Doctoral Thesis

Visual and thermal orientation of Anthonomus pomorum: biological function and application for monitoring

Author(s):
Hausmann, Claudia

Publication Date:
2004

Permanent Link:
https://doi.org/10.3929/ethz-a-004741946

Rights / License:
In Copyright - Non-Commercial Use Permitted
Visual and thermal orientation of
Anthonomus pomorum: biological function
and application for monitoring

A dissertation submitted to the
SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH
for the degree of
Doctor of Natural Sciences

Claudia Hausmann
Dipl. Natw. ETH
born 23 December 1970
Schafisheim (AG), Switzerland

accepted on the recommendation of
Prof. Dr. S. Dorn, examiner
Dr. P.-J. Charmillot, co-examiner
Dr. J. Samietz, co-examiner

2004
1. Summary

The principles of integrated pest management require that action against a pest is only taken once the pest has been shown to be present and proven to be a real threat. Therefore, surveying the presence and abundance of pest insects is highly important in any integrated approach. The apple blossom weevil, *Anthonomus pomorum* (L.), is active early in spring and damages the still closed apple blossom buds. A monitoring tool is needed to verify the need for and proper timing of an intervention. Since a promising approach using the weevils' orientation towards chemical stimuli is missing, this present study concentrates on physical stimuli. The aim of the project is to investigate the visual and thermal orientation of the apple blossom weevil early in the season and to exploit these behaviours to develop such a monitoring tool.

Visual orientation of the apple blossom weevil was examined with regard to the main colours of its supposed visual system, ultraviolet (UV), green and blue. In dual choice experiments female weevils preferred the colours UV, green and blue when offered versus black, while males only preferred UV when offered versus black. These results support the hypothesis that the weevils have a trichromatic visual system with UV, blue, and green receptors. The more discriminating response of females may reflect a greater visual acuity in females than in males due to their possible higher affinity for the host plant. When colours were offered against each other, blue was favoured over green by both sexes. The visual preferences of *A. pomorum* were further investigated in order to evaluate the response to silhouettes of different achromatic contrast in front of green and blue. The weevils' response to these silhouettes was influenced by the background colour, i.e., the higher contrast was preferred in green whereas the lower contrast was preferred in the blue range. The preference for blue over green and the preference for higher contrast in front of green may be related to the weevils' need to detect dark apple trees surrounded by green vegetation. The weevils' preferences during visual orientation did not change with time after termination of diapause (i.e., 2, 4, 6 days). The colour preference seems to be a conservative trait that is persistent during colonisation of orchards in spring.
Thermal orientation of the apple blossom weevil was investigated in a temperature gradient arena. In the experiments carried out during photophase, both sexes chose the warmest part of the arena irrespective of the time that had elapsed since the termination of diapause (2, 4 or 6 days). These results suggest that both sexes benefit from thermoregulation by habitat choice during photophase when the weevils show flight activity to colonise apple trees. However, during scotophase female preference for the warmest part of the gradient decreased with time after termination of diapause while male preference remained for the warmest part of the arena. The thermal orientation during scotophase may be related to the constant status of male gonads and the changing status of post-diapausing female gonads.

In the field, tree colonisation and hiding behaviour of the weevils were studied with different trap types. In order to assess tree colonisation by flight and crawling, Plexiglas flight traps and commercial screen traps were used. A newly designed shelter trap mimicked thermally favourable hiding places. The trap catches were contrasted to the limb jarring technique. Numbers of weevils caught by all three types of traps over the spring season were significantly positively correlated with each other, but in most respects not with the results of the limb jarring. The numbers of weevils colonising the tree by crawling and by flight were in the same order of magnitude. Tree colonisation peaked in the early afternoon. However, the temporal pattern of hiding reveals an accumulation of weevils hiding early in the morning, i.e., during the coldest part of the day. The shelter traps caught a high number of weevils irrespective of weather conditions and thus performed better than both flight and screen traps. The temperature level within the shelter traps was higher than on the trunk, indicating that weevils exploit microclimatic differences and occupy thermally favourable refuges.

The developed shelter trap was validated as a monitoring tool for the apple blossom weevil in 5 different study sites in northern Switzerland. Therefore, the relationships between numbers of weevils caught and absolute injury, i.e., the total number of infested buds on a tree, as well as relative injury, i.e., the percentage of infested buds relative to all buds on the tree, were assessed. The findings with the new shelter traps were contrasted to results from limb jarring. Numbers of weevils caught
with the transparent shelter trap were positively correlated with both absolute and relative injury across the five study sites. The linear relationship allows for predictions of potential damage before oviposition takes place, i.e., early enough for an insecticide application where necessary. Weevil counts with limb jarring were not correlated with absolute injury, but only with relative injury. The newly designed shelter trap is useful for accurately monitoring the seasonal course of colonisation, irrespective of weather conditions, and allows for a prediction of the potential damage to apple blossoms.

This study shows how a monitoring tool can successfully be developed based solely on the use of physical stimuli. Furthermore, it demonstrates the importance of knowledge on the environmental physiology of a target insect species as well as on its orientation behaviour towards physical stimuli.
2. Zusammenfassung


Im Feld wurde mit Hilfe von verschiedenen Fallentypen die Besiedelung der Bäume und das Rückzugsverhalten der Käfer untersucht. Plexiglas-Flugfallen und kommerzielle Drahtgitter-Fallen wurden eingesetzt um die Besiedelung der Bäume durch den Apfelblütenstecher zu studieren. Eine neu entwickelte Falle wurde eingesetzt um thermisch begünstigte Verstecke nachzuahmen, in die sich die Käfer zurückziehen können (Shelter-Falle). Die Fänge mit diesen Fallen wurden zusätzlich mit der Klopfmethode verglichen. Die Fänge aller Fallentypen korrelieren über die Frühjahrsaison signifikant positiv miteinander. Dagegen gab es in den meisten Fällen
keine Korrelation mit den Resultaten der Klopfmethode. Die Besiedelung der Bäume durch Flug und durch Laufverhalten lagen hierbei in der gleichen Größenordnung. Die meisten Käfer besiedelten die Bäume am frühen Nachmittag. Die Mehrheit der Käfer versteckte sich am frühen Morgen in den Shelter-Fallen, d.h. in der kältesten Periode über den Tagesverlauf. Mit der Shelter-Falle konnte eine hohe Anzahl Käfer unabhängig von den Wetterbedingungen erfasst werden, was sich als vorteilhaft gegenüber Flugfalle und Drahtgitter-Falle erweist. Die Temperaturen in den Shelter-Fallen waren durchweg höher als am Stamm. Dies deutet darauf hin, dass die Käfer die mikroklimatischen Unterschiede ausnutzen und entsprechend des Designs der Falle die thermisch günstigeren Verstecke als Rückzugsorte aufsuchen.


In dieser Studie wird zeigt, wie eine Überwachungsmethode rein auf physikalischen Reizen basierend erfolgreich entwickelt werden kann. Zudem wird nachgewiesen, wie wichtig Erkenntnisse über die Umweltphysiologie der Zielarten sowie deren Orientierungsverhalten gegenüber physikalischen Reizen bei der Suche nach geeigneten Überwachungsmethoden sind.
3. General Introduction

Integrated pest management (IPM) was defined by the Food and Agriculture Organisation of the United Nations (FAO 1967) as a pest management system that, in the context of the associated environment and the population dynamics of the pest species, utilises all suitable techniques and methods in as compatible a manner as possible and maintains the pest population at levels below those causing economically injury. An important principle of IPM is to take no action against a pest unless that pest is known to be present and is an actual or potential threat, therefore monitoring of pests as a component of pest management in agroecosystems is of great importance.

At first glance the objectives of monitoring pest insects seem straightforward: to determine if and when a pest is present in order to decide the need for, and timing of, control measures. However, there are many ways in which information from a surveillance technique can be used and it is therefore important that the objectives should be clearly and precisely stated so that a method can be designed to achieve those objectives (Wall 1989). These objectives may be the detection of a particular pest, the timing of control measures, or the risk assessment of the pest. Detection requires a sensitive trapping method that provides qualitative information about the presence or absence of an organism. Timing, on the other hand, needs quantitative information about the insects caught. Quantitative surveys attempt to define numerically the abundance of an insect population in time and space (Pedigo 1996). The timing of control measures often depends on additional biological and meteorological data with which to predict the occurrence of the susceptible stage in the lifecycle. For risk assessment the relationship between trap catch and population density or subsequent damage needs to be established (Howse et al. 1998). The decision made about an intervention may either apply for the current or for the next season.

Surveillance requires knowledge of the insect behaviour and of the factors that trigger the behaviour. A monitoring tool may exploit the behaviour of the insects towards their natural resources by using chemical or physical cues. In contrast to chemical stimuli as related to mates or host plants, use of physical stimuli for
behavioural manipulation has been investigated to a much lesser extent. However, physical stimuli, as for example temperature and visual cues, are important factors influencing insect behaviour (Willmer et al. 2000, Prokopy & Owens 1983) and could therefore have great potential for use in monitoring, especially when abiotic environmental conditions seems to play an important role in a insect species.

The apple blossom weevil, *Anthonomus pomorum* (L.), is considered as insect pest of the apple tree, *Malus domestica* Borkh., for about two centuries (Speyer 1939, Massee 1954). The univoltine herbivore overwinters in the adult stage predominantly in leaf litter of hedges and woods (Wildbolz 1992, Toepfer et al. 2000), as in modern dwarf apple orchards the apple trees themselves offer little shelter. Weevils colonise the orchard during early spring (Hill 1985, Balachowsky 1963, Sattler 1937). After a period of feeding on buds that allows for ovarian maturation, the weevils mate and females lay single eggs into the closed blossom buds (e.g., Brown et al. 1993, Balachowsky 1963). The larvae develop inside the buds and cause the damage, the so-called 'capped' blossoms. After emergence from the capped blossoms the young adults feed on the petals of opened blossom and on the leaves. Weevils are fully fed by the end of June or beginning of July and from this period onwards they start to seek for overwintering sites (Miles 1923, Toepfer et al. 2000). After World War II the weevil disappeared from the orchards, probably as a result of the more intensive spraying programs with broadband insecticides. However, in the last few decades the population of *A. pomorum* increased locally in Switzerland and Southern Germany to damaging levels due to selective control of other major pests in orchards under integrated pest management (IPM) and organic management regime (Wildbolz 1992).

