Journal of Chemical Ecology, Vol. 19, No. 4, 1993

ACCUMULATION OF *Dendrobium superbum* (ORCHIDACEAE) FRAGRANCE IN THE RECTAL GLANDS BY MALES OF THE MELON FLY, *Dacus cucurbitae*

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(Received August 10, 1992; accepted November 23, 1992)

Abstract—4-(4-Hydroxyphenyl)-2-butanone was characterized from flowers of the orchid *Dendrobium superbum* as a specific attractant factor for the male melon fly, *Dacus cucurbitae*. The male flies compulsively licked the flower surface and sequestered the compound in significant quantities in their rectal glands. The compound was detected within 6 hr after ingestion and was retained for more than six days in the rectal gland sacs.

Key Words—*Dacus cucurbitae*, melon fly, Diptera, *Dendrobium superbum*, orchid, 4-(4-hydroxyphenyl)-2-butanone, cue-lure, sequestration, pheromone.

INTRODUCTION

Males of the melon fly, *Dacus (Bactrocera) cucurbitae* Coquillett, show strong affinity to the blossoms of the orchid, *Dendrobium superbum* Rchb. f. (synonym, *D. anosmum* Lindl.) (Flath and Ohinata, 1982; Ichinohe et al., 1983) (Figure 1). Flath and Ohinata (1982) identified several volatile components including benzylacetone (4-phenyl-2-butanone) by a headspace collection of *D. superbum*

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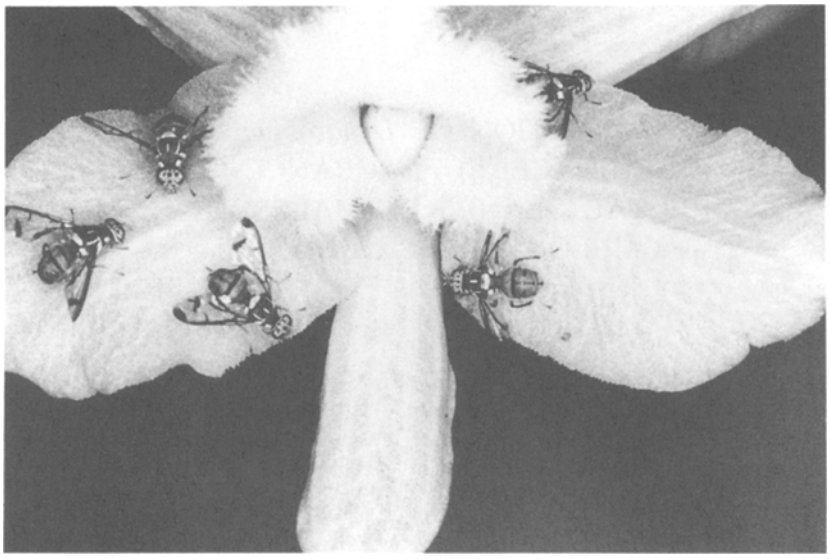

FIG. 1. Males of the melon fly, *Dacus cucurbitae*, congregating and feeding on a *Dendrobium superbum* flower.

flowers. According to our behavioral bioassay, however, the activity of the major attractant chemical in *D. superbum* flowers was more potent than benzylacetone and exhibited a more polar nature on the silica gel chromatography. We have reexamined the attractant chemicals contained in the flowers of *D. superbum*.

Males of *D. cucurbitae* possess a rectal gland complex (Schultz and Boush, 1971) and produce a smokelike substance from the gland during courtship (Kobayashi et al., 1978; Ohinata et al., 1982, Kuba and Sokei, 1988). Alkyl amides, nonan-1,3-diol, 4-hydroxybenzoic esters, and pyrazine derivatives have been identified as the major volatile components of the rectal glands (Baker et al., 1982; Nishida et al., 1990; Perkins et al., 1990). We will demonstrate here the selective accumulation of a *Dendrobium* flower fragrance in the rectal gland, suggesting a possible biological role of the compound in courtship behavior of *D. cucurbitae*.

