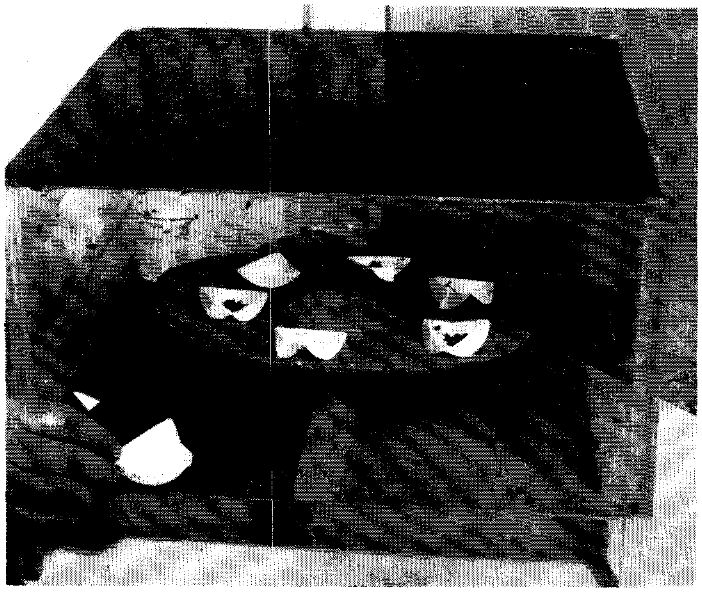
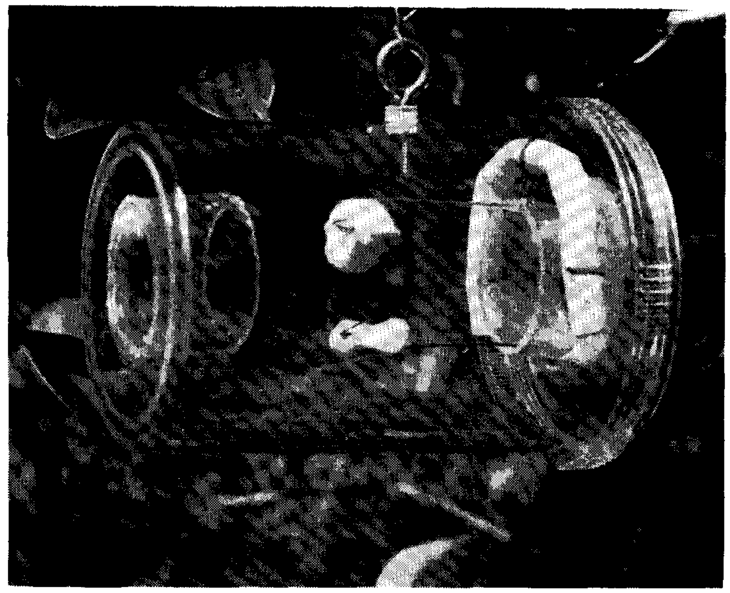




Insect Repellents and Attractants

STANLEY A. HALL, NATHAN GREEN, and MORTON BEROZA,
Entomology Research Division, Agricultural Research Service, USDA

Despite the past year's strides in synthetic repellents and attractants, only the surfaces of many complex phenomena have been scratched

BASICALLY there are two chemical approaches in searching for insect repellents and attractants: the empirical approach, which involves the testing of a wide variety of compounds to develop leads; and the classical approach, which is aimed at isolating in pure form a naturally occurring repellent or attractant, determining its structure, and ultimately synthesizing it or a biologically active analog. Both approaches have been pursued at the Agricultural Research Center, Beltsville, Md.

During the last 15 years several thousand compounds have been synthesized at Beltsville as candidate insect repellents. These compounds were screened against mosquitoes, biting flies, chiggers, fleas, ticks, and disease-carrying arthropods at USDA's Orlando, Fla., laboratories where such insects are reared for this purpose.

