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THE ISOLATING EFFECT OF GREENHOUSES ON ARTHROPOD PESTS:  
A CASE-STUDY ON *CLEPSIS SPECTRANA* (LEPIDOPTERA: TORTRICIDAE)



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Proefschrift

ter verkrijging van de graad van  
doctor in de landbouwwetenschappen  
op gezag van de rector magnificus,  
dr. C.C. Oosterlee,  
hoogleraar in de veeteeltwetenschap,  
in het openbaar te verdedigen  
op vrijdag 28 januari 1983  
des namiddags te vier uur in de aula  
van de Landbouwhogeschool te Wageningen.

## STELLINGEN

1.

Uitdrukkingen als "verbreking van diapauze" en "re-activatie van het diapauzerende insect" zijn misleidend, omdat ze suggereren dat het insect in diapauze fysiologisch inactief is.

Beck, S.D. (1980). Insect photoperiodism 2nd edition.  
Academic Press, New York.

2.

Genetische bestrijding van kaspopulaties van *Clepsis spectrana* door het inkruisen van diapauze heeft geen kans van slagen.

Dit proefschrift.

3.

Succesvolle toepassing van de verstoringstechniek ter bestrijding van schadelijke insecten, is in kasteelten wellicht mogelijk.

Dit proefschrift.

4.

Men kan het bestaan van een afzonderlijk biotype van *Clepsis spectrana* in verwarmde kassen opvatten als fase in een proces dat kan leiden tot de vorming van een nieuwe soort. Men zou dit proces dan moeten omschrijven als soortvorming onder kunstmatig geschapen allopatrische condities.

5.

Een optimale toepasbaarheid van het onderzoek naar geïntegreerde bestrijdingsmethoden van ziekten en plagen kan slechts gegarandeerd worden indien een goede wisselwerking tussen onderzoek, voorlichting en praktijk aanwezig is.

6.

Het zoeken naar bruikbare alternatieven voor methylobromide dient hoge prioriteit te hebben. Toch is een algeheel verbod op het gebruik van dit middel met ingang van 1 september 1983 ongewenst. Een selectief toelatingsbeleid verdient de voorkeur.

7.

Onderzoek naar de mogelijkheden om natuurlijke vijanden van schadelijke insecten en mijten resistent te maken tegen bestrijdingsmiddelen, dient te worden bevorderd.

8.

In het licht van recente ontwikkelingen is de omschrijving van doeltreffende toelatings-eisen voor andere dan chemische bestrijdingsmiddelen dringend gewenst.

9.

Bij de toelating van nieuwe bestrijdingsmiddelen in teelten waarin geïntegreerde bestrijdingsschema's goed functioneren, dient na toetsing de voorkeur uit te gaan naar middelen welke bruikbaar zijn in die schema's.

10.

Financiering van niet-militaire wetenschappelijke activiteiten door de NAVO is ongewenst.

Proefschrift van J. van den Bos

The isolating effect of greenhouses on arthropod pests: a case-study on *Clepsis spectrana* (Lepidoptera: Tortricidae).

Wageningen, 28 januari 1983

Cover photo:

Rose house in Aalsmeer (+ 1905). The walls were only man high, and the glass roof was removed in summer. In early spring, coal stoves were sometimes used for heating, to hasten the flower production.

*Aan mijn Ouders  
Aan Coby*

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## LIST OF ABBREVIATIONS AND SYMBOLS

### STATISTICS

P	probability level
NS	not significant ( $P > .05$ )
S	significant ( $P \leq .05$ )
n	number of observations
S.D.	standard deviation
S.E.	standard error of the mean
r	correlation coefficient (Pearson)
t	t-statistic
$\chi^2$	chi-square statistic

### MEASURES

$^{\circ}\text{C}$	degrees Celsius
cm	centimetre
h.	hours
Hz	Hertz
LD	duration of the light period : duration of the dark period, in the 24-hour cycle
log.	logarithm (base 10)
m	metre
$\mu\text{m}$	micrometre
mm	millimetre
$\text{m}^2$	square metre
sec.	second
W	Watt

### SYMBOLS

♀♀	females
♂♂	males
L1	first larval instar



## 1. INTRODUCTION

### 1.1 THE *CLEPSIS SPECTRANA* PROBLEM

The leaf-roller *Clepsis spectrana* Treitschke (Lepidoptera: Tortricidae, Tortricinae) (syn.: *Cacoecia costana* F., *Tortrix costana* Schiff.) is indigenous in the Netherlands. In the field, it is bivoltine (Graaf Bentinck & Diakonoff 1968). The second generation hibernates in the larval stage (Balachowsky 1966). In the open air, the larvae only occasionally cause damage (Alford 1976). In Dutch greenhouses, however, the species is an important source of economic damage. In greenhouse roses (*Rosa hybrida*) it is even the most important arthropod pest (Van de Vrie 1976). *C. spectrana* is reported to damage greenhouse roses in other countries: Denmark (Pape 1964), Germany (Feiter & Henseler 1971), and England (Burgess & Jarrett 1978), but in contrast to the situation in the Netherlands, in these countries it is considered to be a minor pest.

In greenhouses in the Netherlands, growth and reproduction of *C. spectrana* continue without diapause during winter. This has been observed on roses, *Gerbera*, *Alstroemeria*, and Bromeliaceae (Van de Vrie 1978, and pers. comm.). This may be due to a genetic adaptation to the environmental conditions in these heated greenhouses, where the difference between summer and winter temperatures does not exceed a few degrees, and suitable food is available all the year round. Artificial illumination is not given in these greenhouses, thus the daylength is always the same as outdoors.

Traps baited with synthetic sex pheromone, that are effective in capturing males in the field (Minks et al. 1973), always give very poor results in greenhouses (Van de Vrie 1976). Thus the pheromone released by females occurring in greenhouses may be different.

These phenomena give rise to the hypothesis that a separate greenhouse biotype of *C. spectrana* exists. Whether this biotype can maintain itself depends on the degree of isolation between field and greenhouse populations.

Dutch greenhouse populations of *C. spectrana* have acquired reduced sensitivity to organophosphorus compounds and trichlorophon (M. van de Vrie, pers. comm.). The uninterrupted development and overlapping generations in greenhouses necessitate regular treatments with insecticides.

Another major pest in greenhouse roses, the two-spotted spider mite, *Tetranychus urticae* (Koch), can be controlled effectively in rose houses using phytoseiid predators (M. van de Vrie, pers. comm.). Biological control of *T. urticae* is only applicable if *C. spectrana* is controlled in a way that does not affect the predatory mites.

In order to find answers to the following questions investigations were undertaken: (a) does a separate greenhouse biotype of *C. spectrana* exist, and what are the characteristics of this biotype? (b) What mechanisms govern the isolation of field and greenhouse populations? (c) To what extent are these mechanisms of importance for the non-chemical control of *C. spectrana* populations in greenhouses?

The following discriminating properties were investigated in both field and greenhouse strains: non-diapause development (chapter 4); induction, maintenance, and termination of diapause under both outdoor and greenhouse conditions (chapters 5-7); female sex pheromone and sexual behaviour (chapter 8); and genetic similarity (allozyme frequencies, crossing experiments) (chapter 9).

## 1.2 ROSE CULTURE UNDER GLASS IN THE NETHERLANDS

Rose culture under glass in the Netherlands started at the beginning of this century (Augustijn 1953). The culture was concentrated in the area round Aalsmeer until the end of the Second World War, since then the region round Naaldwijk has also become important, the rose grafts however still come from Aalsmeer (M. van de Vrie, pers. comm.).

Every phase of the modern culture of greenhouse roses takes place under glass. New grafts are produced under glass (Gelein 1965), and new cultivars are developed in propagating houses, mainly in the Netherlands, France, England, and Germany (Van Marsbergen 1968).

Greenhouse roses are cultivated all the year round. Sometimes the roses are rested for 4-6 weeks during winter, but never in all compartments of one rose house at the same time (Gelein 1965). During a resting period a compartment is kept frost free only (W. van Marsbergen Ing., pers. comm.). The earliest cultivar suited for cultivation without any resting period, "Better Times", was introduced in the spring of 1936 (Anonymus 1936). Continuous cultivation became customary, however, only long after the Second World War (W. van Marsbergen Ing., pers. comm.).

## 1.3 THE GREENHOUSE AS AN ECOLOGICAL ISLAND

The ecological conditions in greenhouses differ in many respects from those in the open field. The crops and the climate are different. A free exchange between the fauna of the greenhouses and the open air is hampered by the glass walls and roofs.

The mean daily temperature in heated greenhouses is always higher than outdoors. In rose houses it is about 20°C in summer and 18°C in winter, while outdoors in the Netherlands it ranges from about 17°C in July to 2°C in January (Anonymus 1982).

The difference in temperature in and outside a greenhouse is always greatest around 2 p.m., even when the sky is cloudy (Hiller 1956). During a hot summer day, the surface temperature of leaves oriented perpendicularly to the sun radiation, can rise to 35-37°C in a ventilated greenhouse (Kanthak 1973). If these temperatures last for any length of

time, they are lethal to many arthropod species. In an eggplant nursery in the South of France the number of the peach-potato aphid, *Myzus persicae* (Sulz.), was considerably reduced, without damaging the crop, simply by closing the ventilators for some hours at mid-day in April. The air temperature rose up to 45°C, while the relative humidity remained at 70-80% (Rabasse 1976).

In summer greenhouses are never heated during the day, but incidentally at night in order to maintain the inside temperature above a certain level. Greenhouses are heated continuously in winter. Solar radiation only provides part of the required energy. At night the temperature is maintained at a certain minimum level, depending on the crop (Kanthak 1973). A current minimum night temperature is 15°C.

In greenhouses the relative humidity is usually lower than it is outside. The vapour pressure in a ventilated greenhouse and outdoors is about the same, but because of the higher temperature, relative air humidity is lower in the greenhouse (King 1970). In a closed heated greenhouse relative humidity is also lower than outside. The evaporation rate of the soil and plants is usually not sufficient to keep it higher than 50%. The relative humidity is similar to that outdoors when heating is cut down, and may occasionally approach 100% (Kanthak 1973). The humidity is sometimes increased artificially, depending on the requirements of the crop.

The spectral composition of the radiation inside greenhouses differs from that outdoors. The glass walls transmit about 90% of the radiation from 0.4 to 2.7 µm wavelength, whereas short wave ultra-violet light is almost completely absorbed (Mackroth 1971). The absence of ultra-violet radiation facilitates the use of baculoviruses as control agents against noxious insects in greenhouse cultures (Vlak et al. 1982).

The isolating effect of greenhouses on arthropod pests contributes to the effectiveness of control measures, but also to the development and maintenance of pesticide resistance in greenhouses. Because of the special conditions mentioned above, a specific fauna exists in greenhouses, and the use of exotic predators and parasites for biological control is possible. The greenhouse environment acts as a "sieve" only allowing such species to thrive that are adapted to these conditions.

Sometimes these are exotic species that cannot thrive in the open in the Dutch climate. Some examples of economic importance are:

- a. The beet army worm, *Spodoptera exigua* Hb. (Lepidoptera: Noctuidae), probably was introduced from Florida into Dutch greenhouses on infested *Chrysanthemum* cuttings. At present, it is a serious pest in the Dutch floriculture, especially on *Chrysanthemum* and *Gerbera* (Van de Vrie 1977). The species does not enter diapause (Fye & Carranza 1973). In the open in the Netherlands migrants from the Mediterranean area are occasionally found (Lempke 1963), which have been transported passively in atmospheric depressions (French 1969). Sometimes they reproduce, but the populations invariably do not survive the Dutch winter (Lempke 1963).
- b. The leafminer *Lyriomyza trifolii* Burgess (Diptera: Agromyzidae) is a pest on greenhouse *Chrysanthemum* in North-America (Spencer 1973), and on celery in Florida (Spencer 1982).

It has recently established in greenhouses in the Netherlands, causing economic damage to *Chrysanthemum*, *Gerbera*, and *Gypsophila*. It has also been recorded on tomatoes (M. van de Vrie, pers. comm.). The centre of distribution of *L. trifolii* at present appears to be Florida (Spencer 1973). The species is not known to enter diapause. It can survive in areas where the winters are invariably severe, with sub-zero temperatures for extended periods, but it only thrives in subtropical and tropical conditions. In Western Europe there is little likelihood of it ever becoming established as a pest outside greenhouses (Spencer 1982).

- c. The greenhouse whitefly, *Trialeurodes vaporariorum* Westw. (Homoptera: Aleyrodidae), is a cosmopolitan species of American origin, and a well-known pest insect on a wide variety of greenhouse crops. It is not known to enter diapause. In the open in the Netherlands the species is occasionally recorded, overwintering on frost-hardy plants in sheltered places. It is still not clear to what degree the species can survive the Dutch winter (Bink et al. 1980).

Indigenous species may penetrate into greenhouse cultures, but in order to pass the "sieve" they have to adapt to greenhouse conditions.

An example is the two-spotted spider mite, *Tetranychus urticae* (Koch). Its diapause is induced by short daylength. At 20°C, a regime of short photoperiods evokes only a small percentage of diapause forms in rose house populations, while local populations from wild plants completely enter diapause under these conditions. This genetic adaptation enables year-round development of the mites in heated greenhouses. Rose house populations are resistant to organophosphorus compounds, whereas local populations on wild plants are not. Diapause and resistance are not genetically linked in *T. urticae* (Helle 1962). Resistance to compounds that were used until 1966, was still present in 1973, roughly 100 generations later. This demonstrates that rose house populations of *T. urticae* maintain themselves for many years in spite of intensive chemical control (Overmeer et al. 1980). The natural enemies of *T. urticae* that are found outdoors, do not occur in greenhouses (M. van de Vrie, pers. comm.). *T. urticae* in greenhouse cucumbers is effectively controlled by the release of exotic predators, *Phytoseiulus persimilis* A.-H. (Woets 1976). Genetic exchange between field and rose house populations of *T. urticae* is limited, for several reasons (Overmeer et al. 1980): (a) the greenhouse environment holds an ecological paradox: short days in the autumn induce diapause, but the period of chilling required for diapause termination is absent in heated greenhouses; immigrant mites from outside are trapped by the peculiar environment. (b) Hybrid females exhibit partial sterility. (c) Local mites from wild plants are very sensitive to the current acaricides, and (d) immigration of local mites from the field may be restricted.

Another example is the peach-potato aphid, *Myzus persicae* (Sulz.). It usually is heteroecious, but anholocyclic forms are often found in Western Europe. These forms continue reproducing by parthenogenesis and survive the winter on secondary hosts, either in the field on winter-standing crops such as kale or cabbage, or in more sheltered places such as greenhouses, if suitable host plants are present. Similar life-cycles, in which various overwintering strategies coexist, may apply to a number of other indigenous aphid

species (Blackman 1974). Wyatt (1965, 1966) considers that populations of *M. persicae* in *Chrysanthemum* nurseries are strongly isolated. The same pattern of insecticide resistance was found in colonies from six different nurseries, and from a cutting producer, in the South of England. Probably a single resistant clone, selected by the intense insecticide programmes used by the cutting producers, became distributed throughout the English *Chrysanthemum* industry. Most commercial chrysanthemums are raised from cuttings supplied by a small number of specialist propagators.

Boettger (1929) investigated the fauna of different hothouses in Berlin. Besides indigenous species, he found many species of nearctic, Mediterranean, or tropical/subtropical origin. In the South of Italy, greenhouse faunas mainly consist of indigenous Mediterranean species (Boettger 1930). The adventive fauna in heated greenhouses in the Netherlands has been surveyed by Meeuse (1943), Van Oostrom (1944), Holthuis (1945), Meeuse & Hubert (1949), and Van der Hammen (1949, 1969).

Arthropods from the open field can enter greenhouses in different ways:

- a. Winged adults may enter through the ventilators, or through the doors when they are left open. The likelihood of such an event depends on the species. The chance may be the highest among insects that fly in large numbers and are transported passively by wind during the day, when greenhouses are ventilated (e.g. aphids, Thysanoptera), and among other kinds of insects with a strong flight activity, such as bees and bumblebees.

De Brouwer & Van Dorst (1975) state that aphids of the *Aphis gossypii* (Glover) group can colonize greenhouse cucumbers through the ventilators. Cucumber growers in the past had to protect their crop against bees by screening the ventilators with gauze, to prevent pollination of the crop (De Brouwer & Van Dorst 1975).

- b. Unwinged arthropods may penetrate greenhouses by aerial transport. Barel (1973) states that young larvae of the summerfruit tortrix moth, *Adoxophyes orana* (F.v.R.), floating on their threads in the air, may be transported by wind, and that this mechanism is of importance for the dispersal of the species. This may also apply to other tortricids. Van de Vrie et al. (1972) have reviewed the literature on air-borne transport of tetranychid mites.
- c. Other species, such as predatory mites, and species that live under stones, on old walls etc., and that like to penetrate cellars (Boettger 1932), may enter greenhouses walking, e.g. through crevices between the panes or through the ventilators. Other species may penetrate through the soil.
- d. Arthropods (e.g. eggs and young larvae, that can easily be overlooked) may be brought into greenhouses with plant material, tools or mould. On plant material, species from foreign countries may also gain entry to greenhouses.

Migrations from greenhouse to greenhouse may occur in the following ways:

- a. Transport with plant material from other nurseries, notably propagating houses.
- b. Small leaf-dwelling species that readily attach to clothes (e.g. mites, Thysanoptera,

aphids, and whiteflies), may also be transported by humans.

- c. Occasionally active migration from greenhouse to greenhouse may occur, sometimes with an intermediate generation in the open air. *Lyriomyza trifolii* has been found on beans, and *Spodoptera exigua* on beet close to infested greenhouses (M. van de Vrie, pers. comm.).

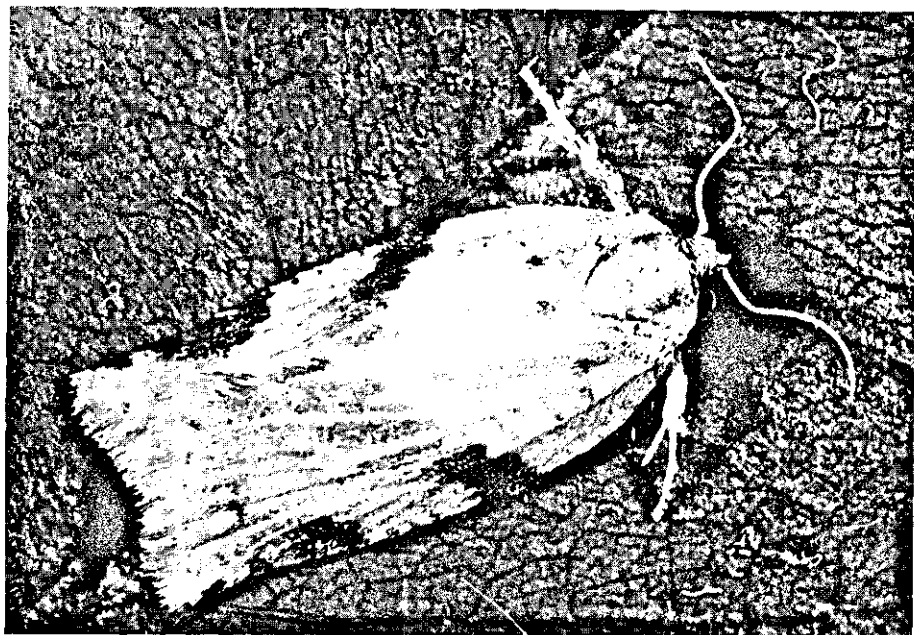


Photo 1. *Clepsia spectrana*, female moth on a rose leaf. Wing span about 20 mm. The species does not show a pronounced sexual dimorphism.

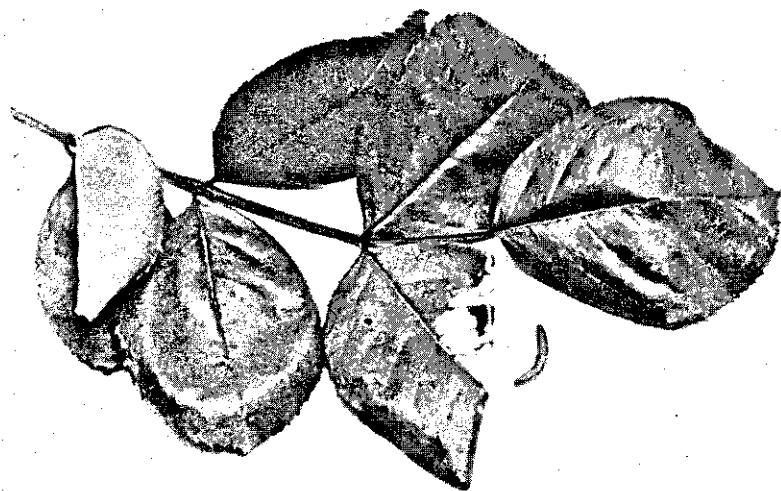


Photo 2. Leaf damage to greenhouse roses, caused by *Clepsia spectrana*.

## 2. LITERATURE

### 2.1 MORPHOLOGICAL ASPECTS

The morphology of *C. spectrana* has been described by: Kennel (1910), Swatschek (1958), Balachowsky (1966), Graaf Bentinck & Diakonoff (1968), Bradley et al. (1973), Razowski (1979), and many others.

The forewing coloration of the adult (photo 1) is extremely variable. The ground colour varies from whitish ochreous to yellowish or reddish brown, and the brown irroration may be either obsolescent or heavy. The forewings usually have dark brown markings, but monochrome specimens also occur. These variations were also observed in field populations from *Urtica dioica*, and in greenhouse populations from roses and *Gerbera* in the Netherlands.

The larvae also vary in colour. Kennel (1910) reports that they are brown-green with whitish pinacula and black head and plates. After Bradley et al. (1973) they are greyish olive-green varying to brown, paler ventrally, with a whitish subspiracular line, cream-white pinacula and a concolorous black irrorated anal plate. The head and the prothoracic plate are black to blackish brown. According to Swatschek (1958) the larvae are brown with black-brown plates and head. In both field and greenhouse populations in the Netherlands, it was observed that the colour of the larval abdomen varies from brown-green to brown.

Wit (1978) compared a population from *Urtica dioica* near Wageningen with a non-diapausing population from a rose house in Aalsmeer. He investigated the morphology of the larvae, pupae and moths, the anatomy of the adult genitalia, and the chaetotaxy of the larvae. He could not find any difference between the two populations.

### 2.2 BIONOMICS

The eggs are deposited in small batches, covered with a gelatinous layer, on the host plant (Bradley et al. 1973). Species of the genus *Clepsis* (Guenée) usually lay their eggs on the upper side of the leaves (preferably on a major leaf vein or other small depressions in the epidermis), on the stems or on the bark (Razowski 1979). This has been observed in both field and greenhouse populations of *C. spectrana*: in the field on stinging nettles (*Urtica dioica* L.), and in greenhouses on roses (Burgess & Jarrett 1978; own observation), *Gerbera*, and *Cyclamen*. The egg masses consist of reticulate oval eggs that overlap like shingles on a roof.

In the field, the larvae feed on the leaves, the leaf stems, the shoots, the





Photo 3. Flower bud of greenhouse roses, attacked by *Clepsis spectrana*.



Photo 4. *Clepsis spectrana* larva. It has spun the top leaves of a rose shoot together.

flowers, the flower buds, the fruits, and the seeds of the host plant (Spuler 1910; Schütze 1931; Graaf Bentinck & Diakonoff 1968; Vernon 1971; Bradley et al. 1973; Razowski 1979). Larval feeding behaviour on greenhouse roses is as follows. The young larvae nibble the superficial tissues at the bottom of the leaves. Older larvae feed on the leaves and the leaf stems. They also burrow in the stems, flower buds, and flowers, causing severe economic damage (Van de Vrie 1978) (photos 2 and 3).

The older larvae build shelters by spinning the leaves or the flowers of the host plant together (Bradley et al. 1973) (photo 4). They make more than one webbing during their development (Burgess & Jarrett 1978; own observation). When young larvae are disturbed, they drop on silken strands on which they may be carried about in the surroundings. Older larvae, when disturbed, can escape abruptly from their webbing, with twisting movements (Balachowsky 1966; Van de Vrie 1978). That is why rose growers in the area round Aalsmeer call tortricid larvae "rozenijltjes", which means "the quick rose caterpillars".

Pupation takes place within the larval habitation (Balachowsky 1966; Bradley et al. 1973; Van de Vrie 1978). This has been observed during our investigations on *Urtica dioica* in the field, and on roses, *Gerbera*, and *Cyclamen* in greenhouses in the Netherlands.

Female moths do not need any food for their oviposition. When newly emerged males and females are brought together at room temperature, oviposition usually starts during the second or third night. Most of the eggs are laid within three nights (Kuperus 1977).

Like all species of the genus *Clepsis* (Guenée), *C. spectrana* overwinters in the larval stage (Razowski 1979). Hibernation starts when the larvae have completed about half their development (Balachowsky 1966). The hibernacula are made in sheltered places, at or close to the host plant (Picard 1912; Van Rossem et al. 1971; Vernon 1971).

The first flight in the Netherlands usually occurs in May and June, and the second in August and September (Graaf Bentinck & Diakonoff 1968). Light trap records from the experimental orchard "Schuilenburg" (from 1975 until 1981), by De Jong (1966), and A. van Frankenhuyzen (from 1954 to 1958, at 10-12 different locations), indicate that the first flight usually starts in the second half of May. Sometimes, in a cold spring, the earliest moths only appear in the first week of June. The first and the second flight sometimes overlap. Flight activity then continues throughout July. The second flight sometimes lasts until mid-October. De Brouwer & Lempke (1973) even caught *C. spectrana* moths as late as the 25 October. During the second flight period always more moths are caught than during the first.