As there is a lack of selective management techniques for the apple blossom weevil, the population has to be controlled with insecticides sprayed during the beginning of spring activity. The most suitable timing for spraying is still not clearly predictable and the abundance of *A. pomorum* can vary widely from year to year (Brown 1993, Toepfer et al. 1999). A monitoring tool for this weevil is not only needed to verify the proper timing of spraying, but also to estimate the population density
which may or may not be beyond the economic damage threshold. An effective insecticide application should take place when the majority of the weevils immigrating from hedges and woods have colonised the orchard or when the individuals which overwintered within the orchard become active but have not yet laid their eggs. The need for an insecticide application can be estimated once the correlation between the abundance of the weevil and the infestation is known, i.e., the number and rate of blossoms infested on a tree. In summary, there are three basic questions that have to be approached by a monitoring tool for *A. pomorum*: (1) when is the period of maximum colonisation of trees by the weevils, (2) how is the abundance of *A. pomorum* at this point in time, and (3) what is the relationship between this abundance and the injury.

A first approach to accurately assess the timing of immigration led to a model based on air temperature data (Toepfer et al. 2002). However, prediction of the start of dispersal into orchards with this model varies greatly and has therefore to be complimented by a monitoring tool. A second approach applied in field studies is repeated sampling by means of the limb jarring technique (Lafleur & Hill 1987). By tapping branches of apple trees, adult weevils are dislodged and can be counted on a white cloth spread out on the ground. Unfortunately, this technique is dependent on favourable weather conditions (Loewel 1936, Toepfer et al. 1999). A third approach is the use of traps developed for other curculionids. The pyramid trap (Tedders & Wood 1994) developed for the pecan weevil, *Curculio caryae*, and a baited trap developed for the boll weevil, *Anthonomus grandis*, were tested with *A. pomorum* (Schärer unpublished diploma thesis, Frey unpublished semester thesis), but promise little success, as the number of weevils caught was very low.

Monitoring techniques may exploit the behaviour of the weevils using chemical or physical cues. Chemical cues applied in traps may include sex pheromones, aggregation pheromones or host plant odours (Foster & Harris 1997). In some curculionid species male-produced aggregation pheromones are known (Hallett et al. 1993, Tinzaara et al. 2003, Eller & Bartelt 1996, Booth et al. 1983, Blight & Wadhams 1987) and even used for surveillance (Eller et al. 1994). However, such pheromones are not yet known for the apple blossom weevil. Plant derived chemical cues might be used by *A. pomorum*.
for orientation in their search for apple buds in early spring (Kalinova et al. 2000),
though experimental evidence on the behavioural effect is lacking. Physical cues, i.e.,
visual, thermal, tactile and acoustic cues, might contribute to insect orientation and
could therefore be used as a base for a monitoring tool. Though, difficulties in the
definition and reproduction of tactile cues limit their use and acoustic cues are relatively
uncommon in insect monitoring (Foster & Harris 1997). In search of a reasonable
means of surveying the spring colonisation of the apple blossom weevil, this study
focuses on visual and thermal cues derived from the biological function of the insects’
orientation in its natural habitat.

It is known from several insect species that visual orientation plays an important
role in host plant location (Prokopy & Owens 1983) and visual cues, such as colour and
contrast, have been shown to be important components of traps developed for other
curculionid species (Cross et al. 1976, Tedders & Wood 1994). However, nothing is
known about the visual orientation of the apple blossom weevil. It is likely that these
herbivores also use visual cues such as the colour of apple trees to find their host plants
during spring activity. If the visual orientation of this weevil is towards the bark of host
trees, *A. pomorum* should prefer brownish or dark colours.

Thermal orientation is especially important for ectotherm insects exposed to
adverse environmental conditions as is the case in *A. pomorum* that is active early in
spring when temperatures are relatively low. The apple blossom weevil is well adapted
to the cold, as it has a low supercooling point of -11°C (Kostal & Simek 1996) and it
has been observed to crawl and feed still at around 0°C (Duan et al. 1996). However,
the activity of the weevil is influenced by temperature and at very low temperatures
activity is significantly suppressed (Duan et al. 1996, Toepfer et al. 2002). Thermal
orientation can be expected by the evolutionary adaptation of thermoregulation by
habitat selection due to climatic selection pressure in spring and the necessity for
mobility early in the season. The apple blossom weevil should prefer microhabitats with
favourable temperature during spring colonisation.
This thesis aims to elucidate the visual and thermal orientation of *A. pomorum* and to combine these findings to develop and validate a monitoring tool for the spring activity of this herbivore. First, the visual orientation of the weevil towards the most likely main colours of the visual system, UV, green and blue, was studied in the laboratory (chapter 4). The effect of the background colours green and blue to the response of weevils to silhouettes of high and low contrast was compared. In a next step of laboratory experiments, the thermoregulation behaviour of the weevil was investigated during photophase and during scotophase, and was analysed with regard to sex and time after termination of diapause (chapter 5). In a field study, the performance of different monitoring techniques, including a newly developed shelter trap, was quantified in the time course of orchard colonisation by the apple blossom weevil. The different methods were evaluated based on efficacy, reliability, weather dependency, and diurnal catching performance (chapter 6). The spatial and temporal activity patterns assessed in the study were used in order to (1) choose the best sites for mounting the developed monitoring tool and to (2) determine the optimum timing for trap assessment during the day. Finally, it was tested if the trap fully complies with the requirements for monitoring, by investigating the relationship between captures of *A. pomorum* with shelter traps and the injury to buds and by analysing the reliability of this method irrespective of biotic and abiotic environmental conditions (chapter 7).
4. Visual orientation of overwintered *Anthonomus pomorum* (Coleoptera: Curculionidae)

4.1. Abstract

Vision is considered to be an important component of the sensory system used by herbivorous insects to locate host plants. We investigated preferences for transmissive colours and contrasts in the apple blossom weevil, *Anthonomus pomorum* (L.), which colonises apple trees, *Malus domestica* Borkh., in early spring. The main components of the supposed visual system, green, blue and ultraviolet light, were offered to the weevils versus black or versus each other in a dual choice arena. Furthermore, silhouettes of two different achromatic contrasts were offered in front of the colours blue and green. We also tested whether visual preferences change with time, i.e., 2, 4 and 6 days after termination of diapause in early spring. Female weevils chose ultraviolet, green and blue over black, while males only chose ultraviolet over black, in a series of dual choice experiments. Both sexes preferred blue to green. Weevil response to silhouettes of different contrasts was influenced by the background colour, i.e., the silhouette with the higher contrast was preferred in front of green whereas the silhouette with the lower contrast was preferred in front of blue. The preferences during visual orientation did not change with time after diapause. The more discriminating response of females may reflect a greater visual acuity or a greater visual responsiveness in females than in males due to a possibly higher affinity for the host plant. Our findings indicate that weevils might have a trichromatic visual system and that they are able to include visual cues for the location of host plants in the field.

4.2. Introduction

All herbivore insects demonstrate some degree of selectivity in terms of host plants, and vision represents an important component of the sensory system involved during this process, often combined with olfaction (Prokopy & Owens 1983, Judd & Borden 1991, Kogan 1994). Accumulated evidence suggests that a distant plant at the horizon is detected by an insect herbivore primarily on the basis of a gross silhouette against a brighter, more uniformly lit sky, with little if any perception of the dominant reflected wavelength, or details of form (Prokopy & Owens 1983, Jermy et al. 1988). When an herbivore is in close proximity of a plant, it appears that the spectral quality of the light emitted by the plant becomes the predominant cue eliciting detection and alightment (Prokopy & Owens 1983). The visual response of an insect may be investigated by its behaviour and by electroretinogram studies, but spectral sensitivity does not imply discrimination of dominant wavelengths and, thus, behavioural evidence is necessary to prove true colour vision.

Most insects studied for their visual systems possess green receptors maximally sensitive at ~ 530 nm, ultraviolet receptors ($\lambda_{\text{max}} \sim 350$ nm) and also blue receptors ($\lambda_{\text{max}} \sim 440$ nm) (Briscoe & Chittka 2001). Chittka (1996) suggested that this set of ultraviolet, blue, and green photoreceptors is ancestral to Insecta, and that some lineages have since lost or added receptors, presumably as a result of different selection pressures.

True colour vision has only been shown for a limited number of insect species, and has not yet been demonstrated in coleopterans (Menzel & Backhaus 1991, Briscoe & Chittka 2001). By far the most studied insect in this regard is the honeybee, *Apis mellifera* L., because of its importance in agriculture, and because it is amenable to training. Proof of colour vision requires, for example, differentiated behavioural response of a species to wavelength cues in combination with results from physiological studies or that the species can be trained for colour cues (Menzel & Backhaus 1991). It is therefore difficult to demonstrate colour vision in many insect species. Therefore, lack of proof of true colour vision in coleopterans does not mean that they cannot
distinguish colours. On the contrary, electroretinograms have shown differential wavelength sensitivity in the eyes of at least eight coleopteran species: A trichromatic visual system with ultraviolet, blue, and green receptors was found in Photuris lucicrescens Barber (Lall et al. 1982) and in Coccinella septempunctata L. (Lin & Wu 1992). Two peaks of spectral sensitivity, in the ultraviolet and green region, were found in six other beetle species, i.e., Liocola brevitarsis (Lewis), Cicindela japonica (Tunberg), Cicindela specularis (Chaudoir) (Lin & Wu 1992), Photuris versicolor F. (Lall 1981), Photuris pyralis (L.) (Lall et al. 1980), and Leptinotarsa decemlineata (Say) (Mischke 1981). Studies on the visual ecology of two weevil species report that Anthonomus grandis Boheman showed colour preference for blue-green in a Y-shaped test chamber in the laboratory (Hollingsworth et al. 1964), and the plum curculio, Conotrachelus nenuphar (Herbst), oriented preferentially to tall sticky green rectangles (Butkewich & Prokopy 1997). Taken together, these studies suggest that beetles have the physiological capability for at least dichromatic vision and that colours may affect behaviour.

The apple blossom weevil, Anthonomus pomorum (L.), is a univoltine herbivore of apple trees, Malus domestica Borkh. Most adults overwinter in leaf litter of forests or hedgerows. Early in the season, weevils immigrate into orchards and colonise apple trees (Toepfer et al. 1999, 2002). Orientation of the weevils towards the host tree may include plant-derived chemical cues (Kalinova et al. 2000), though experimental evidence on the behavioural effect of single or combined compounds is lacking. So far nothing is known about the weevil’s perception of visual cues.

The aim of this study was to investigate the behavioural response of overwintered apple blossom weevils to visual cues. Weevil orientation towards transmissive colours and ultraviolet light was tested in a dual choice arena. The main components of the hypothesised visual system, green, blue, and ultraviolet light, were offered to the weevils versus black and versus each other. Furthermore, the response of the weevils towards silhouettes of different achromatic contrasts in front of the colours blue and green was investigated. In addition, we studied the influence of the physiological state of overwintered weevils on colour preference by testing their
response at different days after termination of diapause. Such an endogenous effect would modify the weevil's success in locating host plants during orchard colonisation.