METHODS AND MATERIALS

Insects. We used two types of melon fly strains, the mass-reared Okinawa strain (Hibino and Iwahashi, 1990) and the Malaysian strain (Nishida et al., 1990). Larvae were reared with an artificial diet (Okinawa strain) (Nakamori

and Kakinohana, 1980) or with cucurbit fruits (Malaysian strain), and adult flies were provided with water and a mixture of protein hydrolysate and sugar. The behavioral bioassay and orchid flower-feeding experiments were conducted with the Okinawa strain. The attractants sequestered in the body tissues were quantified with the Malaysian strain.

Instruments. The gas chromatography-mass spectroscopic (GC-MS) analyses were conducted with a Hitachi M-80 mass spectrometer (20 eV) connected to a GC column (24 m × 0.25 mm fused silica column coated with cross-linked-bonded methyl silicone HP-1, 0.25 μm thick) programmed from 80°C (approximately 2 min holding) to 210°C at a rate of 10°C/min. GC quantifications of volatile chemicals were done on an HP 5790A gas chromatograph with the same capillary column and under the same program conditions by comparing the FID intensities with those of the standard sample of known concentrations and a HP 3390A reporting integrator (Hewlett Packard).

Extraction of Orchid Fragrance. Blossoms of *Dendrobium superbum* were obtained from the cultures grown in Okinawa Island, southeast of Japan, in late April 1990. The flower petals were extracted with absolute ethanol (5 pairs of petals/10 ml). The petal extract, after removal of most of the ethanol in vacuo (20 mm Hg, 32°C), was dissolved in ether (10 ml × 2) and treated with saturated sodium chloride. The ether layer was dried over anhydrous sodium sulfate, and a portion of the concentrated extract was subjected to the GC-MS analysis. To compare the quantities of attractant compound **1** between three parts of the flower—a lip, a petal, and a calyx—each part was extracted with ethanol (10 flower units/50 ml). Portions (100 μl) of the ethanolic extract were concentrated in vacuo (20 mm Hg, 35°C), readjusted to 10 μl with methyl acetate, and a 1-μl portion was injected into the gas chromatograph.

TLC Plate Bioassay. A small portion (50 μl) of the flower petals was subjected to thin-layer chromatography (TLC) on a precoated plate (HPTLC silica gel 60 F_{254}, nano TLC, Merck) and developed with a mixture of benzene and ethyl acetate (4 : 1). The TLC plate was introduced into a small cage containing male flies (approximately 20 males per cage), and the licking behavior was observed for about 10 min.

Detection of Compound 1 from Rectal Gland. Males of *D. cucurbitae* were released in a cage containing a pot of *D. superbum* with flowers. They freely licked the flowers for several hours during the daytime. The feeding was conducted twice, on the 11th and 18th days after adult eclosion (DAE). The male rectal gland sacs were pulled out on the 20th DAE for extraction. Compound **1** in the rectal gland of individual males was quantified by GC analysis.

Quantification of Compound 1 in Body Tissues. Males of *D. cucurbitae* were allowed to feed on pure compound **1** (40 μg/insect offered as a 1-μl solution in 20% ethanol) for 20 min on 14th DAE and then given a normal diet. These males were dissected to remove the rectal gland complex at 6 hr, and one, three,

and six days after treatment. The rectal gland and the body (without rectal gland) of each fly were separately soaked in ethanol (0.25 ml/male). One-microliter portions of the ethanolic extracts after ultrasonication (5-10 min) were used directly for GC analysis. In order to quantify compounds in low concentration, 100-μl portions of the ethanolic solutions were carefully concentrated in vacuo (20 mm Hg, 25°C), readjusted to 10 μl with methyl acetate, and 1 μl was injected into the gas chromatograph.