### 2.3 HOST PLANT RANGE

The species has been recorded on the following plants:

*wild plants*

Fam. AQUIFOLIACEAE: *Ilex aquifolium* (Bradley et al. 1973). Fam. BORAGINACEAE: *Symphytum* spp. (Schütze 1931). Fam. COMPOSITAE: *Artemisia maritima* (Bradley et al. 1973); *Aster tripolium* (Bradley et al. 1973); *Centaurea* spp. (Balachowsky 1966); *Cirsium arvense* (collections Museum for Natural History, Leiden). Fam. CRUCIFERAE: *Lepidium* sp. (collections Museum for Natural History, Leiden); *Nasturtium palustre* (Schütze 1931); *Rorippa* spp. (Kostiuk 1980). Fam. CUPULIFERAE: *Quercus* sp. (Bradley et al. 1973). Fam. CYPERACEAE: *Scirpus lacustris* (Schütze 1931). Fam. EQUISETACEAE: *Equisetum* spp. (stem top) (Kostiuk 1980). Fam. EUPHORBIACEAE: *Euphorbia palustris* (Schütze 1931). Fam. GRAMINEAE: *Arundo phragmites* (Balachowsky 1966); *Glyceria spectabilis* (Schütze 1931); *Glyceria maxima* (Kostiuk 1980); grass (Stange 1899); *Phragmites* spp. (Schütze 1931). Fam. IRIDACEAE: *Iris pseudacorus* (Schütze 1931). Fam. ONAGRACEAE: *Epilobium angustifolium* (Balachowsky 1966); *Epilobium hirsutum* (Schütze 1931); *Epilobium palustre* (Balachowsky 1966). Fam. PAPILIONACEAE: *Medicago* spp. (Kostiuk 1980); *Trifolium* spp. (Kostiuk 1980). Fam. PLUMBAGINACEAE: *Limonium vulgare* (Bradley et al. 1973). Fam. POLYGONACEAE: *Rumex* spp. (Balachowsky 1966). Fam. POMACEAE: *Crataegus* sp. (Van de Vrie 1978). Fam. PRIMULACEAE: *Lysimachia* spp. (Stange 1899). Fam. ROSACEAE: *Comarum palustre* (Balachowsky 1966); *Filipendula ulmaria* (Kostiuk 1980); *Potentilla* spp. (Bradley et al. 1973); *Rubus* sp. (Van de Vrie 1978); *Spiraea* spp. (Schütze 1931). Fam. SALICACEAE: *Salix* sp. (Van Poeteren 1941). Fam. UMBELLIFERAE: *Cicuta virosa* (Balachowsky 1966); *Pastinaca sativa* (collections Museum for Natural History, Leiden). Fam. URTICACEAE: *Urtica dioica* (own observation); *Urtica* spp. (Balachowsky 1966). Fam. VALERIANACEAE: *Valeriana* sp. (collections Museum for Natural History, Leiden). Fam. VIOLACEAE: *Viola* spp. (Balachowsky 1966).

*cultivated plants in the open air*

Fam. CARYOPHYLLACEAE: carnation (*Dianthus barbatus* and *D. caryophyllus*) in the open air in Germany (Hahn 1978), and in the Netherlands (Van Frankenhuyzen & De Jong 1964). Fam. CHENOPODIACEAE: sugar beet (*Beta vulgaris*) in the Netherlands (Van Rossem et al. 1971). Fam. CONIFERAE: larch (*Larix* sp.) in Germany (Bodenstein 1955); *Pinus radiata* seedlings in England (Alford 1976). Fam. CRUCIFERAE: rapeseed (*Brassica napus*) in the Netherlands (Van Rossem 1950); cauliflower (*Brassica oleracea*) in the Netherlands (Van Poeteren 1930); sprouts (*Brassica oleracea*) in the Netherlands (De Brouwer 1970). Fam. IRIDACEAE: *Iris* sp. in the open air in the Netherlands (Van Frankenhuyzen & De Jong 1964). Fam. LILIACEAE: onion (*Allium cepa*) in England (Alford 1976); *Lilium candidum* (Balachowsky 1966). Fam. PAPILIONACEAE: lucerne (*Medicago sativa*) in the Netherlands (Van Poeteren 1935); pole beans (*Phaseolus vulgaris*) in the Netherlands (Van Frankenhuyzen & De Jong 1964); broad beans (*Vicia faba*) in the Netherlands (files Plant Protection Service, Wageningen). Fam. POLYGONACEAE: rhubarb (*Rheum* sp.) in England (Alford 1976). Fam. POMACEAE: pear (*Pyrus communis*) in the Netherlands (Van Rossem et al. 1971); apple (*Pyrus malus*) in the Netherlands (Kuchlein & Helmers 1963; Van Frankenhuyzen & De Jong 1964; De Jong 1966;

Van Rossem et al. 1971). Fam. RIBESIACEAE: black currant (*Ribes nigrum*) in England (Dicker 1972); red currant (*Ribes rubrum*) in the Netherlands (files Plant Protection Service, Wageningen). Fam. ROSACEAE: cultivated strawberry (*Fragaria* sp.) in England (Vernon 1971), in Hungary (Balász 1968), and in the Netherlands (files Plant Protection Service, Wageningen; own observation); cultivated roses (*Rosa hybrida*) in the open air in Germany (Hahn 1978), in Poland near Poznan (Dr. T. Baranowski, pers. comm.), and in the Netherlands (Van Poeteren 1941). Fam. SALICACEAE: poplar (*Populus* sp.) in Germany (Hahn 1978). Fam. URTICACEAE: hops (*Humulus lupulus*) in England (Vernon 1971). Fam. VITACEAE: vines (*Vitis* sp.) in Germany (Schwangart 1911, 1912; Hering 1963), and in the South of France (Picard 1912), mainly in low lying poorly drained vineyards.

#### *plants in greenhouse cultures*

Fam. ALSTROEMERIACEAE: *Alstroemeria* sp. in the Netherlands (Van de Vrie 1978) (Herbert (1837), Schenk (1855), Baker (1888), and Wettstein (1935) attribute *Alstroemeria* to the family Amaryllidaceae; Buxbaum (1951, 1954) attributes it to the family Liliaceae). Fam. AMARYLLIDACEAE: *Hippeastrum* sp. in the Netherlands (files Plant Protection Service, Wageningen). Fam. ARACEAE: *Anthurium* sp. in the Netherlands (Van de Vrie 1976). Fam. ARALIACEAE: *Fatsia japonica* in the Netherlands (files Plant Protection Service, Wageningen). Fam. BALEMINACEAE: balsemina (*Impatiens* sp.) in Germany (Bodenstein 1952). Fam. BEGONIACEAE: *Begonia* sp. in the Netherlands (Van de Vrie 1976). Fam. BROMELIACEAE: Bromeliaceae in the Netherlands (Van de Vrie 1978). Fam. CACTACEAE: *Zygocactus truncatus* in the Netherlands (Van Rossem et al. 1968); *Rhipsalidopsis* sp. in the Netherlands (files Plant Protection Service, Wageningen). Fam. CARYOPHYLLACEAE: carnation (*Dianthus caryophyllus*) under glass in the Netherlands (Van Frankenhuyzen & De Jong 1964). Fam. COMNELINACEAE: *Tradescantia* sp. in Germany (Bodenstein 1952). Fam. COMPOSITAE: greenhouse *Chrysanthemum* in the Netherlands (files Plant Protection Service, Wageningen); *Gerbera* sp. in the Netherlands (Van de Vrie 1976) and in Germany (Hahn 1978); lettuce (*Lactuca sativa*) in the Netherlands (Van Frankenhuyzen & De Jong 1964) and in England (Alford 1976). Fam. CRASSULACEAE: *Kalanchoë* sp. in the Netherlands (Van de Vrie 1978). Fam. CRUCIFERAE: stock (*Matthiola incana*) in the Netherlands (Van Frankenhuyzen & De Jong 1964). Fam. CUPRESSACEAE: *Cupressus macrocarpa* in the Netherlands (files Plant Protection Service, Wageningen). Fam. ERICACEAE: azalea (*Rhododendron* sp.) in the Netherlands (Van de Vrie 1978). Class FILICES: fern in England (Miles & Miles 1948). Fam. GERANIACEAE: geranium (*Pelargonium* sp.) in the Netherlands (files Plant Protection Service, Wageningen), and in England (Vernon 1971). Fam. LILIACEAE: *Asparagus setaceus* in the Netherlands (Van Poeteren 1940); *Asparagus* spp. in Germany (Pape 1955a); *Lilium speciosum* (var. "Rubrum") in the Netherlands (files Plant Protection Service, Wageningen). Fam. MORACEAE: *Ficus* sp. in the Netherlands (Van de Vrie 1976). Fam. MUSACEAE: *Strelitzia reginae* in the Netherlands (Van de Vrie 1976). Fam. OLEACEAE: lilac (*Syringa vulgaris*) in Germany (Hahn 1978). Fam. PRIMULACEAE: cyclamen (*Cyclamen persicum*) in Germany (Bodenstein 1952; Pape 1955a), in England (Vernon 1971), and in the Netherlands (Van Poeteren 1936; Van Frankenhuyzen & De Jong 1964). Fam. ROSACEAE: greenhouse roses (*Rosa hybrida*) in Denmark (Anonymus 1942a;

Pape 1964), in Germany (Feiter & Henseler 1971), in England (Burgess & Jarrett 1978), in Poland near Poznan (Dr. T. Baranowski, pers. comm.), and in the Netherlands (Van Poeteren 1941; Van Frankenhuyzen & De Jong 1964; Van Rossem et al. 1965; Van de Vrie 1976, 1978). Fam. SCROPHULARIACEAE: snapdragon (*Antirrhinum majus*) under glass in the Netherlands (Van Frankenhuyzen & De Jong 1964). Fam. SOLANACEAE: greenhouse tomato (*Solanum lycopersicum*) in the Netherlands (Van Frankenhuyzen & De Jong 1964) and in England (Alford 1976). Fam. VITACEAE: vines (*Vitis* sp.) under glass in the Netherlands (Van Frankenhuyzen & De Jong 1964).

In the cited literature, often only the name of the host plant genus was given. In some cases it was difficult to decide whether the recorded food plant was a true host plant. It was sometimes difficult to ascertain the reliability of the literature data. Still some general conclusions can be drawn.

Many of the wild host plants are from a wet environment. Obviously *C. spectrana* prefers moist biotopes (Spuler 1910; Balachowsky 1966; Razowski 1969, 1979; Vernon 1971; Bradley et al. 1973; Kostiuk 1980). The species certainly is common around Aalsmeer, which is a wet area.

The species of the genus *Clepsis* (Gueneé) usually are oligophagous. The host plants are mainly shrubs and trees including conifers (Razowski 1979). *C. spectrana*, however, is mainly found on herbaceous plants, and is extremely polyphagous, both outdoors and in greenhouse cultures. The list given above shows that the species has been recorded on plants from at least 28 different families in the open air, and 26 different families in greenhouses. The polyphagous character of the species increases its danger as a pest.

## 2.4 DISTRIBUTION

According to Balachowsky (1966) and Bradley et al. (1973) *C. spectrana* occurs in Northern, Central, and Western Europe, and in the South-East of the European part of the Soviet-Union. Kostiuk (1980) adds that the species is widely distributed in the European part of the Soviet-Union, down to the Black Sea coast and the Sea of Azov, and as far as Daghestan, Talysh and the North-Western Ural, and that it also occurs in Asia Minor.

In 1969, Razowski stated that the species is found all over Europe, except in Spain and Portugal, and that it is also found in Syria. However in 1979 Razowski did not include Southern Europe and Syria. The distribution data from 1969 were based on the literature, and probably were in part incorrect. In 1979 he included only the information that had been confirmed. His opinion is that the distribution area of the species does not extend far South. However there may be isolated populations of the species in Northern Yugoslavia and Northern Italy, but this needs to be confirmed (Dr. J. Razowski, pers. comm.).

The species has only been recorded as a noxious insect in countries like Hungary, France, Germany, Denmark, England, and the Netherlands (chapter 2.3). In Italy the species is not known as a noxious insect, and possibly does not occur at all (Prof. Dr. S. Zangheri, pers. comm.). Dr. A. Diakonoff (pers. comm.) remembers that he caught a *C. spectrana* moth in a light trap on the island of Corfu, and that he found the species among

material that was sent to him from Egypt. The species apparently occurs in the Mediterranean region.

When the information from the Museum for Natural History in Leiden, from the Zoological Museum in Amsterdam, from Graaf Bentinck & Diakonoff (1968), from De Jong (1966), from A. van Frankenhuyzen (unpublished), and from the experimental orchard "Schuilenburg" (unpublished) is compared, it is apparent that *C. spectrana* has been caught in all Dutch provinces, at 101 different locations all over the country. Apparently the species is widely distributed in the Netherlands. Kuchlein & Helmers (1963) are of the same opinion. The earliest specimen in the museum in Leiden and Amsterdam was caught by Snellen in 1857 near Rotterdam.

### 3. MATERIALS AND METHODS

#### 3.1 TECHNICAL

Field strains were collected from stinging nettles (*Urtica dioica* L.) near Wageningen, and greenhouse strains from rose houses in the Aalsmeer region, and identified as *C. spectrana* by Dr. A. Diakonoff. In the laboratory at the most 5 generations per strain were reared. At least once a year new larvae and pupae were collected in the field and rose houses, and new mass rearings were started with about 125 specimens per strain.

The larvae were reared singly in glass vials (height 5 cm, diameter 1.5 cm) on artificial diet. Two Philips fluorescent tubes (white light) of 20 Watt each were used as a light source.

The diet was the same as used by Ankersmit (1968) for the summerfruit tortrix moth, *Adoxophyes orana* (F.v.R.). The hot fluid diet was mixed for 10 minutes in a Braun multimix MX 32 kitchen mixer at maximum speed. This was sufficient to grind the solid components and to prevent their precipitation during cooling. The solid diet was cut in pieces. Each vial received one piece and was closed with a wad of cotton wool. The vials with their contents were sterilized by maintaining them at 112°C in a steam container for 20 minutes. The vials were allowed to cool on a Monarch clean bench (type MH-12-6). As soon as the free water inside the vials had evaporated, newly hatched larvae were put on the diet on the clean bench. In this way growth of bacteria and fungi on the diet could for the most part be prevented.

The vials with the larvae were kept in transparent plastic boxes, which prevented the diet from drying out prematurely. Usually it was not necessary to transfer the larvae to a new vial before completion of their development. Moreover in this way two strains could be reared in one room without running the risk of mixing them up.

The pupae were removed from the larval webbing to ensure unhampered emergence of the moths. They were kept on moist filter paper.

The moths of each strain were brought together in a closed polyethylene bag (size ca 15x30x45 cm), containing a fresh rose shoot that was placed in wet Oasis to keep it fresh. The eggs were deposited on the inner side of the bag, and on the upper side of the rose leaves.

The egg masses were collected, and were allowed to hatch on greenhouse rose leaves in petri dishes in closed plastic bags. The larvae were put on the artificial diet within 16 hours after egg hatching.

The head capsule widths of the larval instars, and the pupal body widths, were

measured with a Wild M5 stereomicroscope (ocular x10, total magnification x25, one micrometer unit represented 0.04 mm). Head capsule width was defined as the maximum width of the exterior skeleton of the larval head (dorsally or ventrally). Pupal width was defined as the maximum width of the body (dorsally or ventrally).

### 3.2 EXPERIMENTAL

The rate of development, pupal body width, and mortality, in larvae reared on artificial and natural diets were compared, to test the quality of the artificial diet. The larvae were taken at random from one lot of each strain, and reared singly in glass vials at 25°C and LD 18:6. The bottom of the boxes in which the vials were kept, were covered with filter paper soaked in water, to prevent premature drying out of the natural diets. The diets were renewed twice a week, and the moults were assessed at the same time. Pupation and moth emergence were observed at 24-hour intervals. The number of instars between egg hatching and pupation was variable. The 5-instar growth type strongly predominated in both male and female larvae. For this reason only larvae which had been through 5 instars between egg hatching and pupation (= 5-instar larvae) were considered.

No significant differences in development duration and pupal body width (tested with the t-statistic), and in mortality (tested with the  $\chi^2$ -statistic) were found between the diets in both strains (2-sided test,  $P > .05$ ) (table 1). Differences in development between the strains will be discussed in chapter 4.

Table 1. Duration of larval and pupal development, mortality, and pupal body width of a field strain and a greenhouse strain on artificial and natural diet, at 25°C and LD 18:6.

	field strain		greenhouse strain	
	artificial diet	<i>Urtica dioica</i> leaf	artificial diet	<i>Rosa hybrida</i> leaf
mean development duration of 5-instar larvae (days)	n=55 19.8 (S.D.=2.3)	n=60 20.0 (S.D.=2.0)	n=58 20.2 (S.D.=2.3)	n=48 20.0 (S.D.=2.3)
percentage larval mortality	8%	5%	11%	14%
pupal width (mm) ♂♂	2.41	2.40	2.35	2.37
♀♀	2.82	2.80	2.75	2.75
mean pupal development duration (days)	6.4 (S.D.=0.5)	6.5 (S.D.=0.6)	6.9 (S.D.=0.6)	6.8 (S.D.=0.6)
percentage pupal mortality	7%	10%	5%	8%



#### 4. NON-DIAPAUSE DEVELOPMENT

Developmental rate and diapause may be genetically linked. Hoy (1978a) found that a non-diapausing strain of gypsy moth, *Lymantria dispar* (L.), obtained through purposeful selection in a laboratory culture, exhibited significant differences in developmental rate of some larval instars, when compared to a wild strain from the field.

Greenhouse populations of *C. spectrana* may have adapted to development at higher temperatures. To determine optimum temperature, data on size and weight and also on survival in different temperature conditions are the most often used (Danilevskii 1965, pp. 152-153).

Rate of development and mortality of eggs, larvae, and pupae of field and rose house strains were compared. Moreover the number of larval instars, the head capsule widths of the larvae, and pupal body width were determined.

##### 4.1 VARIABILITY IN THE NUMBER OF LARVAL INSTARS

Variability in the number of larval instars is frequently observed in insect species. A review of the literature on this subject is given by Gruys (1970): besides genetic factors, several environmental factors can influence the number of instars, such as temperature, air humidity, quantity and quality of food, crowding, and photoperiod; rising temperature provokes more moults in some species, while in other species it causes fewer moults; in some species the smallest number of moults is found at a specific medium-range temperature, and it increases at higher and lower temperatures.

The number of larval instars from egg hatching to pupation in *C. spectrana* varied between 4 and 7 in both field and greenhouse strains. In rearings of a greenhouse strain two of the larvae were even observed to go through 8 instars. This variability may modify the rate of larval development.

In the figures 1 and 2 the mean head capsule widths of the separate instars of the different larval growth types are presented. The reliability intervals of the means presented in these and other figures, were calculated according to the following formula:

$$\text{mean} \pm S.E. \times t_n (P=.05, 2\text{-sided test})$$

When the number of observations  $n$  exceeded 100, for  $t_n$  the value 1.96 was taken in the formula.

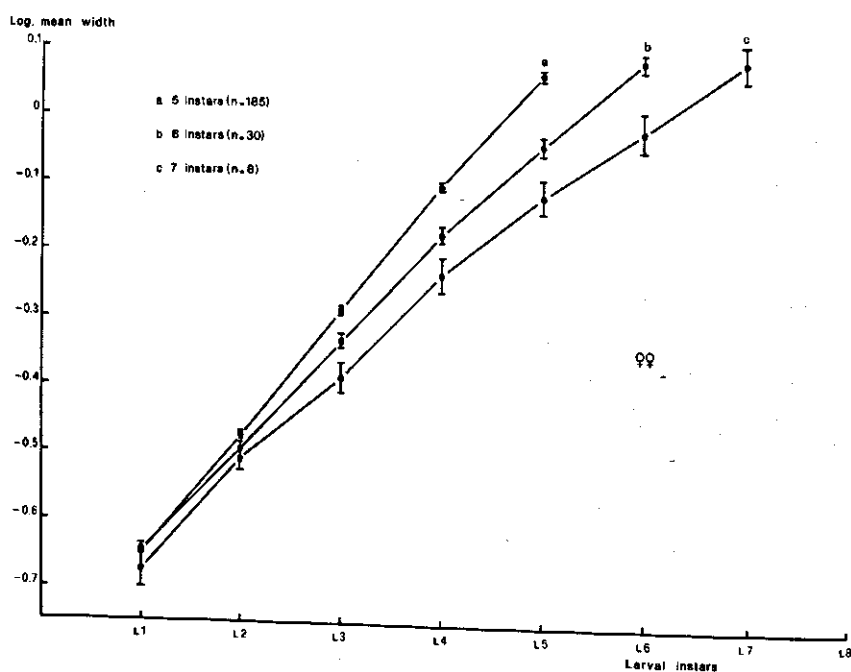
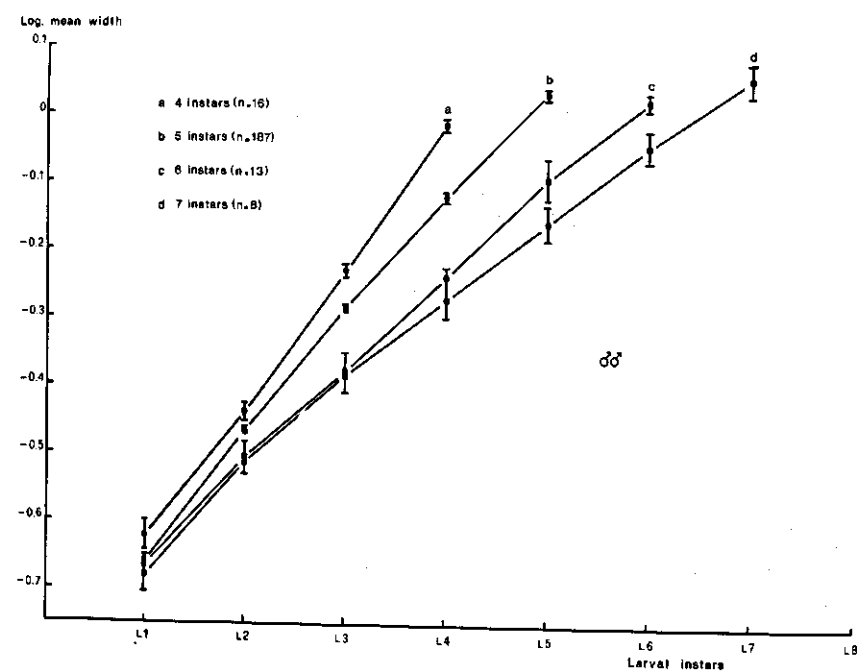


Figure 1. Log. mean head capsule widths (mm) of the separate instars of the 4-instar, 5-instar, 6-instar, and 7-instar larval growth type (field strains). Vertical bars represent the reliability intervals of the means.

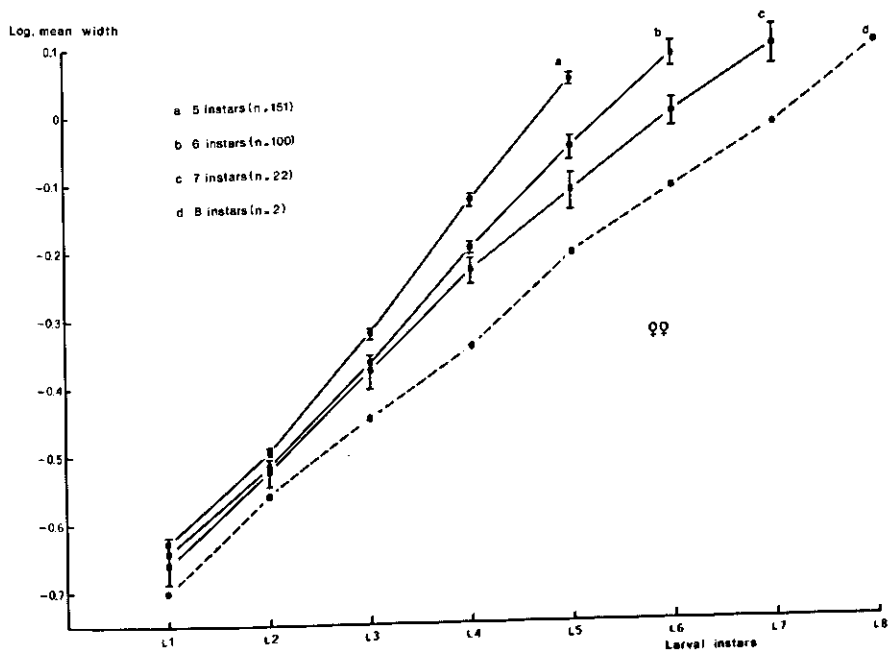
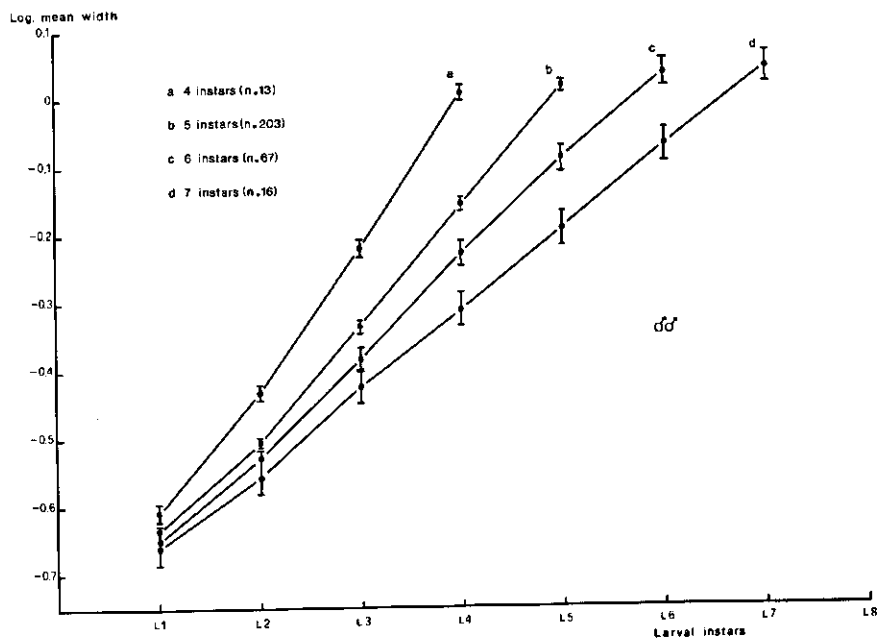


Figure 2. Log. mean head capsule widths (mm) of the separate instars of the 4-instar, 5-instar, 6-instar, 7-instar, and 8-instar larval growth type (greenhouse strains). Vertical bars represent the reliability intervals of the means.

