecifics to suitable new food sources (cf. Chapter 5), but may also act as an arrestant, keeping the insects in already colonized patches, which still have some food value. The low pheromone concentration in dense populations (Smith *et al.*, 1996; cf. Chapter 5) would thereby promote dispersal, while a high concentration (under natural conditions associated with low densities, cf. Chapter 5.2) would have an arrestant effect on the beetles.

In a second experiment, seasonal effects on flight activity and flight initiation were investigated (Chapter 3.3). The numbers of *P. truncatus* caught with pheromone traps and the numbers of *P. truncatus* flying off maize cobs were registered for nine months, covering times of the year with expected high and low flight activity (Borgemeister *et al.*, 1997).

Great seasonal fluctuations of the number of *P. truncatus* caught with pheromone traps were reported by Tigar *et al.* (1994b) in Mexico, by Giles *et al.* (1995) in Kenya and by Borgemeister *et al.* (1997) in Benin. Since a female-biased sex ratio was observed among *P. truncatus* trapped during a period of high flight activity (cf. Chapter 5.5.2), peaks in trap catches may at least partly be explained by an increased number of females dispersing. Therefore, the sex ratios among *P. truncatus* caught with pheromone traps were determined during the course of a year (Chapter 3.4).

Besides investigating the influence of external factors on flight activity, I wanted to determine the physiological characteristics of migrating *P. truncatus* (Chapter 3.5). Physiological age and ovarian developmental stage may provide insight into migration strategies (Johnson, 1969; Ziegler, 1977), and may thereby help to better understand the entire role of dispersal in the life of the beetle. Moreover, the mating status of dispersing *P. truncatus* has so far not been described. As the aggregation pheromone is produced by male *P. truncatus*, males have been regarded as the sex which initiates new colonizations (Hodges, 1994; Fadamiro, 1995). If, however, females mate before dispersal, they may act as colonizers independently of males. The reproductive systems of male and female *P. truncatus*, and the development of a method for age-grading in *P. truncatus* are described in Chapter 3.5. On the basis of these observations, the age composition of, and ovarian development in, the dispersing part of the population were evaluated, and the mating status of the females determined.

3.2. Effect of crowding and aggregation pheromone

3.2.1. Materials and methods

General experimental set-up

Maize grains were deep frozen for two weeks in order to disinfest the maize from hidden insect infestations and afterwards reconditioned at 30 ± 1 °C and 75 ± 5% r.h. for at least 14 days. Eight glass jars (250 ml, screw top with brass gauze) were filled weekly with 175 g maize grains. Two jars at a time were infested with 20, 50, 150 or 300 unsexed *P. truncatus* adults and kept under the same conditions as the basic cultures (cf. Chapter 2.2). After 14 days, all adults were removed in order (a) to separate the parental generation from the F1, (b) to be able to determine the sex ratio in the parental generation and in the F1 separately, and (c) to ensure that all beetles of the F1 were in principle old enough to take flight before the end of the experiment (beetles show little flight activity before day 6 after emergence; Fadamiro, 1995). The sex of all adult beetles was determined for numbers smaller than 100 per jar; for numbers greater than 100, a subsample of 100 beetles was sexed. The sex ratios and the numbers of females per jar were calculated on the basis of these counts.

After 31 days, when the first beetles started to emerge, the jars were transferred into greenhouses (27-31 °C, 40-100% r.h., natural photoperiod *ca.* L12:D12 during the whole experimental period), where the experimental units had been set up. The experimental unit (Fig. 3.1) consisted of a light-permeable, polyethylene bucket, which was inverted and fitted to the top of a funnel. The removable collection container was attached at the exit pipe of the funnel and the open culture jar was placed on two metal bars (2 mm diameter), which had been inserted across the top of the funnel. Beetles flying off the cultures hit the walls and dropped into the collection container, which they could not leave as the surface was too smooth to climb and as the flight behaviour of *P. truncatus* is not accurate enough to permit them to fly out. Before the jars were put into the experimental units, a pheromone vial containing the synthetic analogs of the aggregation pheromone (1 mg of T1 and 1 mg of T2; AgriSense-BCS Ltd., Pontypridd, UK) was wrapped into brass gauze (for protection against boring of the beetles) and inserted in one jar of each initial density. The remaining jars were treated similarly with an empty polyethylene vial. A piece of brass gauze was folded into a square and placed in the center of each jar as a flight platform, since *P. truncatus* cannot climb smooth surfaces and prefers to

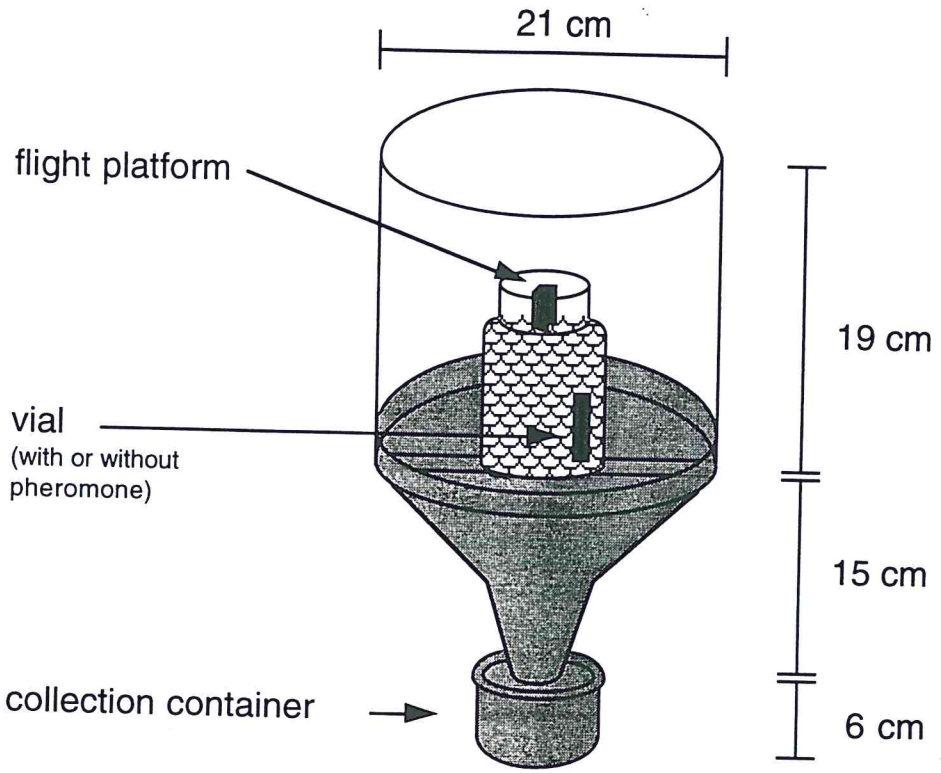


Fig. 3.1. Experimental unit.

take off from elevated locations (Fadamiro, 1995). The jars treated with the pheromone were placed in a different greenhouse from those without the synthetic pheromone. After half of the replicates had been completed, the experimental locations were exchanged.

For the following 28 days, the beetles which had flown off the cultures and had gathered in the collection containers were collected three times per week, counted and deep frozen, separately for each jar. After the final collection on day 28, all *P. truncatus* still left in the jars were counted and deep frozen for each jar separately. The beetles were sexed and the sex ratios determined as described above. Twelve replications, consisting of the eight treatments (four densities × two pheromone treatments), were carried out.

Time-of-day dependent flight initiation

As preliminary observations revealed little flight initiation during the day, and peaks in flight activity were observed at dusk and dawn (e.g., Tigar *et al.*, 1993), the beetles which had flown off the cultures were collected at 5, 6, 7, 8, 9, 17, 18, 19, 20 and 21 hours, and counted. This was done over four successive days for 24 culture jars (four initial densities × two pheromone treatments × three replications) simultaneously.

Statistics

Wilcoxon signed ranks tests were applied (for each initial density separately) to test for differences between the two pheromone treatments in regard to the numbers of beetles flying, the dispersal rates, the total numbers of progeny and the progeny per initial female. The effect of the initial densities on the four measurements was tested using Friedman two-way analysis of variance by ranks, followed by a test for multiple comparison (Siegel & Castellan, 1988). As residual examinations suggested logarithmic transformations of the data (Montgomery & Peck, 1992), linear regression models were applied to the log(number of *P. truncatus* flying) and log(% of *P. truncatus* flying) versus log(number of progeny) as described in Sokal & Rohlf (1995). The regression equations were compared according to the formulas given in Sachs (1991).

The proportions of females which flew off, stayed in the jar and of the total progeny were compared with a Friedman two-way analysis of variance by ranks, followed by a test for multiple comparison ($p<0.05$). The proportions of females in the initial set-ups and of the progeny were compared with a Wilcoxon signed ranks test (Siegel & Castellan, 1988).

3.2.2. Results

Influence of initial densities and pheromone treatment

No significant differences could be detected between the treatments with and without pheromone in regard to the number of *P. truncatus* flying, the dispersal rate (% of progeny taking flight), the number of total progeny and the progeny per initial

Table 3.1. Influence of initial densities on reproduction and on flight initiation of the progeny. Means ± s.e.; means within a row differ significantly when followed by different letters (Friedman two-way analysis of variance by ranks followed by a multiple comparison test, $p<0.05$).

	initial densities (*P. truncatus* adults)			
	20	50	150	300
total progeny	193.5 a (± 19.0)	346.2 a (± 17.9)	661.6 b (± 27.8)	794.3 b (± 39.5)
progeny per initial female	19.3 a (± 1.5)	14.3 a (± 0.8)	8.9 b (± 0.4)	5.1 c (± 0.3)
progeny taking flight	41.7 a (± 7.8)	105.5 a (± 11.1)	224.4 b (± 17.0)	239.6 b (± 17.4)
% progeny taking flight	18.6 a (± 1.9)	28.9 b (± 2.0)	33.3 b (± 1.8)	30.1 b (± 1.5)

female ($p>0.05$, Wilcoxon signed ranks tests for each initial density). Therefore the data of the two treatments were pooled.

The total number of progeny (number of *P. truncatus* flying plus number of *P. truncatus* left in the jar at the end of the experiment) increased with increasing initial densities (Table 3.1). However, significant differences existed only between the lower (20, 50 *P. truncatus*) and the higher initial densities (150, 300 *P. truncatus*). The total progeny per initial female decreased steadily with increasing initial densities (Table 3.1). Significant differences existed between the low density (20, 50 *P. truncatus*), the 150 and the 300 *P. truncatus* treatments.

The number of *P. truncatus* taking flight increased with increasing initial densities (Table 3.1). Again, significant differences existed only between the lower (20, 50 *P. truncatus*) and the higher initial densities (150, 300 *P. truncatus*). Regarding the dispersal rates (% progeny taking flight), only the dispersal rate of the cultures with the lowest initial densities was significantly different from the ones of the three higher initial densities. Approximately 10% more progeny dispersed from the higher density cultures.

Influence of progeny densities and pheromone treatment

A linear regression was performed on the log-transformed data, 'number of *P. truncatus* flying' (log(F)) versus 'total number of progeny' (log(P)), separately for the two pheromone treatments. The two significant ($p<0.0001$) regressions

$\log(F) = -1.607 + 1.392 \log(P)$, $r^2=0.92$ (for the treatment 'without pheromone') and
$\log(F) = -2.008 + 1.534 \log(P)$, $r^2=0.87$ (for the treatment 'with pheromone')

did not differ significantly with respect to slope, intercept and r^2 ($p>0.05$, regression comparison tests). Using the pooled data of both treatments, the relationship between the number of *P. truncatus* flying (F) and the total number of progeny (P) can, therefore, be described with the exponential function (Fig. 3.2 A):

$F = 0.015\ P^{1.465}$,
($p<0.0001$), $r^2=0.92$ for the treatment 'without pheromone' and $r^2=0.86$ for the treatment 'with pheromone'.

Analysing the influence of progeny densities on dispersal rates (% progeny taking flight), a two phase regression model was applied (for progeny densities smaller than 400, consisting of progeny densities deriving from the 20 and 50 insect treatments only, and for progeny densities greater than 400). A linear regression was performed on the log-transformed data: 'percentage of *P. truncatus* flown' (log(%F) versus 'total number of progeny' (log(P)), separately for the two pheromone treatments. For progeny densities greater than 400, the regressions were not significant, either considering the two treatments separately, or for the pooled data (Fig. 3.2 B; $n=56$; $p=0.68$, $r^2=0.006$ for the treatment without pheromone and $p=0.64$, $r^2=0.008$ for the treatment with pheromone). The dispersal rate averaged 32.3%.

For progeny densities of up to 400, regressions of data from both treatments were significant ($p< 0.0001$):

$\log(\%F) = -0.428 + 0.751 \log(P)$, $r^2=0.67$ (for the treatment 'without pheromone') and
$\log(\%F) = -0.523 + 0.755 \log(P)$, $r^2=0.42$ (for the treatment 'with pheromone').

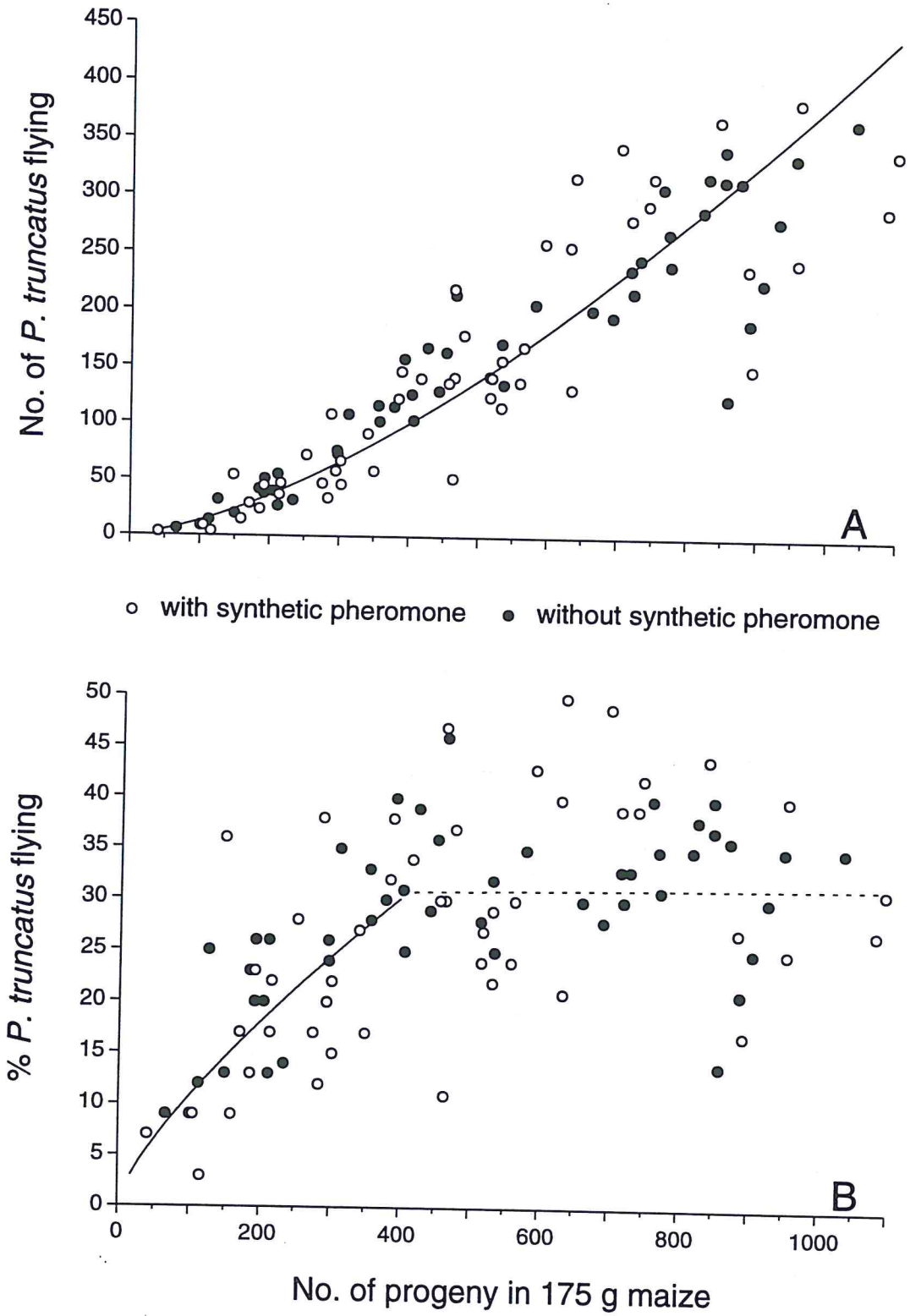

No. of P. truncatus flying
450
400
350
300
250
200
150
100
50
0
A
with synthetic pheromone
without synthetic pheromone
% P. truncatus flying
50
45
40
35
30
25
20
15
10
5
0
B
0
200
400
600
800
1000
No. of progeny in 175 g maize

Fig. 3.2. (previous page) Number of *P. truncatus* taking flight (A) and dispersal rates (B) in relation to progeny densities. (A) The solid line represents the regression curve for the pooled data of both treatments. (B) The solid line represents the significant regression curve for the pooled data of both treatments for progeny densities smaller than 400, the dashed line the non-significant regression curve for progeny densities greater 400.

The regression curves did not differ significantly regarding slope, intercept and r^2 ($p>0.05$, regression comparison tests). Using the pooled data of both treatments ($n=40$), the relationship between the number of *P. truncatus* flown (%F) and the total number of progeny (P) can, therefore, be described with the exponential function (Fig. 3.2 B):

$\%F = 0.343\ P^{0.747}$, with $p<0.0001$, $r^2=0.62$ for the treatment 'without pheromone' and $r^2=0.39$ for the treatment 'with pheromone'.

Sex ratios

The percentage of females in the set-ups (which can be regarded as samples from the basic cultures) averaged 50.2% (s.e.=0.79). This proportion did not differ significantly from the proportion of females of the total progeny (51.5%, s.e.=0.46) ($p=0.43$, Wilcoxon signed ranks test). However, the female proportions of the beetles that flew off, that stayed in the jar and of the total progeny differed significantly (Table 3.2). In 95 of the 96 samples, more females than males migrated.

Table 3.2. Proportions of females among beetles flying, in the jar and of total progeny. Means ± s.e.; means in a row differ significantly when followed by different letters (Friedman two-way analysis of variance by ranks followed by a multiple comparison test, $p<0.05$).

% of females among beetles		
flying	in jar	total progeny
66.6 a (± 0.9)	46.4 b (± 0.6)	51.5 c (± 0.5)

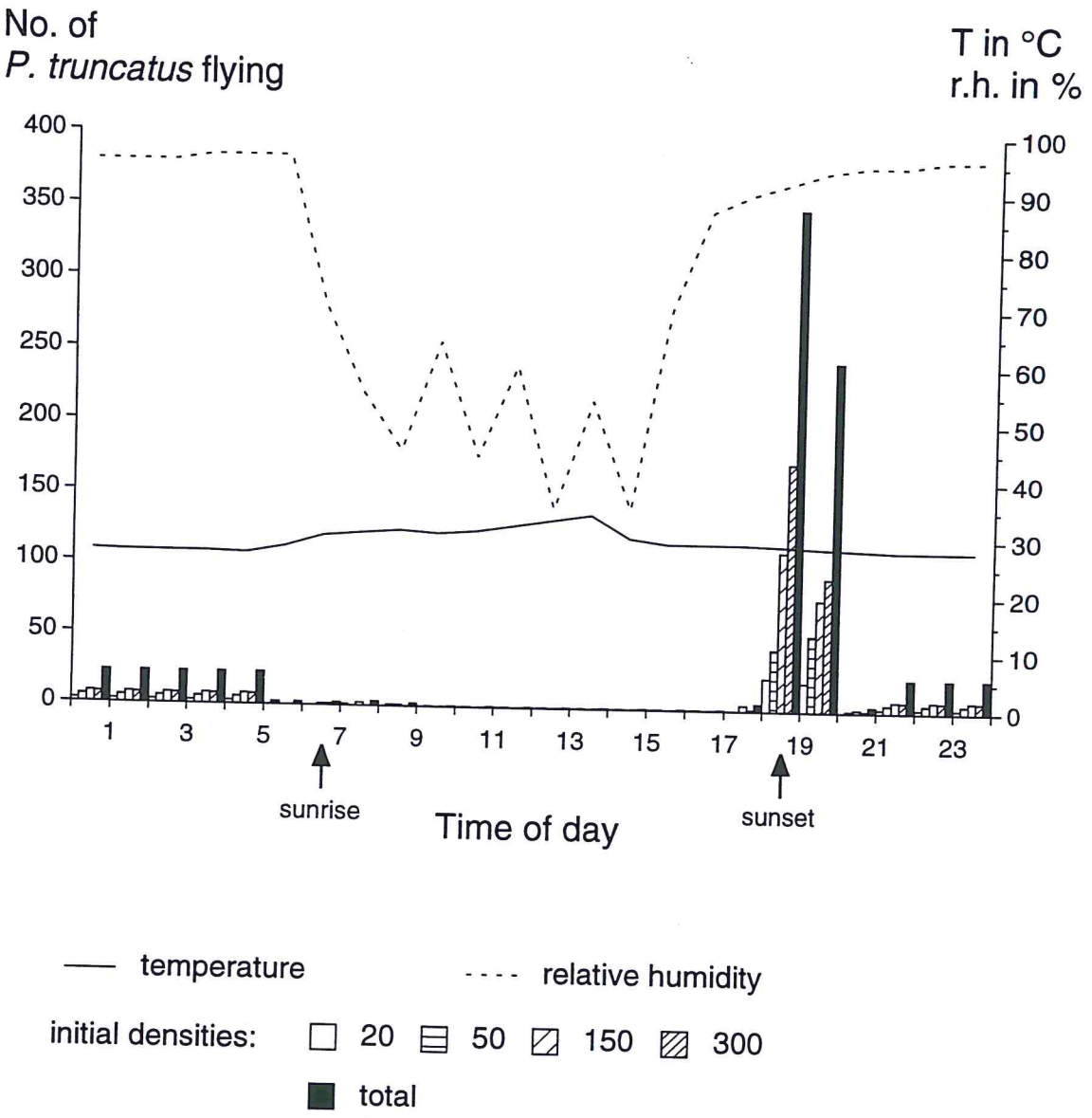


Fig. 3.3. Time-of-day dependent flight initiation. Beetles which had flown out of the cultures were collected at 5, 6, 7, 8, 9, 17, 18, 19, 20, 21 h during four days (six replicates × four densities, n=801). The numbers of beetles collected at 5 h and 17 h, representing the beetles flown during a period of eight hours, are presented equally distributed over this time period.

Time-of-day dependent flight initiation

Figure 3.3 shows the pattern of flight initiation in relation to time of day. The main take off time lay between 18 and 20 hours, which was the time immediately before and after sunset (sunset during the observation period: 18.40 h [information: meteorology department, airport Cotonou], darkness *ca.* 19.00 h). Of the beetles which flew off the cultures, 74% dispersed during these two hours.

3.3. Effect of weather and crowding

3.3.1. Materials and methods

General experimental set-up

Maize cobs (weight: 75-85 g) were deep frozen for two weeks in order to disinfest the maize from hidden insect infestations and afterwards reconditioned at 30 ± 1 °C and 75 ± 5% r.h. for at least 14 days. A hole (*ca.* 0.8 cm wide and 5 cm deep) was drilled in the base of the cobs, and eight cobs were each infested with five female and five male *P. truncatus* weekly. The infested cobs were stored singly in 1000 ml glass jars with rough interior (made with sand and sodium silicate) in an open field laboratory (24-35 °C, 50-100% r.h.) for five weeks. The cobs were then transferred to the experimental units which were set up under a roofed shelter (3 m × 3 m, 2.5 m high), whose sides were covered with shade cloth, providing protection against direct sunlight. Experimental units were identical with the ones used in the previous dispersal experiment (cf. Fig. 3.1 and Chapter 3.2.1), but two 'windows' (10 cm × 10 cm) were cut into the walls of the polyethylene buckets and screened with brass gauze. Instead of a culture jar, a construction resembling a candle holder (Fig. 3.4A) was fixed on the metal bars inside each experimental unit. The holder was made of a cut plastic cup and a screw; the floor of the cup was covered with a mixture of sand and sodium silicate. This design enabled the beetles which fell off the cob to crawl back onto it, but prevented them from climbing the smooth rim of the holder. The maize cobs (including any flour produced) were wrapped in a paper towel and placed on the holder (Fig. 3.4B).

For the following 21 days, the beetles which had flown off the maize cobs and had gathered in the collection containers ('flyers') were collected three times per week and counted. After the final collection on day 21, the cobs were removed from the experimental units and all *P. truncatus* still left on the cobs were counted. Since there were 24 experimental units and eight cobs were set up weekly replacing the removed cobs, in every observation week there were cobs in the first, the second and the third week after set-up ('flight weeks'), except for the two weeks at the beginning and at the end of the experiment (cf. Fig. 3.7). The experiment was run from February to November 1996 over a total of 38 observation weeks.

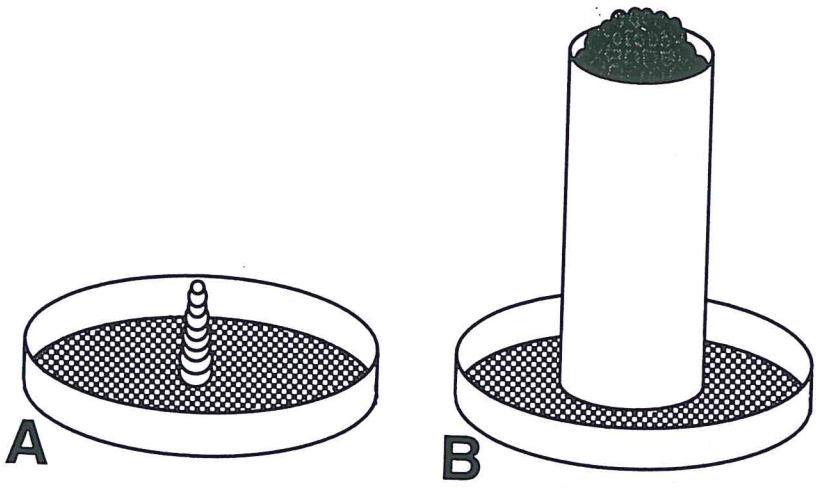


Fig. 3.4. Holder (A) and holder with maize cob wrapped in a paper towel (B).

Simultaneous monitoring with pheromone traps

In order to relate the flight initiation observed in the experiment to natural flight activity in the vicinity of the experimental set-up, the number of *P. truncatus* flying were recorded with pheromone traps. Three Delta sticky traps (Pherocon II trap, Trécé, Inc., USA) baited with polyethylene vials containing the synthetic analogs of the aggregation pheromone of *P. truncatus* (1 mg T1 and 1 mg T2; Agrisense-BCS Ltd., Pontypridd, UK) were set up at nearby locations within the IITA campus; distances between traps were *ca.* 300 m. The traps were collected weekly on the same day when the cobs were exchanged in the experiment, replaced by new traps with fresh pheromone vials and the number of *P. truncatus* caught per trap was recorded.

Determination of temperature and relative humidity

Temperature and relative humidity (r.h.) were measured with thermo-couples and r.h.-probes which were connected to a datalogger (Model 245, Wavetek Inc., San Diego, CA, USA). Temperature and r.h. were measured every 7 sec and the 10-minute-means of the measurements were recorded with the datalogger-specific

programme (Doric) on a personal computer. Four temperature and four r.h. devices were set up. Two empty experimental units were supplied with one temperature and one r.h. device each (to determine the immediate conditions inside the units), and the remaining devices were placed on top of the same experimental units (to determine conditions under the shelter). Weekly means were calculated for daily minimum (Tmin), maximum (Tmax) and mean temperatures and for daily minimum (r.h.min), maximum (r.h.max) and mean r.h. for the measurements inside and outside the experimental units.

Weather data, i.e., maximum and minimum daily temperatures, maximum and minimum r.h. and daily rainfall, were also obtained from the IITA weather station, which was situated *ca.* 400 m from the experimental set-up. Temperature and r.h. were measured with a hygro-thermo graph (Oakton, spring-wound model). Weekly means were calculated for minimum, maximum, and mean [(Tmin+Tmax)/2] daily temperatures and for minimum, maximum and mean [(r.h.min+r.h.max)/2] daily r.h. Total accumulated weekly rainfall was also computed.

Flight initiation observed in the experimental units was to be compared with flight activity, as indicatd by *P. truncatus* caught with pheromone traps, and both parameters were to be assessed in relation to weather parameters. Hence, the relation of temperature and r.h. inside the experimental units, under the shelter and measured in the environment of the IITA station (as measured by the IITA weather station) had to be determined. In addition, the datalogger worked properly only during half of the observation weeks, while data for the whole observation period was available from the IITA weather station. Therefore, it was necessary to verify if the weather station data corresponded well with the datalogger measurements, and could be used to evaluate field flight activity as well as flight initiation in the experimental units.

Weekly means of daily minimum, maximum and mean temperatures as well as weekly means of daily minimum, maximum and mean r.h., measured inside the experimental units, under the shelter and at the IITA weather station were compared (35 weekly observations). As the measurements from the IITA weather station corresponded well with the data obtained with the datalogger (cf. results, Chapter 3.3.2) and provided continuous data for the whole observation period, multiple regression procedures were performed with the data obtained from the IITA weather station.

Sex ratios and density dependent flight initiation in a preliminary experiment

The same experimental procedure as described for the general experimental set-up was also applied in a preliminary experiment, which was conducted from November 1995 to January 1996. However, the eight cobs which were set up weekly were infested with two different densities: Four cobs were infested with three females and three males, and the remaining four cobs with 60 unsexed *P. truncatus*. Beetles which had flown off the cobs and beetles collected from the cobs at the end of each observation period were deep frozen and sexed. When the number of beetles exceeded 100, a subsample of 100 beetles was sexed, while the sex was determined for all beetles for numbers smaller than 100. Ten replications, each consisting of eight cobs, were established initially, however, populations of five cobs from the 6-*P. truncatus*-treatment and of two cobs of the 60-*P. truncatus*-treatment were destroyed by ant attack and were therefore excluded from the analysis.

Time-of-day dependent flight activity

In the previous dispersal experiment carried out in greenhouses (cf. Chapter 3.2.2), a major peak in flight initiation was observed during sunset and some flight during the night hours, while peaks in flight activity registered with pheromone traps in the field have been observed at dusk and dawn (e.g., Tigar *et al.*, 1993). Therefore, an experiment was conducted to compare flight activity in the field with flight initiation in the experimental units, under two sets of artificial conditions. Four experimental units were set up in an insectarium (30 °C, 55-85% r.h.; the light was turned on at 06.00 h and turned off at 18.00 h). Beetles which had taken flight in these units and in the 24 experimental units under the outdoor shelter (in the preliminary experiment) were collected at 1, 5, 6, 7, 8, 9, 13, 17, 18, 19, 20, 21 hours and counted. The number of *P. truncatus* caught with two pheromone traps near the IITA station was recorded at the same times. The experiment was conducted over three successive days, precisely one year after the previous experiment on flight initiation in relation to time of day. During the observation period, sunrise was at 06.50 h and sunset at 18.40 h (information: meteorology department, airport Cotonou), darkness at *ca.* 19.00 h.

Flight initiation among the parental adults

In the experiments described above, insects taking flight may include both the insects initially established on the cobs as adults and their progeny, which have developed during the course of the experiment. An additional treatment was therefore applied to investigate flight initiation solely among insects established on the cobs as adults ('parental adults').

The same experimental procedure as described for the general experimental set-up was applied, but 12 maize cobs were infested with 60 unsexed adult *P. truncatus* and stored in the glass jars for four days to allow the beetles to establish themselves on the cobs. The cobs were then transferred to the experimental units and beetles flying off the cobs were registered for three weeks.

Statistics

The minimum, maximum and mean temperatures as well as the minimum, maximum and mean r.h. measured inside the experimental units, under the shelter and at the IITA weather station were compared using Friedman two-way analyses of variance followed by multiple comparison tests ($p<0.05$; Siegel & Castellan, 1988).

Multiple regression analysis was used to determine the relation between flight activity (*P. truncatus* caught with pheromone traps) and weather (Table 3.3; Montgomery & Peck, 1992; Sokal & Rohlf, 1995). The relation between flight initiation (number of flyers), weather parameters, experimental variables and *P. truncatus* caught with pheromone traps was investigated with a second multiple regression analysis (Table 3.3). In addition, multiple regressions using the same independent variables were performed for each flight week separately. The number of *P. truncatus* caught with pheromone traps, progeny densities (= number of beetles collected from the cob at the end of the observation period plus number off beetles having flown off) and the number of beetles having flown off the cobs were log-transformed (log+1). The mean temperatures were found to be highly correlated ($r>0.7$) with minimum and maximum temperatures, as well as with minimum and mean r.h. Therefore, only the mean weekly temperatures and the mean weekly maximum r.h. were selected as parameters describing temperature and r.h.

Table 3.3. Dependent and independent variables of the multiple regression models.

dependent variable	independent variable
No. of *P. truncatus* caught with pheromone traps (log+1)	mean weekly temperature
	mean maximum relative humidity
	accumulated weekly rainfall
No. of *P. truncatus* flying off in the experiment (log+1)	progeny density (log+1)
	flight week
	cobweight
	mean weekly temperature
	mean weekly maximum relative humidity
	No. of *P. truncatus* caught with pheromone traps (log+1)

To verify if the temperatures during the development periods influenced the final progeny densities (= number of beetles collected from the cob at the end of the observation period plus number off beetles having flown off), temperature sums were calculated for each progeny density (sum of mean weekly temperatures for the eight weeks between cob infestation and removal of the cobs from the experimental units). A correlation analysis was performed on progeny densities versus temperature sums (Sokal & Rohlf, 1995).

The proportions of females which flew off, stayed on the cob and of the total progeny were compared with a Friedman two-way analysis of variance by ranks separately for the treatments with initial densities of six and 60 *P. truncatus* (Siegel & Castellan, 1988). The relation of progeny densities (= number of beetles collected from the cob at the end of the observation period plus number off beetles having flown off) to flyers and dispersal rates [% flyers = (flyers/progeny density) × 100] in the preliminary experiment were investigated with simple linear regressions on the log-transformed data (Montgomery & Peck, 1992; Sokal & Rohlf, 1995).

3.3.2. Results

Comparison of temperatures and relative humidities measured inside the experimental containers, under the shelter and at the IITA weather station

Maximum temperatures at the three locations could be summarized as: in the experimental units > under the shelter > IITA weather station (Table 3.4). Minimum and mean temperatures were only significantly elevated inside the experimental units and differences were relatively small (Table 3.4). The three measurements of minimum and maximum temperature are clearly closely related (Fig. 3.5A).

Minimum r.h. measured inside the experimental units and under the shelter were lower than measured at the IITA weather station (Table 3.4). The higher maximum

Table 3.4. Weekly minimum, maximum and mean temperatures and relative humidities measured inside of the experimental units, under the shelter and at the IITA weather station. Means (± s.e.) of 35 weeks; means within a row differ significantly when followed by different letters (Friedman two-way analysis of variance by ranks followed by a multiple comparison test, $p<0.05$).

	temperature and relative humidity measured		
	in experimental units	under the shelter	at the IITA weather station
T min. (in °C)	24.1 a (± 0.2)	23.6 b (± 0.2)	23.7 b (± 0.2)
T max. (in °C)	35.8 a (± 0.3)	33.0 b (± 0.3)	31.5 c (± 0.3)
T mean (in °C)	28.8 a (± 0.2)	27.6 b (± 0.2)	27.6 b (± 0.2)
r.h. min.	51.0% a (± 1.3)	48.7% b (± 1.1)	62.7% c (± 1.1)
r.h. max.	95.3% a (± 0.4)	92.0% b (± 0.6)	94.8% a (± 0.4)
r.h. mean	77.4% a (± 0.6)	73.7% b (± 0.6)	78.8% a (± 0.6)

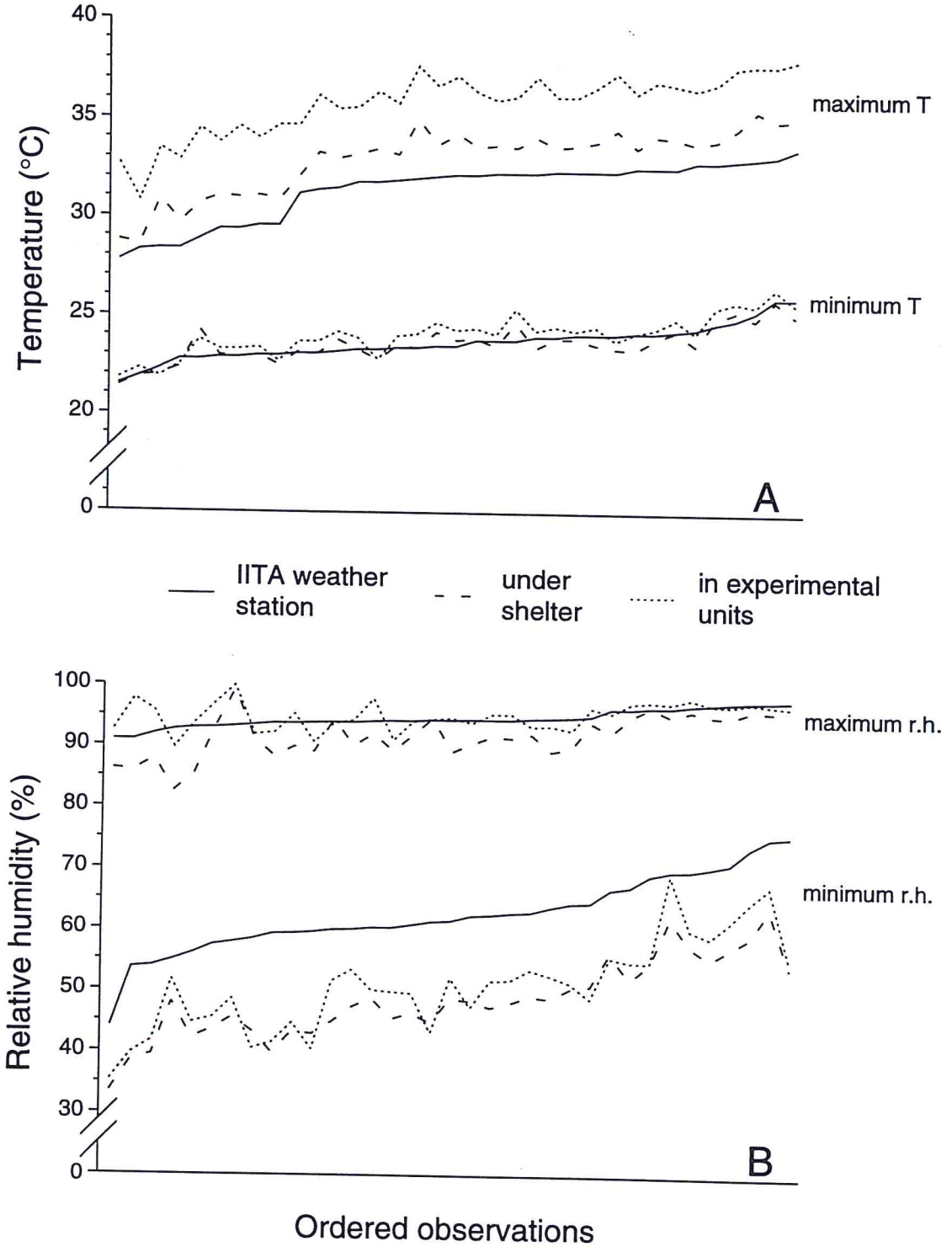


Fig. 3.5. Maximum and minimum temperatures (A) and relative humidities (B) measured at the IITA weather station, under the shelter and inside the experimental units. The 35 observations (weekly means) from the IITA weather station are presented in increasing order with the corresponding data from the other two locations.

temperatures in the experimental units and under the shelter produced lower minimum r.h. Maximum r.h. varied little (Fig. 3.5B), although maximum r.h. under the shelter was in general slightly lower than the two other measurements. As for temperature, there is an obvious relation between minimum and maximum r.h. measurements (Fig. 3.5B).

Factors affecting flight activity in the field and flight initiation under the experimental conditions

Using multiple regression analysis, only the mean weekly temperature proved to have a significant effect on the number of *P. truncatus* caught with pheromone traps (Table 3.5; Fig. 3.6). Approximately 30% of the variance could be explained by this variable.

Analysing factors which may affect the number of flyers in the experiment revealed that flight week, progeny density and mean weekly temperature contributed significantly to the proposed model (Table 3.6; Fig. 3.7). Including any of the other independent variables did not improve the model, which explained *ca.* 70% of the variance. According to the standardized regression coefficients, the contribution of the independent variables to the model can be summarized as: flight week > progeny density > temperature (Table 3.6). Examination of the influence of progeny density and temperature separately for the three flight weeks showed that the influence of temperature decreased steadily from flight week one to flight week three, while the effect of the progeny density increased (Table 3.6; Fig. 3.7).

Final progeny densities were not affected by the temperature differences during the development periods (correlation coefficient of progeny densities and related temperature sums: r=0.1).

