Chapter 1

GENERAL INTRODUCTION

Introduction
Chemicals play an important role in communication between insects. Chemicals that mediate interactions between organisms (inter- or intraspecific) are called semiochemicals. They can be divided into two major groups: allelochemics and pheromones. Allelochemics are chemicals that are secreted by an organism and cause a reaction in a receiving organism of a different species. Pheromones are substances secreted by an organism that cause a specific reaction in a receiving organism of the same species. Pheromones can be classified on the basis of the kind of behaviour they evoke, such as sexual, oviposition, aggregation, dispersion or alarm behaviour (Hendrikse, 1990).

Since Butenandt et al. (1959) identified trans-10, cis-12-hexadecadienol (bombykol) as the female sex pheromone of the silkworm moth *Bombyx mori*, sex pheromones of hundreds of insect species have been identified.

In Diptera, pheromones are often alkenes with a double bond Z-configuration at an odd position. For example, the female sex pheromones of *Drosophila melanogaster*, *Fannia canicularis*, *F. femoralis* and *F. pusio* are (Z,Z)-7,11-heptacosadiene (Antony and Jallon, 1982), (Z)-9-pentacosene, (Z)-11-hentriacosene and (Z)-11-hentriacosene (Uebel et al., 1977, 1978) respectively. In *Musca autumnalis*, however, apart from (Z)-13-nonacosene and (Z)-13-heptacosene also (Z)-14-nonacosene with the double bond at an even position is a component of the sex pheromone (Uebel et al., 1975). It became apparent that these sex pheromones could play an important role in the control of insects.

This thesis focuses on the role semiochemicals play in the behaviour of the
housefly *Musca domestica* L. Differences are described between the cuticular hydrocarbon composition of laboratory and wild-type strains. The reasons for these differences are investigated.

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**Biology of the housefly**

*Musca domestica* L. (Diptera: Muscidae), the common housefly, is one of the most widespread fly species in the world. The insects belong to a group of domestic flies often called “filth flies”. They can be found at almost every place where people live. They have adapted their lifestyle to the human lifestyle by using waste products of human communities to live on and breed in. They can breed in animal faeces, garbage, rotting fruits and vegetables, and in other decomposing organic material. In addition, everything people eat seems to be interesting to houseflies as well. Consequently, houseflies are a nuisance in human and livestock habitations (West, 1951). Moreover, they may be responsible for the transmission of over 100 different pathogens (Pospischil, 1994). They may transmit intestinal worms, or their eggs, and are potential vectors of pathogens of dysentery, gastroenteritis, typhoid, cholera, foot and mouth disease and tuberculosis.

Losses caused by *M. domestica* in poultry houses were reported to be in excess of 60 million US dollars per year in the United States (Anonymous, 1976). High population densities of *M. domestica* in poultry units may not only cause irritation and annoyance to employees but may also considerably reduce egg production (Miller *et al.*, 1993).

The adult fly is about 6-8 mm long. It has a single pair of membranous wings. The hind wings have been modified to balancing organs, the halteres. The fly’s thorax is grey with 4 longitudinal dark stripes. Prominent parts of the head are the non-biting suctorial mouth parts (proboscis) and large compound eyes. The female is usually bigger and has more space between the eyes than the male.

Houseflies are sexually mature 2-3 days after emergence. Females only mate once, whereas males try to mate with several females. A female lays 5-6 batches of about 90 eggs during her lifetime. The eggs hatch 12-24 hours after oviposition. The larvae (maggots), which are yellowish white, reach a length of 8-11 mm in about a
week, the ultimate size depending on the quantity and quality of the food substrate. Then, the larvae pupate and after 7-10 days the flies emerge from the pupae. At 25 °C, the entire development from egg to adult is completed in 14-18 days. As a consequence, several generations can occur during the period of one summer. In our winter houseflies can hibernate as pupae or adults. However, in (sub)tropical countries and in warm environments houseflies remain active and reproduce throughout the year. Organic waste materials and the relatively high temperatures at livestock farms promote rapid development and the continuous presence of flies (Howard and Wall, 1996).