There was a systematic difference in mean head capsule width between the different larval growth types: the higher the number of instars, the smaller the mean head capsule width of each instar. This was usually the case even for the first instar (figures 1 and 2).

The differences in mean head capsule width in each instar, between the larval growth types, were statistically evaluated to determine in which instars they were significantly expressed.

The results are given in table 2. The number of larval instars may be influenced by both genetic and environmental factors. If environmental factors are involved in the determination of the number of instars in *C. spectrana*, the influence of these factors should be felt before the eggs hatch, as both in field and greenhouse strains the difference between the 4-instar and 5-instar larval growth type was already significantly expressed when the eggs hatched (table 2). The difference between the 5-instar and 6-instar type was significantly expressed from the second instar, and the difference between the 6-instar and 7-instar type from the third instar or even later, in both field and greenhouse strains (table 2). The lower the ultimate number of instars, the earlier the difference between the larval growth types was expressed. A similar pattern was found by Gruys (1970) in the pine looper, *Bupalus piniarius* (L.).

Eggs of a field strain and a greenhouse strain (1st laboratory generation) were kept at 21°C and LD 18:6. Larvae were taken at random from one lot of each strain, within 16 hours after hatching. They were reared at different temperatures (at LD 18:6, to prevent induction of diapause).

Table 2. Statistical evaluation of the differences in mean head capsule width between the different larval growth types of a field and greenhouse strain (1-sided t-test)

		field strains		greenhouse strains	
		♂♂	♀♀	♂♂	♀♀
4-instar larvae vs 5-instar larvae	L1	S	--*	S	--*
	L2	S	--	S	--
	L3	S	--	S	--
5-instar larvae vs 6-instar larvae	L4	S	--	S	--
	L1	NS	NS	NS	NS
	L2	S	S	S	S
6-instar larvae vs 7-instar larvae	L3	S	S	S	S
	L4	S	S	S	S
	L5	S	S	S	S
6-instar larvae vs 7-instar larvae	L1	NS	NS	NS	NS
	L2	NS	NS	NS	NS
	L3	NS	S	S	NS
7-instar larvae vs 8-instar larvae	L4	NS	S	S	S
	L5	S	S	S	S
	L6	S	S	S	S

\* no females with 4 instars noticed

Table 3. Fractions of the larval population going through a varying number of instars in a field and greenhouse strain, at different temperatures and LD 18:6.

			number of instars				mean number of instars	total number of larvae observed
			4	5	6	7		
15°C	field	♂♂	5%	87%	8%	---	5.0	63
	greenhouse	♂♂	14%	76%	10%	---	5.0	58
	field	♀♀	---	86%	13%	2%	5.2	64
	greenhouse	♀♀	---	87%	13%	---	5.1	60
20°C	field	♂♂	7%	93%	---	---	4.9	74
	greenhouse	♂♂	10%	90%	---	---	4.9	60
	field	♀♀	---	98%	2%	---	5.0	64
	greenhouse	♀♀	---	97%	3%	---	5.0	70
25°C	field	♂♂	4%	86%	11%	---	5.1	57
	greenhouse	♂♂	3%	90%	8%	---	5.1	78
	field	♀♀	---	75%	25%	---	5.3	69
	greenhouse	♀♀	---	88%	12%	---	5.1	58
30°C	field	♂♂	4%	78%	17%	---	5.1	46
	greenhouse	♂♂	3%	75%	22%	---	5.1	60
	field	♀♀	---	56%	42%	2%	5.5	50
	greenhouse	♀♀	---	70%	30%	---	5.3	44

Table 3 shows the numbers of larvae going through a varying number of instars from egg hatching to pupation, expressed as proportions of the total number of larvae observed at each temperature. The following conclusions can be drawn:

- Larvae going through 5 instars from egg hatching to pupation (5-instar larvae) were predominant at all rearing temperatures in both sexes in both strains.
- Females tended to develop through a higher number of larval instars than males in both strains, but the differences were not significant except in the field strain at 30°C (2-sided  $\chi^2$ -test,  $P>.05$ ).
- The mean number of instars was the lowest at 20°C in both strains, but the only significant difference was between 20°C and 30°C in the females of both strains (2-sided  $\chi^2$ -test,  $P>.05$ ).

The mean developmental times of the different larval growth types of a field and a greenhouse strain, at 25°C and LD 18:6, are given in table 4. Larval development was observed at 24-hour intervals. The higher the number of instars, the longer the larval developmental time (table 4). The same phenomenon was observed in many other insect species with a variable number of larval instars (Gruys 1970). The time required for larval development did not differ significantly between the strains, in each of the larval growth types (2-sided t-test,  $P>.05$ ). Among the larvae of the 4-instar type only males were found, and among the 8-instar type only females. Larval development duration was slightly

Table 4. Mean development duration (days) of the different larval growth types of a field and a greenhouse strain, at 25°C and LD 18:6.

	field strain		greenhouse strain	
	♂♂	♀♀	♂♂	♀♀
4-instar larvae	n=9 16.4 (S.D.=1.5)	----	n=7 15.9 (S.D.=1.3)	----
5-instar larvae	n=69 18.9 (S.D.=2.4)	n=31 20.3 (S.D.=1.6)	n=72 19.8 (S.D.=1.8)	n=41 20.8 (S.D.=2.0)
6-instar larvae	n=12 23.5 (S.D.=2.6)	n=16 25.3 (S.D.=2.0)	n=47 23.8 (S.D.=1.9)	n=76 25.1 (S.D.=2.1)
7-instar larvae	n=8 29.4 (S.D.=2.0)	n=9 31.8 (S.D.=1.8)	n=6 28.3 (S.D.=2.3)	n=12 31.2 (S.D.=1.9)
8-instar larvae	----	----	----	n=2 39.3

longer in females than in males in each of the larval growth types in which both sexes were found. The difference was significant, except in the 5-instar larvae of the greenhouse strain (table 4).

#### 4.2 RATE OF DEVELOPMENT AT DIFFERENT TEMPERATURES

Developmental rates of a field and a greenhouse strain were compared in the egg stage, the larval stage (5-instar larvae, each instar separately), and the pupal stage, at different temperatures and LD 18:6, in the first laboratory generation. Oviposition, egg

Table 5. Mean duration of the egg stage (days) of a field and a greenhouse strain at different temperatures.

	15°C	20°C	25°C	30°C	35°C
field strain	18.5 (S.D.=0.9) n=203	9.1 (S.D.=0.6) n=234	6.5 (S.D.=0.1) n=216	5.6 (S.D.=0.4) n=243	---
greenhouse strain	18.7 (S.D.=0.6) n=226	9.6 (S.D.=0.5) n=241	6.2 (S.D.=0.5) n=233	5.4 (S.D.=0.3) n=201	---
significance of difference (2-sided t-test)	NS	S	NS	NS	

Table 6. Mean development duration (days) of 5-instar larvae of a field and a greenhouse strain at different temperatures (LD 18:6).

	15°C	20°C	25°C	30°C	35°C
field strain ♂♂	54.4 (S.D.=3.2) n=28	28.8 (S.D.=2.8) n=35	18.8 (S.D.=2.4) n=29	18.0 (S.D.=0.8) n=26	----
greenhouse strain ♂♂	58.1 (S.D.=7.3) n=22	30.5 (S.D.=3.8) n=28	19.8 (S.D.=2.8) n=37	17.9 (S.D.=1.3) n=21	----
significance of difference (2-sided t-test)	S	S	NS	NS	
field strain ♀♀	59.7 (S.D.=3.6) n=33	31.7 (S.D.=2.1) n=35	20.2 (S.D.=1.6) n=29	19.9 (S.D.=2.1) n=24	----
greenhouse strain ♀♀	61.0 (S.D.=7.6) n=13	31.5 (S.D.=4.3) n=33	20.6 (S.D.=2.4) n=27	19.4 (S.D.=3.9) n=28	----
significance of difference (2-sided t-test)	NS	NS	NS	NS	

hatching, moults, pupation, and moth emergence were observed at 24-hour intervals. Eggs and larvae, reared at different temperatures, were taken at random from one lot of each strain. The eggs from which the larvae originated, had been reared at 21°C and LD 18:6. The pupae originated from the larvae used in the experiments.

In table 5 the mean durations of the egg stage are given. A temperature of 35°C proved to be lethal to the embryos of both strains. The mean duration of the egg stage decreased from 18-19 days at 15°C to 5-6 days at 30°C. At 20°C the greenhouse strain developed significantly slower than the field strain (table 5).

The mean development duration of 5-instar larvae at various temperatures did not differ significantly between the strains, but greenhouse males at 15°C and 20°C were significantly slower in development (table 6). A temperature of 35°C proved to be lethal to the larvae of both strains. Mean larval development duration decreased from 54-61 days at 15°C to 18-20 days at 30°C (table 6). The mean time required for development was shorter in male larvae than in female larvae, the difference was always significant in the field strain, but only at 30°C in the greenhouse strain (2-sided t-test,  $P<.05$ ) (table 6).

As an example, the mean developmental times of the separate instars of 5-instar larvae, reared at 15°C and LD 18:6, are given in table 7. The mean durations of the separate instars, expressed as proportions of the total mean larval development duration,

Table 7. Mean development duration (days) of the separate instars of 5-instar larvae of a field and a greenhouse strain, at 15°C and LD 18:6.

		field strain	greenhouse strain
♂♂	L1	14.3 (S.D.=2.1)	15.2 (S.D.=5.4)
	L2	9.4 (S.D.=0.9)	9.4 (S.D.=1.1)
	L3	8.0 (S.D.=1.2)	8.8 (S.D.=1.1)
	L4	8.2 (S.D.=1.3)	9.6 (S.D.=1.5)
	L5	14.4 (S.D.=1.3)	15.3 (S.D.=1.7)
	total	54.4 (S.D.=3.2) n=28	58.1 (S.D.=7.3) n=22
♀♀	L1	14.9 (S.D.=2.2)	15.9 (S.D.=5.0)
	L2	9.4 (S.D.=0.8)	9.2 (S.D.=0.7)
	L3	9.2 (S.D.=1.1)	9.0 (S.D.=1.3)
	L4	9.7 (S.D.=1.3)	10.2 (S.D.=1.4)
	L5	16.5 (S.D.=1.4)	16.6 (S.D.=1.7)
	total	59.7 (S.D.=3.6) n=33	61.0 (S.D.=7.6) n=13

did not differ significantly between the strains, and were not significantly influenced by sex or temperature (2-sided  $\chi^2$ -test,  $P>.05$ ).

Table 8. Mean development duration (days) of pupae from 5-instar larvae of a field and a greenhouse strain at different temperatures (LD 18:6).

		15°C	20°C	25°C	30°C
field strain	♂♂	23.6 (S.D.=1.2) n=22	11.7 (S.D.=0.7) n=32	7.2 (S.D.=0.6) n=26	6.1 (S.D.=0.5) n=18
greenhouse strain	♂♂	23.1 (S.D.=1.3) n=19	12.1 (S.D.=0.8) n=27	7.1 (S.D.=0.6) n=34	6.2 (S.D.=0.6) n=16
significance of difference (2-sided t-test)		NS	NS	NS	NS
field strain	♀♀	21.4 (S.D.=1.0) n=26	10.7 (S.D.=0.7) n=29	6.5 (S.D.=0.5) n=27	6.0 (S.D.=0.5) n=16
greenhouse strain	♀♀	21.7 (S.D.=1.2) n=14	11.3 (S.D.=0.7) n=30	6.6 (S.D.=0.6) n=25	5.9 (S.D.=0.6) n=20
significance of difference (2-sided t-test)		NS	NS	NS	NS



In table 8 the mean development durations of pupae from 5-instar larvae at different temperatures are given. The differences were not significant between the strains. The mean time required for pupal development decreased from 21-24 days at 15°C to 5-7 days at 30°C. Male pupae generally had a longer mean development duration than female pupae (table 8). The difference was always significant, except at 30°C (2-sided t-test,  $P < .05$ ).

In the figures 3-5, the mean developmental rates of the egg stage, larval stage (5-instar larvae), and pupal stage are plotted against the temperature. The regression lines in these figures were calculated on the basis of the data at 15°C, 20°C, and 25°C. The regression coefficients did not differ significantly between the strains (t-statistic testing linear regression, 2-sided test,  $P > .05$ ). Extrapolation of the regression lines (figures 3-5) indicated a developmental threshold close to 10°C in both strains. The mean

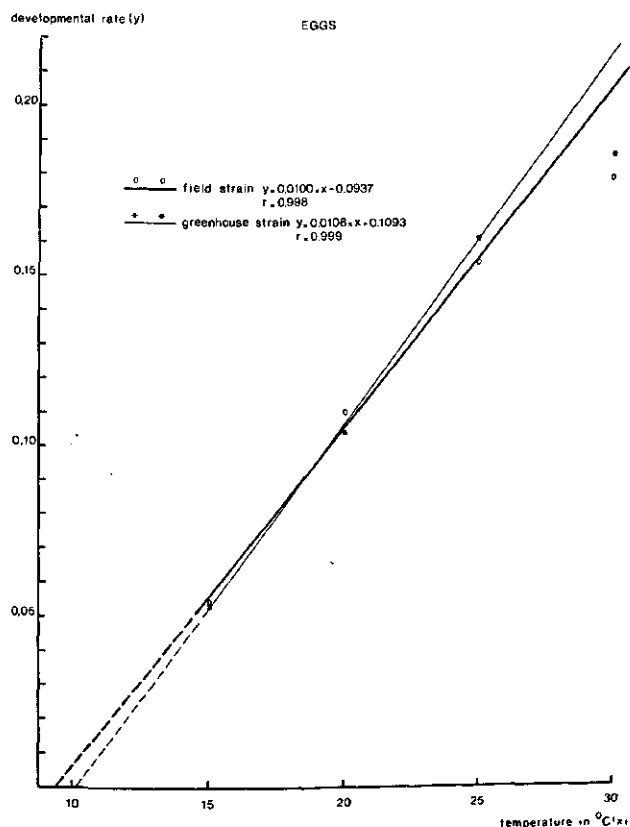


Figure 3. Mean developmental rate ( $\text{day}^{-1}$ ) of eggs of a field and a greenhouse strain, plotted against the temperature ( $^{\circ}\text{C}$ ). Linear regression of developmental rate and temperature.

developmental rate at 30°C was always lower than could be expected on the basis of the regression lines (figures 3-5). This indicates that 30°C was close to the upper thermal limit for development.

Broadly outlined, development duration was the same in the field and the greenhouse strain. Nevertheless sometimes significant differences were found between the strains, in the egg stage and in the larval stage (tables 5 and 6). These differences are difficult to interpret. They may represent characteristic differences between field and greenhouse populations of *C. spectrana*, they may be due to the strain differences one may expect when samples are taken from different greenhouse populations or from different field populations, they may be due to inaccuracy of the thermostats during the experiments, or a combination of these factors.

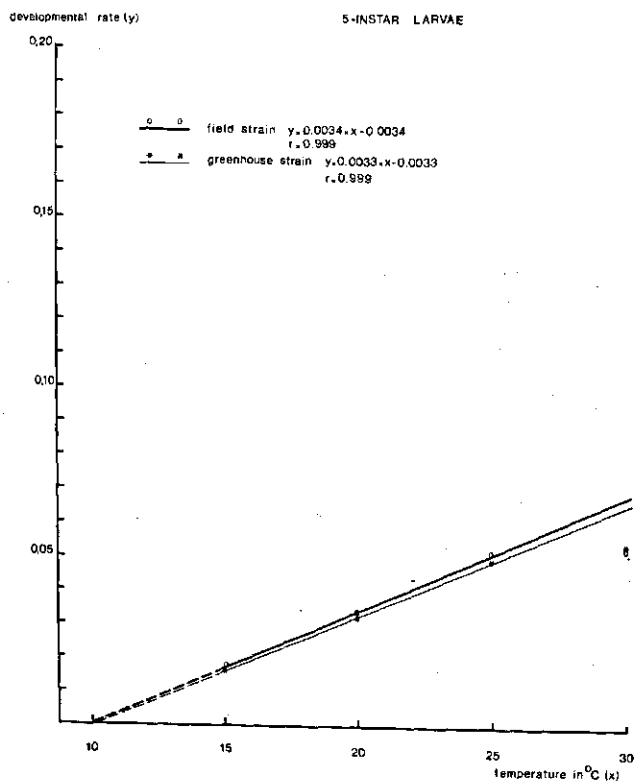


Figure 4. Mean developmental rate ( $\text{day}^{-1}$ ) of 5-instar larvae of a field and a greenhouse strain, plotted against the temperature ( $^{\circ}\text{C}$ ). Linear regression of developmental rate and temperature.

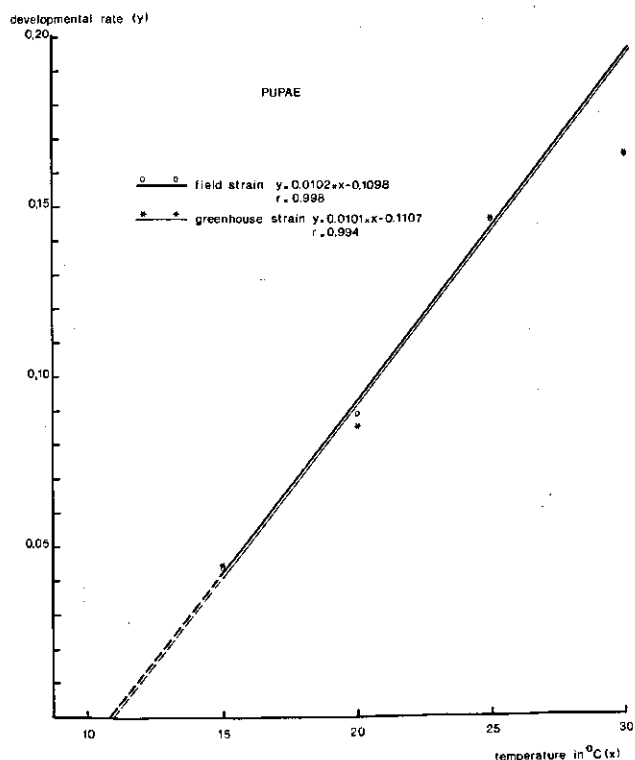


Figure 5. Mean developmental rate ( $\text{day}^{-1}$ ) of pupae of a field and a greenhouse strain, plotted against the temperature ( $^{\circ}\text{C}$ ).  
•Linear regression of developmental rate and temperature.

#### 4.3 MORTALITY AND PUPAL BODY WIDTH AT DIFFERENT TEMPERATURES

In table 9 the mortality of eggs, larvae, and pupae of field and greenhouse strains at different temperatures is given. A temperature of  $35^{\circ}\text{C}$  was lethal to both eggs and larvae. Pupae were not exposed to this temperature. In each of the immature stages, mortality was relatively low at  $20^{\circ}\text{C}$  and  $25^{\circ}\text{C}$ , at  $15^{\circ}\text{C}$  it was higher, and at  $30^{\circ}\text{C}$  it was the highest in both strains. The difference between  $15^{\circ}\text{C}$  and  $20^{\circ}\text{C}$  was significant in the pupae of both strains, and in the greenhouse strain eggs (2-sided  $\chi^2$ -test,  $P < .05$ ). The difference in mortality between  $20^{\circ}\text{C}$  and  $30^{\circ}\text{C}$  was always significant ( $P < .05$ ) (table 9).

The differences in pupal body width were not significant between the strains (table 10). The female pupae were significantly larger than the male pupae (2-sided t-test,  $P < .005$ ). At  $30^{\circ}\text{C}$ , the pupae were significantly smaller than at the other temperatures in both strains ( $P < .05$ ). At  $15^{\circ}\text{C}$ , female field strain pupae were significantly smaller than

Table 9. Percentage mortality of eggs, larvae, and pupae of field and greenhouse strains at different temperatures (LD 18:6)

		15°C	20°C	25°C	30°C	35°C
eggs	field strains	26%	20%	16%	41%	100%
		n=274	n=293	n=257	n=412	n=197
	greenhouse strains	24%	12%	14%	36%	100%
		n=297	n=274	n=271	n=314	n=127
larvae	field strains	15%	10%	13%	35%	100%
		n=148	n=148	n=148	n=148	n=74
	greenhouse strains	18%	12%	10%	30%	100%
		n=148	n=148	n=148	n=148	n=74
pupae	field strains	21%	8%	7%	27%	
		n=109	n=132	n=100	n=64	
	greenhouse strains	14%	5%	7%	19%	
		n=96	n=122	n=121	n=76	

at 20°C ( $P < .05$ ) (table 10).

In figure 6 pupal body width and overall survival egg-adult are plotted against the temperature. Width and survival were related to the temperature in a similar way, in both field and greenhouse strains.

Table 10. Mean body widths (mm) of pupae from 5-instar larvae of field and greenhouse strains at different temperatures (LD 18:6).

		15°C	20°C	25°C	30°C
field strain	♂♂	2.38	2.40	2.39	2.29
		n=55	n=69	n=48	n=36
	greenhouse strain	2.37	2.39	2.36	2.30
		n=44	n=54	n=70	n=45
significance of difference (2-sided t-test)		NS	NS	NS	NS
field strain	♀♀	2.74	2.84	2.79	2.63
		n=54	n=63	n=52	n=28
	greenhouse strain	2.76	2.81	2.76	2.67
		n=52	n=68	n=51	n=31
significance of difference (2-sided t-test)		NS	NS	NS	NS

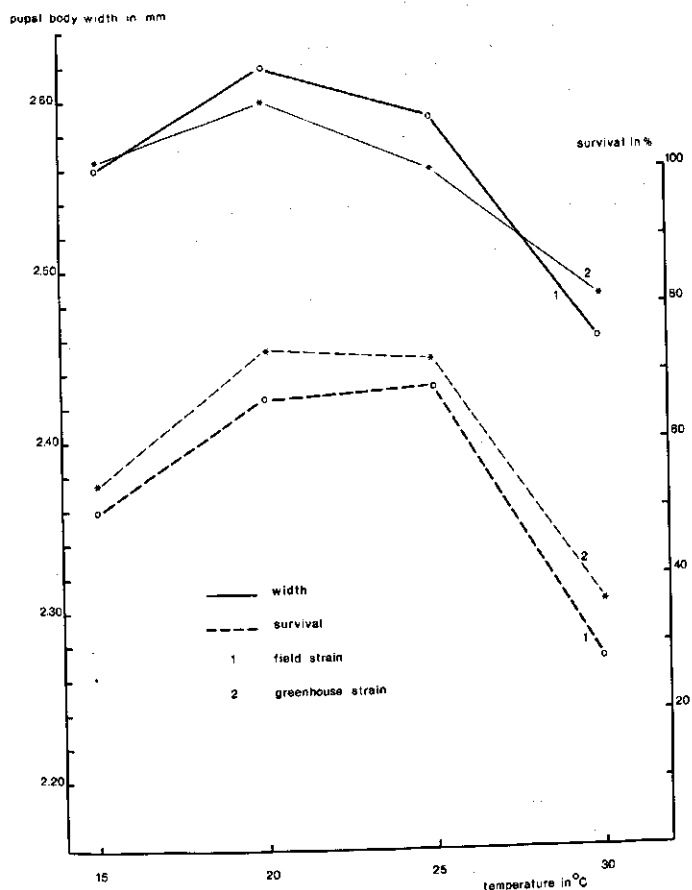


Figure 6. The effect of temperature on the overall survival from oviposition to moth emergence, and the pupal body width, of field and greenhouse strains.

Both in field and in greenhouse strains 35°C was a lethal temperature. Apparently 30°C was close to the upper thermal limit for development:

- At 30°C, mortality was the highest in each of the immature stages. The mortality at 20°C was always significantly lower (table 9).
- At 30°C pupal body width was significantly reduced as compared to the lower rearing temperatures (table 10).
- At 30°C, developmental rate was always lower than could be expected by extrapolating the linear regression of developmental rate and temperature (figures 3-5).

The upper thermal limit for development apparently was between 30°C and 35°C in both field and greenhouse strains.

Survival and pupal body width were similar in field and greenhouse strains in the

temperature range 15°C-30°C (figure 6).

An adaptation of greenhouse populations of *C. spectrana* to development at higher temperatures did not appear. It remains to be ascertained whether there are differences in development between field and greenhouse populations in the temperature range between the developmental threshold and 15°C, as temperatures below 15°C were not investigated. As a rule, the non-dormant stages of insect species show little geographic variation in their temperature requirements for growth and development (Danilevskii 1965, pp. 151-152).

