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1,8-CINEOLE: AN ATTRACTANT FOR THE BANANA WEEVIL, *COSMOPOLITES SORDIDUS*

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Key Word Index—*Musa*; Musaceae; banana; 1,8-cineole; eucalyptol; monoterpenoids; electrophysiological activity; attractant; kairomone; banana weevil; *Cosmopolites sordidus*.

Abstract—1,8-Cineole was identified as one of the electrophysiologically active components of the volatiles from banana cultivars susceptible to the banana weevil. It was also shown to be an attractant for the banana weevil *Comopolites sordidus* in laboratory behavioural bioassays. The resistant cultivar did not contain 1,8-cineole. β-Phellandrene, which exhibited electrophysiological activity, but did not show any attraction to the banana weevil, was found only in the resistant banana cultivar.

INTRODUCTION

The banana weevil, *Cosmopolites sordidus*, (Coleoptera; Curculionidae), is a widely distributed pest of all banana cultivars found in all the banana growing regions of the world [1]. The decrease in banana yields in East Africa has been attributed partly to the effects of this pest [2] as no effective control or monitoring strategy has been found. In our search for an effective control, we have focused on the semiochemical based approach to understand the interaction between this pest and the host plant. This approach has produced tangible results in population monitoring and control of bark beetles.

Although there are reports [3] on the attraction of this insect by both the banana tissue and ethanol, we could not reproduce the attraction by ethanol. We recently reported [4] the composition of air-borne volatiles from the pseudostem of a susceptible cultivar and further demonstrated [5] that the natural blend was attractive to the banana weevil. However, the artificial mixture of the major components (mono- and sesquiterpenes) was not at all attractive. This observation suggested that the active components of the natural blend could be minor components which were missed out during the preparation of the artificial mixture. Critical examination of the natural volatile mixtures revealed that there are 21 minor but electrophysiologically active components in the six susceptible and tolerant cultivars. Most of these compounds were also found in the resistant cultivar. Of particular interest were two compounds: one was present in all the susceptible and tolerant cultivars but absent from the resistant cultivar while the other was present only in the resistant cultivar but conspicuously absent in susceptible and tolerant cultivars. We report on the electrophysiological activity, behavioural bioassay and the identification of these two compounds.

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RESULTS AND DISCUSSION

GC-electroantennography detector (EAD) analysis of the volatiles trapped from the pseudostem of one of the susceptible cultivars, known locally as githumo (AAA-EA) in Kikuyu, using a methylsilicone capillary column revealed many minor and one major electrophysiologically active components. GC-mass spectral analysis of the major component revealed m/z 154, 136 and 121 as the highest mass units for this compound. The presence of m/z 154 and 136, a loss of 18 mass units, in the mass spectrum of the EAG-active compound initially suggested the presence of a terpene alcohol. However, several terpene alcohols such as linalool, geraniol, *cis*- and *trans*-sabinene hydrate, thujyl alcohol, 4-terpeneol, α-terpeneol, β-terpeneol, γ-terpeneol, *p*-menthols, dihydrocarveols, and many others showed higher GC retention times and no EAG-activity. Fortunately, we discovered that the EAG-active compound co-eluted with another EAG-inactive monoterpene hydrocarbon, limonene, which is one of the major components [4] of the banana pseudostem volatiles. The presence of m/z 154 indicated that the compound could be an oxygenated monoterpene. The major components (hydrocarbon monoterpenes) were separated from the minor components (oxygenated monoterpenes) by fractionation of the pseudostem volatiles using Florisil (60–100 mesh).

Analysis of the fractions by GC revealed that the major components (mono- and sesquiterpene hydro-

carbons) were eluted together first and the minor components (oxygenated terpenes) later. GC-mass spectrometry of the combined active fractions revealed that the active compound was actually present as a minor component of the volatile mixture but was swamped by limonene, as revealed in the mass spectrum. Absence of m/z 136 ruled out a terpene alcohol. A cyclic terpene ether was strongly suspected. A computer-assisted mass-spectral data comparison with documented spectra suggested 1,8-cineole. Comparison of the GC retention times and mass spectral data of the authentic sample confirmed that 1,8-cineole was the compound in question. GC-EAD, GC-mass spectrometry and mass spectrometry of the authentic compound and finally behavioural bioassay (Table 1) confirmed that 1,8-cineole is electrophysiologically active and also attractive to the banana weevil. We later found out that 1,8-cineole and limonene in the banana volatiles could be resolved on carbowax 20 M capillary column, as supported by GC-mass spectral and GC-EAD analysis.

