2-Phenylethanol: An Attractant for the Onion and Seed-Corn Flies, *Hylemya antiqua* and *H. platura* (Diptera: Anthomyiidae)¹

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2-Phenylethanol was identified as a potent attractant for onion and seed-corn flies by chemical analysis of aged onion pulp, which was highly attractive for these insects in the field. After filtration, steam-distillation and extraction with ether, the attractive compounds in the aged onion pulp were found to move into the acidic and neutral fraction. Analysis of the neutral fraction by GC and GC-MS revealed the presence of iso-butyl alcohol, ethanol, ethyl acetate, iso-amyl alcohol and 2-phenylethanol, the last two compounds being the major constituents. Only 2-phenylethanol showed moderate attractancy to the onion fly in laboratory bioassay. As 2-phenylethanol alone could not explain the high attractancy of aged onion pulp, some unidentified minor constituents were considered to be important in this manifestation. Despite the moderate attractancy of 2-phenylethanol in laboratory tests, this compound showed high attractancy to onion and seed-corn flies in the field. The attractancy to the onion fly was about the same as the fresh onion pulp, which had about half the attractancy of the aged onion pulp. Attractancy to the seed-corn fly, particularly to the male, was quite high. 2-Phenylethanol caught 6 and 3 times as many male and female seed-corn flies as the fresh onion pulp, respectively.

INTRODUCTION

Onion fly, *Hylemya antiqua* MEIGEN, and the seed-corn fly, *H. platura* M. are important pests of onion and germinating seeds of many crops, respectively. For monitoring the population of the onion fly, onion pieces or macerated onion have been used for many years. Fish meal or molasses are known to have great attractancy to the seed-corn fly. However, the compounds which are attractive to these insects have not been chemically identified in these natural baits. Chemically defined attractants have long been needed for the following reasons: (1) the natural baits are not potent enough to reduce the insect population by mass-trapping; (2) they are short-lived and their attractancy to the insect changes as it ages; (3) since fish meal and molasses of different

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manufacturers differ in attractancy, it is difficult to compare population levels of the seed-corn fly over a broad area.

In our previous study, we revealed that onion pulp increases its attractancy to onion and seed-corn flies after 2 days and 5 days of aging, respectively (ISHIKAWA et al., 1981). In this study, we investigated the compounds that are responsible for the enhanced attractancy of aged onion pulp with a view to developing a potent chemical attractant for both species of flies. Here we report the identification of one component of the attractant, 2-phenylethanol, and the evaluation results of its attractancy in the field.

MATERIALS AND METHODS

Insects. Onion flies used in the laboratory test originated from a strain which was established at the Hokkaido National Agricultural Experiment Station and reared on an artificial diet at the Laboratory of Applied Entomology, University of Tokyo (ISHIKAWA et al., 1983).

Sample preparation. Onion pulp was prepared by grinding 500 g of onion bulbs in a blender for 5 min. The pulp was aged for 8 days at 25°C, then added with distilled water and strained through a sheet of cheesecloth. The liquid obtained was passed through filter paper and the filtrate was steam-distilled under reduced pressure at 50°C. The distillate was then extracted with diethyl ether and fractionated to acidic, neutral and basic fraction in the conventional way. These fractions were concentrated and kept at 0°C in a refrigerator until use. Each fraction was bioassayed against the onion fly in the laboratory. Only the neutral fraction was further subjected to gas chromatographic and GC-MS analysis in this study.

Chemical analysis. A gas chromatograph (Shimadzu GC-4B) was equipped with FID and a stainless steel column (3 mm I.D., 3 m long) packed with celite 545 (60–80 mesh) coated with 20% PEG 20M. Oven temperature was maintained at 30°C for 6 min and then programmed to 210°C at a rate of 4°C/min. Nitrogen flow rate was 35 ml/min. To obtain mass spectra, a GC-MS system (Hitachi 063 and RMU-6) was used under conditions similar to the gas chromatograph analysis by GC-4B. Ionization potential was 70 eV.

Bioassay in laboratory. A transparent plastic cylindrical container (9.5 cm diam. 16 cm high) with a cap was used as a trap. The side wall of the trap was lined with a sheet of black paper and 15 entrance holes (6 mm diam.) were made at the height of 4 cm (Fig. 1A). The test chemicals (1 μ l) were dissolved in 5 ml water in a small petri dish (3 cm diam.), which was set in the bottom of the trap. In a 30 $\times$ 60 $\times$ 30 cm cage, 50 each of male and female onion flies were confined and were starved by supplying only water for 5 hr prior to the experiment. During the experiment also, only water was available to them. The two traps, one with a test chemical and the other with only water, were set 30 cm apart in a cage at 3:00 p.m. The flies trapped were counted at 10:00 a.m. the next morning. The test was replicated 5 times and the attractancy of the chemicals was expressed as the mean number of flies captured in the trap.

