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Sex ratio and female sexual status of the coconut pest, *Oryctes monoceros* (Coleoptera: Dynastidae), differ in feeding galleries and pheromone-baited traps

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Abstract

Oryctes monoceros is a serious coconut pest, causing up to 40% damage in tropical Africa. Synthetic aggregation pheromone, ethyl 4-methyloctanoate, has been used to lure adults to traps. Traps with pheromone plus decaying palm material captured a high proportion of males. This raises the question whether individuals, which damage palms are receptive to the pheromone. We studied the sex ratio of the insects feeding on coconuts and those attracted to pheromone traps. Sixty two percent of adults from feeding galleries on living coconut palms were females. Pheromone with rotting palm material lured 43% females. To investigate the reasons for this difference, we compared the reproductive system of females lured to the odour traps or feeding in coconut galleries, or present in old rotting stems. Ninety six percent of the females trapped by pheromone had mated, and were sexually mature. In the galleries on living palms, 46% of females were immature, and 24% had not mated. In old rotting stems where eggs are laid and larvae develop, a mixture of 52% mated and 48% virgin females was found. Therefore, the pheromone together with the odour of rotting coconut stems signals a reproduction site to beetles, particularly mature females. In practice, the pheromone-baited traps will help in reducing the dissemination of gravid females, but will not affect directly the numbers of immature ones attacking palms. Our results show that when using pheromones for monitoring or controlling insects, the physiological status of the insects may have unexpected effects on the outcome.

Keywords: *Oryctes monoceros*, coconut, pheromone trapping, female reproductive system, sex ratio

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Introduction

Oryctes monoceros (Ol.) and *Oryctes rhinoceros* L. (Coleoptera, Dynastidae) are insects with a long adult life of at least three months (Lepesme, 1947; Hinckley, 1973; Bedford, 1980). Females lay eggs in rotting stems where the larvae develop then build a fibrous cocoon in which pupation takes place.

The behaviour of *O. rhinoceros* adults was studied by Hinckley (1973) and Zelazny & Alfiler (1987). These authors found that virgin females were sexually immature on emergence and moved to palms to feed. Adults had long feeding periods on palms, usually remaining alone there for up to ten days in a gallery, and moving occasionally to the reproduction (larval breeding) sites to mate and lay eggs. Similar behaviour was found in *O. monoceros* (Mariau, 1967).

Since the discovery of the same male-produced aggregation pheromone, ethyl 4-methyloctanoate (e4mo) for both *O. rhinoceros* and *O. monoceros* (Gries *et al*., 1994), numerous trapping trials have been conducted to determine the best way to use synthetic pheromone to control *O. monoceros* (Allou *et al*., 2006). Studies were undertaken to determine how pheromone-based trapping could reduce *Oryctes* populations and damage, but questions remain about which insects are actually receptive to the lure. Indeed, trapping carried out in Indonesia on *O. rhinoceros* and in Ivory Coast on *O. monoceros* in young plantations has revealed that a part of the population does not enter pheromone traps (Morin *et al*., 2001) and burrows galleries in the palms of plots 'protected' by pheromone trap networks. To determine the potential and limitations of pheromone-based mass trapping, it is necessary to characterize the differences between individuals that are receptive and non-receptive to the pheromone and whether unresponsiveness is only temporary.

To tackle this question, we compared the sex-ratio of the beetle and studied the sexual maturity and mating status of females collected in traps baited with pheromone plus decaying palm material, compared to those collected in galleries made on living palms or in reproduction sites (rotting coconut stems). Females were dissected to determine their ovarian development and the presence of spermatophores in the spermatheca.

Material and methods

Study sites

Observations were conducted in Ivory Coast at the Marc Delorme station in Port Bouet village (longitude 05°16W and latitude 03°05N) and at the Robert Michaux plantation in Dabou (04°22W and 05°18N). The climate was equatorial with two rainy seasons.

