Behavioural mechanism and genetic regulation of dispersal
in the tortricid *Cydia pomonella*

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presented by
Sabine Keil
Dipl. Biol.
Albert-Ludwigs University, Freiburg im Breisgau (Germany)
born November 29th, 1965
Germany

accepted on the recommendation of
Prof. Dr. Silvia Dorn, examiner
Prof. Dr. Josef Nösberger, co-examiner
Dr. Hainan Gu, co-examiner

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## Contents

1 Summary 1  
2 Zusammenfassung 5  
3 General Introduction 9  

4 Diel patterns of locomotor activity in *Cydia pomonella*: age and sex related differences and effects of insect hormone mimics 13  

4.1 Abstract 13  
4.2 Introduction 13  
4.3 Materials & methods 15  
  Insects 15  
  Set-up of actograph test 15  
  Application of insect hormone mimics 16  
  Data analysis 18  
4.4 Results 18  
  Diel patterns of locomotor activity 18  
  Sex and age related differences 18  
  Effects of insect hormone mimics 22  
4.5 Discussion 24  
  Diel patterns of locomotor activity 24  
  Sex and age related differences in locomotor activity 25  
  Effects of insect hormone mimics on locomotor activity 27  

5 Response of *Cydia pomonella* to selection on mobility: 29  
  laboratory evaluation and field verification  

5.1 Abstract 29  
5.2 Introduction 30  
5.3 Materials & methods 32  
  Insects 32  
  Actograph test of mobility 32  
  Bi-directional selection 32  
  Field release-recapture experiments with the selected strains 33  
  Data analysis 35
6 Field dispersal study in different strains of *Cydia pomonella*: development of methodology

6.1 Abstract

6.2 Introduction

6.3 Materials & methods
   6.3.1 Preliminary mass release in an unmanaged orchard: 1997 in Schleinikon
       Insects
       Experimental site
       Mark and release
       Trap types and positions
   6.3.2 Mass release in an IP orchard: 1998 in Leuggern
       Insects
       Experimental site
       Mark and release
       Trap types and positions
   6.3.3 Mass release with selected strains in two IP orchards: 1999 in Leuggern and Otelfingen
       Insects
       Experimental site
       Mark and release
       Trap types and positions

6.4 Results
   6.4.1 Mark-release-recapture experiment in Schleinikon 1997
       Recapture rate
       Release sites
       Climatic conditions
   6.4.2 Mark-release-recapture experiment in Leuggern 1998
       Recapture rate
       Release sites
       Efficiency of pheromone traps
       Flight distances of released males
       Relationship between time of release and recapture
       Climatic conditions
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.4.3</td>
<td>Mark-release-recapture with selected strains in Leuggern and Otelfingen 1999</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Recapture profile in released codling moths of selected strains</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Capture profile of wild codling moths</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>Climatic conditions</td>
<td>67</td>
</tr>
<tr>
<td>6.5</td>
<td>Discussion</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Factors involved in short-range dispersal</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Factors involved in long-range dispersal</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>Impact of population dynamics on recapture data</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Efficiency of the trap type: passive versus active traps</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>Conclusions</td>
<td>73</td>
</tr>
<tr>
<td>7</td>
<td>General Discussion</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Actographs in studying insect dispersal: options and limitations</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Genetic regulation of insect dispersal</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>Dispersal biology and pest management in Cydia pomonella</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>Outlook on future research</td>
<td>80</td>
</tr>
<tr>
<td>8</td>
<td>References</td>
<td>83</td>
</tr>
<tr>
<td>9</td>
<td>Acknowledgements</td>
<td>95</td>
</tr>
<tr>
<td>10</td>
<td>Curriculum vitae</td>
<td>97</td>
</tr>
</tbody>
</table>
1 Summary

The codling moth *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae) is regarded as one of the main pest insects in cultivated pome fruit, stone fruit and walnut. A high adaptiveness to changing habitats and deteriorating environmental conditions, together with a hidden larval life-stage accounts for the successful and almost worldwide distribution of this species. The damaging stage of the life cycle is the larva which lives concealed and protected inside the growing fruit. Today's pest management strategies are increasingly reliant on selective methods which spare many beneficial insect species and facilitate environmental protection. Some of these selective strategies against the codling moth target the adult life-stage. Thus, the study of adult behaviour, especially dispersal by flight, in *Cydia pomonella* gains importance. This research project focused on the behavioural mechanism and genetic regulation of dispersal in the codling moth.

The first part of the project emphasised the elucidation of the daily activity pattern in both male and female codling moths. An experimental set-up with infrared actographs was used to measure the locomotor activity under a defined light regime with simulated dusk and dawn phases. Both sexes responded to the change of light intensity with peak activity at dusk and decreasing activity at dawn. Their daily patterns were found to be qualitatively similar between the sexes concerning phases of activity and repose, but quantitatively different as male moths exhibited much higher locomotor activity in comparison with females. The influence of age on the diel pattern of activity was tested in five age classes in both sexes. Results showed that age had a general effect on activity. Males at four days- and females at 12 days-old performed the highest level of locomotor activity while in newly emerged moths females showed higher activity than males. It was hypothesised that adult behaviour might be influenced by the same hormones that direct insect development, and that the mimics of these hormones used in pest management might alter the behaviour of adult codling moths. Thus, behavioural effects of two frequently used insect hormone mimics were studied concerning their influence on female moth activity. The juvenile hormone mimic, fenoxycarb, elicited a higher locomotor activity and triggered an extra peak of activity in virgin and mated female codling moths. Such additional activity occurred at the first few hours of the photophase which is usually
characterised by repose and reduced activity. In contrast to treatment with fenoxycarb, virgin females applied with the ecdysone agonist, tebufenozide, performed a pattern comparable to that of the untreated moths.

The second part of the project concentrated on the genetic control of dispersal in *Cydia pomonella*. The focus was the laboratory selection of two strains differing significantly in mobility. For the establishment of the two strains, an infrared actograph was found to be advantageous. With the actograph technique, a large number of individuals could be tested simultaneously. Furthermore, the test procedure would not interfere with normal breeding in the codling moth, which guaranteed enough offspring in each generation for either actograph tests or selective breeding. The decision for either ‘mobile’ or ‘sedentary’ was made based on the locomotory activity performed in a specified test period. In contrast to previous actograph tests, this period consisted of the time period of maximal activity in both sexes, i.e. a total of 4h including 2h dusk plus one hour for each of the bordering photophase and scotophase, respectively. All movement events taking place during the test period were summed and ranged on a scale from the highest (= mobile) to the lowest value (= sedentary). According to the performed activity level, selective breeding was carried out bi-directionally with each strain functions as a control for the other. Differences were found after the first generation of selection in both directions and in both sexes, with the two strains diverging further in subsequent generations. The response to downward selection occurred faster than that to upward selection, leading to asymmetry of response to bi-directional selection. The realised heritability was calculated for males and females in both directions. Selection in the two strains was continued until the F4-generation when the individual number in both strains was enlarged for mass releases in the field. This led to the third part of the project which was to verify the laboratory findings and to test the correlation between locomotor activity and field dispersal in *Cydia pomonella*. 
Part three comprised the development of a mark-release-recapture method suitable for the study of field dispersal in the codling moth and demonstration of differential dispersal capacity in two selected strains. During three consecutive years, mass releases were undertaken under various experimental conditions, e.g. modification of the orchard type, release site, trap types and positions.

In the first year, releases were based on laboratory-reared, non-selected codling moths of both sexes in an unmanaged orchard consisting of high stem trees. Males were successfully recaptured with pheromone traps while females could not be trapped using passive window traps. Recapure data showed a proportion of the male moths leaving the immediate vicinity of the release site and flew over longer distances, but most moths were attracted by the closest pheromone traps.

Releases in the second year were undertaken in a managed orchard consisting of dwarf apple trees. Recapture data from different release sites, located inside and outside the orchard, confirmed the previous year's findings that a proportion of male moths did not fly to the closest pheromone trap, but left the experimental area heading to high stem trees in the surroundings. Internal and external marking of all released moths made it easier to differentiate release sites and released moths from those of the wild population.

During the third year, codling moths from two laboratory selected strains were released in order to test the hypothesis that moths selected for higher mobility (= mobile strain) would disperse further in the field whereas moths selected for lower mobility (= sedentary strain) showed minor dispersal. Males and females were released in two managed orchards and recaptured with pheromone and light traps, respectively. High recapture rates for males were yielded while female moths could not be trapped. Dispersal in male moths from the mobile strain was found to differ significantly from the sedentary strain, mobile moths dispersed further away from the release site and there was a greater percentage that left the habitat, when compared with sedentary moths.

These findings are of ecological importance as individuals with high mobility can escape unfavourable environmental conditions, fly over long distances and reach new habitats for colonization. The bi-directional selection demonstrated the existence of additive genetic variance for mobility within a codling moth population as confirmed by a significant response to selection. The field release-recapture experiments with the two different strains not only showed a positive correlation...
between laboratory measured mobility and field flight capacity but also verified the differential dispersal potential present in different genotypes of *Cydia pomonella*. 
2 Zusammenfassung


Aktivität der Weibchen überwog. Im weiteren wurde die Hypothese getestet dass Hormone, die für die Insektenentwicklung von Bedeutung sind, auch einen Einfluss auf das adulte Verhalten haben könnten. In diesem Zusammenhang war insbesondere die Frage von Bedeutung inwieweit Insektenhormonpräparate, die im Pflanzenschutz verwendet werden, das Falterverhalten verändern könnten.


Der dritte Teil umfasste die Entwicklung einer geeigneten Freilassungsmethode für die Untersuchung des Ausbreitungsverhaltens des Apfelwicklers im Feld. Ebenfalls stand hier die Veranschaulichung unterschiedlicher Ausbreitungsfähigkeiten der beiden selektionierten Stämme im Vordergrund. Im Verlauf von drei aufeinanderfolgenden Versuchsjahren wurden Freilassungen unter verschiedenen Versuchsbedingungen durchgeführt, unter anderem wurde der Behandlungstyp der Obstanlage, der Freilassungsstandort sowie die Fallenart und die Fallenposition geändert.


Die Freilassungsversuche im zweiten Jahr fanden in einer Intensivobstanlage mit Niederstammbäumen statt. Wie sich anhand der Fangdaten, resultierend aus unterschiedlichen Freilassungsarten innerhalb und ausserhalb der Obstanlage, herausstellte, konnten die Ergebnisse des Vorjahrs bestätigt werden. Wiederum
wählte ein Teil der freigelassenen Falter nicht die nächstgelegenen Fallen sondern verliess die Versuchsanlage um Fallen in Hochstammbäumen der Umgebung anzusteuern. Innerlich und äusserlich angewendete Markierungen aller freigelassener Falter ermöglichten die Differenzierung unterschiedlicher Freilassungsstandorte und garantierten zudem die Abgrenzung freigelassener Falter von denen der lokalen wilden Apfelwicklerpopulation.


Diese Resultate sind ökologisch bedeutend weil Individuen mit einer hohen Flugkapazität ungünstigen Umweltbedingungen entgehen können indem sie weitere Distanzen fliegen und sich dadurch neue Habitate für eine Neubesiedlung erschliessen können. Die bi-direktionale Selektion deckte die Existenz additiver genetischer Varianz hinsichtlich Mobilität innerhalb einer Apfelwicklerpopulation auf, welches anhand einer signifikanten Antwort auf die Selektion bestätigt werden konnte. Im weiteren zeigten die Fangdaten der zwei unterschiedlichen Stämme nicht nur eine positive Korrelation zwischen der im Labor gemessenen Mobilität und der Flugfähigkeit im Feld, sondern ausserdem das Ausbreitungsverhalten zweier unterschiedlicher Genotypen bei Cydia pomonella.
3  General Introduction

The life of an insect consists of a finite number of behavioural sequences with each sequence comprising a long series of events and triggered by an environmental stimulus (Brodsky, 1994). Movement is a major part of an insect's life because it enables dispersal and enlarges the individual radius of activity, e.g. in colonization of new habitats, in searching for mates and suitable host sites. In Lepidoptera, movement occurs most often as a result of adult flight while dispersal of larvae takes place in a short range in order to find an appropriate host plant or pupation site. The impact of mobility in Lepidoptera depends largely upon the status of a given species with regard to its agricultural importance.

The codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) is the major pest species of several pome fruits with a preference for the domestic apple *Malus pumila* Miller whereas secondary hosts include pear *Pyrus communis* Linnaeus, quince *Cydonia oblonga* Miller and walnut *Juglans regia* Linnaeus. In association with the occurrence of its host tree range, *Cydia pomonella* originated from Eurasia and its distribution followed the spread of host tree cultivation (Barnes, 1991). Now, it is a cosmopolitan insect spreading worldwide in orchards. In Central Europe, *Cydia pomonella* has usually one major generation per year and occasionally a second minor one whose occurrence depends on the region and climatic conditions. Diapausing larvae overwinter under the rough bark of apple trees until May when the moths begin to emerge and the flight period starts (Figure 3.1). Adult moths remain in the foliage during day and become active at dusk as mating and flight occur when light, temperature and wind conditions are favourable (Mani & Wildbolz, 1978; Geier, 1981). During oviposition, females lay their eggs singly on fruits (Wildbolz, 1958; Jackson, 1979). Immediately after hatching, first instar larvae (L1) bore themselves to the core of the fruit and pass through 5 instars (L1 to L5) during which the fruit damage occurs. As L5 larvae, they leave the fruit and search on the tree stem for suitable pupation and overwintering sites.
Cydia pomonella has perfectly adapted to its habitat due to the capacity in synchronizing the seasonal activity with the presence of developing fruits on host trees (Geier, 1981). Periods of diapause can last up to two years and enable the species to overcome unfavourable conditions e.g. years with low fruit production (Yothers & Carlson, 1941). The individual susceptibility to signals which induce and terminate diapause can vary largely within (Geier, 1963) and between populations (Shel’deshova 1965; Riedl & Croft, 1978) which makes it rather difficult to predict infestations based on previous years’ population levels and fruit damages.