GC-EAD analysis of volatiles from the pseudostems of seven other banana cultivars, githumo (AAA-EA), mitahato (AAA-EA), nyoro (AAA) which is also known as dwarf cavendish, muraru (AA), njuru (AA), wangae (AB) which is also known as Ney poovan in India and mbuu (ABB) which is also known as pisang awak in Malaysia, revealed a total of 22 EAG-active peaks. Of these, 14 compounds showed weak EAG-responses in all the cultivars in which they were detected. The remaining eight compounds showed strong responses in at least one of the cultivars in which they were detected. Of these eight compounds that evoked strong EAGs, six were found to be universally present in all the cultivars. 1,8-Cineole was found to be present in all the susceptible and tolerant cultivars but was absent in the resistant cultivar, mbuu (ABB). Another compound was found to be exclusively present in the resistant cultivar mbuu (ABB) but universally absent in all the susceptible and tolerant cultivars. This compound gave a mass spectral pattern that was indicative of a monoterpene hydrocarbon, although a monoterpene alcohol could not be ruled out. The presence of m/z 136 and the absence of m/z 139 ruled out the possibility of a cyclic terpene ether. A monoterpene hydrocarbon was strongly suspected. The presence of m/z 93 $[M-43]^+$ as the base peak in the mass spectrum of this compound suggested a monoterpene hydrocarbon structure in which a propyl or isopropyl radical can be easily lost during fragmentation and possibly present as a substituent. This suggested structures such as the terpinenes or phellandrenes. Indeed, computer-assisted mass spectral match suggested α-, β- and γ-terpinenes plus α- and β-phellandrene. The terpinenes and α-phellandrene, gave different GC retention times from that of the unknown. GC-EAD experiment with black pepper (*Piper nigrum*) volatiles, in which β-phellandrene is known to occur [6] revealed an EAG response at exactly the same GC retention time as in the resistant banana (mbuu) pseudostem volatiles. GC-mass spectral analysis of this compound in the black pepper volatiles gave the same mass spectrum as that of the EAD-active peak in the volatiles from the resistant banana cultivar. Confirmation of the structure was done by comparison of GC retention time and mass spectral data with that of the authentic β-phellandrene. Although β-phellandrene showed electrophysiological activity, behavioural bioassays revealed no attraction for the banana weevil.

Table 1. Responses of female *Cosmopolites sordidus* to different doses of 1,8-cineole

Dose (mg)	N	T	C	T/C	(T-C)/N
0	18	7	9	0.78	−0.11±0.20
10^{-3}	18	18	15	1.20	0.17±0.39
10^{-2}	18	19	8	2.38	0.61±0.23*
10^{-1}	18	13	9	1.44	0.22±0.33
1	18	32	6	5.33	1.44±0.33***
10	18	34	3	11.33	1.72±0.41***

N = Replicates; T = visits to treatment; and C = visits to control.

*$P<0.05$, ***$P<0.001$: probability that the number of extra visits to the treatment is different from zero.

Higher plant terpenoids have been reported to play many ecological roles. In particular, 1,8-cineole has been reported as playing a role in direct plant defence against herbivores and pathogens, allelopathy, formation of reactive gases in the troposphere and in pollination [7]. β-Phellandrene has also been reported [8] as a kairomone for two scolytid beetles *Ips latidens* and *I. pini*. Many other scolytid beetles, have been reported [9] to use terpenoids as kairomones, kairomone synergists and pheromone synergists. The phenomenon has also been reported [10] for curculionid beetles such as *Hylobius pales*, *H. abietis*, *Anthonomus grandis*, *Pissodes strobi* and *Cyclas formicarius elegantus*. Similar attraction by terpenoids has also been noted in the cerambycid beetle *Monochamus alternatus* [11]. In addition to acting as kairomones, monoterpenes play other ecological roles. The curcuniolid beetle *Pissodes strobi* [12] uses a variety of terpenoids as synergists for nonvolatile feeding stimulants and feeding deterrents. In the pine beauty moth, *Panolis flammea*, α- and β-pinene mixture has been found to stimulate oviposition [13].