4.3. Materials and methods

Insects

Capped brown blossoms bearing *A. pomorum* pupae were collected in May 2002 in northern Switzerland. Upon emergence, weevils were kept in plastic boxes (28 x 20 x 24 cm) and were fed with fresh apple leaves and pieces of apple fruits until the onset of aestivation (3 – 4 months after emergence). The adults were then sexed by the characteristic differences in the dorsal part of the last abdominal plates (Duan et al. 1999). The weevils were overwintered in a cooling chamber (SR Kältetechnik, Winterthur, Switzerland) at 4 ± 1°C in plastic boxes (19 x 9 x 8 cm). Strips of corrugated cardboard were offered as shelter. Even during aestivo-hibernation, the weevils were provided with fresh pieces of apples and sprayed with water at weekly intervals. Weevils were kept in diapause for a minimum of 20 weeks, as Ctvrtcka & Zdarek (1992) demonstrated that 65% of *A. pomorum* females showed initiation of ovarian development after a 5 month cold treatment (3 - 4°C).

Depending on the time treatment after diapause, two, four, or six days before the experiments weevils were kept for 24 h in a climate chamber (BK 6160, Heraeus, Balingen, Germany) with a constant temperature of 10°C (L:D = 12:12, ca. 80% RH, 3000 Lux), and then they were placed in a climate chamber with a constant temperature of 15°C (L:D = 12:12, ca. 80% RH, 3000 Lux). Such acclimatisation treatment reduced their initial mortality of 80 - 90% to less than 10% (Ctvrtcka & Zdarek 1992). The adults were fed with pieces of apple fruits and provided with water.
Visual test arena

The visual test arena (Figure 4.1) consisted of a cross made of Plexiglas (5 mm thick, Plexiglas XT, Röhm, Darmstadt, Germany). The centre of the arena consisted of a rectangular chamber (15 x 15 x 30 cm). Four arms (one to each sidewall, 15 x 15 x 30 cm) produced channels in each direction. At the end of each channel the arena had an opening (15 x 30 cm) for a frame to hold colour filters. The inner walls of the arena were covered with mirrors to avoid biases due to diffuse reflection of the light falling into the arena. The floor of the arena was coated with black paper (Mi-Teintes, Pastel, Canson, Annonay, France). The Plexiglas top of the arena was covered with grey transparency (acetate sheet, Tektronix Phaser 850, 50% grey) and was provided with a lid of 20 cm diameter to insert the weevils. The colour filter at the end of each channel was lit from the outside with a lamp. The intensity of each lamp was regulated with a dimmer in order to achieve a radiation energy of 150 W/m² in the centre of the test arena. Filters were made of high quality colour transparencies (Göttinger Farbfilter, Göttingen, Germany) with narrow wavelength bands and maximum wavelengths of 350 nm (ultraviolet), 455 nm (green), and 550 nm (blue). Black paper (Mi-Teintes, Pastel, Canson, Annonay, France) was used instead of the filters to provide the black cue. The green and blue colour filters were illuminated with a Tungsten-halogen lamp (78 mm-R7s-150W, Halotone, Royal Philips Electronics, Holland); the ultraviolet filter was illuminated with a ultraviolet-lamp (HPW-125TS-125W/E27, Royal Philips Electronics, Holland). The black paper was also illuminated with a Tungsten-halogen lamp to provide the same temperature in each channel of the arena. Spectral analyses of the colour filters and measurements of radiation energy to set the intensity of lamps were made by placing a fibre-optics reflective spectrometer (Ocean Optics SD 2000 UV-VIS, Dunedin, U.S.A.) at the point where the weevils were released i.e., 22.5 cm from the cues. The white reference for the spectral analyses was measured on a commercial reference standard (WS 1, Ocean Optics).
Figure 4.1. Visual test arena: Two perpendicular chambers bisected each other in the center forming a cross. Each of the 4 arms (15 x 15 x 30 cm) led to one of two visual cues, 1 or 2.

To test the effect of silhouettes of different achromatic contrasts a light or a dark grey transparency (Tektronix Phaser 850, 6% grey, 30% grey, 4.5 cm wide x 30 cm tall) was placed vertically in front of the green or blue filter. The coefficient of transmission of the light grey silhouette was 0.75, i.e., 75% of the radiation penetrated the transparency (low achromatic contrast). For the dark grey silhouette the coefficient of transmission was 0.33, i.e., 33% of the radiation penetrated the transparency (high achromatic contrast).

Bioassay

All bioassays were carried out in a climate chamber (Conviron PVG 36, Controlled Environment Ltd, Winnipeg, Canada). In order to have a temperature of approximately 18 - 20°C in the centre of the visual test arena, the temperature in the
climate chamber during the experiments was adjusted between 10 and 15°C (70% RH). Within the test arena, a choice of two different visual stimuli was presented at the same time. Opposite channels were equipped with the same colour filter to avoid an influence of radiation reflecting from the channels. One weevil at a time was introduced through the lid on the top and placed in a small white plastic dish (1.7 cm diameter) in the middle of the arena. As soon as the weevil crawled into one of the channels, it was scored as choosing a particular visual stimulus and the weevil was removed. Each weevil was only tested once and the black paper on the floor was exchanged for a new unused one after each trial. To exclude possible unexpected influences from the arena or the climate chamber, the colours were changed between the channels after every tested weevil. Each weevil was observed for a maximum time of 30 minutes. If the weevil did not enter a channel within this time it was removed and not scored. All experiments were carried out in the afternoon between 12.00 and 15.00, i.e., approximately 5 to 8 hours after the beginning of the photophase, because in the field it was found that the maximum colonisation of apple trees by flight and by crawling occurred during this time (Hausmann et al. 2004).

The weevils were given the choice between the following pairs of visual stimuli: ultraviolet versus green, ultraviolet versus blue, ultraviolet versus black, green versus blue, green versus black, and blue versus black. Also compared were silhouettes of low and high achromatic contrast in association with green and with blue filters. 45 male and 45 female weevils (15 individuals each 2, 4 and 6 days after termination of diapause) were tested per treatment combination. All choice experiments were analysed by $\chi^2$-tests with the zero hypothesis of homogeneity in the distribution of individuals responding to the choices offered in the bioassays (Zar 1998).
4.4. Results

Female weevils show a preference for ultraviolet, green, and blue over black (ultraviolet vs. black: $\chi^2 = 15.21$, d.f. = 1, $P < 0.001$, green vs. black: $\chi^2 = 8.02$, d.f. = 1, $P = 0.005$; blue vs. black: $\chi^2 = 8.02$, d.f. = 1, $P = 0.005$) (Figure 4.2). Male weevils show a preference for ultraviolet over black (ultraviolet vs. black: $\chi^2 = 10.79$, d.f. = 1, $P = 0.001$) but they show no preference when the colours green versus black or blue versus black are offered (green vs. black: $\chi^2 = 0.20$, d.f. = 1, $P = 0.655$; blue vs. black: $\chi^2 = 3.76$, d.f. = 1, $P = 0.053$) (Figure 4.2). The time after diapause does not influence the colour choice of the males and females (ultraviolet vs. black: females: $\chi^2 = 1.68$, d.f. = 2, $P = 0.431$, males: $\chi^2 = 2.81$, d.f. = 2, $P = 0.245$; green vs. black: females: $\chi^2 = 0.22$, d.f. = 2, $P = 0.898$, males: $\chi^2 = 3.75$, d.f. = 2, $P = 0.153$; blue vs. black: females: $\chi^2 = 1.51$, d.f. = 2, $P = 0.469$, males: $\chi^2 = 3.10$, d.f. = 2, $P = 0.212$).

![Figure 4.2. Colour choice of overwintered female and male apple blossom weevils in the visual test arena when the colours green, blue and ultraviolet (UV) are offered vs. black (n = 45). The asterisks indicate a significant difference ($\chi^2$-test, $p < 0.05$).](image-url)
When the colours green or blue are offered versus ultraviolet light, females and males show no preference (green vs. ultraviolet: female: $\chi^2 = 0.56$, d.f. = 1, $P = 0.456$, male: $\chi^2 = 0.56$, d.f. = 1, $P = 0.456$; blue vs. ultraviolet: female: $\chi^2 = 1.09$, d.f. = 1, $P = 0.206$, male: $\chi^2 = 0.56$, d.f. = 1, $P = 0.456$) (Figure 4.3). Females and males show a preference for blue, when the colours green and blue are offered (females: $\chi^2 = 5.00$, d.f. = 1, $P = 0.025$; males: $\chi^2 = 6.42$, d.f. = 1, $P = 0.011$) (Figure 4.3). The time after diapause does not influence the colour choice of the males and females (green vs. ultraviolet: females: $\chi^2 = 0.72$, d.f. = 2, $P = 0.698$, males: $\chi^2 = 1.26$, d.f. = 2, $P = 0.533$; blue vs. ultraviolet: females: $\chi^2 = 5.10$, d.f. = 2, $P = 0.078$, males: $\chi^2 = 0.72$, d.f. = 2, $P = 0.698$; green vs. blue: females: $\chi^2 = 0.60$, d.f. = 2, $P = 0.741$, males: $\chi^2 = 0.31$, d.f. = 2, $P = 0.902$).

Figure 4.3. Colour choice of overwintered female and male apple blossom weevils in the visual test arena when the colours blue, green and ultraviolet (UV) are tested against each other (n = 45). The asterisks indicate a significant difference ($\chi^2$-test, $p < 0.05$).
When analysed separately, females and males show no preference between silhouettes of a low or a high contrast in blue or green (green low vs. high contrast: female: $\chi^2 = 1.09$, d.f. = 1, $P = 0.297$, male: $\chi^2 = 1.8$, d.f. = 1, $P = 0.180$; blue low vs. high contrast: female: $\chi^2 = 2.69$, d.f. = 1, $P = 0.101$, male: $\chi^2 = 1.8$, d.f. = 1, $P = 0.180$) (Figure 4.4). When the data of females and males are pooled, the response to low and high contrast shows a significant difference when tested in blue and green ($\chi^2 = 7.20$, d.f. = 1, $P = 0.007$). The higher contrast was preferred in green whereas the lower contrast was preferred in the blue range. The time after diapause does not influence the choice of achromatic contrast in males and females (green low vs. high contrast: females: $\chi^2 = 0.18$, d.f. = 2, $P = 0.913$, males: $\chi^2 = 1.67$, d.f. = 2, $P = 0.438$; blue low vs. high contrast: females: $\chi^2 = 0.76$, d.f. = 2, $P = 0.685$, males: $\chi^2 = 2.22$, d.f. = 2, $P = 0.324$).