Table 3.5. Multiple regression analysis for assessing the effect of weather on the number of *P. truncatus* caught with pheromone traps. Data from three pheromone traps collected at the IITA station between December 1995 and November 1996. Documented variables contributed significantly to the model ($p<0.05$); b = standardized regression coefficient, t = t-statistics, r^2 = coefficient of determination.

independent variable	regression statistics			
	b	t	intercept	r^2
mean weekly temperature	0.58	4.75	-3.3	0.30

Table 3.6. Multiple regression analyses for assessing the effect of weather and experimental factors on the number of *P. truncatus* flying. Data were collected between February and November 1996. Documented variables contributed significantly to the models ($p<0.05$), except mean temperature in flight week 3; b = standardized regression coefficient, t = t-statistics, r^2 = coefficient of determination.

model	independent variable	regression statistics			
		b	t	intercept	r^2
complete	flight week	0.66	35.6	-11.2	0.70
	progeny density	0.43	22.7		
	mean weekly temperature	0.27	13.4		
flight week 1	progeny density	0.38	8.8	-7.3	0.47
	mean weekly temperature	0.53	12.3		
flight week 2	progeny density	0.60	15.8	-9.0	0.58
	mean weekly temperature	0.42	10.9		
flight week 3	progeny density	0.72	17.5	-3.9	0.52
	mean weekly temperature	0.03	0.7		

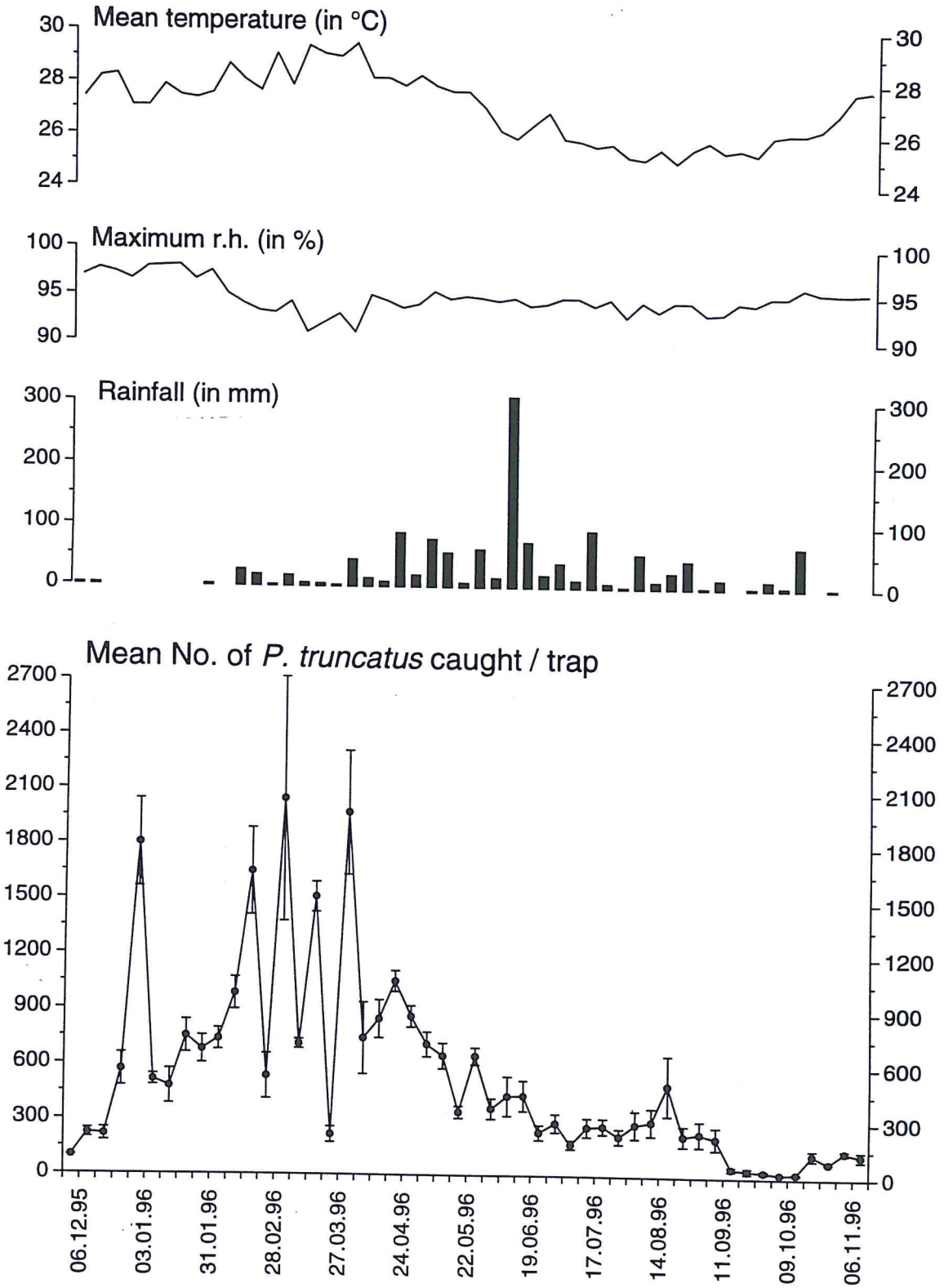


Fig. 3.6. Annual fluctuations of the number of *P. truncatus* caught with pheromone traps and corresponding weather data used in the multiple regression. Error bars represent 1 s.e.

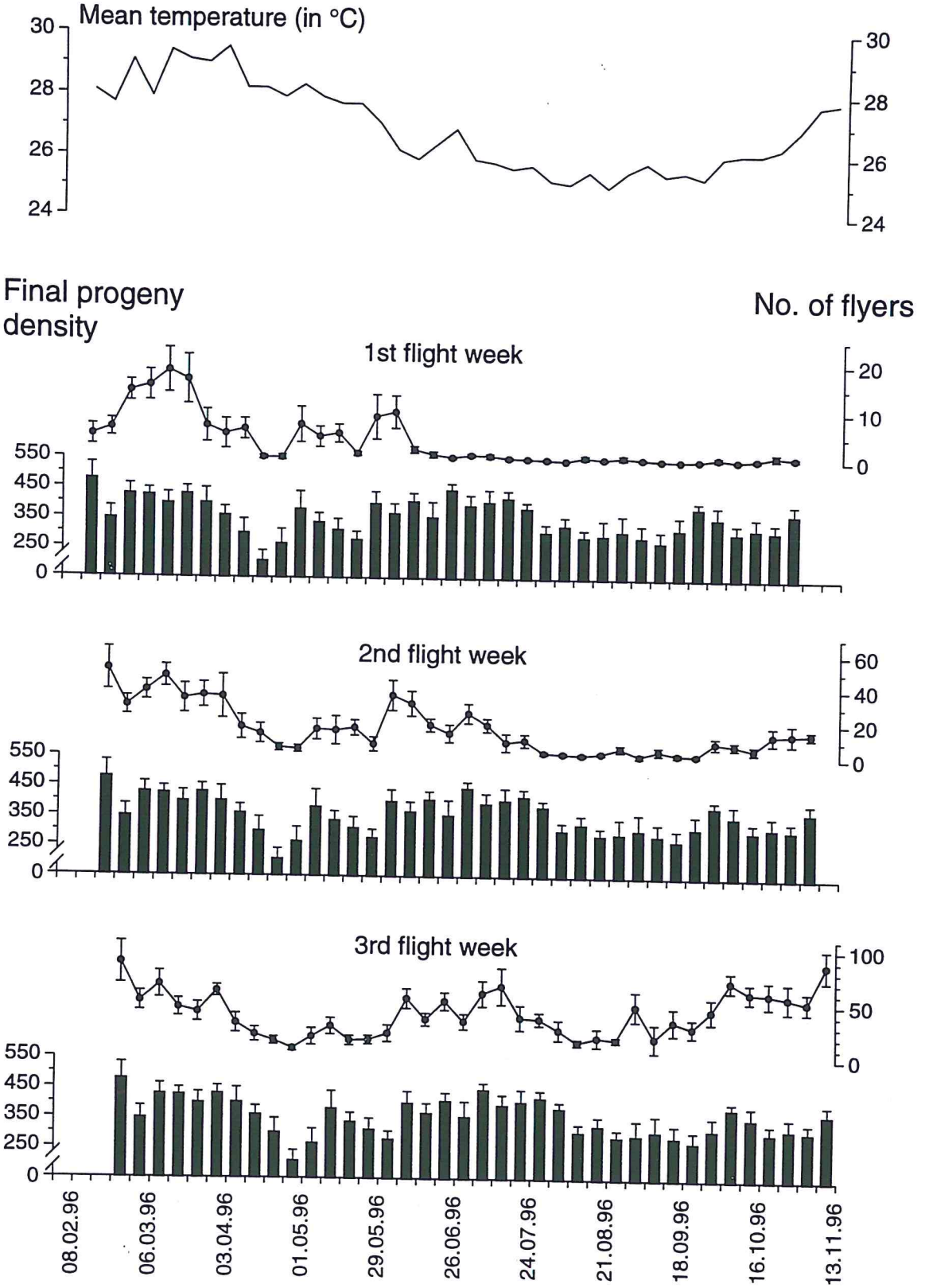


Fig. 3.7. Fluctuations of the number of *P. truncatus* which flew off the cobs in relation to final progeny densities and mean temperatures. The same mean progeny density corresponds with flyers of the first, the second and the third flight week. Error bars represent 1 s.e.

Density dependent flight initiation in the preliminary experiment

Simple linear regressions were performed on the log(number of *P. truncatus* flying) (log(F)) versus log(total number of progeny) (log(P)) and log(% of *P. truncatus* flying) (log(%F)) versus log(total number of progeny) (log(P)), separately for the two initial densities. The final progeny density proved to have significant effects on the number of beetles flying as well as on the dispersal rates (Fig. 3.8):

$F=0.00002\ P^{2.612}$ ($p<0.0001$, $r^2=0.63$) and $\%F=0.00005\ P^{1.867}$ ($p<0.0001$, $r^2=0.41$) for the treatment with initial densities of six *P. truncatus*;

$F=0.008\ P^{1.579}$ ($p<0.0001$, $r^2=0.69$) and $\%F=0.0767\ P^{0.583}$ ($p<0.0001$, $r^2=0.41$) for the treatment with initial densities of 60 *P. truncatus*.

The dispersal rates for initial densities of six *P. truncatus* averaged 17%, the dispersal rates for initial densities of 60 *P. truncatus* averaged 40%. However, it should be noted that these analyses cannot be directly compared to the former experiment (cf. Chapter 3.2.2): The parental beetles in the former experiment had been removed two weeks after infestation, while in this experiment the parental beetles were not removed. It may be assumed that the progeny increased steadily during the observation period.

Sex ratios

The proportion of females was higher among the beetles flying off the cobs than among the beetles which were collected from the cobs at the end of the experiment or among the total progeny (Table 3.7; $p<0.05$, Friedman two-way analysis of variance by ranks followed by a multiple comparison test). The sex ratio of the total progeny was *ca.* 1:1.

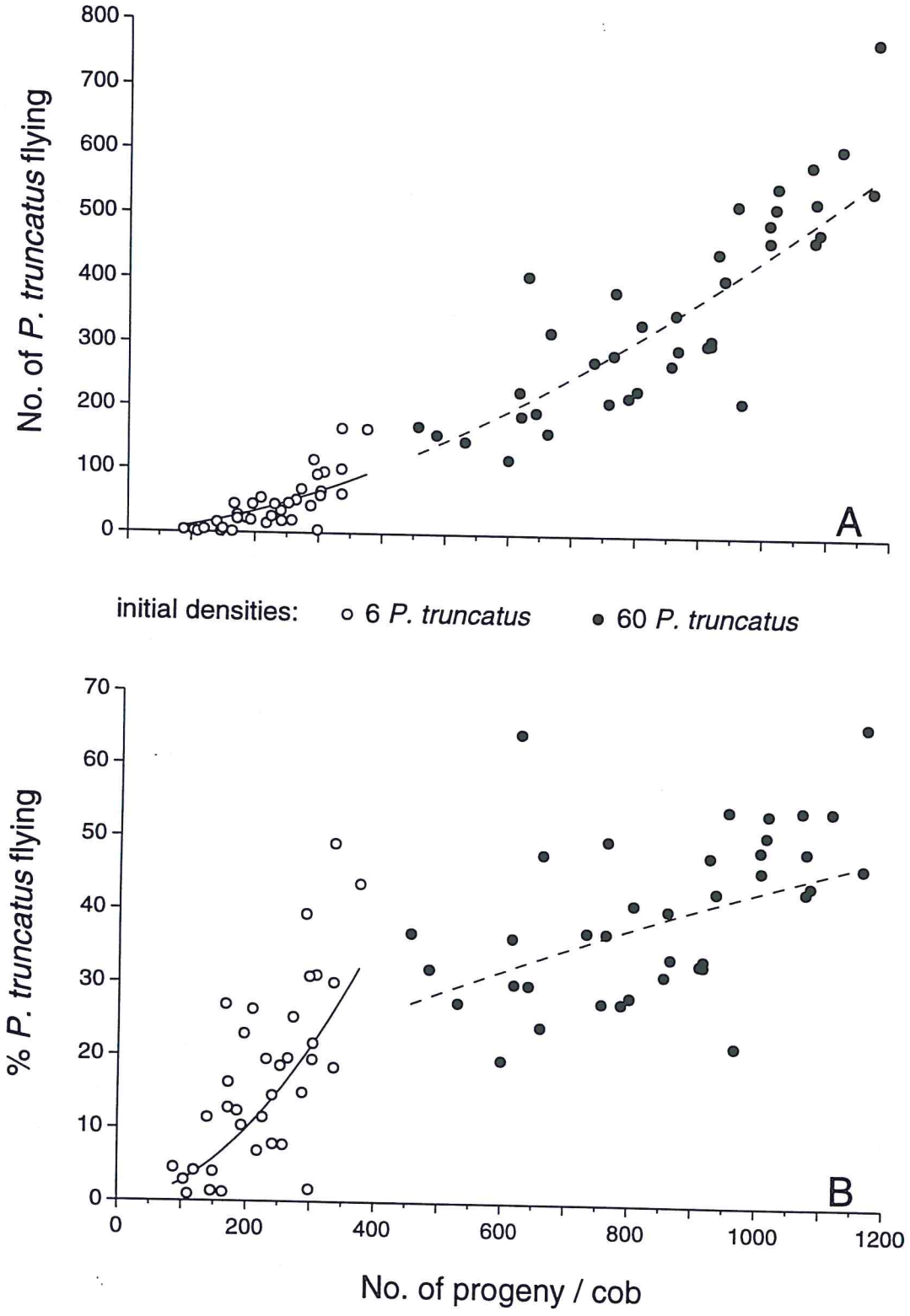


Fig. 3.8. Number of *P. truncatus* taking flight (A) and dispersal rates (B) in relation to progeny densities. Solid lines represent the regression curves for initial densities of six, dashed lines the regression curves for initial densities of 60 *P. truncatus*.

Table 3.7. Proportions of females among beetles flying, collected from the cob and of total progeny. Means ± s.e.; means in a row differ significantly when followed by different letters (Friedman two-way analysis of variance by ranks followed by a multiple comparison test, $p<0.05$).

initial density	% of females among beetles		
	flying	collected from cob	total progeny
6 *P. truncatus*	69.6 a (± 2.5)	46.4 b (± 0.7)	49.8 c (± 0.7)
60 *P. truncatus*	62.7 a (± 1.1)	45.4 b (± 0.8)	51.9 c (± 0.7)

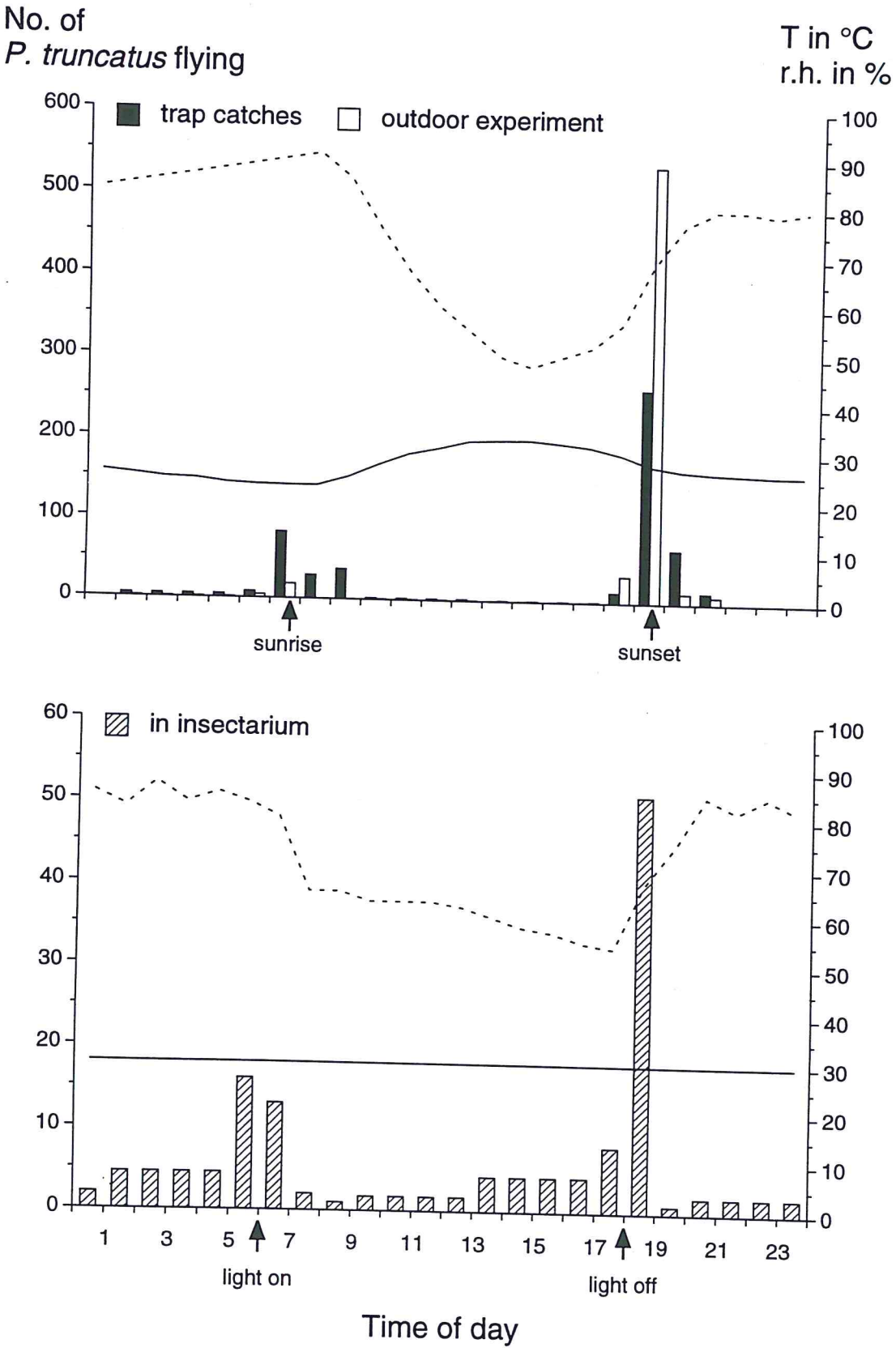
Time-of-day dependent flight initiation

Figure 3.9 shows the pattern of flight initiation in the experiment and accompanying observations in the insectarium as well as flight activity measured with pheromone traps in relation to time of day. The main take off time lay between 18 and 19 hours, which was the time immediately before and after sunset, and immediately after turning off the light. The ratio of morning to evening peak was *ca.* 1:2 for the trap catches and for the flyers in the insectarium, while 85% of the beetles flying in the outdoor experiment left the cob between 18 and 19 hours. It was even observed that the majority of these beetles flew off between sunset (18.40 h) and darkness (*ca.* 19.00 h).

Flight initiation among the parental adults

A mean of 5.3 *P. truncatus* (s.e.=0.91, range: 2-12) flew off the cobs during the three weeks observation period. This is equivalent to a dispersal rate of 9%.

Fig. 3.9. (next page) Time-of-day dependent flight in the experimental units and among trap catches with pheromone traps. Beetles which had flown out of the maize cobs in the outdoor experiment, in the insectarium and beetles caught with pheromone traps were collected at 1, 5, 6, 7, 8, 9, 13, 17, 18, 19, 20 and 21 h during three days. The numbers of beetles collected at 1, 5, 13 h and 17 h, representing the beetles flown during a period of four hours, are presented equally distributed over this time period. Solid line: temperature, dashed line: relative humidity.


No. of
P. truncatus flying
T in °C
r.h. in %
trap catches
outdoor experiment
600
500
400
300
200
100
0
100
90
80
70
60
50
40
30
20
10
0
sunrise
sunset
in insectarium
60
50
40
30
20
10
0
100
90
80
70
60
50
40
30
20
10
0
1
3
5
7
9
11
13
15
17
19
21
23
light on
light off
Time of day

3.4. Sex ratio of the dispersing population

3.4.1. Materials and methods

The sex ratios of *P. truncatus* trapped with in Delta sticky traps (Pherocon II trap, Trécé, Inc., USA) baited with polyethylene vials containing the synthetic analogs of the aggregation pheromone (1 mg T1 and 1 mg T2; Agrisense-BCS Ltd., Pontypridd, UK) were determined from September 1994 to September 1995. The sex ratios were determined for catches from two traps, which were part of a continuous monitoring program in the Mono District in south-western Benin (Borgemeister *et al.*, 1997) and which were located *ca.* 800 m apart. Traps were collected weekly, deep frozen, and replaced by new traps with fresh pheromone vials. The number of *P. truncatus* caught was recorded. The sex of all beetles which were not yet internally decomposed could be determined by the method described in Chapter 2.3. If this number exceeded 100 beetles per trap, only a subsample of 100 beetles was sexed. To verify if there was an association between the recorded sex ratios and the number of beetles trapped, a correlation analysis was performed on the total numbers of *P. truncatus* and the percentages of females (Sokal & Rohlf, 1995).

The evaporating amount of the pheromone components and their ratio have been reported to change over time (Dendy *et al.*, 1991) and may thereby attract different sex ratios at the beginning and at the end of the trapping period. Beetles caught during the first days of the trapping period were already internally decomposed and, therefore, could not be sexed by the method normally applied (Chapter 2.3). It was accordingly necessary to verify, in a preliminary trial, that the selected beetles were a representative sample of all beetles trapped. Over a six week period, sex ratios were determined for *P. truncatus* caught with two traps located near the IITA station, the traps being situated *ca.* 300 m apart. For one trap, the sex ratio was determined for all beetles trapped, collecting the beetles from the traps three times per week. The sex ratio among the beetles from the other trap was determined as described above. The sampling methods applied were changed weekly between the traps. The percentages of females recorded with the different sampling methods were compared using paired t-tests (Sokal & Rohlf, 1995).

To provide comparable samples of non-dispersing beetles, the sex ratios among adult *P. truncatus* in short season maize stores were determined in four monthly samples (January to April 1996; the samples were kindly provided by C. Borge-

meister). In addition, the sex of the adult progeny which emerged from these samples was determined. Sex ratios were compared to an expected 1:1 ratio using Chi-square tests (Siegel & Castellan, 1988).

3.4.2. Results

The mean percentage of females recorded in the preliminary trial was 76.5% (s.e.=1.3) when all trapped beetles were sexed and 78.2% (s.e.=2.2) when the sex was only determined for non-decomposed beetles or a sample of them (the standard method applied in the main experiment). As these means did not differ significantly (paired t-test, p=0.62), the standard method can be regarded as an appropriate procedure to determine the sex ratios in the pheromone traps.

Figure 3.10 presents the fluctuations of trap catches and sex ratios during the 54 weeks of trapping. The proportion of females caught during the whole experimental period averaged 72% and was equal for both traps. There is hardly any correlation between the number of beetles and the percentage of females trapped (trap 1: r=0.026, trap 2: r=0.035).

The proportion of females among the adult *P. truncatus* collected from maize stores was 49.2% (n=3247), and 50.9% of the progeny (n=1441) were females. The sex ratios did not differ from a 1:1 sex ratio (Chi-square tests; p=0.49 for collected adults, p=0.51 for progeny).

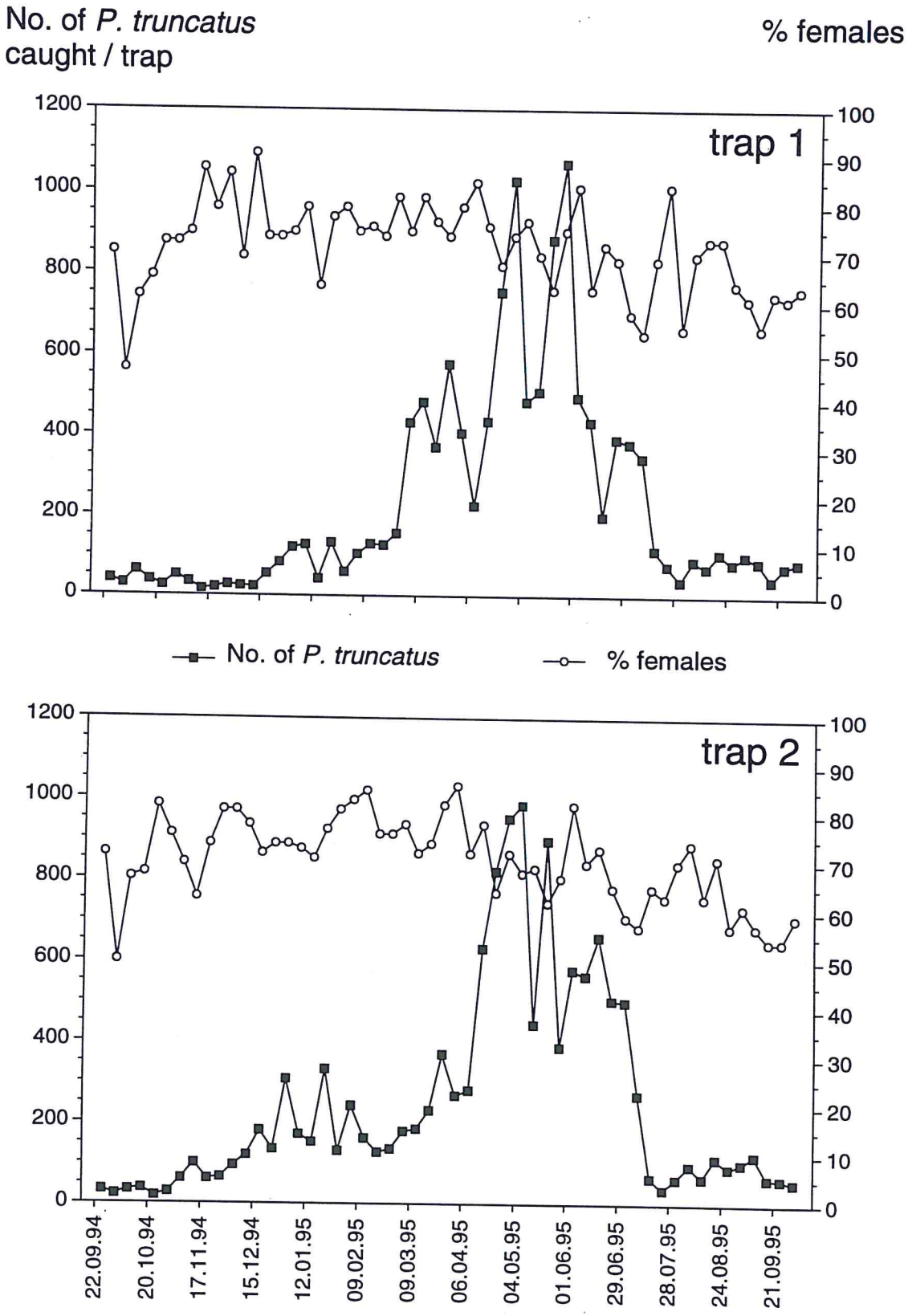


Fig. 3.10. Fluctuations of *P. truncatus* caught with pheromone traps in the Mono District and corresponding sex ratios.

3.5. Physiological age-grading and ovarian physiology

3.5.1. Materials and methods

Insects

Insects of known age and mating status were obtained by introducing two female *P. truncatus* into a glass jar (6 cm high, 2.1 cm inner diameter) filled with a compressed 1:1 mixture of maize flour and cellulose (alpha-cellulose, Sigma Chemical Co., St. Louis, USA), which were kept under the same conditions as the basic cultures (cf. Chapter 2.2). Intact pupal chambers could be obtained by sieving the flour mixture 28 days after infestation. Each pupal chamber was kept separately in a small glass jar until the adult emerged of its own accord. The beetles were either dissected on the day of emergence (day 0; only unmated females tested) or were kept on maize-flour-cubes (compressed 3.5:1 mixture of maize flour and maize starch), either singly (unmated treatments) or with two conspecifics of the opposite sex (mated treatments) for five, ten, 20 or 30 days. To test the effect of starvation, an additional treatment was conducted with mated females which were deprived of food from day six onwards and dissected at the age of ten days. During the four-day starvation period, females were kept in glass petri dishes with a rough flour (made of sand and sodium silicate), so they were able to move. The size of mature eggs was determined using freshly laid eggs, which were collected by the same method as pupal chambers, sieving the flour mixture 24 h after infestation.

Samples of *P. truncatus* originating from the dispersal experiment were collected on the same day, either directly from the cob or from those which had flown off the cob immediately before collection (cf. Chapter 3.3). The beetles were collected at the end of the observation period, eight weeks after the initial infestation. Their age must therefore have been between 0 and 28 days. Dispersing *P. truncatus* were collected with funnel traps baited with the synthetic pheromone (AgriSense-BCS Ltd., Pontypridd, UK). The trap at the IITA station was positioned *ca.* 50 m from the nearest maize store, and only approaching beetles, i.e., beetles which had not yet entered the trap and had not made contact with other conspecifics at this aggregation point, were sampled and dissected immediately afterwards. Beetles collected in the Lama forest (near Bohicon, Zou District) were caught in traps distributed within the forest, at *ca.* 5 km distance from the nearest maize stores, and were dissected 8-24 h after they had been trapped. The beetles were trapped in April 1996, a time of the year generally with high flight activity (Borgemeister *et al.*, 1997).

Dagmar Scholz, 1997: Disperal and Host Finding Behaviour of *Prostephanus truncatus* (Horn) (Coleoptera: Bistrichidae)

Studies on the dispersal and host finding behaviour of *Prostephanus truncatus* (Horn) (Col.: Bostrichidae), a pest of stored maize and dried cassava, have been conducted in cooperation with the Biological Control Center for Africa of the International Institute of Tropical Agriculture in Abomey-Calavi, Republic of Benin.

Dispersal of the beetles was found to be mainly influenced by the immediate population densities (crowding) and accompanying substrate degradation. Among the weather factors considered, only the mean temperature proved to have an effect on the flight activity of the beetles. While sex ratios were *ca.* 1:1 for the resident reproducing part of the population, a higher proportion of females was recorded among dispersing beetles. The reproductive systems of male and female beetles were described, and a method for physiological age-grading of female *P. truncatus* was developed. Females dispersed at varying physiological ages, and their follicle development was suspended. As migrating females were mated, it is suggested that females may act as colonizers independently of males.

Investigations on primary attraction revealed that *P. truncatus* of both sexes react to volatiles from starchy stored commodities and some woody plant species, but only at short range. Long-range attraction may, however, exist in regard to the supposed natural host-plant-complex.

Secondary attraction was confirmed to play an important role in the host finding process of the insect. While it was verified that females do not produce sex or aggregation pheromone, males produced aggregation pheromone only on suitable food substrates, including some woody plants. Both sexes reacted strongly to the aggregation pheromone under all conditions tested. Responses of *P. truncatus* in electroantennogram (EAG) studies were slightly stronger to the second pheromone component, T2, than to T1, while T2 attracted many more beetles in a field experiment. Laboratory and field responses of *Teretriosoma nigrescens* Lewis (Col.: Histeridae), the predator closely adapted to *P. truncatus*, hardly differed in regard to the two pheromone components. Results from EAG experiments suggest that *P. truncatus* and *T. nigrescens*, react with similar sensitivity to the prey-produced pheromone.

Single male *P. truncatus* on maize cobs were capable of attracting conspecifics, but their attractiveness declined after some days, due to an effect of the arrived females. The steep population increase in a maize store can mainly be attributed to the male-attracted initial population and the high reproductive capability of *P. truncatus*, with no continuous immigration necessary.

In this thesis, a scheme for the host finding and dispersal behaviour of *P. truncatus* is developed and the factors acting on various stages in the process discussed, both in terms of their adaptive value for the insect and their implications for efforts to control it.

Dagmar Scholz, 1997: Migration und Wirtsfindungsverhalten von *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae).

Untersuchungen zum Migrations- und Wirtsfindungsverhalten von *Prostephanus truncatus* (Horn) (Col.: Bostrichidae), einem Schädling von gelagertem Mais und getrocknetem Maniok, wurden in Zusammenarbeit mit dem 'Biological Control Center for Africa' des 'International Institute of Tropical Agriculture' in Abomey-Calavi, Benin, durchgeführt.

Die Migration der Käfer wurde durch dichteabhängige Faktoren, d. h. durch den unmittelbaren Bevölkerungsdruck und begleitende Substratverschlechterung, beeinflusst. Von den betrachteten meteorologischen Faktoren hatte nur die durchschnittliche Temperatur eine Wirkung auf die Flugaktivität von *P. truncatus*. Das Geschlechterverhältnis des nicht-fliegenden Teils der Käferpopulation war ca. 1:1, während unter den migrierenden Käfern der Anteil der Weibchen erhöht war. Die Morphologie der inneren Geschlechtsorgane von männlichen und weiblichen *P. truncatus* wurde beschrieben, und eine Methode entwickelt, mit der das physiologische Alter von Weibchen bestimmt werden kann. Die migrierenden Weibchen gehörten unterschiedlichen physiologischen Altersstufen an, und die Follikelentwicklung war eingestellt. Da alle migrierende Weibchen gepaart waren, kann angenommen werden, dass sie Substrate auch in Abwesenheit von Männchen kolonisieren.

Die Untersuchungen zur primären Anziehung ergaben, dass *P. truncatus* beiderlei Geschlechts auf Duftstoffe, die von stärkehaltigen Lagergütern und einigen Hölzern ausgehen, reagieren. Diese olfaktorische Anziehung wirkte aber nur über kurze Distanzen. Anziehung über größere Distanzen könnte u. U. von Duftstoffen ausgelöst werden, die von natürlichen Wirtspflanzenkomplexen ausgehen.

Die Ergebnisse der Untersuchungen bestätigen, dass sekundäre Anziehung eine wichtige Rolle für die Wirtsfindung von *P. truncatus* spielt. Es konnte verifiziert werden, dass Weibchen keine Sex- oder Aggregierungspheromone produzieren. Männchen produzierten Aggregierungspheromon nur, wenn sie sich auf einen geeigneten Nahrungssubstrat befanden, wie z. B. auf Mais oder auf bestimmten Hölzern. Käfer beiderlei Geschlechts reagierten unter alle getesteten Konditionen stark auf das Aggregierungspheromon. *P. truncatus* reagierte in Elektroantennogramm-Versuchen etwas stärker auf die zweite Pheromonkomponente, T2, als auf T1; im Feldversuch, jedoch, wurde mit T2 eine sehr viel höhere Anzahl von *P. truncatus* gefangen als mit T1. Die Reaktionen von *Teretriosoma nigrescens* Lewis (Col.: Histeridae), dem auf *P. truncatus* spezialisierten Prädator, auf die beiden Pheromon-Komponenten unterschieden sich kaum. Die Elektroantennogramm-Untersuchungen belegten, dass *P. truncatus* und *T. nigrescens* nahezu gleich sensibel auf das Aggregierungspheromon reagieren. Sowohl in Olfaktometer- als auch in Feldversuchen waren einzelne *P. truncatus* Männchen auf Maiskolben attraktiv für ihre Artgenossen. Ihre Attraktivität sank aber nach einigen Tagen stark ab, sobald Weibchen eingetroffen waren. Die schnelle Entwicklung einer Schädlingspopulation in einen Maislager kann bereits durch eine geringe Initialbesiedelung ausgelöst werden. Bei der hohen Reproduktionsfähigkeit des Schädlings ist eine zusätzliche Einwanderung während der Lagerperiode nicht bestimmend für die Dynamik der Population.

In dieser Doktorarbeit wird ein Konzept für das Migrations- und Wirtsfindungsverhalten von *P. truncatus* entwickelt. Faktoren, die die einzelnen Schritte des Verhaltens während der Dispersionsphase bestimmen, werden im Hinblick auf deren biologische Bedeutung für den Schädling und in Bezug auf mögliche Maßnahmen zu dessen Kontrolle diskutiert.

General experimental procedures

Dissections were done in Ringer solution using a Wild M3C stereomicroscope (Fa. Heerburg, Switzerland) at 40x magnification, and sizes were determined with an ocular measuring scale, one unit being equivalent to 24 µm. The size of the male genital organs was determined by measuring the length of three testis tubes and the width of one testis tube, as well as the length and the width of both seminal vesicles for 20 males of each age/mating-status group. Since these experiments revealed no age-specific characteristics (cf. results: Chapter 3.5.2), males of the samples from the dispersal experiment and the pheromone traps were not examined.

The reproductive system of a female was described by measuring a) the length and width of one randomly chosen germarium, and b) the length and width of the largest terminal follicle (i.e., oocyte with surrounding follicular cells) in any of the ten ovarioles. In addition, I registered c) the maximum number of follicles per ovariole, d) the presence or absence of white egg yolk in the largest terminal follicle, e) the presence or absence of sperm, using an Axiolab microscope (Fa. Zeiss, Wetzlar, Germany) at 400x magnification, and f) the colour and size of the yellow bodies at the base of the ovariole [categorized as none, stage 1 (just visible, greyish band), stage 2 (greyish spot) and stage 3 (dark brownish/black spot)]. Fifty females of each age/mating-status group (except, only 20 for the treatment: unmated, 0 days old) and of each sampling group were examined. In order to be able to compare follicle sizes with the size of laid eggs, the length and width of 20 eggs (laid 0-24 hours before measuring) were determined.

Statistics

The length (means of three measurements) and widths of testis tubes, as well as seminal vesicle lengths and widths (means of two measurements) of the different age/mating groups were compared with two-way anovas (Sokal & Rohlf, 1995). Age groups of the same mating status and mated versus unmated treatments of the same age were compared with multiple t-tests (Bonferroni method: 0.05/16). Germarium lengths and widths were analysed with multiple t-tests (Bonferroni method: 0.05/22), comparing age groups of the same mating status and mated versus unmated treatments of the same age (including the treatment of starved 10-day-old females). Contingency tables (follicle sizes and yellow bodies) were analysed using Chi-squared tests. As some of the tables did not meet the requirements for

interpretation using the asymptotic p-values (Siegel & Castellan, 1988), exact p-values or approximations of exact p-values (by Monte Carlo algorithms) were calculated using StatXact (Anonymous, 1995). Several Chi-square tests were applied to the same data sets, so the calculated p-values were adjusted by the Bonferroni method (0.05/7 for the standards and 0.05/2 for samples from the dispersal experiment and trap catches). The correlation between follicle length and width was determined according to Sokal & Rohlf (1995).

3.5.2. Results

General description of the male and female reproductive systems

The reproductive system of male *P. truncatus* (Fig. 3.11A) consists of a pair of testes, each of them being composed of five testis tubes, and the long vasa deferentia connecting the testes with the seminal vesicles and the ejaculatory duct. The ejaculatory duct terminates in the aedeagus, which is shielded by the paired parameres. Aedeagus and parameres are of light brownish colour, i.e., lined with sclerotized cuticle. The structure of developing sperm in testis tubes is readily visible at 400x magnification: Roundish bundles of spermatogonia in the distal half and bundles with mature sperm (spermatozoa with head and tail) in the proximal part adjacent to the vas deferens. Three pairs of accessory glands and a pair of seminal vesicles (ventrally situated and filled with sperm) are located at the junction of vasa deferentia and ejaculatory duct. During dissections spermatophores (Fig. 3.11C) were found several times at the tip of the aedeagus and once on the abdomen of a female; the whole lumen was filled with sperm, and a spermatophore measured about the same size as the vagina of a female.

The reproductive system of female *P. truncatus* (Fig. 3.11D) consists of a pair of ovaries, each of them being composed of five ovarioles, which connect with a pair of lateral oviducts. These join to a median oviduct opening dorsally into the vagina. Each ovariole consists of two parts: The germarium where nurse and germ cells are located, and which ends in a terminal filament, and the vitellarium in which the growing oocytes are found in single file in order of increasing volume. The most developed follicle (terminal follicle) is situated at the position closest to the oviduct. Greyish or blackish spots were often found inside the pedicels, which connect the ovarioles to the oviduct (Fig. 3.11D). These spots are follicular relics, so-called yellow bodies, which may develop due to egg resorption or oviposition (Tyndale-

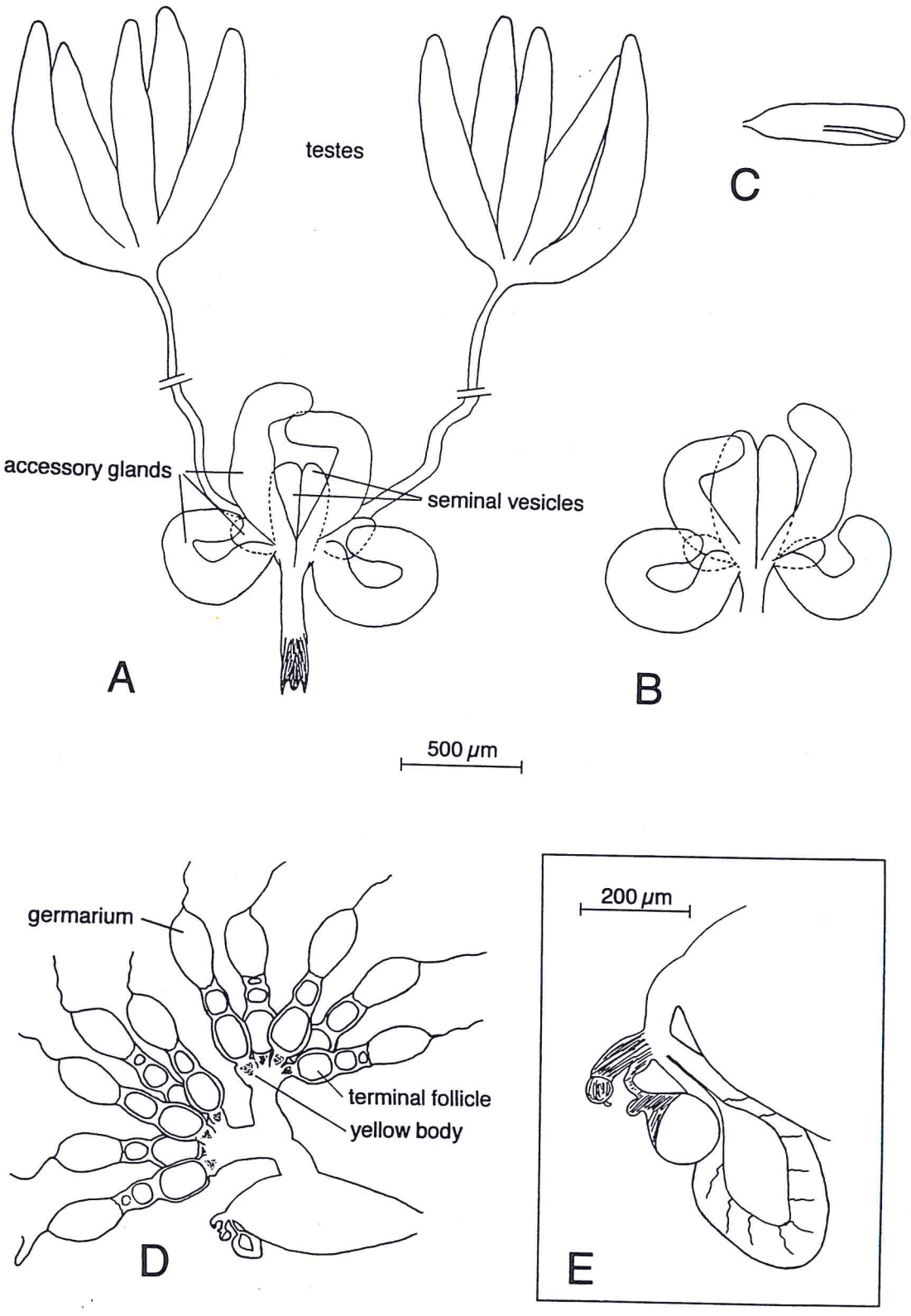


Fig. 3.11. Reproductive systems of male and female *P. truncatus*. (A) male reproductive system (of a male kept with females), (B) enlarged seminal vesicles (incl. accessory glands) of a male kept separated from females for 20 days, (C) spermatophore, (D) female reproductive system, (E) spermatheca with balloon-shaped reservoir and accessory gland.