RESULTS

Observation of Feeding Behavior. The cultivated *Dendrobium superbum* blooms once a year usually around late April to early May in Okinawa. The strong attraction of *D. cucurbitae* males to the blossom can be seen in the open field during this period (Ichinohe et al., 1983). Outdoor observation revealed that the males were attracted to blossoms mostly in the morning (e.g., 10:00-11:00 AM, April 28, 1990, in Okinawa), and they voraciously licked the flower surface. Females paid no attention to the flowers, although Ichinohe et al. (1983) reported that a small number of females were also attracted. The *Dendrobium* flower is composed of a center lip, a pair of petals, and a three-forked calyx. The licking behavior by the males was restricted to the petal area, and the flies seldom visited inside of the lips (Figure 1). Such compulsive feeding did not cause apparent injury to the flowers. The males were also strongly attracted to a piece of filter paper impregnated with an extract of the flowers and licked the filter paper as they did the intact flowers.

Identification of Attractant. The voracious licking behavior of the male flies was observed directly on a developed TLC plate containing the crude extract of the *D. superbum* petals (Figure 2). The males were quickly attracted and licked repeatedly at an R_f value of 0.45. The flies left a clear salivation mark on the plate. The attractant component at $R_f = 0.45$ on the TLC plate corresponded to the peak with a retention time of 11.8 min shown by an arrow in the total ion chromatogram (Figure 3, top). Its mass spectrum exhibited the molecular ion peak at m/z 164 and the base ion peak at m/z 107 (Figure 3, bottom). The compound was identified as 4-(4-hydroxyphenyl)-2-butanone **(1)** by comparison with an authentic sample (Tokyo-Kasei Chemical Industries Co., Ltd.). Petals were found to contain the largest quantity, although the lips and calyxes also contained compound **1** in significant quantities (petal: 60.4 $\pm$ 9.1 μg, lip: 15.4 $\pm$ 0.6 μg, calyx: 20.0 $\pm$ 4.0 μg/flower). Compound **1** was also detected from a variety of *D. superbum* grown in Malaysia (3 μg/whole flower).

Accumulation of Compound 1 in Rectal Glands. D. cucurbitae males incorporated ketone **1** in the rectal glands within a short period after feeding on *D.*

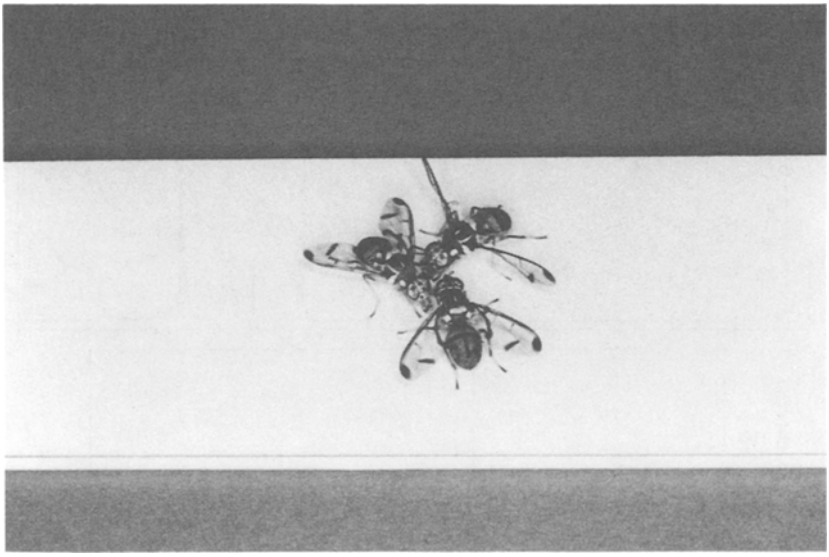

FIG. 2. Males of *Dacus cucurbitae* feeding on an attractive spot ($R_f = 0.45$) in the thin-layer chromatographic bioassay after chromatography of a *Dendrobium* flower extract (HPTLC silica gel 60 F_{254}, nano TLC Merck, developed with benzene–ethyl acetate, 4:1).

superbum flowers. Figure 4 shows the GC-MS trace of rectal gland extracts from *D. cucurbitae* males fed (bottom) and unfed (top) with *D. superbum* flowers. Both were found to contain substantial quantities of *N*-3-methylbutyl acetamide **(2)**, *N*-3-methylbutyl methoxyacetamide **(3)**, 1,3-nonanediol **(4)**, and ethyl 4-hydroxybenzoate **(5)** in common, but ketone **1** was found only in the males fed with *D. superbum* flowers.

