AGGREGATION PHEROMONES OF COLEOPTERAN PESTS OF PALMS

by

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AGGREGATION PHEROMONES OF COLEOPTERAN PESTS OF PALMS

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ABSTRACT

Research in Indonesia, Malaysia and the United Arab Emirates elucidated semiochemical-based communication in the coconut rhinoceros beetle, *Oryctes rhinoceros* (L.), and the Asian palm weevils, *Rhynchophorus ferrugineus* (Olivier) and *R. vulneratus* (Panzer). Coupled gas chromatographic-electroantennographic detection (GC-EAD) analysis and GC-mass spectrometry showed that male palm weevils produce 4-methyl-5-nonanol (ferrugineol) and 4-methyl-5-nonanone (ferrugineone), the first of which is an aggregation pheromone. Both *R. ferrugineus* and *R. vulneratus* produced and responded to the 4*S*,5*S*-isomer of ferrugineol, and production and antennal response to 4*S*-ferrugineone exceeded that of its antipode in both weevils. Trap captures were maximized by placing traps at ground level or 2 m high. Vane traps were superior to bucket traps. Insecticide-free traps containing funnels to prevent weevil escape were equally as effective as traps using insecticide to retain weevils. Synergism occurred between ferrugineol and volatiles released by coconut palm stem. Of several palm-produced compounds identified, ethyl butyrate was both antennally active and attracted weevils in field tests. Male rhinoceros beetles produced ethyl 4-methyloctanoate (oryctelure), ethyl 4-methylheptanoate and methyloctanoic acid. Oryctelure proved to be an aggregation pheromone. In field experiments, 4*S*-oryctelure and the racemic compound were equally attractive and 10 times more effective in attracting beetles than the previously-known attractant, ethyl chrysanthemumate. Addition of oil palm fruit bunches to

pheromone-baited traps significantly enhanced attraction. Newly designed insecticide-free vane traps were more effective in capturing beetles than were barrier or pitfall traps. Short-term mass-trapping trials suggested that rhinoceros beetle populations may be successfully reduced if initial trap density and pheromone dose are high. In morphological, semiochemical, and genetic comparisons no species-specific differences were found between the two palm weevils, which are sympatric in Southeast Asia. Cross-breeding studies showed that hybrid F_1's can develop to the adult stage and produce viable eggs. Given the absence of pre- and post-zygotic isolating mechanisms, *R. ferrugineus* and *R. vulneratus* were judged to be colour morphs of the same species, and by the law of priority are synonymized as *R. ferrugineus*.

DEDICATION

I dedicate this thesis
to my parents, Peter & Helena Hallett, who helped me choose this path,
to my husband, Lawrence Murphy, who has walked beside me and whose
love has sustained me on this journey, and
to my daughter, Asia, who arrived to see its completion.

ACKNOWLEDGEMENTS

There are many people who have contributed in different ways to this thesis work, in particular I would like to express heartfelt thanks to my supervisory committee: Dr. John Borden, for his guidance, insight and belief in me; Dr. Cam Oehlschlager, for numerous discussions and enthusiasm for pheromone-based pest management; Dr. Gerhard Gries for his insights and generous assistance; and Dr. Nello Angerilli for logistical and moral support in Indonesia. I would also like to thank: Dr. Aunu Rauf , *Institut Pertanian Bogor*, for his sponsorship, cooperation and assistance; Alice Perez and Harold Pierce Jr. for their collaboration and chemical expertise; Regine Gries for generously giving her GC-EAD skills; Mr. Wily Baringbing and Mrs. Widi Rumini, *Balai Penelitian Kelapa*, for their companionship and for assistance in so many ways; Wawan Yuandi, IPB, who undertook many collecting expeditions for live insects so that work could proceed at SFU; Dr. R. Syed for helping to arrange my field base at LONSUM; Dr. Hugh Foster, Mr. G. Brown, Mr. P. Baskett, and Mr. A. Saleh, of London Sumatra Indonesia Plantation Company for permitting, assisting and supporting my research at LONSUM plantations; Mr. Rashed Al-Shareqi and Dr. M.S. Gassouma, Ministry of Fisheries & Agriculture, United Arab Emirates for collaborating in field research and showing me the UAE; Dr. M.M. Taher, Near East Regional Office of the FAO for

bringing me to Egypt and to Dr. M.E. El-Garhy and G.M. Mahmoud, Ministry of Agriculture, Egypt, for collaborating in field research and showing me Cairo; Lilly Gonzalez for tirelessly providing me with yet another batch of pheromone lures; Pamela Chung for assistance with weevil rearing; Roger's Sugar for providing sugarcane bagasse for weevil rearing; Ady, Maun, and Maman, for field assistance; Bibi Sumarni Gito for looking after us so well and becoming family; Laurie Pierce, Jim Jarvie, Alison Church and Rob Edmonds for their friendship and for sharing the joys and frustrations of working abroad; and my colleagues in the Borden-Winston lab who made it a great place to come home to. This work was supported by a Natural Science & Engineering Research Council Post-graduate Scholarship, an International Development Research Centre's Young Canadian Researcher's Award, and an SFU President's Research Stipend.

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Preface

Exploring semiochemical-based communication, pheromone identification, species interactions, and pest management implementation is necessarily multidisciplinary and requires expertise of several types. As a result, there are a number of people who have contributed directly to this research project. Pheromone identifications, chemistry, and syntheses were performed by Alice Perez-Sanchez, Gerhard & Regine Gries, Harold Pierce Jr., and Eva Czyzewska, of the Departments of Chemistry and Biological Sciences. Lilly Gonzalez , Chem Tica International, prepared pheromone lures for field testing and trapping trials. Random amplified polymorphic DNA analysis and mitochondrial DNA sequencing were performed by Dr. Bernie Crespi, Department of Biological Sciences. Due to overlap in our thesis topics, some of the information and figures presented in this thesis also appear in the Ph.D. dissertation of Dr. A.L. Perez-Sanchez, "Pheromones of *Rhynchophorus* palm weevils and *Oryctes* rhinoceros beetles." More detailed information about identifications and syntheses of pheromones discussed in this dissertation can also be found in Dr. Perez-Sanchez's thesis.

PART I, CHEMICAL ECOLOGY

CHAPTER 1

INTRODUCTION

The coconut rhinoceros beetle, *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae), and two palm weevils, *Rhynchophorus ferrugineus* (Olivier) and *R. vulneratus* (Panzer) (Coleoptera: Rhynchophoridae), are the most destructive pests of coconut, *Cocos nucifera* L., and oil palms, *Elaieus guineensis* (Jacq.), in South and Southeast Asia (Zelazny & Alfiler, 1986; Sivapragasam *et al.*, 1990; Sadakathulla, 1991).

Adult rhinoceros beetles burrow into the growing point of the palm and feed on unopened fronds, causing reduction in photosynthetic area, damage to inflorescences and decreased nut production (Zelazny, 1979; Young, 1986; Liau & Ahmad, 1991, 1993). Prolonged attacks can kill mature palms by defoliation, and young palms if the growing point is destroyed. The wounds produced by the rhinoceros beetle provide entry points for lethal diseases, such as budrot (Bedford, 1980; Jacob & Bhumannavar, 1991), and provide oviposition sites for the palm weevils, *R. ferrugineus* and *R. vulneratus*.

The Asian palm weevil, *R. ferrugineus*, and red-stripe palm weevil, *R. vulneratus* (= *R. schach*) are sympatric throughout much of their geographic ranges (Wattanapongsiri, 1966). During the last decade, multiple introductions of *R. ferrugineus* to the Middle East from Pakistan

and India have occurred and *R. ferrugineus* is now a serious pest of date palms, *Phoenix sylvestris* Roxb., in the Arab Gulf States (Bokhari & Abuzuhari, 1992) and Egypt (M.M. Taher[1], pers. comm.; pers. obs.). Its previous geographical range encompassed India, Sri Lanka, Thailand, New Guinea, Indonesia and the Philippines (Lever, 1969), where its most important hosts are sago, *Metroxylon sagu* Rottb., coconut, aren, *Arenga pinnata* (Wurmb.), and oil palms (Wattanapongsiri, 1966).

Often palm weevil infestations of coconut and oil palms are not detected until the fronds wilt and the crown collapses suddenly (Kalshoven, 1950; Sivapragasam *et al.*, 1990). By the time infestation is evident, internal damage caused by larval mining in the trunk is extensive, and it is not possible for the tree to recover (Sivapragasam *et al.*, 1990). In date palms, the only visible sign of attack may be oozing of palm sap from the trunk, and infestations are often not discovered until trees are blown over.

***Rhynchophorus* spp.**

Life history. Adult weevils live for 3-4 months. Females lay 200-500 eggs over a period of 5-8 weeks in wounds, slits or soft tissue at 3 mm depth in a hole that the female forms using her rostrum (Kalshoven, 1950). In date palms, oviposition may occur on the soft tissue at the base of vegetative offshoots. The incubation period is three days. The larvae (2-4 months)

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[1]Regional Plant Protection Officer, Regional Office for the Near East, Food & Agriculture Organization of the United Nations, Cairo, Egypt.

can only live in soft moist, tissue such as that in the crown, at the base of the petioles and within the upper part of the trunk of mature trees (Kalshoven, 1950). In young trees, the trunk and base may also be infested. The larvae tunnel into the terminal bud or trunk of the tree leading directly to its death. When infesting leaf scars, the larvae do not penetrate the trunk (Kalshoven, 1950). Pupation (14 days) occurs inside the tree within a cocoon constructed of palm tissues; the adult remains within the cocoon for 11-18 days before emergence. Length of the life cycle varies with the amount of water contained in the tissues eaten (Lever, 1969), and thus may differ from the values above when infestation occurs in date palms. Adults fly during the day and at dusk and can fly 900 m (Kalshoven, 1950), or more (pers. obs.).

Semiochemicals[2]. Both sex and aggregation pheromones have been identified for many curculionid pests, including the boll weevil, *Anthonomus grandis* Boh. (Tumlinson *et al.*, 1968, 1969), the pepper weevil, *A. eugenii* Cano (Eller *et al.*, 1994), the pecan weevil, *Curculio caryae* (Horn) (Mody *et al.*, 1973; Hedin *et al.*, 1979), the sweetpotato weevil, *Cylas formicarius elegantulus* (Summers) (Jansson *et al.*, 1990), the deodar weevil, *Pissodes nemorensis* Germar (Booth & Lanier, 1974;

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[2]Using Nordlund's (1981) widely-accepted terminology, the term semiochemical refers to any chemical involved in a chemical interaction between organisms. Terms for specific semiochemicals used in this thesis are pheromones (a semiochemical that is secreted by an organism that causes a specific reaction in a conspecific receiving organism), and kairomones (a trans-specific chemical messenger that evokes a reaction adaptively favourable to the receiver but not to the emitter).

Booth *et al.*, 1983; Phillips *et al.*, 1984), and the maize, granary and rice weevils, *Sitophilus zeamais* Motschulsky, *S. granarius* (L.) and *S. oryzae* (L.) (Phillips & Burkholder, 1981; Faustini *et al.*, 1982, Walgenbach *et al.*, 1983, 1987). Evidence of aggregation and sex pheromones were found, respectively, in male and female New Guinea sugarcane weevils, *Rhabdoscelus obscurus* (Boisd.), when in contact with fermenting sugarcane (Chang & Curtis, 1972). Prior to the start of this research, an aggregation pheromone was found for the American palm weevil, *Rhynchophorus palmarum* (L.) (Rochat *et al.*, 1991a,b; Oehlschlager *et al.*, 1992), and aggregation pheromones have subsequently been identified in three other congeneric palm weevils: the African palm weevil, *R. phoenicis* F. (Gries *et al.*, 1993), sabal palmetto weevil, *R. cruentatus* Fabr. (Weissling *et al.*, 1994), and black palm weevil, *R. bilineatus* (Montr.) (Oehlschlager *et al.*, 1995a); and in the West Indian sugarcane weevil, *Metamasius hemipterus* Oliv. (Rochat *et al.*, 1993a; Perez-Sanchez, 1996). Evidence of a male-produced pheromone in *R. ferrugineus* was originally found in field trapping experiments with live weevil stimuli in Thailand (Chayophat, 1979), and by laboratory bioassay experiments in India (Abraham, 1987). Mass trapping with rhynchophorol, 6-methyl-2(*E*)-hepten-4-ol, has been shown to reduce *R. palmarum* populations and incidence of associated red ring disease in Costa Rican oil palm plantations (Chinchilla *et al.*, 1993; Oehlschlager *et al.*, 1993a, 1995b).

The palm weevils are likely to use host volatiles in locating suitable oviposition sites as they are known to be attracted to fermenting sap from wounds or cut petioles (Kalshoven, 1950; Lever, 1969; Sadakathulla, 1991). In many countries, traps made of palm stems or petioles have been used to capture *Rhynchophorus* spp. (Abraham & Kurian, 1975; Abraham *et al.*, 1989; Morin *et al.*, 1986; Sivapragasam *et al.*, 1990). Attraction to host volatiles has been reported in the banana weevil, *Cosmopolites sordidus* (Germar) (Budenberg *et al.*, 1993; Koppenhöfer *et al.*, 1994) and host kairomones have been identified for *R. palmarum* (Nagnan *et al.*, 1992), *R. phoenicis* (Gries *et al.*, 1994a) and *R. cruentatus* (Weissling *et al.*, 1992; Giblin-Davis *et al.*, 1994). Young and stressed trees are particularly vulnerable to attack by *Rhynchophorus* spp. (Leong, 1987; Giblin-Davis & Howard, 1989; Sadakathulla, 1991). Newly established or replanted plantations are thus particularly at risk of attack.

Since the palm weevils rely on wounds in order to gain access to the stem tissue for oviposition, it is also possible that *R. ferrugineus* and *R. vulneratus* respond to the pheromones and/or frass produced by feeding *O. rhinoceros* adults in their search for suitable hosts.

Current Control Methods. Current control methods include the detection of infested palms by regular surveys, removal and burning of all infested trees, and application of insecticides to all palms in infested areas. In Egypt, following the detection of infested palms by censusing, infested trees are removed by bulldozer or cutting, transported to an

isolated desert area, and burned. Typically, the burning of infested palms is not complete, so that live larvae and pupae remain within the trunk and can complete development. Delay between detection and destruction of an infested tree may also permit the further emergence of adult weevils prior to destruction. The practice of transporting infested trees to a different area for burning may also result in the introduction of the weevil to other areas.

The widely used tactic of spraying entire tree trunks with insecticides is unlikely to have any effect in controlling weevil populations, as no stage of this insect is commonly present on the outside of the tree. In addition, insecticides applied in this way are not able to penetrate and kill larvae living within the trunk. This practice also results in unnecessarily high volumes of insecticides being used and may have serious negative environmental and public health impacts.

Use of palm stem traps is unsatisfactory since it is labour-intensive, the traps require weekly replacement, and their attractiveness varies considerably with environmental conditions (Chinchilla & Oehlschlager, 1992; Nagnan *et al.*, 1992).

Oryctes rhinoceros.

Life history. Eggs (about 50 per female) are laid in rotting organic material, such as decaying palm trunks, sawdust or compost heaps and garbage dumps (Kalshoven, 1950) and hatch after 8-12 days. There are three larval instars (80-200 days), and pupation (30 days) occurs in

organic material (Khoo *et al.*, 1991). Adults fly to the crowns of palms at dusk, where they feed on sap produced when tunneling into the spear leaf. Mating may occur in palm crowns where beetles remain for 5-10 days (Kalshoven, 1950). Females then move to oviposition sites. Although capable of flying several hundred metres, adults fly only short distances if local conditions are favourable for feeding, mating and oviposition (Kalshoven, 1950).

Semiochemicals. *O. rhinoceros* adults are gregarious. More than one beetle may attack a palm at the same time while a neighboring tree is unattacked (Gressit, 1953). Adults congregate in decaying coconut trunks or compost piles to mate and both single and multiple pairs of adults have been found in the same breeding site (Zelazny & Alfiler, 1991). After a female arrives at a site and lays eggs the male joins her and helps prepare the wood for their eggs and larvae (Zelazny & Alfiler, 1991). Decaying wood is chewed into small pieces and packed around the eggs. These observations suggest that *O. rhinoceros* is attracted to host kairomones and then employs either an aggregation or sex pheromone or both.

Sex pheromones have been identified in a number of scarabaeids belonging to the subfamilies Rutelinae and Melolonthinae, including the grass grub beetle, *Costelytra zealandica* White (Henzell & Lowe, 1970), Japanese beetle, *Popillia japonica* Newman (Tumlinson *et al.*, 1977), soybean beetle, *Anomala rufocuprea* Motschulsky (Tamaki *et* al., 1985),

cupreous chafer beetle, *A. cuprea* Hope (Leal, 1991; Leal *et al.*, 1993a), *A. daimiana* Harold (Leal *et al.*, 1993b), May beetle, *A. schonfeldti* Ohaus (Hasegawa *et al.*, 1993), *A. octiescostata* Burmeister (Leal *et al.*, 1994a), green chafer beetle, *A. albopilosa sakishimana* Nomura (Leal *et al.*, 1994b), the oriental beetles, *Exomala orientalis* (Waterhouse) (= *Blitopertha orientalis* (Waterhouse)) (Leal, 1993; Leal *et al.*, 1994c) and *A. orientalis* (Waterhouse) (Zhang *et al.*, 1994), *A. japonica* (Leal, 1996), the large black chafer, *Holotrichia parallela* Motschulsky (Leal *et al.*, 1993c), and in the dung beetle, *Kheper lamarcki* MacLeay (Scarabaeinae) (Burger *et al.*, 1983). Aggregation induced by semiochemicals released by feeding beetles of either sex was found in the green june beetle, *Cotinis nitida* (L.) (Domek & Johnson, 1988), which also appears to have a female-produced sex pheromone (Domek & Johnson, 1987). An aggregation pheromone was also suspected in the Japanese beetle (Adler & Jacobson, 1971; Iwabuchi & Takahashi, 1983), but the aggregation behaviour may actually be caused by a feeding-induced plant kairomone (Loughrin *et al.*, 1995). Subsequent to the start of my research, an aggregation pheromone, ethyl 4-methyloctanoate, was discovered for *Oryctes monoceros* Olivier (Dynastinae) (Gries *et al.*, 1994b).

Current Control Methods. *O. rhinoceros* breeds in decaying organic matter, such as felled rotting palms, and usually becomes a major problem in newly planted or replanted oil palm plantations. Covering the trunks with a rapidly-growing ground cover (Wood, 1968) or shredding

and burning of the trunks are common practices to minimize the build up of *O. rhinoceros* populations (Liau & Ahmad, 1991). Although effective, shredding and burning is very expensive and has been banned in some parts of Southeast Asia (Tajudin *et al.*, 1993) to lower air pollution from the 4.5 million hectares of oil palm in the region.

Treatment of breeding sites, such as stumps, with insecticidal drenches and routine application of granular insecticides (e.g. carbofuran) to the leaf axils of young oil palms are recommended (Ho & Toh, 1982). These techniques are currently considered economic (Liau & Ahmad, 1991), but are not very effective and present environmental and health risks. Manual removal of beetles from palms and larvae from decomposing trunks are costly and labor-intensive (Ho & Toh, 1982).

Limited success in managing *O. rhinoceros* populations has been achieved through introduction of the baculovirus, *Rhabdionvirus oryctes* Hüger (Bedford, 1986). Introduction of the baculovirus to the Philippines reduced *O. rhinoceros* populations to 10-20% of prerelease levels but even low-level populations of *O. rhinoceros* can cause great damage (Zelazny & Alfiler, 1987, 1991). The baculovirus remains effective only if it infects new larval hosts or is repeatedly introduced, and the potential exists for *O. rhinoceros* to develop resistance to the baculovirus after prolonged exposure (Zelazny & Alfiler, 1991). Several compounds, including ethyl chrysanthemumate (EC, rhinolure) have been recommended as lures for trapping *O. rhinoceros* (Barber *et al.*, 1971; Maddison *et al.*, 1973; Vander Meer *et al.*, 1979) and *O. monoceros* (Julia

& Mariau, 1976), but they are only moderately attractive (Vander Meer *et al.*, 1979; Young, 1986).

Objectives.

The scientific aim of this study was to elucidate semiochemical-mediated behaviour in three important pests of palms. The practical aim was to exploit knowledge of host selection and semiochemical-based communication to develop a management system for these pests, and thereby to increase the efficacy of control. A control program that is directed against adults, such as one based on semiochemicals, has the potential to limit both the number of trees attacked and population levels. Field and laboratory experiments were conducted in Indonesia, Malaysia, the United Arab Emirates, Egypt and Canada in order to accomplish the following specific objectives:

1. to test the hypothesis that pheromone-based communication exists in the three subject species,
2. to identify the pheromones of each species,
3. to utilize pheromone-baited traps in the field to examine differential responses between sexes in terms of pheromone production and response,
4. to develop appropriate protocols for the successful implementation of trapping systems, requiring investigation of bioactivity of synthetic pheromones, pheromone chirality, trap design, pheromone release

rate, distance between traps and the relationship between trap catches and background populations,

5. to test the hypothesis that interspecific semiochemical-based communication occurs,
6. to identify attractive host kairomones and characterize their interaction with pheromones, and
7. to test the hypothesis that semiochemicals have potential for incorporation into semiochemical-based pest management programs for use in palm holdings.

During initial stages of research, lack of differences in responses to natural and synthetic pheromone by *R. ferrugineus* and *R. vulneratus* was repeatedly observed. This result led to an eighth objective: to investigate the species status of these two palm weevils.

CHAPTER 2

AGGREGATION PHEROMONES IN *RHYNCHOPHORUS* SPECIES

INTRODUCTION

Aggregation pheromones are semiochemicals that elicit response by both male and female individuals of the same species regardless of the sex of the signaller, and that thereby cause individuals to join together in groups (Borden, 1985). The formation of these groups may be associated with feeding, protection, mating or a combination of these functions (Borden, 1985). It is likely, however, that aggregation pheromones were initially evolved to send a sex-related message and that this message has subsequently been exploited by individuals of the same sex to locate suitable hosts and breeding sites (Schlyter & Birgersson, 1989). Aggregation pheromones were first identified in the California fivespined ips, *Ips paraconfusus* (Lanier) (Silverstein *et al.*, 1966, 1967).

Aggregation pheromones that have been identified for *Rhynchophorus* species are: 2-methyl-5(*E*)-4-heptenol (*R. palmarum*) (Rochat *et al.*, 1991b; Oehlschlager *et al.*, 1992); 3-methyl-4-octanol (*R. phoenicis*) (Gries *et al.*, 1993); 5-methyl-4-octanol (*R. cruentatus*) (Weissling *et al.*, 1994); and 4-methyl-5-nonanol (*R. bilineatus*) (Oehlschlager *et al.*, 1995a). In all of these species, the pheromone is male-produced. As in the Scolytidae, aggregation in these curculionids

may provide mutual benefit to competing individuals (Alcock, 1982), allowing them to exploit evanescent hosts (Atkins, 1966).

Specificity of aggregation pheromones can contribute to habitat and resource partitioning among competing species in the Coleoptera. Intraspecific attraction and interspecific interruption of response can reside in the specificity of one or more aggregation pheromone components. (*S*)-(+)-Ipsdienol and (*S*)-(-)-ipsenol produced by *I. paraconfusus* interrupt the aggregation of sympatric pine engravers, *I. pini* (Say), to their aggregation pheromone (*R*)-(-)-ipsdienol in California (Birch *et al.*, 1980). The khapra beetle, *Trogoderma granarium* (Everts), uses the (*R*)-(-)-enantiomers of (*Z*)- and (*E*)-trogodermal in a 92: 8 ratio, while beetles in three other *Trogoderma* spp. respond to the (*R*)-(-)-enantiomers of either (*E*)- or (*Z*)-trogodermal alone (Cross *et al.*, 1976). Ratios of both geometrical and optical isomers of brevicomin determine response and pheromonal specificity of sympatric western balsam bark beetles, *Dryocoetes confusus* (Swaine) and *D. affaber* (Mannerheim) (Camacho *et al.*, 1993, 1994).

Experiments were undertaken to test the hypothesis that *R. ferrugineus* and *R. vulneratus* use pheromone-based communication, to identify pheromones produced in both species, and to elucidate species-specific differences in pheromone production, chirality and response. Experimental results presented in this chapter are used, in part, to justify synonymization of *R. ferrugineus* and *R. vulneratus* in Part II.

METHODS

Identification of Candidate Aggregation Pheromone Components

Twenty male and 20 female *R. ferrugineus* and 25 male and 25 female *R. vulneratus* were aerated separately for 1 wk in modified Nalgene desiccators (#5311-0250) containing sugar cane (Oehlschlager *et al.*, 1988, 1992). A vacuum pump was used to draw charcoal-filtered air through the chambers and insect- and host-produced volatiles were captured on Porapak Q held in a glass column (14 cm long x 13 mm OD). Volatiles were eluted from the Porapak Q with 175 mL pentane and the eluant was concentrated by distillation of solvent through a 30 cm Dufton column (Pierce *et al.*, 1981). The concentration of volatiles was adjusted so that 1 μL equaled 1.3 weevil-hours of production. Volatile extracts were analysed and candidate pheromones identified by coupled gas chromatographic-electroantennographic detection (GC-EAD) (Hewlett Packard 5890A) (Arn *et al.*, 1975) utilizing a custom-built amplifier with low-pass filter and a cutoff frequency of 10 kHz, and by GC analyses (Hewlett Packard 5830A) and GC-mass spectrometry (MS) (Hewlett Packard 5985B), employing fused silica columns (30 m x 0.25 mm ID) coated with either DB-5 (J&W Scientific, Folsom, California) or SP-1000 (Supelco, Mississauga, Ontario).

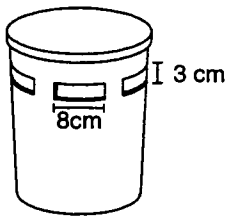
Field Experiments with Candidate Aggregation Pheromone Components

Most field experiments were conducted at the Coconut Research Station (*Balai Penelitian Kelapa, BALITKA*), Pakuwon, West Java, Indonesia. To examine whether geographic differences in pheromonal response exist between Middle Eastern and South East Asian populations of *R. ferrugineus*, additional experiments were run at two locations in the United Arab Emirates (UAE). Unless otherwise indicated, all experiments utilized white bucket traps (20 L) (Fig. 1) attached 2 m high to coconut palms (Indonesia) or 1 m high on date palms (UAE) in randomized complete blocks, with 10 replicates. In all experiments, each treatment was tested once in each block. Intertrap and interblock distances were 24 and 70 m in Indonesia, and 21 and 30 m in the UAE.

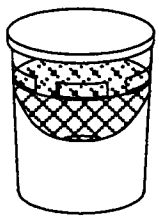
All traps contained pieces of insecticide-treated wood (approx. 5 cm x 5 cm x 20 cm) to enhance the retention of captured weevils. In Indonesia, 2 kg pieces of coconut wood were soaked for 4-5 min in a 0.24% a.i. solution of Basudin 60EC (diazinon 600 g/L; Ciba-Geigy). In the UAE, 1 kg pieces of date palm offshoots were soaked in a 0.3% a.i. solution of Lannate (methomyl 90% w/w; DuPont). Pheromone release devices and release rates for Exps. 1-10 are given in Table 1. All lures were suspended with wire from the underside of the bucket lid. Captured weevils were removed every 2-3 d.

An initial experiment (Exp. 1) to compare attractiveness of palm alone, and with either ferrugineol, ferrugineone, or both, was conducted

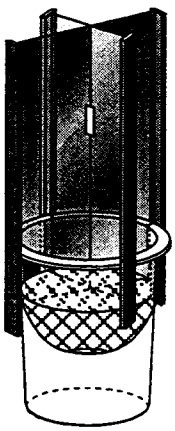
Figure 1. Traps of different designs used for capturing *R. ferrugineus* and *R. vulneratus*. **Standard bucket trap** used in experiments described in this chapter: 20 L white bucket with four slots around rim of bucket just below lid and four openings on lid to allow weevil entry and dissemination of volatiles (Oehlschlager *et al.*, 1993b); lures suspended from lid of bucket. Other traps as follows tested for field efficacy (Chapter 5). **Funnel trap** used in Exps. 27 & 28: same buckets as above except with funnel inserted below side entry slots in order to prevent weevil escape. **Vane trap with funnel** used in Exps. 22, 27, 28 & 37: trap same as that used for *O. rhinoceros*, but fitted with funnel below vanes in order to prevent weevil escape. **Inverted bucket trap** used in Exp. 29: same as standard bucket trap, except trap inverted; four flaps provided around top of trap for weevil entry, and lure suspended from inside top of trap.



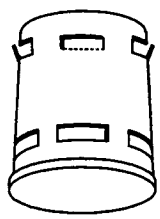
Standard Bucket Trap



Funnel Trap



Vane Trap with Funnel



Inverted Bucket Trap

Table 1. Summary of semiochemical release rates and release devices used in Exps. 1-10.

Exp.	Semiochemical	Release Rate (mg per 24 h)[a]	Release Device	Chemical Source[b]
1	Ferrugineol	0.3	400µL microcentrifuge tube[c]	SFU
	Ferrugineone	1.4	400µL microcentrifuge tube	SFU
2	Ferrugineol	3.0	Three 1.5 mL microcentrifuge tubes	SFU
	Ferrugineone	0.3	Two capillaries (1 mm ID)[d]	SFU
	Ferrugineone	1.4	400µL microcentrifuge tube	SFU
	Ferrugineone	2.8	Two 400µL microcentrifuge tubes	SFU
3	Ferrugineol	3.0	Polymer membrane bag lure[e]	CT
4	Ferrugineol	3.0	Polymer membrane bag lure	CT
	Ferrugineone	0.3	Two capillaries (1 mm ID)	SFU

Exp.	Semiochemical	Release Rate (mg per 24 h)[a]	Release Device	Chemical Source[b]
5	Ferrugineol	0.4	Four capillaries (1 mm ID)	SFU
	Ferrugineol	0.8	Eight capillaries (1 mm ID)	SFU
6	Ferrugineol	0.4	Four capillaries (1 mm ID)	SFU
	(*S,S*)-Ferrugineol[f]	0.1	One capillary (1 mm ID)	SFU
	(*R,R*)-Ferrugineol[f]	0.1	One capillary (1 mm ID)	SFU
7	Ferrugineone	1.6	Two capillaries (1 mm ID)	SFU
	(*S*)-Ferrugineone[g]	0.8	One capillary (1 mm ID)	SFU
	(*R*)-Ferrugineone[g]	0.8	One capillary (1 mm ID)	SFU
8	(*S,S*)-Ferrugineol	0.2	Two capillaries (1 mm ID)	SFU
	Ferrugineone	1.6	Two capillaries (1 mm ID)	SFU
	(*S*)-Ferrugineone	0.8	One capillary (1 mm ID)	SFU
	(*R*)-Ferrugineone	0.8	One capillary (1 mm ID)	SFU
9, 10	(*S,S*)-Ferrugineol	1.0	Ten capillaries (1 mm ID)	SFU
	Ferrugineone	0.2	Two capillaries (0.6 mm ID)	SFU
	(*S*)-Ferrugineone	0.1	One capillary (0.6 mm ID)	SFU
	(*R*)-Ferrugineone	0.1	One capillary (0.6 mm ID)	SFU

a Release rates from capillary tubes and microcentrifuge tubes estimated over 1-2 weeks under laboratory conditions of 25°C and 50% RH. Release rates from polymer membrane bag lures determined by Chem Tica under the same laboratory conditions over 2-5 weeks.

b CT= Chem Tica International, Costa Rica; SFU= Department of Chemistry, Simon Fraser University.

c Capped polyethylene 400 μL and 1.5 mL microcentrifuge tubes (Quality Scientific Plastics, USA, and Gordon Technologies, Mississauga, Ontario, respectively) with two 3 mm diam. holes drilled 1 cm below top.

d All capillary tubes opened 1 cm above liquid meniscus, placed inside microcentrifuge tube as above and suspended 3-4 cm below trap lid.

e Heat-sealed polymer membrane bag lures, Chem Tica International, Costa Rica.

f Enantiomeric excess 98%.

g Enantiomeric excess 95%.

at BALITKA (20 replicates; 16-23 May 1992) and at Lampung, Sumatra (10 replicates; 19-25 May 1992). Ferrugineol and ferrugineone were released at 0.3 and 1.4 mg per 24 h, respectively. At BALITKA, traps were suspended from palm crowns at a height of 8-10 m, while in Lampung traps were attached at the palm crown 2 m high.

A 10 replicate experiment (Exp. 2) was conducted at BALITKA (28 August - 4 September 1992) in order to examine attractiveness of ferrugineol alone and in combination with ferrugineone in ratios of 10:0, 10:1, 10:4.5, and 10:9, respectively.

In a two treatment 10-replicate experiment (Exp. 3) at Ras Al Kaimeh, UAE (15 April - 9 May 1993), attractiveness of ferrugineol in combination with date palm tissue was compared to date palm tissue alone.

In the UAE (Northern Region Research Station, 13 April - 9 May 1993), a 9-replicate experiment (Exp. 4) compared the attractiveness of ferrugineol and ferrugineone individually or in a 10:1 ratio to traps without pheromone.

Pheromone Chirality

Porapak Q-captured ferrugineol and ferrugineone from both weevils (see above) were subjected to GC-EAD (Hewlett Packard 5890A) and GC-MS (Hewlett Packard 5985B) on a fused silica, Cyclodex-B-coated column (30 m x 0.25 mm ID, J&W Scientific, Folsom, CA) which separates optical isomers of both ferrugineol and ferrugineone with baseline resolution.

Chemical ionization (CI, isobutane) GC-MS analyses[1] were conducted in both full scan and selected ion monitoring mode (SIM). A full scan mass spectrum of ferrugineol and ferrugineone, hexane and concentrated weevil-produced pheromone were injected in split mode and analyzed by scanning for diagnostic ions.

Response of *R. ferrugineus* and *R. vulneratus* to chiral isomers of ferrugineol and ferrugineone were investigated in coconut plantations at BALITKA (August-December 1993, July and November-December 1994). Traps were attached 2 m high or at the base of coconut palms in randomized complete blocks. There are no significant differences in trap effectiveness for these two trap placements (Oehlschlager *et al.*, 1993b; Chapter 5, Exp. 26). Minimum intertrap and interblock distances were 16 and 45 m, respectively. All traps contained 1/2 - 1 kg of coconut palm wood treated with a 0.3% a.i. solution of diazinon.

To establish an effective baseline against which scarce chiral isomers could be tested a 9-replicate dose response experiment (Exp. 5) was conducted prior to field testing of chiral compounds at BALITKA (16-26 July 1993) and at Ciemas, Java (7-14 August 1993). Doses of racemic ferrugineol were 0, 0.4 or 0.8 mg per 24 h.

A four-treatment, 20-replicate experiment (Exp. 6) at BALITKA (16-30 August 1993) tested attraction of both *R. ferrugineus* and *R. vulneratus*

[1]Conducted by A.L. Perez-Sanchez, Department of Chemistry, Simon Fraser University.

to coconut tissue alone or in combination with stereoisomeric ferrugineol, (4*S*,5*S*)- or (4*R*,5*R*)-ferrugineol.

A four-treatment, 20-replicate experiment (Exp. 7) (29 November- 4 December, 7- 15 December, 1993) at BALITKA , tested attraction of both *R. ferrugineus* and *R. vulneratus* to coconut stem alone or in combination with (*R,S*)-, (*S*)-, or (*R*)-ferrugineone.

A four-treatment, 20-replicate experiment (Exp. 8) was conducted at BALITKA (15-28 December 1993) to compare response of *R. ferrugineus* and *R. vulneratus* to (4*S*,5*S*)-ferrugineol plus coconut stem alone or in a 1:4 ratio with (*R,S*)-, (*S*)-, or (*R*)-ferrugineone.

In North Sumatra (22 March - 10 April 1994) , a four-treatment, 9-replicate experiment (Exp. 9) was conducted to test attraction of *R. vulneratus* to (4*S*,5*S*)-ferrugineol plus coconut stem alone or in combination with either enantiomeric, (*S*)-, or (*R*)-ferrugineone, approximating the natural 10 : 1 ratio found in the GC analysis of volatiles (above). A final 19-replicate experiment (Exp. 10) with the same treatments as Exp. 9 was conducted at BALITKA (9-21 July and 21 November-6 December 1994), where both *R. vulneratus* and *R. ferrugineus* are found.

Statistical Analysis

If transformation by $\log_e(x + 1)$ did not decrease heteroscedasticity, data for each species were subjected to analysis by X^2 tests ($\alpha = 0.05$) (Zar, 1984). Treatment, sex, and species comparisons

within an experiment were also performed using X^2 tests. Only in Exps. 3 & 4 did normality of the data permit analysis by Analysis of Variance (PROC GLM) and comparison of means by Bonferroni *t*-tests (SAS Institute, 1985). Because no significant differences were found between response patterns of female and male weevils of either species in any experiment (X^2 tests, $P > 0.05$), pooled data for both sexes are presented.

RESULTS

Identification of Candidate Aggregation Pheromone Components

Analyses of volatile extracts by GC-EAD revealed in both species the presence of two male-specific compounds that elicited strong electrical potentials by male and female antennae (Fig. 2). The mass spectrum of the second EAD-active compound [(**2**), Fig. 3] in both *R. ferrugineus* and *R. vulneratus* was identical to that of a minor male-produced compound in the American palm weevil, *R. palmarum* (A.C. Oehlschlager, G. Gries, H.D. Pierce Jr., and E. Czyzewska, unpublished data[2]). It was hypothesized to be a secondary, methyl-branched aliphatic alcohol. Treatment of a male *R. palmarum* extract with Jones reagent (Fieser & Fieser, 1967) and subsequent mass spectroscopy of the oxidized compound gave a ketone with molecular weight 156, identical to that of the first EAD-active compound (**1**) in *R. ferrugineus* and *R. vulneratus*

[2]Departments of Chemistry (ACO, HDP, EC) and Biological Sciences (GG), Simon Fraser University.

Figure 2. Flame ionization detector (FID) and electroantennographic detector (EAD) responses to volatiles obtained from male *R. vulneratus* feeding on sugarcane. **1**= 4-methyl-5-nonanone, **2**= 4-methyl-5-nonanol. FID trace of 1μL extract (= 1.3 weevil hours of pheromone production). The antennal recording presented is that of a single male *R. vulneratus* antenna to 1 μL of extract diluted by 10 with hexane. Chromatography: Hewlett Packard 5890A gas chromatograph equipped with a DB-5 coated, fused silica column (30 m x 0.25 mm ID); 1 min at 70°C, 5°C/min to 240°C.