![Figure 4.4](image)

**Figure 4.4.** Choice of overwintered female and male apple blossom weevils in the visual test arena when the colour blue and green is offered with a vertical silhouette resulting in a high vs. a low achromatic contrast ($n = 45$). Females and males separately: No significant differences. Pooled females and males: Significant difference of choice in the green vs. the blue range ($\chi^2$-test, $p < 0.05$).
4.5. Discussion

Our laboratory results demonstrate that apple blossom weevils orient positively towards transmissive visual stimuli and show preferences between different colours. Furthermore, weevil response to silhouettes of different achromatic contrasts is influenced by the background colour. These results indicate that weevils might include visual cues in their host plant location in the field. Physiological changes within the first six days after diapause do not influence the behavioural responses to the visual stimuli offered in our experiments.

Visual cues are considered to be important for host plant location in insects (Prokopy & Owens 1983) and a variety of solutions have evolved to locate plant resources. For example, host plant location in *Erioischia brassicae* (Bouché) cabbage root flies involves response over a distance of several metres to host volatile compounds, presumably coupled with close range response to plant visual stimuli (Hawkes 1974). The opposite appears to occur in *Trialeurodes vaporariorum* (Westw.) whiteflies, where initial detection of host and nonhost plants from a distance is visual, while host olfactory stimuli are involved only at very close range (Vaishampayan et al. 1975).

The results from our experiments with a choice between a transmissive wavelength cue and black indicate that apple blossom weevils show positive phototaxis. The weevils orient towards light when black serves as a blank in the bioassay design. Female weevils favour ultraviolet, green and blue wavelength cues when offered versus black and therefore have the ability to perceive these cues with their visual system. Males only prefer ultraviolet significantly over black. Nevertheless, this does not exclude the ability of the males to perceive green and blue, as they discriminate between these two colours when offered against each other in a choice test. Both sexes favour the colour blue over green which proves that the weevils can distinguish between green and blue solely on the basis of wavelength differences.

During the colonisation of apple trees in early spring weevils encounter the visual stimuli of sky, green vegetation and brown or grey bark of apple trees. In this period,
weevils need to recognise the dark apple trees still without foliage surrounded by green vegetation. Indeed, they choose a high contrast silhouette in the green wavelength range. In the blue range, the weevils show phototaxis toward the homogeneous transmissive cue and this difference in response to achromatic cues in the blue and green range is significant when analysed for both sexes together. The necessity to orient towards other colours than green during host plant location might lead to the general preference of blue over green in the visual test arena. Nevertheless, the choice of the low contrast silhouette versus high contrast in the blue wavelength range is surprising and would require field tests for further interpretation.

The more discriminating response of the female sex in the apple blossom weevil in the visual test arena may reflect a greater visual acuity or a higher visual responsiveness in females than in males, possibly due to a higher affinity for the host plant. Females should have an advantage of a more critical choice as (1) they have to select oviposition sites, and (2) their choice is final as the larvae cannot abandon the buds into which the eggs were laid. This finding coincides with the visual response of the white pine weevil, *Pissodes strobi*, to cardboard silhouettes in the laboratory: female weevils select only vertical rectangles, whereas males are less critical in their choice (VanderSar & Borden 1977). Similar evidence was found in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say): females took shorter paths to attractive colour stimuli and they were less hesitant than comparable male beetles (Szentesi et al. 2002).

In another curculionid species, the plum curculio, *Conotrachelus nenuphar*, colour does not seem to be a strong visual cue when foraging within the canopy of host trees (Leskey & Prokopy 2002). This is in good agreement with our present findings as the apple blossom weevils showed no preference for green when this colour was offered against other colours. Orientation to green occurred only when green was offered against no colour, i.e., against black. During the search for apple buds in early spring, olfactory cues may contribute to female orientation (Kalinova et al. 2000), though experimental evidence on the behavioural effect of single or combined plant derived chemical compounds is lacking yet in *A. pomorum*. 
Phototaxis is considered to be one of the basic orientations in insects with the ability for visual orientation (Jander 1963). This assumption is supported by our finding that colour preference remained unchanged over a week following termination of diapause. In contrast, thermal choice in female weevils actually changes with time after diapause as females discontinue to prefer warmer sites at night after about one week after termination of diapause (Hausmann et al. in prep.).

Results of our study prove that the apple blossom weevil shows positive phototaxis and a preference for the colour blue. The colour preference seems to be a conservative trait that does not change during colonisation of orchards in spring. Although the physiology of the receptors in curculionids, including A. pomorum, remains to be investigated (Briscoe & Chittka 2001), our findings support the hypothesis that the weevils have a trichromatic visual system with ultraviolet, blue, and green receptors.
5. Thermal orientation of *Anthonomus pomorum* (Coleoptera: Curculionidae) in early spring

5.1. Abstract

Insects are highly sensitive to ambient temperature due to their size and largely ectothermic control of body temperature. Thermoregulation, as for example selection of thermally favourable microhabitats, is particularly important in insect species that are forced to be active under relatively unfavourable conditions during the seasonal cycle. The apple blossom weevil *Anthonomus pomorum* (L.), exploits the closed blossom buds of apple trees, *Malus domestica* Borkh., for oviposition in early spring when the ambient temperatures are still very low. The selection of habitats with favourable temperature by this weevil was investigated in a temperature gradient arena with a range of approximately 0 to 15°C. Single female and male weevils were tested in the arena 2, 4 and 6 days after termination of diapause during photophase and during scotophase. During photophase, weevils of both sexes chose the warmest part of the temperature gradient arena irrespective of the time elapsed after diapause (2, 4 or 6 days). During scotophase, high temperature was favoured by male weevils as well as by females 2 and 4 days after diapause. However, at 6 days after termination of diapause, females discontinued to show any thermal preference in the temperature gradient arena during scotophase. These results suggest that (1) both sexes benefit from thermoregulation by habitat choice during photophase when the weevils show flight activity and colonise the apple trees and that (2) the thermal orientation during scotophase is related to the constant status of male gonads and the changing status of female gonads during the period following termination of diapause.

---

Based on: Hausmann C, Samietz J & Dorn S (submitted) Thermal orientation of *Anthonomus pomorum* (Coleoptera: Curculionidae) in early spring.
5.2. Introduction

Due to their size and tendency to ectothermic control of body temperature insects are highly sensitive to abiotic ambient conditions including temperature (Digby 1955). In insects’ lives the ambient temperature plays an important role since it not only affects their development, survival, abundance and reproduction (e.g., Lapointe 2000, Stathas 2000, Roy et al. 2002) but also their activity (Heinrich 1995, Duan et al. 1996). Consequently, insects have evolved various means to regulate their body temperature for avoiding specific physiologically unsuitable conditions caused by abiotic factors of the environment. In general, these means can be of three types: morphological, physiological and behavioural (Clench 1966, Bursell 1974, Casey & Hegel 1981, Willmer 1982, Schmitz 1994, Kreuger & Potter 2001). Insects from several taxa regulate their body temperature by behavioural adaptations, e.g., by orientation to sun and wind direction (Hamilton 1975), by the position of their wings during basking (Clench 1966), or by the exploitation of the thermal heterogeneity of their environment (Cloudsley-Thompson 1962). Mobile insect stages are capable of avoiding unfavourable conditions by using different microhabitats, choosing particular sites appropriate to their thermal balance (May 1979, Casey 1981). The behaviour of insects in relation to differences in temperature within the habitat may ensure their exposure to conditions far closer to the optimum than would be expected based on ambient air temperature (Bursell 1974). Such thermoregulation by habitat choice could be demonstrated within temperature gradients for many insect species (Deal 1941, Ferguson & Land 1961, Cokendolpher & Francke 1985, Flinn & Hagstrum 1998, Jian et al. 2003).

Benefits from thermoregulation by habitat selection are particularly important in insect species that are forced to be active under relatively unfavourable conditions during the seasonal cycle. When temperatures are relatively low, individuals that choose thermally favourable microclimates should benefit from a higher activity level in immigration, mating, oviposition and development (Willmer 1982).

The apple blossom weevil, *Anthonomus pomorum* (L.), uses closed flower buds of apple trees, *Malus domestica* Borkh., for oviposition in early spring. The adults colonise
the orchard from overwintering sites and have to mature and mate at a time when the blossom buds are still closed. Therefore both sexes of the adult weevils have to be active in early spring under still relatively low ambient temperatures (Central Europe: late February/beginning of March; Toepfer et al. 1999, 2002). Their small body size of a few millimetres precludes endothermic regulation by metabolic heat production that would at least allow for a partial independence of body temperature from environmental conditions (Heinrich 1993). Behavioural studies demonstrated that the ambient temperature of the weevils indeed influences its activity such as crawling, feeding and mating (Duan et al. 1996, Toepfer et al. 2002). Furthermore, the hiding behaviour of the apple blossom weevils in thermal shelters corresponds with the coldest periods of the diurnal cycle (Hausmann et al. 2004).

In the present study we tested the hypothesis that *A. pomorum* weevils use thermoregulation by choosing microhabitats with different temperatures. Benefits from higher body temperatures may differ between males and females as for example due to the need for egg maturation in females, and likewise such benefits may change with time due to developmental processes. Consequently, experiments in a temperature gradient arena tested possible dependency of thermoregulation behaviour on sex and on time after termination of diapause. Furthermore possible behavioural differences were compared between photophase, i.e., the period of highest activity of the weevils, and scotophase, i.e., when the lowest ambient temperatures during the diel cycle may trigger the thermoregulation behaviour.

5.3. Materials and methods

**Insects**

Capped brown blossoms bearing *A. pomorum* pupae were collected in May 2002 in northern Switzerland. After emergence, weevils were kept in plastic boxes (28 x 20 x 24 cm) and were fed with fresh apple leaves and pieces of apple fruit until the onset of
aestivo-hibernation (3 – 4 months after emergence). Then adults were sexed by the characteristic differences in the dorsal part of the last abdominal plates (Duan et al. 1999). Weevils were overwintered in a cooling chamber (SR Kältetechnik, Winterthur, Switzerland) at $4 \pm 1^\circ C$ in plastic boxes (19 x 9 x 8 cm). Strips of corrugated cardboard were offered as shelter. During aestivo-hibernation, weevils were still provided with fresh pieces of apples and sprayed with water at weekly intervals. Weevils were kept in diapause for a minimum of 20 weeks, as Ctvrtecka and Zdarek (1992) demonstrated that 65% of *A. pomorum* females showed initiation of ovarian development after a period of cold treatment ($3 - 4^\circ C$) of 5 months.

The weevils were transferred to a climate chamber (Heraeus, BK 6160, Balingen, Germany) with a temperature of $10^\circ C$ during photophase (light period) and $5^\circ C$ during scotophase (L:D = 12:12 h, ca. 3000 Lux, 60-80% RH) two, four, or six days before the experiments as needed. This photoperiod corresponds to the field conditions in Central Europe when weevils start activity in early spring (beginning of March). Adults were provided with apple fruit and water.