A systems approach to pest management requires knowledge of both the field performance of the insects and their effect on the crop, as well as knowledge of the mechanisms behind these phenomena (Dorn et al., 1999). Novel pest management strategies against the codling moth consider selective methods which demand a thorough knowledge about the species’ biology and ecology. The characteristics of each part of the life cycle in Cydia pomonella cause difficulties in pest management because each stage is vulnerable to any treatment in a different way and duration. Thus, selective biological and biotechnical methods are directed against different life cycle stages. They can be divided in three groups which are either directed against
eggs, larvae or adults. In all groups, certain effects on other life cycle stages than the ones targeted on are possible. The use of insect hormone mimics which are commonly applied in Integrated Pest Management (IPM) against lepidopteran eggs may impose possible side effects on the behaviour of adult codling moths. Changes in insect behaviour after the application of hormone mimics are reported for various species, e.g. after application of the juvenile hormone type (see Walker, 1977; Schneider et al., 1995; Lin & Lee, 1998). The application of biological control agents against neonate larvae is difficult as the newly hatched larva penetrates into the fruit within 24 h and then lives hidden inside what defies any control measures. Therefore, the larval exposure to microbiological insect pathogens like the bacteria *Bacillus thuringiensis* Berliner (B.t.), the granulosis virus and the fungus *Beauveria bassiana* (Balsamo) Vuillemin application is limited to a very short period. This renders control by B.t. and by the virus demanding as these pathogens must be taken up orally by the herbivore. The conventional use of broad-spectrum insecticides against adult codling moths becomes less important for the benefit of sparing pest management strategies with fewer risks for the environment and human health. Methods effective on adult moths include the mating disruption (Bartell, 1982; Charmillot & Bloesch, 1987) and sterile insect techniques (Knipling, 1955; Wildbolz & Mani, 1975; Proverbs et al., 1982). For success in both methods, an understanding of population dynamics in adult codling moths gains importance in contrast to conventional techniques, e.g. the use of broad-spectrum insecticides.

Therefore, much emphasis has been attached to studies on the behaviour of the adult moths, especially their movement. Although the codling moth is often described as a rather sedentary species (Worthley, 1932, Mani & Wildbolz, 1977), reports of newly infested orchards demonstrate the capacity to fly longer distances and to colonize new habitats (White et al., 1973). Field studies using mark-release-recapture techniques have shown the existence of mobile individuals with good flight capacities within a population. Male moths were found at distances up to several kilometers from the release sites (Proverbs, 1971; Howell & Clift, 1974; Mani & Wildbolz, 1977). However, field data on long distance flights were most often restricted to male *Cydia pomonella* due to the inefficiency of a corresponding trap available for females. Female moths were caught most often in or next to release sites because moth density usually decreases with a larger radius and unselective
traps have lower chance to catch females at greater distances. But, recent laboratory studies on tethered flight in flight mills demonstrated that female moths possess the same capacity in flying long distances as males. In addition, these flight capacities were found to be heritable from parent to offspring generation (Schumacher et al., 1997a, 1997b). As those findings were obtained under laboratory conditions it is necessary to make the subsequent step and demonstrate the field relevance. For example, studies on the heritability of insect flight by the use of selection techniques have been available in a few cases (Dingle, 1968; Ritte & Lavie, 1977; Parker & Gatehouse, 1985; Gu & Danthanarayana, 1992). However, previous studies have been limited to laboratory analysis whereas this study represents the expansion of laboratory tests to field experimentation on the subject of insect dispersal.

The objectives of this project are as follows:

1. To investigate the correlation between actograph-measured mobility and flight and dispersal capacity in the codling moth.
2. To determine the influence of insect hormone mimics on the mobility of female codling moths.
3. To analyse the genetic regulation of dispersal in the codling moth by bi-directional selection on mobility in an infrared actograph.
4. To develop a mark-release-recapture method for the study of field dispersal and to verify the dispersal capacity of laboratory-selected strains in the field.
Diel patterns of locomotor activity in *Cydia pomonella*: age and sex related differences and effects of insect hormone mimics

4.1 Abstract

The diel pattern of locomotor activity in the codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) was investigated in the laboratory, using a computer-based infrared actograph. The study focused on sex and age related differences in virgin males and females and effects of insect hormone mimics on females. The level of locomotor activity varied depending on sex and age of codling moths. On the day of emergence, females were more active than males; at 2, 4, 6 and 12 days males were approximately two-fold more active than females. Male codling moths reached their highest activity 4 days after emergence whereas females at the age of 12 days. Both male and female moths, on the other hand, exhibited the peak of locomotor activity during the dusk period. Their diel patterns of locomotor activity at different ages are fitted to a set of polynomial regression models. A single spray on the codling moth with 400 ppm juvenile hormone mimic fenoxycarb stimulated locomotor activity quantitatively and provoked a marked activity peak at dawn in both virgin and mated females. A similar treatment with the ecdysone agonist tebufenozide, however, showed a neutral effect on the locomotor activity of female codling moths. Possible implications of these findings are discussed in relation to the physiology of the codling moth and to its dispersal behaviour.

4.2 Introduction

The codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) is a worldwide key pest of various pome fruit, some stone fruit and walnut. Integrated pest management (IPM) against the codling moth is increasingly reliant on some slow acting management tools, which affect adult moths, such as mating disruption, the
sterile insect technique and the attract & kill technique, i.e. the combination of the codling moth pheromone with an insecticide. Thus, the spatial dynamics of this orchard pest gains more importance in agricultural systems than before; as a result reports on the adult behaviour, especially its mobility, are of special interest (Dorn et al., 1999). So far, studies on the behaviour of adult codling moths, especially in the field, are largely male-biased because of the lack of effective monitoring methods for females (Weissling & Knight, 1994).

In addition to modifying adult behaviour, insect hormone mimics are frequently used as a selective means of IPM in orchards (e.g. Vickers et al., 1998). In many insect species they are applied against larval stages to intervene in the insect life cycle and prevent further development. Fenoxycarb, a juvenile hormone (JH) mimic, has an inhibiting effect on metamorphosis of the last larval instar (Dorn et al., 1981) and also prevents embryogenesis when applied on freshly laid single eggs (Masner et al., 1987). The ecdysone agonist tebufenozide, on the other hand, affects all larval instars and triggers a lethal premature molt (Wing et al., 1988). These insect hormone mimics can also impose a physiological impact on the adult stage of codling moths. For example, topical application of fenoxycarb onto newly emerged female codling moths resulted in a reduction in the number of eggs maturating (Webb et al., 1999). Tebufenozide was found to decrease fecundity and fertility of female codling moths after exposure to treated surfaces (Sun & Barrett, 1999). When applied to apple trees in the field, the effect of fenoxycarb on eggs, and of tebufenozide on neonate larvae of the codling moth, resulted in a comparable efficiency (Charmillot et al., 1994).

Furthermore, JH mimics can have behavioural effects on adult insects, they were reported to influence the circadian rhythm in several species. In the pyralid Diatraea grandiosella the application of the JH mimic methoprene advanced the phase of the eclosion rhythm (Yin et al., 1987). Allatectomy causes a loss of locomotor activity in the German cockroach, Blattella germanica. Such activity is regained, however, following treatment with fenoxycarb (Lin & Lee, 1998). In the locust Schistocerca gregaria, fenoxycarb produced a solitarisation effect in females of the gregarious phase (Schneider et al., 1995). However, the effect of insect hormone mimics on adult behaviour in the codling moth is still unknown.

Insight into the possible effect of insect hormone mimics on the adult behaviour, especially mobility of the codling moth, is meaningful and valuable
because the time of application of insect hormone mimics coincides with the time of increasing flight and thus oviposition activity in this pest species. The present study was carried out in this context. The study, based on the investigation of the sex- and age-related diel patterns of locomotor activity in the codling moth, examined effects of fenoxycarb and tebufenozide on the mobility of female moths. The mobility was measured by locomotor activity using an actograph technique. Actographs have been proven to be useful laboratory tools for studying behavioural effects of insecticides on the codling moth (Dorn & Gu, 1999), as well as for monitoring its diurnal pattern of flight activity (Knight et al., 1994) and for measuring its dispersal potential for laboratory selected strains in the codling moth (Chapter 5).

4.3 Materials & methods

Insects

Codling moths used in the experiments came from a laboratory culture, which originated from diapausing larvae collected in cardboard strips around high stem apple trees in north-eastern Switzerland, and had been maintained for 15 generations. Larvae were reared singly on a semi-artificial medium (Huber et al., 1972) at 26 ± 1°C, a relative humidity of 65 ± 5% and a photoperiod of LD 18:6 with a simulated dusk and dawn period. The 5th instar larvae were sexed; males and females were kept separately in polystyrene boxes (100 x 100 x 80 mm) provided with corrugated cardboard for pupation. Pupation boxes were checked daily for emergence. Newly emerged adult moths were singly held in polystyrene tubes (30 x 60 mm) and supplied daily with water on the perforated tube lid. All developmental stages of the virgin moths tested in the experiment were kept under the above-mentioned conditions. All stages of the mated moths used were reared under the same conditions but without a simulated dusk and dawn phase due to a change of rearing facility.

Set-up of actograph test

Tests for locomotor activity were carried out with infrared actographs. Each unit was composed of a transmitter section emitting an infrared (IR) light beam and a receiver/logic-converter section (Electronic Services Unit, University of New England, Australia) (Figure 4.1). The test insect was placed in a glass vial (28 x 80 mm) and
positioned between the two sections. Every insect movement intercepting the IR beam triggered a signal, which was registered as an activity count by a computer. All tests were conducted within a climatic chamber at a temperature of 23°C ± 1°C and 65 ± 5 % RH. Light intensity at full illumination was 3750 lux during 16 h (photophase) and 0.1 lux during 4 h (scotophase). Between the two phases there were simulated sunset and sunrise periods of 2h each, during which light intensity decreased and increased progressively in 10 min. intervals. Tests were run over 24 h, starting at the last hour of photophase.

**Application of insect hormone mimics**

Commercial formulations of fenoxycarb, registered as Insegar® WG 250 g/kg (Syngenta, formerly Novartis, Basel, Switzerland) and tebufenozide, registered as Mimic® SC 240 g/l (Rohm and Haas, Philadelphia PA, USA) were used. Based on the concentration recommended for field application, a 400 ppm solution for each product was prepared freshly with distilled water. A hand sprayer was used to spray the solution directly onto the female moths. Each moth was subject to a single spray only, and then it was placed on the bottom of a covered plastic cup (200 ml). Control moths were treated with water in the same way. All treated insects were held for five hours under a fume-hood before the actograph test.
Figure 4.1. Experimental set-up of an actograph unit showing the position of a glass vial with test insect in the infrared light beam.
Data analysis

For measuring diel patterns of age-related locomotor activity, a total of 135 males of different ages (n=29 of 0 day; n=30 of 2 day; n=26 of 4 day; n=23 of 6 day and n=27 of 12 day old) and 140 females of different ages (n=28 of 0 day; n=29 of 2 day; n=29 of 4 day; n=29 of 6 day and n=25 of 12 day) were tested. To examine the effect of fenoxycarb and tebufenozide on locomotor activity, a total of 79 virgin female codling moths, 3-4 days old (n=23 fenoxycarb treated, n=22 control; n=16 tebufenozide treated; n=18 control), and 39 mated females, 3-4 days old (n=22 fenoxycarb treated, n=17 control), were tested.

The registered movements during the 24 h period were summed as the total number of activity counts per 30 min intervals. Then, the polynomial regression analysis (Zar, 1999) was performed to determine the sex and age-related differences, as well as the effect of the insect hormone mimics fenoxycarb and tebufenozide on locomotor activity in comparison to the control (water-treated insects).

4.4 Results

Diel patterns of locomotor activity

During the first hour of photophase with full light intensity, almost no activity counts were registered for either male or female codling moths. Locomotor activity in both sexes started and increased as light intensity decreased, reaching the highest level during the dusk period and remained at a lower level during the scotophase (Figure 4.2). At full illumination during photophase, almost no activity occurred. Diel patterns of locomotor activity shown at different ages are fitted to a set of polynomial regression models (Table 4.1).

Sex and age-related differences

Female codling moths apparently showed a second minor peak of activity during the dawn period, whereas this peak was not obvious in the males. Sexual differences, however, were mainly reflected at the activity level. Males at ages of 2, 4, 6 and 12 days after emergence displayed an activity level approximately two-fold higher than females. In the newly emerged moths, females were more active than males. These differences are statistically significant (Table 4.2).
Polynomial regression analyses revealed a general effect of age on the level of locomotor activity in both males (F=16.63, d.f.=12, 6600, P<0.001) and females (F=10.90, d.f.=12, 6844, P<0.001). In males, 2, 4, 6, and 12 day-old moths all showed a significantly higher activity level than newly emerged ones (Table 4.3), with the peak activity occurring at 4 days. In females, 6 day-old moths displayed a similar activity level, and those at ages of 2 and 4 days had a significantly lower activity level in comparison to those on the day of emergence; only the 12-day-old moths showed a significantly higher activity level than the newly emerged ones (Table 4.3).
Figure 4.2. Actograph-measured diel pattern of locomotor activity of adult *Cydia pomonella* depending on sex and age. Identical light regime for females and males.
Table 4.1. Relationships between the level of locomotor activity (y) and time (x) in *Cydia pomonella* of different age classes, as described by polynomial regressions.