The synthesis program started with completely random screening. As structural leads were uncovered and related compounds synthesized, a much higher percentage of biologically active compounds invariably resulted than was obtained by screening a random assortment of chemicals.

As the synthesis and screening program developed, it was found necessary to organize the chemical information on the compounds (e.g., structure, physical properties) and the biological results. McBee Keysort punched cards were adopted for this purpose. Chemical and biological data are recorded on these cards so that a chemical feature may be related to an entomological one. For example, the aliphatic diols may be quickly sorted from stacks of cards by passing the sorting pick through punch 28. The proportion of diols giving good repellents can be determined by sorting the diol cards for class 4 repellents. Insecticides, miticides, and synergists, as well as repellents and attractants, are handled under this system.

References in the literature to repellents and attractants include a multitude of materials. There is frequent mention of poison baits composed of a food attractant mixed with a toxicant. The food may be cornstarch, sugar, molasses, honey, bran or corn, or various other grains. The toxicants that have been used, usually stomach poisons, range from the older inorganic arsenicals, Paris green, sodium fluosilicate, thallium sulfate, and formalin to the newer organic phosphorus and chlorinated hydrocarbon insecticides.

The term "repellent" has been used to include kerosine, creosote, pyridine, certain essential oils, lime-sulfur, Bordeaux mixture, and even lindane or DDT for certain insects. In the older literature, references are made to the use of camel's urine, mixtures of tar and red earth, smoke from wood fires, burning hemp, punk, or joss sticks to ward off insects. The now obsolete odoriferous substances—such as oil of citronella, pennyroyal, cedar, eucalyptus, as well as camphor and cresol—do possess a limited repellency to certain mosquitoes, but the effect in most cases is short-lived.

Attention is now focused on the newer synthetics that have proved so effective in protecting man from biting arthropods and those that are being used for attracting certain species of fruit flies. Repellents for agricultural insect pests are not considered here.

The Search for a Good Repellent

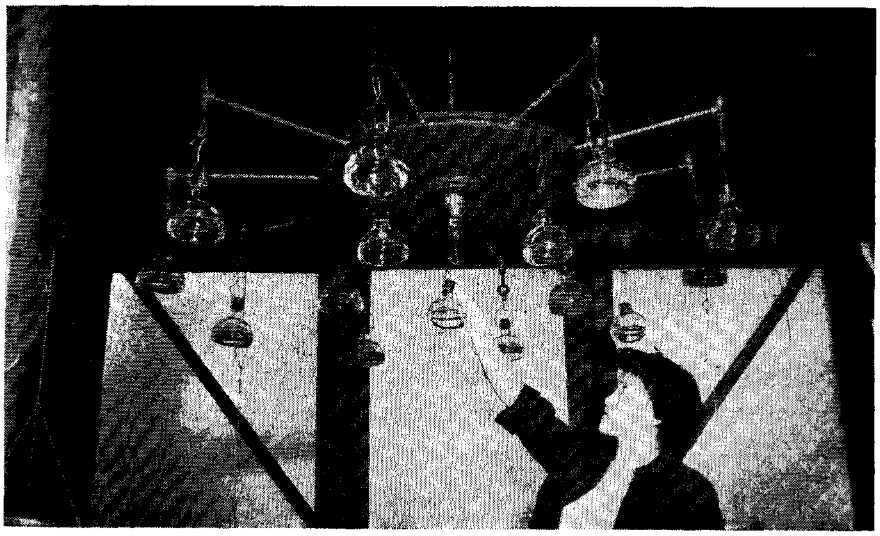
At the start of World War II it became evident that intensive research would have to be undertaken to combat disease-carrying insects if our armed forces were to operate efficiently in the areas of the globe known

Left above: A turntable repellent tester cage. Right: Plastic trap with attractant and toxicant on cotton wick, used in the Florida Medfly campaign

to be infested with malaria-carrying mosquitoes, typhus-carrying body lice, and other arthropod carriers of disease. A crash program was initiated in April 1942 by the then Bureau of Entomology and Plant Quarantine at its Orlando, Fla., laboratory, through a transfer of funds from the Department of Defense (*27*). Thousands of compounds were collected from (or synthesized in) the Beltsville laboratories, several cooperating universities, and industrial sources, for screening against certain test insects. This program comprised the largest systematic screening of compounds against insects ever undertaken. Forty-five different types of tests were carried out against 15 species (*13*).

