Reports

n-Tridecane and trans-2-Heptenal in Scent Gland of the Rice Stink Bug Oebalus pugnax (F.)

Abstract. The scent-gland secretion of the rice stink bug *Oebalus pugnax* (F.) is composed of a liquid two-phase system. The saturated hydrocarbon *n*-tridecane accounts for 60 percent of the secretion. In the other phase, the major organoleptic compound is the *trans* form of 2-heptenal.

A major characteristic of members of the family Pentatomidae is their ability to eject compounds of considerable pungency. Because of this trait they are described appropriately as stink bugs. It is believed that the odoriferous substances ejected by the pentatomids are protective against potential predators (*1*).

The large, round, reddish-orange scent gland is situated on the floor of the body cavity, extending through the metathoracic and first abdominal segments and into the second abdominal segment. The gland is highly developed in both adult males and females but is poorly developed in the nymphs, which do not produce the odoriferous substances. The scent-gland secretion of *Oebalus* is ejected through a pair of small ducts opening onto each side of the metathorax through an ostiole.

The odoriferous secretion was collected by piercing scent glands with fine capillaries. The secretion consists of orange-yellow droplets which are suspended in a clear liquid. The clear liquid phase has been identified as *n*-tridecane, and in the orange-yellow liquid phase one of the main organoleptic compounds has been identified as *trans*-2-heptenal.

Infrared examinations (*2*) were made from a film of the secretion. The following diagnostic bands were present: aldehyde C—H, 3.66 μ; C=O, 5.89 μ; C=C, 6.08 μ; *trans* C=C, 10.25 μ. The C—H/C=O ratio further indicated that a high concentration of a hydrocarbon was present in the mixture.

The scent-gland secretion was analyzed by injection of 1-μl samples into the inlet of a Perkin-Elmer model 154B vapor phase chromatograph; tris-phenoxyphenyl-*n*-dodecyl silane was used as an absorbent. The operating temperature was 160°C, with a helium flow rate of 50 cm^3/min. Three main components were detected. Two were low-boiling and were collected along with several minor components by condensing them in microtubes immersed in liquid nitrogen as they issued from the outlet of the instrument. This fraction was orange and had the typical odor associated with *Oebalus*.

The third major component accounted for 60 percent of the total sample. It was isolated in the same manner as the organoleptic fraction. This component was a clear, odorless liquid which boiled at 233°C and melted at −6.1°C. Infrared examination demonstrated that this material was a saturated hydrocarbon, and elemental analysis established an empirical formula of $C_{13}H_{28}$ (C, 84.91 percent; H, 15.06 percent). These data are all in agreement for *n*-tridecane. The linearity of the compound was demonstrated by its ability to form a urea addition compound (*3*). Confirmation was further established by analysis on a modified Consolidated 21-102 analytic mass spectrometer. The fragmentation pattern was identical with that of a sample on pure *n*-tridecane (Table 1).

The aldehyde-rich organoleptic fraction was dissolved in absolute ethanol and was added to a saturated solution of 2,4-dinitrophenylhydrazine in 2*N* HCl. The resulting 2,4-dinitrophenylhydrazones were separated by filtration and rinsed with boiling ethanol. A portion of the 2, 4-dinitrophenylhydrazone mixture was alcohol-insoluble and was characterized as a dicarbonyl compound. The alcohol-soluble derivatives were chromatographed by the method of Gordon *et al.* (*4*). One major component was isolated; its melting point was 130 to 131°C. The empirical formula of this compound corresponded to a heptenal. (Calculated for $C_{13}H_{16}N_4O_4$: C, 53.42; H, 5.48; N, 19.18 percent. Found: C, 53.61; H, 5.40; N, 19.30 percent.) A melting point in admixture with an authentic sample of the derivative of 2-heptenal produced no melting point depression. The infrared spectrum of the *Oebalus* derivative was identical with that of the corresponding derivative of 2-heptenal. The presence of this enal in the scent-gland secretion was further demonstrated by the facts that the mass spectrometric fragmentation pattern of the organoleptic layer contained a maximum heptenal parent mass of 112 and that the resulting fragmentation pattern contained peak masses identical with those of synthetic 2-heptenal. The grassy odor of this aldehyde is very similar to that of the secretion of *Oebalus*.

Neither *n*-tridecane nor 2-heptenal has been isolated previously from any members of the animal kingdom. However, the grease layer extracted from the cuticle of the cockroach *(Periplaneta)* has been shown to contain a series of aliphatic hydrocarbons in the range C_8 to C_{12} (*5*). *n*-Tridecane occurs in brown coal tar (*6*) and cracked shale tar kerosene (*7*). 2-Heptenal is one of the carbonyls associated with the odor of cooked chicken (*8*) and also occurs in reverted soybean oil (*9*), oxidized skim milk (*10*), and rancid pork (*11*). This enal is closely related to the carbonyl 2-hexenal isolated from the roach *Eurycotis floridana* (Walker) (*12*).

Oebalus pugnax ejects its scent-gland secretion in large droplets. The ejected

Table 1. Mass spectrometric analysis of the *Oebalus* hydrocarbon.

e/*M**	Actual division of spectrum	*n*-Tridecane	Residual
41	3911.0	3910.4	+0.6
42	1030.2	1030.3	−0.1
43	6570.8	6570.8	0
44	210.4	210.3	+0.1
55	1330.0	1330.6	−0.6
56	1086.3	1086.1	+0.2
57	5810.6	5810.3	+0.3
70	839.0	838.4	+0.6
71	2960.1	2960.7	−0.6
72	145.7	145.8	−0.1
84	405.0	405.0	0
85	1821.0	1820.6	+0.4
86	105.6	105.5	+0.1
98	289.1	288.5	+0.6
99	290.3	290.2	+0.1
112	136.9	136.4	+0.5
113	153.4	153.8	−0.4
126	85.4	85.5	−0.1
127	127.8	127.2	+0.6
141	84.1	84.0	+0.1
155	49.5	49.5	0
169	1.7	1.5	+0.2
184	212.0†	212.3†	−0.3

* Charge/mass. † Parent mass.