**Pheromones of houseflies**

The presence of a sex pheromone on female houseflies which induces courtship behaviour in males was first reported by Rogoff et al. (1964). Studies of Carlson et al. (1971) led to the conclusion that this pheromone consists of (Z)-9-tricosene (“muscalure”). Since then, many scientists concentrated their research on several aspects of (Z)-9-tricosene, including its biosynthesis and behavioural functions. Dillwith and Blomquist (1982) and Dillwith et al. (1986) studied the biosynthesis of (Z)-9-tricosene. Using isolated tissue and radio tracer techniques they demonstrated that the female sex pheromone is synthesized by epidermal tissue. (Z)-9-tricosene formation occurs by elongation of oleoyl-CoA to a 24 carbon fatty acyl moiety, which is then converted to an alkene which is one carbon shorter (Blomquist et al., 1993). The highest elongation activity is in the abdominal epidermal tissue (Vaz et al., 1989).

**Figure 1. Components of the sex pheromone of the female housefly.**
Several oxidation products of (Z)-9-tricosene, such as (Z)-9,10 epoxytricosane and (Z)-14-tricosene-10-one and some methylalkanes were also found on female houseflies (Fig. 1). These substances were shown to enhance male sexual activity in combination with (Z)-9-tricosene (Uebel et al., 1978; Rogoff et al., 1980).

Control of houseflies
Several techniques are used in an attempt to control the flies (reviewed by Howard and Wall, 1996).

Chemical control
Since the forties chlorinated hydrocarbons and organophosphates were used for many years as insecticides. These insecticides were applied to surfaces at which flies prefer to rest. DDT and Lindane, for instance, appeared to be highly effective against adults and larvae of M. domestica and other fly species (Lindquist et al., 1945; Tanada et al., 1950). However, these pesticides are toxic to a large spectrum of animal species, also killing non-target organisms. In addition, these substances cannot be metabolized by the organisms and residuals of pesticides persisted in the environment, entered the food chains and accumulated in the body tissue of non-target organisms, including humans (Lancaster and Simco, 1969; Pimental and Perkins, 1980). A further problem is the development of resistance to the killing power of the insecticides (Pospischill et al., 1996). Since a limited number of genetic factors are involved in the development of resistance, which are not strictly bound to specific molecules, cross-resistance to novel insecticides exists (Plapp, 1986). In 1985, Chapman reported that a strain of M. domestica collected from a farm in England was resistant to 18 toxicants. Nowadays synthetic pyrethroids are used as insecticides that are rather safe for mammals, although they may kill crustaceans and fish (Eliot et al., 1978; Hill, 1985). Moreover, several of these substances are biologically degradable.

In the last decades, the search for chemicals other than insecticides has increased. Insect growth regulators (IGRs) have been developed that do not directly act on the nervous system as conventional insecticides do, but principally affect embryonic, larval and nymph development and thus disrupt metamorphosis and reproduction (Howard and Wall, 1996). These so-called third generation pesticides do not usually kill the target pest immediately. Using these substances it takes longer to reduce insect populations than with nerve insecticides. IGRs show some selectivity
(Myamoto et al., 1993) and can be divided into three categories: juvenile hormones, chitin synthesis inhibitors and ‘others’ (Howard and Wall, 1996). Widespread resistance against IGRs, however, also develops (Silhacek et al., 1976; Pap and Farkas, 1994).

**Biological control**

Besides insecticides and IGRs considerable attention has been given to biological control of flies. Especially in livestock units predators, parasitoids and parasites may be used to control fly populations. Renn (1995) studied the mortality of eggs and larvae of houseflies in artificial diet and chicken manure after exposure to encapsulated entomopathogenic nematodes (*Steinernema felitae* and *Heterorhabditis megidis*). It appeared that slow release of the nematodes to the manure may control housefly infestations. However, further investigations are required to determine the optimum application of such a formulation. Renn (1998) also compared the efficacy of the same nematodes with that of the carbamate insecticide methomyl in pig units and showed that nematode baits offer a practical and effective alternative to conventional insecticides for the control of housefly populations in intensive animal units. The nematode *Paraitonchium muscadomesticae* appears to have considerable promise as biological control agent for houseflies. The fly larvae are highly susceptible to infection, resulting in either their death or parasitic castration of the adult fly (Geden, 1977).

Johnson et al. (1998) reported the use of *Bacillus thuringiensis* as a safe and effective way for controlling agricultural pests and especially houseflies. The δ-endotoxins responsible for the activity of the bacteria are members of the so-called Cry IB class of protoxins. These are produced in a few strains of *B. thuringiensis* only.

