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Ecology and genetic control of the onion fly, Delia antiqua (Meigen)

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Abstract

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Literature data on the onion fly's biology are given. The field work on its ecology,
relevant for genetic control, is reported, with an emphasis on dispersal. The methods
used are discussed, especially marking the flies at emergence, releasing sterilized pupae
dug in the soil, recapturing flies by flight interception traps, and sampling of pupae.
Some data are given on the prediction of emergence, the incidence of diapause and
Entomophthora infection of the flies. The distribution of damage and pupae can be descri-
bed by the negative binomial, and problems are encountered in estimating confidence intervals
for the mean density. Densities of pupae normally are some 1 000 -20 000 per ha. From
estimates of the life-span distribution of female flies and the frequency of oviposition
phases the fecundity is estimated. Reproduction factors are found to be about 7 and 3 for
the two flights, respectively. Dispersal is shown to be in general independent of the wind
direction. Several methods of estimating a diffusion coefficient to describe the fly dis-
persal are applied. The best results are obtained by computer simulation with heterogeneity
in time and space, yeilding 2 000 m^2/day in onion fields and 14 000 m^2/day outside these.
Dispersal is age dependent, occurring less in reproductive phases. The logarithm of the
total number recaptured of the released group is about linearity related to the distance
from the release site. Field trials on control by sterile males are described, and data on
competitiveness and reproduction are given. In an onion growing area 1 ha treatment gave
successful control. A release schedule for the practical application of genetic control to
the onion fly is given with estimates for release site density, barrier zone depth, over-
flooding ration, and optimal distribution of available steriles over the two flights and
the subsequent years. For the Netherlands, an estimated mass-rearing output of 1.5 x 10^9
competitive fly equivalents is needed.

Free descriptors: Anthomyiidae, competitiveness, damage distribution, diapause, diffusion,
Diptera, dispersal, flight interception trap, release strategies, reproduction, sampling
methods, simulation, sterile insect technique.

Contents

1 Introduction

The main insect pest of onions in temperate regions of the northern hemisphere is the onion fly, Delia antiqua (Meigen) (e.g. Hennig, 1953; Balachowski & Mesnil, 1935; Essig, 1926; Miller, 1956; Metcalf & Flint, 1962). It may destroy up to 50-100% of the crop. Effective chemical control measures have been developed and applied mainly during the past few decades (Dustan, 1938; Wright, 1938; McLeod, 1946; Maan, 1947).

Research on the onion fly has been done on its general biology (e.g. Eyer, 1922; Kästner, 1929b; Isaev, 1932; Maan, 1945; Miles, 1956, 1958a; Rygg, 1960; Perron and co-workers, 1951-1972; Ellington, 1963) and on special aspects like laboratory rearing (Friend & Patton, 1956; Friend et al., 1957, 1959; Allen & Askew, 1970), attractants (Peterson, 1924; Matsumoto & Thorsteinson, 1968a, 1968b; Matsumoto, 1970) and reproduction (Missonnier & Stengel, 1966). A bibliography has been given by Scott (1969), more selected references are found in Hennig (1974). Literature compilations on its life history have been made by Beirne (1971) and Schnitzler (1967).

Development of resistance against pesticides used (Howitt, 1958; Anonymous, 1967; Brown, 1974) and the hazards that pesticides constitute for the environment caused research to be started aiming at the development of genetic control of the onion fly in the Netherlands (Noordink, 1966) and also in Canada (McClanahan & Simmons, 1966; McEwen et al., 1973). The method of genetic control was first applied by Knipling (1955), Baumhover et al. (1955) and Lindquist (1955). The development of the genetic control method has been reviewed by Proverbs (1969), Smith & von Borstel (1972) and Whitten & Foster (1975).

The ordinary type of genetic control is the sterile insect technique or sterile male technique. By release of sterilized males of the same species in high numbers, the possibility of a wild female to mate with a wild male is lowered to the extent that the average number of reproducing offspring per female is less than two, resulting in a population decline. Other possibilities of genetic control are based on more subtle genetic manipulations, like induced chromosome rearrangements.

This report contains the ecological part of the research for the development of genetic control of the onion fly in the Netherlands, carried out by a research team at Wageningen. Other aspects examined by this research team are:
- mass rearing (Ticheler & Noordink, 1968; Ticheler, 1971; Noorlander, in prep.),
- sterilization (Ticheler & Noordink, 1968; Noordink, 1971),
- use of radioisotopes (Noordink, 1971),
- histopathology (Theunissen, 1971, 1973a, 1973b, 1976),
- sterile-male field trials (Ticheler et al., 1974a; Theunissen et al., 1974, 1975),
- chromosome rearrangements (Wijnands-Stäb & van Heemert, 1974; Robinson & van Heemert, 1975; van Heemert, 1973a, 1973b, 1975; Vosselman, in prep.),

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- simulation of control strategy (Wijnands-Stäb & Frissel, 1973).

The team's research on the sterile insect technique and general aspects is reported in: Jaarverslag Instituut voor Plantenziektenkundig Onderzoek 1964 (1965) and following, and more condensed in Annual Report Institute for Phytopathological Research 1972 (1973) and following. The genetic research part is covered by: Application of atomic energy in agriculture, Annual report 1969 association Euratom-ITAL (1970) and following. Together these data can be found in: Commission of the European Community Euratom, Annual report 1971, programme biology - health protection, Luxembourg (1972) and subsequent issues.

The aim of the study was to provide data on the ecology of the onion fly, necessary for application of genetic control, and to investigate the feasibility of genetic control under normal field conditions. At least partly because of this programme, and because of the limited manpower made available, the data presented are a somewhat unbalanced account of the onion fly's biology. Especially the data on the life cycle and niche are limited to mere incidental observations. More details are presented on densities and reproduction, as these aspects are closely linked to the dispersal which is the main object studied.

Reproduction and mortality were not chosen as the main object because investigations in change of population size have to start by delimiting populations and measuring the degree of exchange among them. Also, the experimental analysis of population dynamics is rather laborious, whereas the data relevant in a sterile male control program can be obtained from only executing and analysing pilot projects with sterile releases, as pointed out by for example Lindquist (1969) and Weidhaas (1973).

The data collected, especially those from a pilot experiment, are used to provide an outlook on the practical application of sterile males in Dutch onion growing.

As the research on chromosomal rearrangements is not yet in a field testing stage, the field work will be considered only in relation to the sterile insect technique.

The experiments were carried out from 1970 to 1974. The field work was done mainly on the former island of Overflakkee in the SW of the Netherlands, with a base at the Foundation Dutch Onion Federation (SNUiF) at Middelharnis. The laboratory experiments were done at the Institute for Phytopathological Research (IPO) at Wageningen.

Also some of the data obtained from a series of field trials on control by sterile males at the experimental farm 'the Schuilenburg' near Wageningen (Ticheler et al., 1974a; Theunissen et al., 1974, 1975) are included in this report.

2 Introductory data on the onion fly and its control

2.1.1 Taxonomy

The onion fly is a dipteron, belonging to the family Anthomyiidae. The synonyms cur-
rently in use are Chortophila antiqua (Meig.), Delia antiqua (Meig.), Phorbia antiqua
(Meig.), Hylemya antiqua (Meig.) and Hylemyia antiqua (Meig.). The last is a later cor-
rection of the orthographical mistake in Hylemya.

Following the recent revision of the Anthomyiidae (Hennig, 1974), Delia is used here.
In earlier publications of the onion fly research team Hylemya has been used because of
its predominance in international use.

In earlier applied entomological literature also the following synonyms occur:
Anthomyia antiqua Meig., A. ceparum Meig., Hylemyia cepetorum (Meade), H. ceparum
(Meig.), Pegomyia cepetorum (Meade), P. ceparum (Meig.), Phorbia cepetorum Meade,
P. ceparum (Meig.) and Leptohylemyia antiqua (Meig.).

2.1.2 Morphology

Adult The species Delia antiqua can mostly be identified from its general appearance
(Fig. 1): its size, its olive grey (males) or slightly yellowish grey (females) dorsal
part of the thorax which is practically unstriated, and the general shape of the male
genitalia in side view. In case of doubt the following characteristics can be used. Males
have a typical irregular row of relatively short hairs on the tibia of their 3rd leg, at
the medial-caudal side (Fig.2). Females have two hairs at the lateral-rostral side of the
2nd tibia, whereas most resembling species have only one hair there. Resembling species
with two hairs there have the pre-alar hair on the thorax as long as the other thoracical
hairs, in contrast to the onion fly and several of its relatives where it is only half as
long (Fig. 3).

The main sex differences are, apart from the thorax colour and the other characteris-
tics mentioned, the abdomen (males: slender, with a black longitudinal line, external ge-
nitalia; females: rounded, light grey) and the size of the eyes (males: eyes nearly tou-
ching each other; females: eyes clearly separated).

For a more detailed description of the adult male morphology, and figures of the male
genitalia, see Hennig (1974). For the morphology of the related bean seed flies,
D. platura and D. florilega, see Hennig (1974) (males) and Ageeva (1968) (males and fe-
males).

The determination characteristics mentioned are rather variable. Once a male onion fly
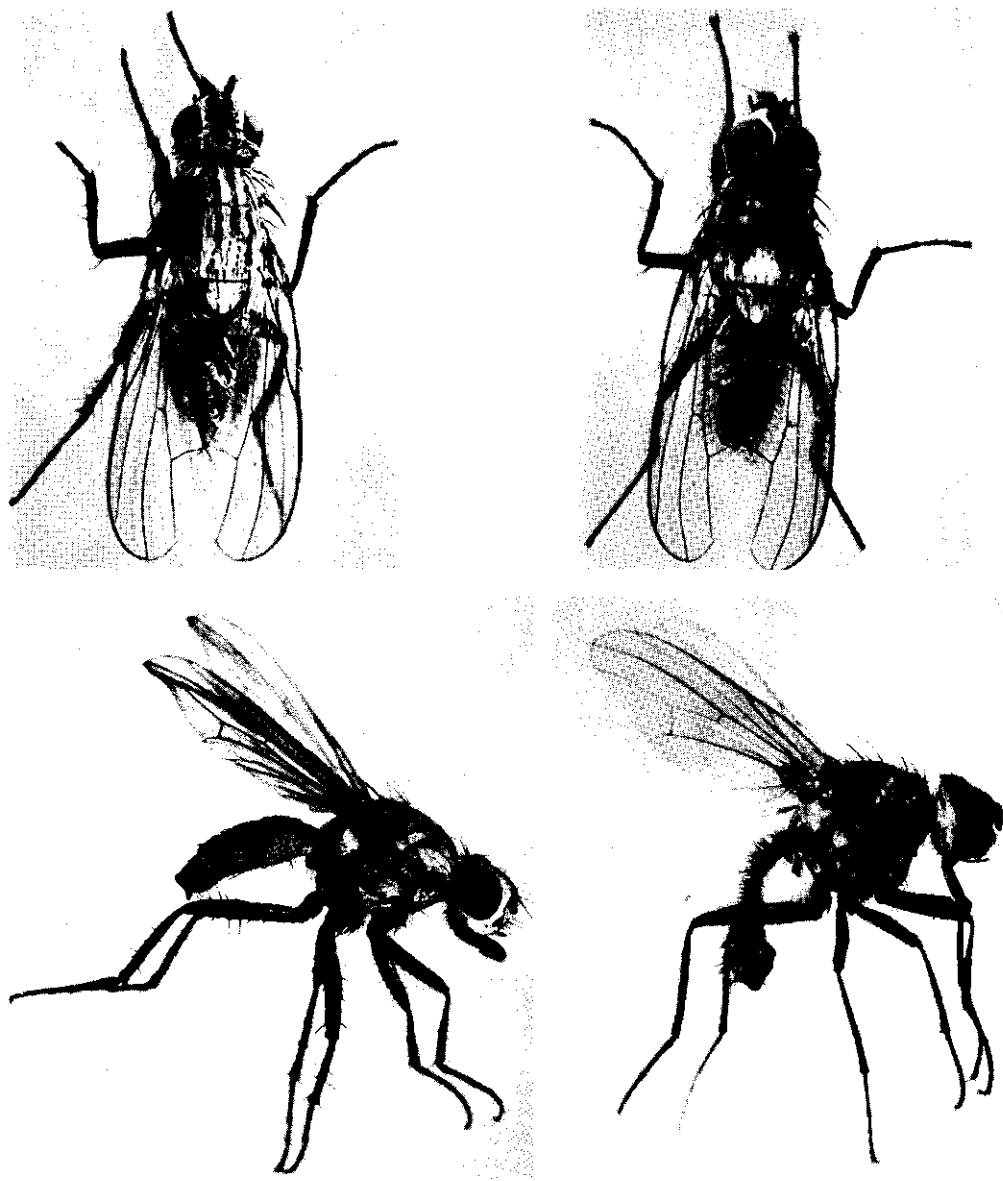
Fig. 1. Onion fly adult. Right male, left female; on top dorsal view, below side view;×6¼.

was found without its left prealare hair. The number of hairs on the female tibia-2 ranged from 1 to 4. Rarely the upper one of the two 'normal' hairs was absent. At either side of this pair of hairs an additional smaller hair could occur.

Several aberrations in wing veins were found. These were mostly appendages and thickenings of the cross veins. They occurred in different populations with frequencies of 5-25%. Similar aberrations have been described for the onion fly by Saager (1959) and for several related species by Sick (1967) in frequencies of 0.01-4.35%.
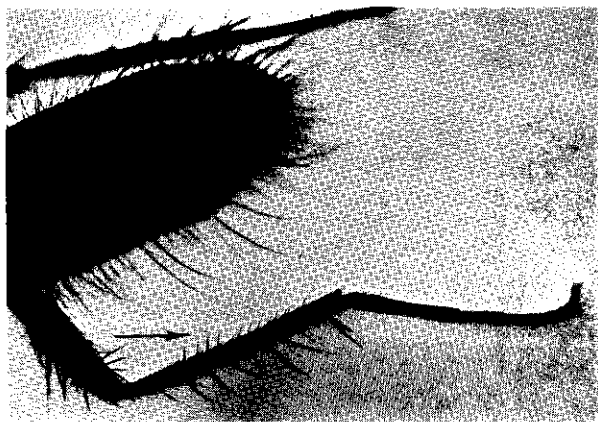
Fig. 2. Male onion fly, ventral view of right 3rd leg, ×20.

Fig. 3. Onion fly, side view of thorax with pre-alar hair indicated, ×20.

The laboratory rearing of the onion fly usually produced a few percent of aberrant males. These had characteristics intermediate between the males and the females: the eyes were separated but not as much as in the females, and the shape of the abdomen was intermediate. Reduced male external genitalia were present. They contained testes and no ovaria. In wild populations 3 such males were found among 7057 individuals. A similar onion fly was mentioned by Tiensuu (1935), also reared in a laboratory. Such aberrations in the sex expression have been mentioned for related species by Sick (1967), Hennig (1974, D. platura), and Smith (1971, 1972, D. brassicae).

Sometimes a considerable fraction, up to 20%, of the laboratory reared females had a misformed ovipositor: it could hardly be extruded. The ovaries of these females only developed until the start of yolk formation (Theunissen, 1973a: stage S5). Also they did not mate.

Egg The egg is 1.1-1.3 mm long, whitish, and its chorion has a characteristic rim structure (Fig. 4). It is opened by the emerging larva along one of the sides of the suture running along the rostral half. Eggs of D. platura are maximally 0.96 mm, significantly shorter than D. antiqua eggs (Miles, 1953; Dušek, 1969; Buth, 1976).

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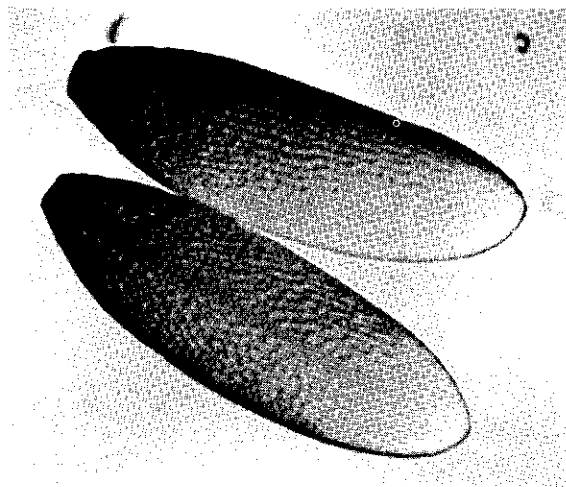
Fig. 4. Onion fly eggs, ×60.

Larva The morphology of the larva has been described in detail by Kästner (1929b), Balachowski & Mesnil (1936), Maan (1945) and Dušek (1969). It develops through three instars. The 1st instar is easy to recognise by the shape of the cephalopharyngeal skeleton (Fig. 5), the difference between the 2nd and the 3rd instar is most easily seen from the number of stigmata per abdominal stigmatophore, 2 and 3, respectively.

The larvae can easily be confused with those of D. platura which are also frequently found in onions. The difference is the number of finger-like processes of the prothoracale stigmata in the 3rd instar: 10-13 in D. antiqua versus 7-9 in D. platura (Dušek, 1969; 5-8 according to Miles, 1953). In the 2nd instar larvae these numbers are two less (Dušek, 1969). First instar larvae can be distinguished by the mandibular part of the cephalopharyngeal skeleton, having two teeth of equal size in D. antiqua and of unequal size in D. platura (Dušek, 1969). Also, there are slight differences in the caudal knobs.
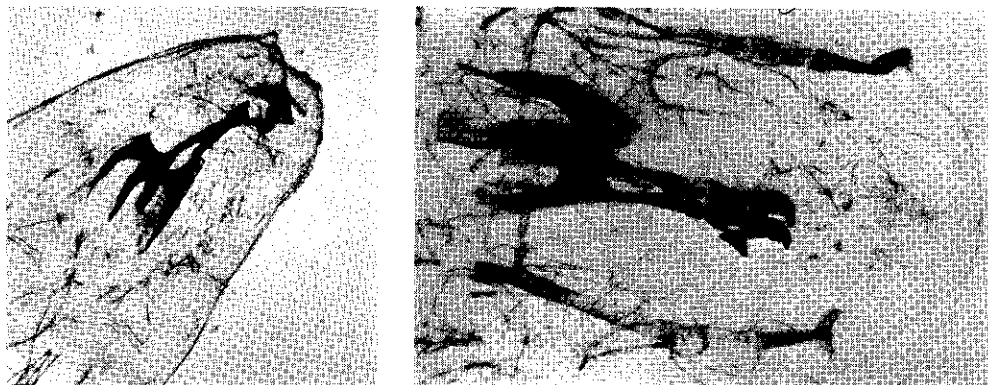
Fig. 5. Cephalopharyngeal skeleton of larva: left 1st instar, right 2nd instar, ×40.

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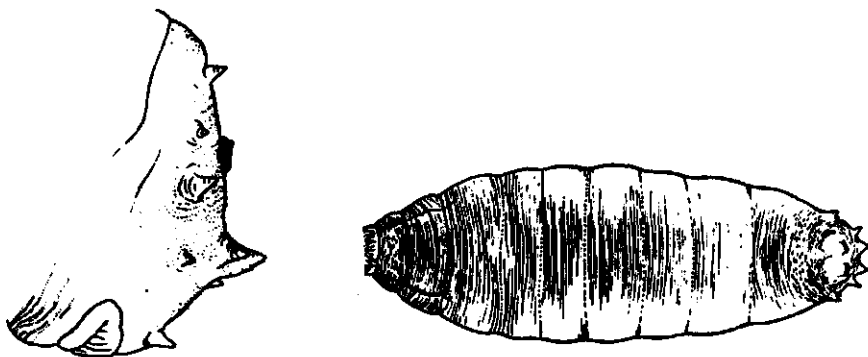
Fig. 6. Onion fly pupa: left lateral view of caudal end, ×45; right dorsal view, ×10. From Dušek, 1969.

Pupa The pupae are 5.5-7 mm long, occasionally reduced to even 4.5 mm. These smaller ones may be confused with D. platura pupae, which are 3-5 mm long or, according to Dušek (1969), 4-5.2 mm. There are slight differences in general appearance and in the larval caudal knobs that are still visible on the pupae. The third pair of knobs, counted from dorsal to ventral, is in the onion fly larvae larger than the 1st, 2nd and 4th pairs, whereas in D. platura they are of about equal size (Dušek, 1969) (Fig. 6). Another difference is the number of finger-like processes of the prothoracale stigmata, if these are still intact. Schnitzler (1969) did not give clear differences between antiqua and platura pupae.

2.1.3 Geographical distribution

The geographical distribution of the onion fly is given in Fig. 7. Earlier references have been mapped by Maan (1945) and the Commonwealth Institute of Entomology (Anonymous, 1957). Additional data were obtained from Hennig (1974), Review of Applied Entomology A up to vol. 62(1974) and Cooperative economical insect report vol. 9(1959)-12(1962). Other information on the distribution has been given by Jones & Mann (1965) (Egypt mentioned), Kuwayama et al. (1960)(Japan) and Vappula (1965)(Finland).

Originally the onion fly was a palaearctic species. The fly was introduced into America and spread there to the west during the 19th Century. In Japan damage was first reported from Hokkaido in 1938, and has spread over all major islands (Kuwayama et al., 1960). The fly has been incidentally intercepted on the Hawaiian islands (Whitney, 1927; possibly also Ehrhorn, 1915).

The onion fly's occurrence in Georgia, Alabama, North and South Carolina and Mississippi (Anonymous, 1957) was not mentioned by Stone et al. (1965); according to Chittenden (1916) such accounts refer to D. platura. Not indicated in Fig. 7 are the references on onion flies from Brasil (in Hennig, 1974) and Columbia (Anonymous, 1917), as these seem questionable. The 'onion fly' or 'onion maggot' in Australian publications refers to D. platura.

Fig. 7. Geographical distribution of the onion fly. ▓▓: general occurrence, ★: local observation. Sources see text.

Data from the Mediterranean countries, where the onion fly is not usually a serious crop pest, are scarce. Also, the information from Siberia and China is certainly incomplete.

2.2 ECOLOGY

2.2.1 Life cycle

The onion fly hibernates as a diapausing pupa. In spring the flies emerge, with a sex ratio of 1:1. Protandry occurs, the males emerging on the average a few days earlier (Maan, 1945; Rygg, 1960). Emergence is said to coincide with the flowering of dandelion (Baker, 1925), or with the pink bud stage of apple trees (Lafrance & Perron, 1959).

Because onions are generally not grown two years in sequence on the same field, the flies have to migrate after emergence. They mate at an age of nearly 1-2 weeks (Miller, 1956; Balachowski & Mesnil, 1936; Ticheler, 1970; Bol, 1972), and not shortly after emergence as reported by Lampa (1905), Eyer (1922), Maan (1945) and Rygg (1960).

Copulation is only rarely observed, even in mass rearings. The onion fly was claimed to be monogamous (Broersma & Luckmann, 1968), but polygamy has been proven clearly to occur (Noordink, 1967). Males may mate at least 5 times in 4 hours and copulation lasts 2-4 minutes (Bol, 1972).

The females are anautogeneous: they have to feed on proteins before egg laying (Missonnier & Stengel, 1966; Missonnier, 1967). The eggs are laid on different Allium species, after a pre-oviposition period of about 1-2 weeks. The duration of the pre-oviposition period is presumably dependent on temperature and on availability of food (Tyndale-

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Biscoe & Kitching, 1974).

The larvae emerge after a few days, and enter the base of the onions. During some weeks the larvae feed there on the onion tissue. Especially when the onions are small the larvae may migrate to other onions, through or over the soil, to get enough food. Full-grown larvae pass down through the soil and pupate there. Pupae are found down to 15 cm and at an average depth of 4-5 cm (Rygg, 1960) or 7-8 cm (Maan, 1945; Isaev, 1932).

Mostly 2 or 3 flights per year occur. In northern Norway this is reduced to one flight (Rygg, 1960), whereas in warmer regions sometimes more than 3 flights are reported (Alma ata 4 (Bundzhe, 1965), Turkey 4 (Keyder & Atak, 1972), SW France 4-5 (Bonnemaison, 1962; but 3 according to Balachowski & Mesnil, 1936), Austria 3-4 flights (Schreier, 1953) but based on insufficient evidence). The later flights are generally incomplete, the other pupae of these generations having gone into hibernation diapause.

Diapause is induced by low temperatures (Miles, 1958b; Missonnier & Brunel, 1972), and also by short days. The relation between the percentage diapause and the rearing conditions of daylength and temperature has been established by McLeod (1965) and in more detail by Ramakers (1973) (Fig. 34). Diapause termination is temperature dependent (Kelderman, 1972). Food quality might also affect the percentage diapause, as found for Pegomyia betae (Malekghassemi, 1969).

Some authors reported diapause frequencies, based on pupae that were held under non-representative circumstances or did not constitute a representative sample (Rygg, 1960; van 't Sant, 1967, 1970; Miles, 1953). Perron & Lafrance (1961) found in a study in field cages in Québec diapause percentages for the three successive generations of: 0-19, 43.5-91 and 99-100. Such data are highly dependent on climate. In warmer regions aestivation occurs, reducing the number of generations in Israel to two and shifting the life cycle by half a year compared to northern Europe (Rivnay, 1958; Yathom, 1963). On Kyushu (southern Japan) both aestivation and diapause occur, with two generations before and one after summer (Kato, 1958). Note that the 2nd and 3rd flight flies are of the 1st and 2nd generation, respectively.

Extensive laboratory data are available on the duration of the developmental stages in relation to temperature. These data often disagree. Factors found to affect the rate of development are larval food (Friend et al., 1959), humidity (Ellington, 1963) and sex (a.o. McClanahan & Simmons, 1966). Other factors causing differences between the data can be temperature measurement at unrepresentative sites, differences in definition of start and end of the stages, and use of genetically different onion fly populations. Average durations of the developmental stages are summarized in Table 1. After completion of diapause the development of the pupae proceeds similar to that of 3-day-old non-diapausing pupae (Theunissen, 1976).

The flies are supposed to live in the field for 2-3 weeks (Balachowski & Mesnil, 1936) or 3-4 weeks (Kästner, 1929b; Maan, 1945).

Dispersal is said to occur with the prevailing wind (Eyer, 1922), but this statement was based on observations of damage which could easily have been explained by other factors. According to Eyer (1922), the fly is capable flying over 3 km of water, provided that the wind is favourable.

	Temperature in °C				
	10	15	20	25	30
egg	12	5	3	2	2
larva 1st instar	10	5	3	2	
2nd instar	10	5	3	2	14
3rd instar	35	20	13	9	
pupa	50	25	16	12	11
pre-oviposition period	22	10	7	5	.

a. Eyer, 1929; Maan, 1945; Miles, 1958b; Friend et al., 1959; Rygg, 1960; Ellington, 1963; Yathom, 1963; McClanahan, 1966; McClanahan & Simmons, 1966; Ticheler & Noordink, 1968; Brunel & Missonnier, 1969; Allen en Askew, 1970; Ticheler, 1971; Kelderman, 1972; Missonnier & Brunel, 1972; Houwing, 1973; Eckenrode et al., 1975b.
b. In the Tables 0 = observed zero, - = essentially zero and · = no observation.

2.2.2 Population dynamics

Quantitative data in the literature on onion fly population dynamics in the field are very scarce. Densities in the field have been given for chemically treated onion fields by McEwen et al. (1973), averaging 3.4 hibernating pupae per m^2, of which 83% emerges. Perron (1972) gave for untreated fields on the average 20 pupae per m^2, of which 70% emerges. The distribution of densities over the fields sampled is a very skew one.

The survival of immature stages, as given by McEwen et al. (1973), 40-60%, seems too high, probably due to the experimental and calculation methods used which are not clearly described. Perron & Lafrance (1961) gave life table data based on a cage population. They found net reproduction factors for the three generations of 17.5, 25 and 10.5, and a fecundity of 58, 36 and 24 eggs per fly, respectively. Such data cannot be extrapolated safely to the field situation.

Perron (1972) reported some data on his extensive samplings of different stages of the onion fly. Unfortunately there is no indication given on how he obtained the given percentages for survival of eggs, larvae and pupae from his samplings, and the sampling procedure applied may have missed a considerable fraction of the eggs. For the first flight he gave mortalities of about 45-78% in the eggs, 74-96% in the larvae and 56-84% in the pupae, and about 30% mortality among hibernating pupae. The differences observed were attributed to the soil type and Allium species, but it is not clear whether any of these differences is significant. Moreover his statements sometimes conflict with the data presented.

For the related Delia brassicae, pupal parasitism has been found to be the factor stabilizing population size (Mukerji, 1971). For the onion fly a similar situation may be expected.

Host plant preference in oviposition   The onion fly is closely associated with different
Allium species by the choice of oviposition site. Species attacked are Allium cepa L.
(onion, shallot), A. ampeloprasum L. (leek, pearl onions), A. fistulosum L. (Welsh onion),
A. sativum L. (garlic) and A. schoenoprasum L. (chives). Shallot is generally referred
to as A. ascalonicum L., but according to Jones & Mann (1963, p.34-35) it should be in-
cluded in A. cepa. Similarly leek is often called A. porrum L. Onions and A. cepa ×
fistulosum are preferred to A. fistulosum (Perron et al., 1958, 1960; Perron & Jasmin,
1963), onions are preferred to A. cepa x fistulosum (de Ponti, 1976), and onions are pre-
ferred to shallots and both to leek (Maan, 1945). According to Labeyrie (1957) these pre-
ferences are highly dependent on the season. Garlic is not seriously attacked (e.g. Jones
& Mann, 1963). Different cultivars of onion have a slightly different preference
(Matthewman et al., 1953), sometimes mentioned as significant (Sleesman, 1934; Huber &
Sleesman, 1935), sometimes not so (Perron et al., 1958), but in the last case probably
due to the limited set-up of the experiment. There is a strong preference for injured
and diseased (rotting) onions (Labeyrie, 1957; Workman, 1958; Armstrong, 1924 and others),
and for onions infested with the onion stem eelworm, Ditylenchus dipsaci (Kühn) (Anonymous,
1973). Undamaged fully developed onions are not attacked (Perron & leRoux, 1962; Perron,
1972). Denser onion growing results in higher egg population densities, without a signif-
icant difference in numbers of eggs per onion (Perron, 1972). Also, onions of heavy flac-
cid growth, such as 2nd year onions that have been planted too deep, are very attractive
(Gray, 1924), and may be used as a trap crop to control onion maggot infestation (see
Section 2.3.3).
    These preferences are, where known, due to oviposition differences (Perron et al.,
1960), and are supposed to originate from chemical differences of Allium odours. Ovipo-
sition is directed by chemical stimuli from components of onion flavour (Matsumoto &
Thorsteinson, 1968a; Matsumoto, 1970), Miller (1969) supposed plant shape and light to be
effective at short distances. In the field, the tactile stimuli required for oviposition
are provided for by the onion and the soil. Eggs are laid on onion plants or very near
to these in earth crevices, and sometimes on leaves.

Larval food   The larvae feed on the subterranean part of the onion stem or, after bulb
formation, the onion bulb tissue. In summer onions may survive onion fly infestation. At-
tacked onions rot by bacterial infection, generally soft rot (Erwinia carotovora (Jones)),
introduced by the larva or having got access by the insects attack (Johnson, 1930;
Gorlenko et al., 1956). Other bacterial and fungus infections may be associated with it as
well (Zečeva & Bečvarov, 1973; Jones & Mann, 1963). This bacterial predigestion is not obli-
gatory, as it is not always present in the field, and aseptic rearing of onion fly larvae in
the laboratory is quite possible (Friend & Patton, 1956; Friend et al., 1957; Allen & Askew,
1970; Ticheler, 1971). However, presence of micro-organisms has a strong positive effect on
the rate of larval development (Friend et al., 1959; Gorlenko et al., 1956). The soft rot
can be reared from the contents of the pupae (Johnson, 1930) and also from the inside of
larvae, flies and even eggs (Gorlenko et al., 1956). The presence of bacteria inside the

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eggs seems to conflict with aseptic rearing after sterilizing the outside of the eggs (Friend et al., 1957) where the bacteria are present too (Gorlenko et al., 1956).

The larvae accept other food as well: they are occasionally found in other plants like cabbage (Maan, 1945; Lundblad, 1933; Kalandadze & Šavkacišvili, 1958), radish and spinach (Miller & McClanahan, 1959), radish (Severin & Severin, 1915) and tulips and lettuce (Smith, 1922) often because of changes in the food available after egg deposition on onions (e.g. Miller & McClanahan, 1959). Also, the larvae can be reared in the laboratory on an artificial diet based on carrot powder (Ticheler, 1971). Severin & Severin (1915) mentioned horse-dung as larval food, but subsequent experiments failed to confirm this (Kästner, 1929b; Maan, 1945). In mass rearing, cannibalism may occur: larvae consume pupae that have been formed in the larval rearing medium (Noorlander, pers commun.).

Adult food and attractants The flies are reported to feed on different flowers, mainly Umbelliferae, Taraxacum and Allium (Baker, 1928; Kästner, 1929b; Maan, 1945; Rygg, 1960). Noordink (1973) did experiments on radioactive label transfer in field cages, and got positive results with some flowering grasses. Baker (1928) observed flies on manure, and supposed its attraction was warmth (on cool days) or its moisture content (for drinking on hot days).

The flies can be attracted by several substances (Howard, 1918; Peterson, 1924; Kästner, 1929a; Yathom, 1963; Niemczyk, 1965; Anonymous, 1965a; Noordink, 1966; Eckenrode et al., 1975b). The most powerful attractant found in the laboratory and in field tests outside onion fields, is n-propyl disulfide (Matsumoto & Thorsteinson, 1968a; Matsumoto, 1970; Noordink, 1967), one of the onions' constituents (Carson & Wong, 1961; Brodnitz et al., 1969; Boelens et al., 1971). It is also one of the attractants for the larvae (Matsumoto & Thorsteinson, 1968b).

Colours have been found to attract several related species, yellow generally being the most attractive (Sick, 1967; Kring, 1968; Brunel & Langouet, 1970; Finch & Skinner, 1972). In the onion fly Müller (1969) found a preference for brightness but not for colour.

Parasitoids, parasites and predators Many species have been reported to parasitize on the onion fly. After elimination of synonyms, incorrect quotations and propably incorrect records, only a limited number is left (Loosjes, in prep.). The common species among these are the parasitoids Trybliographa rapae (Westw.) (Hymenoptera, Cynipidae) which attacks mainly younger larvae (Wishart & Monteith, 1954), Aphaereta minuta (Nees) (Hymenoptera, Braconidae) which attacks larvae (Salkeld, 1959) and Aleochara bilineata (Gyll.) (Coleoptera, Staphylinidae) which attacks pupae (Read, 1962). Heterotylenchus aberrans Bovien (Nematoda) attacks the larvae and leaves the adult females through the genital system, causing sterility (Bovien, 1937).

Entomophthora infection among the flies is common in summer. It has been examined in detail by Perron & Crête (1960). The infection spreads especially with high fly densities and with humid weather. Infected flies show a typical dying position, attached to high points in the vegetation (van 't Sant, 1970: Fig. 5, Miller & McClanahan, 1959: Fig. 1).

The available data on predators are very incomplete, and will be summarized elsewhere

(Loosjes, in prep.). There are no indications that there exists a predator that limits
its activities to the onion fly. Important predators of the immature stages are
Staphilinids (a.o. Aleochara bilineata (Gyll)) and Carabids, and of the flies predatory
flies and insectivorous birds.

Onion coinhabitants Especially in summer, rotting onions may contain a number of differ-
ent species of fly larvae, as well as some other animals (Merrill & Hutson, 1953; Hudon &
Perron, 1954; Schreier, 1953 and others). The species will be listed elsewhere (Loosjes,
in prep.). Most species may be characteristic of decaying organic matter. There are very
few data on their relationships.

The polyphagous bean seed flies, Delia platura and D. florilega, of which the latter
is more prominent in Scandinavia (Rygg, 1960), are sometimes found to be primary attackers
of onions, especially seedlings (Merrill, 1951; Miles, 1956). They have been said to be
attracted for oviposition to disturbed soil surfaces, e.g. due to weeding (Miles, 1953,
1956; Barlow, 1965; Hassan, 1974; Miller & McClanahan, 1960). However, Eckenrode et al.
(1975a) proved that the attraction of bean seed flies to seed and seedlings was due to
the development of some species of micro-organisms present there. The current use of
disinfected seed will thus limit or eliminate the importance of these flies as primary
attackers.

One case of primary attack of Eumerus tuberculatus Rond. has been reported (Merrill
& Hutson, 1953). For larval development this species is, however, obligatorily dependent
on micro-organisms (Creager & Spruijt, 1935).

2.3 ONION GROWING AND PEST CONTROL

2.3.1 Onions in the Netherlands

Onion growing methods widely differ in different countries. Because this study is
about the onion fly in the Netherlands, some data on growing onions there are given.
The methods used are:
1. Spring-sown onions. Sowing is in March at 7 kg seed/ha, in rows 25-40 cm apart.
Harvest is in August/September. They may be stored until the next spring.
2. Onion sets. Sowing in March at about 100 kg seed/ha, in rows 25 cm apart. Harvest
in July. The bulb size is 8-22 mm diameter. These onions are planted the next year.
3. Onions grown from sets. Planting in March, in rows 30-40 cm apart, using about
1200 kg/ha. Harvest in July, no storage.
4. Silverskin onions. Sowing in March/April at 100 kg seed/ha, dispersed over the field.
Harvest in July/August. Used in pickling industry.
5. Autumn-sown onions. Sowing as spring-sown onions but in August. Harvest in July of
the next year.
6. Onion seed. Planting in March, about 20 by 50 cm apart, using full-grown spring-sown
onions. Harvest in September.
The areas commercially grown in the Netherlands are given in Table 2. Onion growing
has been known in the Netherlands at least from the 13th Century, and is at the moment

13

Table 2. Commercial onion growing in the Netherlands, data from 1970-1974.

Species	Crop	Surface in ha	% With chemical onion fly control
Allium cepa L.	spring sown onions	7500 – 10000	100
"	onion sets	600 – 800	100
"	onions grown from sets	500 – 1000	25
"	silverskin onions	700 – 900	100
"	autumn-sown onions	0 – 15	partly
"	onion seed	10 – 30	0
"	shallots	150 – 200	few
Allium ampeloprasum L.	leek	900 – 1400	5
"	pearl onions	2	100
Allium schoenoprasum L.	chives	1 – 2	100
Allium spp.	ornamentals	10 – 20	few
total commercially grown		11000 – 14000	

concentrated mainly on sandy clay on the former islands in the southwest and in the new polders in the centre.

The other Allium species are of minor importance. Shallots are grown locally, especially on sandy soils behind the dunes. Planting is in February/April, and harvest in June/August. Leek is grown in some horticulture areas. It is planted in March/July and harvested August/May. Pearl onions and chives are rare in horticulture; Welsh onion and garlic are not grown commercially. Private growing, mostly leek and further onions and chives, covers a negligible area compared with commercial culture.

Other Allium species in the Netherlands are some wild species: A. vineale L. (wild onion), A. ursinum L. (ramson), A. scorodoprasum L. (rocambole) and A. oleraceum L., and an increasing number of species as ornamental plants in gardens. Except A. vineale all of these can be considered as rather rare and of no possible quantitative importance to the onion fly populations. Richens (1947) in his extended work on A. vineale, had not found records of insect pests.

2.3.2 Damage

In temperate regions the damage to the onion crop by the onion flies is often considerable, unless some control measures are taken. Damage levels of up to 50-95% are reported by Hammond (1924), Flint & Compton (1925), Metcalf & Flint (1939), Smith (1948), Jørgensen (1955), Miller (1956), Bundzhe (1965) and others. Damage is especially severe after wet springs (Metcalf & Flint, 1939; Doane, 1953; Peterson & Noetzel, 1954; Workman, 1958; Beirne, 1971), and in less well drained low-lying fields (Lovett, 1923) because of the higher larval survival in moist soils (Sleesman in Anonymous, 1937). Damage is heavier in sheltered places (e.g. Peterson et al., 1963) and on corners (Wilson & Whitcomb, 1929). More extensive onion growing is said to have caused the onion fly to become a serious pest (Wilson & Whitcomb, 1929). Some local onion growing areas in England are free from onion flies according to Miles (1958b).

The damage depends on soil structure, being more severe on lighter soils (e.g. Dustan,

1932). The cause is supposed to be the preference of egg depositing females (Dustan, 1932; Maan, 1945), lower temperatures and higher moisture content in the top layer of the lighter soils (Dustan, 1932), easier larval migration in lighter soils (Maan, 1945) and earlier 1st flight due to faster warming up of sandy soils (Schnitzler, 1967). Perron (1972), in his population studies, found a higher pupal mortality in heavier soils. He mentioned as important, differences between organic (light) and clay (heavy) soils, higher parasitism and higher number of eggs per female on organic soils, but these assumptions are not supported by the data presented.

When data from different authors are compared it has to be taken into account that in Canada and the United States the main onion growing areas are on muck soil, whereas in Europe sandy clay is normal for onion growing.

Damage by the first generation larvae is characterized by tiny brownish remnants of the subterranean parts of onion seedlings, and still greenish leaves that have fallen over. Desiccation and decomposition quickly make these leaves disappear. This damage can be destructive to the crop especially when the onions were sown late compared with the oviposition period of the first flight flies. When an attacked seedling has been consumed, the larvae migrate, seemingly at random (Workman, 1958), generally to the next seedling. Maan (1945) estimated in leek during the first generation a ratio of 1:15 to 1:20 of seedlings on which egg batches had been laid to seedlings damaged. Kästner (1929b) found this ratio to be 1:11 in onions. One single larva is said to be able to destroy up to 4 (Kendall, 1932) or 8 (Beirne, 1971) seedlings. In the laboratory Workman (1958) found that one larva could eat 28 'seedlings' of 4 mm long. When the number of adjacent seedlings that are damaged is limited, the remaining onions may compensate for this damage.

Typical for damage later in the year is that the central leaf often wilts first, followed by the others. Again the leaves start dying at their bases. Attack causes the onions to rot. If the onion is not dead before the larvae pupated, it may become disformed. The second generation larvae may cause more damage than the mere loss of yield, because onions that are rotting at the time of harvest can prevent economic storage: these have to be picked out or they will infect the other onions.

Damage data from untreated plots in the Netherlands during the last decades, from experiments of the SNUiF, are given in Fig. 8a. The effect of soil type on these data is shown in Fig. 8b.

In interpreting damage data of untreated plots one should be aware that the untreated plot is only representative for its own situation and not for untreated fields of different size and of different situation. It is not just the individual onion flies present at that site that are treated, but a part of a population. Also, the population size present in the area is of influence, and is dependent on the local damage incidence in the preceding year.

The data of the SNUiF are from check plots, generally 3 plots of 2 x 5 m, in tests for chemical control treatments. In these experiments, the second flight flies, emerging mainly from the untreated plots, will have laid eggs on all plots and on any onion fields in the surroundings. So these data underestimate the second flight damage to be expected in untreated areas. On the other hand, these control experiments have often been done at places where infestation could be expected (damage in the preceding year or area with
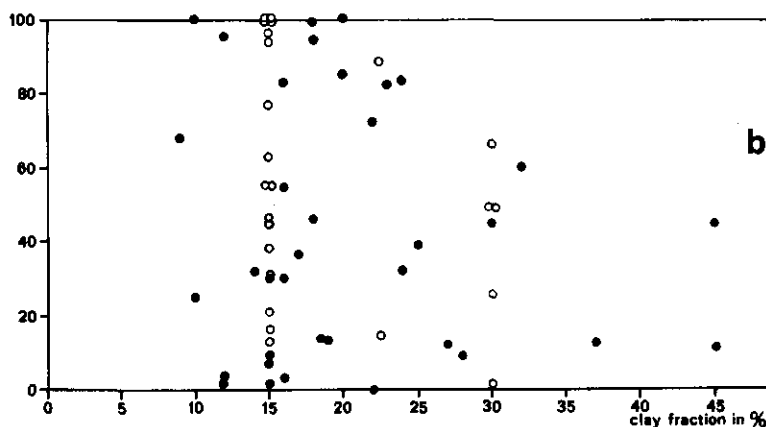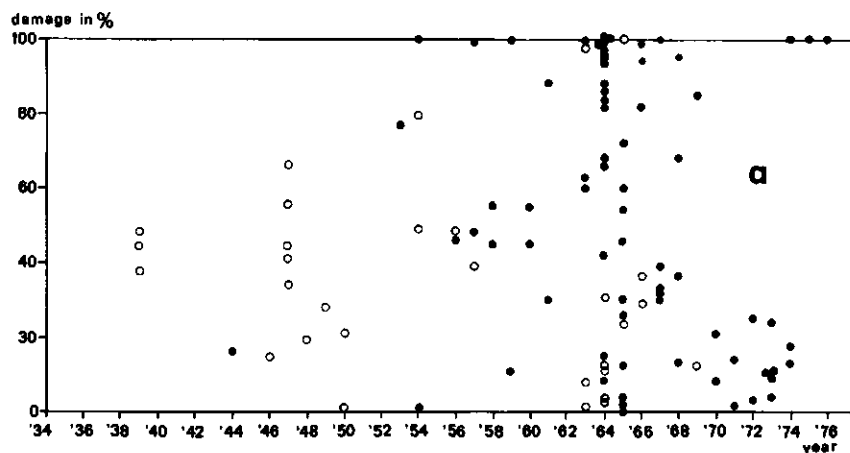
Fig. 8. Percentage damage on untreated plots in the years 1939-1976(a: ○= estimated, ●= observed), and compared with the clay fraction of the soil (b: ○= estimated clay fraction, ●= known clay fraction). Data from SNUiF.

very light soil), resulting in an overestimation. As these factors may cancel each other out, for the time being the observed damage levels on the check plots concerned may be taken as representative for those to be expected when one single farmer does not take effective control measures.

Carden (1960) and Rawlins et al. (1960) both found that every 10% damage results in a 10% yield loss. According to the damage level that causes the farmers to complain or contact the SNUiF, significant yield reduction will occur at over about 10% damage, ex-pressed as percentage of the number of seedlings emerged. The same level was reported by Rawlins et al. (1960). The economic threshold will be about the same, with the low costs of the standard pesticide application. The shift to precision sowing restricts compen-sation for damage by the onions, so it will lower the economic threshold.

Control methods Formerly the onion fly was not controlled. In the Netherlands then onions could hardly be grown on sandy soils, and elsewhere fluctuating damage levels occurred and were accepted. Rotten onions at harvest did not threaten storage so much because they were picked out by hand. Of many onion fly control methods proposed and tested in Europe and America (e.g. Fernald & Bourne, 1914; Kästner, 1929a, 1929c; Balachowski & Mesnil, 1936) only a few seem to have given reasonable results sometimes, like cull onions as a trap crop (a.o. Lovett, 1923; Dudley, 1925), poisoned sweetened bait onions (Kästner, 1930), or naphthalene (Thompson, 1930).

The first very effective method was calomel seed dressing, found by Glasgow (1929) and developed by Dustan (1937, 1938) and Wright (1938). This method was too expensive for general application. Chemical treatment of fields, furrows or seeds became a general practice after 1946, due to the moderate price of the newly discovered organochlorine compounds and to more effective formulations and application methods developed (e.g. McLeod, 1946; Maan, 1947).

Resistance Resistance of the onion fly to organochlorine compounds started in the United States in 1953 (Howitt, 1958) and is now widespread: United States, Canada, Japan, France and the Netherlands (Anonymous, 1967), United States (Finlayson et al., 1959; Peterson et al., 1963), Canada (McClanahan et al., 1958; Harris et al., 1962), Poland (Narkiewicz-Jodko, 1974), England (Gostick et al., 1971) and Finland (Anonymous, 1969b). There were still susceptible strains in some areas in England (Gostick et al., 1971) and France (Missonnier & Brunel, 1972). Where organochlorine resistance has developed, organophosphorous compounds are used for control. However, resistance to dichlofenthion has occurred already (Perron, 1965; van Kampen, 1969; Anonymous, 1969a, 1970).

Organochlorine resistance is based on a single gene (Togwood & Brown, 1962). The effect of this resistance was investigated by Missonnier & Brunel (1972): the larval development was slightly faster and the adult female survival was slightly longer in the resistant strain, but the fecundity was considerably reduced.

After development of resistance the population size and its fluctuations became larger than before the introduction of chemical control. This increase is attributed to the more extensive cropping that was made possible by this control, and to the elimination of predators and parasites (Missonnier & Brunel, 1972; compare the similar case of the cabbage root fly: Morris, 1960; Read, 1964; Coaker, 1966; Mukerji, 1971). Generally very high damage levels (up to 100%) have been reported after the development of resistance (Finlayson et al., 1959; Anonymous, 1964; Beirne, 1971).

Control in the Netherlands Known and estimated percentages of spring-sown onions in the Netherlands treated with different pesticides for onion fly control are given in Fig. 9. Because the area under spring-sown onions is largest and because these onions are the most important for onion fly reproduction, the date can be taken as the parts of the onion fly populations controlled. The effect of chemical treatments is given for the most important chemicals used in Fig. 10.
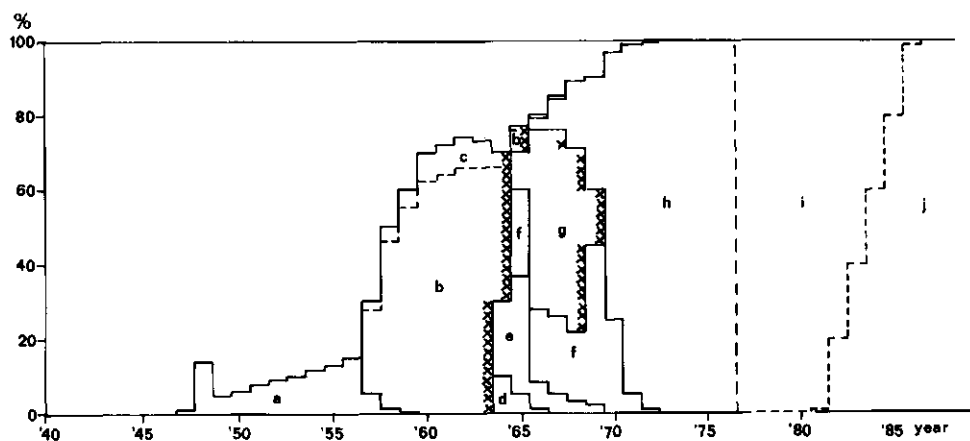
Fig. 9. Chemical control of the onion fly on spring-sown onions in the Netherlands, expressed as percentage of the area. Based on data from SNUiF.
1947-1950 actual data; 1951-1976 estimations; 1977-1986 extrapolation according to a possible programme.
× = loss of market share due to development of resistance; a = DDT; b = dieldrin; c = aldrin and heptachlor; d = ethion; e = diazinon; f = chlorfenvinphos; g = dichlofenthion; h = trichloronate; i = trichloronate, in case of resistance probably carbofuran; j = sterile insect technique.
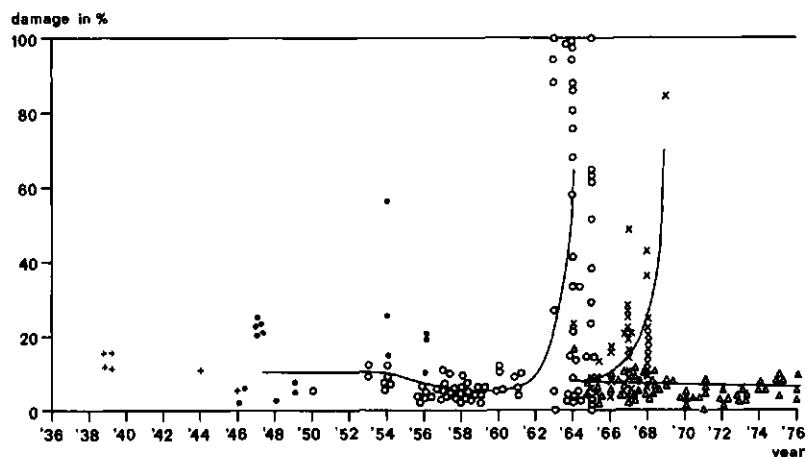
Fig. 10. Effectivity of chemical control of the onion fly in the Netherlands. Data from SNUiF. += calomel; •= DDT; ○ = dieldrin, aldrin and heptachlor; × = dichlofenthion; Δ = trichloronate.

Resistance to organochlorine compounds was noted first in 1962 in three areas (van Kampen, 1963) and had spread to all onion growing areas by 1965 (Anonymous, 1964, 1965b, 1966). Most growers changed to dichlofenthion. During the years 1967-1968 resistance against dichlofenthion developed and was complete in 1969 (van Kampen, 1969; Anonymous, 1969a, 1970). Since then control has been effected with trichloronate. This reached 100% of the market due to its limiting effect on the population increase of the onion stem

eelworm (Kaai & Koert, 1971). In 1976 there was still no sign of resistance to trichloronate.

The possibility of shifting to the sterile insect technique is indicated also in Fig. 9.

Residues Pesticide residues have been analysed only in a few cases. A normal application of dieldrin resulted at harvest in 0.01-0.015 mg/kg in the onions (Ehlers & Liedtke, 1958), or in 0.11 mg/kg in the outer scales, residues in peeled onions being undetectable (Byrdy, 1963). In Canada 0.02 mg/kg dieldrin was found in onions, but no DDT or aldrin although these were also present in the soil (Harris, 1969). In the United States, where onions are often grown for years in succession on the same fields, residues of many pesticides were found in the soil but none of them in the onions (Wiersma et al., 1972). Suett (1974) found residues of pirimiphos of 0.04 mg/kg in onions at harvest. Chlorvenfinphos also gave relatively low residue levels in peeled onions (including breakdown products less than 0.09 mg/kg, Beynon et al., 1968). Where these authors gave data on other vegetables as well, it can be seen that the residue levels in onions are relatively low.

2.4 SUMMARY

According to the recent revision of the Anthomyiidae the onion fly should be called Delia antiqua (Meigen).

The female determination characteristic of two hairs lateral-rostral on the tibia-2 was not always valid. Wing vein aberrations were frequent, and especially in laboratory rearing aberrant males and females occurred. Immature stages of the onion fly may be confused with those of Delia platura which are also common in onions, but differentiating characteristics are available for all stages.

The onion fly is a holarctic species. It hibernates as a diapausing pupa. There are generally one complete and one or two incomplete flights per year. The females are polygamous and anautogeneous, and have a pre-oviposition period of 1-2 weeks. Eggs are laid on Allium species. The larvae feed on these, and pupate in the soil. Diapause is induced by low temperature and short days.

Only few data on population dynamics are available, and most of them may be not representative of the actual situation in the field.

Different onion varieties and related species are attacked to different extents, attributed to odour preferences of ovipositing females. Onion fly attack is accompanied by a non-obligatory bacterial infection. The larvae accept food other than onion.

The adults feed on flowers and flowering grasses. The best attractant for adults known is n-propyl disulfide. Common parasitoids are the staphylinid Aleochara bilineata, and the braconid Aphaereta minuta. Several other, possibly also not host-specific, parasitoids are known. The flies are attacked commonly by a fungus disease. All the predators also seem to be not species specific.

Rotting onions may contain a large variety of different species, mainly fly larvae. They are all secondary pests, except sometimes the bean seed flies on seedlings.

Spring-sown onions are the most common Allium crop in the Netherlands. Of the wild relatives only Allium vineale is common, but seems not to be attacked by the onion fly.

Losses due to onion fly damage may amount to 50-100%, and are partly dependent on soil type. The appearance of damage has typical features that depend on the developmental stage of the onion attacked. The economic threshold is at a damage level of about 10%.

Chemical pest control has become widespread after the introduction of organochlorine insecticides in 1946. Resistance to these compounds developed from 1953 onwards, in the Netherlands in 1962-1965. Since then control has been practised with different organophosphorous compounds. Of these, dichlofenthion has already met with resistance. Currently used in the Netherlands is trichloronate, because of its effect on onion stem eelworm reproduction.

Insecticide residue levels in onions are generally low or undetectable.

3 Environment and discussion of materials and methods

Information is provided on the environment in which the experiments were done and on the prevailing weather conditions. As many experiments were with sterilized flies from a laboratory mass rearing, some data on this rearing and sterilization are given.

The methods that were developed and used in the field work are described and evaluated, especially fly marking, releasing and trapping, and sampling of pupae, as these methods are important in sterile male experiments.

3.1 ENVIRONMENT

3.1.1 Experimental area

The field trials were carried out on the former island of Overflakkee. This area has been one of the main onion growing areas for a long time. The island is nearly completely flat, and partitioned by dikes around polders that have been reclaimed since the 15th Century. Apart from the roads, water, isolated farms and a dozen villages, almost all the land is used for agriculture. Trees and shrubs are scarce except along the dikes, around the farms and in the villages. Meadows are present on most slopes of the dikes and on some slightly lower parts, former creek beds. Typical scenery is shown in Fig. 11.