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

carbonyl compounds are easily detected as orange spots when the bugs are placed on filter papers saturated with 2,4-dinitrophenylhydrazine. The ejection can be either bilateral or unilateral. Unilateral ejection was most commonly observed when the bugs were approached by imported fire ant workers (*Solenopsis saevissima* v. *richteri* Forel). Ants which were exposed to the spray rapidly moved away. This would seem to support the belief that the odoriferous secretions of the pentatomids are at least partially protective.

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Use of Cytoplasmic Male-Sterility in Making Interspecific Crosses in Allium

Abstract. Cytoplasmic male-sterile plants of *Allium cepa* were used in making interspecific crosses with *A. fistulosum.* Some inbred lines of *A. cepa* produce more seed than others. Other *Allium* species could also be used as the pollen parent.

In plant breeding hand emasculation is often slow and painstaking; the results are somewhat disappointing and the number of F_1 progeny is limited. The cytoplasmic male-sterile character in *Allium cepa* L., as reported by Jones and Clarke (*1*), is extremely useful in crossing *A. cepa* and *A. fistulosum* L.

Eight cytoplasmic male-sterile inbred lines of *A. cepa,* each represented by 10 mother bulbs, were placed in an insect-proof isolation cage 3½ by 6 by 6 feet. Though *A. cepa* bloomed much later than *A. fistulosum,* no particular difficulty was encountered. By growing several thousand *A. fistulosum* plants, a sufficient number of late-flowering umbels were obtained. The seedstalks, with stems as long as possible, were cut and placed in a container of water to which 1 part of copper per million in the form of copper sulfate was added to prevent growth of fungi and algae. The container of flowers of *A. fistulosum* was then placed in the cage with the *A. cepa* inbred lines. Honey bees (approximately 3 pounds of workers with a queen, brood, comb, and so forth) were used as the pollinators.

The inbred lines used and the number of seeds from each inbred line are given in Table 1. Of course, the difference in bloom time could account for some but probably not all of the difference noted. I feel that some inbred lines will cross more readily with *A. fistulosum,* although sufficient data are not available for a definite statement. The well-known constancy of bees in pollinating a particular species, strain, or even individual plant, or their preference for plants with high sugar levels in the nectar as reviewed by Grant (*2*) was not a factor in the pollination of the material in this report. The bees were confined to a small volume and were not free to forage. Food was not too plentiful within the cage. The bees visited each and every plant without preference for one or the other. Some of the F_1 progeny were male-fertile, others male-sterile. Ratios were not determined.

The characteristics of *A. fistulosum* are sufficiently distinct from those of *A. cepa* that the two species are readily identified. The hybrid between the two species is intermediate in character. Plants grown from the seeds reported in Table 1 were hybrids between the two species. Emsweller and Jones (*3*) have described the interspecific hybrid.

This system of crossing eliminates emasculation, reduces possible contamination, increases the chance of a cross, and produces a greater number of seeds. When single umbels are being crossed, houseflies or blue-green bottle flies can be used as pollinators. A male-sterile umbel of *A. cepa* can be enclosed in a small cage with the male-fertile umbel of *A. fistulosum.*

Though only a few seeds were produced, they were adequate to grow out the F_1 generation. The F_1 interspecific hybrids produced in the foregoing manner may be either male-fertile or male-sterile. Male-fertile plants may be used as the pollen parents in a backcrossing program with male-sterile *A. cepa* as the recurrent female parent. Male-sterile F_1 interspecific hybrids can be used as the female parent with a male-fertile *A. cepa* as the pollen parent.

Table 1. Number of seeds produced on eight male-sterile *A. cepa* inbred lines pollinated by *A. fistulosum* with honey bees in an insect-proof isolation cage, Parma, Idaho, 1955.

Inbred source	Pedigree	No. of seeds
Early yellow globe	B 2108 A	30
Early yellow globe	B 2117 A	40
Brigham yellow globe	B 2190 A	200
Brigham yellow globe	B 2207 A	75
Brigham yellow globe	B 2217 A	30
Brigham yellow globe	B 2218 A	55
Brigham yellow globe	B 2267 A	40
Yellow sweet Spanish	B 12132 A	20

Although only *A. cepa* was crossed to *A. fistulosum* by this method, other *Allium* species could be used as the pollen parent. The system is simple and effective in making interspecific as well as intraspecific crosses in the *Allium* species, in which a male-sterile *A. cepa* can be used as the seed parent (*4*).

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Pineal Regulation of the Body Lightening Reaction in Amphibian Larvae

Abstract. Body pallor due to contraction of both deep and integumental melanophores occurs when either blinded or normal *Xenopus laevis* and other amphibian larvae are placed in the dark. The reaction is abolished by pinealectomy, but is induced by administration of pineal hormones. It is suggested that the normal body lightening reaction is mediated by the pineal gland.

It has been known for many years that due to melanophore contraction amphibian larvae become pale when subjected to darkness for periods of a few hours (*1–3*). The mechanism of this lightening reaction, however, remains unexplained and our understanding of it has been further complicated by observation that the phenomenon is not abolished in blinded larvae (*2*). With this in mind and as a result of the discovery that the tail darkening reaction of *Xenopus laevis* is due to a direct effect of light on tail melanophores (*4*), it was suggested that a similar photochemical mechanism might mediate the body lightening reaction (*3*). In the course