Since no reliable methods for testing repellency were available at the outset of the investigation, it was necessary to devise procedures to screen large numbers of compounds. These procedures have been described by King (*13*) and by Travis (*25*). Up to the present time more than 16,000 compounds have been tested. A screening report giving results on about 11,000 compounds was issued in 1954 (*13*).

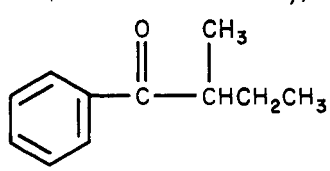
Of the many effective compounds found in this screening program, very few could be put to practical use because of the stringent requirements for a repellent that is to be used on human skin or applied to clothing. It must be nontoxic on topical application, and stable to sunlight. It must be nonirritating to the skin, and nonallergenic. It must be nonstaining, odorless (or possessed of a mild, not-unpleasant odor), and long-lasting in effectiveness against a broad spectrum of mosquitoes and other biting insects. It must withstand copious sweating conditions. A good repellent for use on skin should not wipe off readily. A repellent for application to clothing should preferably withstand laundering. It is also desirable that a repellent not attack plastics commonly used in eyeglass frames, pencils, watch crystals, and similar articles.



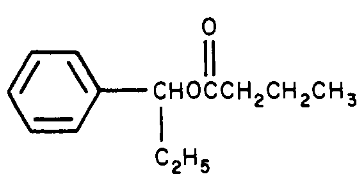
In Hawaii, lures are screened by exposing their water emulsions in small invaginated glass traps in an outdoor olfactometer cage stocked with fruit flies

Attractant Structural Types

Esters (Mediterranean fruit fly)

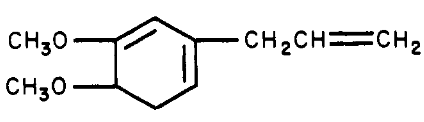


sec-Butyl 6-methyl-3-cyclohexene-1-carboxylate

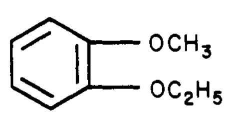


a-Ethylbenzyl butyrate

Alkoxybenzenes (Oriental fruit fly)

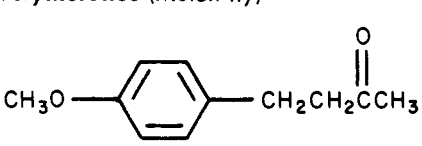


Methyl eugenol

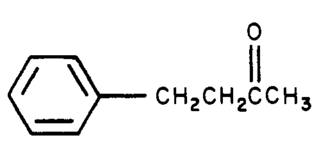


1-Ethoxy-2-methoxybenzene

Arylketones (Melon fly)



Anisylacetone



Benzylacetone

Most of the compounds that the screening report (*13*) indicated to be good repellents were subsequently found to fail in one or more requirements, the biggest stumbling block being mammalian toxicity. Smith (*18*) states that less than a score of the many compounds tested proved highly effective and also safe for unlimited application to the skin.