Biscoe, 1984). The yellow bodies of individual females were always of similar size and coloration, suggesting that the same number of ovulations occurred from each ovariole. The distal end of the vagina terminates in a small, curved, brownish, cuticle-lined spermatheca (Fig. 3.11E), with an additional balloon-shaped reservoir and a plum-shaped accessory gland. Spermatozoa were found within the spermatheca and in the adjacent reservoir (in ordered form, in numerous bundles with the heads joined), but also in the vagina. In general, unmated female *P. truncatus* do not oviposit (Li, 1988), however, in two cases unmated females were observed to have laid several eggs, though no larvae hatched from these eggs.

Determination of physiological criteria for age-grading

The length and width of testis tubes varied little between the groups, with a general tendency of the testis tube length of mated males ten days and older to be smaller than those of unmated males (Table 3.8). A two-way anova showed significant effects of mating status ($p<0.0001$) and factor interaction ($p=0.0032$), but no age effects ($p=0.49$) for testis tube length. However, comparisons within the age groups and between mated and unmated males of the same age revealed significant differences only between 5- and 20-day-old unmated males, and between unmated and mated males 20 days old. For testis tube width, there was only a significant age effect (two-way anova; age effect: $p=0.0002$; mating effect: $p=0.35$; interaction: $p=0.83$), with a single significant difference between unmated 5- and 10-day-old males, and the mean difference was only about one measuring unit.

The length of seminal vesicles increased with age for unmated, but not for mated males (Table 3.8). A two-way anova revealed significant effects ($p<0.0001$) for both factors and interaction. The same tendency was observed for the width of seminal vesicles (Table 3.8), with a two-way anova showing significant effects of mating status ($p<0.0001$), age ($p=0.0024$) and factor interaction ($p=0.0196$).

Mean germarium size (length and width) of just emerged females was significantly smaller than germarium sizes of all other unmated treatments, and mean germarium size of starved 10-day-old females was significantly smaller than germarium sizes of other 10-day treatments (Table 3.9). Germarium length was significantly greater among mated than among unmated females, however, maximum differences between mean lengths were equivalent to only about one measuring unit (=24 μm). The only additional significant difference for germarium width was found between 5- and 30-day-old mated females.

Table 3.8. Testis and seminal vesicle size in relation to age and mating status. Means ± 1 s.e. are presented in µm; n=20 per group; means within a row differ significantly when followed by different small letters, means within age groups when followed by different capital letters [two-way anova followed by multiple t-tests (Bonferroni method, $p<0.05$)].

		age in days			
		5	10	20	30
testis tube length	unmated	1077 a (± 16)	1127 ab (± 23)	1201 b A (± 29)	1142 ab (± 27)
	mated	1117 (± 28)	1050 (± 20)	1051 B (± 25)	1038 (± 31)
testis tube width	unmated	217 a (± 4)	196 b (± 3)	203 ab (± 3)	208 ab (± 6)
	mated	210 (± 4)	196 (± 3)	202 (± 4)	205 (± 5)
seminal vesicle length	unmated	440 a (± 14)	560 b A (± 17)	640 c A (± 18)	630 c A (± 14)
	mated	418 (± 7)	427 B (± 13)	419 B (± 8)	455 B (± 17)
seminal vesicle width	unmated	163 a (± 8)	190 b A (± 5)	213 b A (± 11)	205 b A (± 10)
	mated	137 (± 4)	133 B (± 5)	138 B (± 5)	148 B (± 8)

The size of the largest terminal follicle and the number of follicles in the ovariole is related: No follicles were found in newly emerged females (Fig. 3.12); the first and the following second follicle developed until approximately day five. Thereafter two to four follicles were found in the ovarioles. Among fed females with more than one follicle, the terminal follicle was at least 192 µm long. The width of the follicle is strongly positively correlated with its length ($r=0.91$). Hence, data analysis of follicle width in relation to age and mating status would result in the same interpretation as the analysis of follicle length. The effects of age and mating status on follicle width were therefore not determined. More than 90% of the maturing terminal follicles of mated females (n=200), where deposition of white egg yolk was visible, were at least 384 µm long and freshly laid eggs were on average 740 µm long (range: 624-1008 µm) and 451 µm wide (range: 384-552 µm). The length of the largest terminal follicle and the maximum number of follicles were combined in

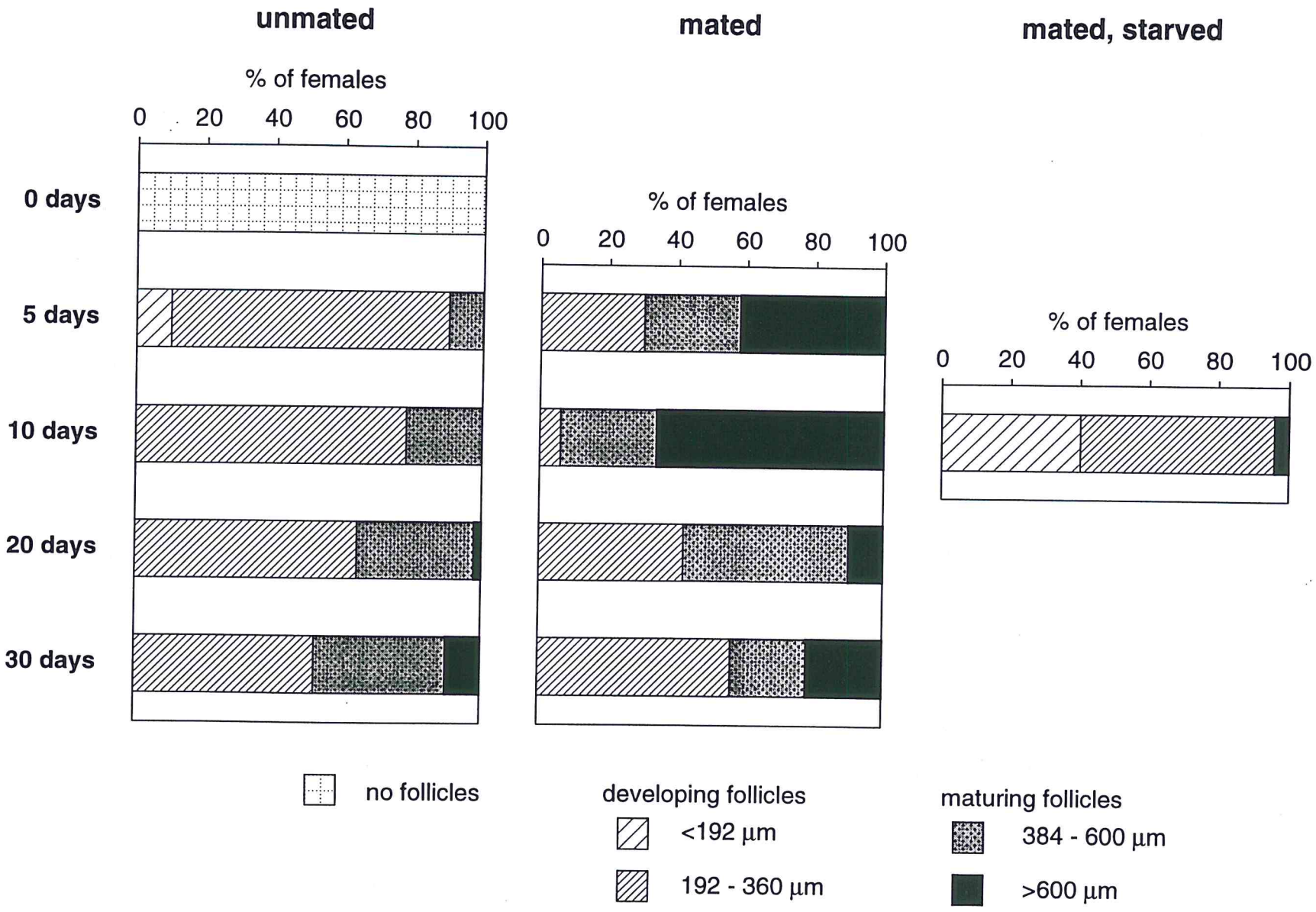
Table 3.9. Germarium size in relation to age, mating status and starvation. Means ± 1 s.e. are presented in µm; n=20 for unmated/0 days, n=50 for all other groups; means within a row differ significantly when followed by different small letters, means within age groups when followed by different capital letters [$p<0.05$; multiple t-tests (Bonferroni method)].

		age in days				
		0	5	10	20	30
germarium length	unmated	217 a (± 7)	324 b A (± 4)	325 b A (± 5)	322 b A (± 5)	322 b A (± 4)
	mated		349 B (± 5)	355 B (± 20)	353 B (± 5)	345 B (± 5)
	mated, starved			283 C (± 5)		
germarium width	unmated	95 a (± 2)	183 b (± 3)	189 b A (± 3)	184 b (± 3)	178 b (± 2)
	mated		183 a (± 3)	179 ab A (± 3)	174 ab (± 5)	169 b (± 3)
	mated, starved			148 B (± 3)		

the following five categories: 1) no follicles present, 2) developing follicles (small): <192 µm (in non-starved females only found when there was only one follicle in the ovariole), 3) developing follicles: 192-360 µm, 4) maturing follicles: 384-600 µm and 5) maturing follicles (ready to be laid): ≥624 µm. The age of the dissected females had an inconsistent effect on the size of the largest follicle in the ovaries (Fig. 3.12): Among unmated females more females with maturing follicles were found with increasing age; among mated females the number of females with maturing follicles decreased from day 10 onwards ($p<0.05$, Chi-square tests among age groups separately for fed mated and unmated females). Comparing mated and unmated females of the same ages showed that follicle development was retarded among unmated females ($p<0.05$ for ages five and ten days, $p>0.05$ for ages 20 and 30 days; Chi-square tests between mated and unmated females of the same age). Starvation had a strong effect on the size of the terminal follicle: There were few maturing and many small follicles (<192 µm), a size not found among fed females with more than one follicle ($p<0.05$; Chi-square test mated versus mated-starved females).

The degree of yellow body formation was compared among age groups for mated and unmated females separately (Fig. 3.13; $p<0.05$, Chi-square tests among age groups separately for mated and unmated females): Hardly any yellow bodies were observed among young females (0- and 5-day-old females), while yellow bodies grew bigger and darker with increasing age (for females ten days and older). Comparisons between mated and unmated females of the same ages showed that yellow body formation was in general independent of mating status and starvation ($p>0.05$; Chi-square tests between mated and unmated females of the same age), the only significant difference was found among 10-day-old females ($p<0.05$), with more unmated females having yellow bodies of stages 2 and 3 than mated and starved ones. During some dissections of young females, I observed no yellow bodies although the ovaries showed definite signs of recent ovulations, i.e., empty cavities between the terminal follicle and the end of the ovariole. Sperm was found in the spermatheca of all females which had been kept with males.

Fig. 3.12. (next page) Number of follicles and length of the largest terminal follicle in relation to age, mating status and starvation. Significant differences existed between females of different ages ($p<0.05$; Chi-square tests among age groups separately for mated and unmated females) and between mated, fed and unmated, fed females five and ten days old ($p<0.05$; Chi-square tests between mated and unmated females of the same age). Follicles of mated, starved females were significantly smaller than those of mated, fed females ($p<0.05$; Chi-square test mated versus mated-starved females); n=20 for unmated/0 days, n=50 for all other groups.


unmated
mated
mated, starved
% of females
0 20 40 60 80 100
0 days
5 days
10 days
20 days
30 days
no follicles
developing follicles
<192 µm
192 - 360 µm
maturing follicles
384 - 600 µm
>600 µm

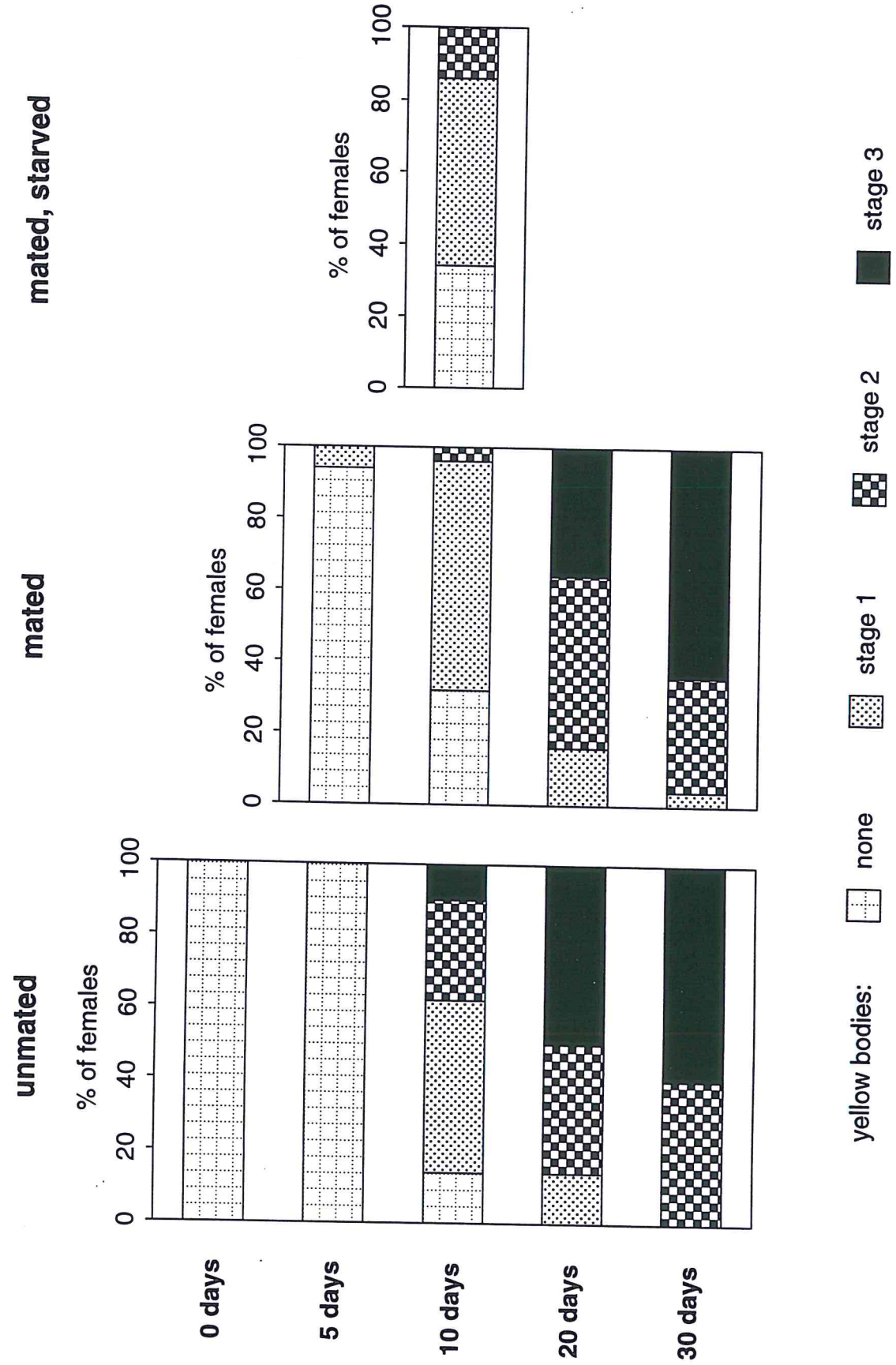

unmated
mated
mated, starved
% of females
0
20
40
60
80
100
0 days
5 days
10 days
20 days
30 days
yellow bodies:
none
stage 1
stage 2
stage 3

Fig. 3.13. (previous page) Yellow body formation in relation to age, mating status and starvation. Significant differences existed between females of different ages ($p<0.05$; Chi-square tests among age groups separately for mated and unmated females) and between mated, fed and unmated, fed females ten days old ($p<0.05$; Chi-square test between mated and unmated females of the same age); n=20 for unmated/0 days, n=50 for all other groups.

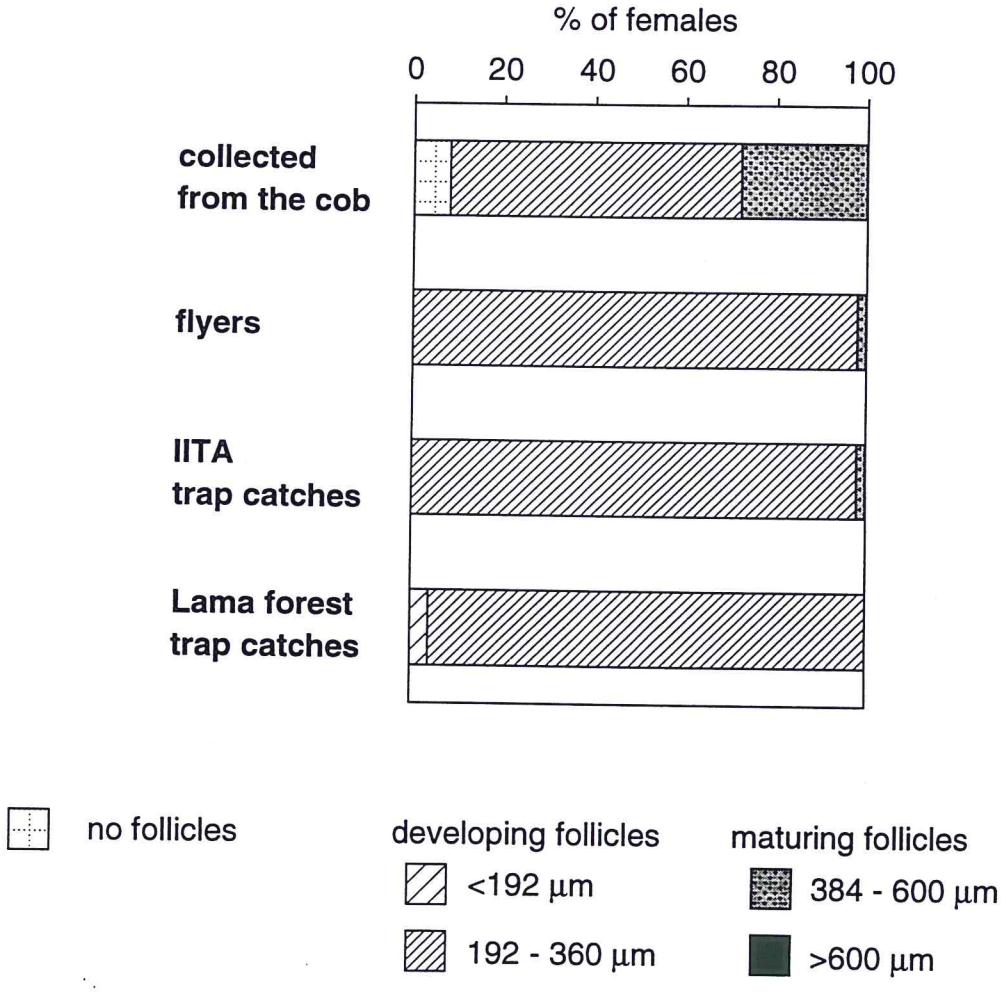


Fig. 3.14. Number of follicles and length of the largest terminal follicle among females collected in a dispersal experiment and caught with pheromone traps. Follicle sizes among beetles collected from the cob differed from follicle sizes of flyers and trap catches (Chi-square test for all four treatments: $p<0.05$; for flyers and trap catches: $p>0.05$); n=50 females per treatment.

Evaluation of follicle and yellow body development and mating status in sampled females

The length of the largest terminal follicle and the maximum numbers of follicles (categorized as described above) of females collected in the dispersal experiment (collected from the cob and flyers) and of females trapped with pheromone traps were compared (Chi-square test for all four treatments: $p<0.05$; for flyers and traps catches: $p>0.05$). Ovarioles without follicles and with developing and maturing follicles were found among the females which were collected from the cob, whereas flyers and trapped females carried hardly any maturing follicles (Fig. 3.14).

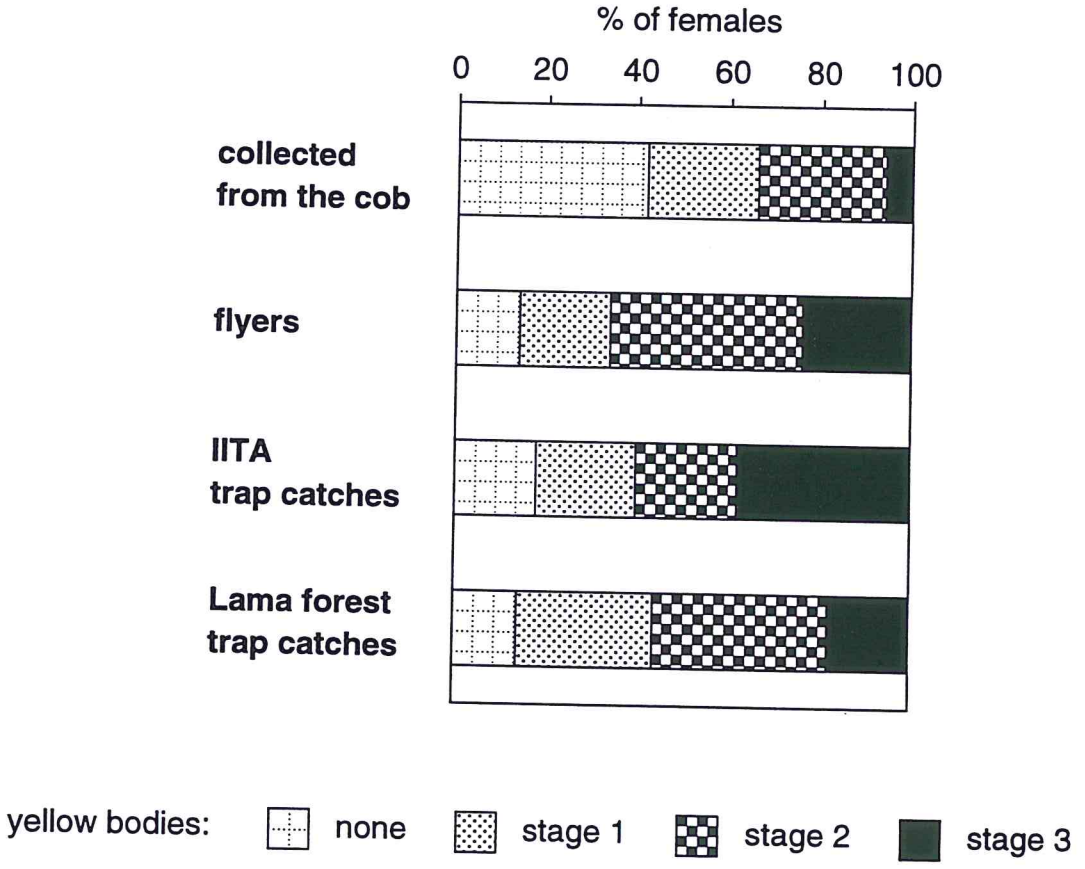


Fig. 3.15. Yellow body formation among females collected in a dispersal experiment and caught with pheromone traps. Yellow body stages among beetles collected from the cob differed from yellow body stages of flyers and trap catches (Chi-square test for all four treatments: $p<0.05$; for flyers and trap catches: $p>0.05$); n=50 females per treatment.

Among females collected from the cobs, there was a high percentage of individuals without any yellow bodies, whereas more yellow bodies of stages 2 and 3 were found among flyers and trap catches (Fig. 3.15; Chi-square test for all four treatments: $p<0.05$; for flyers and traps catches: $p>0.05$). Sperm was found in all females, except in one female collected from the cob which had no follicles developing and no yellow bodies, and so was apparently newly-emerged.

3.6. Discussion

Effects of crowding

Crowding and the accompanying degradation of the food source strongly affected the number of *P. truncatus* dispersing as well as the dispersal rates (percentage of the progeny dispersing). The number of *P. truncatus* dispersing rose steadily with increasing progeny numbers (Fig. 3.2A and Fig. 3.8A), and increasing progeny density as well as flight week (with underlying rising progeny densities) were found to increase the number of *P. truncatus* flying in the second experiment. Also dispersal rates rose with increasing progeny densities. However, in the first experiment, which was designed in such a way that all progeny were at least theoretically old enough to take flight during the observation period, dispersal rates reached a maximum at densities greater 400 insects per 175 g maize, with only a mean of 32% of the population taking flight. The dispersal rates for the higher progeny densities in the preliminary second experiment increased, but at a much lower rate than at lower progeny densities. Thus, a great part of the population stayed in an environment where its reproductive success was declining, and the response of *P. truncatus* to crowding and habitat degradation met therefore only partly the *a priori* expectations of optimal resource assessment (Parker & Stuart, 1976). A high migration threshold may be an appropriate adaptation, however, for an insect whose niche is scarce or difficult to locate (Ziegler, 1978) and whose costs for dispersal are, therefore, high (Rankin & Bruchsted, 1992). *P. truncatus* may meet exactly such a situation in its natural habitat. The ecology of *P. truncatus* outside maize and cassava stores remains ill-defined; however, there seems to be strong evidence that the insect occupies a transient niche in degrading branches of trees and shrubs (Nang'ayo *et al.*, 1993), specifically those girdled by cerambycids (Ramírez-Martínez *et al.*, 1994), which may have enriched nutritional status (Forcella, 1981; 1982; cf. Chapter 4.4: Responses to woody plants). This is also consistent with the observation that *P. truncatus* is only able to reproduce in wood

with a high starch content (Detmers *et al.*, 1993). In these circumstances, it may be advantageous for a great part of the population to remain in a patch of substrate, even if it is already heavily exploited.

The results obtained in the first experiment may be contrasted with those from a similar experiment conducted with *Rhyzopertha dominica* (F.) (Col.: Bostrichidae), a species closely related to *P. truncatus*, which is a cosmopolitan pest of stored cereals (Haines, 1991) and, therefore, probably more adapted to food substrates with high nutritional value. In that study (Barrer *et al.*, 1993), on average 70% of the population migrated at high progeny densities. *P. truncatus* attacks maize and cassava only facultatively, and it is not clear to what extent the results obtained in my studies may have been affected by the fact that maize is richer in nutrients than the woody substrate to which *P. truncatus* is probably evoulutionary adapted. The high nutritional status of the maize may have acted as an arrestant, counteracting the stimulus of crowding to initiate dispersal. Alternatively, it may be that in wood the declining nutritional status of the substrate might normally provide an additional cue to initiate dispersal but on maize a sufficiently low nutritional status might never be reached or only at extremely high densities (cf. Fig. 3.8B).

For aphids, locusts and other insects, an ontogenetic impact on dispersal had been shown, caused by crowding and food deficiency in the parental generation (Johnson, 1966; Dingle, 1985). However, a direct effect of the density in the parental generation on the flight behaviour of the progeny could not be verified for *P. truncatus*; beetles originating from the same parental densities showed varying dispersal behaviour which was strongly related to the current progeny densities. It can therefore be assumed that the beetles reacted directly to their immediate environment. An increasing dispersal rate of *P. truncatus* with increasing initial density (and, therefore, also with increasing progeny density) was also observed by Fadamiro (1995). Fadamiro's experiment was conducted using insects removed from the culture medium (and tested in a wind tunnel), so the crowding effect, caused either by the parental or the progeny density, must have had an endogenous effect, i.e., a physiological change had taken place before dispersal began (cf. discussion on physiological state of the migrating females).

P. truncatus which were used to infest the cobs, i.e., beetles which formed the parental generation in the dispersal experiments, flew off the cobs in low numbers during the whole three-week observation period. This confirms that dispersal can

occur interreproductively at varying ages (cf. discussion on physiological state of the migrating females).

Effect of pheromone

The aggregation pheromone of *P. truncatus* usually functions as an attractant and arrestant for both sexes (e.g., Obeng-Ofori & Coaker, 1990; Ramírez-Martínez *et al.*, 1994). The pheromone production by the males has been found to be reduced in the presence of female conspecifics and no pheromone was produced on a substrate without food value (Smith *et al.*, 1996; cf. Chapter 5.2). It was speculated that a low pheromone concentration might act as a dispersal initiating factor, while a high concentration might discourage dispersal. However, dispersal was not found to be influenced by the addition of synthetic pheromone. This might be due to the fact that in the treatment with additional pheromone two contradictory cues acted simultaneously: The crowding effect which encourages dispersal and the arrestant effect of the pheromone. With tactile cues possibly overriding the olfactory response, dispersal takes place regardless of the pheromone concentration. A similar phenomenon was also observed when investigating the reactions of *P. truncatus* kept under varying conditions: While the pheromone was found to be strongly attractive for beetles separated from food substrates, independent of age and pre-conditioning (Obeng-Ofori & Coaker, 1990; cf. Chapter 5.2), the pheromone did not lure the beetles out of maize stores (Pike, 1993).

Effect of meteorological and environmental factors

Multiple regression analyses revealed that for both *P. truncatus* caught with pheromone traps and beetles flying off the maize cobs only the mean temperature affected the number of beetles dispersing (besides the effect of crowding on the experimental flyers). Since temperature sums during the development period did not influence the size of the final progeny densities, and thereby neither indirectly the number of flyers, it can be assumed that higher temperatures promoted dispersal directly, possibly due to an increased activity of the insects during the twilight flight hours (Johnson, 1969). Also Borgemeister *et al.* (1997) found the mean temperature to be the only meteorological variable to partly explain the fluctuations in flight activity (recorded with pheromone-baited traps) in the surroundings of the IITA station during a three-year observation period. It can be assumed that trap catches were not affected by increased pheromone release rates under warmer conditions, as

higher mean temperatures evoked also increased migration in the dispersal experiment and accompanying trap catches of *T. nigrescens* in the experiment of Borgemeister *et al.* (1997) were not influenced by temperature. Tigar *et al.* (1994b) reported peaks in flight activity to be related to rainfall in the drier but not in the humid regions of Mexico, and Giles *et al.* (1995) observed an increase in flight activity during the minor, but not during the major rainy season in Kenya. However, no association between flight activity and rainfall was observed in the present study.

Although the variance of both dependent variables could partly be explained by variation in temperature, there seems to be no obvious relation between dispersal as observed in the experiment and flight activity in the field; seasonal cues, which have been suggested to initiate dispersal in other insects species (Johnson, 1969; Dingle, 1972, 1985; Mitchell *et al.*, 1972; Forsse & Solbreck, 1985), had no apparent influence on dispersal. Photoperiod or temperature changes may be adequate environmental cues signaling future conditions; in tropical regions, however, these parameters change little and insects may rather respond directly to the suitability of the immediate habitat (Dingle, 1972). Dispersing *P. truncatus* may either have emigrated from maize or cassava stores or from woody plants, the supposed natural habitat, and different factors may influence emigration from these patches. The flight activity pattern as observed between December 1995 and November 1996 at the IITA station (cf. Fig. 3.6) could well be explained in relation to storage practices. Maize stores in the area were established in August/September and subsequently *P. truncatus* populations increased within the stores (Borgemeister *et al.*, 1997). Peaks in flight activity could, therefore, be attributed to increasing population densities in stores. In addition, farmers start to empty stores from approximately November onwards until April/May with the majority of stores being emptied between December and February (Agbaka, 1996), thereby disturbing the breeding populations and eliciting dispersal. Between June and September flight activity remained at a moderate level and varied little, with beetles possibly emigrating from store residues and patches in the natural habitat. When maize cobs matured in the fields and thereby developed suitable breeding conditions for *P. truncatus* (Giles *et al.*, 1995), trap catches decreased to the lowest level, possibly due to the abundant alternative pheromone sources, i.e., male *P. truncatus* on maize.

The area around the IITA station and the Mono District in south-western Benin are both maize growing regions with similar climatic conditions (C. Borgemeister, pers. comm.). However, the annual flight activity pattern differed between these zones (cf. Fig. 3.6 and Fig. 3.10). In both regions, flight activity was lowest in

September/October and increased thereafter, but the major peaks in the Mono District occurred later than around the IITA station, namely during the main rainy season, although storage practices in the two regions coincide (C. Borgemeister, pers. comm.). Hence, flight activity in the Mono District must have been affected by factors different to those which influenced migration near the IITA station. Flight activity in the rural Mono District may be more dependent on migration from natural hosts and associated dispersal initiating factors (Borgemeister *et al.*, 1997). Strong seasonal fluctuations in the flight activity of *P. truncatus* were also observed in non-farmland (Nang'ayo *et al.*, 1993; Giles *et al.*, 1995). Under these conditions migration may be dependent on the degradation and availability of natural host plants in time and space. For instance, heavy rains may destroy breeding sites, thereby initiating migration and, at the same time, reducing progeny production which in turn may lead to the dramatic flight activity drop after the major dispersal period.

Time-of-day dependent flight

The time-of-day dependent flight behaviour observed in all experiments confirms that *P. truncatus* can be regarded as a crepuscular insect (Fadamiro, 1995; Fadamiro & Wyatt, 1995). Flight initiation as observed in the experiments and flight activity in the field (measured as *P. truncatus* caught with pheromone traps) was greatest immediately before and after sunset. Flight initiation was directly linked to light and to an internal clock (Jones, 1996), as beetles in the insectarium flew in increased numbers after turning the light off or on, but also partly just before turning the light on. While the morning peak in flight activity as observed by Novillo Rameix (1991) and Tigar *et al.* (1993) was also evident in the trap catches presented here, the morning peak in flight initiation in the dispersal experiments was very small or not existent. Fadamiro (1995) and Fadamiro & Wyatt (1995) reported a dramatic drop in flight initiation for temperatures lower than 25 °C. Under natural conditions, with relatively low temperatures at night, the vast majority of beetles may fly off just after sunset, when their body temperature is still high. The morning peak observed among trap catches may result from beetles which have left the habitat the evening, or some evenings, before.

Density dependent progeny production

The number of offspring surviving per female parent decreased with increasing initial densities. This result corresponds with the findings of Li (1988) and Vowotor *et al.* (1996), who observed a reduction in progeny production due to egg destruction by tunneling adults, as well as scramble and contest competition between larvae. Another possible factor, reduced oviposition with increasing population densities, as described for *Oryzaephilus surinamensis* (L.) (Coleoptera: Cucujidae) (Pierce *et al.*, 1990), has yet not been demonstrated for *P. truncatus*.

Sex ratios

The proportion of females in the basic cultures (cf. Chapter 3.2.2), among the progeny (cf. Chapter 3.2.2 and 3.3.2) and in the samples and their progeny from the maize stores (cf. Chapter 3.4.2) corresponded to the sex ratio of *ca.* 1:1 reported previously for *P. truncatus* (Shires, 1979; Vowotor *et al.*, 1996). However, in 95 of the 96 samples of the first dispersal experiment and also in the preliminary trial of the second experiment, there were more females dispersing than males. These observations correspond well with the sex ratio observed among *P. truncatus* caught with maize cobs baited with male *P. truncatus*, with the female proportion of *P. truncatus* caught averaging 64% (cf. Chapter 5.5.2) and confirm that females make up the majority of migrating *P. truncatus*. These findings are consistent with the observations of Wekesa (1994) who also caught a majority of females with pheromone traps in Kenya. However, the sex ratios among dispersing *P. truncatus* may depend on the breeding habitat they emigrated from: Trap catches within forests (Ramiréz-Martinéz *et al.*, 1994; Borgemeister, pers. comm.), where the trapped beetles probably emigrated from the natural woody host plants, were nearly balanced. Possibly, the higher nutritional value of stored food products may act as an arrestant, making males (for reasons unknown) less likely to react to other cues, such as crowding, that would otherwise have stimulated them to migrate.

Sex ratios recorded for weekly catches with pheromone traps were always female biased and were not correlated with the numbers of *P. truncatus* caught (cf. Chapter 5.7 for discussion on the evaluation of sex ratios in pheromone traps). It could, therefore, be verified that peaks in flight activity were not caused by an increased proportion of females migrating. In contrast, trap catches of *Pityogenes chalcographus* L. (Col.: Scolytidae) with the synthetic aggregation pheromone of

the beetle were male-biased during high and balanced or female-biased during low flight activity (Zumr, 1988).

Age-grading method

Male and female *P. truncatus* of known age and mating status were dissected to search for and to verify possible age-grading criteria, and to serve as references for the sampled beetles. No suitable age-grading criteria could be determined for *P. truncatus* males. Luff (1973) found testis size of male *Pterostichus madius* (F.) (Col.: Carabidae) to increase with age, and Nordenhem (1989) described testis size of pine weevils, *Hylobius abietis* (L.) to vary according to the reproductive stage. However, in *P. truncatus* testis size varied little among age/mating-status groups and could therefore not to be used as an age-grading parameter. Seminal vesicles increased with increasing age only for males which were separated from females, a situation in which sperm had accumulated in these reservoirs.

Germarium size varied little among the females of varying age and mating status, the size being only reduced for recently emerged and severely starved females. The number of mated females with maturing terminal follicles in their ovaries was higher among younger females than among older ones. Due to a five to ten days preoviposition period (Shires, 1980), there were many females in the 5- and 10-day age groups with follicles developing for the first oviposition, while among older females a higher proportion may have recently oviposited. Among unmated females, follicle development seemed to be retarded; oocytes may have been resorbed before egg yolk deposition started (Bell & Bohm, 1975). With increasing age more follicles matured further, and may have been oviposited unfertilized or resorbed at this later stage. It should be noted that fed females from the age of ten days onwards always had at least two follicles in each ovariole, of which the larger terminal one was at least 192 µm long, while follicles from starved females were obviously in the process of being resorbed. Oosorption due to food shortage has been observed in many insect orders and starts most commonly with the terminal oocyte (Bell & Bohm, 1975; Chapman, 1982).

Follicular relics at the base of ovarioles, so-called yellow bodies, have frequently been found in female adults of different insect species and have been used for physiological age determination (Tyndale-Biscoe, 1984). Yellow body formation was also observed in female *P. truncatus,* and follicular relics grew bigger and

darker with increasing age of the females. This is valid for mated and unmated females, i.e., follicular relics due to oviposition and oosorption are indistinguishable, as also observed in studies with other insect species (Tyndale-Biscoe & Watson, 1977; Tyndale-Biscoe, 1978; 1984). Although the described age-dependent tendency is obvious, females of the same age groups differ in the degree of yellow body development. Oviposition rates were found to differ greatly among individual female *P. truncatus* of the same age (Shires, 1980; Li, 1988). It may be asssumed that oocyte development rates differ accordingly and that this variation would be reflected in the varying degree of yellow body development in individual females. In addition, parts of the follicular relics may have been extruded from the ovariole with subsequent ovulations (Tyndale-Biscoe, 1978). Yellow body stages described for fed and starved 10-day-old mated females were similar. However, the yellow body development was slightly advanced in fed unmated ones, most likely due to oosorption under ample food supply (a situation probably not found under natural conditions), possibly causing an increased turnover of follicles. During some dissections of young females, no yellow bodies were found although the ovaries showed definite signs of recent ovulations. The coloration of the relics developed some time after ovulation, as also described for *Epilachna vigintioctopunctata* Fabr. (Col.: Coccinellidae) (Kulshrestha, 1968). This phenomenon prevents me from distinguishing between nulliparous and parous females. The present evaluation of yellow body formation therefore only permits to determine relative increasing physiological ages of female *P. truncatus*.

Physiological state and age of migrating and non-migrating females

Beetles collected directly from maize cobs represented a population of insects between 0 and 28 days old. There were very young females (no follicles and no yellow bodies, definitely nulliparous) as well as females with developing and maturing terminal follicles, and with and without yellow bodies, i.e., females presumably developing oocytes for the first oviposition and females that had already completed one or several oviposition cycles. Flyers were apparently not a representative sample of this population: Among them more females had already completed several oviposition cycles (supposing that females do not resorb several series of follicles when mated and on a nutritious substrate) and carried hardly any maturing follicles. Females caught with pheromone traps at both locations showed characteristics similar to the flyers from the dispersal experiment. Apparently,

migration occurred at different physiological ages, and follicle development was suspended.