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<thead>
<tr>
<th>Sex</th>
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<th>Regression equation</th>
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<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0</td>
<td>$y = 8.792 - 1.058 x + 0.031 x^2$</td>
<td>76.68</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$y = 31.135 - 3.174 x + 0.078 x^2$</td>
<td>141.92</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>$y = 33.529 - 3.631 x + 0.095 x^2$</td>
<td>95.01</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>$y = 27.186 - 2.901 x + 0.075 x^2$</td>
<td>85.73</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>$y = 26.082 - 2.328 x + 0.049 x^2$</td>
<td>73.92</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>$y = 11.728 - 1.410 x + 0.040 x^2$</td>
<td>74.05</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$y = 8.152 - 0.996 x + 0.029 x^2$</td>
<td>61.50</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>$y = 9.331 - 0.835 x + 0.018 x^2$</td>
<td>64.72</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>$y = 10.971 - 1.077 x + 0.026 x^2$</td>
<td>53.48</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>$y = 17.252 - 1.864 x + 0.050 x^2$</td>
<td>68.24</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Table 4.2. Statistical comparisons of locomotor activity between male and female codling moths, based on the polynomial regression analysis.

<table>
<thead>
<tr>
<th>Age class (days)</th>
<th>F-value</th>
<th>DF</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.10</td>
<td>3, 2787</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>2</td>
<td>79.71</td>
<td>3, 2885</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>4</td>
<td>49.95</td>
<td>3, 2689</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>6</td>
<td>28.71</td>
<td>3, 2542</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>12</td>
<td>10.08</td>
<td>3, 2542</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
Table 4.3. Locomotor activity of male and female codling moths at ages 2, 4, 6 and 12 days, in comparison to 0 days, based on polynomial regression analysis.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age class (days)</th>
<th>F-value</th>
<th>DF</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>2</td>
<td>76.06</td>
<td>3, 2885</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>59.7</td>
<td>3, 2689</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>48.91</td>
<td>3, 2542</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>44.49</td>
<td>3, 2738</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Female</td>
<td>2</td>
<td>4.74</td>
<td>3, 2786</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.63</td>
<td>3, 2786</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.11</td>
<td>3, 2786</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>7.6</td>
<td>3, 2590</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

**Effects of insect hormone mimics**

Virgin female codling moths, treated at age 3 to 4 days with fenoxycarb, exhibited a second peak of activity during the dawn period, which was almost as high as that during the dusk period (Figure 4.3). Thus, the general diel pattern of locomotor activity was significantly different between the fenoxycarb-treated moths and the water-treated counterparts (control) (F=4.05, d.f.=3, 2199, P<0.01). This stimulatory effect was also highlighted in mated females of the same age. The fenoxycarb-treated moths not only displayed a second peak of locomotor activity at and immediately after the dawn period, but also showed a generally higher activity level than the water-treated moths (control) (Figure 4.3). These differences between the fenoxycarb-treated and the water-treated moths were significant (F=5.32, d.f.=3, 1905, P<0.01). In contrast, treatment with tebufenozide on virgin females yielded a diel pattern of locomotor activity similar to the water-treated moths (control) (Figure 4.4). The activity level was also comparable between these two groups (F=0.81, d.f.=3, 1660, P>0.25).
Figure 4.3. Effect of fenoxycarb on the diel pattern of locomotor activity of virgin and mated *Cydia pomonella* females.
Figure 4.4. Effect of tebufenozide on the diel pattern of locomotor activity of virgin *Cydia pomonella* females.

4.5 Discussion

Diel patterns of locomotor activity

The locomotor activity of codling moths occurred largely in the scotophase and peaked during the dusk period, while a period of repose or generally minor activity took place during photophase. This result is in agreement with the previous findings on the flight activity of codling moths from either field or laboratory studies. In field trials, increasing flight activity was correlated with the local sunset and prevailing warm temperatures (Mani *et al.*, 1974). In laboratory trials, visual observations and actograph monitoring showed no increase in activity when illumination occurred without a transition phase (Boving *et al.*, 1978; Castrovillo & Cardé, 1979). Together with previous findings, the result of the present study suggests that locomotor activity in the codling moth occurs in synchronisation with
changes in light intensity, as does the oviposition behaviour of female moths (Riedl & Loher, 1980).

Newly-emerged to 12-day-old female codling moths showed a second minor peak of locomotor activity during dawn which did not occur in males of the same age. Similarly, a second minor peak of activity was found using an ultrasonic actograph, but for both sexes, though this peak apparently did not occur in field monitoring as males were not caught in parallel timing traps at dawn (Knight et al., 1994). Other field studies reported the presence (Borden, 1931; Cutright, 1964) or absence (Wong et al., 1971; Batiste et al., 1973) of this secondary peak. Temperature and/or illumination conditions have been suggested as parameters that favour or suppress this minor peak of activity at dawn (reviewed by Knight et al., 1994). Field observations indicate that sudden temperature decline could prevent flight despite a suitable thermal threshold and cloudy sky with low illumination could shift the flight period to the photophase (Mani et al., 1974). Therefore, the flight and other locomotor activities in the codling moth occur as a behavioural response to changes in these environmental conditions. The impact of the parameters (light and temperature) as triggering impulses for activity is obvious as recent laboratory studies showed that *Cydia pomonella* responded with pupil movement not only to changes in light but also to changes in temperature in the absence of light (Nordstrom & Warrant, 2000). The authors discussed the possibility of using the ambient temperature for pre-adaptation of the superposition eye to different light intensities.

**Sex and age related differences in locomotor activity**

Although diel patterns of locomotor activity were largely similar between male and female codling moths, the present studies revealed a significantly higher locomotor activity in males, except on the day of emergence, than in females. Previous trials with other methods did not reveal such sexually dimorphic behaviours (Knight et al., 1994; Schumacher et al., 1997b). Non-tethered flight has been studied measuring groups of three to five insects in an ultrasonic actograph (Knight et al., 1994) while the present work investigated the locomotor activity of single insects to exclude possible intraspecific interference between individuals. Indeed, codling moth females are able to recognize their own sex pheromone and distinguish from other individuals (Barnes et al., 1992) and the impact of such a response on the individual
behaviour of females within a group is as yet unknown. Furthermore, a possible influence of ultrasound on the activity of moths in the previous study was not ruled out completely although considered as unlikely (Knight et al., 1994).

The tethered flight of the codling moth has been studied on computer-linked flight mills and both sexes showed a similar flight capacity in terms of flight duration and distance (Schumacher et al., 1997b). The tethered flight technique method assesses the flight potential whereas the non-tethered method measures and reveals spontaneous mobility. Available field data indicate that the results found with the two techniques confirm their suitability for the quantification of different aspects of the codling moth's behaviour. Females were regarded generally as rather sedentary but infestation of isolated orchards indicates the potential in females to fly over long distances to colonize new habitats, similar to males (Howell & Clift, 1974; Mani & Wildbolz, 1977; Neumann, 1993).

The lower level of locomotor activity in female codling moths, as compared to males, is probably attributable to their calling behaviour, which usually occurs during the first scotophase after eclosion (Howell et al., 1978; Castrovillo & Cardé, 1979). Female Cydia pomonella remain unwilling to move while calling. Sexual differences in mobility have also been reported for various other lepidopteran species. For example, in the tortricid moth Epiphyas postvittana, males showed a higher mobility than females (Gu & Danthanarayana, 1990). The males of the noctuids Spodoptera depravata, S. exigua and S. litura displayed an activity level several times higher than females (Saito, 2000).

Furthermore, the present actograph tests shed light on the age-related differences in the mobility of codling moths. Males at the ages of 2, 4, 6 and 12 days after emergence showed a significantly higher locomotor activity than newly emerged ones, reaching the highest level in 4 days-old moths. The pattern of age-related changes in locomotor activity coincides with the postemergence development of flight capacity in the codling moth (Schumacher et al., 1997b). A similar coincidence between locomotor activity and flight capacity has been also found in the tortricid moth Epiphyas postvittana (Gu, 1991). Female codling moths at the age of 12 days displayed the highest locomotor activity, while younger females at 2 to 6 days showed a similar or even lower activity level compared to those on the day of emergence. Regarding flight capacity in females, it was found to peak, similarly as for males, four to five days after emergences (Schumacher et al., 1997b). The age-
related changes in mobility in both male and female codling moths seem to correspond to the development of reproductive activities. Three-day old male codling moths were more active and readily attracted to virgin females than newly emerged ones (Benz, 1970), and showed maximal sexual activity when they were denied access to females (Hutt & White, 1973; 1974). Female moths mate usually during the first calling period, i.e. the first 24 hours after eclosion (Howell et al., 1978). The percentage of calling begins to decline in 5 to 6 day-old females (Niemczyk et al., 1977). Maximal mating activity in codling moths occurred at 3 to 4 days (Benz, 1970) or at 2 to 3 days (Deseö, 1971).

Effects of insect hormone mimics on locomotor activity

The ecdysone agonist, tebufenozide, influenced neither the level nor the diel pattern of locomotor activity in virgin female codling moths. Thus, no effects of this hormone mimic on adult codling moth behaviour are known so far, as it was also found to be neutral regarding impact on mortality and longevity (Pons et al., 1999). The juvenile hormone mimic fenoxycarb increased the total locomotor activity of both virgin and mated female moths. It provoked a marked second peak of activity during or shortly after dawn. In both virgin and mated females, this second peak followed the first one at an interval of approximately 7 hours and did not fully reach the level of the first peak. The maximal activity in mated moths was delayed by 2 to 4 hours as compared to virgin moths even in untreated; these mated females were reared under a light regime without the dusk and dawn simulation between the photo- and scotophase which might have caused the noted shift in response. Thus, this apparent difference between virgin and mated females may be partially attributable to the changed experimental conditions.

The present study, along with the previous finding that the topical application of fenoxycarb leads to disrupted egg maturation in virgin codling moth (Webb et al., 1999), suggests that this JH mimic might impose a dual impact on reproduction and mobility in the species. Interestingly, a similar inverse relationship was also demonstrated in the chrysomelid Diabrotica virgifera virgifera, in which the JH mimic methoprene stimulated flight activity and exhibited a negative effect on ovarian development (Coats et al., 1987). More recently, such effects were demonstrated for the curculionid Anthonomus pomorum, which responded to fenoxycarb with a reduction in mature eggs and to methoprene with a marked increase in locomotor
activity (Zdarek et al., 2000). An activational effect of JH on insect behaviour was well documented for larvae but only in few species for adults (see review by Elekonich & Robinson, 2000). In particular, JH can stimulate migratory flight behaviour and suppress ovarian development in some migratory insects, e.g. the milkweed bug Oncopeltus fasciatus (Caldwell & Rankin, 1972) and the monarch butterfly Danaus plexippus (Rankin, 1980, 1986). From studies on the locusts Schistocerca gregaria and Locusta migratoria, Wiesel et al. (1996) concluded that JH played a decisive role in phase-related physiological processes and in coordination of migratory flight and egg maturation in these species. Thus, to some degree the effect of the JH mimic fenoxycarb on the codling moth is analogous to the physiological role of JH in those migratory insects, though the codling moth is often considered as a sedentary insect (e.g. Geier, 1963; Mani & Wildbolz, 1977).
5 Response of *Cydia pomonella* to selection on mobility: laboratory evaluation and field verification

5.1 Abstract

The codling moth *Cydia pomonella* L., largely regarded as a sedentary species, shows great variation in flight capacity among individuals in the laboratory, as has been reported for tethered flight. The occurrence of individuals with the ability to fly over long distances is considered ecologically significant for the colonisation of new habitats in response to deteriorating environmental conditions. The work reported here was designed to investigate the importance of the genetic component in regulating dispersal in *Cydia pomonella*.

Bi-directional selection on mobility measured by actograph was carried out in the laboratory. Both male and female codling moths responded significantly to the upward and downward selections. Divergence between the two selected strains occurred after one generation of selection and increased as further selections continued. The realised heritabilities for mobility were estimated as 0.43 in males and 0.29 in females when averaged from the two selection directions.

The dispersal capacity of the selected mobile and sedentary strains was verified by mark-release-recapture experiments in the field, though only the released male moths were captured effectively. Flight distances by males differed significantly between the two strains, with the mobile strain showing a greater dispersal capacity than the sedentary strain.

This study demonstrated a positive correlation between mobility measured by actograph and field dispersal capacity in the codling moth. Furthermore, it provides the first experimental data to show field performances of different genotypes in insect dispersal.

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2 Based on publication: Keil, S., Gu, H. and Dorn, S. Response of *Cydia pomonella* to selection on mobility: laboratory evaluation and field verification. *Ecological Entomology*, in press.
5.2 Introduction

The codling moth Cydia pomonella is considered largely to be a sedentary insect (Geier, 1963; Mani & Wildbolz, 1977), however comparative studies on the flight capacity of both sexes in the laboratory have demonstrated the existence of individuals with a capacity for both short and long flights (Schumacher et al., 1997b). This variation was shown for a laboratory colony and for moths reared from field-collected larvae of different origins (Dorn et al., 1997, 1999). Field results on flight distances are available mainly for males. Recaptures from pheromone traps have shown that a small proportion of the male moth population is able to disperse up to several kilometres but the majority disperse only within 60 m (Worthley, 1932; Steiner, 1940; Wildbolz & Baggiolini, 1959; White et al., 1973; Mani & Wildbolz, 1977).

Variation in dispersal capacity within populations of Cydia pomonella is presumably adaptive in the context of its specific life history and habitat characteristics (Schumacher et al., 1997b). In Cydia pomonella the number of annual generations varies depending on climate, the year and sometimes the host plant where it occurs, with a certain proportion of larvae entering diapause in each generation (Audemard, 1991). Although the habitat of the species consists of various perennial fruit trees (e.g. apple, pear, quince, walnut, apricot, plum, peach and nectarine), the larval development requires fruits as a food source (Barnes, 1991). Thus, the natural habitat may change in both quality and quantity, since fruit production can be subject to yearly fluctuation, especially when late frosts and severe infestation by some early-season pests occur (Nagy, 1977). To elucidate the possible ecological and evolutionary significance of the variation in dispersal capacity, a genetic analysis is essential, as it is genetic variance that provides the raw material on which evolutionary changes due to natural selection are based (Istock, 1981). Sib analysis and parent-offspring regressions have shown that variation in the flight capacity of the codling moth is under polygenic control, with significant heritabilities for total flight duration and flight distance (Schumacher et al., 1997a). Experimental selection is the most direct way to reveal genetic effects on such a quantitative character. Dingle (1968) was the first to apply intense selection for flight duration in Oncopeltus fasciatus. The proportion of the bugs performing flights of ≥ 30 min in both sexes was increased by one generation of selection,
suggesting that the flight capacities of male and female bugs are heritable. To understand the genetic basis of dispersal ability in the flour beetle *Tribolium castaneum*, Ritte and Lavie (1977) carried out a two-way selection for and against dispersiveness, achieved separation between dispersers and non-dispersers after one generation of selection, and maintained the difference between these lines for subsequent generations of selection. Similarly, bi-directional selections for and against the capacity for prolonged flight confirmed the existence of additive genetic variance in the flight capacity of the noctuid moth *Spodoptera exempta* (Parker & Gatehouse, 1985) and the tortricid moth *Epiphyas postvittana* (Gu & Danthanarayana, 1992), however these studies were restricted to the laboratory. There has been no attempt to investigate the dispersal propensity of artificially selected strains in the field, so the relationship between laboratory flight activity and field dispersal as well as the phenotypic expression of different genotypes in the field is still unknown.

