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Attraction of Several Dipterous Insects to Aliphatic Esters (Diptera: Milichiidae, Chloropidae and Ceratopogonidae)

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The enticement of several dipterous insects to straight chain aliphatic esters was tested under field conditions by means of a filter paper trap permeated with an ascending flow of chemicals. The structurally related compounds were simultaneously exposed in a group and their activities were mutually compared by the numbers of trapped insects. Four insect species respectively gave a precise choice to chemical structures within a limited range, which shifted gradually from species to species; the favored compounds were hexyl butanoate for *Neophyllomyza* sp. (Milichiidae), octyl butanoate for *Siphonella* sp. (Chloropidae), hexyl hexanoate for *Forcipomyia* sp. A (Ceratopogonidae) and decyl hexanoate for *Forcipomyia* sp. B. The attracting activity of the compounds to the insect species varied, in different ways according to the responding species, with respect to the total chain length as well as the length of either the alcohol or acid moiety and the position of the functional group in molecules of the same length. The results are discussed in the light of the current conception on the mechanism of insect olfaction.

INTRODUCTION

While different types of chemically baited traps were tested under field conditions, the adhesion of a large number of small flies to the surface of filter paper traps permeated with an ascending flow of aliphatic esters was observed. Further investigations revealed that the species and numbers of captured insects varied according to the structure of the compounds. A systematic experiment was then performed throughout the insect flight season with saturated, straight chain, aliphatic esters; compounds, which were related in respect to total carbon number or position of an ester group, were simultaneously exposed in various combinations and the relative activity of each group was evaluated on the basis of the number of trapped insects. The discriminative ability of several dipterous insects, which were caught in large numbers and which could be regarded as discrete species of the genera Diptera was indeed highly precise and species specific.

MATERIALS AND METHODS

Test compounds. Straight chain aliphatic esters were synthesized by refluxing an equimolar mixture of an alcohol and an acid in benzene containing a catalytic amount of *p*-toluenesulfonic acid. Refluxing for 16 hr was accomplished by means of Soxhlet's

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extractor to which a layer of anhydrous magnesium sulfate was positioned in the middle part of the assembly. The reaction mixture was treated in a conventional way and distilled. The distillate was checked by GLC and, if necessary, redistilled to single peak purity. Molecular structure was then confirmed by IR spectroscopy.

Trapping procedure. Filter paper strips (100×60 mm) rolled into a cylindrical shape (approx. 30 mm in diam. and 60 mm in height) and set into petri-dishes (15 mm in depth and 32 mm in diam.). Structurally related compounds were simultaneously exposed in a group. These were respectively poured into the petri-dishes, in amounts of 5 ml per dish. Each petri-dish was placed into a larger petri-dish (90 mm in diam.). Fifty ml of water was poured in the larger dish and covered with 10 ml of liquid paraffin.

A shelter covered with a zinc roof (97×97 cm) placed at a height of 100 cm above the ground was erected in the middle of a yard (29×14 m) overgrown with common weeds such as field horsetail, curlydock, common chickweed, and large crabgrass among others, at our campus in Komaba, Tokyo. Three to five inverted flower pots (13 cm in height) were placed in a circle at intervals of 20 cm inside the shelter. The filter paper traps were placed on the pots and left exposed for one or more days. The position of the pots were randomly interchanged at 2 hr intervals for single day experiments and once per day for those experiments greater than one day duration. A constant duration of exposure could not be maintained since those esters containing 8–10 carbon atoms were extremely volatile and this precluded the possibility of any long run testing within this group and also because the period for obtaining catches sufficient for assessment varied according to season, weather and temperature. Nevertheless, the duration of exposure gave no substantial influence on the relative activities of the lures which were simultaneously tried. The chemicals in the petri-dishes were properly replenished as they dissipated for the duration of test. Besides providing a sustained flow of vapor, the wet surface of the filter paper trapped small dipterous insects by their legs or winge. The layer of liquid paraffin in the outer dish retained insects which dropped from the surface of the filter paper and trapped additional numbers of insects flying near the trap. Tests were carried out 35 times during periods of Sept. 1–15, 1970 and May 18-Oct. 25, 1971.