Most migration in insects has been described as a prereproductive behaviour (Johnson, 1963, 1966, 1969; Dingle, 1972; 1985; and references therein), and Johnson (1969) postulated the oogenesis-flight syndrome as the underlying physiological explanation: Adverse or cue environmental factors act ontogenetically and produce migrant adults with a presumably prolonged preoviposition period during which migration occurs. The endocrine effects assumed to be responsible (corpus allatum activity and associated juvenile hormone production) have been investigated (e.g., Tanaka, 1986), but the exact role of hormones and modes of hormonal actions on migration have not yet been clarified (Pener, 1985). However, the oogenesis-flight syndrome can also explain interreproductive flight, as I observed for *P. truncatus* females, since in general it implies that the reproductive system is minimized at a time when the flight system is maximized (Johnson, 1963; Dingle, 1972). This is exactly what was found: Suspended follicle development among migrating females.

Prereproductive dispersal has also been assumed to be adaptive, as migrants should have a high reproductive value, in so far as migrants should be young adults which have their reproductive life ahead, with the ability to rapidly exploit newly colonized sites (Dingle, 1972; Pianka, 1994). Life time fecundity is high among female *P. truncatus* (*ca.* 320 eggs; Li, 1988); expected life span is long and oviposition takes place nearly up to its end (*ca.* 120 days; Li, 1988), and declines only gradually with age (Li, 1988). Migrants, therefore, still possess high reproductive value, even if they disperse after having completed one or several oviposition cycles. In addition, it may be advantageous for the females to leave some offspring behind before setting out on an uncertain search for a new habitat (den Boer, 1968; Pianka, 1994), especially when the risks inherent in migration are high (Ziegler, 1977; 1978). *P. truncatus* may meet exactly such a situation in its natural habitat (cf. discussion on effects of crowding). However, the age-composition of migrating *P. truncatus* may also simply reflect the age-composition of the flight-capable part of resident populations; part of the young females collected directly from the maize cobs may not have yet been capable of flying (Fadamiro, 1995).

So far, only male *P. truncatus* have been regarded as 'pioneers' (Hodges, 1994); they were assumed to be the sex that locates a new breeding site, because the males

produce an aggregation pheromone which attracts conspecifics of both sexes (e.g., Cork *et al.*, 1991). However, females emigrating from maize cobs and female migrants trapped in the field were all mated. Female migrants may, therefore, also act as colonizers. Female *P. truncatus* which mated only once were found to be able to lay fertile eggs for *ca.* 46 days and life time fecundity, compared to females that were able to mate during this period, was not reduced when mating occurred thereafter (Li, 1988). Hence, female fecundity would not necessarily be reduced when a single female acts as an individual colonizer, since they may mate with their male offspring. I therefore postulate a two-fold colonization strategy for discussion: Individual male and female *P. truncatus* migrate, and when a suitable food source, not yet populated with conspecifics, is located, males produce pheromone (Smith *et al.*, 1996) and initiate a new colony by attracting conspecifics (cf. Chapter 5). When females locate such a suitable food and oviposition site, they start to reproduce. In the natural habitat, where breeding sites, such as degrading branches, are only in a suitable condition for a very limited time and where the carrying capacity of such a site is relatively small compared to a maize cob or to a maize store, a single female and its small offspring population may suffice to exploit the entire source. In addition, such female-founded colonies may benefit from beginning their development undetected by the predator *T. nigrescens,* which locates its prey guided by the prey-produced aggregation pheromone (Rees *et al.*, 1990; cf. Chapter 5). However, regarding the colonization of maize stores by the pest, initial infestations by males may be regarded as more important, as they aggregate conspecifics and thereby provide the source for a rapid population increase.

4. Host finding: Primary attraction

4.1. Introduction

Many phytophagous insects use host or host-associated volatiles to locate suitable feeding and oviposition sites (e.g., Fraenkel, 1959; Finch, 1980; Miller & Strickler, 1984; Metcalf, 1987), and host location by olfactory cues has also been reported for stored-product beetles (e.g., Pierce *et al.*, 1981; Barrer, 1983; Walgenbach *et al.*, 1987). Behaviourally active volatiles may act as attractants (Kennedy, 1978), eliciting directional movements towards the odour source (presumably by odor-conditioned anemotaxis at long range and chemotaxis close to the source). Moreover, volatiles may act as arrestants, eliciting non-directional movements like slowing down and stopping (orthokinesis) or changing the rate of turning (klinokinesis) (Kennedy, 1977b; 1978; Baker, 1985). While the former behaviours lead insects directly to the source, the latter arrest insects in close proximity and promote searching behaviour within this area (Kennedy, 1965; Ponsonby & Copland, 1995). Sex and aggregation pheromones have been described as functioning as attractants, while host plant volatiles have been considered to act mainly as arrestants (Thorsteinson, 1960; Finch, 1986). Hence, there are few examples of host-plant finding from distances greater than *ca.* one meter (Miller & Strickler, 1984); however, cabbage root and onion flies (Prokopy, 1986), as well as bark beetles (Beeson, 1930), have been reported to respond to host plant volatiles from at least some metres from the source.

Secondary attraction (attraction mediated by pheromones) is well documented for *P. truncatus* (cf. Chapter 5). However, factors influencing the initial attack (primary attraction), have not yet been investigated satisfactorily. *P. truncatus* responded to volatiles emitted from maize grains and dried cassava in electroantennogram studies and in short-distance bioassays (Pike *et al.*, 1994), but neither the single nor the mixed attractive components identified for maize grains (nonanal, decanal, vanillin, hexanoic and nonanoic acid) nor whole maize grains have been found to be attractive in wind tunnel trials (Fadamiro, 1995). Although there is strong evidence that the beetle is only facultatively associated with stored commodities like maize and cassava (cf. Chapter 1), olfactory responses of *P. truncatus* to assumed woody host plants have not been investigated yet.

For these reasons, I was interested to verify whether the behavioural responses of *P. truncatus* to maize odours are influenced by the beetle's origin or physiological

state and if its reactions are plant specific. The effects of other odour sources associated with maize stores in coastal West Africa [i.e., other storage pests (Gounou *et al.*, 1994; C. Borgemeister, pers. comm.) and *T. nigrescens*, the predator adapted to *P. truncatus*], as well as odours from suspected woody host plants are also evaluated.

4.2. Materials and methods

Insects

Laboratory cultures of *P. truncatus* were set up and maintained as described in Chapter 2.2, and cultures of *S. zeamais* were established and maintained in the same way. Cultures of the predator *T. nigrescens* were started in 1994 with specimens of the same geographic origin as *P. truncatus* (Mono District, south-western Benin). Since only a total of 787 *T. nigrescens* have been released in Benin (Anonymous, 1992) it can be assumed that the specimens used to set up the laboratory cultures were progeny of the population which was released in Togo from 1991 onwards (Biliwa *et al.*, 1992). *T. nigrescens* cultures were set up with 500 adult *P. truncatus*, and 10 days later 50 adult *T. nigrescens* were added, which were removed after two weeks. A prey:predator ratio of 10:1 has been reported to produce maximum numbers of predator offspring, and delaying the introduction of *T. nigrescens* ensures that the preferred prey stages of *P. truncatus* are present when inoculation with *T. nigrescens* takes place (Helbig, 1993; Pöschko, 1993). Basic cultures were mixed several times with insects from the same region and maintained under the same conditions as laboratory cultures of *P. truncatus* (cf. Chapter 2.2). *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) was reared on maize cobs at 27 ± 2 °C and 50-90% r.h. under a L12:D12 photoperiod.

Unless stated otherwise, the *P. truncatus* used as test insects were 0-3 weeks old and were removed from the basic cultures 16-24 h prior to their use. Beetles from the Mono District (maize production region) and near Banté (Zou District, central Benin, an area with predominantly natural savannah woodland vegetation) were collected with funnel traps baited with the synthetic analogs of the aggregation pheromone (AgriSense-BCS Ltd., Pontypridd, UK) and were tested *ca.* 24-48 h after they were trapped. Beetles originating from the dispersal experiment (cf. Chapter 3.2), had flown off maize cultures the evening before they were tested. *P. truncatus* originating from cassava chips (peeled tubers, cut to pieces, washed

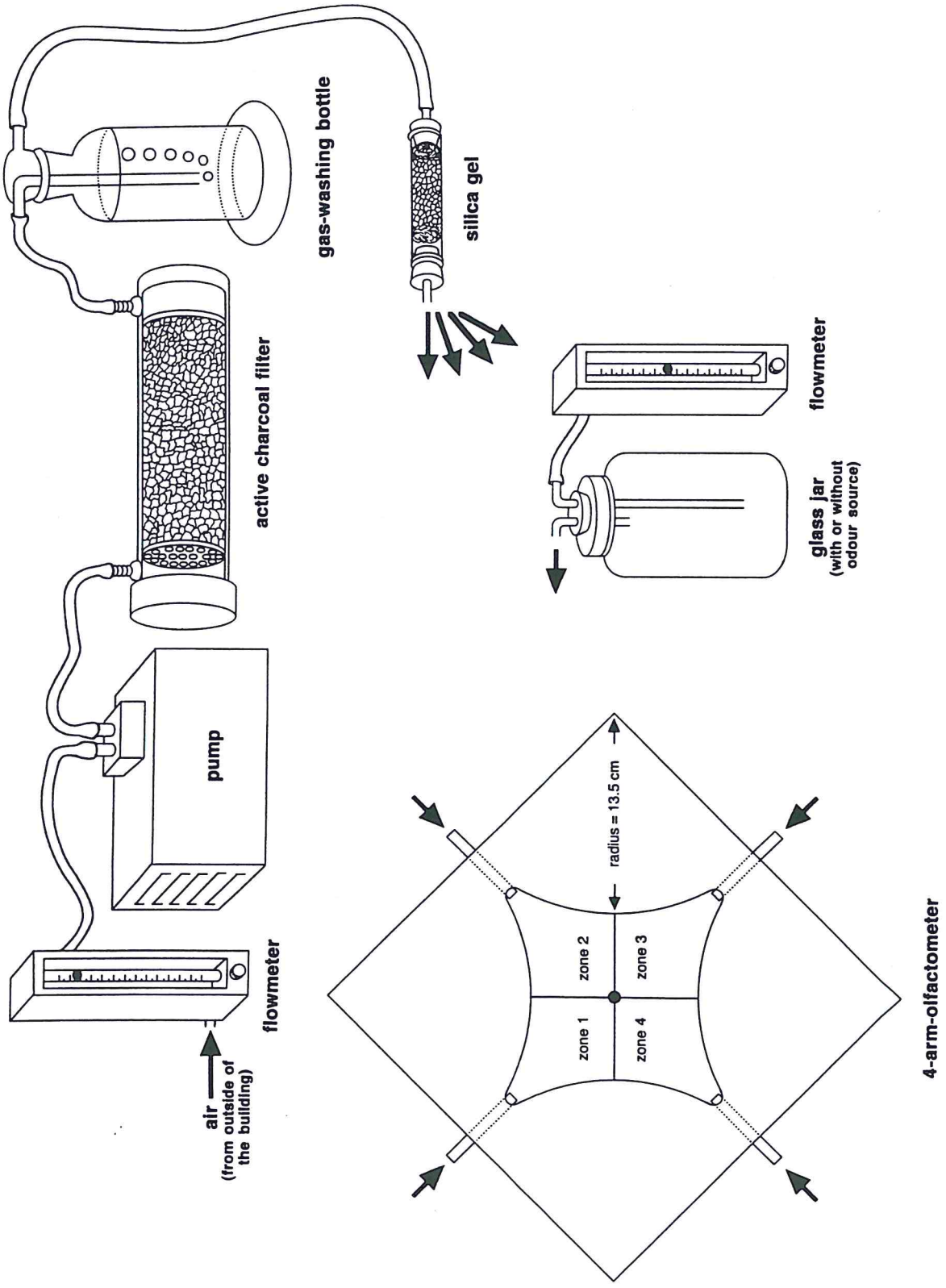
and dried) had been reared on this substrate for one generation. Beetles to be used as experimental subjects were kept in glass petri dishes laid out with brass gauze between collection and use in the experiments.

Olfactometer: Experimental set-up

The basic design of the olfactometer (Fig. 4.1) was similar to that used by Vet *et al.* (1983). The star-shaped four-pointed exposure chamber was milled into an aluminium plate (27 × 27 × 1.2 cm), with a hole (8 mm diameter) drilled into the walls at each rounded point. A second aluminium plate (27 × 27 × 0.3 cm), with a hole in its centre (8 mm diameter), served as the floor, and a glass plate (27 × 27 × 0.8 cm) as cover. Since *P. truncatus* cannot walk on smooth surfaces, a sheet of paper, pierced at the position of the centre hole, was used as floor covering. A small aluminium pipe (1 cm long, 8 mm outer/6 mm inner diameter), covered with plastic screen to prevent beetles from dropping through the hole, was placed inside the centre hole. Aluminium pipes (6 cm, 8 mm outer/6 mm inner diameter) were inserted through the holes of the chamber walls so that the pipes extended into the arena for *ca.* 0.5 cm. The olfactometer was housed in a room with no natural light and illuminated by a fluorescent tube (Sylvania F 18W 154, daylight) centred *ca.* 1 m above the olfactometer.

Air was drawn from the exterior of the building through a *ca.* 10 m long pipe (5 cm inner diameter) by a vacuum pump (Neuberger, type N010 KN18). Total air flow was controlled by a flowmeter (Brooksmeter Sho-rate, 1355, R-2-15-B, sapphire float). The air was pushed through a perspex tube (25 cm long, 6 cm diameter) filled with activated charcoal and a gas-washing bottle filled with distilled water to clean the air from volatiles and to stabilize r.h. As *P. truncatus* avoided high r.h. in preliminary trials (cf. results, Chapter 4.3), the air was then passed through a glass tube (10 cm long, 2.5 cm diameter) filled with equilibrated silica gel to adjust r.h. to 60-70%. (In cases where r.h. was changed by an odour source, e.g., by passing through a jar filled with grains, four tubes, filled with differently equilibrated silica gel, were used to adjust r.h. accordingly.) The single air stream was divided and pushed through four flowmeters (Brooksmeter Sho-rate, 1355, R-2-15-D, stainless steel float) to maintain each flow at 15.2 l/h. The air streams then passed through

Fig. 4.1. (next page) Olfactometer set-up.


gas-washing bottle
silica gel
active charcoal filter
pump
flowmeter
air
(from outside of the building)
flowmeter
glass jar
(with or without odour source)
radius = 13.5 cm
zone 1
zone 2
zone 3
zone 4
4-arm-olfactometer

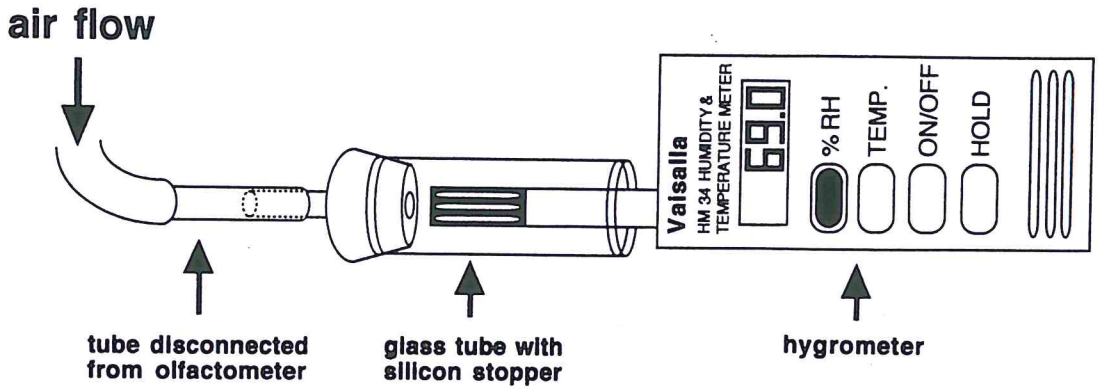


Fig. 4.2. Measuring relative humidity of an air flow.

1000 ml glass jars, each either containing an odour source or serving as control, and each stream entered the arena through one of the four connected aluminium pipes. The air escaped from the arena through the centre hole in the base; the air streams formed four distinct zones within the arena. All tube connections were made with silicon components. NH_4Cl smoke, generated from NaOH and HCl vapour, was used to determine the shape of the odour fields and to check for leaks when the experimental set-up was established.

Olfactometer: Experimental procedure

The beetles were tested individually and each insect was tested only once. The test insect was placed in one of the odour zones (alternating the 'starter zone' with each insect) and the cover plate replaced. Recording started after 20 sec, when the odour fields were properly reestablished. The time of each movement of the beetle from one odour zone to another was recorded for 5 min and later calculated as time spent per quadrant. Afterwards, the beetles were dissected to determine their sex. Unless stated otherwise, each odour situation was tested with 160 insects. Although no directional biases could be found (cf. results), the positions of the odour sources were rotated after a quarter of the replicates had been completed, the odour sources replaced by fresh ones (i.e., four fresh odour sources were tested for each odour situation unless stated otherwise) and the olfactometer cleaned with detergent (Extran neutral), rinsed with distilled water and 75% ethanol and heated overnight at 100 °C. In addition, the air flow was verified with four flowmeters at the four pipes entering the olfactometer at the beginning of each session. Relative humidity

was measured hourly with a hygrometer (Vaisalla HM 34; Fig. 4.2) and adjusted when necessary. The paper floor was changed after every ten insects were tested.

The tracks of female *P. truncatus* (cf. Fig. 4.3) were drawn by placing a transparency on the cover glass of the olfactometer and marking the movements of the beetle.

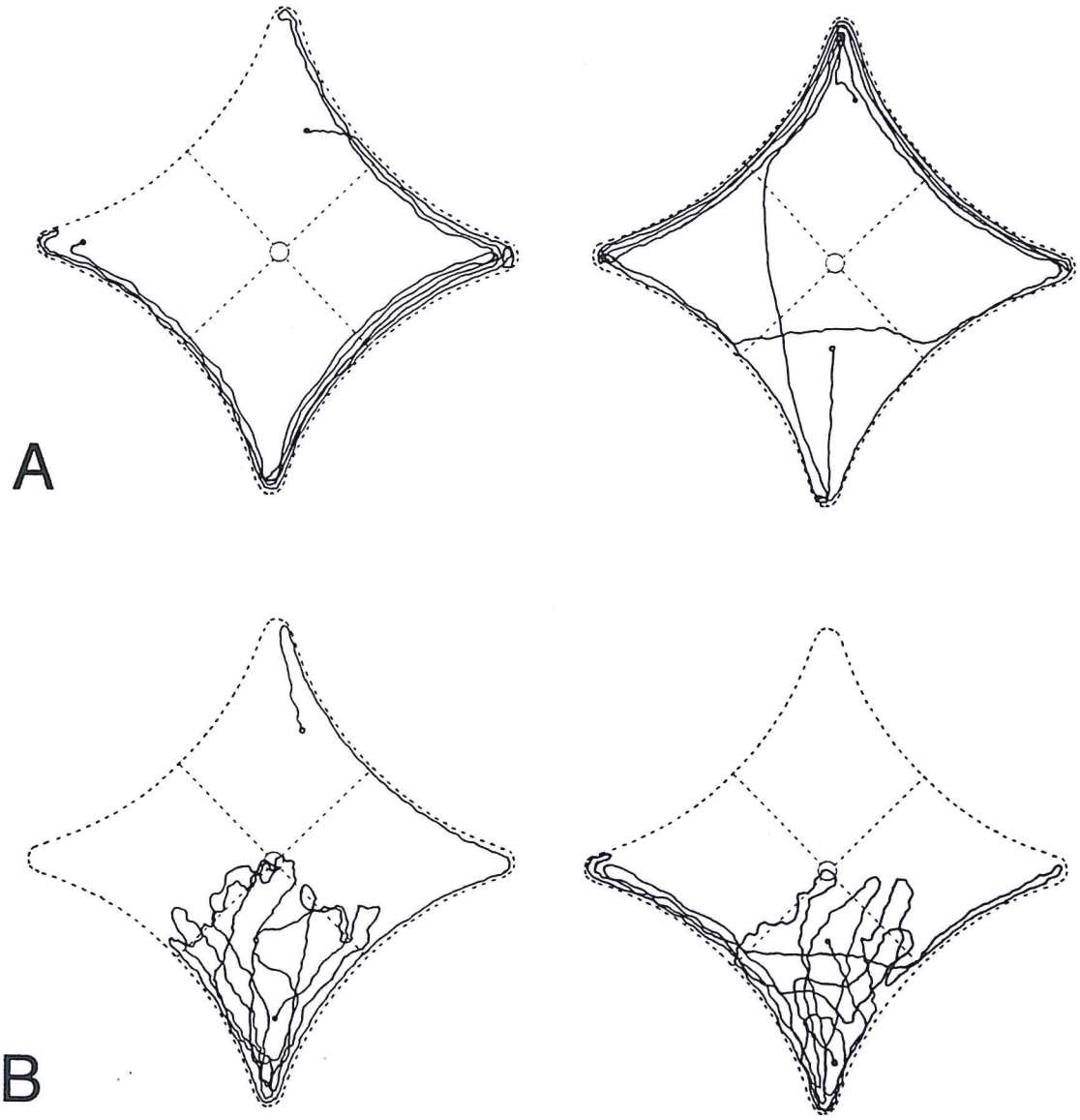


Fig. 4.3. Tracks of female *P. truncatus* in the olfactometer arena. (A) No odours present, (B) synthetic pheromone in one zone; ∘ start of track, • end of track.

Odour situations tested

Preliminary trials were run to determine whether beetles exhibited a bias for any particular quadrant (position control) or for any particular air stream (empty control, taking the rotations of the air streams into account). I also tested the effect of air stream r.h., by testing 90%, 30%, 60% and 60% r.h. simultaneously, on beetle preference. The different relative humidities were created by using differently equilibrated silica gel (cf. Olfactometer: Experimental set-up). The synthetic analogs of the aggregation pheromone of *P. truncatus* (1 mg T1 and 1 mg T2, AgriSense-BCS Ltd., Pontypridd, UK) were used to determine whether the set-up was able to reveal beetle preferences. The pheromone was offered in one and in two odour zones to verify if also the simultaneous presentation of two attractive odours could be evaluated.

Odour sources used and techniques of preparation are listed in Table 4.1. Initial experiments were conducted using odours from maize, wheat, and cassava chips, substrates on which *P. truncatus* reproduction has been demonstrated (Shires, 1977; Hodges *et al.*, 1985), and from cowpea, *Vigna unguiculata* Walpers (Leguminosae), a non-host plant (Shires, 1977). To verify whether the origin or physiological state of the insects influenced their reaction to the odours, *P. truncatus* individuals collected from maize cultures (i.e., non-emigrating), individuals captured emigrating from maize cultures (flyers from the dispersal experiment) and individuals caught in pheromone traps in a maize growing region (Mono) and in a savannah woodland region (Banté) were evaluated for their responses to maize and cowpea odours, as examples of host and non-host plant volatiles. Likewise, *P. truncatus* which had been reared on cassava chips were evaluated for their response to ground maize. The third series of experiments was conducted using odour sources associated with maize stores in coastal West Africa (Gounou *et al.*, 1994; C. Borgemeister, pers. comm.) and included live insects (*S. zeamais*, *M. nigrivenella*, *T. nigrescens*) or the artificial pheromones of *Tribolium* spp. (Coleoptera: Tenebrionidae), *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae) (all pheromones supplied by AgriSense-BCS Ltd., Pontypridd, UK). The sex of live *S. zeamais* was determined according to rostrum differences (Haines, 1991). Woody plant species used in the tests included species which have been found to support reproduction of *P. truncatus* under laboratory conditions (Nang'ayo *et al.*, 1993; F. Nang'ayo, pers. comm.): *Commiphora riparia* Engl. (collected in the Tsavo National Park, Kenya; kindly provided by F. Nang'ayo, Kenyan Agriculture Research Institute) and *Commiphora africana*

Table 4.1. Odour sources tested in the olfactometer experiments.

odour source	detailed description of the odour source
maize cobs	2 maize cobs, deep-frozen and reconditioned at 30 ± 1 °C and 75 ± 5% r.h. for 2 weeks
maize cobs (husks spread)	same cobs as above, but their husks spread to increase the exposed surface
maize grains	1000 ml (*ca.* 730 g) of maize grains, treated as the cobs
maize, ground	1000 ml (*ca.* 680 g), as above, but coarsely ground
wheat grains	1000 ml (*ca.* 770 g) of wheat grains, cv. Kanzler
wheat, ground	1000 ml (*ca.* 710 g), as above, but coarsely ground
cowpea, ground	1000 ml (*ca.* 730 g) of cowpea seeds, local variety, coarsely ground
cassava chips	1000 ml of cassava chips (cassava tubers, peeled, cut to pieces, washed and dried)
S. zeamais (mixed culture)	culture of *S. zeamais* on 125 g maize grains (initiated with 50 unsexed adults 6 weeks prior to the experiment)
S. zeamais (males)	20 male *S. zeamais* on 125 g maize grains, *ca.* 13 days old; kept separately since larval stage and added to maize after emergence
M. nigrivenella (larvae, pupae)	*ca.* 30 larvae and pupae of *M. nigrivenella* on maize cob pieces
T. nigrescens	50 unsexed adult *T. nigrescens*, without substrate (removed from cultures shortly before the experiment)
Tribolium spp.	pheromone dispensers
S. calamistis	pheromone dispensers
O. surinamensis	pheromone dispensers
wood	2 branch pieces (*ca.* 4 × 2 × 2 cm) of *C. riparia*, *C. africana*, *B. fagaroides* or *S. purpurea*; dried in the sun, reconditoned at 30 ± 1 °C and 75 ± 5% r.h. for 14 days; moisture content: 11-12% (as recommended by F. Nang'ayo, pers. comm.)
damaged wood	as above, but the bark cut several time with a knife

(A. Rich.) (Burseraceae) (collected near Ketou, in Benin). Moreover, two neotropical species *Spondias purpurea* L. (Anacardiaceae) and *Bursera fagaroides* (H.B.K.) (Burseraceae) (collected in the Chamela Forest, Mexico; kindly provided by R. H. Markham, IITA), in which larval *P. truncatus* have been found in the field (Ramírez-Martínez *et al.*, 1994), were tested. The wood pieces were either offered undamaged or with the bark being cut several times with a knife, as damaged plants may release more or additional volatiles (Finch, 1980; Metcalf, 1987). The investigations on woody plant species were conducted in cooperation with A. Tchabi (cf. Tchabi, 1996)

Statistics

Friedman two-way analysis of variance by ranks, based on times spent per odour field, was used to test for field preferences, using the data for males and females separately and pooled, followed by a multiple comparison test ($p<0.05$), when the Friedman test indicated significant differences (Siegel & Castellan, 1988). The responses of males and females were compared by Mann-Whitney tests for each of the four odour fields separately (Sokal & Rohlf, 1995).

4.3. Results

Figure 4.3 presents examples for tracks of female *P. truncatus* with no odours present (Fig. 4.3 A) and with pheromone offered in one zone (Fig. 4.3 B). No bias was detected with respect to quadrant (position control) or air flow position (empty control) in the olfactometer. With no attractant or repellent present, the beetles spent approximately equal mean times in any of the odour zones (Fig. 4.4). When a choice was given between high (90%), low (30%) and medium (60%) r.h., *P. truncatus* tended to avoid the high humidity zone. Offering the aggregation pheromone of *P. truncatus* in one or two odour zones, the beetles spent significantly more time within this/these zone/s than within the control fields. In both cases, the response was significantly more pronounced for females than for males (Fig. 4.4).

Odours from maize cobs (husks closed or spread) were not preferred by *P. truncatus* (Fig. 4.5), whereas the beetles spent a significantly greater proportion of the time in the odour field than in the control fields, when ground maize was offered. Also maize grains and cassava chips elicited positive responses (Fig. 4.6).

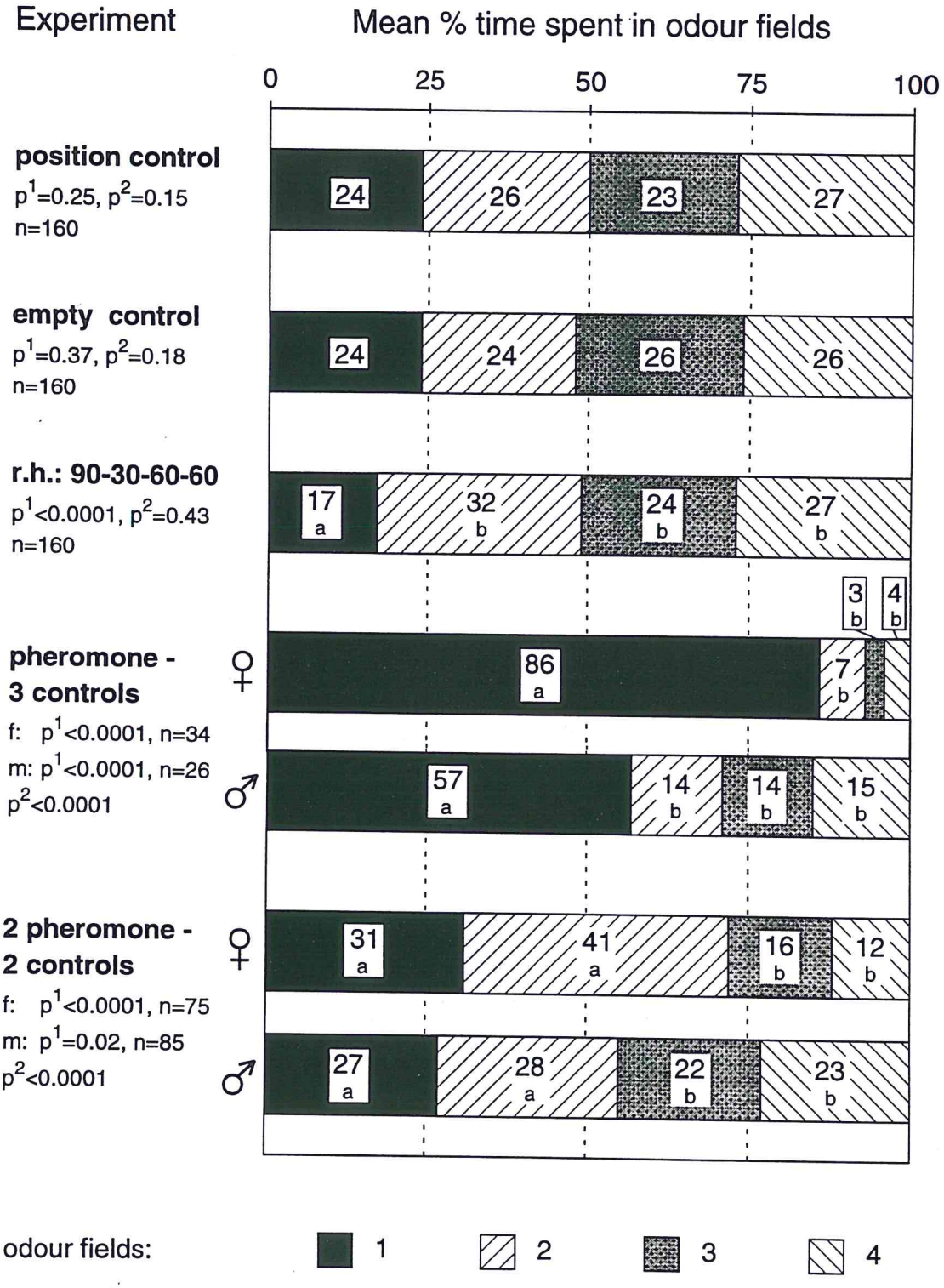


Fig. 4.4. Preliminary olfactometer experiments. p^1 = p-value obtained in Friedman two-way analysis of variance by ranks; p^2 = smallest p-value obtained in Mann-Whitney tests for differences between males and females; n = number of insects tested; means in a bar differ significantly when followed by different letters ($p<0.05$, multiple comparison test).

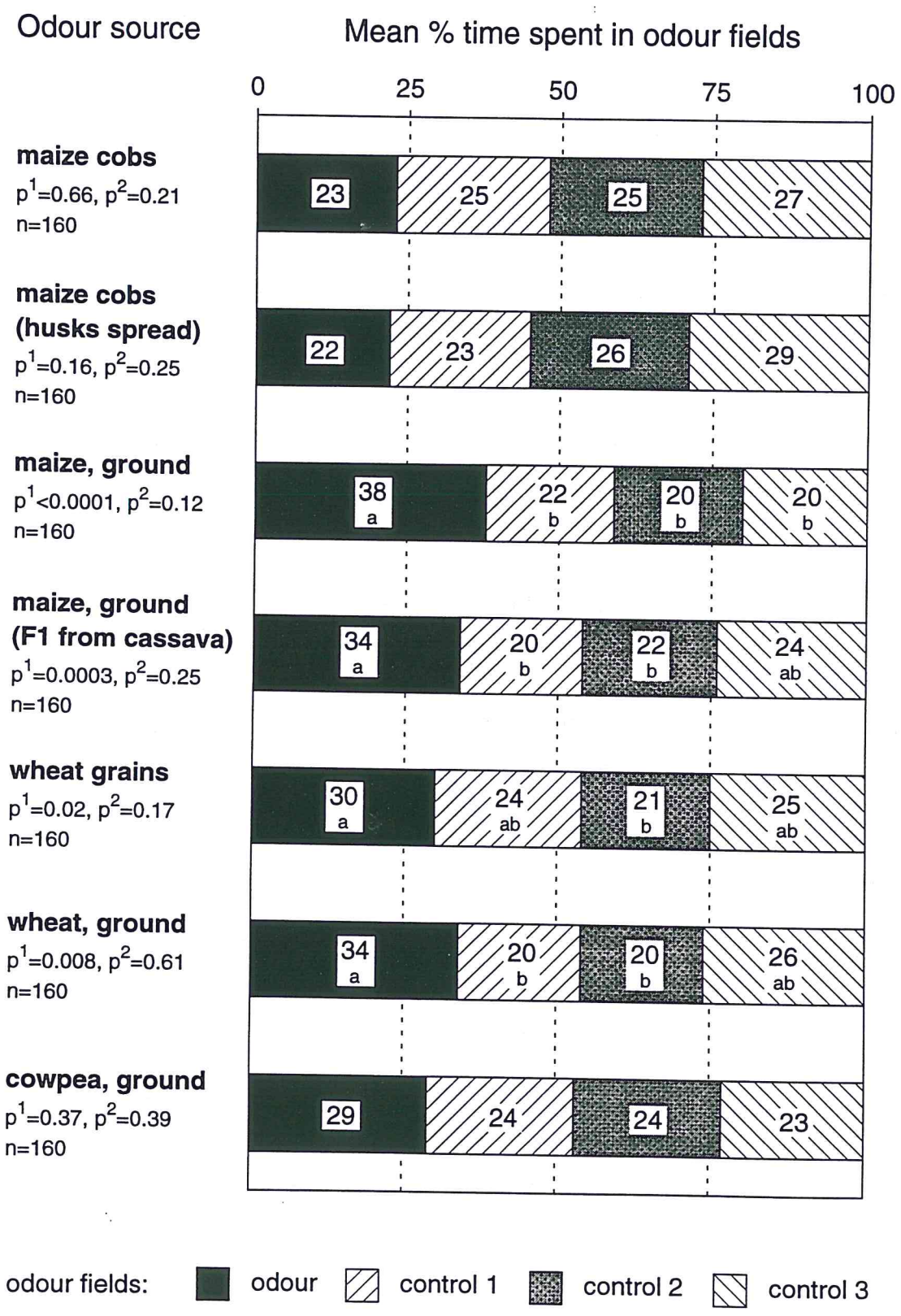


Fig. 4.5. Responses to plant volatiles (one odour source). For details see Fig. 4.4.

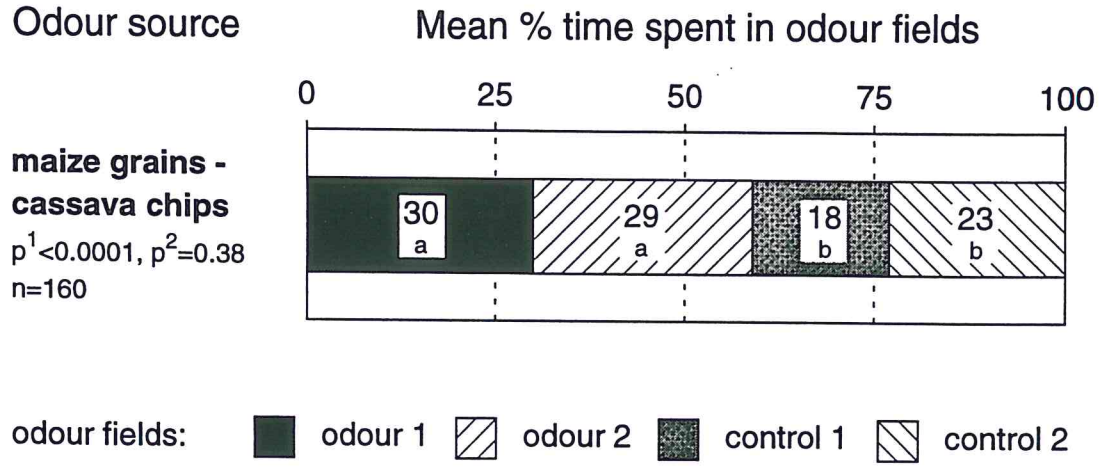


Fig. 4.6. Responses to plant volatiles (two odours offered simultaneously). For details see Fig. 4.4.

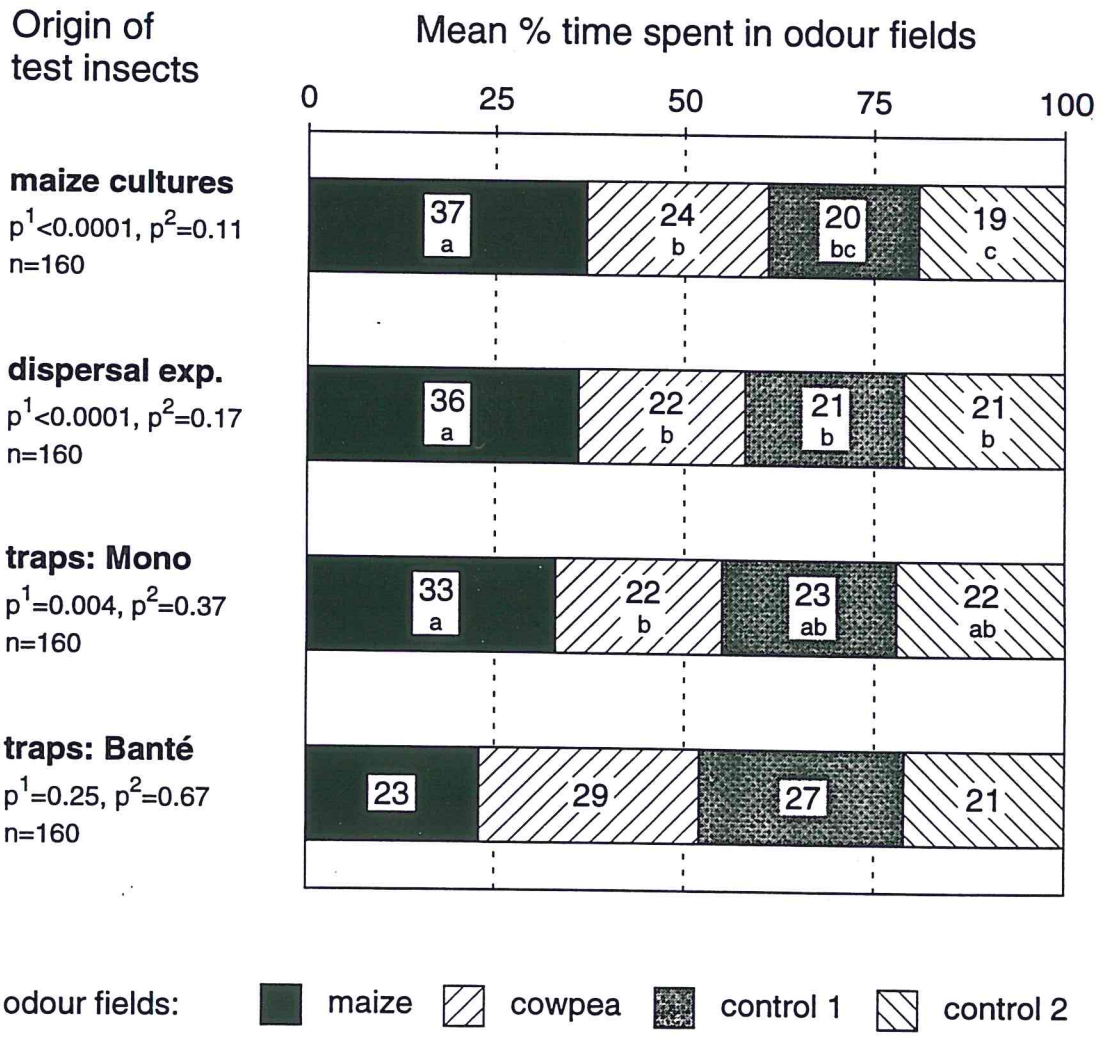


Fig. 4.7. Responses of *P. truncatus* of different origins to maize and cowpea volatiles. For details see Fig. 4.4.

