FINAL TECHNICAL REPORT

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DEVELOPMENT OF PHEROMONES FOR MANAGEMENT OF THE COFFEE WHITE STEMBORER, *XYLOTRECHUS QUADRIPES*

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EXECUTIVE SUMMARY

In India, coffee cultivation provides livelihoods for 140,000 growers and 500,000 workers and their families. Ninetyeight percent of the growers own less than 10ha and produce 60% of total production. In addition to its socioeconomic importance in remote, rural areas, coffee growing makes a major contribution to maintaining an ecological balance in poor hillsides, the coffee and shade trees preventing erosion and preserving biodiversity.

Coffee white stemborer, *Xylotrechus quadripes* Chevrolat (Coleoptera: Cerambycidae), is the most serious insect pest of arabica coffee in India, as well as Sri Lanka, China, Vietnam, Thailand. Loss of production due to *X. quadripes* on arabica is a capital loss caused by the need to uproot and replace infested plants, and it is estimated that the national loss due to this pest is 130m rupees per year (£2m). There are no effective control measures against this pest following the withdrawal of BHC for agricultural use in India. Related stemborers are also major insect pests of coffee in Africa.

In previous work, two components of the male-produced sex pheromone of *X. quadripes* were identified and synthesised and the synthetic blend was shown to attract female beetles in laboratory and field.

The Purpose of the project was to develop improved methods for the management of priority pests and diseases of arabica coffee in order to minimise production losses and optimise yields from agroforestry systems on sloping lands in a sustainable manner. The project aimed to contribute to this Purpose by identifying and synthesising a potential third component of the male pheromone of *X. quadripes*, determining whether it could improve attractiveness of the major component by laboratory and field tests and by initiating trials of mass trapping with pheromone traps to control this pest. This work was carried out in close collaboration with the Central Coffee Research Institute (CCRI) of the Coffee Board of India. The project also aimed to explore the potential for use of pheromones in management of African species of coffee stemborer.

The third component found in volatiles from male *X. quadripes* beetles which elicits an electroantennographic response from females was identified as (*S,S*)-2,3-dihydroxyoctane, and a new synthetic route to this compound was developed. The effect on attractiveness of adding this compound to the major component was tested in laboratory and field. Despite using the same apparatus and procedures as used previously, no significant results were obtained in laboratory bioassays carried out in India and at NRI. Catches in field tests were low, but there was no evidence for the third pheromone component increasing the attractiveness of the major component. If anything, catches were decreased, in agreement with bioassays carried out in the previous project. Dr Jayarama was trained in pheromone synthesis at NRI, and he will initiate field trials with the pheromone produced.

Surveys of smallholders in Malawi carried out during the project "Pesticide and microbial interactions in coffee pest management" (R6807) showed that stemborers are perceived to be the major pests of coffee and that infestation levels are indeed high. The major species is the white coffee stemborer, *Monochamus leuconotus.* The only effective method of control is painting the stem with aldrin, but smallholders cannot afford this and it is being withdrawn from use in agriculture. *M. leuconotus* is also the major pest of arabica coffee in Zimbabwe.

Contact was established with the Coffee Research Station of the Zimbabwe Department of Specialist Services and preliminary bioassay work on *M. leuconotus* carried out there.

The project has contributed to the Purpose by providing the basis for a control of *X. quadripes* by mass trapping with pheromone traps, an approach which should be specific, environmentally-acceptable and appropriate. Links have been established in southern Africa to explore the possibilities of developing pheromones for management of the major coffee pest, *M. leuconotus.*

Further supervisory inputs will be required to assist CCRI staff carry out field trials in India and localise production of the pheromone, traps and dispensers. Technical inputs will be required to identify and develop pheromones for *M. leuconotus*. These inputs could be included in the proposed extension of the project "Pesticide and microbial interactions in coffee pest management" (R6807).

BACKGROUND

Coffee white stemborer, *Xylotrechus quadripes* in India

In India, coffee cultivation provides livelihoods for 140,000 growers and 500,000 workers and their families. Ninetyeight percent of the growers own less than 10ha and produce 60% of total production, a proportion which has increased from 40% twelve years ago and is likely to continue growing. Current national production is 230,000 tonnes per year with 170,000 tonnes exported, earning 1.7 billion rupees (£250m) in foreign exchange.

In addition to its socioeconomic importance in remote, rural areas, coffee growing in India makes a major contribution to maintaining an ecological balance in poor hillsides, the coffee and shade trees preventing erosion and preserving biodiversity.

The area under coffee in India is nearly 300,000 ha with around half arabica and half robusta. However, coffee white stemborer, *Xylotrechus quadripes* Chevrolat (Coleoptera: Cerambycidae), has become a major constraint on production of arabica coffee, and absence of control measures is causing growers to shift from arabica in favour of the lower grade robusta. Loss of production due to *X. quadripes* on arabica is a capital loss caused by the need to uproot and replace infested plants, and it is estimated that the national loss due to this pest is 130m rupees per year (£2m). Arabica has higher production costs than robusta, but the return is nearly 50% greater (1.0 US$/pound *v* 0.7 US$/pound) and it is important that the acreage of arabica is maintained for India's reputation as a quality producer.

X. quadripes is also the most serious insect pest of arabica coffee in Sri Lanka, China, Vietnam and Thailand (Le Pelley, 1968). The adult females lay eggs in crevices in the bark and the hatching larvae tunnel into the trunk and roots, causing death in young plants of seven to eight years old while older bushes may survive for a few seasons but eventually succumb. Current control measures include maintenance of good shade as the adults are more active in sunlight, although this reduces photosynthesis and yield. Removal of loose bark at the base of the tree discourages oviposition and collar pruning of infested trees may be effective if the pest has not reached the roots. BHC applied to the trunk at critical times during the flight periods is still the only effective insecticide against *X. quadripes*, but this has now been banned for agricultural use in India and many other countries.

Male *X. quadripes* were shown to attract female beetles in the field by Venkateshu *et al.* (1986). Production of a sex pheromone by males of a related species, the grape borer *X. pyrrhoderus*, was demonstrated by Iwabuchi (1982), and the chemical structures of the components were identified by Sakai *et al.* (1984). The same components were shown to be produced by the mulberry borer, *X. chinensis* (Kuwahara *et al.*, 1987; Iwabuchi *et al.*, 1987). Further work on the pheromone of *X. quadripes* in India led to proposal of a very different structure for the pheromone of this species (Jayarama and Venkatesha, 1995), and this project arose as a request from the Coffee Board of India for assistance in identification of the pheromone and development of it use in management of the pest. In the previous CPP project (R6928) carried out in collaboration with the Indian Central Coffee Research Institute (CCRI):

- the chemical structure of the major component of the male-produced sex pheromone of *X. quadripes* was established and a synthetic route developed;

- a second, minor component in volatiles from male *X. quadripes* was identified as an isomer of the major component, and this was present at a similar level in the synthetic material;
- controlled release dispensers for the pheromone were developed and characterised;
- laboratory bioassay methods for the pheromone were devised, and the synthetic pheromone shown to be attractive to female *X. quadripes* beetles;
- the synthetic pheromone was shown to attract female *X. quadripes* beetles to traps in the field, and suitable traps were developed;
- two members of the CCRI were trained in aspects of pheromone research at NRI and in India.