The mean content of compound **1** in the rectal sacs in four individual males that had been fed twice on the *Dendrobium* flowers was 0.50 ± 0.26 μg.

Changes in Contents of Ketone 1 in Males. Figure 5 shows the mean contents of ketone **1** in the rectal gland and the rest of the body of *D. cucurbitae* males that had been fed with a pure sample of **1**. The flies appeared to incorporate **1** in the body tissues within 0.25 day after ingestion, and maintained a large portion in the rectal glands for at least six days, with decreasing total contents. The proportion of the content between the gland and body (without gland) at the third day after feeding clearly indicated the selective accumulation of the compound in the gland.

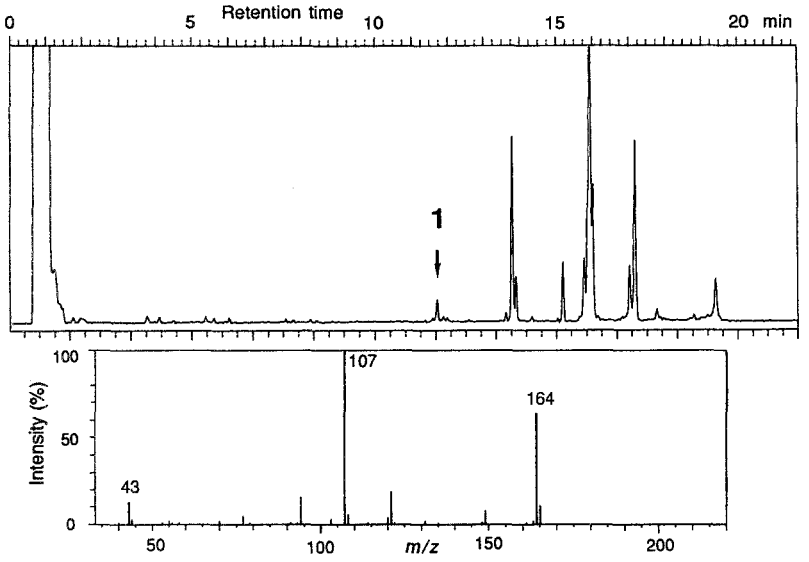

FIG. 3. Top: Mass chromatogram of volatiles in the extract of *Dendrobium superbum* petals. The active peak was found at a retention time of 11.8 min (shown by an arrow). This chromatogram was done on a capillary HP-1 column (24 m × 0.25 mm, programmed from 80 to 210°C, 10°C/min) with a total ion monitor (*m/z* 33–250). Bottom: Mass spectrum of 4-(4-hydroxyphenyl)-2-butanone (**1**), obtained from a scan of the peak with retention time of 11.8 min.

DISCUSSION

4-(4-Hydroxyphenyl)-2-butanone (**1**) has been characterized here as the specific attractant for *Dacus cucurbitae* males from the orchid flower, *Dendrobium superbum*. This compound has already been known as a potent attractant for *D. cucurbitae* males by the name of Willison's lure (Drew, 1974; Drew and Hooper, 1981) and also by the name of raspberry ketone as a characteristic flavor of raspberry (Honkanen et al., 1980; Gallois, 1982). Ketone **1** also has been reported from other plant sources, including Rosaceae, Compositae, and Labiatae (Hirvi et al., 1981; Hirvi and Honkanen, 1984; Lin and Chow, 1984; Marco et al., 1988) and as a fungal metabolite (Ayer and Singer, 1980). We have recently identified the same compound from leaves of the melon fly-attracting coniferous plant, *Juniperus chinensis* (R. Nishida and O. Iwahashi, unpublished). Although our knowledge of the distribution of ketone **1** in the plant kingdom is scanty, it is likely that *D. cucurbitae* males forage ketone **1** from some natural sources wherever available in the field.