## 5. DIAPAUSE IN FIELD POPULATIONS

Diapause is crucial in annual cycles of insects as it largely determines whether a species can survive certain conditions. Field and greenhouse populations of *C. spectrana* seem to differ in this respect. To establish these differences, and to assess how diapause can be eliminated in a greenhouse environment, knowledge about induction, maintenance, and termination of diapause in field populations is required.

### 5.1 PHOTOPERIODIC RESPONSE CURVES

Eggs of a field strain (2nd laboratory generation), and larvae from these eggs, were reared at different combinations of photoperiod and temperature (figure 7). For each combination 165 larvae were used.

Larvae were only assumed to be diapausing if their head capsule width did not exceed the maximum width found among diapausing field strain larvae in the outdoor experiments described in section 5.2 of this chapter (i.e. 0.88 mm).

Extrapolation of the regression lines indicated that the mean development duration of non-diapausing larvae at 13°C is about 3½ months (figure 4). Larvae that did not die or pupate within 6½ months were assumed to be diapausing if their head capsule width was not larger than 0.88 mm.

At 15°C the mean development duration of non-diapausing larvae was about 2 months (table 6). When no diapause was induced, pupae were usually formed within 4 months after egg hatching. Larvae remaining after 4 months were assumed to be diapausing if their head capsule width did not exceed 0.88 mm.

At 20°C the mean developmental time of the larvae was about 1 month (table 6). Those remaining after 2 months were assumed to be diapausing (head capsule width < 0.88 mm).

At 25°C the mean time required for larval development was about 3 weeks (table 6). The larvae remaining after 6 weeks (head capsule width < 0.88 mm) were assumed to be diapausing.

The results are presented in figure 7. At 15°C and 20°C the critical photoperiod was between 16 and 17 hours. In the Netherlands these photoperiods (daylength including civil twilight) occur between mid-July and mid-August (Beck 1980, pp. 3-6). The photoperiodic response of *C. spectrana* was of the "long-day" type. As in many other insect species (Beck 1980, pp. 127-128), high temperatures tended to avert photoperiodic induction of diapause in *C. spectrana* (response curve at 25°C in figure 7).

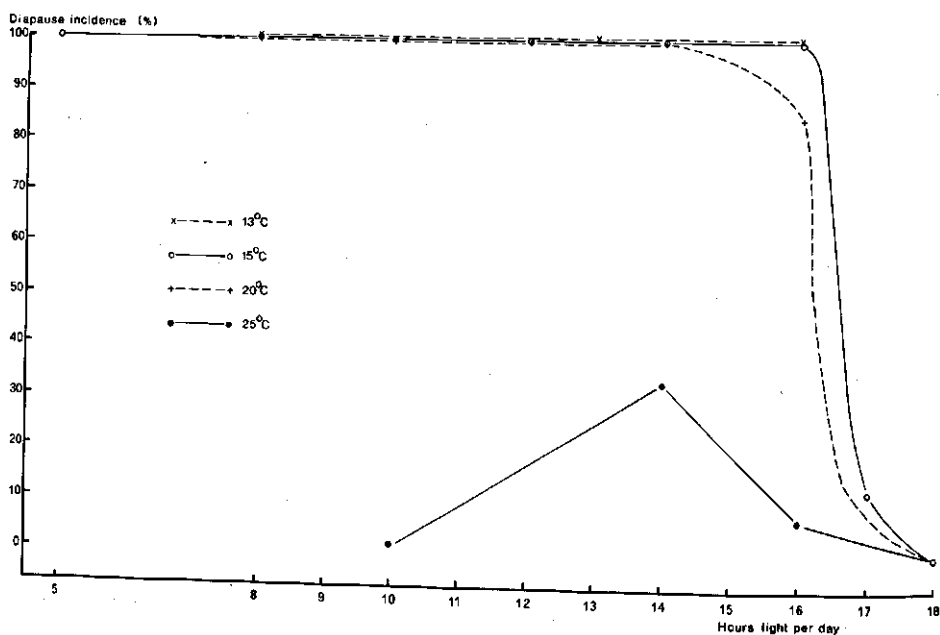


Figure 7. Photoperiodic response curves of a field strain at different temperatures

## 5.2 OUTDOOR EXPERIMENTS

Induction and termination of diapause, and post-diapause development of field strain larvae were studied in outdoor experiments. The larvae (2nd and 3rd laboratory generation) were reared in the open air in a cage that was protected from the sun. Five rearings were performed, with eggs that hatched in the outdoor cage on August 1, August 17, September 9, September 25, and October 14, 1978, respectively. This covers the possible egg hatching period of the second flight in field populations. Each time 80 larvae were reared. The development of the larvae was followed individually. The head capsule width of each instar was measured.

The temperature, recorded with a thermohygrograph placed inside the cage, remained below 9°C from December 25, 1978, until spring. This temperature can be considered as developmental zero (figures 3-5). Therefore no development occurred during this period. After December 25, the larvae were observed once a month. From the beginning of April 1979 daily observations were made to establish when the larvae left their hibernacula. After resumption of feeding and growth, the moults were observed at weekly intervals. The width of the head capsules of each instar, and pupal body width were measured, pupation and moth emergence were observed at 2-day intervals.



### 5.2.1 Induction of diapause

Table 11 shows the mean number of moults up to hibernation, and the mean head capsule width of hibernating larvae. Hibernation was assumed to start on December 25, 1978. Larvae entering diapause could be recognized because they usually made a hibernaculum in the fissure between the wall of their glass vial and the piece of cotton wool with which the vial was closed.

The number of moults up to hibernation was variable. It varied between 1 and 6, but 1, 5, and 6 moults were exceptional. The mean number was lower as the eggs hatched later in the season (table 11). Two explanations are offered:

- The instar in which diapause is entered is fixed, but the larvae undergo "stationary moults" (postecdysial larvae essentially being of the same weight and form as preecdysial larvae) during diapause as long as the temperature is above a certain threshold (compare Burges 1960; Chippendale & Reddy 1972; Scheltes 1978).
- The instar in which diapause is entered is variable, and is correlated with the moment in the season in which the eggs hatch.

Table 11. Number of moults up to hibernation, and mean head capsule width of hibernating larvae (outdoor experiments with a field strain).

date of egg hatching	mean number of moults up to hibernation	percentage of larvae going through different numbers of moults up to hibernation (♂♂ and ♀♀ combined)	mean head capsule width (mm) of hibernating larvae
1978-08-01	♂♂: 4.2 n=31 ♀♀: 3.9 n=39	3 moults: 3% 4 " : 87% 5 " : 7% 6 " : 3%	0.76
1978-08-17	♂♂: 3.4 n=36 ♀♀: 3.2 n=28	3 moults: 70% 4 " : 28% 5 " : 2%	0.62
1978-09-09	♂♂: 2.8 n=47 ♀♀: 2.8 n=29	2 moults: 18% 3 " : 82%	0.54
1978-09-25	♂♂: 2.0 n=43 ♀♀: 2.0 n=33	1 moult : 5% 2 moults: 89% 3 " : 5%	0.42
1978-10-14	♂♂: 1.9 n=36 ♀♀: 2.0 n=35	1 moult : 6% 2 moults: 94%	0.40

The difference ♂♂-♀♀ with respect to the number of moults up to hibernation was not significant (2-sided  $\chi^2$ -test,  $P > .05$ ).

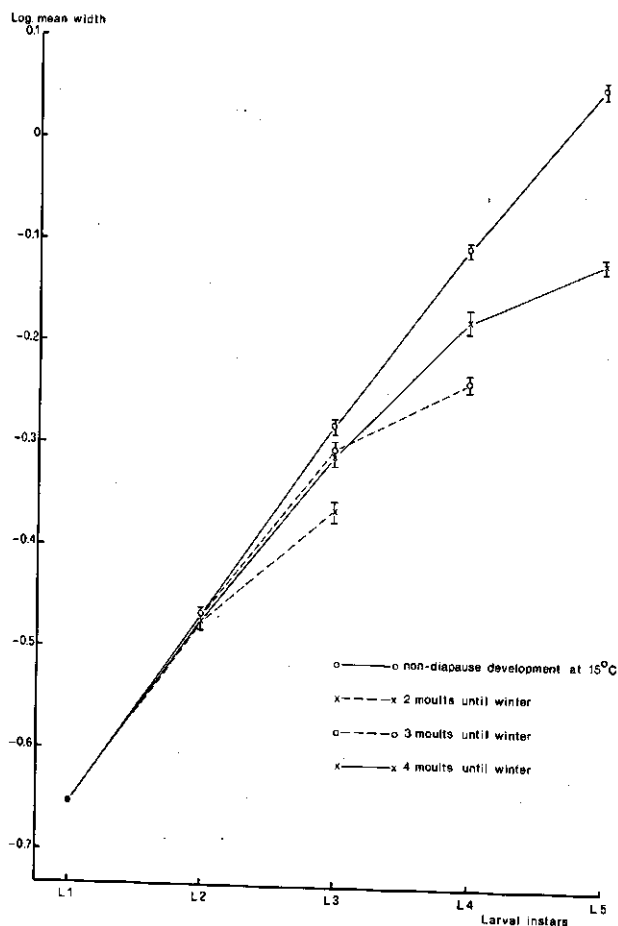


Figure 8. Log. mean widths of the head capsules (mm) of the separate instars of larvae that hibernated after 2-4 moults, and of 5-instar larvae that developed at 15°C without diapause in the laboratory. Outdoor experiments with a field strain. Vertical bars represent the reliability intervals of the means.

The mean head capsule widths of the consecutive instars of larvae entering diapause deviated from a geometrical progression, contrary to those of 5-instar larvae going through non-diapause development (figure 8). The deviation was always most pronounced after the last moult before hibernation. The mean head capsule width of overwintering larvae was larger as they went through more moults up to hibernation (table 11, figure 8). Therefore larvae hibernating after differing numbers of moults respectively all had their own specific progression of head capsule width (figure 8).

Larvae hibernating after 1 moult usually made a hibernaculum, but did not moult within it. Larvae hibernating after 2-6 moults always underwent 1 moult within their

hibernacula. This conflicts with the assumption of "stationary moults" after the onset of diapause.

These observations support the second explanation mentioned above.

### 5.2.2 Resumption of moulting in spring

The maximum temperature remained below 11°C until April 13, 1979, when it rose to 19°C around mid-day. On April 13, larvae from each of the 5 rearings (5 different egg hatching dates) were observed to have left their hibernacula, and were feeding on the diet at the bottom of the glass vials.

Table 12 shows the percentage of larvae that had moulted once between April 13 and 27, or were on the point of moulting on April 27, for each of the 5 rearings. These percentages suggest that the time of resumption of growth and moulting in spring was the same for each of the 5 rearings, and was therefore not correlated with the date of egg hatching in the previous year. It has been demonstrated in many species that post-diapause development is prevented, sometimes for a considerable period, until temperatures rise above the lower thermal threshold for development (Tauber & Tauber 1976).

Table 12. Resumption of moulting in the spring of 1979 (outdoor experiments with a field strain).

date of egg hatching	% larvae that had moulted once between 1979-04-13 and 1979-04-27, or were on the point of moulting (white coloured zone behind the head capsule) on 1979-04-27
1978-08-01	97%
1978-08-17	93%
1978-09-09	92%
1978-09-25	85%
1978-10-14	91%

### 5.2.3 Post-diapause development

In table 13 the mean duration of post-diapause development is presented for each of the 5 dates of egg hatching. In table 14 the mean numbers of moults during pre- and post-diapause development, and the mean pupal widths after diapause are given.

The duration of post-diapause development, both until pupation and until moth emergence, was longer as the eggs had hatched later in the previous year (table 13). The relationship between the developmental period of post-diapause larvae and the date of egg hatching in the previous year was also expressed in the number of moults during post-diapause development (table 14).

The total number of moults (pre-diapause + post-diapause), and pupal body width, were not clearly correlated with the date of egg hatching, and thus with the instar in which diapause was entered (table 14).

Table 13. Mean duration of post-diapause development (days) in 1979 (outdoor experiments with a field strain). Statistical evaluation of the differences between males and females (1-sided t-test). Start of post-diapause development at 1979-04-13.

date of egg hatching in 1978		mean development duration until pupation in 1979	mean development duration of the pupae in 1979	mean development duration until moth emergence in 1979
1978-08-01	♂♂	35.8 (n=28) S	17.8 (n=25) S	53.6 S
	♀♀	42.7 (n=37)	13.9 (n=32)	56.6
1978-08-17	♂♂	53.7 (n=31) S	16.3 (n=27) S	70.0 NS
	♀♀	56.6 (n=25)	15.5 (n=23)	72.1
1978-09-09	♂♂	55.2 (n=45) S	15.4 (n=40) S	70.6 NS
	♀♀	59.6 (n=32)	13.6 (n=29)	73.2
1978-09-25	♂♂	58.3 (n=38) S	18.2 (n=31) S	76.5 S
	♀♀	67.8 (n=29)	17.2 (n=24)	85.0
1978-10-14	♂♂	69.4 (n=22) S	11.1 (n=21) NS	80.5 S
	♀♀	78.6 (n=23)	10.7 (n=20)	89.3

Table 14. Mean number of moults during pre- and post-diapause development. Mean pupal width after diapause (outdoor experiments with a field strain). Statistical evaluation of the differences between males and females (1-sided  $\chi^2$ -test).

date of egg hatching in 1978		mean number of moults during larval post-diapause development in 1979	mean total number of moults (pre-diapause + post-diapause)	mean pupal body width (mm)
1978-08-01	♂♂	1.0 (n=28) S	5.3 NS	2.58
	♀♀	1.6 (n=37)	5.5	2.94
1978-08-17	♂♂	1.9 (n=31) NS	5.3 NS	2.39
	♀♀	2.2 (n=25)	5.4	2.86
1978-09-09	♂♂	2.3 (n=45) NS	5.2 NS	2.46
	♀♀	2.5 (n=32)	5.4	2.95
1978-09-25	♂♂	2.6 (n=38) S	4.6 S	2.50
	♀♀	3.2 (n=29)	5.2	2.86
1978-10-14	♂♂	3.2 (n=22) S	5.1 S	2.46
	♀♀	3.6 (n=23)	5.7	3.01

The variability in the number of larval instars (chapter 4.1) was probably only expressed during post-diapause development, as the number of moults up to the onset of diapause was strongly correlated with the moment in the season in which the eggs hatched (table 11).

The larvae that were diapausing outdoors averaged one more moult than non-diapausing larvae in laboratory rearings (compare the tables 3 and 14). Bonnemaïson (1977) reported a similar phenomenon in the summerfruit tortrix moth, *Adoxophyes orana* (F.v.R.), the larvae going through supernumerary moults after the termination of diapause.

Pupal body width after diapause outdoors was significantly higher than after non-diapause development in laboratory rearings (compare the tables 10 and 14) (2-sided t-test,  $P < .05$ ). This may be connected with the higher number of moults in this diapausing generation (tables 3 and 14).

Diapausing male larvae tended to develop through a lower number of moults than diapausing female larvae. However, the difference in mean total number of moults (pre-diapause + post-diapause) was only significant for the egg hatching dates 1978-09-25, and 1978-10-14 (table 14). The duration of larval post-diapause development was shorter in males, but in the pupal stage male developmental time was longer (table 13). Similar phenomena were observed in the experiments on non-diapause development (tables 4, 6, and 8). The result was a slight protandry in moth emergence. The difference in mean total duration of post-diapause development until moth emergence, between males and females, was significant, except for the egg hatching dates 1978-08-17 and 1978-09-09 (table 13). In Lepidoptera and many other insects there is a general tendency for males to emerge before females (protandry). Different explanations have been advanced. These are summarized by Wiklund & Fagerström (1977). Not only non-diapause, but also post-diapause development can vary with sex (Tauber & Tauber 1976).

### 5.3 INFLUENCE OF TEMPERATURE AND PHOTOPERIOD ON THE INSTAR IN WHICH DIAPAUSE IS ENTERED

From the outdoor experiments described in chapter 5.2 it appeared that the instar in which diapause is entered is variable, and is strongly correlated with the moment in the season in which the eggs hatch. It may therefore be determined by photoperiod and/or temperature. The influence of these two factors was investigated in laboratory experiments.

Eggs of a field strain (4th laboratory generation), and larvae from these eggs, were reared at different combinations of temperature and photoperiod (13°C, 15°C, and 20°C; 5, 8, 13, and 16 hours light per day). In the Netherlands (station De Bilt) the mean daily temperature is 16.4°C in August, 14.0°C in September, and 10.3°C in October (Anonymus 1982). The mean daily temperature in Dutch rose houses is about 18°C in winter and 20°C in summer. Daylength in the Netherlands, including civil twilight, approximately varies between 8 and 18 hours (Beck 1980, pp. 3-6).

The development of the larvae was followed individually. The moults were observed at 24-hour intervals. Diapause was considered to be entered as soon as the first moult within the hibernaculum had occurred. For each experiment 100 larvae were used.

Table 15. Percentage of larvae going through different numbers of moults up to the onset of diapause, at different temperature and photoperiod combinations (laboratory experiments with a field strain).

		diapause entered after				
		2 moults	3 moults	4 moults	5 moults	6 moults
5 hours light per day	13°C 15°C 20°C	n=89 100%	----	----	----	----
8 hours light per day	13°C 15°C 20°C	n=85 100% n=88 100% n=95 100%	---- ---- ----	---- ---- ----	---- ---- ----	---- ---- ----
13 hours light per day	13°C 15°C 20°C	n=90 2% n=81 2% n=92 ----	92% 96% 86%	6% 1% 14%	---- ---- ----	---- ---- ----
16 hours light per day	13°C 15°C 20°C	n=87 ---- n=94 ---- n=89 ----	---- ---- 2%	90% 96% 90%	8% 3% 6%	3% 1% 2%

The results are given in table 15. The photoperiod had a pronounced influence on the number of moults until the onset of diapause. At 16 hours light per day most of the larvae entered diapause after 4 moults, and at 13 hours light per day after 3 moults. At 8 and 5 hours light per day all the larvae entered diapause after 2 moults. Diapause occasionally was entered after 5 or 6 moults (at 16 hours light per day), but never after 1 moult. Some of the larvae in the outdoor experiments (table 11) hibernated after 1 moult. They were probably not able to reach complete diapause on time, before hibernation started. They built a hibernaculum, but did not moult within it. It is remarkable that some of them were able to survive winter and to pupate in spring of the next year.

Temperature, at least within the range investigated (13°C-20°C), did not seem to influence the number of moults after which diapause was entered (table 15).

In table 16 the mean duration from egg hatching until the onset of diapause is given for each investigated combination of temperature and photoperiod. The data in this table confirm that, at any temperature, the time required to enter diapause was shorter as the photoperiod was shorter.

Table 16. Mean development duration from egg hatching up to the onset of diapause (days) at different temperature and photoperiod combinations (laboratory experiments with a field strain).

	5 hours light per day	8 hours light per day	13 hours light per day	16 hours light per day
13°C	----			
15°C	28.2	38.8	51.0	110.1
20°C	----	27.7	35.1	86.0
		19.3	24.9	38.6

\* no experiment conducted

## 5.4 DISCUSSION

Field populations of *C. spectrana* have a facultative, photoperiodically induced larval diapause. The photoperiodic response is of the "long-day" type, and the critical photoperiod is between 16 and 17 hours. The number of moults up to the onset of diapause varies between 2 and 6, and is determined by the photoperiod, at least within the temperature range 13°C-20°C. It is lower as daylength is shorter. Outdoors, the time of resumption of growth and moulting after diapause termination does not vary with the date of egg hatching in the previous year, and thus with the instar in which diapause is entered. The duration of post-diapause development is longer as diapause is entered after a lower number of moults.

The functional significance of these mechanisms might be:

- a. Larvae from eggs that hatch later in the season enter diapause after a lower number of moults, and thus have a better chance of reaching diapause on time, before the leaves of the host plant deteriorate and the temperature drops permanently below the lower thermal threshold for development.
- b. Because the number of moults after which diapause is entered is variable, an additional variation in the duration of post-diapause development is induced. This counteracts the synchronising effect of resumption of growth and moulting in spring, and brings about a longer duration of the first flight period in this bivoltine tortricid. This may be an advantage for the species.

In other Lepidoptera the instar in which larval diapause is entered might also be variable. Sáringer & Szentkirályi (1980) found that larvae of the plum fruit moth, *Grapholitha funebrana* (Tr.), that entered diapause earlier in the year had larger body weights. Geyspits (1965) investigated the pine moths *Dendrolimus pini* (L.) and *D. sibiricus* (Tschetw.), and found that diapause was entered in an earlier larval instar as the temperature was lower and the photoperiod was shorter. This synchronises the onset of diapause. Geyspits argued that a mechanism which prevents premature induction of diapause is advantageous in these species with a life-cycle that exceeds a year, as well as in species with a polycyclic type of development.

## 6. PHOTOPERIODIC RESPONSE IN GREENHOUSE POPULATIONS

### 6.1 LABORATORY EXPERIMENTS

The photoperiodic response of 5 different greenhouse strains (1st laboratory generation) was tested in the same way as that of a field strain (chapter 5.1). The 5 strains had been collected as larvae and pupae from different rose houses at different times of the year (both in winter and summer): from Aalsmeer in November, from De Kaag in January, from Roelofarendsveen in February, from Sloten in June, and from Emmeloord in June. Photoperiods of 8, 10, 12, 14, 16, and 18 hours were tested at 15°C and 20°C. No diapause was induced in any of the greenhouse strains.

### 6.2 OUTDOOR EXPERIMENTS

The photoperiodic response of one of the 5 greenhouse strains mentioned in section 6.1 of this chapter, originating from the rose house in Sloten, was also tested under outdoor conditions, in the 2nd and 3rd laboratory generation. Experiments similar to those with a field strain (chapter 5.2) were done. Field and greenhouse strain larvae were reared in the same outdoor cage. The experiments with the greenhouse strain were done with eggs that hatched on August 1, August 17, September 25, and October 14, 1978, respectively. These egg hatching dates coincided with those of the field strain.

Figures 9 and 10 show the comparative measurements of the mean head capsule width in each rearing of field and greenhouse strain larvae in the course of the season. The curves representing mean head capsule width of field strain larvae leveled off because diapause was entered. The curves of the greenhouse strain always reached a significantly higher level than those of the field strain, and continued to increase until the onset of cold weather in December. This indicates that no, or only some, greenhouse strain larvae entered diapause. This was checked by studying, in more detail, the larval development after each egg hatching date.



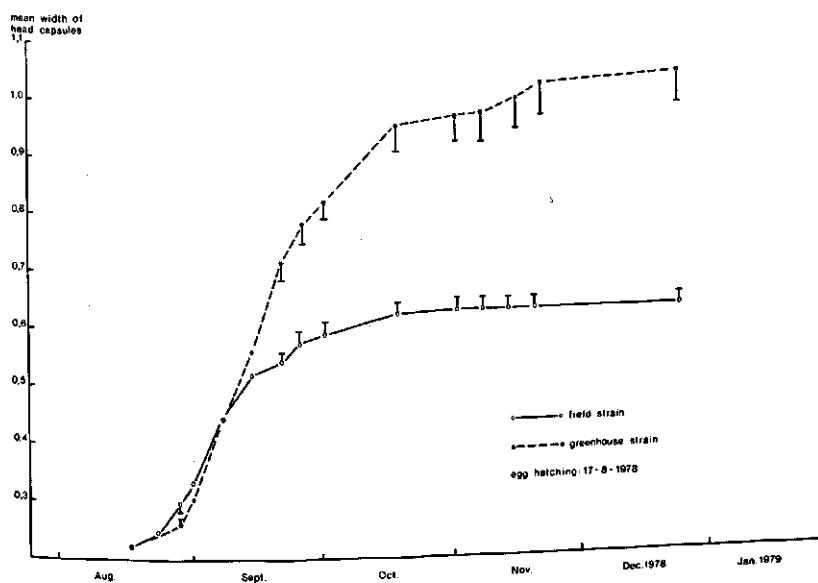
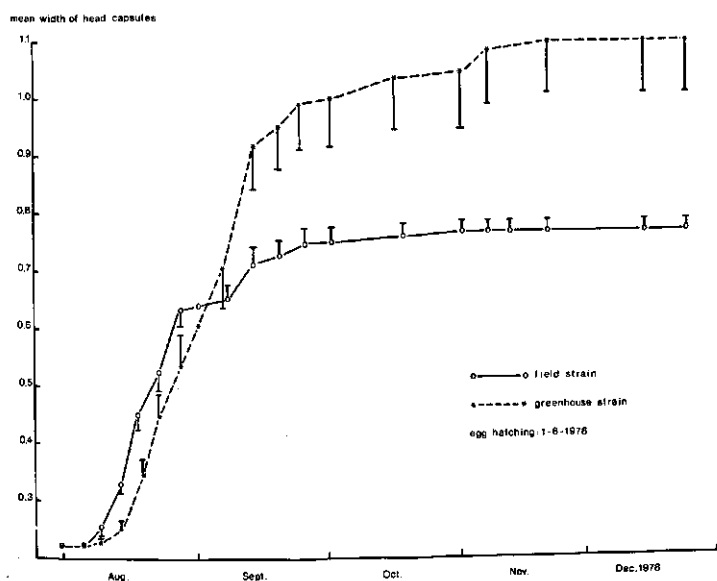


Figure 9. Mean head capsule width (mm) in the course of the season, of larvae of a field and a greenhouse strain, reared outdoors. Egg hatching dates: 1978-08-01 and 1978-08-17. Vertical bars represent the reliability intervals of the means.