Grouping and identification of captured insects. Captured insects were collected with a needle and divided into groups having the same external appearance under a dissecting microscope. The collections were inspected by taxonomic specialists for confirmation of group homogeniety and identified as *Neophyllomyza* sp., *Siphonella* sp., *Forcipomyia* sp. A and sp. B, *Conioscinella* sp. Although the *Siphonella* sp. was captured in a male-female ratio of 50 : 50, males were not caught for the other species.

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RESULTS

The activities of C_{10}–C_{16} esters having the same numbers of carbon atom in both alcohol and acid moieties were initially compared (Fig. 1); run C with the combination of 6.6, 7.7 and 8.8 and run D with combination of 5.5, 6.6 and 7.7. In either case, 6.6, hexyl hexanoate, was the preferred ester for both the *Neophyllomyza* sp. and the *Siphonella* sp., thus the C_{12} esters and their neighbors were selected for further investigation.

Isomers of the C_{12} esters, having functional groups located at different positions

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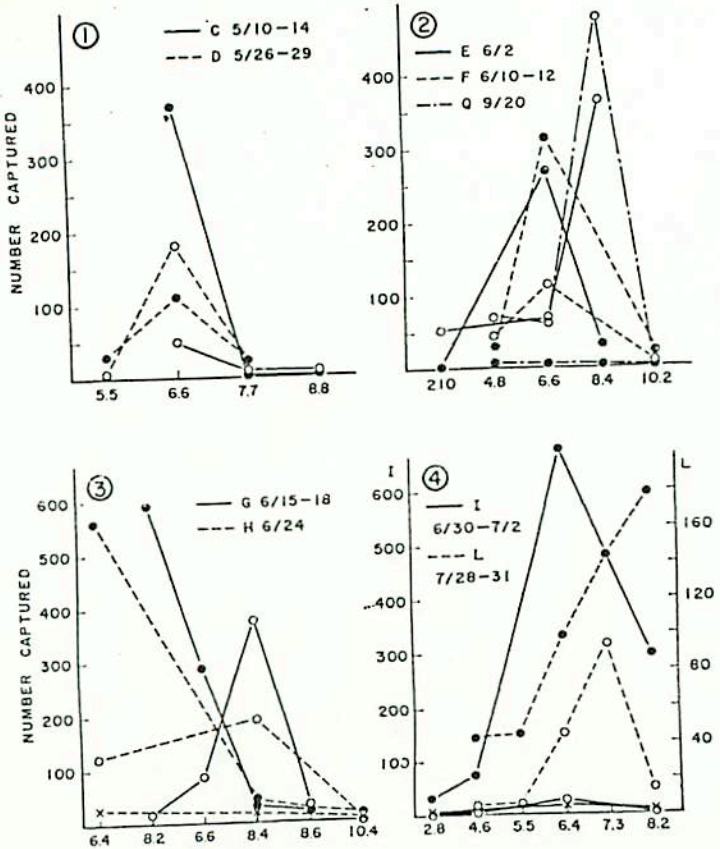

Fig. 1–4. Numbers (ordinate) of *Neophyllomyza* sp. (●) and *Siphonella* sp. (○) captured on and around cylindrical filter-paper traps impregnated with aliphatic, straight chain esters with a general constitution: k.m (abscissa), an ester of C_k alcohol with C_m acid.

were compared (Fig. 2): the 6.6 ester was favored by *Neophllyomyza* sp. while the 8.4 ester was preferred by *Siphonella* sp..

The activity of 8.4, i.e. octyl butanoate to which *Siphonella* sp. responded favorably was compared with those of four homologues, shorter or longer by two carbon atoms, in either alcohol or acid moiety (Fig. 3), with 6.6 included to recheck the result in Fig. 2. While *Siphonella* sp. continued to select the 8.4 esters, *Neophyllomyza* sp. showed a preference for the C_{10} esters.

The activities of a series of C_{10} esters were compared, with the functional group placed in different positions (Fig. 4). *Neophyllomyza* sp. favored the 8.2 and 6.4 esters, while *Siphonella* sp. showed preference for the 7.3.