**Test arena**

The arena consisted of a transparent plastic tube (10 cm diameter, 30 cm length, polystyrene, 0.25 mm thick) that was closed with a plastic lid at one end and with fine gauze at the other end. Inside the tube was a piece of cardboard ($5 \times 29$ cm) for the weevils to crawl on. The cardboard was suspended horizontally in the centre of the tube away from the walls by two loops of 0.8 mm steel wire. In the experiments during scotophase the cardboard was covered with a plastic transparency film to provide the weevils with shelters, as weevils tend to hide during this time of the diel cycle (Hausmann et al. 2004). The arena was placed in a climatic chamber (E15, Conviron, Winnipeg, Canada) at a temperature of $-5^\circ C$ (80% RH) and illumination or darkness. To obtain a temperature gradient, an infrared lamp (PAR 38 IR 100 W, Royal Philips Electronics, Holland) was installed in front of the end of the tube with the plastic lid. The resulting linear temperature gradients ranged from $0.7^\circ C$ to $15.4^\circ C$ (60 ± 10% RH, 6000 Lux) in the experiment during photophase and from $0.2^\circ C$ to $15.2^\circ C$ (60 ± 10%
RH) in the experiment during scotophase. Within the diel cycle of the weevils, the experiments were carried out approximately 5 to 8 hours after the onset of photophase and approximately 4 to 1 hour before the end of scotophase. These two periods correspond (1) to the main activity of the weevils during the day and (2) to the hiding peak of the weevils at night in the field (Hausmann et al. 2004). During each trial, one weevil at a time was placed at a randomly chosen position on the cardboard and its location was assessed again after 60 min. Each weevil was only tested once and the cardboard was exchanged for a new one after each trial. A control experiment was conducted in the same test-arena with a constant temperature of 10°C (80% RH, photophase: 6000 Lux). Thirty male and 30 female weevils per light treatment (photophase/scotophase) were tested for each time treatment after diapause (2, 4, 6 days). Two full replications (Rep. 1, Rep. 2) of the entire experiment and of the control were carried out.

Data analysis

For analysis of the distribution of the weevils in the temperature gradient, the cardboard was divided into 5 sectors of equal size (mean temperature in photophase: sector 1 = 14.3°C, sector 2 = 8.5°C, sector 3 = 6.4°C, sector 4 = 5.3°C, sector 5 = 2.1°C; in scotophase: sector 1 = 13.4°C, sector 2 = 6.5°C, sector 3 = 5.9°C, sector 4 = 5.0°C, sector 5 = 2.7°C). The distribution of the weevils in the temperature gradient (5 sectors) after 60 min was analysed against an equal distribution with a $\chi^2$-test for each of the 3 times after diapause (Zar 1998). For each location a temperature was attributed according to the gradient. Thus individual temperatures could be assigned to the weevils after scoring their position. From these temperatures the median and mean temperature of the weevil's choice was calculated. A Kruskal-Wallis test was performed to test for differences between the temperature choice of weevils 2, 4 and 6 days after termination of diapause. Possible differences in temperature choice between females and males were analysed with a Mann-Whitney U-test including all time treatments.

The data was analysed with the software StatView 5.0.1 for Apple Macintosh (SAS Institute Inc.).
5.3. Results

Photophase

Males chose the warm part of the temperature gradient arena with the following proportion of males in the warmest sector: 2 days after diapause 46.7% (Rep. 1) and 63.3% (Rep. 2), 4 days after diapause 73.3% (Rep. 1) and 66.7% (Rep. 2), 6 days after diapause 46.7% (Rep. 1) and 63.3% (Rep. 2) (Figure 5.1a + b, Table 5.1). The warm part of the temperature gradient arena was also chosen by the females with the following proportion of females in the warmest sector: 2 days after diapause 60.0% (Rep. 1) and 56.7% (Rep. 2), 4 days after diapause 53.3% (Rep. 1) and 60.0% (Rep. 2), 6 days after diapause 73.3% (Rep. 1) and 43.3% (Rep. 2) (Figure 5.1c + d, Table 5.1).

In the control arena with a temperature of 10°C in all sectors, distribution of males and females in all treatments after diapause (2, 4, 6 days) did not deviate significantly from an equal distribution ($\chi^2$-test, $P > 0.05$).

Two days after diapause the mean temperature chosen by males was 9.4°C (Rep. 1) and 10.8°C (Rep. 2) and by females 10.9°C (Rep. 1) and 11.4°C (Rep. 2) (Table 5.1). The mean temperature chosen four days after diapause by males was 12.5°C (Rep. 1) and 12.3°C (Rep. 2) and by females 10.7°C (Rep. 1) and 11.0°C (Rep. 2) (Table 5.1). Six days after diapause the mean temperature chosen by males was 9.2°C (Rep. 1) and 11.5°C (Rep. 2) and by females 12.7°C (Rep. 1) and 9.5°C (Rep. 2) (Table 5.1). There was no difference between the temperatures chosen by males 2, 4 and 6 days after termination of diapause (Kruskal-Wallis test, Rep. 1: $H = 4.28$, d.f. = 2, $P = 0.118$; Rep. 2: $H = 0.95$, d.f. = 2, $P = 0.622$) and by females 2, 4 and 6 days after termination of diapause (Kruskal-Wallis test, Rep. 1: $H = 3.05$, d.f. = 2, $P = 0.217$; Rep. 2: $H = 1.45$, d.f. = 2, $P = 0.485$). No significant difference was found between the temperatures chosen by males and females (Mann-Whitney U-test, Rep. 1: $U = 3704.00$, $P = 0.322$; Rep. 2: $U = 3553.00$, $P = 0.155$).
Figure 5.1. Number of male (a, b) and female (c, d) *A. pomorum* after 60 min in the different sectors of the temperature gradient arena during photophase (n = 30 per treatment). Sector 1 represents the warmest, sector 5 the coolest sector. 'day' refers to the number of days after termination of diapause. The asterisks indicate that the distribution of the weevils is significantly different from an equal distribution ($\chi^2$-test, $P < 0.05$).
Scotophase

Males chose the warm part of the temperature gradient arena with the following proportion of males in the warmest sector: 2 days after diapause 73.3 % (Rep. 1) and 63.3% (Rep. 2), 4 days after diapause 63.3% (Rep. 1) and 40.0% (Rep. 2), 6 days after diapause 53.3% (Rep. 1) and 50.0% (Rep. 2) (Figure 5.2a, b, Table 5.1). The warm part of the temperature gradient arena was also preferred by the females 2 and 4 days after termination of diapause with the following proportion of females in the warmest sector: 2 days after diapause 56.7% (Rep. 1) and 46.7% (Rep. 2), 4 days after diapause 60.0% (Rep. 1) and 53.3% (Rep. 2) (Figure 5.2c, d, Table 5.1). Females showed no thermal preference 6 days after diapause (Table 5.1, Figure 5.2c, d).

In the control arena with a temperature of 10°C in all sectors, the distribution of males and females in all treatments after diapause (2, 4, 6 days) did not deviate significantly from an equal distribution ($\chi^2$-test, $P > 0.05$).

Two days after diapause the mean temperature preferences of males was 11.6°C (Rep. 1) and 10.3°C (Rep. 2) and of females 10.3°C (Rep. 1) and 8.9°C (Rep. 2) (Table 5.1). The mean temperature chosen by males four days after diapause was 10.2°C (Rep. 1) and 8.5°C (Rep. 2) and by females 10.9°C (Rep. 1) and 9.8°C (Rep. 2) (Table 5.1). Six days after diapause the mean temperature chosen by males was 9.8°C (Rep. 1) and 8.9°C (Rep. 2) and by females 7.4°C (Rep. 1) and 8.1°C (Rep. 2) (Table 5.1). In males, there was no difference between the temperatures chosen 2, 4 and 6 days after termination of diapause (Kruskal-Wallis test, Rep. 1: $H = 1.84$, d.f. = 2, $P = 0.398$; Rep. 2: $H = 2.45$, d.f. = 2, $P = 0.293$). In females, there was a significant difference between the temperatures chosen 2, 4 and 6 days after termination of diapause in one replication (Kruskal-Wallis test, Rep. 1: $H = 6.39$, d.f. = 2, $P = 0.041$; Rep. 2: $H = 1.84$, d.f. = 2, $P = 0.398$). No significant difference was found between the temperature chosen by males and females (Mann-Whitney U-test, Rep. 1: $U = 3697.00$, $P = 0.313$; Rep. 2: $U = 3927.00$, $P = 0.725$).
Figure 5.2. Number of male (a, b) and female (c, d) *A. pomorum* after 60 min in the different sectors of the temperature gradient arena during scotophase (n = 30 per treatment). Sector 1 represents the warmest, sector 5 the coldest sector. 'day' refers to the number of days after termination of diapause. The asterisks indicate that the distribution of the weevils is significantly different from an equal distribution ($\chi^2$-test, P < 0.05).
Table 5.1. Temperature chosen by the apple blossom weevils in the temperature gradient arena after 60 min during photophase and during scotophase. A $\chi^2$-test was performed to compare the number of weevils in each sector of the arena with an equal distribution ($n = 30$, d.f. = 4).