In this work, a third component in volatiles collected from the male beetles was shown to elicit an electroantennographic (EAG) response from female beetles, but the chemical structure of this component was not established.

African coffee white stemborer, *Monochamus leuconotus*

Related stemborers are also major pests of coffee in Africa, and surveys carried out as part of the CPP project "Pesticide and microbial interactions in coffee pest management" (R6807) established that the African white coffee stemborer, *Monochamus leuconotus* (Coleoptera: Cerambycidae) is the most important species in Malawi. Ninety percent of smallholders questioned mentioned stemborer as a problem, by far the highest response for any pest or disease, and 60% ranked it as the most important. These views were supported by surveys which found up to 60% of bushes attacked, a very high level considering that one larvae can kill a bush. As with *X. quadripes*, the only effective methods for control of *M. leuconotus* are uprooting of infested bushes or painting the stems with aldrin. Withdrawal of aldrin for agricultural use and declining incomes from coffee growing have meant that smallholders have abandoned control of stemborer or attempted physical methods such as poking wires in the exit holes.

Pheromones

Pheromones are non-toxic to plants and animals, they are specific for the target pest and they are active in very small amounts. They can be used to bait traps to monitor for insect pests and determine the need and optimal timing for application of control measures. Pheromones can also be used to control pests by mass trapping or by permeating the atmosphere with synthetic pheromone to disrupt pheromone-mediated communication and hence prevent mating in the case of sex pheromones. With *X. quadripes*, prospects for successful use of the synthetic pheromone in actual control are particularly good because the pest occurs at very low density and because the pheromone attracts the female beetles preventing egg-laying directly. As they are non-toxic and specific for the target pest, pheromones are compatible with all other methods of pest control, cultural, biological or insecticidal.

PROJECT PURPOSE

The Purpose of the project was to develop improved methods for the management of priority pests and diseases of arabica coffee in order to minimise production losses and optimise yields from agroforestry systems on sloping lands in a sustainable manner. The project aimed to contribute to this Purpose by identifying and synthesising a potential third component of the male pheromone of the major pest of arabica coffee in India and S E Asia, coffee white stemborer, *X. quadripes*, determining whether it could improve attractiveness of the major component by laboratory and field tests and initiating trials of mass trapping with pheromone traps to control this pest.

The geographic focus of the project was India, but *X. quadripes* is a major pest of arabica coffee in several other Asian countries where the results will be directly applicable. Some of the principles developed might also be applicable to other coffee stemborer species which are pests of arabica coffee in other countries, and this project also aimed to investigate the potential of pheromones in management of the major coffee stemborer species in Africa.

RESEARCH ACTIVITIES

Pheromone identification

Gas chromatographic (GC) analyses

GC analyses were carried out on fused silica capillary columns (25 m x 0.32 mm i.d.) coated with polar CPWax52CB (Carbowax 20M equivalent; Chrompack) or non-polar CPSil5CB (methyl silicone; Chrompack). Carrier gas was helium at 0.5 kg/cm^2 , oven temperature was held at 50°C for 2 min then programmed at 6°C/min to 230°C. Injection was splitless or split (50:1) and detection was by flame ionisation detection (FID). GC retention times are reported as Kovats Indices (KI) relative to those of normal hydrocarbons.

Enantiomeric purity was determined by GC analysis on a β-cyclodextrin GC column (50 m x 0.22 mm i.d., SGE,) operated isothermally at 135°C.

Linked Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analyses were carried out with fused silica capillary columns (25 m x 0.2 mm i.d.) coated with polar CPWax52CB or non-polar CPSil5CB linked directly to a Finnigan ITD 700 Ion Trap Detector operated in electron impact (EI) or chemical ionisation with *iso*-butane (CI) mode. GC conditions were as above.

Nuclear Magnetic Resonance (NMR) Spectrometry

NMR spectra were recorded in $CDCl_3$ on a JEOL EX270 machine at 270 MHz for ^1H and 67.8 MHz for ^{13}C.

Synthesis

The major pheromone component, (*S*)-2-hydroxy-3-decanone (I), was synthesised from (*S*)-ethyl lactate by a short, general route developed previously and further optimised in this project (Scheme 3).

(*S*)-2-Hydroxy-3-octanone (V) was synthesised similarly in 62.5% overall yield and reduced to a 2:1 mixture of diastereomers (*S,S*)-2,3-dihydroxyoctane (III) and (*S,R*)-2,3-dihydroxy-octane (IV) with lithium aluminium hydride in ether (Scheme 1).

Novel one-step routes to (*S,S*)-2,3-dihydroxyoctane (III) and (*R,R*)-2,3-dihydroxyoctane (VI) were developed and are described below (Scheme 2).

Bioassay studies

Bioassays were carried out at CCRI, Chikmagulur, during the morning and subsequently at various times during the day using full or shaded natural lighting or artificial lighting at various intensities. Bioassays were also carried out at NRI between 10.00 hr and 12.00hr in an insectary maintained at 27°C with 5,000 lux lighting. Two main types of bioassay apparatus were used, as developed in the previous project and described below.

Insects

Insects were collected as they emerged from infested logs held in a netted room at CCRI. Insects were collected several times during the morning emergence hours to ensure they did not mate, sexed and maintained in separate tubes. Insects used as lures in bioassays were held in plastic, screw-top containers (6 cm x 4 cm) with plastic mesh at both ends.

Pheromone dispensers

Dispensers for synthetic pheromone in bioassay and field trials were sealed polyethylene vials (22 mm x 8 mm x 1.5 mm thick) containing 100μl of pheromone. These were developed during the previous project and released (*S*)-2-hydroxy-3-decanone (I) at 18 μg/hr at 27°C.

"Swastika" bioassay

This consisted of a central circular chamber (19 cm x 5 cm) with four equally-spaced entry/exit openings in the side wall. The openings led to tubes (3.8 cm diameter, total length 55 cm) with a right angle bend in the middle to give a swastika appearance. Sample bottles with fine mesh tops and bottoms were fixed to the distal ends of the tubes. Air was drawn into the apparatus through the arms and out through a tube attached to the centre of the top of the central chamber at 20 litres/min. A single insect was placed at the centre of the central chamber with appropriate treatments in the sidearms. Insect behaviour was observed for 10 min: if it entered one of the sidearms this was scored, otherwise it was scored as remaining in the central chamber. The apparatus was rotated through 90° between each observation to avoid any possible effects due to uneven lighting.

Tube bioassay

This utilised a perspex tube (1 m x 3.8 cm diameter) with a sample bottle with fine gauze top and bottom attached at one end. The test source was put in the sample bottle and a single test beetle placed at the opposite end of the tube and air was drawn in through the source bottle and out through the other end of the tube at 10 litre/min. The tube was marked at 10 cm intervals, and the nearest approach to the source by the test insect during 10 min of observation was scored (1.0 = reached source, 0.0 = no upwind movement).