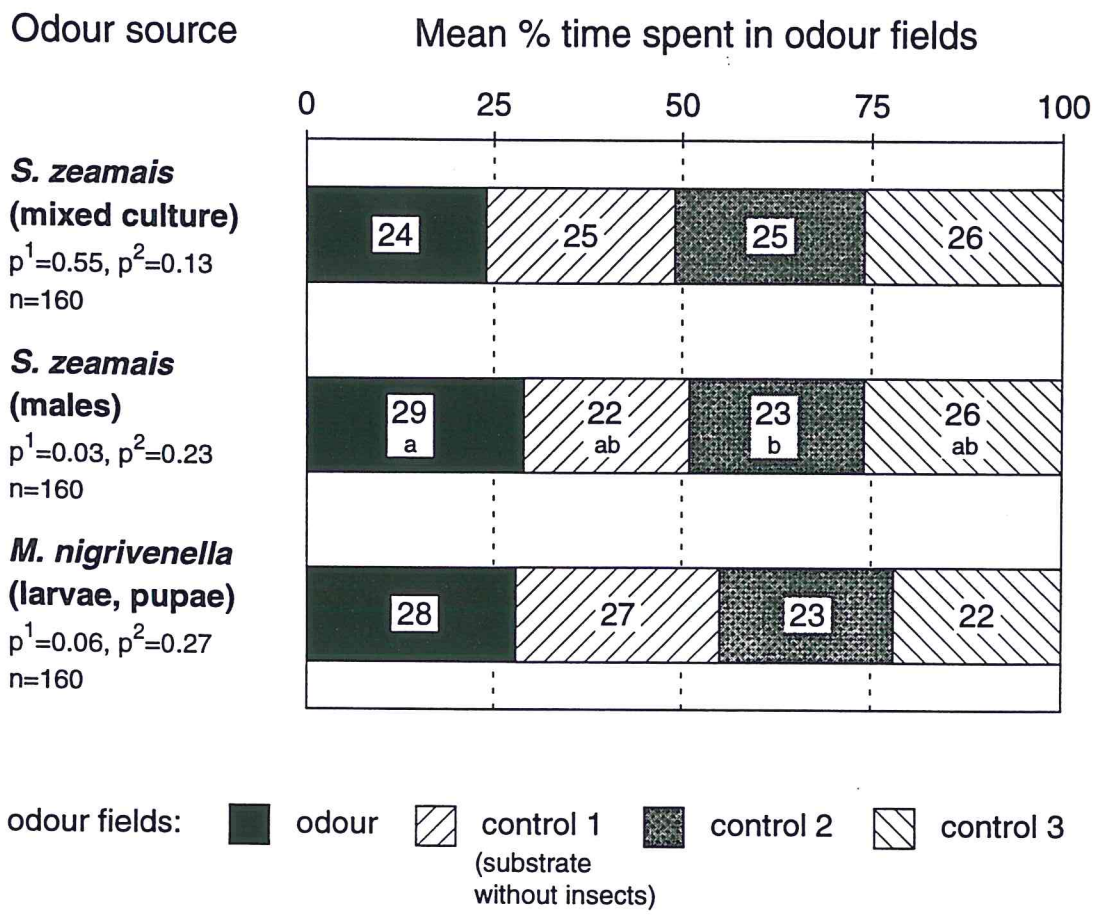


Fig. 4.8. Responses to odours associated with maize stores (control 1: substrate without insects). For details see Fig. 4.4.

Regarding wheat grains and ground wheat, significant differences were only found to one or two of the controls (Fig. 4.5). Odours emitted from cowpea (non-host plant) evoked no significant preference (Fig. 4.5).

The immediate origin of the beetles apparently had an effect on their responses to odours. Beetles collected from maize cultures and captured emigrating from maize cultures preferred ground maize odours to cowpea and the controls (Fig. 4.7). The preference was less pronounced (significant difference only to cowpea) among *P. truncatus* trapped in the maize growing region, and no such preference was observed among beetles from the savannah woodland area. *P. truncatus* which had been reared on cassava chips also preferred odours from ground maize (Fig. 4.5, significant differences to 2 of the 3 controls).

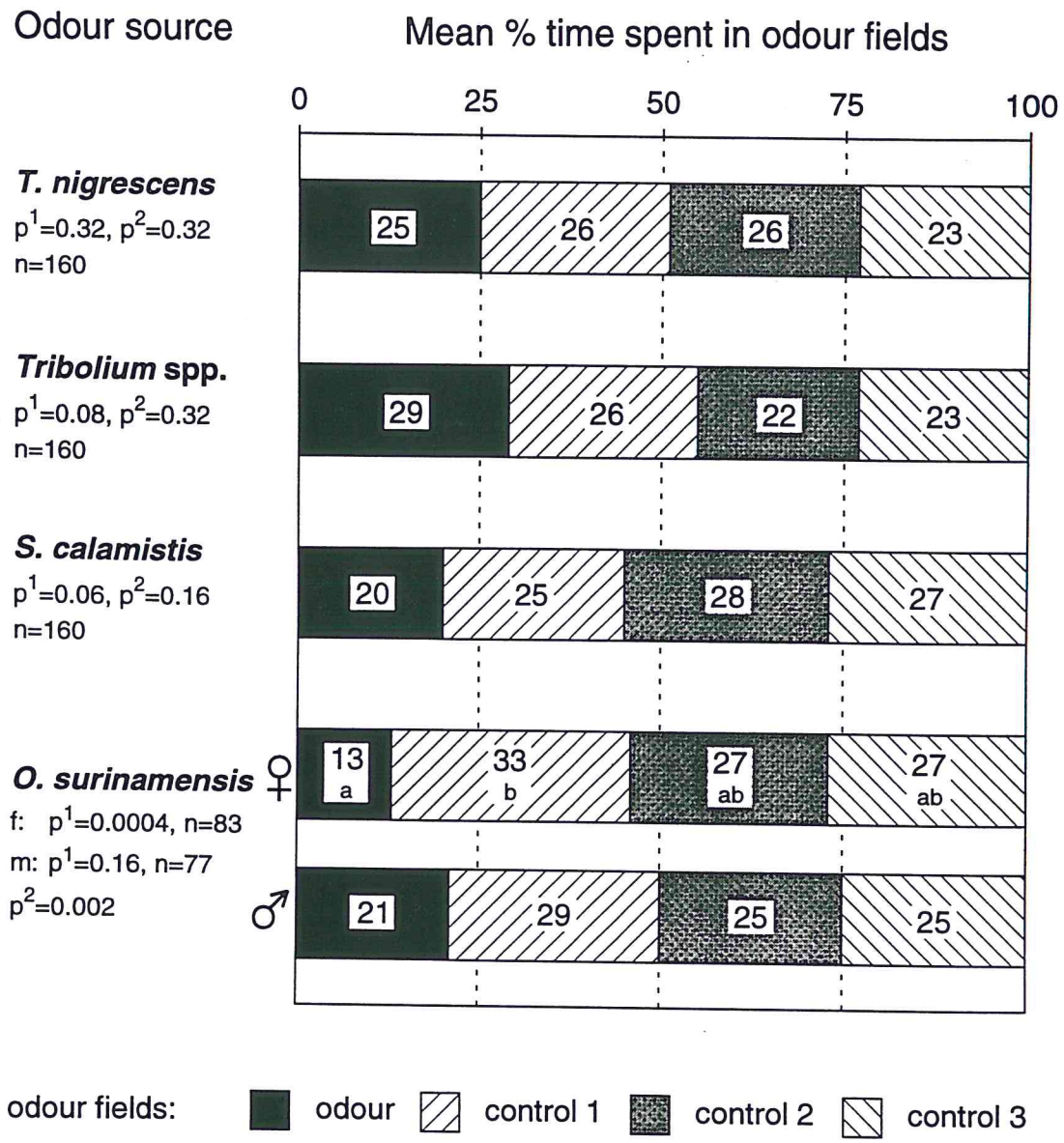


Fig. 4.9. Responses to odours associated with maize stores (one odour source). For details see Fig. 4.4.

Odours emitted from live *S. zeamais* in mixed cultures, *M. nigrivenella* larvae/pupae on maize cob pieces and *T. nigrescens* did not elicit any responses in *P. truncatus* (Fig. 4.8 and Fig. 4.9). With 20 unmated male *S. zeamais* as odour source a significant preference was only observed with respect to the opposite control field. The artificial pheromones of *Tribolium* spp. and *S. calamistis* evoked no responses either, whereas the pheromone of *O. surinamensis* repelled female, but not male *P. truncatus* (Fig. 4.9).

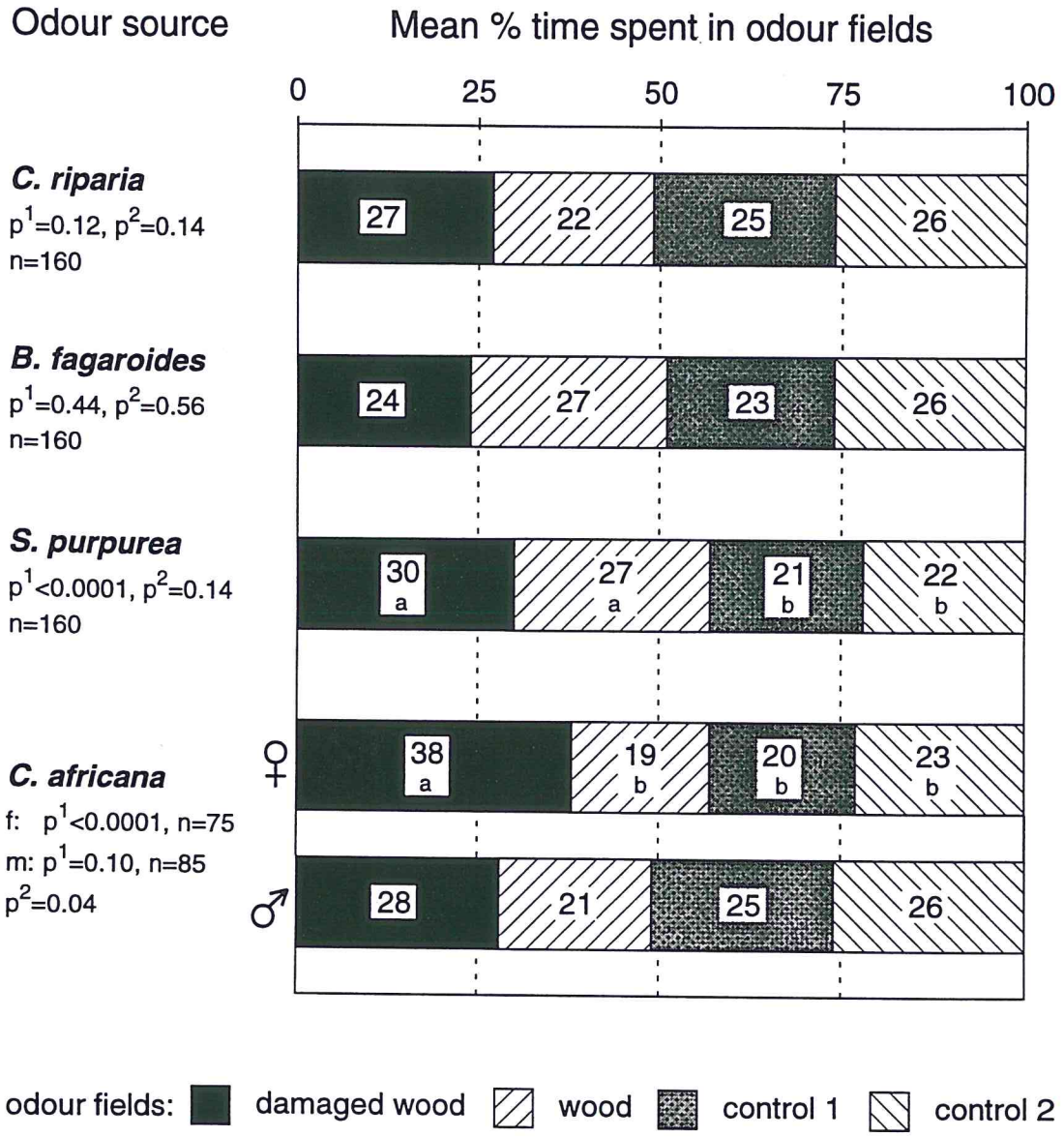


Fig. 4.10. Responses to wood volatiles (two odours offered simultaneously). For details see Fig. 4.4.

Wood volatiles from *C. riparia* and *B. fagaroides* evoked no reactions in *P. truncatus*, while *S. purpurea* odours were preferred to clean air (Fig. 4.10). *P. truncatus* males did not respond to odours emitted from *C. africana*, though females preferred the odour of damaged *C. africana* significantly to the undamaged treatment and to one of the two controls (Fig. 4.10).

4.4. Discussion

Preliminary trials

Preliminary trials proved the olfactometer technique as used here to be suitable to test for olfactory responses of *P. truncatus*: (a) the beetles spent about equal times in each quadrant, when no odours were present, and (b) offering the aggregation pheromone of *P. truncatus*, a known attractant, the beetles preferred this odour significantly. The latter result was especially important since *T. nigrescens*, a specific predator of *P. truncatus*, which is strongly attracted to the pest's aggregation pheromone in the field (Boeye *et al.*, 1992), did not react to this odour source in the same olfactometer set-up (J. N. Ayertey, pers. comm.). A more detailed interpretation of the beetles' reactions to the pheromone is given in Chapter 5. Furthermore, *P. truncatus* was found to avoid high humidity, which corresponds well with the hygronegative behaviour observed among most stored product insects, in choice-chamber (Pertunnen, 1972; Daramola, 1976; Weston & Hoffmann, 1991) as well as in olfactometer experiments (Weston & Hoffmann, 1992).

Responses to stored commodities

The main objective of this study was to investigate whether *P. truncatus* locates its host plants (e.g., maize) guided by volatiles of the hosts or host habitats (primary attraction hypothesis; Tunset *et al.*, 1993), or if the beetles land randomly on plants, trees, etc., perhaps guided by visual cues, and recognize a suitable substrate only after landing, and possibly feeding (random attack hypothesis). Primary attraction may be observed among all individuals, or among all or part of the dispersing population, the so-called pioneers (Payne, 1986). *P. truncatus* reared on maize exhibited positive responses to odours from maize grains, ground maize and cassava chips, which is consistent with the observations of Pike *et al.* (1994), who reported electrophysiological reactions of *P. truncatus* to maize and cassava volatiles and their short-range attraction [i.e., attraction from less than *ca.* 10 cm from the odour source (Kennedy, 1977b; Finch, 1980)]. However, neither the response-eliciting components identified for maize by Pike *et al.* (1994), alone or mixed, nor maize grains were found to be attractive in wind tunnel trials (Fadamiro, 1995). Moreover, maize cobs evoked no response from *P. truncatus* in either the experiment presented here or in those of Wright *et al.* (1993) and M. Ramírez-Martínez (pers. comm.),

who used maize cobs as baits in field trials (cf. also Chapter 5.5.2). Thus, maize odours may only cause short-range attraction, or rather arrestment, as no directional response has been proved so far. This corresponds with the suggestion of Hodges (1994) that host finding behaviour of *P. truncatus* is based on random search, at least at long range. The differences of the responses of *P. truncatus* towards maize grains and ground maize on the one hand, and maize cobs on the other hand, might be caused by a higher amount of volatiles being released from damaged grain. Cracked wheat was also found to be more attractive to *S. zeamais* than whole grains (Walgenbach *et al.*, 1987).

Wheat, which supports the reproduction of *P. truncatus* (Shires, 1977), was slightly preferred as whole or coarsely ground grains, whereas the odour emitted from cowpea, a non-host plant (Shires, 1977), elicited no responses in *P. truncatus*. Thus, attractive volatiles are not restricted to maize and cassava, but are not present in the non-host plant. Response-eliciting volatiles identified for maize grains and cassava chips, are rather general plant volatiles (Pike *et al.*, 1994). Hougen *et al.* (1971) found maize, wheat, rye and rice grains to emit similar volatile mixtures, though the relative quantities emitted were plant and variety specific.

To explore the possibility that behavioural responses may depend at least in part on the insect's physiological state (Borden *et al.*, 1986) or feeding experience (Papaj & Rauscher, 1983; Vet & Groenewald, 1990), insects of different origins were examined in regard to their responses to maize. *P. truncatus* reared on cassava chips for one generation responded only slightly less to ground maize than *P. truncatus* reared on maize, suggesting that feeding experience on another starchy substrate did not influence the behaviour. Beetles captured emigrating from maize cultures were regarded as members of the dispersing population, and, according to Kennedy (in Thorsteinson, 1960), might therefore be expected to respond more vigorously to host volatiles. However, the emigrating beetles were not found to be any more sensitive to maize odours than beetles captured within maize cultures. Dispersing *P. truncatus* caught in the Mono District (maize production area) preferred maize volatiles only slightly, whereas beetles trapped near Banté (savannah woodland area) showed no response. These results were rather unexpected, as insects are known to increase their responses to host volatiles with extended time without food (May & Ahmad, 1983) and distance flown (Lanier, 1983). Either the beetles from Banté may be adapted to woody plants, rather than to maize, or, for unknown reasons, the pheromone traps in Banté attracted a lower percentage of the pioneer insects that would react to plant volatiles.

Responses to store-associated insects

In general, odours from insects commonly found in maize stores in coastal West Africa (Gounou *et al.*, 1994; C. Borgemeister, pers. comm.) elicited few responses from *P. truncatus*, while in other insect species negative or positive responses to associated herbivores or natural enemies have been observed (Price, 1981; Höller *et al.*, 1994). The odours male *S. zeamais*, presumed to be producing pheromone (Walgenbach *et al.*, 1983), were only slightly preferred to the controls; the effect may have been induced by the associated frass. The pheromone of *O. surinamensis*, a facultative predator (Haines, 1991) seems to repel female *P. truncatus*, but the pheromone of *Tribolium* spp., another facultative predator, and odours of live *T. nigrescens*, the predator adapted to *P. truncatus* (Boeye *et al.*, 1992), evoked no reactions. Hence, according to these results, *P. truncatus* has not yet developed the ability to olfactorily perceive the presence of *T. nigrescens* and thereby avoid predation, while females may avoid the immediate vicinity of *O. surinamensis* (or more likely, a species producing similar pheromones which may occur in the natural habitat of *P. truncatus*).

Responses to woody plants

Among the woody plants able to support *P. truncatus* reproduction in the laboratory, only odours of artificially damaged blocks of *C. africana* elicited positive responses from female *P. truncatus*, and among the woody plants found supporting reproduction in the field, only *S. purpurea* was preferred by *P. truncatus* of both sexes equally. The question if these reaction were caused by plant specific volatiles remains open. In the three cases where *P. truncatus* has been found breeding in wood in the forest, it had been in association with girdling cerambycid species (Ramírez-Martínez *et al.*, 1994; F. Nang'ayo, pers. comm.; R. H. Markham, pers. comm.). Co-inhabitants in the girdled branches benefit from the nutrient accumulation above the girdling site (Forcella, 1981; 1982), and bostrichids are commonly found in this habitat (Polk & Ueckert, 1973; Hovore & Penrose, 1982). It may therefore be possible, that *P. truncatus* is rather attracted by odours emitted by the girdling cerambycids themselves, or volatiles in the frass of the cerambycid adults or larvae, than by an undamaged or manually damaged host plant.

Taking the results of this study and the discussed findings of other researchers into account, it can be concluded that volatiles emitted from maize, and presumably also cassava, are general plant volatiles and act as short-range arrestants, especially, when the insects are adapted to the odours. Long-range responses, and thereby primary attraction to stored commodities, could not be demonstrated (cf. also Chapter 5.5). However, it cannot yet be concluded that long-distance chemical attraction plays no part in the host-finding behaviour of *P. truncatus*. Primary attraction would more likely be observed in response to volatiles from the co-evolved natural host-plant-complex, but the behavioural responses to these species remain to be properly investigated.

5. Host finding: Secondary attraction

5.1. Introduction

The role of host plant volatiles in the host finding process (primary attraction) was discussed in the preceding chapter. However, irrespective of whether *P. truncatus* responds at short range to volatiles from starchy stored commodities, and possibly even at longer range to volatiles from its natural host-plant-complex, secondary attraction by the male-produced aggregation pheromone surely plays an important role in the host finding process (Hodges, 1994). While many insects produce sex pheromones to attract the opposite sex for mating in the absence of a food source (Burkholder & Ma, 1985; Levinson & Levinson, 1995), both aggregation and sex pheromones may additionally function to attract conspecifics to a patch suitable for feeding and oviposition (Baker, 1985; Borden, 1985).

On a suitable food source with no female conspecifics present, male *P. truncatus* produce an aggregation pheromone (Smith *et al.*, 1996), which attracts male and female conspecifics (e.g., Obeng-Ofori & Coaker, 1990). In the presence of females, the pheromone production is greatly reduced, and no pheromone could be detected when the males bored in Plaster of Paris (Smith *et al.*, 1996). The two main components of the pheromone, T1 [1-methylethyl-(2E)-2-methyl-2-pentenoate] and T2 [1-methylethyl-(2E, 4E)-2,4-dimethyl-2,4-heptadienoate] have been identified and chemically synthesized (Cork *et al.*, 1991). Traps baited with these pheromone components were also attractive to *T. nigrescens*, suggesting that the predator locates its prey olfactorily, with the prey's pheromone acting as a kairomone (Rees *et al.*, 1990; Helbig *et al.*, 1992).

The occurrence and spread of *P. truncatus* and *T. nigrescens* has been monitored using pheromone traps (e.g., Rees *et al.*, 1990; Markham *et al.*, 1994c). Additionally, these data have been used to interpret the flight activity of the two species in relation to environmental factors (Tigar *et al.*, 1994b; Giles *et al.*, 1995; Borgemeister *et al.*, 1997), and to estimate the ratio of the predator and the prey as well as species-specific sex ratios of the dispersing population. Trap catches have been interpreted, assuming that there are no differences in perception thresholds, either between the two species (Key *et al.*, 1994), or between sexes (Ramírez-Martínez *et al.*, 1994; Giles *et al.*, 1995). This may well not be true: Responses to aggregation pheromones have been found to differ between sexes of the same insect species (Chambers *et al.*, 1990), and it may be assumed that the sensitivity of the

predator and the prey to the prey-produced pheromone may differ as well. Also the role of the two pheromone components, T1 and T2, has not been intensively investigated. While Dendy *et al.* (1991) observed no differences between the numbers of *P. truncatus* attracted to the separate components within stores, Fadamiro (1995) found T2 to be more attractive than T1 in wind tunnel trials. Behavioural responses to the two pheromone components were therefore investigated under laboratory (Chapter 5.2) and field conditions (Chapter 5.3), and are discussed in comparison to electrophysiological reactions (Chapter 5.4).

P. truncatus on maize did not respond to nearby pheromone traps (Pike, 1993), and it was postulated that the beetle's response may be inhibited due to its specific physiological state (Fadamiro, 1995), habituation to the pheromone (Obeng-Ofori & Coaker, 1990) or contact to the female-produced, non-volatile substance that reduces male pheromone production (Pike, 1993; Smith *et al.*, 1996). Therefore, the responses of *P. truncatus* to the synthetic analogs of the pheromone were investigated with beetles of determined preconditioned state (Chapter 5.2).

In the initial studies on pheromone production in *P. truncatus*, Cork *et al.* (1991) tested, in addition to males, only mated females which had been separated from males for up to three days. Based on these results, it was concluded that only male *P. truncatus* produce aggregation pheromone. However, pheromone production may be dependent on mating status (Plarre, 1992), and may only increase with extended separation time from the opposite sex (Smith *et al.*, 1996). I was therefore interested to verify whether or not female *P. truncatus* are olfactorily attractive for male or female conspecifics (Chapter 5.2).

Conditions determining the pheromone production of male *P. truncatus*, measured as attractiveness for conspecifics, were investigated (Chapter 5.2). Males on maize, singly or in groups, on non-nutritive material and suspected woody host plants, were examined. The effect of the presence of females on the pheromone production by males was additionally examined under field conditions, simulating the colonization of a new resource initiated by single males (Chapter 5.5).

A low degree of field infestation of maize cobs by *P. truncatus* has been found by several workers (Henckes, 1992; Giles *et al.*, 1995). However, Borgemeister *et al.* (1994) detected no *P. truncatus* in pre-storage sampling, but observed, nevertheless, lower numbers and delayed population increase of *P. truncatus* in stores which were filled with fumigated maize cobs, in comparison to stores filled with untreated cobs.

Presumably, small initial populations of *P. truncatus* went undetected in the early sampling. Stimulated by the results obtained in the trial on simulated colonization (Chapter 5.5), I wondered if these small numbers of *P. truncatus*, which were possibly attracted by a single male, sufficed to initiate the high infestation levels which were found later on in the storage season, or if the population growth observed in stores depended on additional continuous immigration. For this purpose, data from previous field and laboratory trials were re-examined for any insight they might provide into the importance of initial infestation and subsequent population development in stores (Chapter 5.6).

5.2. Production of, and behavioural responses to, the pheromone under laboratory conditions

5.2.1. Materials and methods

Insects

Unless stated otherwise, beetles used in the experiments originated from the basic laboratory cultures (cf. Chapter 2.2) and were 0-3 weeks old. *P. truncatus* to be used as experimental subjects were kept in glass petri dishes laid out with brass gauze between collection and use in the experiments, i.e., for 16-24 h.

Olfactometer experiments

All tests were carried out using the olfactometer set-up and the experimental procedure described in Chapter 4.2.

To verify that female *P. truncatus* do not attract conspecifics (Cork *et al.*, 1991), the following odour sources were tested:

- 20 virgin female *P. truncatus*, *ca.* 12 days old, on 125 g maize grains, kept separately since pupal stage and added to the maize immediately after emergence.
- 20 mated female *P. truncatus*, *ca.* 17 days old, on 125 g maize grains, kept separated from males for 12 days.

Mechanisms underlying pheromone production of *P. truncatus* males and attractiveness of male-produced pheromone were investigated using:

- 20 male *P. truncatus* on 125 g maize grains, kept separated from females for one, five or 12 days.
- 20 *P. truncatus* males on 125 g maize grains, *ca.* 3-5 weeks old; beetles had flown off maize cultures in the dispersal experiment (cf. Chapter 3.2) and were kept separated from females for 12 days.
- 20 *P. truncatus* males on polystyrene, kept separated from females for one or five days.
- One *P. truncatus* male on 125 g maize grains or a maize cob, kept separated from females for 12 days; 16 different odour sources were tested, instead of only four (cf. Chapter 4.2), as pheromone production may vary between individual beetles (Smith *et al.*, 1996).
- Male and female *P. truncatus* kept in mixed cultures; 125 g maize grains infested with 15 unsexed *P. truncatus* (low density) or 60 unsexed *P. truncatus* (high density) six weeks prior to the experiment.
- 30 male *P. truncatus* kept for 12 days on wood. Tested wood species were *C. riparia* and *C. africana*, which have been found to support reproduction of *P. truncatus* (F. Nang'ayo, pers. comm.; cf. Chapter 4.2) and *Balanites aegyptiaca* (L.) (Zygophyllaceae) which did not support reproduction (Nang'ayo *et al.*, 1993). *C. riparia* and *B. aegyptiaca* were collected in Tsavo National Park, Kenya (kindly provided by F. Nang'ayo, Kenyan Agriculture Research Institute) and *C. africana* near Ketou (Oueme District, Benin). Pieces of branches were dried in the sun and reconditioned at 30 ± 1 °C and 75 ± 5% r.h. for 14 days (final moisture content: 11-12%), as recommended for optimal breeding conditions by F. Nang'ayo (pers. comm.).
- 30 males on 125 g maize and 30 males on *C. africana* offered as two separate odour sources simultaneously to compare pheromone production on maize and wood.

The experiments on pheromone production on wood were carried out in cooperation with A. Tchabi (cf. Tchabi, 1996).

The behavioural responses of *P. truncatus* to the synthetic analogs of the aggregation pheromone (pheromone vial with 1 mg T1 and 1 mg T2; AgriSense-BCS Ltd., Pontypridd, UK) were investigated with beetles which exhibited varying activities prior to the experiment. Test beetles were:

- Beetles which moved actively on the top of the basic cultures, and beetles from the lower levels of the culture jars, removed from within grains. The beetles for both treatments were directly transferred from the cultures to the olfactometer.
- Beetles which had flown off maize cultures in the dispersal experiment the evening before they were tested in the olfactometer (cf. Chapter 3.2).

The effect of habituation to the pheromone was investigated testing :

- Beetles which were kept for 48 h with maize and a pheromone vial in 250 ml glass jars.
- Beetles which were kept for 48 h with a pheromone vial only, i.e., without food, in the glass jars laid out with brass gauze.

Behavioural responses to the separate pheromone components (T1 and T2) were also investigated, offering pheromone vials with 1 mg T1 and 1 mg T2 separately in one- and two-choice experiments. 10 mg pheromone (supplied by AgriSense-BCS Ltd., Pontypridd, UK) were diluted in 100 µl hexane and 10 µl of this solution were applied on filter paper stripes (Whatman No. 1). The solvent was allowed to evaporate for 30 sec before the filter paper was introduced into polyethylene vials. The pheromone vials were stored separately in airtight glass jars at -20 °C until they were used in the experiments.

Nutrients in tested wood samples

Nitrogen [as an indirect parameter for protein content (Brauner & Bukatsch, 1980)] and sugar contents were determined for wood samples taken from the same branches as the wood pieces used in the olfactometer experiments. Analyses were carried out by the Analytical Laboratory of the IITA, Ibadan, Nigeria. Total nitrogen contents was determined by the Kjeldahl method (Anonymous, 1997) and sucrose contents according to the method described in Anonymous (1982).

Statistics

Friedman two-way analysis of variance by ranks, based on times spent per odour field, was used to test for field preferences, using the data separately, for males and females, and pooled, followed by a multiple comparison test ($p<0.05$), when the Friedman test indicated significant differences (Siegel & Castellan, 1988). The

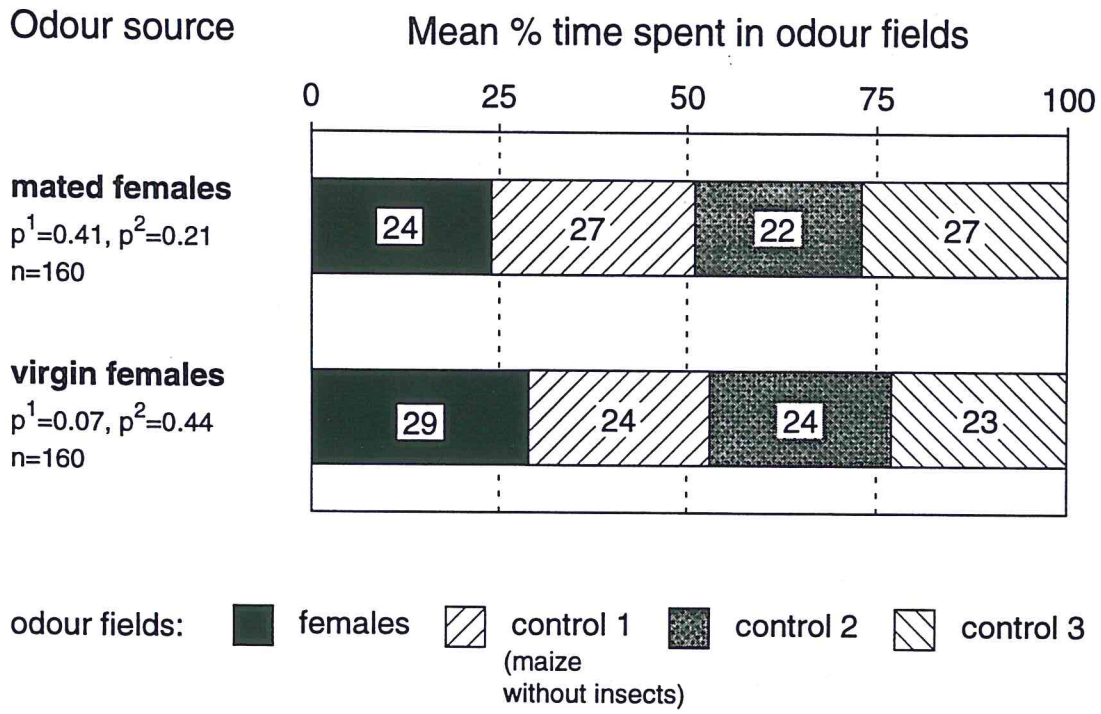


Fig. 5.1. Responses to female *P. truncatus* on maize. p^1 = p-value obtained in Friedman two-way analysis of variance by ranks; p^2 = smallest p-value obtained in Mann-Whitney tests for differences between males and females; n = number of insects tested; means in a bar differ significantly when followed by different letters ($p<0.05$, multiple comparison test).

responses of males and females were compared by Mann-Whitney tests for each of the four odour fields separately (Sokal & Rohlf, 1995).

5.2.2. Results

Pheromone production

Odours from virgin or mated female *P. truncatus* on maize were not preferred by *P. truncatus* (Fig. 5.1), whereas male and female test beetles spent a significantly greater proportion of the time in the odour field than in the control fields when 20 male *P. truncatus* on maize were used as odour sources (Fig. 5.2). The time spent in these odour fields was similar among the male and among the female test beetles for odours from males separated from females for five or 12 days, and for odours from males which had flown off maize cultures in the dispersal experiment. However, the

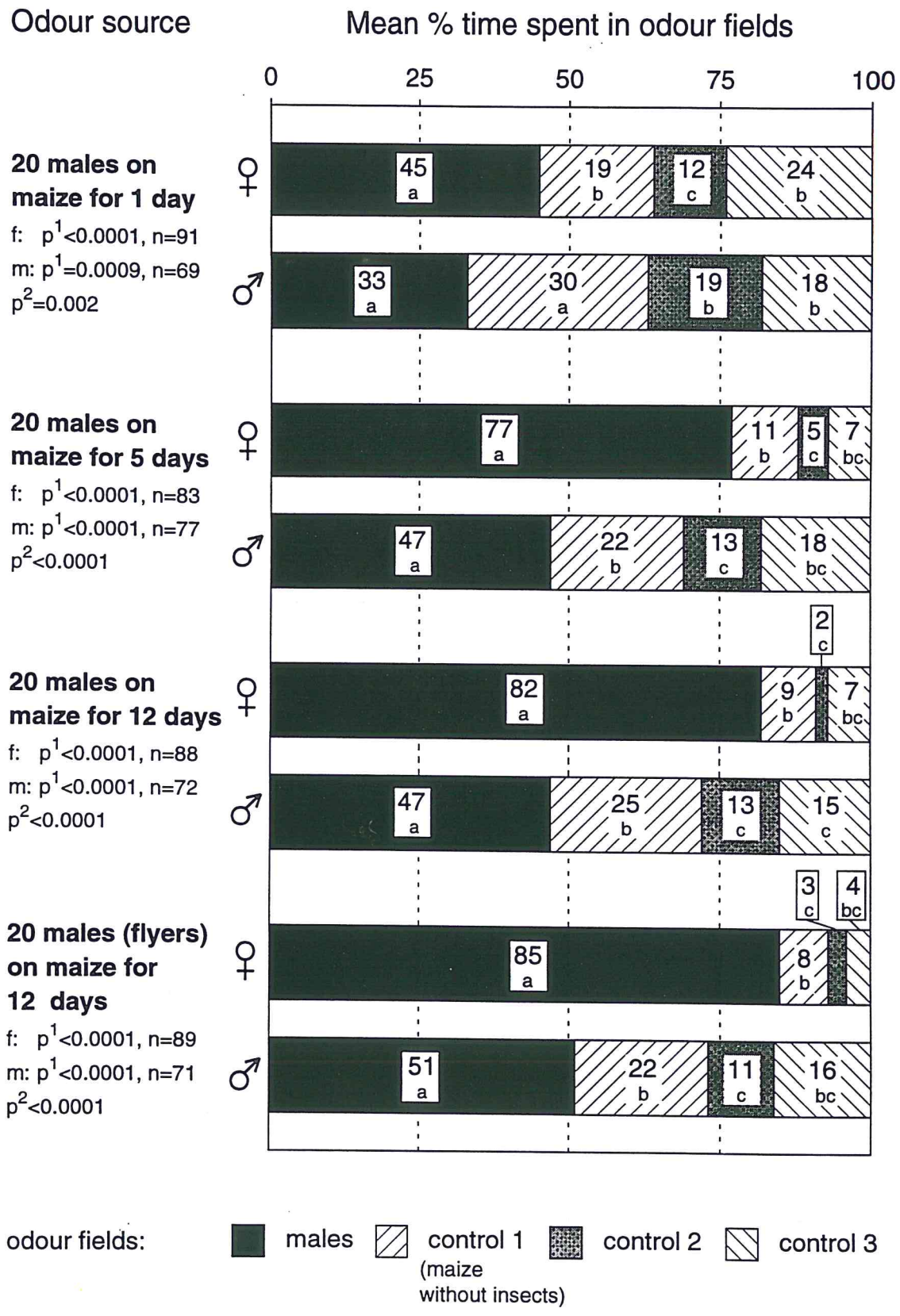


Fig. 5.2. Responses to male *P. truncatus* on maize. For details see Fig. 5.1.

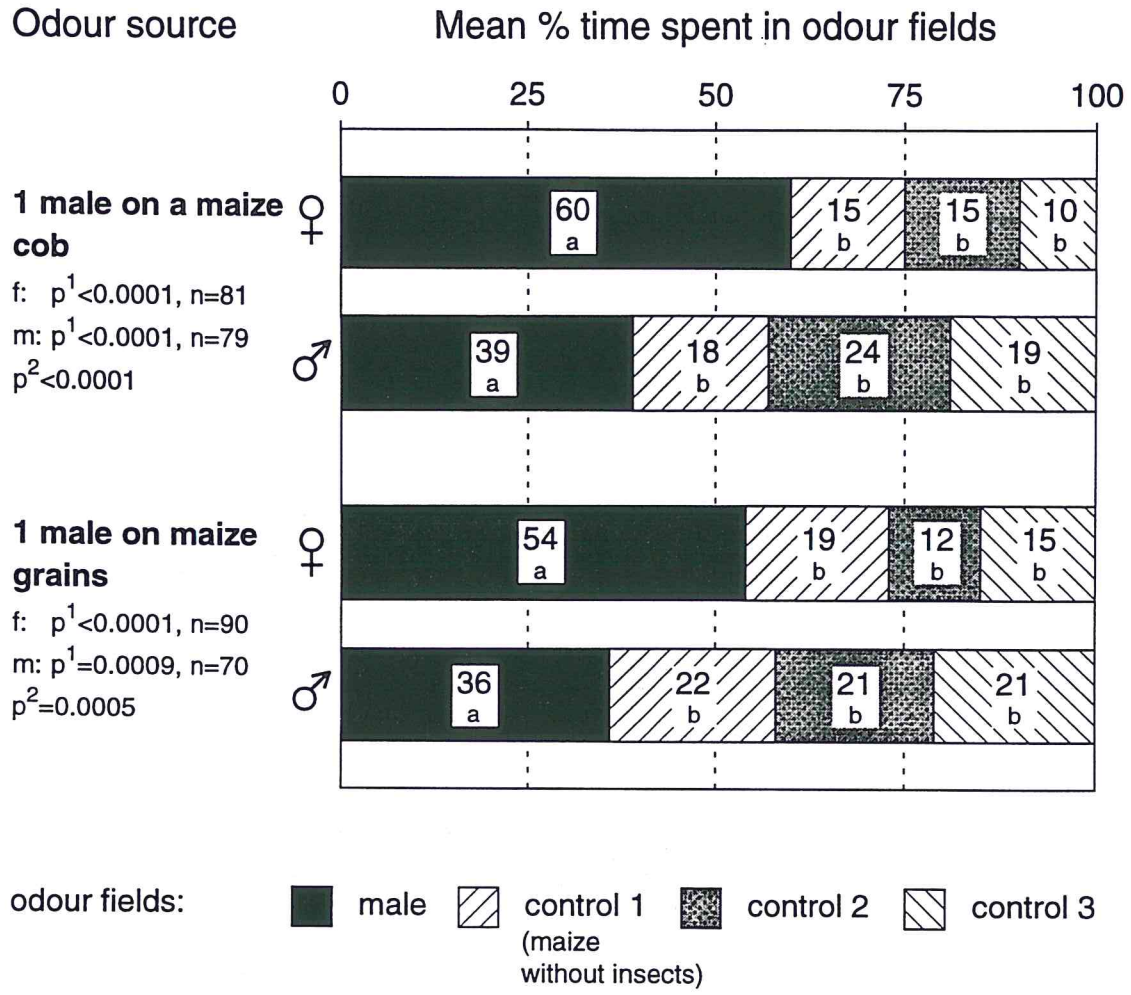


Fig. 5.3. Responses to single male *P. truncatus* on maize. For details see Fig. 5.1.

reactions were less pronounced to males separated from females for only one day (Fig. 5.2). Whenever a significant preference for the odour field was observed, females spent a significantly greater proportion of the time within this field than the males. This is also true for all the subsequent experiments (cf. Fig. 5.3 and Fig. 5.6 - Fig. 5.10), except when mixed cultures were offered (Fig. 5.5). With a single male on maize grains or on a maize cob as odour source, test beetles spent also significantly more time within this zone than within the control fields (Fig. 5.3). Male *P. truncatus* on polystyrene separated from females for one day elicited positive responses in female, but not in male, *P. truncatus*, while after five days polystyrene evoked a slight avoidance by both sexes (Fig. 5.4). Offering two cultures of *P. truncatus* (mixtures of both sexes) simultaneously, the test beetles responded to the low density, but not to the highly populated culture (Fig. 5.5).