It was soon found that there were a number of compounds with little or no odor that were far superior to the older odoriferous repellents. One of the first of these was butyl carbitol acetate. This was marketed until the Food and Drug Administration ruled that it was not safe and it was withdrawn. Safe repellents among the earlier discoveries included dimethyl phthalate, 2-ethyl-1,3-hexanediol, Indalone (butyl 3,4-dihydro-2,2-dimethyl-4-oxo-2*H*-pyran-6-carboxylate), and dimethyl carbate (dimethyl *cis*-bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylate), but they were each effective against a limited number of insect species. In order to obtain a repellent that would protect against a maximum number of species, mixtures were formulated (*19*). Thus, the widely used "6-2-2" skin repellent (*26*) was recommended for military personnel. It had the following composition:

	% by weight
Dimethyl phthalate	60
Indalone	20
2-Ethyl-1,3-hexanediol	20

Later the following skin repellent mixtures were used:

M-2020	*% by weight*
Dimethyl phthalate	40
2-Ethyl-1,3-hexanediol	30
Dimethyl carbate	30

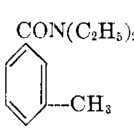
M-2043	*% by weight*
Dimethyl phthalate	40
2-Ethyl-1,3-hexanediol	30
Propyl-*N*,*N*-diethylsuccinamate	30

In the Pacific area during World War II troops needed protection against certain mites that transmit scrub typhus. A nontoxic miticide was sought—one which could be impregnated in uniforms and withstand considerable exposure to sunlight and to laundering. One of the best materials for this purpose was benzyl benzoate. It was subsequently incorporated in the clothing impregnant called M-1960 which was successfully used to impregnate army uniforms during the Korean action. This emulsifiable concentrate had the following composition:

	% *by weight*
Benzyl benzoate	30
N-Butylacetanilide	30
2-Butyl-2-ethyl-1,3-propanediol	30
Emulsifier (Tween 80)	10

The benzyl benzoate protected against mites, the *N*-butylacetanilide against ticks, and 2-butyl-2-ethyl-1,3-propanediol against mosquitoes. Clothing impregnated at the rate of 2 g./sq. ft. was rendered highly repellent for several weeks depending upon environment. M-1960 was also very effective in repelling land leeches in Borneo (*27*).

A statistical study of 4308 chemicals, tested at Orlando as repellents, was made by Travis (*24*) to see which types of molecular structure were associated with repellency. This study revealed that most of the compounds effective for three hours or more against the *Aedes aegypti* mosquito came from four classes: amides, alcohols, esters, and, to a lesser extent, ethers. The incidence of good repellents among the hydrocarbons tested was strikingly low. It was observed that practically all 1,3-diols possessed some degree of repellency. Certain hydroxy esters were also found to be repellent (*5*, *15*). These findings served as a guide for further research. The *N*,*N*-dialkyl amides were selected as being especially worthy of further exploration and for several years synthesis work at Beltsville was steered in this direction. This work culminated in the discovery of the best single insect repellent known, *N*,*N*-diethyl-*m*-toluamide (*8*, *17*):