Bioassay experiments with *Monochamus leuconotus* were carried out at the Coffee Research Station, Chipinge, Zimbabwe, by Mr Kutyawayo. A tube (1 m long) was constructed from perspex overhead projector transparencies. Insects were held in cardboard tubes with muslin over each end, and air was blown through the apparatus with a fan. Times taken for the test insect to emerge from the holding tube, to progress up the tube to the lure insect and to mate were recorded. Fresh insects were used for each replicate, and experiments were run for up to 48 hours.

Field trials

Field trials were carried out in estates round the Central Coffee Research Institute, Chikmagulur, Karnataka, the Coffee Research Sub-Station, Chettalli, Karnataka, and the Regional Coffee Research Station, Thandigudi, Tamil Nadu, India, during April-June and October-December 1998.

Traps were sticky white cross-vane traps (Agrisense; 25 cm x 15 cm) made from Correx sheet and fastened to wooden poles at a height of approximately 1.5 m. Four or five treatments were tested in Latin square layouts at each location, and catches were recorded and discarded every week.

OUTPUTS

Identification of third component of pheromone of male *X. quadripes*

Structural elucidation

In previous work (Hall *et al.*, 1998), analysis of volatiles collected from male *X. quadripes* beetles by GC-EAG on a polar column showed two major components (A) and (B). EAG responses were recorded to (A) and a minor component (C) eluting after (B) (Fig. 1).

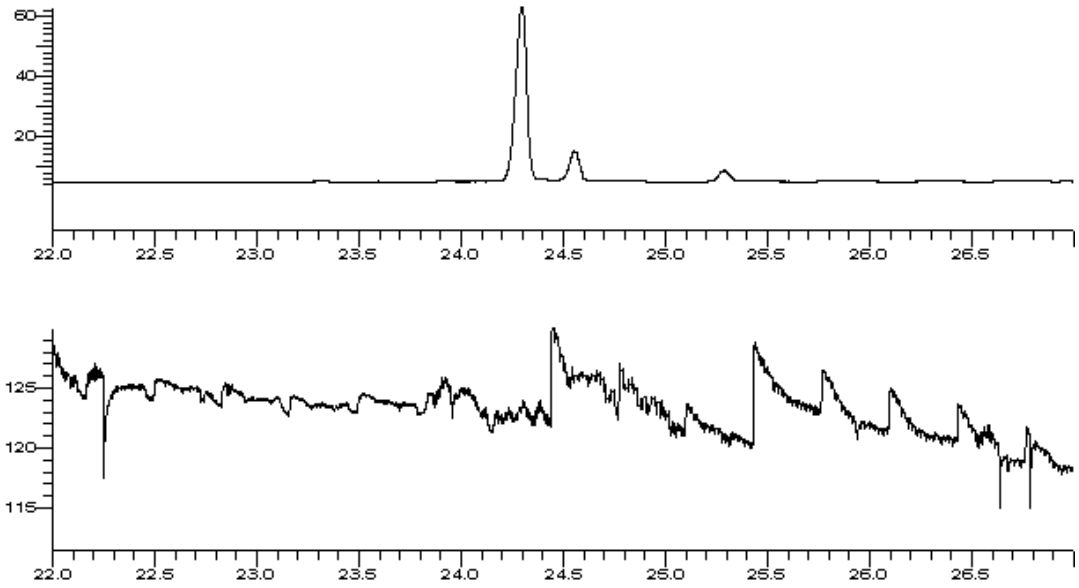
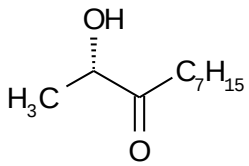
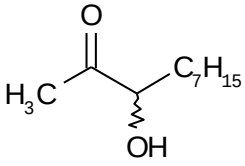


FIG. 1. Linked GC (upper) EAG (lower) analysis of volatiles from male *X. quadripes* on a polar GC column.

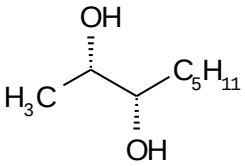
Consideration of their GC retention times and mass spectral data indicated the structures of components (A) and (B) were (*S*)-2-hydroxy-3-decanone (I) and 3-hydroxy-2-decanone (II) respectively. These compounds were synthesised and the synthetic and natural compounds were shown to have identical GC retention times on polar, non-polar and cyclodextrin columns, and to have identical EI and CI mass spectra.



(I)



(II)



(III)

The EI and CI mass spectra of component (C) (Fig. 2) suggested that its structure was related to those of (A) and (B), although probably with a lower molecular weight, possibly 128. The

retention data in terms of the relative retention indices on polar and non-polar columns (Table 1) showed that (C) (Δ = 758) was significantly more polar than either (A) (Δ = 570) or (B) (Δ = 590). Comparison of this data with the literature EI mass spectrum of 2,3-dihydroxyoctane (Kuwahara *et al.*, 1987) and the mass spectrum and retention data for 2,3-dihydroxydecane obtained in the previous work, suggested that component (C) was actually 2,3-dihydroxyoctane (*S*,*S* isomer III).

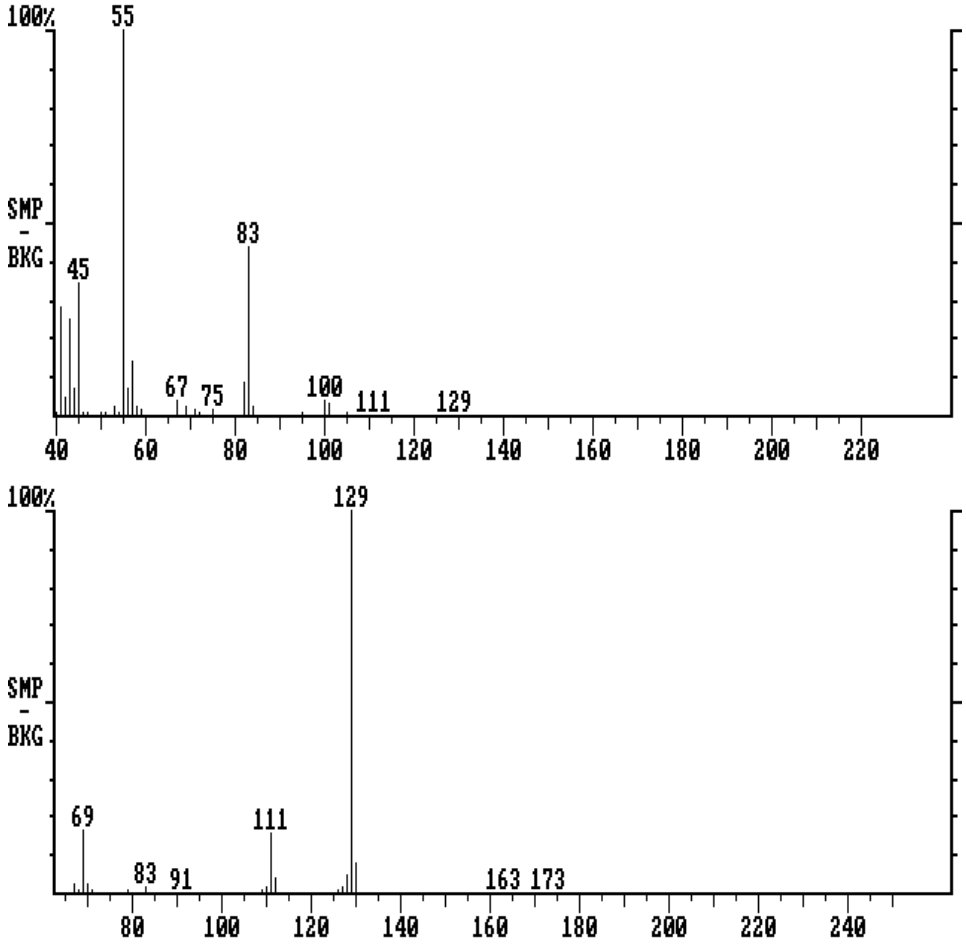
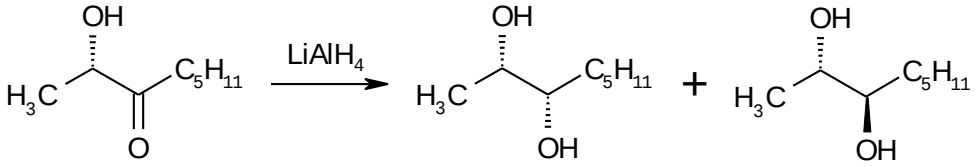