Evaluation of attractancy in the field. Attractancy of 2-phenylethanol, mercaptoethanol and propyl propanethiosulfonate was evaluated in the onion field of the Hokkaido National Agricultural Experiment Station, Sapporo, from the 1st to 18th of

Fig. 1. A: Laboratory bioassay device for evaluating the attractancy of test samples.
B: A trap used in the field test.

August, 1981. The trap, devised by MATSUMOTO (1970), was made of a transparent acryl cylinder (15 cm diam. $\times$ 15 cm high) equipped with a top cover, and a funnel (8 cm high) with an entrance hole (8 mm diam.) was fitted to the bottom of the trap (Fig. 1B). The trap was set on an iron frame so that the bottom of it (funnel opening) was 5 cm above the ground. The traps were set in a Latin square form (4×4) at 8 m intervals in an onion field of about 2 ha. Both onion and seed-corn flies occur simultaneously in this field. As no insecticide spraying has been done for several years, the annual infestation rate by these species exceeds 30%.

Test chemicals were dissolved in 50 ml water in a petri dish (9 cm diam.) so that the final concentration became 0.2%. The dish was covered with a sheet of nylon mesh to prevent flies from drowning and it was set under the trap. Four treatments including water (control) and fresh onion pulp (as a standard) were tested at a time with 4 replications. Fresh onion pulp instead of the more attractive aged one was used as the standard since the latter took much time to prepare and also was more variable in its attractancy. The attractancy of the fresh onion pulp was about the half of the aged one when tested in the field (ISHIKAWA et al., 1981). The traps were set at 1:00 p.m. and the flies trapped were counted, identified as to species and sexed at 10:00 the next morning.

RESULTS

Properties of attractants in the aged onion pulp

In order to clarify the chemical properties of attractants in the aged onion pulp, laboratory bioassay was conducted against the onion fly (Table 1). Using the average of 5 replicates, the trap baited with aged onion pulp filtrate caught 14.9 males and 10.9 females out of 50 each, whereas the control trap baited with water caught only 1.3 males and 1.0 females. This result clearly indicates that this bioassay was valid for evaluating the attractancy of test samples. The chemical properties of the attractant were next investigated conventionally. The steam distillate of the aged onion pulp completely retained the original attractancy; therefore, the attractive compounds were considered to have moderate volatility. The distillate was extracted with ether and fractionated into acidic, neutral and basic fractions. Each fraction was subjected to

bioassay. The acidic and neutral fractions showed high attractancy comparable to the aged onion pulp filtrate. The acidic fraction was somewhat lower in attractancy than the neutral fraction. The basic fraction did not show any attractancy to the onion fly in the laboratory test. Only the neutral fraction was subjected to further chemical analysis in this study.

Table 1. Mean numbers of onion flies captured in traps in a cage:
a laboratory bioassay^a

Tested materials	Treated		Control	
	Male	Female	Male	Female
Aged onion pulp ^b	14.9*	10.9*	1.3	1.0
Steam distillate of aged onion pulp ^b	16.8*	21.2*	4.8	2.6
Ether extract of the distillate, acidic Fr. ^b	10.8*	7.4*	0.8	0.4
do. neutral Fr. ^b	20.2*	13.6*	0.8	0.4
do. basic Fr. ^b	2.4ns	1.9ns	1.2	0.8
2-Phenylethanol ^c	4.6*	4.2*	0.4	0.6
Ethanol ^c	1.3ns	1.6ns	0.5	0.9
iso-Amyl alcohol ^c	2.8ns	1.5ns	3.5	1.2
iso-Butyl alcohol ^c	2.5ns	0.8ns	1.3	0.5
Ethyl acetate ^c	0.6ns	0.3ns	0.7	0.6
Mercaptoethanol ^{c,d}	7.8*	11.0*	0.4	0.6

^a Mean of 5 replications. Water was used as a control.

*: significant at 5% level, ns: not significant.

Tested against 50 each of male and female onion flies in a cage.

^b 5 ml equiv.

^c 1 μ l/5 ml water.

^d Not present in the neutral fraction.