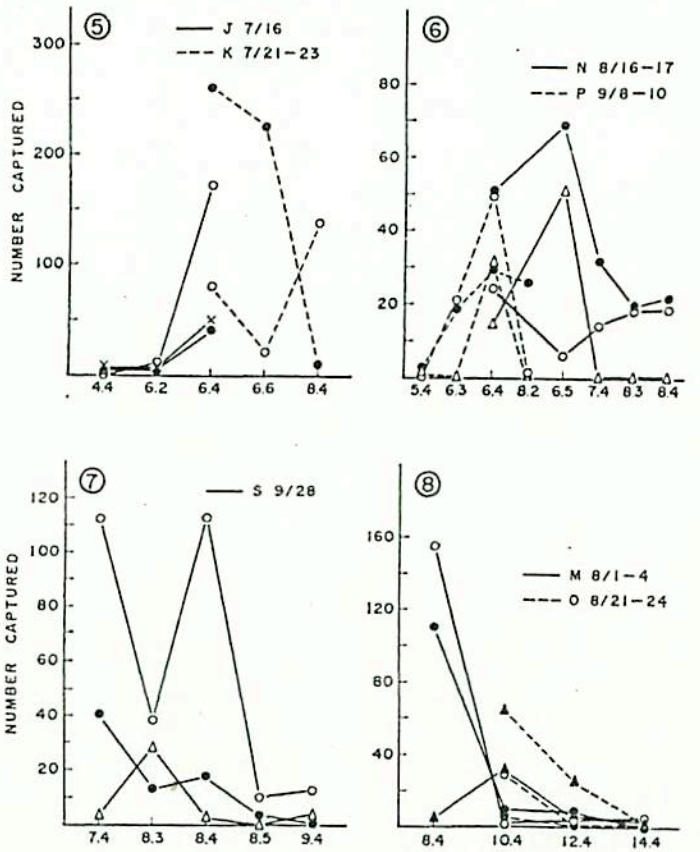

Fig. 5–8. Numbers (ordinate) of *Neophyllomyza* sp. (●) *Siphonella* sp. (○), *Ganioscinella* sp. (×), *Forcipomyia* sp. A (△) and *Forcipomyia* sp. B (▲) captured on and around cylindrical filter-paper traps impregnated with aliphatic, straight chain esters with a general constitution: k.m (abscissa), an ester of C_k alcohol with C_m acid.

The activity of 6.4, a structure favored by *Neophyllomyza* sp. was compared with those of four homologues longer or shorter by a C_2 unit, in either alcohol or acid moiety (Fig. 5). Once again, *Neophyllomyza* sp. and *Siphonella* sp. were highly attracted to the 6.4 and 8.4 esters, respectively.

The activity of 6.4 was compared with that of those homologues longer or shorter by a C_1 unit and the activity of 8.4, with those homologues shorter by a C_1 unit in either alcohol or acid moiety (Fig. 6). Although somewhat complicated, primary choice was given to 6.4–6.5 by *Neophyllomyza* sp. and *Forcipomyia* sp. A, while to 6.4–7.4–8.4 by *Siphonella* sp..

The comparison of the activity of 8.4 with homologues shorter or longer by a C_1

unit in either alcohol or acid moiety was repeated (Fig. 7); 7.4–8.4 esters were unerringly selected by *Siphonella* sp..

Butyric acid residue, seemingly important for *Siphonella* sp., was esterified with C_8–C_{14} alcohols and compared in Fig. 8; 8.4 was selected by *Siphonella* sp., while 10.4 was selected by *Forcipomyia* sp. B.

The activity of hexyl hexanoate, a structure favored by *Forcipomyia* sp. A, was compared with that of those homologues reduced by a C_1 unit in either alcohol or acid moiety or in both and of isomers with an ester group displaced along the main chain (Fig. 9). In either case, 6.6 was selected by that species.