King (1997) investigated the effectiveness of the parasitoid wasps *Spalangia cameroni* and *Muscidifurax raptor* in controlling fly populations. *S. cameroni* alone appeared to be consistently more effective in killing fly pupae than *M. raptor* alone or than the 2 species combined, regardless of fly age and burial (pupae uncovered or covered with 2 cm of larval medium). Greene et al. (1998) reported about mass-released *Spalangia nigraeena*, which attacks the pupae, for biological control of *M. domestica* in western Kansas. The parasitoid-induced mortality (PIM) varied from 23 to 58 % depending on the parasitoid-to-host ratio.
Watson et al. (1996) used the entomopathogenic fungi *Beauveria bassiana* and *Entomophthora muscae*, concurrently with sawdust bedding, to control the housefly in calf hutches on New York dairy farms. Combined mycoses from both fungi infected 60 and 54% of the fly population at two farms respectively. Kuramoto and Shimazu (1997) introduced a small number of flies infected with *E. muscae* in experimental poultry houses and found that 90% of the initially present flies were killed 33 days after introduction. After introduction of conidia-discharging fly cadavers 90% of the flies were killed within 20 days.

Predator flies may also function in the natural control of fly pests. Farkas and Jantnyik (1992) studied the role of *Hydrotaea aenescens* (*Ophyra aenescens*) as predator of housefly larvae. Second and third instar *Hydrotaea* larvae showed to be effective in an experimental setup. One *Hydrotaea* larva is capable of destroying at least 5 *Musca* larvae during its larval development. Tsankova and Luvchiev (1993) and Luvchiev and Tsankova (1994) reported that second and third instar larvae of *Ophyra capensis* can kill up to 17 housefly larvae depending on the larval instar and culture density. Betke et al. (1989) used *O. aenescens* for the control of *M. domestica* in pig fattening units. The authors stated that introduction of laboratory-raised *O. aenescens* resulted in definite elimination of the *M. domestica* stable population. *O. capensis* and *O. aenescens* can, however, become pests themselves (Axtell and Arends, 1990).

Mullens et al. (1996) studied the effect of *Machrocheles* mites and predacious Coleoptera on the presence of *M. domestica* and *Fannia* species. On 3 southern California caged-layer poultry facilities two manure-handling systems were compared during 2 years: all manure rows were removed, or half of the manure was left undisturbed to conserve a part of the predator population. The authors concluded that the slight increase in fly control as a result of alternate manure removal is overshadowed by the required time and effort involved.

**Sterilization**
The release of sterilized male flies to control fly populations (sterile insect technique; SIT) into a wild population may drive the wild population to extinction and was successfully applied in Libya to eradicate the New World screwworm fly, *Cochliomyia homonivora* (Lindquist et al., 1992).
In general, however, the use of SIT is limited by its expense and logistic complexity and, as is the case with houseflies, the release of huge numbers of the pest around human dwellings, although sterile, would exacerbate the nuisance problem at least for a short while. In addition, sterilized males must be sexually competitive with the naturally occurring males (Howard and Wall, 1996).

Howard and Wall (1996a,b) used the chitin synthesis inhibitor triflumuron for autosterilization of the house fly. Sugar-baited targets with this chemical can be applied to reduce fly population in conjunction with the release of insect predators or parasitiods.

**Light traps**

Many insects are sensitive to UV light with a wavelength of approximately 350 nm. Deay and Taylor (1962) showed that wavelengths between 320-380 nm were most attractive to *M. domestica*. However, Burkhard (1962) and McCann and Arnett (1972) found that there were two peaks in the visual systems of houseflies one of 350 nm and the other around 500 nm. Bellingham and Anderson (1993) even found three spectral peaks flies at 350, 450-550 and 630 nm.