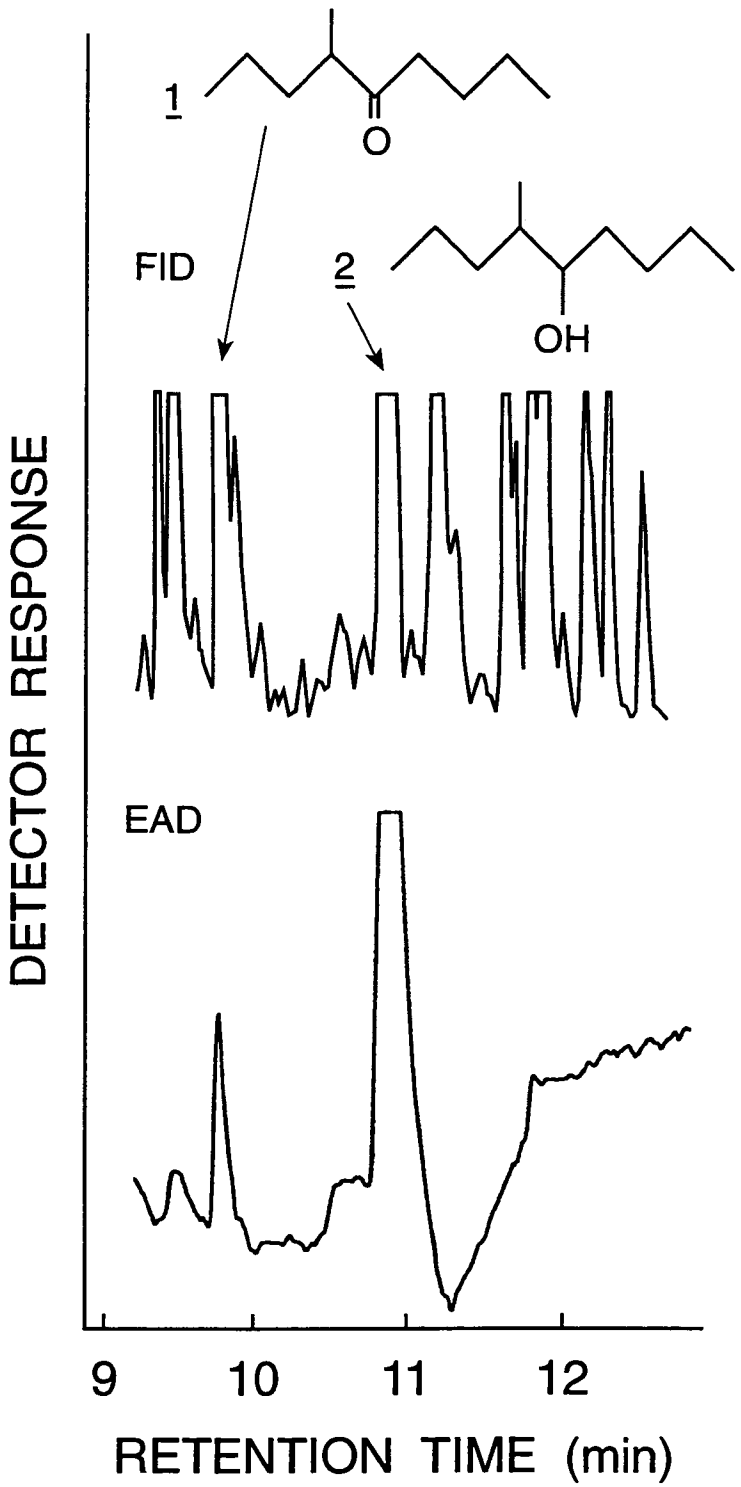
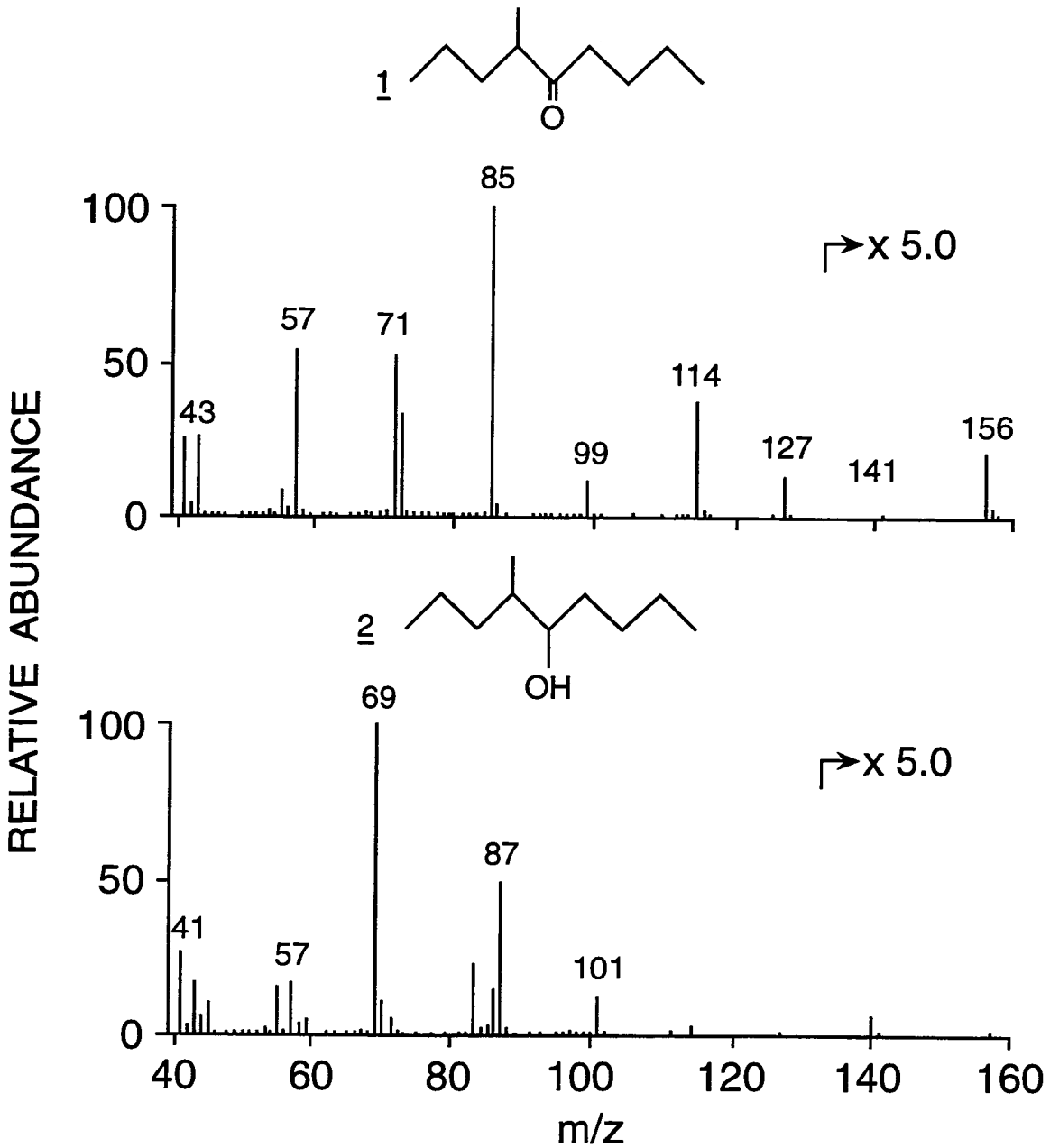

1
O
FID
2
OH
EAD
DETECTOR RESPONSE
9
10
11
12
RETENTION TIME (min)

Figure 3. Mass spectra of 4-methyl-5-nonanone (**1**), and 4-methyl-5-nonanol (**2**). Chromatography: Hewlett Packard 5885B gas chromatograph-mass spectrometer equipped with a SP-1000 coated, fused silica column (30 m x 0.25 mm ID); 1 min at 70°C, 10°C/min to 180°C.


1
O
85
100
x 5.0
57
71
50
114
43
156
99
127
141
0
2
OH
69
100
x 5.0
87
50
41
57
101
0
40
60
80
100
120
140
160
m/z
RELATIVE ABUNDANCE

(Figs. 2, 3). Analysis of the ketone mass spectrum indicated the ketone group at C5. Mass and retention characteristics of authentic 4-methyl-5-nonanol, but not 3-methyl-5-nonanol, were identical with (**2**) in *R. ferrugineus*, *R. vulneratus* and *R. palmarum*. In all three species, equivalent amounts of synthetic (**2**) and the male-produced compound elicited similar antennal responses by male and female weevils. 4-Methyl-5-nonanone (**1**) was present in male *R. ferrugineus* and *R. vulneratus*, but was not detected in male *R. palmarum*. The trivial names ferrugineone and ferrugineol were assigned to 4-methyl-5-nonanone (**1**) and 4-methyl-5-nonanol (**2**), respectively.

Field Experiments with Candidate Aggregation Pheromone Components

Both species were captured at BALITKA in Exp. 1, but only *R. vulneratus* were captured in Lampung (Table 2). No significant differences were found between responses of *R. vulneratus* captured in Lampung and at BALITKA (X^2= 6.8144, df= 3, P> 0.05), or between patterns of response between species (X^2= 6.5802, df= 3, P> 0.05). Analysis of pooled data showed that significantly more weevils were captured in traps containing ferrugineol alone or in combination with ferrugineone than in traps containing ferrugineone alone or the control (X^2= 33.2029, df= 3, P< 0.001).

Significantly more *R. ferrugineus* were captured in Exp. 2 in traps baited with ferrugineol alone or in a 10:1 ratio with ferrugineone than in

Table 2. Total captures in Exp. 1 of *R. ferrugineus* (20 replicates, BALITKA, 16-23 May 1992) and *R. vulneratus* (30 replicates, BALITKA and Lampung, 19-25 May 1992) in traps baited with coconut palm wood alone, or in combination with either ferrugineol, ferrugineone or both.

Treatment	*R. ferrugineus*	*R. vulneratus*	Pooled Data[a]
Ferrugineol + palm	10	25	35 a
Ferrugineone + palm	3	6	9 b
Ferrugineol + ferrugineone + palm	1	20	21 a
Palm alone	0	4	4 b

[a] Values in a column followed by the same letter are not significantly different, pairwise X^2 comparisons, $P > 0.05$.

other treatments (Fig. 4). Increasing amounts of ferrugineone significantly decreased attraction of *R. ferrugineus*, but not *R. vulneratus*. Because the response patterns of *R. ferrugineus* and *R. vulneratus* were not significantly different (X^2 = 5.42, df= 2, $P > 0.05$), data for both species were pooled to examine response patterns over time. The total number of weevils captured on day 5 was significantly higher than that on day 2 or day 7 (Fig. 5). All treatments containing pheromone were more attractive than the palm control. Although ferrugineone was antennally active in both weevils, it induced observable behavioral responses only in *R. ferrugineus* (Fig. 4).

In the UAE, significantly more *R. ferrugineus* were attracted in Exp. 3 to traps baited with ferrugineol than with palm wood alone (Fig. 6). In Exp. 4, ferrugineol alone was more attractive than ferrugineone alone or the control (Fig. 6). Adding ferrugineone to ferrugineol did not significantly enhance or decrease weevil response compared to the response to ferrugineol alone. Ferrugineone alone was no more attractive than the control.

Pheromone Chirality

GC-EAD and GC-MS-SIM analyses on a Cyclodex-B column of weevil-produced ferrugineone, synthetic enantiomeric ferrugineone and each of the two enantiomers indicated that both *R. vulneratus* and *R. ferrugineus* produce and antennally respond more strongly to (*S*)-ferrugineone than to (*R*)-ferrugineone (Figs. 7, 8). Only (4*S*,5*S*)-ferrugineol is produced by and

Figure 4. Total number of *R. ferrugineus* and *R. vulneratus* captured in Exp. 2 in traps baited with palm wood alone (control), in combination with ferrugineol at 3 mg per 24 h (10:0), or with ferrugineol and ferrugineone in three different ratios; BALITKA, 28 Aug - 4 Sept, 1992; N= 10. Bars followed by the same letter are not significantly different, X^2 test, $P > 0.05$.

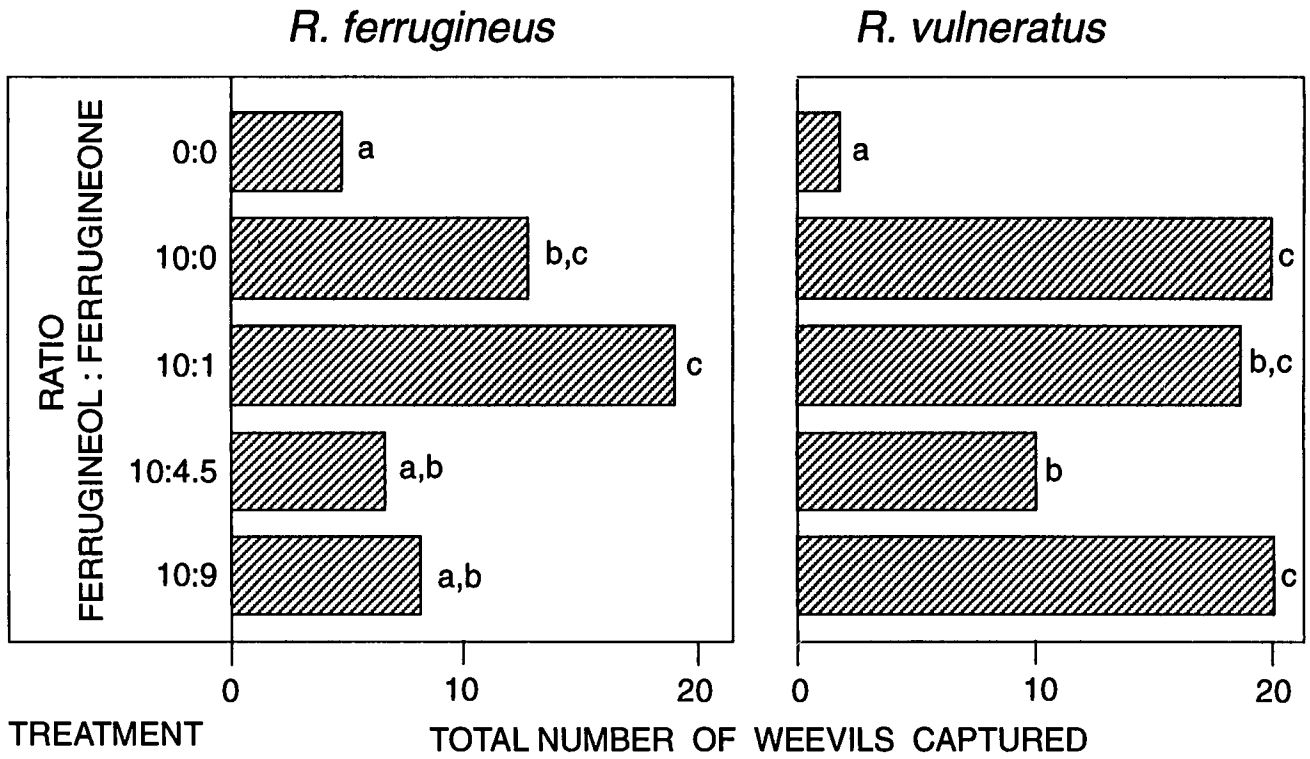

R. ferrugineus
R. vulneratus
RATIO
FERRUGINEOL : FERRUGINEONE
0:0
10:0
10:1
10:4.5
10:9
a
b,c
c
a,b
a,b
a
c
b,c
b
c
0
10
20
0
10
20
TREATMENT
TOTAL NUMBER OF WEEVILS CAPTURED

Figure 5. Time-dependent attraction of both *R. ferrugineus* and *R. vulneratus* in Exp. 2 to coconut wood alone or in combination with ferrugineol or with ferrugineol and ferrugineone. Significantly more weevils were captured on day 5 than on days 2 or 7 (X^2= 17.21, df = 2, P< 0.001).

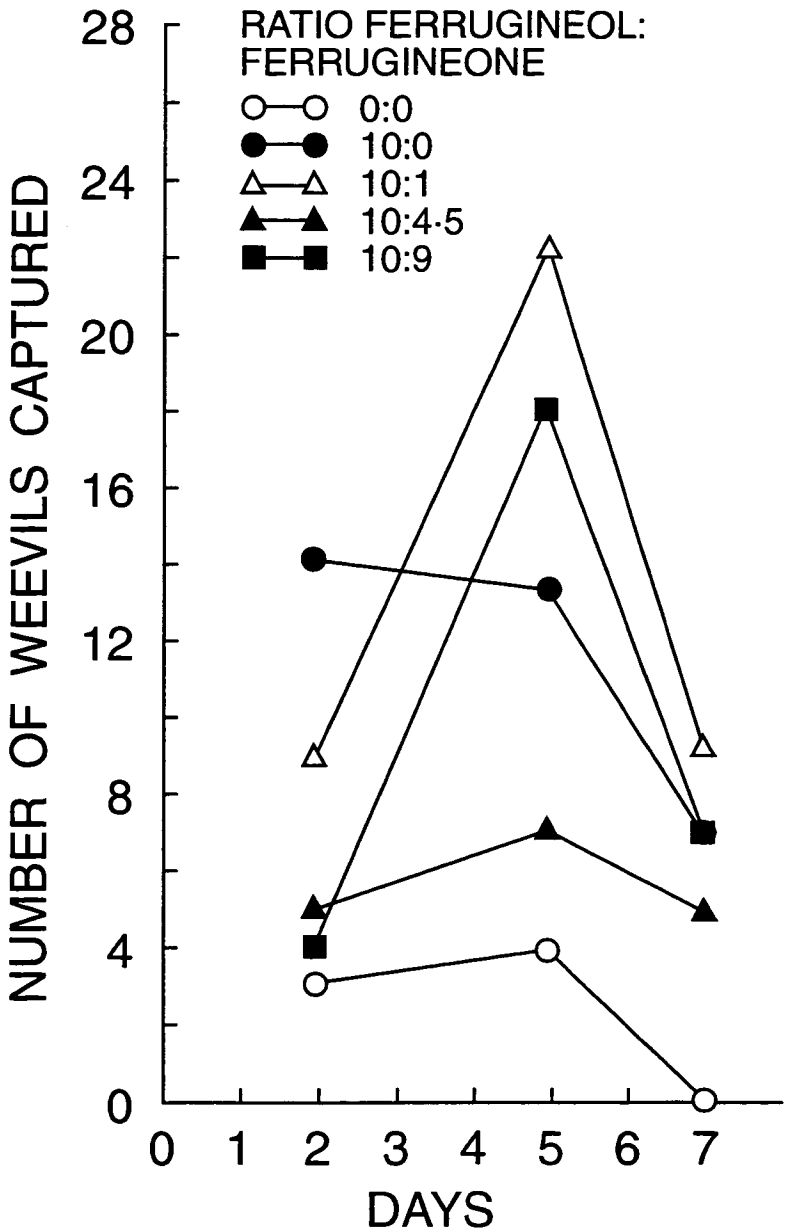
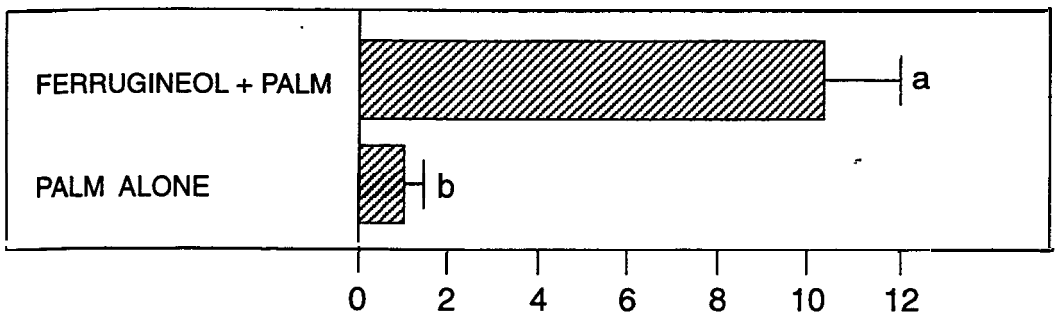

RATIO FERRUGINEOL:
FERRUGINEONE
0:0
10:0
10:1
10:4·5
10:9
NUMBER OF WEEVILS CAPTURED
28
24
20
16
12
8
4
0
0 1 2 3 4 5 6 7
DAYS

Figure 6. Mean number (+ S.E.) of *R. ferrugineus* captured in bucket traps in the UAE. Numbers in parentheses indicate total catch. **Exp. 3:** traps baited with date palm wood alone or in combination with ferrugineol at 3 mg per 24 h; N= 10, 15 April - 9 May 1993, Ras Al Kaimeh; ANOVA, F= 4.76, df= 10,9, $P < 0.01$. **Exp. 4:** traps baited with date palm wood alone or in combination with ferrugineol (3 mg per 24 h), ferrugineone (0.3 mg per 24 h) or both; N= 9, 13 April - 9 May 1993, Northern Region Research Station; ANOVA, F= 3.04, df= 11,24, $P< 0.01$. In each experiment, bars followed by the same letter are not significantly different, Bonferroni t-tests, $P> 0.05$.

Experiment 3



Experiment 4

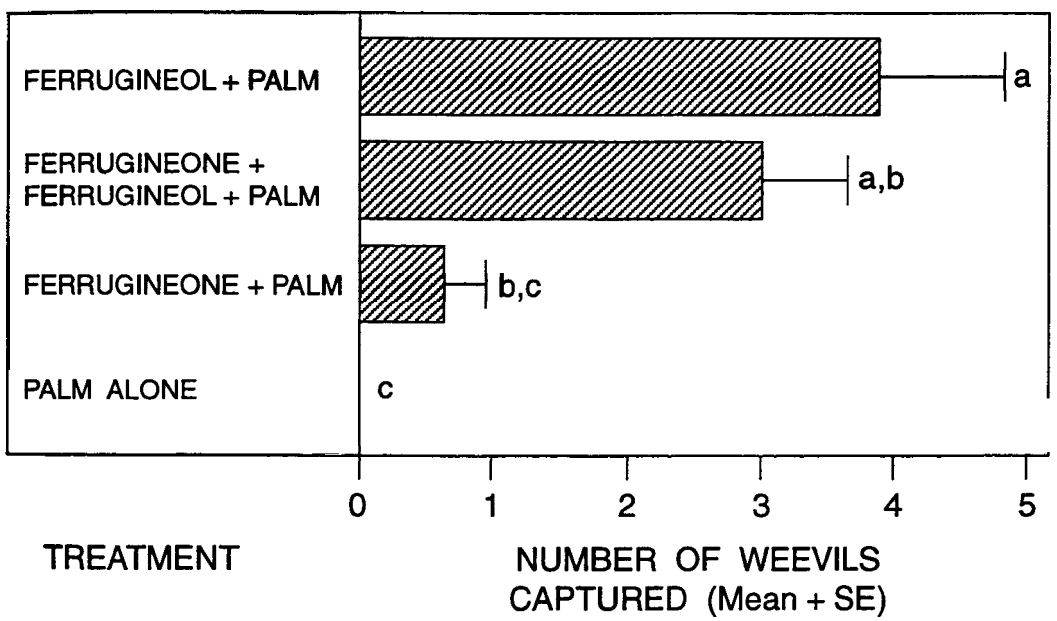


Figure 7. Representative GC-EAD recordings of female *R. ferrugineus* and *R. vulneratus* antennae responding to stereoisomers of 4-methyl-5-nonanone (ferrugineone) and 4-methyl-5-nonanol (ferrugineol) (Hewlett Packard 5890A; Cyclodex-B column; 100°C isothermal; split injector; linear flow velocity of carrier gas: 35 cm/sec; injector temperature: 220°C). Different weevil antennae and chart speeds of 0.5 and 1.0 cm per min were used for analyses of ferrugineone and ferrugineol, respectively.

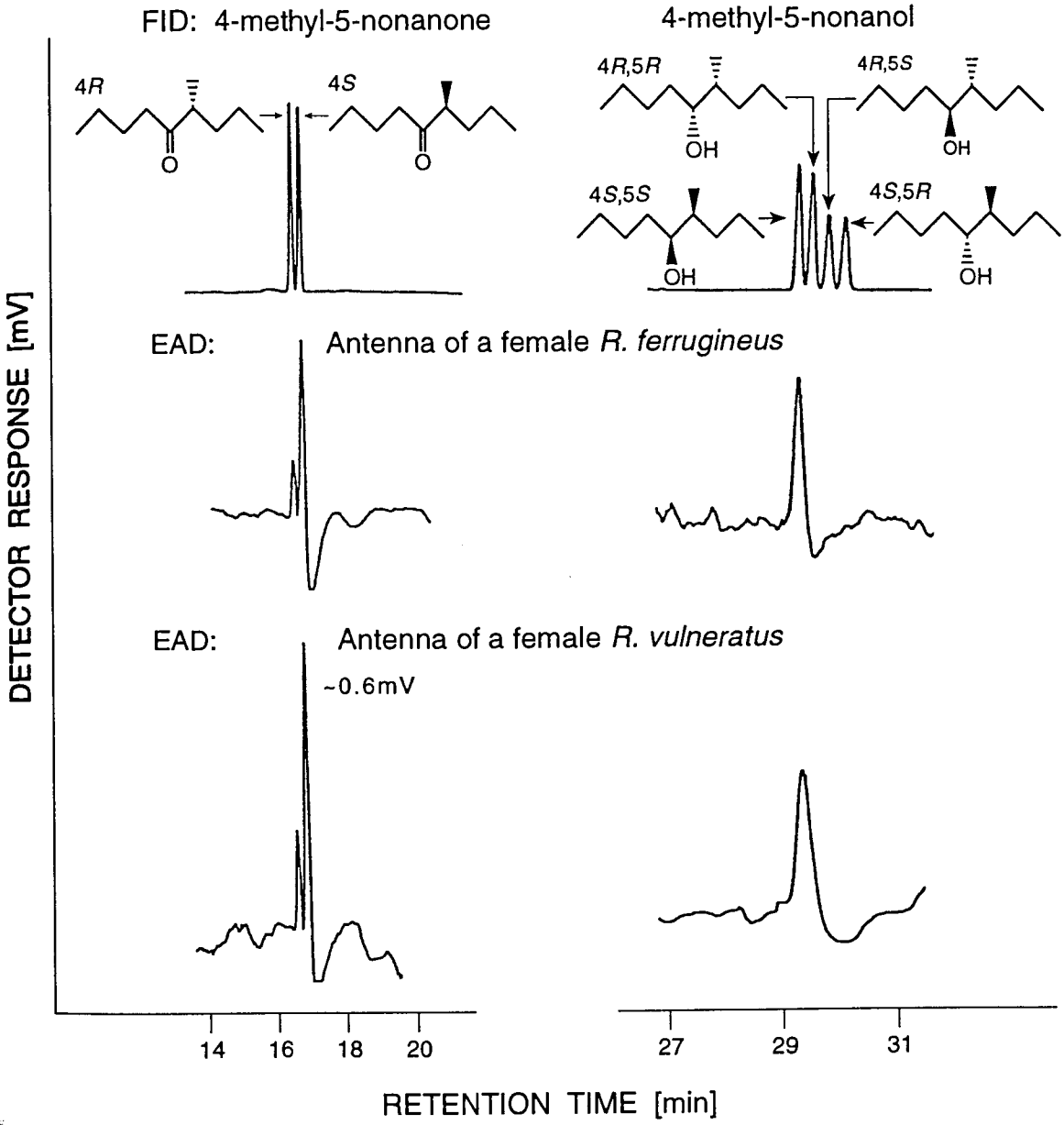
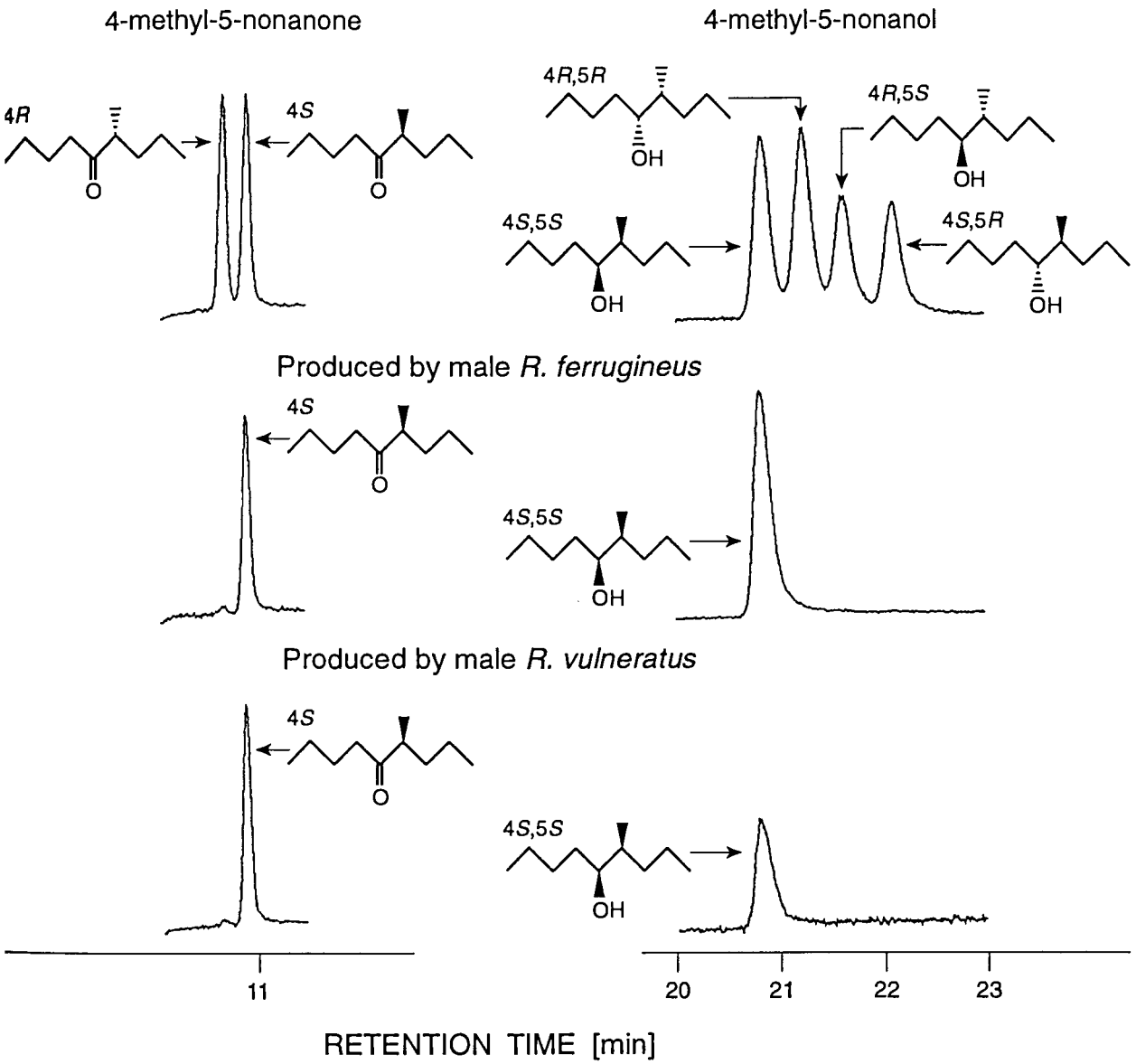

FID: 4-methyl-5-nonanone
4-methyl-5-nonanol
4R
4S
4R,5R
4R,5S
4S,5S
4S,5R
OH
EAD:
Antenna of a female R. ferrugineus
EAD:
Antenna of a female R. vulneratus
~0.6mV
DETECTOR RESPONSE [mV]
14
16
18
20
27
29
31
RETENTION TIME [min]

Figure 8. Selected ion m/z 157 and m/z 141 chromatograms (Hewlett Packard 5985B GC-MS) of stereoisomeric and weevil-produced 4-methyl-5-nonanone (ferrugineone) and 4-methyl-5-nonanol (ferrugineol). m/z 157 and m/z 141 were respectively the parent ions of the full scan mass spectra in CI mode of ferrugineone [$(M^+ + H)$ 157] and ferrugineol [$(M^+ + H)$ 159, $(M^+ - H_2O$ 141] (column and chromatographic conditions as in Figure 7). Relative abundance of ferrugineone and ferrugineol in these ion chromatograms do not represent natural ratios.


4-methyl-5-nonanone
4-methyl-5-nonanol
4R
4S
O
4R,5R
4R,5S
OH
4S,5S
4S,5R
Produced by male R. ferrugineus
Produced by male R. vulneratus
11
20
21
22
23
RETENTION TIME [min]

elicits antennal response by both *R. ferrugineus* and *R. vulneratus* (Figs. 7, 8).

Ferrugineol released at 0.4 mg per 24 h attracted statistically the same number of weevils of both species to traps as it did released at 0.8 mg per 24 h in Exp. 5; at both doses significantly more weevils were attracted than to control traps (X^2= 12.56, df = 1, P< 0.01). Releasing stereoisomeric ferrugineol at 0.4 mg per 24 h in further experiments allowed chiral ferrugineol and ferrugineone to be tested at low doses, thereby conserving these synthetic compounds.

Stereoisomeric ferrugineol or (*4S,5S*)-ferrugineol , but not (*4R,5R*)-ferrugineol, significantly enhanced attraction of both *R. ferrugineus* and *R. vulneratus* in Exp. 6 to traps baited with coconut stem tissue (Fig. 9). No significant differences in attraction were found between the two species (X^2= 0.467, df= 3, P>0.90).

Exp. 7 at BALITKA, West Java, using palm wood alone or in combination with (*R,S*)-ferrugineone, (*S*)-ferrugineone or (*R*)-ferrugineone (Table 3), indicated no significant differences in weevil captures between treatments for *R. ferrugineus* or *R. vulneratus* and no significant differences in response between the two species.

For both *R. ferrugineus* and *R. vulneratus* in Exp. 8, no significant differences were found between traps containing (4*S*,5*S*)-ferrugineol alone or in a 1:4 ratio with either (*S*)-ferrugineone, (*R*)-ferrugineone or (*R,S*)-ferrugineone (Table 4).

Figure 9. Total captures in Exp. 6 of *R. ferrugineus* (X^2= 41.157, df= 3, P< 0.01) and *R. vulneratus* (X^2= 36.987, df= 3, P< 0.01) in bucket traps baited with stereoisomers of ferrugineol and coconut stem tissue (BALITKA, 16-30 August 1993; N= 20). Bars followed by the same letter are not significantly different (X^2 tests, P> 0.05).

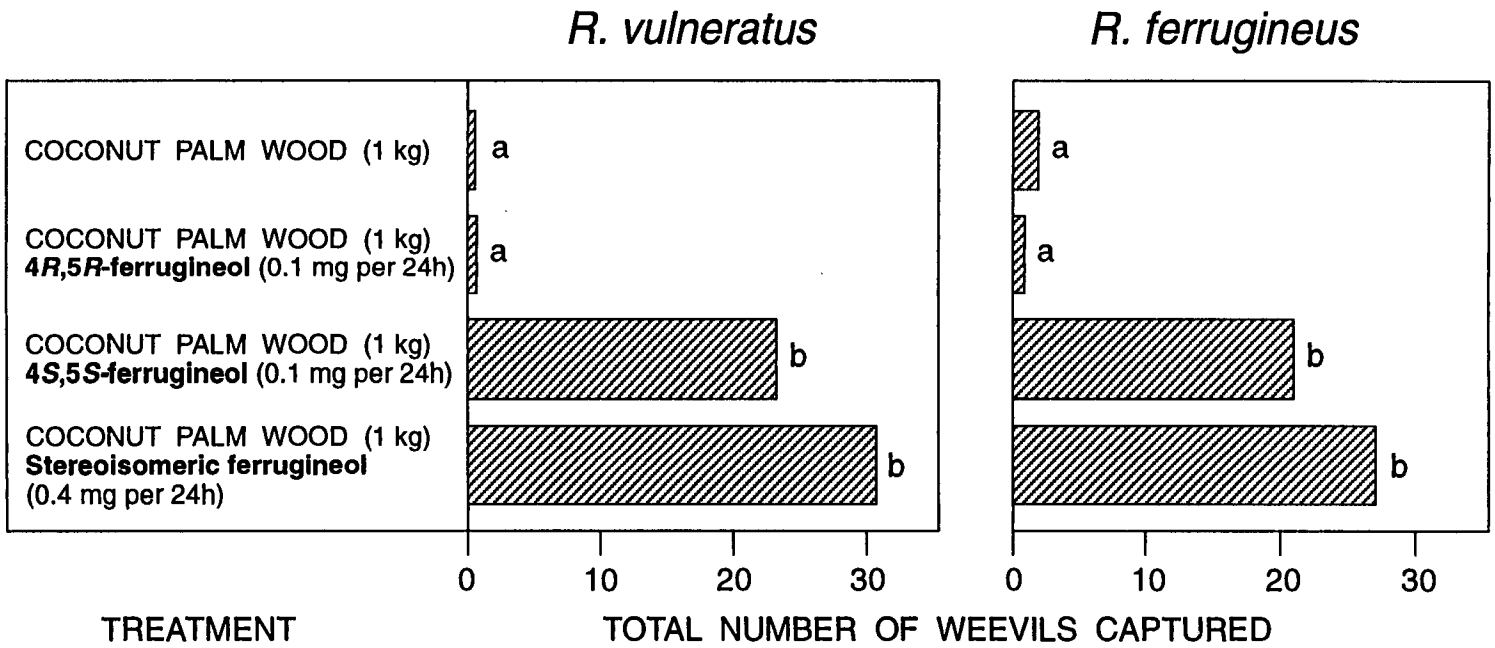

R. vulneratus
R. ferrugineus
COCONUT PALM WOOD (1 kg)
COCONUT PALM WOOD (1 kg)
4R,5R-ferrugineol (0.1 mg per 24h)
COCONUT PALM WOOD (1 kg)
4S,5S-ferrugineol (0.1 mg per 24h)
COCONUT PALM WOOD (1 kg)
Stereoisomeric ferrugineol
(0.4 mg per 24h)
a
a
b
b
a
a
b
b
0
10
20
30
0
10
20
30
TREATMENT
TOTAL NUMBER OF WEEVILS CAPTURED

Table 3. Total captures in Exp. 7 of *R. ferrugineus* and *R. vulneratus* in traps containing palm wood alone, or in combination with (*R*,*S*)-ferrugineone or one of its stereoisomers (BALITKA, 29 November - 4 December, 7-15 December 1993, N= 20).

Treatment	*R. ferrugineus*[a,b]	*R. vulneratus*[a,b]
(*R*,*S*)-Ferrugineone + palm	3	4
(*S*)-Ferrugineone + palm	8	11
(*R*)-Ferrugineone + palm	6	7
Palm alone	3	5

[a] Between-treatment differences were not significant for *R. ferrugineus* (X^2 = 3.60, df = 3, P> 0.25) or *R. vulneratus* (X^2 = 4.259, df = 3, P>0.20).

[b] Patterns of response were not significantly different between species (X^2 = 0.154, df = 3, P> 0.95).

Table 4. Total captures in Exp. 8 of *R. ferrugineus* and *R. vulneratus* in traps baited with (4*S*,5*S*)-ferrugineol alone or in a 1:4 ratio with (*R*,*S*)-ferrugineone, (*S*)-ferrugineone or (*R*)-ferrugineone (BALITKA, 15-28 December 1993, N= 20).

Treatment	*R. ferrugineus*[a]	*R. vulneratus*[a]
(4*S*,5*S*)-Ferrugineol + palm	21	10
(4*S*,5*S*)-Ferrugineol + (*R*,*S*)-ferrugineone + palm	15	21
(4*S*,5*S*)-Ferrugineol + (*S*)-ferrugineone + palm	8	11
(4*S*,5*S*)-Ferrugineol + (*R*)-ferrugineone + palm	10	16

[a] Between-treatment differences were not significant for *R. ferrugineus* (X^2= 7.841, df= 3, P> 0.05) or *R. vulneratus* (X^2= 5.310, df= 3, P> 0.10).

In Exp. 9, no differences were found in response of *R. vulneratus* to traps containing (4*S*,5*S*)-ferrugineol alone or in a 10:1 ratio with either (*R*,*S*)-ferrugineone, (*S*)-ferrugineone, or (*R*)-ferrugineone (X^2= 6.22, df= 3, P> 0.10) (Table 5). However, when treatments were analysed on the basis of whether they contained ferrugineone or not, traps containing ferrugineone captured about twice as many weevils as traps containing (4*S*,5*S*)-ferrugineol alone (X^2= 5.042, df= 1, P< 0.025).

Comparison of captures in traps baited with palm wood and (4*S*,5*S*)-ferrugineol plus either (*R*,*S*)-ferrugineone, (*S*)-ferrugineone or (*R*)-ferrugineone at the natural 10: 1 ratio of ferrugineol : ferrugineone in Exp. 10 failed to confirm any behavioral activity of (*R*,*S*)-ferrugineone or its enantiomers (Fig. 10). No significant differences were found between the two species or between the two time periods in which the experiment was conducted.

DISCUSSION

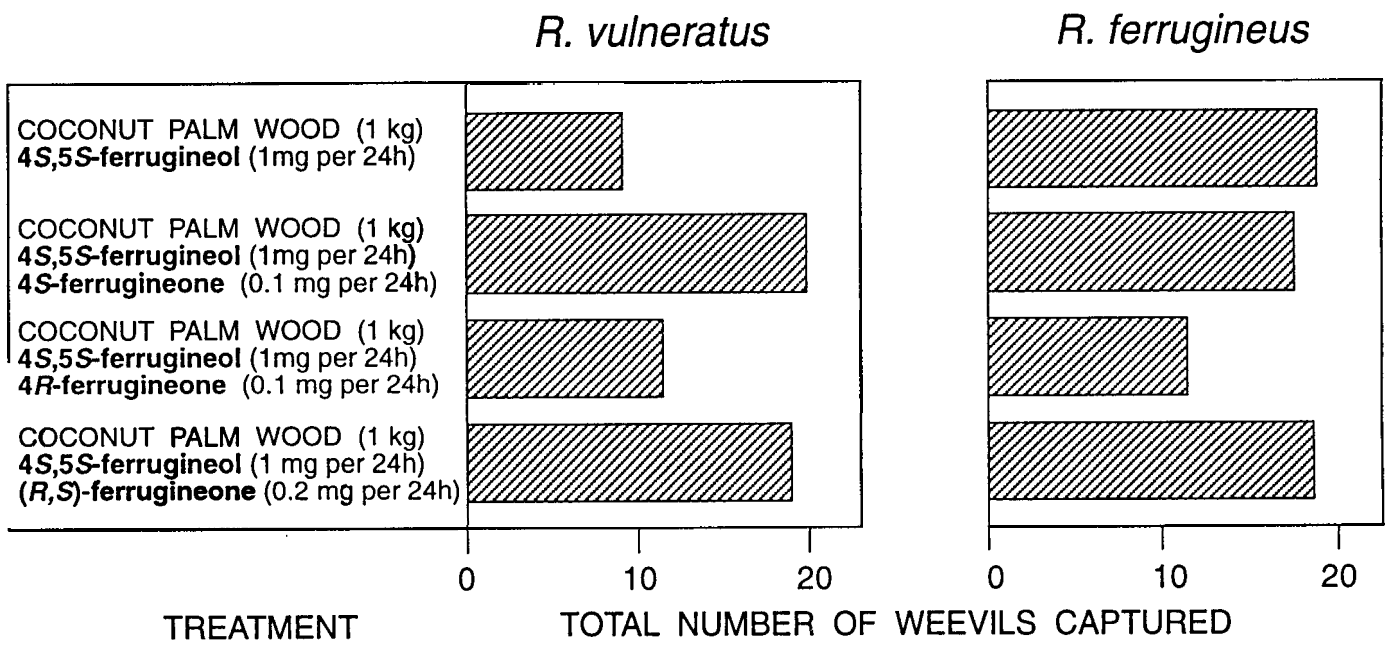
The results of this study uphold the hypothesis that *R. ferrugineus* and *R. vulneratus* use pheromone-based communication. 4-Methyl-5-nonanol (ferrugineol) was produced by male *R. ferrugineus* and *R. vulneratus* and attracted both males and females in the field; thus it can be classified as an aggregation pheromone (Borden, 1985) for both species. 4-Methyl-5-nonanol is also produced by *R. palmarum* (A.C. Oehlschlager, G. Gries, H.D. Pierce Jr., E. Czyzewska, unpublished

Table 5. Total captures in Exp. 9 of *R. vulneratus* in traps baited with (4*S*,5*S*)-ferrugineol alone or in a 10:1 ratio with (*R*,*S*)-ferrugineone, (*S*)-ferrugineone or (*R*)-ferrugineone (Bah Lias Estate, North Sumatra, 22 March - 10 April 1994, N= 9).