The activities of esters of hexanol, an alcohol residue seemingly important for

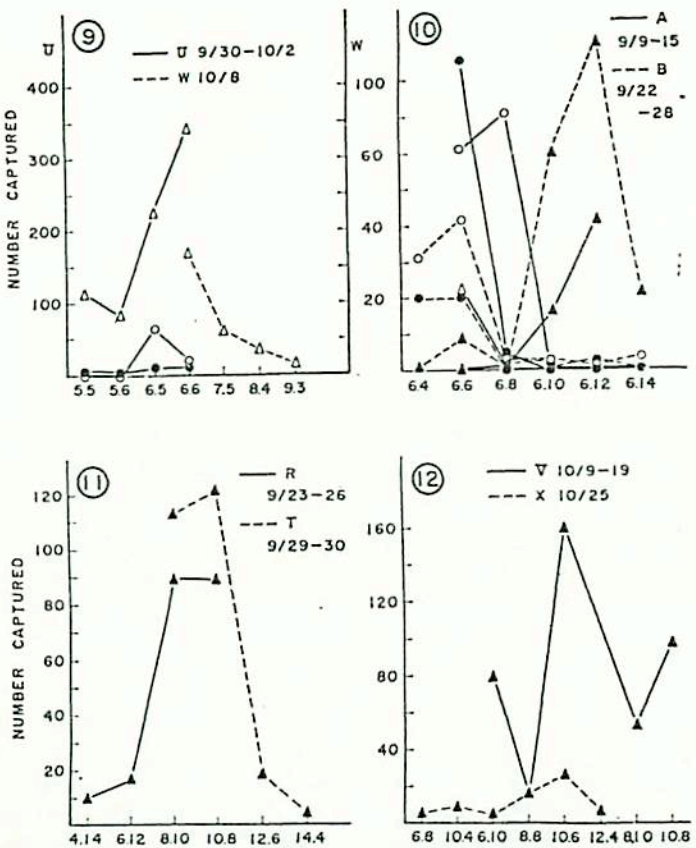

Fig. 9—12. Numbers (ordinate) of *Neophyllomyza* sp. (●), *Siphonella* sp. (○), *Conioscinella* sp. (×), *Forcipomyia* sp. A (△) and *Forcipomyia* sp. B (▲) captured on and around cylindrical filter-paper traps impregnated with aliphatic, straight chain esters with a general constitution: k.m (abscissa), an ester of C_k alcohol with C_m acid.

Neophyllomyza sp. and *Forcipomyia* sp. B, were compared (Fig. 10, test in 1970); the chain length selected by *Neophyllomyza* sp. was C_{10}–C_{12}, somewhat longer for *Siphonella* sp. and still longer for *Forcipomyia* sp. B, respectively.

The activities of C_{18} (carbon number favored by *Forcipomyia* sp. B) comprising the functional group shifting along the main carbon chain were compared (Fig. 12); 8.10–10.8 esters were selected clearly.

Representative members of C_{14-18} esters were compared in Fig. 12; 10.6–10.8 esters were preferred by *Forcipomyia* sp. B.

DISCUSSION

An overall analysis of the results revealed some noteworthy aspects of the interaction between the chemical and biological species.

First, the optimal chain length for each insect species was determined by simultaneously exposing homologues differing by a C_1 or C_2 unit; the optimal length for *Neophyllomyza* sp. are C_{10} from Figs. 3 and 5 and C_{11} from Fig. 6; for *Siphonella* sp., C_{11} from Figs. 7 and 9 and C_{12} from Figs. 3, 5 and 7, although C_{10}, C_{11} and C_{12} were not distinguished well in Run N of Fig. 6; for *Forcipomyia* sp. A, C_{11} from Figs. 6 and 7 and C_{12} from Fig. 9; and for *Forcipomyia* sp. B, C_{16-18} in reference to Fig. 12 together with Figs. 10 and 11. Although the number of captures and repetition of runs were not sufficient for *Conioscinella* sp., the optimal length may be estimated to be about C_{10} from Run J of Fig. 5.

The second noteworthy point is the ability of insects to distinguish the position of the ester group in molecules of the same legnth. In the runs of Fig. 2 performed with C_{12} esters, *Neophyllomyza* sp. gave a definite choice to 6.6, but were not responsive to 8.4 which was, on the contrary, favored by *Siphonella* sp.; in the runs of Fig. 4 using C_{10} esters, the former species selected 6.4–7.3–8.2, while the latter was much less sensitive to 8.2, even though responsive to 6.4–7.3; *Forcipomyia* sp. A, also favoring approximately the same chain lengths as the above two species, was indifferent to both 8.2 and 8.4 and showed a preference for the more symmetrical forms, i.e. 6.4 and 6.5 as shown in Fig. 6; *Forcipomyia* sp. B showed responses to both 8.10 and 10.8 among the C_{18} as indicated in Fig. 11.

The diversity of activity of these compounds dependent on molecular size and shape as revealed in the above analysis seemed to be a new additional expreimental verification of the site-fitting concept of olfaction well documented by Amoore et al. (1969), Klopping and Meade (1971), Tai et al. (1971), Roelofs an Comeau (1971) and Boch and Shearer (1971).