Fig. 2. Gas chromatogram of the neutral ether extract fraction of aged onion pulp. For sample preparation, see text. EtOH: Ethanol, isoBuOH: iso-Butanol, EtAc: Ethyl acetate, isoAmOH: iso-Amyl alcohol, PheEtOH: 2-Phenylethanol.

GC, GC-MS analysis of the neutral fraction

The gas chromatogram of the neutral fraction is shown in Fig. 2. The compounds present in this fraction were identified as ethanol, iso-butanol, ethyl acetate, iso-amyl alcohol and 2-phenylethanol, the last two being the major constituents. Sulfur compounds, which are abundant in fresh onion, were included in only trace amounts. Ethanol and ethyl acetate were found earlier in onions inoculated with a bacterium, *Klebsiella* sp., which was isolated from decaying onion (IKESHOJI et al., 1980). The latter compound was shown to have a synergistic effect on dipropyl disulfide in oviposition stimulation of the onion fly. It was also shown to have attractancy to the onion fly larvae by itself (IKESHOJI et al., 1980). All compounds except 2-phenylethanol are very common as bacterial degradation products of plant tissues. 2-phenylethanol is relatively rare as a bacterial product, however, this compound was the major constituent in the neutral fraction in this study.

Attractancy of compounds in neutral fraction and related compounds in the laboratory

The attractancy of compounds found in the neutral fraction against the onion fly in the laboratory test is shown in Table 1. 2-Phenylethanol showed only moderate attractancy, less than half of the aged onion pulp filtrate. No other compounds showed any attractancy to the onion flies at all. As 2-phenylethanol alone could not explain the high attractancy of the neutral fraction, it was concluded that some unidentified minor compounds have high attractancy by themselves or that a mixture of compounds including 2-phenylethanol is responsible for manifesting the high attractancy.

The attractancy of mercaptoethanol was tested additionally since this compound has molecular property similar to 2-phenylethanol. The compound showed high attractancy to onion flies in laboratory bioassay (Table 1), though its attractancy in the field was low as described below.

Table 2. Numbers of flies captured in traps baited with 2-phenylethanol and other compounds in an onion field in the Hokkaido National Agricultural Experiment Station^a

Treatment	Replication	Total flies captured			
		Onion fly		Seed-corn fly	
		Male	Female	Male	Female
Water	6	10	13	15	10
Fresh onion pulp		75	63	63	73
2-Phenylethanol		82	75	400	208
Water	2	2	0	2	3
Fresh onion pulp		28	12	20	17
Mercaptoethanol		7	6	6	6
Water	3	2	2	2	1
Fresh onion pulp		29	25	15	15
PrSSO ₂ Pr		21	18	14	10

^a Experimental period: August 1 to 18, 1981.

Data were accumulated during the whole series of experiments.

PrSSO₂ Pr: Propyl propanethiosulfonate.

Attractancy of 2-phenylethanol and several other materials in the field

The results of field tests to evaluate the attractancy of 2-phenylethanol and several other materials are shown in Table 2. 2-Phenylethanol showed high attractancy for both onion and seed-corn flies in the field despite only moderate attractancy in the laboratory. Attractancy to the seed-corn fly, particularly to the males, was quite high. The trap baited with 2-phenylethanol caught 6.3 and 2.8 times as many seed-corn fly males and females respectively as the trap with fresh onion pulp. Attractancy to the onion fly was somewhat lower but still comparable to that of the fresh onion pulp.

In this experiment, propyl propanethiosulfonate, an oxidization derivative of dipropyl disulfide, and mercaptoethanol were also tested since the former was known to have higher attractancy to the onion fly than dipropyl disulfide in the field (ISHIKAWA, unpublished data) and as the latter showed high attractancy in laboratory bioassay (Table 1). Mercaptoethanol, which showed substantial attractancy in the laboratory test, showed no significant attractancy in the field. On the other hand, propyl pro-

Table 3. Numbers of flies in traps baited with 2-phenylethanol and other materials in an onion field in the Hokkaido National Agricultural Experiment Station^a