FIG. 2. EI (upper) and CI (lower) mass spectra of pheromone component (C).

2,3-Dihydroxyoctane was synthesised as a 40:60 mixture of the *S*,*S* (III) and *S*,*R* (IV) diastereomers by reduction of (*S*)-2-hydroxy-3-octanone (V) with lithium aluminium hydride (Scheme 1). Compound (V) was synthesised in 62.5% yield from (*S*)-ethyl lactate and 1-bromopentane by the general route to chiral 2-hydroxy-3-ketones developed in the previous work (*cf* Scheme 1 below).



(V) (III) (IV)

SCHEME 1. Reduction of (*S*)-2-hydroxy-3-octanone (V)

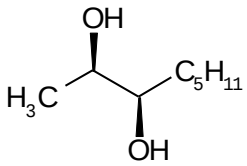
The *S,R* diastereomer (IV) was predicted to be the major product of this reduction, assuming coordination of the aluminium by the 2-hydroxy group.

In the previous work (Hall *et al.*, 1998), it was reported that, although the EI mass spectrum of the 2,3-dihydroxyoctane was essentially identical with that of component (C) in the male *X. quadripes* volatiles, the retention time on the polar column was slightly but significantly different.

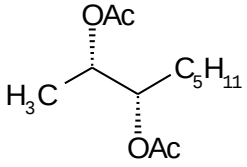
In the current work, it was realised that these 2,3-diols chromatograph poorly on polar GC columns, and, moreover, that the *erythro* diastereomers, e.g. the *S,S* enantiomer (III), seem to chromatograph worse than the *threo*, e.g. the *S,R* enantiomer (IV), such that the former can be completely lost on an older column. Thus in the previous work, only a single peak was observed on analysis of the synthetic mixture of 2,3-dihydroxyoctanes (III) and (IV), in fact corresponding to compound (IV). These observations also explained earlier findings that the minor pheromone component (C) was not always observed in analyses of natural volatile collections.

Once the synthetic diols were available, it was found that - surprisingly - the 2,3-dihydroxy-octanes chromatographed well on a non-polar GC column and that the corresponding diacetates (VII + VIII) chromatographed well on both polar and non-polar columns. Thus the pheromone component (C) was identified as *S,S* (III) or *R,R* (VI) *threo*-2,3-dihydroxyoctane as follows.

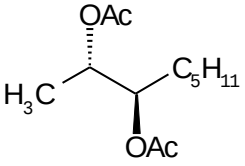
- Pheromone component C had retention times and EI mass spectrum identical with those of the minor component in the above synthetic mixture of 2,3-dihydroxyoctanes (III + IV) on both polar and non-polar GC columns (Table 1).
- After acetylation of the volatiles collected from male *X. quadripes*, a component of the mixture had retention times and EI mass spectrum identical with those of the minor component of the mixture of diacetates (VII + VIII) derived by acetylation of the mixture of 2,3-dihydroxyoctanes (III + IV) (Table 1).



(VI)



(VII)



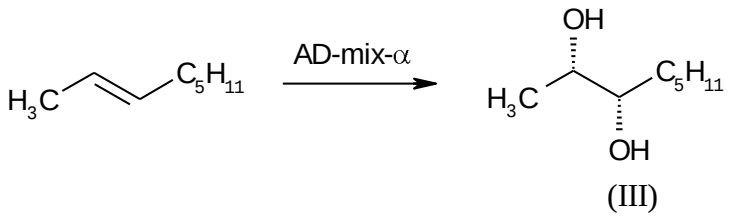
(VIII)

TABLE 1. GC Retention indices (KI) of natural and synthetic compounds

COMPOUND	RETENTION INDEX (KI)	
	NON-POLAR CPSIL5	POLAR CPWAX52
Pheromone component (A)	1272	1848
Pheromone component (B)	1272	1862
Pheromone component (C)	1144	1902
(*S*)-2-hydroxy-3-decanone (I)	1272	1848
3-hydroxy-2-decanone (II)	1272	1862
(*S*,*S*)-2,3-dihydroxyoctane (III)	1144	1902
(*S*,*R*)-2,3-dihydroxyoctane (IV)	1155	1943
(*S*,*S*)-2,3-diacetoxyoctane (VII)	1377	1823
(*S*,*R*)-2,3-acetoxyoctane (VIII)	1366	1779
(*S*)-2-hydroxy-3-octanone (V)	1069	1631
3-hydroxy-2-octanone	1069	1642

Synthesis of enantiomers of 2,3-dihydroxyoctane and chirality of pheromone component (C)

Several multistep syntheses of (*S*,*S*)-2,3-dihydroxyoctane (III) have been reported (Mori *et al.*, 1985; Bel-Rhlid *et al.*, 1989: Chattopadhyay *et al.*, 1990; Kang *et al.*, 1990; Bonini & Righi, 1992; Takahata *et al.*, 1994; Bonini *et al.*, 1995; Paolucci *et al.*, 1995), but a novel, single-step synthesis applicable to both enantiomers was developed for this work (Scheme 2). Reaction of commercially-available (*E*)-2-octene with the commercially-available Sharpless reagent "AD-mix-α" in tert-butyl alcohol in the presence of methanesulphonamide gave (*S*,*S*)-2,3-dihydroxyoctane (III) in 91% yield after chromatography and kugelrohr distillation. Similar reaction of (*E*)-2-octene with the "AD-mix-β" gave (*R*,*R*)-2,3-dihydroxyoctane (VI) in similar yield. Both compounds were completely free (≤ 0.2%) of *threo* diastereomers by GC analysis.