Treatment	*R. vulneratus*[a]
(4*S*,5*S*)-Ferrugineol + palm	14
(4*S*,5*S*)-Ferrugineol + (*R*,*S*)-ferrugineone + palm	25
(4*S*,5*S*)-Ferrugineol + (*S*)-ferrugineone + palm	29
(4*S*,5*S*)-Ferrugineol + (*R*)-ferrugineone + palm	29

[a] Between-treatment differences not significant (X^2= 6.22, df= 3, P> 0.10)

Figure 10. Total captures in Exp. 10 of *R. ferrugineus* and *R. vulneratus* in bucket traps baited with coconut stem tissue, (4*S*,5*S*)-ferrugineol and isomers of ferrugineone in a 10:1 ratio (BALITKA, 9-21 July and 21 November - 6 December 1994, N= 19). Treatment differences not significant for either species, X^2 test, $P > 0.05$. No significant differences were found between the two species ($X^2 = 1.699$, df = 3, $P > 0.50$) or between the two time periods in which the experiment was conducted (*R. ferrugineus*: $X^2 = 6.350$, df = 3, $P > 0.05$; *R. vulneratus:* $X^2 = 3.206$, df = 3, $P > 0.25$).


R. vulneratus
R. ferrugineus
COCONUT PALM WOOD (1 kg)
4S,5S-ferrugineol (1mg per 24h)
COCONUT PALM WOOD (1 kg)
4S,5S-ferrugineol (1mg per 24h)
4S-ferrugineone (0.1 mg per 24h)
COCONUT PALM WOOD (1 kg)
4S,5S-ferrugineol (1mg per 24h)
4R-ferrugineone (0.1 mg per 24h)
COCONUT PALM WOOD (1 kg)
4S,5S-ferrugineol (1 mg per 24h)
(R,S)-ferrugineone (0.2 mg per 24h)
0
10
20
0
10
20
TREATMENT
TOTAL NUMBER OF WEEVILS CAPTURED

data[3]). Ferrugineol is also the major aggregation pheromone for several other Rhynchophorine palm weevils: *Rhynchophorus bilineatus* (Oehlschlager *et al.*, 1995a), *Metamasius hemipterus* (Rochat *et al.*, 1993a; Perez-Sanchez, 1996) and *Dynamis borassi* (R.M. Giblin-Davis, T.J. Weissling, G. Gries, R. Gries, A.L. Perez, A.C. Oehlschlager, unpublished data[4]). The commonality of ferrugineol among different species suggests that it may be a primitive character in the Rhynchophorinae (Giblin-Davis *et al.*, 1996). *R. ferrugineus* and *R. vulneratus* are not sympatric with any of these other species, which may have precluded the need to develop species-specific pheromone components.

Although 4-methyl-5-nonanone (ferrugineone) was antennally active in both Asian species (Fig. 2), its role as a pheromone is unclear. Although it affected the response *of R. ferrugineus*, but not *R. vulneratus* in Java (Fig. 4, Exp. 2), there was some indication that addition of ferrugineone to ferrugineol enhanced response of *R. vulneratus* in North Sumatra to ferrugineol (Exp. 9, Table 5). *R. ferrugineus* from Indonesia and the United Arab Emirates were equally attracted by ferrugineol alone and in a 10:1 ratio with ferrugineone.

Since neither of the two Asian species of palm weevil rely upon mass attack to overcome host defenses and kill the host in order for brood

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production to be successful, it is likely that the aggregation pheromone functions in part to ensure reproduction. To date, there has been little examination of courtship and reproductive behaviour in palm weevils; however it is likely that the major male mating strategy is a version of male dominance polygyny (Emlen & Oring, 1977), Borgia's (1979) "strategy A". In this strategy females have free access to resources, and choose between males on the basis of differences in genetic quality. This strategy may exist in the palm weevils, since other strategies (Emlen & Oring, 1977; Borgia, 1979) involve the procurement and defense of resources or females. If the resource in question is a palm trunk or crown, a single male would not be able to control the entire resource. In addition, this strategy seems to be the most applicable to a system involving an aggregation pheromone. However, it is also possible that the resources that male palm weevils compete for and defend are wound sites. The mating strategy could therefore be resource defense polygyny, and in order to secure use of a wound for oviposition a female must mate with the male guarding it (Emlen & Oring, 1977), Borgia's (1979) "strategy C". This strategy is most prevalent in habitats where resources are unevenly distributed, with the result that there is great variation in quality of male territories, as also occurs for many scolytids (Atkins, 1966). While palms are widespread, suitable wounds for oviposition may be limited and unevenly distributed. The wounds themselves may also vary in quality. In this mating system, females should choose a mate on the basis of both

the quality of the defending male and the resources that he controls (Emlen & Oring, 1977).

Palm weevils probably benefit from other secondary advantages that are common to aggregation pheromones, such as protection from predators and the pooling of information about resource locations (Emlen & Oring, 1977). *Rhynchophorus* adults have dramatic red and black colouration that probably functions aposematically. The formation of groups of these weevils may result in the amplification of the aposematic warning signal directed at potential predators, thereby providing mutualistic protection (Borden, 1985). Secondary advantages such as these are considered to be of the most importance to species that have a long period of sexual activity and aggregate for long periods of time (Emlen & Oring, 1977). Palm weevil adults live for 3-4 months (Lever, 1969), so these benefits are likely to be significant.

Rhynchophorus palm weevils produce single isomers of chiral alcohols as aggregation pheromones (Oehlschlager *et al.*, 1992, 1995a; Perez *et al.*, 1994). As reported in other Rhynchophorinae (Oehlschlager *et al.*, 1992, 1995a; Perez *et al.*, 1993, 1994), non-natural stereoisomers of the aggregation pheromone neither enhanced nor reduced attraction to the natural isomer (Fig. 9, Exp. 6). Lack of pronounced interspecific differences in response to stereoisomeric ferrugineol (Fig. 9, Exp. 6) suggested that pheromone chirality may impart species-specificity of semiochemical communication. Reproductive isolation can be maintained by species-specificity of geometrical and/or chiral pheromone isomers

(Bestmann & Vostrowsky, 1988). In two sympatric bark beetles, *Dryocoetes confusus* and *D. affaber*, reproductive isolation is maintained, at least in part, by species-specific blends of the geometrical and optical isomers of aggregation pheromone components (Camacho *et al.*, 1993, 1994). The ambrosia beetle, *Gnathotrichus sulcatus* produces and responds to a mixture of the (*S*)-(+)- and (*R*)-(-)-enantiomers of its aggregation pheromone, sulcatol, but *G. retusus* responds only to (*S*)-(+)-sulcatol and not to the racemic mixture (Borden *et al.*, 1976, 1980a). The pine engraver, *Ips pini*, responds to (*R*)-(-)-ipsdienol, and is inhibited by (*S*)-(+)-ipsdienol which is a component of the pheromone of the competing species, *I. paraconfusus* (Birch *et al.*, 1980) However, both *R. ferrugineus* and *R. vulneratus* produce (4*S*,5*S*)-ferrugineol as the main aggregation pheromone. *Sitophilus oryzae* and *S. zeamais*, also members of the Rhynchophorinae, utilise the identical stereoisomer of their male-produced aggregation pheromone, (4*S*, 5*R*)-5-hydroxy-4-methyl-3-heptanone (Walgenbach *et al.*, 1987).

I therefore hypothesized that enantiospecific production of and response to the minor component, ferrugineone, may impart species-specificity of pheromone communication in *R. ferrugineus* and *R. vulneratus*. However, GC-EAD and GC-MS-SIM analyses on a Cyclodex-B column of weevil-produced ferrugineone, synthetic (*R*,*S*)-ferrugineone and each of the two enantiomers indicated that both *R. vulneratus* and *R. ferrugineus* produce and antennally respond more strongly to (*S*)- than to (*R*)-ferrugineone (Figs. 7, 8). Furthermore, comparison of capture rates of

traps baited with palm wood and (4*S*,5*S*)-ferrugineol plus either (*R*,*S*)-, (*S*)- or (*R*)-ferrugineone at an artificial 1:4 ratio or at the natural 10:1 ratio did not indicate any consistent behavioral activity of (*R*,*S*)-ferrugineone or its enantiomers (Table 4, Fig. 10). Thus, in Java ferrugineone did not have significant behavioral activity in either weevil and did not impart specificity of pheromone communication. Throughout their wide geographic distribution, populations of *R. ferrugineus* and/or *R. vulneratus* may exist that respond to ferrugineone, as possibly indicated in North Sumatran populations in Exp. 9 (Table 5).

Since ferrugineol attracts both male and female weevils, it has great potential for use in a mass-trapping program against these palm weevils. The apparent lack of pheromonal differences between *R. ferrugineus* and *R. vulneratus*, means that the same traps and pheromone lures can be used to trap both species. In addition, the absence of differences in the pheromonal responses of *R. ferrugineus* from Indonesia and the United Arab Emirates suggests that significant changes have not occurred in pheromone production and response of these widely separated populations. This has important implications for control of this palm weevil as it indicates that the same pheromone lures can be used throughout the entire, expanding geographic range of *R. ferrugineus*.

In some experiments, trap captures may be considered too low to be effective in a mass-trapping control program against these palm pests. However, large insects generally have low population densities, e.g. 23-57 *R. palmarum* per ha in a large Costa Rican oil palm plantation

(Chinchilla *et al.*, 1993). Moreover, the capture of a single female can have a tremendous impact because each female is capable of laying several hundred eggs (Kalshoven, 1950). If trapping is continued over several generations, eventually emerging weevils that have not yet started new infestations may be captured, and damage rates may be reduced rapidly. Finally, various aspects of trapping protocol may need to be improved, e.g. trap design and development of an optimally effective and persistent host kairomonal stimulus.

Lack of pronounced differences in pheromonal production and response present a challenge to Wattanapongsiri's (1966) conclusion that *R. ferrugineus* and *R. vulneratus* are distinct species. Reproductive isolation between species could not be achieved by specificity of pheromone isomers (Figs. 7-10; Tables 3-5). If the species are valid, reproductive isolation must be achieved by other mechanisms.

CHAPTER 3

AGGREGATION PHEROMONE IN *ORYCTES RHINOCEROS*

INTRODUCTION

Oryctes rhinoceros adults are gregarious. More than one beetle may attack a given palm at the same time while a neighboring tree is unattacked (Gressit, 1953). Aggregation of adults in decaying palm trunks to mate, and the occurrence of both single and multiple pairs of adults in the same breeding site (Zelazny & Alfiler, 1991), suggest that *O. rhinoceros* is attracted to host kairomones and employs either an aggregation or sex pheromone or both. Sex pheromones have been identified in a number of scarabaeids. However an aggregation pheromone has been found only in *O. monoceros* (Gries *et al.*, 1994b), and evidence of semiochemically-mediated aggregation has been observed in the green june beetle, *Cotinis nitida* (L.) (Domek & Johnson, 1988).

METHODS

Identification of Candidate Aggregation Pheromone Components

Larvae, pupae or adults of *O. rhinoceros* were collected at Parungkuda, West Java, Indonesia. Fourteen females and 16 males were separately aerated for one week in modified Nalgene desiccators containing sugarcane. Insect- and host-produced volatiles were collected

according to procedures outlined in Chapter 2, with the exception that the airstream (2 L per min) through the aeration chamber was water-driven rather than drawn by a vacuum pump. In a second series of aerations, 10 females and 10 males were aerated separately. The concentration of volatiles was adjusted so that 1 μL equaled 0.6 beetle-hours of production.

Porapak Q-captured volatiles from both males and females were analyzed by GC-EAD, GC, and GC-MS as in Chapter 2 except that columns employed were either fused silica (30 m x 0.25 mm ID) or glass (30 m x 0.5 mm ID) columns coated with SP-1000 (Supelco, Mississauga, Ontario).

Laboratory bioassays were conducted using a white Plexiglas Y-shaped olfactometer (stem 5 x 5 x 20 cm, arms 5 x 5 x 15 cm) with a clear lid. Stimuli were syringed onto filter paper inside of a glass cartridge placed in each arm of the olfactometer. Compressed air at 200 mL per min carried the volatiles through each arm towards the beetles which were released singly into the stem of the olfactometer 10 cm from the Y-junction. A baffle prevented mixing of the two stimuli in the Y-junction. Beetles with intact antennae and walking ability were held 2-3 h in separate containers without food prior to testing. For each bioassay, the paper lining on the olfactometer floor was replaced, and beetle and positions of stimuli were randomly selected. A beetle that did not move forward within 5 min of introduction was classed as a non-responder, whereas complete entrance of a beetle into one of the arms was recorded

as a response. In two experiments, 10 μL of Porapak Q extract from males (Exp. 11) or females (Exp. 12) were tested against 10 μL of hexane. In order to determine behavioral activity of two synthetic male-produced compounds, a 612 ng dose of ethyl 4-methyloctanoate, and ethyl 4-methylheptanoate, in a 100:2 ratio was tested against 10 μL of hexane (Exp. 13).

Field Experiments with Candidate Aggregation Pheromone Components

Experiments were conducted in 1- or 2-year old oil palm plantings at three London Sumatra Indonesia Plantation Co. estates in North Sumatra, Indonesia. Specific locations are given in Figure and Table captions. All experiments were set up as randomized complete blocks with inter-trap and inter-block distances of at least 27 and 54 m, respectively. In all experiments, each treatment was tested once in each block. Traps were checked daily and captured beetles removed.

All compounds were released either from heat-sealed, polymer membrane bag devices (Chem Tica International, Costa Rica) or from capillary tubes. Release devices, release rates, and sources for synthetic compounds, for Exps. 14-18 are given in Table 6.

A 3-treatment, 20-replicate experiment (Exp. 14) determined activity of the candidate pheromone, oryctelure. Black pitfall traps (Fig. 11) were buried in the ground 1 - 1.5 m from palms, and baited with either decomposing oil palm tissue (1 - 2 kg of leaf bases or empty fruit

Table 6. Summary of semiochemical release rates and release devices used in Exps. 14-18.

Exp.	Semiochemical	Release Rate (mg per 24 h)[a]	Release Device	Chemical Source[b]
14	Oryctelure	30	Polymer membrane bag lure[c]	CT
15	Oryctelure	30	Polymer membrane bag lure	CT
	4-Methyloctanoic acid	30	Polymer membrane bag lure	CTC
	Ethyl 4-methylheptanoate	30	Polymer membrane bag lure	CT
	Ethyl chrysanthemumate	30	Polymer membrane bag lure	Aldrich
16	Oryctelure	30	Polymer membrane bag lure	CT
	Ethyl 4-methylheptanoate	0.3	Capillary (1 mm ID)[d]	SFU
	Ethyl 4-methylheptanoate	3	Polymer membrane bag lure	CT
	Ethyl 4-methylheptanoate	30	Polymer membrane bag lure	CT

Exp.	Semiochemical	Release Rate (mg per 24 h)[a]	Release Device	Chemical Source[b]
17	Oryctelure	9	Polymer membrane bag lure	CT
	4-Methyloctanoic acid	30	Polymer membrane bag lure	CTC
	Ethyl 4-methylheptanoate	0.1		SFU
18	Oryctelure	4	16 capillaries (1 mm ID)	SFU
	(*S*)-Oryctelure[e]	2	8 capillaries (1 mm ID)	SFU
	(*R*)-Oryctelure[e]	2	8 capillaries (1 mm ID)	SFU

[a] Release rates determined by ChemTica International over 2-5 weeks under conditions of 25°C and 60% RH.

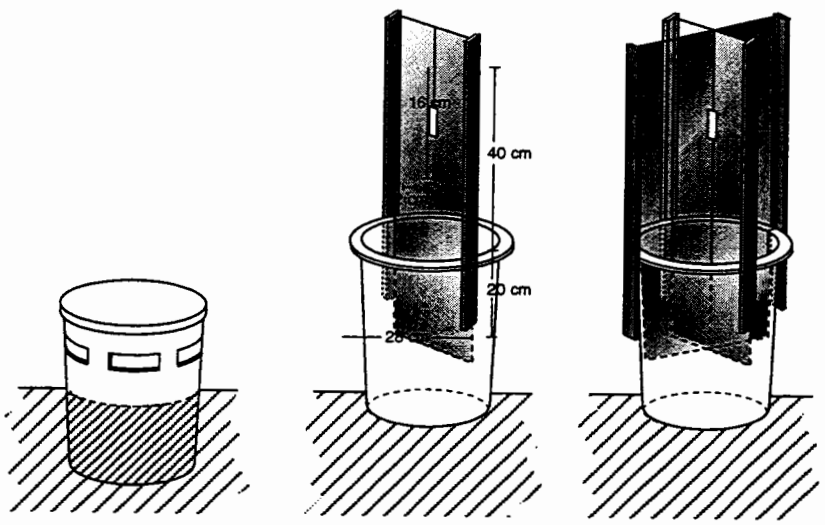
[b] Aldrich= Aldrich Chemical Co., Milwaukee, Wisconsin; CT= Chem Tica International, Costa Rica; SFU= Department of Chemistry, Simon Fraser University; CTC= CTC Organics, Atlanta, Georgia.

[c] Heat-sealed polymer membrane bag lures, Chem Tica International, Costa Rica.

[d] All capillary tubes opened 1 cm above liquid meniscus, placed inside capped polyethylene 400 μL microcentrifuge tubes (Quality Scientific Plastics, USA) with two 3 mm diam. holes drilled 1 cm below top and suspended 3-4 cm below trap lid.

[e] Assumed to be 97.5% ee, i.e. equivalent to chiral citronellol (Aldrich Chemical Co., Milwaukee, Wisconsin) used in synthesis (Perez-Sanchez, 1996).

Figure 11. Traps of three designs used for capturing *O. rhinoceros*. **Pitfall trap** used in Exps. 14 & 32: 19 L black bucket buried in ground to allow beetles to enter through slots below rim; pheromone lure suspended within bucket from plywood lid. **Barrier traps** tested in comparison to other two traps (Chapter 5, Exp. 32): 20 L white bucket with one unpainted sheet metal vane; wooden slats on edge of vane for reinforcement; lures suspended within slot to allow volatile dissemination in all directions. **Vane trap** used in Exps. 15-28, 23-25, 32-37: same as barrier trap, but with two vanes at right angles to each other to increase barrier surface and multidirectionality of trap. Sheet metal in barrier and vane traps extends 20 cm into bucket to interfere with attempts by beetles to fly out of trap.



PitfallTrap Barrier Trap Vane Trap

bunches), oryctelure, or both. Oil palm tissue, or a cloth placed in traps baited with oryctelure only, were treated with 0.3 % a.i. solution of Basudin 60 EC® (diazinon, Ciba-Geigy). Beetles were captured because of contact with the insecticide-treated substrate or inability to exit the traps.

Attraction of beetles to three male-produced compounds, oryctelure, 4-methyloctanoic acid, ethyl 4-methylheptanoate, and to (±)-ethyl chrysanthemumate [95 % pure, mixture of (*Z*) and (*E*)], was compared in Exp. 15 and replicated 10 times. All compounds were released at 30 mg per 24 h.

A 9-replicate experiment (Exp. 16) compared attraction of beetles to oryctelure alone and in combination with ethyl 4-methylheptanoate at ratios of 100:1, 100:10, and 100:100 (Rambong Sialang Estate, 13 - 21 April 1994).

A 10-replicate experiment (Exp. 17) compared attraction of beetles to oryctelure alone, or in approximate natural ratios with either or both of 4-methyloctanoic acid and ethyl 4-methylheptanoate (Rambong Sialang Estate, 9 - 17 June 1994).

Pheromone Chirality

Gas chromatographic and nuclear magnetic resonance analyses of (*S*)- and (*R*)-oryctelure failed to resolve the enantiomers, so that it was impossible to determine chemically which isomer of oryctelure is produced naturally (Perez-Sanchez, 1996).

A 4-treatment, 10-replicate experiment (Exp. 18) at Rambong Sialang Estate (23 May - 8 June 1994) tested attraction of beetles to racemic oryctelure, (*S*)-oryctelure, (*R*)-oryctelure, or a blank control in standard vane traps.

Statistical Analyses

The proportions of beetles responding to each stimulus in laboratory bioassays were compared using the normal approximation to the binomial test, and differences between responses of female and male beetles were compared using X^2 tests (Zar, 1984).

In all field experiments, no significant differences were found in the responses of male and female beetles ($P > 0.05$), so catches were pooled by sex for analysis. Data were transformed by $\log_e (x+1)$ if they were not normally distributed and were subjected to Analysis of Variance (General Linear Modeling) (Minitab, 1989). If replicates were run at different times or locations, data were analyzed for time x treatment or location x treatment interactions. Following ANOVA, multiple pairwise comparisons were made using Bonferroni *t*-tests. If homoscedasticity was not achieved by transformation, data were analyzed by X^2 tests (Zar, 1984).

RESULTS

Identification of Candidate Aggregation Pheromone Components

GC and GC-EAD analyses of Porapak Q-trapped volatiles obtained from aerations of either *O. rhinoceros* males or females revealed two abundant

male-specific components (Fig. 12), of which the early eluting volatile elicited antennal responses by male and female antennae (Fig. 13). Retention and mass spectrometric characteristics of these two compounds were identical to ethyl 4-methyloctanoate and 4-methyloctanoic acid. A second EAD-active compound (not visible in Fig. 13) had a Kovats retention index (RI = 1379) indicative of analogous ethyl 4-methylheptanoate. GC-MS in both electron impact and chemical ionization modes of beetle-produced and authentic ethyl 4-methylheptanoate confirmed this structural assignment.

In laboratory bioassays (Exps. 11 and 12), male-produced volatiles were equally attractive to walking male and female *O. rhinoceros*, but female-produced volatiles were attractive only to males (Table 7). Behavioral activity of synthetic ethyl 4-methyloctanoate plus ethyl 4-methylheptanoate was demonstrated in Exp. 13 (Table 8), and justified field testing of synthetic candidate pheromone components. Ethyl 4-methyloctanoate was assigned the trivial name oryctelure by Gries *et al.* (1994b).

Field Experiments with Candidate Aggregation Pheromone Components

In field experiments, oryctelure at 30 mg per 24 h alone or in combination with decomposing palm tissue attracted more beetles than palm tissue alone (Fig. 14; Exp. 14). Enantiomeric oryctelure was significantly more attractive than 4-methyloctanoic acid, ethyl 4-

Figure 12. Gas chromatograms of volatiles from 10 male and 10 female *O. rhinoceros* maintained in aeration chambers for 1 week with provision of sugarcane as a food source. Chromatography: Hewlett Packard 5830A gas chromatograph equipped with a glass capillary column (30 m x 0.5 mm ID) coated with SP-1000. Early-eluting compound designated by IUPAC formula is ethyl 4-methyloctanoate. Late-eluting compound is 4-methyloctanoic acid.

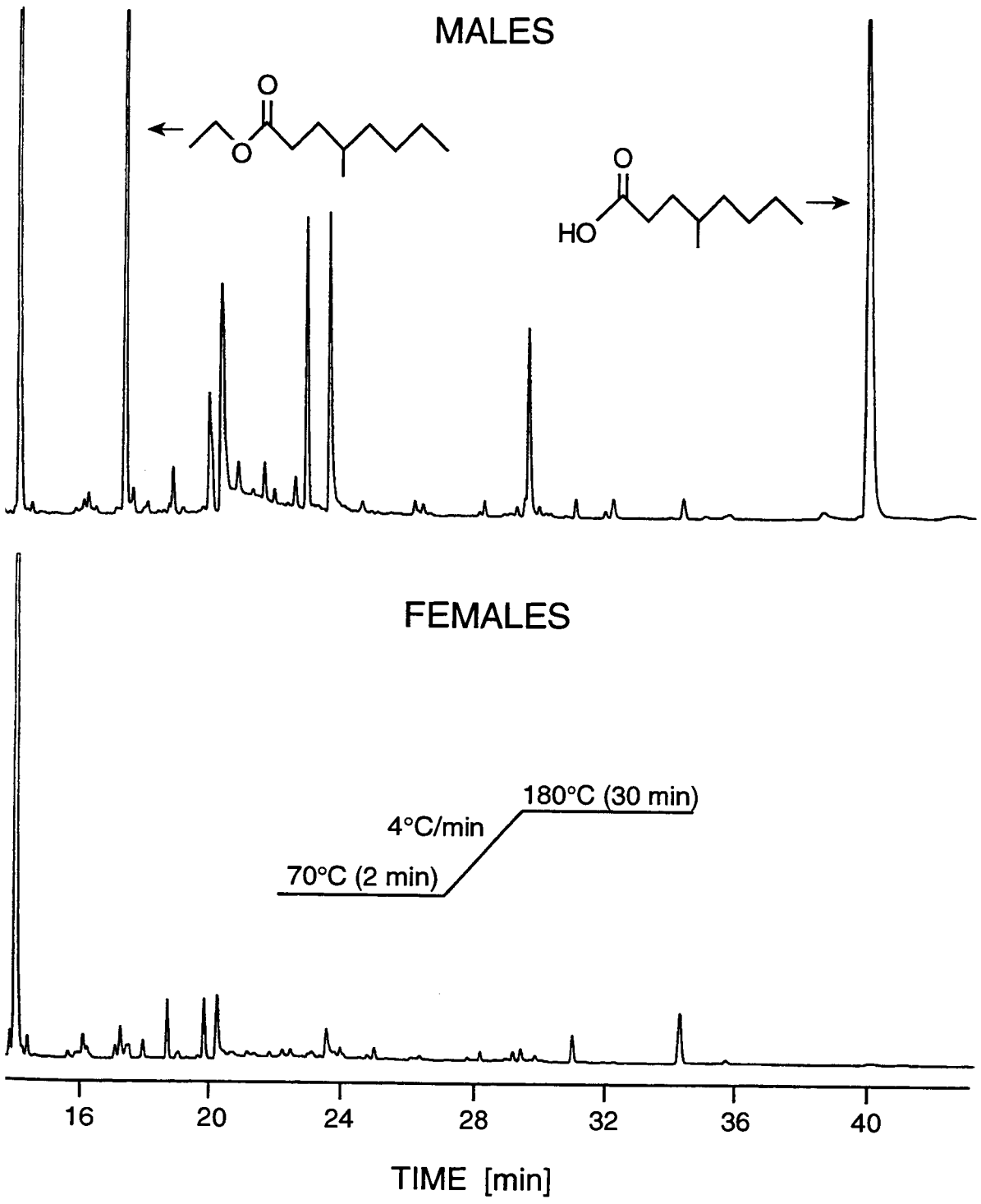

MALES
O
O
O
HO
FEMALES
180°C (30 min)
4°C/min
70°C (2 min)
16
20
24
28
32
36
40
TIME [min]

Figure 13. FID and EAD responses to volatiles obtained from male *O. rhinoceros*. The antennal recording was carried out with an antenna of a female beetle. Chromatography: Hewlett Packard 5890A gas chromatograph equipped with a fused silica column (30 m x 0.25 mm ID) coated with SP-1000. Antennally-active compound with RI= 1477 is ethyl 4-methyloctanoate.

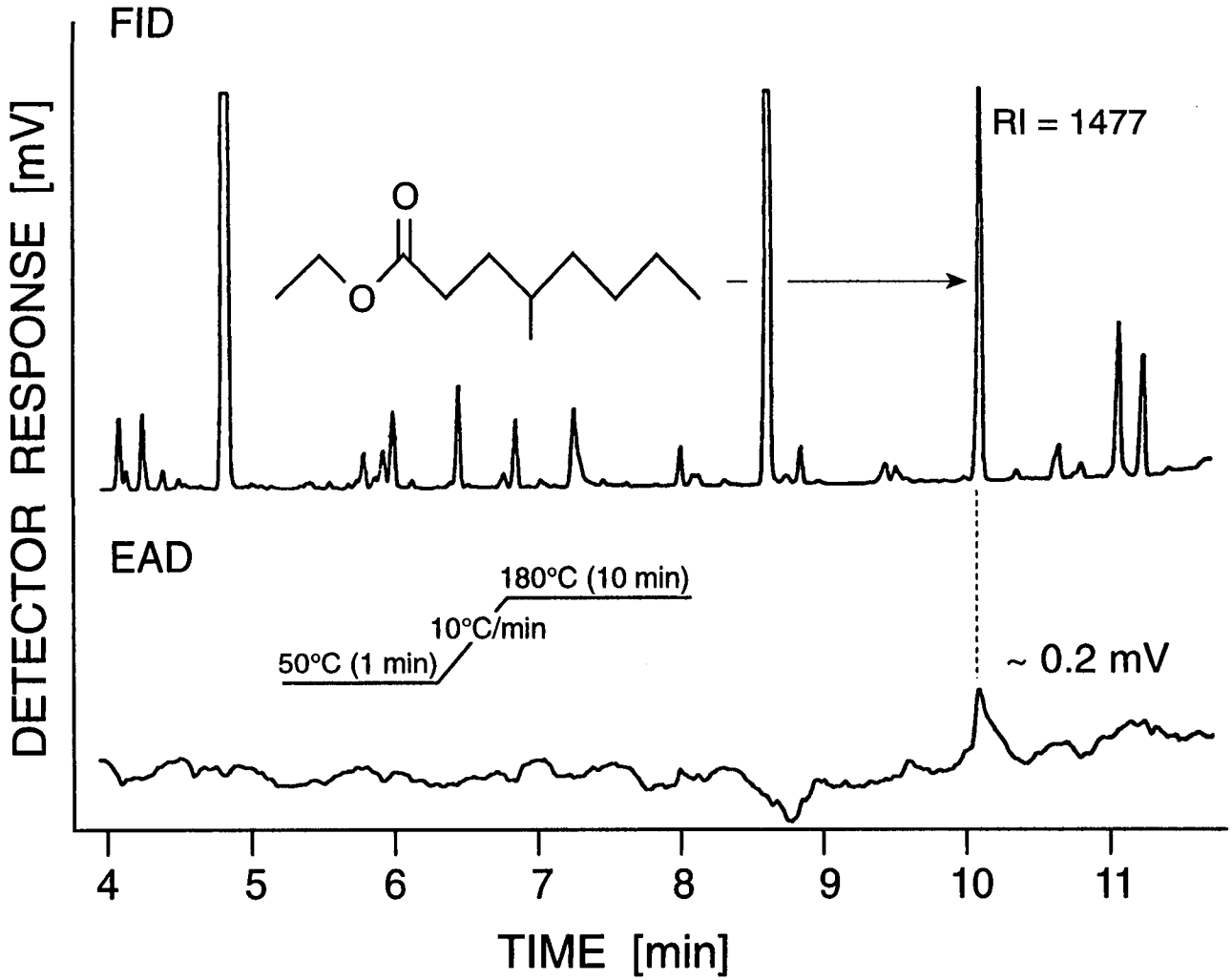

FID
RI = 1477
O
O
EAD
180°C (10 min)
10°C/min
50°C (1 min)
~ 0.2 mV
DETECTOR RESPONSE [mV]
4
5
6
7
8
9
10
11
TIME [min]

Table 7. Responses of male and female *Oryctes rhinoceros* tested individually to pentane extract of male- and female-produced volatiles (10 μL = 5.6 beetle-hours) *versus* 10 μL of hexane control in a Y-shaped olfactometer.

Exp. No. and description	Sex	n	Number of responders to stimuli[a] Extract	Control	*P* of difference between stimuli[b]
11 Female extract *versus* hexane control	Male	20	9	2	0.006
	Female	20	5	8	0.393
12 Male extract *versus* hexane control	Male	15	12	1	0.0001
	Female	17	13	3	0.0014

[a] Responses to female extract in Exp. 11 differ between males and females, X^2= 4.608, df= 1, *P*= 0.032. Responses to male extract in Exp. 12 are not significanlty different between males and females, X^2 = 0.738, df= 1, *P*= 0.39.

[b] Determined by normal approximation to the binomial test (Zar, 1984).

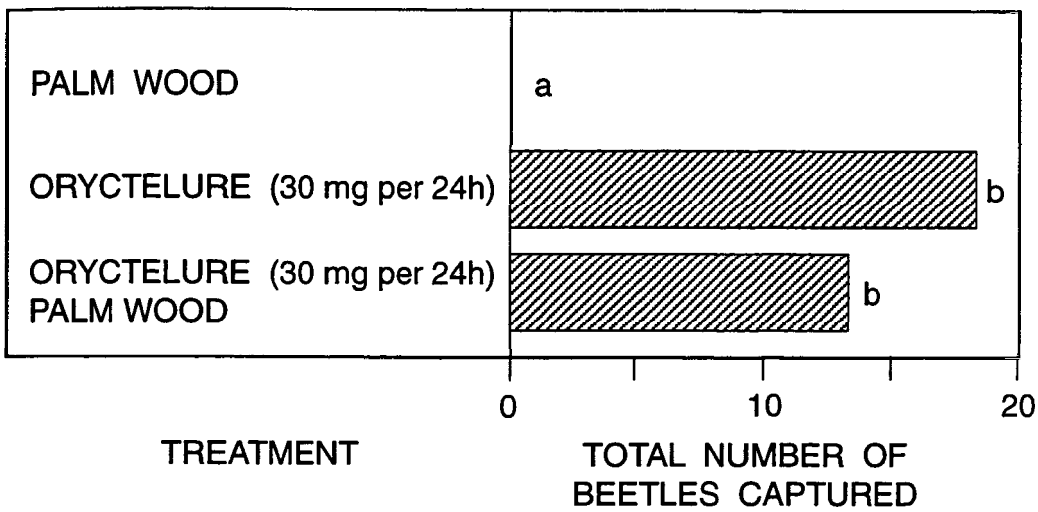
Table 8. Responses of male and female *Oryctes rhinoceros* tested individually to 612 ng of a 100:2 blend of oryctelure and ethyl 4-methylheptanoate *versus* 10 µL of hexane control in a Y-shaped olfactometer (Exp. 13).

Sex tested	n	Number of responders to stimuli[a]		*P* of difference between stimuli[b]
		Synthetic blend	Hexane control	
Male	29	17	7	0.025
Female	29	19	7	0.008

[a] Responses are not significantly different between males and females, $X^2 = 0.031$, df = 1, $P = 0.86$.

[b] Determined by normal approximation to the binomial test (Zar, 1984).

Figure 14. Attraction of *O. rhinoceros* to oryctelure (released at 30 mg per 24 h), decaying oil palm tissue, or both together, in pitfall traps at Bah Lias and Rambong Sialang Estates, North Sumatra, Indonesia (14-22 and 16-23 October 1993, respectively; N= 20) (Exp. 14). Data pooled as no locational differences were found (X^2= 2.2305, df= 1, P> 0.10). Treatment differences significant for pooled data, X^2= 13.036, df= 2, P< 0.01. Bars followed by the same letter are not significantly different, pairwise X^2 tests, P> 0.05.


PALM WOOD
a
ORYCTELURE (30 mg per 24h)
b
ORYCTELURE (30 mg per 24h)
PALM WOOD
b
0
10
20
TREATMENT
TOTAL NUMBER OF
BEETLES CAPTURED

methylheptanoate or ethyl chrysanthemumate (Fig. 15; Exp. 15). Ethyl 4-methylheptanoate was significantly more attractive than 4-methyloctanoic acid, but both compounds did not differ in attraction from ethyl chrysanthemumate. Traps baited with oryctelure alone or with ethyl 4-methylheptanoate in different ratios were equally attractive (Exp. 16) (Table 9), as were traps baited with oryctelure alone, or in binary or tertiary combinations with 4-methyloctanoic acid or ethyl 4-methylheptanoate (Exp. 17) (Table 10).

Pheromone Chirality

Racemic and (*S*)-oryctelure were superior to (*R*)-oryctelure in attracting beetles (Fig. 16; Exp. 18).

DISCUSSION

Semiochemical-based communication in *O. rhinoceros* appears to involve both a male-produced aggregation pheromone and a female-produced sex pheromone (Table 7). Because aggregation pheromones have greater potential than sex pheromones for controlling *Oryctes* populations through mass trapping, research was focused on the identification of aggregation pheromones. Ethyl 4-methyloctanoate (oryctelure) was confirmed in field experiments as the major male-produced aggregation pheromone of *O. rhinoceros* (Exp. 14, Fig. 14). The same compound is a male-produced aggregation pheromone in the

Figure 15. Attraction of *O. rhinoceros* to oryctelure, 4-methyloctanoic acid, ethyl 4-methylheptanoate, and ethyl chrysanthemumate in standard vane traps at Rambong Sialang Estate (30 March - 7 April 1994) (Exp. 15). ANOVA, $\log_e (x+1)$ transformed data, $F= 35.77$, df= 3, $P< 0.001$. Bars followed by the same letter are not significantly different, Bonferroni t-test, $P> 0.05$. Untransformed means presented.

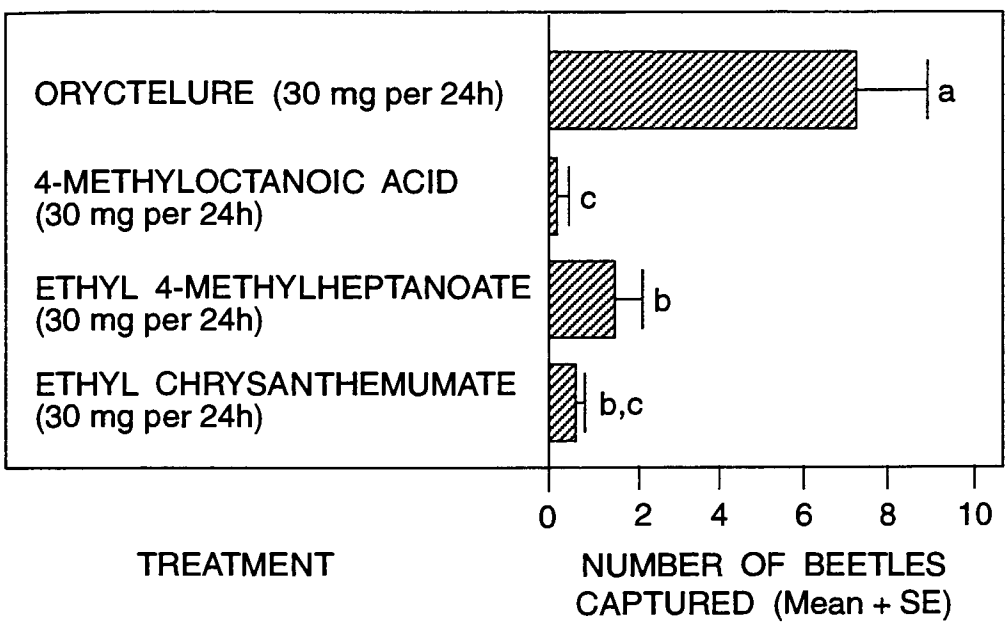

ORYCTELURE (30 mg per 24h)
4-METHYLOCTANOIC ACID (30 mg per 24h)
ETHYL 4-METHYLHEPTANOATE (30 mg per 24h)
ETHYL CHRYSANTHEMUMATE (30 mg per 24h)
a
c
b
b,c
0
2
4
6
8
10
TREATMENT
NUMBER OF BEETLES CAPTURED (Mean + SE)

Table 9. Mean captures in Exp. 16 of *O. rhinoceros* to different ratios of oryctelure and ethyl 4-methylheptanoate in standard vane traps at Rambong Sialang Estate (13 - 21 April 1994, N= 9).

Ratio of Oryctelure : Ethyl 4-methylheptanoate	Mean no. beetles per trap ± S.E.[a]
100 : 0	4.11 ± 0.84
100 : 1	3.33 ± 0.83
100 : 10	3.78 ± 0.80
100 : 100	3.00 ± 0.69

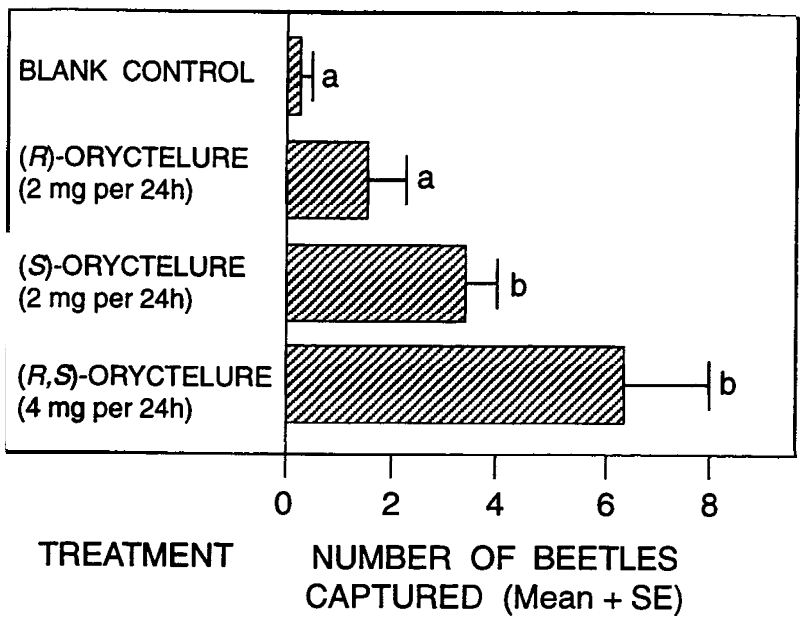
[a] Treatment differences not significant, ANOVA, F = 0.49, df = 3, *P* = 0.693.