In previous studies, including those on the codling moth (Schumacher *et al.*, 1997a, b), the flight capacity of two sexes was determined based on the flight duration and/or flight distance of insects tethered on flight mills. The tethered flight technique has some limitations, such as the negative influence on mating behaviour in the codling moth (Schumacher *et al.*, 1997a, personal observations). An alternative technique is the use of actographs, based either on passive detection of sound through wing vibration (e.g. Hsiao, 1978; Leppla *et al.*, 1979; Luff *et al.*, 1979) or active detection of movement (e.g. Pats & Wiktelius, 1992; Knight *et al.*, 1994; Dorn & Gu, 1999). Actographs minimise the manipulation of test insects by allowing them to move freely and voluntarily. The actograph technique thus seems to be a promising way to study both behavioural and physiological components of dispersal propensity and to use for selection experiments based on phenotypic values.

In this study, a novel approach was used to investigate the genetic regulation of dispersal capacity in the codling moth. Experiments consisted of laboratory selection on mobility measured by actograph and a field mark-release-recapture test on the dispersal capacity of the selected strains. Three questions were addressed. How does the codling moth population respond to the selection on mobility measured by actograph? Is this laboratory-measured mobility correlated with field dispersal capacity? Does the mobile genotype selected in the laboratory show a higher dispersal capacity in the field than the sedentary genotype?
5.3 Materials & methods

Insects

Insects used in the study were obtained from a laboratory culture, which originated from diapausing larvae collected using cardboard strips around high stem apple trees in north-eastern Switzerland and maintained in the laboratory for 20 generations. Larvae were reared singly on a semi-artificial medium (Huber et al., 1972). When they developed into the fifth instar, males and females were distinguished and kept separately in polystyrol boxes (100 x 100 x 80 mm) containing corrugated cardboard strips. On pupation, these boxes were checked daily for emergence of moths. The newly emerged moths were held in polystyrol tubes (60 x 30 mm) closed with perforated lids that were sprayed daily with water. All life stages were reared in a climate chamber at 26 ± 1 °C, 65 ± 5% RH and LD 18:6 h. The light regime was set up with simulated dusk and dawn phases.

Actograph test of mobility

A set of 30-unit infrared actographs (Electronic Services Unit, University of New England, Australia) was used to record the mobility of the moths. Each actograph unit consists of a transmitter section and a receiver/logic-converter section. The transmitter produces a modulated infrared beam. For the test of individual mobility, one moth was enclosed in a glass tube (28 x 80 mm), which was placed between the transmitter and the receiver sections. The operation of all actograph units and data processes was computerised. Movement of a moth gave rise to a positive output pulse, which was registered as a mobility count. All tests were carried out within a climate chamber at a temperature of 23 ± 1 °C and a relative humidity of 65 ± 5%. Tests ran for 4 h and all registered movements were summed as the total number of mobility counts. During this period, the light intensity was changed from 3750 lux (1 h photophase) at the start, over a simulated sunset (2 h dusk phase) with 10 min intervals, to 0.1 lux (1 h scotophase) at the end.

Bi-directional selection

Selection on mobility was based on the phenotypic value of the moth activity counts within the 4 h test period. Initially, 35 male and 35 female individuals were chosen arbitrarily from newly emerged moths and set as single pairs in polystyrol
tubes (30 x 80 mm) provided daily with water. Twenty males and 20 females mated successfully and were considered for the selection procedure. The offspring of each of the 20 pairs was kept separately as a family. Five male and five female moths from each family were tested for mobility on the second day after emergence. The mean value of mobility was calculated based on the ten tested individuals belonging to the same family. According to the mean mobility, these families were ranged and five families showing the highest mobility were selected as mobile strain (strain A) and five families showing the lowest mobility as sedentary strain (strain B). Then 15 males and 15 females were randomly chosen and paired as parents of the F0 generation in each of the two directions. The mass selection was conducted in each of the following generations, that is, individuals were selected based on their own phenotypic performance (Falconer, 1989). Up to 26 male and 26 female moths from each strain were tested for mobility. From these, six males and six females were selected according to their phenotypic values as parents for further breeding in the next generation. This selection procedure was continued from the F1 to F4 generation. Afterwards, the two strains were not subject to selection, only maintained, and the moths were sampled (except for the F5 generation) for testing mobility until the F8 generation.

Field release-recapture experiments with the selected strains

The two selected strains of the F5 generation were used for the field release-recapture experiments. These experiments were carried out for male and female moths separately in two commercial apple orchards (A and B) in north-eastern Switzerland. Both orchards consisted of dwarf apple trees of different varieties. Males were released in orchard A, situated in Leuggern (47°34'N, 8°13'E) at an elevation of 436 m. One side of this 4 ha orchard was bordered to rows of pear trees and the other three sides were adjacent to open fields. Female moths were released in a 5.2 ha orchard (B), located at Otelfingen (47°28'N, 8°23'E) at an elevation of 488 m. Three sides of the orchard were bordered to open fields and one side adjacent to a forest. Both orchards were managed under the Integrated Production guidelines (Boller et al., 1999). The control of codling moth relied completely on insect growth regulators; fenoxycarb (Insegar® WG 250 g/l, Syngenta, formerly Novartis, Basel, Switzerland) was used 7 weeks prior to the release of moths and
diflubenzuron (Dimilin® SC 480g/l, Syngenta, formerly Novartis, Basel, Switzerland) 2 weeks before the release of the moths.

All released moths were marked internally by incorporating the colouring Calco Red Oil into the larval diet (Charmillot, 1979), in order to distinguish them from the wild population. The effect of this colouring on flight behaviour was tested on flight mill tests in the laboratory prior to the releases. No significant differences were detected between the coloured and uncoloured animals (t-test: t = 0.716, d.f. = 38, P = 0.478). Externally, the moths were marked with fluorescent dye (BioQuip, Gardena CA, USA) in two different colours to distinguish between the two selected strains. The newly emerged moths in polystyrol cylinders were transported in a cold bag to the field and released in the morning. All male moths from both strains were released from the same site, which was in the middle of the last apple tree row bordering an open field. The female moths of both strains were handled in the same way. In total, 2024 moths were released, i.e. 450 males and 500 females from the mobile strain, 578 males and 496 females from the sedentary strain. Each release site was checked on trap inspection days for the moths that remained at the site.

Male moths were recaptured using ten commercial pheromone traps, which were set up 2 days after release. Within the orchard, totally six traps were located at 75, 150, and 250 m from the release site. At each of the three distances, two traps were always positioned at a height of 1.7 m in the same row of apple trees, 150 m apart from each other. Outside the orchard, four traps were hung randomly on apple trees at 200 to 750 m from the release site. During three days after the set-up, these traps were inspected once per day for codling moths; thereafter traps were checked every second day until no more marked males were recaptured. All captured codling moths were brought into the laboratory for identification of internal and external marks under a stereomicroscope with an UV-fluorescent filter (Leica Mikroskopie Systeme AG, Glattbrugg, Switzerland). Wild males caught on each trap were also recorded.

For the recapture of female moths, six portable Luminoc light insect traps (Biocom, Quebec, Canada) were set up within orchard B. These light traps were also hung at a height of 1.7 m within rows of apple trees and at 75, 150, and 250 m from the release site. No light traps were set up outside the orchard.
Data analysis

The response of the codling moth to the selection in different generations was analysed for differences in mobility counts between the two strains with ANOVA using the statistical software StatView 4.5 (SAS Institute Inc.). The realised heritability $h^2$ was estimated from the ratio R/S, R being the response to the upward or downward selection, S being the selection differential (Falconer, 1989). Because the released number of moths was not the same for the two strains, recaptures at different distances were calculated as the percentage of the total number of moths released. For the same reason, the overall dispersal distances were compared between the two strains based on the number of moths recaptured at different distances from the release site. The differences in dispersal distances between the two strains were determined using Mann-Whitney U tests in StatView, because the data for recaptures at different distances were not distributed normally for either strain.

5.4 Results

Response to selection

Both male and female codling moths showed significant responses to the bidirectional selection on mobility (Figure 5.1). Divergence between the upward- and downward-selected strains occurred after one generation of selection (F1) and increased with further selection (ANOVA $F = 44.99$, d.f. = 1,7, $P < 0.001$ in females; $F = 61.12$, d.f. = 1,7, $P < 0.001$ in males). Selection was implemented for four generations. From the F0 to F4 generation, the mean activity counts of the selected mobile strain increased from 531 to 817 in males and from 336 to 393 in females. In the selected sedentary strain, the mean activity counts decreased from 531 to 168 in males and from 336 to 107 in females. Differences in mobility between the two selected strains were maintained for subsequent generations without selection.
Figure 5.1. Response of male and female codling moths to selection on mobility measured by actograph in upward and downward directions (mean ± n= 298 males, n = 336 females).
Responses of both male and female moths were more rapid to the downward selection than to the upward selection, which resulted in the asymmetry of responses.

Differences in the response to the two selection directions are substantiated further in the estimated realised heritabilities (Table 5.1). Heritabilities estimated for both male and female moths were much higher from the response to downward selection than from the response to upward selection. When averaged from the two selection directions, the realised heritability for mobility was greater in males than in females.

Table 5.1. Realised heritabilities ($h^2$) in male and female *Cydia pomonella*.

<table>
<thead>
<tr>
<th></th>
<th>Strain A (upward selection)</th>
<th>Strain B (downward selection)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>0.32</td>
<td>0.54</td>
<td>0.43</td>
</tr>
<tr>
<td>Females</td>
<td>0.07</td>
<td>0.52</td>
<td>0.29</td>
</tr>
</tbody>
</table>

*Dispersal patterns of two selected strains in the field*

A total of 1028 male and 996 female codling moths from the two selected strains were released in orchard A and orchard B respectively. Three hundred forty four released male moths were recaptured in the 10 pheromone traps set up within and outside orchard A, yielding a recapture rate of 33.4%. When these marked male moths were distinguished between the two selected strains, the recapture rate was 37.3% for the mobile strain and 30.4% for the sedentary strain. These traps also caught 127 male codling moths from the wild population. No marked female codling moths were recaptured in orchard B due to the ineffectiveness of light traps in attracting female codling moths.
Figure 5.2. Recapture profile of marked male *Cydia pomonella*.
The majority of male recaptures occurred on the first day after the set-up of traps, i.e. three days after the release (Figure 5.2), accounting for 57.7% of recaptures from the mobile strain and 65.9% from the sedentary strain. Recaptures during the first 7 days after release accounted for 97.6% of the total captures in the mobile strain and 98.3% in the sedentary strain. The capture profile of wild moths showed a continuous flight activity during the whole experimental period, indicating that climatic conditions were suitable for flight activity as warm and dry weather prevailed, with evening temperatures considerably above 15 °C (Figure 5.3).

Wild codling moths were captured mainly by the pheromone traps in the middle of the orchard but the recapture pattern of released moths varied depending on the strains and demonstrated a clear preference for traps near the release point. Within the orchard, 50.6% of the recaptured mobile moths and 60.2% of the recaptured sedentary moths were found in the two traps 75 m from the release site.
In contrast, the more distant traps recaptured more mobile moths than sedentary moths. In the traps at the distance of 150 m, 36.3% of the recaptures were from the mobile strain and 32.9% from the sedentary strain. The traps set up at the distance of 250 m accounted for 9.5% of the total recaptures from the mobile strain and 5.7% from the sedentary strain. Furthermore, 3.6% of the mobile moths and 1.1% of the sedentary moths were recaptured by the traps set up outside the orchard (Figure 5.4). One moth from the mobile strain was found to have flown to a group of scattered apple trees at a distance of 750 m from the release site. These results show that the percentages of the released moths that was able to fly beyond the orchard and cross the open field to reach adjacent apple trees differed between the two strains. The overall dispersal distances differed significantly between the two strains (Mann-Whitney $U$ test, $z = 2.091$, $p < 0.05$). On average, the mobile strain dispersed 134 m whereas the sedentary strain dispersed 114 m.
Figure 5.4. Percentages of recaptured marked male *Cydia pomonella* at various distances inside and outside the orchard.
5.5 Discussion

The results of this study focus on three specific issues associated with dispersal in the codling moth: the existence of additive genetic variance in mobility, the positive correlation between the mobility measured by actograph and dispersal capacity, and the differential dispersal performances of the mobile and sedentary genotypes in the field.

The study reported here represents the first attempt to apply an actograph technique in a genetic investigation of insect dispersal, though actographs have been used commonly to monitor the daily pattern of flight activity in insects (e.g. Knight et al., 1994; Dorn & Gu, 1999). Without tethering as in the flight mill technique, the actograph minimises the behavioural manipulation of insects and allows simultaneous measurement of a large number of insects, which is particularly important for the genetic analysis of dispersal capacity in the codling moth. In addition to these experimental advantages, female codling moths were found to reproduce normally following the test using actographs whereas they would most often not be able to copulate and oviposit after being tested on the flight mills (Personal observations; also see Schumacher et al., 1997a).