(III)

SCHEME 2. Synthesis of (*S*,*S*)-2,3-dihydroxyoctane (III)

The asymmetric dihydroxylation mixtures contain potassium osmate, potassium ferricyanide and potassium carbonate mixed with the chiral catalysts 1,4-bis(dihydroquinyl)phthalazine (AD-mix-α) or 1,4-bis(dihydroquinidyl)phthalazine (AD-mix-β) (Sharpless *et al.*, 1992).

The diols were characterised by their NMR spectra (^{1}H (270 MHz, $CDCl_3$): δ 3.58 (1H, quint, *J*=6.3, CH_3-C*H*OH), 3.48-3.46 (1H, br m, C*H*OH), 2.47-2.41 (2H, br m, 2 x OH), 1.50-1.22 (8H, m, 4 x CH_2), 1.18 (3H, d *J* 6.3, C*H*$_3$-CH(OH)-), 0.89 (3H, t *J*=6.6, C*H*$_3$-CH_2-); ^{13}C (67.5 MHz, $CDCl_3$) δ 76.2 and 70.8 (C-2 and C-3), 33.3, 31.8, 25.2, 22.6, 19.4, 14.0). These were identical with literature values, e.g. Mori *et al.*, (1985).

Analyses on a cyclodextrin GC column showed good separation of the two enantiomers at 135°C. The *S,S* enantiomer (III) had an enantiomeric excess (ee) of only 83% while that of the *R,R* enantiomer (VI) was 90%. The lower ee of (III) was thought to be due to the prolonged reaction time (24 h at 0°C and 17 h at room temperature) compared with that used in the second reaction (15 h at RT).

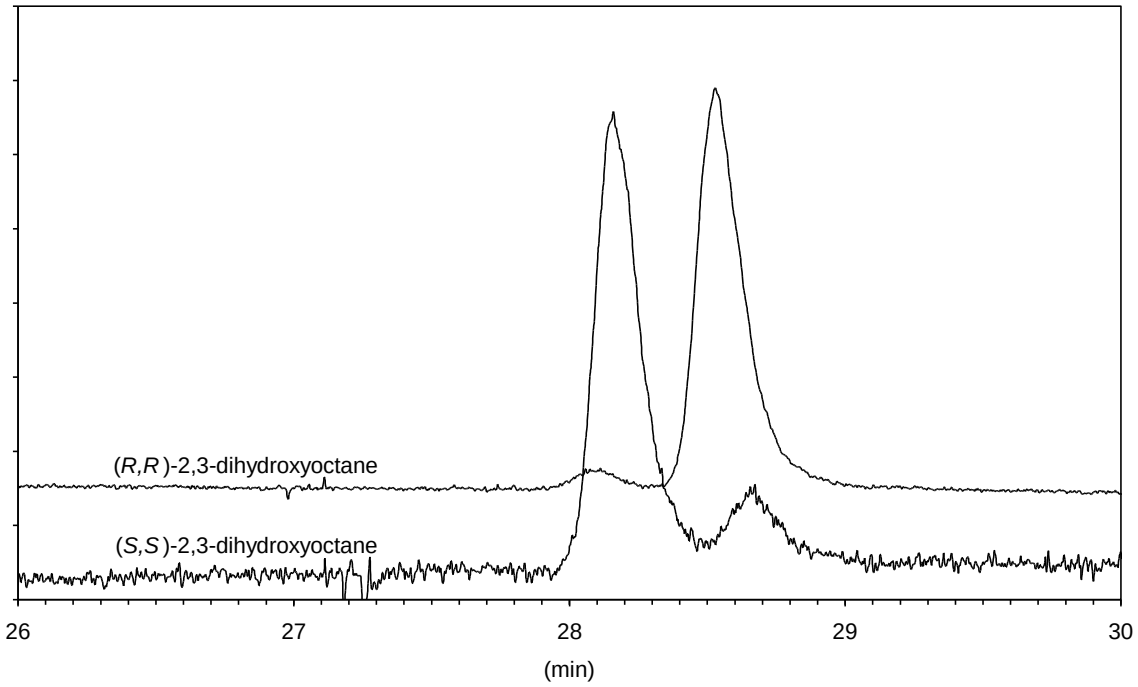


FIG. 3. GC analyses of (S,S)-2,3-dihydroxyoctane (III) and (R,R)-2,3-dihydroxyoctane (VI) on a cyclodextrin column.

Analysis of the volatiles collected from male *X. quadripes* on the cyclodextrin GC column showed a peak at the retention time of the (*S,S*)-2,3-dihydroxyoctane (III), and addition of the synthetic compound confirmed co-chromatography. No significant amounts of the diastereomeric diols were detected ((*S,R*)-2,3-dihydroxyoctane eluting at 31.11 min).

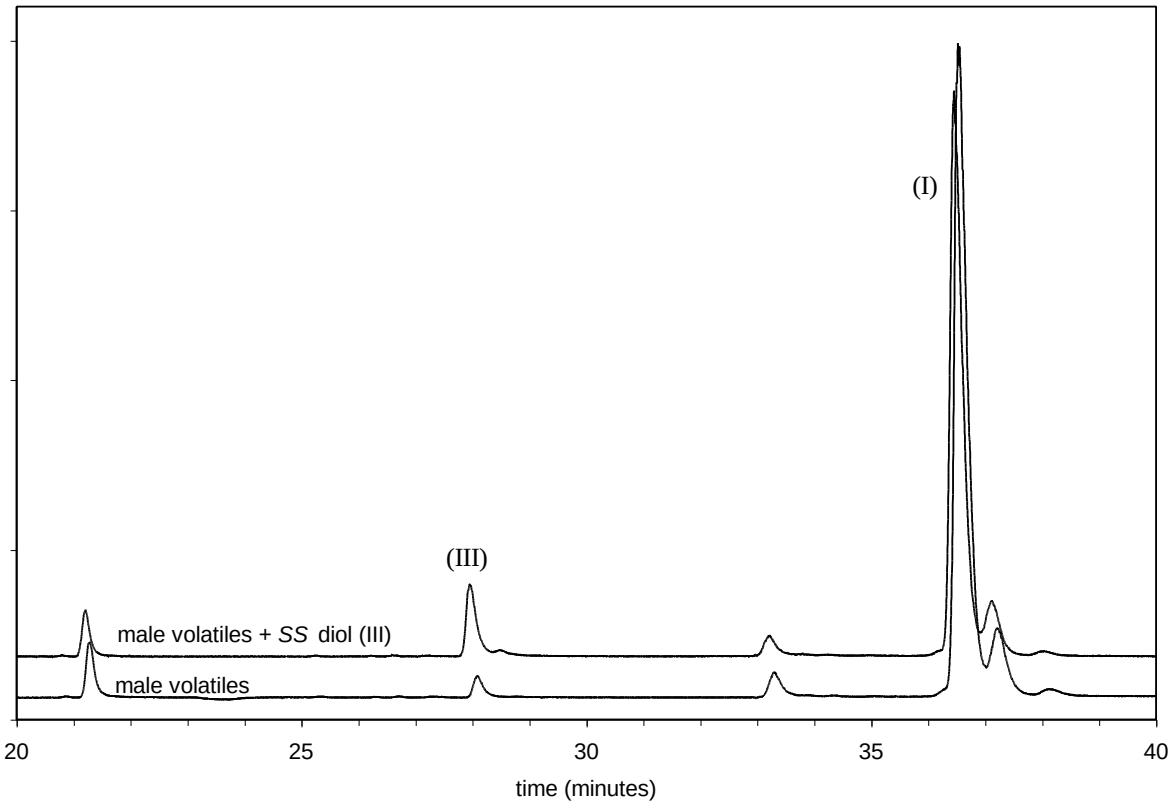


FIG. 4. GC analyses of volatiles from male *X. quadripes* on a cyclodextrin GC column, alone and co-injected with (*S,S*)-2,3-dihydroxyoctane (III).