The crops grown are mainly wheat, potatoes and sugar-beet, with smaller amounts of tulips, gladioli, onions, beans, barley, carrots, chicory, and different agricultural

Fig. 11. Typical scenery of Overflakkee, the former island on which most experiments were carried out.
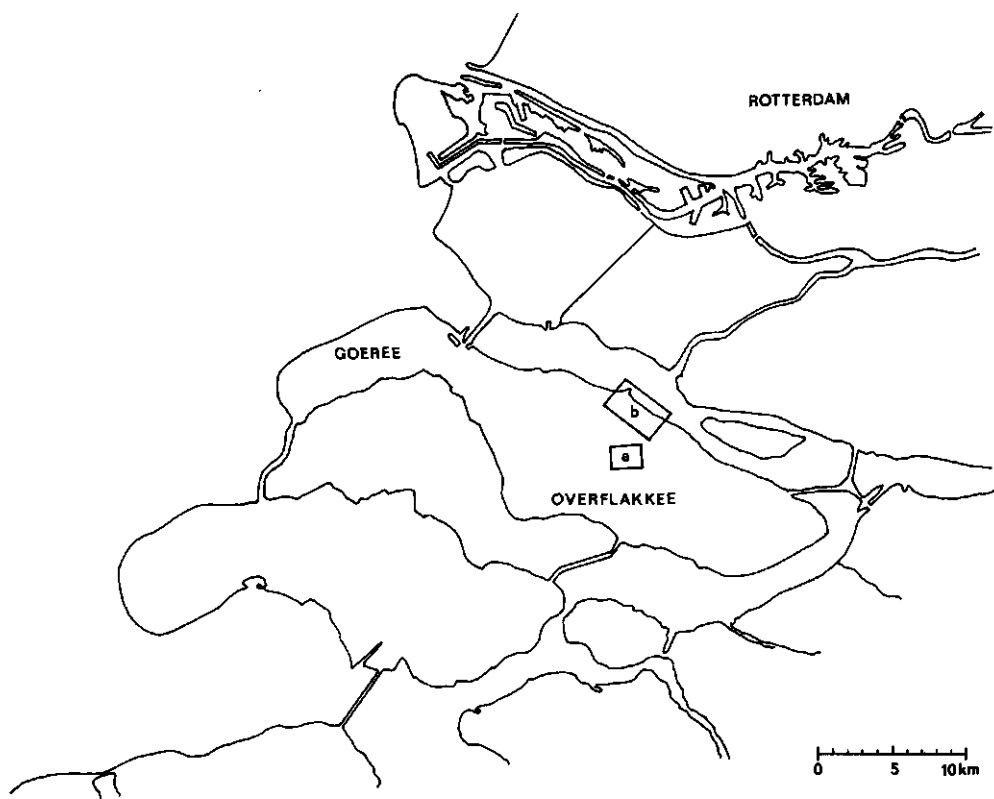
Fig. 12. Map of the former island of Overflakkee and surroundings, showing the location of the main release-recapture experiments. a = Vroegindewei 1972-2 (Fig. 13), b = Mijs 1973 and 1974 (Figs 14 and 15).
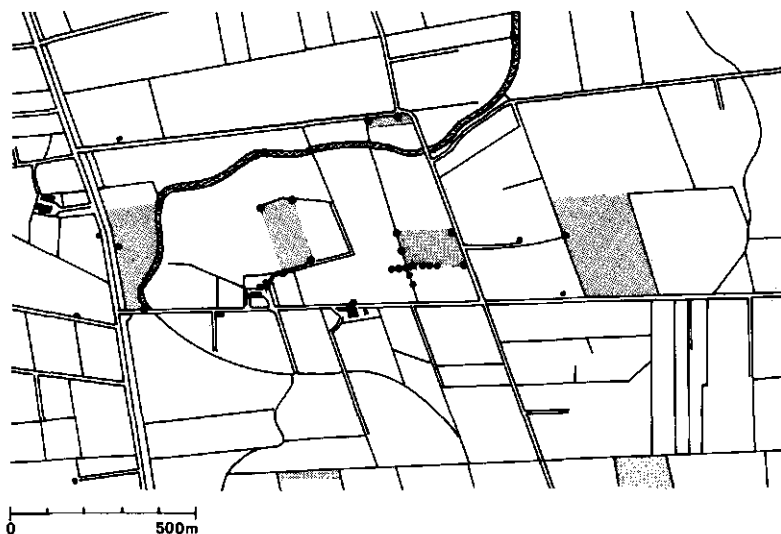
Fig. 13. Map of the release-recapture experiment Vroegindewei 1972-2. For location see Fig. 12. ★ = release site; ● = trap; —— = ditch; ■ = buildings; ▨ = onion field; ≈≈≈ = water.

○   release site
▨   onion field 1972
▧   onion field 1973
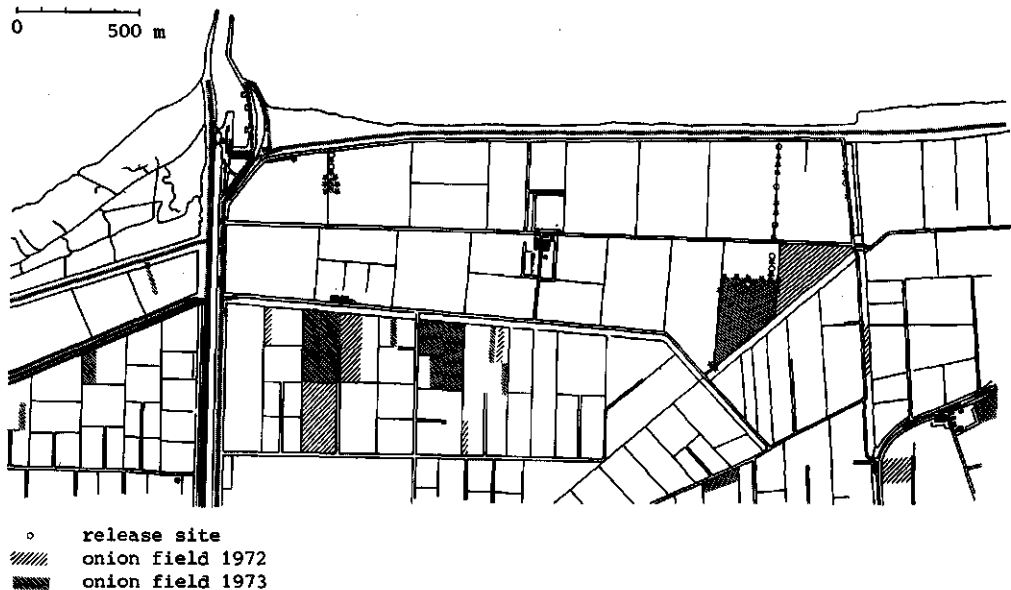
Fig. 14. Map of the release-recapture experiment Mijs 1973-2. For location see Fig.12.

⎯⎯   dike
⎯⎯   ditch
·   trap
▨   onion field 1973
▧   onion field 1974
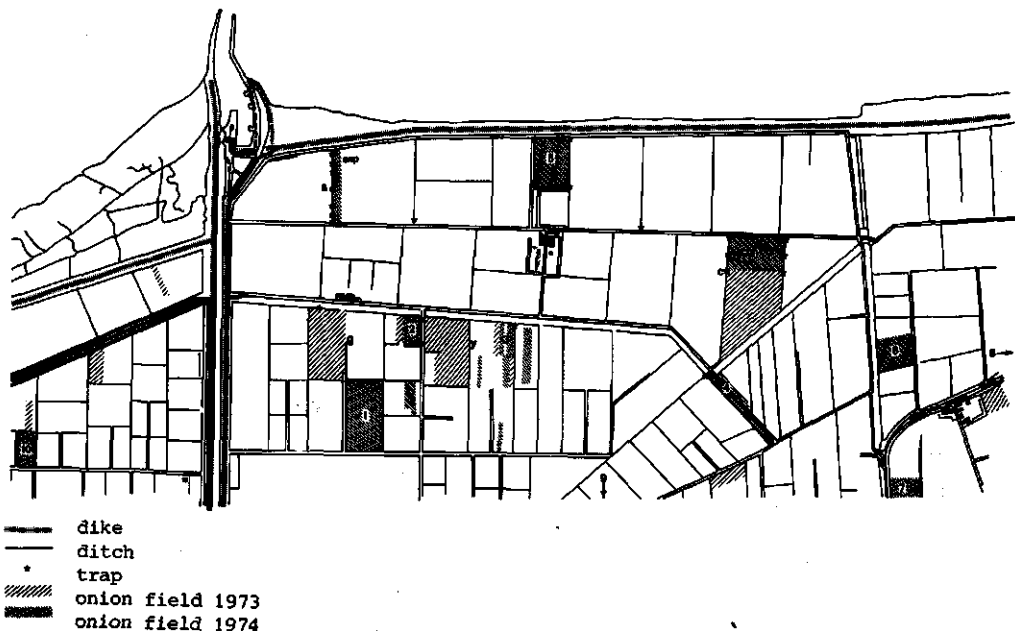
Fig. 15. Map of the release-recapture experiment Mijs 1974. For location see Fig.12. Symbols see Section 4.3.3; 'exp' is the sterile insect technique trial field; left part of 'con' is the untreated control field.

Table 3. General data on release-recapture experiments.

Experiment,year,flight	Number of flies released	Sterile/ fertile[a]		Marks	Pupae in diapause	Number recaptured
van Loon 1970-2	11 450	S	a	$^{32}P, ^{65}Zn, ^{89}Sr$	no	147
Pollemans 1971	23 490	S	b	daily dyes,^{65}Zn	no	394
Buijs 1972-1	16 990	F	c	1 dye/site	yes	71
Vroegindewei 1972-2	26 800	F	d	1 dye	no	409
Mijs,van Es & de Wit 1973-1	24 800	F	e	1 dye/site	yes	191
Mijs 1973-1	61 270	F	f	daily dyes/site	no	1 030
Mijs 1973-2	96 400	F	g	daily dyes	no	2 416
Mijs 1973-2	135 900	F	h	1 dye/site	no	3 263
Mijs 1974-1	73 950	F	i	1 dye/site	yes	642
Mijs 1974	1 411 680	S	j	1-2 dyes/group	no	10 373
Mijs 1974-1	171 610	S	k	1 dye/site	no	688
Mijs 1974-2	57 600	F	l	1 dye	no	682
Schuilenburg 1971-1	5 830	F	m	^{65}Zn	no	80
Schuilenburg 1971	48 100	S	n	^{32}P or 1 dye/group	no	791
Schuilenburg 1972	614 600	S	o	1 dye/group	no	4 655
Schuilenburg 1973	1 580 100	S	p	1 dye/group	no	14 205
Schuilenburg 1974	1 118 500	S	q	1 dye/group	no	4 614

a. Letters indicate location of release sites: a: centre of onion field. b: former onion field and dike near onion field. c: 5 fromer onion fields. d: onion field,near edge. e: 3 untreated experimental plots. f: 100 m from experimental plot in 4 directions. g: onion field, near edge. h: different distances from two onion fields. i: 3 former onion fields. j: onion field edge, 17 weekly releases. k: 2 former onion fields, 5 weekly releases. l: edge of control field.
m: near onion field. n: near(2nd flight:in) onion field, 10 releases. o: in onion field, 19 weekly releases. p: generally in onion field, 19 weekly releases. q: in onion field, 18 weekly releases.

and ornamental plants grown for seed, like grass, carrots and violets. The soil is mainly sandy clay.

During the past decades the farm and field sizes have increased considerably. The average onion field size on Overflakkee during the experiments was 2-2.5 ha. On the average 2/3 of the onion field borders consisted of ditches, generally containing water.

The percentage of land occupied by onion growing in those polders of Overflakkee where the experiments were done ranged from 5 to 15. In 1820 this was only about 1% (Boers, 1843), but before 1880 the onion crop density had probably reached 5% of the area (estimated from data from the SNUiF).

In Fig. 12 the former island of Overflakkee is shown with the location of the areas in which the main release-recapture experiments were done. Detailed maps of these areas are given in Fig. 13-15.

General data on all release-recapture experiments are given in Table 3.

3.1.2 Weather

The maximum daily temperature and the daily rainfall are given in Fig. 16 and Table 4. Observations are from different sites on or near Overflakkee, depending on their availability. The number of monthly values, outside the relevant 80 or 90% confi-
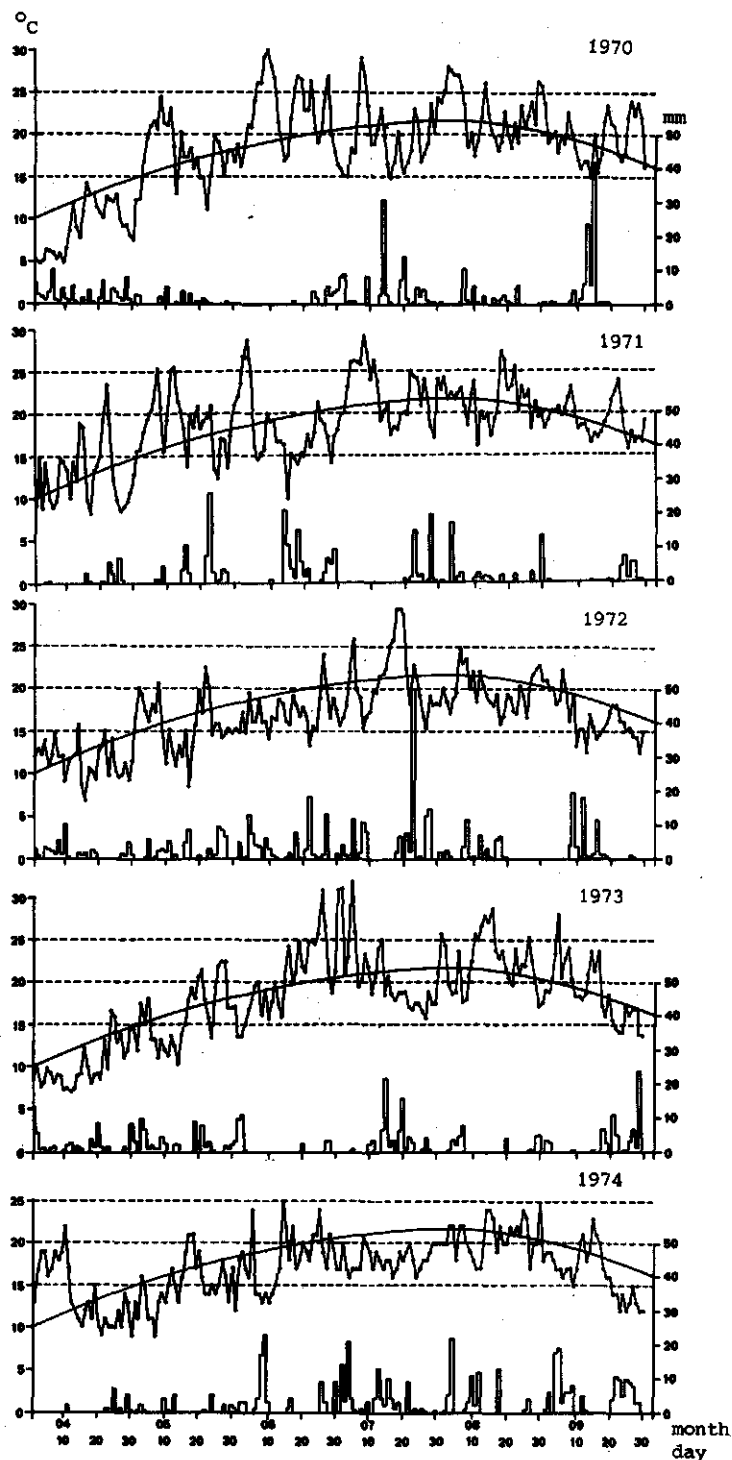
Fig. 16. Weather data from the seasons during which the experiments were done. Data from sources as indicated in Table 4.

-•- = Maximum temperature per day in °C.
— = Average maximum temperature per day from 1931-1960, smoothed curve.
⊓ = Rainfall per day in mm.

Table 4. Monthly weather data, Overflakkee 1970-1974. Data from the Royal Netherlands Meteorological Institute (KNMI) (Anonymous 1972; pers. commun.).

	1970[a]	1971[a]	1972[a]	1973[b]	1974[b]	Average 1970-1974	Average 1931-1960[c]	Confidence interval 80%[d]	Confidence interval 90%[d]
Maximum temperature per day in °C									
April	9.0**	12.9	11.5	10.2*	13.1	11.3	12.7	10.5-14.9	9.9-15.5
May	17.8	19.0*	15.8	16.3	14.9*	16.8	16.8	15.0-18.6	14.5-19.1
June	22.8**	17.9	17.2**	20.5	18.2	19.3	19.5	17.8-21.2	17.3-21.7
July	19.8	22.5	20.8	21.0	18.2**	20.5	21.2	19.5-22.9	19.0-23.4
August	22.6	21.5	19.9	22.6	20.7	21.5	21.3	19.3-23.3	18.7-23.9
September	19.5	19.2	16.7*	19.0	16.5*	18.2	18.8	16.8-20.8	16.3-21.3
October							14.2	12.7-15.7	12.3-16.1
Rainfall per month in mm									
April	73	21	51	50	16*	42	41	20 - 75	
May	20*	71	68	70	22	50	45	(21 - 80)	
June	17*	90	92	33	72	61	55	23 - 93	
July	99	41	135*	73	109	91	70	(26 -120)	
August	30	60	43	31	74	48	73	28 -135	
September	85	25*	62	68	136	75	76	(29 -138)	
October							72	23 -132	

Testing for significance:
temperature: 30% outside 80% confidence interval (n.s.), 13% outside 90% confidence interval (n.s.);
rainfall: 17% outside 80% confidence interval (n.s.).

a. Nieuwe Tonge (Overflakkee).
b. Temperature data from Hellevoetsluis (Voorne), rainfall data from Dirksland (Overflakkee).
c. Middelharnis (Overflakkee).
d. Temperature: calculated with standard deviation from Vlissingen (Walcheren),1931-1960; rainfall: interval from Middelharnis (Overflakkee), 1931-1960. Interpolated values between brackets.

dence intervals, did not significantly differ from that expected. So the weather of the summers of 1970-1974, during which the experiments were done, can be taken as reasonably representative. From the five-year averages it can be seen that only April was colder than average.

3.2 MATERIALS: FLIES USED FOR THE EXPERIMENTS

3.2.1 Mass rearing

The flies used in the release experiments were obtained from the mass rearing at the IPO in Wageningen. For details on the rearing methods see Ticheler (1971) and Noorlander (1974).

The larvae were reared on an aseptic artificial medium, based on carrot powder, at a constant temperature of 22°C or 17°C, under daylength conditions of 16 hours or 10 hours light per 24 hours, for obtaining non-diapausing or diapausing pupae, respectively. Non-diapausing pupae can be stored for at least a year at 3°C, but the quality of the resulting flies decreases. They should be at 15-20% of their age when transferred to cold storage.

The flies emerge 12-13 days after transfer of stored pupae to 21°C. The flies were kept in cages, generally 60 x 40 x 40 cm perspex, with 2500 flies each, at 21°C. They were given water, dry food and onion odour. Eggs were deposited on devices which provided the necessary chemotropic and tactile stimuli. The net reproduction factor per generation was during the years concerned about 10 x. The top production amounted to 2.10^{6} pupae per month.

As mentioned (Section 2.1.2) the rearing produced aberrant males in frequencies of about 2-10%.

3.2.2 *Sterilization*

Pupae were sterilized at on the average 85% of their age, being one day before the start of emergence. The sterilizing agent was 3 kR gamma-radiation from a ^{60}Co-source, or X-rays from an electron generator. This dose causes complete infecundity in the females. About 0.6% of the eggs of non irradiated females mated to irradiated males hatch (Noordink, pers. commun.). For more details on sterilization see Ticheler & Noordink (1968) and Noordink (1971).

For more details on sterilization see Ticheler & Noordink (1968) and Noordink (1971).

According to common usage, the term sterility and related terms are used throughout this report. Often however the term sterility is used to indicate absence of sperm, whereas in sterilized onion flies, competitive sperm is present but contains dominant lethal factors so that eggs 'fertilized' with this sperm do not develop. The controversy about the effect of multiple matings on the feasibility of the sterile male method for insect pest control, arising from the use of the term sterile in this context, has been elaborated by von Borstel (1960). The effect on genetic control of different assumptions about the effect of sterilization on the presence and the competitiveness of sperm, also in respect to the number of matings, has been formulated by Berryman (1967).

The percentages sterility given in this report always refer to populations, not to the degree of sterility of individuals.

Fertile females, when mated to males irradiated with at least 1.5-2 kR, lived 1.3 times longer and had their fecundity increased by a factor 1.6-1.8 (Noordink, 1974), provided that irradiation had taken place at the right moment (see Ticheler & Noordink, 1968). A similar effect can be found in the data of McClanahan & Simmons (1966). It is interesting that a similar increase in fecundity resulted from mating with ^{65}Zn-labelled males (Noordink, 1974). A possible cause is a radiation effect on the male accessory gland substance, which has an effect on oviposition (e.g. Leahy, 1967; Riemann & Thorson, 1969; Swailes, 1971).

Competitiveness is a measure of the difference between certain individuals or populations and the wild type of the same species. It is expressed as the number of individuals that are equivalent to one wild individual.

To calculate the competitiveness of sterile males from egg sterility data obtained in competition experiments, these data have to be corrected for the natural sterility of about 5% on the average, for the 0.6% hatch of 'sterile' eggs, and for the surplus of eggs laid mentioned above. Thus, a part of the sterile eggs, to be calculated from a

fertile control group, has to be considered as originally fertile, and the number of
sterile eggs has to be corrected by a factor 1/1.7 (the factor 1/0.994 can be neglected).
When the data of the competition experiments, given by Noordink (1971) and Ticheler (1969)
are corrected in this way, mating competitiveness values of 0.79-1.04 are found, with on
the average 0.87. Because of the high dependency of the fecundity increase on the age at
irradiation (Ticheler & Noordink, 1968), this competitiveness can be considered to be not
significantly different from 1.

When egg sterility data have been corrected, if needed, like indicated above, the
competitiveness c of a type present in the population in a fraction p, and with effect
in a fraction e, can be found from $e = cp/(cp + (1-p))$. This equation can be written as

$$c = (e-ep)/(p-ep) \qquad\qquad (1)$$

The same results can be obtained with the formulae that are given and amply explained
by Fried (1971).

3.3 DISCUSSION OF METHODS

3.3.1 Marking

Flies can be marked in many different ways (see e.g. Southwood, 1966). Because high
numbers of marked flies were needed, and because in most experiments it is inadvisable
to handle them, methods of applying recognisable materials to the flies were not attrac-
tive. The remaining possibilities are marking the flies externally at emergence, inter-
nally by larval ingestion, or changing the genotype (markers, sterility) or the phenotype
(by means of the rearing circumstances). Genetic markers were not available. The flies
were usually marked with daylight fluorescent dyes at emergence.

3.3.1.1 Marking larvae

Theunissen (1969) mixed rhodamine B and acridine orange through the larval diet.
Both substances were shown to be toxic to the larvae, and did not mark the adults. I did
similar experiments with Helecon 2266, fluorescein, DayGlo red, neutral red and Calco oil
R-1700. Fluorescein and neutral red could be recognised in the adults, but they were toxic.
The other substances, of which the Calco oil was toxic, did not mark the adults.
Noordink (1973) mixed several rare earth elements through the larval diet, in order to
recognise the adults after neutron activation. The incorporated elements, however, dis-
appeared from the larvae at pupation. Moreover, this method produces much radioactive
waste, so its application should be restricted.
Radioactive labelling was made effective by Noordink (1971). The most useful labels
found were ^{32}P for short-term experiments and ^{65}Zn for long-term experiments. The ^{65}Zn is
transferred very well to the next generation (Noordink,1973),so that it is especially
suitable for experiments on reproduction.
The half-life of ^{32}P is 14.3 days. Noordink (1970) found 9-10 days as effective

(biological and physical) half-life in onion flies in the laboratory. The effective half-life of ^{32}P in onion flies in the field was calculated from data of two release-recapture experiments, under almost identical temperature conditions. Effective half-lives of 6.3 and 5.8 days were found. The difference can be attributed to chance, so the effective half-life of ^{32}P in onion flies in the field can be taken as 6 days.

The physical half-life of ^{65}Zn is 245 days, its biological half-life may be even longer apart from loss in oviposition.

3.3.1.2 Marking emerging flies

Application In most of the experiments to be described here, the marking method developed by Norris (1957) was applied: the flies are forced to pass a dye layer when they are moving to the soil surface after emergence. They become coloured all over. After reaching the surface they retract their ptilinum, on which the dye is retained. This method combines several advantages: (a) the flies are not handled, (b) no substances need to be incorporated in the larvae that might be harmful, (c) it can easily be applied to large numbers of individuals, (d) it can easily be checked immediately after recapture, (e) several different dyes are available, and (f) the dye cannot be lost.

A disadvantage is that the flies generally have to be killed before the mark can be checked. Furthermore the head should be present, and the fly should preferably not have been in a fluid.

Fig. 17. Construction of dye and release unit for emergence of flies, cross-section.
The pupae, mixed with earth, were put in a container of PVC tubing, o.d. 8 cm, which is standard rain pipe. The bottom was closed by polyethylene foil, tightened with a PVC ring. On the container a dye unit was placed, which was originally together with the ring a rain pipe connecting piece. To its bottom plastic gauze, mesh 2 mm, was glued. It was filled partly with earth, the undermost part of which was garden mould because the little roots and clods in it prevent the earth falling down through the gauze. This earth was slightly compressed and covered with fine earth granules with a small sieve to get a smooth surface. Over this a thin layer of dye powder was applied, which was covered again with a few millimeters of sieved earth. Each dye unit needed about 10 g of dye powder. This amount covered the 50 cm^2 of earth completely, and resulted in a 100% marking of at least the first 5000 flies. Some grass or a stick was laid on the dye unit so that the young flies could climb out easily.
The earth in the pupae-container should be compressed and reach to just under the upper rim, so that after fitting the dye unit there is no empty space left between. Also, the earth should be somewhat clayish so that it will not settle down when flies have emerged. Even though many flies used the same holes for reaching the surface the quality of the dye mark they obtained was not impaired.
To check emergence, traps were placed on certain units. These were of extruded PMMA (plexigum) tubing. Plastic gauze, mesh 2 mm, was glued to the base. For emptying, the top side was provided with a funnel of plankton gauze, that could be closed with a wad of paper tissue.
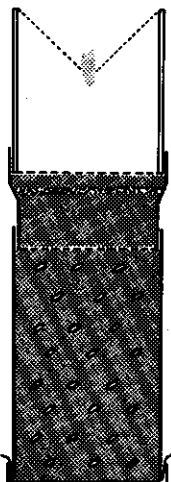
The dyes I used were several RadGlo pigments, a type of daylight fluorescent powder, with a particle size of 5 μm. The dye was usually applied according to the principle described in Fig. 17. This modification allows the dye colour to be changed during the emergence period, also permitting reusage of the dye layer.

A modification of the marking of emerging flies, often used in sterile male programmes, consists of mixing the pupae with the dye powder, and letting them emerge. Because the confinement of young flies may change their normal behaviour, this method was only used incidentally. However, no effect on the dispersal of the flies was found (Section 5.2.4). The release cages, used with this method, are described in Section 3.3.2.2.

Recognisable dyes Fourteen different RadGlo colours with particle size of 5, 2-4 and 1-2 μm were tested for distinguishability. All resulted in well marked flies. Clearly recognizable were the set consisting of blue (B), green (G), yellow (Y), orange (O) and carmine (magenta) (C) with all their combinations in pairs except OC. Moreover some combinations of three different colours were also clearly recognizable: BYO, BYC, GYO and GYC. In these combinations OC cannot be used, and BG is difficult to distinguish from BY in the presence of O or C. These difficulties arise because the dye powders, when mixed, partly show even with 80 x magnification their combined colour, like green for BY. Therefore the mixing should not be done too well. Y proved to be a weaker colour, so more than 1:1 should be used of it in mixtures, whereas of B and C less may be used.

Fitness of dyed flies A possible effect of the dye on the fitness of the flies under field circumstances was looked for in one of the release-recapture experiments (exp. Pollemans 1971). Pupae labelled with ^{65}Zn were dug in the field under dye units that were changed daily. One day a dye unit without dye layer was provided. The recaptures give no reason to assume any diminished fitness of dyed flies. The observed decrease in number of recaptures with time of emergence will have been due to predation (see Section 3.3.2.1) and occurred as well in a simultaneous release of dyed flies, emerging at a distance of about 100 m from the labelled group.

The flies clean themselves the first few days after emergence. So any effect of a changed colour of the flies on their life-span or competitiveness, as observed for red dyed medflies by Holbrook et al. (1970), is not to be expected.

3.3.1.3 Marking by sterilization

Obviously sterilization is a marking method in sterile male programmes. In the sterile male experiment Mijs 1974 (Section 6.2) sterility of the females was assessed, apart from the dye marks used, by their ovary development. In females sterilization stops ovary development (Theunissen, 1971). It was shown, by inspecting fertile females (wild and released) that 95.3% of the fertile females caught had a yolk formation visible by checking with 80 x magnification under a microscope, and could thus be recognised as being fertile. This yolk formation corresponds with the stages S5 and higher in the classification of Theunissen (1973a). The high percentage of fertile females identifiable as such, is due to the relative low probability of capturing young ones (Table 25).

30

Theunissen (1972) found that safe identification of males as being sterilized was only possible histologically. This method is too time-consuming for practical application in field work. Thus sterility is only a useful tag for females, especially when the steriles are in the majority.

3.3.1.4 Wing vein aberrations

Wing vein aberrations (Section 2.1.2) could be used for a marking method. Different populations screened for this character had different wing vein aberration patterns and frequencies. A preliminary laboratory experiment failed to show any heredity. According to Sick (1967) thickenings of the vein r_{2+3} may be the result of environmental conditions during the pupal stage. However, the wide variability offers the possibility for selection, or by inbreeding heritable wing vein aberrations may be found.

When in laboratory rearing certain aberrations can be selected for, or when these can be induced in the phenotypes, they can be used as a natural marker in field studies. Because of apparent lack of function and their high natural frequency, these aberrations probably have no deleterious side-effects. The identification is very simple and can in clear cases be done with the naked eye.

3.3.1.5 Size

In principle fly size can be used as a feature for recognition. As a measure of the fly's size, the length of the wing vein r_{2+3} was chosen for practical reasons (relatively long and seldom damaged). The inner angles that the vein makes with the other veins are considered as its beginning and end. Its length is well correlated with the pupal weight (correlation coefficient for females: $r = 0.929$). The wing vein length fitted the normal distribution for the males, but not for the females due to relatively high numbers of shorter wing vein lengths.

Flies can be reared that are on the average different in size from the wild populations, generally smaller. Because partly overlapping normal distributions can be separated statistically, size can be used as a mark for males, or for females if larger than normal, when the numbers recaptured are large and one is not interested in the individual recaptures. This may be so, for instance, in determining the percentage sterility among flies.

The danger in using flies of a deviating size lies in the possible effect on their competitiveness, and on the fact that their behaviour, for example in dispersal, is not representative for the wild flies. The latter aspect is considered in Section 5.2.4. Reduced competitiveness due to size has been reported for smaller males by Riedel (1967) with the cabbage root fly, Delia brassicae (Bouché), and by Alley & Hightower (1966) with the screw worm, Cochliomyia hominivorax (Cqrl.).

3.3.2.1 Releasing pupae

The method used in releasing pupae has been described mainly in Section 3.3.1.2. Ploughing causes wild pupae to become dispersed throughout the upper 25-cm layer of the soil. Thus, to get an emergence curve of diapausing laboratory-reared flies that is identical to that of the wild ones, containers of 25 cm length were buried in the soil.

The emergence of the flies in the spring is dependent on the soil temperature, so the
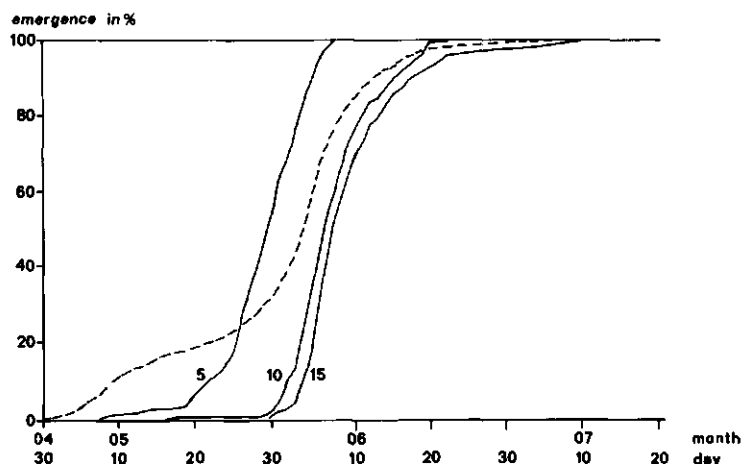
Fig. 18. Emergence of diapausing pupae from different depths under bare soil, Schuilenburg 1972. ———— 5, 10 or 15 cm depth,- - - pupae mixed over 0-15 cm depth.
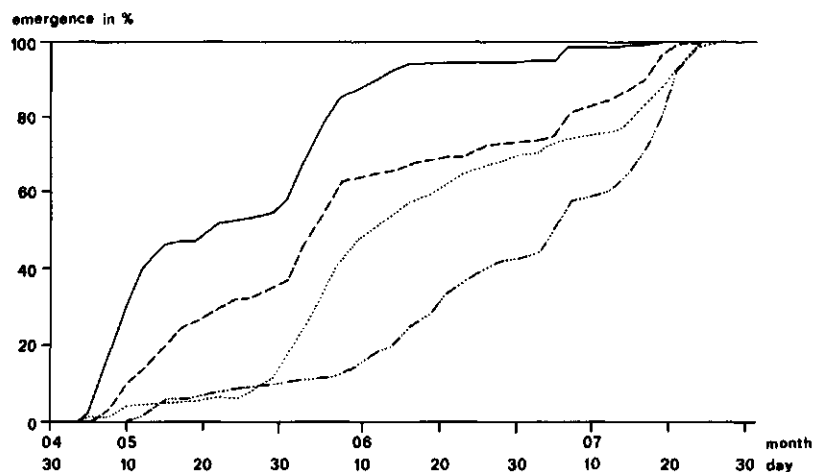
Fig. 19. Emergence of diapausing pupae under different vegetations. Pupae mixed over 0-25 cm depth. Experiment Buijs 1972.
———— under silverskin onions; - - - under sugar-beet; ····· under winter wheat, rather low crop; —— ···— under winter wheat, rather high crop.

pupae that are less deep in the soil will emerge earlier. Experimental data on the effect of depth are given in Fig. 18 and also by Rygg (1960). It is clear that the vegetation will have an influence too by keeping the soil temperature lower. This effect, i.e. of the crop following the onions, is shown in Fig. 19. Some similar data have been given by Ticheler et al. (1974b).

For experiments in which the emergence curve should be preferably short, pupae were generally put in containers of 12 cm length, and dug in only partly to prevent falling over. The release units were placed in the crop or in the vegetation in the ditch.

To limit predation of the newly emerged flies by birds, the release units were covered with a few m^2 of iron gauze, mesh 3 cm. Pieces of polyethylene foil were attached to this as a protection against rain. Predation by mice on the pupae was prevented by the plastic gauze bottom of the dye unit.

The emergence was not influenced by the presence of a dye layer, but a cover from rain proved to be essential. The bottom of the pupae container should be closed with foil and not with gauze. A gauze bottom caused 5-10% of the flies to move downwards and die or (if mesh of 1 mm or more is used) escape and emerge without dye. With rainy weather and without cover from rain this percentage reached 40.

The recapture rates of daily cohorts released without protection against birds (see Section 3.3.1.2) show a decline, especially evident in the group released in the more exposed site (sugar-beet field versus grassy slope of dike). This will be the result of a learning process of insectivorous birds. The prevailing cold weather will have affected the activity levels of birds and flies differently, probably resulting in a high predation level.

The recapture rates of successive weekly releases of flies (Fig. 20) also indicate the effect of predation. The first two groups emerged in rather cold weather, resulting in a widely spread emergence and probably lower fly survival. The obvious decline in recapture rates at the end of May will be the result of a learning process of insectivorous birds that were regularly observed near the release sites. The later recapture rates were more or less stable. The reason for the high rate of the group emerged 21 July is not known.

During this series of releases two experiments were carried out to find ways to diminish predation. In the first experiment the effect of different numbers of flies emerging per release site was analysed. On two occasions the flies were released in two groups, one emerging in high numbers at one site, the other in low numbers at several sites (Table 32). The flies from crowded release sites had a 35-45% higher recapture rate (Fig. 20, upper points on 13 and 29 July). This difference, if significant, may be due to the birds having already learned to find the release sites by eye. Thus at the more crowded sites the flies had the advantage of crowding under a constant predation pressure. An effect of density on the initial dispersal activity may have played a role as well.

The other experiment consisted of the standard release of large numbers on one site combined with the simultaneous release of flies from cages as described in the next section, 1-2 days after their emergence, also on one site. The release of flies from cages resulted in an immediate spread of the flies up to about 10 m distance, whereas the flies that emerged in the field after having left the wire gauze cover mostly stayed at first
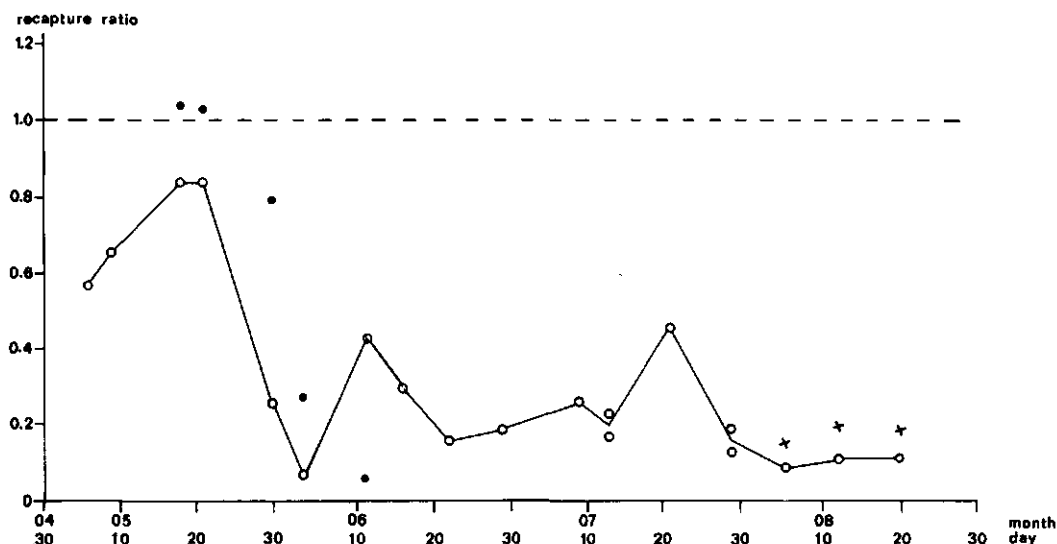
recapture ratio

Fig. 20. Competitiveness of successive releases of sterile onion flies, expressed as the ratio of observed recapture rate to the recapture rate of the diffusely emerging wild flies, versus the date of 50% emergence. For the flies released outside the experimental field the percentage of the recapture rate of fertile flies, released from diapausing laboratory-reared pupae, has been used as the best possible approximation. Experiment Mijs 1974. Data see Table 32.
o, X = data from experimental field(11 June less reliable observation); X= flies released from cages; ● = data from commercial onion fields.

within a few metres. There was a rather consistent difference, the recapture rate being 60-70% higher for those released as flies.

In the Schuilenburg experiments, especially in 1973 and 1974, predation of birds on the newly emerged flies was obvious. Satisfactory results are claimed from the use of a web of artificial fibres (Starex) over the release units (Ticheler et al., 1975). The only safe method, however, will be to prevent the occurrence of high initial concentrations of released flies.

Depots of pupae to check the emergence were constructed as described in Section 3.3.1.2. On two occasions the emergence during the day was checked. The results are given in Fig. 21. The depots thus should be visited preferably in the late afternoon. For practical reasons this has generally been done between 16 h 00 and 18 h 00.
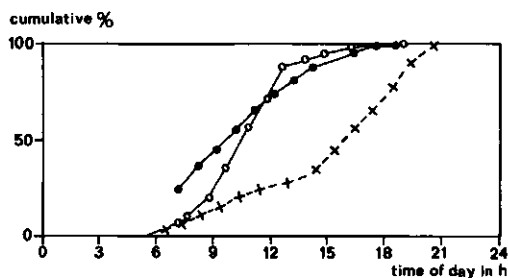
Fig. 21. Onion fly emergence from depots, and catches in flight interception traps, during a day.
● = emergence, 25 May 1972;
o = emergence, 27 June 1972;
+ = trap catches, 27 June 1972.

3.3.2.2 Releasing flies

Flies were released according to two methods. The first, used in the experiment Mijs
1973-2, was designed to permit the flies to be kept caged if necessary for several days.
The flies emerged, marked by the method described in Section 3.3.1.2, in the perspex
rearing cages of 60 x 40 x 40 cm, at a density of 6240 flies/cage. Food and water were
provided. In due time the cages were transported into the field by night, and covered
with black polyethylene foil except for one side which was left open. By the next after-
noon all healthy flies had left and the cages were removed. Application of this method
may have had an influence on the dispersal speed (see Section 5.2.4).

A less voluminous method was used at the end of the experiment Mijs 1974, as mentio-
ned in the preceding section. The flies emerged on small trays placed in 'cages' of
60 x 40 x 1.2 cm, at a density of about 3 flies/cm^3, or nearly 2 flies/cm^2 of resting
surface. These 'cages' were 60 x 40 cm wooden frames, on the underside of which iron gauze,
mesh 1 mm, had been stapled, thus forming a kind of tray. The upper side of the frame
was provided with a band of plastic foam (draught-stopper). These trays were piled up,
the one covering the other. This pile of 'cages' was kept in complete darkness to pre-
vent local aggregation of the flies, and occasionally some water was dripped through the
cages. The flies had to be released at an average age of 1-2 days to prevent excessive
mortality. The percentage mortality, including flies not yet emerged at the moment of
release, was dependent on the fly concentration: densities of 1.6, 3.2, 4.8 and 6.5
flies/cm^3 resulted in mortalities of 19, 18, 24 and 34%, respectively. These values are
not exact because the gauze used was too coarse for the flies of the group concerned
(average pupal weight 14.4 mg): some hundreds of flies came through the gauze and hundreds
died in trying this. This did not occur in another group with 16.2 mg average pupal weight,
so the percentage of flies not released from this group, 13%, will be inevitable with the
current spread in emergence. The maximum concentration to be used may be estimated at
about 5 flies/cm^3.

The flies were released by putting the pile of cages uncovered in the field as
shown in Fig. 22 for about half an hour.
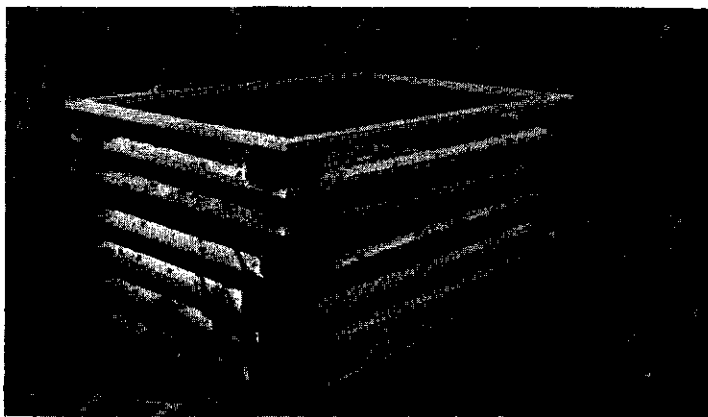
Fig. 22: Release of flies from cages.

Flies can be trapped in many different ways (e.g. Southwood, 1966). For trapping onion flies generally bait traps have been used in which the flies drown (Noordink, 1967 and others), or in which bait was used to get the flies in a funnel placed over it (Peterson, 1924; Matsumoto, 1970; Eckenrode et al., 1975b and others) or into some other device (Lafrance, 1951). Sticky traps have been used by Roesler (1953), Shirck (1957) and Ellington (1963).

To inspect the ptilinum for the dye mark, and the ovaries for sterility, the flies had to be fresh and dry. For assessing egg sterility, female flies were needed alive. Therefore generally flight interception traps were used, in which many of the flies stayed alive unless there were some predators present. The flight interception trap was developed according to the specific needs encountered, and was generally used without attractant so that the number of variables influencing the catch size was limited. Later some experiments were done on attraction, especially to increase the catch of the flight interception traps.

3.3.3.1 Flight interception traps

Construction The behaviour of the flies near different gauze screens put in the field has been observed. The flies seldom fly above the local vegetation. In an onion field this is up to 50 cm, and on ditch banks generally 10-30 cm, depending on the mowing intensity.

Trapping equipment higher than 50 cm, placed in onion fields, is easily damaged by the spraying booms, necessary for herbicides and fungicides.

After experience with a preliminary trap model, developed from the principle of Malaise (1937) (Loosjes, 1971), a simple flight interception trap was made (Fig. 23), comparable to those described by e.g. Gressit & Gressit (1962), Butler (1965) and Aubert et al. (1969). The trap consisted of a plastic gauze screen, stretched by three nylon ropes over two aluminium poles. Collectors of plexigum tubing fitted into plexigum rings glued on the gauze roof in both upper corners. The screen height was 50 cm, in some cases 75 or 100 cm, the entrance height was 10 cm less. When traps of different height were placed side by side there was no systematic difference among their catches, provided that the vegetation was clearly below 40 cm.

Location The location of traps has a considerable influence on the numbers of flies caught. Onion fly populations aggregate near onion fields. So the number of flies trapped is a function of the distance from an onion field and, during the 1st flight, also from the distance from an infested onion field the year before. The effect of the trap position is shown in Table 5, comparing catches in traps placed within a radius of about 100 m of each other. Clearly the optimum position is along the side of an onion field, bordering a ditch or a crop (cereals) which provides shelter, and with the trap entrance turned away from the onion field.

The variation among the catches in traps that have seemingly similar positions can
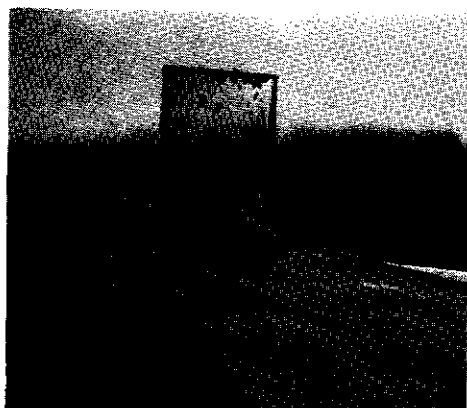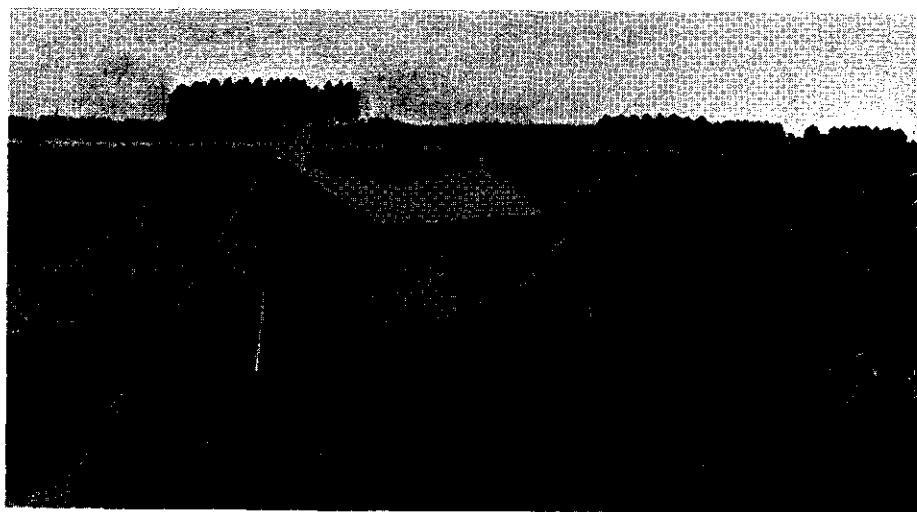
Fig. 23. Flight interception trap as used in most release-recapture experiments. On top: trap in optimal position in the field; below: collector.

Table 5. Effect of trap position on catch size. Data expressed as relative catches per trap, for standard position = 100. Between brackets data from one trap only. Other data based on 2-12 traps.

	Trap position code[a]									
	1	2	3	4	5	6	7	8	9	10
Pollemans 1971-1	100	.	15	.	.	.	.	.	.	.
Pollemans 1971-2	100	.	62	36	.	.	.	43	18	30
Vroegindewei 1972-2	100	.	.	(20)	(32)	.	(138)	61	27	21
Schuilenburg 1972	.	100	45	.	.	75	.	45	.	36
van Es 1973-1	100	.	80	.	.	.	.	.	.	.
Mijs 1973-1	100	93	.	.	.	.	.	.	.	.
Mijs 1973-2a	100	.	26	.	.	.	.	.	.	20
Mijs 1973-2b	100	.	72	.	.	.	.	.	(5)	9

a. Position code: 1 = edge of onion field, ditch bank, open side away from onions; 2 = edge of onion field, cereals; 3 = edge of onion field, others (potatoes, sugar-beet); 4 = edge of onion field, ditch bank, open side towards onions; 5 = as 4, but halfway down ditch bank; 6 = as 1, but a 3 m wide path between trap and onions; 7 = as 2, but placed at right angles to edge of field; 8 = in onion field; 9 = outside onion field, ditch bank; 10 = outside onion field, in other field.

still be considerable. For ten traps in an onion field the mean and confidence interval for one year's catch were 444 and 299-589, respectively.

One would expect that the number of flies caught in the traps depends on their flight direction. No effect of trap position on catch size with respect to the direction of dispersal was found. The flight directions therefore have a low correlation with direction of dispersal, which can be due to predominantly random movement.

Daily rhythm In order to know the period for which the weather could be correlated to the catch size, the moment of checking the traps in relation to the daily rhythm of trapping should be known. Therefore the activity of the flies and the occurrence of captures were checked.

The results of the most extensive data set obtained are given in Fig. 21. Many incidental observations indicated that the daily rhythm as found here, with a maximum catch towards the end of the day, was representative of the normal situation. A similar rhythm has been found, like in many other insects, for the activity rhythm of the onion fly in the laboratory (Brunel & Rahn, 1971).

Thus the best time for changing the collectors is either after sunset or early in the morning. For practical reasons the collectors were changed generally between 8 h 00 and 10 h 00, and all flies were considered to have been trapped the day before.

Influence of weather The daily catch size is proportional to the local population density and to the activity of the flies. The former factor causes long-term changes, the latter depends on the age distribution of the flies causing also long-term changes, as well as on the weather which causes short-term fluctuations. The change in numbers of the flies caught from one day to another will mainly reflect the influence of the weather on the activity of the flies.

To estimate this weather effect from the trapping data, catches on consecutive days were analysed in relation to the corresponding maximum temperatures per day, measured at the standard height of 1.5 m above the soil surface. This weather parameter was chosen because it is easily available, and is independent of night temperatures which are not relevant here. The method used is similar to that of Williams (e.g. C.B. Williams, 1961; see also Johnson, 1969, p. 249). The average factor with which catches changed was calculated per $0.5^{\circ}C$ change in maximum temperatures from one day to the next. Fig. 24 gives the percentage change in catch size, with 100% put at a maximum temperature of $20^{\circ}C$, as calculated from the data of 249 pairs of consecutive days in 1970-1973.

The relation shown in Fig. 24 was used to correct trap catches, and other data dependent on or correlated with fly activity, to yield those expected at 'standard days', i.e. days with a maximum temperature of $20^{\circ}C$. Thus the presumably larger part of the influence of the weather is eliminated.

Rain and strong winds diminish captures, but because they are correlated with lower temperatures, little would have been gained by including these factors as well. Moreover the moment of occurrence is very important, and this could not be traced from the available weather data.

One would expect that catches of flight interception traps are dependent on their

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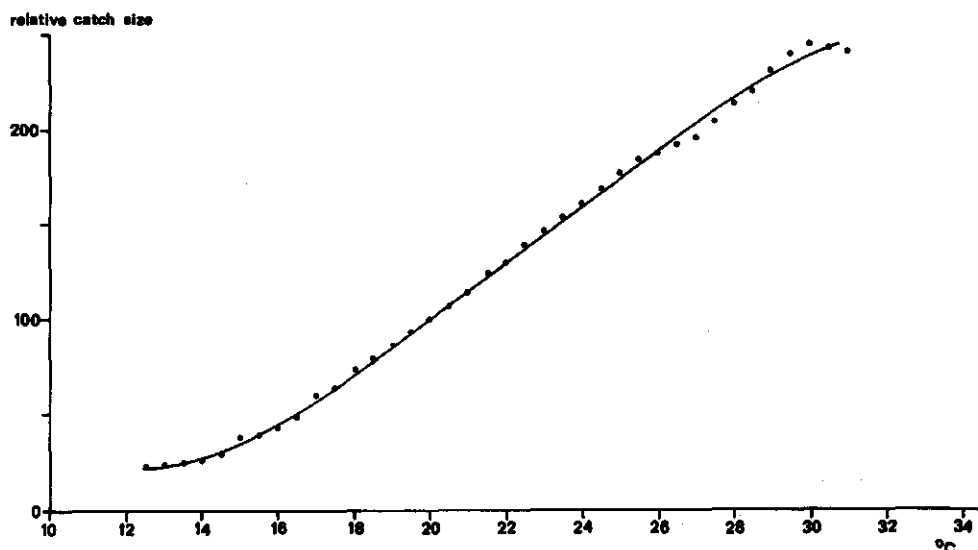
Fig. 24. Relation between catches in flight interception traps and maximum temperature per day in °C. Catches expressed as percentage of catch on standard days (20°C maximum temperature). Data from Overflakkee and the Schuilenburg, 1970-1973.

position relative to the wind direction. Data from the Schuilenburg experiment in 1971 (Section 6.1) were analysed for a wind direction effect. Five pairs of traps were placed in an onion field, three traps facing NW and three SE, and two facing NE and two SW. For all days with the wind blowing in a certain direction, the difference in catch in each pair of traps facing upwind and downwind has been taken into consideration. The distribution of this difference was about normal, with mean and 95% confidence interval of 1.31 ± 9.48, in which positive values denote more flies in traps facing downwind. This is significantly different from the expected mean of zero ($t_{168}= 3.53$). Thus more onion flies were trapped in traps facing downwind probably because flights were predominantly upwind. Also traps facing downwind may offer more shelter against the wind. The average numbers caught per trap per day were for traps facing downwind, upwind and to the two sides: 5.65, 4.34, 4.89 and 4.89, respectively.

For traps placed along the edges of onion fields the wind effect seemed even less, but this impression could not be checked because a situation in which this could be tested is not usually encountered. Moreover local differences in the vegetation or other factors cause the differences between traps at onion field edges to be higher than between traps in onion fields, thus obscuring a possible wind direction effect.

3.3.3.2 Attraction

Some known attractants (Section 2.2.3) were tested for use in water traps. These traps were yellowish square plastic trays, 16 x 16 cm, containing 500 cm^3 water with attractant and a few drops of detergent. A preliminary test was done to check the attractiveness of onion juice, obtained with a juice centrifuge. The onion juice putrified, and under the

Table 6. Attractivity of different concentrations of some attractants. Experiment Mijs 1973.

Number of traps	Attractant	Average number of flies per trap per day
6	beer 4%, 20% and 100%	0.2
6	onion juice 2%, 10% and 50%	0.2
8	onion juice 10%, renewed every 2-3 days	0.1
4	beer 20% + onion juice 10%	0.3
2	n-propyl disulfide 1%	8.8
2	n-propyl disulfide 0.2%	13.2
2	n-propyl disulfide 0.04%	10.4
2	control (water only)	0.0
12	flight interception traps	2.7

prevailing weather was optimally attractive during the second week.

A 12-day test with onion juice, beer and n-propyl disulfide was done subsequently. The n-propyl disulfide was dissolved in 25 cm^3 paraffin oil, floating on water in the tray. The traps were placed 3 m from each other in a row along the edge of an onion field. The results are given in Table 6. Obviously n-propyl disulfide was most attractive to onion flies. The effect of the concentrations used was never significant. The ratio males to females in the n-propyl disulfide baited traps was 4:1, whereas in the other traps it was 1:1.

Preliminary observations, August 1973, indicated that a n-propyl disulfide source placed under the roof of a flight interception trap had a positive effect on the catch size. This was studied in more detail in the experiment Mijs 1974. Flight interception traps, both with and without attractant, were placed along the edges of the experimental field, and in two places at over 300 m distance from the nearest onion field. The attractant, 12.5 ml of 0.4% solution of n-propyl disulfide in paraffin oil, was put in a tray as before, then covered with plastic gauze, mesh 1 mm. Several times new attractant was added.One trap was provided with rotting onion juice.

Fig. 25 shows the ratio of the catches in traps with attractant to those without, as a function of time since addition of attractant. Far from onion fields attractants were much more effective: the first application augmented the catches by a factor of over 10 because there were no competing onion odours. The effects of subsequent applications of attractant appeared to diminish with time. The most probable explanation is that, by not cleaning the tray when adding new attractant, certain decomposition reactions occurred that counteracted the effect of the n-propyl disulfide.

Over all the periods during which the attractant did show an effect, the catches in traps with and without attractant have been totalled for the sexes separately and combined. The results are given in Table 7. Both attractants have a larger effect on the catches of the males than on the catches of females.

Matsumoto (1970) found that 90% of the onion flies in n-propyl disulfide baited traps were females with eggs. As he did his experiments on fallow land the bait will have attracted the females for oviposition. The present experiments were mainly in onion fields. The limited data collected outside onion fields do not permit any conclusion on the sex ratio there.
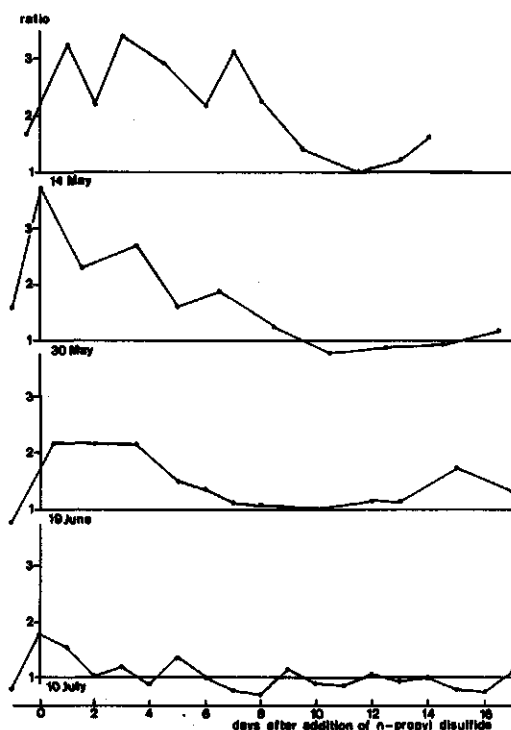
Fig. 25. Increase in catches, due to placing a tray with n-propyl disulfide under flight interception trap, expressed as ratio to catches in unbaited traps. Traps on onion field edges, experiment Mijs 1974.

Table 7. Effect of attractant on catches by flight interception traps on edges of an onion field, corrected for temperature. Aberrant males included in total only. Experiment Mijs 1974.