Table 10. Mean captures in Exp. 17 of *O. rhinoceros* to different combinations of oryctelure, ethyl 4-methylheptanoate and 4-methyloctanoic acid in standard vane traps at Rambong Sialang Estate (9 - 17 June 1994, N= 10).

Ratio of Oryctelure : Ethyl 4-methylheptanoate : 4-Methyloctanoic acid	Mean no. beetles per trap ± S.E.[a]
100 : 0 : 0	2.90 ± 0.50
100 : 1 : 0	5.30 ± 1.25
100 : 0 : 300	4.90 ± 0.74
100 : 1 : 300	4.60 ± 0.81

[a] Treatment differences not significant, ANOVA, $F = 2.28$, df = 3, $P = 0.105$.

Figure 16. Attraction of *O. rhinoceros* to standard vane traps containing enantiomers of oryctelure at Rambong Sialang Estate (23 May - 8 June 1994) (Exp. 18). ANOVA $\log_e$ (x+1) transformed data, $F = 24.04$, df = 3, $P < 0.001$. Bars followed by the same letter are not significantly different, Bonferroni t-test, $P > 0.05$. Untransformed means presented.


BLANK CONTROL
a
(R)-ORYCTELURE
(2 mg per 24h)
a
(S)-ORYCTELURE
(2 mg per 24h)
b
(R,S)-ORYCTELURE
(4 mg per 24h)
b
0
2
4
6
8
TREATMENT
NUMBER OF BEETLES
CAPTURED (Mean + SE)

African rhinoceros beetle, *O. monoceros*, and was given the trivial name oryctelure (Gries *et al.*, 1994b). Other geographically or temporally isolated scarabaeid beetles also utilize identical sex pheromones (Leal *et al.*, 1993a,b; Tóth *et al.*, 1994). When related species are spatially or temporally isolated from one another there is little selection pressure for specificity of pheromone blends. The eight-component male-produced aggregation pheromone of the Australian sap beetle, *Carpophilus davidsoni* Dobson (Nitidulidae), is qualitatively identical to that of the North American species, *C. freemani* Dobson (Bartelt & James, 1994).

Neither of the other components (4-methyloctanoic acid or ethyl 4-methylheptanoate) identified from male extracts could be confirmed as pheromones in field experiments (Exps. 15-17, Figs. 15, Tables 9, 10). Ethyl 4-methylheptanoate was more attractive than 4-methyloctanoic acid, but neither compound was more attractive than the previously known attractant ethyl chrysanthemumate (Exp. 15, Fig. 15). Oryctelure was 10 times more attractive than ethyl chrysanthemumate (Exp. 15, Fig. 15). In the large black chafer, *Holotrichia parallela*, a minor sex pheromone component, (*R*)-(-)-linalool, enhanced attractiveness of the major sex pheromone, L-isoleucine methyl ester (Leal *et al.*, 1993c), but neither ethyl 4-methylheptanoate nor 4-methyloctanoic acid enhanced attraction of the main aggregation pheromone of *O. rhinoceros* (Exps. 16, 17, Tables 9,10).

In the Japanese beetle, pheromonal attraction is strongly inhibited by the presence of the non-natural enantiomer of its sex pheromone,

(*R,Z*)-5-(1-decenyl)dihydro-2(3H)-furanone (Tumlinson *et al.*, 1977). The cupreous chafer beetle, *Anomala cuprea*, also utilizes an optically active sex pheromone, (*R,Z*)-5-(-)-(oct-1-enyl)oxacyclopentan-2-one, which is inhibited by the presence of its non-natural enantiomer (Leal, 1991; Leal & Mochizuki, 1993). I therefore investigated the response of *O. rhinoceros* to chiral isomers of oryctelure (Exp. 18). Racemic and (*S*)-oryctelure were similarly attractive to *O. rhinoceros* (Fig. 16), indicating that (*S*)-oryctelure is a naturally produced isomer and (*R*)-oryctelure is not repellent. Similarly, response of *Holotrichia parallela* to its major and minor pheromone components was not inhibited by the presence of the non-natural isomers (Matsuyama *et al.*, 1994). Behavioral activity of (*R*)-oryctelure and apparently enhanced attraction of *O. rhinoceros* to racemic oryctelure rather than (*S*)-oryctelure (Fig. 16) may indicate that both isomers are produced naturally. Thus, accessible racemic oryctelure can be used operationally to trap *O. rhinoceros*.

Trapping has long been suggested as a control measure for rhinoceros beetles (Barber *et al.*, 1971; Maddison *et al.*, 1973; Julia & Mariau, 1976; Vander Meer *et al.*, 1979; Young, 1986). Trapping of rhinoceros beetles, particularly in traps baited with aggregation pheromone, has great potential for population control. Frequently, among insect pests, it is the larval stage that causes damage, so that levels of control achieved by trapping of adults cannot be assessed until the next generation (Bestmann & Vostrowsky, 1988). However, in trapping adult *O. rhinoceros*, the benefits may be felt in a much shorter time span, since it

is the adult that causes damage. A common source of emerging beetles in oil palm plantations is the felled trunks of previous oil palm stands. In the past, burning of these trunks has helped to reduce the breeding potential of *O. rhinoceros* (Liau & Ahmad, 1991). However it is often difficult to achieve a complete burn and concerns about atmospheric pollution have led some regions to ban this practice (Tajudin *et al.*, 1993). The use of border traps to intercept arriving beetles has great potential to reduce the number of females that are able to oviposit in the trunks prior to full cover being achieved by cover crops. Traps located within the new planting may intercept emerging beetles prior to attack of young oil palms. Oryctelure has been found to be much more effective in trapping beetles than the previously recommended attractant, ethyl chrysanthemumate (Fig. 15). Pheromone-based trapping has the potential to both reduce beetle population levels and damage to palms.

CHAPTER 4

HOST KAIROMONES

INTRODUCTION

Synergism between an insect pheromone and its host kairomone was first reported for the Western pine beetle, *Dendroctonus brevicomis* LeConte (Bedard *et al.*, 1969, 1980). In many examples since then, host kairomones have been found to have either an additive or synergistic effect upon insect response to their pheromones. *Rhynchophorus* spp. are known to respond to damaged or dying palms, split palm trunks and to fermenting palm sap (Kalshoven, 1950; Lever, 1969). Split coconut petioles are used to trap *R. ferrugineus* in Sri Lanka (Sivapragasam *et al.*, 1990). Therefore, host selection is likely to be due to primary attraction rather than random landing.

Wounded cabbage palmettos, *Sabal palmetto* (Walter), are vulnerable to attack by *R. cruentatus* while healthy palmettos are not (Giblin-Davis & Howard, 1989). *R. cruentatus* can be captured in traps containing sabal palmetto as bait for at least 35 d (Weissling *et al.*, 1992). Volatiles most attractive to weevils could be most easily collected 24 - 72 h after harvest of a healthy tree (Weissling *et al.*, 1992). In a further study, sabal palmetto, sugar cane or pineapple, all in combination with 0.4 mg per 24 h of the aggregation pheromone, cruentol, were equally attractive *to R. cruentatus* (Giblin-Davis *et al.*, 1994). *R. cruentatus* was equally attracted by traps containing cruentol and either of two compounds

identified in sabal palmetto volatiles, ethyl acetate (at 480 -1840 mg per 24 h) or ethyl (*S*)-(-)-lactate (release rate unknown), as to traps containing cruentol and 0.5 kg sabal palmetto, but not to traps containing 1.5 kg of either sabal palmetto or sugarcane alone.

One week old traps made from banana pseudostems were found to be more attractive to the banana weevil, *Cosmopolites sordidus*, than traps 2-3 weeks old (Koppenhöfer *et al.*, 1994). This attraction was not to the major compounds (i.e. mono- and sesquiterpenes) present in banana pseudostem volatiles, and is hypothesized to be due to minor components (Budenberg *et al.*, 1993).

In field tests with several volatiles identified from oil palm, *E. guineensis*, only ethyl propionate was found to increase captures *of R. phoenicis* in traps baited with the aggregation pheromone, phoenicol (Gries *et al.*, 1994a). Volatiles collected from fermenting oil palm sap were found to reach their maximum diversity and concentrations in extracts collected 18 and 24 h after sap collection (Nagnan *et al.*, 1992). In addition, increasing amounts of acetic acid in the blend of fermented volatiles 72 h after sap collection may be the cause of the rapid decline in attractiveness of stem traps (Nagnan *et al.*, 1992). Volatiles from sugar cane, heart of coconut palm and oil palm sap elicited strong antennal responses by both male and female *R. palmarum*, but responses to individual compounds were not tested (Rochat *et al.*, 1993c).

Three six-carbon alcohols (green leaf volatiles) are known to synergize attraction of the boll weevil, *Anthonomus grandis*, to its

aggregation pheromone, grandlure (Dickens, 1989). Green leaf volatiles were also reported to enhance pheromone response in the smaller European elm bark beetle, *Scolytus multistriatus* (Marsham), and the Mediterranean fruit fly, *Ceratitis capitata* Weid. (Dickens *et al.*, 1990).

Fermented sugarcane (Abraham, 1987) and coconut toddy (palm wine) (Kurian *et al.*, 1982) are attractive to *R. ferrugineus*. Attraction of weevils to coconut toddy was enhanced by addition of yeast and acetic acid (Kurian *et al.*, 1982). Volatiles associated with fermentation or stress (Kimmerer & Kozlowski, 1982) (e.g. ethanol and acetaldehyde) are known to be attractive to insects, e.g. fruit flies *Drosophila* spp. (Hoffman & Parsons, 1984) and *Carpophilus* beetles (Lin & Phelan, 1991; James *et al.*, 1994), that feed or develop in fermenting media. Ethanol is also an attractant or pheromone synergist for several beetle species that develop in moribund trees (Cade *et al.*, 1970; Moeck, 1970; Borden *et al.*, 1980b; Schroeder & Lindelöw, 1989; Dunn *et al.*, 1986; Phillips *et al.*, 1988), as well as root-feeding weevils (Rieske & Raffa, 1991), and has been reported as an attractant for *R. palmarum* (Hagley, 1965) and *R. ferrugineus* (Gunatilake & Gunawardena, 1986).

The scarab beetle, *Anomala octiescostata* Burmeister, has been found to be attracted to a blend of volatiles produced by dandelion, *Taraxacum officinale* Weber (Leal *et al.*, 1994d). The kairomone also had a synergistic effect on attraction of the synthetic sex pheromone. Aggregation in the scarabaeid, *Maladera matrida* Argaman, appears to be mediated by host volatiles released through mechanical damage (Harari

et al., 1994; Yarden & Shani, 1994). A role for plant volatiles has also been suggested in semiochemical communication of *Anomala albopilosa sakishimana* (Leal *et al.*, 1994b).

Evidence for a time-dependent synergistic effect of palm tissue on activity of the aggregation pheromone of *R. ferrugineus* and *R. vulneratus* was seen in Fig. 4. Similar patterns of response have been found in *R. palmarum* (Oehlschlager *et al.*, 1993b), *R. phoenicis* (Gries *et al.*, 1993), and *R. cruentatus* (F.) (Weissling *et al.*, 1992), and are hypothesized to be due to the peak production of attractive host volatiles several days after cutting. Although trap captures were low, attraction to palm wood alone (Fig. 5) peaked 4-6 d after cutting. The accentuation of this pattern by the addition of pheromone may be indicative of synergism between the insect- and host-produced compounds. However, direct comparison of the attractiveness of pheromone alone, palm wood alone, and the two in combination must be made before it can be concluded that synergism occurs.

For the palm weevils, experiments were undertaken in order to examine this effect and to attempt to identify attractive host volatiles. Initial experiments with the aggregation pheromone of *O. rhinoceros* and highly decayed oil palm tissue indicated that no synergism was occurring (Fig. 14.). For the rhinoceros beetle, experiments were undertaken to test the hypothesis that synergism might occur between the volatiles produced by freshly rotting plant material and the aggregation pheromone.

METHODS

Release devices and rates for Exp. 19-25 were as given in Table 11.

RHYNCHOPHORUS SPECIES

Pheromone-Host Volatile Interactions

To determine whether host tissue acts synergistically with ferrugineol, a four treatment, 20-replicate experiment (Exp. 19) was conducted at BALITKA (4 - 11 August 1993 and 8-14 October 1993) using standard bucket traps baited with ferrugineol and palm tissue (1 kg), ferrugineol and insecticide-treated sponge (or towel), palm tissue alone, and sponge (or towel) alone. In all cases, palm tissue, sponges and towels were treated with a 0.3% a.i. solution of diazinon. Sponges and towels were used in 10 replicates each.

Exp. 20 was conducted in Ras Al Kaimeh (3-12 September 1993), UAE, to assess the attractiveness of plant materials other than date tissue and their possible synergistic effect upon activity of ferrugineol. White bucket traps (Fig. 1) were baited with ferrugineol alone (3 mg per 24 h) or in combination with date palm tissue (1/2 kg), sugar cane (1/2 kg) or two apple halves.

Exp. 21 tested whether weevils would enter and remain in a trap when they could not feed on palm tissue; bucket traps containing either palm wood suspended 5 cm below the lid of the trap, or palm wood on the bottom of the trap, were compared. All traps contained ferrugineol (3 mg

Table 11. Summary of semiochemical release rates and release devices used in Exps. 19-25.

Exp.	Semiochemical	Release Rate (mg per 24 h)[a]	Release Device	Chemical Source[b]
19-21	Ferrugineol	3	Polymer membrane bag lure[c]	CT
22	Ferrugineol	0.3	3 capillaries (1 mm ID)	SFU
	Ethyl acetate	30	Sealed plastic tube[d], no hole	Aldrich
	Ethyl butyrate	30	Sealed plastic tube, 1 hole	Aldrich
	Ethyl isobutyrate	30	Sealed plastic tube, 1 hole	Aldrich
	Ethyl propionate	30	Sealed plastic tube, 1 hole	Aldrich
	Isobutyl acetate	30	Sealed plastic tube, 1 hole	Aldrich
	Isobutyl propionate	30	Two sealed plastic tube, no holes	Aldrich
	Dioxane	30	Sealed plastic tube, no hole	Aldrich

Exp.	Semiochemical	Release Rate (mg per 24 h)[a]	Release Device	Chemical Source[b]
23, 24	Oryctelure	9	Polymer membrane bag lure	CT
25	Oryctelure	9	Polymer membrane bag lure	CT
	Ethanol	200	Two sealed plastic tubes, no holes	

[a] Release rates from plastic tubes estimated under laboratory conditions of 25°C and 50% RH. Release rate for ethanol estimated under field conditions by decline in liquid meniscus over time.

[b] Aldrich= Aldrich Chemical Co., Milwaukee, Wisconsin; CT= Chem Tica International, Costa Rica; SFU= Department of Chemistry, Simon Fraser University.

[c] Heat-sealed polymer membrane bag lures, Chem Tica International, Costa Rica.

[d] Capped polyethylene 8.5 mL Polyvials (Bel-Art) with no, one or two pin holes 1 cm below top.

per 24 h), and black rat glue was spread on the inside bottom of the trap for weevil retention. Traps were placed on the ground at the base of coconut palms in a cocoa-coconut intercropped field at Bah Lias Estate (13 January - 4 February 1994). Treatments were rerandomized (total 20 replicates) and palm tissue renewed on 20 January 1994.

Electrophysiological and Behavioural Activity of Palm Volatiles

Aerations of coconut stem tissue and oil palm fruit bunches were conducted by passing air over 4.6 kg of coconut stem tissue or 3 kg oil palm fruit bunches for 72 h. Host-produced volatiles were collected in Porapak Q traps changed every 24 h. Volatiles were eluted from the traps with 175 mL of pentane and concentrated by distillation. Volatile extracts were analysed by GC and GC-EAD, and electrophysiologically active compounds were identified by GC-MS and by comparison with authentic standards. Electrophysiological activity of synthetic compounds was determined by GC-EAD using antennae of *R. ferrugineus* and *R. vulneratus*.

Attractiveness of synthetic palm volatiles was examined in an 8-treatment 6-replicate experiment (Exp. 22) at Bah Lias Estate, 14-25 November 1994. All traps contained ferrugineol (0.3 mg per 24 h) alone or in combination with one of: ethyl acetate, ethyl butyrate, ethyl isobutyrate, ethyl propionate, isobutyl acetate, isobutyl propionate, or dioxane (all 98% chemically pure). Dioxane (diethylene dioxide) is not found in palm volatiles, but is a general insect attractant and has been reported to be

attractive to *R. ferrugineus* (Abraham, 1987). All synthetic volatiles were released at approximately 30 mg per 24 h. Pheromone and palm volatiles were suspended from the trap lid. Vane traps (Fig. 1) fitted with a metal funnel to prevent weevil escape were placed between coconut palm trees in a cocoa-coconut intercrop field. Inter-trap and inter-block distances were 27 m and 54 m, respectively.

ORYCTES RHINOCEROS

Pheromone-Host Volatile Interactions

Experiments were conducted in a field of one-year old oil palms at Rambong Sialang Estate, unless otherwise noted. In all experiments vane traps with white buckets (Fig. 11) were used, and inter-trap and inter-block distances were 27 m and 54 m respectively.

The attraction of beetles to oryctelure (9 mg per 24 h) alone, freshly milled empty fruit bunches (1/3 to 1/2 bunch or approx. 1-1.5 kg, per trap) or to both was examined in a 3-treatment, 12-replicate experiment (Exp. 23) at Rambong Sialang (25 April - 11 May 1994) and Dolok (3-18 May 1994) Estates.

A comparison was made in Exp. 24 between attraction of beetles to oryctelure alone (9 mg per 24 h) or in combination with either freshly milled fruit bunches (1/3 to 1/2 bunch per trap) or cut oil palm seedling tissue (one 6 month old seedling per trap) at Rambong Sialang Estate (19-30 March 1994; 12 replicates). Intertrap and interblock distances were 27 and 77 m, respectively.

Exp. 25 tested whether ethanol was attractive to *O. rhinoceros* and was conducted with 9 replicates in a one year old oil palm planting at Dolok Estate from 26 February - 11 March 1994. There were three treatments: oryctelure alone (9 mg per 24 h), ethanol alone (approx. 200 mg per 24 h released from 2 sealed plastic tubes), and oryctelure plus ethanol. Minimum intertrap and interblock distances were 54 m and 72 m, respectively.

STATISTICAL ANALYSES

In all field experiments, no significant differences were found in the responses of male and female beetles ($P > 0.05$), so catches were pooled by sex for analysis. Data were transformed by $\log_e (x+1)$ if they were not normally distributed and were subjected to Analysis of Variance (General Linear Modeling) (Minitab, 1989). If replicates were run at different times or locations, data were analyzed for time x treatment or location x treatment interactions. Following ANOVA, multiple pairwise comparisons were made using Bonferroni *t*-tests. If homoscedasticity was not achieved by transformation, data were analyzed by X^2 tests (Zar, 1984). In these cases, treatment, sex, and species comparisons were also performed using X^2 tests.

RESULTS

RHYNCHOPHORUS SPECIES

Pheromone-Host Volatile Interactions

Captures in Exp. 19 of both *R. ferrugineus* and *R. vulneratus* were greatest in traps containing both ferrugineol and palm (Table 12), and were more than the sum of catches in traps containing these components individually. Captures were highest 3-5 days after cutting of palm tissue (Table 13).

No significant differences were found in Exp. 20 between the attractiveness of date palm wood, sugarcane and apples (X^2= 1.4706, df= 2, P> 0.30). Captures in traps containing palm wood or sugarcane dropped over the 8 day trapping period, while captures increased in traps containing apple halves (Table 14). However, less than half as many weevils were captured in traps containing apple as in those containing palm wood or sugarcane. Nine weevils were caught in each of the two treatments in Exp. 21, indicating no difference in attractiveness of traps in which weevils could feed on palm tissue and those in which the palm was suspended.

Electrophysiological and Behavioural Activity of Palm Volatiles

Volatile production was greatest during the second 24 h aeration period (Fig. 17). The volatile spectrum was essentially the same during each of the three 24 h time periods, but some differences in volatile ratios were seen. Compounds identified from coconut palm wood included ethyl

Table 12. Total captures in Exp. 19 of *R. ferrugineus* and *R. vulneratus* in traps containing ferrugineol and insecticide-treated palm tissue or an inert material (sponge or towel), or palm or inert material alone.

Treatment	*R. ferrugineus*[a]	*R. vulneratus*[b]
Ferrugineol + Palm	41	31
Ferrugineol + Sponge or Towel	1	0
Palm alone	0	8
Sponge or Towel alone	0	0

[a] Treatment differences significant for *R. ferrugineus*, X^2= 118.190, df= 3, P< 0.001.

[b] Treatment differences significant for *R. vulneratus*, X^2= 66.128, df= 3, P< 0.001.

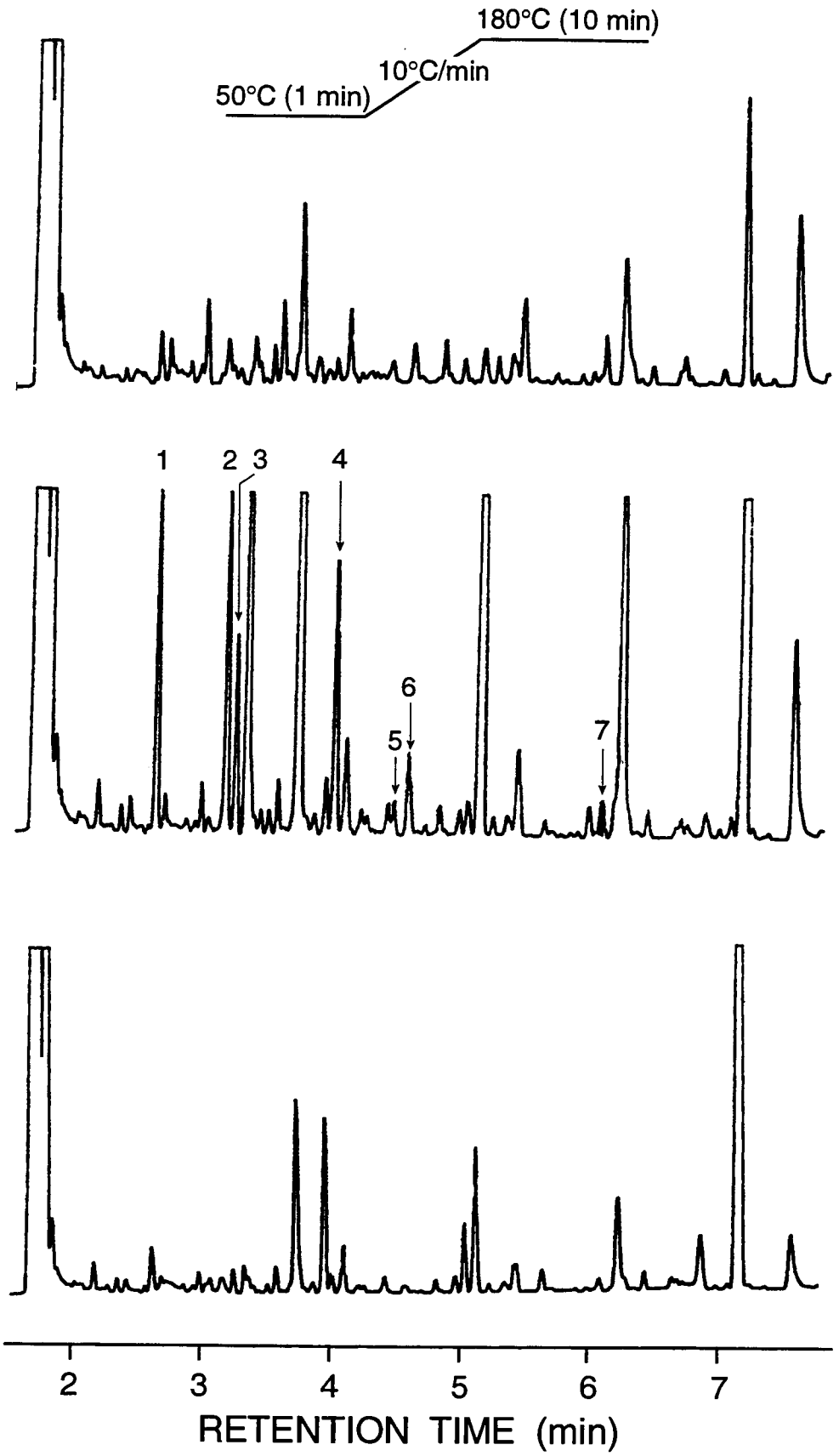
Table 13. Daily captures of *R. ferrugineus* and *R. vulneratus* in traps containing ferrugineol and palm tissue (Exp. 19). Trap captures from days 7-8 standardized to 24 h period.

	Day						
Species	1	2	3	4	5	6	7-8
R. ferrugineus	3	0	10	17	7	3	0.5
R. vulneratus	1	9	7	6	6	0	0.5

Table 14. Daily captures of *R. ferrugineus* in traps containing ferrugineol in combination with date palm tissue, sugarcane or apple (Exp. 20). Trap captures standardized to 24 h period.

	Day			Total weevils
Treatment	1-2	3-4	5-8	captured
Apple	0.5	0.5	1.5	8
Sugarcane	3.0	2.0	0.8	13
Date palm wood	2.5	2.0	1.0	13

Figure 17. Gas chromatograms of volatiles produced by coconut palm wood over three successive 24 h periods (top to bottom). Compounds: **1**= ethyl acetate, **2**= ethyl propionate, **3**= ethyl isobutyrate, **4**= ethyl butyrate, **5**= butyl acetate, **6**= isobutyl propionate, **7**= amylacetate. Chromatography: Hewlett Packard 5890A gas chromatograph equipped with an SP-1000 coated fused silica column (30 m x 0.25 mm ID).


180°C (10 min)
10°C/min
50°C (1 min)
1
2
3
4
5
6
7
2
3
4
5
6
7
RETENTION TIME (min)

acetate, ethyl propionate, ethyl isobutyrate, ethyl butyrate, butyl acetate, isobutyl propionate and amylacetate. Antennal responses of *R. ferrugineus* and *R. vulneratus* to palm-produced volatiles were nearly identical (Fig. 18). Both *R. ferrugineus* and *R. vulneratus* gave antennal responses to ethyl acetate, ethyl propionate, ethyl isobutyrate, ethyl butyrate and isobutyl propionate. *R. vulneratus* also gave a minor antennal response to butyl acetate.

Captures of weevils in Exp. 22 were too low to permit analysis; however more weevils were captured in traps containing ethyl butyrate than in any other treatment (Table 15).

ORYCTES RHINOCEROS

Pheromone-Host Volatile Interactions

Freshly milled oil palm fruit bunches alone were unattractive to rhinoceros beetles in Exp. 23, but in combination with ethyl 4-methyloctanoate significantly enhanced pheromonal activity (Fig. 19).

No differences in attractiveness occurred in Exp. 24 between traps containing oryctelure alone or in combination with either oil palm seedlings or freshly milled empty fruit bunches (ANOVA, F= 3.45, df= 2, P= 0.054). However, a significant effect of time occurred, apparently because different ages of EFB were used in different time periods (Table 16). If time periods were analysed separately, EFBs milled 2 days prior to placement in traps were found to be significantly more attractive than

Figure 18. FID and EAD responses of male *Rhynchophorus ferrugineus* and *R. vulneratus* to coconut palm volatiles produced 24-48 h after cutting. Compounds: **1**= ethyl acetate, **2**= ethyl propionate, **3**= ethyl isobutyrate, **4**= ethyl butyrate, **5**= butyl acetate, **6**= isobutyl propionate, **7**= amylacetate. Chromatography: Hewlett Packard 5890A gas chromatograph equipped with an SP-1000 fused silica column (30 m x 0.25 mm ID).

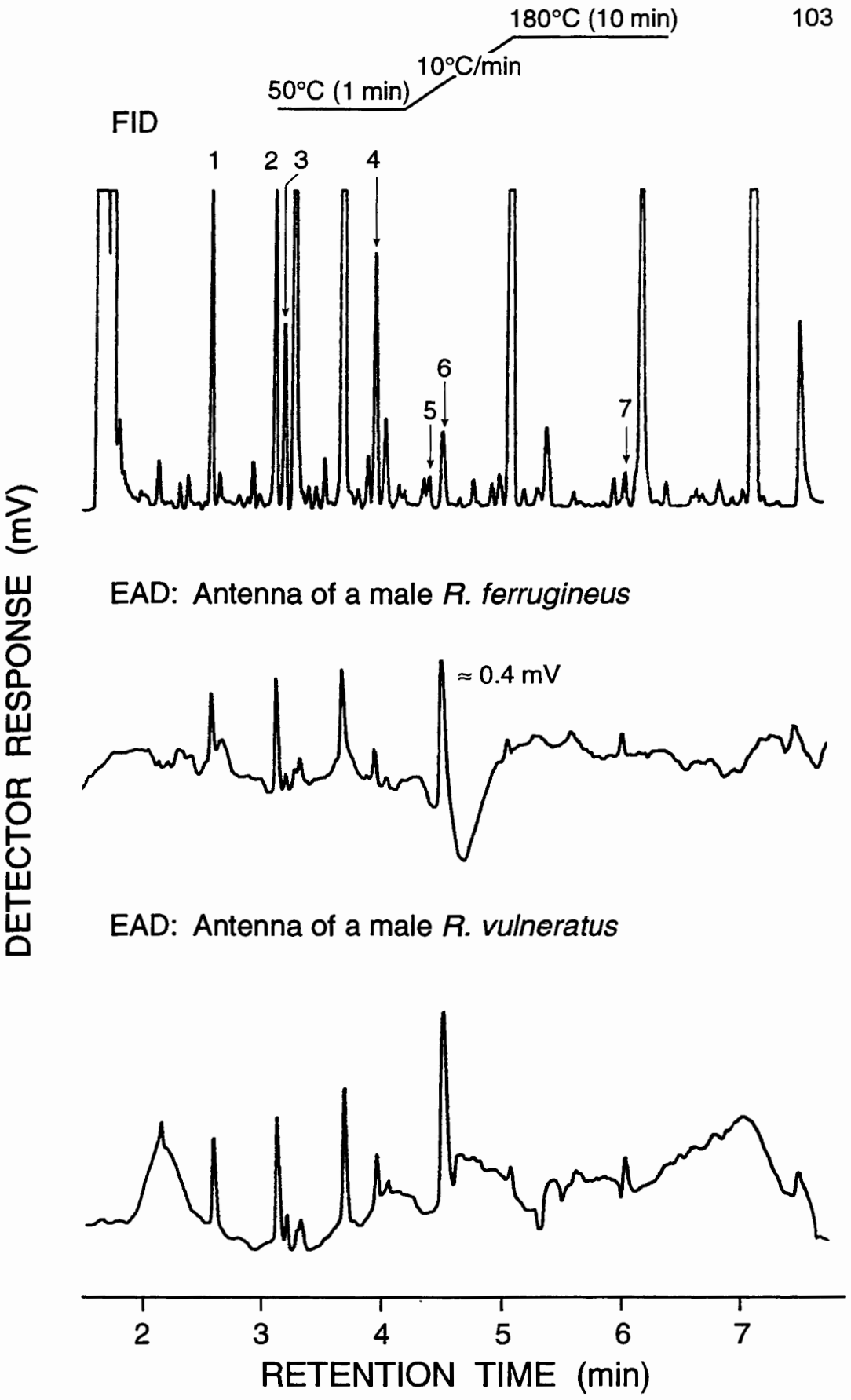

180°C (10 min)
10°C/min
50°C (1 min)
FID
1
2
3
4
5
6
7
EAD: Antenna of a male R. ferrugineus
≈ 0.4 mV
EAD: Antenna of a male R. vulneratus
DETECTOR RESPONSE (mV)
2
3
4
5
6
7
RETENTION TIME (min)

Table 15. Captures of *R. vulneratus* in traps containing ferrugineol (0.3 mg per 24 h) alone or in combination with a synthetic palm volatile (all released at 30 mg per 24 h) (Exp. 22).

Treatment	Total weevils captured
Ethyl acetate + ferrugineol	0
Ethyl butyrate + ferrugineol	4
Ethyl isobutyrate + ferrugineol	0
Ethyl propionate + ferrugineol	1
Isobutyl acetate + ferrugineol	0
Isobutyl propionate + ferrugineol	0
Dioxane + ferrugineol	1
Ferrugineol alone	0

Figure 19. Attraction of *O. rhinoceros* to standard vane traps containing oryctelure (released at 9 mg per 24 h), freshly milled empty fruit bunches (EFB), or both together at Rambong Sialang and Dolok Estates (26 April - 11 May and 3 -18 May 1994, respectively) (Exp. 23). ANOVA, F= 46.60, df= 2, $P < 0.001$. Bars followed by the same letter are not significantly different, Bonferroni t-test, $P > 0.05$.

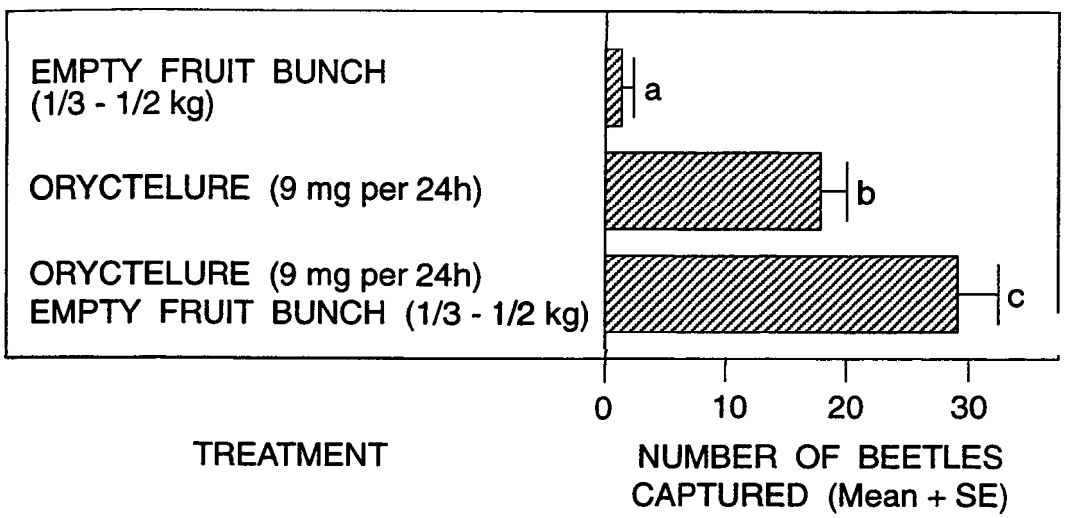

EMPTY FRUIT BUNCH
(1/3 - 1/2 kg)
ORYCTELURE (9 mg per 24h)
ORYCTELURE (9 mg per 24h)
EMPTY FRUIT BUNCH (1/3 - 1/2 kg)
a
b
c
0
10
20
30
TREATMENT
NUMBER OF BEETLES
CAPTURED (Mean + SE)

Table 16. Captures of *O. rhinoceros* in Exp. 24 in traps containing oryctelure alone or in combination with either oil palm seedlings or freshly milled empty fruit bunches (EFB) of three different ages post-milling at start of experiment.

Days post-milling of empty fruit bunches[a]	Treatment	*Oryctes* captured (Mean ± S.E.)[b]
1 - 4	Oryctelure alone	3.5 ± 1.0 a
	Oryctelure + seedling	1.8 ± 0.9 a
	Oryctelure + EFB	0.8 ± 0.8 a
2 - 5	Oryctelure alone	2.8 ± 0.9 a
	Oryctelure + seedling	3.0 ± 1.1 a
	Oryctelure + EFB	7.8 ± 1.8 b
5 - 10	Oryctelure alone	3.5 ± 1.0 a
	Oryctelure + seedling	2.5 ± 0.9 a
	Oryctelure + EFB	5.5 ± 1.7 a

[a] Significant difference between experimental time periods was found, ANOVA, $F= 4.47$, df= 2,18, $P< 0.03$.

[b] Within a time period, means followed by the same letter are not significantly different, Bonferroni *t*-tests, $P > 0.05$.

traps containing seedling tissue or oryctelure alone (replicates 5-8) (Table 16). EFBs milled five days prior to use (replicates 9-12) were not attractive presumably because attractive volatiles were no longer being released. It is unclear why EFBs milled 1 day prior to placement in traps were unattractive (replicates 1-4). In no case was seedling tissue attractive.

Significantly more beetles were captured in Exp. 25 in traps containing oryctelure alone or in combination with ethanol than in traps containing ethanol alone (Table 17). However, there was no significant difference in attractiveness of traps containing oryctelure or oryctelure plus ethanol indicating that ethanol has no synergistic effect upon pheromone activity.

DISCUSSION

In many Coleoptera, pheromones interact synergistically with host volatiles (Borden, 1985). Synergism between aggregation pheromones and host compounds has recently been demonstrated for *A. octiescostata* (Leal *et al.*, 1994d), and palm weevils, *Rhynchophorus* spp. (Oehlschlager *et al.*, 1992; Gries *et al.*, 1994a; Giblin-Davis *et al.*, 1994).

Captures of palm weevils in traps containing ferrugineol and palm wood were far greater than the sum of weevils captured in traps containing these components individually (Exp. 19, Table 12), confirming

Table 17. Captures of *O. rhinoceros* in Exp. 25 to traps baited with oryctelure (9 mg per 24 h) or ethanol (200 mg per 24 h) alone or in combination.

Treatment	*Oryctes* captured (Mean ± S.E.)[a]
Ethanol	0.1 ± 0.1 a
Oryctelure	13.0 ± 2.0 b
Ethanol + oryctelure	10.2 ± 1.8 b

[a] Means followed by the same letter are not significantly different, Bonferroni t-test, $P > 0.05$.

the synergistic effect (Figs. 3, 5) between ferrugineol and host kairomones. The greatest synergistic effect occurred 3-5 days after cutting of palm tissue (Table 13). Similar age-dependent attractiveness of palm tissue was not seen in the UAE (Exp. 20), possibly due to rapid drying of palm tissue under desert conditions. While numbers were too low to permit analysis, apple increased in attraction over time (Table 14), indicating that apple or some other stimulus may be a more suitable and persistent host material under desert conditions than palm or sugarcane.

No differences were found in catches between traps in which weevils could feed on palm tissue and those in which palm tissue was suspended (Exp. 21), indicating that weevils could be retained in traps even if they were unable to feed. Consequently, funnel traps that prevented weevil escape were used in testing synthetic host volatiles. In GC-EAD analyses, both *R. ferrugineus* and *R. vulneratus* gave antennal responses to a number of coconut palm-produced volatiles (Fig. 18). In a field test, captures of *R. vulneratus* to synthetic host volatiles were very low, but appeared to indicate that ethyl butyrate is bioactive (Exp. 22, Table 15). Ethyl butyrate elicited antennal response by *R. phoenicis* in GC-EAD analyses, but did not enhance attraction to its aggregation pheromone, phoenicol, in field tests (Gries *et al.*, 1994a). Tested individually, ethyl butyrate, ethyl acetate and ethyl propionate each increased attraction of *Metamasius hemipterus sericeus* (Olivier) to a 8:1 blend of ferrugineol and 2-methyl-4-heptanol, but were not as attractive

as the pheromone blend in combination with sugarcane (Perez-Sanchez, 1996).

Freshly milled oil palm fruit bunches had a synergistic effect on response of *O. rhinoceros* to its aggregation pheromone, oryctelure (Exp. 23, Fig. 19; Exp. 24, Table 16). Synergistic oil palm volatiles are apparently produced early in the decomposition (fermentation) process, because freshly milled fruit bunches (Exp. 23, Fig. 19) but not decomposed palm tissue (Exp. 14, Fig. 14) enhanced pheromone attraction. Ethanol was not responsible for this synergistic effect (Exp. 25, Table 17). In order to minimize trap maintenance, it is preferable to use synthetic host compounds in a trap rather than the fruit bunches themselves. Several compounds, including naphthalene, were tentatively identified by GC-EAD and GC-MS of Porapak Q-captured volatiles of oil palm fruit bunches (R. Gries, pers. comm[1]), but they were not tested because of time constraints. It is interesting to note the presence of naphthalene in fruit bunch volatiles as placement of naphthalene moth balls in young oil palm leaf axils has been recommended to repel *O. rhinoceros* (Ho & Toh, 1982). Milled fruit bunches are routinely used around young oil palms as a mulch, which may have unexpected adverse effects upon *O. rhinoceros* damage.