<table>
<thead>
<tr>
<th>Days after diapause</th>
<th>Temperature chosen (°C)</th>
<th>Median</th>
<th>Mean</th>
<th>SD</th>
<th>$\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Photophase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rep. 1</td>
<td>2</td>
<td>7.8</td>
<td>9.4</td>
<td>5.82</td>
<td>15.67</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>15.4</td>
<td>12.5</td>
<td>4.69</td>
<td>54.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>10.6</td>
<td>9.2</td>
<td>6.51</td>
<td>21.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rep. 2</td>
<td>2</td>
<td>15.4</td>
<td>10.8</td>
<td>6.06</td>
<td>37.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>15.4</td>
<td>12.3</td>
<td>4.99</td>
<td>41.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>15.4</td>
<td>11.5</td>
<td>4.50</td>
<td>35.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rep. 1</td>
<td>2</td>
<td>13.4</td>
<td>10.9</td>
<td>5.22</td>
<td>30.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>12.5</td>
<td>10.7</td>
<td>5.14</td>
<td>21.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>15.4</td>
<td>12.7</td>
<td>4.82</td>
<td>56.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rep. 2</td>
<td>2</td>
<td>13.4</td>
<td>11.4</td>
<td>4.70</td>
<td>30.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>13.9</td>
<td>11.0</td>
<td>5.50</td>
<td>33.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>9.4</td>
<td>9.5</td>
<td>5.56</td>
<td>11.33</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Scotophase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rep. 1</td>
<td>2</td>
<td>15.0</td>
<td>11.6</td>
<td>5.24</td>
<td>55.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>13.6</td>
<td>10.2</td>
<td>5.89</td>
<td>40.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>12.9</td>
<td>9.8</td>
<td>5.74</td>
<td>24.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rep. 2</td>
<td>2</td>
<td>12.4</td>
<td>10.3</td>
<td>5.32</td>
<td>37.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>8.6</td>
<td>8.5</td>
<td>4.46</td>
<td>9.67</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>8.1</td>
<td>8.9</td>
<td>5.59</td>
<td>19.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rep. 1</td>
<td>2</td>
<td>11.8</td>
<td>10.3</td>
<td>5.14</td>
<td>26.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>14.7</td>
<td>10.9</td>
<td>5.30</td>
<td>30.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5.9</td>
<td>7.4</td>
<td>5.23</td>
<td>6.00</td>
<td>n.s.</td>
</tr>
<tr>
<td>Rep. 2</td>
<td>2</td>
<td>8.6</td>
<td>8.9</td>
<td>5.34</td>
<td>15.00</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>11.8</td>
<td>9.8</td>
<td>5.31</td>
<td>22.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6.0</td>
<td>8.1</td>
<td>5.23</td>
<td>7.67</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
5.4. Discussion

Our study demonstrates that overwintered apple blossom weevils perform active behavioural thermoregulation by selection of warm microhabitats. Early in spring when weevils terminate their diapause, they are forced to be active under unfavourable conditions at relatively low temperatures since they use apple blossom buds in an early stage for ovarian maturation and for oviposition. Thermal orientation is crucial for an insect that becomes active so early in the spring. Higher body temperature allows for a higher activity level and thus the weevils can benefit in colonising trees, finding mating partners, oviposition and feeding. Disappearance of a thermal preference in females 6 days after termination of diapause could indicate a change in behavioural priority related to the status of gonad development.

The supercooling point of the apple blossom weevil is low (-11°C) in feeding individuals (Kostal & Simek 1996), and the weevils have been observed to crawl and feed at temperatures around 0°C (Duan et al. 1996). Even if weevils show this physiological attribute necessary for the exploitation of early stages of apple bud development, their total activity, i.e., crawling, feeding and mating, was significantly suppressed at lower temperatures (below 5°C) (Duan et al. 1996, Toepfer et al. 2002). 25 to 30% of the weevils were active at temperatures between 0 and 12°C; 85% of the weevils were active at temperatures above 12°C (Toepfer et al. 2002).

During photophase female and male apple blossom weevils may benefit from higher temperatures with a higher activity level. Tree colonisation occurs mainly during photophase and colonisation by flight in particular was observed solely during photophase (Hausmann et al. 2004). Flight initiation in the laboratory rarely occurred at temperatures of 12°C or below (Duan et al. 1996). This dependency of relatively high temperatures in spring was demonstrated in our study by the preference of weevils for the warmer sectors of the arena during photophase. Adults of the Colorado potato beetle, *Leptinotarsa decemlineata*, show a similar thermoregulation behaviour during photophase. They apparently modify body temperature by seeking sunlit substrates at low ambient temperatures and by moving into the shade at high ambient temperatures.
during daylight hours (May 1982). Other coleopteran species also showed thermal orientation in a temperature gradient, e.g., adults of *Tribolium castaneum* (Herbst) (Hagstrum et al. 1998), *Cryptolestes ferrugineus* (Stephens) (Jian et al. 2003), and *Dermestes maculates* Degeer (Osuji 1975).

During scotophase male apple blossom weevils favoured the warmer sectors of the temperature gradient on all days tested after termination of diapause. Females also preferred the warmer sectors 2 and 4 days after diapause. However, 6 days after diapause they showed no thermal preference during scotophase. This pattern of thermal orientation corresponds with data gathered in a field study whereby weevils were found in shelter traps that mimic relatively warm hiding sites only on the first days after colonisation (Hausmann et al. 2004). Upon termination of diapause females have only slightly developed gonads (Ctvrtcka & Zdarek 1992). It is possible that their feeding status and the degree of egg maturation determines their behavioural priority between thermal orientation and oviposition-site selection. Indeed, oviposition-oriented behaviour is affected by endogenous factors, and often correlates with conditions of the ovaries (Browne 1993). Further investigations will have to evaluate whether the time when thermal orientation in female weevils discontinues coincides with a distinct nutritional status and/or egg development stage. Our findings on the timing of this behavioural change, about one week after the termination of diapause, both under laboratory (this study) and field conditions (Hausmann et al. 2004) is compatible with the hypothesis that the endogenous factor regulating female behaviour is of hormonal nature. Males, on the other hand, have sperm in their spermatic ducts already on the first day after termination of diapause (Ctvrtcka & Zdarek 1992). They may benefit from higher temperatures in general by having a higher level of activity, e.g., for searching females and for mating.

This study highlights the importance of behavioural thermoregulation in an insect species that is active early in the year when temperatures are still relatively low. The apple blossom weevil was shown to perform active thermoregulation by choosing warm microhabitats in a temperature gradient. In the field, weevils might choose sunny spots during photophase and warm shelters during scotophase. Our study provides, for the
first time, data indicating that the thermal choice of female *A. pomorum* changes with time after the termination of diapause.
6. Monitoring the dynamics of orchard colonisation by *Anthonomus pomorum* in spring

6.1. Abstract

Overwintered adult apple blossom weevils, *Anthonomus pomorum* (L.) (Coleoptera: Curculionidae), colonise apple trees, *Malus domestica* Borkh. (Rosaceae), in early spring. Information gained from a suitable monitoring technique could serve as a guide to determine the accurate timing for control measures. To assess tree colonisation by flight and crawling, Plexiglas flight traps and commercial screen traps developed for different curculionid species were used. Refuges were mimicked by a newly designed shelter trap based on transparent bubble wrap. The trap catches were contrasted to the limb jarring technique. Microclimate and weather conditions were determined over the trial period of one and a half months. The shelter traps caught a high number of weevils irrespective of weather conditions, and thus performed better than both flight and screen traps. The temperature level within the shelter traps was slightly, but significantly, higher than on the trunk, indicating that weevils exploit microclimatic differences and occupy thermally favourable refuges. The seasonal culmination of colonisation as determined by shelter traps coincided with that as determined by the two methods used to monitor weevil movement towards host trees (flight traps, screen traps). The data indicated that not only flight, but also crawling as quantified by the screen traps contributes substantially to spring colonisation. Captures by all three types of traps over the spring season were significantly positively correlated with each other, but in most respects not with the results of the limb jarring. We conclude that the newly designed shelter trap will be useful for accurately monitoring the seasonal course of colonisation and holds promise for determining timing of interventions.

---

6.2. Introduction

The development of an effective trap for monitoring an adult herbivore pest ultimately relies on the pest's needs for food resources and oviposition sites, for mates, or for refuges. Flight and crawling are typical modes by which an adult approaches its host plant, and passive flight traps, trapping devices wrapped around the base of the plant (Prokopy et al. 2000), as well as traps baited with attractive host-plant odours (Natale et al. 2003) take advantage of this fact. The need for mates can be exploited once a volatile sex pheromone has been identified for the given insect species. A refuge can be mimicked by offering the insects a shelter such as a cardboard band around a tree trunk (Massee 1925).

The apple blossom weevil, *Anthonomus pomorum* (L.) (Coleoptera: Curculionidae), is a univoltine herbivore and often a pest of apple trees, *Malus domestica* Borkh. Most adults overwinter in the leaf litter of forests or hedgerows, and migrate into orchards early in the season, as the smooth bark of dwarf apple trees in modern orchards offers little shelter (Toepfer et al. 2000). Weevils have been observed to colonise host trees by flying, although flight proclivity was limited (Toepfer et al. 1999, 2002). Crawling is thought to be an additional means of movement towards the tree and can already be observed at temperatures around freezing point (Duan et al. 1996). The orientation of the weevils towards the host tree may include plant-derived chemical cues (Kalinova et al. 2000), although experimental evidence on the behavioural effect of single or combined compounds is lacking, as is knowledge on pheromones in this species. After a period of feeding that allows ovarian maturation (maturation feeding) and mating, females injure the closed blossom buds and deposit single eggs therein. Hatched larvae feed inside the bud. This eventually leads to capped blossoms and, in many instances, to substantial yield loss (Balachowsky 1963). In the past decades, *A. pomorum* populations have reached damaging levels in many regions in Europe. This increase is attributed to the selective control of the codling moth and of aphids in orchards under organic and integrated crop management (Wildbolz 1992).
To date, insecticide sprays are the only effective means controlling *A. pomorum*, irrespective of management regime. The level of control required can be derived from damage in the preceding year (Anonymous 2001). One of the greatest challenges is timing an insecticide application to avoid unnecessary multiple treatments. The intervention should take place in the period between colonisation of the tree by a large number of individuals and the oviposition injury by female adults. Maturation feeding of the females takes about 2 weeks (Ctvrtecka & Zdarek 1992). Thus information gained from suitable monitoring could serve as a guide for accurately timing an insecticide application. Thus far, two different approaches have been taken to characterising the dynamics of spring colonisation by *A. pomorum*, and we propose to test further options.

One approach involves use of a cumulative heat unit model designed to predict the onset of colonisation by *A. pomorum*. Toepfer et al. (2002) found that the dispersal of overwintered weevils into an orchard starts when the thermal time totals reach 161 ± 27 degree-days (DD above 0°C, mean ± SD, calculated from 1 January). The period of highest dispersal speed began at 210 ± 26 DD (Toepfer et al. 2002). A principal drawback of this model is the relatively high standard deviation, corresponding to a range of approximately 2 weeks in early spring covering the 90% confidence intervals of the prediction. Consequently, this day-degree model must be complemented by additional monitoring.

A second approach, practised in recent field studies (Brown et al. 1993, Toepfer et al. 1999, 2000, 2002) involves limb jarring. Adult weevils dislodged from the branches of apple trees by tapping are subsequently counted on the white cloth onto which they fall. Recapture rates under dry and sunny conditions were 75 ± 19% (mean ± SD) of the weevils dwelling in the canopy (Toepfer et al. 1999). The principal shortcomings of this approach are its dependency on favourable weather conditions (Loewel 1936, Toepfer et al. 1999), and an inconvenient mode of sampling.