Observations of insects arriving on coconut palms

Observations were carried out in plot 050 (6.5 ha) at the Marc Delorme station, in genetic trials set up to replace the old coconut plantings that were older than 50 years old. The presence of rotting fallen stems increased *Oryctes* development. In order to protect the young crops planted in 2002, regular phytosanitary inspections were carried out to remove *O. monoceros* adults from the galleries burrowed in the young palms. Inspections were made every three days, recording the reference number of the attacked palm and the number and sex of the beetles found in the galleries. We determined the distribution of *Oryctes* beetles on the young coconut palms, along with the sex ratio, from these data.

Study of insects collected in pheromone-baited traps

The sex ratio of insects responding to the aggregation pheromone with decaying palm material was determined from collections made during trials conducted between 2001 and 2003 at the Robert Michaux plantation. The trials were done to assess the attraction of synthetic pheromone, e4mo, formulated in a sachet (25 × 50 mm, made from a high-density polyethylene 200-μm-thick film, which emitted

Table 1. Distribution of *O. monoceros* on coconut palms attacked in plot P050 (6.5 ha) at Port Bouet village in 2004.

Month	No. of attacked palms	No. of palms with						Total			
		1 insect		2 insects			⩾3 insects[1]	male	female	m + f	% female[2]
		m	f	1m + 1f	2 m	2 f					
Jan	65	14	47	2	0	2	0	16	53	69	78.8
Feb	84	24	52	5	2	1	0	33	59	92	64.1
Mar	141	45	66	14	6	9	1	74	99	173	57.2
Apr	100	26	62	5	1	5	1	38	87	125	69.6
May	66	34	26	4	0	2	0	38	34	72	47.2
Jun	51	19	24	3	3	2	0	28	31	59	52.5
Jul	39	11	26	1	0	1	0	12	29	41	70.7
Aug	27	20	6	0	1	0	0	22	6	28	21.4
Sep	18	4	9	0	0	5	0	4	19	23	82.6
Oct	39	10	29	0	0	0	0	10	29	39	74.4
Nov	27	4	23	0	0	0	0	4	23	27	85.2
Dec	26	11	14	1	0	0	0	12	15	27	55.6
Total	683	222	384	35	13	27	2	291	484	775	62.5*
	Total	606			75		2				
	%	88.7			11.0		0.3				

m, male; f, female.
[1] March, 1 coconut palm with 3m + 1f; April, 1 coconut palm with 5m + 10f.
[2] Mean sex-ratio of monthly collected beetles is significantly female-biased (Wilcoxon signed-rank test; $n = 12$; *, $P = 0.017$).

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Table 2. Numbers and sex-ratio of *O. monoceros* collected during six pheromone trapping trials in Dabou (Allou *et al.*, 2006).

Trial no.	Lures[1]	Date[2]	No. of males[3]	No. of females	Total	% female[4]
1	EFB + e4mo	Jan–Feb 2002	188	104	292	36%*
2	EFB or CW + e4mo	Sep–Oct 2002	207	133	340	39%*
3	EFB + e4mo and/or 4-methyloctanoic acid	Aug 2002	72	34	106	32%*
4	EFB + e4mo and/or 4-methyloctanoic acid	Feb 2003	41	15	56	27%*
5	EFB or CW + e4mo	Sep–Oct 2003	113	104	217	48%
		Oct–Nov 2003	119	120	239	50%
		Nov–Dec 2003	116	133	249	53%
		Total	348	357	705	51%*
6	EFB or CW + e4mo	Apr 2004	170	113	283	40%
		May	207	191	398	48%
		Jun	224	166	390	43%
		Jul	263	191	454	42%
		Aug	261	145	406	36%
		Sep	186	132	318	42%
		Oct	126	111	237	47%
		Nov	280	191	471	41%
		Dec	222	188	410	46%
		Jan 2005	119	62	181	34%
		Feb	171	106	277	38%
		Mar	110	77	187	41%
		Apr	100	84	184	46%
		May	214	188	402	47%
		Jun	180	211	391	54%
		Total	2833^{w}*	2156	4989	43%*
1–6	Mainly EFB and CW + e4mo	2002–05	3689	2799	6488	43%

[1] CW, split coconut stem wood; e4mo, synthetic aggregation pheromone, ethyl 4-methyloctanoate; EFB, empty oil palm fruit bunches.
[2] Captures per line correspond to monthly totals per assay irrespective of the number of traps (see Allou *et al.*, 2006 for detail).
[3] Mean sex-ratio of monthly collected beetles of trial six is significantly male-biased (Wilcoxon signed-rank test; $n = 15$, ^{w}*, $P = 0.002$).
[4] The percent females in the traps and in the attacked palms (sample described in table 1) differ significantly with *, $P < 0.001$ (Khi^2 tests with 1 df).