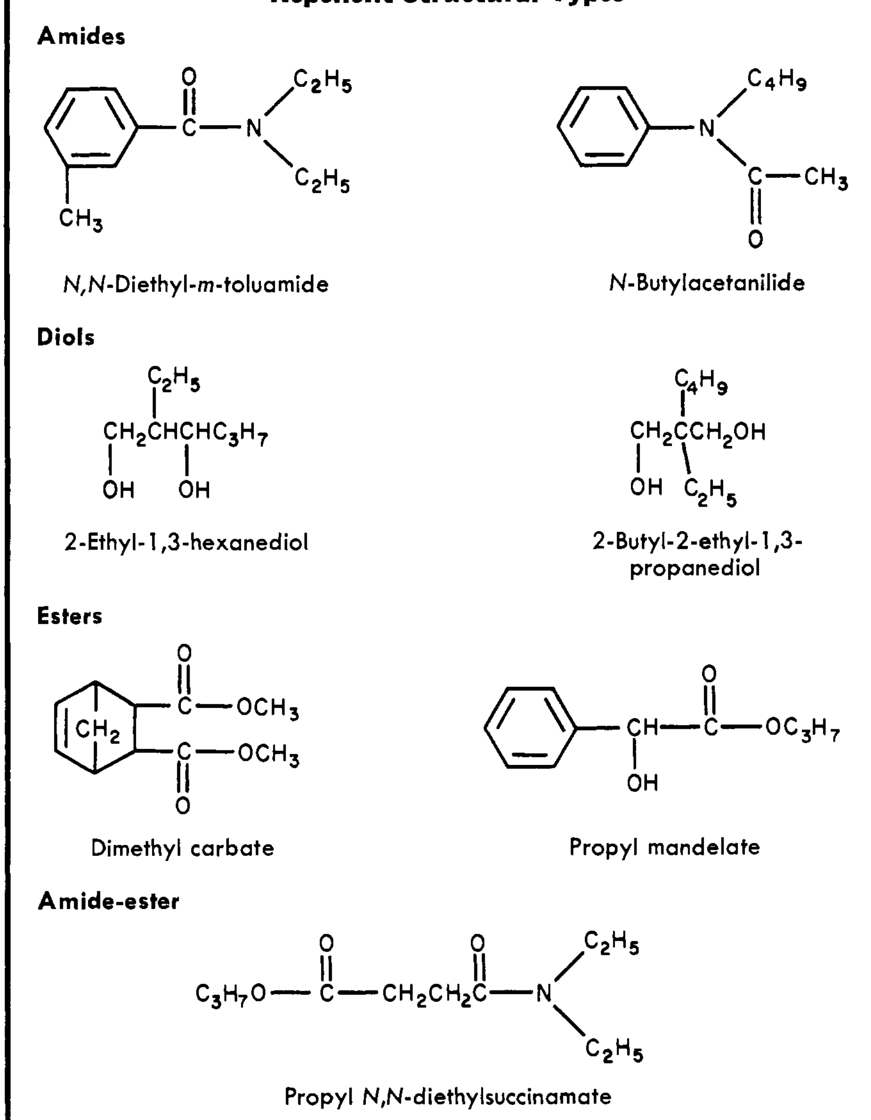


This repellent, commonly called diethyltoluamide, is effective against a wide range of insects that attack man, including mosquitoes, fleas, chiggers, ticks, and biting flies, for a longer time than any other material now known. When applied to the skin at full strength it repels most species of mosquitoes for three to eight hours. The period of effectiveness against deer flies is somewhat shorter. When applied on clothing, diethyltoluamide provides protection against chiggers, ticks, and fleas for several days or more. It is notably resistant to removal by wiping and it persists even under sweating conditions. It has good properties from a cosmetic standpoint, not being oily and possessing a slight, not-unpleasant odor. It is safe to use on skin and clothing when applied according to instructions. Like all repellents, however, it may cause irritation if it gets into the eyes. It is generally nonirritating to the skin, but under severe sweating conditions a slight redness may appear temporarily. However, diethyltoluamide is not recommended for use on pets, livestock, or plants.

Unquestionably many complex phenomena are involved in repelling or attracting insects. An attractant must represent to an insect something needed or desired—e.g., food, the opposite sex, or an ovipositional host; a repellent represents something to be avoided. Although we do not yet understand the fundamental mechanisms of repellency or attractancy, it is clearly evident that chemistry plays an important role in this field. A repellent should keep away a maximum number of insect species; an attractant should be specific for one species. Both should be effective for long periods.

One of the striking properties of repellents is their short range; a flying insect must approach a repellent chemical closely or under certain conditions even touch it before being repelled. In other words, a repellent must cover the surface of the host it is protecting. Skin in an uncovered

Repellent Structural Types



area will be sought out and penetrated by a blood-thirsty mosquito. Experiments by Travis (*23*) using impregnated nets of different mesh have shown that even a very effective repellent does not act at a distance of more than a few centimeters at most.

Such is decidedly not the case with attractants. The effects of a good insect attractant are evident at a considerable distance. This is especially true of sex attractants.