Both male and female codling moths showed rapid responses to selection on the mobility measured by actograph. The two selected strains diverged substantially after the first generation of selection. A significant response to the selection reveals the existence of additive genetic variance for mobility in the population of C. pomonella. As in other cases where a bi-directional selection is implemented (e.g. Falconer, 1953; Baptist & Robertson, 1976), however, the response of the codling moth to selection on mobility was asymmetrical, with response to downward selection being faster than response to upward selection. The asymmetry of response to selection on mobility is confirmed by the differential realised heritabilities estimated from the two selected strains. This asymmetry may suggest that the character subject to selection is a fitness component because selection for fitness is normally less successful than selection against fitness (Falconer, 1989). The realised heritability for mobility was calculated as 0.43 for males and 0.29 for females. It is interesting that these estimates are very close to those for flight duration and flight distance found in flight mill tests. For example, heritability for flight distance was estimated as 0.57 for a field population and 0.37 for a laboratory
population (Schumacher et al., 1997a). Heritabilities for mobility in the codling moth are also within a range determined for flight capacity in other insects, e.g. the milkweed bug *Lygaeus kalmii* (0.20-0.40) (Caldwell & Hegmann, 1969), the tortricid moth *Epiphyas postvittana* (0.23-0.56) (Gu & Danthanarayana, 1992), the noctuid moth *Mythimna separata* (0.27) (Han & Gatehouse, 1993), and the fruitflies *Drosophila buzzatii* (0.284) and *D. aldrichi* (0.214) (Gu & Barker, 1995). Different insect species apparently have a similar and moderate heritability for either flight capacity or mobility, even though an estimate of heritability applies only to a specific population in a particular environment (Falconer, 1989).

The present study extended, for the first time, the laboratory analysis of genetic variation in insect dispersal to field verification. Laboratory selection on either flight capacity or dispersiveness has been undertaken on a range of insect species in different orders (e.g. Dingle, 1968; Ritte & Lavie, 1977; Parker & Gatehouse, 1985; Gu & Danthanarayana, 1992). Previous studies have generated a large amount of data to document the contribution of additive genetic variance to the phenotypic variation in this character, however such genetic studies on insect dispersal have so far been limited to laboratory analysis, and thus the importance of the genetic component in regulating insect dispersal in the field is still not beyond speculation. The release-recapture experiments demonstrated that the mobile genotype selected in the laboratory dispersed further than the sedentary genotype in the field. The positive correlation between the mobility measured by actograph and dispersal capacity in the codling moth gives further support to the use of actographs in the quantification of dispersal capacity in insects.

Field release-recapture experiments showed that some male codling moths left the orchard and were able to fly over open fields to the pheromone traps set up in the scattered high-stem apple trees, though the majority moved only within the orchard. This concurs with findings from other studies (e.g. Worthley, 1932; Steiner, 1940; Mani & Wildbolz, 1977). Among the moths that flew beyond the experimental orchard, the mobile strain was found to account for a higher percentage than the sedentary strain. Although it was not possible to assess dispersal distances for female codling moths due to the lack of an effective recapture technique, they are assumed to show similar patterns as the males. Females from a laboratory population contained a proportion of long-flyers similar to the males, as has been demonstrated on flight mills (Schumacher et al., 1997b). Further, moths originating
directly from larvae collected in four different regions of Switzerland contained an even higher proportion of long-flyers in both sexes (Dorn et al., 1999). In this context, the genetic component plays an important role in regulating dispersal in the field, as the mobile genotype will facilitate the colonisation of new habitats. The quantification of female dispersal in the field has so far relied either on indirect techniques such as scoring fruit damage caused by larval injuries after releasing female moths within orchards (Wildbolz & Baggiolini, 1959) or estimating recaptures at short distances, e.g. with passive window traps (Weissling & Knight, 1994; Bloem et al., 1998). The development of host-plant derived attractants may provide new opportunities for future investigations (Hern & Dorn, 1999).

Genetic variation in dispersal capacity within populations of the codling moth raises a question about the factors responsible for the maintenance of such variation. The species-specific life history along with the habitat characteristics of *Cydia pomonella* has been suggested as one of these factors (Schumacher et al., 1997a,b). The habitat of this species consists principally of perennial apple trees, and selection may favour the sedentary genotype, however the amount of food resource (i.e. fruit) of the codling moth is subject to fluctuations among years due to the biennial tendency in bearing, late frosts, or severe attack by early-season pests, such as *Anthonomus pomorum* in Europe (Schumacher et al., 1997b; Töpfer et al., 1999). Also, the habitat exists in a patchy landscape in which orchards and uncultivated areas alternate. Therefore, long-distance flights by some individuals as a response to environmental deterioration would allow a part of the population to escape from unfavourable conditions and colonise new habitats, and hence enable the insect to survive and reproduce in heterogeneous environments (Southwood, 1962; Wellington, 1964; Den Boer, 1970; Kimura, 1992). In this sense, the presence of both sedentary and mobile genotypes within populations of *Cydia pomonella* is of adaptive significance because it may facilitate an optimal exploitation of its habitats.
6 Field dispersal study in different strains of *Cydia pomonella*: development of methodology

6.1 Abstract

This study investigated the effectiveness of different trap designs in order to optimize the mark-release-recapture method for studying field dispersal in two laboratory selected strains of the codling moth *Cydia pomonella*. Methodological modifications were made concerning the location of the release sites as well as the trap types and positions. Trials were conducted in different habitats, including an unmanaged orchard with high stem apple trees and in two IP orchards consisting of dwarf apple trees. The codling moths were marked both internally and externally with different colouring dyes and released. Active and passive traps served for the recapture of male and female moths, whereby much effort was undertaken to find an effective trap for the recapture of females. During all releases wild male codling moths were monitored to gain insight into natural population dynamics. The results obtained and the experience gained in the field during three consecutive years are discussed with particular reference to factors involved in the field dispersal of *Cydia pomonella*.

6.2 Introduction

Insect flight can be investigated by means of various laboratory methods, e.g. direct visual observations at close range, actographs, flight mills and wind tunnel experiments, but insight into insect dispersal can be obtained only by means of field studies under natural conditions. Field dispersal studies are often associated with the term 'mark-release-recapture' which covers a chain of events closely affiliated with each other. For each insect species the optimal method needs to be found. In *Cydia pomonella*, previous dispersal studies were conducted either with bait traps (Yothers, 1927; Worthley, 1932; Steiner 1940; van Leeuven, 1940) or with live virgin females (Vakenti & Madsen, 1976). The discovery of the codling moth pheromone by Roelofs *et al.* (1971) facilitated and improved monitoring. In pest management, the pheromone trap soon became an important tool to provide information on codling moth field populations for fruit growers. The number of captured males indicates
population levels and, hence, serious infestations can be prevented by well timed applications of control measures. In release experiments, the pheromone trap is a helpful tool to trace the dispersal pattern and the flight distances of male *Cydia pomonella* (Mani & Wildbolz, 1977, Mani *et al.*, 1978; Riedl *et al.*, 1979; Howell *et al.*, 1990).

Data on the dispersal of females are much more difficult to obtain due to the lack of a suitable trap. Recapture of females by use of different types of traps, e.g. molasses baited, light and window traps, was often low and only useful in a short range from the release point (Hamilton & Steiner, 1939; Weissling & Knight, 1994; Bloem *et al.*, 1998). Therefore, data on the dispersal of females were obtained frequently indirectly by survey and counting of infested apples (Wildbolz & Baggio, 1959; White *et al.*, 1973). However, these studies inevitably produce a certain inaccuracy because the infestation caused by marked and wild females cannot be distinguished. As an alternative approach, laboratory studies using flight mills may provide indications of flight capacity in the field. Such studies with tethered flight using a flight mill yielded no differences in flight distances between male and female codling moth (Schumacher *et al.*, 1997b). Without consideration of the possible influences the tethered flight technique might exert on the moths, both sexes are theoretically supposed to disperse similarly. Selective breeding of the codling moths, individually tested for their locomotor activity in an infrared actograph, yielded two strains which differed significantly in their mobility (Chapter 5). To verify laboratory findings in the natural environment, i.e. to investigate whether there is a relationship between laboratory selected mobility and field dispersal capacity, a field mark-release-recapture method was needed.

During three consecutive years (1997-1999) field releases were carried out with the intention of finding a suitable method reflecting the spatial and temporal dispersal pattern of the selected strains in *Cydia pomonella*. In the first trial (1997) the emphasis was put on the technical details of the release, whereas the second (1998) and third (1999) trials were carried out to gain reasonable recapture rates and to obtain information on the dispersal of the codling moth. The objective of these field experiments was to develop a mark-release-recapture method for examining differences in field dispersal patterns of two selected strains in *Cydia pomonella*.
6.3 Materials & methods

6.3.1 Preliminary mass release in an unmanaged orchard: 1997 in Schleinikon

*Insects*

Insects used in this release experiment originated from a commercial breeding station in Switzerland. They were reared on a semi-artificial medium containing water, agar, maize grits, wheat flakes, brewers yeast and preservatives. They were obtained as pupae, sexed at the pupal stage and held apart under long day conditions (LD 18:6 h, 65% ± 5% RH) in a climatic chamber until emergence. 600 male and 600 female codling moths were used for the release. The emergence of adult moths occurred over several days, thus animals differed slightly in age.

*Experimental site*

The release was conducted in a private untreated orchard in Schleinikon (Canton Aargau (AG)) at an elevation of 472 m (47°30' N, 8°24' E). High stem apple trees (> 4m) of different varieties (e.g. Bemerrosen, Golden Delicious, Tobiasler) and other host and non-host fruit trees (plum, pear, cherry and walnut) were irregularly distributed over an area of 2.4 ha which was divided into two parts (A and B). Northern, eastern and southern sides of the total area were surrounded by open fields whereas the western side was bordered by hedgerows. A weather station with a data logger installed inside the orchard recorded climatic data regularly during the experimental period. The experimental design is illustrated in Figure 6.1.
Figure 6.1. Experimental design of the field release in Schleinikon in 1997.
Mark and release

The experimental period lasted from the 19th of August to the 1st of October. Moths were marked externally with fluorescent dye (Bioquip, Gardena, CA, USA) immediately before release in the field. External marking facilitated distinguishing released from wild moths. In addition, different colours were used to distinguish two release sites. These release sites were located vis-à-vis at the western and eastern border of Part A in the experimental area. After the moths were marked they were put in plastic jars that served as release shelters (Charmillot, 1979) protecting them against climatic influences until they left to disperse. The shelters were fastened to potted dwarf trees.

Trap types and positions

Two types of traps were used for the recapture of released moths. For catching male codling moths, five pheromone traps, labelled ph 1 to 5, were positioned two days after the release, at different distances (between 51 m and 390 m) from the release points according to the natural distribution of the fruit trees in the orchard. These traps acted as active traps and each of them consisted of a dispenser and a sticky pad. For the recapture of female and male codling moths, 51 window traps were used as passive traps according to the method of Weissling & Knight (1994) and labelled wi 1, wi 2, etc. Traps were made of perspex (0.3 m x 0.3 m) and hung on tree limbs with wire looped through two holes at the upper edge of the trap and at a height of 2 m above ground. They were covered on both sides with a thin layer of sunflower oil. Tests carried out in a climate chamber showed sunflower oil to be suitable for catching those codling moths that coincidentally flew at the window trap. The surfaces of the window traps covered with oil remained sticky and transparent for several days. Transparency was an important factor for the passive trap as insect contact should be incidental and not visually influenced. This was not necessarily the case with the insect glue ‘TangleTrap®’ (The Tanglefoot Company, Michigan, MI, USA) which was tested as well. TangleTrap glue turned white with time and the sticky layer was difficult to renew after having been covered with insects.

Pheromone traps were arranged in block A and block B, while window traps were only set up in block A where the release sites were situated. The position of the window traps depended strongly on the natural distribution of the apple trees as very
high trees had to be excluded for technical reasons. Distances between release sites and window traps ranged from 11 to 135 m. Both trap types were checked daily and the captured animals were removed to identify their markings in the laboratory. The oil layer on the window traps was renewed once a week.

6.3.2 Mass release in an IP orchard: 1998 in Leuggern

*Insects*

Codling moths used in this release originated from two sources, from a laboratory population and from a commercial breeding station in Switzerland. Both populations were reared on a semi-artificial medium, the laboratory population according to Huber et al. (1972), the other as described in Section 6.3.1. A total of 1745 moths were used for the release, 1180 males (713 self-reared, 467 purchased) and 565 females (333 self-reared, 232 purchased).

*Experimental site*

A commercial IP orchard in Leuggern (AG) was chosen as the experimental area. It was situated at an elevation of 436 m (47°34' N, 8°13' E) and consisted of 53 rows of dwarf apple trees of different varieties (e.g. Elstar, Jonagold, Golden Delicious, Gala etc.). The total area of 4 ha was bordered on one side by pear trees and on the other sides by open fields. There were high stem cherry trees in an adjacent meadow and single high stem apple trees widely spread in the surroundings. For periodical recording of weather data, a station with a data logger was situated inside the orchard. The experimental design of the first release is shown in Figure 6.2, that of the second and third releases in Figure 6.3.
Figure 6.2. Experimental design of the first field release in Leuggern in 1998.
Figure 6.3. Experimental design of the second and third field release in Leuggern in 1998.
Mark and release

The experimental period lasted from the 13th of July to the 11th of September. Three consecutive releases were undertaken from the following release sites, i.e. in the centre of the orchard, at a border adjacent to an open field and outside the orchard, 105 m away from the last row of apples (Figures 6.2 and 6.3). Codling moths which originated from the laboratory population were all marked internally with 'Calco Red Oil' that had been mixed into the larval diet. These moths and those purchased from the commercial breeding station were marked externally with one of three different colours of fluorescent dye (BioQuip, Gardena, CA, USA). Moths from two populations and from three different releases sites could thus be distinguished. All moths were marked with the colour dye immediately before release in the shelters as in the previous year. The shelters were fastened directly to the limbs of dwarf trees 1.7 m above ground.