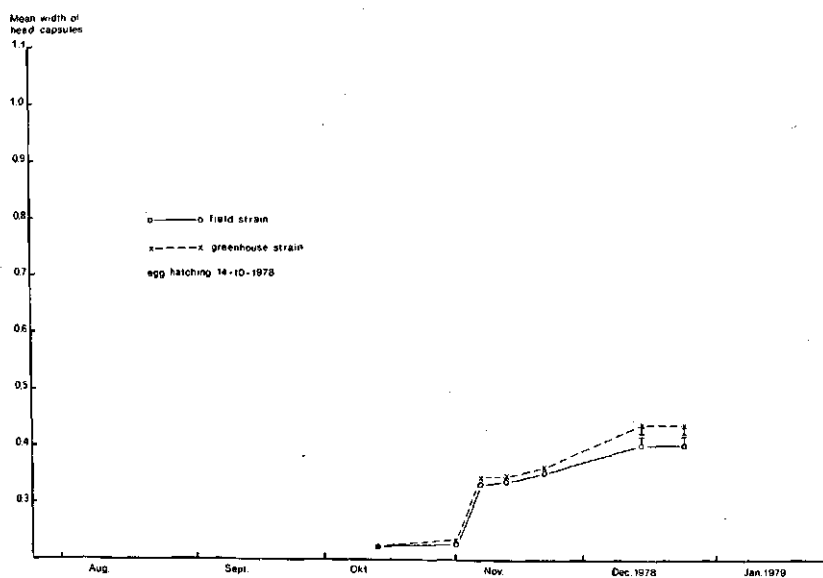
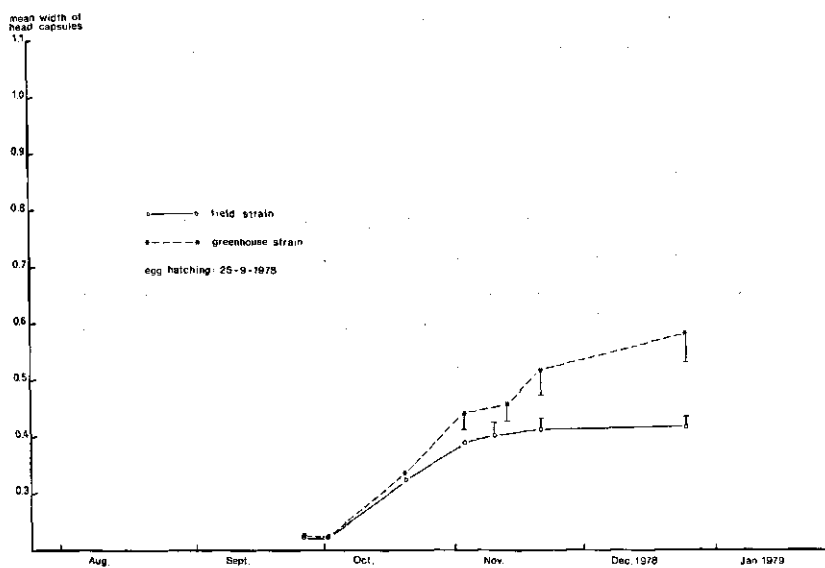


Figure 10. Mean head capsule width (mm) in the course of the season, of larvae of a field and a greenhouse strain, reared outdoors. Egg hatching dates: 1978-09-25 and 1978-10-14. Vertical bars represent the reliability intervals of the means.

*Egg hatching 1978-08-01*

By December 25, 1978 (onset of hibernation, see chapter 5.2), 90% of the greenhouse strain larvae had pupated. The largest head capsule width found among diapausing larvae of the field strain was 0.88 mm. However, the head capsule width of the remaining greenhouse strain larvae was at least 1.04 mm. Probably they were not diapausing, but had reached the last instar before pupation. They all died during winter.

*Egg hatching 1978-08-17*

By December 25, 1978, 31% of the greenhouse strain larvae had pupated. The head capsule width of 46% of the larvae was  $\geq 1.04$  mm. They had gone through 4 or 5 moults, and had probably reached the last instar before pupation. The rest of the greenhouse strain larvae (head capsule width  $< 1.04$  mm) hibernated after 3 or 4 moults. The mean head capsule widths of the successive instars of these larvae are given in the figures 11 and 12.

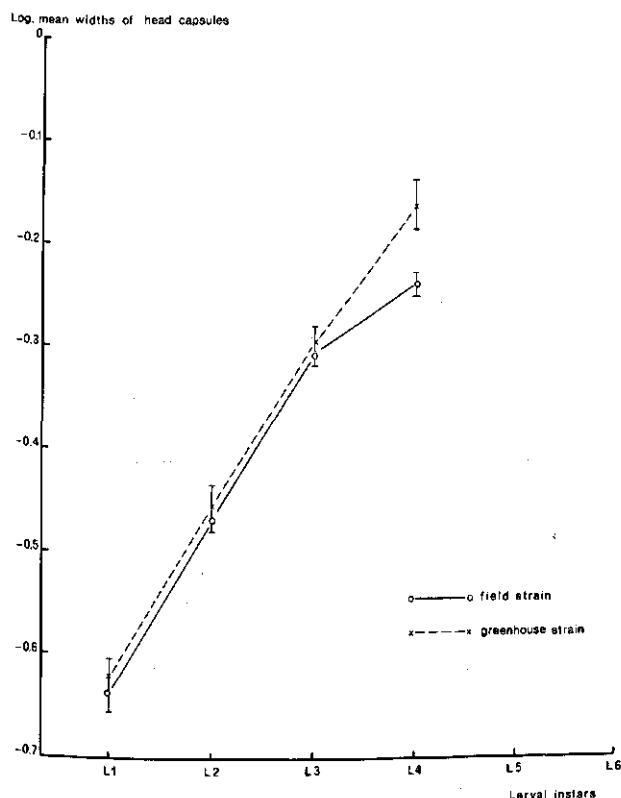


Figure 11. Log. mean widths of the head capsules (mm) of the separate instars of larvae of a field and a greenhouse strain, hibernating after 3 moults. Egg hatching: 1978-08-17. Vertical bars represent the reliability intervals of the means.

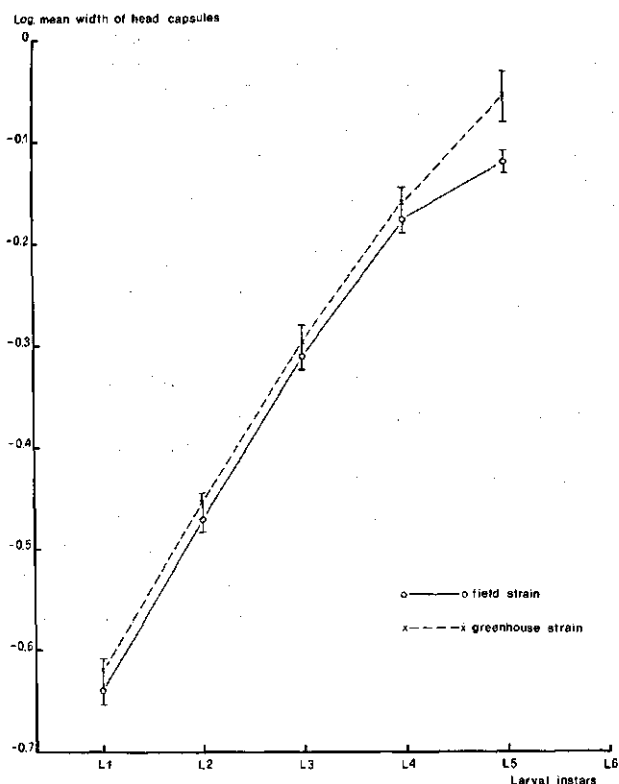


Figure 12. Log. mean widths of the head capsules (mm) of the separate instars of larvae of a field and a greenhouse strain, hibernating after 4 moults. Egg hatching: 1978-08-17. Vertical bars represent the reliability intervals of the means.

They did not essentially deviate from a geometrical progression, contrary to those of diapausing field strain larvae. Probably these greenhouse strain larvae were of the 6- and 7-instar growth type, and had reached the 4th or 5th instar by the onset of hibernation, without entering diapause. The 5-instar larvae of the greenhouse strain had by this time pupated or reached the prepupal instar. The winter mortality of the hibernating greenhouse strain larvae (egg hatching 1978-08-17) was not determined.

#### *Egg hatching 1978-09-25*

No pupation occurred in the greenhouse strain before December 25, 1978. The frequencies of the different numbers of moults that occurred until hibernation, and the mortality of hibernating larvae, are given in table 17.

Table 17. Frequencies of the numbers of moults occurring until hibernation in a greenhouse strain, and winter mortality (outdoor rearing, eggs hatched 1978-09-25).

number of moults until hibernation (greenhouse strain)	proportion of the total number of larvae (greenhouse strain)	mortality of hibernating larvae until pupation in the next spring
1	20%	
2	33%	greenhouse strain: 94%
3	46%	n=69
		field strain : 12%
		n=76

Field populations of *C. spectrana* enter diapause after at least 2 moults (chapter 5.3). Thus the greenhouse strain larvae hibernating after 1 moult (table 17) were probably not diapausing. The mean head capsule widths of the successive instars of greenhouse strain larvae hibernating after 2 or 3 moults (table 17) did not essentially deviate from a geometrical progression, contrary to those of field strain larvae entering diapause after 2 or 3 moults (figures 13 and 14). This indicates that at least most of these greenhouse strain larvae did not enter diapause. The mortality of hibernating larvae was much higher in the greenhouse strain than in the diapausing field strain (table 17).

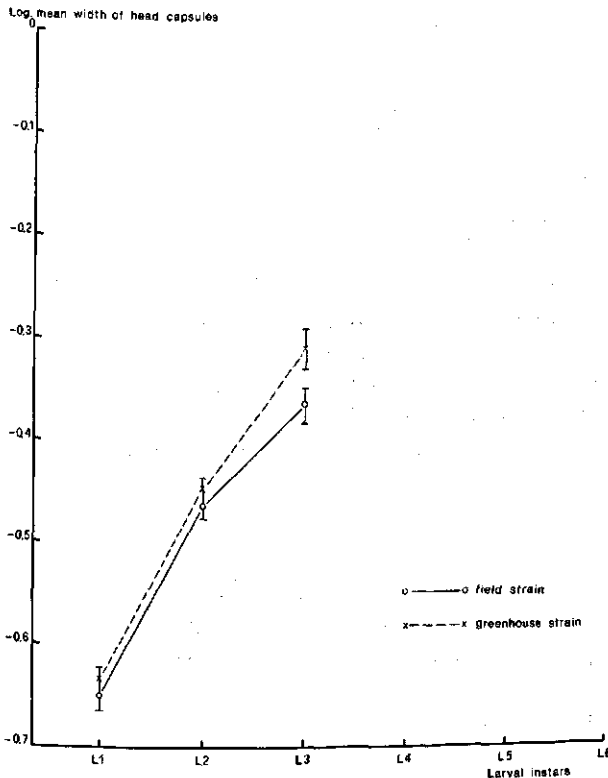


Figure 13. Log. mean widths of the head capsules (mm) of the separate instars of larvae of a field and a greenhouse strain, hibernating after 2 moults. Egg hatching: 1978-09-25. Vertical bars represent the reliability intervals of the means.

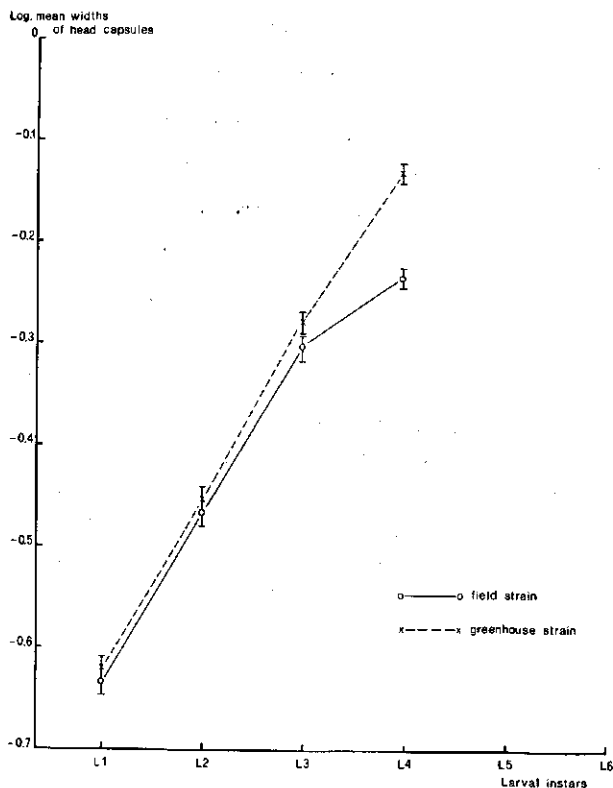


Figure 14. Log. mean widths of the head capsules (mm) of the separate instars of larvae of a field and a greenhouse strain, hibernating after 3 moults. Egg hatching: 1978-09-25. Vertical bars represent the reliability intervals of the means.

Larval development of both strains was compared with a simulated non-diapause development of 5-instar larvae. The use of this simulation model is justified in this case, as the larvae in these outdoor rearings (egg hatching 1978-09-25) did not develop further than the 4th instar until the onset of hibernation (tables 11 and 17), and the difference in development duration between the 5-, 6-, and 7-instar growth type is strongly expressed only from the 5th instar on. The simulations were done according to a method designed by De Wit & Goudriaan (1974) ("boxcar train with controlled dispersion"). The simulations were done on the basis of the temperature recorded by the thermohygrograph, and of the recorded temperature + 1°C, to allow for the inaccuracy of the temperature measurement. The temperature indicated by the thermohygrograph was compared once a week with the temperature indicated by a gauged mercury thermometer. After some time the thermohygrograph showed a lower temperature. The deviation was never larger than 1°C.

The simulation results are presented in figure 15. Larval development of the field and the greenhouse strain, and the simulated non-diapause development, coincided fairly well in the 1st and 2nd instar. In the 3rd and 4th instar, the development of the field strain deviated appreciably from the greenhouse strain development, and from the simulated non-diapause development, because diapause was entered. The major part of the field strain larvae interrupted its development and entered diapause after 2 moults.

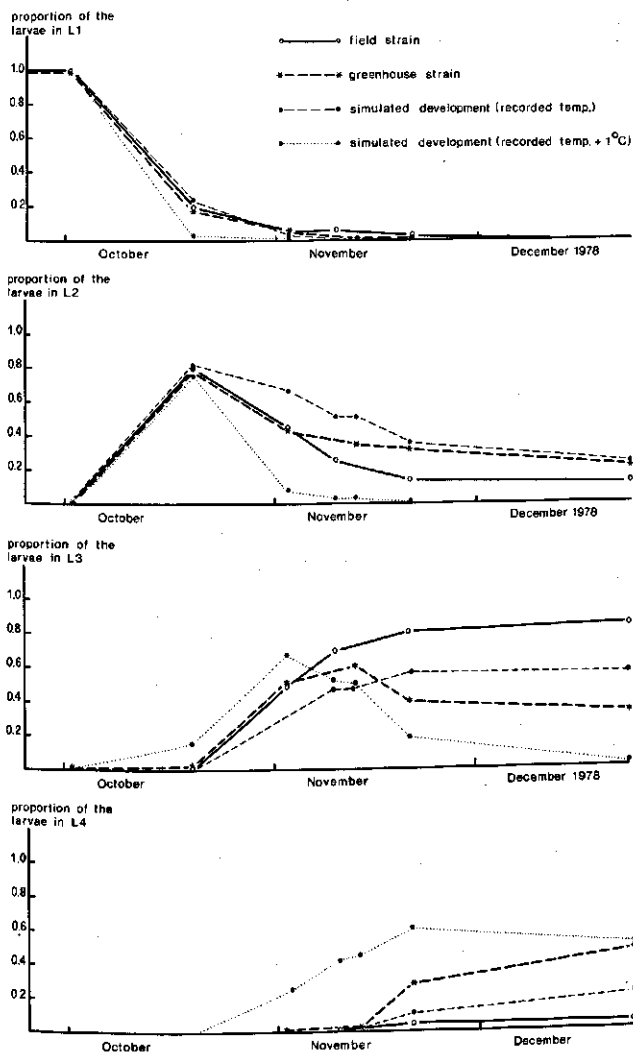


Figure 15. Larval development of a field and a greenhouse strain, and simulated non-diapause development, in outdoor rearings. Egg hatching: 1978-09-25.

*Egg hatching 1978-10-14*

No pupation occurred in the greenhouse strain before December 25, 1978. The frequencies of the different numbers of moults that occurred until the onset of hibernation, and the mortality of hibernating larvae, are given in table 18. The greenhouse strain larvae underwent 1 or 2 moults until hibernation. The larvae hibernating after 1 moult (table 18) probably were not diapausing. If there were diapausing greenhouse strain larvae among those hibernating after 2 moults, they could in the first place be expected

Table 18. Frequencies of the numbers of moults occurring until hibernation in the greenhouse strain, and winter mortality (outdoor rearing, eggs hatched 1978-10-14).

number of moults until hibernation (greenhouse strain)	proportion of the total number of larvae (greenhouse strain)	mortality of hibernating larvae until pupation in the next spring
1	7%	greenhouse strain: 72%
2	93%	n=74
		field strain : 44%
		n=71

among those that were able to reach the pupal stage in the next spring. The mean head capsule widths of the successive instars of these larvae are given in figure 16. They did not essentially deviate from a geometrical progression, contrary to those of the field strain larvae diapausing after 2 moults. Thus at least the major part of these greenhouse strain larvae did not enter diapause. They obviously survived a temperature-induced quiescence that lasted several months. Quiescence is a direct response to adverse physical conditions, and diapause an indirect response induced by periodic token stimuli such as photoperiod (Beck 1980, pp. 119-120). The mortality of hibernating greenhouse strain larvae was about twice that of field strain larvae (table 18).

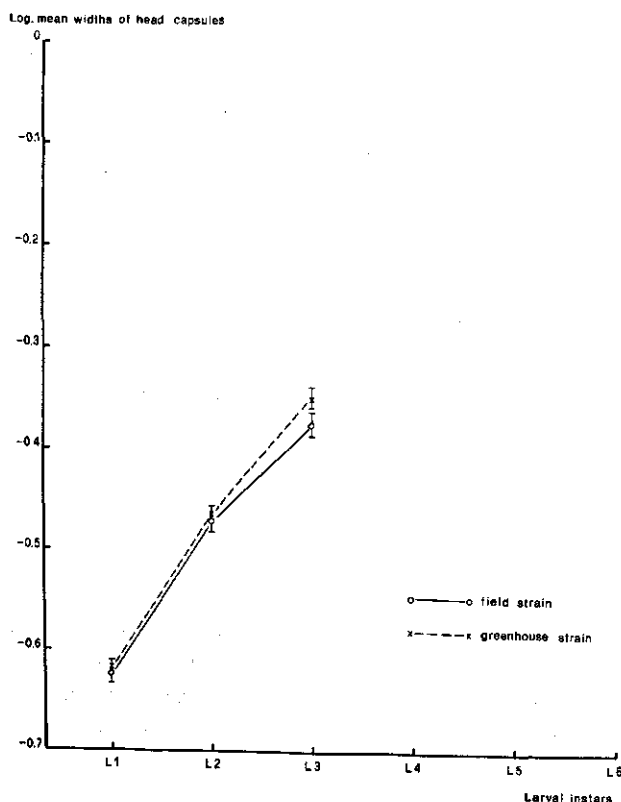


Figure 16. Log. mean widths of the head capsules (mm) of the separate instars of larvae of a field and a greenhouse strain, that hibernated after 2 moults and that were able to reach the pupal stage in the next spring. Egg hatching: 1978-10-14. Vertical bars represent the reliability intervals of the means.



### 6.3 DISCUSSION

Five different rose house strains did not enter diapause at any photoperiod at 20°C and 15°C on artificial diet in the laboratory.

The mean head capsule widths of the successive instars of outdoor reared hibernating larvae of one of these greenhouse strains never appreciably deviated from a geometrical progression, contrary to those of diapausing field strain larvae. This was valid even for the greenhouse strain larvae from eggs that hatched in mid-October, and that were able to reach the pupal stage during the next spring. Thus for every egg hatching date in the outdoor rearings it was proved that at least the major part of the greenhouse strain larvae did not enter diapause. Apparently this greenhouse strain had to a large extent lost its ability to enter diapause, not only in laboratory experiments but also under the tested outdoor conditions, on artificial diet.

In their natural environment, rose house strains of *C. spectrana* are almost certainly non-diapausing. Even under natural conditions in the open field no diapause may be induced in these populations.

## 7. DIAPAUSE OF A FIELD STRAIN UNDER GREENHOUSE CONDITIONS

The photoperiodic response of field populations of *C. spectrana* is different from that of greenhouse populations (chapter 6). Possibly field populations changed their photoperiodic response under the influence of specific selection pressures, after immigration into heated greenhouses. To discern how this selection may take place, knowledge about induction, maintenance, and termination of diapause in field populations introduced into greenhouse cultures is needed.

The climate in heated greenhouses is characterised by the lack of a period of chilling during winter. Many insects in a state of diapause die without developing, or grow in a protracted and irregular manner, when exposed to temperatures that would be expected to favour non-diapause morphogenesis (Lees 1955, p. 50).

### 7.1 MAINTENANCE AND TERMINATION OF DIAPAUSE WITHOUT A PERIOD OF CHILLING

#### 7.1.1 Greenhouse experiments

Field strain eggs (3rd and 4th laboratory generation), and larvae that hatched from these eggs, were reared in a *Gerbera* house on artificial diet, in a cage that was protected from the sun. The greenhouse was heated in winter (mean daily temperature 18°C, minimum night temperature 15°C). Pupation was observed at 1-3 day intervals. The experiment was done twice, during the second flight period of field populations, with eggs that hatched on August 24, and October 22, 1979. In each trial 200 larvae were used.

Larval development under these conditions was compared with:

- a. Non-diapause development in the same *Gerbera* house (100 field strain larvae from eggs that hatched on July 6, 1980; pupation was observed at 24-hour intervals).
- b. Development of field strain larvae with diapause outdoors (chapter 5).
- c. Non-diapause development outdoors (100 field strain larvae from eggs that hatched on July 6, 1980; pupation was observed at 24-hour intervals).

In figure 17 pupation rate in the course of time of the larvae from eggs that hatched on August 24, and on October 22, 1979 in the *Gerbera* house, is given. About 6 weeks after egg hatching all the larvae had made a hibernaculum. The first pupation occurred at least 150 days after egg hatching. Thus it was certain that all the larvae entered diapause.

The time elapsing between the first and the last pupation was extremely long. The larvae from eggs that hatched on August 24, 1979, pupated between January 28 and July 15 of the following year (figure 17). The larvae from eggs that hatched on October 22, 1979, pupated between March 21 and July 18 of the following year (figure 17). The number of

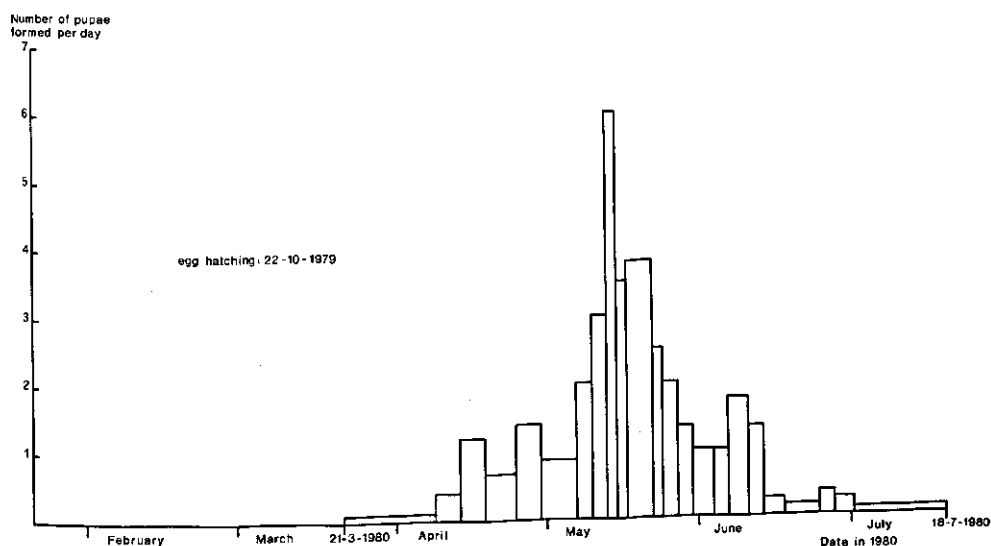
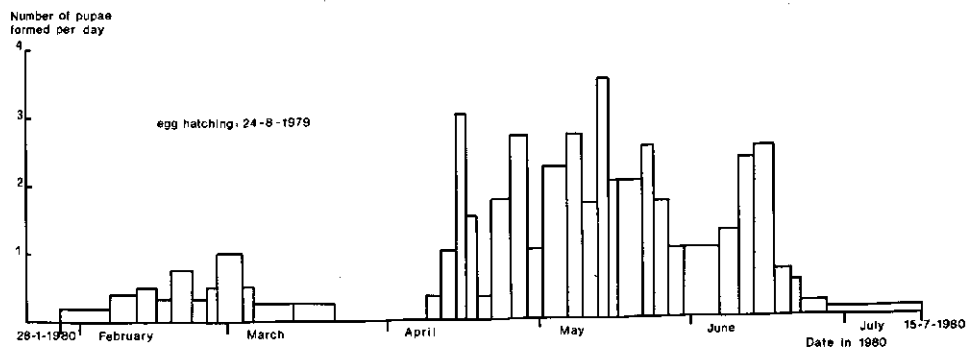


Figure 17. Pupation rate of a field strain after diapause in a heated greenhouse. Egg hatching: 1979-08-24 and 1979-10-22.

pupae formed per day was the highest around mid-May in both trials, but a large part of the larvae pupated before or after May 1980 (figure 17).