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CHAPTER 5

TRAPPING PROTOCOLS AND MASS-TRAPPING TRIALS

INTRODUCTION

Traps used in the past for *O. rhinoceros* include: tin cans placed on the top of up-ended palm logs (Bedford, 1975), vane traps equipped with or without funnel (Barber *et al.*, 1971; Maddison *et al.*, 1973), and a small plastic bucket fixed to the top of a post (Julia & Mariau, 1976). Gries *et al.* (1994b) used a pitfall trap to capture *O. monoceros* in Africa. Traps developed for *Rhynchophorus* spp. include traps from palm logs or split palm petioles (Sivapragasam *et al.*, 1990), sandwich traps and bucket traps (Oehlschlager *et al.*, 1993b). Oehlschlager *et al.* (1993b) also evaluated the efficacy of McPhail traps (White & Elson-Harris, 1992) and multiple funnel traps (Lindgren, 1983), but found that newly designed bucket traps were most effective in trapping *R. palmarum*.

Bucket traps similar to those used by Oehlschlager *et al.* (1993b) were used in experiments for *R. ferrugineus* and *R. vulneratus* (Chapters 2, 4), and pitfall, barrier and vane traps (Fig. 11) in experiments for *O. rhinoceros* (Chapters 3, 4).

Cross attraction of secondary species to the pheromone of a primary tree-killing species, with which they co-colonize a living host occurs in the Scolytidae (Borden 1985, Setter & Borden, 1992; Poland & Borden, 1994a,b). For example, presence of the pheromone of *Ips grandicollis* Eichh. causes a synergistic effect on the response of *I.*

avulsus Eichh. to its own pheromone (Hedden *et al.*, 1976). Because *R. ferrugineus* and *R. vulneratus* may oviposit in wounds created *by O. rhinoceros* (Khoo *et al.*, 1991), I hypothesized that the weevils may be cross-attracted to *O. rhinoceros* pheromone. Alternately, feeding-induced volatiles released due to *Oryctes* damage may be different from those released by other types of damage (Loughrin *et al.*, 1995), and be more attractive to *Rhynchophorus* spp. Regardless, knowledge of interactions between palm weevils and rhinoceros beetles may influence how operational trapping programs are developed and implemented.

My objectives were to: evaluate trapping protocols for all three species; develop methods for trapping without using insecticide-treated materials; determine whether pheromone-based trapping systems for *Rhynchophorus* spp. and *O. rhinoceros* can be superimposed; and to determine whether pheromone-based mass trapping programs are feasible for these species.

METHODS

Release devices and release rates for Exps. 26-37 are given in Table 18.

RHYNCHOPHORUS SPECIES

Trapping Protocols

To determine optimum trap height Exp. 26 was conducted at BALITKA (30 October - 13 November 1993; N = 10). Standard white

Table 18. Summary of semiochemical release rates and release devices used in Exps. 26-37.

Exp.	Semiochemical	Release Rate (mg per 24 h)[a]	Release Device	Chemical Source[b]
26-29	Ferrugineol	3	Polymer membrane bag lure[c]	CT
30	Ferrugineol	0.3	400µL microcentrifuge tube	SFU
	Ferrugineol	1	1.5 mL microcentrifuge tube	SFU
	Ferrugineol	3	Three 1.5 mL microcentrifuge tubes	SFU
31	Ferrugineol	3	Polymer membrane bag lure	CT
	Ferrugineol Desert Lure	3 mg at 30°C	Polymer membrane bag lure	CT
32	Oryctelure	30	Polymer membrane bag lure	CT
33	Oryctelure	30	Polymer membrane bag lure	CT
34	Oryctelure	0.3	Capillary (1 mm ID)[e]	SFU

Exp.	Semiochemical	Release Rate (mg per 24 h)[a]	Release Device	Chemical Source[b]
34 cont'd	Oryctelure	3	Polymer membrane bag lure	CT
	Oryctelure	30	Polymer membrane bag lure	CT
35	Oryctelure	3	Polymer membrane bag lure	CT
	Oryctelure	6	Polymer membrane bag lure	CT
	Oryctelure	9	Polymer membrane bag lure	CT
	Oryctelure	30	Polymer membrane bag lure	CT
36	Oryctelure	6	Polymer membrane bag lure	CT
	Oryctelure	9	Polymer membrane bag lure	CT
	Oryctelure	18	2 Polymer membrane bag lures (9 mg per 24 h)	CT
	Oryctelure	30	Polymer membrane bag lure	CT

Exp.	Semiochemical	Release Rate (mg per 24 h)[a]	Release Device	Chemical Source[b]
37	Oryctelure	3	Polymer membrane bag lure	CT
	Ferrugineol	3	Polymer membrane bag lure	CT

[a] Release rates of ferrugineol from microcentrifuge tubes estimated under laboratory conditions of 25°C and 50% RH. All other release rates determined by ChemTica International under conditions of 25°C and 60% RH, unless otherwise noted.

[b] CT= Chem Tica International, Costa Rica; SFU= Department of Chemistry, Simon Fraser University.

[c] Heat-sealed polymer membrane bag lures, Chem Tica International, Costa Rica.

[d] Capped polyethylene 400 μL and 1.5 mL microcentrifuge tubes (Quality Scientific Plastics, USA, and Gordon Technologies, Mississauga, Ontario, respectively) with two 3 mm diam. holes drilled 1 cm below top.

[e] All capillary tubes opened 1 cm above liquid meniscus, placed inside microcentrifuge tube as above and suspended 3-4 cm below trap lid.

bucket traps (Fig. 1) (Oehlschlager *et al.*, 1993b) were placed at ground level, or at 2, 5, or 10 m above ground. All traps were attached to coconut palms and contained ferrugineol and 1 kg palm wood treated with 0.3 % a.i. solution of diazinon.

Efficacy of the standard white bucket trap was compared in Exp. 27 to traps of three other designs (funnel trap; vane trap with funnel and low pheromone placement, vane trap with funnel and high pheromone placement) (Fig. 1) in order to test whether trap captures could be improved and an insecticide-free trap developed. Traps with high pheromone placement had lures suspended in a slot 24 cm above the bucket rim, while those with low pheromone placement had lures suspended in a slot at the level of the bucket rim. This 4-treatment, 10-replicate experiment was conducted at Bah Lias Estate (18 February - 19 March 1994). All traps contained ferrugineol and half coconut husks (fresh, young coconuts with meat removed). Only coconut husks in the standard bucket traps were treated with insecticide (0.3 % a.i. diazinon).

The funnel and vane + funnel traps were compared in Exp. 28 to vane + funnel traps with black painted vanes at Bah Lias Estate, Sumatra (11-25 April 1994; N = 15) in a mixed cocoa-coconut planting. Intertrap and interblock distances were 45 m. All traps contained ferrugineol and half coconut husks.

In short date palms, presence of leaf axils minimized contact between standard bucket traps and the trunk. Black buckets were more readily obtainable than white buckets in the UAE. Consequently, the

standard trap (white upright) was compared to three other trap designs in Exp. 29 (inverted white bucket, upright black bucket and inverted black bucket) (Fig. 1) in Ras Al Kaimeh, UAE (12-20 June 1993; N= 10). Inverted bucket traps had entry holes near the top and bottom of bucket and all traps contained ferrugineol and date palm tissue soaked in 0.3% a.i. solution of Lannate.

A four treatment 20-replicate experiment (Exp. 30) was conducted to determine the activity and optimal dose of ferrugineol at BALITKA (14-27 August 1992). Ferrugineol was released at 0, 0.3, 1.0, and 3.0 mg per 24 h from standard white bucket traps attached 2 m high to coconut palms in randomized blocks with traps at 24 m intervals and blocks 70 m apart. All traps in each experiment contained 2 kg of one day old coconut palm wood pieces (approx. 5 x 5 x 20 cm) treated with Basudin 60EC (diazinon; Ciba-Geigy), 0.24% a.i. in water, to retain captured weevils.

In Egypt, Exp. 31 was conducted at Abu Negai, New El Kassassin, Ismailiya Governorate (7-13 November 1993; N = 5) to compare the attractiveness of ferrugineol at two release rates with that of date palm tissue alone. Ferrugineol was released from bag lures designed to allow the release of 3 mg per 24 h ferrugineol at 25°C (high or normal lure) and at 30°C (low or desert lure). All traps contained approx. 0.5 kg of chopped date palm wood treated with a 0.2 % a.i. solution of Lannate. Intertrap and interreplicate distances at each site were 20-25 and 50-70 m, respectively.

ORYCTES RHINOCEROS

Trapping Protocols

A 3-treatment, 10-replicate experiment (Exp. 32) compared trapping efficacies of pitfall, barrier, and vane traps (Fig. 11) at Rambong Sialang Estate (2-10 February 1994). All traps were baited with oryctelure and were placed about 1-1.5 m away from palm base at edge of covercrop-free area.

In a further experiment (Exp. 33), four types of vane traps were compared: unpainted (standard) vane traps (Fig. 11), vane traps fitted with an internal funnel (15 cm opening) to prevent beetle escape, traps with matte black-painted vanes, and traps with both funnels and black-painted vanes (Dolok Estate, 26 February - 11 March, 1994; N = 9). Non-reflective black vane traps were tested because *Oryctes* are known to respond to vertical silhouettes (Bedford, 1980).

In a 4-treatment, 18-replicate experiment (Exp. 34) (Dolok Estate, 12-26 February 1994, and Rambong Sialang Estate, 21 February - 1 March 1994) with standard vane traps at 36 m intervals, oryctelure was released at 0, 0.3, 3 or 30 mg per 24 h. To examine release rates between 3 and 30 mg per 24 h, standard vane traps were baited in Exp. 35 with oryctelure released at 0, 3, 6, 9, or 30 mg per 24 h (Dolok Estate, 11-21 March 1994; N = 15). Release rates between 6 and 30 mg per 24 h were further examined in Exp. 36. Standard vane traps were baited with oryctelure released at 0, 6, 9, 18, or 30 mg per 24 h (Dolok Estate, 24 May - 8 June 1994; N = 12). To minimize interference between

treatments in Exps. 35 and 36, intertrap and interblock distances were increased to 54 and $\geq$ 63 m, respectively.

To determine whether both *Oryctes* and *Rhynchophorus* spp. could be caught in the same traps, Exp. 37 tested vane + funnel traps containing either oryctelure, ferrugineol or both, set out in a mixed cocoa/coconut planting at Bah Lias Estate (27 April - 7 June 1994; N = 28).

Mass-Trapping Trials

Small trials were conducted to assess the potential impact of semiochemical-based trapping on damage levels by *O. rhinoceros* in young oil palm plantings.

Trial 1. At Rambong Sialang, a trapping trial using a density of one trap per 0.75 ha was set up in Egaharap Division. The number of trees attacked was recorded daily in two adjacent 10 ha plots, and after one week, traps containing pheromone lures that released 9 mg per 24 h were set up in the plot experiencing the highest damage levels.

Trial 2. In a second trapping trial, at Dolok, traps were set out at a density of 1 trap per 2 ha in a 24 ha section of a 41 ha field. The surrounding 17 ha were used as the control. Initially 30 mg per 24 h pheromone lures were used, and after week 2 these were replaced by 9 mg per 24 h lures.

STATISTICAL ANALYSIS

In all field experiments, no significant differences were found in the responses of male and female beetles ($P > 0.05$), so catches were pooled by sex for analysis. Data were transformed by $\log_e (x+1)$ if they were not normally distributed and were subjected to Analysis of Variance (General Linear Modeling) (Minitab, 1989). If replicates were run at different times or locations, data were analyzed for time x treatment or location x treatment interactions. Following ANOVA, multiple pairwise comparisons were made using Bonferroni *t*-tests. If homoscedasticity was not achieved by transformation, data were analyzed by X^2 tests (Zar, 1984). In these cases, treatment, sex, and species comparisons were also performed using X^2 tests.

Mass-trapping trial data were normalized by dividing weekly damage data for each plot by the level of damage found in that plot prior to pheromone deployment (i.e. in Week 0).

RESULTS

RHYNCHOPHORUS SPECIES

Trapping Protocols

No significant difference ($X^2 = 0.1745$, df= 2, $P > 0.70$) was found between species in Exp. 26, so results were pooled for further analyses. Bucket traps at ground level caught significantly more weevils than traps 5 or 10 m high, while those at 2 m were intermediate in effectiveness

between those at ground level and 5 m high (Fig. 20). Traps at 10 m were significantly less effective than those at all lower positions.

All four types of traps examined in Exp. 27 were equally effective (Fig. 21). Painting the sheet metal vanes black in Exp. 28 resulted in significantly more weevils being captured than in funnel traps (Fig. 22). Unpainted vane traps were intermediate in effectiveness.

Black inverted bucket traps were most attractive in Exp. 29, followed by black upright traps (Fig. 22). No weevils were captured in white bucket traps regardless of their position.

At 3 mg per 24 h, ferrugineol in Exp. 30 was significantly more attractive to *R. ferrugineus* than all other stimuli (Fig. 23), and even at a 10-fold lower release rate (0.3 mg per 24 h), attraction exceeded that of the palm control. All doses of ferrugineol were significantly and equally more attractive to *R. vulneratus* than palm alone (Fig. 23).

After 6 days, 10 weevils were captured in Exp. 31 in traps at Abu Negai, Egypt, eight of which were female. Three weevils were captured in traps containing the normal lure, while 6 were captured in traps containing the "desert lure", and only one weevil was captured in traps baited with palm tissue alone. Weevil captures were too low to permit accurate statistical analysis, but the results indicate the superior attraction of pheromone-baited traps over food material alone. The high pheromone dose also appears to be more attractive than the low dose.

Figure 20. Efficacy of traps at different heights in capturing *R. vulneratus* and *R. ferrugineus* (Exp. 26). Traps contained ferrugineol (3 mg per 24 h) and palm wood; BALITKA, 30 October - 3 November 1993, N = 10. Bars followed by the same letter are not significantly different, X^2 tests, $P > 0.05$. Trap captures were too low to permit comparison of traps at 5 and 10 m, but all other pairwise comparisons were made.

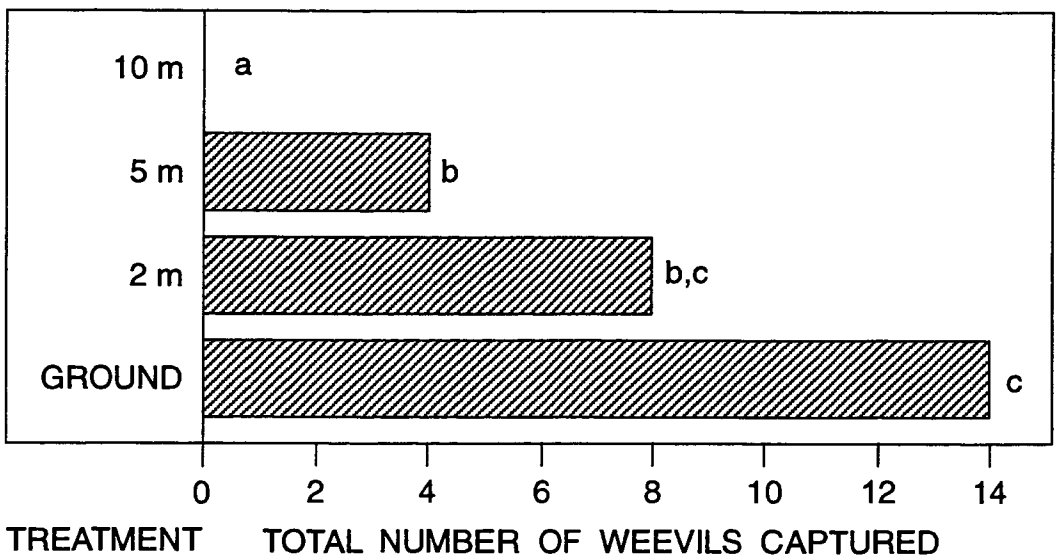
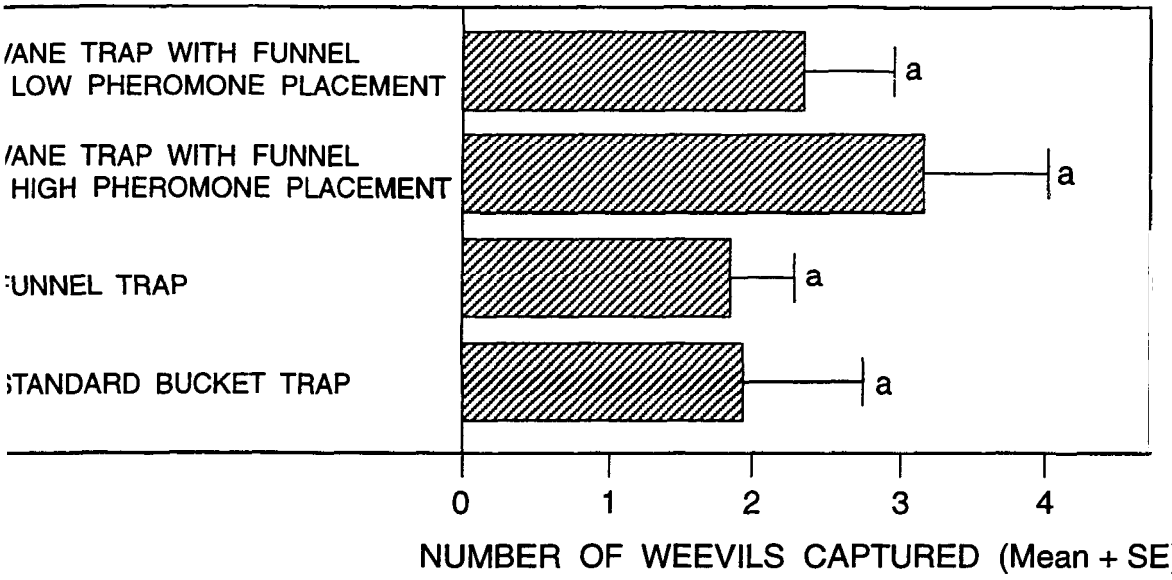

10 m
a
5 m
b
2 m
b,c
GROUND
c
0
2
4
6
8
10
12
14
TREATMENT
TOTAL NUMBER OF WEEVILS CAPTURED

Figure 21. Efficacy of different trap types in capturing *R. vulneratus* at Bah Lias Estate, North Sumatra. **Exp. 27:** Vane + funnel traps with high or low pheromone placement compared to funnel and standard bucket traps, 18 February - 19 March 1994, N = 10. Between treatment differences not significant, ANOVA, $\log_e$ (x+1)-transformed data, F= 0.56, df= 3,15, P= 0.653. **Exp. 28:** Black or unpainted vane + funnel traps compared to funnel traps, 11-25 April 1994, N = 15. Between treatment differences significant, ANOVA, $\log_e$ (x+1)-transformed data, F= 3.47, df= 2,24, $P<$ 0.05. All traps contained ferrugineol (3 mg per 24 h) and fresh halved coconut husks. Bars followed by the same letter are not significantly different, Bonferroni *t*-tests, P> 0.05.

Experiment 27



Experiment 28

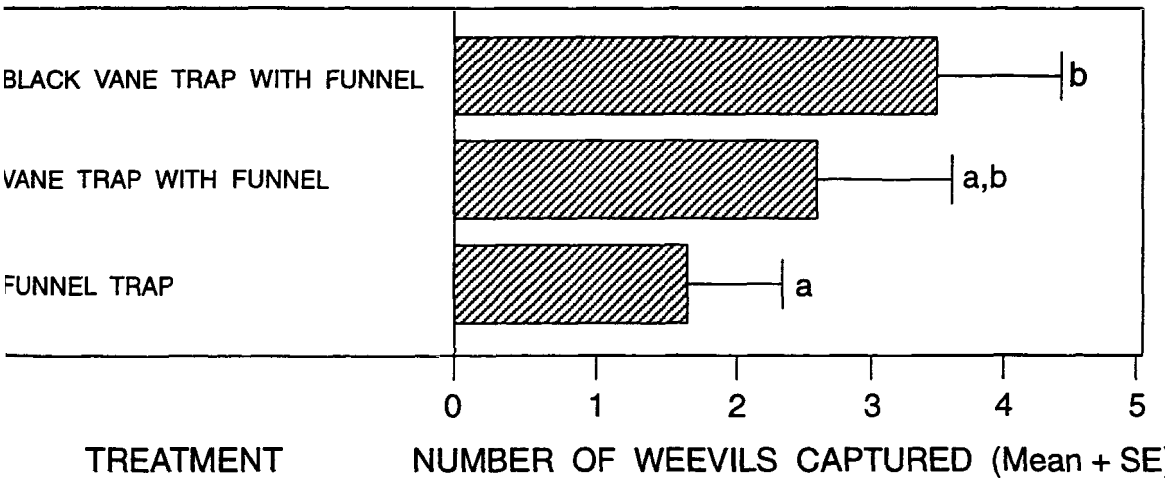


Figure 22. Comparison of trapping efficacy of traps of different colour and orientation in capturing *R. ferrugineus* in Ras Al Kaimeh, UAE, 12-20 June 1993; N= 10 (Exp. 29). Bars followed by the same letter are not significantly different, X^2-tests, $P > 0.05$.

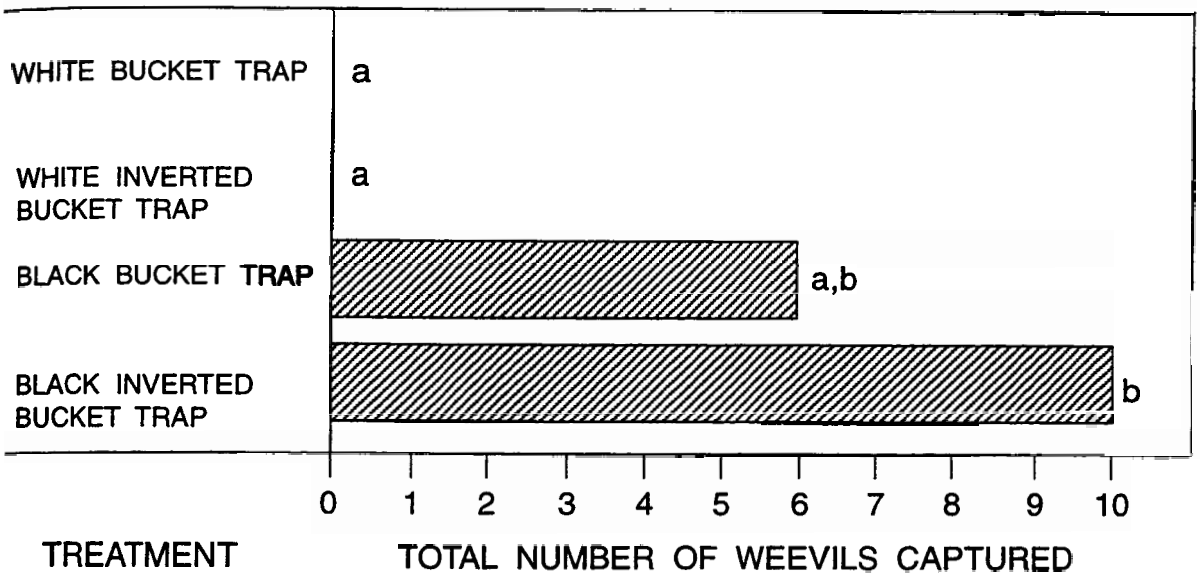
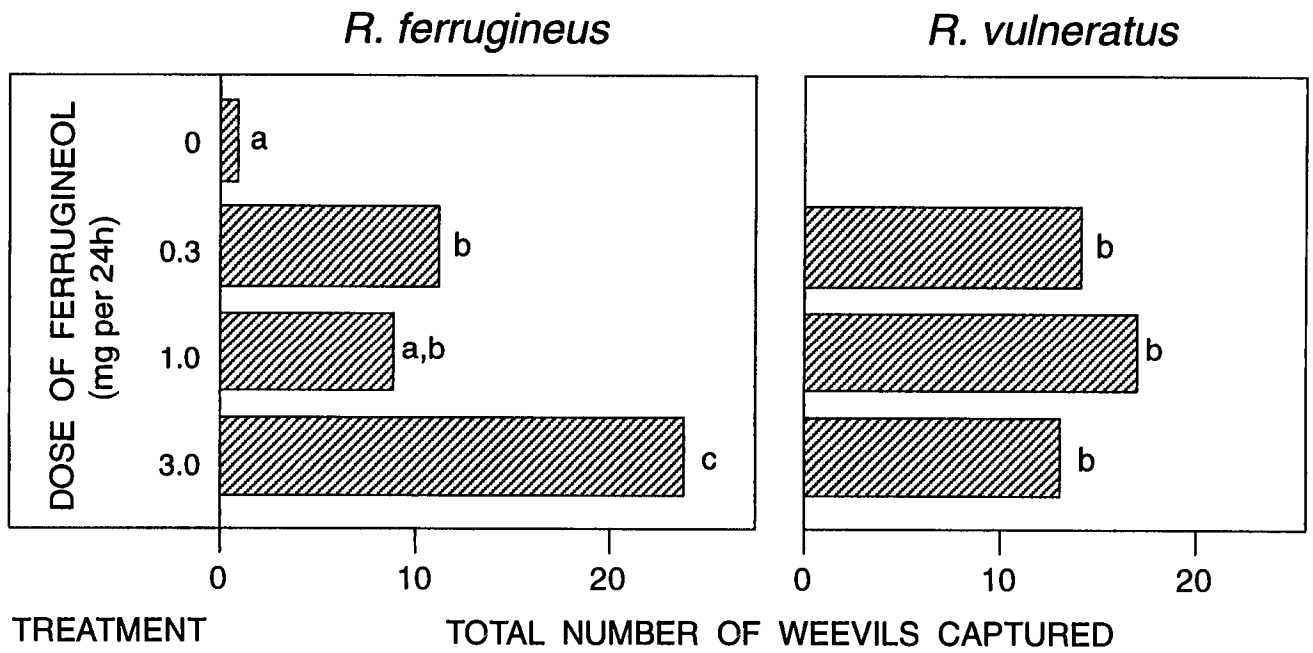

WHITE BUCKET TRAP
a
WHITE INVERTED BUCKET TRAP
a
BLACK BUCKET TRAP
a,b
BLACK INVERTED BUCKET TRAP
b
0 1 2 3 4 5 6 7 8 9 10
TREATMENT
TOTAL NUMBER OF WEEVILS CAPTURED

Figure 23. Total number of captured *R. ferrugineus* and *R. vulneratus* in traps baited with palm wood alone (control) and in combination with ferrugineol at 3 release rates; BALITKA, 22-27 August 1992; N= 10 (Exp. 30). Bars followed by the same letter are not significantly different, X^2 test, $P > 0.05$.


R. ferrugineus
R. vulneratus
DOSE OF FERRUGINEOL
(mg per 24h)
0
0.3
1.0
3.0
a
b
a,b
c
b
b
b
0
10
20
0
10
20
TREATMENT
TOTAL NUMBER OF WEEVILS CAPTURED

ORYCTES RHINOCEROS

Trapping Protocols

Both of the above ground traps (Fig. 11) were superior in trapping *O. rhinoceros* to the pitfall trap in Exp. 32. Both vane and barrier traps were superior to the pitfall trap, with vane traps capturing about three times more beetles than pitfall traps (Fig. 24).

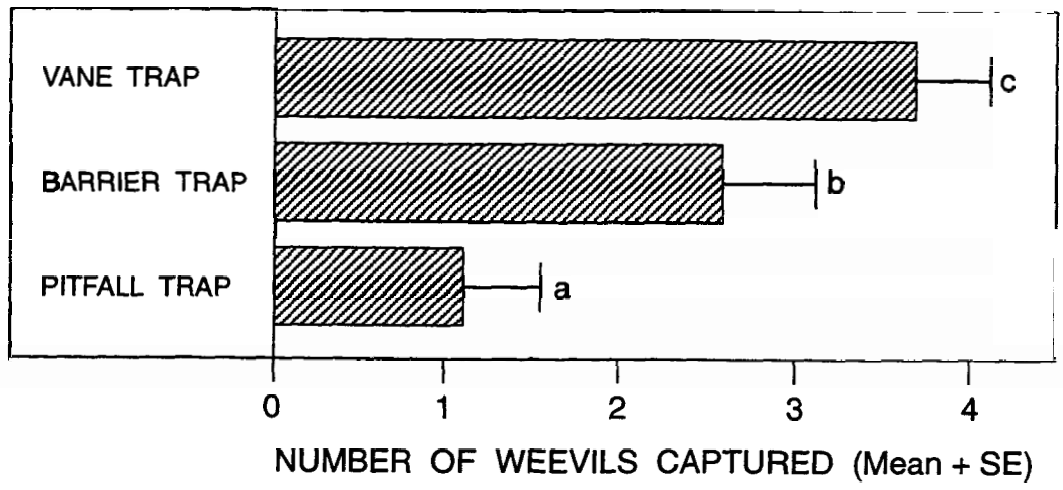
Addition of a funnel to the vane trap and painting vanes black in Exp. 33 did not alter trap efficacy (Fig. 24). Therefore, the original unpainted funnel-less vane trap was used in all subsequent experiments.

Catches of *O. rhinoceros* increased with increasing release rates of oryctelure in Exp. 34 (Fig. 25). A significant location x dose interaction was observed in Exp. 34 ($F = 18.39$, df = 1, $P < 0.001$), which arose from the 3 mg per 24 h lure being more attractive relative to the 30 mg per 24 h lure at Rambong Sialang than at Dolok (mean catches in 3 mg per 24 h traps were 64 % and 32 % of those in 30 mg per 24 h traps, respectively). This result along with the prohibitive cost of 30 mg per 24 h lures, prompted us to examine release rates between 3 and 30 mg per 24 h. In Exps. 35 and 36, lures releasing oryctelure at 6, 9, and 18 mg per 24 h were competitive with 30 mg per 24 h lures (Fig. 25).

While there were significant differences between treatments for both *R. vulneratus* and *O. rhinoceros*, for both species no differences were found in Exp. 37 between traps containing their own pheromone and those containing both pheromones (Fig. 26). Traps containing the

Figure 24. Efficacy of insecticide-free traps for capturing O. rhinoceros in North Sumatra. **Exp. 32:** Comparison of vane, barrier and pitfall traps, Rambong Sialang Estate, 2-10 February 1994, N= 10, ANOVA, F= 6.58, df = 2, P = 0.007. **Exp. 33:** Comparison of black or unpainted metal vane traps either fitted with a funnel or not, Dolok Estate, 26 February - 11 March 1994, N= 9, ANOVA, F = 1.19, df = 3, P = 0.341. All traps baited with oryctelure released at 30 mg per 24 h. Bars followed by the same letter are not significantly different, Bonferroni t-test, P> 0.05.

Experiment 32



Experiment 33

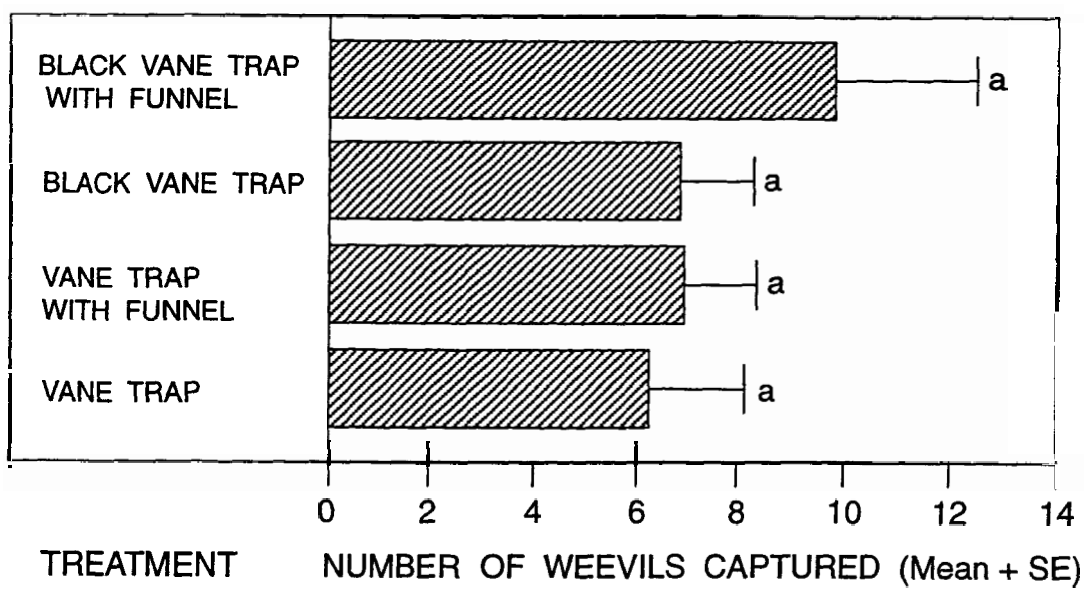


Figure 25. Attraction of *O. rhinoceros* to oryctelure released at various rates from standard vane traps. **Exp. 34:** 12-26 February and 21 February - 4 March 1994, Dolok and Rambong Sialang Estates, respectively; N= 18, ANOVA, F= 44.89, df = 3, P< 0.001. **Exp. 35:** 11-17 March 1994, Dolok; N= 15, ANOVA $\log_e$ (x+1) transformed data, F= 12.13, df= 4, P< 0.001. **Exp. 36:** 24 May - 8 June 1994, Dolok; N= 12, ANOVA $\log_e$ (x+1) transformed data, F= 49.84, df= 4, P< 0.001. Bars followed by same letter are not significantly different, Bonferroni t-test, P> 0.05. Untransformed means presented.

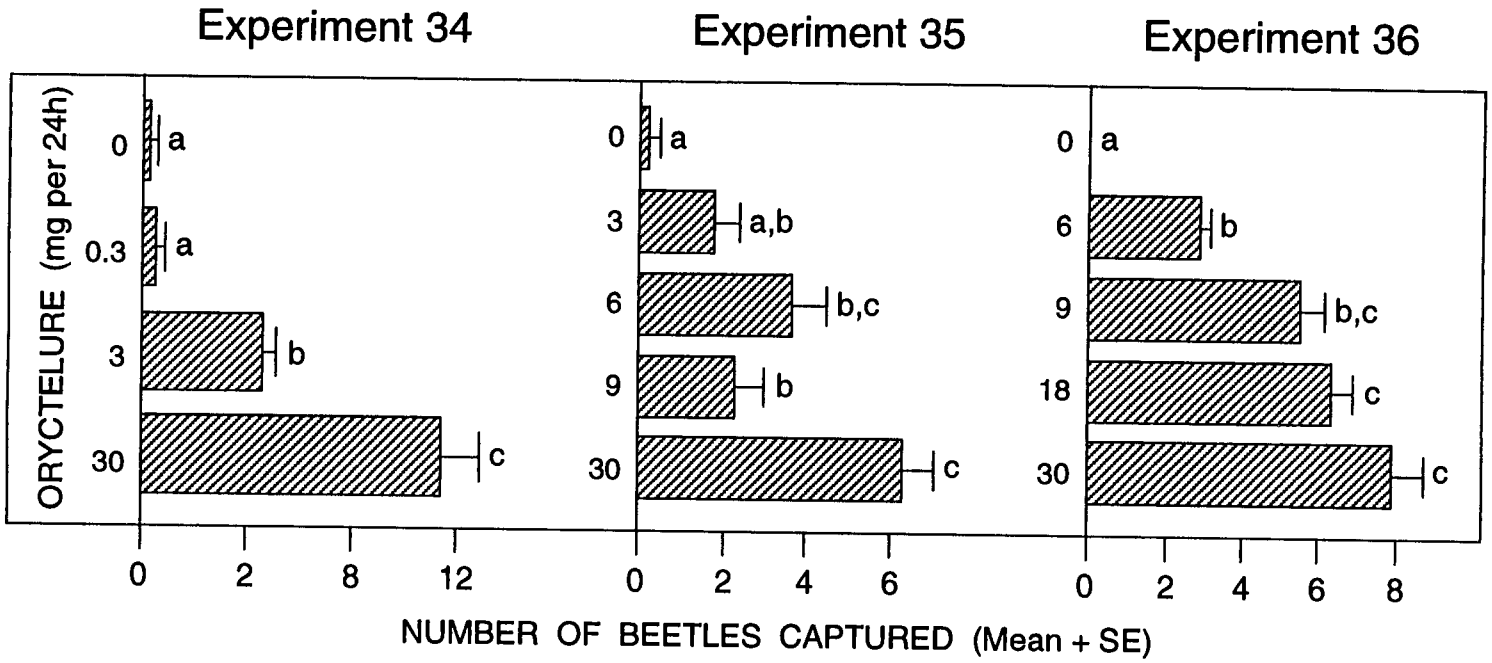
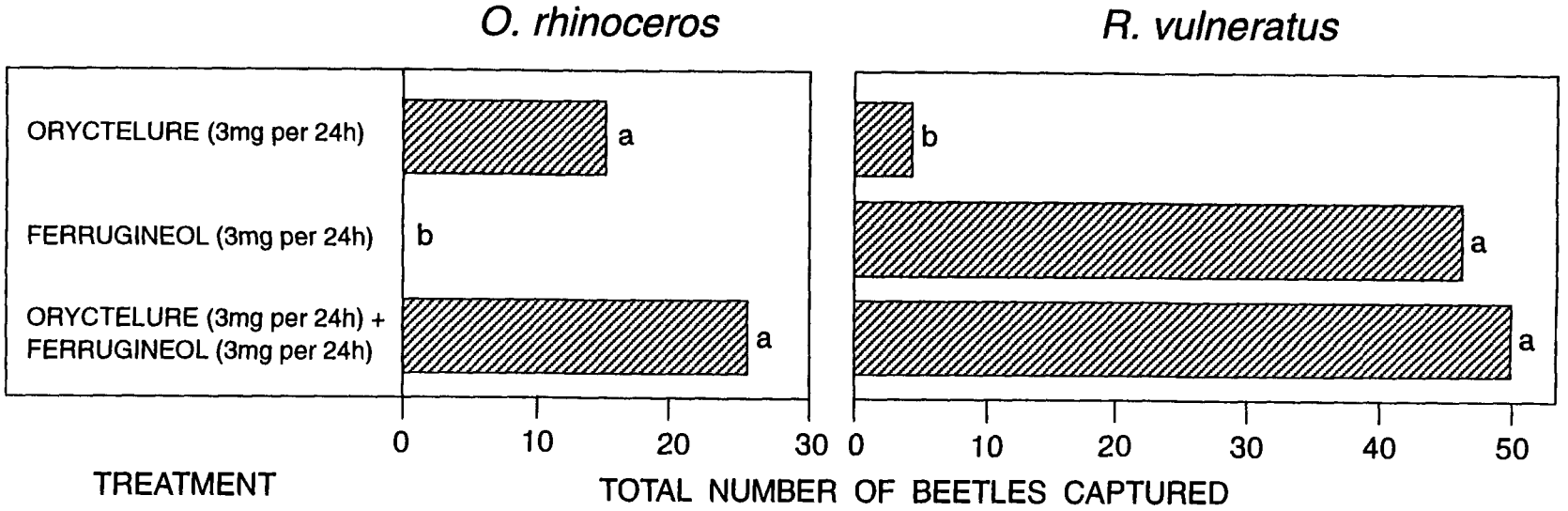

Experiment 34
Experiment 35
Experiment 36
ORYCTELURE (mg per 24h)
0
0.3
3
30
a
a
b
c
0
3
6
9
30
a
a,b
b,c
b
c
0
6
9
18
30
a
b
b,c
c
c
0
2
8
12
0
2
4
6
0
2
4
6
8
NUMBER OF BEETLES CAPTURED (Mean + SE)

Figure 26. Attraction of *R. vulneratus* and *O. rhinoceros* to traps baited with oryctelure (3 mg per 24 h), ferrugineol (3 mg per 24 h) or both (Exp. 37, Bah Lias Estate, 27 April - 7 June 1994, N= 28). Between treatment differences significant for *R. vulneratus* (X^2= 39.347, df= 2, P< 0.001) and *O. rhinoceros* (X^2= 25.721, df= 2, P< 0.001). Bars followed by the same letter are not significantly different, X^2 test, P> 0.05.


O. rhinoceros
R. vulneratus
ORYCTELURE (3mg per 24h)
FERRUGINEOL (3mg per 24h)
ORYCTELURE (3mg per 24h) +
FERRUGINEOL (3mg per 24h)
a
b
a
b
a
a
0
10
20
30
0
10
20
30
40
50
TREATMENT
TOTAL NUMBER OF BEETLES CAPTURED

pheromone of the other species were not attractive to either *R. vulneratus* or *O. rhinoceros*.

Mass-Trapping Trials

In neither mass trapping trial did the treatment appear to have a major effect on the normalized trends in damage in relation to the damage levels at the start of the trapping programs (Figs. 27, 28). In the first trial there were no striking differences in the normalized damage trends between treatment and control plots (Fig. 27), possibly because the 9 mg per 24 h release rate was too low to have a significant effect. In the second trial, in which 30 mg per 24 h lures were used for the first two weeks, damage levels decreased more during that period in the trap plot than in the control plot (Fig. 28). However, after the third week damage levels rose proportionally more in the trap plot than in the control area. This may have been partly due to the use of devices that released the pheromone at a lower dose (9 mg per 24 h).