13–14 mg d^{-1} under field conditions) and combined with plant synergists (oil palm empty fruit bunches and/or coconut stem wood). Those trials were described by Allou *et al*. (2006).

Physiological status of females

Initial work consisted of dissecting several females reared from ten pupae in the laboratory, to describe the reproductive system and observe its development in young females (one and two weeks old) and in females that had mated. As reported by Hinckley (1973) for *O. rhinoceros*, we found that *O. monoceros* females were still sexually immature on adult emergence, with slightly developed ovaries. Mating first occurred after an initial feeding period lasting a minimum of 2–3 weeks.

Ovarian development and its variability were then compared for females from three sources: (i) taken from galleries on young living coconut palms in plot 050; (ii) collected in traps using e4mo with coconut stem wood as the attractant; and (iii) collected from natural reproduction sites formed by old rotting coconut stems around Port Bouet village.

For each origin, we dissected 50 females. We categorized the ovarian development and mating status as follows: bursa copulatrix: empty or full of spermatophores; ovaries: (i) undeveloped without oocytes; (ii) slightly developed with small oocytes, <2.5 mm; (iii) fully developed with large oocytes, > 2.5 mm, and few eggs (≤ 9); and (iv) fully developed with large oocytes, > 2.5 mm, and many eggs (>9).

Sexual maturity was not studied in males because we found no typical phase of testicle development related to sexual activity.

Statistical analyses

The balance of the sex-ratio of the beetles was analysed using Wilcoxon signed-rank tests. Differences in the sex-ratios of the beetles collected from two different situations were compared by χ^2 tests on contingency tables. The proportions of mated females and their ovarian development category from two different origins were also compared using χ^2 tests on contingency tables.

Results

Insects arriving on coconut palms vs. insects collected by pheromone-baited traps

In 2004, 484 females were collected (62.4% out of a total of 775) on coconut palms in plot 050 (table 1). There was a significant female bias in the monthly collections ($U = 70.0$, $n = 12$, $P < 0.05$). Inspection showed that the palms were mostly attacked by a single male or a single female (89%). Two insects (all combinations of sexes) on the same plant were observed in 11% of the checks.

The percentage of females collected during the six pheromone trials, listed in table 2 (Allou *et al.*, 2006), varied

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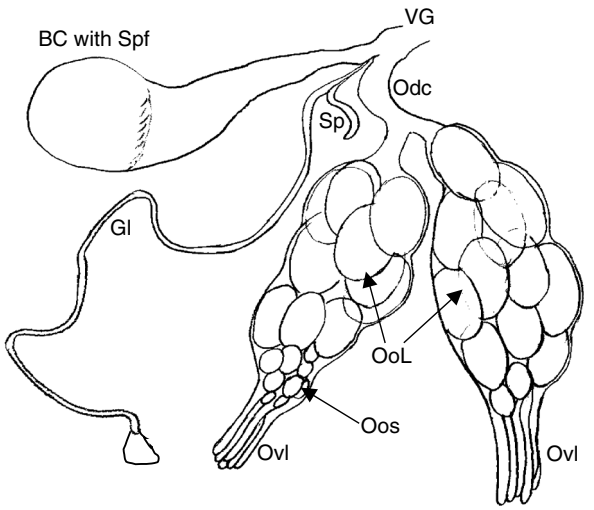

Fig. 1. Reproductive apparatus of a mature and mated *O. monoceros* female showing developed ovaries with large oocytes and a *bursa copulatrix* with a spermatophore.