Trap types and positions

Pheromone traps were used during all three release periods, while window traps were set up only in the first one. A total of 6 pheromone traps (ph 1 to 6) were set up two days after each release inside the orchard and in the surroundings. Since the moths were always released from a different site, the distances between release site and pheromone trap differed between 75 and 275 m. As the field release in 1997 yielded no recapture of females, two major modifications were made. The first was to increase the total number of traps and the second was rearrangement of the trap design. In the first release, 251 passive window traps, labelled wi 1, wi 2, etc. and made of PVC (0.3 x 0.3 m), were set up inside the orchard around the central release point. In contrast to the previous year, the window traps were hung on the wire in three squares 5, 25 and 50 m from the release site (Figure 6.2). A high density of traps restricted to three distances was set up to increase the chance of catching female moths. The traps were fastened at one corner with a metal hook while the opposite corner was weighted down with a metal ring of 2.5 cm diameter. The rhomboid shaped trap was thus held in a movable but still stable position. The idea was to enhance the catchability of the window trap by allowing it to turn with the wind. At each check, traps were cleaned by removing the insects caught, thus avoiding visual influences on the codling moths. In the second trial, differently marked males were released from the centre, border and outside the orchard.
Pheromone traps were hung up as in the first release but pheromone trap ph 5 was removed as no marked males had been caught there during the first trial. In addition, males in subsequent trials were released close to that trap position. The third release was realized with the same trap design but the release site was transferred outside the orchard to a group of non-host trees (Figure 6.3).

6.3.3 Mass release with selected strains in two IP orchards: 1999 in Leuggern and Otelfingen

The mass release with the selected strains was described in the section entitled 'Field verification' following the selection procedure as specified in Chapter 5. For clarity, and for the understanding of the whole methodological evaluation, the description follows the structure of the previous releases and facilitates comparison of the three years. However, conformable aspects are discussed only briefly here, whereas technical modifications are emphasized in detail.

Insects

Codling moths used in the mass release were reared under the same climatic conditions as described before (see Section 6.3.2). In contrast to the previous year, they did not belong to a homogenous laboratory population but came from two strains selectively bred, based on phenotypic values of mobility. Each strain showed behavioural differences in locomotor activity measured by the actograph, i.e. the mobile strain was characterized by high mean activity whereas the sedentary strain by low mean activity. A total of 2024 codling moths were released of which 450 males and 500 females belonged to the mobile strain and 578 males and 496 females to the sedentary strain.

Experimental site

Based on the previous two years' experience, the decision to release male and female codling moths separately was made in order to increase the general recapture rate and, moreover, the chance of catching females. Males were released in the IP orchard located in Leuggern as in the previous year and females in a second IP orchard of comparable structure situated in Otelfingen (Chapter 5). Thus, interference between the two sexes of experimental moths in the release area could
be avoided. Climatic data were periodically recorded in both orchards by two weather stations each equipped with a data logger. Figure 6.4 and 6.5 illustrate the experimental design in the two orchards.
Figure 6.4. Experimental design of the field release with males of the selected strains in Leuggern in 1999.
Figure 6.5. Experimental design of the field release with females of the selected strains in Otelfingen in 1999.
Mark and release

The experimental period lasted from the 24th of July to the 17th of August. In accordance with previous releases, all released moths of either strain were marked internally to distinguish them from wild codling moths. Because moths from both strains were released from the same site it was necessary to mark each strain in addition externally with a separate colour. Codling moths of the mobile strain were coloured with red fluorescent dye, while moths of the sedentary strain were marked yellow. Thus, recaptured moths of each strain could later be easily identified under a UV-light stereomicroscope. The release site in both orchards was chosen as in the second release of last year, in both orchards from a bordering apple tree row to gain more information on immigration into the orchard and on dispersal out of the orchard.

Trap types and positions

For the recapture of male codling moths, pheromone traps were used as in previous years but the trap number was increased within an extended experimental area. Recaptures in 1997 and 1998 demonstrated that a certain proportion of the male moths left the release area. Based on these results and especially in the consideration of the mobile strain, recaptures in larger distances could be assumed. Ten traps were set up two days after the release. Of these, 6 were positioned pairwise inside the orchard at 75 m, 150 m and 250 m from the release site, while 4 pheromone traps were set up outside the release area at distances between 200 m and 750 m in high stem trees that were randomly distributed in the surroundings.

For the female codling moths, a modification in trap type was necessary to increase chances of recapture. As observed in previous years, passive window traps are an inefficient tool to monitor female flight. Light traps offered an encouraging alternative, as they belong to the active trap type. Six light traps (Luminoc®, Biocom, Quebec, QC, Canada) equipped with a blue bulb, an automatic timer and a container for collecting the insects were set up inside the orchard at 75 m, 150 m and 250 m from the release site. The efficiency of the light traps was tested first in the laboratory and outside within a group of enclosed dwarf apple trees.
6.4 Results

6.4.1 Mark-release-recapture experiment in Schleinikon 1997

Recapture rate

A total of 62 male codling moths was found in the pheromone traps, 45 marked and 17 wild animals. The recapture rate for the released male moths was 7.5%. On one of the window traps on the apple tree with the central pheromone trap ph 1, only two male moths were found. No female moths were caught with the window traps. The recapture profile of marked and wild male codling moths is shown in Figure 6.6. Ph 1 which was situated between the two release sites in block A was most attractive to both wild and marked moths and caught 45 animals. The pheromone traps ph 2 and ph 3 which were in block B and at a larger distance from the release sites caught 14 and 3 males, respectively. At traps ph 4 and ph 5, which were located on the borders of block B and farthest away from the release sites, no moths were found. These findings show that most males caught (77.8%) flew only a short distance, i.e. 51 m from the release site. Figure 6.7 gives an overview of the flight distances travelled by the marked male *Cydia pomonella*.

Release sites

Regarding recaptures in trap ph 1, percentages of males caught from the two sites were comparable with 42.2% coming from the west and 35.6% from the east release site. About a quarter (22.2%) of the marked moths caught dispersed further away from the release sites. Despite the fact that distances from the two release sites to these pheromone traps were comparable, 20% of the males were found in ph 2 i.e. 205 m from the release site compared to 2.2% males trapped in ph 3 at 190 m from the release site. The pheromone trap ph 2 was on a cherry tree, a non-host tree of *Cydia pomonella* with an exposed position in one corner of the experimental area. The silhouette of the tree was clearly visible to bordering open fields. In contrast, trap ph 3 was in a walnut tree, a host tree adjacent to hedgerows and surmounted by higher trees.
Figure 6.6. Recapture profile of male codling moths in Schleinikon in 1997.

Figure 6.7. Flight distances of marked males in Schleinikon in 1997.
Climatic conditions

To understand the field results in trap captures of released and wild codling moths, the weather conditions are summarized to frame the environmental conditions in each field experiment.

The release experiment in Schleinikon lasted from 19\textsuperscript{th} of August - 1\textsuperscript{st} of October, during which a daily mean temperature of 15.2 °C was recorded. Evening temperatures measured within an hour after sunset, i.e. the main activity period of *Cydia pomonella*, ranged from 10.2 ° to 23 °C. The wind direction was mainly from NW with a mean wind speed of 0.7 m/s. During the experimental period, rainfall occurred on 13 days with a mean of 3.1 mm.

6.4.2 Mark-release-recapture experiment in Leuggern 1998

Recapture rate

During the whole experimental period, a total of 314 male codling moths was found in the pheromone traps, 161 released and 153 wild males. Figures 6.8 and 6.9 summarize the catches of marked and wild males from the different release sites. Since the released moths came from two different sources, their relative dispersal performances were first investigated. Of the 713 self-reared male moths 90 were recaptured while 71 of 467 purchased moths were recaptured. This yielded a recapture rate of 12.6 % for self-reared males compared to 15.2 % for the purchased males. For the passive window traps used during the first release period, only one female codling moth was found at a distance of 5 m. Thus these traps were no longer used during subsequent releases. Table 6.1 gives an overview of male recapture rates from the two different codling moth sources. The moths of both sources showed comparable recapture rates. The highest total recapture rate (23.1%) was during the second experimental period whereas the third period had the lowest rate (3.9%).
Figure 6.8. Recapture profile of marked males during the first release in Leuggern in 1998.

Figure 6.9. Recapture profile of marked males during the second and third release in Leuggern in 1998.
Table 6.1 Summary of recaptures of marked male codling moths during the experimental period in 1998.

<table>
<thead>
<tr>
<th>Experimental period</th>
<th>Release site: orchard</th>
<th>Released moths: sex</th>
<th>Recapt. rate: total males</th>
<th>Recapt. rate: self-reared males</th>
<th>Recapt. rate: purchased males</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) 13.7.-29.7. centre males, females</td>
<td>7.9 %</td>
<td>7.7 %</td>
<td>8.3 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2) 12.8.-25.8. centre, border males + outside</td>
<td>23.1 %</td>
<td>23.5 %</td>
<td>22.7 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) 27.8.-11.9. outside males</td>
<td>3.9 %</td>
<td>3.9 %</td>
<td>not released</td>
<td></td>
<td></td>
</tr>
<tr>
<td>whole period all males</td>
<td>13.6 %</td>
<td>12.6 %</td>
<td>15.2 %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Release sites

The field experiment in 1998 comprised three release periods, in which moths were released from three different sites. The first release site was situated in the centre of the orchard, the second on a border and the third outside the area (Table 6.1, Figures 6.2 and 6.3). Concerning the central release site, the recapture data showed that most males (93.2 %) flew to the closest pheromone traps. A few (3.4 %) left the orchard and were found in a pheromone trap (ph 3) situated in a group of cherry trees i.e. a non-host tree of the codling moth. Another 3.4 % of the caught male codling moths were attracted to the pheromone traps in the corners of the orchard (ph 4 and ph 6). In Figure 6.8, the marked and wild male moths caught are shown for the first release and recapture period. Regarding the release site at the border of the orchard during the second release period, moths were found to immigrate into the orchard rather than to fly to the closest pheromone traps. 74.2 % of the males were found in the traps positioned in the central part (ph 1 and 2) and 25.8 % of the males in those in the corners of the orchard (ph 4 and ph 6). When the release site was shifted further outside of the orchard, 70 % of recaptured moths were found in the centrally situated pheromone traps (ph 1 and ph 2) in the orchard and 20 % were trapped in the corners of the area (ph 4 and ph 6). Another 10 % did not immigrate into the apple orchard but flew to trap ph 3 within a group of non-host trees adjacent to the orchard. During the last experiment, moths were only released
from the most distant release site, outside the orchard, to investigate the proportion immigrating into the orchard. 50 % of the marked moths flew to the centrally located traps (ph 1 and ph 2) in the orchard and 50 % were heading to the closest pheromone sources (ph 4 and ph 6).

**Efficiency of pheromone traps**

With respect to the efficiency of all pheromone traps during all releases, regardless of release site, the traps in the central part of the orchard (ph 1 and ph 2) were definitely the most attractive, accounting for 86.8 % of the caught male *Cydia pomonella*. Pheromone traps in the corners of the orchard (ph 4 and ph 6) accounted for 10 % and a trap inside a group of non-host trees (ph 3) for 3.2 % of all recaptured moths. Trap ph 5 which was used in the first experimental period caught no marked moths and was removed thereafter.

Catches of the local *Cydia pomonella* population showed a similar preference for pheromone traps in the central part of the orchard (ph 1 and ph 2). 49.1 % of the wild males were caught by these centrally located traps in contrast to 11.7 % by those traps in the corners of the orchard (ph 4 and ph 6). The two traps located in the surroundings of the orchard, on non-host trees, accounted for 33.3 % wild males at trap ph 3 and 5.9 % wild males at ph 5.

**Flight distances of released males**

Regarding the flight distance of the marked males, for all releases, the catches in the pheromone traps showed that most males (72 %) flew to the closest trap i.e. within 75 m of the release site. More than a quarter of the trapped moths (28 %) did not fly to the closest pheromone source, but to one of the traps at a greater distance. The distances travelled by marked males are shown in Figure 6.10. Constant catches of wild codling moths in all pheromone traps during the whole experimental period indicated that weather conditions were generally suitable for moth flight. Only during the third experimental period were the evenings often chilly and catches of both marked and wild moths decreased when compared with those of the first and second period (Figures 6.8 and 6.9).
Concerning the time of recapture after all releases, most catches of marked males (87.6 %) occurred within the first week after release. Due to asynchronous emergences of the released moths in the laboratory, a precise determination of the age at the day of recapture was not possible. During the whole experimental period, catches were always highest on the first days after release and then decreased with time (Figure 6.8 and 6.9). During the second period recapture was higher than during the third period, in which especially low catches were obtained for both marked and wild male codling moths.

Climatic conditions

During the first trial period (13\textsuperscript{th} of July - 31\textsuperscript{st} of July), the mean daily temperature was 19.6 °C and the mean daily evening temperatures ranged from 14.3 ° to 25.5 °C. Mean wind speed was 1 m/s predominantly from SW. Rainfall occurred during 13 days in this period with a mean of 3.2 mm. In the second trial period (12\textsuperscript{th} of August - 26\textsuperscript{th} of August), the mean temperature was 18.9 °C and the evening temperatures were between 14 ° and 25.2 °C. The mean wind speed was
1 m/s mainly from SW. Rainfall was registered during 6 days with a mean of 4.4 mm. In the last trial period (27th of August - 11th of September) the mean temperature decreased to 14.8 °C whereas the evening temperatures were between 9.5 ° and 19.3 °C. Mean wind speed was 0.7 m/s from SW. Rainfall was measured during 9 days with a mean of 4.8 mm.