DISCUSSION

The decline in efficacy of trapping *R. ferrugineus* and *R. vulneratus* when traps were > 2 m high (Fig. 20) appears to be in contrast to findings for *R. palmarum*, for which no differences in trapping efficacy were found for bucket traps placed on palms at heights between 0 and 3.3 m

Figure 27. Mean number of rhinoceros beetles captured per ha per week in pheromone traps in the 10 ha mass-trapping plot, Egaharap Division, Rambong Sialang Estate, 7 April - 8 June 1994, compared to the normalized damage levels in trapping and control plots expressed as a proportion of the damage in week 0. Arrow indicates start of trapping with traps baited with oryctelure released at 9 mg per 24h.

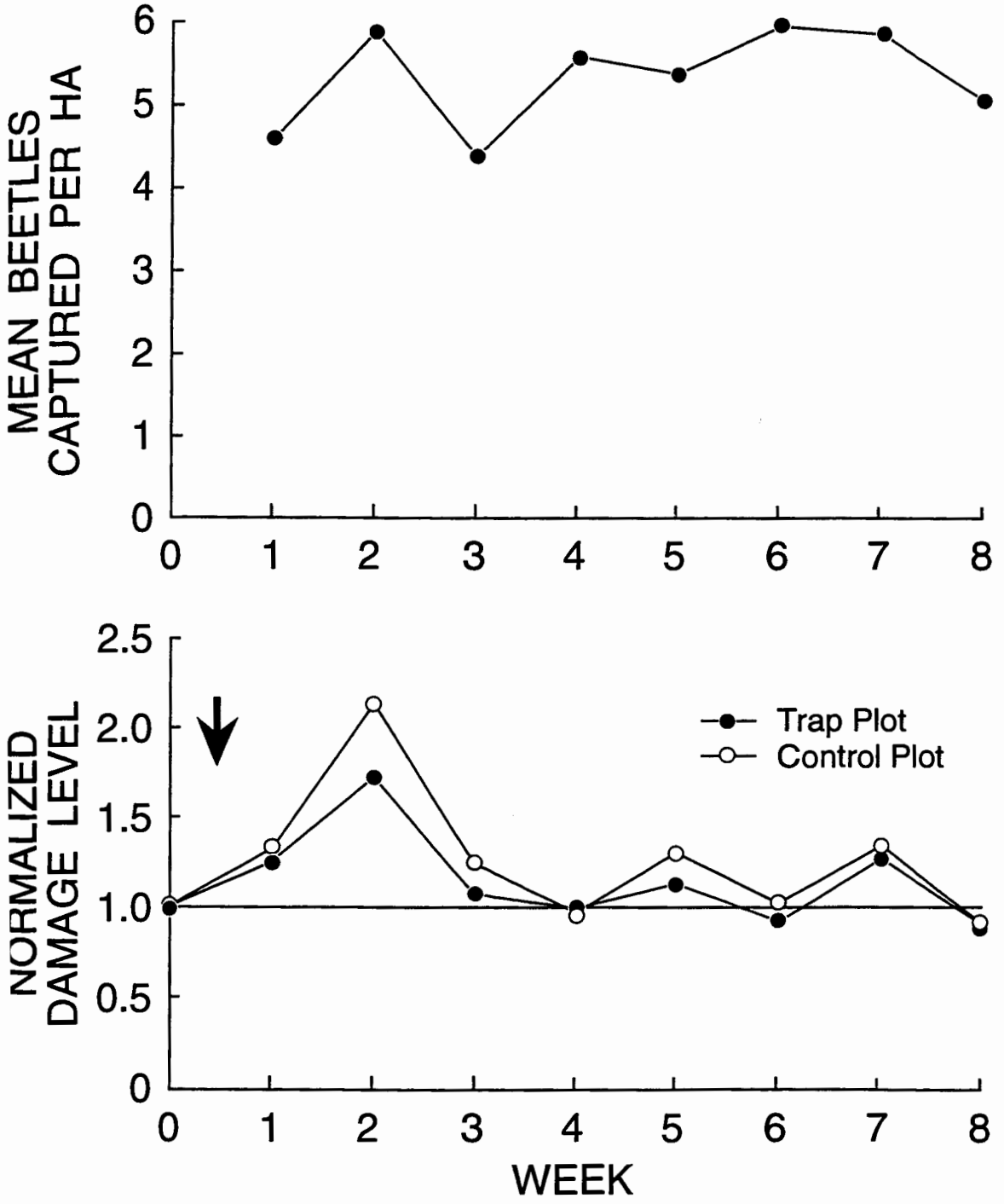
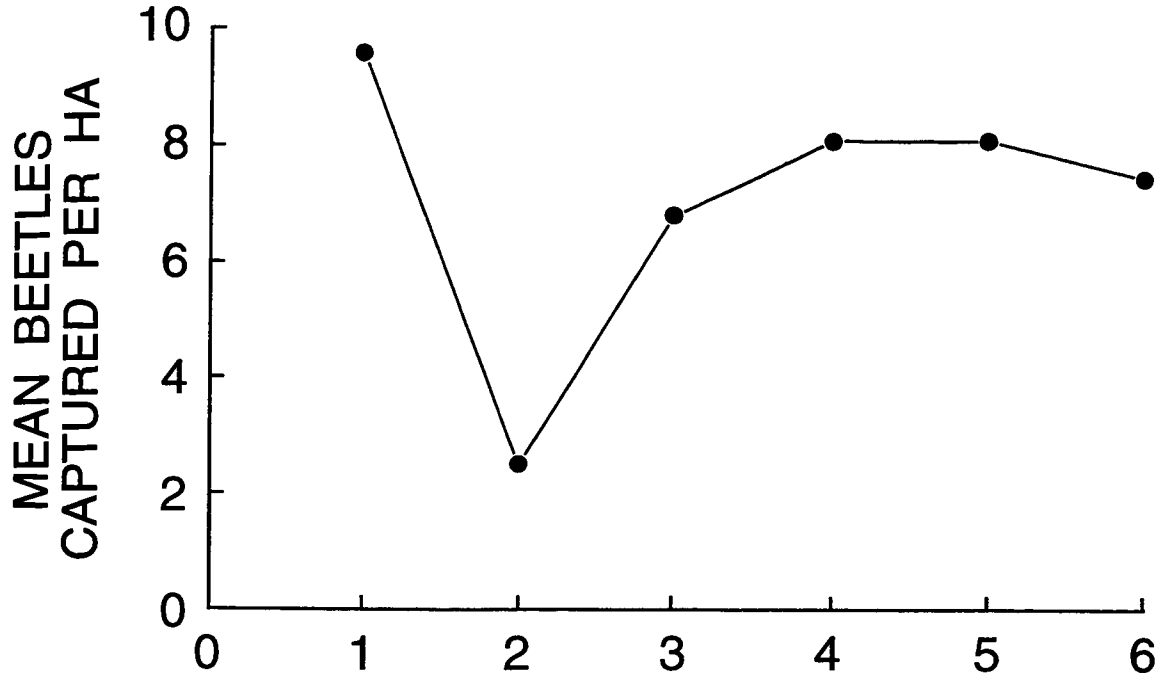
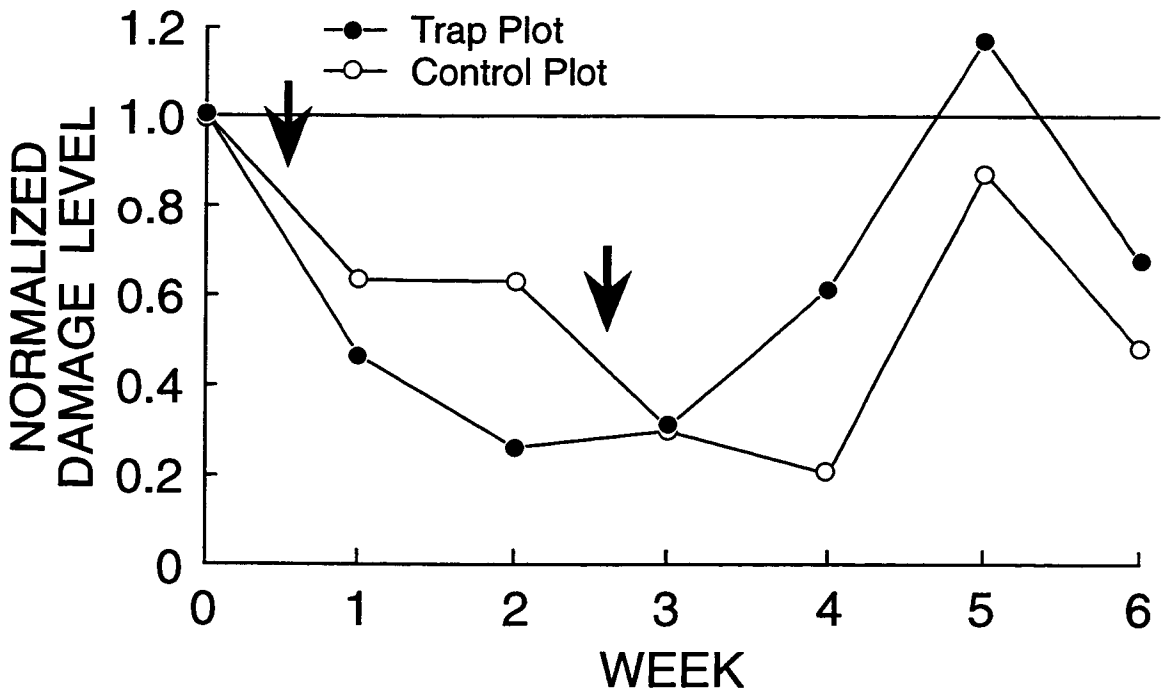

MEAN BEETLES CAPTURED PER HA
6
5
4
3
2
1
0
0 1 2 3 4 5 6 7 8
NORMALIZED DAMAGE LEVEL
2.5
2.0
1.5
1.0
0.5
0
Trap Plot
Control Plot
0 1 2 3 4 5 6 7 8
WEEK

Figure 28. Mean number of rhinoceros beetles captured per ha per week in pheromone traps in the 10 ha mass-trapping plot, Dolok Estate, 7 April - 8 June 1994, compared to the normalized damage levels in trapping and control plots expressed as a proportion of the damage in week 0. Arrows indicate start of trapping with traps baited with oryctelure released at 30 mg per 24 h and then at 9 mg per 24h.


MEAN BEETLES CAPTURED PER HA
10
8
6
4
2
0
0
1
2
3
4
5
6


NORMALIZED DAMAGE LEVEL
1.2
1.0
0.8
0.6
0.4
0.2
0
Trap Plot
Control Plot
0
1
2
3
4
5
6
WEEK

(Oehlschlager *et al.*, 1993b). However, decreased trapping efficacy may have been found in that study, if trap heights above 3.3 m had been tested. The greater response of *R. ferrugineus* and *R. vulneratus* to black than white buckets (Fig. 21) and to vane traps with black-painted vanes over those with reflective unpainted vanes (Fig. 21) suggests that vision is important in host selection by these species. Visual discrimination between acceptable and unacceptable host silhouettes has been hypothesized in the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Borden *et al.*, 1986) and demonstrated in *Pissodes strobi* (VanderSar & Borden, 1977a), which selects feeding and oviposition sites by positive phototaxis and negative geotaxis (VanderSar & Borden, 1977b).

Oryctes spp. also respond to vertical silhouettes (Bedford, 1980). Although volatile plumes may be different when emitted from pitfall or above-ground traps (Lewis & Macaulay, 1976; McNeil, 1991) it is likely that the superior efficacy of vane and barrier traps for capturing *O. rhinoceros* (Fig. 24) can be attributed to the vertical trap silhouettes which are lacking in the pitfall trap. Because response by *O. rhinoceros* to the vertical silhouette was not enhanced further by use of non-reflective black vanes (Fig. 24), and the use of funnels did not improve retention of captured beetles in traps, insecticide-free, unpainted vane traps were adopted as standard for *O. rhinoceros*.

Because ferrugineol released at 3 mg per 24 h was significantly more attractive to *R. ferrugineus* than at lower release rates (Fig. 23), this

release rate was adopted as a standard in subsequent experiments and is recommended for operational use. As in *A. octiescostata* (Leal *et al.*, 1994a), increase of pheromone release rate resulted in increasing numbers of captured *O. rhinoceros* (Fig. 25). Because traps baited with oryctelure released at 6, 9, 18 or 30 mg per 24 h were similarly attractive (Fig. 25), a release rate of 9 mg per 24 h was adopted for operational trapping trials.

Both *R. vulneratus* and *O. rhinoceros* were captured in equal numbers in traps containing pheromones of both species as in traps containing only their own pheromones (Fig. 26). This result indicates that the pheromones of one species do not interfere with those of the other. The capture of several *R. vulneratus* in traps containing only oryctelure may be indicative of a slight cross-attraction. Cross-attraction would be expected when one species exploits the pheromone of a co-inhabiting species in order to colonize a host most efficiently (Birch, 1984). The absence of an inhibiting effect of either the pheromone of *R. vulneratus* or *O. rhinoceros* on the other species indicates that these species do not interact competitively when colonizing the same host. Presumably this is because only the palm weevils utilize living palms for brood production, while the pioneer species, *O. rhinoceros*, uses live palms as a food source for adult beetles only. Strong cross-attraction may not occur because *O. rhinoceros* may also release aggregation pheromone in its breeding sites (e.g. rotting trunks, compost piles), which are unsuitable for palm weevil brood production. The lack of interference between

pheromones also means that wherever both *Oryctes* and *Rhynchophorus* spp. are both considered to be problems, the same traps can be used to capture both species, thereby avoiding the need for two separate types of traps and without any increase in the cost of trapping system maintenance. Lack of interference between heterospecific pheromones also permits simultaneous monitoring of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) and the red flour beetle, *Tribolium castaneum* (Herbst) (Lindgren *et al.*, 1985).

The impact of pheromone-based mass-trapping on damage to young oil palms by *O. rhinoceros* was not clearly demonstrated in pilot trials (Figs. 27, 28). However, these data result from only 6 - 8 weeks of trapping, and since *O. rhinoceros* is capable of moving over large distances the population of beetles in a given area is not likely to be affected very greatly over the short term. Captures of *O. rhinoceros* to coconut trunk traps were also found to be highest when undergrowth was slashed and at the new moon, and to be depressed by heavy rains (Bedford, 1975). Mixed results have been experienced in attempts to control pest populations by mass-trapping with pheromones. In a single season trial, *Dendroctonus brevicomis* damage was greatly reduced in mass-trapping plots in a small isolated infestation at Bass Lake, California (Bedard & Wood, 1974). However, in Norway, reductions in *Ips typographus* (L.) populations were attributed more to weather conditions than to the capture of 4.9 billion beetles in 1980 (Bakke, 1983). Incidence of Dutch elm disease increased in trap plots despite the capture of 3.8

million smaller European elm bark beetles, *Scolytus multistriatus* (Marsham), in one trapping season in Detroit (Lanier *et al.*, 1976). It was presumed that increased disease rates were the result of immigration of beetles to the trapping area.

Because large spatial and temporal variations exist in populations of *Oryctes* spp. and damage caused by them, some difficulty was experienced in establishing appropriate controls for comparison. Because of these variations, it was concluded that trapping may have to be conducted over a full year before its full impact can be clearly evaluated. Additional studies on the effective distance of pheromone lures and population estimates would also provide valuable information for evaluating the impact of mass-trapping on *O. rhinoceros* populations and damage. Population estimates of overwintering *Trypodendron lineatum* enabled Lindgren & Borden (1983) to determine that mass-trapping led to an average of 36% suppression of populations per year over 1 to 3 years. The impact of mass-trapping programs is difficult to assess or may not even become apparent in short-term or small scale trials (Borden, 1995). Following 12 years of mass-trapping at one location, damage caused by *T. lineatum* and *Gnathotrichus sulcatus* declined by 90 % and yielded an estimated benefit:cost ratio of at least 5:1 (Lindgren & Fraser, 1994). Information is not currently available on the extent to which *O. rhinoceros* damage reduces oil palm fruit bunch or oil production due to tree stunting and delayed time to maturation. Such information is vital to obtaining a

realistic estimate of the benefit:cost ratio of a pheromone mass-trapping program.

Long-term trapping trials were established in several 1994 oil palm plantings in October 1994, using the relatively inexpensive, but still effective, release rate of 9 mg per 24 h for oryctelure. However, the results of the second trial suggest that the higher release rate of 30 mg per 24 h may have had some impact on the numbers of beetles captured and the normalized damage trend (Fig. 28). Therefore, in the long run, it may be more cost-effective to initially use a high release rate or high density of traps, or both, e.g. 18 mg per 24 h and 4 traps per ha, and then change to a release rate of 9 mg per 24 h and 1 trap per ha. In this manner a population might be reduced in the first period of trapping and then maintained at a low level thereafter. Currently, *O. rhinoceros* is being trapped operationally in Malaysia with a trapping density of 1 trap per 2 ha (A.C. Oehlschlager[1], pers. comm.). These programs utilize pheromone traps with black vanes placed 2 m high, which is superior to the ground level traps that I used (Teh Chong Lay[2], pers. comm.). However my short-term trials and initial results of long-term trials in Sumatra, Indonesia, and Sabah, Malaysia, suggest that greater trap densities than 1 per 2 ha are required.

Mass-trapping trials against *R. ferrugineus* and *R. vulneratus* were not conducted as part of this study as they were not considered to be

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[1]Department of Chemistry, Simon Fraser University, Burnaby, B.C., Canada
[2]BAL Plantations, Tawau, Sabah, Malaysia

significant pests in Indonesia and Malaysia. However, in the United Arab Emirates, Saudi Arabia, and Egypt, where introduced *R. ferrugineus* is causing severe damage to date palms, mass-trapping is considered to be a viable method of control and is being conducted and supported by local Ministries of Agriculture and by the Food & Agriculture Organization of the United Nations (A.C. Oehlschlager[3] , pers. comm.). Mass-trapping of the American palm weevil, *R. palmarum*, has been credited with reducing weevil populations and maintaining them at a low density (Chinchilla *et al.*, 1993; Oehlschlager *et al.*, 1995b). In addition, incidence of the nematode-caused red ring disease, which is vectored by *R. palmarum*, decreased in the trapping areas, while it increased in other parts of the test plantation (Chinchilla *et al.*, 1993; Oehlschlager *et al.*, 1995b). The potential for control of *R. ferrugineus* and *R. vulneratus* by mass-trapping with pheromones is thus very great.

[3]Department of Chemistry, Simon Fraser University, Burnaby, B.C., Canada

PART II, SYNONYMIZATION OF *RHYNCHOPHORUS* SPECIES

The concept of a species, while obvious intellectually, has not been easy to define in practical terms. The most widely accepted definition of a species has been that of the "biological species concept", in which species are defined as "groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups" (Mayr, 1969). This concept, also referred to as the "isolation species concept" (Templeton, 1989), has been criticized because it is defined in terms of isolating mechanisms which may arise as a byproduct of evolution, but are not necessarily an active part of the process of speciation (Templeton, 1989). Two further concepts, the "recognition species concept" (Paterson, 1985) and the "cohesion species concept" (Templeton, 1989) have thus been proposed. However, if sympatric species are actually sibling species rather than a single species, some form of isolating mechanism must be present. In examining the species status of *R. ferrugineus* and *R. vulneratus*, it is therefore convenient to consider the isolation species concept, and to determine what isolating mechanism(s) is in operation, if any. Synonymies for *R. ferrugineus* and *R. vulneratus* are presented in Table 19.

Several observations made while investigating production of and response to aggregation pheromone by *R. ferrugineus* and *R. vulneratus*

Table 19. Synonymies for *Rhynchophorus ferrugineus* (Olivier) and *R. vulneratus* (Panzer).

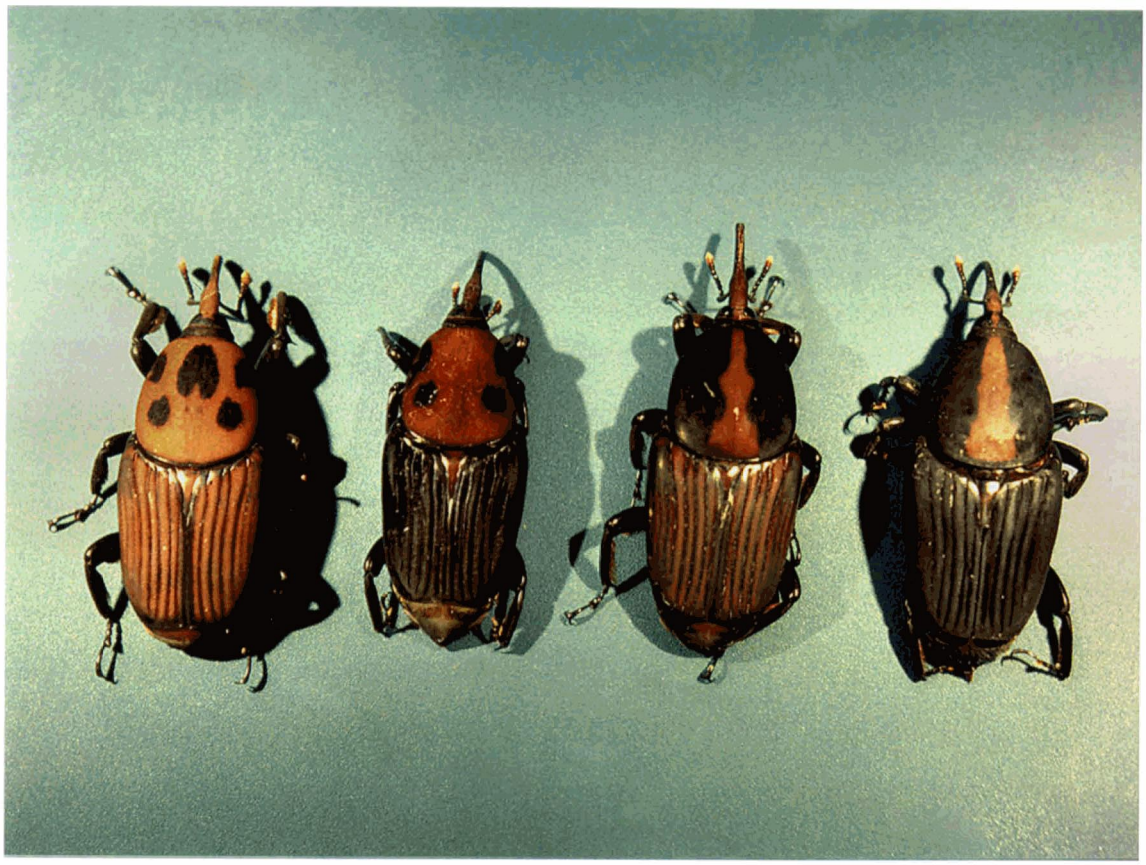
Species	Synonym	Author	Date
R. ferrugineus	*Cossus saguarios*	Rhumpf	1750-55
	Curculio hemipterus	Sulzer	1776
	Curculio ferrugineus	Olivier	1790
	Rhynchophorus ferrugineus	Herbst	1795
	Cordyle sexmaculatus	Thunberg	1797
	Calandra ferruginea	Fabricius	1801
	Rhynchophorus indostanus	Chevrolat	1882
	Rhynchophorus signaticollis	Chevrolat	1882
	Rhynchophorus pascha var. *cinctus*	Faust	1892
	Rhynchophorus ferrugineus var. *semínger*	Faust	1894
	Rhynchophorus signaticollis var. *dimidiatus*	Faust	1894
	Rhynchophorus glabrirostris	Schaufuss	1885
R. vulneratus	*Curculio vulneratus*	Panzer	1798
	Calandra schach	Fabricius	1801
	Rhynchophorus schach	Schoenherr	1826
	Rhynchophorus vulneratus (Panzer)	Gyllenhal	1838
	Rhynchophorus schach var. β, γ, δ	Boheman	1845
	Rhynchophorus pascha	Boheman	1845
	Rhynchophorus ferrugineus var. *tenuirostris*	Chevrolat	1882
	Rhynchophorus glabrirostris	Schaufuss	1885

(Chapters 2,5) led me to question the validity of designating these weevils as separate species. In three experiments, 27 captured weevils (10.6% of total captured) had colour markings intermediate between those of *R. ferrugineus* and *R. vulneratus* (Fig. 29). These individuals had the black pronotum with a red stripe, characteristic of *R. vulneratus*, but their elytra and abdomens were reddish-brown, like *R. ferrugineus*. A single field-collected individual had the characteristic pronotum of *R. ferrugineus*, but the black elytra and abdomen typical of *R. vulneratus*.

Another observation was the lack of pronounced differences in the chemical composition or chirality of any antennally- or behaviourally-active compound produced by either species (Figs. 2, 7, 8), and yet another was the lack of differences in response to these compounds (Figs. 4, 9, 10, Tables 2-5).

These observations alone do not constitute a basis for the synonymization of the species. Colour variation could occur within a species. Other compounds not detected in my experiments could impart species specificity to communication mechanisms. Moreover, there are other pre-mating mechanisms that could ensure reproductive isolation (Lanier & Burkholder, 1974). These include: ecological stratification of habitat, differences in flight periods or diel activity, differences in host compounds used as synergists, food preferences, specificity of stridulation or courtship rituals, and genital incompatibility. Potential

Figure 29. Specimens of *R. ferrugineus* (far left) and *R. vulneratus* (far right) and two intermediate colour forms found in Indonesia.



postmating mechanisms of isolation include nonfertility of eggs and sterility of hybrids (Lanier & Burkholder, 1974).

Although there are reports that *R. ferrugineus* more often attacks the trunk and *R. vulneratus* the terminal bud of the tree (Sivapragasam *et al.*, 1990), both attack the same variety of palm types (Wattanapongsiri, 1966), and coattacked trees have been observed (Banks, 1906). Antennal responses of *R. ferrugineus* and *R. vulneratus* to synthetic host volatiles were nearly identical (Fig. 18). Differences in food preferences or the use of specific host compounds as pheromone synergists, therefore, do not appear to be functioning to achieve reproductive isolation.

In terms of differences in flight periods or diel activity, no formal study was made, but I have observed *R. ferrugineus* and *R. vulneratus* flying at the same times of day and in experiments conducted throughout the year where the two species are sympatric in Indonesia, individuals of both species have always been captured.

Rochat and Zagatti (1993) reported evidence for a female-produced cuticular pheromone for *R. palmarum*, which may be important in sex recognition and mating behavior. However, interspecific mating pairs of *R. ferrugineus* and *R. vulneratus* are often observed in caged populations, suggesting that incompatibilities in courtship rituals or genitalia may not exist or may not be sufficient on their own to maintain isolation.

My objective was to challenge in a definitive manner, the null hypothesis that despite the existence of two distinct colour morphs, they

do not constitute valid species. My investigations included a reexamination of morphological characters used in part to justify separation of *R. vulneratus* from *R. ferrugineus* (Wattanapongsiri, 1966), an experiment testing con- and heterospecific responses to male weevils, comparative analysis of DNA variability, and a cross-breeding study.

METHODS

Specimens of *R. ferrugineus* and *R. vulneratus* were collected from four locations (Bogor, Bojongkalong, Cikancana and Pakuwon) in West Java (Fig. 30), and specimens of *R. ferrugineus* were also collected in the UAE. Voucher specimens of weevils used in morphological analyses and F_1's from the cross-breeding study have been deposited in the collection of the Canadian Museum of Nature, Ottawa.

Morphological Comparisons.

Submentum and subgenal sutures. Twenty six specimens of *R. ferrugineus*, 34 *R. vulneratus* and 10 *R. vulneratus* colour intermorphs (Wattanapongsiri, 1966) from Indonesia were mounted on Styrofoam blocks with the ventral surface of the rostrum exposed. The basal area of the rostrum (Fig. 31) was drawn under the microscope (500 x) using a camera lucida (Wild M5, Heerbrug, Switzerland). Because the weevils were mounted in an inverted position and specimens of both species were mixed in a single group, I could not determine which species of weevil was being drawn. Several measurements were taken from the drawings

Figure 30. Map of the island of Java, Indonesia, with enlarged view of West Java and locations in which specimens of *R. ferrugineus* and *R. vulneratus* were collected: Bogor (6° 30'S, 106° 15'E), Bojongkalong (6° 48'S, 106° 58'E), Cikancana (6° 50'S, 107° 02'E), and Pakuwon (6° 45'S, 106° 45'E).

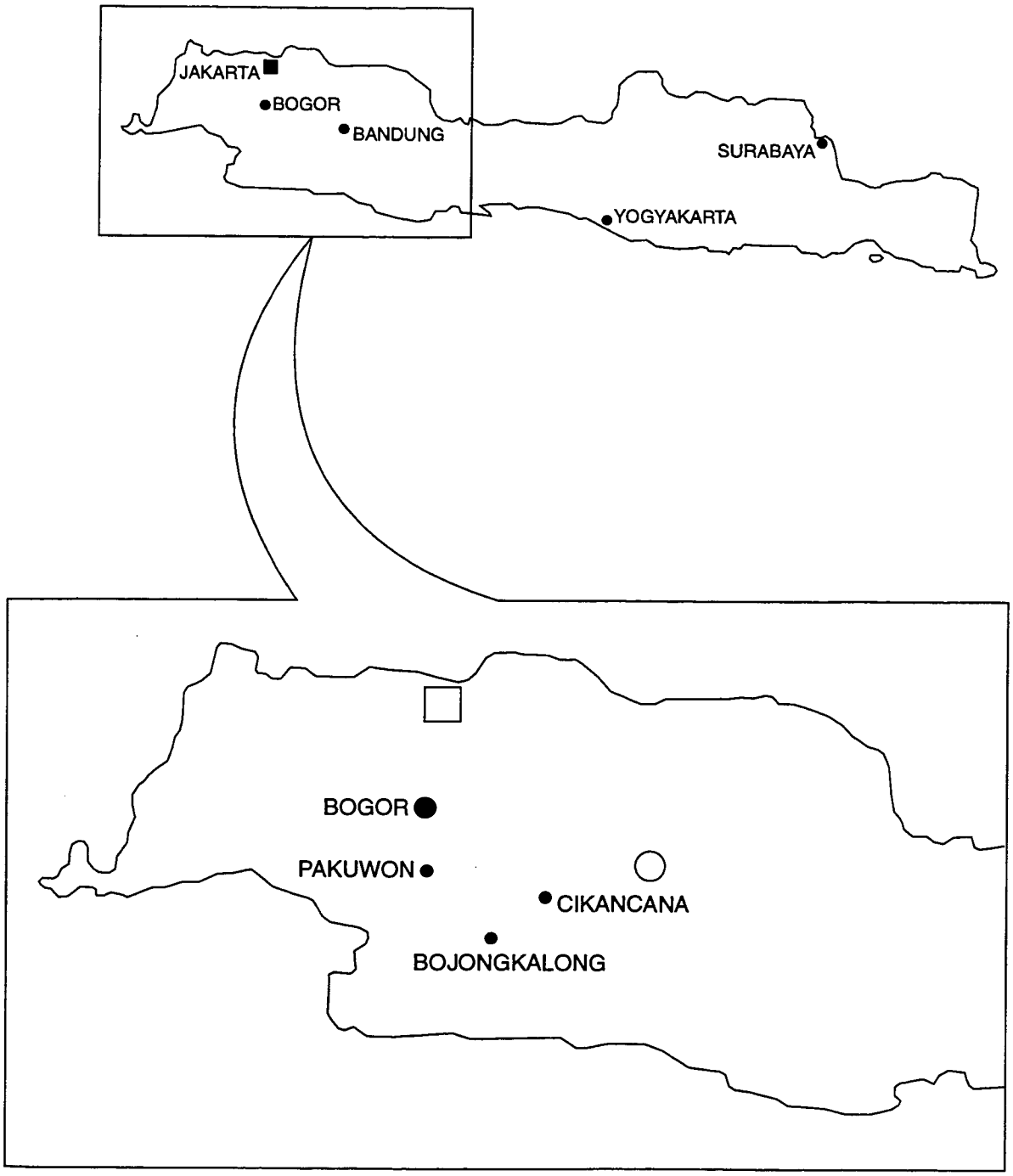
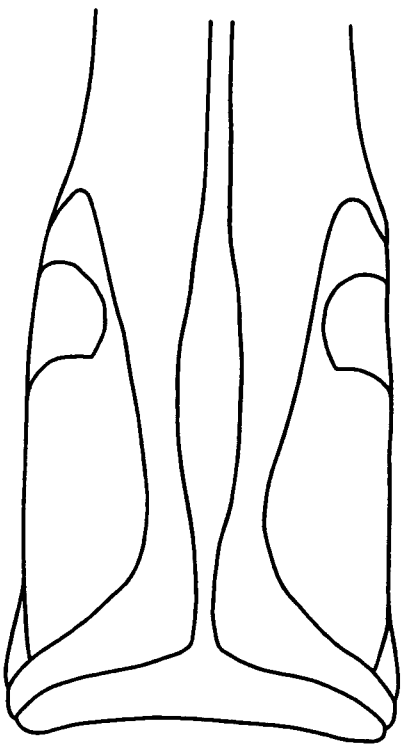
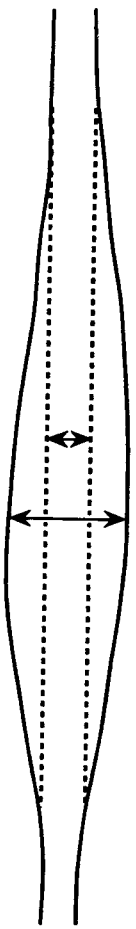

JAKARTA
BOGOR
BANDUNG
SURABAYA
YOGYAKARTA
BOGOR
PAKUWON
CIKANCANA
BOJONGKALONG

Figure 31. Representative diagram of ventral view of basal area of rostrum of *R. ferrugineus* or *R. vulneratus* as drawn by camera lucida (500x). Measurements depicted in enlarged drawing are the central width of the submentum (short arrowed line) and maximum distance between the subgenal sutures (longer arrowed line). Wattanapongsiri (1966) used the term gular suture in place of subgenal suture (Lyal, 1995). Wattanapongsiri (1966) described *R. vulneratus* as having the gular suture concave at both sides medially before reaching base of rostrum, and *R. ferrugineus* as having a gular suture oval at base but less concave than in *R. vulneratus*.





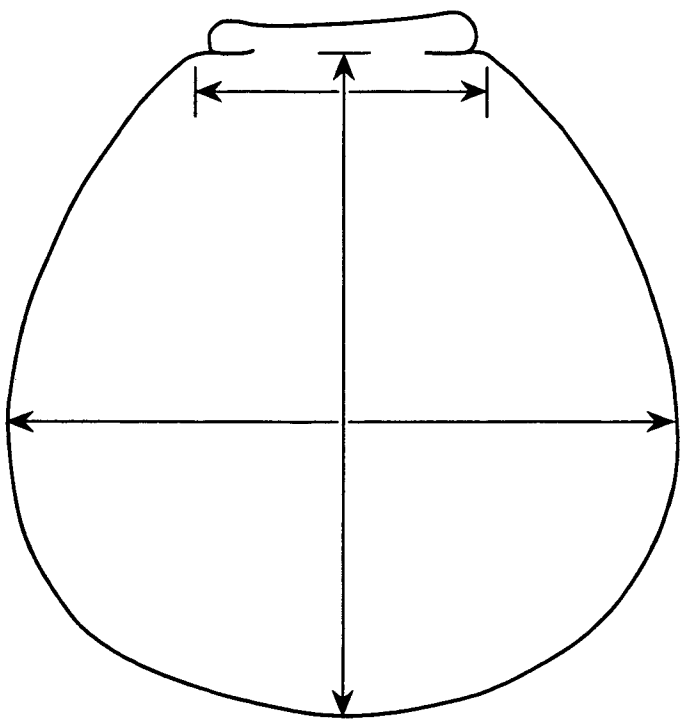
(length of concave section of submentum, width of submentum and maximum distance between suture lines), and an estimate of concavity was developed: (central width of submentum) / (maximum distance between subgenal sutures) (Fig. 31). By this estimate, a submentum with subgenal sutures more concave medially would receive a lower score than one less concave medially.

Pronotal shape. Fifty specimens of *R. ferrugineus* (24 from Indonesia and 26 from United Arab Emirates), 35 *R. vulneratus* pure colour types and 10 intermediate *R. vulneratus* colour types, all from Indonesia, were mounted with the dorsal side up, and drawings were made of the shape of the pronotum using a microscope (60 x) and camera lucida. Three measurements were taken from the drawings: maximum and minimum width of the pronotum and pronotal length (Fig. 32). Two estimates of pronotal shape were then devised: ratio of minimum to maximum pronotal width, and ratio of minimum pronotal width to length.

Cross-Attraction of Live Males.

The con- and interspecific attractiveness of *R. ferrugineus and R. vulneratus* males was compared with the activity of steresoisomeric ferrugineol and enantiomeric ferrugineone in a 10:1 ratio in a five-replicate experiment conducted at BALITKA (5-9 September 1992). Weevil-baited bucket traps contained a glass jar half-filled with cut pieces of coconut palm petiole and 10 males of either *R. ferrugineus* and *R.*

Figure 32. Representative pronotum of *R. ferrugineus* or *R. vulneratus* as drawn by camera lucida (60x), and showing three measurements used in calculating measures of pronotal shape: maximum and minimum width of pronotum (long and short horizontal arrowed lines) and pronotal length (vertical arrowed line).



vulneratus collected approximately 30 km from BALITKA. Twenty 3 mm diam. holes in the jar lid provided ventilation and allowed natural pheromones to be released. Synthetic ferrugineol and ferrugineone were released at 3 mg per 24 h (three 1.5 mL polyethylene microcentrifuge tubes) and 0.3 mg per 24 h (two capillary tubes, 1 mm ID), respectively.

Genetic Comparisons[1].

Live adult weevils of both *R. ferrugineus* and *R. vulneratus* were collected at 4 locations in West Java, Indonesia and shipped to SFU. Weevils were frozen at -80° C until needed for analysis.

One middle leg of each beetle was ground in liquid nitrogen with a glass pipette and a small amount of Lifton buffer in a 1.5 mL microtube and suspended in 0.9 mL Lifton buffer (0.2M sucrose, 0.05M EDTA, 0.1M Tris, 0.5% sodium dodecyl sulphate, pH 9.0). After 15 min on ice, 100 µL 8M potassium hydroxide was added, the suspension vortexed briefly, returned to ice for another 15 min, and centrifuged at high speed (14,000 rpm) for 15 min. The supernatant was transferred to a new 1.5 mL microtube, 100 µL 1:24 isoamyl alcohol : chloroform and 1.3 mL equilibrated phenol was added and the mixture was centrifuged at 14,000 rpm for 10 min. The aqueous phase was transferred to a new tube, 1:24 isoamyl alcohol : chloroform was added and centrifuged as above. The aqueous phase was then removed to a new tube and 30 µL ammonium

[1]All genetic analyses conducted by Dr. Bernard J. Crespi, Department of Biological Sciences, Simon Fraser University.

acetate and 1/2 current volume isopropanol was added. After 1 h at -20° C, solutions were centrifuged for 30 min to pellet the DNA. The pellet was washed twice in 1 mL cold 70% ethanol, air-dried for 1 h, resuspended in 100 μL distilled deionized water at room temperature, and stored at -20° C. Prior to polymerase chain reaction (PCR) amplifications, DNA was quantified in a spectrophotometer and amount adjusted so that equal amounts of DNA were used in each amplification.

Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) analyses. RAPD amplification reactions each contained 22 μL water, 0.5 μL of 10 μM dNTPs, 2.5 μL 10x ultratherm buffer (100 mmol/L TrisHCl, 15 mmol/L $MgCl_2$, 500 mmol/L KCl, pH 8.3), 0.33 μL 10 μM primer (University of British Columbia oligonucleotide facility RAPD primer set), 0.125 μL ultratherm *Taq* DNA polymerase and 0.83 μL of weevil genomic DNA. All 24 primers used were random 10-base oligomers, the sequences of which are shown in Table 20. Amplifications were performed in a Perkin-Elmer-Cetus 480 thermocycler programmed for 35 cycles with denaturation at 94° C for 1 min, annealing at 35° C for 1 min, and extension at 72° C for 2 min, for specimens of both *R. ferrugineus* and *R. vulneratus* from Bojongkalong. Amplified DNA was analyzed by electrophoresis on 1.5% agarose gels, stained with ethidium bromide, and photographed.

Table 20. Sequences of the oligomer primers (University of British Columbia oligonucleotide facility RAPD primer set) used in RAPD analyses of *R. ferrugineus* and *R. vulneratus*.