Legend: **BC**: *bursa copulatrix*, **Gl**: accessory gland, **Odc**: common oviduct, **OoL**: large developed oocytes (>2.5 mm), **Oos**: small oocyte (<1 mm), **Ovl**: ovarioles, **Sp**: spermatheca, **Spf**: spermatophore, and **VG**: vagina.

from 27% to 54%. Overall, 2799 of the 6488 trapped beetles (43%) were females. The analysis of the collections in trial six on a monthly basis (total = 4989 beetles) showed that the average sex-ratio was biased towards males during 15 months (#: U = 5.0, $n = 15$, $P < 0.01$). For all six trapping trials, the sex-ratio of the trapped beetles differed significantly from that of the insects collected in the feeding galleries on coconuts (*: χ^2 values ranging from 25.7 to 100.0, 1 df, $P < 0.001$).

General organization of the female reproductive system

Figure 1 shows the female reproductive apparatus; it consisted of one pair of six ovarioles. In the mature female, many fully developed oocytes (2.5–3 mm) were found in the paired oviducts and the common oviduct, which occupied a large part of the abdomen. The bursa copulatrix was orange in colour and swollen by spermatophores. Near its connection with the vagina, there was the spermatheca and a long accessory gland. In the immature female, the ovarioles were slightly developed with only small oocytes and the bursa copulatrix was flat and contained no spermatophores. Nematodes were very often found in the bursa copulatrix of females. Spermatozoids from the spermatheca, placed in a drop of physiological saline, could be seen easily under the microscope.

Physiological status of females from pheromone traps (table 3)

All females except two (96%) had a bursa copulatrix containing spermatophores, indicating that they had mated. All individuals contained well-developed oocytes and 96% had large oocytes and eggs. The females were, therefore, all sexually mature.

Physiological status of females from feeding galleries

Out of 50 females observed, 76% had a bursa copulatrix containing spermatophores and 24% had an empty one. Almost half of the insects (46%) had slightly developed ovaries. The proportion of mated females and of ovarian development differed with females collected from pheromone traps ($\chi^2 = 8.31$, 1 df, $P < 0.01$; and $\chi^2 = 52.1$, 2 df, $P < 0.01$, respectively).

Physiological status of females from rotting coconut stems (reproduction site)

We observed as many mated (52%) as unmated (48%) females based on the content of the bursa copulatrix. Half of the females observed had slightly developed ovaries (56%) and 24% of them contained no oocytes. The proportion of mated individuals and the ovarian development differed with those of females collected from pheromone traps ($\chi^2 = 6.25$, 1 df, $P < 0.05$; and $\chi^2 = 60.0$, 3 df, $P < 0.001$, respectively), and also from those collected from living coconuts ($\chi^2 = 25.2$, 1 df, $P < 0.001$; and $\chi^2 = 13.8$, 3 df, $P < 0.01$, respectively).

Discussion

Our data showed that the beetle populations, which damaged the coconut palms by burrowing feeding galleries and which were collected in pheromone-baited traps, were

Table 3. Physiological status of *O. monoceros* females depending on their origin in the coconut plot with characteristics of the bursa copulatrix and the ovaries.

Origin of insects ($n = 50$)	Bursa copulatrix (%)		Ovaries (%)			
	empty	with spermatophores	undeveloped with very small oocytes	slightly developed with oocytes <2.5 mm	developed with <9 large oocytes (>2.5 mm)	developed with >9 large oocytes (>2.5 mm)
Pheromone traps (p)	4	96	0	0	4	96
Living palms	24	76 $^{P**, S*}$	0	46	28	26 $^{P***, S**}$
Rotting stems (s) (=reproduction site)	48	52 ^{P***}	24	32	24	20 ^{P***}

Proportions between origins are compared by Khi^2 tests. Comparisons: ^{S} living palms vs. rotting stem; and ^{P} living palms vs. pheromone traps. Differences are significant with *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

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different regarding the sex-ratio and the sexual maturity of the females. This result confirms that the coconut plots shelter populations with heterogeneous reproductive status, as suggested by several previous studies; larvae develop in rotting stems and other palm debris (reproduction sites) where females mate and lay eggs. The adult is immature on emergence (Hurpin & Fresneau, 1970) and has to spend 2–3 weeks feeding to mature its muscles and reproductive apparatus. It then passes through reproduction cycles (mating + egg laying at reproduction sites), each likely alternating with feeding periods on fresh palm tissue to complete the oogenesis processes for females (Mariau, 1968).