Treatment	Date	Total flies captured			
		Onion fly		Seed-corn fly	
		Male	Female	Male	Female
Water	Aug. 5 '81	10	7	56	41
Fresh onion pulp		16	19	65	52
PheEtOH + HSEtOH		20	11	340	183
PheEtOH + PrSSO ₂ Pr		26	19	335	153
Water	Aug. 8	2	1	5	1
Fresh onion pulp		25	19	21	19
PrSSO ₂ Pr + HSEtOH		22	24	15	10
PrSSO ₂ Pr + HSEtOH + PheEtOH		13	11	40	40
Water	Aug. 9, 17	7	7	5	4
Fresh onion pulp		31	41	28	24
PheEtOH		32	46	112	85
PheEtOH + PrSSO ₂ Pr		45	22	115	105
Water	Aug. 10	1	0	1	0
Fresh onion pulp		16	7	5	4
HSEtOH		4	6	2	0
HSEtOH + PheEtOH		6	2	23	8
Water	Aug. 11	1	2	1	1
Fresh onion pulp		21	23	11	8
PrSSO ₂ Pr		19	16	6	6
PrSSO ₂ Pr + HSEtOH		9	15	4	6
Water	Aug. 15, 16	0	2	3	0
Fresh onion pulp		7	4	4	13
Fish meal		0	4	10	4
PheEtOH		9	9	60	16

^a PheEtOH: 2-Phenylethanol, HSEtOH: Mercaptoethanol, PrSSO₂Pr: Propyl propanethiosulfonate.

panethiosulfonate showed high attractancy for the onion fly comparable to the fresh onion pulp, though the attractancy to the seed-corn fly was somewhat lower.

Synergism among attractants

Several combinations of attractive compounds were tested against the flies in the field to see if the attractancy of the mixture was enhanced. However, no clear synergism was shown as far as the combinations tested were concerned (Table 3). For instance, addition of propyl propanethiosulfonate did not seem to affect the attractancy of 2-phenylethanol at all. It seemed that those combinations which contained 2-phenylethanol showed high attractancy regardless of the other constituents.

Finally, the attractancy of 2-phenylethanol was compared with that of fish meal, a commonly used bait for the seed-corn fly. Though the seed-corn fly population seemed low, 2-phenylethanol showed much higher attractancy than fish meal (Table 3).

DISCUSSION

Discrepancy between attractancy in laboratory and field

2-Phenylethanol showed only moderate attractancy to the onion fly in the laboratory, whereas its attractancy in the field was very high. Conversely, mercaptoethanol, which showed higher attractancy than 2-phenylethanol in the laboratory test did not show significant attractancy in the field. Although the cause of this discrepancy is not clear, difference in the design of traps used in the laboratory and the field, and differences in the air stream around the traps and thus dispersion of attractants are considered possible causes of this phenomena. Detailed analysis of the cause of this discrepancy is under investigation.

2-Phenylethanol as a bioactive compound

2-Phenylethanol is principally known as the odor of the rose, and it is used in perfumes in large quantities. As to the biological activity, it is known to be present in "hair pencil" substances of several Noctuidae moths (BIRCH, 1974). More recently, this compound was identified as a component of the male-secreted sex pheromone of a pyralid moth, *Aphomia gularis* (KUWAHARA, 1980). Moreover, it is also known as a food attractant in rice and corn for the rice weevil, *Sitophilus zeamais* (YAMAMOTO et al., 1971). 2-Phenylethanol is thus not novel as a bioactive compound. However, its attractancy to dipteran species was first discovered in this study. 2-Phenylethanol is considered to be a food attractant for onion and seed-corn flies from this study because both sexes were attracted and the flies were observed to probe around the petri dish which contained the attractant in the field. It may also play a role in host selection as described below.

Ecological significance of 2-phenylethanol

The importance of microbes in the rhizosphere of the host plants has recently been stressed in relation to the host selection of the two fly species by several workers (ECKENRODE et al., 1976; HOUGH et al., 1981, 1982; IKESHOJI et al., 1980). In this regard, 2-phenylethanol may play an important role in the host selection by *Hylemya* species since there is a possibility that it is produced by microbes in the rhizosphere of their host plants by working on the hosts exudates. Moreover, 2-phenylethanol may

have a synergistic effect on the propylthio compounds, which are oviposition stimulants emanating from onion (ISHIKAWA et al., 1978). An investigation into the host selection by *Hylemya* from these points of view is under way.

Synergism among attractants

Recently, DINDONIS and MILLER (1981) tested the attractancy of several microbial products against onion flies in the field. Though they could not find a potent attractant, they found that a mixture of several compounds showed higher attractancy than each component alone. Therefore, there is a high possibility that mixing some compounds with 2-phenylethanol may render a much higher attractancy. Though no clear synergism was observed among the compounds tested in this work, further experiment, particularly analysis of the acidic fraction which showed high attractancy to the onion fly in the laboratory, is needed. This analysis and the field evaluation of the attractancy of acidic compounds will be dealt with in a following paper (ISHIKAWA, in preparation).

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