Primer Code	Nucleotide Sequence (5' - 3')	Primer Code	Nucleotide Sequence (5' - 3')
601	CCG CCC ACT G	613	TGC ACC CAC G
602	GCG AAG ACT A	614	GTA GTC TCG C
603	ACC CAC CGC G	615	CGT CGA GCG G
604	GGC CCA TTG C	616	CGG AAG AAA C
605	CCG ATC ATT C	617	CGG ACT ATG T
606	CGG TCG GCC A	618	CGG ACT ATG T
607	AGT GTC GTC G	619	TTC CCT AGC G
608	GAG CCC GAA A	620	TTG CGC CCG G
609	ACA GCA CCA T	621	GTC TGC GCT A
610	TTT GCC GCC C	622	ACA GGT GGT T
611	CCA TCG TAC C	623	TGC GGG ACT G
612	CCG TGA GTA T	624	GTG ATA AGC C

Mitochondrial DNA sequencing. PCR amplification of the mitochondrial cytochrome oxidase subunit 1 (CO1) gene was conducted with specimens of both *R. ferrugineus* and *R. vulneratus* from three locations (Bogor, Cikancana and Pakuwon). Double- and single-stranded mitochondrial DNA (mtDNA) amplifications used C1-J-1718 and TL2-N-3014, and sequencing was conducted by annealing with TL2-N-3014 and C1-J-2441 (Simon *et al.*, 1994). Double-stranded mtDNA amplifications each contained 39 μL water, 1 μL dNTPs, 5 μL 10X buffer, 2.5 μL each of 10 μM primers (C1-J-1718 and TL2-N-3014), 0.25 μL ultratherm *Taq* DNA polymerase and 0.7 μL of weevil genomic DNA. Amplifications were performed in a Perkin-Elmer-Cetus 480 thermocycler programmed for 30 cycles with denaturation at 94° C for 1 min, annealing at 47° C for 1 min, and extension at 72° C for 1 min. Single-stranded amplifications were then conducted using 3 μL of amplified double-stranded DNA, 39 μL water, 1 μL dNTPs, 5 μL 10X buffer, 0.6 μL 10 μM primer (C1-J-1718 or TL2-N-3014) and 0.25 μL ultratherm *Taq* DNA polymerase. There were 30 amplification cycles with denaturation at 94° C for 1 min, annealing at 52° C for 1 min, and extension at 72° C for 1 min. In order to remove dNTPs, primers and enzymes, amplified DNA was centrifuged in Millipore Ultrafree MC 30,000 NMWL polysulfone membrane tubes (Millipore, Bedford, Massachusetts) and resuspended in 20 μL water prior to sequencing by annealing with TL2-N-3014 or C1-J-2441. PCR amplifications and sequencing of mtDNA was also performed on specimens of the black palm weevil, *R. bilineatus*, from Rabaul, Papua

New Guinea. Double-stranded mtDNA amplification of the CO1 gene was conducted with the primers C1-J-1718 and TL2-N-3014 and sequenced by annealing with C1-J-2441 and TL2-N-3014. Amplifications each contained 17 μL water, 2.5 μL 10X buffer, 0.6 μL bovine serum albumin (100 ng/μL), 0.1 μL *Taq* DNA polymerase, and 0.7 μL of weevil genomic DNA. The thermocycler was programmed for 30 cycles with denaturation at 94° C for 1 min, annealing at 47° C for 1 min, and extension at 72° C for 45 sec in the first six cycles with duration increased by 10 sec in each subsequent cycle. Prior to sequencing, 3 μL of double-stranded DNA was treated with exonuclease I and shrimp alkaline phosphatase to digest the primers and to deactivate the dNTPs.

Cross-Breeding Studies.

Pupal chambers containing live pupae were collected in Bojong Kalong, Indonesia and brought to Simon Fraser University. All newly-eclosed adults used in this study had the distinctive colour markings of either *R. ferrugineus* and *R. vulneratus*. They were kept separately for at least 3 days before pairing with another adult. All possible con- and heterospecific pairings were performed (female *ferrugineus* x male *ferrugineus*, female *ferrugineus* x male *vulneratus*, female *vulneratus* x male *vulneratus*, female *vulneratus* x male *ferrugineus*). The conspecific pairing between female and male *R. ferrugineus* was made only once. All other pairings were performed with two pairs of weevils.

Adult pairs were kept in 150 mm tissue culture dishes (Corning Inc., Corning, New York) and given slices of apple on which to feed and oviposit. Two pieces of paper towelling (one under and one over the apple slices) were provided to help any overturned weevil to right itself. Three times a week, paper towelling and apple slices were replaced and inspected for eggs and early instar larvae. Eggs were removed, soaked for 2 min in a 1:840 solution of benzalkonium chloride (Sigma Chemical Co., St. Louis, Missouri), and dried on filter paper. Both eggs and larvae were placed individually in 29.6 mL plastic containers (Solo Cup Co., Urbana, Illinois) containing an artificial rearing medium adapted from Rahalkar *et al.* (1985) (Table 21). Containers were inspected on a weekly basis for signs of feeding and larvae were transferred into new containers of diet as required. Later instars were transferred to 100 mL glass containers, and as larvae approached pupation they were given diet that contained jute fibers for construction of the pupal chamber. The rearing chamber was maintained at 27-30°C and 90-95% R.H.

Upon emergence, F_1 adults were held individually with apples until they could be mated. Due to the long time span over which F_1 adults emerged and high mortality, only four F_1 weevils were subsequently paired for mating (female *vulneratus-ferrugineus* x male *vulneratus-vulneratus*, and female x male *vulneratus-vulneratus*). Paper towelling and apple slices were examined for eggs and early instar larvae as above. Containers of artificial diet were examined for early instar larvae five and eight weeks after the last egg was laid.

Table 21. Recipe for artificial diet used in rearing *R. ferrugineus* and *R. vulneratus* larvae. Adapted from Rahalkar *et al.* (1985); modifications indicated by asterisks.

Ingredient	Quantity
Sugarcane bagasse	53 g
Desiccated coconut (medium)*	60 g
Brewer's yeast	20 g
Granulated sugar*	76 g
Agar	20 g
Salt Wesson	2 g
Vitamin Diet Fortification*	12 g
Distilled water	760 mL
4M Potassium hydroxide	3 mL
Methyl para-hydroxybenzoate, 14% solution in 95% ethanol	10 mL
Sorbic acid, 12.5% solution in 95% ethanol	15 mL

Statistical Analyses.

Comparisons between morphological measurements were made with Analysis of Variance and Bonferroni t-tests (PROC GLM, SAS Institute, 1985). Data on numbers of weevils captured in bucket traps were analyzed for each species using Chi-square tests, $\alpha = 0.05$ (SAS Institute, 1985). Low sample size permitted pairwise comparisons only between the synthetic pheromone treatment and all other treatments.

RESULTS

Morphological Comparisons.

Submentum and subgenal sutures. No significant differences were found between the estimates of concavity for *R. ferrugineus* and *R. vulneratus* (Table 22).

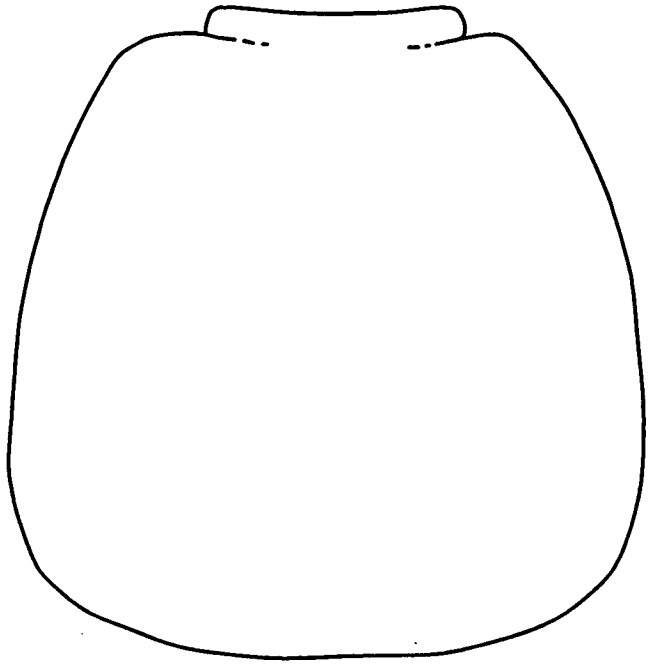
Pronotal Shape. According to Wattanapongsiri's (1966) figures (Fig. 33), *R. ferrugineus* would be expected to have a score of 0.6 for both measures of pronotal shape, while *R. vulneratus* should have scores of around 0.4 for both (Table 23). However, for both ratios, no significant differences were found between *R. ferrugineus* and *R. vulneratus* specimens of both the characteristic and intermediate colour types, all collected from the same locality (Table 23). However, specimens of *R. ferrugineus* from the UAE differed significantly from specimens of *R. ferrugineus* from Indonesia for both measures of pronotal shape (Table

Table 22. Estimates of concavity of subgenal sutures of specimens of *R. ferrugineus* and *R. vulneratus*.

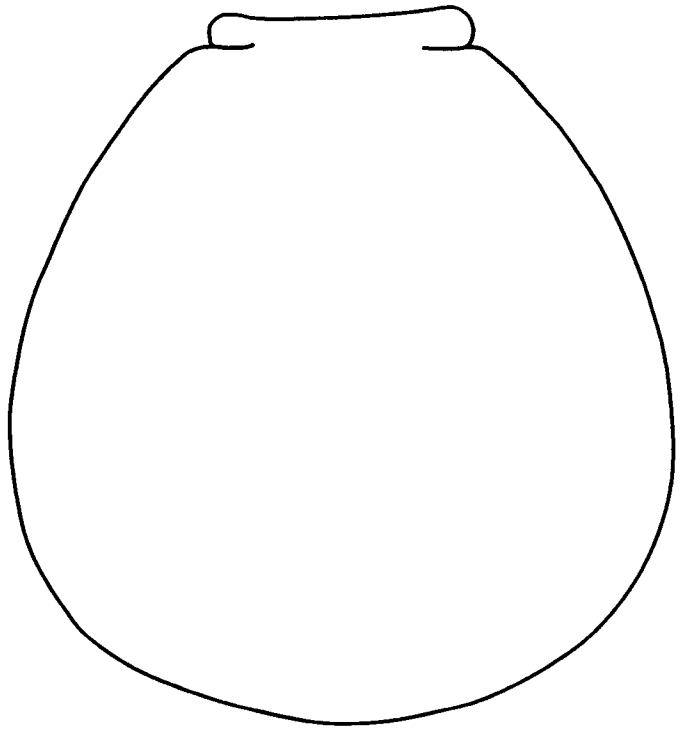
Specimen type	n	Estimate of concavity (Mean ± S.E.)[a]
R. ferrugineus	22	0.33 ± 0.03
R. vulneratus	31	0.35 ± 0.02
R. vulneratus intermediate colour morphs	7	0.37 ± 0.03

[a] No significant differences between means: ANOVA, $F = 0.42$, df = 2, 57, $P = 0.66$.

Figure 33. Pronotal shapes for *R. ferrugineus* and *R. vulneratus* adapted from Wattanapongsiri (1966). *R. ferrugineus* was described by Wattanapongsiri (1966) as having the sides of the pronotum gradually curved to the apex and then abruptly constricted anteriolaterally, whereas the pronotum of *R. vulneratus* was described as broadly rounded at the base and then strongly narrowed to the apex.



R. ferrugineus



R. vulneratus

Table 23. Estimates of pronotal shape of specimens of *R. ferrugineus* and *R. vulneratus* compared to measures taken from Wattanapongsiri's (1966) drawings. Means in a column followed by the same letter are not significantly different, Bonferroni t-tests, $P > 0.05$.

Specimen type & origin	n	Ratio of minimum width to length (Mean ± S.E.)[a]	Ratio of minimum to maximum width (Mean ± S.E.)[b]
R. ferrugineus			
Indonesia	24	0.409 ± 0.006 a	0.450 ± 0.006 a
United Arab Emirates	26	0.488 ± 0.005 b	0.530 ± 0.005 b
Taxonomic standard[c]		0.63	0.61
R. vulneratus			
Pure type	35	0.420 ± 0.006 a	0.460 ± 0.007 a
Intermediate colour type	10	0.421 ± 0.012 a	0.466 ± 0.001 a
Taxonomic standard[c]		0.43	0.42

[a] ANOVA, $F = 31.12$, df = 3, 91, $P < 0.001$

[b] ANOVA, $F = 30.29$, df = 3, 91, $P < 0.001$

[c] Based on drawings from Wattanapongsiri (1966).

23). Thus there was more variation within *R. ferrugineus* than between the species.

Cross-Attraction of Live Males.

Male *R. ferrugineus* were attractive at approximately the same levels to both *R. ferrugineus* and *R. vulneratus* (Fig. 34). Male *R. vulneratus* were not attractive to either species.

Genetic Comparisons.

RAPD-PCR analysis. RAPD banding patterns were visualized for 14 primers (601, 602, 603, 604, 607, 610, 612, 613, 615, 617, 620, 621, 622, 623) (Figs. 35-37). In nine of these (604, 607, 610, 612, 613, 615, 617, 622, 623), no differences in banding patterns were observed between *R. ferrugineus* and *R. vulneratus* from Bojong Kalong, Java (Figs. 35-37). For all five of the remaining primers (601, 602, 603, 620, 621), some amplification fragments were common to both *R. ferrugineus* and *R. vulneratus* (Figs. 35, 37).

Mitochondrial DNA sequencing. Clear sequences were obtained in two segments of the CO1 gene, corresponding to nucleotides 2563-2661 on the sense (or major) strand and 2765-2877 on the antisense (or minor) strand of the mitochondrial DNA molecule of *Drosophila yakuba* (Clary & Wolstenholme, 1985). *R. ferrugineus* and *R. vulneratus* from Bogor, Cikancana and Pakuwon were identical in the sequence of these

Figure 34. Total number of *R. ferrugineus* and *R. vulneratus* captured in traps containing palm wood alone (control), or in combination with a 10:1 ratio of ferrugineol : ferrugineone, or with 10 live males of either *R. ferrugineus* or *R. vulneratus*; N= 5; 5-9 September 1992. Bars followed by * and ** are significantly different from 10:1 ferrugineol : ferrugineone, X^2 test, at $P < 0.05$ and $P < 0.005$, respectively.

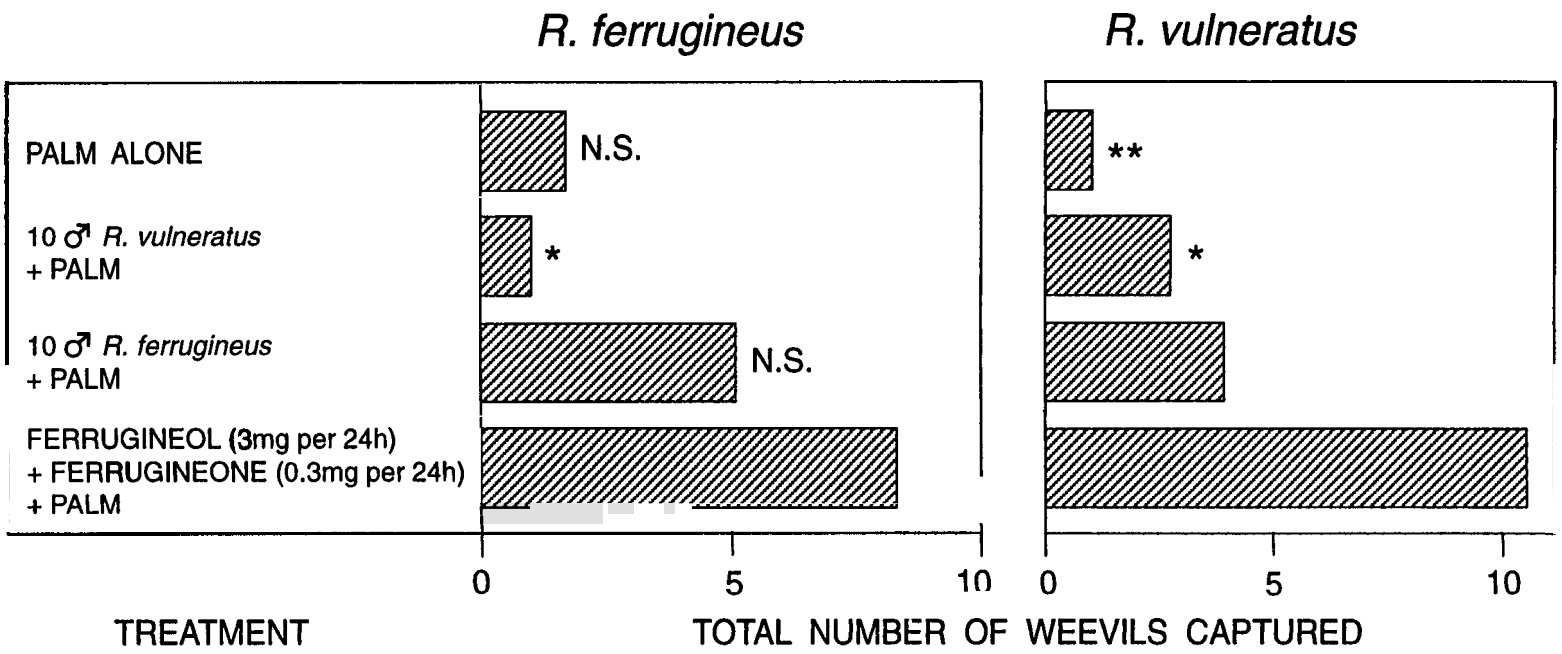

R. ferrugineus
R. vulneratus
PALM ALONE
10 ♂ R. vulneratus
+ PALM
10 ♂ R. ferrugineus
+ PALM
FERRUGINEOL (3mg per 24h)
+ FERRUGINEONE (0.3mg per 24h)
+ PALM
N.S.
*
N.S.
**
*
0
5
10
0
5
10
TREATMENT
TOTAL NUMBER OF WEEVILS CAPTURED

Figure 35. RAPD fragments amplified using primers 601-608 (for sequences refer to Table 22) on DNA from *R. ferrugineus* (**F**) and *R. vulneratus* (**V**) specimens from Bojong Kalong, Java. Lanes 1 and 10 contain a 100 bp ladder for reference.

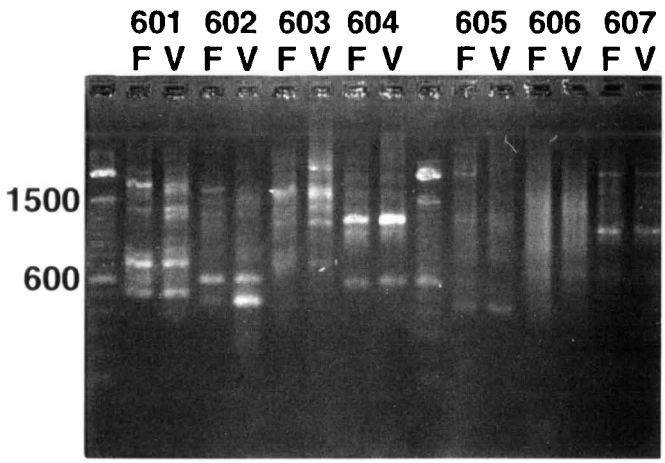

601 602 603 604 605 606 607
F V F V F V F V F V F V F V
1500
600

Figure 36. RAPD fragments amplified using primers 609-616 (for sequences refer to Table 22) on DNA from *R. ferrugineus* (**F**) and *R. vulneratus* (**V**) specimens from Bojong Kalong, Java. Lanes 7, 12 and 13 contain a 100 bp ladder for reference.

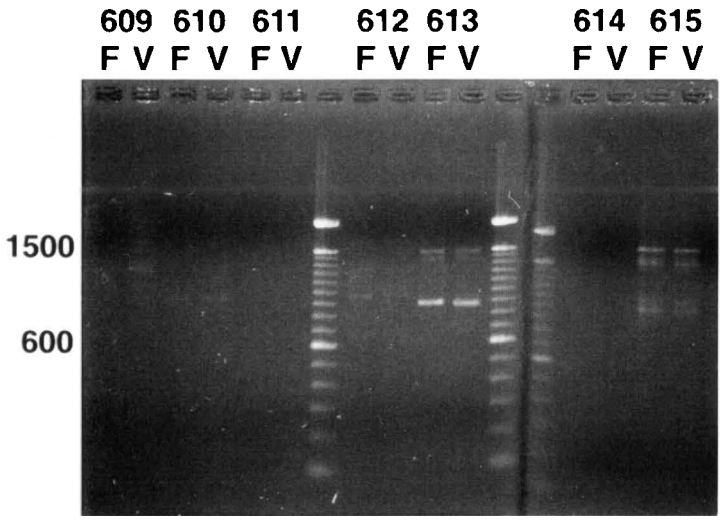
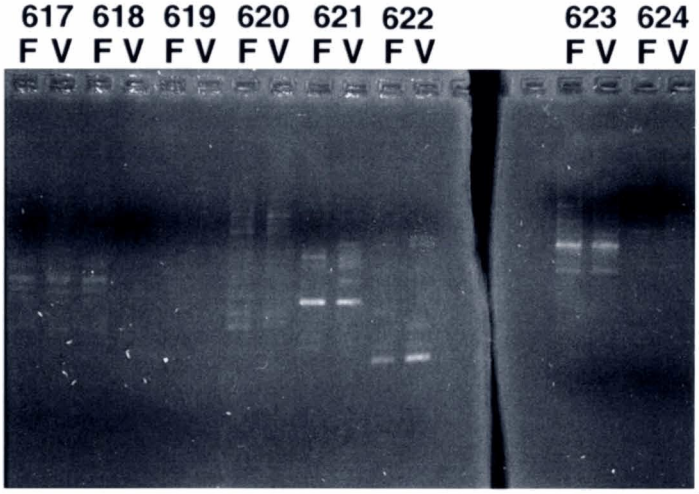

609 610 611 612 613 614 615
F V F V F V F V F V F V F V
1500
600

Figure 37. RAPD fragments amplified using primers 617-624 (for sequences refer to Table 22) on DNA from *R. ferrugineus* (**F**) and *R. vulneratus* (**V**) specimens from Bojong Kalong, Java.


617 618 619 620 621 622 623 624
F V F V F V F V F V F V F V F V

segments (over 201 base pairs read) of the CO1 gene (Figs. 38, 39). Over the same segments, *R. bilineatus* differed from *R. ferrugineus* and *R. vulneratus* at 21 nucleotides (Figs. 38, 39). All but one of these differences were point substitutions at third codon positions.

Cross-Breeding Studies.

Numbers of eggs laid, hatching success and survival to pupal and adult stages were comparable regardless of whether weevils were paired with con- or heterospecific mates (Table 24).

Seventeen adult F_1's were obtained from the crosses, of which nine arose from paired conspecific *R. vulneratus*. The four heterospecific pairings produced six hybrid adults. In all cases, colouration of the F_1 adults was identical to that of the male parent. The F_1 female x male *vulneratus-vulneratus* pair both died within 5 days of being paired and no eggs were laid. The F_1 female *vulneratus-ferrugineus* x male *vulneratus-vulneratus* pair produced 66 eggs, from which 24 larvae emerged, demonstrating that a hybrid female could produce viable eggs.

DISCUSSION

None of my investigations produced evidence that could be used to invalidate the hypothesis that *R. ferrugineus* and *R. vulneratus* are the same species. The cross attraction experiment (Fig. 34), indicated no

Figure 38. A 97 nucleotide segment of the sense strand of the mitochondrial cytochrome oxidase subunit 1 gene as sequenced in *R. ferrugineus*, *R. vulneratus* and *R. bilineatus*, by annealing with C1-J-2441 (Simon *et al.*, 1994). This segment corresponds to nucleotides 2563-2661 of the mtDNA molecule of *Drosophila yakuba* (Clary & Wolstenholme, 1985). Double underlining indicates nucleotide at which some difference exists between species.

Species	Base Pair Sequence (5' to 3')
	...2312312312312312312312312312312312-
R. ferrugineus	...TTCTCCATGACACATATTATGTTGTTGCCCACTTTG-
R. vulneratus	...TTCTCCATGACACATATTATGTTGTTGCCCACTTTG-
R. bilineatus	...TTCTCCATGATACATATTATGTTGTTGCTCATTTTC-
	2312312312312312312312312312312312312-
R. ferrugineus	ATTATGTGCTTTCTATGGGAGCAGTATTTGCTATTATTG-
R. vulneratus	ATTATGTGCTTTCTATGGGAGCAGTATTTGCTATTATTG-
R. bilineatus	ATTATGTTCTTTCTATAGGTGCAGTATTTGCTATTATTG-
	2312312312312312312312...
R. ferrugineus	CGGGTTTTATTCAATGATTCCC...
R. vulneratus	CGGGTTTTATTCAATGATTCCC...
R. bilineatus	CAGGTTTTATTCAATGATTTCC...

Figure 39. A 104 nucleotide segment of the anti-sense strand of the mitochondrial cytochrome oxidase subunit 1 gene as sequenced in *R. ferrugineus*, *R. vulneratus* and *R. bilineatus*, by annealing with TL2-N-3014 (Simon *et al.*, 1994). This segment corresponds to nucleotides 2765-2877 of the mtDNA molecule of *Drosophila yakuba* (Clary & Wolstenholme, 1985). Double underlining indicates nucleotide at which some difference exists between species.

Species	Base Pair Sequence (5' to 3')
	...3123123123123123123123123123123123-
R. ferrugineus	...CTATAAAATACTTATTAAAGAGATTATTCTACCGAT-
R. vulneratus	...CTATAAAATACTTATTAAAGAGATTATTCTACCGAT-
R. bilineatus	...ATATAAAATACTTATTAAAGAGATTATTCTACCGAT-
	3123123123123123123123123123123123123-
R. ferrugineus	AGAAGAGATAGAATTTCAAAGGGTCTATGCGTCAGGGTA-
R. vulneratus	AGAAGAGATAGAATTTCAAAGGGTCTATGCGTCAGGGTA-
R. bilineatus	AGAAGAAATAGAATTTCATAGGGTGTAGGCATCTGGGTA-
	312312312312312312312312312312...
R. ferrugineus	GTCGGAGTATCGCCGAGGTATGCCTCTTA...
R. vulneratus	GTCGGAGTATCGCCGAGGTATGCCTCTTA...
R. bilineatus	GTCAGAATATCGTCGAGGCATACCTCTTA...

Table 24. Summary of eggs produced, number of emerged larvae, number of pupal chambers produced and number of adult F_1's obtained from inter- and intraspecific crosses between *R. ferrugineus* (**F**) and *R. vulneratus* (**V**). Numbers in parentheses indicate percent of individuals to successfully reach that stage from previous stage.

Pair (Female-Male)	Total eggs laid	Total larvae emerged	Total pupal chambers produced	Total F_1 adults emerged
FF-1	414	208	3	2
		(50.2%)	(1.4%)	(67%)
VV-1	355	148	4	3
		(41.7%)	(2.7%)	(75%)
VV-2	401	159	9	6
		(39.7%)	(5.7%)	(67%)
FV-1	341	159	1	1
		(46.6%)	(0.6%)	(100%)
FV-2	359	135	2	1
		(37.6%)	(1.5%)	(50%)
VF-1	260	105	5	2
		(40.4%)	(4.8%)	(40%)
VF-2	296	169	4	2
		(50.1%)	(2.4%)	(50%)

differences in the natural pheromone blends produced by males of either species. In the scolytid genus *Ips*, closely related species are cross attractive, but unlike *R. ferrugineus* and *R. vulneratus* maintain reproductive isolation through allopatric or parapatric distributions (Lanier & Wood, 1975). In two sympatric *Gnathotrichus* spp., specificity in aggregation pheromones is maintained by chirality of a single compound, one species requiring both enantiomers and the other only one enantiomer, the antipode of which is repellent (Borden *et al.*, 1976, 1980a). Despite extensive investigation, no evidence for species specificity imparted by blends of compounds or chirality was disclosed for sympatric *R. ferrugineus* and *R. vulneratus* (Figs. 4, 7-10; Tables 2-5).

Major morphological differences between *R. ferrugineus* and *R. vulneratus* were described by Wattanapongsiri (1966) in his revision of the genus *Rhynchophorus*. Foremost amongst these were differences in the shapes of the submentum and the pronotum. Wattanapongsiri (1966) erroneously referred to the subgenal sutures (Lyal, 1995) delineating the submentum and running down the ventral surface of the rostrum as the "gular sutures". These sutures were described in *R. vulneratus* as "concave at both sides medially before reaching base of rostrum", and in *R. ferrugineus* as "oval at base, but less concave than in *vulneratus*" (Wattanapongsiri, 1966). According to the estimate of concavity that I devised, *R. ferrugineus* would be expected to have a higher score than *R. vulneratus*, but I found no such differences (Table 24).

R. ferrugineus was described by Wattanapongsiri (1966) as having the sides of the pronotum gradually curved to the apex and then abruptly constricted anteriolaterally, whereas the pronotum of *R. vulneratus* was described as broadly rounded at the base and then strongly narrowed to the apex (Fig. 33). The lack of differences in measures of pronotal shape between *R. ferrugineus* and *R. vulneratus* and the existence of differences in these traits between specimens of *R. ferrugineus* from Indonesia and the UAE (Table 23), suggest that pronotal shape is more variable within than between species. *R. ferrugineus* and *R. vulneratus* cannot be differentiated on the basis of these morphological characters.

Analysis of random amplified polymorphic DNA (RAPDs) (Williams *et al.*, 1990) is very useful in determining the existence of different species and populations (Ballinger-Crabtree *et al.*, 1992; Chalmers *et al.*, 1992; Kambhampati *et al.*, 1992; Perring *et al.*, 1993; Puterka *et al.*, 1993). RAPD analyses can detect higher levels of genetic variation than detected by allozyme analysis (Kambhampati *et al.*, 1992; Black, 1993; Puterka *et al.*, 1993), used in part by deGroot (1992) to support synonymization of two sympatric scolytid cone beetles, *Conophthorus resinosae* Hopkins and *C. banksianae* McPherson. Differences in RAPD banding patterns observed in amplification products of primers 601-603 and 620-621 (Figs. 35, 37) may be due to weakly staining bands or non-discrete adjacent fragments, or may be the result of differences between either species or individuals. In order to determine the source of this variation, a much more comprehensive study is required, examining

RAPD banding patterns of many individuals. However, the identical banding patterns seen for both *R. ferrugineus* and *R. vulneratus* from nine other primers (604, 607, 610, 612, 613, 615, 617, 622, 623) (Figs. 35-37) suggests that these weevils are very closely related and possibly a single species.

The complete absence of differences in mtDNA sequencing between *R. ferrugineus* and *R. vulneratus* from three different locations (Figs. 38, 39) provides strong evidence that they are not distinct species. The parapatric species, *R. bilineatus*, differed from *R. ferrugineus* and *R. vulneratus* at 10 % of nucleotides examined. Sequence divergence estimates of 1.7 to 2.3 % per million years have been determined for a number of arthropod species (Martin & Simon, 1990; Knowlton *et al.*, 1993; Boyce *et al.*, 1994; Brower, 1994; Funk *et al.*, 1995). This degree of sequence divergence observed between *R. bilineatus* and *R. ferrugineus/R. vulneratus* suggests that divergence between these species may have occurred between 4-6 million years ago. However, almost all sequence differences found were at third codon positions, and are therefore silent substitutions as they do not cause alteration of the amino acid (Simon *et al.*, 1994). Silent substitutions are less constrained than changes at first and second codon positions (Simon *et al.*, 1994), and as a result it may be argued that divergence between these species occurred more recently than the given estimate. *R. bilineatus* is morphologically distinct from *R. ferrugineus* and *R. vulneratus* (Wattanapongsiri, 1966), but also utilizes ferrugineol as its major

aggregation pheromone. This suggests that geographical separation precluded the need for evolution of pheromone specificity and that morphological differences may be sufficient to maintain reproductive isolation should members of these species ever meet.

Adult F_1's were obtained from all crosses performed, indicating that genital mismatch and nonfertility of eggs are not operating as isolating mechanisms between *R. ferrugineus* and *R. vulneratus*. The influence of the male parent on colouration suggests that it is sex-linked, although this result may be an artefact of the small number of adult F_1's obtained. *R. ferrugineus* has a typical curculionid chromosome formula of 10 A + Xy_p (Bartlett & Rananavare, 1983). Due to the presence of intermediate colour morphs in the wild, it is likely that colouration is controlled by more than one gene locus. High mortality and loss of limbs among adult F_1's may have been the result of deficiencies in the artificial diet. The production of viable eggs from the female *vulneratus-ferrugineus* x male *vulneratus-vulneratus* pair, demonstrates that hybrid F_1's can be fertile and that reproductive isolation maintained by hybrid sterility is unlikely.

deGroot (1992) supported synonymization of the sympatric scolytid species *C. resinosae* and *C. banksianae* on the basis of similarities in morphology (Wood, 1982), cuticular hydrocarbons (Page *et al.*, 1990), karyology (deGroot & Ennis, 1990), life history (deGroot & Borden, 1991), host selection behaviour (deGroot & Borden, 1992), allozymes (deGroot *et al.*, 1992), and pheromone production and

response (Pierce *et al.*, 1995). Synonymization of the bark beetles, *Dendroctonus ponderosae* Hopkins and *D. monticolae* Hopkins (Wood 1963) was confirmed by mating experiments, developmental rates, karyology and morphological similarities (Lanier & Wood, 1968). Synonymization of sympatric *Ips cibricollis* (Eich.) and *I. grandicollis* (Eich.) was disputed on the basis of morphological and chromosomal differences, and their inability to interbreed (Lanier, 1987). The bark weevils, *Pissodes approximatus* Hopkins and *P. nemorensis* Germar were synonymized as *P. nemorensis* on the basis of identical pheromones (Phillips *et al.*, 1984), cross-attraction (Phillips & Lanier, 1986), and similarities in allozymes, morphology and behaviour (Phillips *et al.*, 1987). The sibling species, *P. strobi* (Peck), has been upheld as a distinct species due to evidence of reproductive isolation (Phillips & Lanier, 1983) and mtDNA sequence divergence (Boyce *et al.*, 1994).

In comparison, *R. ferrugineus* and *R. vulneratus* are alike in morphological characters (Tables 22, 23), host plant preference (Fig. 18), RAPD banding patterns (Figs. 35-37), mitochondrial DNA sequencing (Figs. 38, 39) and pheromone production and response (Figs. 4, 7-10, 34, Tables 2-5). Given these similarities, the lack of reproductive isolating mechanisms and the existence of colour intermorphs it is unlikely that *R. ferrugineus* and *R. vulneratus* constitute two valid species. On the basis of accumulated evidence, I propose that *R. ferrugineus* and *R. vulneratus* be considered as colour morphs of the same species and that by the law of priority (International Congress of Zoology, 1964; Table 19) they be

synonymized under the name *Rhynchophorus ferrugineus* (Olivier), with *R. vulneratus* becoming a junior synonym.

LITERATURE CITED

Abraham, V.A. 1987. Study of sex pheromones and other attractants for the management of major pests of coconut, March 1983 to December 1985, Final Report of Research Project No. Ent. IX (231), P1-83/25 -ICI-H10/0311. Central Plantation Crops Research Institute, Kerala, India.

Abraham, V.A., K.M. Abdulla Koya, and C. Kurian. 1989. Integrated management of red palm weevil (*Rhynchophorus ferrugineus* F.) in coconut gardens. *J. Plant. Crops.*, **16** (suppl.): 159-162.

Abraham, V.A., and C. Kurian. 1975. An integrated approach to the control of *Rhynchophorus ferrugineus* F. The red weevil of coconut palm. Fourth Session of the FAO Technical Working Party on Coconut Production, Protection and Processing. Kingston, Jamaica, 14 - 25 September 1975.

Adler, V.E., and M. Jacobson. 1971. Electroantennogram responses of adult male and female Japanese beetles to their extracts. *J. Econ. Entomol.*, **64**: 1561-1562.

Alcock, J. 1982. Natural selection and communication among bark beetles. *Fla. Entomol.*, **65**: 17-32.

Arn, H., E. Städler, and S. Rauscher. 1975. The electroantennographic detector - a selective and sensitive tool in the gas chromatographic analysis of insect pheromones. *Z. Naturforsch.*, **30c**: 722-725.

Atkins, M.D. 1966. Behavioural variation among scolytids in relation to their habitat. *Can. Entomol.*, **98**: 285-288.

Bakke, A. 1983. Host tree and bark beetle interaction during a mass outbreak of *Ips typographus* in Norway. *Z. Angew. Entomol.*, **96**: 118-125.

Ballinger-Crabtree, M.E., W.C. Black IV, and B.R. Miller. 1992. Use of genetic polymorphisms detected by the random-amplified polymorphic DNA

polymerase chain reaction (RAPD-PCR) for differentiation and identification of *Aedes aegypti* subspecies and populations. *Am. J. Trop. Med. Hyg.*, **47**: 893-901.

Banks, C. 1906. *The Principal Insects Attacking the Coconut Palm. Parts I and II, New Philippine Insects.* Bureau of Printing, Manila.

Barber, I.A., T.P. McGovern, M. Beroza, C.P. Hoyt, and A. Walker. 1971. Attractant for the coconut rhinoceros beetle. *J. Econ. Entomol.*, **64**: 1041-1044.

Bartelt, R.J., and D.G James. 1994. Aggregation pheromone of Australian sap beetle, *Carpophilus davidsoni* (Coleoptera: Nitidulidae). *J. Chem. Ecol.*, **20**: 3207-3219.

Bartlett, A.C., and H.D. Rananavare. 1983. Karyotype and sperm of the red palm weevil (Coleoptera: Curculionidae). *Ann. Entomol. Soc. Am.*, **76**: 1011-1013.

Bedard, W.D., P.E. Tilden, D.L. Wood, R.M. Silverstein, and J.O. Rodin. 1969. Western pine beetle: field response to its sex pheromone and a synergistic host terpene, myrcene. *Science*, **168**: 1284-1285.

Bedard, W.D., and D.L. Wood. 1974. Programs utilizing pheromones in survey of control. Bark beetles - the western pine beetle. pp. 441-449 in M.C. Birch (ed.) *Pheromones.* North-Holland Publishing, Amsterdam. 495 pp.

Bedard, W.D., D.L. Wood, P.E. Tilden, K.Q. Lindahl, Jr., R.M. Silverstein, and J.O. Rodin. 1980. Field responses of the western pine beetle and one of its predators to host- and beetle-produced compounds. *J. Chem. Ecol.*, **6**: 625-641.

Bedford, G.O. 1975. Trap catches of the coconut rhinoceros beetle *Oryctes rhinoceros* (L.) (Coleoptera, Scarabaeidae, Dynastinae) in New Britain. *Bull. Entomol. Res.*, **65**: 443-451.

Bedford, G.O. 1980. Biology, ecology and control of palm rhinoceros beetles. *Ann. Rev. Entomol.*, **25**: 309-39.

Bedford, G.O. 1986. Biological control of the rhinoceros beetle (*Oryctes rhinoceros*) in the South Pacific by baculovirus. *Agric. Ecosyst. Environ.*, **15**: 141-147.

Bestmann, H.J., and O. Vostrowsky. 1988. Pheromones of the Coleoptera. pp. 95-183 in E.D. Morgan & N.B. Mandava (eds.), *Handbook of Natural Pesticides*, CRC Press, Boca Raton. 203pp.

Birch, M.C. 1984. Aggregation in bark beetles. pp. 331-353 in W.J. Bell & R.T. Carde (eds.), *Chemical Ecology of Insects.* Chapman & Hall Ltd.

Birch, M.C., D.M. Light, D.L. Wood, L.E. Browne, R.M. Silverstein, B.J. Bergot, G. Ohloff, J.R. West, and J.C. Young. 1980. Pheromonal attraction and allomonal interruption of *Ips pini* in California by the two enantiomers of ipsdienol. *J. Chem. Ecol.*, **6**: 703-713.

Black, W.C., IV. 1993. PCR with arbitrary primers: approach with care. *Insect Mol. Biol.*, **2**: 1-6.

Bokhari, U.G., and R. Abuzuhari. 1992. Diagnostic tests for redpalm weevil, *Rhynchophorus ferrugineus* infested datepalm trees. *Arab Gulf J. Scient. Res.*, **10**: 93-104.

Booth, D.C., and G.N. Lanier. 1974. Evidence of an aggregating pheromone in *Pissodes approximatus* and *P. strobi. Ann. Entomol. Soc. Am.*, **67**: 992-994.

Booth, D.C., T.W. Phillips, A. Claesson, R.M. Silverstein, G.N. Lanier, and J.R. West. 1983. Aggregation pheromone components of two species of *Pissodes* weevils (Coleoptera: Curculionidae): isolation, identification, and field activity. *J. Chem. Ecol.*, **9**: 1-12.

Borden, J.H. 1985. Aggregation pheromones. pp. 257-285 in G.A. Kerkut & L.I. Gilbert (eds.), *Comprehensive Insect Physiology, Biochemistry and Pharmacology,* Pergamon Press, Oxford.

Borden, J.H. 1995. From identifying semiochemicals to developing a suppression tactic: a historical review. pp. 3-10 in S.M. Salom & K.R. Hobson (tech. eds.), *Application of Semiochemicals for Management of Bark Beetle Infestations- Proceedings of an Informal Conference.* Annual Meeting of the Entomological Society of America, December 12-16, 1993, Indianapolis, Indiana. USDA Forest Service, Gen. Tech. Rep. INT-GTR-318, Ogden, Utah.

Borden, J.H., L.J. Chong, J.A. McLean, K.N. Slessor, and K. Mori. 1976. *Gnathotrichus sulcatus*: synergistic response to enantiomers of the aggregation pheromone sulcatol. *Science*, **192**: 894-896.