Thus the minor EAG-active component (C) in volatiles collected from male *X. quadripes* is confirmed as (*S,S*)-2,3-dihydroxyoctane (III). The amount present assayed at 7.8% of the major component (I) on the non-polar GC column, 7.0% on a polar column and 2.6% on the cyclodextrin column.

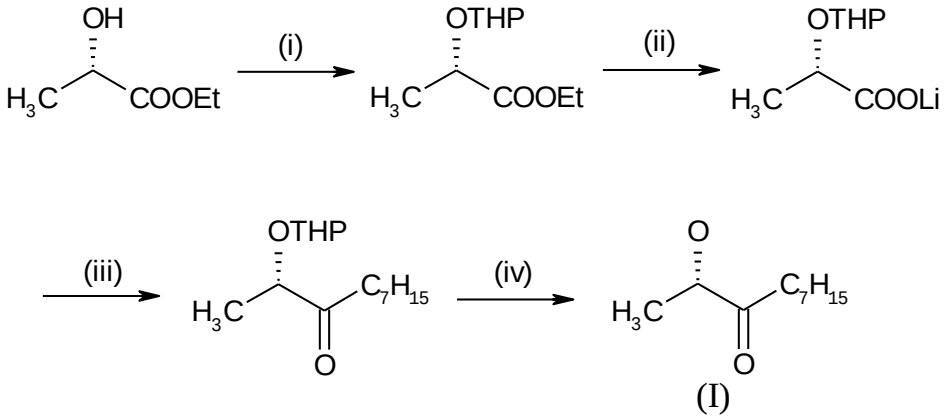
During this work, the corresponding 8-carbon analogue of the major pheromone component (I), i.e. (*S*)-2-hydroxy-3-octanone (V) was found to be present at 0.2-0.5% of (I) in the volatiles collected from male *X. quadripes*.

Training in pheromone synthesis

During a two-week visit to NRI in March 1999, Dr Jayarama was trained in synthesis of the major pheromone component, (S)-2-hydroxy-3-decanone (I). During this visit, the route (Scheme 3) was further scaled up and optimised:

- the route was easily scaled up to 0.25M using appropriate apparatus and procedures;
- distillation of the the intermediate 2-(2'-tetrahydopyranyloxy)-3-decanone (bp 100°C/0.07 mBar) gave improved separation from minor by-products, particularly tetradecane;

- the final product (I) can be distilled (60°C/0.04 mBar) but suffers significant decomposition and it is thus preferable to distill the precursor and not the final product.



SCHEME 3. Synthesis of (*S*)-2-hydroxy-3-decanone (I): (i) 2,3-dihydropyran/*p*TSA (100%); (ii) LiOH.H_2O/EtOH (91%); (iii) C_7H_{15}Li/ether (74%); (iv) MeOH/PPTSA

Laboratory bioassays

In previous bioassay work carried out by Dr Venkatesh of the CCRI while working at NRI during March 1998, swastika and tube bioassays were used to demonstrate:

- attraction of female *X. quadripes* beetles to male but not to female beetles;
- no attraction of male beetles to either male or female beetles;
- attraction of female beetles to synthetic (*S*)-2-hydroxy-3-decanone (I);
- greatly reduced attrction of female beetles to a blend of (I) with 10% of the 40:60 mixture of (*S*,*S*)-2,3-dihydroxyoctane (III) and (*S*,*R*)-2,3-dihydroxyoctane (IV).

Bioassay work was repeated during 10 days in November 1998 at CCRI by Drs Hall and Jayarama and during a subsequent 7 days in December 1998 at NRI by Drs Hall and Phythian. No significant responses were obtained from male or female *X. quadripes* beetles to live insects of either sex or a range of blends of synthetic pheromone components.

At NRI exactly the same apparatus and insectary conditions were used as used by Dr Venkatesh. At CCRI, similar apparatus was used under a range of conditions using natural or artificial sunlight at various times throughout the day.

The reasons for these failures are unknown. Unfortunately Dr Venkatesh had left CCRI to take up a position at the University of Bangalore, and it was not possible to contact him during the experiments. When subsequently contacted, he suggested that the beetles used were not virgin. However, every effort was made by insectary staff at CCRI to collect the beetles as soon after emergence as possible under essentially the same conditions as used

previously by Dr Venkatesh, and, even if a few had mated, it is considered unlikely that all would have done so.

It has also been suggested that the beetles show differenr responses during the two flight seasons, i.e. during March-June when Dr Venkatesh' experiments were carried out, and September-December when the subsequent experiments were carried out. This is to be checked by Dr Jayarama during April-June 1999, but results are not yet available.

Field trials

Field trials were carried out by Dr Jayarama during April-May 1998 to compare catches in traps baited with the major pheromone component, (*S*)-2-hydroxy-3-decanone (I), with those in unbaited traps. Results from sites in and around the CCRI farm are shown in Table 2.

TABLE 2. Catches of Cerambycid beetles in sticky crossvane traps, April-May 1998 (4 replicates at each site)

LOCATION	*X. quadripes*		*Demonax*	OTHER
TREATMENT	FEMALE	MALE	*balyi*	CERAMBYCIDS
CCRI Farm				
(I)	12	3	27	3
unbaited	2	5	1	3
Nethrakonda-Gundikhan				
(I)	5	0	37	3
unbaited	0	1	2	1
Nethrakonda Estate				
(I)	1	1	56	
unbaited	0	0	4	
TOTAL (12 reps, 2 months)				
(I)	18	3	120	6
unbaited	2	6	7	4

Catches of female *X. quadripes* were higher in the baited traps than in the unbaited, although catches were low (18 in 720 trap days). Catches of male *X. quadripes* were low and actually higher in the unbaited traps than the baited. Larger numbers of females of another Cerambycid were caught at all sites, and these were identified by the CABI Identification Services as *Demonax balyi* Pascoe.

Trials later in 1998 were set up to investigate the effect of adding 10% of the minor pheromone component, (*S,S*)-2,3-dihydroxyoctane (III) to the major component (I). Experiments were set up at ten sites, and results from four sites in Karnataka and two in Tamil Nadu are shown in Table 3. Catches were very low, but more female beetles were caught in traps baited with the major pheromone component (I) alone than in traps baited with the blend or unbaited traps, and this difference was actually significant from all the other

catches at the 1% level after transformation of the mean total catches to log(x+1) and analysis of variance. Results were not received from three of the other sites and no beetles were caught at a fourth.