Further options for monitoring *A. pomorum* include exploitation of the different modes of tree colonisation, namely flight and crawling. These modes of motion have been exploited for developing traps in other curculionid pests of fruit and nut trees.
Adult flight can be observed with sticky clear Plexiglas traps designed for monitoring the migration of the plum curculio, *Conotrachelus nenuphar* (Herbst.) (Coleoptera: Curculionidae) (Prokopy et al. 2003). Adults colonising a tree by crawling up the trunk into the canopy can be caught with screen traps, developed by the Kansas pecan grower Edmund Circle and depicted and described in Mulder et al. (1997), for monitoring plum curculio and pecan weevil, *Curculio caryae* (Hinds) (Coleoptera: Curculionidae). Commercially available screen traps consist of an aluminium screen that can be wrapped tightly around the tree trunk, intercepting weevils crawling up the trunk. The weevils are guided to an attached inverted screen funnel where they are captured (Prokopy et al. 2003). Flight and screen traps have not yet been examined for *A. pomorum*.

An additional approach for monitoring increasing numbers of overwintered weevils makes use of their expected need for shelter. In general, insects, with their small size and proportionally large outer surface, are highly sensitive to changes in abiotic ambient conditions including temperature. They tend to escape from harsh ambient conditions to more favourable microniches (Willmer 1982). The most common mechanism of thermoregulation by insects is believed to be the short-term selection of thermally favoured microclimates (May 1979) such as the occupation of thermally favourable refuges (Willmer et al. 2000). *Anthonomus pomorum* has been found to seek shelter for hibernation, and rough bark provides an excellent refuge based on weevil preference and survival (Toepfer et al. 2000). Modern commercial orchards with dwarf trees are largely lacking in such hiding sites, and behavioural studies have indicated a relatively low adaptation of the weevil to dwarf trees with smooth bark (Toepfer et al. 2000). We postulate that artificial shelters mimicking rough bark are promising tools for monitoring *A. pomorum*. Indeed, early observations by Duan et al. (1996) indicated that the frequency of weevils occupying shelters or refuges increases when temperatures drop to low values.

The principal objective of this study was to quantify the performance of different monitoring techniques in the time course of orchard colonisation by *A. pomorum*. To monitor the temporal dynamics of the species in spring we developed a transparent
shelter trap and applied a Plexiglas flight trap as well as a commercial screen trap. The trap catches were contrasted to the limb jarring technique. An evaluation of the different methods was based on the need for a technique that allows for effective and reliable monitoring of *A. pomorum* irrespective of the weather conditions.

6.3. Materials and methods

*Study site*

All experiments were carried out in an organic apple orchard with an area of 0.9 ha located in Niederwil, Aargau (47°N, 8°E, elevation 440 m, Northern Switzerland). No insecticides were applied during the experimental period. The rows of dwarf trees in the orchard (4 m row spacing) were oriented perpendicular to an adjacent forest. The observations concerning ‘flight’ and ‘hiding’ were conducted in a part of the orchard bordered to the north by a mixed forest approximately 20 m high, to the south and west by apple trees, and to the east by fallow land. This part of the orchard consisted of a section of trees of the variety Retina, Idared, Florina, Liberty and Priam (2.5 m high), and a section of trees of the variety Jonathan, Spartan, and Roter Marlet (4 m high). Limb jarring and the observations with screen traps were carried out in an adjacent part of the orchard with dwarf apple trees of the variety Empire, Maigold, Glockenapfel, Florina, Renora, Rubinola, Resi, Rewene, and Liberty. The first six rows adjacent to the forest were used for the observations, as infestation levels at the boarder is typically highest (Brown et al. 1993, Toepfer et al. 1999). Trees were assigned to different treatments using a randomised plot design.

Climate data at the experimental site (air temperature and RH measured 2 m above ground level, solar radiation, wind speed and direction, and precipitation) were recorded every 10 min with an automatic weather station (Campell Scientific Ltd., Shepshed, Leicestershire, UK).
**Hiding dynamics**

A new design of shelter trap was developed to mimic relatively warm sites on the tree trunk. Each trap consisted of a 10 cm wide strip of transparent air bubble wrap (Folag AG, Sempach, Switzerland) wound twice around the trunk and attached with sticky tape. A brown plastic band of the same width was attached to protect the weevils from light. To prevent weevils from hiding in the bark beneath the trap, the bark was tightly covered with masking tape (Scotch 230, 3M, St. Paul, USA). Shelter traps were installed on 40 trees. Half of these traps were set up 40 cm above the ground, i.e., below the lowest branches ('low trap'), and the other half in mid-canopy ('high trap'). To assess the effect of exposition (cardinal direction), each shelter trap was divided into two equal parts, one facing the adjacent forest (north) and one the centre of the orchard (south). To test the effect of temperature, 10 trees with low traps and 10 trees with high traps were equipped with a piece of electric heating film (70 x 110 mm, 12 V, 12 W, Conrad Electronic, Hirschau, Germany) under the bubble wrap material that increased the within-trap temperature by 1.9 ± 0.6°C (mean ± SD, n = 218, measured at 15:00 h) during the day and by 2.1 ± 0.4°C (mean ± SD, n = 218, measured at 03:00 h) during the night.

To assess an influence of tree size, the diameter and height of the canopy was recorded and the volume of each canopy was then calculated as a rotation ellipsoid with the height as major axis and the diameter as minor axis. Trees with a canopy larger than 5 m³ were regarded as large trees, and those with a lower canopy size as small trees.

Observation of weevils hiding in the traps was made during 11 diel cycles at 3-4 day intervals (25/26 February, 28 February/1 March, 3/4 March, 7/8 March, 11/12 March, 14/15 March, 17/18 March, 20/21 March, 23/24 March, 26/27 March, 3/4 April 2002). Starting at 12:00 h each day, the traps were examined every 3 h by removing the brown plastic cover and counting the weevils hiding in the transparent bubble wrap. As the bubble wrap material did not have to be removed, the weevils hiding inside were not disturbed and the temperature in the trap was not altered. Temperatures both in the trap and on the surface of the trunk above the trap were measured each time using an
electronic thermometer with a type-T thermocouple probe (testoterm 901, Testo, Lenzkirch, Germany).

**Flight dynamics**

Clear circular Plexiglas discs (200 cm², 2 mm thick, Plexiglas XT, Röhm, Darmstadt, Germany) were used as flight traps. Twenty trees without shelter traps were equipped with flight traps fixed with plastic-coated iron wires. To assess the effect of exposition (cardinal direction), each tree was equipped with four discs each on a branch in mid-canopy (north – facing the forest, south – facing the orchard, east, west), and with two discs on the trunk 1 m above the ground (north – facing the forest, south – facing the orchard). The distal surfaces of the discs were covered with transparent insect glue (Tangle-Trap, The Tanglefoot Company, MI, USA). Prior to each observation cycle all insects were carefully removed from the discs with tweezers.

Weevils on the flight traps were counted and removed in 3-h intervals. Eleven diel observation cycles (24 h) were carried out, each starting at 12:00 h on the same dates as described above for the shelter traps. Additionally, 11 observations during the photophase (from 09:00 to 18:00 h) were conducted on days with an ambient air temperature of ≥ 10°C (i.e., 6, 9, 12, 13, 14, 16, 17, 18, 20, 21, and 22 March 2002). To estimate the total number of weevils colonising the tree by flying, the total projection area of the tree was calculated and compared with the area of the flight traps exposed in the four directions. For this estimation we assumed an equal distribution of weevils across the strata of the trees (cf. results with shelter traps: no difference between 'high' and 'low' traps). The height of the trees was recorded and a picture was taken of each tree. Subsequently, the projection area was calculated with the program Scion Image 1.62c (Scion Corporation, Frederick, USA).

**Dynamics of colonisation by crawling**

Screen traps (Great Lakes IPM, Vestaburg, MI, USA) are small-sized, commercially available traps developed for pecan weevils and plum curculio to catch all insects crawling up a tree trunk (Mulder et al. 1997). The trap consists of a wire screen
cage (33 cm wide, 20 cm high) capped with an inverted screen funnel for catching the insects. Screen traps were used on 20 trees without any other treatment and on the 20 trees equipped with flight traps. They were installed on the trunk approximately 40 cm above the ground (which is below the lowest branch), and examined every 3 h during the diel observation cycles on the same dates as the flight and shelter traps. The weevils in the screen traps were counted, and then released by placing them onto the lowest branch just above the screen trap.

*Limb jarring*

Limb jarring was carried out alongside the other methods, irrespective of weather conditions, although it is only recommended for dry and sunny afternoons (Lafleur & Hill 1987, Toepfer et al. 1999). Two white sheets (160 x 220 cm) were laid out on the ground close to the tree trunk. Each tree was beaten three times with the end of a wooden stick (diameter 5 cm, 90 cm long), wrapped in a 3 cm thick layer of rubber foam fastened with an elastic band. At each sampling time, five trees that were not being used for any other treatment were randomly chosen within the first six rows. The weevils that dropped off a tree were collected from the sheets, counted and then released again at the bottom of the tree trunk.

*Data analysis*

The captures with all methods tested were related to the following climatic parameters by Pearson’s correlation: mean temperature, maximum daily temperature, precipitation, and daily sunshine period.

The temporal dynamics of weevils hiding in shelter traps were tested for significant deviation from random distribution during the diurnal cycles using \( \chi^2 \)-tests (Zar 1998). Temporal dynamics of captures on the flight traps were analysed for deviation from random distribution by a Friedman test according to the high number of zero counts in the data set (Zar 1998). The spatial dynamics of captures on the flight traps were analysed by \( \chi^2 \)-tests with respect to orientation on the branches and on the tree trunk.
The relationships between ambient air temperature (averaged for 3 h) and the number of weevils in shelter traps, flight traps, and screen traps were analysed by Pearson’s correlation and tested for significance with Fisher’s P. The same correlation analysis was applied to test for significant relationships between the differences in numbers of weevils and the temperature differences between: (a) the shelter traps facing the forest (north) and facing the orchard (south), (b) the traps with and without heating, and (c) the low and high traps. Furthermore, Pearson’s correlation coefficient was also used to investigate relationships between the numbers of adults caught with the different methods over the entire season and over the critical colonisation period, i.e., the first six of the 11 diel cycles studied.

Detailed statistical analyses for the shelter traps were carried out for those three diel observation cycles in which more than 50 weevils had been caught. The spatial dynamics of weevils hiding in the shelter traps was investigated by analyses of variance with repeated measurements (RM ANOVA). The counts during the diel cycle were considered as repeated measurements with the factors: exposition of the trap (north, south), heating (with, without), position of the trap (low, high), position of the tree (row 1-6), and size of the tree (large, small). Accordingly, the temperature difference between the trunk next to the shelter trap and inside the trap was analysed by RM ANOVA with respect to the same factors: exposition of the trap (north, south), heating (with, without), position of the trap (low, high), position of the tree (row 1-6), and size of the tree (large, small). Over all measurements, the temperature differences between tree trunk and shelter traps were analysed separately by one-sample t-tests for heated and unheated traps.

In order to estimate the number of weevils colonising trees by flying, a ratio of the total vertical projection area of each tree to the area of the flight traps in a certain direction was calculated and multiplied with the number of weevils caught in that direction.