Nowadays light traps (lamps emitting attractive wavelengths) in combination with electric grids which kill the flies) are commonly used for capturing flies. Morgan and Pickens (1968) tested several types of lamps with spectra between 310 and 720 nm at temperatures between 19 and 32 °C for their attractiveness to houseflies. Males were shown to be most responsive to green and orange light at lower temperatures. Females responded best to green, blue and ultraviolet light at 32 °C. According to Syms and Goodman (1987) flickering UV light (100 or 120 Hz) is more attractive to *M. domestica* than non-flickering light. Rutz et al. (1988) evaluated the effectiveness of insect-electrocutor black light devices in cage-layer poultry facilities. The addition of (Z)-9-tricosene (25 to 100 mg/device) increased the total number of flies caught by about 30%. The authors concluded that these devices, particularly when operated with (Z)-9-tricosene, could be an effective component in an integrated fly management programme in poultry facilities. Veal et al. (1995) carried out experiments to compare the efficiency of electrocuting traps in which a green and ultraviolet lamp were combined against traps containing lamps emitting blue and ultraviolet. They found that the green + ultraviolet lamp caught 30% more houseflies than the blue + ultraviolet lamp. However, the authors also concluded that it is hardly possible to
predict the effectiveness of electrocuting traps because many different factors are involved, such as design and siting of the traps, and light wavelengths and brightness.

**The role of (Z)-9-tricosene (muscalure) in controlling *Musca domestica.***

*Laboratory studies*

Rogoff *et al.* (1964) first demonstrated the presence of a sex pheromone on female houseflies. Olfactometers baited with live females or frozen females attracted significantly more males than olfactometers without females or baited with males. Both males and females did not attract females. Female-extract-impregnated pseudo flies (a piece of shoelace) caused sexual excitation in male flies. These results were confirmed by Murvosh *et al.* (1965) and Mayer and Thaggard (1966). The latter authors showed that dead females remained attractive for 8 days.

Carlson *et al.* (1971) were able to isolate, identify and synthesize the female pheromone. This substance, (Z)-9-tricosene, was called muscalure. The attractant was obtained from sexually mature, laboratory-reared, female houseflies by surface washing with hexane or ether. A 50 µg sample of the synthetic (Z) isomer attracted more flies than 200 µg of the (E) isomer. Other cuticular monoolefins (C27 and C29) were weakly active. The authors conclude: “Though not a potent attractant as compared to some sex pheromones, (Z)-9-tricosene is expected to be inexpensive to manufacture and it may have good potential for reducing the amount of insecticide needed to control the ubiquitous housefly.”

Mansingh *et al.* (1972) investigated the effects of a series of (Z)-9-alkenes with 19-25 carbon atoms on the behaviour of male houseflies. It appeared that a large number of these substances showed biological activity. The most potent was a 7:3 mixture of (Z)-9-tricosene and (Z)-9-heneicosene, which induced and maintained high excitement and mating behaviour in most male flies. However, Richter (1974) found that moving dummies loaded with (Z)-9-heneicosene or a mixture of (Z)-9-heneicosene and (Z)-9-tricosene did not induce more mating strikes in males than unloaded moving dummies.

Carlson *et al.* (1974) determined the effects of structural changes of the (Z)-9-tricosene molecule on the activity of male flies in olfactometers. Structural changes included variations in carbon chain length and in the position of the double bond, substitution of double bond by triple bond, cis-trans isomerism and methyl
branching at different points in the molecule. Compounds with double bonds at the 7 and 11 positions and those with a trans configuration rated poorly. The length of the longer chain next to the unsaturated bond seemed to be crucial. The most active compounds contained in their longer chains mostly C14 and some C13 groups. (Z)-9-heneicosene, having a C12 chain, showed low activity. Methyl branching at the second position on the short chain elicited high activity. The finding of Mansingh et al. (1972) that a 7:3 mixture of (Z)-9-tricosene and (Z)-9-heneicosene increased courtship behaviour in males could not be confirmed.

In contrast to Carlson et al. (1974), Uebel et al. (1976) observed little response in male flies to pseudo-flies when these were loaded with (Z)-9-tricosene alone. Unfractionated cuticular hydrocarbon fractions washed from females induced much higher activity (mating strikes) in males. Washings from males were ineffective. Saturated and unsaturated female cuticular hydrocarbons tested separately elicited low responses. However, these substances were combined at a ratio of 65%:35% saturated:unsaturated, induced male activity. When methylheptacosanes and methylnonacosenes were combined with (Z)-9-tricosene male activity strongly increased compared to (Z)-9-tricosene alone. The two compounds producing the highest activity in combination with (Z)-9-tricosene were 4,8-dimethylheptacosene and 13-methylnonacosene.