Apparently the development duration of these diapausing larvae was extremely variable, and neither a period of chilling nor long-day conditions were prerequisites for diapause termination.

In table 19 larval development (diapause and non-diapause) outdoors and in the *Gerbera* house is compared. The extremely long period which elapsed between the first and the last pupation after the field strain larvae had terminated their diapause in the greenhouse is also evident from the data in table 19. After diapause outdoors this period

Table 19. Larval development (diapause and non-diapause) outdoors and in a *Gerbera* house, on artificial diet, of a field strain.

rearing situations	date of egg hatching	larval mortality (egg hatching up to pupation)	time between first and last pupation (days)	mean developmental time of female larvae minus male larvae (days)
diapause in a <i>Gerbera</i> house	1979-08-24	23%	168	18
	1979-10-22	27%	119	21
diapause outdoors	1978-08-01	19%	38	7
	1978-08-17	30%	52	3
	1978-09-09	10%	67	4
	1978-09-25	16%	36	10
non-diapause in a <i>Gerbera</i> house	1980-07-06	17%	8	1
non-diapause outdoors	1980-07-06	33%	16	2

was much shorter, although the mean daily temperature was considerably lower than in the greenhouse.

The extreme variability in development duration of diapausing larvae in the greenhouse coincided with an increase in the difference in mean developmental time between male and female larvae (table 19). Thus, especially in the beginning and at the end of the period during which pupation took place, the chance of emerging field strain moths to find a mating partner was considerably reduced.

Larval mortality during development with diapause was not appreciably increased in the greenhouse compared to the other rearing situations mentioned in table 19. About  $\frac{1}{3}$  of the larvae in the greenhouse reached the pupal stage, although they had to terminate their diapause without a period of chilling. The fertility of the female moths after termination of diapause was not investigated.

When a field strain is introduced into cultures in heated greenhouses, diapause will be entered as soon as the daylength is sufficiently short. Diapause will considerably decrease the multiplication rate of the population per annum, but the absence of a period of chilling will not essentially affect the survival rate of the diapausing larvae.

#### 7.1.2 Laboratory experiments

Additional experiments were done in the laboratory, in which the development of individual diapausing larvae, without the interference of a period of chilling, was studied.

Field strain eggs (4th laboratory generation), and 250 larvae that hatched from these eggs, were reared at 20°C and 16 h. light per day until pupation. Larvae that did not die or pupate within 2 months after egg hatching, were considered to be diapausing. Moults and pupation of the diapausing larvae were observed at 2-day intervals.

Table 20. Frequencies of the numbers of moults of diapausing larvae, from egg hatching up to death or pupation, larval mortality, and mean time needed by diapausing larvae to reach the pupal stage, in different rearing situations, of field strains.

number of moults from egg hatching until death or pupation of diapausing larvae	rearing situations					
	outdoors (egg hatching Aug. 1 and 17, Sept. 9 and 25, 1978)		laboratory (20°C and 16 h. light per day)		laboratory (20°C and 16 h. light per day; after 2 months: 20°C and 18 h. light per day)	
	larvae pupating	larvae dying	larvae pupating	larvae dying	larvae pupating	larvae dying
2	---	6%	---	---	---	---
3	---	3%	---	---	---	5%
4	8%	64%	---	4%	1%	11%
5	61%	18%	7%	16%	9%	11%
6	27%	6%	18%	16%	41%	23%
7	3%	3%	11%	9%	24%	11%
8	---	---	18%	17%	15%	11%
9	---	---	22%	9%	5%	11%
10	---	---	9%	6%	2%	11%
11	---	---	9%	6%	2%	2%
12	---	---	---	4%	---	2%
13	---	---	---	3%	---	---
14	---	---	7%	6%	---	---
15	---	---	---	3%	---	---
16	---	---	---	---	---	---
17	---	---	---	---	---	---
18	---	---	---	2%	---	---
mean number of moults	5.2 n=260	4.2 n=33	8.6 n=45	8.3 n=90	6.7 n=147	7.0 n=44
mortality from egg hatching up to pupation	10-30%		77%		33%	
mean time needed by the diapausing larvae to reach the pupal stage (days)			98 (S.D.=48)		70 (S.D.=48)	

In a second experiment field strain eggs (4th laboratory generation), and 250 larvae that hatched from these eggs, were reared at 20°C and 16 h. light per day. The larvae that did not die or pupate within 2 months after egg hatching, were considered to be diapausing and transferred to 20°C and LD 18:6 (conditions that do not induce any diapause). Moults and pupation of these larvae were also observed at 2-day intervals.

Larval development under the conditions described above was compared with the development of larvae diapausing outdoors (experiments in chapter 5.2). The results are presented in table 20.

The mean number of moults from egg hatching until pupation of diapausing larvae was 5.2 under outdoor conditions. The number of moults outdoors never exceeded 7 (table 20).

The mean number of moults increased to 6.7 at 20°C and LD 18:6, and to 8.6 at 20°C and 16 h. light per day in the laboratory. The variability in the number of moults was much greater in the laboratory than outdoors. It is remarkable that, contrary to the situation outdoors, some of the laboratory reared diapausing larvae that died before pupation, underwent a higher number of moults than any of the diapausing larvae that were able to reach the pupal stage. Some of these larvae died after 18 moults (table 20).

The absence of a period of chilling apparently causes protracted and variable development in diapausing larvae of *C. spectrana*.

At 20°C and LD 18:6, diapausing larvae on the average needed 70 days to reach the pupal stage, while they needed 98 days at 20°C and 16 h. light per day (table 20). The difference was significant (2-sided t-test,  $P < .05$ ).

Long-day conditions apparently hasten the termination of diapause.

## 7.2 HEREDITY OF DEVELOPMENT DURATION IN DIAPAUSING LARVAE

Under greenhouse conditions, without a period of chilling, a strong variability in the time required for diapausing larvae to reach the pupal stage is expressed (section 7.1 of this chapter). If this variability is genetically determined, selection in favour of a shorter diapause duration may operate in heated greenhouses if a field strain is introduced. Experiments were set up to determine whether the development duration of diapausing larvae can be shortened by selection under conditions without a cold period.

From the greenhouse experiment described in section 7.1.1 of this chapter (egg hatching 1979-08-24) three groups of pupae were taken:  
group 1: pupae formed between 1980-01-28 and 1980-03-03  
group 2: pupae formed between 1980-05-01 and 1980-05-14  
group 3: pupae formed between 1980-06-12 and 1980-06-17  
Offspring of each group was reared at 20°C and LD 16:8. Each time 220 larvae were used, from eggs that had hatched on the same day. The larvae that did not die or pupate within 2 months were considered to be diapausing, and transferred to 25°C and LD 18:6 (long-day conditions that do not induce any diapause). Pupation was observed at 24-hour intervals to determine the length of time the diapausing larvae needed to pupate.

Group 1 had pupated earliest after diapause in the greenhouse. From their diapausing offspring the earliest 3 male pupae and the earliest 3 female pupae were taken (group 1-1), and their offspring was reared as the parental generation. From the diapausing offspring of group 1-1, again the earliest 3 male pupae and the earliest 3 female pupae were taken (group 1-1-1), and their offspring was reared as the parental generation.

The results of these experiments are given in table 21.

Table 21. Photoperiodic response of field strain larvae at diapause induction, and time required for diapausing larvae to reach the pupal stage, in selection experiments.

		proportion of the larvae entering diapause at 20°C and 16 h. light per day	mean time required by the diapausing larvae to reach the pupal stage (days) at 25°C and LD 18:6
1st generation	offspring group 1	93%	71 (S.D.=39) n=130
	offspring group 2	96%	73 (S.D.=56) n=51
	offspring group 3	64%	74 (S.D.=33) n=54
2nd generation	offspring group 1-1	100%	54 (S.D.=26) n=147
3rd generation	offspring group 1-1-1	94%	43 (S.D.=23) n=117

Within the 1st generation (offspring groups 1-3), a difference in the time needed for diapausing larvae to reach the pupal stage did not appear (table 21). The differences between the offspring of the groups 1, 2, and 3 were not significant (2-sided t-test,  $P>.05$ ).

In the 2nd generation (offspring group 1-1), the mean time required for diapausing larvae to reach the pupal stage was significantly shorter than in the previous generation (1-sided t-test,  $P<.005$ ).

In the 3rd generation (offspring group 1-1-1) the mean number of days needed by diapausing larvae to reach the pupal stage was again significantly shorter than in the previous generation (1-sided t-test,  $P<.005$ ).

The shortened development duration of diapausing larvae in the 2nd and 3rd generation did not coincide with a change of the photoperiodic response at 20°C and 16 h. light per day (table 21).

Genetically determined intrapopulation variation in the duration of diapause is found in many insect species (Tauber & Tauber 1976). The results in table 21 indicate such a genetically determined variation in *C. spectrana*. The mean time required for diapausing larvae to reach the pupal stage under conditions without a period of chilling, rapidly responded to selection. Consequently, if a field population of *C. spectrana* is introduced into cultures in heated greenhouses, the mean time required for diapausing larvae to reach the pupal stage will gradually be shortened, larvae pupating earlier contributing sooner to the reproduction of the population. If within the field populations sufficient genetic variation is present, the above mechanism may, after a sufficient number of generations is passed in the greenhouse, lead to a loss of diapausing behaviour of the whole population under these conditions.

## 8. FEMALE SEX PHEROMONE

Female sex pheromone plays an important role in arousing mating behaviour in many insect species. The composition of the pheromone is usually specific for each species, thus contributing essentially to their reproductive isolation.

To detect possible isolation mechanisms between field and greenhouse populations of *C. spectrana*, studies on sex pheromone and calling behaviour of the female moths are important. The female sex pheromone of field populations of *C. spectrana* comprises at least 2 components, cis-9- and cis-11-tetradecen-1-ol acetate (cis-9- and cis-11-TDA). Cis-11-TDA is the main component (Minks et al. 1973). Optimal attraction in the field is obtained with a mixture of cis-11- and cis-9-TDA in the ratio 9:1 (Minks et al. 1974). Poor results were obtained when this mixture was used in greenhouses (Van de Vrie 1976). This may indicate a deviating composition of the pheromone produced by greenhouse strain females. It may also be caused by the deviating nature of the air movements in greenhouses.

### 8.1 COMPOSITION OF THE FEMALE SEX PHEROMONE

The contents of a number of pheromone glands of a field strain (2nd laboratory generation) and a greenhouse strain (3rd laboratory generation) were analysed by gas chromatography. The cooperation of W.J. Nooijen Ing. (Division of Technology for Society TNO, Department of Chemistry, Delft) in performing these analyses, is gratefully acknowledged. Fifty glands of each strain were used. The females were allowed to emerge at 20±1°C under artificial white light: light period 17 hours, dusk 1.5 hour, dark period 4 hours, dawn 1.5 hour. The light intensity changed linearly during dawn and dusk. Observations during the dark period were made possible by the use of red fluorescent lights. The glands were collected during the second half of the dark period when the females were 4 days old. The glands, together with an internal standard (octadecane C18 alcohol), were kept in an aluminium capsule in fluid nitrogen (-196°C). The contents of the glands, together with the internal standard, were injected with a Perkin Elmer MS 41 solid injector (Décoins & Gallois 1979) into a stainless steel 3% ov-101 column on chromosorb WHP, 2 m long and an inner diameter of 4 mm. The effluents were collected in the C14 acetate area, and with a falling needle injector put into a glass cp wax 51 capillary, 50 m long and an inner diameter of 0.25 mm.

The female sex pheromone of both strains contained two components in the C14 acetate area. The retention times of these components coincided with those of cis-9-TDA and cis-11-TDA. The ratio cis-9:cis-11 in the pheromone glands was 2:100 in the field strain and 1:100 in the greenhouse strain.



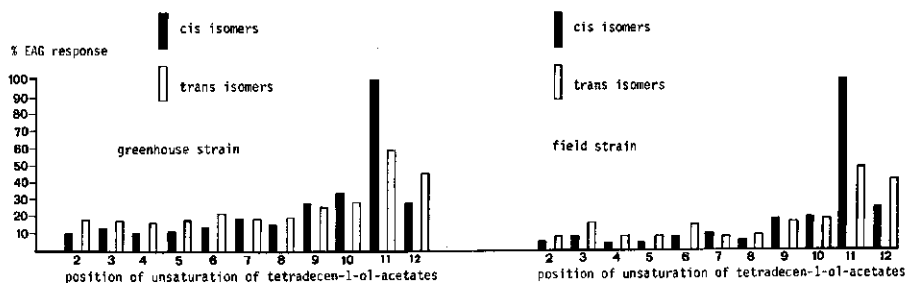


Figure 18. Male antennal response of a field and a greenhouse strain to mono-unsaturated tetradecen-1-ol acetate standards. EAG, electro-antennogram. Average control response is 0 to 1 percent.

The male antennal response to a series of mono-unsaturated TDA standards was determined by electro-antennography (EAG). Thanks are due to Dr. Ir. C.J. Persoons (Division of Technology for Society TNO, Department of Chemistry, Delft) for carrying out these experiments. Eight males of each strain were used.

The results are presented in figure 18. The electro-antennogram pattern was identical for the males of both strains. This confirms that the ratio cis-9-TDA:cis-11-TDA was not or slightly different in the two strains.

## 8.2 CALLING BEHAVIOUR OF VIRGIN FEMALES IN RELATION TO THE LIGHT-DARK CYCLE

Even if the composition of the female sex pheromone is identical in both strains, mutual attraction may be inhibited if the females call at different times during the night.

The calling behaviour of virgin females in relation to the light-dark cycle was investigated in an experimental room under standardized conditions: light period 17 hours, "dusk" 1.5 hour, dark period 4 hours, and "dawn" 1.5 hour; light was given with 2 bulbs (white light) of 200 W each; the light intensity changed linearly during "dusk" and "dawn"; during the light period 2 fluorescent tubes (white light) of 65 W and 50 Hz each, were additionally used; the light sources were placed at a height of 1 m above the moths, and were arranged in such a way that both strains received the same light intensity; observations during the dark period were made possible by the use of red fluorescent lights; temperature 23°C (light period) and 21°C (dark period); relative air humidity 70-80%.

Female pupae were collected from a mass rearing at 25°C and LD 18:6 within 16 hours after pupation and transferred to the experimental room. The moths were allowed to emerge individually in glass vials and transferred to perspex cylinders (length 15 cm, diameter 10 cm) closed with gauze at both ends (5 moths per cylinder). Wet cotton wool was used as a water supply for the females. Calling behaviour was observed during 2 successive "nights" with 3-4 days old moths (50 per strain per "night"). The observations were made at 15-



### 8.3.1 Outdoor trials

One set of release-recapture trials was conducted in an outdoor cage (area  $7 \times 6 = 42 \text{ m}^2$ , height 2.5 m) of polyamide gauze (mesh width 1.25 mm, thread diameter 0.315 mm), planted with rows of *Prunus triloba* (L.). Air currents in the cage resembled those in a natural situation.

Three kinds of traps, each in duplicate, were used:

- Sticky traps (delta shape) baited with 2 virgin females of a field strain, that were kept inside the trap in a tube (length 5 cm, diameter 2.5 cm), closed at both ends with gauze. Wet cotton wool was used as a water supply for the females.
  - Traps of the same type baited with 2 virgin females of a greenhouse strain.
  - Unbaited traps of the same type were used to assess what proportion of the catches in the female-baited traps had to be attributed to other factors than pheromonal attraction.
- The traps were placed 1 m from the walls of the cage, at a height of 0.5 m. The distance between adjacent traps was 2.5-4 m.

Male and female moths were taken from a mass rearing at  $25^{\circ}\text{C}$  and LD 18:6 (greenhouse strain 2nd and 3rd, field strain 3rd and 4th laboratory generation). The moths were allowed to emerge individually in glass tubes. Females were transferred to the traps within 16 hours after emergence. Males were released inside the cage within 16 hours after emergence when the females in the traps were at least 2 days old. Field strain and greenhouse strain males were released alternately at intervals of at least 2 weeks, to prevent them getting mixed up. Recaptures in the traps were recorded daily, and dead or dying females were replaced. The arrangement of the traps was changed daily to prevent effects of position in the catches. The trials lasted from June until October 1980.

The results are presented in table 22. Differences in the release-recapture trials were tested by the  $\chi^2$ -method (1-sided test). Recaptures in the female-baited traps were always significantly larger than in the traps without bait ( $P < .005$ ) (table 22). This demonstrates the role of pheromonal attraction in these experiments. There were no

Table 22. Percentage recaptured males in female-baited and unbaited traps in a field cage.

	traps baited with virgin females of a greenhouse strain	traps baited with virgin females of a field strain	unbaited traps
males greenhouse strain	3.8% n=27	9.2% n=66	0.6% n=4
males field strain	4.2% n=18	8.5% n=36	0.7% n=3
total number of males released: greenhouse strain: 715 field strain : 426			

significant differences in recaptures between the males of both strains ( $P>.05$ ), thus females of both strains attracted males of both strains in equal proportions (table 22). This confirms the unhampered mutual attraction between the strains, when brought together under outdoor conditions.

Field strain females attracted about twice as many males of each strain than greenhouse strain females (table 22). At present, an exact explanation for this phenomenon cannot be given. The greenhouse strain females might for example have called less frequently than the field strain females, or the amount of pheromone produced per female might have been lower in the greenhouse strain.

### 8.3.2 Greenhouse trials

Another series of release-recapture trials was conducted in a *Gerbera* house (area  $8 \times 10 = 80 \text{ m}^2$ , ridge height 5 m). The ventilators were screened with gauze to prevent moth escape. The trials were set up in exactly the same way as those in the outdoor cage. The number of traps per  $\text{m}^2$  was about the same as in the outdoor cage: 2 traps of each kind were placed between the *Gerbera* beds, and another 2 of each kind along the walls inside the greenhouse, at a height of 0.5 m. The distance between adjacent traps was 3-4 m. The trials were conducted during 3 weeks in January 1981, when the greenhouse was continuously heated (greenhouse strain 1st, field strain 5th laboratory generation), and during 3 weeks in June 1981, when the greenhouse was only incidentally heated during the coldest part of the night (greenhouse strain 3rd, and field strain 1st laboratory generation). Released male moths all belonged to a greenhouse strain. The recaptures were compared with those of greenhouse strain males in the outdoor cage (section 8.3.1 of this chapter). The results are presented in the tables 23 and 24.

Table 23. Percentage recaptured males (greenhouse strain) in female-baited and unbaited traps in a greenhouse.

		traps baited with virgin females of a greenhouse strain	traps baited with virgin females of a field strain	unbaited traps
January 1981	along the walls	1.4% n=14	2.0% n=20	1.0% n=10
	between the plant beds	0.4% n=4	0.6% n=6	0.2% n=2
June 1981	along the walls	3.4% n=25	1.7% n=12	0.8% n=6
	between the plant beds	0.8% n=6	0.8% n=6	0.3% n=2

total number of greenhouse strain males released:

January 1981: 1010

June 1981: 726

The recaptures of greenhouse strain males in the greenhouse were low compared to those of greenhouse strain males in the outdoor cage (tables 22 and 23). Although the recaptures in the traps baited with greenhouse strain females did not even differ significantly from those in the unbaited traps in the trials of January 1981 ( $P > .05$ ) (table 23), it is justifiable to conclude that both greenhouse strain females and field strain females attracted greenhouse strain males in a greenhouse environment.

In January 1981, field strain females attracted more males than greenhouse strain females, but the difference was not significant ( $P > .05$ ) (table 23). In June 1981, field strain females attracted significantly less males than greenhouse strain females ( $P < .05$ ) (table 23). The recaptures in the greenhouse were significantly higher along the walls than between the plant beds ( $P < .05$ ) (table 23). This was the case for both female-baited and unbaited traps. At present, an exact explanation for these phenomena cannot be given.

In table 24 recaptures of greenhouse strain males in the greenhouse and in the outdoor cage are compared. A significantly lower proportion of males was recaptured in the greenhouse compared to the outdoor cage, both before and after correction for the catches in unbaited traps ( $P < .005$ ). Apparently pheromonal attraction by virgin females was less effective in the greenhouse than in the outdoor cage.

Air movements in greenhouses differ greatly from those outdoors. In greenhouses there are no horizontal air currents, but the air only circulates by warm air rising and cold air falling (Kanthak 1973). The wind speed is very low. It usually varies between 5 and 20  $\text{cm/sec}^{-1}$  when the ventilators are closed, and between 5 and 40  $\text{cm/sec}^{-1}$  when open (Ir. G.P.A. Bot, Ir. G.A. van den Berg, pers. comm.).

The reduced effectiveness of pheromonal attraction in the greenhouse may have different causes. Basic male flight activity (not elicited by pheromone) may be lower in greenhouses than in the open air, and females may call less frequently. A more likely explanation is that pheromone-mediated long-range behaviour of the males is disturbed in a greenhouse environment. In the field, males react by flying up wind when they encounter pheromone molecules (Kennedy & Marsh 1974). The specific nature of the air movements in

Table 24. Comparison between recaptures of male moths (greenhouse strain) in an outdoor cage and in a greenhouse (release-recapture trials using female-baited and unbaited traps).

		greenhouse (January)	greenhouse (June)	outdoor cage (summer)
% males recaptured	along the walls	4.4%	5.9%	
	between the plant beds	1.2%	1.9%	
	total recapture	5.6%	7.8%	13.6%
% males recaptured after correction for the catches in unbaited traps		2.0%	4.5%	11.9%
number of males released		1010	726	715

greenhouses may reduce the effectiveness of this anemotactic behaviour. This may be the reason for the poor catches of *C. spectrana* in traps in greenhouses that were baited with synthetic pheromone, as reported by Van de Vrie (1976). However, male sexual behaviour in short-range orientation, close to calling females, may be unaffected in greenhouses.

*C. spectrana* is a serious pest in greenhouses. The chance of a male getting close to a calling female, with or without pheromone-mediated anemotactic flight, and therefore the chance of a female getting fertilized, is apparently sufficiently high to ensure a considerable population increase. Behavioural observations should reveal whether greenhouse populations of *C. spectrana* have adapted their sexual behaviour to the specific greenhouse conditions, e.g. in the sense that other than pheromonal stimuli play a more important role than in field populations.

#### 8.4 MATING PREFERENCE

Roelofs & Cardé (1977) suggested that the function of secondary pheromone components may be to release male short-range responses (landing, wing fanning, gland extrusion, mating). The female sex pheromone of the summerfruit tortrix moth, *Adoxophyes orana* (F.v. R.), and that of *C. spectrana*, are thought to contain the same components (cis-11-TDA and cis-9-TDA), but in a different ratio (Minks et al. 1973). Den Otter et al. (1978) and Den Otter & Klijnsma (1980) suggested that the pheromone released by females of *A. orana* contains one or more additional components that are needed to induce the copulatory act. Thus the fact that a field strain and a greenhouse strain of *C. spectrana* mutually attract each other when they are brought together, does not necessarily imply that inter-strain matings can occur. Experiments with a field strain and a greenhouse strain were set up to check whether inter-strain matings are possible.

Male and female moths of a field strain (1st laboratory generation) and a greenhouse strain (3rd laboratory generation) were taken at random from one group of each strain, reared at 25°C and LD 18:6. They were brought together within 16 hours after emergence in polyethylene bags (size ca 10x15x30 cm, each containing a fresh rose shoot placed in wet Oasis). The bags were kept at 25°C and LD 18:6, and each received 1 female and 3 males, in the following combinations: greenhouse ♀ x greenhouse ♂♂; greenhouse ♀ x field ♂♂; field ♀ x greenhouse ♂♂; field ♀ x field ♂♂. There were 10 bags per combination. Mating was considered to have occurred if a female laid fertilized eggs.

Table 25. Occurrence of intra-strain and inter-strain matings between a field and a greenhouse strain.

female x males	number of females laying fertilized eggs
greenhouse x greenhouse	6 (n=10)
greenhouse x field	5 (n=9)
field x greenhouse	5 (n=10)
field x field	7 (n=10)

Successful inter-strain matings readily occurred (table 25). In these experiments, a difference in mating preference between the strains did not appear. If only a minor difference exists, it may only be detected in a situation in which males could actually choose between females of both strains.

## 9. HYBRIDIZATION

No isolating mechanisms were found in the pheromonal attraction or mating habits between field and greenhouse populations of *C. spectrana* (chapter 8). Allozyme frequencies were determined in order to get an indication of the degree of genetic differentiation between these populations. Crossing experiments were done to determine whether hybrid offspring are viable and fertile. The photoperiodic response of F1 hybrids was determined.

### 9.1 ALLOZYME FREQUENCIES

One hundred larvae were collected from stinging nettles, *Urtica dioica* (L.), near Wageningen in May 1979, and another 100 larvae from a rose house in Roelofarendsveen (near Leiden) in August 1979. Fifty larvae of each population were preserved at -25°C. The rest of the larvae were reared on artificial diet, and the moths were also preserved at -25°C. Allozyme (electromorph) frequencies of 18 different loci in both strains were determined by electrophoresis. The cooperation of Dr. S.B.J. Menken (Department of Zoogeography, Free University, Amsterdam) in performing these experiments is gratefully acknowledged. The methods applied by Menken (1980) for *Yponomeuta* spp. (Lepidoptera: Yponomeutidae) were used. The abbreviations, names and Enzyme Commission Numbers for the enzymes assayed are as follows: ACPH, acid phosphatase, E.C.3.1.3.2; ALD, aldolase, E.C.4.1.2.13; EST- $\beta$ -1, esterase, E.C.3.1.1.2; FUDH, fucose dehydrogenase, E.C.1.1.1.122; GLUO, glucose oxidase, E.C.1.1.3.4; GOT-1 and GOT-2, glutamic-oxaloacetic transaminase, E.C.2.6.1.1; G6PDH, glucose-6-phosphate dehydrogenase, E.C.1.1.1.49; HDBH, hydroxybutyrate dehydrogenase (runs cathodally), E.C.1.1.1.30; LAP-1, leucine aminopeptidase, E.C.3.4.11.1; MDH-1 (runs anodally) and MDH-2 (runs cathodally), malate dehydrogenase, E.C.1.1.1.37; ME, malic enzyme, E.C.1.1.1.40; NDH, "nothing dehydrogenase" (runs cathodally); PGI, phosphoglucose isomerase, E.C.5.3.1.9; PGM, phosphoglucomutase, E.C.2.7.5.1; PT-1 and PT-2, "general proteins" with unknown function.