It might deserve attention to mention that the paired compounds are related by exchanging carbon and oxygen atoms next to a carbonyl group and thus similar contours of molecules are precisely discriminated by the insects: *Neophyllomyza* sp. preferred 6.6 to 4.8 (Fig. 2, Run F), 6.4 to 2.8 (Fig. 4, Run I); *Siphonella* sp., 8.4 to 2.10 (Fig. 2, Run E) and 6.6 to 4.8 (Fig. 2, Run F); and, *Forcipomyia* sp. B, 10.8 to 6.12 (Fig. 11, Run R). Such a significant effect of the position of –O– in respect to –C– on the activity suggests that some physical and/or chemical interaction between

a possible receptor site and an attractant molecule, which has successfully landed there by virtue of its well fitted complementarity to the receptor face, must intervene to

trigger effective impulses, as has been referred to by KAFKA (1970) in his comprehensive discussion; in this stage of interaction, the vibration of molecules, particularly in the far infra-red region as proposed by WRIGHT (1963) or conformational distortion of the receptor surface might play a role. For *Neophyllomyza* sp., the decrease in activity from 6.4 to 6.6, from 6.4 to 6.5 and from 6.4 to 6.3 is moderate as compared with that from 6.4 to 8.4, from 6.4 to 7.4 and from 6.4 to 5.4 respectively, when read from Runs G (Fig. 3) and K (Fig. 5), Run N (Fig. 6) and Run P (Fig. 6). This would implicate the alcohol moiety as the more important in alluring ability. On the other hand, for *Siphonella* sp., the acid residue seems to be more significant as indicated by a decrease of activity from 8.4 to 6.4 and from 8.4 to 7.4 less than that from 8.4 to 8.2 and from 8.4 to 8.3 in Runs G and H (Fig. 3) and Run S (Fig. 7) as well as the activities of 8.4, 6.4 and 6.6, decreasing in this order (Fig. 5, Run K). Interestingly, of the two kinds of insects highly sensitive to 6.4, *Neophyllomyza* sp. became indifferent to 8.4 which had been extended in alcohol chain length but was favored by *Siphonella* sp., while in turn the latter species became insensitive to 8.2 that had been shortened in its acid chain length but was favored by the former species. Also, for *Forcipomyia* spp., the alcohol moiety is more important, as judged by decreasing orders of activity: for A, 6.5, 6.4 and 7.4 (Fig. 6, Run N) and 6.6, 6.5 and 5.6 (Fig. 9, Run U); and for B, 10.6, 10.8 and 12.6 (Fig. 11, Run T and Fig. 12, Run V).

A similar trend may be found in the following works: TASHIRO et al. (1964) showed the acid residue to be essential for the attractancy of butyl sorbat in the European chafer. The activity of octyl butyrate to *Vespula* spp., more tolerant to the change in alcohol moiety than that in acid moiety is revealed by the analysis of the data by MCGOVERN et al. (1970). ROELOFS and COMEAU (1971) pointed out the acetyl group to be essential and the alcohol moiety to be somewhat variable in their discussion on synergists for RiBLuRe. Also, BLUM et al. (1971) showed that shortening of the longer alkyl chain by a C_1 unit does not impair the ant alarm-releasing activity of natural 4-methyl-3 heptanone as contrasted to a marked decrease in activity following a similar shortening in the shorter alkyl side. Our results likewise indicate that the activity of ester homologues is more closely related not only with the acid moiety, as was shown in the response of *Siphonella* sp., but also with the alcohol moiety as induced from the above analysis of the responses of *Neophyllomyza* sp. and *Forcipomyia* spp. Compounds, even if optimal in total carbon number, frequently succumb in activity to congeners, though next to optimum in the total chain length but more suited in the length of the more important moiety; the activity may be dominated by the length of the more important moiety no less than by the total chain length. Furthermore, it is speculated that access of the ester group and the terminal methyl group of the more important moiety to the corresponding active sites of a receptor would be a necessary (but not sufficient) condition to elicit a response in insects, even if the physiological events following such a favorable orientation of an attractant molecule over the receptor surface should be another problem.

Finally, the molecules of choice for each insect, varying from one species to another lead us to imagine for the odor receptor, like a variety of morphological traits characterizing insect species, a macromolecular structure under genic control, as has been indicated experimentally by formalin denaturation by RIDDIFORD (1970). It is expected as well that the above mentioned aspects of the interaction between aliphatic esters with the simplest constitution and the Diptera species remote from human life

would be tenable in still other combinations of chemicals and insects.

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