Rogoff et al. (1980) confirmed the findings of Uebel et al. (1976) that the several synthetics tested, being less active than (Z)-9-tricosene when tested alone, markedly increased male sexual activity when combined with (Z)-9-tricosene. However, they found that this also occurred without (Z)-9-tricosene when three of the materials were mixed together. They conclude that: “It appears that what has been referred to as “the” housefly sex pheromone may actually be a complex mixture of materials.”

Blomquist et al. (1984) showed that on all bodyparts of both male and female houseflies (Z)-9-tricosene is metabolized to an epoxide and ketone. Adams and Holt (1987) found that these substances had different roles in male courtship behaviour. (Z)-9-tricosene increased male mating strike activity towards females and other males. The non-hydrocarbon fraction including both the epoxide and the ketone decreased the number of homosexual mating strikes when (Z)-9-tricosene was present. The authors thus concluded that the non-hydrocarbon fraction contained sex recognition factors. Both the methylalkanes and the non-hydrocarbon fraction increased the number of
copulatory attempts made by males. It was concluded that the methylalkane fraction acted as an arrestant and increased the amount of time spent with a treated model.

La-France et al. (1989) confirmed that (Z)-9-tricosene is the main active component of the sex pheromone produced by the female housefly, initiating striking activity in males. The (Z)-9-alkenes present in the cuticular lipid layer probably act synergistic to (Z)-9-tricosene. The n-alkanes and methylalkanes showed low to medium activity and the addition of (Z)-9-tricosene did not enhance it.

Field studies
Carlson and Beroza (1973) carried out a study on the use of (Z)-9-tricosene as an attractant for *M. domestica* in the field. Panels containing adhesive paper strips and sugar bait in pans and electric grids were used as traps. The addition of (Z)-9-tricosene increased the number of flies caught 3 to 12 times. (Z)-9-tricosene-baited traps caught about equal numbers of males and females. This was in contrast to olfactometer studies in the laboratory in which only males were attracted to (Z)-9-tricosene.

In experiments in open poultry houses, Mitchell et al. (1975) showed that (Z)-9-tricosene-baited traps caught 2-14 times more flies than unbaited traps. The traps were most effective when situated on the ground adjacent to manure. The sex ratios of the flies caught were the same in baited and unbaited traps. Traps containing both (Z)-9-tricosene and toxic substances (pheromone-toxicant devices; PTD’s) were evaluated in the field for control of the housefly by Carlson and Leibold (1981). Traps loaded with both permethrin and (Z)-9-tricosene were used, and their catches were compared with those of traps containing permethrin only. It appeared that the (Z)-9-tricosene treated devices captured 1.4 to 2.0 more flies than the untreated controls. However, the pheromone apparently attracted flies from surrounding areas in such numbers that the flies were not effectively controlled by the toxicant. All (Z)-9-tricosene in the traps had disappeared within 2 to 3 weeks and all permethrin around 60 days after the start of the experiment. Chapman et al. (1998) carried out field trials comparing the effectiveness of toxic targets impregnated with different formulations of (Z)-9-tricosene. Targets baited with (Z)-9-tricosene caught significantly larger numbers of males and females of *M. domestica* than control targets. The introduction of these toxic targets suppressed the density of adult *M. domestica* populations up to 13 weeks.
General conclusions
Control of houseflies requires an integrated pest management approach, considering all available pest control tactics and evaluating the potential interaction among them (Axtell and Arends, 1990). In stables the manure should be kept as dry as possible. The manure should also not be totally removed in a brief span of time. Some old manure should be left to conserve fly predators and parasites. Chemical techniques can be used as a supplement to cultural and biological methods. However, one should exercise due care in applying insecticides. The use of insecticides should be limited to small areas of manure containing high numbers of fly larvae, because most insecticides are toxic to predators and parasites. In addition, there is the risk of the development of resistance to insecticides, even against the degradable pyrethroids and specific IGRs. Finally, a good monitoring system to evaluate re-invasion and to depict the moment of control measures as well as fly dispersal should be incorporated in integrated pest management (IPM).

The role of semiochemicals and particularly (Z)-9-tricosene in the behaviour of houseflies is not unambiguous. Moreover, behavioural studies have almost exclusively been carried out on flies of laboratory cultures. Therefore, if semiochemicals are used for controlling houseflies, one should also focus on possible differences in behaviour between laboratory and wild-type strains.