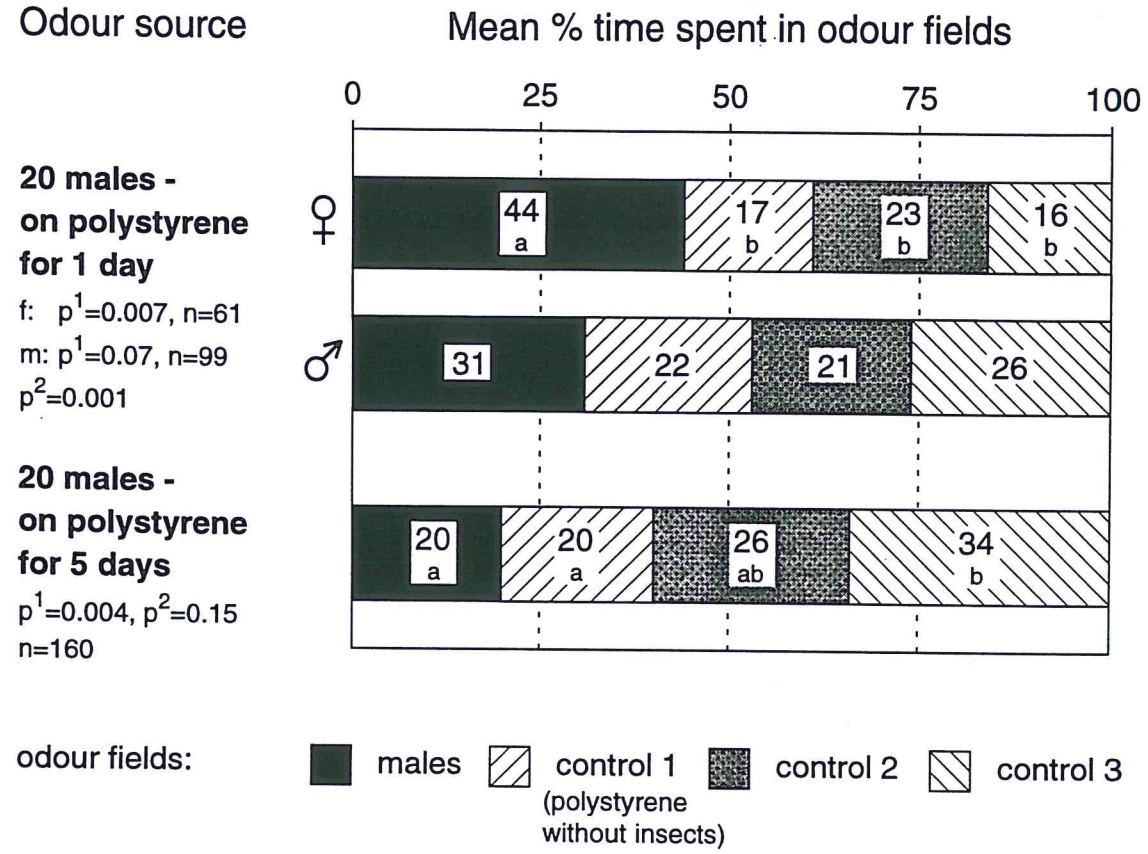


Fig. 5.4. Responses to male *P. truncatus* on polystyrene. For details see Fig. 5.1.

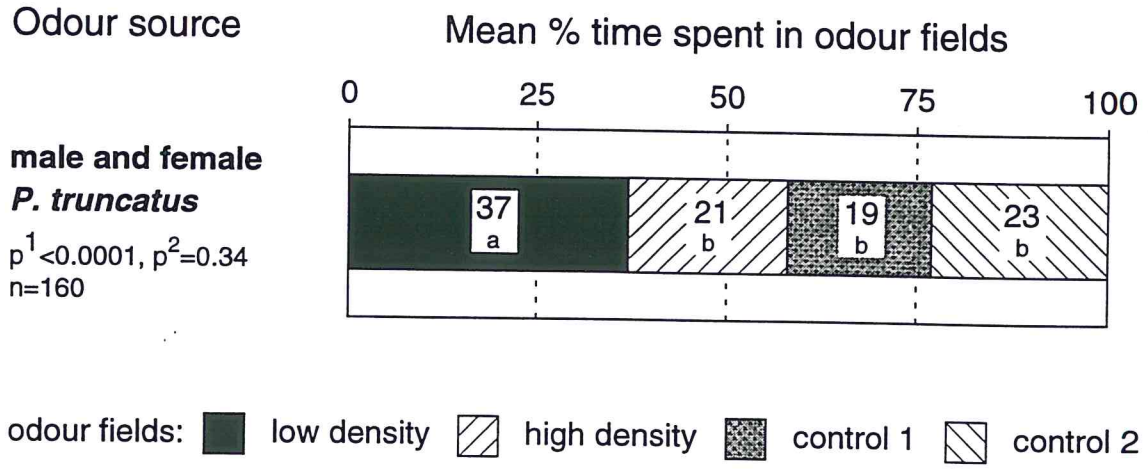


Fig. 5.5. Responses to mixed cultures of *P. truncatus* on maize (two odours offered simultaneously). For details see Fig. 5.1.

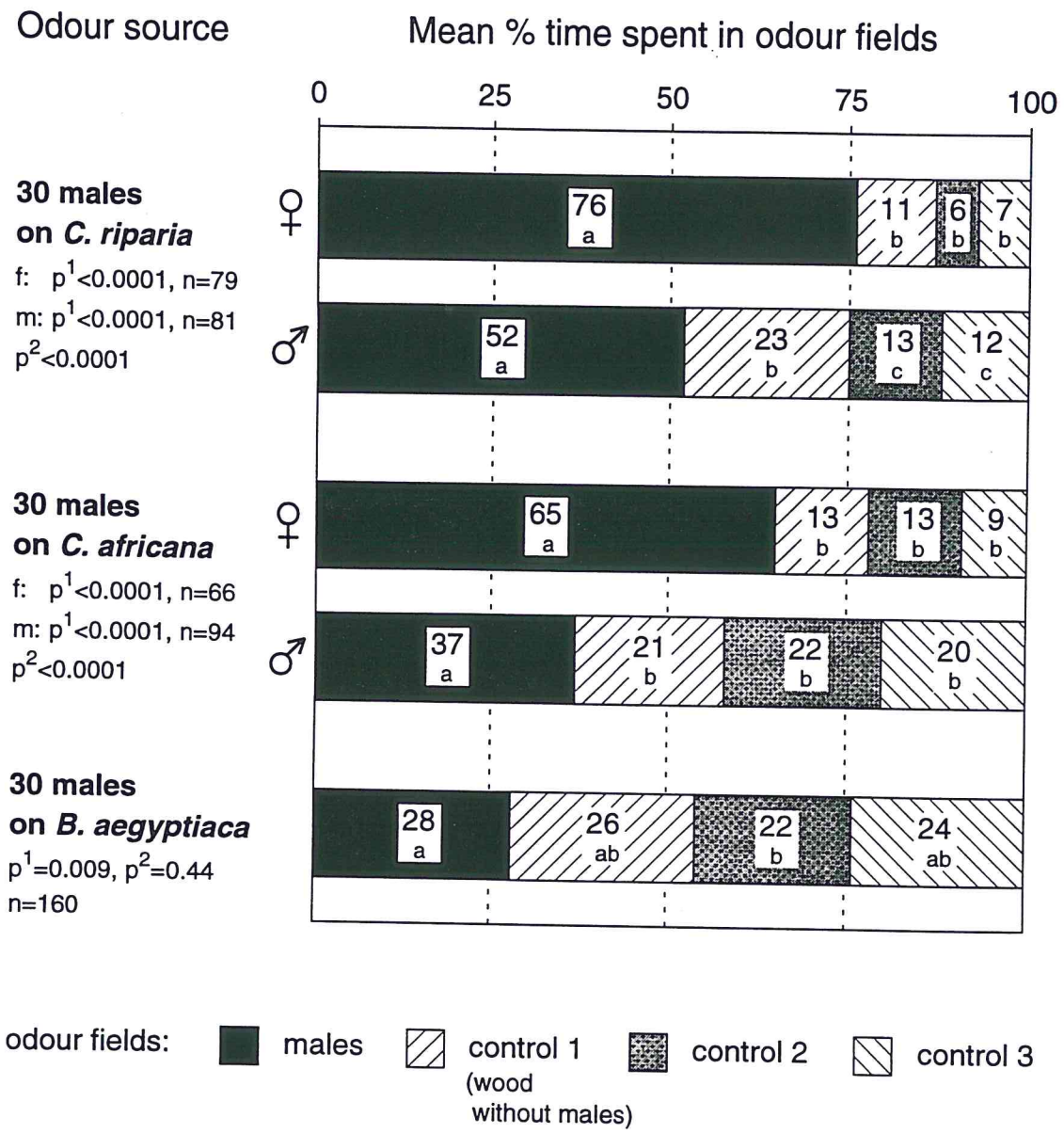


Fig. 5.6. Responses to male *P. truncatus* on wood. For details see Fig. 5.1.

Volatiles emitted from males on *C. africana* and on *C. riparia* were significantly preferred to the controls; however, *P. truncatus* did not respond to males on *B. aegyptiaca* [no significant differences between wood with and without males (Fig. 5.6)]. Males on *C. africana* and on maize were similarly attractive (Fig. 5.7).

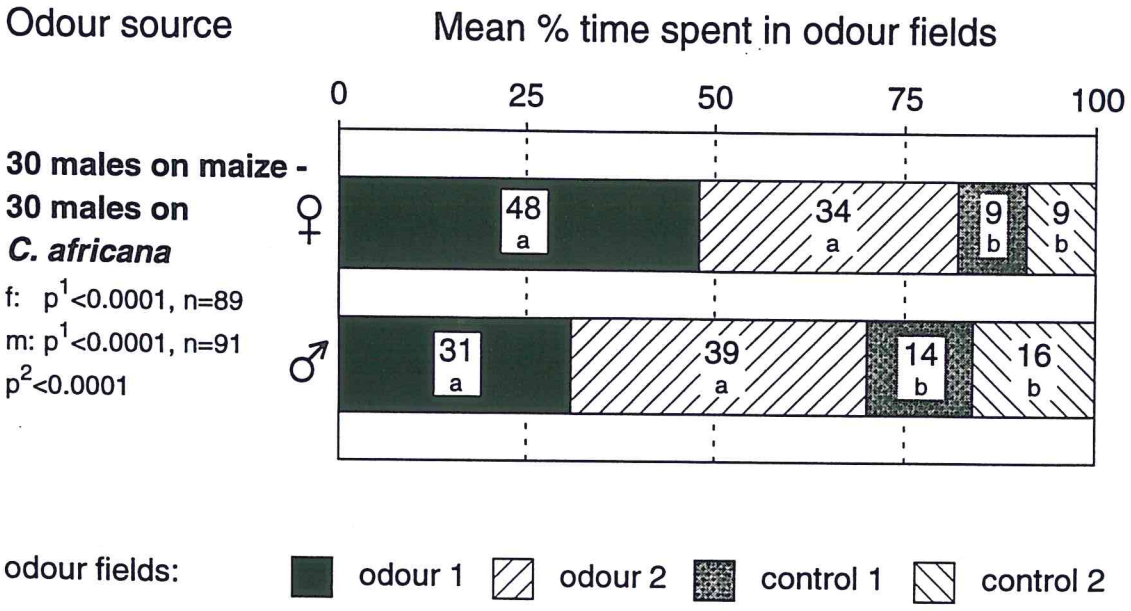


Fig. 5.7. Responses to male *P. truncatus* on maize and wood (two odours offered simultaneously). For details see Fig. 5.1.

Responses to pheromone

P. truncatus always reacted strongly to the synthetic pheromone (Fig. 5.8 and Fig. 5.9), and the time spent within this odour zone was nearly always similar among the male and among the female test beetles. Only for the treatment, when the insects were kept with pheromone but without food for 48 h before the experiment, was the time the females spent within this odour zone reduced (Fig. 5.9). Testing the two pheromone components, T1 and T2, in separate one-choice experiments elicited positive responses of male and female beetles to both components (Fig. 5.10). When, however, the two components were offered simultaneously in separate air streams, female *P. truncatus* reacted more strongly to T2 and less intensely to T1, while males exhibited positive reactions to T2 only.

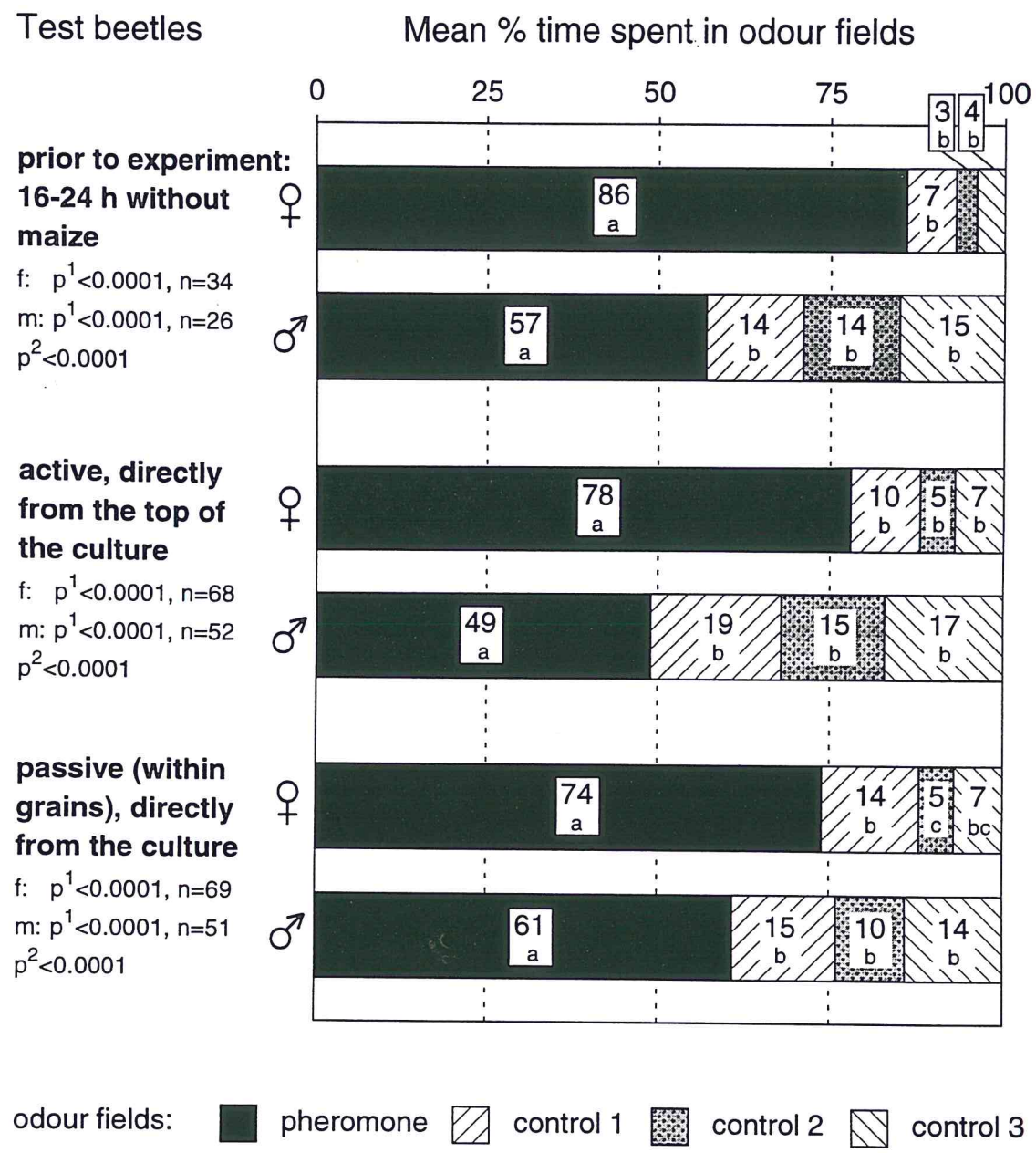


Fig. 5.8. Responses to the synthetic pheromone (1). For details see Fig. 5.1.

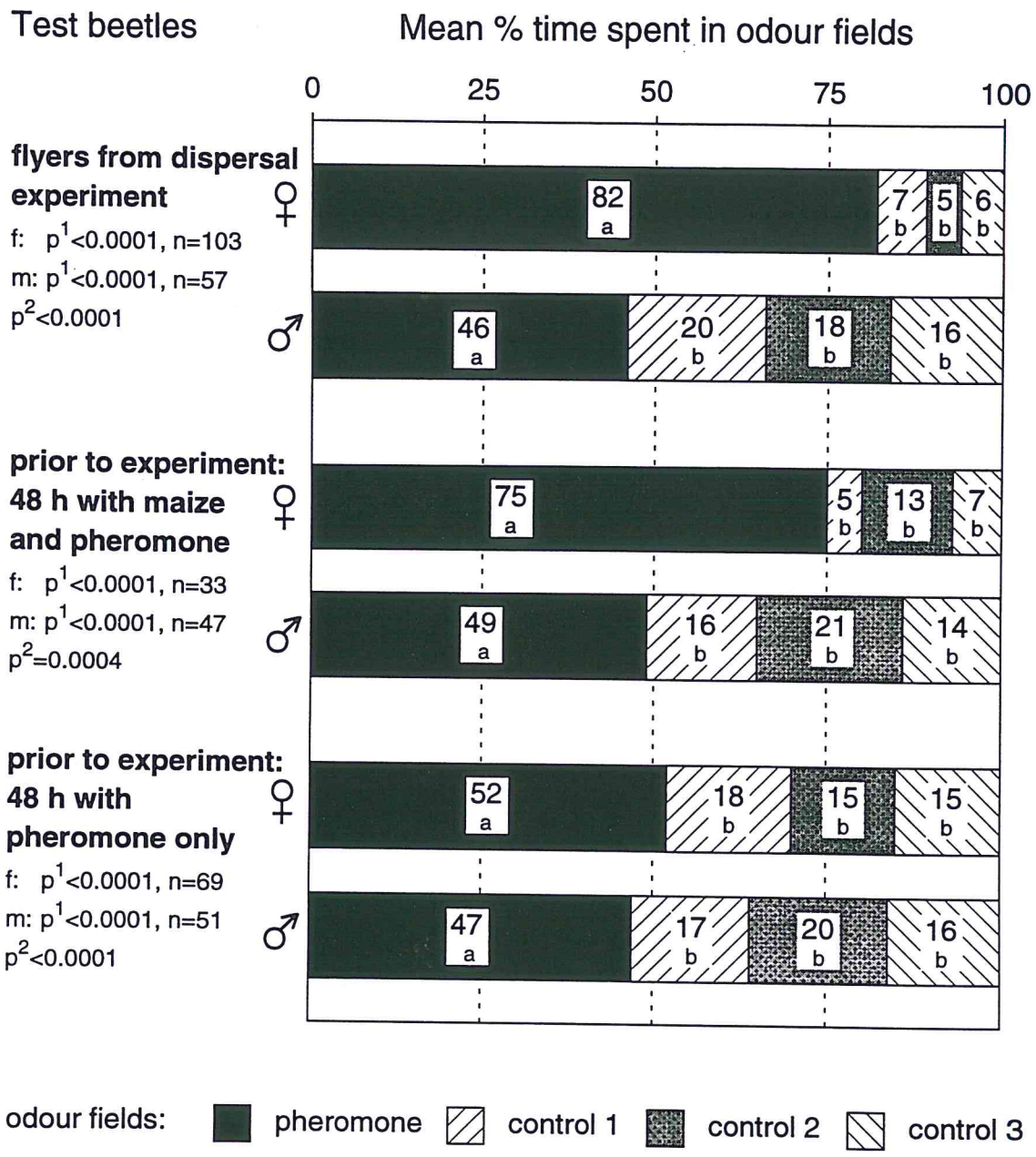


Fig. 5.9. Responses to the synthetic pheromone (2). For details see Fig. 5.1.

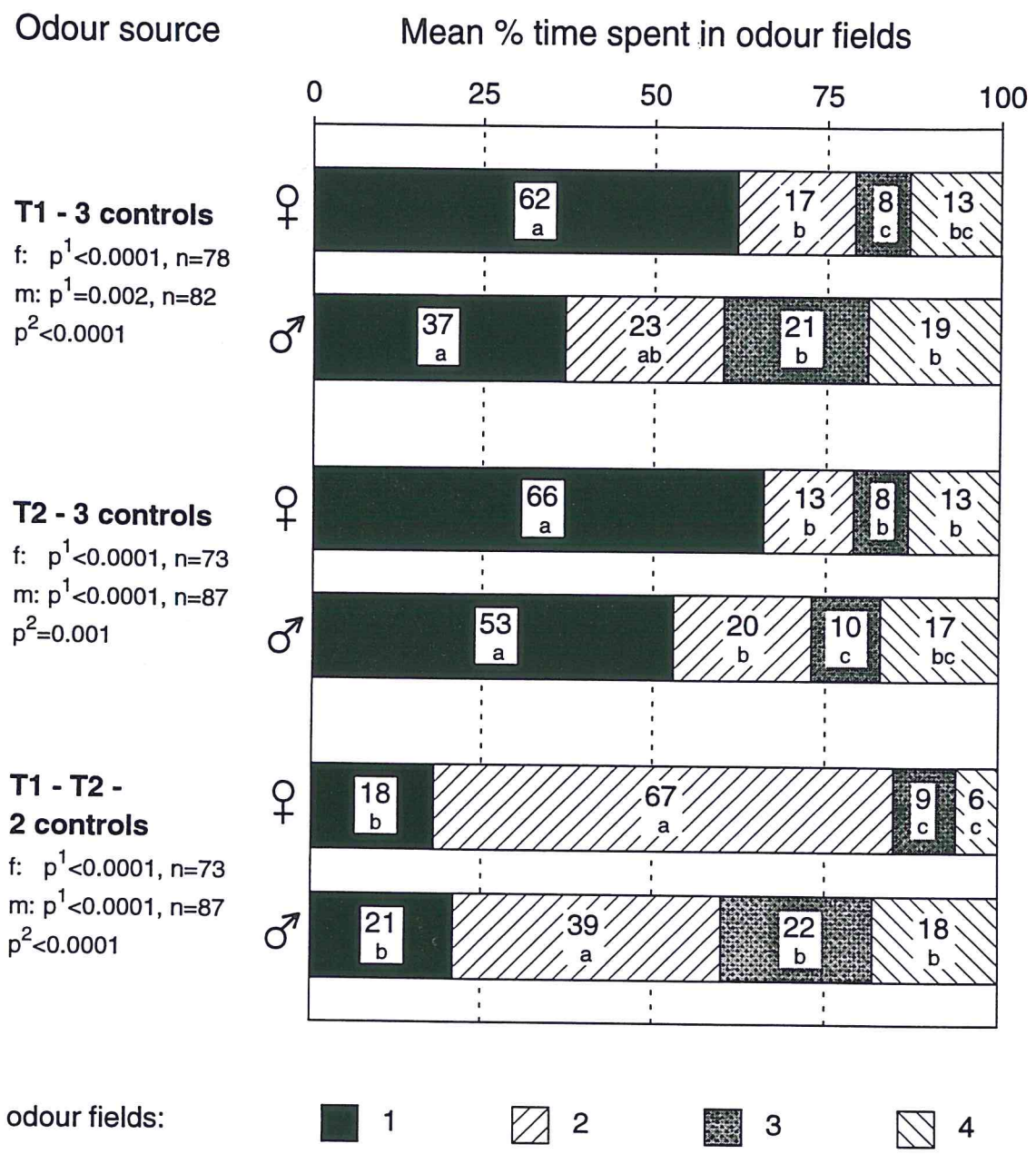


Fig. 5.10. Responses to the synthetic pheromone components, T1 and T2. For details see Fig. 5.1.

Nutrients in tested wood samples

The results of the wood nutrient analyses are presented in Table 5.1. Nitrogen content was highest in *C. riparia*, while nitrogen contents differed little between *C. africana* and *B. aegyptiaca*. The sucrose levels in the wood species may be summarised as: *B. aegyptiaca* > *C. africana* > *C. riparia*.

Table 5.1. Nitrogen and sucrose contents of wood samples used in olfactometer experiments on pheromone production of males on wood. Means ± s.e. of three samples of each species; nitrogen and sucrose content in % of dry matter.

wood species	nitrogen	sucrose
C. riparia	0.81 (± 0.06)	0.12 (± 0.01)
B. aegyptiaca	0.44 (± 0.03)	0.46 (± 0.05)
C. africana	0.39 (± 0.08)	0.26 (± 0.04)

5.3. Behavioural responses of male and female *P. truncatus* and *T. nigrescens* to the pheromone components T1 and T2 under field conditions

5.3.1. Materials and methods

General experimental procedure

Behavioural responses to the pheromone components (T1 and T2) in the field were investigated by distributing pheromone traps baited with (A) 1 mg T1, (B) 1 mg T2 or (C) 1 mg T1 and 1 mg T2 near the IITA station. Pheromone vials were prepared by diluting 10 mg T1 or T2 (supplied by AgriSense-BCS Ltd., Pontypridd, UK) in 100 µl hexane and applying 10 µl of this solution on filter paper stripes (Whatman No. 1). The solvent was allowed to evaporate for 30 sec before the filter paper was placed into a polyethylene vial. The pheromone vials were kept separately in airtight glass jars at -20 °C until used in the experiments. Control baits were prepared with hexane only.

In a first experiment, sticky flight traps (Pherocon II trap, Trécé, Inc., Salinas, California, USA) were distributed over four locations, the locations being *ca.* 100 m apart. Traps were baited with (A) 1 mg T1, (B) 1 mg T2, (C) 1 mg T1 and 1 mg T2 or (D) with a control bait, i.e., with hexane. The traps and pheromone vials were exchanged and replaced by new ones every two days at the same time of the day. All four baits were tested simultaneously, and the position of treatments changed in the scheme of a Latin square design over time. Four replications were conducted in succession, each lasting eight days. The number of *P. truncatus* and *T. nigrescens*

trapped with each trap were recorded and the sex of all trapped beetles determined (cf. Chapter 2.3). *T. nigrescens* were sexed by by dissection of the genitals (Pöschko, 1993).

Probably due to the heavy rains during the first experiment, the numbers of beetles trapped were relatively low. Therefore a second experiment was conducted. Since control baits caught hardly any beetles (cf. Fig. 5.11), the second experiment was conducted without control baits, with only three treatments distributed over three locations. Two replications were conducted, each lasting six days.

The trapping was carried out between 25 May and 8 July, 1996, a time of the year when constant high flight activity of *P. truncatus* and *T. nigrescens* could be expected (Borgemeister *et al.*, 1997).

Statistics

Due to the extremely low catches with the control baits (first experiment) homogeneity of the variances, an assumption for analysis of variance, could also not be obtained by transformations. Therefore, trap catches of the first experiment were analysed using Friedman two-way analysis of variance by ranks followed by a multiple comparison test ($p<0.05$; Siegel & Castellan, 1988). Trap data of the second experiment were square-root transformed [$\sqrt{(x+0.5)}$] and analysed by Latin square anova followed by the Tukey-test (SAS Institute, 1985). Overall sex ratios of the catches with the different pheromone treatments were compared with Chi-square tests (Siegel & Castellan, 1988).

5.3.2. Results

Control baits, containing hexane only, caught hardly any beetles (one *P. truncatus* and three *T. nigrescens* during the whole first experiment). Significantly less *P. truncatus* were caught with T1 than with T2 or with the mixture of T1 and T2 in both experiments, while the number of *P. truncatus* caught with T2 and the mixture did not differ significantly (Fig. 5.11). There were no significant differences between the numbers of *T. nigrescens* trapped with the three pheromone treatments (Fig. 5.11).

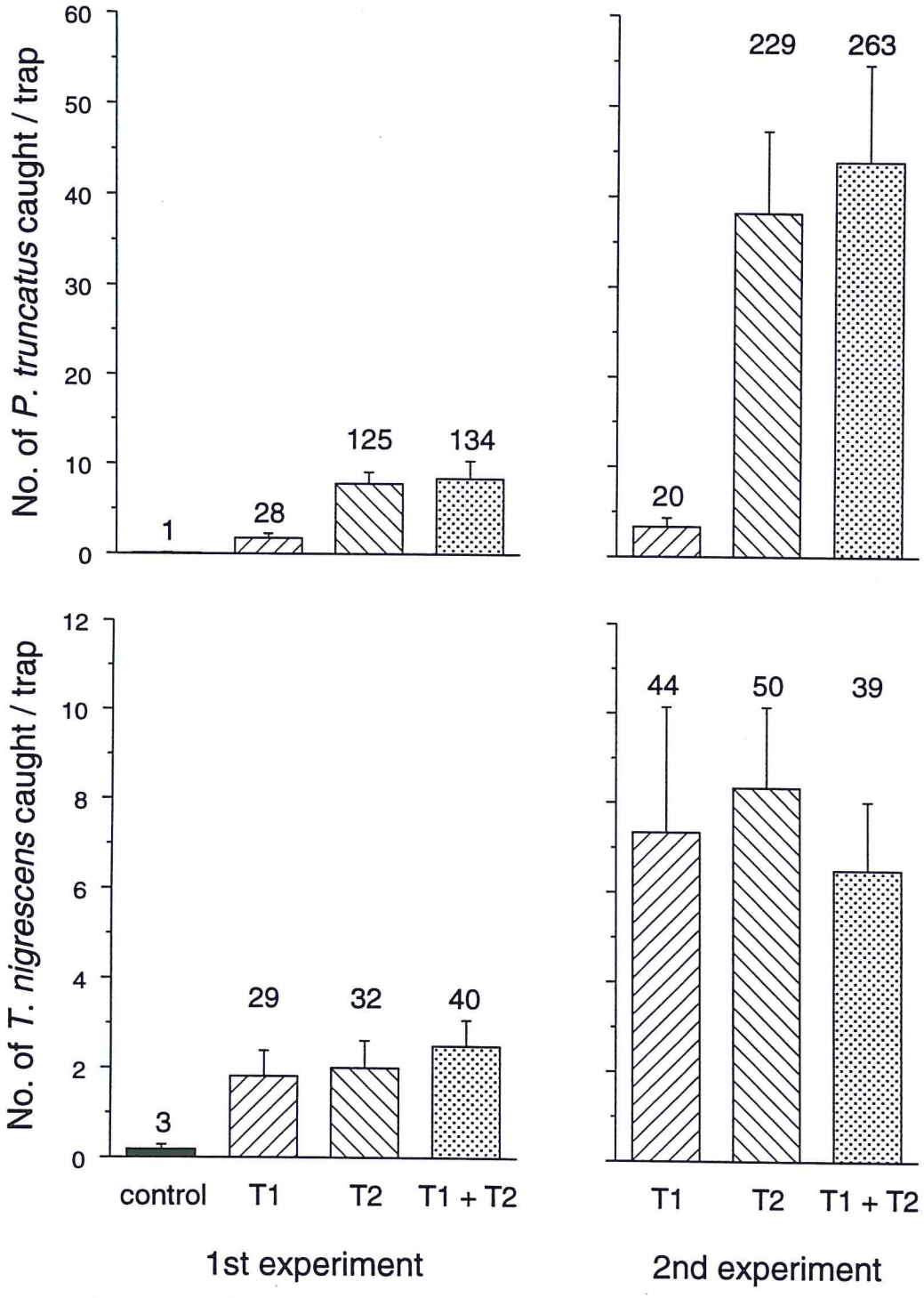


Fig. 5.11. Mean numbers of *P. truncatus* and *T. nigrescens* caught per trap during two days. Numbers in the graph document the total number of beetles caught; error bars represent 1 s.e.

Sex ratios of *P. truncatus* trapped with the different pheromone baits did not differ significantly in the first experiment (Table 5.2; p=0.83), but differed just significantly in the second experiment (Table 5.2; p=0.04). Sex ratios of *T. nigrescens* trapped with the different pheromone baits did not differ significantly (Table 5.2; 1st experiment: p=0.77, 2nd experiment: p=0.95).

Table 5.2. Proportions of female *P. truncatus* and *T. nigrescens* among trap catches in regard to pheromone components. P-values were obtained in Chi-square tests for differences between the sex ratios of the three treatments.

		T1	T2	T1 + T2	p-value
P. truncatus	1st experiment	57%	54%	58%	0.83
	2nd experiment	50%	55%	65%	0.04
T. nigrescens	1st experiment	38%	38%	45%	0.77
	2nd experiment	41%	38%	38%	0.95

5.4. Electrophysiological responses of male and female *P. truncatus* and *T. nigrescens* to the pheromone components T1 and T2

5.4.1. Materials and methods

Experiments were conducted at the Institute of Phytopathology, University of Kiel, Germany.

Insects

Initial laboratory cultures of *P. truncatus* and *T. nigrescens* were established as described in Chapter 2.2 and in Chapter 4.2. Test insects for this experiment were reared on 170 g maize grains in 250 ml glass jars covered with brass gauze and maintained at 28 ± 3 °C and 75 ± 5% r.h. *P. truncatus* cultures were inoculated with 50 adults, which were removed after two weeks. *T. nigrescens* cultures were set up with 200 adult *P. truncatus* and 10 days later 20 adult *T. nigrescens* were added. The adult *T. nigrescens* were removed after two weeks. Test insects were 0-3 weeks old. They were removed from the cultures 16-24 h prior to the experiment and kept in glass petri dishes laid out with brass gauze.

Electroantennogram recordings

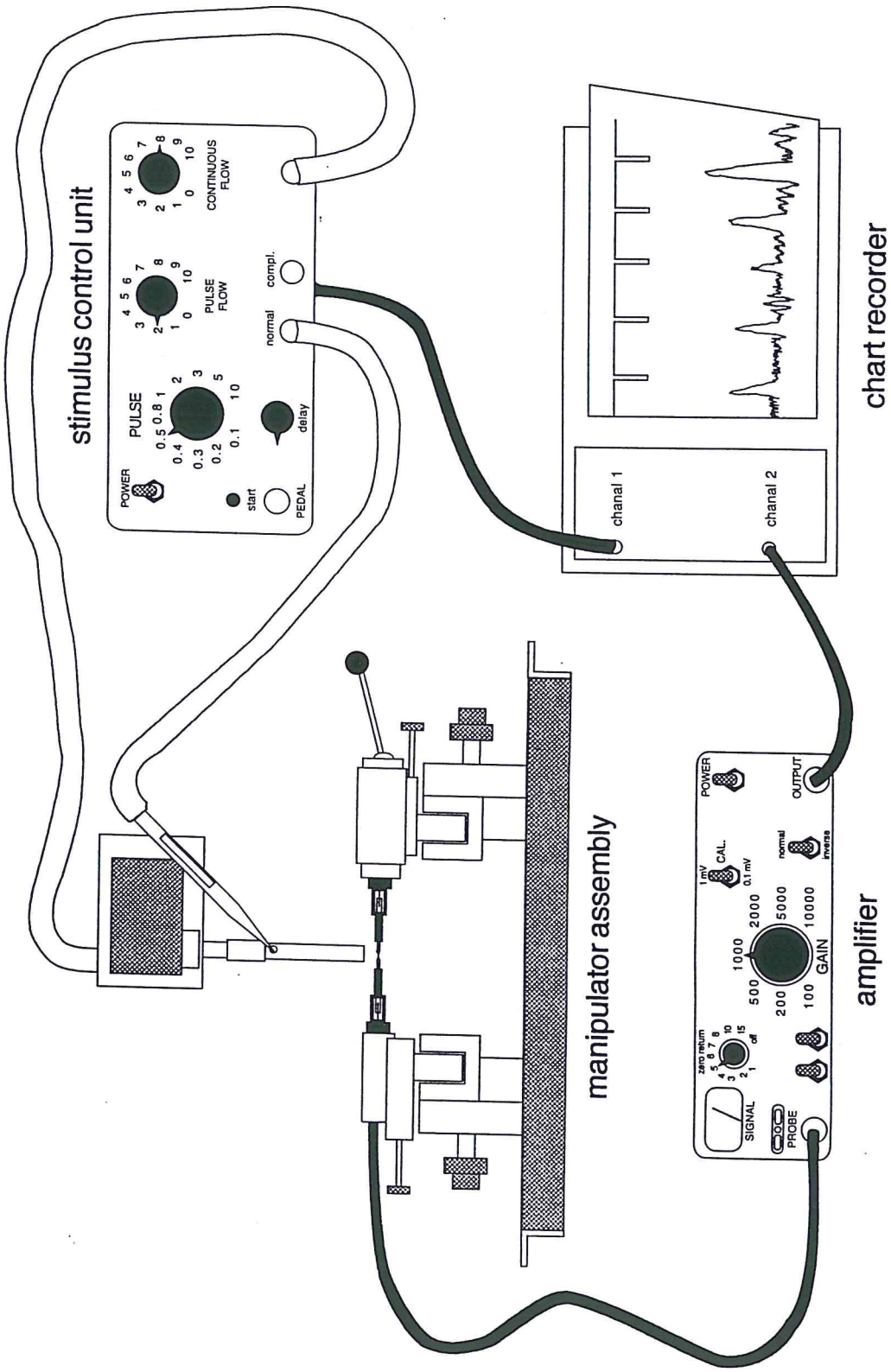
The electroantennogram (EAG) techniques applied were similar to the ones described by Dickens (1981) (Fig. 5.12). Antennal preparations proved to be difficult to make for *P. truncatus* and are generally short lasting, so head preparations were used instead (Fig. 5.13). For *P. truncatus*, the head was cut off including the first thorax segment, whereas for *T. nigrescens* only the head was excised. Two glass capillaries, drawn to a fine point, were filled with Ringer solution (Merck), and one of them was carefully inserted through the base of the head, such that the tip lodged close to the base of the antennae. This preparation was mounted on the indifferent electrode (silver wire) of the manipulator assembly (MP-12, Syntech, Hilversum, The Netherlands). The tip of one antenna was brought into contact with the solution of the second capillary, which had been slit onto the recording electrode. The guarded probe on the manipulator assembly was connected to an amplifier (AM-05/b, Syntech) via a shielded cable. EAGs were recorded on a chart recorder (ABB Metrawatt SE 120) and measured as the maximum amplitude of depolarization elicited by a stimulus.

Odour stimuli

The two components of the aggregation pheromone, T1 and T2 (AgriSense-BCS Ltd., Pontypridd, UK), were tested separately. Serial dilutions in hexane were made for each pheromone component separately, resulting in stock solutions of concentrations of 1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} µg pheromone/µl hexane.

In preliminary trials, *P. truncatus* showed adequate EAG responses to 10 µg T1 applied on filter paper, so 10 µl of the 1 µg/µl T1-solution were chosen as the standard for *P. truncatus*. Depolarizations in the antennae of *T. nigrescens* in response to this pheromone quantity were too high for a standard, so for *T. nigrescens* 10 µl of the 10^{-2} µg/µl T1-solution (i.e., 0.1 µg T1 applied on the filter paper) were used instead.

Fig. 5.12. (next page) Electroantennogram: Experimental set-up.


stimulus control unit
POWER
PULSE
start
PEDAL
delay
PULSE FLOW
normal
compl.
CONTINUOUS FLOW
chart recorder
chanal 1
chanal 2
manipulator assembly
amplifier
POWER
CAL.
OUTPUT
GAIN
SIGNAL
PROBE
zero return
normal
inverse

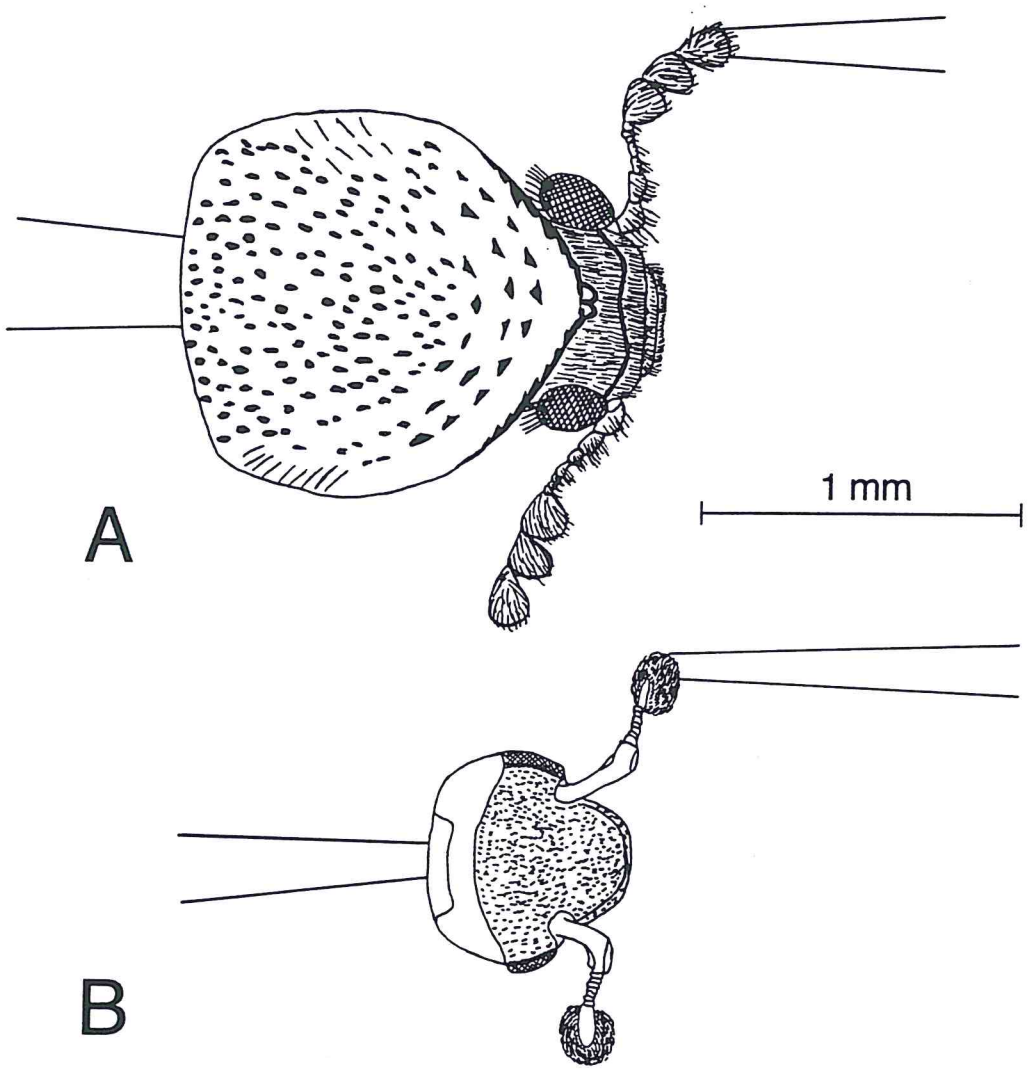


Fig. 5.13. Head preparations of *P. truncatus* (A) and *T. nigrescens* (B).

Solutions were stored at -20 °C, taken out of the freezer singly, only to extract the solution to be applied in the particular stimulation, and replaced immediately afterwards. This procedure was chosen to avoid temperature changes and thereby changes in the volatility of the solutions during the course of the experiment. As each insect was stimulated seven times with the control (hexane) and seven times with the standard, but only once with each of the 13 test solutions, seven standard and seven control solutions were prepared for the above mentioned reasons. All solutions were renewed four times in the course of the experiments.