Attractant	Total number of flies captured						Ratio with/without attractant		
	with attractant			without attractant					
	males	females	total	males	females	total	males	females	total
n-propyl disulfide	397.3	238.8	668.6	169.5	132.6	314.0	2.34	1.80	2.13
rotting onion juice	431.0	239.0	698.0	148.7	123.9	284.3	2.90	1.93	2.46

3.3.4 *Obtaining eggs*

In a sterile male programme, the sterility of the eggs is the earliest and safest indication whether the sterile flies have behaved as they were expected to do. Therefore methods of obtaining this information are required. In the Schuilenburg experiments near Wageningen, because laboratory room and manpower were available, the female onion flies caught alive were kept in the laboratory to lay their eggs. On Overflakkee an attempt was made to concentrate the oviposition in the field on artificial egg deposition devices, in short, egg traps. The latter work was done mainly by the students J.J.L. Huber, J. Roest and G.J. Buth.

When eggs were wanted from wild females, these were picked out of the catches under CO_2 anaesthetization, and put into separate cages for different trapping sites and days.

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They were kept there for one week. After that they were killed, and if needed checked for dye mark, ovary development and presence of sperm. The cages were 8 or 15 cm Ø plexigum tubing, standing in a Petri dish, with a plastic gauze roof, mesh 1 mm. Water, dry food and Noorlander's oviposition device (Ticheler, 1971) were provided.

The egg were collected twice a week, and kept at 22°C and 100% relative humidity for at least three days. After that the sterility of the eggs was checked. The results were rather limited. The majority of the females trapped were sterile, and these do not lay any eggs. The fertile flies did not lay their eggs as readily as expected: 88% of the females contained mature eggs at the end of the week, compared with 55-60% in the field (Section 4.5.2). This drawback may be circumvened by using a different cage or oviposition device. A general and inevitable problem with this method is that at the low densities, at which the information is needed, very few fertile flies are caught (cf. the data in Theunissen et al., 1975).

Several different egg traps were tested. As attractants rotting onion juice, freshly cut onion pieces and n-propyl disulfide were used. All experiments were done in and around onion fields. Notched earthenware, notched plastic, sand, filter paper and several combinations of these provided tactile stimuli.

The numbers of eggs obtained by the egg traps used have remained too low to be useful at low densities (Huber, 1973; Buth, 1976; Roest, pers. commun.). Incidentally, when there were high densities of mature females, there was a substantial accumulation of eggs in the egg traps, up to hundreds of eggs per trap. Social facilitation of oviposition is well known form mass rearing. It is possible that the females were more conspicuous when depositing eggs in the egg traps than on onions.

Searching for or sampling of eggs is only feasible at rather high densities of onion flies. Searching may give an idea of the change in numbers with time, but up to 50% may be overlooked easily. Samples should contain the onion bulb and the lower part of all the leaves, and the earth up to about 2 cm around the onion plant and at least 2 cm deep. It depends on the soil structure and the sampling method whether deeper samples are necessary because the eggs can slide down. The soil samples were taken with a spoon or with a little grasper like that of a crane (Webley, 1957). The grasper took half of a sphere of 8 cm Ø.

Eggs were extracted from the soil samples by simple flotation. This method gives clearly somewhat less than 100% recovery. The onion plant parts were searched, especially the leaf sheaths.

3.3.5 Sampling flies, larvae and damage

Flies Especially when onion plants are small, the number of flies in the field can be counted to estimate absolute density. The major drawback of this method is that it cannot be used in the grassy verges of the fields where most onion flies occur.

For fly samples that are presumably independent of the vegetation, the method of MacLeod & Donnelly (1957) was used. Tents of cloth were stretched over an iron wire frame. They had a top trap made of 8 cm Ø plexigum tube, covered with 1 mm mesh plastic gauze and as an entrance a plankton gauze funnel. The tent's bottom surface was 0.5 x 0.5 m.

These tents were placed in the field after sunset, and covered with black polyethylene foil. With fair weather the traps were inspected the next afternoon. The same type of tent was used to sample emerging flies. Used as a depot trap for pupae dug into the soil, it yielded the same percentage emergence as the normal type depot trap, indicating that no young flies were lost. Older flies are less positively phototropic, so flies may be missed in sampling.

Larvae Larvae sometimes were sampled by sampling attacked onions and dissecting them. First instar larvae are very difficult to find in the onion tissue without the use of a microscope. When sampling the onions one must take care to include in the sample larvae that are just under the onions in the soil.

Damage Damage was sampled in 1973 on regularly distributed plots of 0.5 m onion row, and in 1974 on random plots of 0.25 m row. Attacked onions were indicated with a small stick exactly beside them so that after they had disappeared their original positions remained clear.

The average duration of the visibility of damage was estimated by noting at each inspection the visibility of onions found damaged earlier. This average duration increased linearily from about 20 days for damage that became visible in early June to 45 days for damage that became visible half July. All damage was still clearly recognisable during the first 2/3 part of these periods. Young seedlings should be checked twice a week when damage is to be expected. As a rule of thumb the next check should preferably be within a number of days that is equal to the average onion height in cm.

The effect of plot length on the data obtained is discussed in Section 4.3.1.4.

3.3.6 Sampling pupae

Pupae are present only near attacked onions. They were generally sampled at harvest. Full pupae then are diapausing, empty pupae represent the second flight. Both can belong to a third flight if present, dependent on the time of sampling and of this flight. Another strategy followed was to sample pupae one or two weeks after attack had been noted, in order to obtain a representative sample of 1st generation pupae. The onions in the sample that still might contain larvae were put back. This method can only be applied with small soil samples, i.e. of 5 cm Ø, because otherwise many healthy onions have to be destroyed. Then an onion in the neighbouring row was taken at random as a replacement.

Distribution of pupae in the soil In two places, one on sand and another on sandy clay, the soil around attacked onions was carefully dug out and the position of each pupa from the centre of the onion's row was measured. The sideward distribution was identical in both soil types, and conformed to a normal distribution (Fig. 26). The depth was dependent on the soil type (Fig. 27). The bottom of onion bulbs is normally at a depth of 1-3 cm.

Sample size Samples were taken with diameters of 5 or 20 cm, centered on attacked onions. The fraction of pupae that is sampled with a certain sampling core diameter is dependent
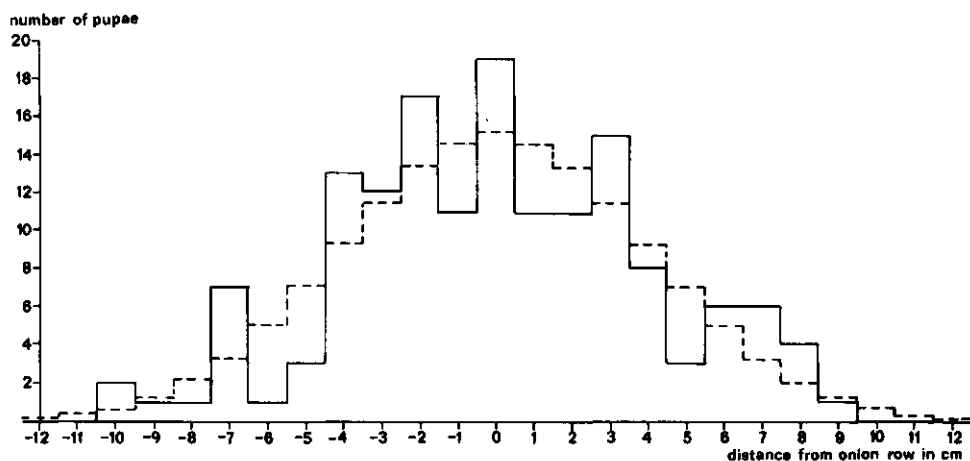
Fig. 26. Sideward distribution of pupae in the soil, as measured from the onion row. Data combined from sand ($m = 0.1$, $s = 4.0$) and sandy clay ($m = -0.1$, $s = 4.1$). Tested against normal distribution: $\chi^2_{14} = 17.2$, n.s. Data from Goeree and Overflakkee, 1970.
— observed distribution; - - - normal distribution with $m = 0$, $s = 4.0$.
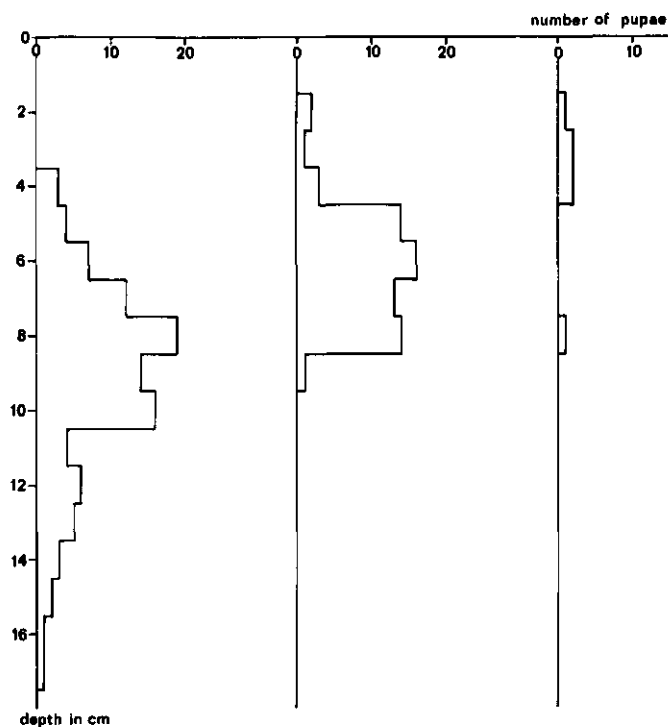
Fig. 27. Depth distribution of pupae in the soil, for different soil types.
Left: clay fraction 10%, average depth 9.1 cm; Goeree 1970;
middle: clay fraction 15%, average depth 6.2 cm; Overflakkee 1970;
right: clay fraction 20%; Overflakkee 1970.

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on the aggregation of the samples. At first consider a row of onions which is 100% infested, and of which a part, the predetermined sampling site, is being sampled. The fraction of the pupae sampled can then directly be read off from the sideward distribution of pupae as given in Fig. 26. Displacement of larvae parallel to the onion row before pupation does not influence this distribution. The part to be sampled is in fact a rectangular surface with the width of the sample core diameter, and the length of the sampling site.

Now consider the case of a single attacked onion. It has been proven that the horizontal component of the larval movement prior to pupation is independent of the direction of the onion row. Thus the dispersion of pupae, originating from one onion, will be the circular normal distribution corresponding to the normal one given in Fig. 26. Percentage points for the circular normal distribution have been tabulated (Owen, 1962).

Thus with a 5 cm $\emptyset$ sample the fractions sampled, for the two extreme cases mentioned above, were: from the normal distribution 46.7% and from the circular normal distribution 17.8%. For 20 cm $\emptyset$ samples these values were 98.7% and 95.6%, respectively.

In the field damage is highly aggregated (Section 4.3.1.2). Moreover the majority of damage cannot be subdivided into attacked onions which larvae left in order to pupate or which larvae left to attack a neighbouring onion. Therefore samples mostly contain only slightly less than the % of pupae estimated from the normal distribution. Every sample from an onion of the latter type will cause the percentage sampled to come closer to the one estimated from the normal distribution, that is for the case of 100% infested.

Data on the pupae sampled with 5 and 20 cm $\emptyset$ are given in Fig. 28. The fact that not 100% is contained in 20 cm $\emptyset$ samples can be neglected here because its effect on the percentage sampled with 5 cm $\emptyset$ is less than 1%. There are two sets of data deviating from the theoretical model given above. These deviations correspond with deviating soil structure, as indicated in the figure. When soil structure is the cause, the agreement of data from different soils in Fig. 26 is due to chance, and the variances will actually be different. The 95% confidence limits of the standard deviations of the sideward dis-
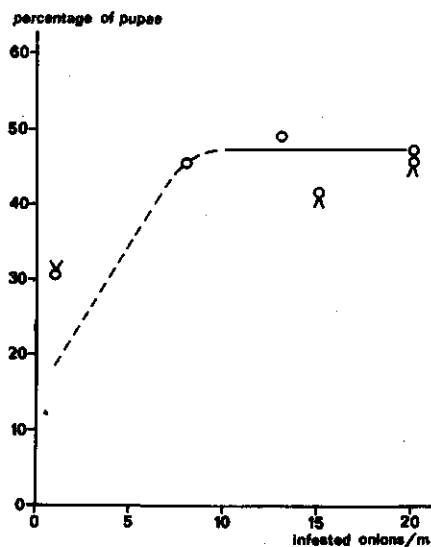
Fig. 28. Percentage of pupae sampled with a 5 cm $\emptyset$ sampling core, as a function of infestation intensity. Expressed as percentage of the number of pupae obtained with a 20 cm $\emptyset$ sampling core. Infestation in number of onions attacked per plot of 0.5 m row, so 20 onions correspond with about 100% damage.
—— Relation estimated from the values calculated for the extreme cases (see text).
O = Data from sandy clay (clay fraction 15%);
Я = data from sand (clay fraction 10%; the Я at 20 onions damaged corresponds with the data of Fig. 26);
Ƌ = data from clay (clay fraction about 30%).

tributions of pupae in sand and sandy clay are 3.5-4.5 and 3.5-5.0, respectively, wide
enough to make deviations as found in Fig. 28 probable (e.g. a circular normal distribu-
tion with 30% in 5 cm ∅ has a standard deviation of 3.1).

The line drawn in Fig. 28 is a 'safe' supposed relation for sandy clay. Data to be
collected for 2-5 attacked onions per 0.5 m onion row may yield a steeper initial increase
in the percentage sampled, due to aggregation of infestation.

Samples were taken as the total soil core, generally also when the centre was less
than the core diameter from the end of a sampling site. This procedure will result in an
overestimation of pupae when attack occurs up to the end of the sampling site and not
beyond it. It is compensated for by the underestimation of pupae in the opposite case
which will occur as frequently. With damage on both sides of the sampling site's end,
the number of pupae thus sampled will be too high. However, when there was damage across
the end, visible at harvest, the samples were taken exactly up to the sampling site's
end. So the number of pupae will have only been overestimated with damage no longer vi-
sible at harvest, which corresponds with low numbers of pupae per attacked onion (Fig. 47),
and will thus have been negligible.

Extraction The pupae were extracted from the samples by flotation. The samples were
crumbled into buckets with water, stirred, and then the water was poured through a 1 mm
mesh plastic gauze. This procedure was repeated once or twice. By washing the mud through
the gauze any pupae on the gauze became conspicuous. All pupae float after having reached
an age of about 16 hours (22°C), that is 5% of the pupal stage duration. The pupae not
yet floating and any larvae present will become suspended by the stirring and settle down
on the mud's surface, where they are also recovered. Empty pupae may not be so easily re-
covered. Subsequent stirrings and washings yielded the numbers of pupae given in Table 8.
From these data the number recoverable with an infinite number of stirrings may be esti-
mated. Recovery of pupae from depots indicated that the fraction of unrecoverable pupae,
if any, will be negligible.

3.3.7 Fly inspection

Generally the flies to be inspected came from flight interception traps, so they had
to be killed first. The total catch was anaesthetized with CO_2 in the trap collectors.
Then the plankton gauze funnel of the collector was turned outwards and the contents were
shaken into a Petri dish. A piece of paper tissue with a little bit of acetaldehyde was

Table 8. Pupae recovered by subsequent extractions. Extrapolated values between brackets.
In 1974 the extraction was more careful.

		Number of pupae extracted					% Recovered	
		1st	2nd	3rd	4th	5th	in 1st	in 1st+2nd
Mijs 1973	full pupae	79	5	2	0	(0)	91.8	97.7
	empty pupae	400	159	26	3	0	68.0	95.1
Mijs 1974	full pupae	143	5	(0.2)	(0)	(0)	(96.5)	(99.9)
	empty pupae	241	39	(6.3)	(1)	(0.2)	(83.8)	(97.4)

added for killing. If the female onion flies were wanted alive the collectors' contents were shaken into a sieve with a plankton gauze bottom, up through which a slow current of CO_2 was passed. The female onion flies could be identified and selected there.

Onion flies can be identified and sexed by their general appearance. Wet flies are much more difficult to identify.

To see whether the onion fly females have mated, pull out the ovipositor with the spermatheca, put this in a drop of water on a slide and if needed pull the spermathecae free from other tissue, and squeeze them with a cover-glass under the microscope. Then with all females that have copulated a lot of sperm will burst out, as can be seen at about 100 x magnification and the diaphragm nearly closed (Theunissen, 1974). To check ovarian development squeeze the ovaries out of the abdomen after removal of the ovipositor, and inspect them under about 80 x magnification. The stages of ovary development are described by Theunissen (1973a). Checking for the presence of sperm and ovarian development takes 2-3 minutes per fly.

The larger wing vein aberrations can be seen with the naked eye. For finding small aberrations and for measuring the wing vein length, take the wing off, put it between two slides and inspect with a microscope, magnification up to 20 x.

The dye mark was generally checked with a microscope at 40-80 x magnification. Take the flies' abdomen, legs and mouth parts between left thumb and forefinger, and squeeze the fly slightly. Then the ptilinum, and not the proboscis, will come out without the fly getting damaged. Keep or make the fly's head dry, for when the ptilinum becomes wet the colours become very difficult to distinguish. To look at the ptilinum from dessiccated flies keep the fly's head between left thumb and forefinger, introduce both points of very sharp-pointed tweezers together through an eye, and allow the points then to separate slowly.

Sorting of flies and checking them for dye mark takes 1 hour for 200 fresh dead flies or for 100 dessiccated flies.

3.3.8 Field observation of fly behaviour

The flight behaviour of the flies was observed in the field, to get an indication of its dispersal.

When a fly was found in the field, its behaviour was recorded on a portable tape recorder, with the aid of a stopwatch. A record ended after a predetermined period, but actually often earlier because the fly was lost sight of.

The behavioural data noted were: type of sitting place, orientation, direction and distance of walk or flight, causes for alighting if identified, and remarks on feeding, preening and presence of other onion flies or other animals within the estimated sight range of the individual recorded. Other data collected were the flies' sex, dye marks if any visible, direction and distance of the observer to the fly recorded, and the presence of sunshine and rain on the fly. Moreover regularly temperature and humidity were measured at soil level with an Assmann thermohygrometer, and average, maximum and minimum wind speed at 20 cm height were estimated from a cup anemometer with wind speed indicator.

observer

number of flights
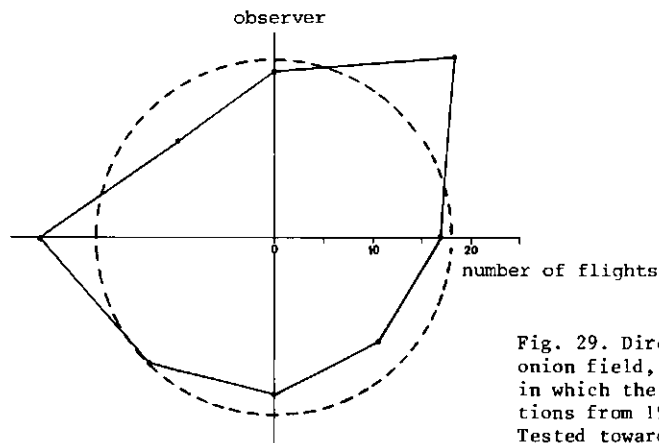
Fig. 29. Direction of onion fly flight in
onion field, with respect to the direction
in which the observer was situated. Observa-
tions from 1971-1973, $n = 147$.
Tested towards/away from observer: $\chi^2_1 = 0.6$, n.s.

Nearly all data had to be collected in onion fields, because of the very low chance
of finding onion flies outside onion fields. Thus the results are only representative
for the situation inside these fields.

The flight direction was independent of the direction where the observer was, although
he was generally 0.5-2 m from the fly (Fig. 29).

3.4 SUMMARY

The field experiments were done in a typical Dutch onion growing area, with 5-15%
occupied by onions. The prevailing weather was not abnormal.

Flies used for releases were obtained from a laboratory mass-rearing. When needed
the flies were sterilized with 3 kR irradiation treatment, causing 99.4% sterility in eggs
from untreated females mated to treated males. The mating competitiveness of the sterile
males, as measured in competition experiments in the laboratory, was not significantly
different from 1.

Flies were marked sometimes by incorporating a radioactive label in the larvae, but
generally by forcing the emerging flies to pass through a dye layer which could be
changed during the emergence period. Radiation induced sterility is a good tag for
females, especially when steriles are in the majority. Fly size, as measured from wing
vein length, can be used as a mark especially for males, provided that recaptures are
large.

Generally flies were released from containers with pupae buried in the soil. Depth
and vegetation affect the temperature and thereby the speed of emergence. Predation by
birds on the concentrations of newly emerged flies, which is inevitable with this
method, is supposed to have been the cause of up to 90% loss. Flies could also be re-
leased from cages with up to 5 flies per cm^3.

Flight interception traps were used. The catches could be more than doubled when
combined with the use of attractants (rotting onion juice or n-propyl disulfide). The
optimum trap position is at onion field edges which provide shelter, and facing out-
ward. Most flies were trapped in the afternoon. The daily catch was strongly related to

the maximum temperature, but only slightly dependent on the wind direction.

Eggs from captured wild females were obtained by keeping them for some time alive in the laboratory. This laborious method often gives only little information. No efficient egg trap has been developed yet.

Data on sampling methods used for flies, eggs, larvae and damage are mentioned. For the sampling of pupae, the distribution of pupae in the soil under attacked onions was examined. The horizontal distribution conformed to a normal distribution, the average depth was clearly dependent on the soil type. The fractions of the numbers of pupae, obtained with sampling cores of 5 or 20 cm Ø, are estimated as a function of the damage level. Extraction was done by flotation and subsequent sieving, with an estimated recovery of over 95%.

The methods for killing and sexing the flies, and of inspection for dye mark, mated status of females and ovary development, are described.

Fly behaviour in onion fields was recorded. The observed flight directions were independent of the position of the observer.

4 Onion fly ecology

In this chapter at first the data are reported on the onion fly's life cycle and niche. Only some aspects like the prediction of emergence and the impact of Entomophthora infection are discussed in some detail. The other aspects that were considered only superficially are reported nevertheless, as they may help to provide a better understanding of the experiments.

In pest control by sterile males, the pest densities and reproduction factors need to be estimated. As will be shown, the low densities of damage and pupae and their high degree of aggregation cause problems in the determination of the confidence intervals of population density estimates.

The fecundity of the flies is estimated from their survival and the frequency of deposition of egg batches. Reproduction data, obtained from sterile male field experiments, are compared.

4.1 LIFE CYCLE

4.1.1 *Prediction of emergence*

Prediction from temperature records   The possibilities for predicting the emergence of wild populations from soil temperatures, for use in sterile male programmes, were analysed. The pupae were distributed evenly throughout the top 25 cm of the soil (Section 3.3.2.1). Temperatures were recorded from the standard depth of 10 cm only, measured between the depots. Because a temperature sum (accumulated daydegrees above a certain threshold) needed by the pupae is reached later at greater depth, the 10 cm records should predict optimally the point of about 40% emergence.

Laboratory experiments by Ticheler (1975) indicated that the temperature threshold to be assumed for pupal development could best be placed at 6°C. Houwing (1973) had estimated this threshold to be 9°C, but his data did not exclude the possibility of lower values. Eckenrode et al. (1975b) found 4.5°C as developmental threshold, Missonnier & Boulle (1964) mentioned 5°C.

Fig. 30 gives the emergence curves versus the temperature sum. As a reference laboratory data are also given. These differ from the field data because all pupae were kept at the same constant temperature which was rather high, compared with the field situation. There is considerable difference between the various sets of field data. These differences can originate from variation in one or more of the following factors: distribution of viable pupae throughout the soil, especially in the top layer; soil structure and surface reflection; amount of direct sunshine and of humidity; and, as the temperatures had to be measured outside the depots, the duration of the presence of the depot traps before emer-

50
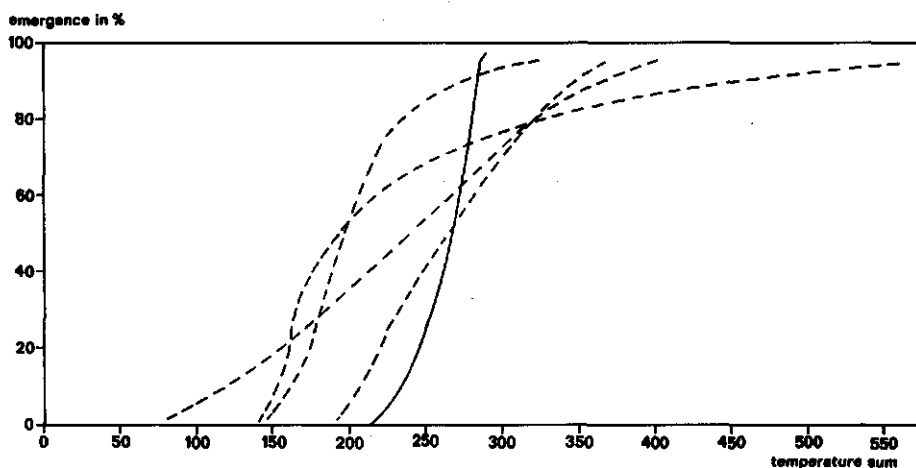
emergence in %

Fig. 30. Onion fly emergence from diapausing pupae, plotted against the temperature sum, for field data as measured at 10 cm depth.
- - - Field data, Overflakkee and the Schuilenburg, 1973 and 1974; —— Laboratory data at 20°C (Noorlander, pers. commun.).

gence started. Moreover in the Schuilenburg experiments the pupae were mixed through the upper 15 cm only.

For genetic control measures one would like to adjust the release schedule as much as possible to the emergence of the wild flies. The first point to be considered is when to start with releases. For this the date of 5% emergence from bare soil was taken. Now let us assume that by recording temperatures inside depots and taking other precautions, the temperature sum needed for 5% emergence is fixed with reasonable accuracy at 150 daydegrees. It can now be determined whether a prediction based on this would be useful. A prediction needs to be made at least 10 days in advance, because of the time needed between taking the pupae from the storage and the emergence of the flies. The 5% emergence will occur somewhere between 20 April and 20 May, and prediction should result in a narrower range.

Eckenrode et al. (1975b) used air temperature sums to predict the start and median of the first flight, but their data do not provide convincing evidence that this prediction is more accurate than when average date is used.

In the following calculations temperature records from 10 cm depth were used. Temperature data from lesser depths would have yielded a better 5% emergence estimate, but on the other hand the accuracy of such measurements diminishes due to the steeper temperature gradients.

Fig. 31 gives the data and daydegrees 10 days before 150 daydegrees was reached. The lower 95% probability limit is given by assuming that the deviations from the calculated regression line are independent of the date. From the temperature records it can be found that the cumulating temperature sum crosses this line 13±3 days before it reaches 150 daydegrees. Actually, also after years of data collection, the inaccuracy of the temperature sum required will remain noticeable. The assumption that the temperature sum is known to be within a range of n daydegrees, causes the possible deviation of the predicted from the actual 5% date to increase by $n/3$ days, as can be estimated from the accumu-
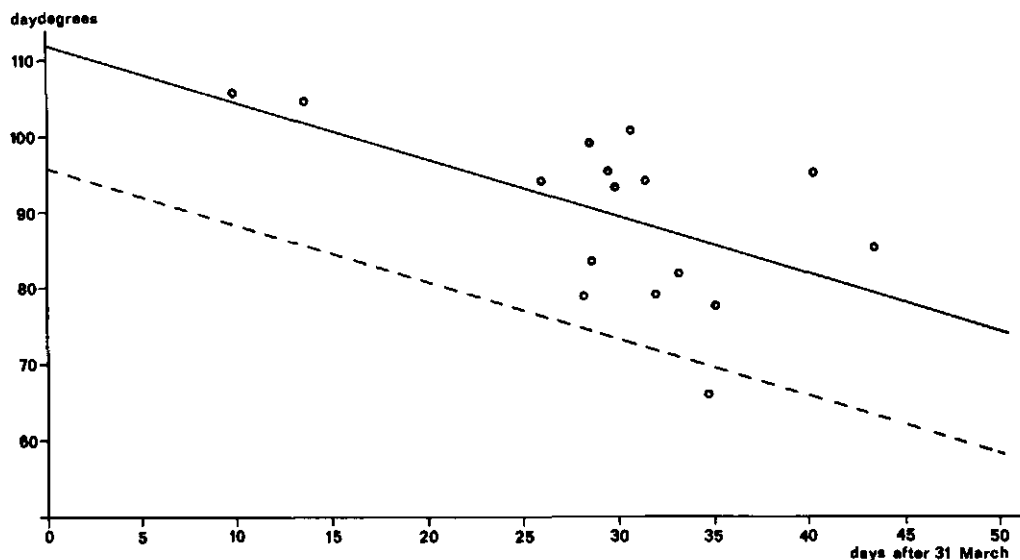
Fig. 31. Date and temperature sum 10 days before the temperature sum of 150 daydegrees is reached. Calculated from data from 10 cm depth, Nieuwe Tonge (Overflakkee), from KNMI. —— Regression line: $y = -0.75x + 111.8$; - - - 95% one-sided lower confidence limit.

lation of daydegrees in the relevant period.

Other points of the emergence curve can be predicted in the same way. However, because of the increasing influence of the vegetation such estimates will be less representative for the actual situation.

Ticheler et al. (1975), considering the pupal development after diapause versus temperature in detail, for instance, using a slight temperature shock, found a complicated reaction pattern. Thus a simple prediction method with temperature sums may be impossible.

Prediction from phenological parallels Another prediction possibility is the use of phenological parallels (Section 2.2). Among the data available (Fisscher, 1964) the leaf budding of the oak, Quercus robur L., seemed to be the most appropriate because of its time of occurrence and the commonness of the species. It buds on the average on 2 May, with 95% confidence limits of 17 April and 17 May (calculated from Fisscher, 1964). The 5% emergence data for the onion fly occurred on the average some 12 days later (Table 9).

Table 9. Experimental data on the time of 5% emergence of onion flies in spring. Independent evidence is available that the emergence from the depot was later (Mijs 1974) and earlier (Schuilenburg 1974) than that of the wild population.

	5% Emergence from depot	Days after oak leaf budding	Temperature sum when 5% had emerged from depot
de Wit 1973	24 May	12	154
Mijs 1974	6 May	21	199
Schuilenburg 1973	11 May	13	98
Schuilenburg 1974	17 April	2	148

The choice of more representative reference oaks and a more representative depot location may confine the confidence interval of the number of days between leaf budding and fly emergence to a useful length. Also care should be taken that the oak leaf budding is observed as a certain percentage-point, because first observations of an event in a population of unknown size are not very useful.

Because of the limited number of trees, their microclimate which is different from that of arable land, and the age dependency of their phenological events (Nienstaedt, 1974), it would be useful to look for common annuals with a similar average date for phenological parallels. Such plants should enable an even better prediction.

Flies released 'too early' are not useless because the emergence of the wild flies will have already started. The 2% emergence date lies 1-4 days before the 5% date.

The need of the first releases to coincide with the first emergence of wild flies should not be overstressed. It is not a question of 'starting the sterile male programme at low densities', which is sensible only if these densities refer to whole populations. The point here is which part of the population may be missed without harming the effect of the releases. Because due to the lower temperatures an earlier start of the emergence will be followed by a less fast emergence curve and because the early emerged flies will develop more slowly, a week delay will hardly ever have a discernable effect.

4.1.2 *Flight curves*

Flight curves for the various years and locations are given in Fig. 32.

The 1970-1972 data refer to wild flies captured on normally treated onion fields on sandy clay, except for the series of Goeree 1972 that is from an untreated experimental plot on sandy soil and some adjacent untreated shallot fields, and for the last part of the 1971 graph, which is from the only commercially grown onion field on the island that received no chemical treatment against the onion fly. The data of 1973 originate from three untreated plots. The plot Mijs was somewhat isolated from 1972 onion fields, whereas the other two plots lay adjacent to such onion fly sources. Also indicated are the fertile flies released on the plot Mijs during the first flight, because they contributed to the second flight population. The 1974 data are given separately for different onion fields which had different onion fly control and different degrees of isolation. All these fields were in the same area (Fig. 15). For details about this experiment see Section 6.2. The catches, expected when parathion was not sprayed on some of these fields, are indicated.

Differences between the curves are partly attributable to control measures and degree of isolation from onion fly sources. Lack of control on lighter soils (Fig. 32, d, g) caused a considerable population in summer, whereas on heavier soils (Fig. 32, c, e, f) there has been little effect. A higher degree of isolation resulted in a slower buildup of the population in spring (Fig. 32, g, j). The remaining differences have to be ascribed to the weather circumstances (especially the short-term changes in the catches) and to the population level. The latter may be higher in areas with more extensive onion growing, i.e. Fig. 32, a, b (1st flight).

The population decrease that resulted from the successive sterile male treatments done
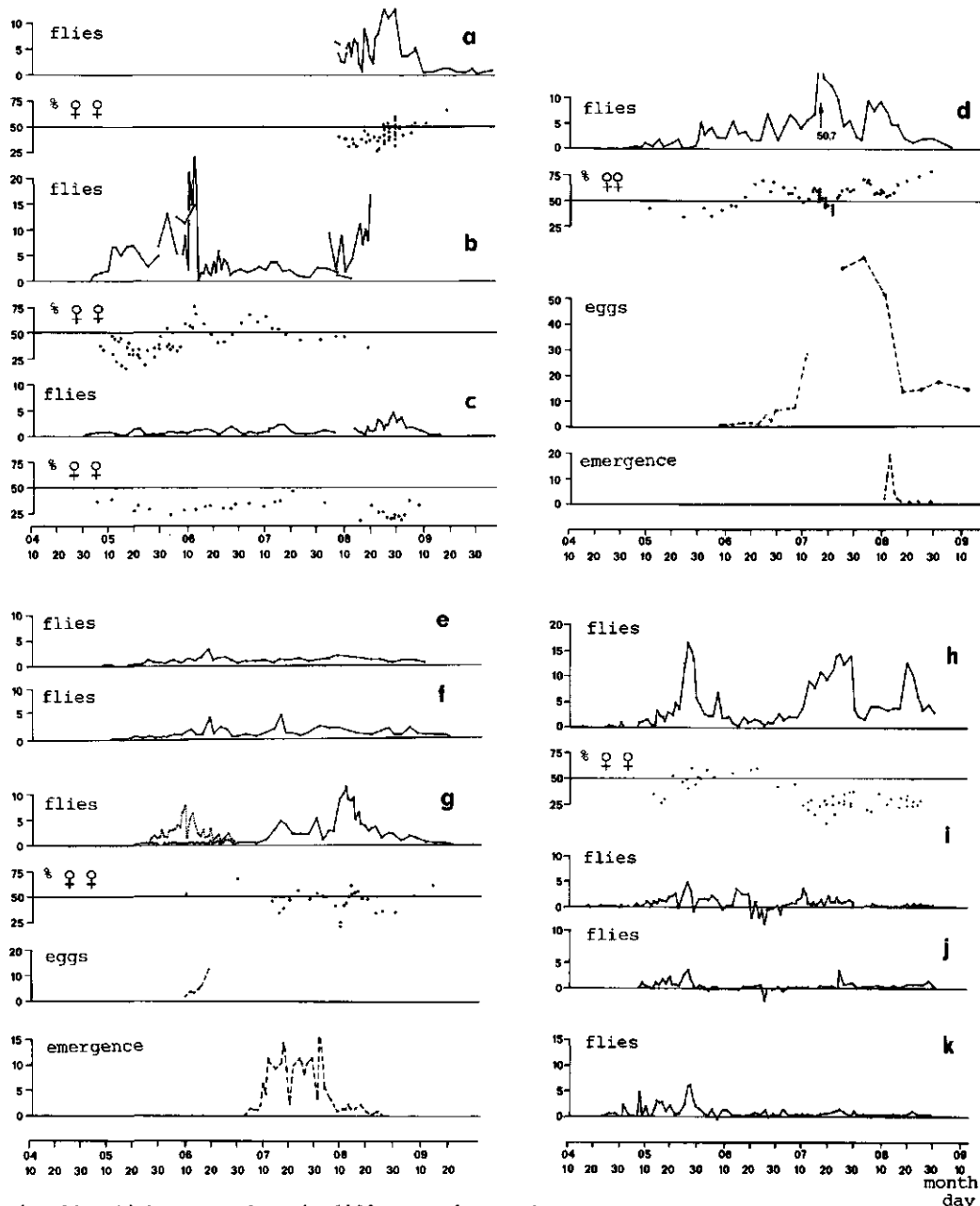
Fig. 32. Flight curve data in different sites and years.
flies: Flight curves in flies per trap per day; %♀♀: percentage females per 100 flies
captured; emergence: emergence in summer of a representative sample of pupae; eggs:
eggs per m onion row.
a. 1970, Overflakkee (van Loon);
b. 1971, Overflakkee (Pollemans);
c. 1972, Overflakkee (Buijs, Vroegindewei);
d. 1972, Goeree;
e. 1973, Overflakkee (de Wit);
f. 1973, Overflakkee (van Es);
g. 1973, Overflakkee (Mijs; - - - 1st flight released fertiles);
h. 1974, Overflakkee (Mijs, control field);
i. 1974, Overflakkee (Mijs, trial field);
j. 1974, Overflakkee (Mijs, field 5, chemically controlled and relatively isolated);
k. 1974, Overflakkee (Mijs, fields 1,2 and 3, chemically controlled);

1. 1971, Schuilenburg (only females);
m. 1972, Schuilenburg (× = only females);
n. 1973, Schuilenburg;
o. 1974, Schuilenburg.

at the Schuilenburg, is also clear from the trapping data (Fig. 32, 1, m, n, o).

The relation with the weather (Fig. 16) is clear. The effect of the daily maximum temperature and of attraction were eliminated from the data of Mijs 1974. The incidentally negative values were due to corrections that had to be applied (Section 6.2.2).

4.1.3 Incidence of diapause

The induction of diapause has been studied by Kelderman (1972) and Ramakers (1973). It was found to be strongly age dependent: short daylength induced diapause in the third larval instar, and low temperatures induced diapause in the first days of pupal develop- ment (Fig. 33).

The combined effect of temperature and daylength on diapause induction is shown in Fig. 34; the data of McLeod (1965) are in reasonably good agreement with this. The curves are of the normal type for long-day species (Danilevskii, 1965). The estimated courses of the percentage diapause induced at different temperatures versus daylength suggest a strong selection pressure in favour of a low percentage diapause at longer daylengths, provided the temperature is at least $18^{\circ}C$, and a high percentage diapause with shorter daylengths even with high temperatures. The adaptive value of this is clear. The first generation will be susceptible to diapause induction very much dependent on soil tempe- rature, yielding an incomplete second flight when soil temperatures are relatively low. This susceptibility will be partly balanced by a later first flight with low soil tempera- tures in spring. The second generation will go into diapause mainly dependent on daylength,

diapause in %

Fig. 33. Developmental stages sensitive to diapause induction. Age in days at 18°C.
L1,L2,L3: larval instars, P:pupal stage; — — transition between larval instars;
— - - — pupation.
a. Effect of photoperiod on diapause, expressed as the percentage diapause induced when
the photoperiod is changed at different moments in the larval and pupal development
(Ramakers, 1973). × = Short day (10 h light per 24 h) changed to long day (continuous
light); O = long day changed to short day.
b. Effect of temperature on diapause, expressed as the percentage diapause induced when
the temperature is changed at different moments in the larval and pupal development
(Kelderman, 1972). × = Cold (12°C) changed to warm (18°C); O = warm changed to cold.

diapause in %
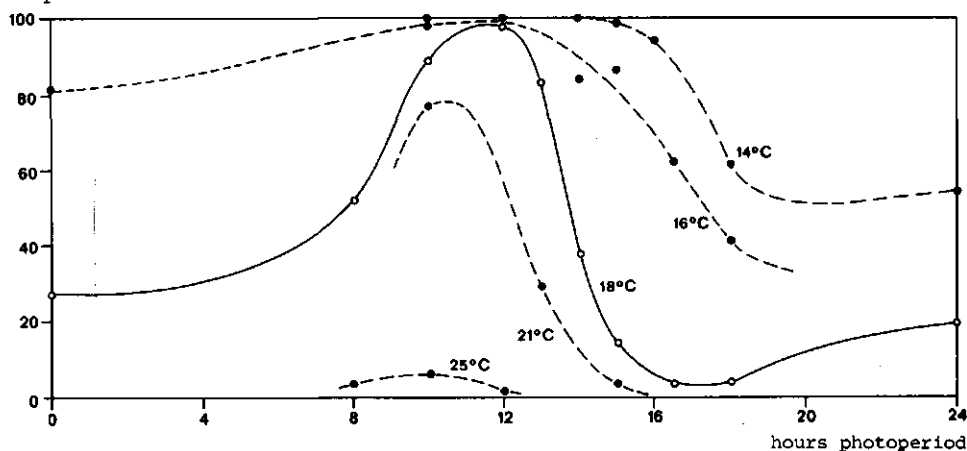
Fig. 34. Diapause induction as a function of temperature and photoperiod, photoperiod
expressed as hours light per 24 hours (Ramakers, 1973).

so a warm autumn will not cause many flies to emerge when the chance of reproduction is small.

To check whether these laboratory data can be extrapolated to the field, one has to know at what time the stages sensitive for induction are present, and to what daylength and temperature they are exposed.

In Fig. 37 it is indicated that pupation mainly occurs in the periods 10 June – 30 July, 10 Aug. – 10 Oct., for the two generations respectively. The larvae pass down through the soil to 5-8 cm deep (in sandy clay) and pupate there. Thus use of the soil temperature data for 6.5 cm depth would be optimal for correlation with the laboratory data. Temperature measurements are available for depths of 5 and 10 cm. By interpolation in Fig. 34 the expected percentages of diapausing pupae were estimated for the average soil temperatures per decade. Fig. 35 gives these expected percentages diapause versus time, for different daylengths.

It is not known what daylength is effective. Instead the length of the dark period may induce diapause, as all laboratory experiments were done with a 24 hour cycle. The photoperiod is effective on average about a week earlier than the temperature, and on larvae that are generally found inside onions. The photoperiod cannot be effective through changes in the onions because it is also effective on larvae living in an artificial medium in the laboratory. The larvae may perceive the light directly through the onion, during visits to the outside of the bulb, and during migration to other onions. The latter occurs too infrequently to provide sufficient information, especially in the second generation larvae when daylength is the crucial factor.

Infrequent presence of the larvae near the bulb's outside will tend to result in
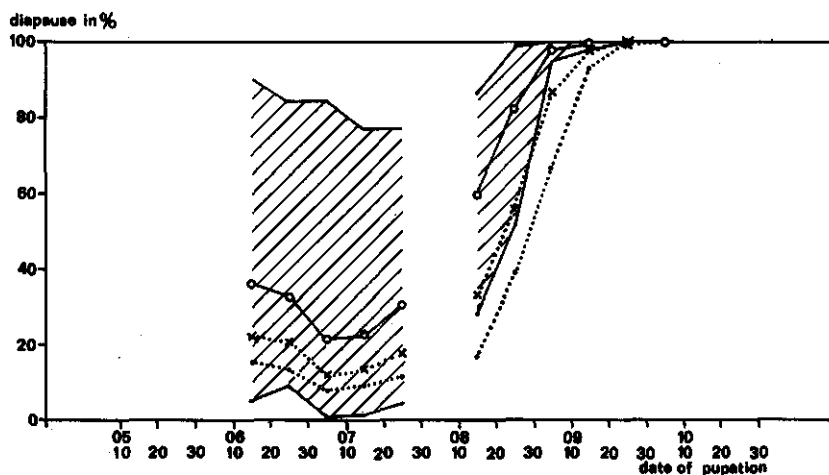
Fig. 35. Expected percentage diapause from soil temperature at 5 cm depth and for different photoperiods, Overflakkee 1962-1972.
● = astronomical daylength, average values; X = astronomical daylength minus 1 hour, average values; O = astronomical daylength minus 2 hours, average values and range (shaded).

Expected average and range of percentage diapause per flight:	1st flight	2nd flight
astronomical daylength	10 (1-32)	75 (61-93)
astronomical daylength minus 1 hour	16 (3-42)	86 (78-95)
astronomical daylength minus 2 hours	27 (10-55)	94 (90-99)

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Table 10. Percentages of pupae diapausing in the field.

	1st generation	2nd generation
Schuilenburg 1973	8	83
Schuilenburg 1974	18	87
de Wit 1973	19	.
van Es 1973	23	.
Mijs 1973	29	85
Mijs 1974 (control)	25	78
Mijs 1974 (experiment)	0	
weighted average	21.9	84.6

shorter light periods than the generally biologically effective daylength, which is the astronomical daylength (sunrise-sunset) plus half an hour in morning and evening (Danilevskii, 1965).

Diapause percentages in representative samples are available for some years, as given in Table 10. As shown in the next section, a 3rd flight may occur in September if the 2nd flight was not too late. Pupation after August gives 100% diapause. These data suggest that the soil temperatures at 5 cm depth, together with a photoperiod with the light period 1-2 hours shorter than the astronomical daylength are probably the most relevant factors for the induction of diapause under field conditions. This has to be tested in future experiments, including more detailed laboratory tests on diapause induction in the relevant temperature-photoperiod space.

4.1.4 Number of flights

For estimation of the number of flights, first the duration of a generation needs to be estimated. The set of data most appropriate for this are the ^{65}Zn labelled recaptures in the sterile male experiment on the Schuilenburg in 1971. In winter labelled pupae had been dug into the soil. They emerged in the same period as the 1st flight wild flies. The offspring of ^{65}Zn labelled females is slightly labelled and can be identified by autoradiography. By screening the recaptures for labelling the recapture curves for the flights were found to be about 50 days apart. Because May 1971 was relatively warm, the period between the 1st and the 2nd flight will generally be somewhat longer. Temperatures in July and August are higher, so between the 2nd and 3rd flight there may be less time if the 2nd flight occurs before August. The later in the year a flight occurs, the more it will be the offspring of only the first part of the preceding flight, because the percentage of diapausing pupae increases with time. Consequently, the 3rd flight comes earlier than expected from the total of the 2nd flight.

Additional information on the occurrence of a 2nd or 3rd flight is given by the emergence data (Fig. 32, d, g) and the egg deposition data (Fig. 32, d).Both in 1973 and 1974 the sampling of pupae at harvest indicated that there was a small 3rd flight.

Another indication on the delimitation of the flights is given by the percentage of females (Fig. 32). Fig. 36 shows that this percentage in the recaptures changes with age because the female flies live longer. Roughly it can be said that the percentage of fe-

percentage of females
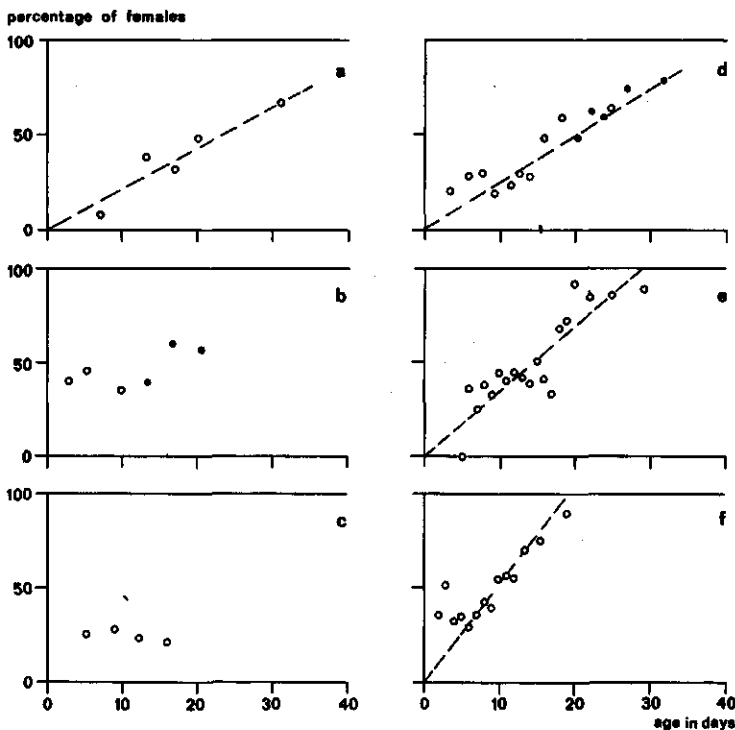
Fig. 36. Percentage females in recaptures versus age, for different release-recapture experiments. O = 100 flies, • = 25 flies.
Experiments: a. van Loon 1970-2; b. Pollemans 1971; c. Vroegindewei 1972-2; d. Mijs 1973-1; e. Mijs 1974-1 (O = 5 or more flies per trap per km sheltered edge of onion field); f. Mijs 1974-2 (O as in e.).

males is linearily correlated with age. Extrapolating this correlation to wild populations, the speed of change of the sex ratio is a measure of the difference between the birth and death rate. A decrease of the percentage of females indicates a higher birth rate, an increase occurs at higher death rates.

With these data and those given in the preceding sections the flights can be assumed to have been as indicated in Fig. 37.

4.2 NICHE

4.2.1 *Oviposition site*

As mentioned in Section 2.3.1, there are several wild host plants that are possible oviposition sites of the onion fly. Allium vineale is by far the most common species in the Netherlands, so its probable role for supporting wild populations was investigated, as this might result in a lack of isolation between onion fly populations.

Some attention to the role of A. vineale could be paid during the experiment Mijs 1973. This experiment was done mainly on and around a small 0.1 ha onion field, with a

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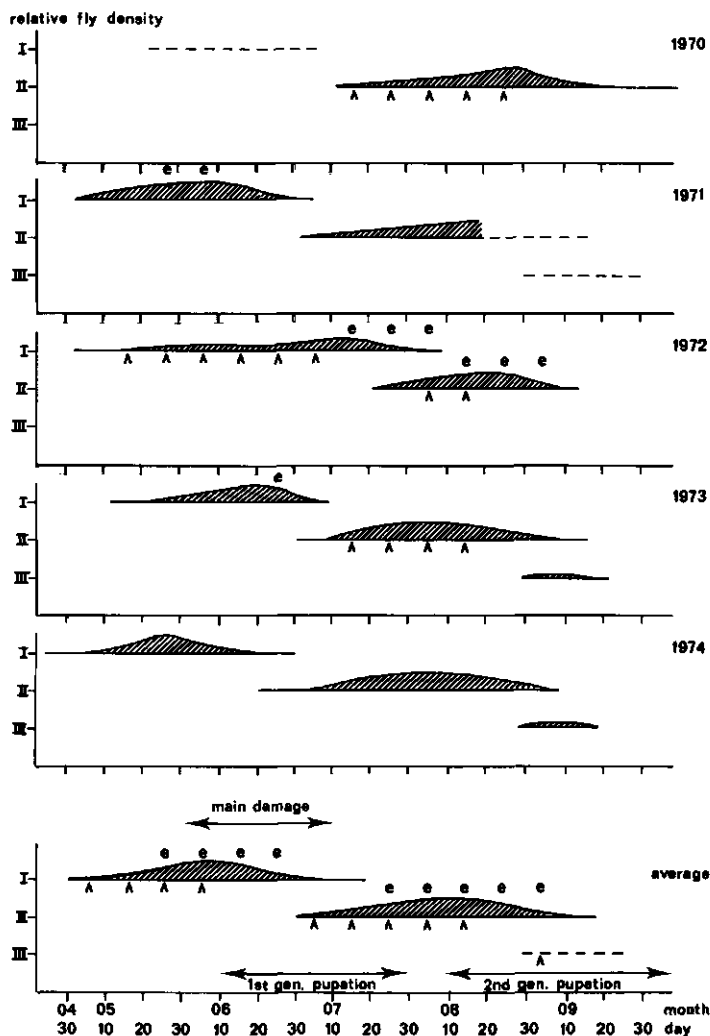
Fig. 37. Estimated occurrence of flights, 1970-1974 and average. I,II and III: flights; e: oviposition; A: emergence; - - - supposed flight occurrence.

high concentration of fertile flies present (Fig. 32, g). About 100 m from this field, and about 10 m from a release site of fertile flies, there were road verges with a vege- tation locally dominated, even in summer, by A. vineale. Trap catches there failed to show any accumulation of the flies. Moreover no infestation could be found.

Wolff (1973) did some experiments on the role of A. vineale. In the laboratory female onion flies would lay their eggs on this species, but the larvae failed to pe- netrate into the bulbs unless these were cut. Obviously the hard skin of the bulbs prevents attack by onion fly larvae. In the field in the surroundings of Wageningen he could not find infestation of A. vineale, nor pupae in the soil around bulbs.

The exact places of oviposition can be inferred from egg searching data, although such results are affected by the influence of the site on the probability of the eggs

being found by research workers and other predators. Eggs were found generally in the leaf-axils of the lower leafs, and with young seedlings mainly on or in the ground close to the plant.

Ovipositing flies strongly prefer rotten or damaged onions to normal ones (Section 2.2.3). In a field with about 7% rotting onions 98.4% of the eggs were laid on these (Roest, pers. commun.), the difference in egg density between rotting and normal onions being a factor 800. It seems very probable that the increase in onion fly damage among onions injured, for instance, by weeding or tractor wheels is at least to some extent due to egg deposition preference. The other probable cause is increased survival among newly emerged larvae

It was investigated to what extent residue onions tipped on waste ground were onion fly sources. Especially on the smaller or less official rubbish dumps, irregularly sprayed with different pesticides or not al all, heaps of rotting onions might contain high population densities. The catches of two traps placed at such sites were compared with data from traps around onion fields adjacent to onion fields the year before (Fig. 38). The densities were similar, but the waste areas were very small compared with onion fields. The lower percentage of females near the waste onions indicates that these are not preferred for oviposition. Thus onion tipping sites are unimportant in the population dynamics of the onion fly. In the United States tipped onions are occasionally said to be the most important onion fly sources in spring (Anonymous, 1933), but Merrill & Hutson (1953) stated that onion flies did not breed in older cull piles. Some other Diptera related to rotting onions showed a higher concentration on these sites (Fig. 38).
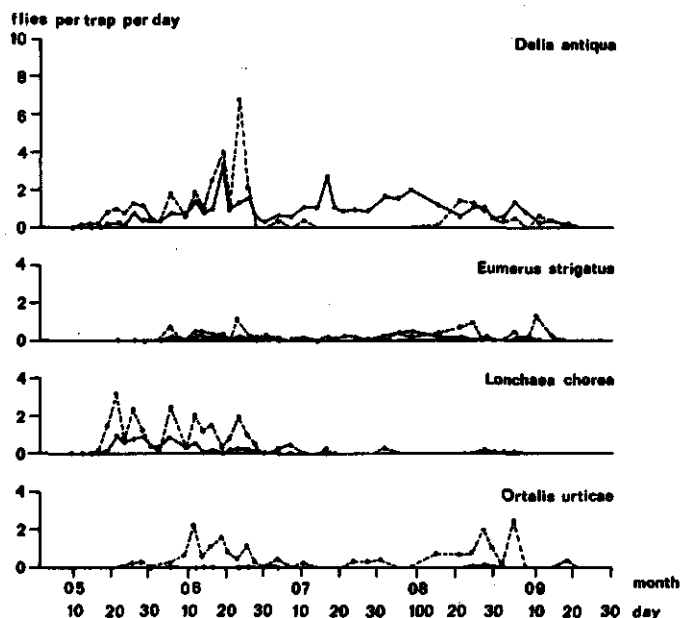
Fig. 38. Catches at onion tipping sites compared with catches on onion fields, in flies per trap per day for different species. Experiments van Es and de Wit 1973.
——— onion fields (18 traps); – – – tipping sites (2 traps).

The onion fly larvae may eat from fresh onion bulb tissue, but generally the attacked onions putrify. Also larvae are regularly found in onions that have been attacked by onion stem eelworm.

During the first flight especially, onions with only larvae of Delia platura were found several times. Of course it is not certain whether any onion fly larva had been present before or not. To determine whether D. platura should be treated as a primary or a secondary pest, the population densities of both Delia species were compared on the fields sampled. If D. platura is an effective primary pest its numbers will be relatively high when D. antiqua is selectively eliminated by genetic control. Such a phenomenon was observed with tortricids in the genetic control of the summer fruit tortrix, Adoxophyes orana F.R. (Ankersmit, 1975). Fig. 39 shows that the numbers of D. platura diminished even more. Note that the highest point in the graph is from the same location the year before, whereas the next highest point is from the same year but from the control field at nearly 2 km distance. This reduction in numbers does not imply that D. platura cannot be a primary attacker of onions, but it shows that its population buildup is dependent on some factor(s) associated with D. antiqua. Effectively D. platura is a secondary pest of onions. Because of the oviposition preferences of D. platura (Section 2.2.3) and the fact that all onion seed is treated with a fungicide, the observed situation may not hold for untreated seed.

Possible primary attackers, although on untreated fields still quantitatively unimportant, are cutworm larvae (Lepidoptera: Noctuidae) and wireworms (Coleoptera: Elateridae). Damage due to these species is rare.

Rotten onions, especially in summer when they have a reasonable size, often contain several other insect species (Table 11).
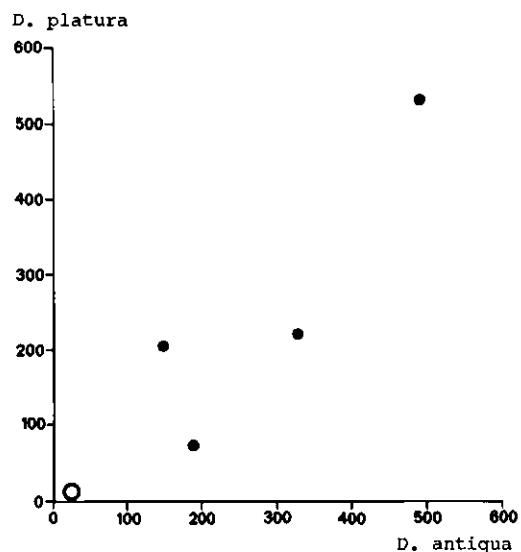
Fig. 39. Populations of Delia antiqua and D. platura, in thousands of pupae formed per ha per year.
● = untreated field; O = onion fly under genetic control.

Table 11. Diptera and Coleoptera found in onion bulbs attacked by onion larvae.