Living coconut palms were mostly attacked by isolated males or females. This suggests that the beetles came for feeding and not for mating, and agrees with reports of aggregation for mating only in reproduction sites (Lepesme, 1947). Conversely, male and female *Scapanes australis* (Boisduval) (another dynastid coconut pest in Papua New Guinea) mate and feed in a gallery burrowed in living palms and only females go to the larval breeding sites (Prior *et al*., 2000). Therefore, there is not a single scheme common to the palm Dynast species for the meeting of the sexes on the host plant, as previously suggested by Rochat *et al*. (2004) for *Oryctes elegans* Prell, a date palm species. Whether the few cases of several beetles found feeding on one palm were a random event or the expression of a particular suitability of the plants or of the site is not clear, but based on our regional experience, such occurrences are very rare.

On coconut palms, the sex-ratio of beetles was female-biased, whereas it was male-biased for beetles collected in the traps baited with synthetic aggregation pheromone and decaying palm woody material. Thus, beetles responsive to the male aggregation pheromone with decaying palm material were not the same population as those responding to the fresh palm odour. Half the females found feeding in galleries on living coconuts were sexually immature, and even virgin, while such females were rarely found in pheromone-baited traps. This suggests that young females were not responsive to the male aggregation pheromone and to the odour of decaying palm wood. They might also be more receptive to volatile cues from green fresh palm tissues. The female biased sex-ratio in feeding galleries is also consistent with a longer feeding activity due to higher energy requirements for oocyte and egg maturation and subsequent flight to reproduction sites as compared to the males. The latter could find enough food in the reproduction sites where they stay to recruit females by emitting aggregation pheromone. Thus, the male pheromone appears essentially to promote colonization of reproduction sites in females as reported for *O. rhinoceros* (Morin, 2002; Norman & Basri, 2004). As shown by our dissections, most females collected in pheromone-baited traps had fully developed eggs and were likely seeking sites to lay them. A strictly sexual role of this pheromone is not consistent with the male-biased sex-ratio of the pheromone-trapped populations and further investigation must be carried out to clarify this point.

In rotting woody palm material, the larval breeding sites, we found more sexually immature females, which had no doubt just emerged, than in the pheromone traps and the feeding galleries. Thus, *O. monoceros* females were not sexually mature on emergence as documented for *O. rhinoceros* (Hinckley, 1973; Bedford, 1980; Zelazny & Alfiler, 1987).

Finally, our observations confirm that using synthetic pheromone and rotting plant matter as the co-attractant mimics the beetle's reproduction sites, where males are emitting their pheromone. From a practical point of view, the potential for trapping *O. monoceros* with the aggregation pheromone is restricted to insects responsive to the male pheromone, which is synergistic with the odour from decaying woody material (Allou *et al*., 2006). Trapping as the main method of pest management is worthwhile in an isolated area and/or in old coconut plantings where adult coconut palms can afford one or more gallery that are not fatal to the plant, contrary to young palms, which can be killed by a single attack. Allou *et al*. (2006) showed trapping to efficiently reduce the number of attacks over two to three years in such a situation. However, other trials in replantings in village farming with young coconut palms were not as conclusive (Morin, unpublished data). Unresponsive females, which are either immature or have just laid eggs and which need to feed on fresh palm tissue to initiate a new oogenesis process, cannot be targeted by the pheromone lure. Consequently, trapping can only have a partial effect in reducing damage on young coconut palms depending on the population size and dynamics. It remains important to determine which males are responsive to the pheromone, since this could not be determine using our approach. Laboratory experiments, using an olfactometer with laboratory bred beetles of known age and reproductive status, should provide such information. Finally, it will be also very important to determine which cues are used by insects searching for fresh palm tissue to feed and propose an additional tool, possibly based in part on fresh palm odours, to eliminate or divert them from damaging palms.

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