TABLE 3. Catches of *X. quadripes* beetles in sticky cross-vane traps at five sites, October 1998 - January 1999 (S = (*S*)-2-hydroxy-3-decanone (I); S+SS = (I) + 10% (*S,S*)-2,3-dihydroxy-octane (III); C = unbaited)

LOCATION	DATES treatment (no. traps)	DAYS	FEMALE			MALE		
			S (4)	S+SS (2)	C (2)	S (4)	S+SS (2)	C (2)
CCRI Chikmagulur	30/10/98-5/1/99	66	5	0	0	0	0	0
Hireguda Estate	29/10/98-31/12/98	62	2	0	0	4	1	0
CRSS Chettalli	10/11/98-19/1/99	70	10	1	1	1	0	0
Hemevathi Estate	1/11/98-31/12/98	61	3	0	0	0	0	1
Pillavali Estate, Tamil Nadu	1/12/98-29/1/99	60	2	2	0	0	0	0
CDF, Perumparai, Tamil Nadu	1/12/98-29/1/99	60	14	3	0	0	0	0
TOTAL			36	6	1	5	1	0
CATCH/TRAP/SITE			1.50	0.50	0.08	0.21	0.08	0.08
S.E			0.50	0.26	0.08	0.16	0.08	0.08

Further trials were set up at three sites to test the effect of adding 10% of either the (*S,S*)-2,3-dihydroxyoctane (III) or (*R,R*)-2,3-dihydroxyoctane (VI) to the major pheromone component (I). Results are shown in Table 4. Catches of *X. quadripes* beetles were even lower than in the other experiments, and, although more beetles were caught in baited traps than in unbaited and these were almost all females, there was no indication of any differences in attractiveness between the different blends.

TABLE 4. Catches of *X. quadripes* beetles in sticky cross-vane traps at three sites, December 1998 - January 1999 ((S = (*S*)-2-hydroxy-3-decanone (I); S+SS = (I) + 10% (*S,S*)-2,3-dihydroxy-octane (III); S+RR = (I) + 10% (*R,R*)-2,3-dihydroxy-octane (VI); C = unbaited)

LOCATION	DATES	DAYS	REPS	FEMALES (MALES)			
				S	S+SS	S+RR	C
CCRI, Chikmagulur	9/12/98-5/1/99	27	2	0	1	0	0
Gundikan Estate	4/12/98-5/1/99	28	4	3	1	0	0
BBTC, Sidhapura	1/12/99-10/2/99	71	2	0	2	3(1)	0
TOTAL				3	4	3	0

Investigation of the potential for use of pheromones in management of African coffee stemborers.

Liaison with project "Pesticide and microbial interactions in coffee pest management" (R6807)

Discussions were held with Dr Rory Hillocks (NRI), Dr Sarah Simons (CABI, Nairobi) and Dr Bernie Briscoe (CABI, Egham) and information exchanged. As mentioned in the Background, results from project surveys indicate that stemborers are perceived to be the most important of all pests and diseases by smallholders in Malawi and that they are a serious source of loss of productivity with currently no effective methods of control that smallholders can use. The main species is the white stemborer, *M. leuconotus*, with the yellow stemborer, *Dirphya nigricornis*, as minor species. Both are Cerambycid beetles, likely to have similar pheromone systems to that of *X. quadripes*.

Visit to Zimbabwe

During a visit to Zimbabwe by Dr Hall, contact was made with the entomologist and Officer-in-Charge of the Coffee Research Station of the Department of Research and Specialist Services, Mr Dumisani Kutyawayo.

He confirmed that coffee is grown as both smallholder and estate crop in th e Eastern Highlands of Zimbabwe, and *M. leuconotus* is the major pest problem. Current control methods rely on painting a band of insecticide 1.5 m high on the trunk of the bush. Dieldrin is the most effective, but, as this is now banned, chlorpyrifos and fipronil have been tested. Mr Kutyawayo was very enthusiastic about developing a pheromone for this pest, and also mentioned that it would be a good subject for his PhD studies. He thought it would have value as a monitoring tool since application of insecticide is labour-intensive and somewhat hazardous, and anything that made it more effective and minimised its use would be valuable. Assuming that this species is similar to that in India, *X. quadripes*, where the male produces a pheromone that attracts female beetles, then prospects for mass trapping would be good.

The current state of work on the pheromone of *X. quadripes* was discussed and suggestions made for laboratory and field work to determine whether pheromones are involved in mating behaviour of *M. leuconotus*.

Bioassays with the African coffee white stemborer, Monochamus leuconotus

Following Dr Hall's visit, laboratory bioassays for pheromone-mediated mating behaviour in *M. leuconotus* were carried out in Zimbabwe by Mr Kutyawayo. Using field-collected insects, he showed that females moved upwind towards males (Table 5) and also that males moved upwind towards females (Table 6). In view of the uncertain mated status of the insects and the absence of control experiments to check whether the beetles moved upwind in the absence of any possible pheromone source, these results must be considered very preliminary. One interesting feature of these experiments is that response times were quite long. These experiments were run for 24 hours, while bioassay experiments with *X. quadripes* were generally terminated after 30 minutes.

TABLE 5. Responses of female *M. leuconotus* to male beetles (data from D Kutyawayo, Zimbabwe, March 1999).

	TIME (MINUTES)		
REPLICATE	EMERGENCE	ARRIVAL	MATING
1	-	-	-
2	27	131	420
3	123	125	-
4	31	33	-
5	-	-	-
6	85	88	135
7	-	-	-
8	347	349	-
9	4	15	18
10	130	132	-

TABLE 6. Responses of male *M. leuconotus* to female beetles (data from D Kutyawayo, Zimbabwe, March 1999).

	TIME (MINUTES)		
REPLICATE	EMERGENCE	ARRIVAL	MATING
1	13	15	-
2	24	27	42
3	22	29	33
4	100	101	106
5	13	22	-

CONTRIBUTION OF OUTPUTS

Progress to Purpose

Completion of identification of male sex pheromone of X. quadripes

In the previous project, the major component of the male sex pheromone of the coffee white stemborer, *X. quadripes* was identified as (*S*)-2-hydroxy-3-decanone (I). Both natural and synthetic compounds were accompanied by 5-10% of the isomeric 3-hydroxy-2-decanone (II), although it is not certain whether this is an essential component of the pheromone. A synthetic route to compound (I) was developed and the synthetic compound shown to attract female *X. quadripes* beetles in laboratory bioassays and trapping tests in the field. Polyethylene vials were shown to be suitable slow release dispensers for the synthetic pheromone with a field life of several months, and commercially-available, sticky cross-vane trps were found to be effective for this pest.

In this project, the third compound in volatiles from male *X. quadripes* which elicits an EAG response from female *X. quadripes* beetles was identified as (*S,S*)-2,3-dihydroxyoctane (III). Although the major component (I) is a novel pheromone component, related 6- and 8-carbon compounds have been reported in the pheromones of other Cerambycidae (Sakai *et al.*, 1984; Kuwahara *et al.*, 1987; Iwabuchi *et al.*, 1987; Leal *et al.*, 1995; Fettköther *et al.*, 1995), and compound (III) is a pheromone component produced by male *X. pyrrhoderus* (Sakai *et al.*, 1984) and male *X. chinensis* (Kuwahara *et al.*, 1987).