	Stage[a]	frequency[b]		Stage[a]	frequency[b]
DIPTERA:			COLEOPTERA:		
Syrphidae			Curculionidae		
Eumerus strigatus Fall.	1, p	+++	Ceuthorrhynchus		
Lonchaeidae			suturalis F.	1	+
Lonchaea chorea (Fabr.)	1	++	Nitidulidae		
Muscidae			Glischrochilus		
Fannia canicularis L.	1, p	++	quadripustulatus	a	++
Muscina assimilis Fall.	1	++	Staphylinidae		
Cordiluridae			Dinaraea angustula Gyll.	1, a	+++
Cnemopogon apicalis Wied.	1	++	Atheta fungi Grav.	a	+
Otiididae			Oxytelus rugosus F.	1, a	+++
Ortalis urticae	1	++	Aleochara bilineata Gyll.	a	++
Chloropidae			Trogophloeus corticinus		
Elachiptera cornuta Fall.	1	+	Grav.	a	+
Sarcophagidae			Catopidae		
Sarcophaga carnaria L.	1	+	Ptomophagus medius Rey	a	+
Anthomyiidae			Lathridiidae		
Delia platura (Meig.)	1	+++	Lathridius lardarius Deg.	a	+
Delia pullula (Zett.)	1	++	Elateridae		
Pegomyia sp.	1	+	Corymbites sp. or		
			Athous sp.	1	++

a. 1 = larva, p = pupa, a = adult.
b. +++ = common, ++ = regular, + = incidental.

Observations on feeding of adults are very incidental. They were seen feeding on flowers of Taraxacum vulgare, Allium cepa, Euphorbia peplus and E. helioscopis. On several occasions no feeding onion flies could be found on any of the flowers in the neighbourhood of an onion field although many flies were seen in the field. Grasses were not checked, but these may be important food sources (Section 2.2.3). There are several observations of mainly females, also at low onion fly densities, eating or drinking from bird excrements and sometimes also from rotten onions. Once, inside a trap, an onion fly female was seen to feed on the pollen carried by a bumble bee.

4.2.3 Parasitoids

Some parasitoids were reared from pupae. The species observed are Aleochara bilineata (Gyll.) (Coleoptera: Staphilinidae), Aphaereta minuta (Nees) (Hymenoptera: Braconidae) and Phygadeuon sp. (Hymenoptera: Ichneumonidae). Aphaereta minuta and the Cynipid Trybliographa diaphana (Hartig) were reared from D. platura.

Parasitoids emerged only from pupae from the last pupal samplings in a year, August-September. Their frequencies are given in Table 12. Aphaereta minuta emerged with 5-12 wasps per pupa, on average 8. The frequency of Aleochara and Phygadeuon may have been underestimated because they parasitize on pupae, and these were sampled before this stage ended.

The lower degree of parasitism in the onion growing area (data from Overflakkee as compared with those of the Schuilenburg) may be due to the more intensive chemical control

Table 12. Frequencies of parasitoids.

	Percentage of full pupae at harvest with:				Total number of parasitized pupae
	Aleochara bilineata	Phygadeuon sp.	Aphaereta minuta	total	
Schuilenburg 1970	10	11	0	21	19
Schuilenburg 1971	≥3	≤19	0	22	7
Schuilenburg 1972	0	20	0	20	13
Nieuwe Tonge 1971	0	0	16	16	13
van Es 1973	0	1	1	2	2
de Wit 1973	0	0	2	2	2
Mijs 1973	0.6	1.2	1.4	3.2	16
Mijs 1974	≥0	3	≥1	7	6

there. In the cabbage root fly such control is known to diminish parasitization consider-
ably (e.g. Coaker, 1966) and this could apply to the onion fly too.

4.2.4 Entomophthora

An important parasite of the second flight flies especially is the fungus Entomophthora
sp. Some data on the increase of the infection during the season are given in Fig. 40. In-
fected flies with already externally visible signs move too slowly to get trapped. Visibly
infected flies were found in the traps' collectors because the flies generally stayed there
for half to one day. After having kept flies for one week in cages at the laboratory, the
fungus was clearly visible on many more flies (Fig. 40a). In the cages no new infection
occurs that results in externally visible signs within this week: the percentage visibly
infected flies did not rise with the number of flies per cage increasing from 1 to over 20
(r = -0.316 with n = 15, data from a period with a reasonable constant percentage infected).
Flies died about one day after the infection had become visible. Both sexes, including
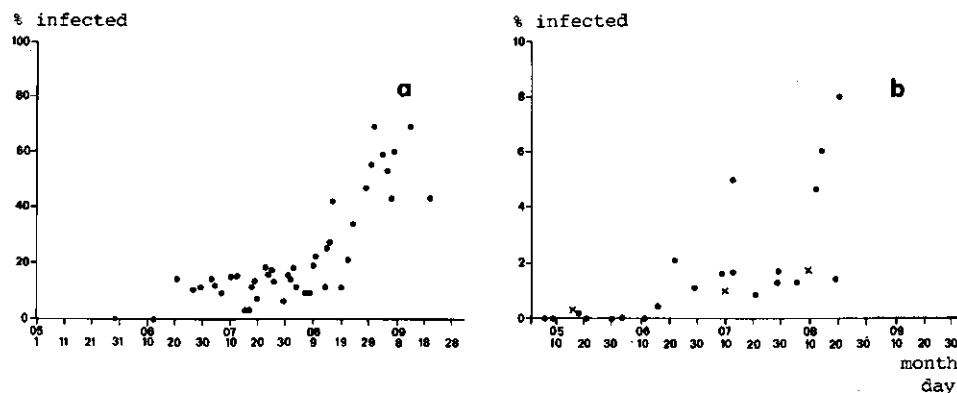
Fig. 40. Development of Entomophthora infection during the season, expressed as the
percentage of flies which has visible infection.
a. Schuilenburg 1972. Females checked after one week in the laboratory, versus day of
trapping. Each point represents at least 50 females.
b. Mijs 1974. Flies checked one day after having been trapped, versus average day of
emergence. Each point represents the recaptures of a released cohort of steriles (•) or
a part of the wild population (×).

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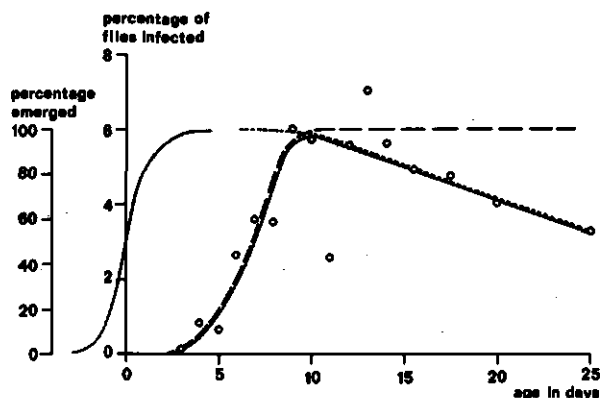
Fig. 41. Percentage flies visibly infected by Entomophthora one day after having been trapped, versus age when trapped. Experiment Mijs 1974. Average of both sexes, second flight steriles.
—— Average emergence; ━○━ experimental data on fungus infection and estimated average percentage infected; - - - expected fungus infection with a constant infection rate of 6% per day; ······ actual rate of infection, occurring 6 days earlier than indicated on the abscissa.

aberrant males, were equally attacked (experiment Mijs 1974: 161 infected flies, χ^2_{17}= 19.76, n.s.).

The percentage flies visibly infected one day after having been trapped, versus their age, is shown in Fig. 41. This percentage, when the spread in emergence is taken into account, rises steeply from zero at an age of 6 days to about 6% at 7 days of age, and decreases afterwards slowly. The infection will have started from emergence onwards, the fungus taking 6 days to develop (Perron & Crête, 1960: one week). The infection rate versus age was thus about 6% during the first day of adult life, and decreased thereafter identically with the decrease as shown by the graph for the visibly infected percentage of flies, but 6 days earlier. A much higher susceptibility of very young flies due to soft chitin, as suggested by Ticheler et al. (1974a) did not occur. The higher infection rate of younger flies may very well be ascribed to the fly density. According to these data, the number of flies visibly infected will be 18% of the total number of infected flies alive.

In the first sterile male field experiment, Schuilenburg 1971, the sterility among the eggs dropped considerably compared with fly sterility. Because this drop coincided with the increase of fungus infection, and because the flies had been released weekly on the same spots, Ticheler (1973) and Ticheler et al. (1974a) supposed that these repeated concentrations of steriles were preferentially attacked by the fungus, decreasing competitiveness. These authors suggested that such a preference had been found. The very few -unpublished- data on this subject did not support this hypothesis (June 1971: 75 steriles checked, expected from total catch: 6 infected, observed: 5 infected). Moreover, it is not clear how the fungus infection could explain the observed phenomenon. The sterility was assessed from the ovary development in the females that had been kept in the laboratory for one week. The females with visible fungus attack could no longer be checked for sterility, because the ovarioles had already completely disintegrated. If the fungus was the cause of a lower competitiveness of the males, it has to be assumed that the fraction

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of sterile males that were made ineffective was much higher than the fraction of sterile females which had visible fungus infection 8 days after entering the trap. From the infection rate versus age and the lack of sex difference, this assumption seems to be unreasonable.

4.2.5 Predators

Preliminary experiments were done to evaluate the suitability of using radioactive labels to analyse the role of predators on eggs and larvae, and to find the species involved. Feeding experiments, by confinement of individual potential predators with some ^{65}Zn labelled eggs for one or more days in little cages, have been done mainly by Buth (1976). The results are given in Table 13.

From rotten onions on which we had deposited labelled eggs, several potential predators were collected. They may have eaten eggs or larvae, or they may be scavengers, having ingested the label indirectly. The results of checking these potential predators for the presence of label by autoradiography are given in Table 14.

In the experimental fields some 175 potential predators, belonging to over 20 species, were collected. No label could be found in these, probably because of the limited number

Table 13. Results of feeding experiments with single potential predators confined with ^{65}Zn-labelled onion fly eggs. Most data from Buth (1976).

	Number of individuals used	Number in which label demonstrated
COLEOPTERA:		
Staphylinidae: Paederinae	1	1
Carabidae: Trechus quadristriatus	3	3
Bembidion ustulatum	1	0
Agonum dorsale	3	2
Asaphidion flavipes	1	1
Carabus granulatus	1	0
Harpalus pubescens	7	1
Calathus melanocephalus	1	1
ARANEIDA:		
Micryphantidae	7	3
Opilionidae	1	0
ACARI:		
Trombidiidae	1	1

Table 14. Presence of ^{65}Zn in potential predators (Coleoptera), collected from rotten onions on which labelled eggs had been deposited. Experiment Mijs 1974.

	Number of individuals	
	collected	labelled
Staphylinidae: Dinaraea angustula (larvae)	8	−
Dinaraea angustula (adults)	16	3
Oxytelus rugosus	14	2
Elateridae: Corymbites sp. or Athous sp (larvae)	13	3
Carabidae: Trechus quadristriatus	1	−

of labelled eggs used (7000 eggs in two onion fields), in relation to the population den-
sity and mobility of the potential predators. Loss of label by predators will have been
negligible, as indicated by the experiments of Breymeyer & Odum (1969) who found a biolo-
gical half-life for ^{65}Zn in predatory spiders of 12-120 days, dependent on species and
oviposition frequency.

From these results one may conclude that, for analysing predation on onion fly eggs,
labels can be useful with experiments in confined environments.

In some experiments concentrations of pupae, buried in the soil for release and not
yet covered with gauze, were very effectively preyed upon by mice, pheasants and patrid-
ges, as could be seen from their tracks.

Generally near release sites, the following birds were observed preying on adult
onion flies, in order of frequency of occurrence in the Overflakkee experimental areas:
meadow pipit (Anthus pratensis (L.)), sky lark (Alauda arvensis L.), blue-headed wagtail
(Motacilla flava flava L.), starling (Sturnus vulgaris L.), white wagtail (Motacilla alba
L.) and reed bunting (Emberiza schoeniclus (L.)). Most probably also the less common
winchat (Saxicola rubetra (L.)) preyed on onion flies. Because onion flies seldom fly hig-
her than the vegetation, predation on them by the different swallow species present, as
supposed by Kästner (1929b), will not have occurred.

The dragon fly Ischnura elegans (Vanderl.) was observed capturing onion flies in the
field. Supposed predators are the common toad (Bufo bufo L.) and the predatory fly
Scatophaga stercoraria (L.). The latter was often observed killing onion flies in the
traps' collectors.

4.3 ESTIMATION OF DAMAGE INTENSITY AND POPULATION DENSITY

4.3.1 *Damage intensity*

The pattern of damage over onion fields will be considered to indicate how differen-
ces in damage level may be tested for, and what amounts of samples need to be taken to
obtain certain levels of precision in estimating damage intensity. Then the preferable
plot size will be indicated, and finally the distribution of the percentage damaged per
onion field is given.

4.3.1.1 Damage pattern

Onion fly damage is not equally distributed over onion fields. It was investigated
whether some stratification of the fields is advisable. The damage at the heads of the
fields, as delimited by the direction of the onion rows, was found to be up to ten times
the damage level of the rest of the fields. The difference was on the average a factor
of about 4, so that stratification is advisable. In the present analysis this has not
yet been done. The increased damage levels on the heads may be attributed to the fre-
quent occurrence of onions being damaged by tractor wheels, as such onions are prefer-
red for egg deposition. Border effects in crop damage are quite common and have been
mentioned for the onion fly (Kästner, 1930), although uniform distribution of damage

has been reported also (Perron & leRoux, 1962). A small experimental plot in 1972 gave some indication of such an effect (Huber, 1973), but data from a much larger field failed to show any border effect.

4.3.1.2 Distribution of damage

Damage occurs in a highly aggregated way (Table 15). This originates from:
1. clustered egg deposition, and larval migration over only short distances,
2. some preference of egg depositing females for certain types of healthy onions according to the onions' developmental stage, size or plant density, and for artificially damaged onions (mainly by weeding) (Section 2.2.3),
3. a strong preference of egg depositing females for onions already attacked by onion larvae or fungal diseases (Section 4.2.1; Workman, 1958),

Table 15. Frequency distributions of damage and parameters of the distributions.

Number of onions damaged	Experiment no.											
	1	2	3	4	5	6	7	8	9	10	11	12
0	5	22	0	48	190	28	32	50	449	132	225	50
1	5	16	1	33	54	21	26	17	26	31	23	27
2	4	7	1	10	11	16	8	12	12	27	2	17
3	3	2	1	6	2	10	5	9	5	19	1	8
4	2	1	0	3	0	6	4	2	4	8	0	1
5	2	1	2	0	0	7	1	1	1	9	0	0
6	1	1	1	0	0	6	4	1	2	7	0	0
7	0	0	1	0	0	6	7	3	0	9	0	0
8	1	0	1	0	0	2	1	1	0	2	0	0
9	1	0	1	0	0	4	1	3	0	1	0	0
10	1	0	0	0	0	4	0	1	0	4	0	0
11-15	5	0	2	0	0	11	2	1	0	6	0	0
16-20	3	0	0	0	0	4	0	0	0	0	0	0
21-25	1	0	1	0	0	1	0	0	0	0	0	0
26-30	0	0	0	0	0	1	1	0	0	0	0	0

Exp no.	Location & Year	Plot length in m	Plot pattern	n	m	s^2	m^*	k	χ^2_d	d	P
1	Lombok 1972	1/2	regular	34	6.235	40.79	11.78	0.903	1.77	3	0.62
2	Schuilenburg 1970	3	random	50	1.02	1.775	1.76	1.606	0.42	1	0.52
3	" 1971	5	regular	12	7.83	33.42	11.10	2.731			
4	" 1972	5	"	100	0.830	1.072	1.12	2.809	1.44	1	0.23
5	" 1973	5	"	257	0.319	0.351	0.42	3.144	0.05	1	0.83
6	Mijs 1973	1/2	"	127	4.528	28.89	9.91	0.774	4.09	9	0.90
7	van Es 1973	1/2	"	92	2.391	14.42	7.42	0.609	10.78	5	0.06
8	de Wit 1973	1/2	"	101	1.653	7.129	4.97	0.466	5.97	4	0.20
9	Mijs 1974 (exp)	1/4	random	499	0.196	0.524	1.87	0.0944	0.57	2	0.75
10	Mijs 1974 (con)	1/4	"	255	1.804	7.804	5.13	0.404	8.49	7	0.29
11	Schuilenburg 1974 (exp)	5	regular	251	0.120	0.146	0.34	0.562			
12	" 1974 (con)	5	"	103	0.864	1.040	1.07	3.382	1.71	1	0.19
tested simultaneously									35.29	34	0.41

n number of samples
m mean frequency of damage
s^2 variance
m^* mean crowding

k parameter of the negative binomial distribution
d degrees of freedom
P probability of the observed χ^2 value.

4. possible differences in survival of larvae at different levels of larval density.

From this combination of factors that cause aggregation, no specified distribution for the damage can be deduced. Thus the negative binomial distribution, which can be derived from a wide variety of models (e.g. Anscombe, 1950; Southwood, 1966), seems the most appropriate.

The fit of the damage data to the negative binomial distribution was tested according to the method given by Bliss (1971). Because of the low efficiencies of approximate estimates of the parameter k for the present distributions (Anscombe, 1950), the maximum likelihood estimator of k was determined by iteratively solving

$$\ln(1 + \tfrac{m}{k})\ N = \sum_i \frac{f(x_i+1)+f(x_i+2)+\ldots}{k + x_i} \tag{2}$$

(Bliss, 1953; Fisher, 1953), in which $f(x)$ is the observed frequency of x, and N is the number of samples. The expected frequencies $\phi(x)$ were calculated from

$$\phi(0) = N/(1 + \tfrac{m}{k})^k \tag{3}$$

and

$$\phi(x) = \frac{\phi(x-1)m(k+x-1)}{x(m+k)} \tag{4}$$

(Bliss, 1953, 1971). Tested with x^2_{n-3} a good fit was obtained for all 12 distributions (Table 15).

Gurland & Hinz (1971) give a method of x^2 analysis comparable with analysis of variance, applicable to untransfromed data of four generalized Poisson distributions, such as the negative binomial. This method may thus be used when differences between damage distributions have to be tested. The generally advocated procedure of transforming the data to conform to a normal distribution and applying analysis of variance (e.g. Taylor, 1961, 1965; Southwood, 1966; Harcourt, 1967; Iwao & Kuno, 1968) may not be exact (Gurland & Hinz, 1971). Especially with the present type of distributions, with low mean and high aggregation, the distributions will remain monotonously decreasing and not become like a normal distribution, whatever transformation is applied (cf. Taylor, 1961). As the k values obtained were correlated to the mean for the data of Overflakkee, calculation of a common k (Bliss, 1953) was not useful. The Schuilenburg data gave a wide scatter of k values. Bliss (1971) found that in several cases where no common k was present, a linear relation existed between $\lg\left(\tfrac{1}{k}\right)$ and $\lg(m)$, with a slope of -0.5. This relation fits for the Overflakkee data, assuming a dependency on plot length (Fig. 42). The Schuilenburg data may confirm this, when account is taken of some underestimation in the aggregation in 1970 (damage only checked at harvest) and in 1973 (heads of fields not sampled).

Because of the high number of zero counts the truncated negative binomial distribution was considered (Bliss, 1971), but this did not give a clearly better fit. Therefore there is no reason to assume that parts of the onion fields were not liable to be attacked.

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4.3.1.3 Precision estimation

The precision, D, is a measure of the reliability of m as an estimate for μ, that is, a measure of the width of the confidence interval. For contagious distributions generally the measure $D = s_m/m$ is used (e.g. Kuno, 1969). It can be useful, for instance, in making population estimates at different densities with an equal precision, thus optimalizing the sampling effort allocation.

For contagious distributions there are some methods of estimating the number of samples needed for a certain degree of precision, based on one of the following relations between the variance and the mean:

1. from the negative binomial distribution (Rojas, 1964 (in: Southwood, 1966); Kuno et al., 1963 (in: Iwao & Kuno, 1968); Gérard & Berthet, 1971):

$$s^2 = m + m^2/k \tag{5}$$

which is a special case of

2. from the regression of mean crowding ($m^* = m + s^2/m -1$; Lloyd, 1967) and the mean: $m^* = \alpha + \beta m$ (Iwao & Kuno, 1968, 1971; Kuno, 1969, 1972):

$$s^2 = (\alpha +1)m + (\beta -1)m^2 \tag{6}$$

and

3. Taylor's power law (Green, 1970):

$$s^2 = am^b \tag{7}$$

Kuno (1972) criticized Taylor's power law (Taylor, 1961, 1965, 1971; Southwood, 1966; Bliss, 1971) as being purely empirical, and not describing the biological reality because it assumes random distribution when m approaches zero. On the other hand, the relation between the mean crowding and the mean is empirical as well. This relation has been criticized by Taylor (1971) as it would not be exactly linear: it should contain a factor with m^2. Incorporating such a factor results in a relation between s^2 and m which cannot be distinguished from the power law even with the most extensive data set available (Taylor, 1971). In fact generally the negative binomial distribution is also an empirical description of the observed distributions, and thus of the relation between s^2 and m.

With Equations (6) and (7) the variance is calculated from $s^2 = \sum_i (x_i-\bar{x})/(n-1)$, and used, either directly or via $m^* = m + s^2/m -1$, in estimating the relation between the variance and the mean. On the other hand, with Eqn (5) the parameter k is calculated from (2) using all x_i, and the variance is calculated from (5).

Sabelis & de Reede (1975) applied all three relations for estimating precision in orchard mite distributions, and showed that clear differences may occur. They assumed Eqn (5) to be the most reliable, because then the estimated variance is not subject to the severe instability caused by incidental high values of x_i. However, in with Eqn (5)
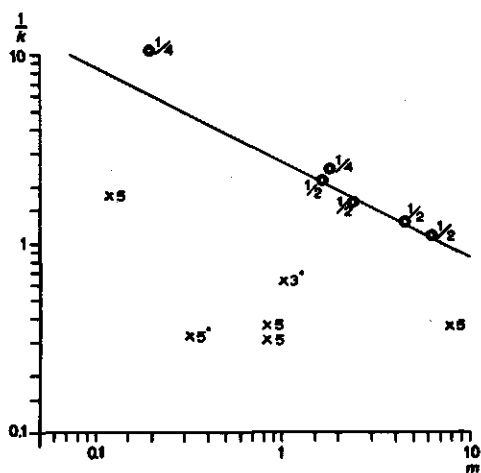
Fig. 42. Damage distributions: relation between $1/k$ from the negative binomial distribution and the mean m, with plot size indicated in m. Regression line for 0.5 m plots: $k = 0.37\sqrt{m}$. O :Overflakkee; X: Schuilenburg; * :case where k has been underestimated.
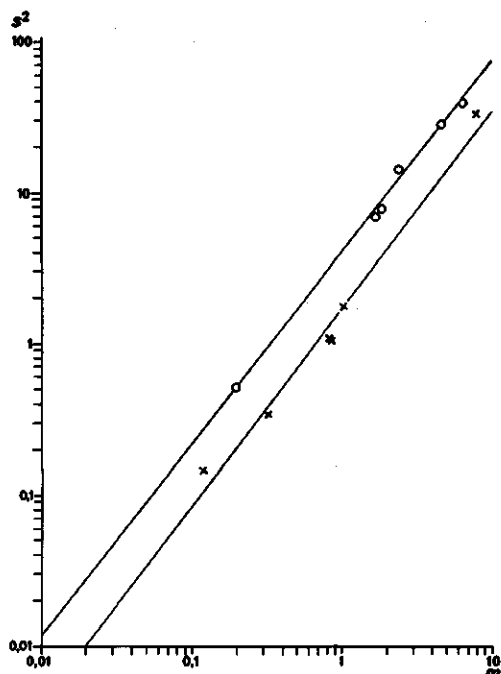
Fig. 44. Damage distributions: Taylor's power law.
O: Overflakkee, $s^2 = 4.079m^{1.273}$;
X: Schuilenburg, $s^2 = 1.709m^{1.318}$.
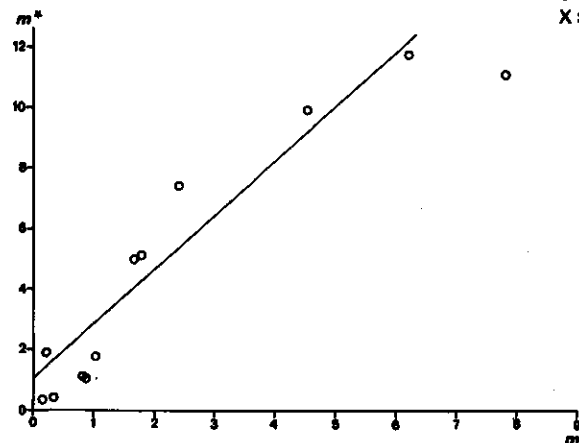
Fig. 43. Damage distributions: relation of the mean crowding m^* to the mean m. Regression line $m^* = 1.60m + 1.04$.

the variance is not independent of the mean, so the advantage claimed is not obvious. Thus there is no reason to prefer one of these relations beforehand. For the onion fly damage, all three models may describe the situation observed (Figs. 42, 43 and 44), although it seems that m^* is curvilinearily related to m (Fig. 43).

Now the number of samples required for a given precision can be calculated from the mean and the variance (e.g. Southwood, 1966). In practice it may be easier to use a

'stop line' in a graph of T_n, the number of individuals in n samples, versus n (Kuno, 1969). The stop line is a line connecting points of equal precision. When in sequential sampling the subsequently obtained results are plotted in the graph of T_n versus n, the required precision is reached when the corresponding stop line is crossed. The use of such a graph can have an advantage over just calculating the precision of the sample taken. This is so when the graph is based on a model that has been shown to fit many extensive data sets, and is used for estimating the precision in relatively small samples from which the variance, and thus the precision, can be estimated much less reliably due to the skewness of the distributions as indicated above.

With $s^2 = f(m) = f(T_n/n)$, the stop line can be found by putting $D^2 = (n/T_n^2)\ f(T_n/n)$ in the form $T_n = f(n)$. For the relation (6) this results in (Kuno, 1969):

$$T_n = \frac{\alpha + 1}{D^2 - (\beta - 1)n} \tag{8}$$

reducing for the negative binomial distribution to (Kuno, 1969):

$$T_n = \frac{1}{D^2 - 1/nk} \tag{9}$$

For the Overflakkee data $k = c\sqrt{m}$ (from Fig. 42, c being a factor dependent on plot size), and incorporating this gives:

$$T_n = \frac{\gamma + 1 + \sqrt{2\gamma + 1}}{D^2\gamma} \tag{10}$$

in which $\gamma = 2c^2 n D^2$. Using Taylor's power law one finds (cf. Green, 1970):

$$T_n = \sqrt[2-b]{\alpha\ n^{1-b} D^{-2}} \tag{11}$$

Introduction of a limit N for the population sampled is done by multiplying s^2 by $1 - n/N$ (Kuno, 1969). For a 1 ha onion field, sampled with 0.5 m plots, N is 60 000, so this correction can be neglected here.

The stop lines calculated are given in Fig. 45. The problem in interpreting these lines is that this precision cannot be related to some exact probability because the probability distribution of the mean is calculated with Student's t, requiring a stable variance and stochastical independence of s^2 and m. Either one or the other requirement is not met, as mentioned before. The bias so introduced is unknown, so the precisions in Fig. 45 must be considered as some approximate measure. This may become serious especially at the smaller sample sizes that have to be used in practice.

When the mean is calculated from data obtained in sequential sampling, this is biased. It has to be diminished by a factor $f(m)/(n(m-b))$ (Kuno, 1972) in which m is the population mean, and n and b are the corresponding values of the number of samples needed and of the slope of the stop line for the precision considered. The corresponding bias in the variance is, according to Kuno (1972) negligible.

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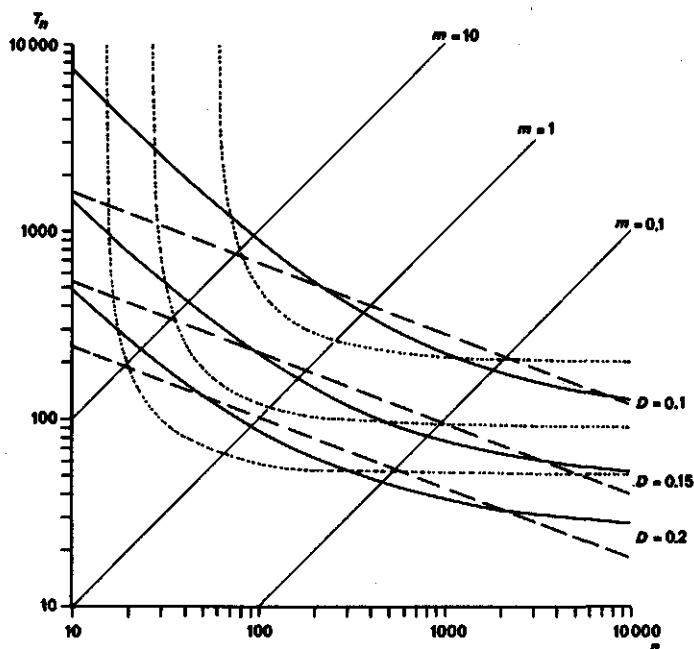
Fig. 45. Damage distributions: stop lines for certain levels of precision $(D = s_m/m)$, in sequential sampling.
Underlying model: —— negative binomial distribution, $k = 0.37\sqrt{m}$;
— — — Taylor's power law, $s^2 = 4.079m^{1.273}$;
· · · · mean crowding $m^* = 1.60m + 1.04$.

4.3.1.4 Plot size

Different plot sizes were used. In an onion field a certain number of damaged onions T_n are present on the area inspected. When T_n is kept constant, more smaller plots increase the precision (Fig. 45). At lower densities this increase is less, as can be imagined from the high zero frequencies expected then. Taking more smaller plots has on the other hand three disadvantages:

1. The amount of time lost in going from one plot to another. With very low numbers of plots this factor will become important, but quantitative information on this is not yet available.

2. The variance in plot length, as staked out in the field, will be rather constant, and thus of increasing importance in smaller plots. This factor is felt to become serious in plots of under 25 cm length.

3. A more serious objection to smaller plots is the limited number of onions that is present in them. The aggregation will be underestimated if the number of damaged onions in a plot falls in the range of the total number of onions per plot. On the average there are about 35 onion seedlings per metre plot length. Thus, as can be seen from Table 15, the aggregation will have been slightly underestimated in some experiments on Overflakkee.

The distribution of damage at harvest in commercially grown onion fields, with chemical control of the onion fly, is given in Table 16. From data of the SNUiF it could be

Table 16. Frequency distribution of damage level in spring-sown onions at harvest.
Data from 49 fields, 84.5 ha. Overflakkee 1971.

estimated % damage	% surface checked	estimated % damage	% surface checked
0	21.9	2.0	0.6
0.1	24.9	3.0	4.2
0.2	7.7	3.5	4.1
0.5	4.7	5.0	7.1
1.0	10.0	10.0	4.7
1.5	9.5	15.0	0.6

Weighted average percentage damage 1.5%

estimated that the total damage, except at high damage levels (over 60%), is less than
twice the damage observable at harvest. Thus normal levels of total damage in practice
are under 5% or 2 onions damaged per metre row, and one metre plots should be sufficient
(cf. Table 15: exp. 7 & 10). For lower damage levels 0.5 metre plots could be used. For
higher levels, plots should be larger and, as can be seen from Fig. 42, the distribution
will become approximately random, i.e. a Poisson distribution.

4.3.1.5 Damage frequency in time

The cumulative percentage damaged versus time is shown in Fig. 46. Most damage was
due to 1st generation larvae, feeding on the onion seedlings. Of the damage due to 2nd
generation larvae, only a part became visible during the regular checking. The remaining
damage was found at harvest when the onions were pulled up and inspected. Therefore in the
graphs the data before the last check are somewhat too low.
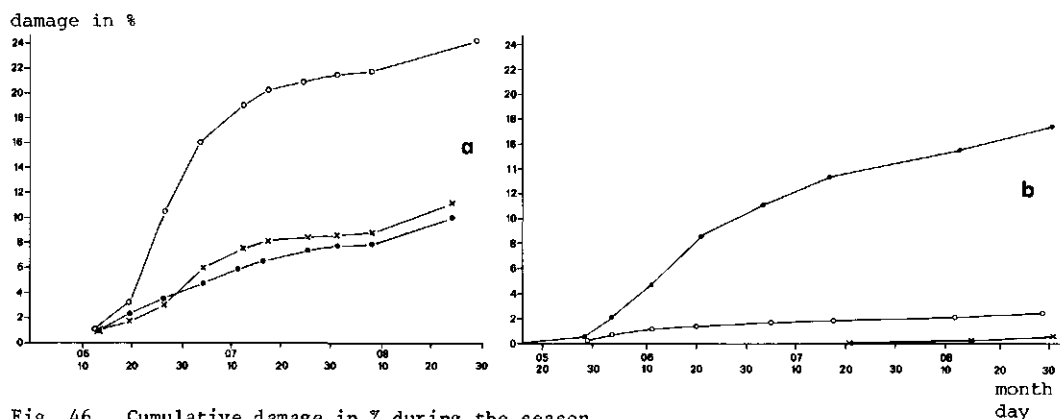

Fig. 46. Cumulative damage in % during the season.
a. 1973. O :Mijs; X :van Es; ● :de Wit.
b. 1974, Mijs. O :trial field; X :control field (with a reduced 2nd flight, see Section
6.2.7); ● :field receiving normal chemical treatment.
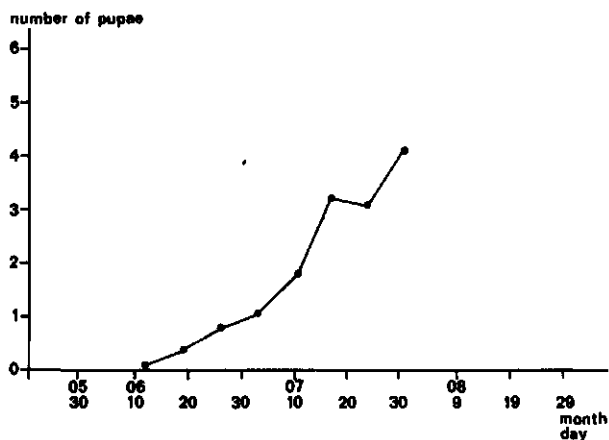
number of pupae

Fig. 47. Number of pupae per onion
attacked, versus data of finding
damage. Experiment Mijs 1973.

4.3.2 Pupal population density

Pupae distribution The number of pupae per infested onion depends on the developmental
state of the onion at the time of attack (Fig. 47) and gives an indication of the rela-
tive amount of food available per onion during the year. Clearly, neglecting damage in
May and the first half of June will hardly affect the population estimates.

The heads of the fields had pupal densities that deviated less than densities of
damage. The density of pupae was in the experiment Mijs 1974 2.1 and 1.6 times higher
on the heads of the experimental and control field, respectively, than on the rest of
these fields, whereas the damage was 4 times higher.

The distributions of pupae per sample are very skew (Table 17). The data were tested
for fit to the negative binomial distribution (see Section 4.3.1.2). The fit was good
except for two distributions with some irregularities in the tails. Both these distri-
butions could be fitted well to the negative binomial when split up over two separate
parts. In the first case (experiment Lombok 1972) the damage, and thus the occurrence
of pupae, was concentrated near the sides of the field, and the distribution was esta-
blished separately for the part of the field more than 8 m from the fields border and
the part less so. The other case (Mijs 1974, control field) was spatially homogenous.
Division of the data between those from the first and those from the second generation
pupae gave distributions with different k values.

The k values were correlated to the mean, but in a different way from those for the
damage. A rather linear relation was found on a lg/lg plot of k versus m (Fig. 48).

When k is estimated by maximum likelihood, the test quantities calculated to test
the fit are not asymptotically x^2 distributed. Gurland & Hinz (1971) gave another method
of estimation of k, which tests for fit with test quantities that are, according to these
authors, asymptotically x^2 distributed. This method will not necessarily give better re-
sults but has the advantage that no lumping of frequencies is required. Thus the informa-
tion from the tail of the distribution is used better. Some distributions of pupae were
tested according to this method (Table 17). The k values obtained are similar, but the

probabilities differ greatly, and in general the fit of the pupae distributions to the negative binomial is not very convincing.

Table 17. Frequency distributions of pupae and parameters of the distributions. Explanation of symbols in Table 15.

Number of pupae	Experiment no.[c]															
	1	2	3	4	5	6	7	8	9	10	11	12	1a	1b	10a	10b
0^a	0	22	0	48	190	28	32	50	449	132	225	50	0	0	135	135
0^b	2	7	3	14	35	36	32	24	19	47	19	23	0	2	68	53
1	2	9	1	8	10	14	8	8	16	21	3	7	0	2	13	21
2	4	0	1	7	5	11	4	4	7	5	3	6	1	3	8	11
3	1	3	2	4	3	2	4	3	5	6	1	3	0	1	9	10
4	1	2	0	3	2	4	0	2	2	5	0	5	0	1	2	6
5	1	2	1	1	2	2	1	3	1	10	0	2	0	1	6	4
6	0	1	0	2	2	4	1	0	0	5	0	0	0	0	0	2
7	0	1	0	1	1	4	3	0	0	2	0	1	0	0	3	2
8	0	0	0	4	0	1	3	1	0	1	0	3	0	0	4	1
9	1	0	1	1	0	0	1	2	0	2	0	1	0	1	1	4
10	1	1	2	2	1	5	0	0	0	2	0	0	0	1	2	1
11-15	2	1	0	2	2	7	2	2	0	10	0	1	0	2	3	3
16-20	6	0	0	3	1	2	0	2	0	5	0	1	2	4	0	0
21-25	7	1	1	0	1	5	0	0	0	1	0	0	3	4	0	0
26-30	4	0	0	0	0	1	0	0	0	0	0	0	3	1	0	0
31-40	6	0	0	0	1	1	1	0	0	1	0	0	4	2	1	0
41-50	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
51-110	7	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0

Exp. no.	Sample Ø in cm	n	m	s^2	m^*	k	χ^2_d	d	P	(Gurland & Hinz, 1971)	
										k	$P(\chi^2_1)$
1	20	46	26.02	543.89	45.92	1.023	16.44	5	0.006	1.223	0.43
2	20	50	1.84	15.08	9.03	0.255	1.89	1	0.17		
3	20	12	5.50	45.00	12.68	0.677					
4	20	100	2.08	16.24	8.89	0.205	1.38	3	0.71		
5	20	257	1.05	17.01	16.29	0.040	1.27	3	0.74		
6	5	127	3.89	49.56	15.63	0.247	5.92	6	0.43	0.277	0.09
7	5	92	1.66	18.34	11.69	0.145	4.33	3	0.23	0.149	0.51
8	5	101	1.35	12.15	9.37	0.129	0.68	2	0.71	0.145	0.26
9	20	499	0.12	0.28	1.53	0.060	0.86	1	0.35	0.079	0.08
10	5	255	1.89	19.69	11.33	0.132	19.68	7	0.006	0.148	0.007
11	20	251	0.05	0.09	1.01	0.029					
12	20	103	1.21	7.21	6.15	0.168	2.03	2	0.37		
1a	20	21	41.14	628.43	55.42	2.737	0.43	1	0.51		
1b	20	25	13.32	127.98	21.93	0.970	3.10	2	0.21		
10a	5	255	0.97	8.90	9.19	0.096	3.91	5	0.56	0.095	0.25
10b	5	255	0.92	5.05	5.40	0.153	2.89	5	0.72	0.177	0.03
tested simultaneously[d]							28.69	34	0.73		0.05

a. Plots without damage.
b. Plots with damage.
c. Division of experiments 1 and 10 into 1a/1b and 10a/10b see text.
d. Experiments 1 and 10: undivided data sets not included where divided sets available.
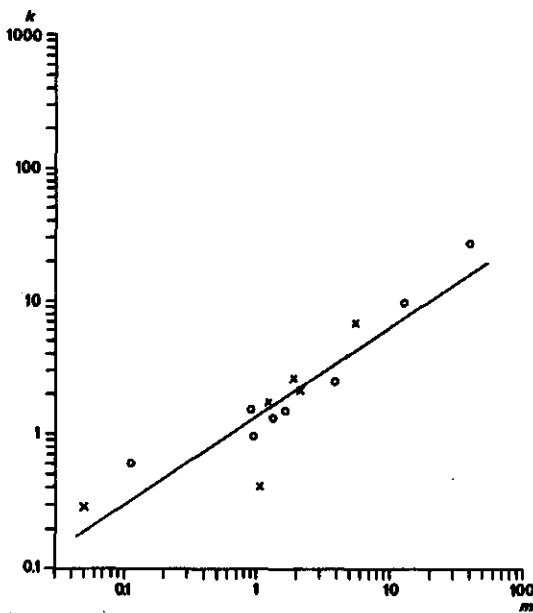
Fig. 48. Pupa distributions: relation
between k from the negative binomial
distribution and the mean m. Regression
line $k = 0.14m^{0.66}$.
O :Overflakkee; X :Schuilenburg.
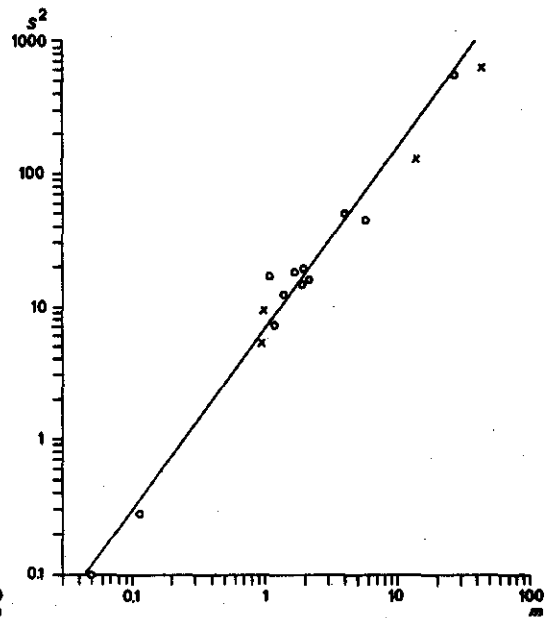
Fig. 49. Pupa distributions: Taylor's
power law. $s^2 = 7.006m^{1.373}$.
X : Data of experiments 1 and 10 (Table
17) after having been split up into two
parts; not used in the regression
calculation.
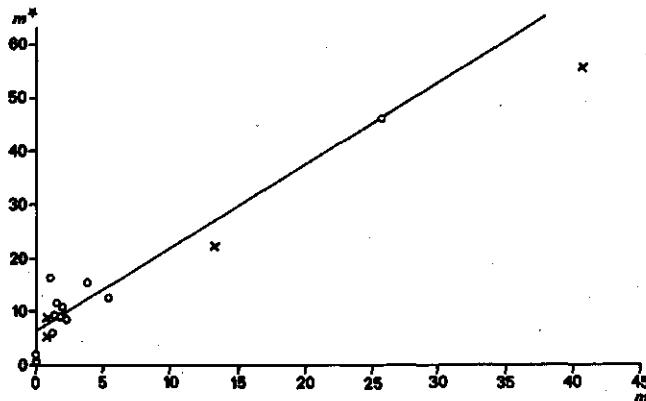
Fig. 50. Pupa distributions: relation
of the mean crowding m^* to the mean m.
Regression line $m^* = 1.53m + 6.50$.
X : As in Fig. 49.

Population estimation Precision was calculated in the same way as was done before for
the damage. Again the linearity of the relation between m^* and m was not very good
(Fig. 50). The power law fitted to the data, even without an effect of plot size or
sampling core diameter (Fig. 49). Stop lines were calculated as for damage (Fig. 51), in
the case of the negative binomial distribution with $k = 0.14m^{0.66}$. Again, the correspond-
ing probabilities could not be indicated exactly.
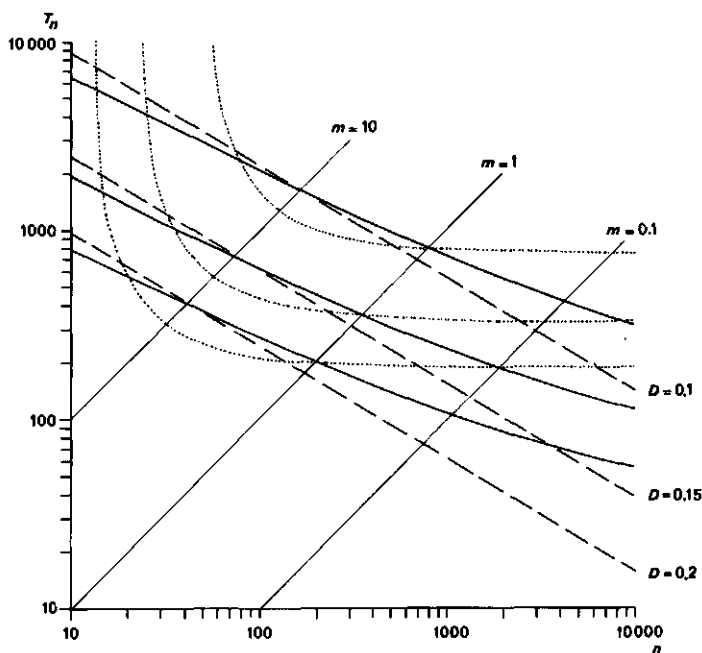
Fig. 51. Pupa distributions: stop lines for certain levels of precision ($D = s_m/m$), in sequential sampling.
Underlying model: —— negative binomial distribution, $k = 0.14m^{0.66}$;
– – –Taylor's power law, $s^2 = 7.006m^{1.373}$;
···· mean crowding $m^* = 1.53m + 6.50$.

Now with practical application of the sterile insect technique, the sample size will have to be low because of the large number of fields to be sampled, and confidence limits, especially the upper one, are required for the population size. As mentioned before (Section 4.3.1.3), usage of Student's t for calculating these is not very reliable. Data obtained in this way are given in Fig. 52, based on the Eqns (5), (6) and (7). For low values of n they can for the moment best be considered as indications of the order of the confidence interval.

It was investigated whether the amount of work in sampling pupae could be reduced by observing more plots for damage than those used also for sampling. For several representative data sets used, the calculations showed that this procedure would not be useful.

4.3.3 *Fly population density*

Direct estimation of fly densities Onion flies can be counted in fields where the onion plants are still small. In an onion field where, from trapping, population density was known to be high, 0.25 fly/m^2 was found. Simultaneously the density on the ditch banks was estimated from samples with tents (Section 3.3.5) to be about 5 flies/m^2. Both methods may yield serious underestimations.

Sampling with these tents was done several times in and around the 1974 experimental field. In onions nearly 2 (range 0.5-4) flies/m^2 were found, and nearly 3 (range 0.5-8) on the ditch banks. These numbers correspond to 20 000 - 25 000 flies (sterile and wild)
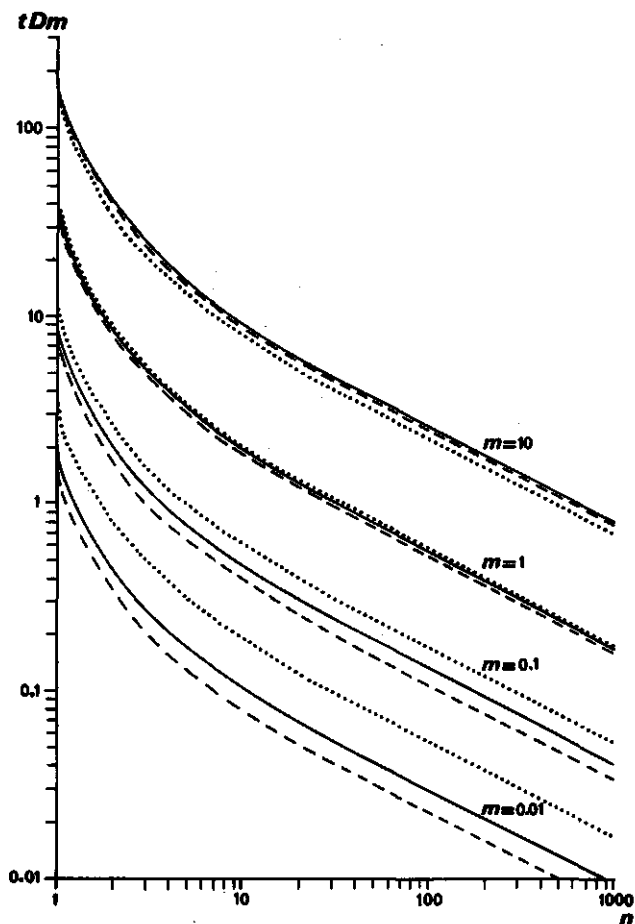
Fig. 52. Pupa distributions: half width of the 95% confidence interval (*tDm*) for different values of the mean, as a function of the number of samples n. Underlying models as in Fig. 51.

on this field. Or, 2000 - 2500 wild flies present at the end of May and beginning of July, on a field where 21 000 wild flies had emerged (compare Fig. 32i).

Release recapture experiments To permit estimation of wild populations according to the principle of release-recapture and application of the Lincoln-index, fertile diapausing laboratory-reared pupae were dug into the soil in early spring, in fields that were onion-fly sources because of the presence of onion fields with some damage the year before. These flies were expected to show about normal emergence curves, and mix with the local populations. At emergence the flies were marked. Traps were placed around the emergence fields to trap the flies when migrating to onion fields. Indeed these flies were trapped there as could be seen from the low percentage mated and the limited ovary development as compared with catches at other sites. The results of the simultaneous experiments in 1972 are given in Table 18. These fly densities relate to the few fields found to have some damage at harvest in 1971.

Table 18. Estimation of populations from release-recapture experiments, 1st flight 1972.

Situation	Number of flies			Wild population	ha Onions in 1971	Average number of flies emerging per m^2
	released	trapped				
		wild	marked			
Vroegindewei	5960	325	29	66 800	1.85	3.6
van Rossum	1590	172	6	45 600	1.9	2.4
Buijs	1000	198	8	25 000	1.5	1.7

Large scale release-recapture experiment Populations were estimated from release-recapture data, obtained during the first and second flight in the experiment Mijs 1974. For the first flight estimation, fertile diapausing laboratory-reared pupae were put in the field in early spring on three sites indicated by G, Y and C on Fig. 15 according to the dye marks used.

From the recaptures it appeared that the emergence of the wild flies took place on the average some three weeks earlier than these 'mixing groups', and also more concentrated in time. The reason for this difference will have been the habitat differences (cf. Fig. 19). These could not have been avoided because the wild populations emerged from fields planted with crops whose mechanical treatment did not allow the dye mark units to be placed there.

As a comparable 'mixing group' for the field on which a sterile male treatment was done (Section 6.2), the steriles released during the first flight were taken (i.e. the groups with dye marks YO-BYO). This group is indicated on Fig. 15 by S .

The recapture data showed that the migration pattern of groups G and Y was similar to that of sterile flies that were also released on these sites. For the subsequent calculations therefore these steriles were incorporated in the respective mixing groups.

As in the other calculations with recapture data from the experiment Mijs 1974, these data have been corrected for incomplete dye-marking of some released cohorts (Section 6.2.1), for locally and temporary increased catches due to the use of an attractant (Section 3.3.3.2), and for the effect of temperature on catch size as given by Fig. 24.

The numbers of first-flight wild flies and of the four mixing groups caught are given in Table 19 for the different onion fields separately. As indicated these data can be treated as a set of equations with four unknowns: the factors converting the numbers of competitive flies per mixing group into the numbers of wild flies emerged in the same field (including the immigrants that had arrived there from outside the area in which trapping was done. Of course these immigrants count here only for that part of their lifetime that took place after immigration into the field concerned. As shown in Section 5.6.3, the numbers of these immigrants will have been insignificant. The equations have been solved, using those pertaining to the fields nearest to where the mixing groups originated. The resulting conversion factors, filled in in the equations, give relative average densities of the catchable fractions of populations on the onion fields considered, split up according to their fields of origin.

An underlying assumption in this calculation is that equal densities in different onion fields result in the same number of flies per trap. Because trap position severely

Table 19. Calculation of conversion factors of mixing groups to wild flies, from numbers of flies trapped in one trap/onion field. Experiment Mijs 1974, 1st flight. Explanation see text.

Trapping site	Wild flies	Mixing groups			
		S	G	Y	C
exp	59.04 =	577.35 s +	11.29 g +	4.34 y +	0.22 c
5	26.06 =	37.92 s +	4.35 g +	5.05 y +	3.93 c
con	169.59 =	6.31 s +	0.94 g +	1.47 y +	45.51 c
1	15.84 =	10.57 s +	63.34 g +	4.24 y +	0.45 c
2	74.00 =	22.72 s +	71.02 g +	34.23 y +	0.52 c
3	115.86 =	42.04 s +	55.85 g +	147.94 y +	0.50 c
4	178.28 =	4.21 s +	0.00 g +	1.80 y +	1.32 c
conversion factors:		s = 0.0925	g = 0.163	y = 0.683	c = 3.688

affects the catch size (Section 3.3.3.1), deviations may be expected especially in fields with a low number of traps. This factor may be responsible for the high numbers of flies found on onion fields 3 and 4, each with only one or two traps which were in a very sheltered position.

The wild populations cannot be calculated directly from the numbers released and the corresponding conversion factors, as the competitiveness of the released flies and the predation on them are unknown. Thus a more complicated approach was needed.

Now the fly densities, as indicated by the trapping, did not occur over the whole onion fields, but only at the edges where these offered shelter. In the present experiment all the traps were situated on such edges. So the relative population densities apply to these, and multiplication of the densities by the length of sheltered edge of the onion field concerned yields values that are approximately proportional to the catchable populations (Table 20).

In these calculations it is assumed that the dispersal of the wild flies is the same as that of the released flies. If the wild flies had dispersed faster, many more flies from 'elsewhere' would have appeared for fields 2 and 5. As the numbers found there are readily explained as having originated from the 1973-onion field near 2, there is no reason to assume that the released flies dispersed differently from the wild ones.

Because not all the flies are concentrated along the onion field edges that provide shelter, the populations on the fields with a larger surface/sheltered edge ratio could have been underrated. When, to prevent such underrating, the relative densities are converted to relative populations on the basis of the onion field surfaces, the populations emerging from G, Y and C increase with factors 1.5, 1.2 and 2.1, respectively. In Section 6.2.2 the effect of this change on the calculated competitiveness values is given: some competitiveness values rise to over 1, especially on field 1. This are highly improbable values, so the incorporation of onion field surfaces in the calculations will be no improvement.

From the data given here, the amount of emigration from the area where flies were trapped was estimated (Section 5.5). When the emigrated fractions are included, the sum of columns represent the total catch for 1 trap/km of all sheltered onion field edges, for each origin. Now for the experimental field there is a reasonable estimation of the

Table 20. Estimated relative catchable populations, expressed as numbers of wild flies
per trap per km sheltered edge of onion field, distributed by origin and trapping site.
Calculated from Table 19, extrapolations to fields 6-10 calculated in Section 5.5.
Experiment Mijs 1974, 1st flight.

Trapping site	Edge in km	Origin[b]					
		exp	1(+2)	3(+2)	con	elsewhere	total
exp	0.38	20.30	0.70	1.13	0.31	0[c]	22.44
5	0.67	2.35	0.47	2.31	9.71	2.61[a]	17.46
con	0.55	0.32	0.08	0.55	91.48	0[c]	92.43
1	0.88	0.86	9.07	2.55	1.46	0[c]	13.94
2	0.28	0.59	3.24	6.55	0.54	9.81[a]	20.72
3	0.54	2.11	4.91	54.55	0.99	0[c]	62.56
4	0.85	0.33	0.00	1.04	4.14	146.02	151.54
6-8		0.08	0.10	0.93	7.79	.	.
9		0.16	0.27	1.57	1.65	.	.
10		0.22	0.21	0.29	0.04	.	.
total		27.32	19.04	71.47	118.08		

Absolute population estimates, calculated from 21 000 on onion field exp:

population	21 000	79 000	90 800
ha onions (1973)	0.1	9.1	5.9
emerged flies/ha	210 000	8 700	15 400

a. Flies supposed to have originated from near onion field 2.
b. The 1974-onion fields nearest to the emergence sites are indicated as origin.
c. These values are zero because of the calculation method.

number of pupae: 23 560 (Section 6.2.1), of which about 90% can be assumed to have
emerged. That is about 21 000 emerging flies. From this the other emerging populations
were estimated (Table 20), and their densities are expressed as numbers emerging per ha
onion field of the preceding year. Note that the experimental field was not treated with
insecticides, and that on both the experimental and control fields fertile flies were
released during the preceding year.

The same calculations were made for the second flight. During this flight a major
disturbance occurred due to the parathion spraying on 31 July. For population calcula-
tions from release-recapture data, only catches after this date could be used. At first
the same procedure was followed as for the first flight data. This gave populations of
2400 and 76 400 on the experimental and control field, respectively. Obviously, there
had been onion fly sources during the second flight in the trapped area that had not
been provided with a mixing group. To get a more complete picture a different calcula-
tion was made subsequently. The migration pattern during this part of the second flight
did not differ essentially from that during the first flight (Fig. 81). Therefore from
this graph the percentages migrated were read for supposed populations originating from
the fields 1 to 5 inclusive. The percentages not migrated were obtained by subtraction
of all calculated migrated percentages from 100. Together with the observed percentages
of the mixing groups on the experimental field (exp) and the control field (con), these
data can be used as a new set of seven equations with seven unknowns, as shown in Table
21. At the left of each equation the relative population size at that trapping site is
mentioned, that is the relative density multiplied by the number of km of sheltered
onion field border.

Table 21. Estimation of populations in experiment Mijs 1974, 2nd flight after 31 July.

Percentage distributions of 2nd flight populations over their trapping sites.