Eight enzyme loci were fixed for the same allele in both populations: ACPH, FUDH, GOT-1, GOT-2, G6PDH, ME, NDH, and PT-2. The allozyme frequencies of the other 10 loci are given in table 26.

These electrophoretic data were used to estimate the average heterozygosity per locus (H), the genetic distance (D), and the genetic identity (I) of the two populations.

H estimates the proportion of heterozygous loci in a single individual or population. It amounted to 0.098 in the field population, while the greenhouse population had a



Table 26. Allozyme frequencies at 10 loci in a field population and a greenhouse population.

locus	allozymes	field population	greenhouse population
ALD	100	97%	96%
	103	3%	4%
		n=38	n=28
EST- $\beta$ -1	97	9%	---
	100	91%	100%
		n=38	n=28
GLUO	100	71%	73%
	101	---	2%
	102	29%	23%
	104	---	2%
		n=38	n=28
HDBH	- 94	9%	---
	-100	91%	100%
		n=23	n=28
LAP-1	98	8%	4%
	100	92%	91%
	102	---	5%
		n=38	n=28
MDH-1	97	1%	---
	100	84%	75%
	107	14%	25%
		n=38	n=28
MDH-2	- 98	1%	---
	-100	99%	82%
	-102	---	18%
		n=38	n=28
PGI	94.5	---	10%
	96.5	7%	---
	100	88%	90%
	105.5	5%	---
		n=88	n=66
PGM	94	---	1%
	97	1%	12%
	100	91%	78%
	106	8%	9%
		n=68	n=43
PT-1	100	92%	100%
	102	8%	---
		n=38	n=28

similar value (0.104).

D estimates the number of net codon differences per locus between populations (Nei 1971, 1972). The value was  $0.006 \pm 0.018$  in the present investigation. The mean distance values ( $\bar{D}$ ) in the genus *Speyeria* (Lepidoptera: Nymphalidae) are  $0.013 \pm 0.003$  for conspecific populations, and  $0.182 \pm 0.013$  between species (Brittnacher et al. 1978).

Compared with similar studies of species groups not belonging to the order Lepidoptera, the *Speyeria* values are low. They correspond very well with the distance values found in the genus *Yponomeuta* (Menken 1980). The value of D in the present investigation indicates a conspecific status of field and greenhouse populations of *C. spectrana*.

The index I measures the mean genetic similarity between two groups (Nei 1971, 1972). The value was 0.994 in the present investigation. For sibling species I generally ranges from 0.98 to less than 0.70 (Ayala 1975). The genetic identity of conspecific local populations ranges from 0.97 to 1.00 in *Drosophila* spp. (Powell 1975), from 0.94 to 0.98 in *Aedes aegypti* (Munstermann 1979), and from 0.989 to 0.997 in *Aedes triseriatus* (Matthews & Craig 1980). The mean identity values ( $\bar{I}$ ) in the genus *Speyeria* are  $0.987 \pm 0.003$  for conspecific populations, and  $0.833 \pm 0.011$  between species (Brittnacher et al. 1978). These correspond very well with the identity values in the genus *Yponomeuta* (Menken 1980). When conspecific populations are panmictic, their genetic similarity is expected to be greater than 0.99 (Matthews & Craig 1980). Thus the genetic identity of the two populations in the present investigation was at the level of panmictic populations of one species.

Field and greenhouse populations of *C. spectrana* are almost certainly still conspecific.

## 9.2 CROSSING EXPERIMENTS

A field and a greenhouse strain were crossed in the F1 and F2 generation at 25°C and LD 18:6. The field strain (1st laboratory generation) originated from larvae and pupae collected on *Urtica dioica* near Wageningen, and the greenhouse strain (3rd laboratory generation) from larvae and pupae collected in a rose house in De Kaag (near Leiden). The strains had been reared at 15°C and LD 18:6.

In each F1 and F2 crossing 15 male and 15 female moths were, within 16 hours after emergence, taken at random and confined in one polyethylene bag (size ca 15x30x45 cm), containing a fresh rose shoot placed in wet Oasis.

The duration of development of a number of eggs laid on the inner side of the polyethylene bag was determined (at least 200 viable eggs per crossing). The egg masses were cut out within 16 hours after laying and put on moist filter paper in a petri dish in a closed plastic bag. Egg hatching was observed at 24-hour intervals.

The rest of the egg masses were divided at random over petri dishes containing fresh rose leaves. The dishes were kept in closed plastic bags. Within 16 hours after egg hatching, 220 larvae were collected at random and transferred to glass vials containing artificial diet. The vials were kept in closed transparent plastic boxes.

Egg mortality was determined by counting the empty chorions (representing hatched eggs), as well as the non-hatched eggs.

Pupation and moth emergence were observed at 24 h. intervals, after pupation the diets and larval webs were removed to ensure unhampered moth emergence. The bottom of the boxes were covered with wet filter paper in order to ensure a high air humidity.

### 9.2.1 F1 crosses

Table 27. Fecundity, fertility, and sex ratio in F1 crosses at 25°C and LD 18:6.

females x males	number of eggs laid per female	number of eggs hatched per female	% male pupae
F x F	467	322	45%
F x G	476	324	50%
G x F	394	256	49%
G x G	375	236	59%

F=field strain      G=greenhouse strain

The first set of crosses involved the field and greenhouse strain matings, and the two possible hybrid crosses. The results are presented in the tables 27, 28, and 29.

Fecundity and fertility did not seem to be affected by the strain of male participating in the mating (table 27). The sex ratios of the hybrid and pure strain progeny did not significantly deviate from 1:1 (2-sided  $\chi^2$ -test,  $P>.05$ ). On the basis of these experiments it cannot be decided whether the pure strains significantly differed in fecundity or fertility, as the number of eggs laid by each separate female involved in a crossing was not known. The number of eggs per female was calculated by dividing the total number of eggs per crossing by 15 (the number of females involved in each crossing).

Mortality of the progeny in the F1 crosses did not differ significantly in each of the immature life stages (2-sided  $\chi^2$ -test,  $P>.05$ ) (table 28).

Compared to the field strain (FxF), the mean larval development duration in the greenhouse strain (GxG) was significantly longer (2-sided t-test,  $P<.005$ ) (table 29). Possibly the mean number of instars up to pupation was higher in the greenhouse strain. The number of instars was not determined in these experiments. Larval development durations in the two hybrid classes (FxG and GxF) ranged between those of the pure strains (table 29). In the egg stage and pupal stage no significant differences between the F1 crosses were found ( $P>.05$ ) (table 29).

Table 28. Percentage mortality of various life stages in F1 crosses at 25°C and LD 18:6.

females x males	% non hatched eggs	% larval mortality	% pupal mortality	% overall mortality from egg to adult
F x F	31%	5%	4%	37%
F x G	32%	3%	5%	37%
G x F	35%	4%	4%	40%
G x G	37%	7%	3%	44%

F=field strain      G=greenhouse strain

Table 29. Mean duration of development (days) in F1 crosses at 25°C and LD 18:6.

females x males	eggs	larvae		pupae		total egg-adult	
		♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
F x F	6.2	20.9	23.5	6.2	5.8	33.3	35.5
F x G	6.5	22.4	25.4	6.4	6.0	35.3	37.9
G x F	6.4	21.1	23.5	6.1	5.9	33.6	35.8
G x G	6.9	26.4	28.4	6.4	5.9	39.7	41.2

F=field strain      G=greenhouse strain

### 9.2.2 F2 crosses

Six different F2 crosses were set up to test the reproductive capacity of hybrid females and the viability of the progeny. The results are presented in the tables 30, 31, and 32.

Fecundity and fertility in the F2 crosses were variable (table 30). Sometimes they were higher, and sometimes lower than in the F1 crosses (tables 27 and 30). The numbers of male and female pupae never significantly deviated from the 1:1 ratio in the F2 crosses (2-sided  $\chi^2$ -test,  $P>.05$ ) (table 30).

Mortality in the 2nd generation crosses was variable in the egg stage and in the pupal stage (table 31). Total mortality up to moth emergence was sometimes significantly lower, and sometimes significantly higher than in the pooled control crosses (FxF and GxG combined). The mean total mortality amounted to 48% in the pooled F2 crosses of hybrids, which was significantly higher than in the pooled control crosses ( $P<.05$ ). In the pooled F2 backcrosses it was 39%, which was about the same as in the pooled control crosses.

Development duration did not differ significantly between all F2 and F1 crosses in the egg and pupal stage (2-sided t-test,  $P>.05$ ) (tables 29 and 32). In the larval stage,

Table 30. Fecundity, fertility, and sex ratio in F2 crosses at 25°C and LD 18:6.

females x males		number of eggs laid per female	number of eggs hatched per female	% male pupae
back- crosses	F x (FxG)	428	321	51%
	F x (GxF)	392	188	52%
	(FxG) x G	499	429	47%
	(GxF) x G	464	329	57%
crosses of hybrids	(FxG) x (FxG)	478	311	52%
	(GxF) x (GxF)	295	162	48%

F=field strain      G=greenhouse strain

Table 31. Percentage mortality of various life stages in F2 crosses at 25°C and LD 18:6.

females x males		% non hatched eggs	% larval mortality	% pupal mortality	% overall mortality egg-adult
back-crosses	F x (FxG)	25%*	5%+	5%+	32%+
	F x (GxF)	52%*	4%+	9%*	58%*
	(FxG) x G	14%*	3%+	18%*	31%*
	(GxF) x G	29%+	4%+	5%+	36%+
crosses of hybrids	(FxG) x (FxG)	35%+	4%+	18%*	49%*
	(GxF) x (GxF)	45%*	8%+	19%*	58%*
pooled control crosses (FxF + GxG)		34%	6%	4%	40%

F=field strain  
 G=greenhouse strain

+ does not differ significantly from pooled control cross data (2-sided  $\chi^2$ -test,  $P>.05$ )  
 \* differs significantly from pooled control cross data ( $P<.05$ )

the development duration in the F2 crosses ranged between those of the pure strains or was even shorter (tables 29 and 32). An interpretation of these differences cannot be given, because the number of larval instars was not determined in these experiments.

Table 32. Mean duration of development (days) in F2 crosses at 25°C and LD 18:6.

females x males		eggs	larvae		pupae		total egg-adult	
			♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
back-crosses	F x (FxG)	6.3	19.5	22.0	6.6	6.2	32.4	34.5
	F x (GxF)	6.8	19.6	22.2	6.6	6.2	33.0	35.2
	(FxG) x G	6.4	20.4	22.3	6.5	6.3	33.3	35.0
	(GxF) x G	6.4	21.8	23.5	6.7	6.4	34.9	36.3
crosses of hybrids	(FxG) x (FxG)	6.5	22.3	25.4	6.7	6.5	35.5	38.4
	(GxF) x (GxF)	6.7	23.2	25.5	6.4	6.1	36.3	38.3

F=field strain      G=greenhouse strain

### 9.2.3 Discussion

When heterosis of F1 hybrids occurs, it is caused by increased genetic variability of the hybrids compared to the control crosses, allowing the most efficient developmental pathways to predominate during growth. It is possibly expressed by a faster development and a higher survival rate when compared to the control crosses (Liebherr & Roelofs 1975). The F1 hybrid progeny did not exhibit a higher survival rate than the control progeny in any of the immature stages (table 28). The duration of development did not differ

significantly from the control progeny in the egg and pupal stage, but a definite conclusion about the rate of development of the larvae is hampered by possible variations in the mean number of instars.

Oliver (1972) considers that hybrid incompatibility in a species cross is shown by reduced fecundity, reduced embryo viability, a distorted sex ratio and more asynchronous eclosion of the two sexes in the hybrid crosses when compared with the control crosses.

More asynchronous eclosion of the two sexes did occur neither in F1 nor in F2 hybrids (tables 29 and 32).

The sex ratio never significantly deviated from 1:1 (tables 27 and 30). Reduced embryo viability occurred in some of the F2 crosses, but in others it was significantly increased (table 31).

Mean fecundity of pure strain females amounted to 422, and mean fertility 275 (derived from the tables 27 and 30). For hybrid females, these values were 434 and 301 respectively (derived from table 30). Fecundity and fertility of hybrid females did not seem to be reduced, but a definite conclusion is hampered by the fact that the number of eggs laid by each separate female involved in a crossing, was not known.

The question whether hybrid incompatibility in any form exists, cannot be answered, but when a field strain and a greenhouse strain are brought together, hybridization and introgression certainly will occur.

### 9.3 PHOTOPERIODIC RESPONSE IN F1 HYBRIDS

Field and greenhouse populations of *C. spestrana* can readily be intercrossed (section 9.2 of this chapter). Thus diapause characteristics can be crossed into greenhouse populations by introducing field strain moths. To determine the effectiveness of this procedure as a genetic control method, knowledge about the photoperiodic response of F1 hybrids is needed.

Photoperiodic response of the reciprocal F1 hybrids was determined at 20°C and 16 h. light per day in crossing experiments using a field strain (3rd laboratory generation) and a greenhouse strain (2nd laboratory generation). The strains had been reared at 15°C and LD 18:6. For each crossing 15 male moths and 15 female moths were taken at random from one lot of each strain, and confined in one polyethylene bag, containing a fresh rose shoot. Eggs were collected and kept in petri dishes on fresh rose leaves. A sample of 220 larvae was taken at random within 16 hours after egg hatching, and reared individually on artificial diet. Larvae that did not die or pupate within 2 months after egg hatching were considered to be diapausing.

The percentage of diapausing larvae in each of the 4 possible F1 crosses, is given in table 33. The reciprocal F1 hybrids showed an intermediate photoperiodic response compared to the pure strains. Their reaction inclined towards the maternal characteristics.

Table 33. Percentage of larvae entering diapause at 20°C and 16 h. light per day, in F1 crosses.

	females x males			
	F x F	F x G	G x F	G x G
% diapause	86% n=208	52% n=203	18% n=214	0% n=210

## 10. DISCUSSION

Field and rose house populations of *C. spectrana* differ essentially with respect to diapause. Field populations have a facultative, photoperiodically induced diapause in the larval stage (chapter 5). Rose house populations lacked the ability to enter diapause under the conditions tested, both in laboratory and outdoor experiments (chapter 6). Non-diapausing behaviour is advantageous in heated greenhouses, where suitable food is available throughout the year, and the temperature always allows for growth and reproduction.

Morphological differences between field and rose house populations did not appear (Wit 1978). No differences were found in the composition of the female sex pheromone, the pheromone-mediated behaviour of the males, the calling behaviour of virgin females in relation to the light-dark cycle, and mating preference (chapter 8). Field and rose house populations can be intercrossed with a viable F2 generation (chapter 9.2). Their genetic identity (I) amounted to 0.994, which is the level for panmictic populations of one species (chapter 9.1).

Field and greenhouse populations of *C. spectrana* are certainly still conspecific. It is adequate to speak of a "field biotype" and a "greenhouse biotype".

When the biotypes are brought together, they seem to interbreed freely, without a great deal of hybrid incompatibility (chapter 9.2). Development duration and temperature-dependent mortality did not differ between the biotypes (chapter 4). The survival of diapausing larvae was not essentially affected by the absence of a period of chilling in a heated greenhouse, and was not lower in comparison with the survival of larvae going through non-diapause development in a greenhouse and outdoors. Thus the survival of the biotypes may not differ essentially in a greenhouse environment (chapter 7.1.1). A difference in sensitivity to the synthetic pyrethroids that are commonly used for leaf-roller control in greenhouses in the last few years, is at present unlikely to exist (M. van de Vrie, pers. comm.).

Diapause appreciably decreases the multiplication rate per annum in heated greenhouses (chapter 7.1.1). This seems to be the only factor limiting genetic exchange between the biotypes, after the immigration of local wild leaf-rollers.

Greenhouse populations have overlapping generations. All developmental stages are always found simultaneously (Van de Vrie 1978). This means that the flight periods of the biotypes overlap each other. Even their phenology does not prohibit interbreeding.

Yet the genetic exchange between field and greenhouse populations is sufficiently low to maintain a separate greenhouse biotype. Samples from rose house populations lack the ability to enter diapause, regardless of whether they are taken in summer or in winter



(chapter 6). Apparently the glass walls and roofs to a large extent limit free introgression of the field biotype into resident greenhouse populations, first of all by prohibiting immigrations into the greenhouse, and also by creating a different environment, in which a period of chilling is lacking.

The origin of the greenhouse biotype might be a non-diapausing geographic race of *C. spectrana*, from the warmest parts of its distribution area, imported with plant material into the Dutch greenhouse cultures, or the field biotype was introduced into heated greenhouses, and subsequently lost its ability to enter diapause under the influence of specific selection pressures in the greenhouse environment. These two possibilities will now be discussed.

*C. spectrana* is a palaearctic species. The area of distribution probably includes the Mediterranean region, but the species is only known to be noxious in countries with a colder climate (chapter 2.3). *C. spectrana* belongs to the group of arthropod species that diapauses in winter, diapause development taking place at temperatures below the threshold for active development. These species usually do enter diapause in the Mediterranean region (Danilevskii 1965, pp. 22-24). In some of these species Mediterranean populations have been shown to be partly non-diapausing, e.g. in the lacewing *Chrysoperla carnea* Steph. (Alrouechdi & Canard 1979). *C. spectrana* has been recorded in vineyards in the Hérault district in the South of France. This district has a Mediterranean climate, but the larvae enter diapause in autumn (Picard 1912). Nothing is known about the existence of non-diapausing populations of *C. spectrana* under natural conditions. An introduction of such a population into the Dutch greenhouse cultures is therefore unlikely.

Hoy (1978b) reviews an impressive number of arthropod species in which a non-diapausing strain was obtained by inadvertent or purposeful selection in laboratory cultures. Some striking examples are: Lyon et al. (1972) found that a colony of the Western spruce bud worm, *Choristoneura occidentalis* (Freeman), had inadvertently lost its ability to enter diapause after it had been reared for 20 generations under controlled, non diapause-inducing conditions. Hoy (1977) selected a strain of gypsy moth, *Lymantria dispar* (L.), that could be reared continuously without its normal "obligatory" diapause within 8 generations.

Roses belong to the earliest cutflowers that were reared under glass in Aalsmeer. The first rose house was constructed in 1898 (Augustijn 1953). The flower auction in Aalsmeer was founded in 1912, rose houses were then already often heated in winter (Anonymus 1930). Tortricid larvae on roses are called "rozenijltjes" by the growers in the Aalsmeer region. Old rose growers state that the name is as old as the rose culture in this region. From the literature it is certain that the name was already commonly used in 1941 (Van Poeteren 1941). It may be assumed that tortricids have occurred on greenhouse roses since the start of the culture under glass. The earliest mention of tortricid damage (species unspecified) in Dutch rose houses that could be found in the literature, was in 1929 (Van Poeteren 1929). The first time *C. spectrana* was recorded in greenhouses by the Dutch Plant Protection Service was in 1936, on *Cyclamen* (Van Poeteren 1936). In 1941, *C. spectrana*

caused considerable damage to greenhouse roses. Until that time the species had been considered a rare insect in floriculture (Van Poeteren 1941). Since then the name "rozen-ijltjes" has regularly appeared in the literature: Anonymus (1942b), Augustijn et al. (1945), Van Dort (1948), Van Raalte (1950), Gelein (1965). In February 1964, *C. spectrana* caused heavy damage to greenhouse roses (Van Rossem et al. 1965), indicating that in 1964 a non-diapausing biotype already existed. If it is assumed that selection in favour of non-diapause was possible as soon as the rose culture under glass was started, greenhouse populations of *C. spectrana* have had a period of at least 60 years to adapt to their environment. This period comprises 360-540 generations of the greenhouse biotype. It is likely that a non-diapausing biotype can be selected out within such a period. Development duration of diapausing larvae could be shortened rapidly by selection under conditions without a period of chilling (chapter 7.2). As the genetic identity (I) of the biotypes was on the level of panmictic populations of one species (chapter 9.1), it may be speculated that the greenhouse biotype is not of exotic origin.

It seems to be quite possible that the field biotype immigrated into heated greenhouses, and subsequently lost its ability to enter diapause.

If it is assumed that the greenhouse biotype originates from the local field populations, four questions arise:

1. How can field populations enter a greenhouse?
2. How can a non-diapausing biotype be selected for in a heated greenhouse?
3. How many generations are required to obtain this biotype?
4. How does the non-diapausing biotype maintain itself, and how does it migrate from greenhouse to greenhouse?

Question 1: field populations can enter a greenhouse in different ways:

- a. Female moths or airborne young larvae may penetrate through the ventilators. This probably happens rarely.

Field populations mainly feed on low herbaceous plants (chapter 2.3), which limits the chance that the young larvae are transported aerially over longer distances.

The flight activity of *C. spectrana* is likely to be low. Male flight range of the summerfruit tortrix moth, *Adoxophyes orana* (F.v.R.), a related species with a similar life-cycle and about the same wing span (Graaf Bentinck & Diakonoff 1968), is usually not more than 200 m, and often less than 100 m. Females probably fly even less (Barel 1973). A difference between dispersing and migrating flight behaviour (Johnson 1969) was not found in *A. orana* (Barel 1973). Modern rose houses only have ventilators at ridge height ( $\pm$  5 m) (Van Marsbergen 1968), and these are not always open at night.

Several tortricid species regularly cause damage to cultivated roses in the open air in Western Europe (e.g. Richter von Binnenthal 1903; Pape 1955b). At present, none of these species occurs on greenhouse roses in the Netherlands (Van de Vrie 1978), which illustrates the isolating effect of modern rose houses on tortricid species.

Isolation may have been less in the past. The walls of the earliest rose houses were only man high, and the glass roof was removed in summer (Spaargaren 1908). Rose houses with a permanent glass roof were introduced later, with ventilators both at

ridge height and in the side-walls (Anonymus 1926). This type prevailed until long after the Second World War (W. van Marsbergen Ing., pers. comm.).

- b. Eggs, larvae, and pupae may be brought into a greenhouse with plant material from the open air. This probably happens rarely as well. Rose shrubs are kept 5-7 years in a greenhouse before they are replaced (W. van Marsbergen Ing., pers. comm.). Formerly this period was 7-10 years (Wasscher 1955; Gelein 1965). New shrubs are produced by grafting under glass, the grafts originating from greenhouse roses (Gelein 1965). A second method is by inoculation, the inoculated plants are kept in the open air for a year before they are brought into a greenhouse (Van Marsbergen 1968). The field biotype may occasionally penetrate a rose house with these plants. Most greenhouse crops are reared completely under glass.

Question 2: studies on the genetic basis of diapause in arthropod species have often been fragmentary, and have sometimes yielded conflicting results. No clear conclusions can be drawn about the genetic basis of diapause in the broad sense, although usually several genes seem to be involved (Hoy 1978b).

The genetic variability exhibited with respect to diapause by most arthropods, is impressive. Several elements of diapause are genetically determined. The critical photophase, the duration of diapause, the day degrees required for diapause termination, and the degree of cold-hardiness exhibited during diapause all respond to selection, as demonstrated by several species (Hoy 1978b).

The question arises how a non-diapausing biotype of *C. spectrana* may have been selected for in a heated greenhouse. The answer can only be given if more information about the genetic basis of diapause in this species is available. Only some suggestions can be given.

As in many other arthropods, the photoperiodic response of *C. spectrana* is modified by the temperature (chapter 5.1). Genotypes that lack photoperiodic induction of diapause under the temperature conditions in a heated greenhouse are at an advantage over diapausing genotypes, as they contribute more frequently to the reproduction of the population. In this way the photoperiodic response of immigrated wild leaf-rollers may shift towards non-diapause.

The duration of development of diapausing *C. spectrana* larvae in heated greenhouses is strongly variable and genetically determined. It can rapidly be shortened by selection (chapter 7.2). When a field population is introduced into a heated greenhouse, diapause may be terminated more quickly after a certain number of generations, specimens with a shorter diapause duration contributing sooner to the reproduction of the population.

These mechanisms can only give rise to a loss of diapausing behaviour if within the field populations sufficient genetic variation is present, which would enable these populations to adapt to an extremely wide range of environmental conditions. The cost, in terms of reduced fitness of a population associated with the production of less than optimally fit individuals, is called the "genetic load". As Haldane (1957) reflected, "This is the price a population must pay for the privilege of evolution".

Another possible mechanism presupposes the existence of a "switch gene" controlling the ability to enter diapause. The action of such a gene causes a switch of the epigenotype

to a different developmental pathway (Mettler & Gregg 1969, p. 62). If the ability to enter diapause is controlled by the action of one single gene, a non-diapausing biotype will be formed simply because non-diapausing specimens contribute sooner to the reproduction of the population.

Question 3: the number of generations required to obtain a non-diapausing population, cannot be estimated exactly, although the duration of development of diapausing larvae can be shortened rapidly by selection under conditions without a period of chilling (chapter 7.2).

Question 4: the non-diapausing biotype may migrate from greenhouse to greenhouse on young plants produced in propagating houses, in spite of the spray programmes applied by the propagators. Active migration from greenhouse to greenhouse, and transport by humans (young larvae attached to clothes), may only play a role if the greenhouses are adjacent and belong to the same grower.