Attractants serve two main purposes: to survey insect populations, and to control insects by being mixed with a toxicant. Traps baited with an attractant may be set out to detect the presence of certain insects or, should insects be known to be present, to follow the progress made in control programs. It is highly desirable that these attractants should lure only one or two species; a nondiscriminating attractant results in a tedious job of sorting out the desired insects from many species, and the task becomes too time-consuming. If a control program is aimed at eradication, survey traps help attain this goal. The value of using an attractant plus a toxicant as a control measure is obvious.

STANLEY A. HALL, director of Pesticide Chemicals Research Laboratories, Entomology Research Division, ARS, Beltsville, Md. Mr. Hall received his B.S. from Columbia's school of engineering (1931) and a B.S. cum laude in chemistry from Brooklyn Poly. (1939). In 1939 he joined USDA, and since 1943 has been with the Division, where he was in charge of chemical investigations on synthetic insecticides, attractants, and repellents before promotion to his present position in 1956.

NATHAN GREEN, chemist for the Pesticide Chemicals Research Laboratories, Entomology Research Division, ARS, Beltsville, Md. Mr. Green received his B.S. degree from George Washington University in 1944. He was employed at the National Bureau of Standards from 1935 until 1944, when he joined the Entomology Research Division. There his chief field of interest has been synthesis of insecticides and insect attractants.

MORTON BEROZA, head of Synthesis Investigations, Pesticide Chemicals Research Laboratories, Entomology Research Division, ARS, Beltsville, Md. Dr. Beroza received his B.S. degree from George Washington University (1943), and the M.S. and Ph.D. in 1946 and 1950 from Georgetown University. He joined USDA in 1948. His fields of interest have included isolation and determination of structure of insecticidal constituents from plants, methods of analysis, and synthesis of insecticides, insect repellents, and attractants.

Sex Attractants

The spatial (distance) effect of the sex attractants is in many cases striking. The female gypsy moth [*Porthetria dispar* (L.)] does not fly, but the male is a strong flier and is able to detect through its sensitive antennae a minute amount of sex attractant given off by the female. Collins and Potts (*6*) reported that this natural sex attractant lured males from more than two miles downwind.

Acree (*1, 2, 3, 11*) spent several years at Beltsville in isolating and attempting to characterize chemically the sex attractant that he extracted from the abdominal tips of virgin females of the gypsy moth. This type of study illustrates the classical approach mentioned earlier. Acree (*3*) succeeded in isolating a very potent fraction to which he gave the name gyptol. This investigation was discontinued for a time but was recently reactivated in the hope of isolating the sex attractant in pure form and determining its structure.

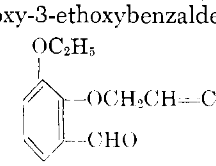
Hecker (*12*) in Germany has followed Acree's methods in a similar attempt to isolate the sex attractant from the abdominal tips of female silkworm moths [*Bombyx mori* (L.)]. Makino *et al.* (*16*), working independently in Japan on this problem, obtained from the unsaponifiable portion of an alcoholic extract of female silkworms a viscous, faintly yellowish oil distilling at 100° to 110° C. at 0.06 mm. of mercury. The Japanese chemists tried to purify this yellow oil through ascending paper strip chromatography. The paper was passed slowly in front of a male moth, and when the spot of the sex attractant on the strip was approached, the moth became excited, shaking his wings violently. The Japanese investigators reported that they could get such a response from as little as 3×10^{-5} γ of material.

Hecker reported that as little as 10^{-6} γ of his most potent fraction on a glass rod would excite 10 out of 20 caged male silkworm moths. He calculated that a definite response could be obtained from only 1000 to 10,000 molecules! One would not hesitate to say that sex attractants are among the most fantastically potent biologically active materials ever discovered.