Trapping site	Relative population	Origin exp.	5	con	1	2	3	4
exp	2.92	76.1	3.4	0.5	4.1	5.0	3.4	0.7
5	10.62	7.4	79.0	8.0	1.5	3.4	5.0	2.3
con	79.62	0.7	3.4	80.2	0.7	1.5	3.4	7.5
1	2.26	7.7	1.5	1.3	76.1	9.0	5.0	1.0
2	1.39	2.6	3.4	0.2	9.0	68.6	9.0	1.5
3	6.25	3.8	5.0	0.0	5.0	9.0	66.7	4.1
4	1.90	0.0	2.3	2.0	1.0	1.5	4.1	71.6
elsewhere	.	1.7	2.0	7.8	2.6	2.0	3.4	11.3

Solving these equations as in Table 19 gives the relative population sizes per origin:

| | | 2.64 | 2.63 | 98.82 | 0.35 | 0.31 | 8.92 | -0.72 |

With the unrealistic negative value eliminated, the absolute population estimates, calculated from 21 000 flies corresponding with an estimated relative population of 27.32
(Table 20) are:

| | | 2 000 | 2 000 | 75 400 | 300 | 200 | 6 900 | 0 |

Absolute population estimates of total 2nd flight, from the empty pupae sampled
(Tables 36, 37, 38):

| | | 9 300 | | 109 500 |

Percentages of catches of 2nd flight after 31 July (Fig. 32 h, i):

| | | 21% | | 38% |

Estimated populations of 2nd flight after 31 July, from the empty pupae sampled:

| | | 2 000 | | 42 000 |

With the same ratio of emergence to number caught per trap per km of sheltered onion field border as was found for the first flight (21 000/27.32), the absolute populations in the second flight after 31 July were estimated (Table 21). The difference with the first calculation is small. The accuracy of the population estimates of the control field may be low as can be seen from comparison with the estimate obtained from sampled empty pupae and the distribution of catches before and after 31 July (Table 21).

4.3.4 Relation between pest density and damage

The available information on the relation between the onion fly pupal density and the damage level is summarized in Fig. 53. Useful in a sterile male control programme is knowledge of a relation between the damage level at harvest and the population of diapausing pupae. This cannot yet be estimated with reasonable accuracy.

As the weighted average damage level, as found in spring-sown onions at harvest, was about 1.5% (Table 16), the average population per hectare will be of the order of 10 000.

4.4 MORTALITY

Eggs and larvae A preliminary experiment was done to assess the mortality of marked eggs and larvae. A number of ^{65}Zn-labelled eggs were put on onions in the field, and these
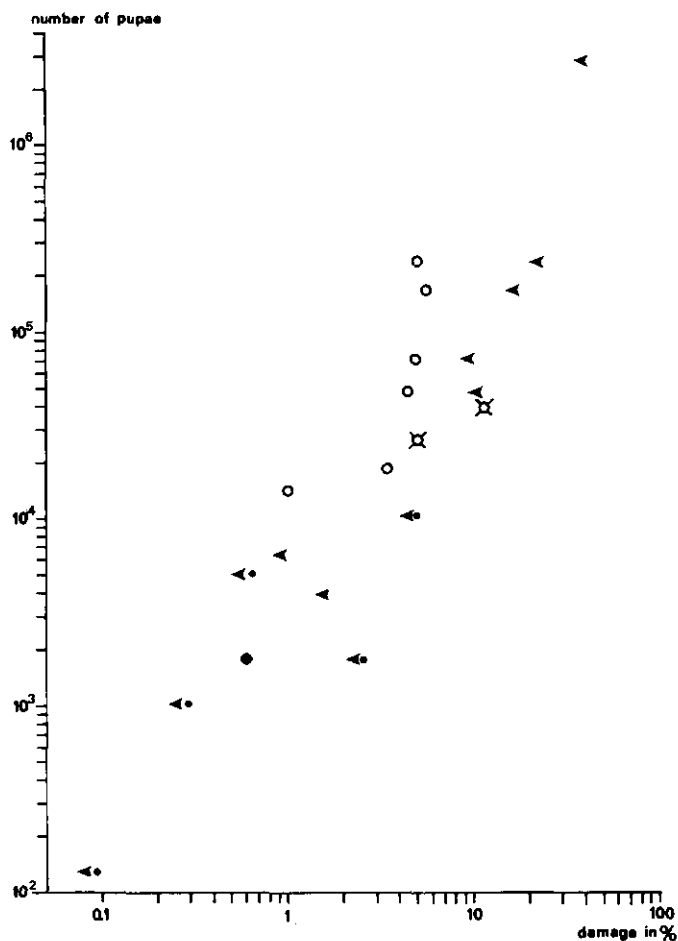
83

Fig. 53. Pest density expressed as number of diapausing pupae per ha, versus damage in %.
○ = Damage at harvest only; ◀ = total damage; • = sterile insect technique; ✕ = chemical
control; other data no control.

onions were sampled at different intervals. The experiment was done both on undamaged
and on infested onions on a field with a high population, and only on undamaged onions
on a field with a low population. The eggs were laid in the leaf axils or between the
onion and the soil, in batches of 2-64 eggs per onion, on different days, with in total
36 replicates.

The emergence of the eggs, as checked with depot groups, was low due to manipulation
and transport. Corrected for emergence, and omitting the 8 replicates that were in the
egg stage in the field during an unexpected parathion spraying, 2876 eggs were used.

The label was found in 25 3rd instar larvae that were sampled as 1st instar larvae
and reared in the laboratory, all survivors being found in already infested onions (9.6%
survival; Buth, 1976). The label was found also in 16 3rd instar larvae sampled as such,
again only from eggs laid on infested onions (2.5% survival). Labelled pupae were not
found, nor any larvae on undamaged onions.

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Pupae Parasitization is discussed in Section 4.2.3. For Overflakkee an average of 5%
parasitized seems a reasonable estimation.

Predation on artificial concentrations of pupae is known from birds, mice and ants,
the latter actually on the emerging flies. As found by van 't Sant (1970) such concen-
trations may also be destroyed by fungi. For wild populations these mortality factors
are unimportant unless population densities are very high. Only the pupae that are brought
to the surface by ploughing are probably reduced considerably by predation. If all pupae
in the upper 1 cm of soil are eaten, mortality would be about 4%.

The weather does not seem to be detrimental to pupae. Pupae brought into the field in
December had a percentage emergence in spring similar to that in the laboratory, where
the average emergence was 85-95%. Ellington (1963) found in field cages a winter morta-
lity of 0-4%.

The total mortality in the pupal stage thus may be estimated roughly at about 10-20%.

Adults Data on Entomophthora infection as a mortality factor of second flight flies have
been given in Section 4.2.4. The effect of other mortality factors such as birds and
predatory flies in unknown. Predation on artificial concentrations of newly emerged flies,
as mentioned in Section 3.3.2.1, will occur with wild flies only at very high population
densities. Such a case has been observed by Peterson et al. (1963).

The sex ratio changes with age (Fig. 36). The released steriles were generally caught
with a sex ratio similar to that of wild flies. Therefore it seems justifiable to assume
that the survival curve for wild flies is similar to that of steriles, except for morta-
lity on release sites.

The survival curves of released flies can be read from recapture curves when migration
has been taken into account. This approach is disturbed by the young flies being seriously
underrepresented in the catches (see e.g. Table 25). Comparison of trap catches with tent
samples taken simultaneously showed that the catching probability can be assumed to be
constant from about an age of less than one week onward, so decrease in catches thereafter
will reflect mortality.

The most extensive set of data available are the relative catchable population esti-
mates of sterile flies in experiment Mijs 1974 (Table 25). As can be seen from Table 20
under exp, the fact that no data from the onion fields 6-10 were available can be neglec-
ted for these flies. Flights and sexes were examined separately. The population sizes at
age zero, without the flies that were lost by predation on concentrations at the release
site, were estimated by extrapolation on graphs of lg(population) versus age, after which
these graphs were shifted along the y axis to get coincidence of the relative population
sizes at age zero at 1000 flies (Fig. 54). The resulting survival curves look reasonably
like those of laboratory data from onion flies (Noorlander, pers. commun.) and related
flies (e.g. Rockstein & Miquel, 1973).

The survival of the flies in the two flights is compared in Fig. 55, after synchro-
nization of the physiological ages by getting the ages at the first mating to coincide
(determined from Fig. 57 and average temperatures of 12.8 and 16.5°C, respectively). For
both sexes and flights the curves are in accordance with a normal distribution of ages
reached. Obviously mortality during the second flight is higher. The difference can only

partially be explained by mortality from infection with Entomophthora during the second flight, according to the infection model given in Fig. 41.
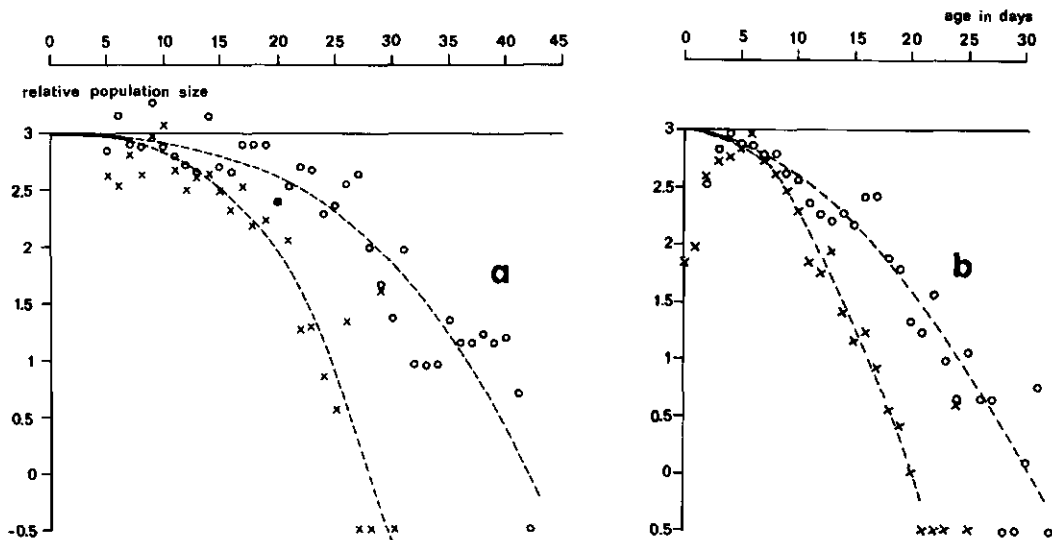
Fig. 54. Relative population size of released steriles versus age in days. Experiment Mijs 1974. Corrected to an estimated number of 1000 at age zero.
O: Females; X: males; a: 1st flight; b: 2nd flight. Curves drawn in by eye.
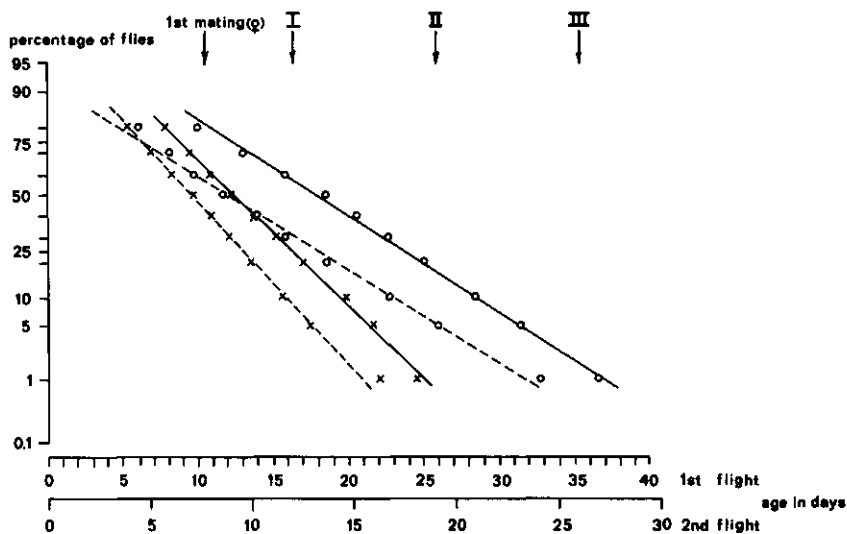
Fig. 55. Survival of adult onion flies, released steriles. Experiment Mijs 1974. Data obtained from the curves in Fig. 54. 1st and 2nd flight age scales adjusted to synchronize ages of females at 1st mating.
I,II and III: oviposition periods; O :females; X:males; ——— 1st flight; - - - 2nd flight.

percentage of females mated
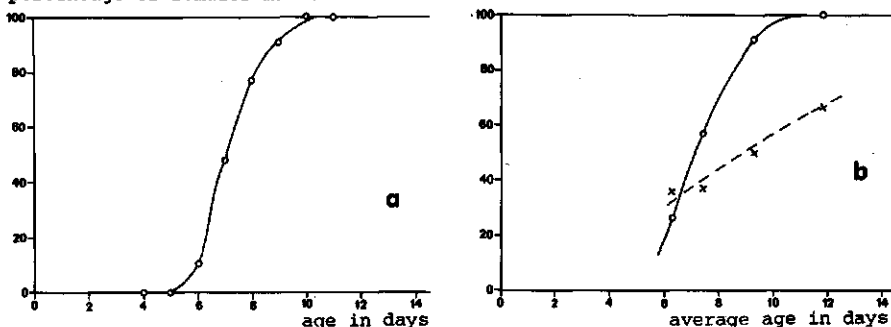
Fig. 56. Mating of fertile released females versus age. Experiment Mijs 1973-2.
a. Flies released and recaptured in a large onion field. Each point represents 30-35
females checked.
b. Flies released and recaptured at over 150 m from any onion field, as compared with the
percentage expected from Fig. 56a. X : 6-12 females outside onion fields; O : 30-35
females in onion fields (different from Fig.56a because the data have been adjusted to the
age distribution at release of the flies outside onion fields).

4.5 REPRODUCTION

4.5.1 Mating

All females mate within a relatively short period, starting at an age of about a week
(Fig. 56). This period was significantly prolonged for females that were released and
recaptured at distances of over 150 m upwind from an onion field and without onion fields
within 4.5 km upwind (Section 5.2.1 , see Figs. 12 and 14). Absence of onion odour was
found to retard ovary development and mating (Ticheler 1970). Conforming to the data of
Table 1, a linear relation between rate of mating and temperature was assumed (Fig. 57).

In the experiment Schuilenburg 1971, the age of most recaptured flies was not known.
The average mating age could be estimated then from the percentage of females that had
mated: because all females mate, the 34% unmated recaptured females were the younger ones.
In the average cumulative recapture curve of marked females versus age in this experiment
34% was reached in 8.2 days, this being an estimate of the average age at first mating.

The onion fly female is polygamous. Experiments of Noordink (pers. commun.; 1967)
indicate that remating is generally within a week after the first mating. When not given
the possibility to remate, a mating may permit a female to lay fertile eggs during some
20 days thereafter (Table IV in Noordink, 1971).

The frequency of multiple matings can be estimated from the percentages sterility in
egg depositions of females caught in the Schuilenburg experiments. By natural sterility
of some 10% the information on the males involved is obscured in the data obtained. If
there was no natural sterility, the number of egg batches with a sterility of 1 to 49%
inclusive would equal that of batches with a sterility of 51 to 99% inclusive, because
the probability of a female mating with a sterile and a fertile male is independent of
the sequence of these matings (full competitiveness of sperm from sterilized males assumed,
and provided that the sterility among the males is not changing fast). If one takes the
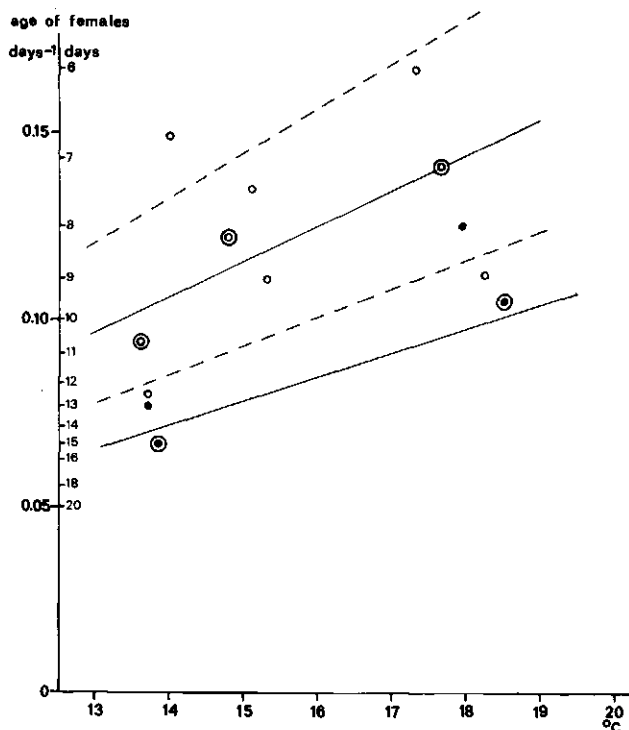
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Fig. 57. Age of females at mating and egg ripening versus average daily temperature at 150 cm height. Experiments Mijs 1973-1(left), Vroegindewei 1972-2(middle) and Mijs 1973-2 (right).

o :10% (upper point) and 90%(lower point) mated; ⊚: 50% mated; •: 10% with eggs; ⊙ : 50% with eggs.

natural sterility into account, the number of batches with eggs that are 'fertilized' partly by sperm from sterilized males and partly by sperm from normal males can be estimated as twice the number of batches with an observed sterility of 56 to 99% inclusive.

The second mating has a dominating effect, and it can eliminate the effect of the first mating after a week (Noordink, pers. commun.). For the moment let us assume that all multiple matings result in a detectable percentage of offspring from all males concerned. The data on the sterility of egg depositions, corrected as indicated above, are:

	sterile	fertile	mixed
1st flight	21%	29%	50%
2nd flight	23%	53%	24%

A reasonable mating model is that females will remate a certain number of days, d, after the previous mating, mating occurring at ages t_1, t_2, ... From Fig. 55 the fractions alive a_t have been taken, for different values of d. Let the probability of mating with a fertile male be f and with a sterile male $1-f$. The fraction fertile egg depositions is the sum of the fractions mating n times multiplied by the probability of mating only with fertile males, and this sum corrected for the non-mated fraction. Or:

fraction fertile $= (1/a_{t_1}) \sum_n (a_{t_n} - a_{t_{n+1}}) f^n$, and similarly

fraction sterile $= (1/a_{t_1}) \sum_n (a_{t_n} - a_{t_{n+1}}) (1-f)^n$

fraction mixed $= (1/a_{t_1}) \sum_n (a_{t_n} - a_{t_{n+1}}) (1-(f^n+(1-f)^n))$.

As a_t is a function of d, d and f can be estimated with these formulae by iteration:
1st flight $f = 0.55$ $d = 5.7$
2nd flight $f = 0.66$ $d = 5.1$

The rate of mating in the second flight is thus $5.7/5.1 = 1.1$ times higher than in the first flight, whereas the speed of physiological ageing, as measured from the age at the first mating, is 1.35 times higher in the second flight (Fig. 55). The cause may be, apart from chance and a bias in d to be mentioned, the fact that data from different years were mixed: the sterility data were from 1971 and 1972, and the survival curves used were from 1974. To obtain the d values that fit these survival curves best, the difference between the d values was increased to a factor 1.35, by changing both values with a factor $\sqrt{1.1/1.35}$ in opposite directions, resulting in d values of 6.3 (1st flight) and 4.6 (2nd flight). The average numbers of matings per female emerged, calculated from Fig. 55, are: 1.7 (1st flight) and 1.0 (2nd flight).

Because the effect of prior matings is eliminated to some extent, the actual rate of mating will be somewhat higher. On the other hand the rate of mating will have been actually lower, because the probability of mating with a fertile or sterile male changed considerably during the experiments concerned. The effect of such a change on the 1st flight calculations was investigated. If we assume, for example, instead of a constant f value of 0.55, two equal fractions with values of 0.85 and 0.25, this change has to be compensated for by increasing d from 0.57 to 0.85.

The probabilities $(1-f)$ of mating with a sterile male, 0.45 and 0.34, correspond reasonably well to the observed effective sterilities among the flies trapped in the corresponding periods: 0.57 and 0.43. The difference can be explained assuming a competitiveness of steriles of 0.65, but chance may be responsible as well.

4.5.2 Oviposition

It was investigated whether the oviposition frequency could be derived from the frequencies of ovary developmental stages. At first the ovary development classification of Theunissen (1973a, 1976) was used, stages S5 to S10, based on the amount of yolk expressed as a percentage of the ovariole length occupied. To eliminate biased estimations, the yolk and ovariole lengths were measured (one representative ovariole per female). The frequency distribution of these percentages (Fig. 58) suggests 33% and 66% as more realistic transitions between stages than the 25%, 50% and 75% used by Theunissen.

The eggs are generally not laid in one batch. Frequency distributions of the number of ovarioles and the number of ripe eggs present are given in Fig. 59. From this the females with eggs could be divided into three stages: not yet ovipositing (over 36 eggs), oviposition started (19-36 eggs) and oviposition ending (1-18 eggs). For practical rea-
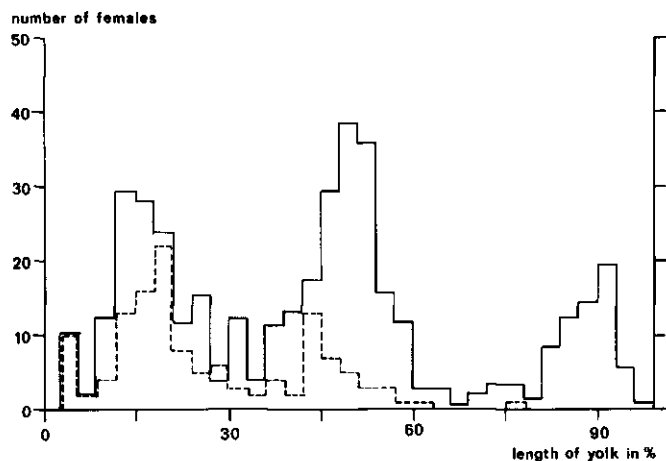
number of females

Fig. 58. Frequency distribu-
tion of length of yolk as per-
centage of ovariole length.
Experiments Mijs 1973-1 and
1973-2, flies of all ages
combined.
——— Oldest egg chamber;
- - next oldest egg chamber.

length of yolk in %

number of females

number of ovarioles

number of eggs
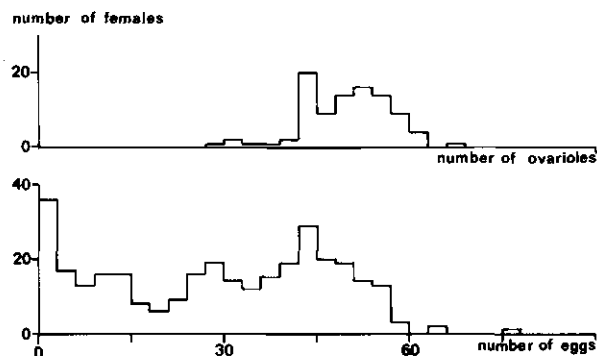
Fig. 59. Frequency distributions of
numbers of ovarioles and eggs.
Experiments Mijs 1973-1 and 1973-2.

sons the stages with 34-66% yolk and 67-99% yolk were taken together, as well as the
stages with 1-18 and 19-36 eggs.

The relative average duration of each stage, assuming equal catching probabilities
for the different stages, was derived from the frequencies of females in these stages
after the first oviposition had started.

The percentage distribution of the females over these stages, versus their age as
found in experiments Mijs 1973-1 and Mijs 1973-2, is given in Fig. 60. The ages indicated
were adjusted to the scale of the 1974 2nd flight mortality data (Fig. 55), with the li-
nearity of the relation of rate of ovary development to temperature (Theunissen, 1974) to
eliminate differences between days, yielding relative ages, and with the age at the aver-
age time of first mating to calculate the absolute ages. Theunissens' data could not be
used to calculate absolute ages because his experiment was done under clearly suboptimal
circumstances that retarded development.

The distance between the graphs of each pair of stages was made to correlate with the
average relative duration of these stages as estimated above. Due to the decreasing num-
bers caught, the graph becomes less reliable towards the right.

In Fig. 60, the average development is represented by a straight line which has to
pass as much as possible through the relative maximal frequencies in the graphs. The

Fig. 60. Distribution of females over different stages of ovary development versus age, from which the duration of gonado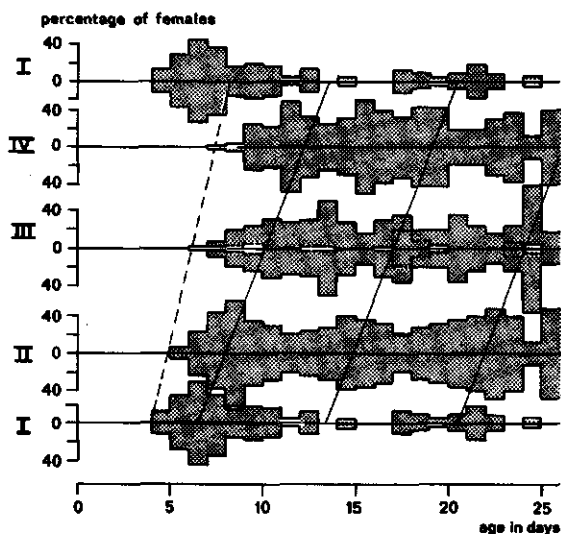trophic cycles is estimated. Age expressed in days at an average temperature of 16.5°C(situation in 2nd flight 1974). Experiments Mijs 1973-1 and 1973-2.
- - - Fastest female; ⸺ estimated average female; ⟨⟩ ovaria partly with not yet fully developed eggs (data incomplete up to age of 14 days).
Stage of ovary development: I= 1-33% yolk, II= 34-99% yolk, III= over 36 eggs, IV= 1-36 eggs.

fastest developing females oviposit every 4 days. The average female is considerably slower, as can be inferred from the diminishing rate of initial increase in frequencies of further developed stages. A reasonable estimate of average oviposition rate seems to be once every 7 days, with first oviposition at an age of about 12 days. Or, a cycle duration of 115 daydegrees and a pre-oviposition period of 200 daydegrees. The fastest developing female will start the second cycle before the slowest female starts with the first. Thus the relative maxima in the frequencies corresponding to the second and higher gonadotrophic cycles are less conspicuous.

There is some additional support for this estimation. The egg traps present during experiment Mijs 1973-2 (but on another field) caught very high numbers of eggs at about 130 and 260-310 daydegrees after release of large numbers of flies of 20-40 daydegrees old.

In many species gonadotrophic cycles can be identified from the presence and size of follicular relics (e.g. Detinova, 1968; Vogt et al., 1974). Follicular relics are present in multiparous onion flies, but with the available information they cannot be used reliably to distinguish nulliparous from parous females, as is also the case in some related species (Anderson, 1964). It may become possible to identify the gonadotrophic cycles by developing more elaborate and time-consuming observation methods. In the laboratory, parous females without eggs could be distinguished as such by the presence of no longer functional ovarioles (smaller and of a different appearance), or by variance of the percentage of yolk present, which is significantly higher after deposition of the first eggs. Both aspects however, still do not enable a sound discrimination between nulliparous and parous onion fly females.

The numbers of eggs laid per female can now be estimated from the mortality and the rate of oviposition. The percentage of females taking part in the subsequent ovipositions is estimated in Table 22.

The difference between the two flights is a factor 2.1. This can be attributed to differences in fly survival, partly because of the fungus disease. Because females will stop egg laying before they die from fungus infection (Theunissen, pers. commun.), the 2nd flight fecundities will be actually lower than the calculated 18.5 eggs per female.

The reproduction factors in the sterile male experiments, the effect of the steriles being eliminated, are summarized in Table 23. The third flight's reproduction was estimated at zero or negligible.

Reproduction was clearly higher on the lighter soils of Overflakkee than on the Schuilenburg, by a factor 2 per generation or 2.5 for a year. The difference between the two flights was a rather constant factor, 2.3 on the Overflakkee control plot. The difference in fecundity was estimated at 2.1 (Section 4.5.3), or less when a sterilizing effect of Entomophthora was taken into account. From the fecundities and reproduction factors as estimated above, the total mortality in the egg, larval and pupal stages could be estimated at 65% and less than 68% in the two flights on the Overflakkee control plot, and 82% and less than 85% in the Schuilenburg field trials.

It is not yet known what effect the density has on reproduction. This effect may increase reproduction when genetic control reduces population levels to lower values than previously occurred (Geier, 1969; Berryman et al., 1973; Lawson, 1967; Pal & LaChance, 1974). Such an increase occurred in a genetic control field test with Culex (Weidhaas et al., 1971).

In the onion fly, chemical control keeps the population far below the level at which

Table 22. Calculation of fecundity per flight, data from Figs 55 and 60

Gonadotrophic cycle no.	Average moment of oviposition			
	1st flight		2nd flight	
	age (d)	% alive	age (d)	% alive
1	16	58	12	31
2	26	17	19	5.5
3	35	1.7	26	0.3
4	45	0.1	33	0.0
number of gonadotrophic cycles per flight	0.77		0.37	
fecundity	39		18.5	

Table 23. Reproduction factors in field trials on sterile insect technique, effect of the sterile males excluded. Details see Tables 27, 37 and 38.

	1st flight	2nd flight	for a year
Mijs 1974 (exp)	6.01	2.49	11.97
Mijs 1974 (con)	6.97	2.98	12.78
Schuilenburg (1971–1974 average)	3.55	1.44	4.99

mortality from competition can be expected. Also, parasites and predators that could cause density dependent mortality are probably quantitatively unimportant as they are not species specific and because onion flies are a minority among the Anthomyiids present in the agricultural areas concerned. Thus population levels lower than at present will most probably not show an increased reproduction. One might even expect reproduction to decrease at lower densities due to scarcity of rotten onions on which survival is higher. At very low densities reproduction will be limited also when the chance of a female to meet a male becomes less than 1. When we consider the reproduction factors observed in the sterile insect technique experiment Mijs 1974, factors of 6-8 in the 1st flight and 2-4 in the 2nd flight seem reasonable assumptions.

4.6 SUMMARY

The possibilities of predicting emergence from soil temperature records or from oak leaf budding are evaluated. Both methods seem promising.

Flight curves for different years and circumstances are given. Diapause is induced by daylength and temperature. Extrapolation of these laboratory data to field circumstances gives a good agreement when the effective daylength is assumed to be 1-2 hours shorter than the astronomical daylength. Percentages diapausing are on the average 20% for first generation pupae and 85% for second generation pupae. So the second flight is usually a partial one, and a third flight is small or absent. The sex ratio in the captures changes about linearily with the average age, because females live longer.

Allium vineale does not support a wild population of onion flies, so wild host plants can be considered to be absent. The preference of ovipositing females for rotting onions is quantified. Onion waste disposal sites were found to be unimportant for the population dynamics of the onion fly.

Under the prevailing circumstances, the bean seed fly proved to be a secondary pest of onion seedlings, as its population was nearly eliminated by an effective genetic control of the onion fly.

The species of diptere larvae and beetles, observed in onions, are listed. Incidental observations of feeding onion flies are mentioned. The parasitoids Aleochara bilineata, Aphaereta minuta and Phygadeuon sp. were observed.

Infection of flies with Entomophthora is common in summer. The infection rate was found to be 6% per day, with increasing age of the flies decreasing to about 3%. This fungus infection could not explain the reduced competitiveness observed in the first field trial on sterile insect technique at the Schuilenburg.

Preliminary experiments with radioactive label transfer revealed some actual and potential predators of eggs and young larvae. Some predators of pupae and adults are mentioned.

Onion fly damage in untreated fields occurs mainly in June, due to the first generation of larvae. Damage was more severe at the heads of the fields; an edge effect was not found. The occurrence of damage fitted the negative binomial distribution, with k dependent on the mean. The number of samples as a function of the mean, needed for a certain level of precision, was calculated with the variance estimated from the negative binomial distribution,

from Taylor's power law, or from the relation of mean crowding to the mean. These precision levels are approximate values because of some bias in these calculations. Plot size for damage estimation should be in practice 0.5-1 m.

The occurrence of pupae also conformed to the negative binomial distribution, with k dependent on the mean. However, tested with another method, the fit was questionable. The numbers of samples needed for certain levels of precision were calculated as for damage. Confidence limits estimated from these data will be rather unreliable for the small sample sizes and low means to be expected in practice.

Most fly population estimates were obtained from release-recapture experiments. With marked flies, emerged from reared diapausing pupae and mixed with the first flight flies in the field, populations of 1.7-3.6 flies emerging per m^2 were calculated for some fields with relatively high damage levels. From recapture data of a release experiment with releases on different fields, with one population known from pupa samplings, other populations could be estimated.

Data on the relation of the numbers of diapausing pupae to damage at harvest are given. The variation is considerable. Densities on chemically treated fields were about 1000-20 000 pupae/ha.

From an experiment on survival of labelled eggs and larvae, a high mortality in these stages was calculated. The mortality of diapausing pupae was estimated at about 10-20%. Survival curves of flies were estimated from recaptures, taking into account migration, and underrepresentation of very young flies in the catches. The life-spans conformed to a normal distribution, with mean values for females that were about 1.4 times larger than for males.

The relation of the age distribution at the time of the first mating versus temperature is given. From sterility data of the eggs laid by fertile females in a field trial on sterile insect technique, the frequency of matings could be estimated at about once every 5-6 days.

From the frequencies of different phases in the gonadotrophic cycle versus time, the frequency of oviposition periods was estimated at about once a week. Combination of these data with the mortality curves gave average fecundities of 39 and less than 18.5 eggs per female for the 1st and 2nd flight, respectively.

Reproduction factors were calculated from the sterile insect technique experiments. The 2nd flight reproduction was nearly a factor 2.5 lower than the 1st flight reproduction. Reproduction factors under normal onion growing circumstances, onion fly control excepted, were 7 (1st flight), 3 (2nd flight) and 13 (for a year). In the Schuilenburg experiments these values were half as high. From the calculated fecundities and reproduction factors, mortality in the pre-adult stages could be estimated at about 65% (light soil) and about 85% (heavy soil).

5 Dispersal

Dispersal is a critical factor in genetic control: it conditions the delimitation of populations and thereby the minimal area to be treated, and it determines the release site distances. Also, when the reproduction of populations is measured, exchange with other populations needs to be taken into account.

Most information on dispersal has been collected from release-recapture experiments. The general data on these experiments, including the sterile male releases on the Schuilenburg, are summarized in Section 3.1.1 (Table 3, Figs 13-15). In Section 4.3.3 support is given for the assumption that the dispersal, as observed in these experiments with laboratory-reared flies, can be taken as representative for the wild fly dispersal.

First the effect of some factors like weather and onions on dispersal is examined, then the rate of dispersal is estimated in different ways, assuming that the dispersal can be considered as a diffusion process. Also an example of another approach in dispersal analysis is given. To overcome the problems in estimating the rate of dispersal from the field data, that arose from heterogeneity in time and space, a simulation model was used to estimate fly diffusion parameters.

The impact of wild populations, emerging near a field treated with sterile males, on the outcome of this control, is estimated from the average mating age, the age distribution of immigrating flies, the distribution of flies over different sites versus age, and the relation of the numbers caught versus distance. These data were obtained from an extensive release-recapture experiment done mainly as a field test on sterile insect technique. Also it is calculated from these data how pre-reproductive dispersal changed the numbers emerged and released into those actually reproducing on the onion fields concerned.

5.1 PRELIMINARY DISPERSAL EXPERIMENTS

A few preliminary experiments were done on colonization by placing untreated onions, that had been slightly damaged artificially, as a trap crop in the field. In May 1970, trays with greenhouse-grown onion seedlings were placed at different sites in Oostelijk Flevoland, an area reclaimed in the sixties, on which cultivation was still spreading by on the average about 1 km/year westwards. Three trays were placed near the westernmost onion fields, and two along the western dike separating the polder from a more recently reclaimed still uncultivated area (Fig. 61). By the end of August 1970 onion fly larvae or pupae were found in two of the trays near onion fields. Thus the flies could keep pace with the spread of onion growing.

Another experiment was done on the dikes connecting the former islands of Overflakkee to other former islands. These are surrounded by water or tidal mudflats, and are about
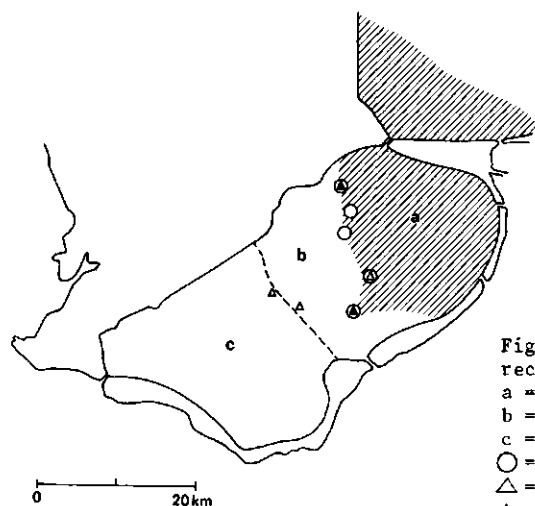
Fig. 61. Occurrence of onion flies in a recently reclaimed area.
a = Under cultivation, with about 3% onions;
b = mainly large-scale cultivation, no onions;
c = not yet cultivated;
○ = westernmost onion fields (1970);
△ = trap onions placed, no larvae found;
▲ = trap onions placed, some larvae found.

50 m wide and about 6 km long. Some young onions were planted in little groups at distances of 0-2100 m from the island, every 100 or 200 m, in the grassy verges, in May 1972. Subsequent checking revealed only at the first control date, 3 weeks after planting, one onion fly larva at 200 m from the island, on one of the two dikes considered. Clearly these dikes are for the flies not the crowded migration funnels they constitute for birds and cars.

5.2 FACTORS AFFECTING DISPERSAL

5.2.1 Weather and onions

The rate and direction of fly dispersal may be dependent on several factors. Before a more general approach to dispersal analysis is made, the effect of these factors will be considered.

To analyse such effects, the rate of dispersal was measured under different circumstances. This rate can be measured from the time it takes from emergence to the first recapture at a certain distance. Such a measurement strongly depends on the size of the released population and on the trapping intensity. To obtain a measurement independent of these factors, the dispersal rate was measured from the time it takes from emergence to the moment that a certain percentage (i.e. 5 or 10) of the total number of flies recaptured at a certain distance is reached. This measurement is proportional to the average net displacement.

The weather affects fly displacement measured by flight interception trapping, as analysed for a temperature parameter in Section 3.3.3.1. The rate of dispersal as defined above should thus conform to the same relation as is indeed found (Fig. 62). The relation estimated in Section 3.3.3.1 is based on more, and more accurate, data compared with the relation of Fig. 62. Therefore this relation, as given in Fig. 24, was used to

rate of migration in m/day
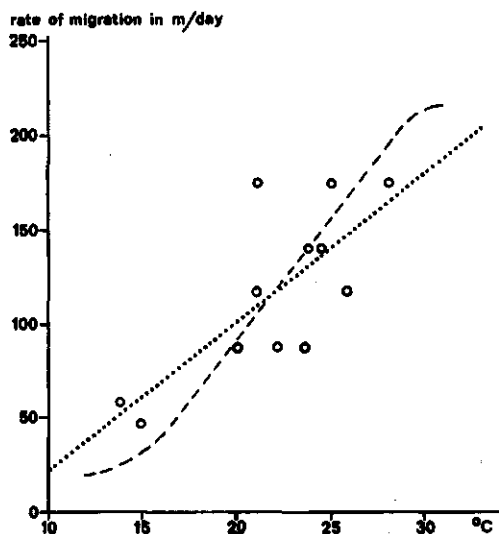
Fig. 62. Relative rate of migration versus
temperature, as calculated from the days
between average emergence to 10% recaptured
on an onion field at 700 m distance.
Experiment Schuilenburg 1973.
····· Regression line $y = 7.91x - 57.2$;
- - - trapping probability (Fig. 24).

eliminate the probably larger part of weather influence on dispersal rate, by transfor-
ming the data to those expected on standard days (max. $20^{\circ}C$).

Another important effect of weather may be expected from wind direction. This may af-
fect the preferred flight direction and thereby the dispersal rate as observed in a cer-
tain direction. The effect of wind direction has to be analysed in connection with the
presence or absence of onion odour. In an area like Overflakkee nearly all upwind move-
ment is somehow towards an onion field. During one experiment data could be collected
on upwind dispersal in a direction where the nearest onion fields were at a distance of
at least 4.5 km (on the next island), and there in a very low density. It was found that
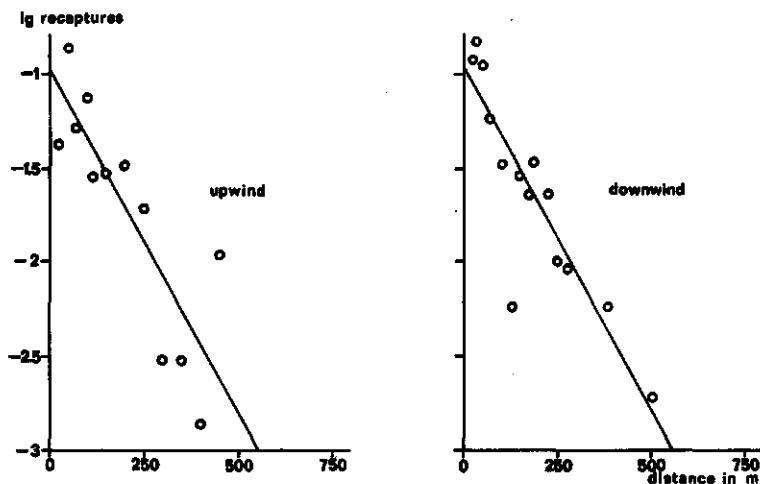
Fig. 63. Effect of wind direction on onion fly dispersal, in absence of onion odour, shown
from the lg(% recaptured per trap) versus distance from the release site. Experiment Mijs
1973-2.
Regression line: upwind $y = -0.00360x - 0.992$; downwind $y = -0.00356x - 0.989$.

in this case, with the wind presumably carrying no detectable quantity of onion odour, dispersal was independent of wind direction (Fig. 63).

Data on the effect of wind direction on rate of dispersal, in the presence of onion odour, are summarized in Fig. 64, separately for dispersal through onion fields and other vegetations. There does not seem to be an effect of wind direction.

Fig. 64 shows that outside onion fields the rate of dispersal is about a factor 2.5 faster than that through the onion crop, possibly because the onion odour had surpassed an upper limit for induction of activity. The presence of such a limit was demonstrated by Wolff (1973). Onion fields constitute unnaturally large and concentrated sources of onion odour, so the concentration may very well be too high. Another explanation is the probably different composition of the odour near onions, due to the instability of several of its components (Carson & Wong, 1961; Saghir et al., 1964; Brodnitz et al., 1969; Boelens et al., 1971). The latter mechanism may work very well under natural circumstances to keep the flies near onions.

The fly behaviour observations in onion fields gave some indication of an upwind preference (Fig. 65). Also, the trapping probabilities in an onion field were dependent on wind direction, which may be attributed to a preferential dispersal direction (Section 3.3.3.1).


rate of migration in m/day
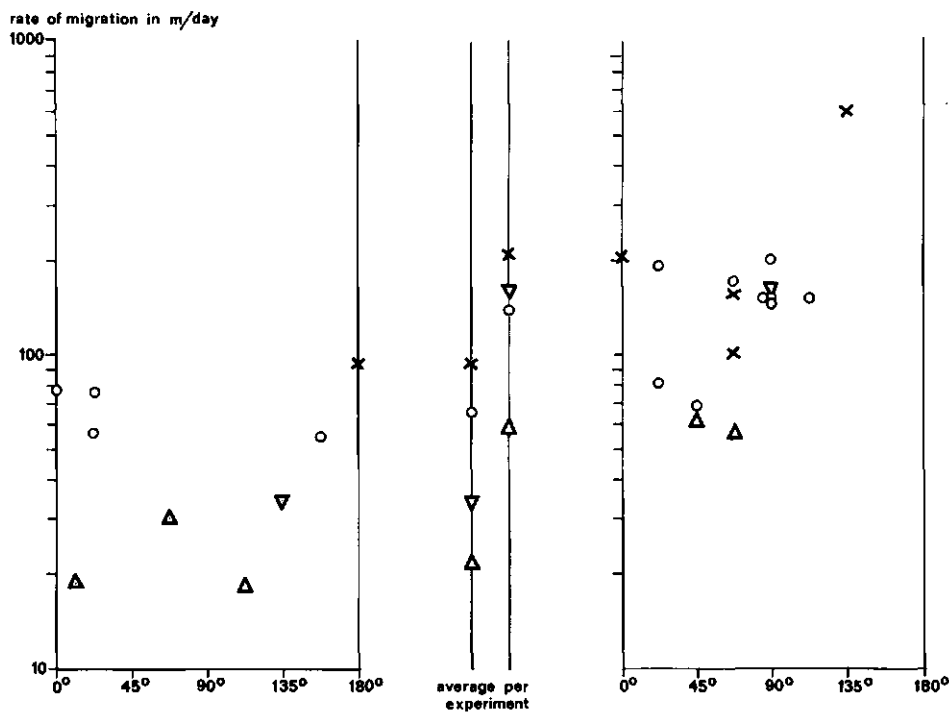
Fig. 64. Effect of onion crop and wind direction on relative rate of migration, calculated from the days between average emergence to 5% recaptured on a site, time corrected to standard days. Abscissa: average angle between dispersal direction and upwind direction. Left: through onion crop, right: outside onion crop. Weighted difference 2.46. Experiments: X Mijs 1974, flies released on trial field; O Mijs 1974, pupae released on trial field; Δ Vroegindewei 1972-2; ▽ Mijs 1974, pupae released on control field.
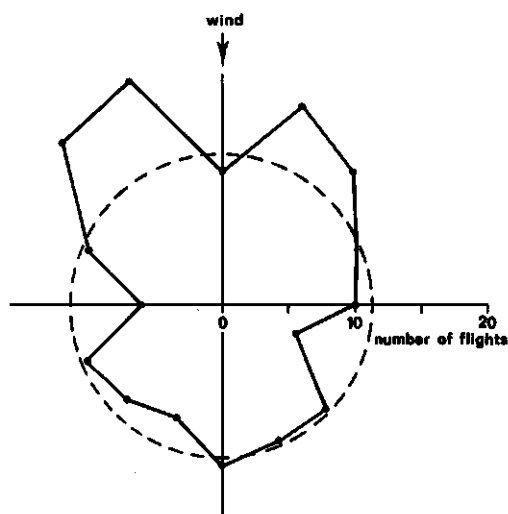
Fig. 65. Flight direction with respect to wind direction. Observations from 1971-1973, $n=183$. Tested against/with wind direction: $\chi^2_1 = 4.36$, $P = 0.04$, just significant.

Recaptures throughout the year on a distant field, expressed as percentage of recaptures on the field of origin on the same days (to eliminate effects of population density and weather-dependent differences in trapping probability) were compared with the wind direction. There was no correlation so that migration towards another onion field is independent of wind direction.

5.2.2 Barriers

Dispersal can be disturbed by barriers. These can be either absorbing (killing the flies) or reflecting (halting the flies). A water of about 20 m wide was no barrier for the dispersing flies: the recaptures across the water conformed to the expectation from the linear relation of lg(recaptures) versus distance which is generally found (see Section 5.5; Fig. 80c).

Several experiments were done near a water of 3-4 km wide. It was investigated whether its effect could be best considered as absorbing or reflecting. If flies fly easily over the water, some may cross it and others may return, giving intermediate results, as when some of the flies are washed ashore alive. A reflecting barrier increases the population densities with a factor approaching 2 near the barrier and also with a factor approaching 2 over the whole area when the release point is near to the barrier. An absorbing barrier will severely reduce the population densities near it, approaching population elimination when the release point is close to the barrier.

Consider the oblong trial field in 1974, lying perpendicular to the water (Fig. 15). Flies were released a few times near one or both ends of this field. Fig. 66 compares the average recapture sites of such groups against time on this field with those of wild flies and flies that emerged all along the edges of the field. The dispersal of the groups released nearer to the water seemed slower, as could be explained by a steeper population density gradient caused by the water as a reflecting barrier. An absorbing barrier would have caused a seemingly faster dispersal. Also the recapture rates were
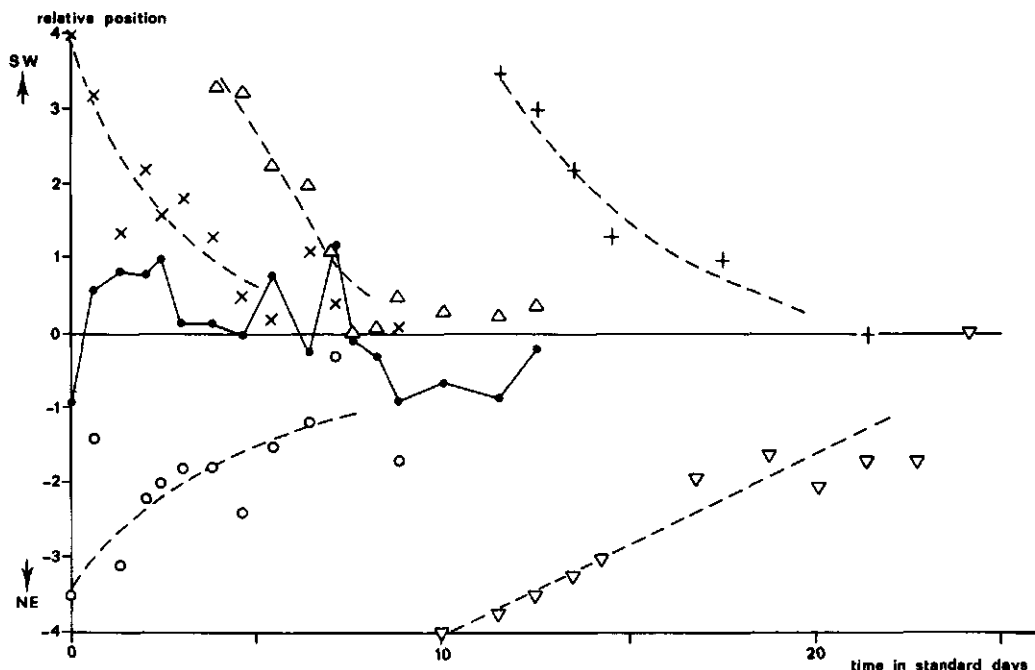
Fig. 66. Average recapture sites along an oblong onion field, of flies released at one end of the field. Experiment Mijs 1974.
——— Wild flies and flies released evenly distributed along the long side of the field; ×,△,+: flies released near the SW end of the field at different times; O,▽: flies released near the NE end of the field at different times.
Day zero is 13 July.

not dependent on the release site positions (see Table 32 and Fig. 20 : released near the water: GC_2, BYC_2, GY_2; at the other end: BO_2, BY_2, B_2, BG_2, C_2). Thus the wide water can best be considered as a reflecting barrier.

The assumption was checked by releasing some hundred flies 0.1 m from the water, during fine weather with a SW wind blowing offshore, and in absence of onion odour. Flights started in all directions, but the flies that flew over the water returned within 0.2 m of the shore, with only one exception, a fly that landed on the water some metres offshore. Only a few flies landed on the water when flying back to the shore. Most of these were washed ashore undamaged probably because the water was very calm. The others floated away quickly due to the wind. They may have reached the other side of the water (cf. Norlin, 1964).

5.2.3 *Passive dispersal*

Any traffic may serve for transport of onion flies. Several times onion flies were transported on the outside of the author's car, over at least 1 km. Onion flies are found on cars regularly, probably feeding on dead insects or 'sunning'. Especially onion transport may provide long distance transport for the flies. Because the number of flies transported will be very small compared with the populations present, this dispersal is unim-

portant except for causing reinfestation after eradication, or for spreading resistance.

Another more or less passive transport may occur by aerial currents. In data of aerial catches Anthomyiidae are mentioned in rather small numbers (e.g. Hardy & Milne, 1938; Glick, 1939, 1960; Glick & Noble, 1961). It is impossible to assess from these data the quantitative importance for the onion fly, one of the larger Anthomyiids. From field observations it does not seem probable that onion flies enter the aerial plankton: flights were generally at the level of the top of the vegetation and never much higher. With strong winds the flies remained lower; with a wind of 15 m/sec the flies were obviously very well able to choose their own flight direction.

. Any loss of released flies to the aerial plankton would appear in the data as a mortality factor for these flies, and cannot be distinguished from it. The shape of the mortality curve (Fig. 55) can hardly be interpreted as being caused mainly by such a type of emigration. However, it may be that only very young flies show this migration, which is a common and logical phenomenon (Johnson, 1960). Whether young onion flies exhibit such behaviour cannot be ascertained from the recapture data of the released flies. Now a quantitatively important aerial migration will remove a considerable fraction of the populations, and add a considerable amount of wild flies per area. Such an amount is important in 'isolated' onion fields with low populations, causing the apparent reproduction factors there to rise when the populations of emerging flies are reduced e.g. by sterile insect technique. As can be seen in Table 27, the reproduction factors on such an 'isolated' field do not show a clear density dependence. Thus a long distance dispersal would not be quantitatively important.

Another argument against such long distance dispersal is the rather gradual spread of resistance (Section 2.3.3): it took at least three years or about six generations for resistance to become established in all Dutch onion growing areas, starting from at least three sources. Also, more than ten years after the start of resistance in the Netherlands, susceptible strains were still present in England (Gostick et al., 1971), France (Missonnier & Brunel, 1972) and Poland (Narkiewicz-Jodko, 1974). Gostick et al. (1971) even found strains of which 3% and 0% survived a discriminating dose of dieldrin, at distances of 0.8 and 1.6 km, respectively, from the site where a strain was found which had 55% survival in this test. It seems that the spread of resistance is more the result of the control history than of dispersal of resistant flies.

5.2.4 *Fly properties*

Fly size was found to have an effect on recapture probability (Fig. 67). It can be seen from the experiment Pollemans 1971 that this is not an effect of a higher mortality rate. There the very small flies (average 9.2 mg pupae) showed very limited dispersal (Fig. 80b), but their mortality seemed fairly normal as they were caught up to an age of about 40 days (corrected to standard days; that is comparable to the 2nd flight data in Fig. 55). Thus the observed decrease of the fraction recaptured with distance will have been due to a slower dispersal.

Differences between the sexes will be considered in Section 5.3.2 and, together with the effect of age, in Section 5.6.
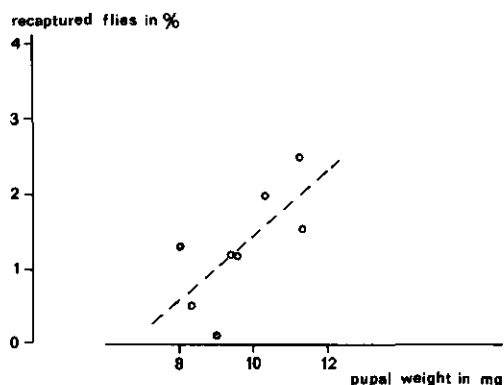
101

Fig. 67. Percentage flies recaptured versus average pupal weight. Experiment Schuilenburg 1971.

The release method can influence the rate of dispersal because of interaction between the flies at very high initial densities. Density has been found to increase migration activity in a wide variety of species (e.g. Wolfenbarger et al., 1974), including onion fly larvae (Robinson, pers. commun.). The effect of different concentrations at the emergence site did not affect the percentage of flies that emigrated from the experimental field (Table 32), nor the rate of dispersal. Recapture data from flies released from crowded cages resulted in a dispersal rate which was at least initially increased. However, with another type of cage (Section 3.3.2.2) an even more intense crowding caused no significant deviation. The high rate of dispersal in the former releases can be explained by the absence of onion odour.

5.3 DIFFUSION

5.3.1 *Introduction*

Especially when pest eradication is aimed at, the maximum distances that can be reached by dispersing flies are of interest because these affect the probability of re-infestation and the depth of the barrier zone that has to be retained around an emptied area. Of course the oldest fly and the fastest flight observed are rather meaningless, and any 'dispersal maximum' should be connected to some probability of the occurrence of that value or a higher one.

The most useful dispersal parameter for calculating this is the diffusion speed as developed in physics for particles. The advantage of a diffusion model for dispersal is its simplicity, its disadvantage being the often rigorous simplification of the biological reality. It has been used to describe many biological dispersal phenomena often with sufficient accuracy (e.g. McIntyre et al., 1946; Skellam, 1951; Broadbent & Kendall, 1953; Beverton & Holt, 1957; Jones, 1959; Richardson, 1970; Scotter et al., 1971). Although some complications can be coped with (e.g. Holt, 1955; E.J. Williams, 1961; Holgate, 1971; Skellam, 1973), a more detailed adjustment of the diffusion model ɔ biological heterogeneities quickly complicates the formulae.

The diffusion speed is measured by the diffusion constant, diffusion coefficient or diffusivity D, or, after Skellam (1951), by the mean square displacement $a^2 = 4D$. Beverton

& Holt (1957) use a dispersion coefficient D that equals a^2. The dimensions are speed ×
distance, here in m^2/day.

5.3.2 Calculation of diffusion coefficient from field observations of fly behaviour

The diffusion coefficient can be calculated from the number of flights n per unit
time, with independent directions, and the average flight distance d, by $D = nd^2/4$
(Beverton & Holt, 1957, p.138; Pielou, 1969, p.130). These data could be estimated for
dispersal in onion flies from the observations of fly behaviour. In the determination of
both flight direction and distance flown, any deviations from the straight path connec-
ting the beginning and end point have been ignored. The subsequent flight directions were
independent of each other (Fig. 68), as observed also for the cabbage root fly (Hawkes,
1972). However, Skellam (1973) showed that this is no prerequisite for the diffusion for-
mula to be applicable.