6.4.3 Mark-release-recapture with selected strains in Leuggern and Otelfingen 1999

The method used for the final field release experiments in Leuggern and Otelfingen was based on experience of the two previous years. The field releases in 1999 with the mobile and sedentary strains are documented in detail in Chapter 5, with results of the dispersal pattern, i.e. the recapture rate, the temporal distribution and the flight distance in both strains. Thus, the results presented here represent a brief comparison of the selected strains, with major focus on the methodological modifications. The recapture profiles of the two selected strains and the wild population are described.

Recapture profile in released codling moths of selected strains

In comparison with the previous releases, three main modifications in the experimental design were made. These were the spatially separated releases of males and females of both strains in two different orchards, modifications in pheromone trap number and position, as well as the use of light traps for the recapture of female moths. The recapture rates for male moths increased, with the mobile strain accounting for 37.3 % and the sedentary strain for 30.4 %. The pheromone traps closest to the release point captured the largest proportion of male moths of both strains, but here the proportion in the sedentary was higher than that in the mobile strain. In contrast, pheromone traps which were positioned in the surroundings, caught more males from the mobile than from the sedentary strain (Figures 5.2 and 5.4). Females released in the orchard in Otelfingen were not recaptured.

Capture profile of wild codling moths

The capture data of the wild males caught in pheromone traps showed the presence of a wild *Cydia pomonella* population in the orchard and in its
surroundings. During the whole experimental period 127 wild males were found (Figure 5.3). The central traps, which were 150 m and 250 m from the release site, accounted for 22.8 % and 40.1 %, respectively of the wild males caught. In contrast to the high density of marked males found in the traps close to the release site, no wild male flew to one of these traps. Furthermore, 15 % of the captured wild males were found outside the release area, in the trap 750 m away which was situated in a group of high stem apple trees. Another 13.4 % of the captured wild male codling moths were caught in the trap 200 m from the release site, whereas the rest (8.7 %) was caught at distances up to 550 m from the release site.

Climatic conditions

The release periods in Leuggern and Otelfingen lasted from the 24th of July until the 11th of August. During the experimental period in Leuggern the mean daily temperature was 19.8 °C, evening temperatures were between 14.3° and 24.8 °C. Wind speeds had a mean of 0.7 m/s mainly from SE. Rainfall occurred during 11 days with a mean of 2.3 mm. In the experimental orchard in Otelfingen, temperatures during the evenings were between 16.3 and 23.9 °C with a mean of 18 °C. Wind came predominantly from S with a mean speed of 0.7 m/s. Rainfall occurred during 16 days with a mean of 2.5 mm.

6.5 Discussion

The results of the mark-release-recapture experiments in three consecutive years (1997 to 1999) reflect the complexity of dispersal in Cydia pomonella. Recapture data were apparently influenced by different factors, e.g. orchard type, pheromone trap density and position, environment, the presence of high stem host and non-host trees and time in the season. However, in all releases in the years 1997 to 1999, inspite of different orchard types, release sites and pheromone trap positions, one finding was noticeable: a major proportion of Cydia pomonella males did not disperse far when released within or near an orchard as demonstrated by high recapture numbers in pheromone traps close to the release site. This finding is in accordance with field releases of Worthley (1932) in which 51 % of moths were caught not more than 60 m from the release site. Field studies of Charmillot (1979)
showed a mean flight distance of 65.4 m for male codling moths released in the central part of the orchard. Another finding, common in all releases, was that about 25% of the recaptured moths were not attracted by the closest trap but flew further away. This phenomenon in the codling moths’ flight pattern might suggest the presence of two types of dispersal, namely short- and long-range.

Factors involved in short-range dispersal

The short-range dispersal, which is probably responsible for trap captures close to the release site, may be found in the orientation of the species to host plants and possibility of finding females. Codling moth orientation tends to be along tree rows and moths avoid leaving the canopy (Mani et al., 1995). Probably, due to this behaviour, males found close to the release site were attracted directly to the first pheromone source available when they moved inside the canopy of trees. Avoidance of leaving the canopy could also explain why pheromone traps at the corner of the orchard and fastened to a fence, seldom trapped males (as shown in Leuggern in 1998). This apparently unattractive characteristic of these traps is also confirmed by the lack of catches of the wild population. Higher infestations of codling moths at the border of orchards/open field point in the same direction as moths prefer to swarm inside the canopy and return to the foliage when faced with adjacent open fields. This may explain that when moths of both sexes are released in the central site, larval attacks decrease around the release site but increase at the border between apple trees and open fields (Wildbolz & Baggioni, 1959).

Other lepidopteran species were found to exhibit a behaviour similar to that of Cydia pomonella. Males of Eucosma gloriola, a tortricid species, prefer pheromone traps attached directly to the tree and avoid traps 1.5 m away from the tree (De Groot et al., 1998). Two other tortricid species, Choristoneura occidentalis and Choristoneura retiniana, were more attracted by pheromone traps in the canopy of host and non-host trees than by traps at a distance of up to 6m in the open field (Liebhold & Volney, 1984).

Short-range orientation might occur, in addition, to a major degree in populations maintained over many generations in the laboratory. Comparison of the flight capacity of field and laboratory strains of Cydia pomonella showed that the field strain had a significantly higher flight capacity than the laboratory strain, although the
latter was found to have a small contingent of long-flying individuals (Dorn et al., 1999).

Factors involved in long-range dispersal

Despite the generally sedentary character of the codling moth, a certain percentage left the orchard as shown in the mark-release-recapture experiments in 1998 and 1999. The existence of a large variation within a codling moth population maybe one of the factors likely responsible for the long-range dispersal demonstrated in field releases. A large variation in flight capacity was reported by Schumacher et al. (1997b) who found that both tethered male and female moths are able to undertake long-distance flights of up to several kilometres when tested in flight mill laboratory studies. In addition, it was found that this flight trait is heritable from parent to offspring generation (Schumacher et al., 1997a). Similarly, field studies conducted by various authors confirmed that a certain proportion of the codling moths left the orchard and flew over long distances as reported here for releases in 1998 and 1999 in Leuggern. Steiner (1940) found codling moths at distances of up to 630 m and van Leeuven (1940) recaptured males at a distance of 800 m. Single male codling moths were reported to fly longer distances. Howell & Clift (1974) caught marked males 9 km from the release site and Mani & Wildbolz (1977) trapped a few moths between 8 and 11 km. In the field releases in 1998 and 1999, some males left the orchard and had to cross open fields and meadows to fly to pheromone traps positioned at greater distances. These males most probably belonged to the proportion of long-flyers within a population and they were attracted visually by silhouettes of high stem trees. The position of host and non-host trees plays a role. Isolated high stem trees with a clear-cut silhouette could be attractive to the codling moth as reported by Mani et al. (1974), who observed *Cydia pomonella* moths flying to single pear trees. During the release in 1997 there was a higher number of males found on a non-host but isolated tree, compared to a host tree at a similar distance from the release site. In 1999, a proportion of males was probably first visually attracted by a group of high stem apple trees. After installation of pheromone traps at all locations two days after the release, these marked males were olfactorily attracted to the trap in the immediate vicinity.
Impact of population dynamics on recapture data

Interference of marked male recaptures caused by a local wild female population is a possible factor influencing trap data. The presence of a wild codling moth population was evident since 15% of the captured wild males were trapped within a group of high stem apple trees 750 m from the release site. Thus, the percentage of males from the mobile strain, which left the orchard to fly over greater distances, is perhaps underestimated as a part of the mobile males might have been more attracted by wild females than by the synthetic pheromone source. Those wild females could have competed with the pheromone traps and decreased the number of caught males. Riedl et al. (1976) assumed that competition and interaction with a female codling moth population were responsible, as the efficiency of the pheromone trap was different between the spring and summer generations. Knight & Croft (1987) found that competition for males of the tortricid *Argyrotaenia citrana* between a synthetic pheromone in a trap and the natural pheromone exuded by a female population in the same locality could influence catches in pheromone traps. Other studies showed that the addition of virgin females to wild populations reduced trap catch due to competition (Muirhead-Thomson, 1991). This phenomenon could account for the recapture data in males of the mobile strain which were found at a greatest distance in 1999. The situation of the trap area outside the release orchard and the existence of a wild codling moth population indicated the presence of wild female moths attracting released males.

The release area within the orchard in Leuggern was treated, but the surroundings were not, suggesting a very limited number of wild codling moths within the area of interest. Therefore, it was unlikely that calling wild females were rivalling synthetic pheromone sources in traps. High recapture numbers of released males in the traps inside the orchard gave an indication that males were not attracted by wild females. Here, competition between the synthetic pheromone in traps and the natural pheromone of wild females can be assumed to play only a minor role for male codling moths. However, males of the wild population were presumably present in the areas neighbouring the release orchard since they were found on pheromone traps inside the orchard. But, those wild males had apparently not been present beforehand inside the treated orchard as, in contrast to marked males, no wild males were caught in traps at the corners of the orchard. Presumably, wild males migrated
from neighbouring high stem host trees into the orchard and then were attracted by
the synthetic pheromone in the traps.

In addition to the level of population dynamics, for a short time, overcrowding
at the release site might be another reason for a set of marked codling moths flying
over longer distances. Avoidance of overcrowding is one of the reasons generally
accepted for dispersal, because it is significant for the survival of the species (Stein,
1986). Concerning field experiments in general, a high moth density automatically
occurs at the beginning of a mass release and an initial thinning effect can be
assumed to lead to a normal dispersal pattern.

Efficiency of the trap type: passive versus active traps

The recapture data from the 1997-1999 field releases might be useful for
further releases in the future. As far as the recapture of female codling moths is
concerned, the application of either passive window traps or active light traps failed.
What reasons can be assumed? The 'passive' window trap implies that insects are
catched incidentally, because the surface is transparent and without any visually
attracting or repelling stimulus. Thus, insects become trapped when they fly against
the sticky pane. During the releases in 1997 and the first trial in 1998 in which such
window traps were used, dipteran species were often caught indicating that such
species belong to the strong and frequently flying insects. In comparison,
lepidopteran species, e.g. moths, are more often weaker, less frequent flyers.
Another reason to account for the inefficiency of the window traps might be the
general mobility behaviour of the females. The actograph study (Chapter 4) showed
clearly that generally, female codling moths exhibited up to three times lower
locomotor activity when compared with male codling moths. Such reduced mobility
could be the reason why females were not trapped by window traps in 1997 and in
the first trial in 1998.

Light traps were found to be an effective capture technique for two groups of
Before pheromone traps became available, codling moths were often caught by light
lures. White & Hutt (1971) conducted mark-release-recapture trials with light traps
and got recapture rates of up to 2.6 % for female and 9.9 % for male moths. During
the field experiment in 1999 in Otelfingen, where only females were released in the
orchard, recapture with light traps was not successful. The failure in recapture might
have been caused by a higher sedentariness due to modified mating behaviour leading to reduced mobility. Actograph studies in the laboratory (Chapter 4) showed that the immobility of females as compared to males was linked with the calling behaviour, which usually occurs immediately after emergence. Female *Cydia pomonella* moths remain inactive when they call and release pheromones to attract males (Castrovillo & Cardé, 1979).

During the field release in 1999, such calling behaviour of the released virgin females could probably lead to an augmented sedentariness and less dispersal for the following reasons. Firstly, the IP orchard in Otelfingen, in which marked females were released, had been treated and thus a low wild male codling moth population could be assumed. This held true for the surrounding areas too, because high stem apple trees were observed to be lacking. Secondly, marked male codling moths were released spatially separated in another orchard in Leuggern. Therefore, the released females continued to call, albeit without response, and did not disperse. The actograph study showed that 2 and 4 days-old females had a significantly lower locomotor activity than newly emerged ones, in contrast to females at the age of 12 days, which exhibited a significantly higher activity (Chapter 4). As codling moths in the field are subjected to increased risk of mortality than in the laboratory, it is conceivable that the released females did not reach the age of 12 days. Most marked males, on the other hand, were caught shortly after the release, e.g. 87.6 % of the marked males were recaptured within one week after the release in 1998. Thereafter, recaptures of older moths waned.

For future studies on female dispersal, a more attractive trap comparable to the pheromone trap for males, seems indispensable. Conventional traps most often yield low recapture rates and moth catches only within a short distance from the release sites. Bloem *et al.* (1998) used passive traps in mark-release-recapture experiments and found low recapture rates between 0.08 and 1.63 % for female codling moths. Weissling & Knight (1994) reported higher recapture rates (16-24 %) for female *Cydia pomonella* caught by passive window traps, but they used small experimental areas with 3-by-3-tree arrangements (0.03 ha) to investigate trap efficiency and did not consider the flight distance of the codling moth. As moth recaptures usually decrease with increasing distance from a release site, a reliable trap is needed to verify flight distances found for the selected mobile and sedentary strains of male *Cydia pomonella*. 
Pheromone traps were successful in recapturing male codling moths with recapture rates of up to 37.3 %. Advantages of the pheromone trap are high species selectivity and easy handling. Due to the selective character of the trap, catches of other insects e.g. beneficial insects can be excluded. After identification of the pheromone (Roelofs et al., 1971) the trap has seen success in pest management and provides fruit growers and consultant entomologists with information on the population levels of *Cydia pomonella* (Howell, 1972; Westigard & Graves, 1976, Charmillot, 1980). Studies on trap density showed that an increase in the number of traps in relation to tree density led to a higher total number of insects caught, but simultaneously to a reduced number in moths per trap (McNally & Barnes, 1981). The pheromone trap is a useful tool to monitor dispersal of male *Cydia pomonella*. This was shown in various mark-release-recapture studies of long-range dispersal (Howell & Clift, 1974; Mani & Wildbolz, 1974; Mani et al., 1978; Thwaite & Madsen, 1983). A sizeable area is especially important for investigating flight distances of different strains, e.g. mobile and sedentary strains. On the other hand, any active trap like the pheromone trap may elicit a behavioural response by the insect (Muirhead-Thomson, 1991). But, during the field releases in 1997 to 1999, attractance by the synthetic pheromone was held at a minimum, as the traps were set up two days after the releases. Thus, codling moths could disperse immediately after release according to their natural flight pattern without pheromone influence. After the pheromone traps were installed, the males were most likely to fly to the closest trap.