Although it is the amount of pheromone passing over the antennae that elicits the EAG response, I will hereafter be referring to the amount of pheromone applied to the filter paper when discussing quantities of pheromones. The amount of the pheromone components actually evaporating and reaching the antennae was not measured.

Odour delivery and stimulation procedure

A stimulus control unit (CS-05/b, Syntech) was used for air and odour delivery (Fig. 5.12). During the course of an experiment, a constant flow (2 l/min) of charcoal-filtered air was passed over the antenna, delivered through a stainless steel tube, which ended about 1 cm from the antennal preparation. For stimulation, a Pasteur pipette containing the stimulus was connected to a hose delivering pulses of air, and its tip inserted into the constant airstream through a small hole in the metal tube.

The same procedure for stimulation was used in all experiments. The stock solution (10 µl) was pipetted onto a piece of filter paper (4 mm × 6 mm, Whatman No. 1), and the solvent allowed to evaporate for 25 sec before placing the filter paper into a glass Pasteur pipette. After another 25 sec, a pulse of air of 1 sec duration (air flow: 0.85 l/min, i.e., a volume of *ca.* 14 ml) was passed through the pipette, transferring the volatiles into the constant air flow. Stimuli were given at 2 min intervals to allow recovery of the olfactory sensilla. The onset and duration of the stimulation were recorded simultaneously with the EAG on the chart recorder.

In order to avoid the sequence of the stimuli presented influencing the interpretation of the data, the following stimulation procedure was applied:

- Stimuli presentations of T1 and T2 (of the same dosage) were preceded and followed by a standard and a control stimulation. T2 of the same concentration as the standard was incorporated in one of these sequences.
- The sequence of T1 and T2 between standard/control stimulations was exchanged in the next experiment with an insect of the same species and same sex.
- The sequence of dosages offered during one experiment was changed randomly.
- Males and females of the same species were tested alternately, applying the same stimuli and dosage sequences to each sex.

- Each insect was presented with all dosages (10 µg - 10^{-5} µg of both pheromone components).

Ten insects of each species and sex were tested.

Data evaluation

Absolute net EAG responses towards the test components (in mV) were obtained by subtracting the mean absolute EAG response of the control stimulations immediately preceding ($control_x$) and following ($control_{x+1}$) the presentation of the test component from the absolute EAG response of the test component:

- net EAG_x (in mV) = EAG_x - [($control_x$ + $control_{x+1}$)/2]

Relative net EAG responses towards the test components (in % of standard) were calculated by:

- net EAG_x (in %) = net EAG_x × 100/[($standard_x$ + $standard_{x+1}$)/2], with $standard_x$ and $standard_{x+1}$ being the adjacent net standard response amplitudes.

Perception threshold was defined as the lowest pheromone quantity at which the mean absolute response (in mV) was significantly higher than the mean of the responses elicited by the adjacent control stimuli:

- EAG_x > [($control_x$ + $control_{x+1}$)/2]

Volatility

T1 is known to be more volatile than T2 (A. Cork in Dendy *et al.*, 1991 and in Fadamiro, 1995). EAG responses of both species to the same quantities of the pheromone components were relatively higher to T2 than to T1 (cf. results: Chapter 5.4.2). I wondered if this observation was due to a high loss of T1 before the insertion of the filter paper into the pipette. To evaluate if this was the case, *P. truncatus* and *T. nigrescens* females were stimulated with 1 µg of T1 and T2 separately; the time of exposure before the filter paper was put into the pipette was either 5 or 25 sec. Two control stimuli (hexane evaporated for 5 or 25 sec) preceded

and followed the four stimulations with the pheromone components. This procedure was replicated three times per test insect. Four females of each species were tested. The sequence of the four stimuli was changed in each replicate, but the same sequences were applied to both species.

The net EAG response (in mV) elicited by T2 within each sequence (of four test stimuli) was compared with the corresponding net EAG response elicited by T1, and the response to T1 defined as 100%. If the EAG responses elicited by T2 were higher than the EAG responses elicited by T1, because more T1 than T2 evaporated before insertion of the filter paper into the pipette, then the relation of T1:T2 should be closer in the 5 sec than in the 25 sec treatment: By decreasing the evaporation time, the differences between the amounts of T1 and T2 evaporated should be reduced.

Statistics

In order to determine perception thresholds, absolute EAG responses to test stimuli (EAG_x) were compared with the mean of the absolute responses to the adjacent control stimuli [($control_x$ + $control_{x+1}$)/2] using Wilcoxon signed ranks tests. Responses of males or females to T1 and T2 above threshold were also compared with Wilcoxon signed ranks tests. Mann-Whitney-tests were used to compare the responses of males and females to the same pheromone quantities above threshold (Siegel & Castellan, 1988). In each of three test series, Holm's sequentially rejective procedures (a variation of the Bonferroni procedures) were applied to adjust the experimentwise error rate (Hochberg & Tamhane, 1987). Mann-Whitney tests were also used to test for differences between EAG responses of males and females of the same species to standard and control stimuli, and Wilcoxon signed ranks tests to compare the relative differences of the EAG responses in the experiment to evaluate volatility. Responses were regarded as significantly different, when $p<0.05$.

5.4.2. Results

Responses to standards and controls

There were no significant differences between *P. truncatus* females and males regarding the absolute responses to the standard (i.e., 10 µg T1), the responses to

the control or the absolute net EAG responses to the standard (Table 5.3). Also between *T. nigrescens* females and males there were no significant differences, either in terms of the absolute EAG responses to the standard (i.e., 0.1 µg T1), or in terms of their amplitudes to the control stimuli (Table 5.3). The absolute net EAG responses to the standard did not differ significantly between sexes neither.

Table 5.3. EAG responses (in mV) of male and female *P. truncatus* and *T. nigrescens* to standards and controls (hexane). Standards were 1 µg T1 for *P. truncatus* and 0.1 µg T1 for *T. nigrescens*. Means ± s.e.; p-values were obtained in Mann-Whitney tests for differences between males and females.

	response to	females	males	p-value
P. truncatus	standard (absolute)	3.1 (± 0.2)	3.3 (± 0.2)	0.40
	control	0.9 (± 0.1)	1.0 (± 0.1)	0.49
	standard (net)	2.2 (± 0.2)	2.3 (± 0.1)	0.40
T. nigrescens	standard (absolute)	6.8 (± 0.4)	7.7 (± 0.5)	0.27
	control	2.3 (± 0.2)	2.7 (± 0.3)	0.42
	standard (net)	4.5 (± 0.2)	5.0 (± 0.4)	0.79

Responses to T1 and T2

Perception thresholds (the smallest quantities of the pheromone components which elicited EAG responses significantly different from the EAG responses elicited by the controls) are presented in Table 5.4. Female and male *P. truncatus* had equal response thresholds for T2 (0.001 µg). Significant EAG responses to T1 were elicited from females at a 10 times higher pheromone quantity (0.01 µg), and for males the threshold quantity of T1 was 100 times higher (0.1 µg). There were no significant differences between the responses of males and females with respect to the different pheromone quantities above thresholds (Fig. 5.14). Comparisons of the responses to the two pheromone components revealed significant differences between the EAG responses elicited by T1 and T2 for all concentrations equal to, or higher than, 0.001 µg (= threshold of T2): T2 evoked higher depolarizations than the same quantities of T1, in males as well as in females (Fig. 5.14).

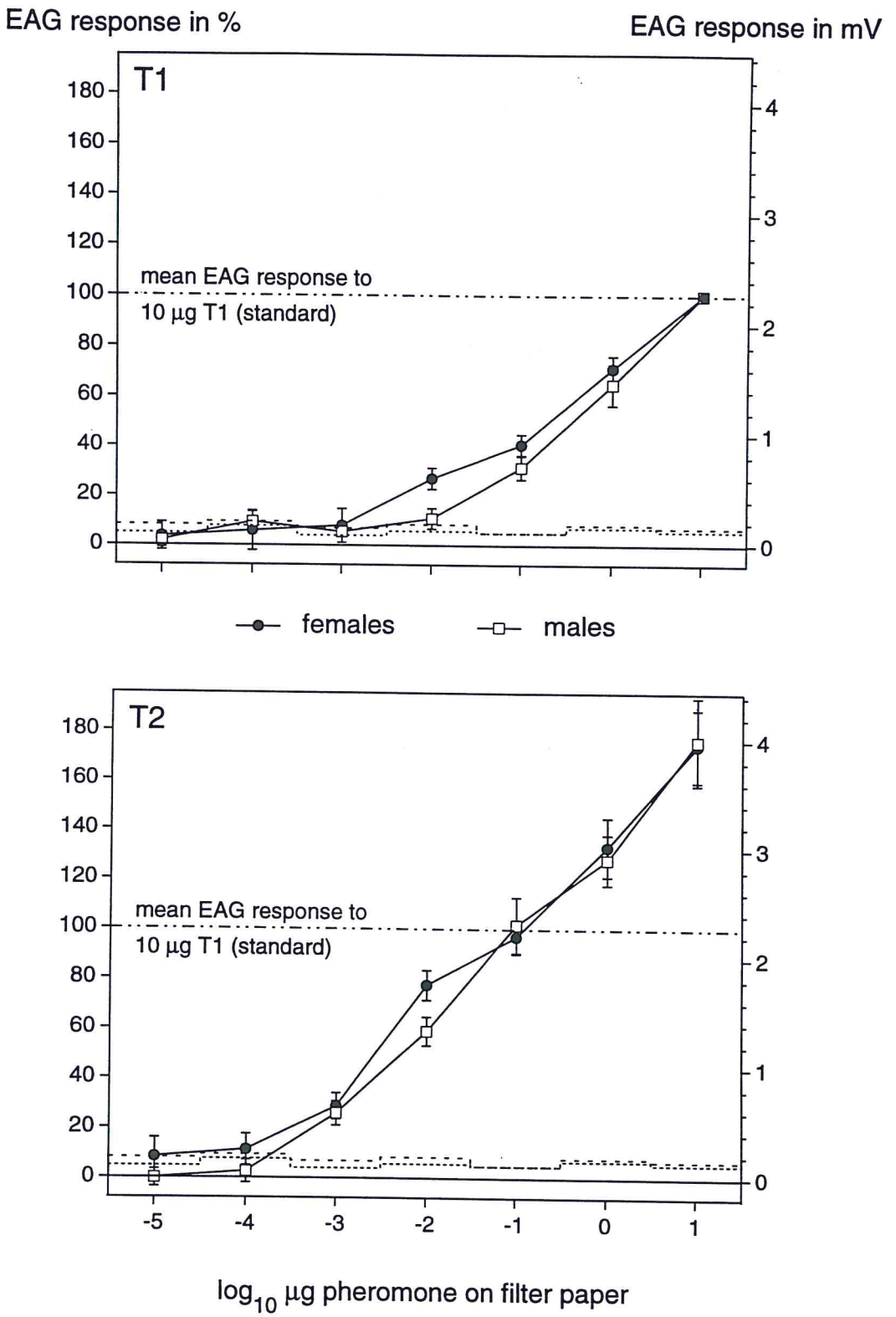


Fig. 5.14. EAG dose-response curves of male and female *P. truncatus* to T1 and T2. Perception threshold: lowest pheromone quantity at which the absolute responses to the test stimuli were significantly higher than responses to the adjacent controls. Dashed lines (steps) represent the standard errors of the responses to the controls for the specific pheromone quantities (short dashes represent the control standard errors for females, longer dashes the control standard errors for males). Error bars represent 1 s.e.

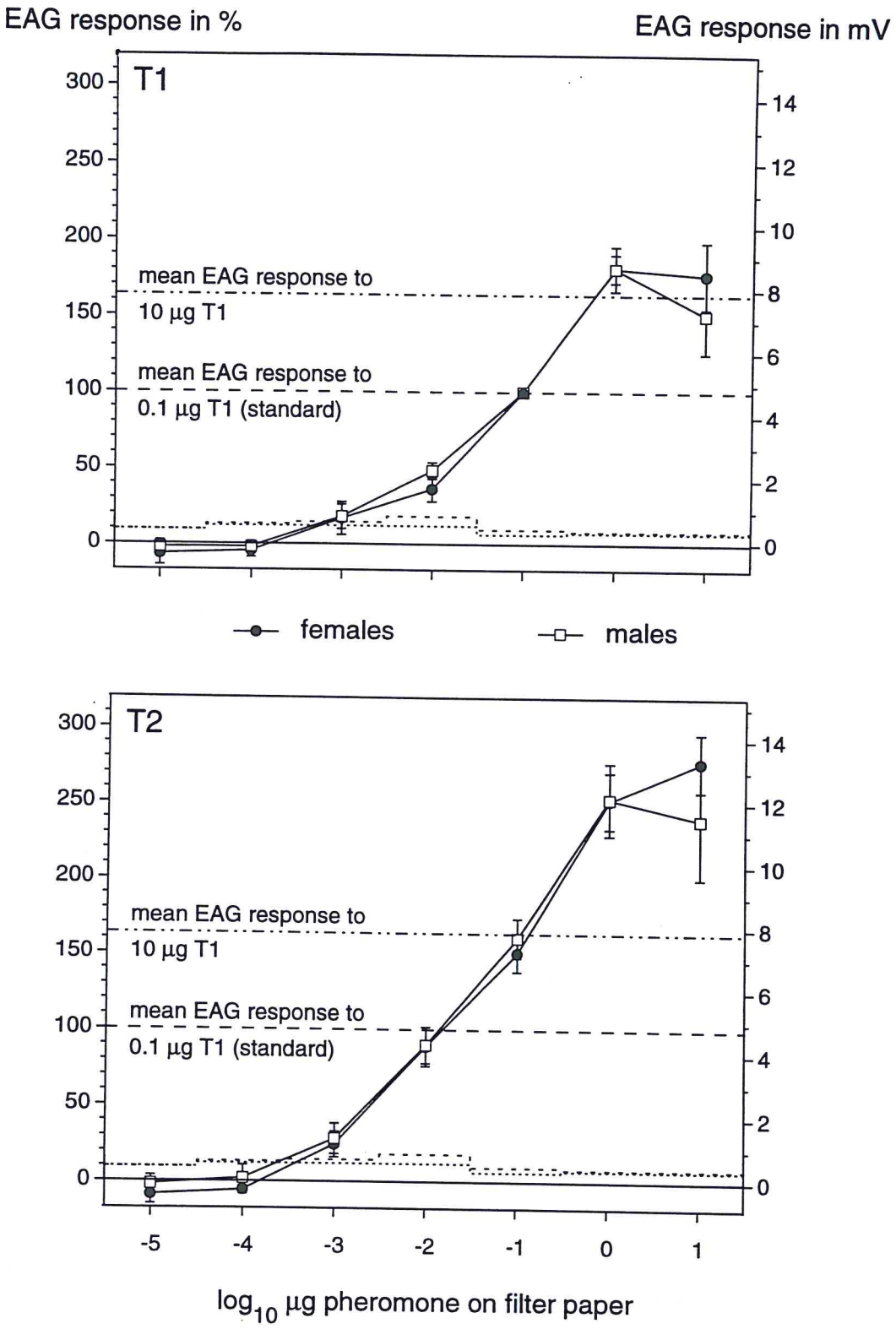


Fig. 5.15. EAG dose-response curves of male and female *T. nigrescens* to T1 and T2. For details see Fig. 5.14.

Table 5.4. Perception thresholds of male and female *P. truncatus* and *T. nigrescens*.

	sex	T1	T2
P. truncatus	female	10^{-2} µg	10^{-3} µg
	male	10^{-1} µg	10^{-3} µg
T. nigrescens	female	10^{-2} µg	10^{-3} µg
	male	10^{-2} µg	10^{-3} µg

For *T. nigrescens*, male and female perception thresholds were 0.001 µg for T2 and 0.01 µg for T1 (Table 5.4). However, the magnitude of the responses differed only slightly at 0.001 µg (Fig. 5.15). There were no significant differences between the responses of males and females towards the different pheromone quantities (Fig. 5.15). The responses evoked by T1 and T2 differed significantly for all pheromone quantities from 0.01 µg onwards, with responses to T2 always higher.

Depolarizations caused by the pheromone components, but also by the control (hexane) were much higher in *T. nigrescens* than in *P. truncatus* (Table 5.3; Fig. 5.14 and 5.15). Nevertheless for T1 alone the threshold quantity was 10 times lower in male *T. nigrescens*, i.e., males of the predator reacted to a lower quantity of T1 than did male *P. truncatus* (Table 5.4).

Volatility

By reducing the evaporation time of the solutions (1 µg pheromone) before the filter paper was placed into the pipette, the ratios of the EAG responses T1:T2 became slightly closer for *P. truncatus* as well as for *T. nigrescens* (Fig. 5.16). However, these differences were not significant (ratios: *P. truncatus*: 1:1.85 for 25 sec, 1:1.61 for 5 sec; *T. nigrescens*: 1:1.73 for 25 sec, 1:1.57 for 5 sec).

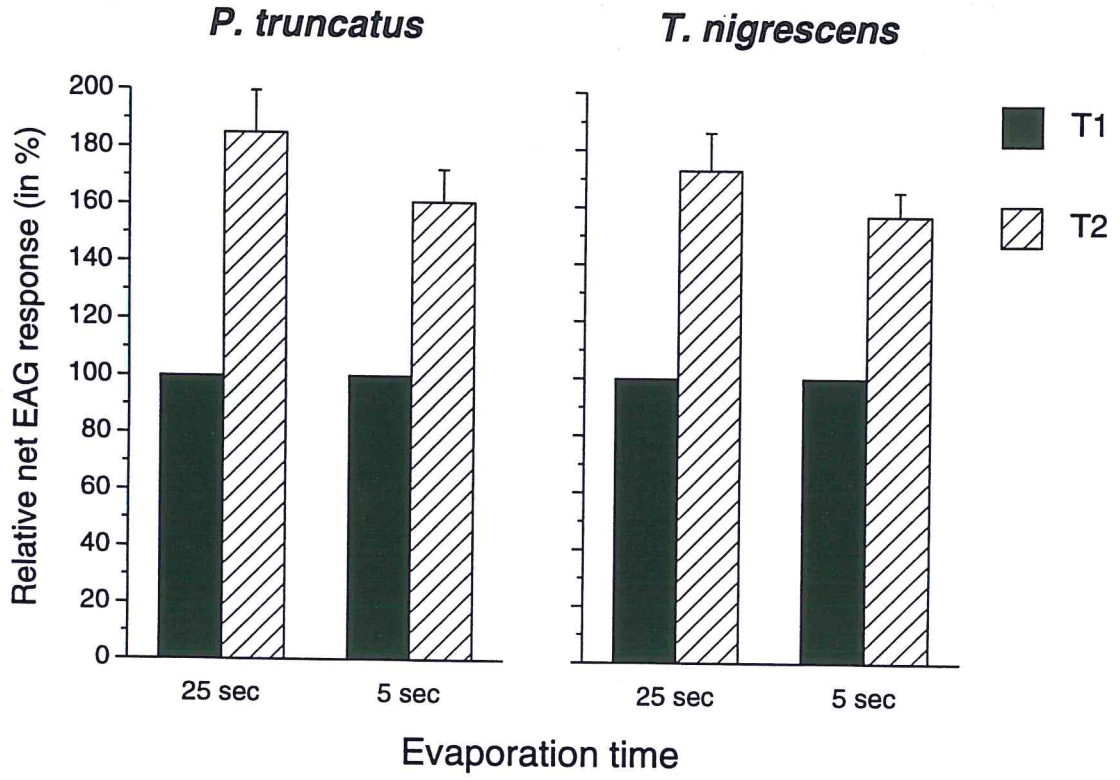


Fig. 5.16. Volatility comparison of T1 and T2. Relative net EAG responses of *P. truncatus* and *T. nigrescens* to 1 µg of the pheromone components after 25 and 5 sec evaporation time before insertion into the pipette. Error bars represent 1 s.e.

5.5. Attraction capacity of a single male *P. truncatus* under field conditions

5.5.1. Materials and methods

Preliminary experiment

To verify if *P. truncatus* can also be caught with non-baited traps or may be attracted to traps baited with maize cobs, a preliminary trial was run with Delta sticky traps (Pherocon II trap, Trécé, Inc., USA) either exposed empty, with an undamaged maize cob or with a maize cob infested with one male *P. truncatus* five days prior to the experiment (cf. 'general experimental procedures' for exact description of the infestation method). Six traps (two traps of each treatment) were distributed over six locations near the IITA station, the locations being *ca.* 100 m

apart. Traps and baits were exchanged and replaced by new ones every seven days. All three baits were tested simultaneously, and the position of treatments changed in the scheme of two Latin square designs over time, the whole trial lasting 21 days. The trapping was carried out in September 1995, a time of the year with relatively low flight activity (Borgemeister *et al.*, 1997; cf. also Fig. 3.6)

General experimental procedure

Undamaged, dehusked maize cobs were deep frozen and afterwards conditioned at 30 ± 1 °C and 70 ± 5% r.h. for at least 14 days. Each cob was weighed, wrapped in a paper towel (to facilitate penetration by *P. truncatus*) and infested with one male *P. truncatus*, which was up to three weeks old and originated from the basic cultures (cf. Chapter 2.2). These cobs were then stored singly for five days under the same condition as the basic cultures (cf. Chapter 2.2). This time was sufficiently long to allow the beetles to bore into the cobs and to increase their pheromone production (cf. Chapter 5.2.2). As the number of beetles caught is dependent on the type of trap used (Dendy *et al.*, 1989) and since I wanted the number of *P. truncatus* trapped with the baited cobs to be comparable with the trap catches obtained with synthetic pheromone, I covered the sticky inner surface of Delta sticky traps (Pherocon II trap, Trécé, Inc., USA) with cardboard, so that the arriving beetles could enter the cobs without adhering to the glue. One cob was placed in each trap and secured with a wire, after the paper towel had been removed. The strings with which the traps were fixed to trees and posts were covered with insect glue to prevent ant attack. The traps were hung out at 20 different locations (eight being situated near the IITA station and 12 in the Mono District in south-western Benin). Traps were placed at least 100 m apart. The cobs were collected after one, two, three or four weeks. Since maize cobs by themselves have been reported not to attract *P. truncatus* (Wright *et al.*, 1993; M. Ramírez-Martínez, pers. comm.) and traps with cobs but without males did not catch more beetles than empty traps in the preliminary trial (cf. Fig. 5.17), only male-baited cobs were used in this experiment. Routine monitoring data at the IITA had always shown a strong location and time biased dependency of trap catches (Borgemeister *et al.*, 1997), so the four time treatments were conducted at each location in succession, with four different treatment sequences being distributed over the 20 locations (80 traps = 4 time treatments × 4 treatment sequences × 5 replications; in a Latin square design with 5 replications). All adult *P. truncatus* were then removed from the cobs, counted, and their sex determined (cf. Chapter 2.3). The residues of the cobs of the

one week treatment were placed in glass jars and stored under the same conditions as the basic cultures. After 31 days all *P. truncatus* which had emerged were counted and the sex determined (for all, if less than 100, or for a sample of 100 beetles).

The trapping was carried out between 30 March and 8 June, 1995, a time of the year when constant high flight activity of *P. truncatus* could be expected (Borgemeister *et al.*, 1997; cf. also Figs. 3.6 and 3.10).

Simultaneous monitoring with pheromone traps

P. truncatus flight activity was assessed using three Delta sticky traps (baited with polyethylene vials containing 1 mg T1 and 1 mg T2, AgriSense-BCS Ltd., Pontypridd, UK) simultaneously with the experiment described above. The traps were distributed near the IITA station; distances between pheromone traps were *ca.* 300 m, while the distance between a pheromone trap and the closest male-baited trap measured about 100 m. Traps were collected weekly (on the same day as the cob traps), replaced by new traps with fresh pheromone vials and the number of *P. truncatus* caught recorded. The sex ratio among *P. truncatus* caught in pheromone traps was determined for catches from two additional traps, set up in the vicinity of two traps with baited cobs in the Mono District. A sample of 100 beetles per trap was sexed weekly during the whole period of investigation, i.e., a total of 2000 beetles.

Statistics

Trap catches of the main experiment (square root-transformed) and percentages of females caught (arcsine-transformed), in regard to the different trapping durations, were analysed by using analysis of variance procedures (Latin square) of general linear models in SAS (SAS Institute, 1985). Chi-square tests were used to compare overall sex ratios of the trap catches and of the progeny to an expected 1:1 ratio and to compare sex ratios in pheromone traps and traps with baited cobs (Siegel & Castellan, 1988).

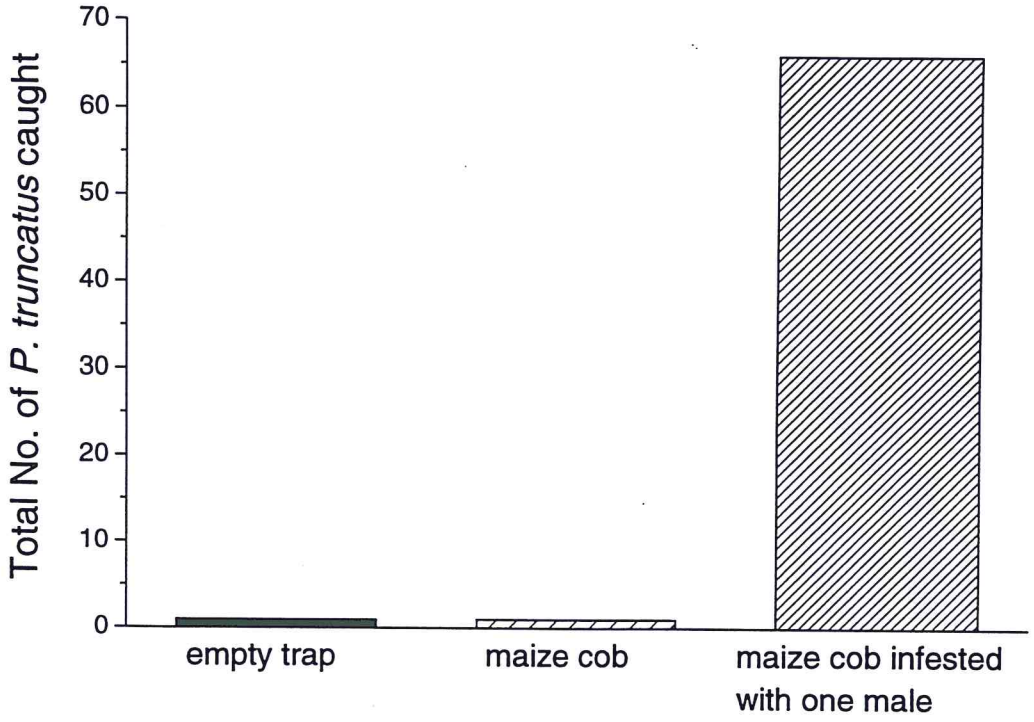


Fig. 5.17. Total numbers of *P. truncatus* caught in the preliminary experiment. Accumulated trap catches of six traps (two traps × three weeks) per treatment .

5.5.2. Results

In the preliminary trial, empty traps and traps baited with uninfested maize cobs caught hardly any *P. truncatus* (one beetle each during the whole trapping period), while traps baited with infested cobs caught a total of 66 *P. truncatus* (Fig. 5.17).

The number of *P. truncatus* caught during the trapping periods of one, two, three and four weeks duration did not differ significantly (Fig. 5.18; Latin square anova, treatment effect, p=0.67). Sequence order and position within the sequence had no effect, either (p=0.30 and p=0.35). A mean of 58.7 (s.e.=5.3) *P. truncatus* was caught per trap, catches ranging from 2 to 210 beetles. The frequency distribution of the trap catches was left-skewed, the median being 39.5.

Also the proportions of the female *P. truncatus* caught during the four trapping periods did not differ significantly (Latin square anova, treatment effect, p=0.43;

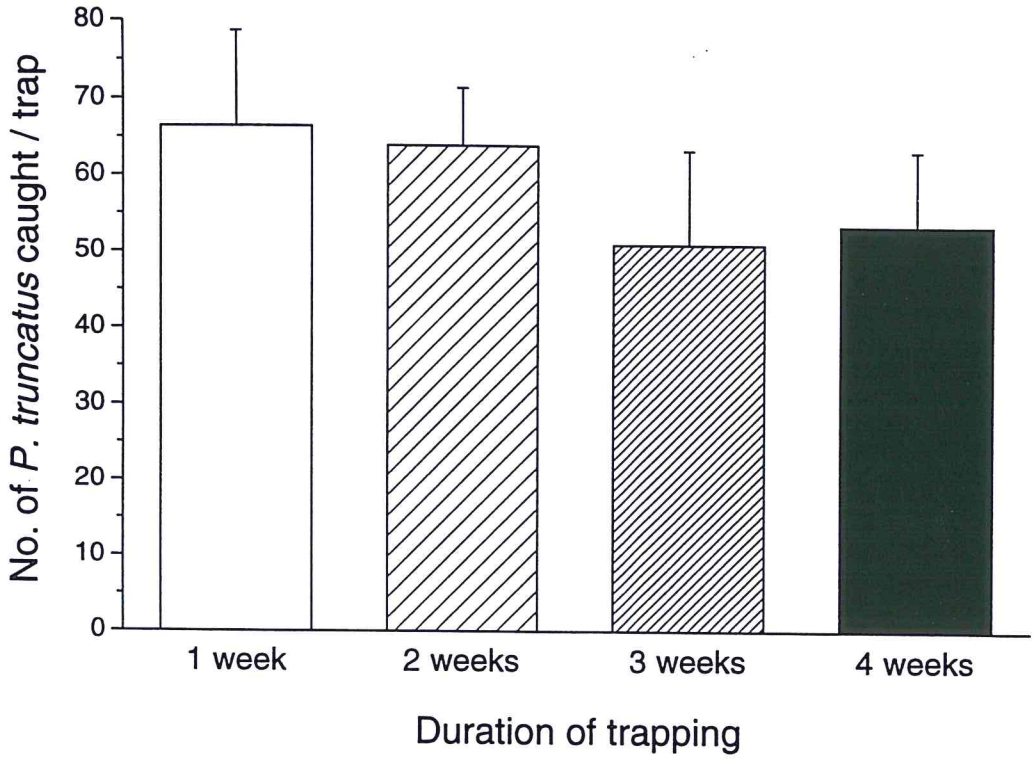


Fig. 5.18. Mean numbers of *P. truncatus* caught with traps baited with one maize cob infested with one male *P. truncatus*. The numbers of beetles caught during the one, two, three and four week trapping periods did not differ significantly (Latin square anova, p=0.67). Error bars represent 1 s.e.

sequence-order and position-in-sequence effects, p=0.99 and p=0.62). Of the 4693 beetles caught, 64.1% were females (sex ratio: 1:1.8). This sex ratio differed from a 1:1 ratio (Chi-square test, p<0.0001). The percentage of females caught with the two pheromone traps averaged 73.6% (significantly different to a 1:1 ratio; Chi-square test, p<0.0001), while the two baited-cob traps in their vicinity caught 67.4% females (n=561). These two proportions were significantly different (Chi-square test, p=0.0004).

The attracted females produced a mean of 6.9 (s.e.=1.5) progeny during the seven days trapping period. The female proportion of the F1 was 49.2%, the sex ratio therefore not differing significantly from 1:1 (Chi-square test, p=0.35).

As the catches did not differ significantly with the duration of the trapping period, it can be assumed that most *P. truncatus* were caught during the first week of exposure. Therefore, the weekly pheromone trap catches were compared with the

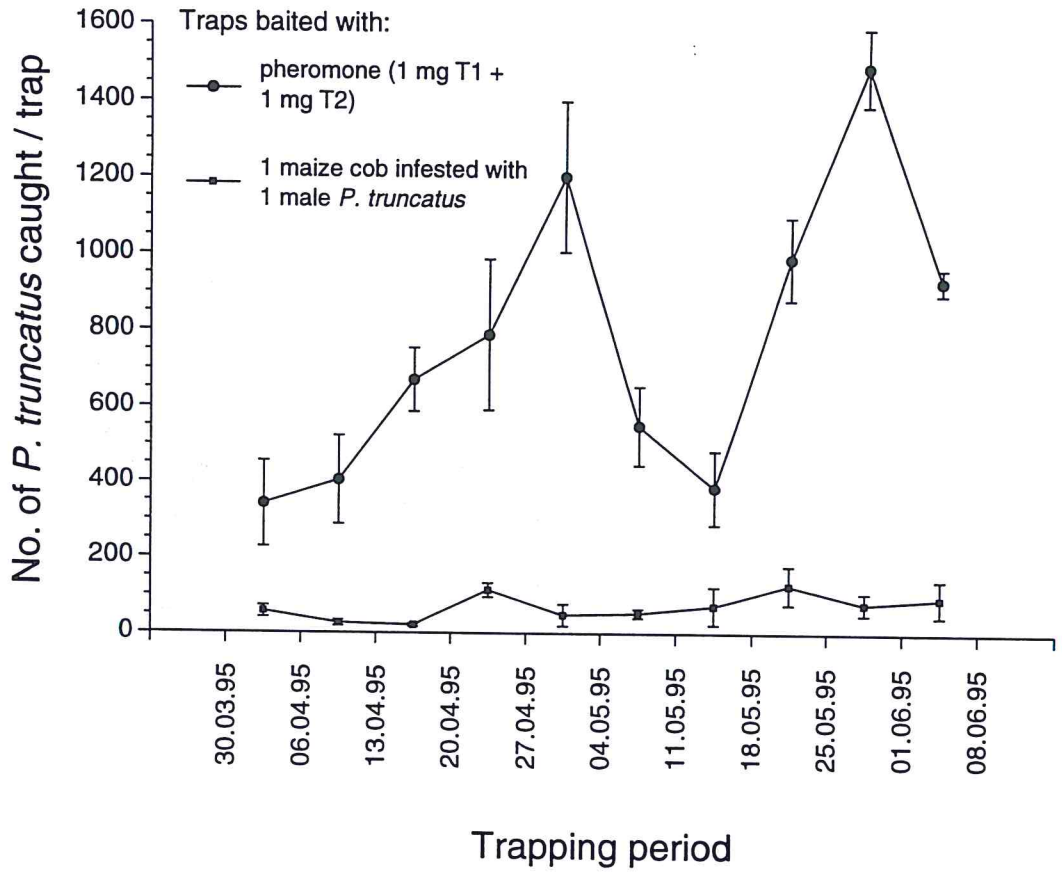


Fig. 5.19. Mean weekly catches of *P. truncatus* with pheromone traps and with traps baited with one maize cob infested with one male *P. truncatus*. Pheromone traps: means of three traps; cob-baited traps: means of eight traps on the first and the sixth sampling occasions, of two traps every other week; only traps which were distributed near the IITA station. Error bars represent 1 s.e.

numbers of *P. truncatus* caught with the baited cobs (only traps which were distributed near the IITA station) in such a way that the numbers of beetles caught with the infested cobs were all attributed to the first week of the trapping period (Fig. 5.19). This comparison showed that traps baited with synthetic pheromone caught on average 13 times more *P. truncatus* (range: 5 - 30 times) than the traps with baited cobs. Figure 5.20 demonstrates the variation in fluctuations of the numbers of *P. truncatus* caught in the individual pheromone traps.

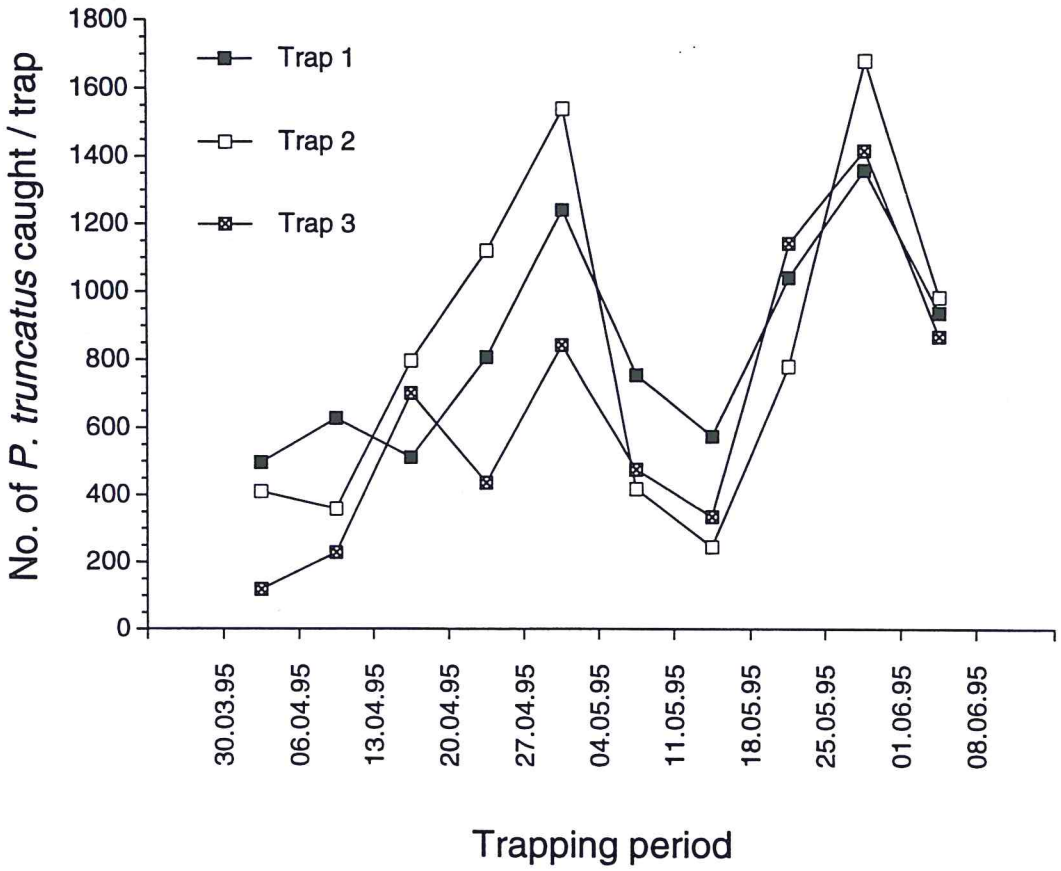


Fig. 5.20. Weekly catches of *P. truncatus* with traps baited with synthetic pheromone at the IITA station. Trapping locations were *ca.* 300 m apart.

5.6. Estimation of initial population size in a maize store

5.6.1. Materials and methods

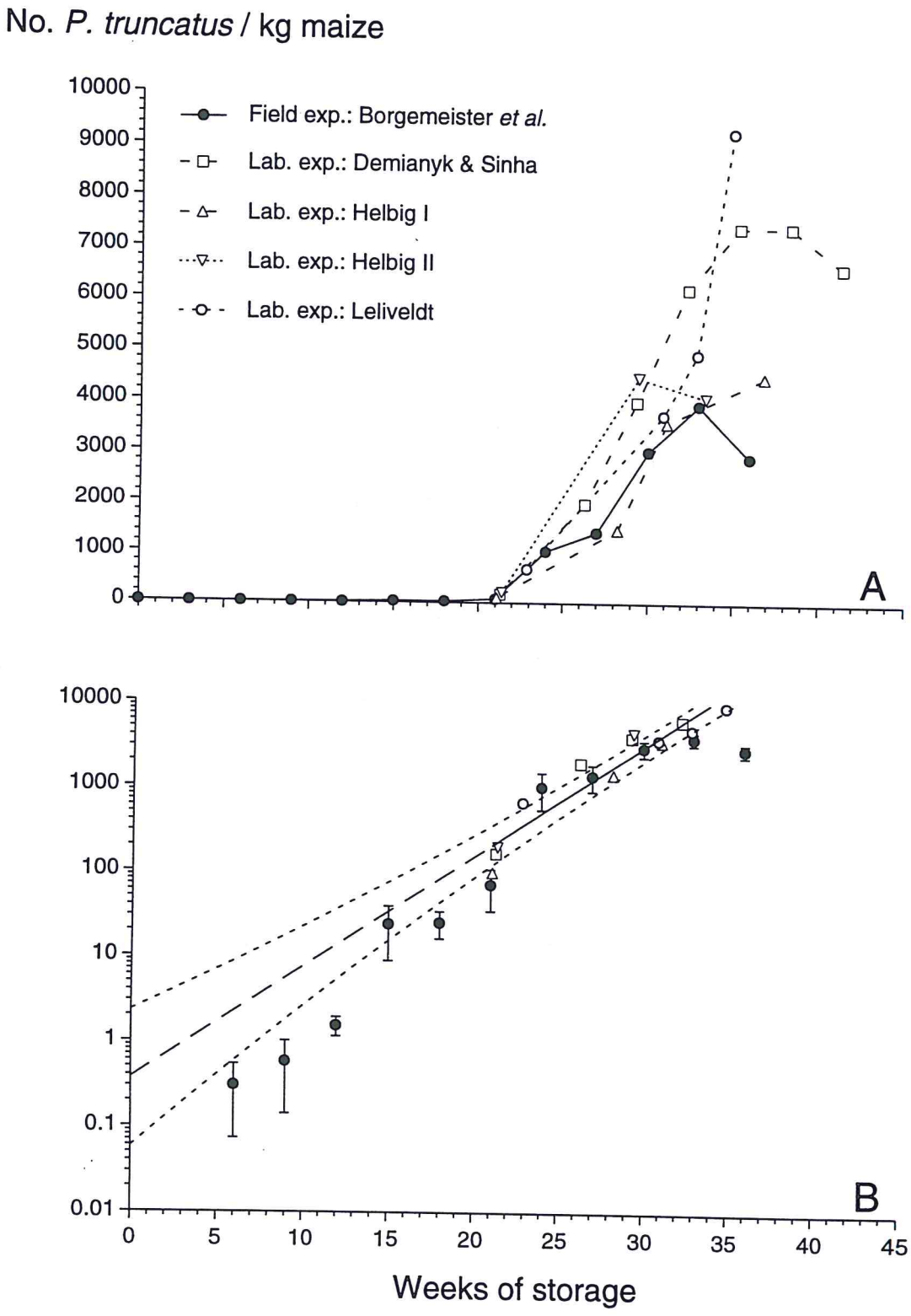
P. truncatus population growth curves taken from three studies (Demianyk & Sinha, 1987; Leliveldt, 1990; Helbig, 1993) conducted under laboratory conditions (hence no immigration or emigration possible) were compared to data from a field storage experiment (Borgemeister *et al.*, 1994) in order to indirectly examine the role of immigration in store colonization. The growth curve (mean numbers of live and dead *P. truncatus* adults/kg maize) which was used to describe the population development in a store was based on samples which had been taken at three weeks intervals from four maize stores [960 cobs per store, 160 g mean grain weight/per cob (W. G. Meikle, pers. comm.), *ca.* 150 kg maize per store]. The stores had not been fumigated or otherwise treated to prevent insect infestation and the presence of

P. truncatus had been observed from early on in the storage season (6th or 9th week of storage), so that infestation by *P. truncatus* at harvest or at the beginning of the storage period could be assumed. Laboratory growth curves were based on different initial densities on varying quantities of maize grains and were therefore recalculated to numbers of adult *P. truncatus* (dead and alive)/kg maize (initial densities: Demianyk & Sinha: 20 *P. truncatus*/120 g; Helbig I: 20 *P. truncatus*/200 g; Helbig II: 100 *P. truncatus*/500 g; Leliveldt: 100 *P. truncatus*/150 g). The growth curves were arranged in such a way that the initial densities of the laboratory curves lay on the corresponding data points of the storage curve. A straight line was fitted to the log-transformed laboratory data, which had been adjusted to the storage curve in the above-described way, by linear regression (Sokal & Rohlf, 1995). Maximum data points of the experiments Helbig I (one value), Helbig II (one value), Demianyk & Sinha (three values) were excluded from this calculation, as at these high densities the growth rates were declining or even becoming negative, whereas, for present purposes, I was interested in growth rates achieved at lower population densities. The fitted line was extrapolated to lower densities to investigate whether a simple exponential growth curve would suffice to explain population levels encountered in the field experiment.