The average distance flown could not be determined directly, because several times
a fly was lost sight of and could be said only to have flown, for example, at least 5 or
at least 10 metres. Generally this occurred with the longer flights. Now it was found
that the flight distances conformed to the log-normal distribution over the observable
range, and were not contradictory with this distribution over longer distances as was
determined from the extreme possible values of partly known flight distances. These ex-
treme possible values were: either all flights ceased at the moment the flies were lost
sight of (upper points in Fig. 69) or all flights continued indefinitely (lower points
in Fig. 69). By linear extrapolation, the frequencies of the longer distances were es-
timated as a preliminary model. The mean and variance of the transformed distribution
can be read off the graph: $m(\lg(x)) = \lg 1.48 = 0.17$ and $s^2(\lg(x)) = (\lg 5.1 - \lg 1.48)^2 =$

preceding flight

number
of flights
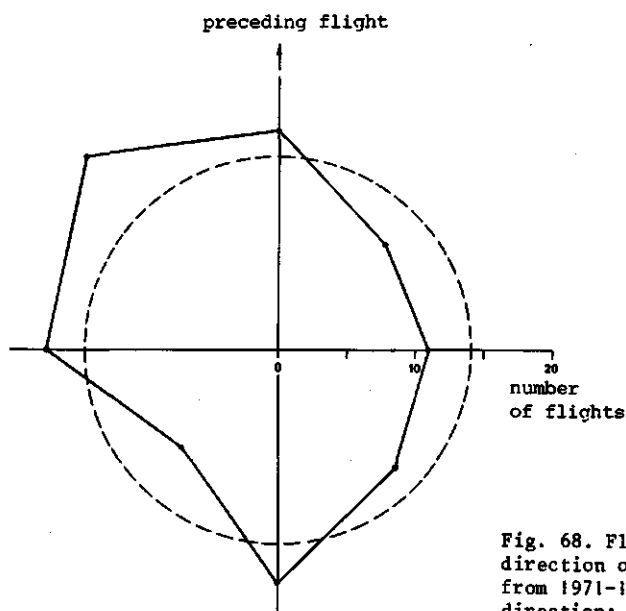
Fig. 68. Flight directions with respect to the
direction of the preceding flight. Observations
from 1971-1973, $n=114$. Tested same/opposite
direction: $\chi^2_1 = 0.74$, n.s.

103
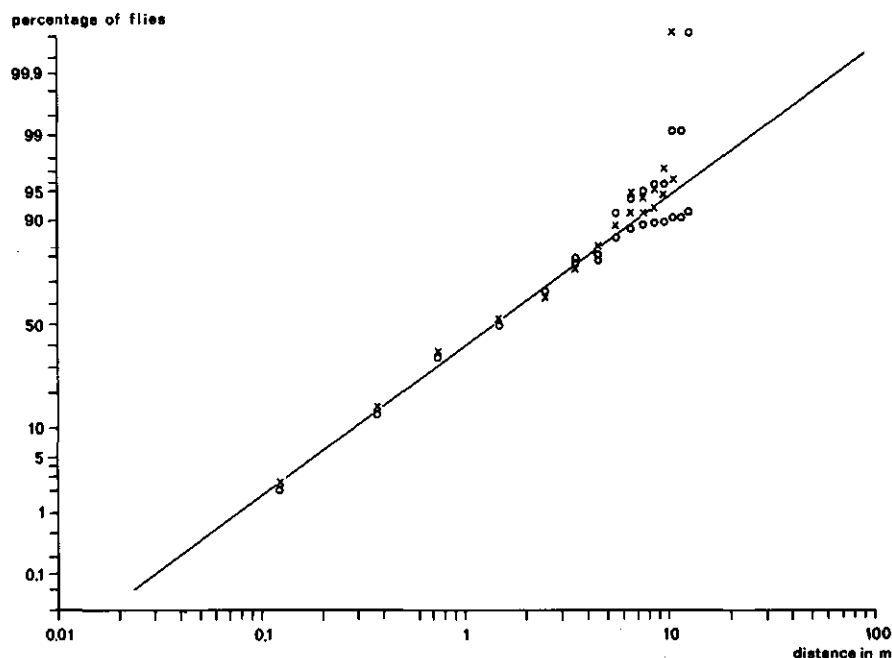
Fig. 69. Distribution of flight distances from observations in onion fields: cumulative
frequency versus distance flown.
O : Females, 112 flights; X : males, 97 flights.
Two symbols at one distance indicate the possible range of the percentage, because of
individuals that were lost sight of.

0.29. Transforming back to the log-normal distribution the original mean can be obtained
from

$$m(x) = 10^{m(\lg(x)) + \frac{1}{2} s^2 (\lg(x))}$$ (12)

or, $m(x) = 2.06$ m.

The males provide, moreover, another problem. They show two types of flights:
flights similar to those of the females, thus contributing to dispersal, and short
flights consisting of crossing at the vegetation top level, the fly often returning to
exactly the same site. This behaviour is interpreted as being flights in search of a
mate (compare the similar behaviour of related species: Downes, 1969; Gruhl, 1924, 1959;
Vogel, 1957; Miller & McClanahan, 1960; Hawkes, 1972). No eliciting stimuli were identi-
fied, apart from the fact that males sitting near each other usually flew up at nearly
the same moment. These flights were excluded from the data by assuming for males an
equal percentage of dispersal flights with zero displacement as was observed for the fe-
males. The resulting flight distance distribution of the males is similar to that of the
females.

The frequency of flights was calculated also for the sexes separately. In the 15.3
hours of observation of females, 122 flights were recorded (flights at the beginning or
end of an observation period are counted for half). Thus a female fly flies every 7.5 mi-
nutes, or 8 times an hour. The daylength may be put at about 14 hours (Fig. 21), yielding
about 110 flights a day. With a measured flying speed of 2-3 m/sec, over such short dis-

tances as observed the duration of the flights is negligible. Flight distance and duration of the preceding resting period are not correlated.

The diffusion coefficient in onion fields can now be estimated at $D = 110 \times 2.06^2/4$, or about 120 m^2/day for females.

For males, after eliminating the same fraction of flights (17.7%) as done in the calculation of flight distances, the data are: in 9.8 hours of observation 117 flights, that is one every 5 minutes or about 167 flights per day, and $D = 175$ m^2/day. The dispersal in an onion field is thus for the males 1.5 times higher than for the females, due to a higher frequency of flights.

5.3.3 Calculation of diffusion coefficient from release-recapture experiments

The diffusion coefficient can also be calculated from recapture data. When the pattern of the population versus time can be estimated, the relation $p_t = \exp(-r_t^2/4Dt)$ can be used to estimate D (Scotter et al., 1971; Pielou, 1969), where p_t is the fraction of the population outside a circle with radius r_t around the origin at time t. Thus, with the median distance $r_t^2 = 4Dt.\ln 2$, and D can be found from the regression of r^2 on t.

This approach could be directly applied in the experiment Pollemans 1971, where due to the cold and wet weather the flies moved only little and thus could be counted around the release site. The result was $D = 1.75$ m^2/day.

The population distribution around the origin can be calculated from the recapture data when a correction is made to obtain an equal trapping intensity over the whole area concerned. The data of some experiments were corrected accordingly. Because of the local accumulations in onion fields and the considerable areas for which the low numbers recaptured at larger distances were taken to be representative, the corrected data obtained are rather unreliable. Data can be used only up to the moment when considerable numbers of flies have supposedly left the area where flies were trapped.
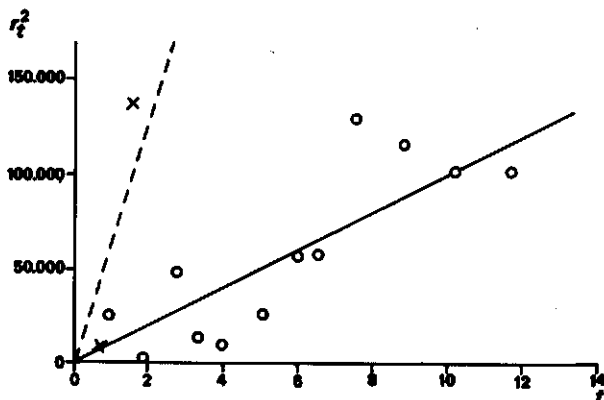
Fig. 70. Estimation of diffusion coefficient from the regression of the squared median distance of the released population on the time in standard days.
X : Experiment Mijs 1973-2, releases from fly cages over 150 m from any onion field, onion odour absent; $r_t^2 = 65\ 000t$, $D = 23\ 400$ m^2/d.
O : Experiment Vroegindewei 1972-2, releases from pupae, dispersal through and between onion fields; $r_t^2 = 10\ 000t$, $D = 3\ 600$ m^2/d.

As shown in Fig. 70, in an area with a usual onion field density, D is found to be 3600 m^2/day, and a much higher value is found for dispersal outside onion fields after release from cages: about 23 400 m^2/day.

Another method was as follows. The density at a given point at distance r is given by (e.g. Pielou, 1969)

$$f(r,t) = \frac{1}{4D\pi t} \cdot \exp \frac{-r^2}{4Dt} \tag{13}$$

A certain threshold density will result in an expected number of one fly per trap per day. This threshold will be reached on different places at distances r_n and times t_n. Thus, by putting $f(r_1,t_1) = f(r_2,t_2)$ for the first recaptures per distance, it follows that

$$D = \frac{r_1^2/t_1 - r_2^2/t_2}{4\ln(t_2/t_1)} \tag{14}$$

like the similar formulae for one and three dimensional cases given by Bossert & Wilson (1963). When applied in experiment Vroegindewei 1972, widely differing values were found, including negative ones. Application of Eqn (14) to the output of a dispersal simulation model (Section 5.4) revealed that this instability was caused by the distribution of emergence over the mornings of several consecutive days, and by the concentration of the flies on onion fields. The method is thus very sensitive to heterogeneities, so it will be generally inapplicable to biological data.

With a regular spacing of the traps over the area over which the flies disperse, the diffusion coefficient can be found from the increase of the variance ($2Dt$) with time (Richardson, 1971). This can give indications about the effect of age or time dependent factors on dispersal (e.g. weather, Dobzhansky & Wright, 1943). Due to the fast dispersal, the limited number of traps available and the heterogeneity of the experimental areas this approach could not be followed in the onion fly experiments.

The considerable differences between the diffusion coefficients obtained above require some comment. The values calculated from the observations of fly behaviour are rather unreliable, as the quantitatively more important flights, those over longer distances, were estimated by extrapolation, without having any indication on the reliability of such an extrapolation. The actual value may be somewhat lower or very much higher, as can be seen from the possible non-linear extrapolations in Fig. 69. The figure of 1.75 m^2/day obtained above refers to slowly dispersing flies (Section 5.2.4; Fig. 80b), during cold weather, so it will not be representative at all. Also, the method of direct observation of the fly distributions can easily lead to underestimation, as the very low densities at higher distances are difficult to observe.

As mentioned, the figures of 3 600 and 23 400 m^2/day are not very reliable either, but they can at least be supposed to indicate the real magnitude, as there is no important bias in the observation or in the calculation towards higher or lower values.

5.4.1 *Simulation model*

An attempt was made to get more reliable estimates for the diffusion coefficient of onion flies, by computer simulation of their dispersal.

In co-operation with Dr. M.J. Frissel (ITAL, Wageningen), a simulation model was developed, for analysing the dispersal of the onion fly. The computer language used was CSMP-3. With the computer program, given in the Appendix, fly densities in cells of a two-dimensional grid-cell system were calculated as a function of time, under different assumptions about the dispersal of the flies. The model is deterministic. For low numbers of dispersing flies a stochastic model is preferable (Wehrhahn, 1973).

Included features The length of the time steps, the grid cell size and the grid size can be chosen, the latter within the limits set by the computer's capacities. Emergence or releases of flies can be simulated in any amount in any cell at any time. In the version presented here, mortality is a function of time or place, so age-dependent mortality can only be simulated with instantaneous emergence.

Dispersal, as far as trivial movement is concerned, is simulated by calculating the diffusion of flies between cells. The computer program was checked by making runs assuming a spatially and temporally homogeneous environment. The results closely fitted the data calculated with a conventional analytical approach.

Sites that are preferred by the flies are represented by preferred grid cells, the accuracy of this representation depending on the coarseness of the grid. Preference is expressed in one or both of two possible ways: arresting, that is a reduction of the diffusion coefficient of the flies present in the preferred cells, or attracting, causing the flies to exhibit an additional migration in the direction of attractive cells. Attraction was introduced by transferring flies in upwind direction to the adjacent grid cell(s). Parameters were the fraction transferred per unit time, and the grid cells in which anemotaxis was assumed to occur, the latter depending on the wind direction. For simplicity the attraction was kept constant over these cells, thus in this respect there was only a yes or no situation.

The influence of weather was introduced in the model in two ways. Wind direction was used for the anemotaxis, and temperature for modifying the diffusion coefficient. A relation between temperature and diffusion coefficient can be found from the relation between temperature and relative number of flies caught in the flight interception traps (Fig. 24). The number of flies caught will be linearly related to the average displacement $\overline{dx}$. A diffusion coefficient can be defined by $D=(\overline{dx})^2/2t$, where t is time, in analogy with the diffusion from particles (e.g. Kruyt, 1952). Thus the diffusion coefficient is proportional to the square of the catch size. In the simulation runs made so far, generally the diffusion coefficient was kept independent of the temperature.

Barriers, reflecting or killing either all or a part of the flies, can be provided for a every grid-cell boundary. In the runs made, only a completely reflecting or killing barrier was used along one of the grid borders.

Because the densities outside the grid cell system are per definition unknown, some extrapolation was needed to calculate the numbers crossing the system's border. To estimate the bias caused by this extrapolation, some runs were done both with smaller and larger grid sizes. The results indicated that, with the parameters usually used here, the underestimation of the number of flies present in the border cells is up to 5%, and much less further from the border. The runs with a small grid size were nearly all done with a suboptimal extrapolation equation for calculating the diffusion over the systems border. This method caused overestimations up to 15% in the border cells.

Diffusion equation The equation used in the model to calculate the amount of flies diffusing will be explained here, additional remarks being made in the model itself (Appendix 1).

Fick's diffusion equation can be written as

$$dm = D \frac{dc}{dx} q \, dt \tag{15}$$

in which dm is the amount diffusing, D is the diffusion coefficient, dc is the concentration difference, dx the distance between these concentrations, q the surface through which the diffusion takes place, and dt the infinitely short period over which measurements are made. The amount diffusing is represented in the computer program by IN. The numbers of flies per grid cell are QC, grid-cell length is DX, thus the difference in concentrations QC/DX^2 is $(QC_1-QC_2)/DX^2$. The distance and the surface, of which the vertical dimension is only 1 unit, through which diffusion takes place are both DX. Thus the equation in the model for diffusion from cell H-1, V to cell H, V (see Fig. 71; H and V are grid-cell indices) is:

$$\text{IN } (H,V,2) = D*(QC(H-1,V)-QC(H,V))/DX**2 \tag{16}$$

where the west to east displacements, from H,V-1 to H,V, are given in IN(H,V,1), and the south to north displacements, from H-1,V to H,V, are given in IN(H,V,2). The discrete time-steps over which diffusion was calculated were 1/16 or 1/32 day.

The arresting preference was introduced by multiplying the concentrations by the fractions of the populations assumed to take part in the diffusion (QPREF).

Now consider the northern border of the grid, for horizontal IN, with a grid size of 11 × V as an example. The normal equation should have contained H = 12. However, QC (12,V) is per definition unknown, and QPREF (12,V) is therefore obsolete. The difference between the densities in (11,V) and (12,V) is thus unknown, and is now taken proportional to the difference of the densities between (10,V) and (11,V).
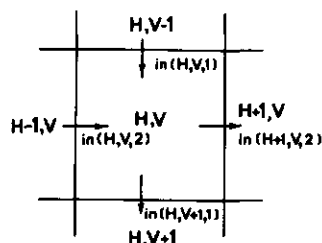

Fig. 71. Simulation model grid-cell with codes of cells and fly moves.

A reflecting barrier is introduced in the example of the northern border used above, by putting IN(12,V,2) = 0. ; a killing barrier is provided by IN(12,V,2) = (D*QC(11,V)* QPREF(11,V))/DX**2, as here QC(12,V) remains zero.

5.4.2 *Analysis of a release-recapture experiment*

For different parameter values, the model's output was compared with the experimental data by comparing the distributions of the recaptured number of flies over relevant groups of traps or grid cells for each trapping period (day) (Table 24; cf. Fig. 76). This procedure reduces the influence of the location of individual traps, and eliminates the effect of the unknown survival and of the weather on the numbers recaptured per day. The model's degree of dissimilarity with the experiment was expressed as the sum of squared deviations divided by their expectations, χ^2. Because there were many small expected (simulated) values, considerable lumping of data was needed to apply the χ^2 test. But the goal here was optimalization, i.e. minimalization of χ^2, and for this lumping of data can be omitted.

The results can be used to evaluate which set of diffusion parameters describes the actual situation most successfully. Simulation of several experiments is needed to confirm whether the parameter values obtained are realistic.

The simulation of the experiment Vroegindewei 1972-2 is reported here. The grid is shown in Fig. 72 (compare Fig. 13), with positive anemotaxis over 150 m at NE wind.

The relative dissimilarity of the model with the experimental data is given in Figs 73 and 74, for different parameter values. Diffusion alone obviously is not a good descriptor of the dispersal behaviour of the onion fly. The expected recaptures were concentrated on onion fields by a reduced diffusion in preferred grid cells (Fig. 73). A good fit was obtained, optimum at a diffusion coefficient of about 7 000 m^2/day, reduced to about 2 500 m^2/day inside onion fields. These values can easily be found from the estimated lines along which the deviations of the model from the observation to the positive and the negative side, as measured by the χ^2 values, are in balance for a certain trap group.

An indication of the results of the model at these values, using the smaller grid

Table 24. Hypothetical example of how the model's output was compared with experimental data.

	Grid cells (sites)				
	a	b	c	d	total
fly density at time t,					
from simulation	100	10	1	0.1	
number of traps per grid cell	2	5	1	1	
expected relative catch	200	50	1	0.1	251.1
actual catch in trapping period					
concerned	29	20	1	0	50
expected catch	39.8	10.0	0.2	0.0	50
χ^2	2.9	10.0	3.2	0.0	16.1
χ^2 from other trapping periods, calculated similarly					23.4
total deviation					39.5
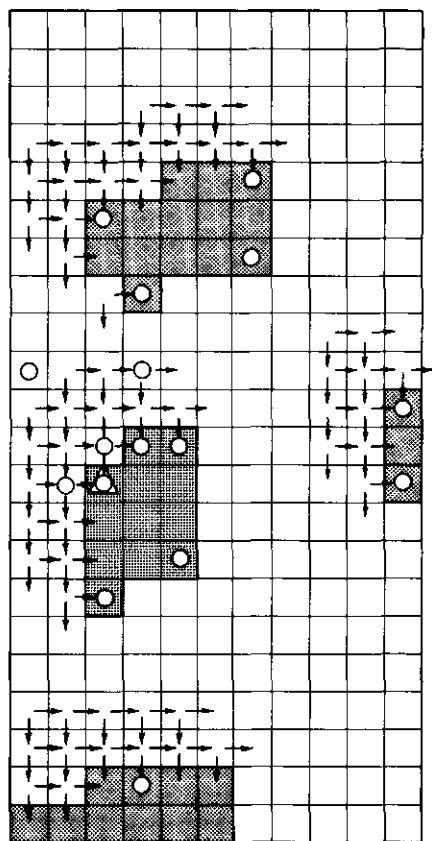
Fig. 72. Map used in simulation of experiment Vroegindewei 1972-2, small grid size. Grid cell length 50 m.
▓:Onion fields; △:release site;
○:trap(s); ⟶ :extra move due to positive anemotaxis, shown for NE wind.

Fig. 73. Simulation of experiment Vroegindewei 1972-2, χ^2 values for different combinations of diffusion coefficients inside and outside onion fields, small grid size. ⟶ Lines along which positive and negative deviations for a group of traps are in balance; R = release field, O = other onion field, X = outside onion fields.

Fig. 74. Simulation of experiment Vroegindewei 1972-2, χ^2 values for different combinations of diffusion coefficients outside onion fields and extra number of flies transferred daily by positive anemotaxis. Diffusion coefficient inside onion fields kept constant at 2000 m^2/day. Explanation see Fig. 73.
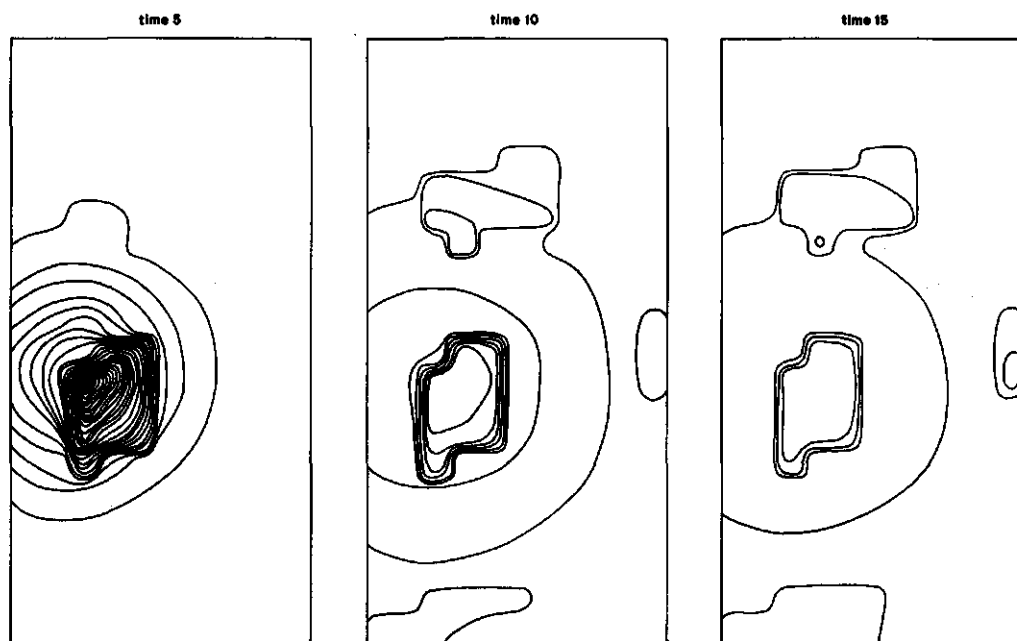
Fig. 75. Simulation of experiment Vroegindewei 1972-2. Isodensity lines, for a diffusion coefficient of 7000 m^2/day, inside onion fields 2500 m^2/day. Lines at multiples of 50 flies per grid cell.

size, is given in Fig. 75. The concentric circles, isodensity lines of flies, are not disturbed outside the onion fields. Thus accumulation of flies on a field does not decrease the fly density in the area further away in the same direction.

The percentage recaptured within the release onion field drops rather steadily according to the model (Fig. 76). The slight irregularity in the observed data is in conformity with Fig. 84: a concentration of flies on onion fields at the age of mating. The irregularities are not those that are obtained when attraction is introduced.

The result of introducing attraction is shown in Fig. 74. The attraction distance used was 150 m. Smaller values would have little influence on the output. Of the larger values only 400 m was tried, but this gave obviously unrealistic results.

Optimum fit was found at no attraction, but the possibility of occurrence of some attraction cannot be excluded.

When account was taken of the increased probability of flies being caught outside onion fields due to the higher flight activity there (Section 5.2.1), the results changed considerably. Fig. 77 gives the χ^2 values obtained in this case. The optimum fit can be estimated at diffusion coefficients of 13 800 m^2/day and 1 900 m^2/day outside and inside onion fields, respectively. The preference for onion fields is thus a factor 7-7.5. As mentioned before, a change in trap catches is proportional to a change in displacement, and the corresponding change in diffusion coefficient is the square of the change in catches. Thus a preference factor of 6.25 was expected. From Fig. 77 it can be seen that the observed value does not conflict with the expected one.

From the average daily maximum temperature during this experiment (18.7°C) it can be

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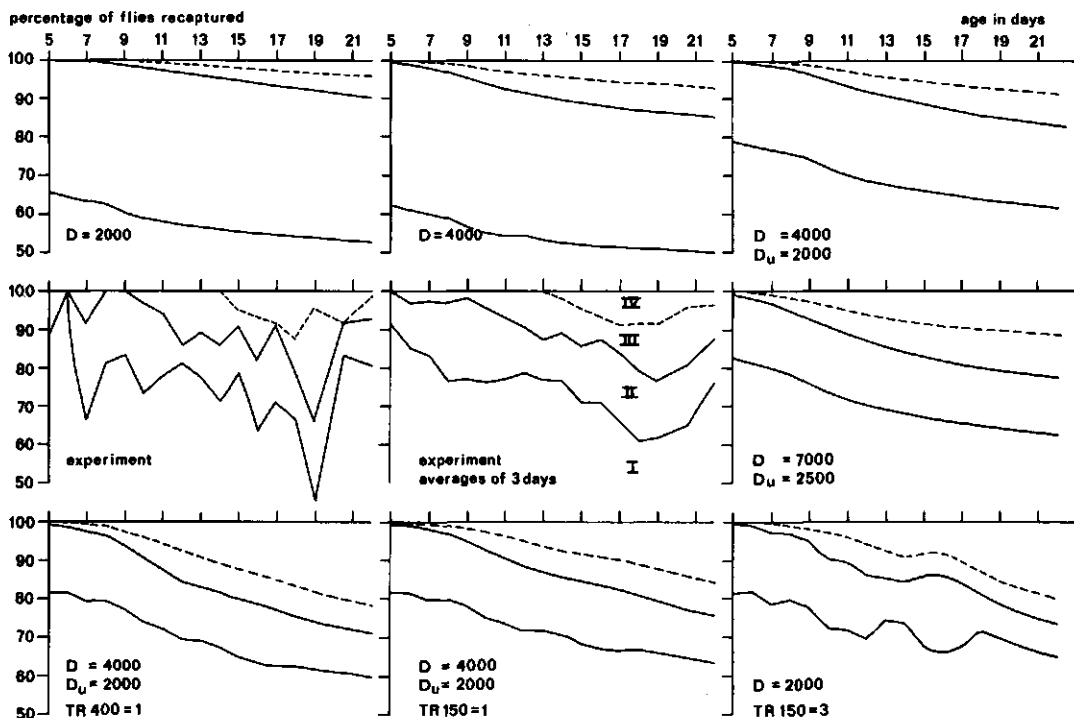
Fig. 76. Simulation of experiment Vroegindewei 1972-2. Percentage distribution of recaptures over four groups of traps versus day after average emergence.
I: release field; II: outside onion fields (uncorrected data, see text), III: onion field to the west (see Fig. 72), IV: onion fields to the north and east (see Fig. 72).
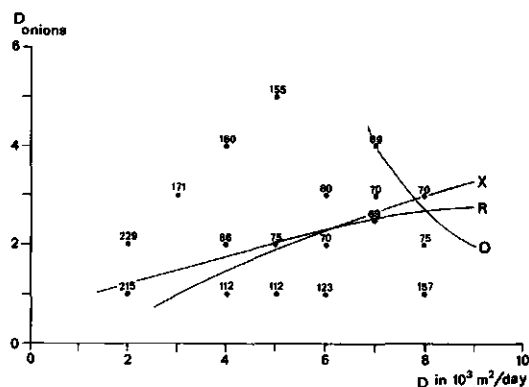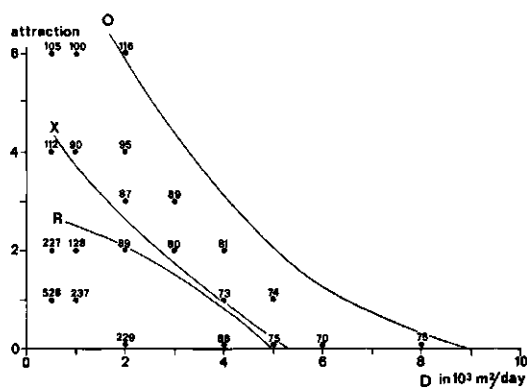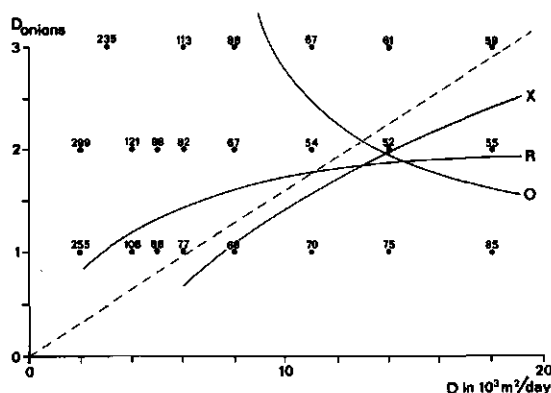

Fig. 77. Simulation of experiment Vroegindewei 1972-2, χ^2 values for different combinations of diffusion coefficients inside and outside onion fields, large grid size. Captures outside onion fields corrected by $1/2.5$ (see text).
- - - $D_{onions} = D/6.25$; other symbols as in Fig. 73.

estimated that the trap catches were 82% of those for standard days (max. $20^{\circ}C$; Fig. 24). The diffusion coefficients for standard days would then be about 20 000 m^2/day, and in onion fields about 3 000 m^2/day.

These values can be considered the best estimates available at the moment, as by such
13   simulation all recapture data are used and heterogeneity in time and space is accounted for.

Theoretical model When the logarithm of the total number of onion flies recaptured per trap was plotted against the distance from the origin, generally a linear relation was observed. It was investigated whether this would follow from the assumptions on survival and dispersal made above.

Let the dispersal be sufficiently approximated by a simple diffusion process. The density at a given point at distance r from the origin is then given by $f(r,t)$ (Eqn 13). Let the flies have normally distributed life-spans (Section 4.4), the fraction alive at time t being given by $g(t)$. The density at time t and distance r is thus $f(r,t)g(t)$. Assuming a certain fraction of the flies present on a unit area to be trapped, independent of t and r, then the total of recaptures per distance is proportional to $\sum_t f(r,t)g(t)$. This was calculated for some parameter values, and gave very slightly concave curves (Fig. 78). As for the moment only the degree of linearity and the slope need to be considered, the recaptures were corrected in this figure to get 100 flies at 100 m distance. The slope is dependent on the life-span distribution and on the diffusion coefficient. Given the former, the latter can be estimated from the slope (Fig. 79).

Experimental data The experiments differ considerably in the slope of the regression of total recaptures versus distance on a semi-log graph (Fig. 80). In the experiment Pollemans 1971 the very slow dispersal will have been due to cold weather and small flies (Section 5.2.4).
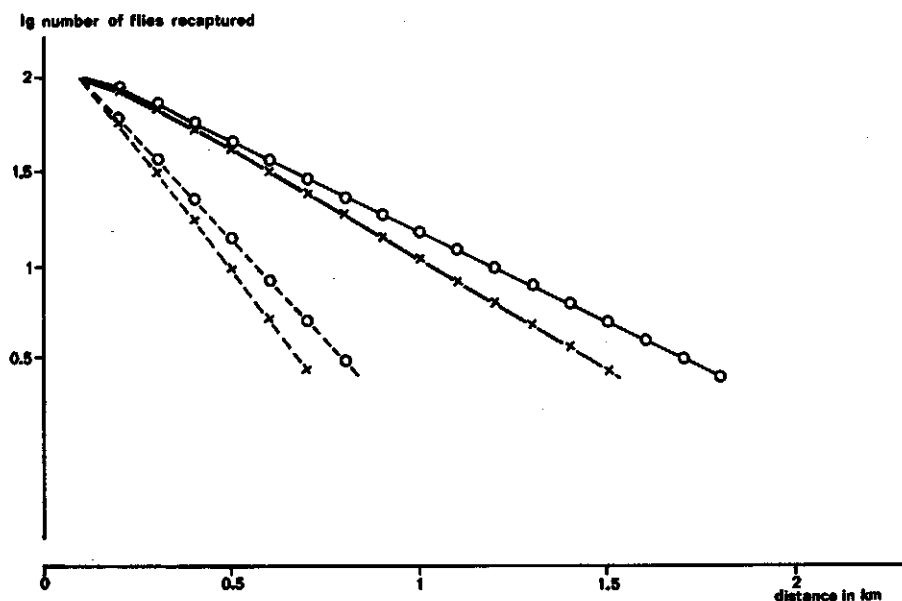
Fig. 78. Logarithm of relative recaptures versus distance, according to a theoretical model (see text), for two values of the diffusion coefficient.
X = males; O = females; —— D = 25 000 m^2/day; - - - D = 5 000 m^2/day.
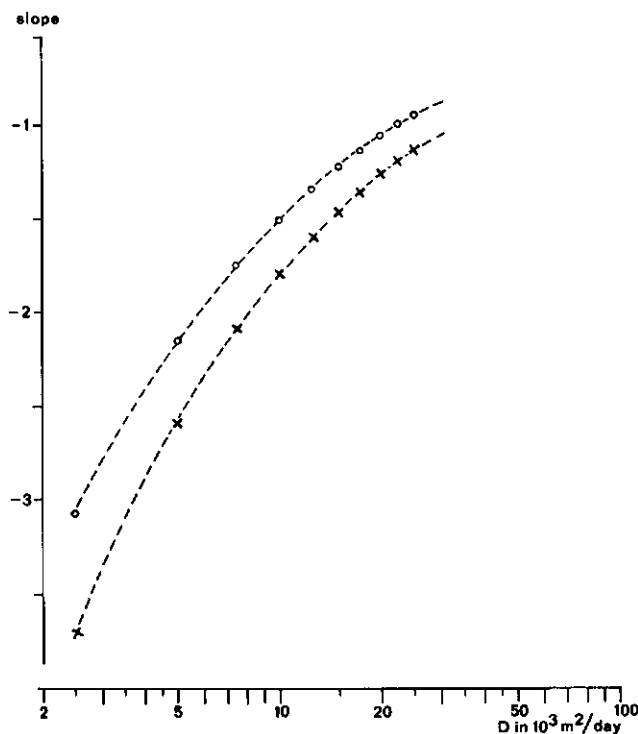
Fig. 79. Estimated slope of the approximately linear relation of the lg (relative recaptures) versus distance (Fig. 78), in relation to the diffusion coefficient assumed in the underlying model. X = males; O = females.

The data of experiment Mijs 1974 were analysed for the flights and sexes separately (Fig. 80 e, f). The slopes were equal for the sexes, but steeper in the 2nd flight. During the 2nd flight the average maximum temperature was higher than during the 1st flight, about 19 versus 17°C. Higher temperatures cause faster dispersal (Section 5.2.1), but the 2nd flight flies live shorter, also when a correction for temperature differences was applied to the survival curves (Fig. 55). So less dispersal is expected, and explanation of the available data does not require the assumption of diffusion coefficient differences between the flights.

As Fig. 79 is based on the life-spans of the 1st flight steriles in the experiment Mijs 1974, the slopes of the regression lines of these flies (Fig. 80 e) can be used

Fig. 80. Linearity of the relation of the lg (% recaptured) versus distance.

Experiments	Regression lines
a. van Loon 1970-2	$y = -0.93x -1.09$
b. Pollemans 1971	$y = -8.17x -0.86$
c. Buijs 1972-1	$y = -2.91x -0.42$
d. Vroegindewei 1972-2	$y = -1.82x -1.11$
e. Mijs 1974-1, steriles on exp	$y = -1.15x -0.95$ males $(+)$
	$y = -1.13x -1.01$ females $(\bullet)$
f. Mijs 1974-2, steriles on exp	$y = -1.50x -1.28$ males $(+)$
	$y = -1.54x -1.38$ females $(\bullet)$

e and f: Encircled points based on over 10 traps, regression line weighted according to number of traps.

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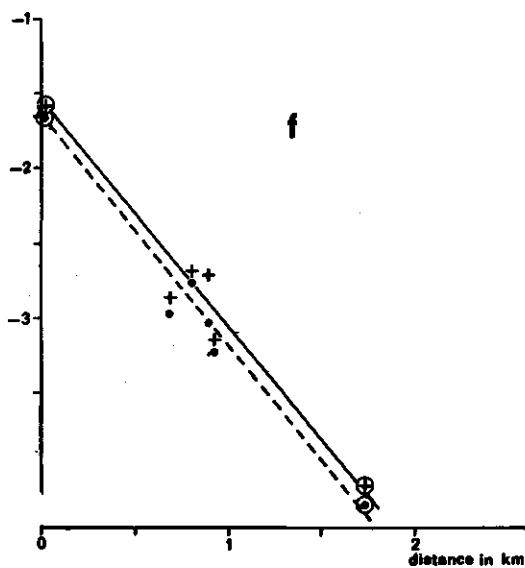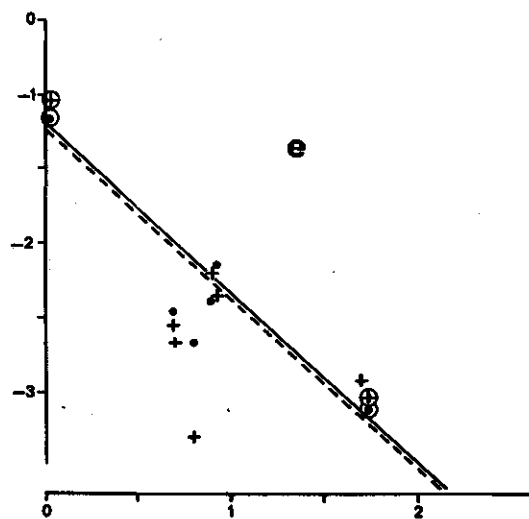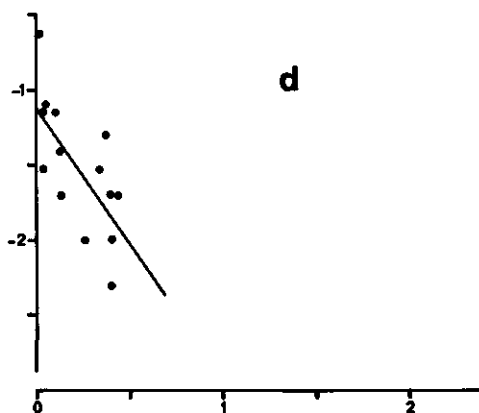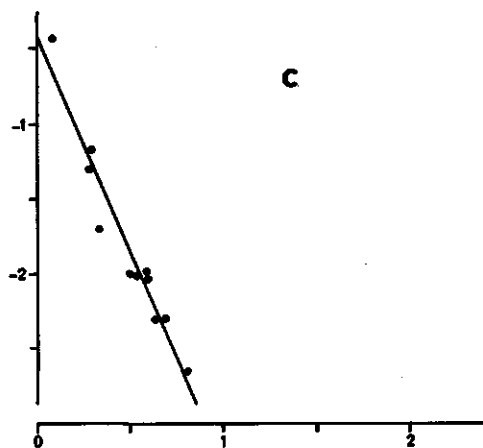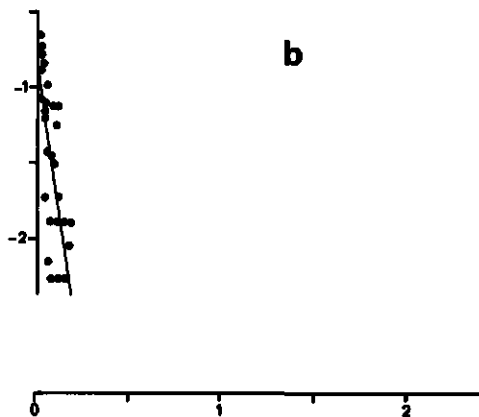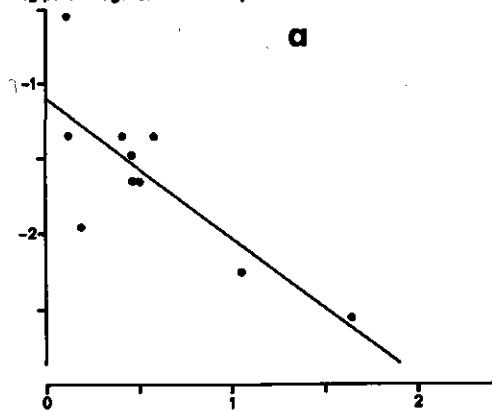
to find diffusion coefficients: 25 000 m^2/day for males and 18 000 m^2/day for females. These values are somewhat higher than those obtained by simulation of experiment Vroegindewei 1972-2, especially as dispersal through onion fields is included.

Migrated fractions of catchable populations, in relation to distance For the experiment Mijs 1974 the percentage distribution of the released flies over the different trapping sites can be derived from the data given in Table 19. As captures outside the onion fields are neglected, the flies which are outside onion fields can best be thought of as being on the nearest onion field. These percentages are compared with the distance migrated (Fig. 81). In conformity with Fig. 80, a linear relation can be assumed between the distance migrated (x) and $\lg(y)$, the logarithm of the percentage migrated:
$\lg(y) = -1.145\ x + 1.59.$

Obviously this relation does not give realistic figures for the percentages migrated to the onion fields nearest to the release sites (4 points in the left top corner), presumably because onion flies concentrate on onion fields: the flies accumulating on the field nearest to the release site become less available for migration to other fields.
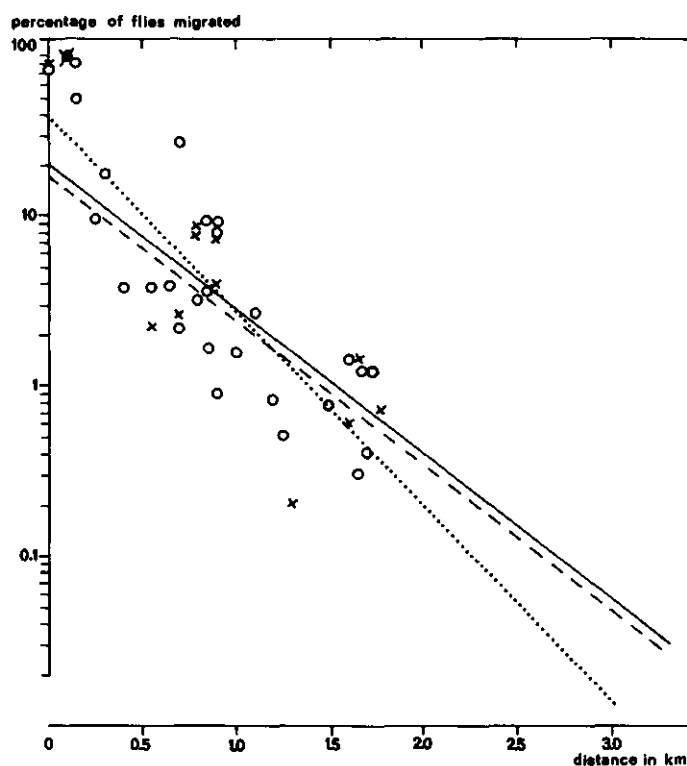
Fig. 81. Percentage of flies migrated versus distance, catchable populations. Experiment Mijs 1974. Regression lines of 1st flight data:
····· $\lg(y) = -1.145x + 1.59$ (based on all points);
- - - $\lg(y) = -0.851x + 1.23$ (recaptures on field at origin excluded);
——— $\lg(y) = -0.851x + 1.29$ (as before but corrected to the average percentage on a linear scale).
O = 1st flight; X = 2nd flight.

So onion fields that are farther away lie 'in the shadow' of nearer fields. As was seen from dispersal simulation (Fig. 75) the direction in which these fields lie is not very important. Thus irregularity of the onion field distribution over the recapture distances will disturb the linearity of the relation between $\lg(y)$ and x. A shadow effect of fields that are farther away cannot be seen clearly from the data, because data from different release sites are combined here, smaller numbers of flies are accumulating on these fields and chance fluctuations become dominant. As a reasonable approximation of $\lg(y)$ versus distance the linear relation was calculated without the data of the nearest onion fields: $\lg(y) = -0.851\ x + 1.23$.

Because not the mean values of $\lg(y)$ versus distance but the mean percentages themselves are of interest, the line has to be raised slightly. From the vertical distribution of the data around the regression line, the untransformed mean is found from Eqn (12) to be 1.15 times higher than the transformed mean. The regression line to be used for extrapolations thus becomes: $\lg(y) = -0.851\ x + 1.29$.

In these calculations the absence of flies from G on field 4 would have given difficulties. Because on field 4 one fly from the comparable group Y resulted in 1.5%, and on the control field the percentage of Y was twice as high as that of G, it was assumed that from G 0.75% came on field 4.

From Fig. 81 the percentages migrated to onion fields outside the area where was trapped were read off. For each released group the total of percentages had to be made equal again to 100%. The figures did not change so much that the regression had to be recalculated with these corrected values. The resulting migration pattern is given in relative numbers in Table 20 and in absolute numbers in Fig. 82.
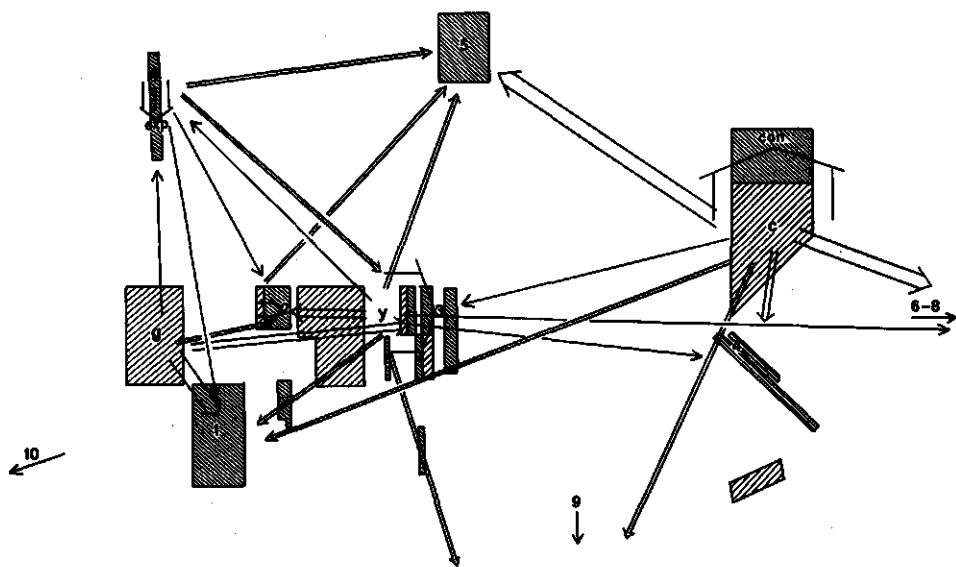
Fig. 82. Migration pattern of catchable populations. Experiment Mijs 1974, 1st flight. Symbols see Section 4.3.3.
1 mm width = 2000 flies, ——— = 1000-2000 flies; lightly shaded = emergence site, heavily shaded = onion field.

Note that the considerable dispersal of flies from the sources G and Y to onion field 2, and the low fraction remaining near the origin of especially G, will at least to some extent be caused by flies originating from a possible source near field 2, because this source had no mixing group of its own.

The flies originating from source C will have become mixed incompletely with the C-dyed mixing group, because this group for practical reasons had to be released near the control field (Fig. 15). Therefore the mixing group C is underrepresented among the flies caught on field 4 that emerged from source C. The wild flies there, not 'explained' by the mixing groups will thus have originated partly from source C.

The corresponding data of the 2nd flight after 31 July do not differ in migration essentially from the 1st flight data, as shown in Fig. 81.

It should be kept in mind that the migration pattern, as derived here, describes the average distribution of the populations insofar they are catchable. As mentioned in Section 4.4, very young flies are underrepresented in such figures. The migration pattern of all flies alive can be obtained by adequate correction: the percentages of migrated flies would be about halved and on the fields adjacent to the emergence sites they would rise by over 10%. A more useful measure of migration is given by the distribution of the populations during the period that they are reproducing (see Section 5.6.4).

Deviations from linearity Up to now dispersal has been considered in experiments that had a somewhat homogeneous distribution of onion fields versus distance, except around the origin. When flies are migrating in an area where onion fields are very scarce, one might suppose that the accumulation of the flies on these onion fields would result in a higher percentage recaptured at larger distances than expected from a linear relation. This situation was present in experiment Mijs 1973-2 (Fig. 14). The percentage recaptured at about 1.75 km distance was indeed relatively high (Fig. 83), causing the relation lg(% recaptured) versus distance to become hollow, especially for flies that had been released outside onion fields (a and b in Fig. 83).

In the small onion field (Fig. 14, left) this accumulation was more pronounced: the recaptures per trap of the groups released 50 and 100 m from the small onion field were about 3.5 times higher than when released in the same way near the large onion field (Fig. 14, right). When the data are corrected for the extra accumulation on the small field, the points a and c come 0.5 lower in the graph. Also the point d will come somewhat lower. The fact that the points a and b are still relatively high then, can be due to an initial high dispersal speed (Fig. 70a).

One pair of traps (crosses in Fig. 83) had consistently higher recaptures than the others. The general level can be higher because of a favourable position of the traps, which results in a larger fraction of the population present being captured, or because of favourable features of the site, causing a larger fraction of the population being present. The latter was here the case, as the site was very sheltered (a deep ditch, and trees in the directions of the main winds). Note that data of different release sites are combined in this figure.

In general it can be stated that a higher fraction than can be deduced from Fig. 78 will be recaptured
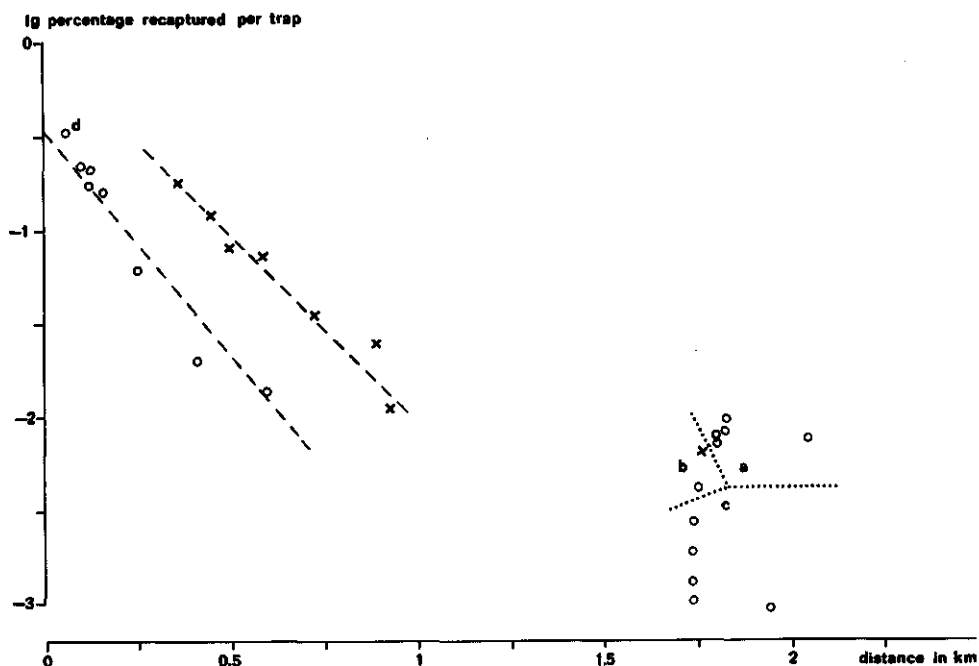
Fig. 83. Logarithm of the percentage recaptured per trap versus distance. Experiment Mijs 1973-2. Explanation see text.
x = One pair of traps, O= other traps; a,b,c and d = deviating data referred to in text.

1. in an isolated onion field (less competition for flies with other onion fields),
2. in a smaller onion field (the concentration of flies on the smaller field is more important than the lower attractiveness or chance of encountering the field due to its smaller size),
3. at very favourable sites as more flies are present,
4. when the trap location is more favourable.
Lower fractions than expected from the linear relation will be found in the opposite situations. As mentioned in Section 5.2.2, location of release or recapture sites at different distances from a reflecting barrier can also disturb the expected linearity.

5.6 AGE-DEPENDENT DISPERSAL

5.6.1 Trapping place versus age

    The age at which dispersal takes place is relevant when the impact of dispersal on reproduction is considered. At first it is established where the steriles, released during experiment Mijs 1974, were trapped in relation to their average age. For this purpose the steriles have been divided into two groups, i.e. with dye marks YO-BYO and with dye marks O-B$_2$ (viz. the release data, Section 6.2.1). This division was made because these groups emerged and were present during periods with different average temperatures, whereas the temperatures within these periods were relatively constant (Fig. 16: periods

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6 May - 13 June and 14 June - 31 August, 1974). Also the males and females were treated separately, because the details of their migration patterns might differ.

At first the percentage recaptured versus age was calculated. For each released group the date of 50% emergence was estimated graphically. The daily recaptures of the flies per trap were listed with their dates of 50% emergence synchronized. Then the data of the different released groups were taken together, for each site separately, and converted to cumulative percentages recaptured. Also their average cumulative percentage emergence curve was calculated, weighted according to the numbers released. With this emergence curve the recapture curves were corrected, thus eliminating the effect of the spread in emergence, giving the cumulative percentage recapture curves against age. From these the fractions recaptured per day were read. These fractions were converted to relative densities by multiplying them by the total number of flies of the corresponding sex and dye marks trapped on the site concerned. These data were converted to relative population sizes by multiplication by the length of sheltered onion field border, as was done in Section 4.3.3. The results are given in Table 25.

Here the catches outside onion fields, made on sheltered edges of non-onion fields, are incorporated in the calculations. The densities were converted to relative population sizes by multiplication by the length of sheltered edges of non-onion fields in the area for which the catches could be supposed to be representative, and divided by 2.5 to correct for the higher trapping probability (Section 5.2.1). These figures are less accurate than the figures from onion fields, because they are derived from only six traps with very low catches, which traps are taken as representative for about 10 km of sheltered field edge.

Fig. 84 summarizes the data obtained, expressed as the percentage distribution of flies over the experimental field, outside onion fields and on other onion fields, against age. As in Fig. 55, the age scales have been adjusted to get coincidence of the females' ages at first mating. For 2nd flight females the ages at first mating and at first and second oviposition are indicated as in Fig. 55. They fit very well to the periods in which the females are concentrated on the onion fields. Exactly the same irregular pattern of dispersal is seen in the independently derived data on the 1st flight females. Therefore it has to be assumed that the physiological ageing in the 1st flight is slower than that of the 2nd flight by a factor 1.5 instead of 1.35. The factor 1.5 is not seriously conflicting with the data in Fig. 57, from which the factor 1.35 was estimated.

Using the females' ages at mating and oviposition, thus calculated and given in Fig. 84, to calculate the fecundity (Section 4.5.3), yields 30.5 eggs per female for the 1st flight.

The graphs for the males have a similar irregularity, i.e. a concentration on onion fields, occurring in the two flights with an age difference of about 1.5. The most obvious explanation is that this concentration on onion fields indicates the average age of mating of the males. Thus males probably mate at an age of about 1.4 times the age of the females at their first mating. It is a common phenomenon, especially in Muscidae, to find dispersal between phases of oviposition (Chapter 8 in Johnson, 1969).

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Table 25. Relative catchable populations against age, per trapping site. Experiment Mijs 1974, steriles released on experimental field.

Age in days	Trapping site[a]																
	males									females							
	exp	5	con	1	2	3	4	no	total	exp	5	con	1	2	3	no	total
1st flight																	
4	22	0	0	0	0	0	0	0	22	0	0	0	0	0	0	0	0
5	1031	0	0	0	0	0	0	0	1031	0	0	0	0	0	0	0	0
6	856	0	0	0	0	0	0	0	856	496	0	0	0	0	0	48	544
7	1580	0	0	0	0	0	0	0	1580	530	0	0	0	0	0	569	1099
8	878	0	3	0	0	0	0	194	1075	547	0	0	0	0	0	70	617
9	1009	0	40	0	19	0	0	1196	2264	496	0	0	0	32	0	63	591
10	812	15	31	0	64	151	0	1810	2883	496	183	0	0	1	184	588	1452
11	439	12	7	0	96	133	54	388	1129	428	49	0	0	11	8	88	584
12	417	103	11	10	38	0	0	210	789	308	12	0	103	8	33	25	489
13	329	173	0	0	0	0	57	501	1060	291	49	13	15	17	13	6	404
14	307	164	1	0	0	42	42	517	1073	274	43	5	0	11	18	0	351
15	285	127	3	0	0	11	0	356	782	291	6	14	28	34	72	626	1071
16	329	157	12	0	4	0	0	16	518	308	12	17	0	4	0	50	391
17	198	254	0	0	0	67	161	146	826	291	41	7	0	0	0	13	352
18	110	82	0	0	0	97	0	97	386	308	224	13	3	0	72	0	620
19	110	61	0	60	0	2	0	194	427	291	16	33	18	34	223	0	615
20	13	33	0	0	0	9	0	566	621	274	28	0	9	40	251	0	602
21	9	6	0	0	0	0	0	275	290	154	4	1	0	7	26	0	192
22	9	6	7	20	0	5	0	0	47	171	6	2	13	6	5	57	260
23	9	9	6	19	0	6	0	0	49	137	59	0	32	3	3	158	392
24	9	9	0	0	0	0	0	0	18	120	4	3	18	2	10	215	372
25	9	0	0	0	0	0	0	0	9	120	0	7	15	3	5	0	150
26	9	0	0	46	0	0	0	0	55	137	0	7	32	1	0	0	177
27	0	0	0	0	0	0	0	0	0	137	0	1	28	6	102	0	274
28 et seq.	0	0	0	0	0	104	0	0	104	240	77	9	274	34	0	0	634
2nd flight																	
0	0	0	0	0	0	0	0	271	271	0	0	0	0	0	0	0	0
1	0	0	0	0	0	16	0	348	364	0	0	0	0	0	0	0	0
2	883	0	0	0	0	13	0	620	1516	516	0	0	0	0	0	0	516
3	817	65	0	54	8	1	0	1123	2068	994	0	0	30	8	0	0	1032
4	1368	85	1	310	8	34	0	503	2309	810	7	0	31	4	32	592	1476
5	1236	60	2	194	37	14	0	1084	2627	700	29	0	50	17	60	294	1150
6	1346	47	1	419	30	36	0	1975	3854	645	54	0	111	4	7	298	1119
7	1148	67	0	178	41	19	0	639	2092	608	103	5	85	5	17	130	953
8	795	169	5	163	63	22	0	329	1546	700	100	4	67	15	47	10	943
9	530	172	4	62	57	23	0	310	1158	442	44	2	7	15	44	91	645
10	309	70	2	0	14	12	0	348	755	424	17	1	30	13	12	77	574
11	177	37	3	11	48	0	0	0	276	295	13	2	30	19	0	0	359
12	132	40	0	43	5	0	0	0	220	239	9	0	20	15	0	0	283
13	22	27	2	78	4	0	0	194	327	203	20	0	24	7	0	0	254
14	22	32	3	39	5	0	0	0	101	166	20	0	96	13	0	0	195
15	9	45	3	0	0	0	0	0	57	129	10	3	44	9	0	38	233
16	9	55	3	0	0	0	0	0	67	129	2	6	33	4	15	212	401
17	9	20	3	0	0	0	0	0	32	129	0	0	59	21	30	183	422
18	9	5	0	0	0	0	0	0	14	74	7	0	22	3	14	0	120
19	9	0	1	0	0	0	0	0	10	74	13	0	0	0	11	0	98
20 et seq.	0	0	4	0	15	0	0	0	19	91	41	5	0	20	27	0	184

a. Onion fields see Fig.15; no = outside onion fields. On field 4 no females were recaptured.
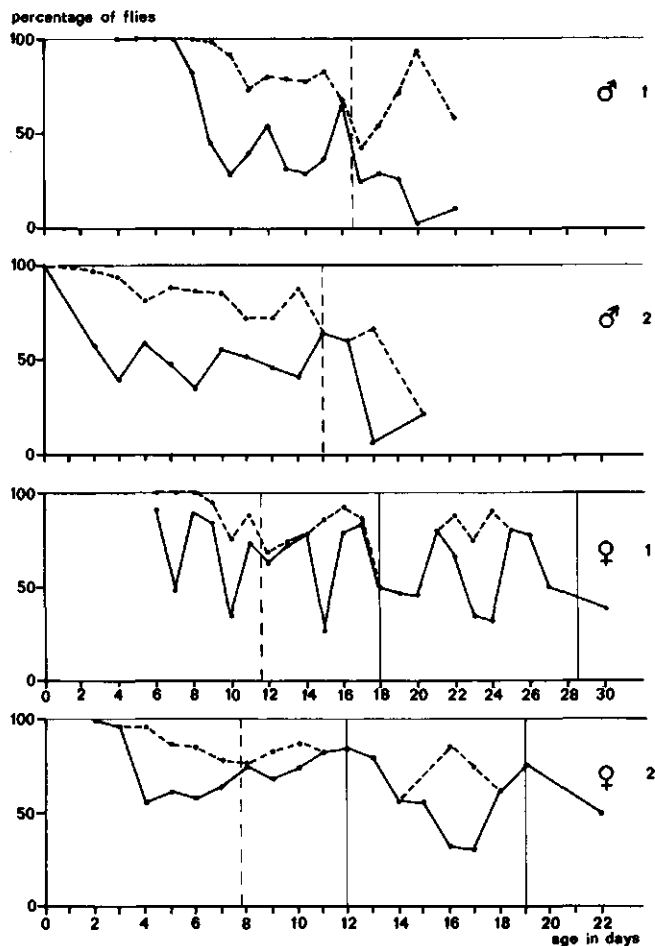
Fig. 84. Percentage distribution of catchable populations over onion field where emerged, other onion fields and elsewhere, versus age. Sex and flight indicated. Experiment Mijs 1974.
——— Under this line in onion field where emerged;
     between these lines outside onion fields;
- - - above this line in other onion fields.
⸽ = Supposed age of mating; | = supposed age of oviposition.