EXPERIMENTAL

Plant material and collection of volatiles. Pseudostems of githumo, mitahato, nyoro, muraru, njuru, wangae and mbuu were collected immediately after the harvest of the banana bunches from Mika farm in Garden Estate, Nairobi. Volatiles were collected from the headspace of a 5-l flask filled with 2 kg of chopped pseudostem pieces. Air was drawn from the flask at 40 ml min^{-1} for 24 h via an inlet of activated charcoal, over the pseudostem pieces and through and outlet of an activated charcoal trap using a vacuum pump. The outlet trap was eluted with 4 ml of CH_2Cl_2 and the eluant reduced to 2 ml under a single stream of N_2 (white spot). The eluant was used for GC, GC-MS,

behavioural bioassay, electroantennography (EAG), and GC-EAD.

Insects. The banana weevil, *C. sordidus*, used for behavioural bioassays and electroantennogram experiments were collected from split pseudostem traps in a banana plantation at Mika farm in Garden Estate, Nairobi, Kenya. The weevils were sexed and the sexes kept separately on 0–10-day old githumo pseudostem in 4.5-l plastic jars in batches of 120 individuals. The plastic jars with the weevils were kept at 24°, 12 h light/12 hr dark with the scotophase starting at 09.00 hours. Before use in behavioural bioassay, the weevils were removed from the pseudostem and put in groups of five individuals in small tubes with damp tissue paper.

Behavioural bioassays were run between 10.30 and 16.30 hours after which the weevils were returned to their holding jars. The same weevils were never used for bioassays on two consecutive days. Weevils used in electrophysiology were removed from the pseudostem and kept with moist tissue paper overnight to ensure that the sensillae on the antenna were not covered by any deposit material.

Fractionation of githumo volatiles. Six lots of volatile collections from githumo pseudostem were combined and reduced to 0.5 ml, under a single stream of N_2 (white spot) while being cooled in ice/H_2O. The reduced volatile solution was loaded onto a florisil column (60–100 mesh, 15 × 3 cm) and the column eluted with 30 ml of hexane followed by 20 ml each of 5, 10, 20, 30, 40, 50, 60, 70, 80 and 90% CH_2Cl_2 in hexane. The column was finally washed with 20 ml CH_2Cl_2. Frs of 5 ml each were collected, reduced to 0.2 ml and analysed by EAG and GC.

Analysis of volatiles

Gas chromatography. GC analysis was carried out on a HP 5890A using ultra 1 crosslinked methylsilicone (50 m × 0.32 mm × 0.32 μm) or carbowax 20 M (50 m × 0.21 mm × 0.17 μm) capillary column with a FID and N_2 as the carrier gas. The GC oven temp. was programmed from 40° for 5 min to 270° for 10 min at 5° min^{-1} for the methylsilicone capillary column. For the carbowax column, the GC oven temperature was programmed from 60° for 5 min to 220° for 20 min at 5° min^{-1} or from 60° for 2 min to 220° for 20 min at 10° min^{-1}.

GC-MS. GC-MS analysis was carried out on a HP 5790A GC (with a carbowax 20 M capillary column) linked to a VG Mass Lab. 12–1250 mass spectrometer detector. Oven temp. was programmed from 60° for 2 min to 220° for 20 min at 10° min^{-1}. For the preliminary identification of the compounds from MS data a computer-assisted search of the NBS library of mass spectra was performed. To confirm the identity of the compounds their MS data and GC R_t values were compared with those of the authentic samples.

EAG and behavioural bioassay: These were performed according to the procedures of ref. [5].

GC-EA. GC-EA analysis was done using a carbowax 20 M capillary column (50 m × 0.21 mm × 0.17 μm) in a HP 5890 GC linked to an electroantennogram detector supplied by Syntech. The effluent from the GC column was split into two (1:1); one flowed to the FID and the other to the EAD, arranged as described above. The reference stimulus was applied periodically during the run to test the sensitivity of the electroantennogram detector with time. GC oven temp. was programmed from 60° for 2 min to 220° for 20 min at 10°/min^{-1}.

Statistical analysis. For each run of bioassay, the number excess visits to treatment was calculated (number of visits to treatment hole, less number of visits to control hole). These were then tested using a non-parametric signed-rank statistic to determine whether they were different from zero (the expected number if there were no visits to the treatment). Comparisons were made between number of excess visits by males and females to treatments. In all cases differences were deemed significant at $P < 0.05$.

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