Several syntheses of compound (III) have been reported, but in this project a novel, one-step synthesis from commercially-available starting materials was developed which is applicable to both enantiomers. Asymmetric dihydroxylation of (*E*)-2-octene with "AD-mix-α" or "AD-mix-β" (Sharpless *et al.*, 1992) gave (*S,S*)-2,3-dihydroxyoctane (III) or (*R,R*)-2,3-dihydroxyoctane respectively in >90% yield and high enantiomeric excess.

During this work, the 8-carbon analogue of the major pheromone component (I), i.e. (*S*)-2-hydroxy-3-octanone (V) was also detected in volatiles produced by male *X. quadripes* at 0.2-0.5% of the major component (I).

Bioassay and field testing of synthetic pheromone components

Although effective bioassay methods were apparently developed in the previous project, it was subsequently impossible to reproduce the results obtained in India or at NRI using exactly the same apparatus and seemingly identical conditions. Previous work was done by Dr Venkatesh, but he has now left CCRI and was not able to assist in this work. Reasons for these failures are unknown. It is conceivable that experimental beetles were already mated, but every care was taken to ensure they were virgin, and it is considered highly unlikely that all were mated even if a few were. Furthermore, Visitpanich (1994) reported that both male and female *X. quadripes* mated more than once. It was also suggested that there is a seasonal difference in behavioural responses: bioassay experiments were unsuccessful in November 1997, successful during April-June 1998 and unsuccessful again during the September-December 1998 flight. This possibility will be checked by Dr Jayarama at CCRI during the April-June 1999 flight.

It was thus not possible to check the effect of adding the minor pheromone component (III) to the major component in the laboratory bioassay. Field tests were carried out at 13 locations in southern India during October 1998 - January 1999. Catches of *X. quadripes* beetles were low in all cases, but the results obtained suggested that addition of minor component (III) at 10% of the major component (I) did not increase catches. In the one experiment where catches were sufficient to do a statistical analysis, more beetles were caught in traps baited with major component (I) alone than with any other blends. This would suggest addition of the new pheromone component (III) reduces attractiveness of (I), in agreement with bioassay results obtained in the previous project.

These and experiments carried out during April-May 1998 also confirmed the attractiveness of major pheromone component (I) to female *X. quadripes* beetles, and it is recommended that this compound alone is used in future trials.

Attraction of females of another Cerambycid beetle, *Demonax balyi*, to the synthetic pheromone was observed in these experiments.

Initiation of trials of mass trapping for control of X. quadripes

A convenient synthetic route to compound (I) was developed in the previous project. In this project, Dr Jayarama was trained at NRI to carry out this synthesis, and the procedure was further optimised and scaled up. With this knowledge, Dr Jayarama will be able to approach organisations in India to carry out large-scale synthesis and also be able to quality control their work. NRI has good contacts with Dr Narasimhan of the SPIC Centre for Agricultural Research in Madras, and he will be asked to quote for production of the pheromone.

Dr Jayarama is also investigating local production of the cross-vane traps and supply of the polybutene sticky material.

Sufficient material was synthesised during Dr Jayarama's visit to enable him to initiate field trials of mass trapping as a control measure for *X. quadripes*. At two sites, 10-acre blocks will be treated with 40, 20, 10 or 0 traps, using. Trap catches will be recorded weekly and stemborer damage estimated by standard visual observation procedures.

Investigation of the potential for pheromones in management of African coffee stemborer species

Some progress was made towards assessing the potential for pheromones for management of coffee stemborers in Africa. The African coffee white stemborer, *M. leuconotus*, is perceived as the major pest in Malawi and Zimbabwe, and surveys carried out under the CPP project "Pesticide and microbial interactions in coffee pest management" (R6807) have confirmed high levels of infestation in Malawi. Withdrawal of subsidies and credit facilities for coffee growers in Malawi during the last four years have meant smallholders have essentially abandoned any attempts to control stemborers more sophisticated than poking wires down boreholes in the stem - which are probably exit holes and unlikely to contain stemborer larvae. It is thus unlikely that smallholders would be able to take up any pheromone technology at present even if it was available. However, with the establishment of a Coffee Cooperative Union to replace the Smallholder Coffee Authority, improvements in yields will require reintroduction of credit schemes for input purchase.

In Zimbabwe, insecticidal control is still used against stemborers, and this could be replaced by pheromone-based control if available and cost-effective. Mr Kutyawayo, entomologist and Officer-in-Charge of the Coffee Research Station of the Zimbabwe Department of Research and Specialist Services, Chipinge, was very enthusiastic about the possibilities for pheromones. He thought they could have value even as a monitoring tool since application of insecticide is labour-intensive and hazardous, and anything that made it more effective and minimised its use would be valuable. He has already carried out some preliminary bioassay work to investigate whether *M. leuconotus* uses pheromones in mating.

Promotion pathways

This work was requested by and carried out in close collaboration with staff of the Central Coffee Research Institute (CCRI) of the Coffee Board of India which will be the main promotion pathway. Drs Jayarama and Venkatesha of the CCRI spent time at NRI playing major parts in the identification and bioassay work respectively, and Dr Jayarama was trained in pheromone synthesis at NRI. Drs Cork and Hall of NRI assisted in field work at CCRI and the Coffee Research Sub-Station, Chettalli.

Dr Naidu, Director of Research at CCRI, is initiating an Asian Coffee Network with representatives in Papua New Guinea, Vietnam, Thailand, Australia, Vanuatu, Honolulu, Singapore and China as well as India. This network will serve as a promotion pathway for the pheromone work in the region, and it is planned to use the network to apply for funding for a major IPM project in the region.

Further work

Further work is required to:

- localise production of pheromone and traps for *X. quadripes* in India;
- establish whether pheromone traps provide effective and cost-effective control of *X. quadripes*;

- establish whether a similar pheromone system exists in the African stemborer, *M. leuconotus* and characterise this.

The CCRI has Government of India funding for work on *X. quadripes* until 2001, and Dr Jayarama will be following up localisation of the pheromone technology and carrying out field trials of mass trapping against *X. quadripes*. He has requested continued NRI involvement with this work.

The current phase of the project "Pesticide and microbial interactions in coffee pest management" (R6807) ends in September 1999, and it is proposed to bid for CPP funds for a second phase. Discussions are in progress as to whether to include investigation of the pheromone of *M. leuconotus* in this second phase. Continued advisory inputs for the work on *X. quadripes* in India might also be included in such a project, with the possibility of also transferring some of the outputs on control of coffee pests and diseases in Africa to India and Asia.

Dissemination

A paper on identification of the pheromone of *X. quadripes* is being drafted.

Dr Hall attended the National Coffee Conference organised by the Coffee Board of India and the National Bank for Agriculture and Rural Development in Bangalore, 12-13 December 1998. This was attended by over 200 planters, extension workers, accountants, marketing people, scientists, etc. The pheromone work and collaboration with NRI was mentioned in at least five of the presentations, including the inaugural address by the Chairman of the Coffee Board.

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