Outline of the thesis
The present work is part of the project “Environmentally friendly control of houseflies using combined chemical and visual stimuli” which comprises behavioural studies on the responses of Musca domestica to different light sources and olfactory stimuli, electrophysiological studies on the responses of olfactory cells of M. domestica to natural and synthetic volatiles, and studies on the production of cuticular hydrocarbons by M. domestica and the role of these in the behaviour of the flies. The work reported in this thesis focuses on the latter part.

Almost all knowledge on the production of cuticular hydrocarbons and on their role in the behaviour of houseflies has been collected from laboratory strains of houseflies. However, in order to be able to control the flies in their natural environments, studies on wild-type flies are also necessary. In this thesis attention is paid to houseflies which had been kept in culture in the laboratory for 40 years (WHO strain of flies) and flies obtained from a poultry breeding (Van Diermen strain) and a
cow-house with pig-sty (Pesse strain). The latter two strains have been cultured in the laboratory for several generations.

In Chapter 2 gas chromatographical studies are described which showed striking differences between the cuticular hydrocarbon composition of females of the WHO strain and females of the Van Diermen and Pesse strains. On WHO females hydrocarbons with 23-25 C atoms constituted about 65% of the total hydrocarbons, whereas on first-generation laboratory wild-type females these compounds made up less than 2% of the total amount. (Z)-9-tricosene (‘muscalure’), the alleged sex pheromone of the female housefly, comprised up to 20-30% of the total hydrocarbons on 5-20-day-old WHO females, whereas less than 0.5% of the total hydrocarbons on the wild-type females consisted of muscalure. Furthermore, it appeared that on the wild-type strains the amounts of muscalure had increased considerably after some tens of generations in the laboratory.

In Chapter 3 we investigated whether these differences in muscalure quantities were reflected in the sexual activity of the males. We found that sexual activity of males of all three strains was higher towards females with higher amounts of muscalure. In addition, males from strains with higher amounts of muscalure on the females appeared to be more sexually active. EAG recordings indicated that both males and females of all three strains were able to perceive (Z)-9-tricosene, which suggested that differences in sexual behaviour were not due to differences in ability to smell these substances.

The next step was to find out whether environmental circumstances like temperature, humidity and population density affected the hydrocarbon composition on the cuticle of the flies with special attention to the production of (Z)-9-tricosene. The results of this study are presented in Chapter 4. Male and female flies produced more hydrocarbons at 35 °C than at 20 °C. However, no indication was found that the relative humidity had a distinct effect on the production of (Z)-9-tricosene by females than on the production of the other hydrocarbons. On females the relative amounts of nonacosane, and methyl- and dimethylnonacosanes were significantly higher at 35 °C than at 20 °C. Female flies produced some (Z)-9-tricosene after 8 generations at low population density, in contrast to females at high population density which did not produce muscalure.

In Chapter 5 a new technique is introduced to apply semiochemicals on test flies in a more natural way than is commonly used. It appeared that hydrocarbons were
taken up by flies walking on a filter paper onto which the pure chemicals had been pipetted. In this way, the substances were distributed in a more natural way over the body than on flies onto which the chemicals, solved in hexane, had been pipetted. Using this new ‘self-loading’ technique it appeared that (Z)-9-heptacosene stimulated copulation when present in relatively high amounts on females, whereas (Z)-9-pentacosene did not affect male sexual behaviour.

Another new technique is proposed in Chapter 6. A radar-Doppler actometer is described which allows the recordings of movements of individual body parts of the flies. It is shown that head movements of the fly can be used as a behavioural detection mechanism for semiochemicals. Although flies can smell both (Z)-9-heneicosene and (Z)-9-tricosene as shown by EAG studies, head movement reactions to these chemicals, however, occur to (Z)-9-tricosene but not to (Z)-9-heneicosene, which is in accordance with male sexual responses to these substances. Comparison of our results with those of field experiments described in the literature lead us to suggest that (Z)-9-tricosene may function as an aggregation pheromone bringing males and females together and as a female sex pheromone inducing mating behaviour in males.

Finally in Chapter 7 we present strong indications that an oviposition pheromone is deposited together with the eggs. This pheromone appears to disappear within a short period of time after oviposition either because it is very volatile or disintegrates.