Once a greenhouse population has become established, it may maintain itself for many years by moving from one part of the greenhouse to another, or to adjacent greenhouses of the same grower. A flower grower never replaces all his plants at the same time.

## 11. PROSPECTS OF NON-CHEMICAL CONTROL OF GREENHOUSE POPULATIONS

The existence of a non-diapausing biotype of *C. spectrana* indicates a strong isolating effect of greenhouses on this species. The greenhouse acts as an "ecological island". This situation offers prospects for some control techniques that can only be effective at low immigration rates, such as the communication disruption technique and the sterile-male technique. Moreover biological control by use of natural enemies may offer prospects in this island situation.

Pheromonal trapping of *C. spectrana* is less effective in greenhouses than in the open air (chapter 8.3). Similar phenomena have been observed in other Lepidoptera (Van den Bos 1982). Probably the long-range pheromone-mediated behaviour of males is less effective in a greenhouse environment because of the specific nature of the air movements. However, male sexual behaviour in short-range orientation, close to calling females, may be unaffected (chapter 8.3.2).

This decreases the effectiveness of synthetic pheromones used for monitoring and mass trapping. The question arises whether pheromone-baited traps can be effective at all, when used against insects living in enclosed spaces. Hoppe & Levinson (1979) and Levinson & Levinson (1980 a,b) state that pheromone-baited traps can be used successfully for long-term monitoring and mass trapping of storage moths (Phycitinae) and *Trogoderma* spp. in granaries, flour mills and food factories.

Decrease of the effectiveness of the male's long-range sexual responses in greenhouses may, on the contrary, contribute to the effect of application of the communication disruption technique:

in nocturnal moths, the male's responses to female sex pheromone usually consist of a sequence of distinctive behavioural steps. There is evidence that each step requires a higher pheromone concentration. This has been demonstrated in the laboratory by Schwinck (1955) in the Chinese silkworm moth, *Bombyx mori* (L.), and by Bartell & Shorey (1969) in the light-brown apple moth, *Epiphyas postvittana* (Walker). It is also known to occur in male American cockroaches, *Periplaneta americana* (L.) (Rust 1976; Silverman 1977). In laboratory essays Den Otter & Klijnsstra (1980) pointed out that female sex pheromone is indispensable to evoke mating attempts in males of the summerfruit tortrix moth, *Adoxophyes orana* (F.v.R.). Shimizu & Tamaki (1980) studied the mating behaviour of the smaller tea tortrix (*Adoxophyes* sp.). They stated that the female sex pheromone is indispensable to release male sexual behaviour in short-range orientation, even after initial contact with a female. Castrovillo & Cardé (1980) stated that only female sex pheromone may evoke male short-range sexual behaviour in the codling moth, *Laspeyresia pomonella* (L.). Female sex pheromone may be indispensable in every step of the sequence of male sexual behaviour,

both in and outside greenhouses.

When the communication disruption technique is applied in the field, the overall concentration of the synthetic pheromone in the air is usually insufficient to overflow the pheromone plumes produced by calling females. In greenhouses, the male's sexual responses in long-range orientation are already less effective without application of the communication disruption technique. In greenhouses, it may be possible to make the overall concentration of synthetic pheromone sufficiently high to overflow the pheromone plumes produced by calling females, and thereby to achieve communication disruption. Greenhouses seem to be well-suited for the application of the communication disruption technique, as a control measure against noxious moth species, provided moth density is not too high to rule out the chance of random encounters and matings. Studies on application of the communication disruption technique in greenhouses should be encouraged.

Application of the sterile-male technique against *C. spectrana* has several advantages in a greenhouse environment. Immigration of new adults from outside almost certainly is negligible. At present, *C. spectrana* is the only tortricid species occurring in the Dutch floricultural greenhouses (Van de Vrie 1978). The danger that other leaf-rollers will take over when chemical control against *C. spectrana* is stopped, seems therefore small.

Application of the sterile-male technique against the summerfruit tortrix moth, *Adoxophyes orana* (F.v.R.), in orchards is too costly. There are two bottlenecks. By releasing sterile males the population can be reduced to an extremely low level, it remains necessary, however, to control other leaf-rollers chemically, which defeats the purpose. Moreover, the method requires a continuous release of large numbers of sterile males per generation, to deal with immigrants. As this polyphagous species thrives on a wide variety of perennials, immigration from the surrounding wild vegetation cannot be prevented (Ankersmit 1975, 1980).

Genetic control of *C. spectrana* by the release of sterile males however is not impeded by these two factors. In the rose culture under glass higher pest control costs, but lower levels of damage can be tolerated than in fruit growing. The following procedure may be practicable: firstly, a greenhouse population should be reduced to low numbers by insecticides to prevent economic damage; this should be followed by the release of large numbers of sterile males, in order to achieve eradication of the pest separately in each greenhouse. Studies on the application of the sterile-male technique against greenhouse populations of *C. spectrana* are recommended.

Interference with the life-cycle of insect pests by manipulation of the diapause response has been suggested as a means of control (LaChance & Knipling 1962; Klassen et al. 1970 a,b; Hogan 1971, 1974; Foster & Whitten 1974).

Diapause characteristics can be crossed into greenhouse populations of *C. spectrana*, without increasing the infestation, by releasing males of the field biotype. This probably is ineffective to control these populations, and even insufficient to suppress growth and reproduction of the pest during the winter season: (a) the absence of a period of chilling does not affect the survival rate of diapausing larvae (chapter 7.1.1). (b) The F1

progeny exhibits an intermediate photoperiodic response, inclining towards the maternal characteristics (chapter 9.3), thus only part of the offspring will enter diapause, and (c) the diapause characteristics are probably soon lost.

Biological control of *C. spectrana* in greenhouses may have interesting prospects. A rose house in Roelofarendsveen was visited in the first week of February, and in August 1979. In February, 26% of *C. spectrana* larvae were parasitised, and in August 52%. The larvae had been continuously present between February and August, but they remained at a low population density. Chemical control was not necessary during this period. The parasites, which were not identified, were also active during winter. Apparently these parasites were not diapausing. A survey of the parasite fauna of *C. spectrana* in greenhouse cultures should be made.

## SUMMARY

Chapter 1: the environmental conditions in greenhouses differ in many respects from those in the open field. Both the climate and the crops are different. A free exchange between the fauna of the greenhouses and the open air is hampered by the glass walls and roofs. The isolating effect of greenhouses on arthropod pests contributes to the effectiveness of control measures, but also to the development and maintenance of pesticide resistance in greenhouses. Because of the special conditions a specific fauna exists in greenhouses, and the use of exotic predators and parasites for biological control is possible. The greenhouse environment acts as a "sieve" only allowing such species to thrive that are adapted to these special conditions. These are sometimes exotic species that cannot thrive in the open in the Dutch climate. Native species may penetrate into greenhouse cultures, but to pass the "sieve" they have to adapt to greenhouse conditions.

The leaf-roller *Clepsis spectrana* Tr. is native in the Netherlands. It gives an example of the development of a greenhouse-adapted biotype. In Dutch greenhouses, especially on roses, it causes much damage. In heated greenhouses, where artificial illumination is not given, growth and reproduction of *C. spectrana* continue without diapause during winter, which is advantageous for the species in this environment, where the difference between summer and winter temperatures does not exceed a few degrees and suitable food is available all the year round.

Chapter 2 deals with the morphology, bionomics, host plant range, and distribution area of *C. spectrana*. Chapter 3 describes the materials and methods.

Chapter 4: the number of larval instars from egg hatching to pupation varied between 4 and 8 in both field and greenhouse strains. Each larval growth type had its own specific progression of head capsule width. The difference in head capsule width between the 4- and 5-instar type was already evident in the 1st instar, between the 5- and 6-instar type in the 2nd instar, and between the 6- and 7-instar type only in the 3rd instar or even later. Females tended to develop through a higher number of instars than males, but 5 instars was the most usual in both sexes. The development duration was the same in field and greenhouse strains, in each of the immature stages. The upper thermal limit for development was between 30°C and 35°C in both field and greenhouse strains, whereas the developmental threshold was close to 10°C. An adaptation of greenhouse populations of *C. spectrana* to development at higher temperatures did not appear.

Chapter 5: short daylength induced diapause in field strains in the larval stage. The critical photoperiod was between 16 and 17 hours. The number of moults up to the onset



of diapause varied between 2 and 6, and was determined by the photoperiod. Larvae from eggs hatching later in the season entered diapause after a lower number of moults. The time of resumption of growth after diapause termination in spring was not correlated with the date of egg hatching in the previous year, in outdoor experiments. The larvae underwent supernumerary moults after termination of diapause. The duration of post-diapause development was longer as diapause had been entered after less moults. The number of moults after termination of diapause, and the duration of post-diapause development, were sex-linked. The functional significance of these phenomena is discussed.

Chapter 6: the photoperiodic response of 5 different greenhouse strains, originating from larvae and pupae collected in different rose houses at different times of the year (both in summer and in winter), was tested in the laboratory. These greenhouse strains did not enter diapause, at both 20°C and 15°C, regardless of the photoperiod. One greenhouse strain was also reared outdoors. Egg hatching dates were August 1, August 17, September 25, and October 14. At least the major part of the larvae did not enter diapause.

Chapter 7: field strain larvae entered diapause in a heated greenhouse, but the absence of a period of chilling caused an abnormal growth pattern in these larvae, compared to larvae terminating their diapause outdoors: (a) larval development duration was extremely variable (larvae from field strain eggs that hatched at August 24 in the greenhouse, pupated between January 28 and July 15 of the following year). (b) The difference in mean developmental time between male and female larvae was greatly increased, and (c) the mean number of moults, and the variation in the number of moults, were increased. The survival of diapausing larvae, however, was not essentially affected by the absence of a period of chilling. The time required for diapausing larvae to reach the pupal stage under conditions without a cold period, was genetically determined, and could be shortened rapidly by selection.

Chapter 8: a difference in the composition of the female sex pheromone between field and greenhouse strains could not be shown, nor in the calling behaviour of virgin females in relation to the light-dark cycle. Release-recapture trials revealed that females of both strains attracted males of both strains in equal proportions. There did not appear to be a difference in mating preference.

Chapter 9: the index of genetic identity (I) of a field population on stinging nettles from the middle of the country, and a rose house population from the West of the country, amounted to 0.994 (based on allozyme frequencies). This is the level for panmictic populations of one species. Field and greenhouse strains could readily be intercrossed, with a viable F2 generation.

Chapter 10: field and greenhouse populations of *C. spectrana* are certainly still conspecific. It is adequate to speak of a "field biotype" and a "greenhouse biotype". The greenhouse biotype is characterised by absence of the ability to enter diapause. When the biotypes are brought together, hybridization and introgression certainly will occur.

Immigration of the field biotype into heated greenhouses apparently is sufficiently low to maintain a separate greenhouse biotype with constant characteristics. The origin of the greenhouse biotype might be a non-diapausing geographic race of *C. spectrana*, from the warmest parts of its distribution area, or the field biotype immigrated into heated greenhouses, and subsequently lost its ability to enter diapause. The last possibility seems the most likely.

Chapter 11: pheromonal trapping of *C. spectrana* is less effective in greenhouses than in the open air. Probably the specific nature of the air movements in greenhouses reduces the effectiveness of the long-range pheromone-mediated behaviour of the males. However, male pheromone-mediated behaviour in short-range orientation, close to calling females, may be unaffected (chapter 8.3). This decreases the effect of synthetic pheromone used for monitoring and mass trapping. However, application of the communication disruption technique may be successful under glass, because the overall concentration of synthetic pheromone in the air can be made much higher in greenhouses than in the open field. Further research is recommended.

Controlling *C. spectrana* in greenhouses by releasing sterile males seems feasible, as: (a) immigration of new adults from outside almost certainly is negligible, and (b) *C. spectrana* is the only tortricid occurring in the Dutch floriculture; the danger that other leaf-rollers will take over when chemical control of *C. spectrana* ceases seems therefore small.

Diapause can be crossed into greenhouse populations of *C. spectrana* by releasing males of the field biotype. This probably is an ineffective way of controlling these populations.

Parasitised *C. spectrana* larvae were found in several greenhouses, even in winter. The parasites, which were not identified, apparently were not diapausing. It is recommended to make a survey of the parasite fauna of *C. spectrana* in greenhouse cultures.

## SAMENVATTING

Hoofdstuk 1: het kasmilieu verschilt in veel opzichten van dat in het vrije veld. Er worden andere gewassen gekweekt, en het klimaat is anders. Een vrije uitwisseling tussen kasfauna's en veldfauna's wordt belemmerd door de wanden en het dak van de kas. Het isolerende effect van kassen op insecten- en mijtenplagen kan bijdragen aan de doeltreffendheid van bestrijdingsmaatregelen, maar ook aan de ontwikkeling en handhaving van resistentie tegen bestrijdingsmiddelen in kassen. Als gevolg van het speciale kasmilieu bestaat een specifieke fauna in kassen, en wordt het gebruik van uitheemse parasieten en predatoren voor biologische bestrijding mogelijk. Het kasmilieu fungeert als een "zeef" die alleen die soorten "doorlaat" die aan de specifieke omstandigheden zijn aangepast. In kassen worden tropische en subtropische insecten- en mijtensoorten gevonden die zich in de buitenlucht in het Nederlandse klimaat niet kunnen handhaven. Soms komen inheemse soorten binnen, maar om de "zeef" te kunnen passeren moeten ze zich aanpassen aan de kasomstandigheden.

De bladroller *Clepsia spectrana* (Tr.) is inheems in Nederland, en heeft een afzonderlijk kasbiotype ontwikkeld. In kassen is hij een belangrijke plaag, met name op rozen. In verwarmde kassen, waar geen kunstmatige belichting wordt gegeven, gaat zijn ontwikkeling ook gedurende de winter verder, niet onderbroken door diapauze (winterrust). Dit is voordelig voor de soort in dit milieu, waar het verschil tussen zomer- en wintertemperaturen slechts enkele graden bedraagt, en het gehele jaar door geschikt voedsel aanwezig is.

In hoofdstuk 2 worden de uiterlijke kenmerken, de levenswijze, de waardplantreeks en het verspreidingsgebied van *C. spectrana* besproken. In hoofdstuk 3 worden de materialen en methoden beschreven.

Hoofdstuk 4: het aantal larvenstadia vanaf het uitkomen van het ei tot de verpopping, liep uiteen van 4 tot 8 in zowel veld- als kasstammen. Elk van deze groeitypen had zijn eigen specifieke verloop van kopkapselbreedtes (ieder larvenstadium heeft zijn eigen specifieke gemiddelde kopkapselbreedte). Het verschil in kopkapselbreedte tussen 4- en 5-stadia rupsen kwam al in het eerste larvenstadium tot uiting, tussen 5- en 6-stadia rupsen in het tweede stadium, en tussen 6- en 7-stadia rupsen pas in het derde stadium of later. De vrouwtjes maakten gemiddeld meer vervellingen door dan de mannetjes, maar het 5-stadia type was overheersend in beide geslachten. Er was geen verschil in ontwikkelingsduur tussen veld- en kasstammen. Dit gold voor zowel eieren, rupsen, als poppen. De bovengrens voor actieve ontwikkeling lag tussen 30°C en 35°C in veld- en kasstammen, terwijl de ontwikkelingsdrempel in de buurt van 10°C lag. Een aanpassing van kaspopulaties van *C. spectrana* aan ontwikkeling bij hogere temperaturen was niet aantoonbaar.

Hoofdstuk 5: veldstammen hadden een diapauze in het larvenstadium, geïnduceerd door korte daglengte. De kritische daglengte lag tussen 16 en 17 uur. Het aantal vervellingen tot het intreden van de diapauze liep uiteen van 2 tot 6, afhankelijk van de daglengte. Rupsen die later in het seizoen uit het ei kwamen, gingen na een geringer aantal vervellingen in diapauze. Het tijdstip van groeihervatting na beëindiging van de diapauze in het voorjaar was niet afhankelijk van de datum waarop de eieren in het voorgaande jaar waren uitgekomen, bij proeven in de buitenlucht. De rupsen maakten extra vervellingen door na beëindiging van de diapauze. De duur van de post-diapauze ontwikkeling was langer naarmate de diapauze na een geringer aantal vervellingen ingetreden was. Het aantal vervellingen na beëindiging van de diapauze, en de duur van de post-diapauze ontwikkeling, verschilden tussen mannetjes en vrouwtjes. De biologische betekenis van deze verschijnselen wordt besproken.

Hoofdstuk 6: de reactie op daglengte van 5 verschillende kasstammen, afkomstig van rupsen en poppen die in verschillende rozenkassen in verschillende tijden van het jaar (zowel in de zomer als in de winter) verzameld waren, werd in het laboratorium onder geconditioneerde omstandigheden getoetst. Deze kasstammen gingen bij zowel 20°C als 15°C bij geen enkele daglengte in diapauze. Een van deze kasstammen werd ook in een kooi in de buitenlucht gekweekt. De eieren kwamen uit op 1 augustus, 17 augustus, 25 september en 14 oktober. Er trad geen, of nagenoeg geen, diapauze in.

Hoofdstuk 7: rupsen van een veldstam gingen in een verwarmde kas in diapauze, maar de afwezigheid van een koudeperiode veroorzaakte een abnormaal groeipatroon in deze rupsen, vergeleken met rupsen die hun diapauze in de buitenlucht beëindigden: (a) de variatie in de ontwikkelingsduur van de rupsen nam sterk toe (rupsen die op 24 augustus uit het ei kwamen, verpopten tussen 28 januari en 15 juli van het volgende jaar). (b) Het verschil in ontwikkelingsduur tussen mannelijke en vrouwelijke rupsen nam sterk toe, en (c) het gemiddeld aantal vervellingen, en de variatie in het aantal vervellingen, namen sterk toe. De overlevingskans van diapauzerende rupsen werd evenwel niet noemenswaardig beïnvloed door het ontbreken van een koudeperiode. De tijd die diapauzerende rupsen onder kasomstandigheden nodig hadden om het popstadium te bereiken, was erfelijk bepaald, en werd onder invloed van selectie snel korter.

Hoofdstuk 8: een verschil in de samenstelling van het vrouwelijke sex-feromoon tussen veld- en kasstammen kon niet aangetoond worden, noch in het lokgedrag van maagdelijke wijfjes in relatie tot de licht-donker cyclus. Uit terugvangproeven met wijfjesvallen bleek dat vrouwtjes van beide stammen mannetjes van beide stammen in gelijke mate aanlokken. Een verschil in paringsvoorkeur kwam niet naar voren.

Hoofdstuk 9: de index van genetische identiteit (I) van een veldpopulatie op brandnetels in het midden van het land, en van een populatie op kasrozen in het westen van het land, bedroeg 0,994 (gebaseerd op allozym frequenties). Deze waarde komt overeen met die van parmicetische populaties van één soort. Veld- en kasstammen konden gemakkelijk onderling gekruist worden, en de F2 generatie was levensvatbaar.

Hoofdstuk 10: veld- en kaspopulaties van *C. spectrana* behoren zeker nog tot één soort. Men kan het beste spreken van een "veldbiotype" en een "kasbiotype". Het kasbiotype wordt gekenmerkt door het ontbreken van het vermogen om in diapauze te gaan. Wanneer de biotypen samen gebracht worden, zullen ze zeker onderling kruisen. De immigratie van het veldbiotype in verwarmde kassen is kennelijk gering genoeg om een afzonderlijk kasbiotype met constante eigenschappen te handhaven. De oorsprong van het kasbiotype kan een diapauze-vrij geografisch ras van *C. spectrana* zijn, uit de warmste delen van zijn verspreidingsgebied. Het is ook mogelijk dat het veldbiotype in verwarmde kassen immigreerde, en vervolgens zijn vermogen om in diapauze te gaan verloor. De laatste mogelijkheid lijkt het meest waarschijnlijk.

Hoofdstuk 11: de toepassing van feromoonvallen tegen *C. spectrana* is in kassen minder effectief dan in de buitenlucht. Vermoedelijk is als gevolg van de specifieke aard van de luchtbewegingen in kassen de effectiviteit van het door feromoon opgewekte lange-afstands gedrag van de mannetjes verminderd. Het door feromoon opgewekte korte-afstands gedrag, dicht bij lokkende wijfjes, zal echter niet gestoord zijn (hoofdstuk 8.3). Dit doet afbreuk aan de effectiviteit van feromoonvallen voor het signaleren van een aantasting in een vroegtijdig stadium, of voor het massaal wegvangen van mannetjes. Toepassing van de verstorings techniek in kasteelten biedt waarschijnlijk wel kans op succes, omdat de algehele concentratie van het synthetische feromoon in de lucht, in kassen veel hoger gemaakt kan worden dan in het vrije veld. Verder onderzoek wordt aanbevolen.

Toepassing van de steriele-mannetjes techniek tegen *C. spectrana* in kassen lijkt veelbelovend: immigratie van nieuwe motjes van buiten is vrijwel zeker te verwaarlozen, en (b) *C. spectrana* is de enige bladrollersoort in de Nederlandse bloementelt onder glas, zodat er geen gevaar bestaat dat andere soorten in aantal toenemen als de chemische bestrijding van *C. spectrana* gestaakt wordt.

Diapauze kan in kaspopulaties van *C. spectrana* ingekruist worden door mannetjes van het veldbiotype los te laten. Dit is ontoereikend als bestrijdingsmethode.

In verschillende rozenkassen bleek een deel van de *C. spectrana* rupsen geparasiteerd te zijn, zelfs in de winter. De parasieten, die niet gedetermineerd werden, gingen blijkbaar niet in diapauze. Het verdient aanbeveling een inventarisatie te maken van de parasietenfauna van *C. spectrana* in kasteelten.

## ACKNOWLEDGEMENTS

The research for this thesis was proposed by Prof.Dr. J. de Wilde, Mr. M. van de Vrie, and Dr.Ir. G.W. Ankersmit. It was carried out for the Laboratory of Entomology (Agricultural University Wageningen), while I was seconded to the Research Station for Floriculture, Aalsmeer, the Netherlands.

I wish to thank sincerely and profoundly all those who have contributed in any way to this publication. I would like to mention some of them in particular.

First of all I would like to thank my promotor, Prof.Dr. J. de Wilde, for giving me the opportunity to do this work. His interest, continuous encouragement, and comments on the manuscript were of great help.

Thanks are also due to the board of the research station in Aalsmeer for their hospitality and cooperation.

The interest and support of Mr. M. van de Vrie and Dr.Ir. G.W. Ankersmit throughout the work, their valuable suggestions and comments on the manuscript, are gratefully acknowledged.

I am indebted to Dr.Ir. C.J. Persoons for making the electro-antennograms, to W.J. Nooijen Ing. for gaschromatographical analysis of the contents of pheromone glands, to Dr. S.B.J. Menken for providing the electrophoretic data, to Dr. A. Diakonoff for taxonomic work and providing literature on host plants and distribution of *C. spectrana*, to Dr. A.A. Evenhuis for taxonomic work, to Dr. G. van Rossem for allowing me to use the files of the Plant Protection Service, to Ir. A.K.H. Wit for assistance in collecting leaf-rollers in the field, and to Dr.Ir. R. Rabbinge for assistance in computer modelling and reading parts of the manuscript.

Among others Dr.Ir. A.K. Minks, Drs. C. van der Kraan, Dr. L. Blommers, Dr. W.M. Herrebout, Drs. A. Hendrikse, Prof.Dr. J.C. van Lenteren, Ir. J. Woets, Ir. P.M.J. Ramakers, Dr. D. Hille Ris Lambers, and Dr.Ir. M.W. Sabelis have contributed by their interest and stimulating discussion.

I received indispensable assistance from Mr. M. Boogaard and Mrs. B. Overdevest (taking over of routine observations during my holidays), Miss C.C.M. de Boer (drawings), Mr. M.J. 't Hart (advice on lay-out), Mr. H. Stephan (photographs), and Mrs. S. Kerkhoven-Hodges (improvement of the English text).

Last but not least I would like to thank my wife, Coby van der Lubbe, without whose care and mental support this work would not have been completed.

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## CURRICULUM VITAE

Hans van den Bos werd op 19 mei 1948 te Enschede geboren. Na de lagere school bezocht hij het Enschedees Lyceum. In 1966 legde hij het eindexamen gymnasium-8 met succes af. In het zelfde jaar begon hij zijn studie aan de Landbouwhogeschool, studierichting Planteziektenkunde. Zijn praktijktijd bracht hij door bij de Arbeitsgemeinschaft zur Erforschung der Populationsdynamik des Grauen Lärchenwicklers van de E.T.H. Zürich, in het Oberengadin, Zwitserland. In 1975 studeerde hij cum laude af, met het volgende vakkenpakket: Entomologie (hoofdvak), Dierkunde (dieroecologie), Erfelijkheidsleer (populatiegenetica), en Theoretische Teeltkunde (computersimulatie van oecologische systemen). Hij behaalde daarbij tevens de eerste graads onderwijsbevoegdheid voor biologie. Van maart 1975 tot juli 1976 vervulde hij zijn militaire dienstplicht. Van augustus 1976 tot augustus 1977 was hij verbonden aan het Institut für Forstzoologie van de universiteit te Göttingen (West-Duitsland). Hij verrichtte daar een onderzoek naar de mogelijkheden tot manipulatie van het eileggedrag van zweefvliegen als natuurlijke vijanden van bladluizen. Vanaf februari 1978 was hij als promotie-assistent van de Landbouwhogeschool verbonden aan het Proefstation voor de Bloemisterij in Nederland te Aalsmeer, alwaar het onderzoek voor deze dissertatie verricht werd.

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