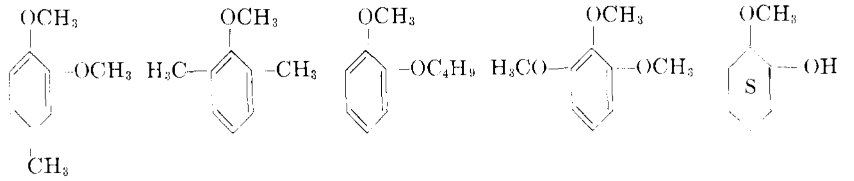
And yet we know next to nothing about their chemistry. Makino and coworkers reported that there is probably a primary hydroxyl group in their silkworm sex attractant. Hecker stated that the sex attractant is a molecule of 10 or 12 carbon atoms with at least two conjugated double bonds, and that there is a primary or secondary hydroxyl group. Acree's work on gyptol indicates that it may be an alcohol, possibly a diol, with one of the hydroxyl groups esterified by a long-chain fatty acid. That sums up about all that is known about these interesting materials at present. Certainly much more will be learned in the coming years, and it will be the first revelation of the chemistry of nature's sex attractants.

We do know of some simple compounds having the properties of sex attractants; however, they were not isolated from female insects, but were discovered by screening organic compounds. The entomological literature of the past 50 years contains a number of scattered references to attractants found in this manner. As an example, Lehman (*14*) 25 years ago reported on some lures for click beetles, which are adults of the Pacific Coast wireworm [*Limonius canus* (Lec.)]. He screened 146 materials using an olfactometer that he constructed for this purpose. Most of the compounds were simple esters, alcohols, or acids, but about 25 essential oils also were included. He found some strong sex attractants among the aliphatic acids. Caproic acid, which was the most potent, caused the males to attempt copulation. It seems quite remarkable that compounds having this type of biological activity can be found by this purely empirical method.

Some chemicals have been found to attract preponderantly the males of several species of fruit flies, but whether or not they are true sex attractants is a moot question. Methyl eugenol is the most powerful known attractant for the male oriental fruit fly [*Dacus dorsalis* Hendel] (*20*), being effective one half mile or more downwind. This compound is sought out and so greedily devoured by the male insects that they will, if permitted, engorge themselves until they die. We have found that some compounds attract both sexes; one such is 2-allyloxy-3-ethoxybenzaldehyde:

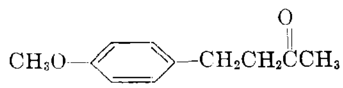


An uncanny ability to detect dialkoxybenzenes seems to be possessed by the males of this species. They are especially attracted to aromatic molecules containing one or more methoxy groups, such as the following:



Each of the methoxybenzenes has a different odor; yet the insect responds to all of them.

Indeed, there is evidence in support of the theory that the compound's chemical structure rather than its odor (as perceived by humans) is what counts. Thus, Barthel *et al.* (*4*) discovered that a strong attractant for the male melon fly [*Dacus cucurbitae* Coq.] exists in anisylacetone:



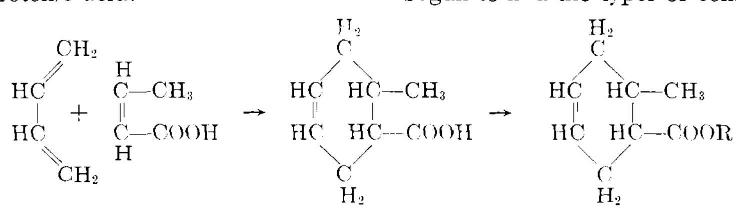
In preparing analogs they found that one could eliminate the *p*-methoxy group, changing the odor, and still retain a good measure of the attractancy. Just as methoxy groups and dialkoxybenzenes are detected by the oriental fruit fly, the butanone side chain seems to be detected by the melon fly. Anisylacetone was used effectively in southern California in the summer of 1956 to scout for a suspected melon fly infestation.

Perhaps the greatest public interest in attractants was aroused last year in connection with the Mediterranean fruit fly, or Medfly [*Ceratitis capitata* (Wied.)]. Angelica seed oil played a vital role in the intensive program to eradicate this serious pest from Florida. The attractancy of this oil to the males was discovered by Steiner *et al.* (*21*) at USDA's Honolulu laboratory. This discovery came at a most fortunate time, just prior to the finding of the Medfly infestation in Florida. The oil was put to immediate use for survey purposes.