5.6.2 *Age distribution of immigrating flies*

    Another aspect of age-dependent dispersal is the age distribution of immigrants at the time of immigration. In this context migration is used to indicate dispersal from one onion field to another. To estimate this age distribution, the percentage distributions of catches against average age on the different onion fields, as derived above, were used. These distributions can be thought of as the sum of recapture distributions of cohorts of immigrants immigrating at different ages. A cohort of size x_i, arriving at age n, will yield on the field where it immigrated a recapture curve that is similar in shape to the recapture curve on the field of origin for flies of age n and onwards, when one

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percentage of immigrants recaptured
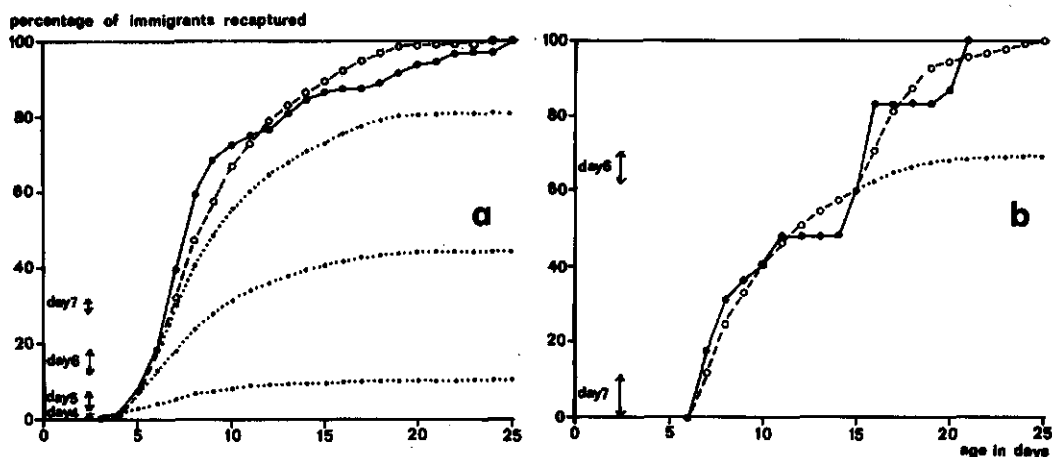
Fig. 85. Cumulative percentage of recaptures of immigrants versus age, and imitated curve derived from estimated recapture curves of immigrated cohorts. Experiment Mijs 1974. As examples: a: females 2nd flight, steriles immigrating to field 5;
　　　　　b: females 2nd flight, steriles immigrating to control field;
● = recaptures; o = imitated curve from sum of estimated recaptures of immigrating cohorts; ····■ immigrating cohorts separately; arrows are relative cohort sizes with age when immigrated indicated.

assumes that the catching probability of flies is independent of their migration. When the number of flies, present on the field of origin at age n, is x_o, the recaptures of the immigrating cohort are x_i/x_o times the recaptures on the field of origin. Now the cumulative distributions of catches against average age on fields where the flies immigrated, have, by trial and error, been optimally approximated by the sum of cumulative distributions of immigrating cohorts; an example is given in Fig. 85.

As the first immigrants arrive at ages when the low initial catching probabilities of young flies have passed, the size of the immigrating cohorts will be proportional to the relative number caught on the first day of their presence, as indicated in Fig. 85. The actual number of flies recaptured on the field concerned is distributed over the different immigration days according to these relative captures on the first day of presence.

The numbers of flies are totalled per immigration age and per distance migrated. The resulting cumulative percentage distributions are given in Fig. 86 for the migration trajects encountered. The data for migration over a distance of about 1700 m were much less accurate due to the lower numbers, and did not differ essentially from the data on migration over a distance of about 800 m. To some extent independent of previous findings (Fig. 84), there are indications of reduced female migration during the reproductive phases.

5.6.3 Dispersal with regard to genetic control

The most interesting aspects of dispersal for application of sterile insect technique are:
1. What is the percentage emigration of sterile males from the treated area before mating?

percentage of immigrants arrived
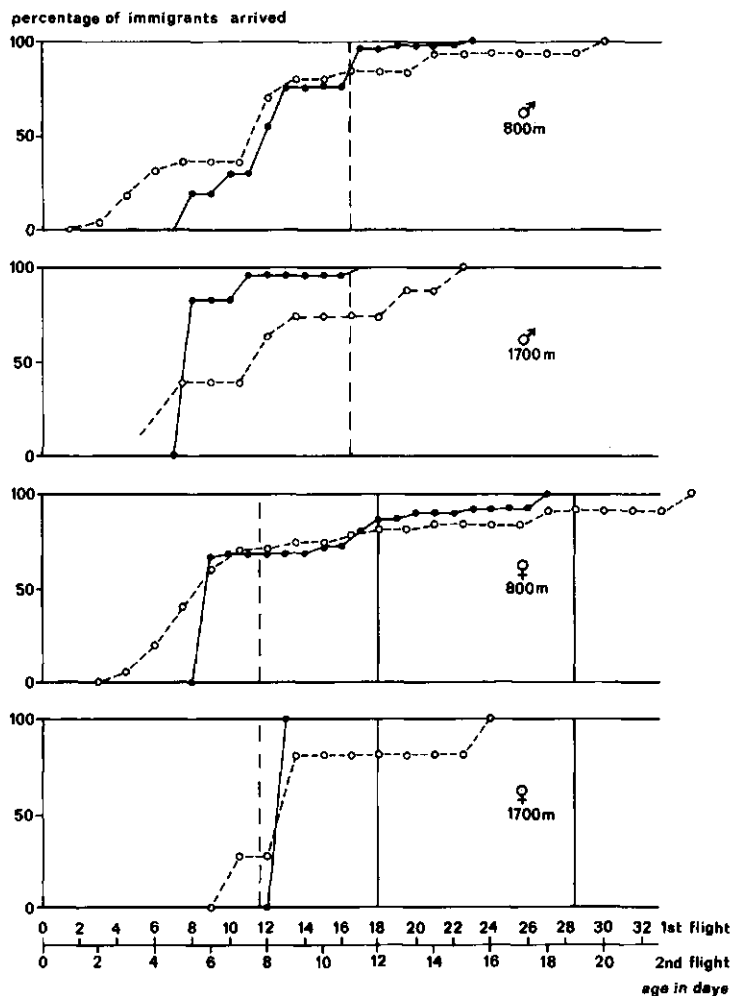
Fig. 86. Cumulative percentage of migrants arrived versus age. Experiment Mijs 1974. Sex and distance indicated.
——— 1st flight, recaptured over 800 m: 156 males,109 females; over 1700 m: 30 and 26;
· · · · · 2nd flight, recaptured over 800 m: 176 males, 89 females; over 1700 m: 10 and 13.

2. What is the number of immigrating fertile males in the treated area before mating, as a function of distance and size of the population they originate from?
3. What is the number of immigrating fertile females in the treated area between mating and oviposition, as a function of distance and size of the population they originate from?
These aspects will be considered for one treated onion field.

As can be seen from Fig. 84, the percentage of sterile males that emigrated before mating is about 50. The survival at the average mating age is some 25% (Fig. 55). The fraction of a population emerging at a given distance that migrates into the field considered cannot be found directly from Fig. 81 or Table 20, as these figures refer to catchable populations, summed over their whole lifetime. At the moment of mating about 40%

of males alive have migrated to other fields (Fig. 84). The percentages that have migra-
ted to other fields, as given in Table 20, are 15-25%. Now how far they have migrated
is not very important with respect to the age distribution at arrival (Fig. 86). Thus,
the distribution of the 40% emigrated flies will be similar to that of the about 20%
emigrated flies (Table 20). With the survival at 25% these figures can roughly be halved
to find the percentage of the emerged population that has migrated to other fields at the
time of mating, versus distance. Or, with a regression line as in Fig. 81, lg(%) =
-0.85(distance in km) + 1.0. What this means for populations of different sizes at dif-
ferent distances is visualized in Fig. 87, assuming a higher percentage immigrated for
short distances (under 0.5 km; Fig. 81).

As a rule of thumb, the magnitude of the number of males migrated before mating is
found by dividing 1/4 of the original population by a factor 10 for each 0.8 km distance.

Of the immigrating females, 10-15% immigrate between mating and oviposition, as can
be estimated from Fig. 86. In total 30-40% of the females alive emigrate from their emer-
gence site (Fig. 84). So 10-15% of these, or about 4% of the females alive immigrate to
other fields between mating and oviposition. Such 'disturbing' females are about 2% of
the females of emerging populations, as about half of the females die before the first
oviposition (Fig. 55). The same line of argument as for the males above leads to a si-
milar rule of thumb, only a factor 5 lower. As these females will have encountered alrea-

Fig. 87. Immigration of fertile males as a function of distance and population size at
the origin of the immigrants. Figures indicate the number of immigrating fertile males;
between brackets the sterile:fertile ratio resulting from immigration in a field where
10 000 fertile males emerge and 100 000 sterile males are released, their emigration
taken into account.

dy several sterile males on the field where they mated, their potential harmful effect will be reduced. In Section 6.2.4 the effect of these females on egg fertility was calculated for experiment Mijs 1974 on control by sterile males.
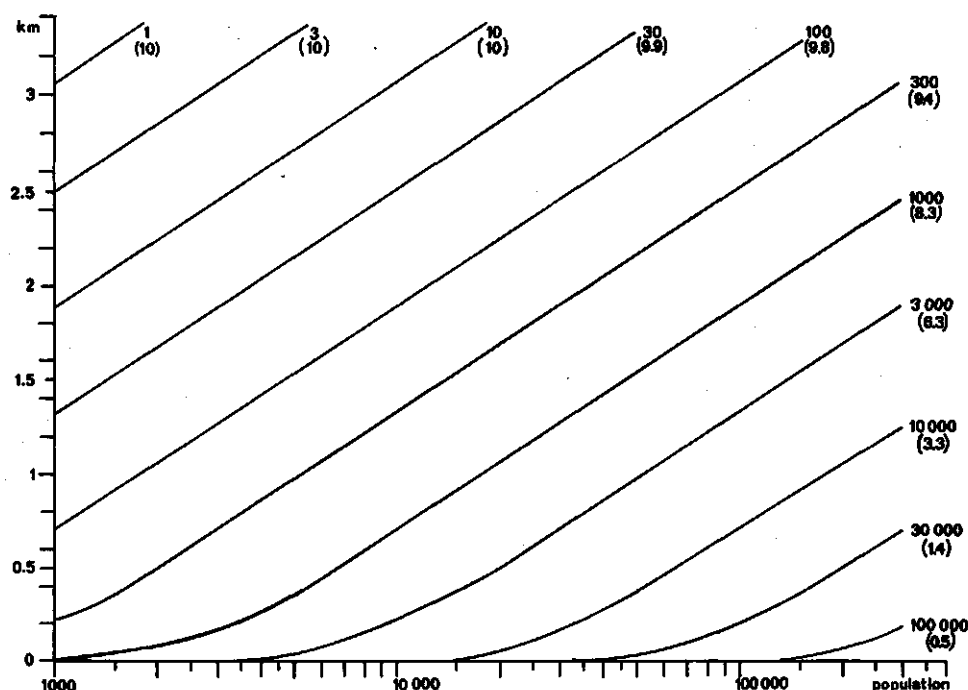
5.6.4   Migrated fractions of reproducing populations, in relation to distance

In reproducing populations, the number of reproducing females is important and, for sterile male control, the sterile/fertile ratio in the males. The numbers of males are not so important here because they are very polygamous. The ratio in the males can be found from the data given in the preceding section, the numbers of reproducing females will be considered here.

For experiment Mijs 1974, the migrated fractions of catchable populations, as given in Section 5.5, can be converted to estimated migrated fractions of reproducing populations. The calculations are given in Table 26. The total populations of emerging females are found from Tables 20 and 21, divided by 2 because at emergence the sex ratio is 1:1. The average fractions alive when reproducing (ovipositing) are found in Table 22   for subsequent ovipositions, and the correction given in Section 5.6.1 has to be applied to 1st flight data. These data were simplified to assumed percentages of 60% of the 1st flight females and 37% of the 2nd flight females ovipositing once. Thus the numbers of female flies reproducing were calculated (Table 26). How these numbers were distributed between the field of emergence and other onion fields can be found from Table 25, by adding up all data from days during which the females are supposed to oviposit, that is when females are concentrated on the onion fields. The result is for the 1st flight (days 17-21, 25 et seq.): 46% emigrated, and for the 2nd flight (days 11-14, 18 seq.): 28% emigrated. As no such data are available for the other onion fields, these percentages from the experimental field are applied there.

For estimating the distribution of these emigrated females over the different onion fields, the data of Tables 20 and 21 were used. The results are given in Table 26, as estimated numbers of females reproducing, divided by origin and reproduction site. The pre-reproductive dispersal, that can be seen from comparison of the bottom row (totals emerged) with the right hand column (totals reproducing), is levelling the populations to some extent.

5.7 SUMMARY

Onion flies could keep pace with the 1 km/year spread of onion cultivation in a newly reclaimed area. Dikes connecting the former islands in the SW of the Netherlands did not constitute migration routes for the flies.

The rate of dispersal is dependent on temperature, but was found to be in general independent of wind direction. More detailed data on dispersal inside onion flies revealed some preference for upwind flights. Net displacement outside an onion crop is 2.5 times faster than through onion fields.

The wide waters around Overflakkee can be considered as reflecting barriers to the flies, as indicated by recapture data and direct observation. Incidentally, long range

Table 26. Distribution of females at the average age of oviposition over the onion fields, different sources seperately. Experiment Mijs 1974.

a. 1st flight

	Onion field where emerged					
	exp	con	1(+2)	3(+2)	2	total
Number of flies						
emerging population	21 000	90 800	14 600	54 900	9 500	
females alive (60%)	6 300	27 240	4 380	16 470	2 850	
females emigrated (46%)	2 898	12 530	2 015	7 576	1 311	

Number of ovipositing females $x\ 10^3$

onion field where ovipositing:

	exp	con	1(+2)	3(+2)	2	total
exp	3.4	0.1	0.1	0.5	-	4.2
5	1.0	4.6	0.1	1.0	1.3	8.0
con	0.1	14.7	0.0	0.2	-	15.1
1	0.4	0.7	2.4	1.1	-	4.5
2	0.2	.3	0.7	2.9	1.5	5.6
3	0.9	.5	1.0	8.9	-	11.2
4	0.1	1.9	0.0	0.5	-	.
6-8	0.0	3.7	0.0	0.4	-	.
9	0.1	.8	0.1	0.7	-	.
10	0.1	.0	0.0	0.1	-	.
total	6.3	27.2	4.4	16.5	2.9	

b. 2nd flight

	Onion field where emerged						
	exp	5	con	1	2	3	total
Number of flies							
emerging population	2 000	2 000	75 400	300	200	6 900	
females alive (37%)	370	370	13 949	55	37	1 277	
females emigrated (28%)	104	104	3 906	15	10	358	

Number of ovipositing females $x\ 10^3$

onion field where ovipositing:

	exp	5	con	1	2	3	total
exp	0.2	0.0	0.1	0.0	0.0	0.0	0.3
5	0.0	0.2	1.6	0.0	0.0	0.1	1.8
con	0.0	0.0	10.0	0.0	0.0	0.0	10.1
1	0.0	0.0	0.3	0.0	0.0	0.1	0.4
2	0.0	0.0	0.0	0.0	0.0	0.1	0.2
3	0.0	0.0	0.0	0.0	0.0	0.9	1.0
4	0.0	0.0	0.4	0.0	0.0	0.0	.
6-10	0.0	0.0	1.5	0.0	0.0	0.0	.
total	0.4	0.4	13.9	0.1	0.0	1.3	.

dispersal may result from being carried by traffic. It is not probable that significant numbers of onion flies join the aerial plankton for long distance dispersal.

Small onion flies show a slower dispersal. High concentrations at the release sites did not seem to result in a faster dispersal.

The onion fly dispersal was described by a diffusion process. The diffusion coefficient was calculated from field observations of the frequencies and distances of flights,

and was found to be about 120 m^2/day for females and 175 m^2/day for males inside onion fields. Calculation of the diffusion coefficient from the increase of the median distance of the flies from the origin versus time gave 3600 m^2/day in an area with an average onion field density.

A dispersal experiment was simulated, essentially by assuming diffusion of the flies. Introduction of positive anemotaxis did not improve the fit of the model's output to the experimental data. After correcting the trapping probability outside onion fields for its higher value due to the increased flight activity of flies there, the optimal fit of the model's output could be estimated to be obtained with a diffusion coefficient of about 14 000 m^2/day, reduced to about 2 000 m^2/day inside onion fields.

The relation of the logarithm of recaptures versus distance was found to be about linear. When it is assumed that the flies diffuse in a homogeneous environment and have normally distributed life-spans, a nearly linear relation is expected. A linear relationship was used to obtain by extrapolation some missing data in a large-scale release-recapture experiment. When there are few onion fields, recapture rates were higher at greater distances compared with those estimated from the linear model. Possible causes for deviations from the linearity are indicated.

From the distribution of recaptured flies on different sites versus their age, it was found that migration in females was reduced during the periods of mating and oviposition. In the males there was a similar temporary concentration on onion fields, supposedly for mating. The age distribution of immigrants at the moment of immigration gave a similar picture. About 70% of the immigrants arrive before the average age of mating.

At the moment of mating about 50% of the males alive have left the onion field where they emerged. From this the impact of wild populations on a single field treated with sterile males is shown, dependent on the distance and the relative size of such populations. The fraction of females alive that has mated but not yet oviposited after migrating was estimated at 2% of the number emerged. From the distribution of females over the fields where they emerged and the fields where they oviposited, the effect of pre-reproductive migration was derived.

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6 Genetic control

Field trials on the use of sterile males for onion fly control are described here. These experiments were the final goal of the research phase of the project, and were done to demonstrate ecological feasibility of the sterile insect technique for the onion fly. At the same time, these experiments provided the opportunity to estimate some parameters of population dynamics relevant to releases of sterile onion flies in practice.

First a series of experiments are reviewed briefly; these were done mainly by other members of the onion fly research team, in an area remote from the commercial onion growing areas. Then, an experiment in one of the main Dutch onion growing areas is described in detail. Finally estimations are given, insofar as possible from the available information, on how the sterile insect technique should be applied in practice.

6.1 FIELD TRIALS AT THE SCHUILENBURG

6.1.1 Introduction

A series of field trials on sterile insect technique were carried out from 1971 to 1974 by the onion fly team on the experimental farm the Schuilenburg, some 10 km SW of Wageningen. Some data have been reported by Ticheler et al. (1974a) and Theunissen et al. (1974, 1975).

The experiments were done in this area because, at the time they were set up, the dispersal properties of the onion fly were not yet known, and it was thought that the site chosen would be isolated from other onion fly populations, as there was no commercial onion growing in this area. The isolation may not have been very good, as onions were grown in kitchen gardens within a few km distance. The location was not representative for onion growing areas as the soil was rather heavy (clay fraction 30-45%), and low damage levels are therefore expected. In this preliminary field trial such levels were an advantage because they limited the demands on the mass-rearing output.

The reproduction factors have been discussed in Section 4.5.3, and damage data have been given in Table 15.

The numbers of steriles released were correlated with the subsequent egg sterility. Optimum correlation was found for egg sterility with the sum of the numbers of steriles released one week and two weeks earlier. Thus the average age of mating can be estimated at 1-2 weeks. The existence of a correlation indicated that the sterile insect technique worked in the field, as a desired egg sterility could be obtained by releasing the required number of steriles.

Both sexes were released. Sterilized females impede the control in species with a low mating frequency (Ailam & Galun, 1967; Cuellar, 1973). In the onion fly male, the mating

Table 27. Populations, sterility and reproduction, Schuilenburg field trials on
sterile insect technique, 1971-1974. Guessed values are between brackets; the other
values, given in italic, were calculated from these and observations.

Year	Populations, sterility and diapause[a]		Reproduction factors		
			flies[b]	eggs[b]	migration[b]
1970		14 400			
1971		s_1=0.675	r_1= 5.87	5.69	10.54
	20 600 · 20 600 d_1=(0.25) 6 870				
	s_2=0.413		r_2= 0.30	0.21	0.29
	0 d_2=(1) 3 580				
	10 450		r_y= 2.79	2.32	4.93
1972		s_1=0.470	r_1= 1.56	1.58	2.92
	6 900 · 6 900 d_1=(0.2) 1 725				
	s_2=0.438		r_2= 1.08	1.24	1.73
	0 d_2=1 4 175				
	5 900		r_y= 1.35	1.88	4.63
1973		s_1=0.857	r_1= 6.78	1.64	3.03
	5 500 · 5 265 d_1=0.08 460				
	s_2=0.828		r_2= 1.52	1.16	1.61
	235 d_2=0.83 1 140				
	1 600 + 2 750[c]		r_y= 8.41	1.58	3.97
1974		s_1=0.915	r_1= 0.92		1.71
	300 · 280 d_1=0.18 60				
	s_2=0.852		r_2= 3.84		5.36
	20 d_2=0.87 140				
	200		r_y= 2.52		6.85
Average reproduction factors:			r_1= 2.75	2.45	3.55
			r_2= 1.17	0.67	1.44
			r_y= 2.99	1.90	4.99

a. s=effective sterility, d=diapausing fraction, subscripts denote flights.
b. r_1=1st flight reproduction, r_2=2nd flight reproduction, r_y=reproduction for a year
$(r_y=r_1 d_1+(1-d_1)r_1 r_2 d_2)$. First column calculated with the sterilities among the flies as
given, second column calculated with the sterilities among the eggs, third column with
corrections for emigration included and, where available, based on sterility among eggs.
c. From 9000 laboratory-reared pupae with a competitiveness of 0.31 as estimated from
recapture data. These pupae were fertile and introduced to increase the wild population.

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frequency is rather high (Section 2.2.1). The related cabbage root fly males can mate effectively about 50 times (J.L. Smith, 1973).

6.1.2 Reproduction

Data on reproduction are given in Table 27. The populations of diapausing pupae and the percentages of sterility differ slightly from those given in the literature mentioned in the preceding section, because those data are based on preliminary calculations.

The aim of sterile releases is to mate fertile females with sterile males. Thus, the relevant figure is the sterility among the males met by fertile females; this figure is called here the effective sterility. Estimation of the percentage sterility among the flies from the total of recaptures per flight of steriles and fertiles will tend to yield too high values, as the flight curves of steriles and fertiles will often not be congruent. Because of lack of knowledge on prediction of flight curves, a constant release rate was chosen, thus deliberately not trying to attain congruency of flight curves. The effective sterility can be estimated from the percentage of daily sterility among the males during a flight by taking the number of fertile females that were caught on the same day as weighting factor. In some experiments considered here the sterility was checked in only one sex, and then this sterility was extrapolated to the other.

In the 1971 and 1972 data the number of fertiles had to be calculated from the percentage of sterility in a sample of the catch, and this resulted in biased values of the effective sterility. Comparison of weighted and unweighted percentages sterility (Table 28) shows that the difference between these as found in 1971 is similar to the differences found in 1973 and 1974. The larger deviation in 1972 can easily be explained by the more important differences during that season between the sterile and fertile flight curves. So the bias in the calculation of the effective sterility in 1971 and 1972 can be considered as unimportant. Note that the differences between weighted and unweighted sterility are larger in the 2nd flights, which are thus less well mimicked by the releases.

The reproduction factors calculated for 1971 are not very reliable because of the low number of samples (12) and for 1974 because of the low number of pupae present. It seems probable that the 2nd flight population in 1971 was overestimated and that in 1974 this population was underestimated.

Table 28. Comparison of weighted and unweighted percentage sterility among the flies. Experiments Schuilenburg 1971-1974.

Year	Flight	Weighted	Unweighted	Difference
1971	1	73.8	67.5	6.3
	2	51.5	41.3	10.2
1972	1	62.5	47.0	15.5
	2	67.4	43.8	23.6
1973	1	94.0	85.7	8.3
	2	94.8	82.8	12.0
1974	1	94.9	91.5	3.4
	2	89.8	85.2	4.6

In 1971 there will probably have been a third flight (Fig. 37). If the diapause frequency of the 2nd generation pupae had been at the low value of 0.8, there would have been a 3rd generation of 1000 flies. Then the reproduction factors would have been $r_1 = 5.58$, $r_2 = 0.43$ and $r_y = 2.83$. The third flights are assumed not to have reproduced because of the generally unfavourable weather at that time and the scarcity of onions as they had already been harvested.

The Schuilenburg populations are considered above to be confined to the experimental field. In reality both steriles and fertiles will have emigrated from the experimental field, and fertiles may have immigrated. The immigration will not have been very important quantitatively, as estimated in Section 6.1.3. Another indication as to the relative importance of immigration to be expected in this area can be found from the control field in 1973, at a distance of 0.7 km from the experimental field. This field was provided with ^{65}Zn labelled flies from diapausing pupae buried in early spring. If one excludes these labelled flies from the captures, the 1st flight sterility on the control field is still lower than that observed in the experimental field. The difference can be explained by assuming another source of wild flies, and the numbers immigrated from there are estimated at 700. Similar numbers can be expected to have migrated from there into the experimental field. Expressed as a fraction of the wild flies caught on the experimental field, these can be estimated as 14%.

This number of extra immigrating wild flies on the control field can also be explained by assuming the dispersal of the wild flies to be faster than that of the laboratory reared flies by a factor of about 2.5. But, if one considers the low numbers of flies concerned, and the presence of non-commercial onion growing in the near surroundings, the latter explanation does not seem very probably.

Figures on pre-reproductive emigration can be obtained as follows. The recaptures of steriles per trap at 0.7 km distance in 1973 and at 2.3 km distance in 1974 were 3.1 and 0.3% of those on the corresponding experimental fields, which data fit rather well in Fig. 81. So the emigration from the experimental field will have been comparable to the emigration from the experimental field of Mijs 1974, which was of equal size and also rather isolated. Thus the pre-reproductive emigration can be taken as 46% during the 1st flight and 28% during the 2nd flight (Section 5.6.4).

The effect of immigrating fertiles is included in the observed sterility. The effect of emigrants has to be corrected for by reducing the wild populations by the percentages given above. The resulting reproduction factors are given in Table 27, right column.

6.1.3 Competitiveness

During the Schuilenburg experiments there were considerable losses of sterile flies as were reported for the Overflakkee experiments on sterile insect technique (Fig. 20, Table 32). The losses were attributed to predation by insectivorous birds, which was actually observed here. These losses appear in the data as lowered competitiveness values (Table 29).

Another aspect of the competitiveness is seen from the difference between sterility among the flies and sterility among the eggs. For each flight the effective sterility

Table 29. Competitiveness of sterile flies found from recapture rate. Experiments
Schuilenburg 1971-1974.

Year	Flight	Number of flies		Ratio sterile/fertile		Competitiveness calculated
		emerged fertile	released sterile	expected	observed[a]	
1971	1	14 400	318 000	22	2.82	0.14
	2	20 600	157 000	7.6	1.06	0.13
1972	1	10 450	383 200	37	1.67	0.05[b]
	2	6 900	231 400	34	2.07	0.06[b]
1973	1	5 900	1 013 900	172	15.67	0.09
	2	5 500	566 200	103	18.23	0.18
1974	1	4 350	683 400	157	18.61	0.12
	2	300	435 100	1450	8.80	0.01[b]

a. Unweighted percentage sterility.
b. Sterility assessed from dye marks, and as not all released steriles were well dyed,
   the figures are too low.
c. Rather unreliable because a few immigrating females will have had a serious impact
   on the relative size of the fertile population, and because the estimated population
   is probably too low (Section 6.1.2).

Table 30. Mating competitiveness of sterile flies. Experiments Schuilenburg 1971-1973.

Year	Flight	Effective sterility among flies	Observed sterility among eggs	Competitiveness calculated	Number of eggs	Estimated number of females that laid these eggs[a]
1971	1	67.5	66.5	0.96	5210	155
	2	41.3	16.9	0.29	6437	227
1972	1	47.0	47.6	1.02[b]	6692	207
	2	43.8	61.3	2.03[b]	5448	196
1973	1	85.7	40.7	0.12	938	23
	2	82.8	77.4	0.71	1759	57

a. Estimated separately for each cage in which females were kept.
b. Sterility assessed from dye marks, and as not all released steriles were well dyed,
   the figures are too high, cf. Table 29.

(Section 6.1.2) is compared with the sterility among the eggs laid by captured fertile
females in the laboratory (Theunissen et al., 1974), and the competitiveness is calcu-
lated from these data (Table 30). The observed egg sterilities were corrected for a
natural sterility of an estimated 10%, but not for the increase in egg deposition of
females after mating with sterilized males (Section 3.2), because this effect is highly
dependent on the pupal age at irradiation, which varied somewhat during these experiments.
The competitiveness values of Table 30 will thus at least in part be too high, e.g. the
2nd flight 1972 value. Especially in the 1971 2nd flight and the 1973 1st flight, the
calculated competitiveness values are probably significantly below 1, and need some
explanation.

As the methods used for assessing sterility in eggs and females were essentially the
same throughout these years, they cannot be a source of incidental deviations. Possible

Table 31. Possible causes of low values for mating competitiveness: weighted average duration of storage of pupae and average pupal weight. Experiments Schuilenburg 1971-1974.

Year	Flight	Duration of storage in days	Weight in mg
1971	1	21	9.11
	2	40	10.74
1972	1	49[a]	12.73
	2	13	13.04
1973	1	80	12.58
	2	69	12.21
1974	1	193[b]	12.89
	2	87[c]	14.14

a. 31% 113-212 days, 69% 0-15 days.
b. 75% stored in diapause.
c. 100% stored in diapause.

causes are immigration of wild females, or reduced competitiveness of sterile males.

In 1971, 30% of all females caught during the 2nd flight would have had to be immigrated mated females to account for the low competitiveness. This percentage is clearly unrealistic, as these females would be only a minor fraction of all immigrating females (see Fig. 86; Section 5.6.3).

A reduced competitiveness of the males could be due to a lower pupal weight. Also, the duration of storage of pupae at 3^OC was checked, because Vosselman (pers. commun.) found recently that this storage, although not affecting fly emergence, did reduce the fly performance in single pair matings. The pupal weights and storage periods of the steriles released are indicated in Table 31. The reduced competitiveness values can be explained by assuming a combined effect of low pupal weights and longer storage periods.

6.2 GENETIC CONTROL FIELD EXPERIMENT ON OVERFLAKKEE

6.2.1 Introduction

Experimental area The experimental area is shown in Fig. 15. The experimental and control field were cultivated by the same farmer, ensuring a minimum of differences in treatment between these fields except in onion fly control.

The experimental field was about 1 ha (30 x 300 m). An untreated control plot of about 0.5 ha (42 x 132 m), about 1.7 km from the experimental field, was part of a larger onion field, the other part receiving the normal insecticide treatment.

The small 1973 onion field, situated in the centre of the 1974 experimental field, was not treated against onion fly. The damage there was 24%, whereas the other 1973 onion fields in the surroundings had no noticeable damage. The experimental field was supposed to be relatively isolated because of its distance from other 1973 and 1974 onion fields. The soil around the area in which the experiment was done is heavier, reducing the likelihood of significant populations which might cause immigration on the

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Table 32. Releases and recaptures of steriles. Experiment Mijs 1974.

Number of pupae	Average weight in mg	% Emergence	Date of 50% emergence	Release method[a]		Dye mark[b]	Number of flies released	Recaptured actual	Recaptured corr.[c]	% Recaptures on exp
experimental field										
76 922	12.0	84.8	6 May	a	6.5	YO	65 230	1123	47.42	71
104 482	11.8	95.8	9 May	a	9.9	GC	98 786	1914	83.60	70
97 888	12.6	90.8	18 May	a	4.5	YC	44 895	800	95.74	79
				b	-	none	43 987	.	.	.
107 546	13.2	52.4	21 May	a'	2.5	BYC	28 274	485	42.26	77
				b	-	none	11 205	.	.	.
97 853	13.7	52.4	30 May	a'	2.5	B	25 627	158	16.87	86
				b	-	none	25 627	.	.	.
98 810	14.5	50.4	3 June	a'	2.5	BYO	24 895	41	4.38	92
				b	-	none	24 895	.	.	.
97 770	13.6	74.4	11 June	a'	3.6	O	36 363	50	19.95	53
				c	0.0	none	0	-	-	-
187 036	12.5	65.2	16 June	a'	24.4	GYO	40 537	365	47.34	78
				d	.	none	81 397	.	.	.
90 245	13.7	86.0	22 June	a	7.6	GYC	76 102	363	16.29	80
125 227	14.0	85.0	29 June	a	.	BG	100 253	456	24.20	84
89 523	13.4	85.4	9 July	a	.	YO_2	75 517	615	25.09	86
95 818	13.4	93.6	13 July	a	5.0	GC_2	44 748	231	9.78	86
					44.7	BO_2	44 748	294	13.34	87
87 512	13.7	90.0	21 July	a	.	BY_2	78 402	1021	46.49	82
99 873	14.5	93.4	29 July	a	83.9	BYC_2	83 862	532	20.43	77
					0.45	BC_2	9 340	45	1.54	85
110 983	14.3	86.2	5 Aug	a	93.2	YC_2	93 179	380	10.77	94
			3 Aug[d]	e	1.2	Y_2	1 225	10	0.24	100
185 985	16.2	93.0	12 Aug	a	102.5	GY_2	102 536	484	14.62	78
			12 Aug[d]	e	61.1	B_2	61 074	518	15.09	79
109 319	14.4	93.6	20 Aug	a	52.9	BG_2	52 861	202	7.77	72
			17 Aug[d]	e	36.1	C_2	36 115	286	8.24	85
1862 792	13.6	80.5	total steriles				1411 680	10373	571.45	
23 560			wild 1st flight				(21 000)		26.86	
other onion fields										Field
49 444	12.6	90	18 May	a	42.8	BY	42 800	261	67.16	1
53 512	13.2	71.0	21 May	a'	38.0	BC	37 962	194	60.45	3
48 926	13.7	46.0	30 May	a'	22.5	BO	22 495	124	26.88	1
49 404	14.5	61.6	3 June	a'	30.4	GY	30 428	71	12.74	3
48 885	13.6	77.6	11 June	a'	37.9	GO	37 929	38	3.60	3

a. Method of release and number emerging per release site in thousands of flies. Method a: standard method of releasing pupae with dye mark, see Section 3.3.2.1; a': without cover against the rain. Method b: scattering pupae by hand in the grassy verges of the field, evenly dispersed over the length of the field. Method c: pupae buried in the soil, 2-5 cm deep, between the onions near the edge of the field. Method d: as c, but covered with gauze, mesh 3 cm. Method e: released as flies from a pile of flat cages, see Section 3.3.2.2.
b. Dye marks see Section 3.3.1.2.
c. Recaptures per trap per km sheltered edge of onion field, corrected for unmarked steriles, for temperature and for attraction.
d. Flies released on 6, 14 and 19 August, respectively.

experimental field.

Apart from the experimental and control fields all onion fields were treated with trichloronate, either as a seed dressing or applied as granulates. The onions on the experimental and control fields emerged about 15 April, were harvested on 2 September, and taken from the fields in October. The chemical treatments were rather normal, and consisted of insecticide (parathion, 31 July, preventive against the onion moth Acrolepia assectella (Zeller) and the onion thrips Thrips tabaci Lind.), fungicide (zineb/maneb, four times during July) and herbicides (propachloor in April and 27 May; ioxynil on the control plot only, 11 June). The herbicides may have affected the onion fly populations (cf. Godan, 1969).

Releases of steriles In principle the pest control treatment on the experimental field consisted of weekly releases of 100 000 sterilized pupae. The actual number of pupae received deviated generally somewhat from this, sometimes on purpose. There were changes in the release method, because of lack of time or because improvements were developed. Thus, mortality before and during emergence was variable as were the numbers of steriles released per week. The data on the releases are summarized in Table 32. As can be seen there, with proper release methods an average emergence of about 90% can be expected.

During the 1st flight, steriles were released also at the two nearest sources of wild flies, in order to diminish a possible disturbing influence of immigrating wild flies on the experimental field.

Fertile populations present The wild population of hibernating pupae in the experimental field was estimated from pupae samplings on 2.25% of the area at 23 560, with a parasitism of 3.2%. It is supposed that about 90% will have yielded flies in spring. The populations near the control and other fields were unknown and were therefore estimated by mixing them with fertile flies released from diapausing laboratory-reared pupae. The population sizes are given in Section 4.3.3. Because they were calculated from the population in the experimental field, any bias in the estimation of this population is the same in the other populations.

6.2.2 Fly sterility

Sterility data Sterility was indicated by the dye mark applied at release, and for the females also by their ovary development (Section 3.3.1.3). Sometimes because of practical limitations a part of the steriles were released without a dye mark. To account for these a correction had to be applied to the data for the males, which was estimated from the ratio marked:unmarked among the sterile females. During the recapture period of the group dyed BYC there were less unmarked sterile females than was expected, during the recapture period of 0-dyed flies there were more. These numbers of unmarked sterile females captured could be explained by an assumed predation of about 60% on the pupae that had been released simultaneously with the BYC-dyed ones, and an assumed dye recognition of only 1/7 part of the 0-dyed flies. This correction, applied to the unmarked sterile males was an important factor in the period 23 May - 1 July, and it did affect the accuracy of the data.

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Table 33. Average percentage sterility among the flies during the 1st and 2nd flight. Experiment Mijs 1974, Aberrant males excluded.

Site	Flight	% Sterility			% Sterility(releases other than steriles on trial field excluded)		
		males and females	males	effective	males and females	males	effective
exp	1	91.65	89.92	88.54	91.84	90.13	88.83
	2	92.10	90.36	88.44	92.27	90.43	88.09
5	1	57.5	52.4	55.2	60.7	55.7	57.5
	2	42.9	43.3	44.4	47.8	48.7	44.1
con	1(May)	4.3	4.5	5.4	3.9	4.4	5.4
	1	3.5	3.9		3.2	3.9	
	2	0.4	0.3		0.4	0.3	
1	1	71.6	71.6		41.5	40.8	
	2	63.3	49.2		67.9	52.9	
2	1	51.5	41.1		24.9	16.9	
	2	51.3	44.8		49.3	43.5	
3	1	56.9	55.9		27.5	20.8	
	2	32.4	32.7		31.8	32.7	
4	1	2.8	4.2		2.3	3.5	
	2	0.0	0.0		0.0	0.0	
exp/5	1	85.2	97.8		85.4	100.0	
	2	100.0	100.0		100.0	100.0	
exp/1	1	58.5	60.4		43.5	70.4	
	2	99.2	99.0		99.2	99.0	
5/con	1	4.8	12.1		1.2	0.0	
	2	0.0	0.0		0.0	0.0	

The average sterility per field during the 1st and 2nd flight is given in Table 33. The values are also given as obtained after elimination of all releases except those of steriles on the experimental field.

The sterility per 100 flies captured is given in Fig. 88. The figures for the females are less relevant for genetic control, but because of their much higher reliability, as no corrections had to be made, they are also given.

Sex differences Data for both sexes appear to be in moderately good agreement with each other, the sterility of the males on the experimental field being consistently somewhat lower than that of the females. Such a sex difference could have been caused by a higher male or sterile male dispersal activity, but the data did not support this view. Selective predation on sterile males may occur if these remain for a longer period near the emergence site than females. Whether they do so is not known, but the effect would be counteracted by protandry, because insectivorous birds learn where the release sites are. The most probable explanation is found in the difference between the sexes in preference for onion fields, as shown in Fig. 84. Between the two flights the male sterility dropped to between 80 and 85%, whereas the female sterility remained constant. The reasons for this difference are not clear. It may at least partly have been caused by the corrections that were applied. Male sterility decreased at the end of the season because
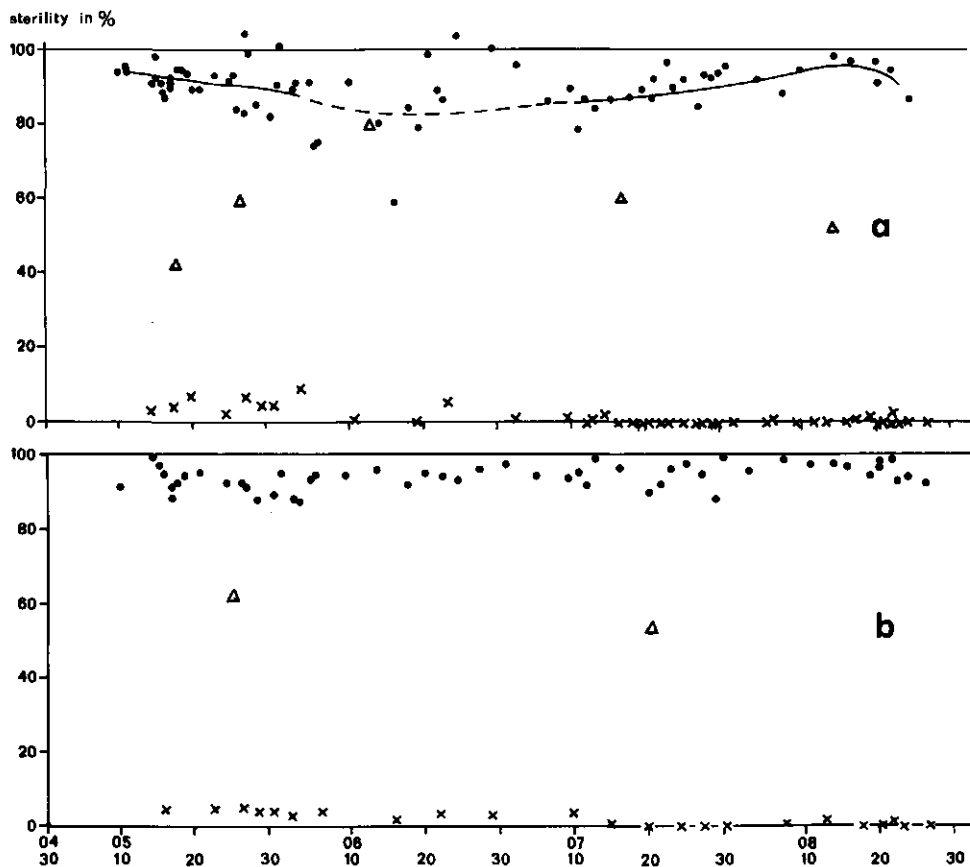
Fig. 88. Percentage sterility versus time. Experiment Mijs 1974. Each point represents 100 flies caught. ● = trial field; △ = onion field 5 (between trial and control field); ✕ = control field.
a. Males, aberrant males excluded. During June corrections for unmarked sterile males are quantitatively important.
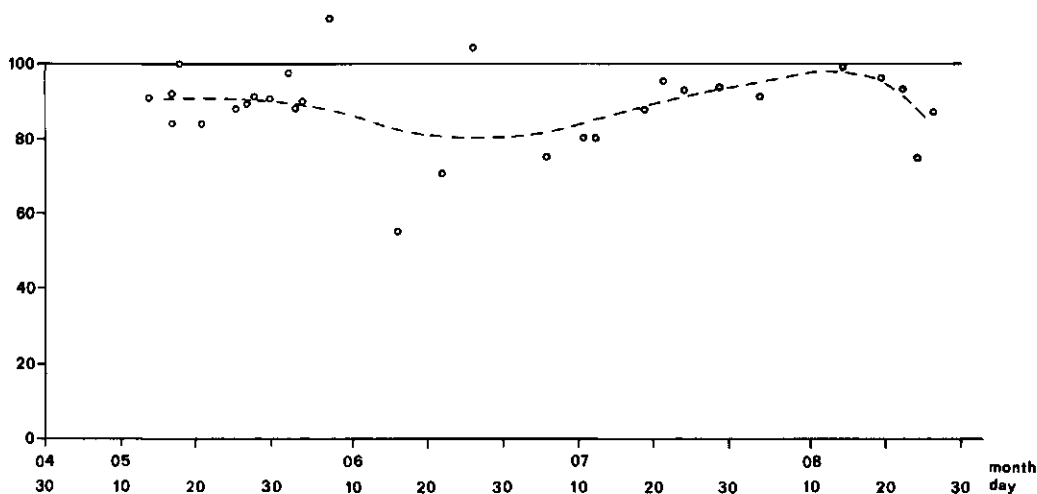b. Females.

Fig. 89. Percentage effective sterility versus time, average values per 10 fertile females caught. Experiment Mijs 1974, trial field. During June corrections for unmarked sterile males are quantitatively important.

of the shorter life-span of males and because releases ended earlier than emergence of wild flies.

Effective sterility The effective sterility (Section 6.1.2) was measured by calculating the percentage sterility among the males for each day and each pair of traps (the traps were usually in pairs close to one another) separately, and the average was weighted according to the numbers of fertile females in these catches. Fertility of females was assessed from ovary development, so young fertile females were not included as such in this calculation. Because they have not yet reached the age of mating, they should be left out of the calculation.

The course of the percentage of effective sterility during the season is given for the trial field in Fig. 89. The flight averages are given in Table 33. During the two flights on the trial field, the effective sterility is on the average 1.2 and 2.2%, respectively lower than the unweighted sterility among the males. On field 5 and on the control field, the effective sterility was not consistently lower than the corresponding unweighted sterility. This result is due to the sensitivity of this method of calculation to random perturbations at lower absolute numbers of males caught per trap location per day, combined with the corrections for the unmarked sterile males.

Because the checking of ovary development had to be stopped in June on most fields as manpower was lacking and because unmarked sterile females were present, often the effective sterility could not be calculated. From Table 28 it can be seen that the difference between weighted and unweighted sterility is not very large. Thus the unweighted sterility has been used where the weighted was not available.

6.2.3 Competitiveness

When the released sterile flies are fully competitive with the wild flies, the sterility among the flies can be calculated from the numbers released and the wild population estimates, after correction for migration. To verify whether the competitiveness was impeded, the number of fully competitive sterile fly equivalents was calculated from the corresponding wild population and the percentage sterility observed. Division by the actually released number gives the competitiveness (Table 34).

Because in this experiment sterility was measured only in the flies caught, and not in the eggs, the competitiveness values calculated apply to the catchable flies only. The mating competitiveness (fly sterility versus egg sterility) was supposed to be 1 (Sections 3.2 and 6.1.3)

The competitiveness of the fertile and sterile flies released elsewhere were calculated in the same way, but these data are less reliable because of the less reliable population estimates involved.

The aberrant males (Section 2.1.2), constituting on the average 4% of the flies, are included in the numbers released. For mass rearing they are a fully incompetitive fraction, lowering the competitiveness in this experiment by 0.01-0.05 (Table 34).

Several competitiveness values are well below 1, especially on the trial field. The values over 1 indicate most probably the inaccuracy of the data rather than a superior

Table 34. Calculation of competitiveness. Experiment Mijs 1974.

Onion field & flight	Wild flies present	Released type[a]		Ratio released to wild	Competitive flies released				Competitiveness[b]		
					present number	%[c]	released calc.	actual	(a)	(b)	(c)
exp 1	17 249	YO-GYO	S	11.255	194 137	74.3	261 288	551 718	0.47	0.49	
2[d]	9 125	GYC-C$_2$[d]	S	11.937	108 925	76.1	143 134	859 963	0.17	0.17	
1[d]	17 249	(YO-O)[d]	S	6.098	105 184	74.3	141 567	249 065	0.57		
con 1[e]	71 048	C	F	0.268	19 041	77.6	24 537	48 191	0.51	0.53	1.15
2[e]	61 201	G$_2$	F	0.390	23 868	80.2	29 761	57 600	0.52	0.54	
1 1	10 715	BY,BO	S	3.030	32 466	47.8	67 921	65 295	1.04	1.08	1.78
1	10 715	G	F	0.545	5 840	47.8	12 218	11 797	1.04	1.08	1.77
3 1	48 088	BC-GO	S	0.935	44 962	76.6	58 697	106 319	0.55	0.57	0.60
1	48 088	Y	F	0.258	12 407	76.6	16 197	13 959	1.16	1.21	1.26

a. Dye marks (see Table 32 and Section 4.3.3), and sterile or fertile.
b. (a) Calculated from preceding figures, (b) aberrant males not included in number released, (c) wild populations calculated from onion field surfaces instead of from sheltered edges of onion fields, see text.
c. From Tables 20 and 21.
d. Steriles only insofar as their combined emergence curve was similar to that of the wild flies.
e. Only after 31 July.

quality of the reared flies. Serious underestimation of the winter mortality would have increased all competitiveness values, resulting in some rather improbably high values. As mentioned before (Section 4.3.3), the use of onion field surfaces instead of sheltered edges in calculating populations from densities also yields unrealistically high competitiveness figures.

The competitiveness could have been low due to different causes: (a) the suboptimal sterile release schedule: a more or less continuous release of steriles compared with a more or less concentrated emergence of wild flies, (b) the rearing history, affecting the performance of the flies and (c) the fate of the flies after emergence, affecting their survival.

a The effect of the suboptimal release schedule will be low, in view of the slight difference between effective and unweighted sterility. Its impact was analysed by using for the calculation of the competitiveness only the sum of fractions of different released cohorts instead of all the first flight steriles. These fractions were chosen in such a way that the combined emergence of this group was about equal to the estimated emergence curve of the wild flies. Then the competitiveness increases from 0.47 to 0.57. This difference might be due to differences in recapture rate of the different sterile cohorts, rather than recaptures of flies released at the wrong moment. The recapture data are summarized in Table 32. The percentage recaptured per trap per km sheltered edge of onion field is for all 1st flight steriles combined 6.48, and for those emerging synchronously with the wild flies 7.63, that is a factor 1.18 higher. The difference in competitiveness was a factor 1.21. Thus it is clear that the asynchronous emergence did not seriously affect the competitiveness.

Another argument stems from the fact that the emergence curve for the 2nd flight is

normally more spread than that for the 1st flight. Then the competitiveness, if lowered because of emergence differences, should be higher during the second flight. Actually it was much lower.

b Often mass rearing affects performance of the flies produced. The only data available on the quality of the reared flies used are those of the pupal weight. The laboratory-reared pupae often weighed less than the wild ones, and the smaller flies emerging from these may have a lowered competitiveness (Section 3.3.1.5). The rather constant average pupal weight and the recapture rate did not show any correlation in the present experiment, so this quality aspect of the flies cannot have been the origin of the diminished competitiveness. It is possible that other, not size-related, quality aspects affecting the competitiveness occur, although it does not seem probable: one has to assume that severe changes in the laboratory conditions occurred which did not affect pupal weight.

c To analyse the effect of the release method, two experiments were carried out during this control experiment: one on the effect of the number of flies emerging per release site, and another on the effect of releasing flies instead of burying pupae in the field. These experiments are described earlier (Section 3.3.2.1). As mentioned there, the changing recapture rate among the sterile cohorts can very well be interpreted as an effect of changing predation pressure. This effect depends on the season, on learning by predators and on the local fly densities produced by the release method. The competitiveness values of the successive cohorts of steriles are given in Fig. 20.

The fertile released groups do not have equal competitiveness values, probably also due to predation: they had a prolonged emergence curve, and the numbers released at C were about four times as high as at G and Y. Moreover, at the release point of C insectivorous birds were observed regularly.

6.2.4 *Egg sterility*

The data on egg sterility, obtained when developing an egg trap, are too scarce to permit any conclusions. The egg sterility can be lower than the fly sterility because of females immigrating between mating and deposition of eggs fertilized by sperm from this mating, and because of diminished mating competitiveness of the males. Because of lack of data the latter factor was assumed to equal 1 (cf. Section 6.2.7).

The effect of immigration on egg sterility can be estimated. From Fig. 86, concerning the female immigrants coming from about 800 m distance, one can deduce that 10-15% of the immigrants immigrate between the first mating and the first oviposition. If for the second reproductive cycle the same ratio is assumed between immigrating before (70 out of 80-85) or after (10-15 out of 80-85) mating preceding egg laying, there will be another 20à15 × 10à15 / 80à85 or about 2.5% of such immigrants. As the first mating may have an effect on the second oviposition, which effect is still unknown, calculations were made with the extreme values of zero and 100% influence, independent of the male being fertile or sterile. Hence a total of 10à15 + 2.5 + (0 or 1) × (20à15 − 2.5) = 12.5 à 17.5% or 30% of the egg depositions can be estimated to originate from matings

outside the experimental field. The about 15% might reflect the actual situation, but 30% is a theoretical upper limit that will not be reached because it implies complete ineffectiveness of subsequent matings, which is not so.

Third and higher reproductive cycles can be neglected (Sections 4.5.2 and 4.5.3). The figures derived above can be applied to all migration distances, because the data from different distances did not differ significantly (Section 5.6.2). The 2.5% of females immigrating after having mated for the second time outside the experimental field were assumed to have mated on an onion field halfway. The egg sterility now can be calculated from the immigrants' origins and the actual fly sterility in the different onion fields, and is given in Table 35.

The differences between fly sterility and the calculated egg sterility are very small during the 1st flight. The effect of the steriles released elsewhere, which was partly nullified by the fertiles released there, was negligible. During the 2nd flight, due to the smaller local population and the rising population on the control plot, the effect of immigrants on the egg sterility was somewhat higher and might even have become serious especially if the 1st mating had a strong effect on the 2nd oviposition.

The seriousness of a reduction of the sterility by migration from 88.44% in the flies to 84.97% or less in the eggs becomes clear when the percentage distribution of the fertile eggs over the fields from which their mothers emerged is looked at: without immigrants the resulting egg population would have been halved, and over a quarter up to one third of the fertile eggs would have been laid by females that came from the big population on the control field: nearly 100 000 flies emerging at nearly 2 km distance. Thus with small local populations immigration may become important in counteracting the population reduction aimed at by sterile male releases (see Fig. 87).

Table 35. Egg sterility on trial field, calculated from the fly sterility in Table 33 and assuming that 15% of the immigrants on the experimental field arrived after having mated elsewhere. Assumed mating competitiveness of 1. Experiment Mijs 1974.

Field of origin	% Of reproducing females coming from this field[b]	Contribution[c] to the total % of egg		% Egg sterility under different assumptions	
		sterility	fertility	(a)[a]	(b)[a]
first flight					
exp	81.1	71.8	9.3		
1	3.4	2.9	0.5		
3	12.0	10.0	2.0		
con	3.5	2.7	0.8		
total		87.5	12.5	86.9	86.3
second flight					
exp	51.2	45.3	5.9		
1	0.9	0.8	0.2		
2	0.6	0.5	0.1		
3	11.4	9.1	2.3		
5	5.3	4.3	1.0		
con	30.6	23.3	7.3		
total		83.3	16.7	82.5	77.6

a. (a) Releases other than of steriles on the experimental field excluded.
   (b) Assuming no effect of 2nd and further matings.
b. From Table 26.

The results of regular damage assessment on the experimental and control field and on a normally chemically treated field are shown in Fig. 46b.

The damage on the control field was clearly above the economic threshold. The onions from this field could still be harvested, but the rotten infested onions made storage inappropriate. The chemical treatment of the adjacent onion field caused the 1st flight offspring to be largely eliminated, so a real control field would have had a much higher damage during the 2nd flight.

The damage on the experimental field remained well below the economic threshold, so from a practical viewpoint the experiment was successful.

6.2.6 Sampling of pupae

In the autumn of 1974 populations were estimated by sampling pupae on the trial and control fields. Of the control field, an area of 30 m wide was sampled, 72% of the untreated field. The results are given in Table 36. Especially the diapausing population on the trial field could not be estimated very accurately due to the very low population: 9 pupae were found, which meant 9 x 191 pupae present.