Borden, J.H., J.R. Handley, J.A. McLean, R.M. Silverstein, L.J. Chong, K.N. Slessor, B.D. Johnston, and H.R. Schuler. 1980. Enantiomer-based specificity in pheromone communication by two sympatric *Gnathotrichus* species (Coleoptera: Scolytidae). *J. Chem. Ecol.*, **6**: 445-456.

Borden, J.H., D.W.A. Hunt, D.R. Miller, and K.N. Slessor. 1986. Orientation in forest Coleoptera: an uncertain outcome of responses by individual beetles to variable stimuli. pp. 97-109 in T.L. Payne, M.C. Birch, C.E.J. Kennedy (eds.) *Mechanisms in Insect Olfaction.* Clarendon Press, Oxford.

Borden, J.H., B.S. Lindgren, and L. Chong. 1980b. Ethanol and α-pinene as synergists for the aggregation pheromones of two *Gnathotrichus* species. *Can. J. For. Res.*, **10**: 290-292.

Borgia, G. 1979. Sexual selection and the evolution of mating systems. pp. 19-80 in M.S. Blum & N.A. Blum (eds.), *Sexual Selection and Reproductive Competition in Insects.* Academic Press, New York. 463pp.

Boyce, T.M., M.E. Zwick, and C.F. Aquadro. 1994. Mitochondrial DNA in the bark weevils: phylogeny and evolution in the *Pissodes strobi* species group (Coleoptera: Curculionidae). *Mol. Biol. Evol.*, **11**: 183-194.

Brower, A.V.Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proc. Natl. Acad. Sci. USA*, **91**: 6491-6495.

Budenberg, W.J., I.O. Ndiege, F.W. Karago, and B.S. Hansson. 1993. Behavioral and electrophysiological responses of the banana weevil *Cosmopolites sordidus* to host plant volatiles. *J. Chem. Ecol.*, **19**: 267-277.

Burger, B.V., Z. Munro, M. Roth, H.S.C. Spies, V. Truter, G.D. Tribe, and R.M. Crewe. 1983. Composition of the heterogeneous sex attracting secretion of the dung beetle, *Kheper lamarcki. Z. Naturforsch.*, **38c**: 848-855.

Cade, S.C., B.F. Hrutfiord, and R.I. Gara. 1970. Identification of a primary attractant for *Gnathotrichus sulcatus* isolated from western hemlock logs. *J. Econ. Entomol.*, **63**: 1014-1015.

Camacho, A.D., H.D. Pierce Jr., and J.H. Borden. 1993. Geometrical and optical isomerism of pheromones in two sympatric *Dryocoetes* species (Coleoptera: Scolytidae), mediates species specificity and response level. *J. Chem. Ecol.*, **19**: 2169-2182.

Camacho, A.D., H.D. Pierce Jr., and J.H. Borden. 1994. Aggregation pheromones in *Dryocoetes affaber* (Mann.) (Coleoptera: Scolytidae): stereoisomerism and species specificity. *J. Chem. Ecol.*, **20**: 111-124.

Chalmers, K.J., R. Waugh, J.I. Sprent, A.J. Simons, and W. Powell. 1992. Detection of genetic variation between and within populations of *Gliricidia sepium* and *G. maculata* using RAPD markers. *Heredity*, **69**: 465-472.

Chang, V.C.S., and G.A. Curtis. 1972. Pheromone production by the New Guinea sugarcane weevil. *Environ. Entomol.*, **1**: 476-481.

Chayophat, T. 1979. Study on the coconut weevil, *Rhynchophorus ferrugineus* Oliv., that attracts other coconut weevils in the field. *Research Report 1979*, Entomology & Zoology Division, Department of Agriculture, Bangkok. p.159.

Chinchilla, C.M., and A.C. Oehlschlager. 1992. Captures of *Rhynchophorus palmarum* in traps baited with the male-produced aggregation pheromone. *ASD Oil Palm Papers*, **5**:1-8.

Chinchilla, C.M., A.C. Oehlschlager, and L.M. Gonzalez. 1993. Management of red ring disease in oil palm through pheromone-based trapping of *Rhynchophorus palmarum*. Proceedings, 1993 PORIM International Oil Palm Congress, 20-25 September 1993, Kuala Lumpur, Malaysia.

Clary, D.O., and D.R. Wolstenholme. 1985. The mitochondrial DNA molecule of *Drosophila yakuba*: nucleotide sequence, gene organization, and genetic code. *J. Mol. Evol.*, **22**: 252-271.

Cross, J.H., R.C. Byler, R.F. Cassidy Jr., R.M. Silverstein, R.E. Greenblatt, W.E. Burkholder, A.R. Levinson, and H.Z. Levinson. 1976. Porapak-Q collection of pheromone components and isolation of (*Z*)- and (*E*)-14-methyl-8-hexadecanal, sex pheromone components, from the females of four species of *Trogoderma* (Coleoptera: Dermestidae). *J. Chem. Ecol.*, **2**: 457-468.

de Groot, P. 1992. *Biosystematics of Conophthorus Hopkins (Coleoptera: Scolytidae) in Eastern North America*. Ph.D. thesis, Simon Fraser University, Burnaby, BC, Canada. 210pp.

de Groot, P., and J.H Borden. 1991. Life history of the jack pine tip beetle, *Conophthorus banksianae* McPherson (Coleoptera: Scolytidae): implications for species status. *Can. Entomol.*, **123**: 211-217.

de Groot, P., and J.H. Borden. 1992. Host acceptance behaviour of the jack pine tip beetle, *Conophthorus banksianae* and the red pine cone beetle, *C. resinosae*. *Entomol. exp. appl.*, **65**: 149-155.

de Groot, P., and T.J. Ennis. 1990. Cytotaxonomy of *Conophthorus* (Coleoptera: Scolytidae) in eastern North America. *Can. Entomol.*, **122**: 1131-1135.

de Groot, P., G.T. Harvey, and P.M. Roden. 1992. Genetic divergence among eastern North American cone beetles, *Conophthorus* (Coleoptera: Scolytidae). *Can. Entomol.*, **124**: 189-199.

Dickens, J.C. 1989. Green leaf volatiles enhance aggregation pheromone of boll weevil, *Anthonomus grandis*. *Entomol. exp. appl.*, **52**: 191-203.

Dickens, J.C., E.B. Jang, D.M. Light, and A.R. Alford. 1990. Enhancement of insect pheromone response by green leaf volatiles. *Naturwissenschaften*, **77**: 29-31.

Domek, J.M., and D.T. Johnson. 1987. Evidence of a sex pheromone in the green june beetle, *Cotinis nitida* (Coleoptera: Scarabaeidae). *J. Entomol. Sci.*, **22**: 264-267.

Domek, J.M., and D.T. Johnson. 1988. Demonstration of semiochemically induced aggregation in the green june beetle, *Cotinis nitida* (L.) (Coleoptera: Scarabaeidae). *Environ. Entomol.*, **17**: 147-149.

Dunn, J.P., J.W. Kimmerer, and G.L. Nordin. 1986. Attraction of the twolined chestnut borer, *Agrilus bilineatus* (Weber) (Coleoptera: Buprestidae), and associated borers to volatiles of stressed white oak. *Can. Entomol.*, **118**: 503-509.

Eller, F.J., R.J. Bartelt, B.S. Shasha, D.J. Schuster, D.G. Riley, P.A. Stansly, T.F. Mueller, K.D. Shuler, B. Johnson, J.H. Davis, and C.A. Sutherland. 1994. Aggregation pheromone for the pepper weevil, *Anthonomus eugenii* Cano (Coleoptera: Curculionidae): identification and field activity. *J. Chem. Ecol.*, **20**: 1537-1555.

Emlen, S.T., and L.W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science*, **197**: 215-223.

Faustini, D.L., W.L. Giese, J.K. Phillips, and W.E. Burkholder. 1982. Aggregation pheromone of the male granary weevil, *Sitophilus granarius* (L.). *J. Chem. Ecol.*, **8**: 679-687.

Fieser, L.F., and M. Fieser. 1967. *Reagents for Organic Synthesis, Volume I.* New York: John Wiley & Sons.

Funk, D.J., D.J. Futuyama, G. Orti, and A. Meyer. 1995. A history of host associations and evolutionary diversification for *Ophraella* (Coleoptera: Chrysomelidae): new evidence from mitochondrial DNA. *Evolution*, **49**: 1008-1017.

Giblin-Davis, R.M., and F.W. Howard. 1989. Vulnerability of stressed palms to attack by *Rhynchophorus cruentatus* (Coleoptera: Curculionidae) and insecticidal control of the pest. *J. Econ. Entomol.*, **82**: 1185-1190.

Giblin-Davis, R.M., A.C. Oehlschlager, A.L. Perez, G. Gries, R. Gries, T.J. Weissling, C.M. Chinchilla, J.E. Pena, R.H. Hallett, H.D. Pierce Jr., and L.M. Gonzalez. 1996. Chemical and behavioral ecology of palm-associated weevils. *Fla. Entomol.*, in press.

Giblin-Davis, R.M., T.J. Weissling, A.C. Oehlschlager, and L.M. Gonzalez. 1994. Field response of *Rhynchophorus cruentatus* (Coleoptera: Curculionidae) to its aggregation pheromone and fermenting plant volatiles. *Fla. Entomol.*, **77**: 164-177.

Gressit, J.L. 1953. *The Coconut Rhinoceros Beetle* (Oryctes rhinoceros) *with particular reference to the Palau Islands*. Bernice P. Bishop Museum Honolulu, Hawaii, Bulletin 212.

Gries, G., R. Gries, A.L. Perez, L.M. Gonzalez, H.D. Pierce, Jr., A.C. Oehlschlager, M. Rhainds, M. Zebeyou, and B. Kouame. 1994a. Ethyl propionate: synergistic kairomone for African palm weevil, *Rhynchophorus phoenicis* L. (Coleoptera: Curculionidae). *J. Chem. Ecol.*, **20**: 889-897.

Gries, G., R. Gries, A.L. Perez, A.C. Oehlschlager, L.M. Gonzalez, H.D. Pierce, Jr., M. Kouda, M. Zebeyou, and N. Nanou. 1993. Aggregation pheromone of the African palm weevil, *Rhynchophorus phoenicis* F. *Naturwissenschaften*, **80**: 90-91.

Gries, G., R. Gries, A.L. Perez, A.C. Oehlschlager, L.M. Gonzalez, H.D. Pierce, Jr., M. Zebeyou, and B. Kouame. 1994b. Aggregation pheromone of the African rhinoceros beetle, *Oryctes monoceros* (Olivier) (Coleoptera: Scarabaeidae). *Z. Naturforsch.*, **49c**: 363-366.

Gunatilake, R., and N.E. Gunawardena. 1986. Ethyl alcohol: a major attractant of red weevil (*Rhynchophorus ferrugineus*). *Proceedings, Sri Lanka Association for the Advancement of Science, 42nd Annual Session*. p.70.

Hagley, E.A.C. 1965. Tests of attractants for the palm weevil. *J. Econ. Entomol.*, **58**: 1002-1003.

Harari, A.R., D. Ben-Yakir, and D. Rosen. 1994. Mechanism of aggregation behavior in *Maladera matrida* Argaman (Coleoptera: Scarabaeidae). *J. Chem. Ecol.*, **20**: 361-371.

Hasegawa, M., W.S. Leal, and M. Sawada. 1993. Field evaluation of *Anomala schonfeldti* Ohaus (Coleoptera: Scarabaeidae) synthetic sex pheromone. *J. Chem. Ecol.*, **19**: 1453-1459.

Hedden, R., J.P. Vite, and K. Mori. 1976. Synergistic effect of a pheromone and kairomone on host selection and colonisation by *Ips avulsus*. *Nature*, **261**: 696-697.

Hedin, P.A., J.A. Payne, T.L. Carpenter, and W. Neel. 1979. Sex pheromones of the male and female pecan weevil, *Curculio caryae*: behavioural and chemical studies. *Environ. Entomol.*, **8**: 521-523.

Henzell, R.F., and M.D. Lowe. 1970. Sex attractant of the grass grub beetle. *Science*, **168**: 1005-1006.

Ho, C.T., and P.Y. Toh. 1982. Some investigations into the control of *Oryctes rhinoceros* L. in coconut plantings. *Planter*, **58**: 492-506.

Hoffman, A.A., and P.A. Parsons. 1984. Olfactory response and resource utilization in *Drosophila*: interspecific comparisons. *Biol. J. Linn. Soc.*, **22**: 43-53.

International Congress of Zoology. 1964. *International Code of Zoological Nomenclature adopted by the XV International Congress of Zoology, London, July 1958*. Published in 1961. Revised edition 1964. International Trust for Zoological Nomenclature, London.

Iwabuchi, K., and J. Takahashi. 1983. Aggregative distribution pattern of the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), and the role of former occupants in the formation of an aggregation. *Appl. Entomol. Zool.*, **18**: 324-329.

Jacob, T.K., and B.S. Bhumannavar. 1991. The coconut rhinoceros beetle *Oryctes rhinoceros* L. - its incidence and extent of damage in the Andaman and Nicobar Islands (India). *Tropical Pest Management*, **7**: 80-84.

James, D.G., R.J. Bartelt, and R.J. Faulder. 1994. Attraction of *Carpophilus* spp. (Coleoptera: Nitidulidae) to synthetic aggregation pheromones and host-

related coattractants in Australian stone fruit orchards: beetle phenology and pheromone dose studies. *J. Chem. Ecol.*, **20**: 2805-2819.

Jansson, R.K., F.I. Proshold, L.J. Mason, R.R. Heath, and S.H. Lecrone. 1990. Monitoring sweetpotato weevil (Coleoptera: Curculionidae) with sex pheromone: effects of dosage and age of septa. *Tropical Pest Management*, **36**: 263-269.

Julia, J.F., and D. Mariau. 1976. Research on *Oryctes monoceros* Ol. in Ivory Coast. III.- Olfactory trapping with ethyl chrysanthemumate. *Oléagineux*, **31**: 263-272.

Kalshoven, L.G.E. 1950. *Pests of Crops in Indonesia.* P.T. Ichtiar Baru-Van Hoeve, Jakarta. Revised and translated by P.A. Van der Laan (1981). 701pp.

Kambhampati, S., W.C. Black IV, and K.S. Rai. 1992. Random amplified polymorphic DNA of mosquito species and populations (Diptera: Culicidae): techniques, statistical analysis, and applications. *J. Med. Entomol.*, **29**: 939-945.

Khoo, K.C., P.A.C. Ooi, and C.T. Ho. 1991. *Crop Pests and their Management in Malaysia*. Tropical Press, Kuala Lumpur. 242 pp.

Kimmerer, T.W., and T.T. Kozlowski. 1982. Ethylene, ethane, acetaldehyde, and ethanol production by plants under stress. *Plant Physiol.*, **69**: 840-847.

Knowlton, N., L.A. Weigt, L.A. Solórzano, D.K. Mills, and E. Bermingham. 1993. Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. *Science*, **260**: 1629-1632.

Koppenhöfer, A.M., K.V. Seshu Reddy, and R.A. Sikora. 1994. Reduction of banana weevil populations with pseudostem traps. *International Journal of Pest Management*, **40**: 300-304.

Kurian, C., V.A. Abraham, and K.N. Ponnamma. 1982. Attractants - an aid in red palm weevil management. *PLACROSYM-V*, Proc. 5th Ann. Symposium on Plantation Crops, CPCRI, Kasaragod, India, 15-18 December 1982. pp. 581-585.

Lanier, G.N. 1987. The validity of *Ips cribricollis* (Eich.) (Coleoptera: Scolytidae) as distinct from *I. grandicollis* (Eich.) and the occurrence of both species in Central America. *Can. Entomol.*, **119**: 179-187.

Lanier, G.N., and E. Burkholder. 1974. Pheromones in speciation of Coleoptera. pp. 161-189 in M.C. Birch (ed.), *Pheromones*. North-Holland Publishing Co., Amsterdam. 495 pp.

Lanier, G.N., R.M. Silverstein, and J.W. Peacock. 1976. Attractant pheromone of the European elm bark beetle (*Scolytus multistriatus*): isolation, identification, synthesis and utilization studies. pp. 149-175 in J.F. Anderson & H.K. Kaya (eds.) *Bark Beetle Pheromones*. Academic Press, New York.

Lanier, G.N., and D.L. Wood. 1968. Controlled mating, karyology, morphology, and sex-ratio in the *Dendroctonus ponderosae* complex. *Ann. Entomol. Soc. Am.*, **61**: 517-526.

Lanier, G.N., and D.L. Wood. 1975. Specificity of response to pheromones in the genus *Ips* (Coleoptera: Scolytidae). *J. Chem. Ecol.*, **1**: 9-23.

Leal, W.S. 1991. (*R,Z*)-5-(-)-(Oct-1-enyl)oxacyclopentan-2-one, the sex pheromone of the scarab beetle *Anomala cuprea*. *Naturwissenschaften*, **78**: 521-523.

Leal, W.S. 1993. (*Z*)- and (*E*)-Tetradec-7-en-2-one, a new type of sex pheromone from Oriental beetle. *Naturwissenschaften*, **80**: 86-87.

Leal, W.S. 1996. Evolution of sex pheromone communication in plant-feeding scarab beetles. In R. Carde & A. Minks (eds.), *Pheromone Research: New Directions*. Chapman & Hall, New York, in press.

Leal, W.S., M. Hasegawa, M. Sawada, and M. Ono. 1994c. Sex pheromone of oriental beetle, *Exomala orientalis*: identification and field evaluation. *J. Chem. Ecol.*, **20**: 1705-1718.

Leal, W.S., M. Hasegawa, M. Sawada, M. Ono, and Y. Ueda. 1994a. Identification and field evaluation of *Anomala octiescostata* (Coleoptera: Scarabaeidae) sex pheromone. *J. Chem. Ecol.*, **20**: 1643-1655.

Leal, W.S., F. Kawamura, and M. Ono. 1994b. The scarab beetle *Anomala albopilosa sakishimana* utilizes the same sex pheromone blend as a closely related and geographically isolated species, *Anomala cuprea. J. Chem. Ecol.*, **20**: 1667-1676.

Leal, W.S., and F. Mochizuki. 1993. Sex pheromone reception in the scarab beetle *Anomala cuprea*: enantiomeric discrimination by sensilla placodea. *Naturwissenschaften*, **80**: 278-281.

Leal, W.S., M. Ono, M. Hasegawa, and M. Sawada. 1994d. Kairomone from dandelion, *Taraxacum officinale*, attractant for scarab beetle *Anomala octiescostata. J. Chem. Ecol.*, **20**: 1697-1704.

Leal, W.S., M. Sawada, and M. Hasegawa. 1993a. The scarab beetle *Anomala cuprea* utilizes the sex pheromone of *Popillia japonica* as a minor component. *J. Chem. Ecol.*, **19**: 1303-1313.

Leal, W.S., M. Sawada, and M. Hasegawa. 1993b. The scarab beetle *Anomala daimiana* utilizes a blend of two other *Anomala* spp. sex pheromones. *Naturwissenschaften*, **80**: 181-183.

Leal, W.S., M. Sawada, S. Matsuyama, Y. Kuwahara, and M. Hasegawa. 1993c. Unusual periodicity of sex pheromone production in the large black chafer *Holotrichia parallela*. *J. Chem. Ecol.*, **19**: 1381-1391.

Leong, C.T. 1987. Sago worm culture. *Annual Report of the Research Branch, Department of Agriculture for the year 1986.* Sarawak, Ministry of Agriculture and Community Development.

Lever, R.J.A.W. 1969. *Pests of the Coconut Palm.* FAO Agricultural Studies No. 77, Food and Agriculture Organization of the United Nations, Rome. 190pp.

Lewis, T., and E.D.M. Macaulay. 1976. Design and evaluation of sex-attractant traps for pea moth, *Cydia nigricana* (Steph.) and the effect of plume shape on catches. *Ecol. Entomol.*, **1**: 175-187.

Liau, S.S., and A. Ahmad. 1991. The control of *Oryctes rhinoceros* by clean clearing and its effect on early yield in palm-to-palm replants. *Proceedings*, PORIM International Palm Oil Congress, 9-14 September 1991, Kuala Lumpur, Malaysia.

Liau, S.S., and A. Ahmad. 1993. Simulated defoliation and crop loss in young oil palm. *Proceedings*, PORIM International Palm Oil Congress, 20-25 September 1993, Kuala Lumpur, Malaysia.

Lin, H., and P.L. Phelan. 1991. Identification of food volatiles attractive to dusky sap beetle, *Carpophilus lugubris* (Coleoptera: Nitidulidae). *J. Chem. Ecol.*, **17**: 1273-1286.

Lindgren, B.S. 1983. A multiple funnel trap for scolytid beetles (Coleoptera). *Can. Entomol.*, **115**: 299-302.

Lindgren, B.S., and J.H. Borden. 1983. Survey and mass trapping of ambrosia beetles (Coleoptera: Scolytidae) in timber processing areas on Vancouver Island. *Can. J. For. Res.*, **13**: 481-493.

Lindgren, B.S., J.H. Borden, A.M. Pierce, H.D. Pierce Jr., A.C. Oehlschlager, and J.W. Wong. 1985. A potential method for simultaneous, semiochemical-based monitoring of *Cryptolestes ferrugineus* and *Tribolium castaneum* (Coleoptera: Cucujidae and Tenebrionidae). *J. stored Prod. Res.*, **21**: 83-87.

Lindgren, B.S., and R.G. Fraser. 1994. Control of ambrosia beetle damage by mass trapping at a dryland sorting area in British Columbia. *Forestry Chronicle*, **70**: 159-163.

Loughrin, J.H., D.A. Potter, and T.R. Hamilton-Kemp. 1995. Volatile compounds induced by herbivory act as aggregation kairomones for the Japanese beetle (*Popillia japonica* Newman). *J. Chem. Ecol.*, **21**: 1457-1467.

Lyal, C.H.C. 1995. The ventral structures of the weevil head (Coleoptera: Curculionidae). *Memoir Entomol. Soc. Wash.*, **14**: 35-51.

Maddison, P.A., M. Beroza, and T.P. McGovern. 1973. Ethyl chrysanthemumate as an attractant for the coconut rhinoceros beetle. *J. Econ. Entomol.*, **66**: 591-592.

Martin, A., and C. Simon. 1990. Differing levels of among-population divergence in the mitochondrial DNA of periodical cicadas related to historical biogeography. *Evolution*, **44**: 1066-1080.

Matsuyama, S., W.S. Leal, M. Hasegawa, and M. Sawada. 1994. Is chirality essential for pheromonal activity of scarab beetles ? The case of the large black chafer, *H. parallela*. *Proceedings, 38th Annual Meeting of the Japan Society for Bioscience, Biotechnology and Agrochemistry. Nogeikagakukaishi*, **68**: 58.

Mayr, E. 1969. *Principles of Systematic Zoology*. McGraw-Hill, New York. 428 pp.

McNeil, J.N. 1991. Behavioral ecology of pheromone-mediated communication in moths and its importance in the use of pheromone traps. *Ann. Rev. Entomol.*, **36**: 407-430.

Minitab. 1989. Release 7.1, Standard Version, Minitab Inc.

Mody, N.V., D.H. Miles, W.W. Neel, P.A. Hedin, A.C. Thompson, and R.C. Gueldner. 1973. Pecan weevil sex attractant: bioassay and chemical studies. *J. Insect Physiol.*, **19**: 2063-2071.

Moeck, H.A. 1970. Ethanol as the primary attractant for the ambrosia beetle *Trypondendron lineatum* (Coleoptera: Scolytidae). *Can. Entomol.*, **102**: 985-995.

Morin, J.P., F. Luchini, J.C.A. Araujo, J.M.S. Ferreira and L.S. Fraga. 1986. *Rhynchophorus palmarum* control using traps made from oil palm cubes. *Oléagineux*, **41**: 57-62.

Nagnan, P., A.H. Cain, and D. Rochat. 1992. Extraction and identification of volatile compounds of fermented oil palm sap (palm wine), candidate attractants for the palm weevil. *Oléagineux*, **47**: 135-142.

Nordlund, D.A. 1981. A glossary of terms used to describe chemicals that mediate intra- and interspecific interactions. pp. 495-497 in E.R. Mitchell (ed.), *Management of Insect Pests with Semiochemicals.* Plenum Press, New York. 514 pp.

Oehlschlager, A.C., C.M. Chinchilla, and L.M. Gonzalez. 1993a. Optimization of a pheromone-baited trap for the American palm weevil *Rhynchophorus palmarum* (L.). Proceedings, 1993 PORIM International Oil Palm Congress, 20-25 September 1993, Kuala Lumpur, Malaysia.

Oehlschlager, A.C., C.M. Chinchilla, L.M. Gonzalez, L.F. Jiron, R. Mexzon, and B. Morgan. 1993b. Development of a pheromone-based trapping system

for the American palm weevil, *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae). *J. Econ. Entomol.*, **86**: 1381-1392.

Oehlschlager, A.C., R.S. McDonald, C.M. Chinchilla, and S.N. Patschke. 1995b. Influence of a pheromone-based mass trapping system on the distribution of *Rhynchophorus palmarum* (Coleoptera: Curculionidae) in oil palm. *Envir. Entomol.*, 24: 1005-1012.

Oehlschlager, A.C., A.M. Pierce, H.D. Pierce, Jr., and J.H. Borden. 1988. Chemical communication in Cucujid grain beetles. *J. Chem. Ecol.* **14**: 2071-2098.

Oehlschlager, A.C., H.D. Pierce, Jr., B. Morgan, P.D.C. Wimalaratne, K.N. Slessor, G.G.S. King, G. Gries, R. Gries, J.H. Borden, L.J. Jiron, C.M. Chinchilla, and R.G. Mexzon. 1992. Chirality and field activity of rhynchophorol, the aggregation pheromone of the American palm weevil. *Naturwissenschaften*, **79**: 134-135.

Oehlschlager, A.C., R.N.B. Prior, A.L. Perez, R. Gries, G. Gries, H.D. Pierce Jr., and S. Laup. 1995a. Structure, chirality, and field testing of a male-produced aggregation pheromone of Asian palm weevil *Rhynchophorus bilineatus* (Montr.) (Coleoptera: Curculionidae). *J. Chem. Ecol.*, **21**: 1619-1629.

Page, M., L.J. Nelson, M.I. Haverty, and G.J. Blomquist. 1990. Cuticular hydrocarbons of eight species of North American cone beetles, *Conophthorus* Hopkins. *J. Chem. Ecol.*, **16**: 1173-1198.

Paterson, H.E.H. 1985. The recognition concept of species. pp. 21-29 in E.S. Vrba (ed.) *Species and Speciation.* Transvaal Museum Monograph No. 4, Pretoria.

Perez, A.L., G. Gries, R. Gries, R.M. Giblin-Davis, and A.C. Oehlschlager. 1994. Pheromone chirality of African palm weevil, *Rhynchophorus phoenicis* (F.)

and palmetto weevil, *Rhynchophorus cruentatus* (F.) (Coleoptera: Curculionidae). *J. Chem. Ecol.*, **20**: 2653-2671.

Perez, A.L., R. Gries, G. Gries, R.H. Hallett, A.C. Oehlschlager, H.D. Pierce, Jr., L.M. Gonzalez, J.H. Borden, and R.M. Giblin-Davis. 1993. Pheromones of *Rhynchophorus* palm weevils. Paper presented at the International Society of Chemical Ecology, July 31 - August 4, 1993, Tampa, Florida.

Perez-Sanchez, A.L. 1996. *Chemical Ecology of Rhynchophorus Palm Weevils and Oryctes Coconut Beetles.* Ph.D. Thesis, Simon Fraser University, Burnaby, B.C., Canada. 293 pp.

Perring, T.M., A.D. Cooper, R.J. Rodriguez, C.A. Farrar, and T.S. Bellows Jr. 1993. Identification of a whitefly species by genomic and behavioral studies. *Science*, **259**: 74-77.

Phillips, J.K., and Burkholder, W.E. 1981. Evidence for a male-produced aggregation pheromone in the rice weevil. *J. Econ. Entomol.*, **74**: 539-542.

Phillips, T.W., and G.N. Lanier. 1983. Biosystematics of *Pissodes* Germar (Coleoptera: Curculionidae): feeding preferences and breeding site specificity of *P. strobi* and *P. approximatus*. *Can. Entomol.*, **115**: 1627-1636.

Phillips, T.W., and G.N. Lanier. 1986. Interspecific activity of semiochemicals among sibling species of *Pissodes* (Coleoptera: Curculionidae). *J. Chem. Ecol.*, **12**: 1587-1601.

Phillips, T.W., S.A. Teale, and G.N. Lanier. 1987. Biosystematics of *Pissodes* Germar (Coleoptera: Curculionidae): seasonality, morphology, and synonymy of *P. approximatus* Hopkins and *P. nemorensis* Germar. *Can. Entomol.*, **119**: 465-480.

Phillips, T.W., J.R. West, J.L. Foltz, R.M. Silverstein, and G.N. Lanier. 1984. Aggregation pheromone of the deodar weevil, *Pissodes nemorensis* (Coleoptera: Curculionidae): isolation and activity of grandisol and grandisal. *J. Chem. Ecol.*, **10**: 1417-1423.

Phillips, T.W., A.J. Wilkening, T.H. Atkinson, J.L. Nation, C. Wilkinson, and J.L. Foltz. 1988. Synergism of turpentine and ethanol as attractants for certain pine infesting beetles (Coleoptera). *Environ. Entomol.*, **17**: 456-462.

Pierce, A.M., J.H. Borden, and A.C. Oehlschlager. 1981. Olfactory response to beetle-produced volatiles and host-food attractants by *Oryzaephilus surinamensis* and *O. mercator*. *Can. J. Zool.*, **59**: 1980-1990.

Pierce, H.D., Jr., P. de Groot, J.H. Borden, S. Ramaswamy, and A.C. Oehlschlager. 1995. Pheromones in red pine cone beetle, *Conophthorus resinosae* Hopkins, and its synonym, *C. banksianae* McPherson (Coleoptera: Scolytidae). *J. Chem. Ecol.*, **21**: 169-185.

Poland, T.M., and J.H. Borden. 1994a. Semiochemical-based communication in interspecific interactions between *Ips pini* (Say) and *Pityogenes knechteli* (Swaine) (Coleoptera: Scolytidae) in lodgepole pine. *Can. Entomol.*, **126**: 269-276.

Poland, T.M., and J.H. Borden. 1994b. Attack dynamics of *Ips pini* (Say) and *Pityogenes knechteli* (Swaine (Col., Scolytidae) in windthrown lodgepole pine trees. *J. Appl. Entomol.*, **117**: 434-443.

Puterka, G.J., W.C. Black IV, W.M. Steiner, and R.L. Burton. 1993. Genetic variation and phylogenetic relationships among worldwide collections of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), inferred from allozyme and RAPD-PCR markers. *Heredity*, **70**: 604-618.

Rahalkar, G.W., M.R. Harwalkar, H.D. Rananavare, A.J. Tamhankar, and K. Shanthram. 1985. *Rhynchophorus ferrugineus*. pp. 279-286 in P. Singh &

R.F. Moore (eds.), *Handbook of Insect Rearing Vol. I.* Elsevier Science Publishers, Amsterdam. 488 pp.

Rieske, L.K., and K.F. Raffa. 1991. Effects of varying ethanol and turpentine levels on attraction of two pine root weevil species, *Hylobius pales* and *Pachylobius picivorus* (Coleoptera: Curculionidae). *Environ. Entomol.*, **20**: 48-52.

Rochat, D., C. Descoins, C. Malosse, P. Nagnan, P. Zagatti, F. Akamou, and D. Mariau. 1993b. Ecologie chimique des charançons des palmiers, *Rhynchophorus* spp. (Coleoptera). *Oléagineux*, **48**: 225-236.

Rochat, D., A. Gonzalez, D. Mariau, A. Villanueva, and P. Zagatti. 1991a. Evidence for male-produced aggregation pheromone in American palm weevil, *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae). *J. Chem. Ecol.*, **17**: 1221-1230.

Rochat, D., C. Malosse, M. Lettere, P.-H. Ducrot, P. Zagatti, M. Renou, and C. Descoins. 1991b. Male-produced aggregation pheromone of the American palm weevil, *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae): collection, identification, electrophysiological activity, and laboratory bioassay. *J. Chem. Ecol.*, **17**: 2127-2141.

Rochat D., C. Malosse, M. Lettere, P. Ramirez-Lucas, J. Einhorn, and P. Zagatti. 1993a. Identification of new pheromone-related compounds from volatiles produced by males of four Rhynchophorinae weevils (Coleoptera, Curculionidae). *C.R. Acad. Sci. Paris Ser. II.*, **316**: 1737-1742.

Rochat, D., and P. Zagatti. 1993. Mise en évidence d'une phéromone cuticulaire chez les femelles de *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae). *C.R. Acad. Sci. Paris Ser. III.* , xxx: xxx-xxx.

Sadakathulla, S. 1991. Management of red palm weevil, *Rhynchophorus ferrugineus* F., in coconut plantations. *Planter*, **67**(786): 415-419.

SAS Institute. 1985. SAS User's Guide: Statistics. Version 5. SAS Institute Inc., Cary, N.C.

Schlyter, F., and G. Birgersson. 1989. Individual variation in bark beetle and moth pheromones - a comparison and an evolutionary background. *Hol. Ecol.*, **12**: 457-465.

Schroeder, L.M., and Å. Lindelöw. 1989. Attraction of scolytids and associated beetles by different absolute amounts and proportions of α-pinene and ethanol. *J. Chem. Ecol.*, **15**: 807-817.

Setter, R.R., and J.H. Borden. 1992. Response by the striped ambrosia beetle, *Trypondendron lineatum* (Oliver), to the bark beetle pheromone, frontalin. *Can. Entomol.*, **124**:559-560.

Silverstein, R.M., J.O. Rodin, and D.L. Wood. 1966. Sex attractants in frass produced by male *Ips paraconfusus* in ponderosa pine. *Science*, **154**: 509-510.

Silverstein, R.M., J.O. Rodin, and D.L. Wood. 1967. Methodology for isolation and identification of insect pheromones with reference to studies on California five-spined ips. *J. Econ. Entomol.*, **60**: 944-949.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Lui, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.*, **87**: 651-701.

Sivapragasam, A., A. Arikiah, and C.A. Ranjit. 1990. The red stripe weevil, *Rhynchophorus schach* Olivier (Coleoptera: Curculionidae): an increasing menace to coconut palms in Hilir Perak. *Planter*, **66**: 113-123.

Tajudin, M.H., C.H. Teoh, K. Aribi, and M. Ali. 1993. Zero-burning - an environmentally friendly replanting technique. *Proceedings*, PORIM

International Palm Oil Congress, 20-25 September 1993, Kuala Lumpur, Malaysia.

Tamaki, Y., H. Sugie, and H. Noguchi. 1985. Methyl (*Z*)-5-tetradecenoate: sex-attractant pheromone of the soybean beetle, *Anomala rufocuprea* Motschulsky (Coleoptera: Scarabaeidae). *Appl. Entomol. Zool.*, **20**: 359-361.

Templeton, A.R. 1989. The meaning of species and speciation: a genetic perspective. pp. 3-27 in D. Otte & J.A. Endler (eds.) *Speciation and Its Consequences*. Sinaver Associates, Massachusetts. 679 pp.

Tóth, M., W.S. Leal, I. Szárukán, M. Lesznyák, and G. Szöcs. 1994. 2-(*E*)-Nonen-1-ol: male attractant for chafers *Anomala vitis* Fabr. and *A. dubia* Scop. (Coleoptera: Scarabaeidae). *J. Chem. Ecol.*, **20**: 2481-2487.

Tumlinson, J.H., D.D. Hardee, J.P. Minyard, A.C. Thompson, R.T. Gast, and P.A. Hedin. 1968. Boll weevil sex attractant: isolation studies. *J. Econ. Entomol.*, **61**: 470-474.

Tumlinson, J.H., D.D. Hardee, R.C. Gueldner, A.C. Thompson, P.A. Hedin, and J.P. Minyard. 1969. Sex pheromones produced by male boll weevil: isolation, identification, and synthesis. *Science*, **166**: 1010-1012.

Tumlinson, J.H., M.G. Klein, R.E. Doolittle, T.L. Ladd, and A.T. Proveaux. 1977. Identification of the female Japanese beetle sex pheromone: inhibition of male response by an enantiomer. *Science*, **197**: 789-792.

Vander Meer, R.K., U.R. Ghatak, S.K. Alam, and P.C. Chakraborti. 1979. (±)-Des-N-Morphinan: a unique bridged hydrocarbon attractant for the rhinoceros beetle, *Oryctes rhinoceros*, and development of an olfactometer. *Environ. Entomol.*, **8**: 6-10.

VanderSar, T.J.D., and J.H. Borden. 1977a. Visual orientation of *Pissodes strobi* Peck (Coleoptera: Curculionidae) in relation to host selection behaviour. *Can. J. Zool.*, **55**: 2042-2049.

VanderSar, T.J.D., and J.H. Borden. 1977b. Role of geotaxis and phototaxis in the feeding and oviposition behavior of overwintered *Pissodes strobi*. *Envir. Entomol.*, **6**: 743-749.

Walgenbach, C.A., J.K. Phillips, W.E. Burkholder, G.G.S. King, K.N. Slessor, and K. Mori. 1987. Determination of chirality in 5-hydroxy-4-methyl-3-heptanone, the aggregation pheromone of *Sitophilus oryzae* (L.) and *S. zeamais* Motschultsky. *J. Chem. Ecol.*, **13**: 2159-2169.

Walgenbach, C.A., J.K. Phillips, D.L. Faustini, and W.E. Burkholder. 1983. Male-produced aggregation pheromone of the maize weevil, *Sitophilus zeamais*, and interspecific attraction between three *Sitophilus* species. *J. Chem. Ecol.*, **9**: 831-841.

Wattanapongsiri, A. 1966. *A Revision of the Genera Rhynchophorus and Dynamis (Coleoptera: Curculionidae).* Department of Agriculture Science Bulletin 1, Department of Agriculture, Bangkok.

Weissling, T.J., R.M. Giblin-Davis, G. Gries, R. Gries, A.L. Perez, H.D. Pierce, Jr., and A.C. Oehlschlager. 1994. Aggregation pheromone of the palmetto weevil, *Rhynchophorus cruentatus* (F.) (Coleoptera: Curculionidae). *J. Chem. Ecol.*, **20**: 505-515.

Weissling, T.J., R.M. Giblin-Davis, R.H. Scheffrahn, and N.M. Mendoza. 1992. Traps for capturing and retaining *Rhynchophorus cruentatus* (Coleoptera: Curculionidae) adults using *Sabal palmetto* as bait. *Fla. Entomol.*, **75**: 212-221.

White, J.M., and M.M. Elson-Harris. 1992. *Fruit Flies of Economic Significance.* CAB International, Oxford. 601pp.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.*, **18**: 6531-6535.

Wood, B.J. 1968. Studies on the effect of ground vegetation on infestations of *Oryctes rhinoceros* (L.) (Col., Dynastinae) in young oil palm replantings in Malaysia. *Bull. Entomol. Res.*, **59**: 85-96.

Wood, S.L. 1963. A revision of bark beetle genus *Dendroctonus* Erichson (Coleoptera: Scolytidae). *Great Basin Natur.*, **23**: 1-117.

Wood, S.L. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae). A taxonomic monograph. *Great Basin Nat. Mem.*, **6**: 1359pp.

Yarden, G., and A. Shani. 1994. Evidence for volatile chemical attractants in the beetle *Maladera matrida* Argaman (Coleoptera: Scarabaeidae). *J. Chem. Ecol.*, **20**: 2673-2685.

Young, E.C. 1986. The Rhinoceros Beetle Project: history and review of the research programme. *Agric. Ecosyst. Environ.*, **15**: 149-166.

Zagatti, P., R. Desmier de Chenon, P. Genty, D. Rochat, and D. Mariau. 1993. The pheromonal substances of some oil palm insect pest species: initial results. Proceedings, 1993 PORIM International Oil Palm Congress, 20-25 September 1993, Kuala Lumpur, Malaysia.

Zar, J.H. 1984. *Biostatistical Analysis*. Prentice-Hall, New Jersey.

Zelazny, B. 1979. Loss in coconut yield due to *Oryctes rhinoceros* damage. *FAO Plant Prot. Bull.*, **27**(3): 65-70.

Zelazny, B., and A.R. Alfiler. 1986. *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) larval abundance and mortality factors in the Philippines. *Environ. Entomol.*, **15**: 84-87.

Zelazny, B. and A.R. Alfiler. 1987. Ecological methods for adult populations of *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). *Ecol. Entomol.*, **12**: 227-238.

Zelazny, B., and A.R. Alfiler. 1991. Ecology of baculovirus-infected and healthy adults of *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) on coconut palms in the Philippines. *Ecol. Entomol.*, **16**: 253-259.

Zhang, A., H.T. Facundo, P.S. Robbins, C.E. Linn, Jr., J.L. Hanula, M.G. Villani, and W.L. Roelofs. 1994. Identification and synthesis of female sex pheromone of Oriental beetle, *Anomala orientalis* (Coleoptera: Scarabaeidae). *J. Chem. Ecol.*, **20**: 2415-2427.