Males of *D. cucurbitae* congregate at dusk to form a lek (Kuba et al., 1984;

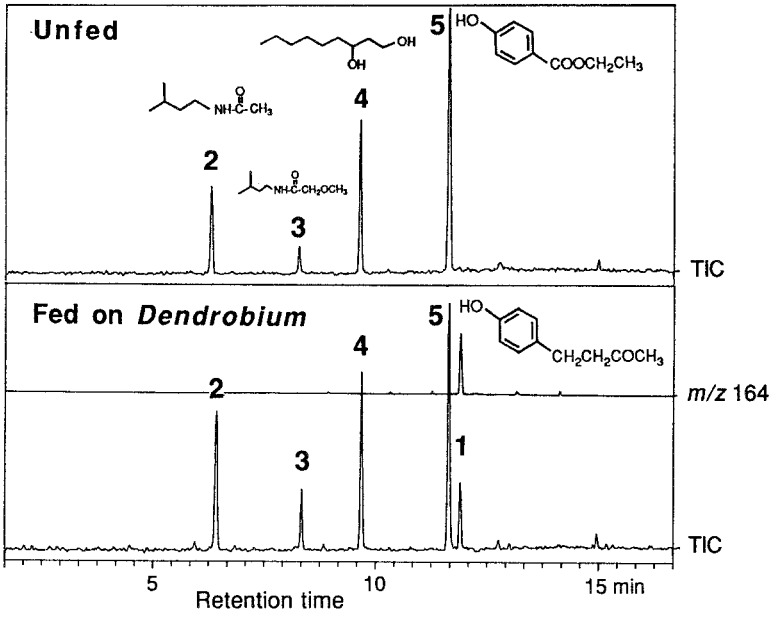

FIG. 4. Gas chromatograms [total ion monitor (TIC *m/z* 33–250); selective ion monitor, *m/z* 164] of volatiles in the rectal gland complex of *Dacus cucurbitae* males fed with *Dendrobium* flowers (bottom) and unfed control (top). Assignments were given by the diagnostic mass spectra: *N*-3-methylbutyl acetamide **(2)** (R_t = 6.4 min), *N*-3-methylbutyl methoxyacetamide **(3)** (R_t = 8.3), 1,3-nonanediol **(4)** (R_t = 9.7), ethyl 4-hydroxybenzoate **(5)** (R_t = 11.7), 4-(4-hydroxyphenyl)-2-butanone **(1)** (R_t = 11.9).

Iwahashi and Majima, 1986) and produce a smokelike substance that originates from the rectal glands during the courtship period (Kobayashi et al., 1978; Ohinata et al., 1982; Kuba and Sokei, 1988). The rectal secretion was attractive to the conspecific females at close range (Kobayashi et al., 1978), but its behavioral role is not fully understood. The volatile portion of the rectal secretion is composed of tetramethylpyrazine, *N*-3-methylbutyl acetamide, *N*-3-methylbutyl methoxyacetamide, 1,3-nonanediol, and methyl, ethyl, and propyl 4-hydroxybenzoates (Baker et al., 1982; Nishida et al., 1990; Perkins et al., 1990). In addition, the rectal sacs incorporated ketone **1** when males were fed either with cue-lure (Nishida et al., 1990) or *Dendrobium superbum* flowers (this study). The selective accumulation of orchid fragrance **1** in the male rectal glands suggests an additional pheromonal function of the compound in nature where the chemical source is available. Neither the rectal volatile mixture nor the intact ketone **1** induced any apparent behavioral response from females (Nishida et al., 1990). We observed that the male flies that had been fed either