**Conclusions**

Experience of three years field releases confirm that pheromone traps are suitable for monitoring dispersal of male codling moths. Pheromone traps in an IP area, where insect hormone mimics against Lepidoptera were applied, caught more males than in an untreated area. Thus, the IP orchard was more suitable for the study of flight distances in male codling moths than the unmanaged orchard, although the latter reflects the natural habitat to a greater extent. The field release in 1997 in the untreated area yielded a low recapture rate of 7.5 % compared to the releases in 1998 and 1999 in the treated orchard with recapture rates up to 37.3 %. Various trap methods for catching female codling moths were found to be unsuitable for monitoring female flight dispersal in the field. Both passive window and light traps
were too inefficient and unselective for this purpose. Instead of releasing the insects from the central part of the orchard, greater trap efficiency in 1998 (second release) and 1999 may have been observed by release from the border, as confirmed by the satisfactory recapture results for male moths. In addition, the release site situated on the border allowed a maximum immigration and thus monitoring of within-orchard dispersal of male codling moths as well as of dispersal outside orchard. The latter reflected the proportion of moths leaving the habitat to fly over larger distances, for instance, males of the mobile strain in 1999. Captures of wild males in pheromone traps in the surroundings during all releases confirmed the presence of a wild codling moth population. The influence of wild females competing with synthetic pheromone sources in traps, which can be assumed to be higher in an untreated area than in a treated one, cannot be excluded.
7 General Discussion

This study investigated the mobility and dispersal behaviour of the codling moth *Cydia pomonella* by both laboratory experiments and field release trials. The emphasis was firstly the compilation of the daily pattern of general locomotor activity by use of an infrared actograph (Chapter 4). Therein, mobility differences between male and female codling moths were acquired with a focus on the effect of age on mobility. Possible behavioural effects of two frequently used insect hormone mimics on the mobility in females were studied additionally. In Chapter 5, the importance of the genetic component in regulating dispersal in the insect was investigated. A bi-directional selection using an infrared actograph led to the establishment of a mobile and a sedentary strain in both male and female moths. Laboratory findings were verified in a mass release in the field. Chapter 6 demonstrated the development of a field method by comparing different mark-release-recapture techniques for the study of codling moth dispersal in general and flight distances of selected strains in particular.

With regard to the findings of this study, I would like to focus my discussion on the following three aspects: (1) actographs in studying insect dispersal, (2) genetic regulation of insect dispersal, and (3) dispersal biology and pest management of *Cydia pomonella*. Finally, challenges in future research on the subject are pointed out in the outlook.

*Actographs in studying insect dispersal: options and limitations*

The codling moth *Cydia pomonella* is a nocturnal species with a relatively short adult lifespan. Therefore, most main activities like mating, host plant searching and oviposition occur early in the adult life and the success of such activities depend strongly on the insect's ability to change the location by movement in general and flight in particular. Consequently, a thorough knowledge of the relationship between age and mobility seems essential for the determination of adult activity and thus the pest's dispersal within a population. The importance to characterize male and female activity pattern is obvious as both are influenced by endogenous circadian rhythms and external stimuli. For example, the females perform calling behaviour for the release of pheromone to attract male codling moths and stimulate mating behaviour.
Female codling moths are also able to detect their own pheromone (Barnes et al., 1992) which could trigger a behavioural modification. In both sexes, the decrease of light intensity causes an increase in general locomotor activity (Knight et al., 1994).

The decision for the infrared actograph as a measuring means was due to its suitability for behavioural studies on general mobility in male and female codling moths. The method allows the insect to move freely during measurements, which is advantageous for behavioural studies. In addition, sublethal effects of insecticides on adult behaviour (Dorn & Gu, 1999) and side effects of insect hormone mimics on the moths’ activity can be tested in an actograph as the mechanical manipulation of animals is minimized and the behavioural response is solely attributable to the treatment. The actograph study with male and female moths demonstrated a significant difference in mobility between the two sexes and that age has a significant effect on the mobility. Females were found to exhibit much lower locomotor activity as compared to males (Chapter 4).

In virgin females, the JH mimic, fenoxycarb, caused an activity peak and prolonged the total activity period during 24 h. Similarly to virgin females, mated ones responded to fenoxycarb with a generally higher activity when compared to the untreated control. This was not the case after a treatment with the ecdysone agonist, tebufenozide, on virgin female codling moths (Chapter 4). The role of the two insect hormones is decisive in immature larval development, which led to their successful use in pest management. But, their involvement in regulating adult life history traits is poorly understood. Recent findings demonstrate that the progression of oviposition and egg maturation pivots around JH titers in Cydia pomonella and that the latter is affected by fenoxycarb (Webb et al., 1999). A relationship between flight and oogenesis as postulated by Johnson (1969) has been confirmed for several migrating insect species. Although Cydia pomonella is a non-migrating species, the findings of the study give hints to a positive correlation between mobility and the level of JH in this species. The mobility in the codling moth was clearly stimulated by the application of the JH mimic fenoxycarb. To what degree flight behaviour and therefore dispersal in the field is affected still remains open as the actograph technique could not record specifically flight movements.
The infrared actograph was favoured for the selection procedure as it facilitated the detection of mobile and sedentary individuals without disturbance of the tested insects by the measurement procedure (Chapter 5). Flight mills, which were first used as a selection means, were found to cause behavioural modifications in most tethered moths and thus prevented further breeding. In contrast, after the measurement in the actograph, mating and oviposition behaviour could occur normally and further direct selective breeding among tested individuals was possible for the establishment of two selected strains differing in mobility. On the other hand, infrared actographs have their limitations in the detection of specified activities of the test insect, as different activities, e.g. flying, crawling, fluttering etc. can not be distinguished. Therefore, the recorded activity level should be regarded as relative mobility of the test insect. From this point of view, the potential in flight capacity in the mobile strain might not be estimated completely as flight could not be measured separately from other movements. A more precise actograph method, which allows simultaneously the distinguished detection of flight activity meanwhile further selective breeding is not affected should have more advantages for studies of insect flight capacity.

Genetic regulation of insect dispersal

An organism should disperse if the chance of reaching a better site exceeds the expected loss from the risk of death during dispersal or the chance of reaching a poorer habitat (Gadgil, 1971). This cost-benefit equation seems trivial but masks a complicated system of factors involved in the decision whether it pays to emigrate or not. A mixed strategy is most likely within a population which means a proportion of individuals staying on in the same habitat while the rest disperse (Macdonald & Smith, 1990). Such a strategy seems also to function well in Cydia pomonella as the findings of mass field releases have shown (Chapter 6). Recaptures of marked male codling moths demonstrated in all releases a proportion leaving the habitat to disperse further. Concerning the field release with the selected strains, this proportion tending for dispersal was shifted towards the mobile strain (Chapter 5).

The combination of the demonstrated laboratory selection and subsequent field verification is a new approach in the study of the genetic regulation of flight capacity in insects as bi-directional selection was usually limited to laboratory
experimentation (e.g. Parker & Gatehouse, 1985; Gu & Danthanayarana, 1992). The field release with the two selected strains showed a larger dispersal capacity in the mobile genotype as compared with the sedentary genotype. Therefore, the mobile genotype existing in a population can be assumed to colonize new habitats to a greater extent in case of poor conditions. In natural habitats, of course, responses to changing environmental conditions occur over multiple generations. In addition, natural selection happens not as vectored as under laboratory conditions because a wild codling moth population is subject to several biotic and abiotic factors. However, responses to deteriorating habitats can occur from one generation to another, e.g. the percentage of diapausing larvae which is usually around 50% within a population can increase in subsequent generations in case of fruitless years. Regular absence of fruit has a direct physiological effect on the growth of larvae and an indirect genetical effect as it leads gradually to a higher proportion of overwintering diapausing larvae in the next generation (Audemard, 1991).

The artificial selection by means of the actograph demonstrated the presence of additive genetic variance in the natural population of codling moths (Chapter 5). Thus, it is imaginable that under a certain set of conditions the proportion of the mobile or the sedentary genotype within a population can increase. Facing deteriorating habitats, only mobile individuals with high flight capacity can reach an isolated formerly unoccupied habitat to found a new population. This situation would impose pressure on the population to select for the mobile genotype. Under the assumption of stable conditions within the habitat, the proportion of the mobile genotype would probably decrease as the capacity to fly over long distances would be of no advantage in contrast to the strategy of sedentariness. However, the relative advantages of mobile versus sedentary genotypes remain to be verified by comparison of their fitness characteristics in the ecological context.

Dispersal biology and pest management in Cydia pomonella

The success of a selective pest management method depends to a large degree on the species' characteristics, particularly those relevant to the corresponding part of the life cycle in consideration. The mating disruption and the sterile insect technique are two strategies selectively against the codling moth. Both
methods are directed towards adult moths and require a detailed knowledge of the flight behaviour because they intervene in the relatively short part of the adult life cycle related to flight. The results on flight distances of the mobile and the sedentary strain in the field have shown that mobile male moths disperse to a larger extent than sedentary males. Insect populations are nonrigid and susceptible systems which are subject to influences of forces acting within the population as well as forces operating from the environment. The laboratory selection with the establishment of two strains differing significantly in their mobility simulated the effect of two selective forces acting under the assumption that selection for mobility and for sedentariness are both optimal strategies to ensure the survival of the species under different environmental conditions.

What does this mean in particular for selective pest management strategies against the codling moth? The mating disruption technique in *Cydia pomonella* is based on masking the endangered area with sufficient dispensers, thus a closed pheromone cloud makes it difficult for the male moths to localize females in the treated area. Knowledge of the variation of dispersal capacity existing in natural populations is important. The success depends strongly on an area-wide treatment without interruption, whilst patchy unmanaged areas can endanger this method of control. Male moths dispersing over larger distances require a larger area covered with pheromone dispensers than moths with low dispersal potential. Such males with good flight capacities mate to a larger extent with wild females as their activity radius increases. Those mated wild females, for their part, are consequently of importance as they can immigrate and increase fruit infestation. In particular, gravid females with high dispersal potential are likely to disperse and deposit their eggs also in orchards in the vicinity. Such immigration and thus fruit infestation cannot be prevented by the mating disruption technique.

The sterile insect technique (SIT) used for codling moth control is based on the interference between the natural population and released sterile males mass-reared from a laboratory population, thus mating between sterile males and fertile females results in infertile eggs. For the success of this selective technique it is important to cover the endangered crop area as much as possible with sterile male codling moths to avoid mating and subsequently oviposition of mated females and fruit damage by larvae. However, in case of failure, i.e. if sterile males do not
disperse far enough in the treated area, fertile males from the natural population win the competition against sterile males and infestation increases. The findings of the field release with the selected strains open up new possibilities in the use of SIT. Concerning SIT programmes, male codling moths might be selected for high mobility in the laboratory, mass-reared, sterilized and released. Thus, the dispersal radius could expand and consequently, the proportion of infertile laboratory males competing with fertile males of the natural population could increase.

Outlook on future research

Future studies on codling moth dispersal behaviour in the field should be carried out with focus on the flight distances of females, as satisfactory data are still not available due to the lack of proper trapping methods. Laboratory studies based on tethered flight in flight mill experiments showed an equal potential in female's flight capacity as compared with males (Schumacher et al., 1997b). On the other hand, the actograph experiments in the presented study, based on the detection of free movements, showed a significantly reduced locomotor activity in females in comparison to males. The results are not contradictory as the two methods measure distinct behavioural traits of the codling moth in different ways. However, for verification of female's flight potential in the field, the development of an attractive and selective substance comparable to the synthetic pheromone in the males trap seems indispensible. In future field studies on male dispersal, the extension of trap positions at greater distances, i.e. up to several kilometers, could shed more light on flight distances travelled by the selected mobile and sedentary strain.

At this point, the improvement of the selection technique would be a helpful tool to clarify the individual's flight behaviour. An elegant improvement in the selection procedure would be gained by the use of an infrared actograph based on a video-track system with sensors covering all three spatial dimensions in the test unit instead of one single infrared light beam. Botteau & Osborn (1997) have used such a motion analyser to study the behavioural effect of insecticides on aphids and could measure the individual travelled distance. In a tortricid species, recent studies have shown the applicability of a video system to study the flight capacity in the grapevine moth Lobsisia botrana (El-Sayed et al., 2000). With this system, movements in Cydia pomonella might be specified more precisely and the 'good' and 'bad' flyers could be
easily detected and selected for further breeding without negative effects. Although the actograph selection presented in this study already yielded a positive response in both the mobile and sedentary strain, an improved selection system tracing only flights instead of the general mobility can be assumed to be an encouraging tool for future both laboratory selection and field verification investigating the inherited flight capacity in *Cydia pomonella*.
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8 References


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Curriculum vitae

Personal data

Sabine Keil
Born on the 29th of November, 1965 in Bremen (Germany)

Education

1996-2000 PhD study at the Swiss Federal Institute of Technology (ETH) in Zurich at the Institute of Plant Sciences, Applied Entomology under the guidance of Prof. Dr. Silvia Dorn. Assistant in entomology courses and excursions.

1995-1996 Research assistant at the department of Zoology at the Federal Grapevine Institute of Freiburg (Germany).

1990-1995 University grade (Diplom-Biologin) in biology at the Albert-Ludwigs University of Freiburg (Germany). Diploma thesis at the Federal Grapevine Institute of Freiburg (Germany) under the guidance of Dr. Günther Schruft. Title: Experimentelle Untersuchungen über Unterschiede in Aufnahme und Wirkung von Bacillus thuringiensis beim Einbindigen (Eupoecilia ambiguella Hb.) und Bekreuzten Traubenwickler (Lobesia botrana Schiff.).

1986-1990 Studies in biology and chemistry (2 sem.) at the Technical University (TU) of Braunschweig (Germany).

1985-1986 Language and au-pair stays in London (Great Britain) and Valencia (Spain).

1985 Abitur at the secondary school Lohfeld in Bad Salzuflen (Germany).