5.6.2. Results

Figure 5.21 shows the population growth curves of the field and laboratory experiments. Visual examination revealed that the laboratory growth rates differed only slightly from one another and corresponded more or less with the slope of the storage curve at similar densities, though higher final densities were reached under laboratory conditions (Fig. 5.21A). The curve fitted to the laboratory data is described by the equation: No. *P. truncatus*/kg maize = 0.371×1.35^{x}, $r^2=0.91$, with x being the week of storage (Fig. 5.21B). By extrapolating the fitted curve to points

Fig. 5.21. (next page) *P. truncatus* population development in maize stores and laboratory cultures (A) and population development curve fitted to data from laboratory trials by linear regression of the log 'No. of *P. truncatus*' on weeks of storage (B). The laboratory growth curves were arranged in such a way that their initial densities lay on the corresponding data points of the storage curve. Data originated from Demianyk & Sinha (1987), Leliveldt (1990), Helbig (1993) and Borgemeister *et al.* (1994). (B) solid line: curve fitted to laboratory data; long-dashed line: extrapolated part of the fitted curve; short-dashed lines: 95% confidence limits of the mean.


No. P. truncatus / kg maize
Field exp.: Borgemeister et al.
Lab. exp.: Demianyk & Sinha
Lab. exp.: Helbig I
Lab. exp.: Helbig II
Lab. exp.: Leliveldt
A
B
Weeks of storage

earlier in the season, and assuming population growth rates similar to those observed in the laboratory trials, then at the beginning of the storage period the mean initial population density would have been 0.37 *P. truncatus*/kg maize, with the 95% confidence interval of the mean ranging from 0.07 to 2.11 *P. truncatus*/kg maize. If, as according to Borgemeister *et al.* (1994), there were 960 cobs per store with a mean cob weight of *ca.* 160 g, then the initial population size of the entire 150 kg store would have been 57 *P. truncatus* (95% confidence interval: 11 - 317), or approximately one *P. truncatus* per 17 cobs, assuming that the individual beetles were evenly distributed over all cobs in the store.

5.7. Discussion

Secondary attraction mediated by pheromones is a well known mechanism in the host finding behaviour of many insects, especially Coleoptera (Borden, 1985). Burkholder and Ma (1985) postulated that species producing sex pheromones for mating only may be short-lived, many requiring no feeding for reproduction, and may spread their eggs over many oviposition sites. The majority of these pheromones were found to be produced by females. On the other hand, the majority of long-lived adults (i.e., living longer than one month) released aggregation pheromones, which were generally male-produced. Levinson and Levinson (1995) suggested that aggregation pheromones in Coleoptera developed as a mechanism to cause aggregation at a suitable food source, and implied that the sexual function arose secondarily. However, except for certain bark beetle species which have to overcome a tree's resistance by rapid colonization (Birch, 1984), there appears to be no selective advantage for a male to attract other males, potential mating competitors (Walgenbach *et al.*, 1983). Walgenbach *et al.* (1983) postulated, 'that the males developed the ability to produce sex pheromone first. The ability of a male to perceive the pheromone may have evolved as a secondary sexual strategy. A male could benefit by mating with females already drawn to an area by other males.' This strategy has also been suggested for male *P. truncatus* (Hodges, 1994), as the males reduce their pheromone production greatly in the presence of females (Smith *et al.*, 1996). Under these conditions the pheromone producing sex, in our cases the males, may possibly react less sensitively to the pheromone, as observed by Chambers *et al.* (1990) for the rusty and the flat grain beetle [*Cryptolestes ferrugineus* (Stephens) and *C. pusillus* (Schönherr) (Col.: Cucujidae)].

Responses to the pheromone

The higher EAG responses of *P. truncatus* and *T. nigrescens* to T2 compared to the same quantity of T1 were not (or only to a small extent) caused by the higher evaporation of T1 during the 25 sec between solution application on the filter paper and insertion of the filter paper into the Pasteur pipette. Consequently, it can be assumed that the relatively higher responses to T2 were not due to a decreased EAG response to T1. However, I did not evaluate whether the higher volatility of T1 caused an relative increase of the EAG responses to T1. The EAG results are therefore discussed without corrections for volatility differences.

A sex ratio of *ca.* 1:1 was observed for *P. truncatus* in maize laboratory cultures (Shires, 1979; cf. also Chapters 3.2 and 3.3), in maize stores (cf. Chapter 3.4) and for the progeny produced by the *P. truncatus* caught with the baited cobs. However, the traps with baited cobs trapped more females than males (64% females, sex ratio 1:1.8). These figures and the observation that males reacted less strongly to the pheromone in the olfactometer experiments would support the above-described hypothesis that *P. truncatus* males may be less sensitive to the pheromone and were, consequently, caught in fewer numbers than females. However, threshold concentrations for eliciting EAG responses of male and female *P. truncatus* were equal for T2, and the males were only slightly less sensitive to T1 than the females. In addition, there were hardly any differences between the sex ratios of *P. truncatus* attracted to the two separate pheromone components in the field. The female biased sex ratio among trap catches was therefore probably not caused by a lower male pheromone sensitivity, but was rather due to females making up the majority of the dispersing part of the population (cf. Chapter 3.6: Sex ratios). This interpretation is also supported by data from the dispersal experiments, where 63-70% of the *P. truncatus* flying off maize cultures or cobs were females (cf. Chapters 3.2 and 3.3). Traps baited with the synthetic pheromone caught an even higher proportion of females than baited cobs (74%; cf. also Chapter 3.4: annual average: 72%, sex ratio 1:2.5). Although both sexes may be equally attracted from a distance, flying males approaching a strong pheromone source may reach a threshold concentration eliciting landing further away from the release point than females (Schlyter *et al.*, 1987a; 1987b). The sex ratios among beetles attracted to baits with low pheromone release rates, i.e., in our case the cobs baited with one male, may represent the sex ratio of the dispersing population (Schlyter *et al.*, 1987a), while sex ratios in pheromone traps may be more female biased due to the source avoidance by males. This explanation may also be supported by the observation that the mixture of T1

and T2 (twice the quantity of the single substances) in the second trapping experiment (cf. Table 5.2) caught a relatively lower proportion of males. The less pronounced reaction of the males, compared to the females, in the olfactometer experiments may be related to this phenomenon: Males may be as sensitive to the pheromone as the females but less focussed on the immediate pheromone source (Borden, 1985), searching a greater area, thus also the vicinity of the pheromone odour field. Correspondingly, the behaviour of walking bark beetle males has been described by Wood and Bushing (1963) as 'area orientation' compared to a 'point orientation' of the females.

In the olfactometer experiments, male and female *P. truncatus* responded strongly to the synthetic pheromone, no matter if the test beetles had been taken directly out of maize cultures or starved for 16-24 hours. Also flyers from the dispersal experiment and beetles which had been kept in an environment saturated with pheromone reacted with similar response intensities in the olfactometer. Only responses of females which were habituated to the pheromone without food, a situation not found under natural conditions, responded slightly less vigorously. In contrast to other insect species, which have been found to respond to pheromone only after some time of starvation (Borden *et al.*, 1986) or flight (Kennedy, 1986), male and female *P. truncatus* reacted to the pheromone under all situations tested. It can therefore be assumed that contact stimuli from the maize, rather than the physiological state of feeding beetles or habituation to the pheromone, prevented *P. truncatus* on maize from responding to nearby pheromone traps (Pike, 1993).

Both pheromone components were highly attractive when tested separately in one-choice experiments in the olfactometer, but T2 was greatly preferred when both components were offered simultaneously. In the field trial, the pheromone component T2 and the mixture of T1 and T2 caught many more *P. truncatus* than T1 alone. A preference for T2 over T1 at short range has also been observed in wind tunnel experiments (Fadamiro, 1995) and in field trials with the traps positioned close to each other, i.e., with presumably overlapping pheromone plumes (Leos-Martínez *et al.*, 1995). However, the data from my field trial suggest that, even without interference from the other pheromone component, T2 has a greater attraction capacity than T1. This may partly be due to the lower perception thresholds for T2, i.e., T2 can be perceived at a greater distance from the source than T1. On the other hand, it is doubtful whether the differences in attraction capacity can solely be attributed to the relatively small difference between perception thresholds. Separate pheromone components or specific concentrations

of the same component may mediate specific steps in the behavioural sequence of the resource location behaviour of an insect, for instance, long range attraction, arrestment or landing (Prokopy, 1986; Schlyter *et al.*, 1987b). It is suggested that both pheromone components of *P. truncatus* act as long range attractants, with T2 possessing a slightly greater attraction range, and at the same time playing a more important role in close range orientation, which may be facilitated by the steeper dose-response gradient of T2 (Dickens, 1981).

The number of *P. truncatus* caught with the cobs baited with one male was much lower than catches with the pheromone traps (on average 13 times lower). The amount of artificial pheromone evaporating from the vials must therefore be greater than the quantity released by a single male. Comparing pheromone release rates from pheromone dispensers (1 mg T1 and 1 mg T2), estimated from data reported by Dendy *et al.* (1991), with pheromone production rates, as stated in Smith *et al.* (1996), suggests that dispenser release rates for T1 were *ca.* 64 times higher, and for T2 *ca.* 333 times higher, than the quantities emitted by a single male *P. truncatus*. A similar level of attraction was observed between naturally produced pheromone and pheromone dispensers when 100 *P. truncatus* males were used as the natural pheromone source (Wekesa, 1994; Giles *et al.*, 1995), an observation, which corresponds well with my estimates. For *P. truncatus,* the maximum sampling range (with traps baited with synthetic pheromone; 1 mg T1 and 1 mg T2) varied from 300 m (Novillo Rameix, 1991) to 340 m (Farrell 1990; Farrell & Key, 1992). As the sampling range describes the maximum distance over which animals are attracted to an attractive source within a given time period, it consists of the distance of actual attraction (attraction range) plus the distance the animal covers in random search before entering the attraction range (Schlyter, 1992). It can therefore be assumed that the attraction range of the artificial pheromone lures is less than 300 m. Farrell (1990) observed directional flight of *P. truncatus* towards an artificial pheromone source (1 mg T1 and 1 mg T2) from up to 20 m. The sampling range and attraction range of the pheromone quantity emitted by a single male should be proportionately several times smaller.

There were no significant differences between the EAG responses of male and female *T. nigrescens* elicited by the two pheromone components or between the perception thresholds, neither did the sex ratios among trap catches with the separate components or their mixture differ significantly. The sex ratio of *T. nigrescens* cultures has been found to be *ca.* 1:0.9 (48% females; Pöschko, 1993), and the percentage of females caught with pheromone traps was only slightly lower.

These results suggest that both sexes of *T. nigrescens* show a similar tendency to disperse. *T. nigrescens* was more sensitive to T2 than to T1 (thresholds: T1=0.01 µg; T2=0.001 µg), however, the actual responses to 0.001 µg of T1 and T2 differed only little. It is therefore not surprising that, in contrast to the pheromone trap catches of *P. truncatus*, there were no significant differences in the mean numbers of *T. nigrescens* caught with T1, T2 and the mixture of the components in the field. Hence, the behavioural differences, as postulated for *P. truncatus*, seemed not to be exhibited by *T. nigrescens*: Both sexes approach the source of the pheromone directly (cf. Key *et al.*, 1994), and the predator's behaviour does not differ in regard to the pheromone components.

Any comparisons between *P. truncatus* and *T. nigrescens* on the basis of the EAG data must be made on the assumption that the different morphology of their antennae did not influence the results. The depolarizations in the antenna, i.e., the EAG responses in mV, were much higher for *T. nigrescens* than for *P. truncatus*. The magnitude of antennal depolarizations elicited by a given dose of a compound may vary between species or sexes of a species but such differences may not reflect differences in sensitivity of the insects to the compounds (Guerin & Visser, 1980). However, the relative sensitivity of insects to a compound can be indirectly measured by comparing the perception thresholds. Perception thresholds were equal for both species in regard to the pheromone components except that male *T. nigrescens* responded already to a 10 times lower concentration of T1 than male *P. truncatus*. The relative dose-response curves of *P. truncatus* and *T. nigrescens* were very similar, with the only evident difference being the threshold discrepancy regarding T1. This means that the predator is as sensitive as or even more sensitive (with regard to the males' responses to T1) than the prey to the prey-produced pheromone. Similar results were obtained for *Thanasimus formicarius* (L.), a predaceous clerid, which uses the aggregation pheromone of bark beetles to locate its prey (Hansen, 1983). It may therefore be assumed that the attraction range of a given pheromone source is of similar size for *P. truncatus* and *T. nigrescens*.

Pheromone production

Pheromone production of *P. truncatus* was investigated in olfactometer experiments and the attraction capacity of single males evaluated in a field trial. Neither mated nor unmated females were attractive for conspecifics, although the females had been separated from males for considerable time. It was therefore verified that only males

possess the ability to produce aggregation pheromone. While in many species aggregation pheromone is produced by one sex only (e.g., Walgenbach *et al.*, 1983; Birch 1984; Chang *et al.*, 1989), there are also examples where both sexes release aggregation pheromone (Pierce *et al.*, 1981) or where males produce aggregation pheromone, while the female conspecifics produce sex pheromone (Borden, 1985).

Male *P. truncatus* on maize, single individuals or in groups, were highly attractive to male and female conspecifics in the olfactometer experiments, suggesting that the males produced pheromone when on a suitable substrate (Smith *et al.*, 1996). Responses were low to males separated from females for only one day, but increased when the males were kept without females for five or 12 days, indicating that pheromone production rose with extended time of separation. These results, and the observation that only low density cultures were attractive, are consistent with the findings of Smith *et al.* (1996) who reported that pheromone production of male *P. truncatus* is greatly reduced by a non-volatile substance produced by female conspecifics. The effect of this mechanism on resource colonization was investigated under field conditions, simultaneously with the determination of the attraction capacity of a single male *P. truncatus* on a suitable food source. The mean number of *P. truncatus* caught during the one, two, three, and four week trapping periods did not differ. Hence, most *P. truncatus* were attracted during the first week of exposure as pheromone production declined due to the effect above described. However, this is a reversible process: The males used as odour sources in the olfactometer experiments were taken from mixed cultures and resumed pheromone production when kept separately from females. The high variability of trap catches with baited cobs may be caused by several factors. Long term monitoring with artificial pheromone showed overall similar flight activity tendencies within a certain geographic area, however, single trap catches may vary strongly from week to week and between locations (Borgemeister *et al.*, 1997). The numbers of beetles caught varied greatly even between neighbouring traps, baited with the same quantities of artificial pheromone (Fig. 5.20). In addition, it can be assumed that the amount of pheromone produced by individual males varied as well (Smith *et al.*, 1996), as has also been shown for *Carpophilus obsoletus* Erichson (Col.: Nitulidae) (Petroski *et al.*, 1994) and for bark beetles (Borden *et al.*, 1986).

The attractiveness of a single male in the olfactometer experiments was lower than the attractiveness of 20 males, suggesting that the total amount of pheromone produced by a group was higher than the pheromone production by a single male, although individual production may be reduced in groups, as has been reported for

C. obsoletus (Petroski *et al.*, 1994). In contrast to the postulation of Walgenbach *et al.* (1983) that attracting male conspecifics is disadvantageous (cf. first paragraph of this discussion), this observation suggests that attracting other males may have an advantage for the pheromone-producing male. After a single male has located and infested a suitable new food source, it starts to produce pheromone. In an area or during a season with low flight activity, the chances of conspecifics entering the attraction range of the pheromone may be low. When, however, one or several males enter the attraction range and locate the food with the pheromone producing male, they also start to produce pheromone and extend thereby the attraction range and the probability of attracting more conspecifics, including females. Wekesa (1994) failed to catch any *P. truncatus* with cobs baited with single males, while cobs baited with groups of males attracted male and female conspecifics.

When males were kept on polystyrene, a substrate suitable for boring but without any nutritive value, instead of on maize, they were not attractive (except slightly for females on the first day after infestation). This observation verifies that males do not produce aggregation pheromone in absence of suitable food (cf. Chapter 5.1: Smith *et al.*, 1996). Males on certain wood species (*C. africana* and *C. riparia*), were highly attractive, apparently as attractive as males on maize, indicating that these woody substrates encouraged pheromone production. However, males on *B. aegyptiaca* were not more attractive than the wood without beetles, suggesting that the males did not produce aggregation pheromone. *P. truncatus* was reported to be able to reproduce on the former wood species (Nang'ayo *et al.*, 1993; F. Nang'ayo, pers. comm.), even though the progeny were much fewer than on maize, while *B. aegyptiaca* did not support reproduction (Nang'ayo *et al.*, 1993). Many carbohydrates, especially sucrose, and amino acids have been found to be phagostimulants for insects (e.g., Thorsteinson, 1960; Hsiao & Fraenkel, 1968; Hsiao, 1985) and rising contents of these stimulants (until optimum concentrations) promoted feeding (Thorsteinson, 1960; Hsiao, 1985). Food acceptance may be assumed to be the behavioural step preceding pheromone production; the nutrient contents in a substrate may therefore also determine pheromone production. However, the nutritive qualities of the wood samples, measured as nitrogen and sucrose contents, did not differ in such a way that they could explain the support or inhibition of pheromone production. Sucrose content was highest in *B. aegyptiaca*, but did not exceed concentrations found in maize [sucrose content in maize grains: *ca.* 2% (King, 1992)] and thus did not exceed the sucrose concentration in a substrate which promotes pheromone production. Nitrogen contents of *B. aegyptiaca* and *C. africana* hardly differed; nitrogen contents could therefore not

have determined pheromone production. For some insects, specific amino acids or secondary plant substances had to be present in the food to promote feeding (Hsiao, 1985); possibly such substances also elicit pheromone production by *P. truncatus* males. In addition, Dethier (1982) postulated that food acceptance is dependent on the ratio of phagostimulants and feeding deterrents present in a substrate. All tested wood samples may contain suitable stimulants in sufficient concentrations to induce feeding and pheromone production, but *B. aegyptiaca* may possess more or higher amounts of inhibiting substances: Saponins extracted from the bark of *Balanites roxburghii* Planch, a closely related species to *B. aegyptiaca*, have been reported to exhibit antifeedant activity against caterpillars of *Diacresia (Spilosoma) obliqua* Walker (Lep.: Arctiidae) (Jain, 1987; Jain & Tripathi, 1991).

Store colonization

Having determined the attraction capacity of a single male *P. truncatus* on a maize cob and having observed that at least during the first weeks of colonization hardly any additional conspecifics had been attracted, I wondered how many *P. truncatus* had to be present at the beginning of the storage season to generate population growth curves as observed in field experiments (e.g., Henckes, 1992; Helbig, 1993; Borgemeister *et al.*, 1994). Thereby it could be evaluated whether further beetle immigration was an important factor in explaining population dynamics later in the season. Reports on the population development in stores which had been artificially infested (Tigar *et al.*, 1994a), or where low rates of field infestation had been observed at the time of harvest (Henckes, 1992), showed that small initial populations sufficed to induce high numbers of *P. truncatus* later in the storage period. The curve-fitting model appears to fit field data well later in the storage season, and reasonably well earlier in the season (extrapolated part of the model). This suggests that maximum storage densities could have resulted (although not necessarily) from a very low population density at the beginning of the storage season, such as might be initiated by individual males, and that further immigration was not a necessary assumption. Such low initial population densities, at *ca.* one insect per 17 cobs, would have been below the resolution of, and therefore undetected by, many sampling plans. In a store containing 1000 cobs, 10% of the capacity of the store, i.e., 100 cobs, would have needed to be sampled for a reasonable chance of finding five beetles, assuming that the beetles were randomly distributed. However, *P. truncatus* distribution among cobs in stores tends to be highly aggregated during the first months after infestation (W. G. Meikle, pers.

comm.), which would reduce the expected number of infested cobs. It is therefore not surprising that such low densities at the beginning of the storage season would go undetected.

It should be emphasized that my approach uses a simple exponential model of population growth, parameterized by laboratory data, to help evaluate assumptions of initial and continuous immigration in maize stores. The model is not intended to fit data from other circumstances. Rather, these results should be considered as arguments to support the hypothesis that few *P. truncatus* are needed at the beginning of the season to generate high, damaging populations later in the season.

In the experiment with baited cobs, I observed the colonization of a new food source during the first four weeks after infestation. Additional conspecifics may arrive at a maize store later on in the storage season either by chance (cf. Chapter 4), as the first colonizers, or possibly attracted by pheromone production of progeny males that spread within the store. However, these numbers would be negligibly small, compared to the size of the already existing population, and would therefore have little influence on the overall population development in the store. Also the actual number of *P. truncatus* attracted to the stores at the beginning of the storage season does not seem to be important, as long as a small population is able to establish itself. On this basis, it may be concluded that it is rather the factor 'if and when' a store is infested, which determines the damage level caused by *P. truncatus* at the end of the storage season. The damage level at the end of the storage period can therefore be reduced by preventing early infestation, but not by inhibiting further immigration.

6. General Discussion

The main aims of this study were to investigate the principles underlying dispersal and host finding of *P. truncatus*. A holistic approach considering the insect's biology not only during the resident reproductive stage, which has been thoroughly investigated in regard to stored commodities (cf. Markham *et al.*, 1991; Henning-Helbig, 1994), but also during migration was thought to contribute to a better understanding of the entire life history of *P. truncatus*. Such an approach could provide the basic knowledge which is needed for the development of successful IPM strategies. In addition, the insights obtained helped to interpret previous experimental results of other scientists, and part of the data collected in this study were directly and indirectly incorporated in a population model being developed for *P. truncatus* (Meikle *et al.*, 1997b). The subjects investigated (dispersal, primary and secondary attraction) have been discussed separately in the relevant chapters. In this general discussion, I give an overall picture of the beetle's life history during the dispersal phase.

The general concept of dispersal and host finding behaviour (cf. Fig. 1.2) can now be specified (Fig. 6.1). Dispersal from stored commodities is mainly dependent on population density and accompanying food degradation. Similar effects can be assumed to act in the beetle's natural habitat, although due to the presumably inferior nutritional value of these natural, woody host plants, migration may already occur at much lower population densities and possible also at higher rates. Weather factors, as suggested by Tigar *et al.* (1994b), Giles *et al.* (1995) and Borgemeister *et al.* (1997), may affect flight activity only partly or indirectly: a) higher mean temperatures increase the beetle's general activity and thereby induce dispersal from low density populations, but crowding overrides this effect at high densities, b) changes temperature and rainfall may coincide with population increase and habitat availability in particular situations, and c) habitat suitability may be reduced by humid conditions [e.g., due to mold grow in moist substrates which increases larval mortality (Bell & Watters, 1982)] and dispersal thereby induced. Beetles have been observed to react to the immediate conditions at the breeding site, migration taking place at varying physiological ages. However, a change in the physiological state of the insect takes place before dispersal starts. Variations in flight activity, which have been related to the abundance of *P. truncatus* in an area (Tigar *et al.*, 1994b), cannot simply be interpreted by considering the suitability of conditions for reproduction (such as preferred temperature and relative humidity), but must take into account local specifities in storage practices and changes in the availability of

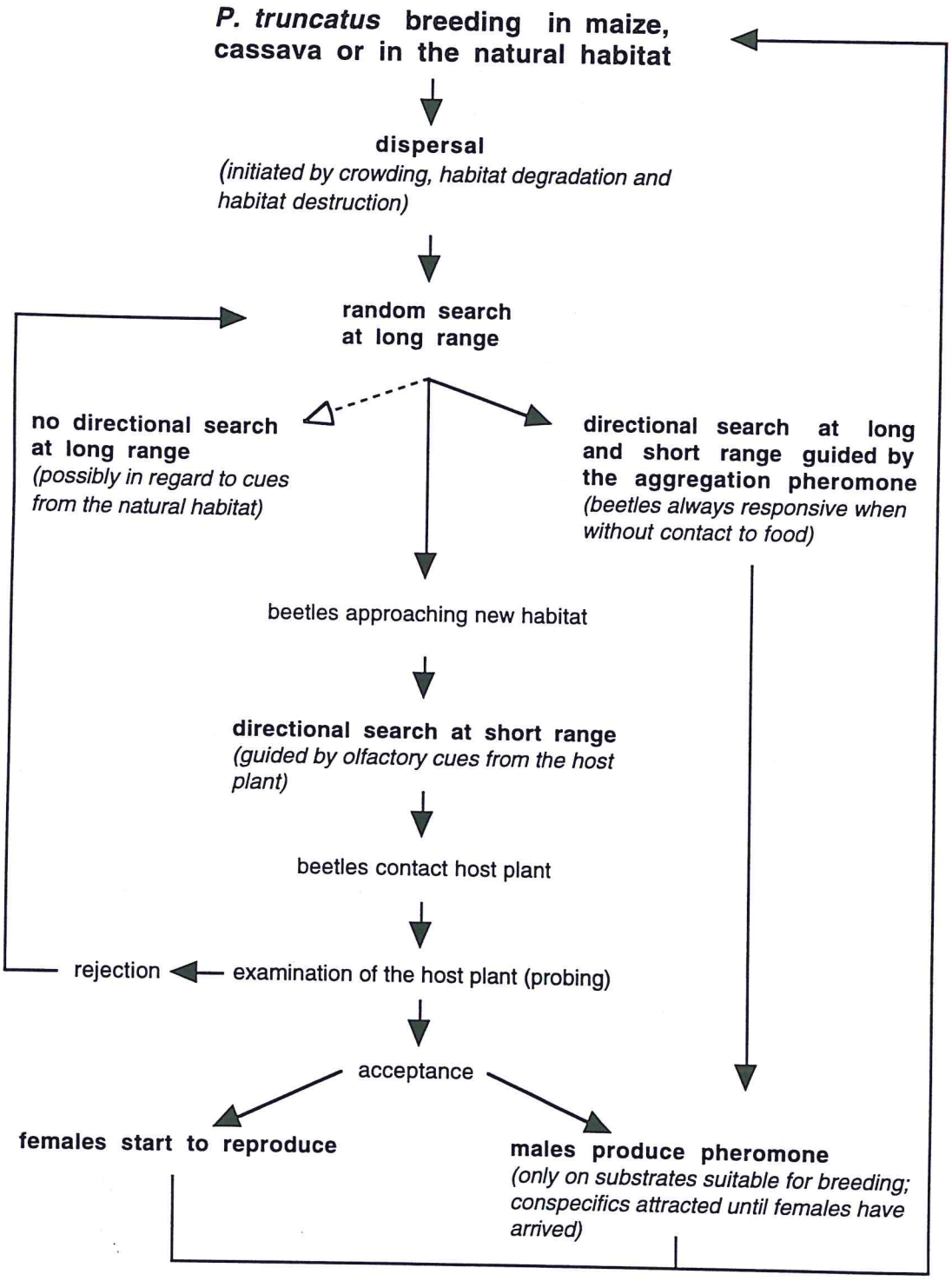


Fig. 6.1. Specified scheme of dispersal and host finding of *P. truncatus*.

alternative host plants. Observed low flight activity may reflect a momentary low abundance of the beetles in an area, but may also result from an increased number of suitable food sources present.

Olfactory primary attraction to stored commodities and some woody plants was only observed at short range; *P. truncatus* was found to be arrested by volatiles of potential hosts in the immediate vicinity of the odor sources. Correspondingly, Pike *et al.* (1994) observed positive responses to maize in petri dish experiments, while Fadamiro (1995) could not verify the attractiveness of maize in wind tunnel trials. It can therefore be concluded that beetles approach maize cobs, maize stores and dried cassava by random flight or guided by the male-produced pheromone. The selection of plant varieties which do not release attractive plant volatiles (Finch, 1980; Renwick, 1992) can therefore not be adopted as part of an IPM strategy. However, since the natural host-plant-complex has not yet been precisely determined, it is possible that primary attraction exists in regard to the habitats to which the beetle is adapted.

Evidently, secondary attraction plays an important role in resource colonization. Beetles of both sexes always reacted strongly to the pheromone. The non-response of beetles in stores to nearby pheromone sources (Pike, 1993) was not based on the physiological state or reduced sensitivity of the insects, but on the contact to a suitable food source. In contrast to other insect species (e.g., Borden, 1985; Kennedy, 1986), *P. truncatus* does not have to fly or be in a specific responsive physiological state before it becomes sensitive to this stimulus. Male *P. truncatus* produce pheromone only when on a suitable food source, including woody plants. Pheromone production may be dependent on the presence of sufficient amounts of adequate stimulants, like nutrients, as well as on the absence of inhibiting secondary plant substances.

A single male *P. truncatus* was found to be able to attract conspecifics and thereby found initial populations. Low field infestation of maize cobs are difficult to detect (Borgemeister *et al.*, 1994), and one or several maize cobs infested by males may provide the source for high population densities and severe damage of maize stores at the end of the storage season. Identifying and applying the female-produced substance which reduces the pheromone production by male *P. truncatus* and thereby inhibiting secondary attraction and store colonization, was considered as a possible method to control the pest (Smith *et al.*, 1996). However, even if the chemical structure of this substance could be identified and the substance produced

cheaply enough to be used on a broad scale, its application would not achieve the intended results. Firstly, the chemical could not be used to prevent secondary attraction in the field, unless it could be sprayed on the growing maize, which would require large quantities. Secondly, the substance could only be applied on the surface of the cobs and would therefore probably not inhibit pheromone production of the males inside the cobs. Thirdly, although the attractiveness of maize cobs infested with single males diminished with time in the field experiment, mixed population at moderate densities were found to be slightly attractive for conspecifics. The latter, laboratory, observation may be supported by results from a field trial (C. Borgemeister, pers. comm.): Faster store colonization, higher population densities and consequently increased damage levels were not only observed in maize stores which had been artificially infested at the beginning of the storage season, but also in stores which had initially been baited with infested cobs inside insect-proof gauze envelopes. Thus, in the latter treatment, the artificially-introduced beetles could not contribute to the population development within the store, but simply attracted conspecifics.

Female *P. truncatus* are not able to attract male *P. truncatus* via pheromone production. However, since females disperse mated, they are able to colonize suitable breeding sites independently of males. Such female-initiated colonies may develop undetected by the predator *T. nigrescens*, at least until progeny males emerge and pheromone is produced. Male *P. truncatus* which have located a suitable food source produce pheromone and attract thereby, besides other males, females as potential mating partners, as well as assuring an adequate food supply for their offspring. The response of the females to the pheromone increases their chance of detecting an appropriate breeding site and enables them to increase the fitness of their offspring by mating with several non-kin males.

The insights provided by this study of dispersal and host finding, combined with previous research on the behaviour of the beetle, go a long way to completing our understanding of the dynamics and biology of *P. truncatus* as a pest of stored products. To round off our knowledge of this species, however, further investigations of the beetle's biology in its natural, woody habitat are essential. Such studies should focus on identifying the natural host-plant-complex of *P. truncatus* and elucidating factors which determine host finding and host acceptance within this context.

7. Glossary

Words in italics (except *P. truncatus* and *et al.*) refer to the corresponding terms in the list.

Abdomen: rear group of segments (tagmata) of an insect's body. Other tagmata are head and thorax (Evans, 1994).

Aedeagus: copulatory organ of a male insect; as an ectodermal derivative usually at least partly lined with sclerotized cuticle (Davey, 1985b).

Arrestment: non-directional movement (*kinesis*) which increases the chance of encountering the source of the arrestant (Kennedy, 1965).

Attraction: directional movement (*taxis*) towards the source of the attractant (Kennedy, 1978). In cases where the type of movements are not specified also as a general term expressing attractiveness. **Primary attraction**: attraction to host or host-associated stimuli, which do not originate from and are not evoked by members of the same species, e.g., of insects to host plant volatiles (Tunset *et al.*, 1993). **Secondary attraction:** attraction to a host caused by signals emitted from or evoked by members of the same species, e.g., to *pheromones* (Tunset *et al.*, 1993). → *host finding*

Attraction range: maximum distance over which animals can be shown to direct their movement to the attractive source; area in which the stimuli concentration is high enough to elicit a directional response (Schlyter, 1992).

Biological control: the use of living organisms as pest control agents. **Classical biological control:** selection of natural enemies in the pest's area of origin and their introduction to a new outbreak area of the pest for establishment and long-term control (Markham *et al.*, 1992a). → *integrated pest management*

Clypeus: a sclerite (cuticular plate) on the front of the head of an insect, above the labrum (flaplike structure anterior to the mouthparts) (Evans, 1994). **Clypeal tubercles:** small tubercles on the clypeus of *P. truncatus*, used for external sex determination (Shires & McCarthy, 1976).

Corpus allatum: endocrine gland which secrets juvenile hormone; situated just behind the insect's brain. The corpora allata may occur paired, or fused to a median structure, the corpus allatum (Lawrence, 1995).

Crepuscular: pertaining to organisms which are active in twilight hours (Hale *et al.*, 1995).

Deterrent: a substance which inhibits a specific behaviour, e.g., feeding or oviposition (Nordlund, 1981). → *repellent, stimulant*

Dispersal: in this thesis used synonymously with migration in the sense of adaptive dispersal; describing a specialized behaviour for the displacement of an individual in space (Dingle, 1985), an adaptive change of the breeding habitat (Johnson, 1969).

Ejaculatory duct: usually a single sperm duct connecting the *vasa deferentia* with the lumen of the *aedeagus* (Davey, 1985b).

Electroantennogram (EAG): recordings of the summed antennal receptor potentials. The amplitudes increase with increasing concentrations of active chemical stimuli until saturation level is reached (Roelofs, 1984).

F1: first filial generation (Lawrence, 1995).

Flight activity: number of insects in the air; dependent on the number and sizes of populations in an area and proportion of them in flight; the latter depends on rate of take-offs (*flight initiation*) and the time the individuals spend flying (Johnson, 1969).

Flight initiation: start of flight; number of insects taking off (Dowdy, 1994). → *flight activity*

Follicle: an *oocyte* with its surrounding follicular epithelium (Chapman, 1982). Also used for the group of cells surrounding the *oocyte* (follicular epithelium) and probably concerned with its nutrition. In general terms, a small sac-like structure (Lawrence, 1995).

Germarium: → *ovariole*

Habituation: the progressive loss of a behavioural response as a result of continued stimulation (Hale *et al.*, 1995).

Hormone: a substance produced by tissue or endocrine glands which controls physiological processes within an organism (Nordlund, 1981). **Juvenile hormone:** secreted by the *corpus allatum*; maintains juvenile features in immature insects and controls certain aspects of adult physiology and behaviour (Evans, 1994). → *pheromone*

Host acceptance: second step(s) of the *host selection* process when the insect contacts the host; may be induced by visual, tactile, olfactory, and/or gustatory stimuli (Dethier, 1982). → *host finding, host selection*

Host finding: random and orientated (directional) searching for a host (Miller & Strickler, 1984); first step(s) of the *host selection* process (Thorsteinson, 1960). Directional search may be mediated by visual and/or olfactory cues from the host or host-associated organisms. → *attraction, attraction range, host acceptance, host selection*

Host selection: behaviour with leads to the location and acceptance of a host (Thorsteinson, 1960). → *host finding, host acceptance*

Integrated pest management (IPM): integration of available techniques to reduce pest populations and maintain them below the levels causing economic injury (economic thresholds) in a way which avoids harmful side effects, i.e., minimizing the use of pesticides (Markham *et al.*, 1992a). → *biological control*

Kairomone: a substance which evokes a behaviour or physiological reaction in an organism of a species different from the producer (i.e., an allelochemic). The response elicited in the specific interaction benefits rather the receiver than the emitter, e.g., the attraction of an enemy to its prey by prey-specific volatiles (Whittaker & Feeny, 1971). → *pheromone*

Kinesis: non-directional movement induced by a source of stimulation. **Orthokinesis:** change in the speed of movement, e.g., slowing down, stopping or speeding up. **Klinokinesis:** change in the rate or frequency of turning (Kennedy, 1977b). → *taxis*

Migration: → *dispersal*

Monitoring: determining the occurrence and spread of insects (Markham *et al.*, 1992b).

Nulliparous: describes females which have not laid any eggs (Phoofolo *et al.*, 1995). → *parous*

Ontogenetic: determined by or dependent on **ontogeny**, i.e., the development of an individual organism from fertilization of the egg to adulthood (Allaby, 1996)

Oocyte: female germ cell in which meiosis occurs to form the egg (Lawrence, 1995), i.e., an immature egg in the *ovary* before fertilization. Oocytes are produced from oogonia (precursors of oocytes) in the germarium of each *ovariole* and grow in the vitellarium (Chapman, 1982).

Oosorption: egg resorption in the ovary (Bell & Blom, 1975).

Ovary: female organ where germ cells are produced (i.e., female gonad) (Lawrence, 1995). In insects, usually a pair of ovaries, each being composed of a set of *ovarioles,* in which the *oocytes* develop (Davey, 1985a).

Ovariole: one of the egg-tubes, making up the *ovary*. Each consisting of a distal germarium, in which *oocytes* are produced from oogonia, and a proximal vitellarium, in which the oocytes grow and get enriched with yolk (Chapman, 1982).

Oviduct: one of the ducts connecting the *ovary* with the vagina; usually a pair of lateral oviducts (one from each ovary) which join to a single median oviduct (Davey, 1985a).

Ovulation: passage of an *oocyte* from the *ovary* to the *oviduct* (Chapman, 1982).

Oviposition: passage of an egg from the external genital opening to the outside of the female (Davey, 1985a).

Parameres: a pair of appendices, exterior to the *aedeagus* (Davey, 1985b).

Parous: describes females which have oviposited at least once (Phoofolo *et al.*, 1995).

Pedicel: short connective duct joining an *ovariole* to the lateral *oviduct* (Chapman, 1982). In general terms, foot-stalk of a fixed organ or organism (Lawrence, 1995).

Pheromone: a substance which is excreted by an organism to the outside and causes specific reactions in a receiving organism of the same species (Nordlund, 1981). **Aggregation pheromones** attract conspecifics of both sexes. **Sex pheromones** are produced by one sex and attract only conspecifics of the opposite sex (Levinson & Levinson, 1995). → *hormone, kairomone*

Photoperiod: the length of light as compared to the length of darkness in each 24-hour cycle (Hale *et al.*, 1995).

Pioneers: insects which initially find a host without the aid of secondary attractants (Payne, 1986). → *attraction, host finding*

Pupal chamber: a capsule formed by the *P. truncatus* larva before pupation, by coating surrounding food particles with a secretion (Bell & Watters, 1982).

Repellent: a substance which causes directional movements away from the source of stimulation (Nordlund, 1981). → *attraction, deterrent, stimulant*

Reproductive value: index of the extent to which members of a given age group contribute to the next generation between that age and death (Lawrence, 1995).

Seminal vesicle: in male insects, expansion (sac-like structure) of the *vas deferens* which stores mature sperm (Evans, 1994).

Sex ratio: proportion of males in a population; e.g., a sex ratio of 1:3 describes a population with 25% males and 75% females (Pianka, 1994).

Sperm: usually mobile, male germ cells (= **spermatozoa**). (Lawrence, 1995).

Spermatheca: in female insects, a sac-like structure for the storage of sperm. As an ectodermal derivative it may be lined with sclerotized cuticle (Davey, 1985a).

Spermatophore: structure produced by the male which encloses sperm and transferred as this to the female (Davey, 1985b).

Stimulant: a substance which elicits a specific behaviour, e.g., a phagostimulant eliciting feeding (Nordlund, 1981). → *deterrent*

Taxis: directional movement towards or away from the source of stimulation. **Chemotaxis:** orientation towards or away from an odour source following concentration gradients. **Anemotaxis:** directional movements with respect to currents in air. **Odour-conditioned anemotaxis:** anemotaxis induced by olfactory cues present in the air current (Kennedy, 1977a). → *kinesis*

Testis: male organ where germ cells are produced (i.e., male gonad). In insects, usually a pair of testes, each may be subdivided into a set of **testis tubes** (Evans, 1994).

Vas deferens: sperm duct, connecting the testis with the ejaculatory duct (Davey, 1985b).

Vitellarium: → *ovariole*

Yellow body: follicular relics found at the base of the ovariole, irrespectively of colour; may develop due to *oviposition* (laying bodies) or *oosorption* (resorption bodies) (Tyndale-Biscoe, 1984).

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