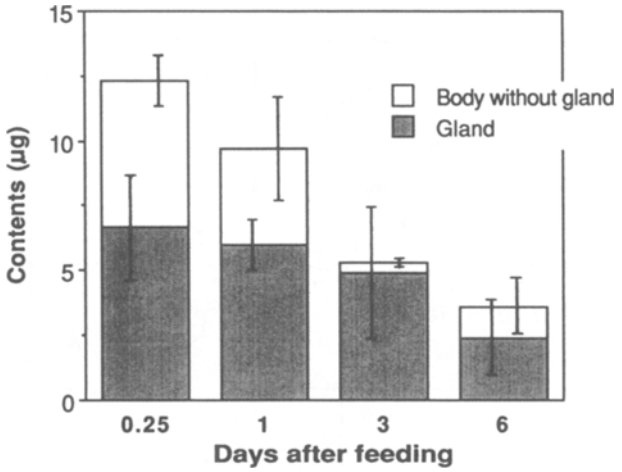

FIG. 5. Mean contents (±SD, μg/male) of 4-(4-hydroxyphenyl)-2-butanone (**1**) in the rectal gland complex and the rest of the body of *Dacus cucurbitae* males fed with compound **1**.

with **1** or cue-lure were persistently chased by unfed males. Many unfed males eagerly licked the surface of the container where the fed males deposited the secretion in spite of the presence of a female in the vicinity. The behavior might be associated with male-to-male competition in the sexual process. Further behavioral bioassay is needed to clarify the significance of ketone **1** together with other rectal volatiles, including male-to-female interactions in terms of direct attraction or aphrodisiac induction, and/or male-to-male interactions in the context of lek formation and mating disruption against competitors.

A parallel investigation has been conducted for the Oriental fruit fly, *Dacus (Bactrocera) dorsalis*. These males selectively accumulated phenylpropanoid metabolites in the rectal glands by foraging from methyl eugenol-containing flowers in the field. They released the rectal components during the courtship period, which suggests a very similar role as pheromone (Nishida et al., 1988; Nishida and Fukami, 1990). It was also suggested that the phenylpropanoids sequestered by *D. dorsalis* males could serve as allomones against predatory animals, since one of the components, 2-allyl-4,5-methoxyphenol, significantly deterred feeding of the sparrow, *Passer montanus* (Nishida et al., 1988; Nishida and Fukami, 1990). In the case of *D. cucurbitae*, however, ketone **1** did not deter feeding of sparrows (R. Nishida, unpublished). Instead, one of the endogenous rectal gland components, 1,3-nonanediol, was found to exhibit some deterrent activity against a lizard, *Hemidactylus frenatus*. Moreover, ketone **1** on the body of fed males was caused by the rectal gland secretion, which was

discharged via reflex action under stress, e.g., during immobilization (K.H. Tan, unpublished).

The flowers also might gain an ecological advantage by attracting a specific pollinator with the fragrant signal (Nishida et al., 1988). Similarly, male euglossine bees pollinate orchids while collecting floral fragrance components that are subsequently utilized to form lek where mating takes place (Dodson, 1975). *D. cucurbitae* males were arrested within the flower petal area and seldom visited inside the lip where pollination occurs, although a significant quantity of ketone **1** also was present in the lips. In contrast, Ichinohe et al. (1983) reported that lips of a variety of *D. superbum* attracted the male flies as well. *D. superbum* is distributed from Laos to the Malay Peninsula, the Philippines, Indonesia, and New Guinea (Karasawa, 1986). Observation of the native *Dendrobium* flowers instead of the cultivated varieties will be necessary to understand the association of the melon fly with the orchid plant in nature.

Acknowledgments—We wish to thank Mr. T. Sasaki of Ocean Exposition Memorial Park Management Foundation, Okinawa, for useful information about *D. superbum*. This work was supported in part by Grants-in-Aid for Scientific Research (Nos. 01560052 and 03269210) from the Ministry of Education, Science and Culture of Japan, and the Malaysian Government R&D grant IRPA-1-07-06-03-03.

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