The diapausing pupae can be offspring either from the 1st of from the 2nd flight. The diapausing fraction of the 1st generation, as determined in plots where only damage was observed before 10 July, was estimated at 25% on the control field and 0% on the trial field. The diapausing fraction of the 2nd flight, determined from plots with only damage after 10 July, was estimated at 80% (data of both fields combined; each diapause percentage given here is based on but about 30 pupae). The remaining 20% must have given rise to a 3rd flight. This 3rd flight cannot be seen clearly in the fly trapping data, both because of the large overlap between the 2nd and the 3rd flight, and also because this flight must have occurred from the end of August onwards, so mainly after trapping had to stop because of the harvest. Half September the weather turned cold and wet and remained so for months. Thus the 3rd flight will have stopped half September, without real reproduction possibilities. The pupae that gave rise to the 3rd flight were found as empty pupae because the samples, taken in the beginning of September, remained in the field and the pupae could not be rinsed out until the second half of September.

This experiment was the first case of genetic control of onion flies in an onion growing area, so the first case where the possibility of niche replenishment by other species could be checked adequately. As mentioned (Fig. 39), the severe selective re-

Table 36. Populations from pupa samples, experiment Mijs 1974.

	Field	Pupae	Allocation to generations
empty pupae at harvest:	exp	9 760 = 1	x 1st gen. + 0.2 x 2nd gen.
	con	125 060 = 0.75 x 1st gen. + 0.2 x 2nd gen.	
diapausing population	exp	1 725 =	0.8 x 2nd gen.
	con	98 820 = 0.25 x 1st gen. + 0.8 x 2nd gen.	

duction of the onion fly population also resulted in a reduction of the bean seed fly population. No other pest filled the emptied niche of the onion fly.

6.2.7 Reproduction

Reproduction on the control field Table 37 contains the relevant data and computation of the reproduction of the flies on the control field. The correction for the flies reproducing on the adjacent chemically controlled field is made according to the lengths of the sheltered edges of the fields. This correction is right when females do not lay their eggs more than 42 m (the control field width) away from these edges. If females do lay their eggs further away, then the correction applied should rather be based on the field surfaces. Then the estimation of the population reproducing on the control plot would be reduced, thus enlarging the reproduction factor. On the adjacent chemically controlled field the 1st generation young larvae were all killed, as can be seen from the damage data (Fig. 46b).

   During the 2nd flight the correction for the effect of the chemically controlled field is less evident. At first it might be questioned whether the flies show a preference for the untreated side of the field, because of attraction by the rotten infested onions there. Comparison of the recaptures of females on both sides of the field indicated no preference. The attractiveness of rotten onions thus will only act at short distances. Secondly, the

Table 37. Reproduction on the control field. Experiment Mijs 1974. Release sites of fertile dyed flies see Fig.15 and Section 4.3.3.

1st flight

populations emerging	wild: 90 800	C: 48 200		
corrected for competitiveness	90 800	24 540		
after pre-reproductive dispersal	49 000	13 250	G&Y: 240	
	total: 62 500			
reproducing on control field	21 800			
corrected for male sterility	20 950			
1st generation pupae	146 000			
1st flight reproduction factor				× 6.97

2nd flight

populations emerging	wild:109 500	G_2:57 600		
corrected for competitiveness	109 500	29 750		
after pre-reproductive dispersal	78 800	21 450		
	total:100 250			
reproducing on control field	34 950			
corrected for male sterility	34 850			
2nd generation pupae	77 900			
2nd flight reproduction factor				× 2.24
when parathion killed 25% of the 2nd flight				× 2.98

3rd flight

population emerging on control field	15 600	
population emerging on chemically treated part	2 400	
assumed reproduction factor 3rd flight		× 0

Total reproduction for a year, assuming 90% emergence of pupae in spring	× 9.98
when parathion killed 25% of the 2nd flight	×12.78

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chemical control is no longer completely effective in summer: Hennequin & Lacroix (1966) found a 100% effectivity of trichloronate during some 100 days. Thus a 3rd flight can have emerged from the chemically controlled field. But as this flight can safely be assumed not to have reproduced, it has no effect on the calculations of reproduction. So the same correction has been applied as in the 1st flight calculations, again keeping in mind that this might slightly underestimate the reproduction factor.

The data are presented with and without taking into account the effect of the parathion spraying, to indicate both the actual reproduction and the reproduction that would have occurred without this disturbance of the experiment.

Reproduction on the trial field Table 38 contains the relevant data and computation of the reproduction of the flies on the trial field. The reproduction factors, after elimination of the effect of the steriles, are only slightly less than on the control field. For the 2nd flight this may seem strange as the reproduction on rotten onions is much better than on normal ones (Section 4.4) and rotten onions were very scarce on the trial field but common on the control field. As mentioned in the preceding section, females do not react to rotten onions over large distances, so they might have had difficulties in finding any. It may be that this adverse effect of low onion fly densities is balanced by a positively density dependent factor as for example predation could be. Assumption of a lowered mating competitiveness of the steriles would result in lower repro-

Table 38. Reproduction on the trial field. Experiment Mijs 1974. Release sites of fertile dyed flies see Fig. 15 and Section 4.3.3.

1st flight				with steriles
population emerging	wild: 21 000			
after pre-reproductive dispersal	11 340 G: 380 Y: 430 C: 260			
	total: 12 400			
corrected for egg sterility(Table 35)	1 550			
1st generation pupae	9 330			
1st flight reproduction factor			× 6.01	× 0.75
2nd flight				
population emerging	wild: 9 330			
after pre-reproductive dispersal	6 720 G₂: 210			
	total: 6 930			
corrected for egg sterility(Table 35)	1 160			
2nd generation pupae	2 160			
2nd flight reproduction factor			× 1.87	× 0.31
when parathion killed 25% of the 2nd flight			× 2.49	× 0.41
3rd flight				
population emerging	wild: 430			
assumed reproduction factor 3rd flight			× 0	× 0
Total reproduction for a year, assuming 90% emergence of pupae in spring			× 8.99	× 0.19
when parathion killed 25% of the 2nd flight			×11.97	× 0.25

reduction in experiment versus control:	1st flight	2nd flight	year
	0.108	0.139	0.020

duction factors calculated for the experimental field, so it can be assumed that the mating competitiveness was not seriously affected.

The reproduction factor on the control field for a year was about 13, which on the trial field could be reduced to 0.25 due to a sterility of about 90% for both generations.

6.3 A NORMALIZED FIELD EXPERIMENT

When the genetic control experiment Mijs 1974 has to be used to predict how sterile males would perform in practical circumstances, it is useful to convert the results into those that can be expected from a more ideal experiment, done as a large area treatment.

In large area treatments, the sterile and fertile populations can be assumed to be distributed more or less homogeneously, so migration has only an effect near the border of the treated area. Table 39 gives results of the experiment Mijs 1974, compared with the results when all migration was eliminated. Absence of pre-reproductive migration causes the reduction to be much more severe.

If a release method were used that prevents initial concentrations of flies, and that has no adverse effect on the emergence, emergence would be 90% and competitiveness about 1. Then the same reduction as in the actual field experiment could have been attained with 405 000 pupae instead of the 1 863 000 actually used. With all migration eliminated, the number of pupae needed would have been 215 000.

Obviously the continuous release of a fixed number of steriles per week is not the most economic. It was realized before, that to obtain an optimal effect the releases during the 1st flight should be higher than in the 2nd flight, because of the decreasing population, the diapausing fraction and the lower reproduction of the 2nd flight. The present partition, 62.4% in the 1st flight, gave a reproduction factor for a year nearly identical to the factor that would have been obtained with the optimum partition.

Table 39. Reproduction on the trial field, experiment Mijs 1974, compared with results to expected if no migration occurred.

	1st Flight		2nd Flight	
	migration present	migration absent	migration present	migration absent
emerging wild population	21 000	21 000	9 330	9 450
reproducing wild population (Table 38)	12 400	21 000	6 930	9 450
competitive steriles present (Table 34)		261 300		143 100
reproduction factor, without steriles (Table 38)	× 6.01	× 6.01	× 2.49	× 2.49
reproduction with steriles, observed (Table 38)	× 0.75		× 0.41	
percentage fly sterility, observed (Table 33)	91.65		92.10	
percentage fly sterility, calculated		92.56		93.81
percentage egg sterility, calculated (Table 35)	87.48		83.32	
percentage egg sterility, calculated		92.56		93.81
reproduction with steriles, calculated		× 0.45		× 0.15
reproduction factor for a year, migration present (Table 38)		× 0.25		
reproduction factor for a year, migration absent		× 0.05		

When applying the sterile insect technique for fly control, some essential informa-
tion is needed about places and times of release and the numbers that should be released.
Most of this information, with regard to the onion fly in the Netherlands, is not yet
available with sufficient accuracy to apply the method reasonably. The relevant para-
meters and their magnitude, however, can be estimated from the data presented.

Only onion growing will be considered, leek growing is not included because the
onion fly is a less important pest on it, and because leek growing is concentrated in
other parts of the country.

Release sites Release sites should be diffuse, to prevent concentrations of steriles
susceptible to predation. Thus it is best to release the flies from some vehicle along
certain release lines. Assuming straight lines, one has to find how far these lines can
be apart so that the density of steriles throughout the area is more or less constant at
the moment they mate. A rough estimate can be obtained from the data in Table 26 and the
distances from Fig. 15. With 50% of the flies in fields nearest to the emergence sites
(up to say 100 m distance), and thereafter a logarithmic decrease (cf. Fig. 81), the re-
lative densities over a transect square on two release lines are shown in Fig. 90 for
different release line distances. Release lines thus should be at less than 1 km distance
of each other. It has to be taken into account that, when releases are made by car in-
stead of by aeroplane, it is even more dangerous to have sites that do not get the mini-
mum safe level of steriles, because the release lines (roads) will have to be about the
same each year.

For estimating the depth of the barrier zone to be treated around an area where con-
trol is by sterile releases, Fig. 87 is useful. In an area with populations of equal size,
an onion free zone of less than about 1 km will cause a noticeable decline in the sterile:
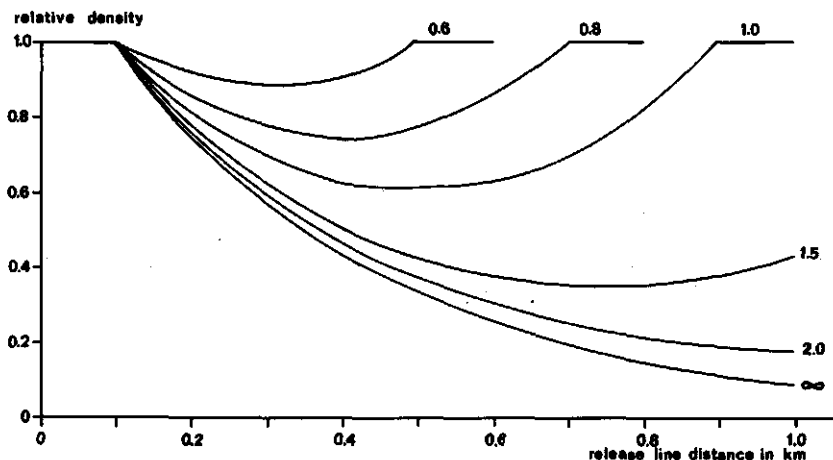fertile ratio among the flies. During the next year of control the barrier should be

Fig. 90. Relative densities of steriles between two release lines, for some release line
distances. Assumptions see text.

shifted outwards, as the difference between the sterile male treated and chemically trea-
ted populations will be increased considerably.

Frequency The minimum frequency of releases needed to get a more or less constant ste-
rile population density should be known. Pupae that were buried in the field weekly gave
the rather regular percentage sterility among the males as observed in the experiment Mijs
1974 (Figs 88, 89). In the Schuilenburg experiments the pupae were also buried weekly and
no cyclic changes were observed in egg sterility. Releases of flies cause much less
spread in emergence as only flies of 1-2 days old can be released (Section 3.3.2.2).
Weekly releases of flies may be not enough as the survival of the males is rather short
especially during the 2nd flight (Fig. 55). It cannot yet be determined how low the
release frequency can be made before the savings in the release costs overrule the expen-
diture on rearing the numbers needed extra to keep the minimum density at a safe level.

Distribution over the flights The partial 2nd flight creates the problem how to distri-
bute the releases over the two flights to obtain an optimum population reduction. The 3rd
flight is neglected here. The reduction has been calculated for different values of the
percentage diapause in the 1st generation (Fig. 91) and for the reproduction factors of
the 1st and 2nd flight (Fig. 92). The reproduction factors are of little influence on the
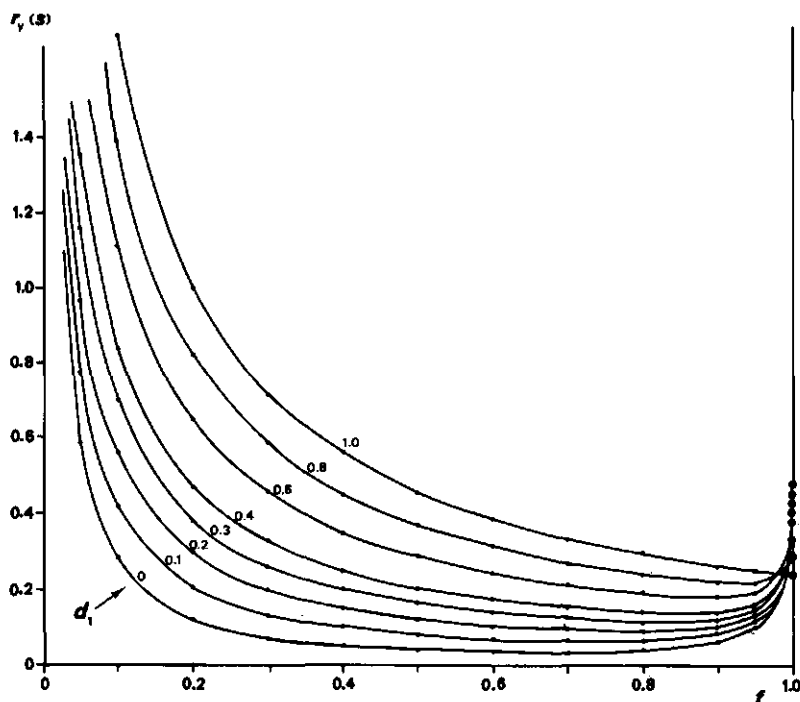

Fig. 91. Graph for choosing a convenient f in relation to d_1.
Actual reproduction for a year, $r_y(s)$, versus fraction of available steriles released in
1st flight (f), for different percentages of diapausing pupae of 1st generation (d_1),
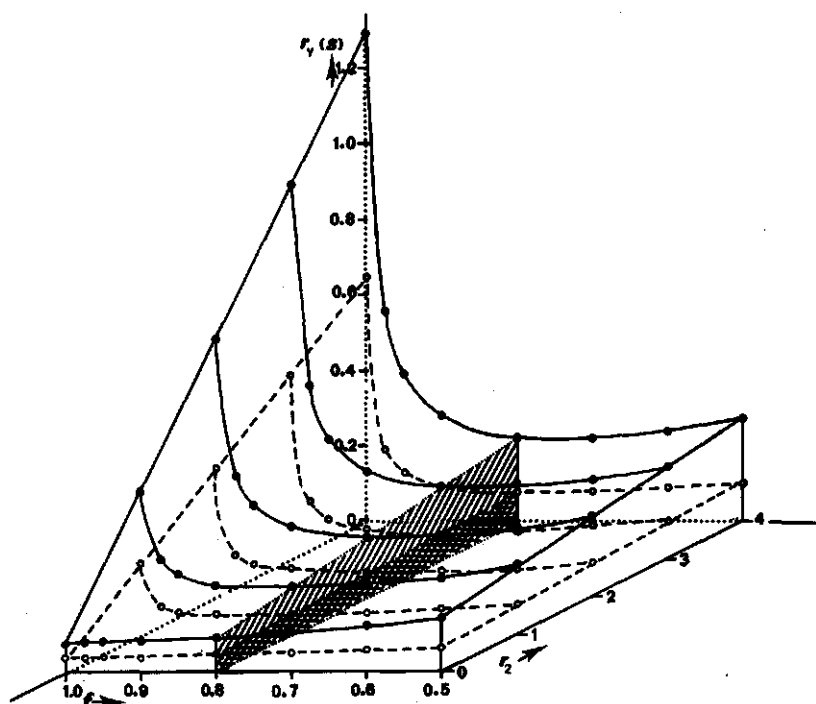assuming available steriles 20x wild population, and reproduction factors $r_1 = 5$, $r_2 = 2$.

Fig. 92. Graph for choosing a convenient f in relation to r_1 and r_2.
Actual reproduction for a year, $r_y(s)$, versus 2nd flight reproduction (r_2) and fraction
of available steriles released in the 1st flight (f), for two levels of the 1st flight
reproduction (r_1), assuming 20% diapause in the 1st generation.
– – – $r_1 = 4$, ——— $r_1 = 8$; cross-section at $f = 0.8$ shaded.

optimum distribution in the range between 60 and 85% of the available flies released in
the 1st flight. As the percentage diapause is normally in the range of 0-30% (Table 10),
about 80% seems the best value.

Number of flies needed At first it will be established how many flies per year are nee-
ded to obtain a certain reduction at the end of the year. The number is expressed as a
ratio to the diapausing population to be treated. It has been assumed that 80% of the
steriles are released during the 1st flight.

    For some assumptions about reproduction factors and the fraction of the 1st genera-
tion that goes into diapause, the effect of different release ratios is shown in Fig. 93.
For the moment let us assume that a release ratio of 20 steriles per fertile is safe
enough and economically feasible. The effect of reproduction factors and 1st generation
diapause on the reduction attained with this ratio is shown in more detail in Fig. 94,
with the reproduction factors observed so far in the field indicated. Obviously, planning
for sterile male releases requires more detailed information on the actual parameter va-
lues.

    Another problem in planning releases is how to distribute the control effort optimal-
ly over subsequent years. The best is to aim at a constant output from mass rearing whilst
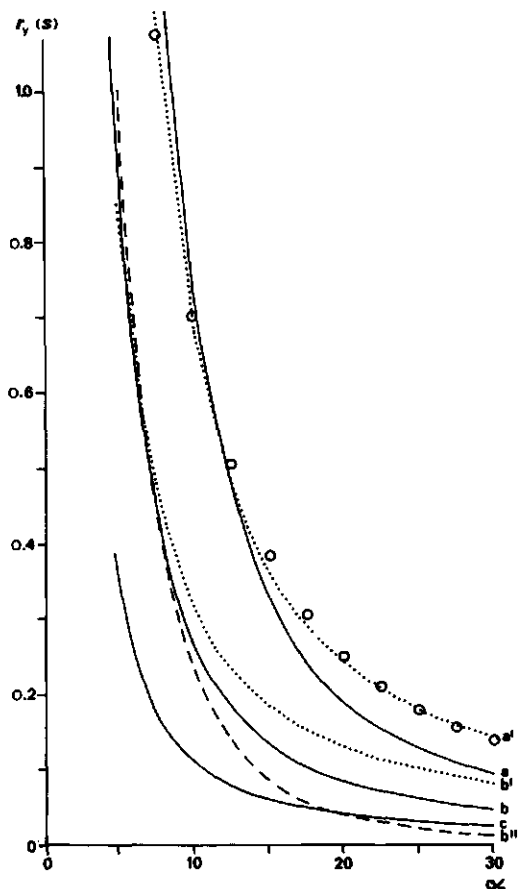the sterile insect technique is introduced in the area under consideration, i.e. the

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Fig. 93. Graph for choosing a convenient α. Actual reproduction for a year, $r_y(s)$, versus the overflooding ratio α (available steriles versus initial wild population), for different assumptions of r_1, r_2 and the diapausing fraction of the 1st generation (d_1), for f=0.8.
a: r_1=8, r_2=3, d_1=0.2; a': d_1=0.4;
b: r_1=5, r_2=2, d_1=0.2; b': d_1=0.4; b": d_1=0.0;
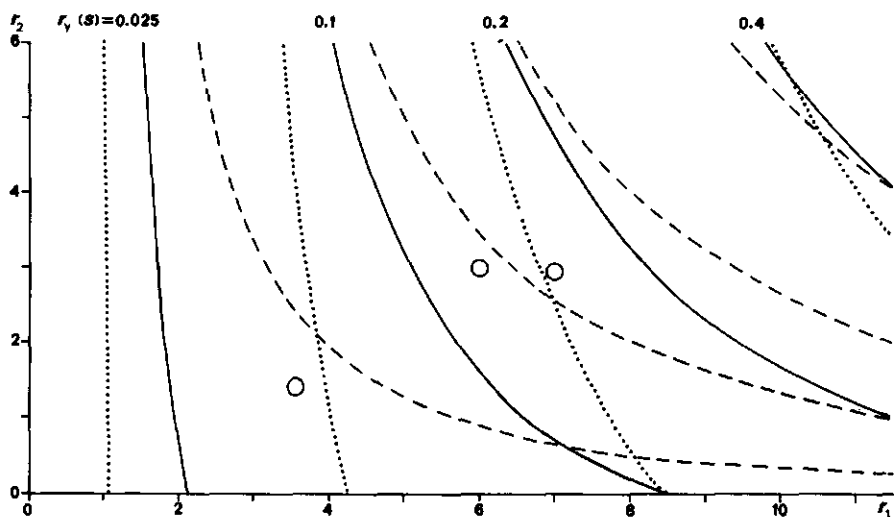c: r_1=3, r_2=1$\frac{1}{2}$, d_1=0.2.

$\bigcirc$ $r_y(s) = 21.432\alpha^{-1.485}$

Fig. 94. Graph visualizing the effects to be attained with $f = 0.8$ and $\alpha = 20$. Actual reproduction for a year, $r_y(s)$, as function of the reproduction factors r_1 and r_2, for three levels of the 1st generation diapause (d_1).
······ d_1= 0.4; ——— d_1= 0.2; − − − d_1= 0.0. Observed reproduction values indicated.

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Netherlands. Let the Allium crop to be treated cover A hectares, with a pest density of p viable diapausing pupae per hectare, and a reproduction factor per year of r. Let the reproduction factor, including the effect of the steriles, be chosen as r', and the number of years during which the method is introduced in practice as n.

Note that here a constant population reduction factor is chosen instead of the often advocated constant release rate. The latter method is the cause of the statement that the sterile insect technique becomes more efficient at low pest densities, as the population is reduced at an increasing rate. However, this application is generally a waste of steriles, unless there are urgent reasons reduce the pest quickly to very low levels.

At first it has to be established what part of the total area should receive its first sterile male treatment per year, when the mass-rearing output is kept at a constant level. The relative numbers needed per area are 1 in the 1st year, r' in the 2nd year, $(r')^2$ in the 3rd year and so on. Assuming the method to be introduced each year in a new area, and of course to be continued where it has been introduced, one finds the fraction a of the total area that is to be treated in the first year:

$$a_1 = 1/(n-(n-1)r') \tag{17}$$

which area is increased every year by

$$a_i = (1-r')a_1 \tag{18}$$

The total amount of flies needed per year is thus $Ap/(n-(n-1)r')$, multiplied by the overflooding ratio required.

Now consider at first the case of a pest with a single generation per year, with population size F and the number of steriles released S. Than $r' = rF/(S+F)$, so the overflooding ratio $S/F = (r/r')-1$. The number required annually, N, is:

$$N = \frac{Ap(r-r')}{(n-(n-1)r')r'} \tag{19}$$

However, due to the presence of a partial 2nd generation this model has to be adjusted. The overflooding ratio, α, is now expressed as the ratio of the total numbers of steriles to be released versus the diapausing population of fertiles. To have a safe value, the upper line in Fig. 93 will be used. This line can very well be approximated by the relation $r' = 21.432 \, \alpha^{-1.485}$ (correlation coefficient 0.998), or, $\alpha = r'^{-0.67}/0.127$. The mass-rearing output required now can be found from:

$$N = \frac{Apr'^{-0.67}}{0.127(n-(n-1)r')} \tag{20}$$

For the situation in the Netherlands, the parameters are estimated as follows. The onion crop covers about 12 000 ha, with fluctuations of about 10% (Table 2). The population density of diapausing pupae has to be fixed at a rather safe level, say 20 000 per ha (Fig. 53). Now N has been calculated according to Eqn (20) for different values of r' and for different options for the number of years during which the method will be intro-
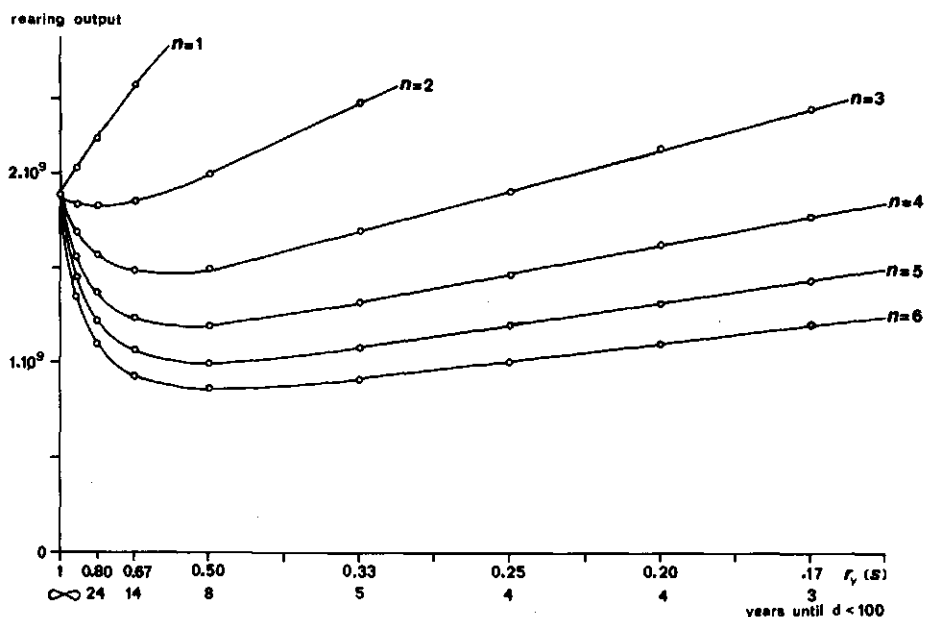
Fig. 95. Required mass-rearing output per year in competitive fly equivalents versus the reduction factor to be chosen, for some numbers of years (n) over which the method is introduced in the Dutch onion growing areas.

duced (Fig. 95). From this graph a reasonable scheme for introduction of the sterile insect technique can be derived, especially as N is directly proportional to the other variables, A and p.

A choice for a higher number of years reduces the required mass-rearing capacity. On the other hand the preferability of the sterile insect technique to the current chemical control, because of its selectivity and lack of pollution and resistance development, will shift the preference to a shortest possible introduction period. A balanced choice may be about 4 years. The annual reduction factor should be safely above 1, and can be chosen at 4. This would mean a mass-rearing output of 1.5 x 10^9 flies annually for 4 years, declining thereafter. Dependent on the competitiveness of the flies, the release method and the percentage emergence of the pupae, the number of pupae to be reared has to be higher by a factor between 1.2 and 20. As indicated at the bottom of Fig. 95 it will take in total 4 years until a diapausing population is reduced to a level of less than 100 flies per ha. After that time probably the most economic procedure is to continue indefinitely with release of standardized low numbers by simplified release procedures. The aim should not be eradication, as this is an unstable situation (Kojima, 1971), unless it is worldwide.

A favourable circumstance is that for the onion fly the sterile insect technique is not disturbed by the current chemical control because with the latter the newly emerged larvae are killed, whereas sterile males prevent the occurrence of many such larvae. As the sterile males result in a more severe suppression of the populations, chemical control will be useless already in the first year of mass releases of sterilized onion flies.

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As indicated in Tables 37 and 38, the parathion spraying, which is generally done once in July or August, reduces the 2nd flight reproduction. Because it will equally kill steriles, it does not impede the sterile insect technique.

6.5 SUMMARY

In the series of experiments on sterile insect technique done in 1971-1974 on the Schuilenburg, the wild population was severely reduced. Reproduction factors were calculated per flight. When pre-reproductive dispersal was taken into account, an average factor of 5 for a year was found. The numbers of sterile flies caught were about 10% of the numbers expected, probably due to predation by insectivorous birds. The egg sterility was generally lower than the fly sterility. This may have been the result of low pupal weights and long storage periods of non-diapausing pupae.

On Overflakkee the experiment Mijs 1974 demonstrated the ecological feasibility of the sterile insect technique for the onion fly in normal practice. The effective sterility on the experimental field was 88.5%. The competitiveness of the steriles, as judged from the recaptures, was well below 1, and is attributed to predation. Egg sterility data are not known, but when equal mating competitiveness of the steriles is assumed, this sterility is expected to have been about 88% in the 1st and 85% in the 2nd flight. Damage on the trial field was only 2.4%, on the control field it was 17.4% although the majority of the 2nd flight did not reproduce on the control field itself because of the set-up of the experiment.

The reproduction factor for a year was 13 on the control field and 0.25 on the trial field. If the sterile flies had a full mating competitiveness, the latter factor would have been 12 with no steriles applied. As this factor can be expected to have been actually lower than the reproduction on the control field, the mating competitiveness of the steriles will have been good.

From the experiment it was calculated that without the pre-reproductive migration, the annual reproduction factor on the trial field would have been 0.05 instead of 0.25.

When the sterile insect technique is applied in practice, release routes are needed and these should be less than 1 km apart. Between treated and untreated populations of equal size a barrier of about 1 km can be considered to be sufficient. The release frequency of once a week worked well when pupae were released.

For an optimum effect, about 80% of the steriles available in a year should be released during the 1st flight. A ratio of steriles released per year to wild populations in spring of 20:1 will be sufficient to achieve reduction by a factor of at least 4. An efficient programme, with a constant mass-rearing output and aiming at a constant reduction factor of 4, requires a mass-rearing capacity of 1.5×10^9 competitive fly equivalents per year. This is based on a safe estimate for the reproduction factor, and on a spread of the introduction of genetic control over 4 years.

7 Concluding remarks

The dispersal pattern of a species inhabiting a discontinuous environment will have
evolved to balance the advantage of reproducing on a not yet inhabited favourable site
with the disadvantage of failure to find a site for reproduction. With a certain rate of
dispersal, scarcity of sites for reproduction will result in more mortality from migra-
tion and thus lower population levels (Huffaker, 1965; Gadgil, 1971). It may be that the
onion fly does not become a serious pest until a certain intensity of onion growing is
reached.

The onion fly dispersal is relatively easy to analyse as the environment can be
clearly subdivided into two levels of average attractiveness: onion fields and other
places. In fly species such a division often cannot be made so easily, as when animals
or wild plant species are important hosts. Dispersal of these species has often been
described in rather general terms, like 'initial wandering flight, resulting in accumu-
lation on attractive sites', and 'most flies remain near the release site whereas few
reach considerable distances' (e.g. Musca domestica: Schoof & Siverly, 1954; Simuliidae:
Baldwin et al., 1975; Delia brassicae: Hawkes, 1972; Rhagoletis cerasi: Boller, 1969;
Lucilia sericata: MacLeod & Donnelly, 1963; Cochliomyia hominivorax: Hightower et al.,
1965).

It is probable that the dispersal of these species is in principle similar to that
of the onion fly. Johnston & Heed (1975) found for Drosophila nigrospiracula dispersal
rate to be highly dependent on the distances between attractive sites, and state that
dispersal should be determined separately within and between attractive areas. As can
be expected, in those cases where the 'attractive sites' are more or less evenly dis-
tributed over the area considered, a pure diffusion model reasonably describes the fly
dispersal observed (e.g. Lucilia cuprina: McIntyre et al., 1946; Drosophila pseudoobscura:
Dobzhansky & Wright, 1943).

The observed degree of dispersal of the onion fly is favourable to genetic control,
as it requires a reasonably coarse release site pattern, and provides on the other hand
a reasonable degree of isolation by distance.

In the Netherlands the onion fly is a serious pest of onions, and it is the only im-
portant insect pest of onions in this country, so that it is relatively easy to introduce
integrated or biological control measures.

Apart from the sterile insect technique, cultural and biological control measures

have not shown much promise for future application. Growing winter wheat after onions (cf. Fig. 19) is the only measure, that may be feasible to reduce damage and the pest density. Increasing the distance between onion fields of subsequent years is not possible for the time being, as it requires less extensive onion growing or stringent co-operation or governmental regulation. The use of cull onions (Section 2.3.3) is only applicable on a small scale or in labour-intensive cropping methods which are for the present too expensive for general use. Positive results from breeding onions for resistance to onion flies (de Ponti, 1976) are not expected in the near future. The non-specific predators and parasites may not be of much help. On the other hand, because of the relatively high damage level that can be tolerated, density dependent factors should in principle be manipulatable to keep populations below the required level. Probably the most promising one, the predator-parasite Aleochara bilineata, has been tested in mass releases in the USSR against the cabbage root fly and the onion fly, but for the latter with insufficient results (Šumakov, unpubl.).

But, whatever control method is applied in future to the onion fly, most of the information presented here will be useful as all control should be based on an understanding of the pest's biology.

7.3 GENETIC CONTROL

The sterile insect technique is sometimes criticized because of some failures in field tests encountered after its successful introduction for the screw worm. However, there are already some seven species in which the method is applied successfully (Lindquist et al., 1974). As indicated by Helle (1972), the reasons for the failures were probably due to haste in developing this method for practice: the conditions which a pest should satisfy were not met, rearing and sterilization methods were inadequate, knowledge of the pest's biology was insufficient or logistic problems in large-scale applications arose. In fact field experiments were done in which, wrongly or prematurely, control was expected instead of information on the feasibility of the method and the way of application. Also, the breakdown of the screw worm control in 1972 was not a failure of the method but of its application: ineffectiveness of the biologically suboptimally reared flies and a decline in intensity of checking for infestation, coinciding with a favourable environment for the pest (much wildlife, mild winter) are supposed causes (R.H. Smith, 1973; LaChance, 1974; Bush et al., 1976).

Fortunately the sterile insect technique as applied on Overflakkee in 1974 was successful as a control measure. From the field work done already and from the Schuilenburg experiments which had indicated the effectiveness of the method, it was clear that insight into the numbers to be released in practice could be gained from releasing steriles in an onion growing area. But no guarantee could be given that the planned releases would indeed result in the degree of control needed.

It is often claimed that no resistance can develop against genetic control. However, as soon as differences between mass-reared individuals and wild ones occur, preferential mating may develop. The establishment of such 'resistance' will be very difficult in situations where wild local populations regularly become extinct, and where survivors are

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constantly mixed with laboratory-reared flies by the residual fertility of the sterilized males (here 0.6%, Section 3.2). In the sterile insect technique programme for the onion fly, new stocks are regularly established from material collected in the field, to reduce inbreeding and to limit effects of selection pressure in the laboratory that are diffe-rent from the field situation.

Nevertheless, should some preferential mating develop, then it can be eliminated by replacing the wild population with a laboratory-reared strain, using the principle of negative heterosis with, for example, chromosomal rearrangements (Robinson & Curtis, 1973; Fitz-Earle et al., 1975). Because of the work on chromosomal rearrangements in the onion fly, this method can be expected to be operational in due time. It can be applied with the type of resistance concerned because only a limited overflooding ratio is re-quired over local and still limited populations.

Another possibility of resistance is that against the sterilizing agent. Resistance to chemosterilants has been found already in an early stage (Hazard et al., 1964; see also Proverbs, 1969 for later cases). Some resistance to sterilization by irradiation could be induced in Aedes aegypti (Stahler, 1971), but in similar experiments with Tribolium castaneum no resistance could be induced within 25 generations (Brower, 1974). Moreover, any such resistance cannot be extrapolated to genetic control as irradiation is applied there only once on a certain population.

A more general problem related to resistance is the creation of empty niches, which are unstable situations in evolution. This is an inevitable circumstance and will employ future generations of research workers.

Genetic control is criticized as not being safe for the environment. This applies especially to chemosterilants, which for that reason are not used in the current onion fly programme although they are effective (Luckmann et al., 1967). There is an important quantitative difference between the radiation used for sterilization and the pesticides used directly in the environment. For practical reasons the possibility of usage of other less controversial sterilizing agents for the onion fly, as heat or u.v. treatments (Riordan, 1964; Proverbs, 1969), have not yet been investigated.

Major limitations in the application of the sterile insect technique are its initial costs and its species specifity. Also, it relies on a reasonable degree of technological development, but with scientific aid and using aerial releases it can in principle be applied everywhere. The costs limit its use to major pests in economically important crops or to medically important disease vectors. The costs will exceed the returns, as with insecticides, in most food crops in developing countries (Ticheler, 1975). The spe-cies specifity is an ecological advantage, but makes the economical aspect even more im-portant. On the other hand, Brader (1975) indicated that the development of integrated control methods is not especially expensive or time consuming when compared to pesticide development.

In Dutch onion growing, about 1.8×10^6 Dfl. is spent annually on insecticides and their application against the onion fly, in a crop yielding, due to fluctuating prices, $30-120 \times 10^6$ Dfl. annually (price level 1974). It has been calculated (de Vries, inter-nal report LEI, the Hague), based on a mass-rearing output of 2.5×10^9 pupae annually during the years of introduction of the method, that genetic control is economically com-

petitive with the current chemical control. Since then, mass rearing has been improved (Noorlander, in prep.), and the introduction strategies were refined (Section 6.4). So the economic prospects are good. However, as it is the first control method in the Netherlands of this type to be applied over larger areas, it still meets objections from fund providers, as can be expected in any new development (Helle, 1972).

Summary

The aim of the study was to provide data on the ecology of the onion fly which is necessary for application of genetic control, with an emphasis on dispersal, and to investigate the feasibility of genetic control under normal field conditions.

Literature data on the onion fly's biology are given, along with some general data on onion growing and onion fly control in the Netherlands.

Most experiments were done on Overflakkee, an onion growing area in the SW of the Netherlands. The prevailing weather could be considered as representative. The flies used in releases were obtained from a laboratory mass-rearing and generally were sterilized. This did not impede their competitiveness.

The flies were marked at emergence with daylight-fluorescent dye-powders. Other marking possibilities were also considered. Generally pupae were released, buried in the soil. This method increased predation on newly emerged flies. Recaptures were made by flight interception traps. Where damage was noted, soil samples were taken for estimating pupal populations. The effect of the sample core diameter was analysed. Other field research methods are treated briefly.

Limited data on the onion fly's niche are given. The possibilities for the prediction of pupal emergence in spring were analysed. Data are presented on the incidence of diapause and on the occurrence of flights. Allium vineale did not sustain an onion fly population. Delia platura was a secondary pest as could be seen from its severe reduction by the selective elimination of the onion fly. The infection rate of adult onion flies by Entomophthora was estimated.

The distribution of damage and pupae conformed to the negative binomial. From this distribution, from Taylor's power law and from the relation of the mean crowding to the mean, precision was calculated. The values obtained, and the confidence limits calculated from these, are not reliable for the current low population levels and high degrees of aggregation, and for the low numbers of samples needed in practice. Most densities of diapausing pupae in chemically treated fields are between 1 000 and 20 000 per ha.

The life-spans of the flies conformed to normal distributions. From these data, combined with the frequencies of oviposition periods, as estimated from the frequencies of different phases in the gonadotrophic cycle versus age of the females, fecundities were calculated at 39 and less than 18.5 eggs per female for the two flights, respectively. The corresponding reproduction factors, estimated from field trials with sterilized insects, were 7 and 3.

Dispersal was found to be dependent on temperature, but in general independent of wind direction. Dispersal was described by a diffusion process. The diffusion coefficient has been estimated in different ways. The best results were obtained from simulation of fly diffusion with heterogeneity in time and space. This yielded diffusion coefficients

of about 2 000 m^2/day in onion fields and 14 000 m^2/day outside onion fields.

The relation of the logarithm of the total numbers recaptured versus distance was found to be about linear, both from field observations and from a simple model in which diffusion and normally distributed life-spans were assumed. Migration was reduced during periods of mating and oviposition. At the age of mating nearly half of the males had emigrated from the release field. Immigration of females between mating and oviposition was rare. The impact of surrounding wild populations on a single field treated by sterile males is quantified.

From field trials on sterile insect technique, done near Wageningen, the reproduction of wild flies was estimated at about 5 for a year. There were indications of severe predation on newly emerged sterile flies, and of a reduced mating competitiveness.

The sterile insect application under normal practice circumstances on Overflakkee is described in detail. The reproduction factor on the check plot was 13 for a year, and was reduced on the trial field to 0.25 by an effective sterility of 88.5%. Predation on the newly emerged steriles was severe, but the mating competitiveness of the sterile flies seemed good. As a control the steriles were successful.

For practical application of sterilized onion flies, release routes should be less than 1 km apart, and barriers between equal populations should be about 1 km wide. Per year, 20 times the wild diapausing population should be released, 80% during the 1st flight. An efficient introduction of the sterile insect technique for the onion fly in the Netherlands will require 1.5 × 10^9 competitive fly equivalents for four years, and less thereafter.

Some criticism against genetic control is considered, and possibilities for onion fly control in the Netherlands are indicated.

Samenvatting

Doel van het onderzoek was het verzamelen van gegevens over de ecologie van de uievlieg, Delia antiqua (Meig.), ten behoeve van de ontwikkeling van de bestrijding met gesteriliseerde mannetjes, en tevens om deze methode onder praktijkomstandigheden te testen. De nadruk lag op onderzoek naar de migratie van de vliegen.

De uievlieg overwintert als pop in diapauze en heeft meestal één volledige en één onvolledige vlucht per jaar. De eieren worden gelegd op uien. De bol wordt gegeten door de larven en gaat dan rotten waardoor een oogst geheel verloren kan gaan. De gangbare bestrijding is chemisch en de uievlieg heeft al tegen verschillende middelen resistentie ontwikkeld.

Het veldwerk werd verricht in de jaren 1970-1974 op Overflakkee, waar op 5-15% van het land uien geteeld worden. De weersomstandigheden waren normaal. De vliegen die gebruikt werden voor 'loslaatproeven' werden massaal gekweekt op het laboratorium. Desgewenst werden ze gesteriliseerd door bestraling. Hierdoor werd 99.4% steriel van de eieren gelegd door onbehandelde vrouwtjes na paring met gesteriliseerde mannetjes.

De vliegen werden gemerkt door ze bij het ontpoppen een laagje daglicht-fluorescerende kleurpoeder te laten passeren. Gesteriliseerde vrouwtjes kunnen worden herkend aan de niet verder ontwikkelde ovariën. Meestal werden de vliegen in het veld gebracht door de poppen in een berm van het uieveld in te graven.

Voor het vangen van vliegen werden gazen vangschermen gebruikt. Met een lokstof kon de vangst hierin meer dan verdubbeld worden. De beste plaats voor vangschermen is aan beschutte randen van de uienvelden, met de opening van het veld af. De grootte der vangst was sterk afhankelijk van de temperatuur; de windrichting was van weinig invloed.

Een grondmonster van 20 cm doorsnee, rondom een aangetaste ui, bevat vrijwel alle poppen die als larf uit die ui gekropen zijn om zich te verpoppen. Een monster van 5 cm doorsnee bevat de helft of minder van die poppen, afhankelijk van de mate van schade.

Bij het waarnemen van uievliegen in het veld bleek hun vliegrichting onafhankelijk te zijn van de richting waarin de waarnemer zich bevond.

Het voorspellen van het begin van de eerste vlucht leek mogelijk, zowel op grond van bodemtemperaturen als op grond van fenologische paralellen (b.v. uitlopen van de zomereik). Laboratoriumgegevens over de inductie van diapauze door middel van korte daglengte en lage temperatuur komen overeen met veldwaarnemingen wanneer wordt aangenomen dat de effectieve daglengte 1-2 uur korter is dan de tijd tussen zonsopgang en zonsondergang. Van de eerste vlucht gaat gemiddeld 20% in diapauze, van de tweede vlucht 85%.

De bonevlieg, Delia platura, bleek in uien onder de huidige praktijkomstandigheden een secundaire plaag te zijn. De parasitoiden Aleochara bilineata, Aphaereta minuta en Phygadeuon sp. werden in kleine percentages uit uievliegpoppen gekweekt. s'Zomers worden vliegen aangetast door de schimmel Entomophthora sp. In 1974 werd per dag 6% van de jonge

vliegen hierdoor geïnfecteerd, voor oudere vliegen liep dit terug tot 3%.

De schade aan uien wordt vooral veroorzaakt door de larven van de eerste generatie in juni. De verdelingen over de proefstroken, zowel van schade als van poppen, konden redelijk worden beschreven als negatief binomiale verdelingen met de parameter k afhankelijk van het gemiddelde. Het aantal monsters dat nodig is om een zekere graad van nauwkeurigheid te krijgen is berekend als functie van dit gemiddelde. Hierbij is de variantie geschat op verschillende manieren: met de negatief binomiale verdeling, met Taylor's 'power law' en met het verband tussen de 'mean crowding' en het gemiddelde. Dit zijn allen benaderingen die voor de beperkte aantallen monsters en de lage gemiddelden die in de praktijk verwacht kunnen worden, vrij onbetrouwbare betrouwbaarheidsintervallen opleveren.

Met verschillende terugvangproeven met gemerkte vliegen werden populaties geschat. Gezien de schade op praktijkvelden kunnen de populaties overwinterende poppen daar geschat worden op 1000 à 20 000 per ha. Rekening houdende met migratie en met de geringe kans op het vangen van jonge vliegen zijn uit de terugvangsten overlevingscurven bepaald. De vrouwtjes leefden gemiddeld bijna anderhalf maal zolang als de mannetjes, en wel gemiddeld 18 dagen in de eerste vlucht (mei-juni) en 9 dagen in de tweede vlucht (juli-augustus) en maximaal één à anderhalve maand.

De vrouwtjes paren vanaf ongeveer een week na uitkomst. Uit de verdeling van het percentage steriliteit van eieren, gelegd door wilde vrouwtjes in veldproeven met gesteriliseerde uievliegen, kon geschat worden dat ze elke 5 à 6 dagen paren. In de cyclische ontwikkeling van de ovarien kunnen verschillende fasen worden onderscheiden. Uit het verloop van de frequenties van deze fasen in de tijd is gevonden dat de eilegperioden één tot anderhalve week uiteen liggen. Gecombineerd met de overleving volgt hieruit, dat in de eerste vlucht een vrouwtje gemiddeld 39 eieren legt, en minder dan half zo veel in de tweede vlucht.

Uit de gegevens van de veldproeven met de steriele-insektenmethode kon de reproduktie (van pop tot pop) worden geschat. Op Overflakkee werden reproduktiefactoren gevonden van 7 (eerste vlucht) en 3 (tweede vlucht). Op zware grond bij Wageningen lagen deze faktoren half zo hoog.

De netto verplaatsingssnelheid van de vliegen was wel afhankelijk van de temperatuur, maar slechts in geringe mate van de windrichting. Buiten uievelden lag deze snelheid 2,5 maal zo hoog als binnen uievelden. Het water rond Overflakkee kon worden opgevat als een spiegelende barrière voor de vliegen. Het rondvliegen van de vliegen werd beschouwd als een diffusieproces. De diffusiecoëfficiënt is onder andere geschat uit de frequenties en de afstanden van in het veld waargenomen vluchten, en uit de toename van de afstand van het loslaatpunt waarbinnen de helft van de populatie zich bevond. Nauwkeuriger schattingen waren mogelijk met een simulatiemodel, waarin rekening kon worden gehouden met onregelmatigheden in de ruimte (uievelden) en in de tijd (uitkomstcurve, weersomstandigheden). Er werden diffusiecoëfficiënten gevonden van 14 000 m^2/dag, verminderd tot 2 000 m^2/dag in uievelden.

De logaritme van de totale terugvangsten, uitgezet tegen de afstand vanaf het loslaatpunt, gaf bij benadering rechtlijnige verbanden. Dit werd ook gevonden uitgaande van een normale verdeling van de levensduren en een diffusie van de vliegen in een homogeen milieu. Onder andere een onregelmatige verdeling van uievelden geeft afwijkingen van dit

verband.

Uit de verdeling van de vliegen over verschillende plaatsen, uitgezet tegen hun leeftijd, werd gevonden dat de vrouwtjes zich vooral concentreren op uienvelden gedurende perioden van paren of eieren leggen. Uit de leeftijdsverdeling van vliegen op het tijdstip dat ze van elders een uieveld binnen kwamen, volgde dat ongeveer 70% van deze immigranten aankwam voordat ze gepaard hadden. Wanneer ze paren heeft inmiddels 50% van de dan nog levende mannetjes het uienveld waarin ze uitkwamen of werden losgelaten verlaten. Ruwweg kan het aantal vóór paring geïmmigreerde mannetjes op een uienveld gevonden worden door een kwart van het aantal elders uitgekomen mannetjes te delen door een factor 10 voor elke 0,8 km afstand van waar ze uitkwamen. Het aantal vrouwtjes dat immigreert tussen paring en eileg is 1/5 van dit aantal mannetjes.

Uit de serie veldproeven bij Wageningen met de steriele-insektenmethode ter bestrijding van de uievlieg bleek dat de methode in het veld aan de verwachtingen voldeed. De veldproef op Overflakkee in 1974 toonde de bruikbaarheid aan van deze methode onder praktijkomstandigheden. De schade op het proefveld was 2,4% en op het onbehandelde controleveld 17,4%, ondanks het feit dat daar de tweede vlucht ten gevolge van de proefopzet grotendeels uitgeschakeld werd. De economische schadedrempel ligt omtrent 10%. De reproduktiefactor op het controleveld was 13 op jaarbasis, op het proefveld beperkt tot 0,25 ten gevolge van een effectieve steriliteit van 88,5%. Afgezien van predatie door vogels ten gevolge van de toegepaste loslaatmethode, waren de in het veld gebrachte steriele vliegen competitief met de wilde.

Bij praktijktoepassing van de steriele-insektenmethode ter bestrijding van de uievlieg zijn loslaatroutes nodig op minder dan 1 km afstand van elkaar. Een bufferzone van ongeveer 1 km breedte om het met steriele vliegen behandelde gebied zal in het algemeen voldoende zijn. Een optimaal effect wordt verkregen wanneer 80% van de in een jaar beschikbare steriele vliegen in de eerste vlucht wordt losgelaten. Met een verhouding van steriele (per jaar) tegen fertiele vliegen (in de eerste vlucht) van 20:1 kan de populatie in één jaar tot een kwart of minder worden teruggebracht. Een efficiënt programma waarbij de methode verdeeld over 4 jaar in Nederland wordt geïntroduceerd, vereist een produktie van 1.5×10^9 vliegen per jaar.

De vliegen vertonen een verspreidingsgedrag dat geschikt is voor het toepassen van de steriele-insektenmethode: het maakt een redelijk grof loslaatpatroon en een eenvoudige isolatie door afstand mogelijk. Andere niet-chemische bestrijdingsmethoden voor de uievlieg tonen nog niet veel perspectief. De steriele-insektenmethode heeft het voordeel dat resistentie en verstoring van het milieu hierbij geen probleem vormen. Beperkingen zijn echter de afhankelijkheid van een behoorlijke technologische ontwikkeling, de soortspecificiteit, de beginkosten, en op het ogenblik ook nog de problemen van het introduceren van een nieuwe methode.

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Appendix

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TITLE    MIGRATION ONIONFLY
*        LOOSJES-FRISSEL MAY 1976 VERSION 5 GRID 11 * 26
INITIAL
NOSORT

PARAMETER DX =50
FUNCTION EMERGT = 0.,1., 0.001,0., 30., 0.
* EMERGENCE AS F(TIME)
FIXED NC
STORAGE NC(286)
STORAGE SOURCE(286)
*      NUMBER OF EMERGING FLIES PER GRID CELL PER FLIGHT
TABLE SOURCE(1-286) = 193*0., 100000., 92*0.

STORAGE PREF(286)
*      PREFERENCE FOR CELLS
FIXED N
     DO 2 N = 1,286
2       PREF(N) = 1.
* PREFERENCE FOLLOWING FIELDS REDEFINED
PARAM PR = 0.1
PREF(106) = PR
PREF(194) = PR
PREF(205) = PR

PARAM DD = 7000.
*      DIFFUSION COEFFICIENT, M**2 PER DAY

FUNCTION DECAYT = 0., 0., 18., 0.
*      DECAY = MORTALITY FRACTION PER DAY, A F(TIME)
STORAGE TEMP(18)
TABLE TEMP(1-18) = 18*20.
FUNCTION DISPFT = 0.,1., 18.,1.
* DISPERSION FACTOR ACCOUNTS FOR VARIATION IN SPEED DUE TO TEMPARTURE VARIATIONS

STORAGE OUTT(5)
FIXED J
     J = 1
*      OUTT=INDICATOR FOR OUTPUT IN MAP
TABLE OUTT(1-5) = 3.5, 4.5, 5.5, 6.5, 7.

C = INTGRL(0., DC,286)

                                          TELLER = 0
PARAM SKIP = -1.
DYNAMIC
NOSORT
                                          TELLER=TELLER+1.
FIXED JJ
JJ = TIME + 1.00001
DISPF = AFGEN(DISPFT, TEMP(JJ))
D = DD*DISPF

*      CALCULATION OF EMERGENCE
*      ========================
*      EMERGENCE ONLY ONCE PER DAY
EMERG = 1./DELT*AFGEN(EMERGT, TIME)
STORAGE EMER(286)
DECAY = AFGEN(DECAYT, TIME)
          SEMERG = 0.
       DO 10 N = 1,286
          EMER(N) = EMERG*SOURCE(N)
10        SEMERG  = SEMERG+EMER(N)
                          TEMERG = INTGRL(0.,(SEMERG-DECAY*TEMERG))
* TEMERG = POPULATION ALIVE
```

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```
*      CALCULATION OF DIFFUSION                    (SYMBOL IN)
*      =================================
PARAM WATER = 0.
FIXED H, V, HV

* HORIZONTAL IN (POSITIVE LEFT-TO-RIGHT)
      DO 9 H = 1,12
      DO 9 V = 1,27
          DO 9 HV= 1,2
9         IN(H,V,HV) = 0.
* LEFT SIDE
      DO 11 V = 1,26
          IN(1,V,2)=(D*(QC(1,V)*QPREF(1,V)-QC(2,V)*QPREF(2,V))/DX**2)...
          *INSW(QC(1,V),0.,1.)*(QC(1,V)*QPREF(1,V)/(QC(2,V)*QPREF     ...
          (2,V)+0.00001)
* INSW TO PREVENT NEGATIVE QC VALUES
11        IN(1,V,2) = FCNSW(IN(1,V,2), IN(1,V,2),0.,0.)
* FCNSW TO PREVENT ACCIDENTAL IMMIGRATION

* CENTRAL
      DO 12 H = 2,11
      DO 12 V = 1,26
12        IN(H,V,2) = (D*(QC((H-1),V)*QPREF((H-1),V)-QC(H,V)*QPREF   ...
                       (H,V))/DX**2)
* RIGHT SIDE
      DO 13 V = 1,26
      IN(12,V,2)=(D*(QC(10,V)*QPREF(10,V)-QC(1,V)*QPREF(11,V))/     ...
      DX**2)*INSW(QC(11,V),0.,1.)*(QC(11,V)*QPREF(11,V))/(QC(10,V)  ...
      *QPREF(10,V)+0.00001)
      IF(WATER .EQ. 1.) IN(12,V,2) = 0.
      IF (WATER .EQ. -1) IN(12,V,2) = (D*(QC(11,V)*QPREF(11,V) -0.) / ...
      DX**2)
13        IN(12,V,2) = FCNSW(IN(12,V,2),0.,0.,IN(12,V,2))

* VERTICAL IN (POSITIVE TOP-TO-BOTTOM)
* TOP SIDE
      DO 14 H = 1,11
      IN(H,1,1)=(D*(QC(H,1)*QPREF(H,1)-QC(H,2)*QPREF(H,2))/          ...
      DX**2)*INSW(QC(H,1),0.,1.)*(QC(H,1)*QPREF(H,1))/(QC(H,2)       ...
      *QPREF(H,2)+0.00001)
14        IN(H,1,1) = FCNSW(IN(H,1,1),IN(H,1,1) ,0.,0.)
* CENTRAL
      DO 15 V = 2,26
      DO 15 H = 1,11
15        IN(H,V,1) = (D*(QC(H,(V-1))*QPREF(H,(V-1))-QC(H,V)*        ...
                       QPREF(H,V))/DX**2)
* BOTTOM SIDE
      DO 16 H = 1,11
      IN(H,27,1)=(D*(QC(H,25)*QPREF(H,25)-QC(H,26)*QPREF(H,26))/     ...
      DX**2)*INSW(QC(H,26),0.,1.)*(QC(H,26)*QPREF(H,26))/(QC(H,25)   ...
      *QPREF(H,25)+0.00001)
16        IN(H,27,1) = FCNSW(IN(H,27,1),0.,0.,IN(H,27,1))

*      DEATH RATE
*      ==========
      DO 31 H = 1,11
      DO 31 V = 1,26
31        DR(H,V) = DECAY*QC(H,V)

*      CALCULATION TOTAL CHANGES
*      =========================
      DO 32 H = 1,11
      DO 32 V = 1,26
      N = H + (V-1)*11
32        DC(N)   = IN(H,V,1)+IN(H,V,2)-IN(H+1,V,2)-IN(H,V+1,1)     ...
          -DR(H,V) + EMER(N)

*    PREPARE OUTPUT
*    ==============
IF(KEEP .NE. 1) GO TO 301
IF (TIME .GE. (OUTT(J)-0.0001)) GO TO 300
GO TO 301
300 CONTINUE
      IF((FLOAT(J)/2.-J/2)   .GT. 0.) WRITE(6,306)
      WRITE(6,302)
      WRITE(6,303)TIME , TELLER,   TEMERG
      WRITE(6,302)
DO 333 N = 1,286
333 NC(N) = C(N)+0.5
      WRITE(6,305)NC
      306 FORMAT(1H1)
      302 FORMAT(1H )
      305 FORMAT(11(I8  ))
      303 FORMAT(29H FLIES PER GRID CELL AT TIME  F6.2,9X,6HTELLERF7.0,
     $ 5X,16HTOTAL POPULATION   F7.0)
      WRITE(6,302)
  J = J+1
 301 CONTINUE
```

```
METHOD RECT
TIMER FINTIM = 18., OUTDEL = 1., DELT = 0.125
TERMINAL
NOSORT

IF(SKIP .LT. 0.) GO TO 500
* MAPS OF INPUT CONDITIONS IF WANTED
500   CONTINUE
END
* OTHER PARAMETER VALUES IF WANTED, FOR SUBSEQUENT RUNS
END
STOP
```

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