Angelica seed oil is a product of a biennial plant that is cultivated in Belgium and to a lesser extent in other countries. Guenther (*10*) has described its culture and the extraction and properties of the oil. The annual production of the oil has been reported to be only about 600 pounds, a quantity insufficient to meet the needs of the Florida eradication program. As supplies dwindled the price of the oil rose from \$56 to about \$250 a pound. The identity of the attractant in the oil is not known, but it is believed to be a sesquiterpene, which constitutes less than 5% by weight of the total oil. A rapid empirical color test was devised (*9*) to predict whether a given lot of angelica seed oil will attract Medflies; the intensity of the blue color was found to be roughly proportional to attractancy.

Aside from its scarcity and high cost, angelica seed oil has other disadvantages. Since it is a natural material, its composition is variable, and its adulteration is difficult to detect. Oddly enough, freshly prepared samples are less attractive than aged ones, the best thus far encountered being an 18-year-old product that was much darker and more viscous than the freshly prepared oil.

Keeping 50,000 survey traps in Florida supplied with attractant completely exhausted all stocks of angelica seed oil by December 1956. Substitute concoctions made up by the perfumery trade, some of which closely resembled angelica seed oil in odor, were not attractive to the Medfly. In the meantime the synthesis program at Beltsville, closely coordinated with a screening program on fruit flies in Hawaii, led to the discovery by Gertler *et al.* (*7*) of a series of attractants for the male Medfly. These were esters of 6-methyl-3-cyclohexene-1-carboxylic acid, which is made by a Diels-Alder condensation of butadiene and crotonic acid:



The attractants in increasing order of effectiveness are the propyl, isopropyl, and secondary butyl esters. The isopropyl ester was the first produced in commercial quantities to replace angelica seed oil for the traps in Florida. Subsequent field tests in Hawaii pointed to the superiority of the secondary butyl ester, which is now in production. The field data indicate that this ester is longer lasting than the average sample of angelica seed oil. It is possible to vary the alcohol moiety considerably and yet retain most of the attractancy, but most of the modifications of the acid thus far have given an inferior product. As an example, the corresponding esters of *o*-toluic and cyclohexanecarboxylic acids are only weak attractants for the Medfly.

We are now looking for an attractant for the Mexican fruit fly [*Anastrepha ludens* (Loew)], which is a serious threat to citrus in Texas and California. So far we have selected or synthesized about 1500 compounds for sending to our Mexico City laboratory to be screened in an olfactometer for this species. Thus far only one compound, diacetyl, has caused the Mexican fruit fly to become excited—although unfortunately not attracted. However, we are turning up some structural leads on an occasional compound that is feebly attractive to this species. By synthesizing related compounds we hope to build a molecule that is a good attractant for this pest.

On a smaller scale we have begun to screen compounds as attractants for the boll weevil [*Anthonomus grandis* (Boh.)], the pink boolworm [*Pectinophora gossypiella* (Saund.)], the gypsy moth [*Porthetria dispar* (L.)], the European chafer [*Amphimallon majalis* (Raz.)], and the hickory shuckworm [*Laspeyresia caryona* (Fitch)] which attacks pecans.

In our studies of attractants we have made one observation that is most encouraging: compounds that are structurally related to a strong attractant usually show some degree of attractancy. This principle does not seem to hold in the case of insecticides. It should, therefore, be easier to find attractants by the empirical approach than it is to find insecticides.

While the pest control chemicals obtained as a result of the screening program are helping to solve some of our insect problems, we have hardly begun to find the types of compounds that we urgently need. It is now common knowledge that we are faced with very serious problems arising from insect resistance to insecticides and we must find new ways to combat insects if we are ultimately to prevail over them. Attractants and repellents may play an important role in this quest. The surface has only been scratched.

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