The thermal time totals above 0°C, calculated from 1 January, for a prediction of the onset of spring dispersal and of maximum dispersal speed of *A. pomorum* were calculated following the heat unit model of Toepfer et al. (2002).
The data was analysed with the software StatView 5.0.1 for Apple Macintosh (SAS Institute Inc.).

6.4. Results

**Hiding dynamics**

Shelter trap captures peaked at 2.4 ± 3.4 weevils per tree (mean ± SD) on 11/12 March at 253 DD (above 0°C, calculated from 1 January). Weevils were first detected in shelter traps at 222 DD on 7 March. There was no significant correlation between numbers of weevils caught during all diel observations and any of the climatic parameters considered: daily mean temperature ($r = 0.13, n = 11, P = 0.722$), maximum temperature ($r = 0.40, n = 11, P = 0.228$), precipitation ($r = -0.21, n = 11, P = 0.548$), or daily sunshine period ($r = 0.21, n = 11, P = 0.551$).

The more detailed analyses of hiding dynamics presented below focussed on the three diel cycles in which 88.9% of all captures were made (cycle A: 11/12 March, 354 weevils; cycle B: 14/15 March, 106 weevils; cycle C: 17/18 March, 61 weevils).

A clear diurnal cycle was observed in trap captures ($\chi^2$-test, $\chi^2 = 38.51$, d.f. = 7, $P < 0.001$), culminating in the morning between 03:00 and 06:00 h (Figure 6.1). The number of weevils caught was negatively correlated to the ambient air temperature ($r = -0.52, n = 24, P = 0.008$). The temperature in the unheated shelter traps was on average 0.26 ± 0.54°C higher than the temperature on the surface of the trunk (t-test, $t = 10.71$, d.f. = 479, $P < 0.001$).

Heating of the shelter traps did not influence the number of weevils caught (RM ANOVA, cycle A: $F_{1,78} = 0.31, P = 0.558$; cycle B: $F_{1,78} = 0.44, P = 0.511$; cycle C: $F_{1,78} = 0.21, P = 0.645$). It resulted in a temperature within the trap which was 1.90 ± 0.99°C higher than the temperature on the surface of the trunk (t-test, $t = 42.24$, d.f. = 479, $P < 0.001$), and significantly higher than in the unheated shelter trap (RM ANOVA, cycle A: $F_{1,78} = 186.11, P < 0.001$; cycle B: $F_{1,78} = 222.57, P < 0.001$; cycle
Comparing the traps with and without heating, no significant correlation was noted between the differences in numbers of weevils trapped and the temperature differences of the traps during the 3 h periods (cycle A: $r = 0.09$, $n = 8$, $P = 0.847$; cycle B: $r = 0.28$, $n = 8$, $P = 0.520$; cycle C: $r = 0.16$, $n = 8$, $P = 0.726$).

Figure 6.1. Average proportion (± SE) of *A. pomorum* hiding in shelter traps (bars) during the different time intervals on the three diel observation cycles with more than 50 weevils caught compared to the total number of weevils found during these diel observation cycles ($n = 523$ weevils). Average difference (± SE) between the ambient air temperature and the minimum temperature (■) during a diel cycle period (averaged over 3 h).

No significant influence of exposition, that is the proportion of weevils hiding underneath that part of the shelter oriented either towards the north or south, was noted in two out of three diel cycles considered. In diel cycle C, however, a higher number of
weevils was counted in that part of the trap facing north, i.e., the forest (RM ANOVA, $F_{1,78} = 5.04, P = 0.028$), and mean values of captures during diel cycles A and B were higher for the part facing the forest (Figure 6.2), but these differences were not significant. Exposition changed the difference of temperature between trap and open tree trunk only in one of the diel cycles in a significant way (RM ANOVA, cycle A: $F_{1,78} = 2.26, P = 0.137$, cycle B: $F_{1,78} = 5.04, P = 0.028$, cycle C: $F_{1,78} = 3.93, P = 0.051$). No significant correlation was found during any 3 h period between the differences in numbers of weevils in the traps facing north (forest) and south (orchard) and the corresponding differences in temperatures (cycle A: $r = -0.23, n = 8, P = 0.593$; cycle B: $r = 0.02, n = 8, P = 0.969$; cycle C: $r = -0.38, n = 8, P = 0.366$).

Trap position, either on the trunk below or above the branching point, did not influence the number of weevils nor the temperature differences between the trap and the open tree trunk (RM ANOVA, $P > 0.1$). The numbers of weevils hiding in the low traps vs. those in the high traps were 215 against 140 (cycle A), 63 against 43 (cycle B), and 25 against 36 (cycle C). The position of the traps, either low or high, did not significantly influence captures (RM ANOVA, cycle A: $F_{1,78} = 1.20, P = 0.277$; cycle B: $F_{1,78} = 0.68, P = 0.411$; cycle C: $F_{1,78} = 0.53, P = 0.469$). Comparing the low and high traps, no significant correlation was found between the differences in numbers of weevils and the temperature differences between them (cycle A: $r = 0.62, n = 8, P = 0.107$; cycle B: $r = 0.16, n = 8, P = 0.723$; cycle C: $r = -0.15, n = 8, P = 0.731$).

The position of the tree with respect to distance to the forest border did not influence the number of weevils caught (RM ANOVA, cycle A: $F_{5,74} = 1.33, P = 0.261$; cycle B: $F_{5,74} = 1.73, P = 0.138$; cycle C: $F_{5,74} = 0.71, P = 0.619$), but it influenced the temperature differences between trap and open tree trunk (RM ANOVA, cycle A: $F_{5,74} = 3.56, P = 0.006$; cycle B: $F_{5,74} = 3.76, P = 0.004$; cycle C: $F_{5,74} = 3.21, P = 0.011$).

Tree size did not influence the temperature differences between trap and open tree trunk (RM ANOVA, cycle A: $F_{1,78} = 0.02, P = 0.880$; cycle B: $F_{1,78} = 0.04, P = 0.853$; cycle C: $F_{1,78} = 0.92, P = 0.340$), but during cycles A and B significantly more weevils were caught on the large trees (RM ANOVA, cycle A: $F_{1,78} = 22.48, P < 0.001$; cycle B: $F_{1,78} = 14.48, P < 0.001$; cycle C: $F_{1,78} = 3.50, P = 0.065$).
Figure 6.2 (a-c). Effect of exposition on the proportion of *A. pomorum* hiding in shelter traps compared to the total number of weevils hiding during one diel observation cycle (cycle A: 354 weevils, cycle B: 106 weevils, cycle C: 61 weevils). The black bars indicate the number of weevils hiding in that part of the shelter trap facing the forest (north) and the grey bars indicate the number of weevils in the part facing the orchard (south). The differences between ambient air temperature and minimum daily temperature (*T*<sub>min</sub>) are indicated by ▲ (averaged over 3 h). 

Factor exposition: forest vs. orchard

**Cycle A**

- *T*<sub>min</sub> = 1.8°C
- Proportion of weevils hiding: 25%

**Cycle B**

- *T*<sub>min</sub> = 0.01°C
- Proportion of weevils hiding: 25%

**Cycle C**

- *T*<sub>min</sub> = 2.9°C
- Proportion of weevils hiding: 25%
Flight dynamics

Flight trap captures peaked at 0.35 ± 0.6 weevils per tree (mean ± SD) on 11/12 March at 253 DD. Weevils were first detected in flight traps at 238 DD on 9 March. Considering all diel observation cycles, there was no significant correlation of the number of weevils on the flight traps and any of the climatic parameters measured: daily mean temperature ($r = 0.23, n = 11, P = 0.515$), maximum temperature ($r = 0.53, n = 11, P = 0.099$), precipitation ($r = -0.22, n = 11, P = 0.533$), or daily sunshine period ($r = 0.50, n = 11, P = 0.121$).

A clear diurnal cycle was observed in trap captures (Friedman, $\chi^2 = 2.46, \text{d.f.} = 7, P = 0.017$), as all captures were made between 09:00 and 15:00 h, i.e., during photophase. In observations restricted to days with a temperature maximum above 10°C and to the photophase, the number of weevils caught was positively correlated with ambient temperature ($r = 0.36, n = 33, P = 0.042$). A total of eight weevils were caught between 09:00 and 12:00 h, 16 between 12:00 and 15:00 h, and 10 between 15:00 and 18:00 h, but the variation in flight with respect to those three time intervals was not significant ($\chi^2$-test, $\chi^2 = 3.06, \text{d.f.} = 2, P = 0.217$).

Exposition influenced the captures on flight traps, both at the level of the canopy and at the level of the tree trunk (Figure 6.3, $\chi^2$-test, trunk: $\chi^2 = 4.26, \text{d.f.} = 1, P = 0.039$; branches: $\chi^2 = 29.35, \text{d.f.} = 3, P < 0.001$). The highest proportion of weevils (73.8%) was caught on the flight traps facing north, i.e., the forest. Twenty-three weevils were caught in traps at the level of branches and 19 in the trunk zone.

By extrapolating the size of the traps to cover the complete vertical projection of each tree, the total number of weevils colonising a tree by flight was estimated as 23.7 weevils per tree.
Figure 6.3. Spatial distribution of *A. pomorum* captures on flight traps during spring colonisation. Different letters (upper case for tree trunk, lower case for branches) indicate significant differences at the 0.05-level according to the χ²-test. n = 42 weevils.

**Dynamics of colonisation by crawling**

Screen trap captures peaked at 1.1 ± 1.7 weevils per tree (mean ± SD) on 11/12 March at 253 DD. Weevils were first detected in screen traps at 222 DD on 7 March (Figure 6.4). Considering the captures during the entire diel cycles, the maximum daily temperature was positively correlated with the captures in screen traps ($r = 0.68$, $n = 11$, $P = 0.019$). Mean temperature ($r = 0.54$, $n = 11$, $P = 0.086$), precipitation ($r = -0.14$, $n = 11$, $P = 0.692$), and daily sunshine period ($r = 0.14$, $n = 11$, $P = 0.686$) were not significantly correlated with the number of weevils caught in the screen traps. Captures by screen traps on the 20 trees equipped with both screen and flight traps did not differ significantly from captures on the 20 trees equipped with screen traps only (t-test, $t = 1.67$, d.f. = 38, $P = 0.104$).
There were no significant diurnal cycles in screen trap captures noted in the diel cycle experiments ($\chi^2$-test, $\chi^2 = 10.79$, d.f. = 7, $P = 0.148$), although average numbers of weevils caught were highest between 12:00 and 15:00 h. In observations restricted to days with a temperature maximum above 10°C and to the photophase, differences in weevil abundance in the traps were significant between the three sampling periods ($\chi^2$-test, $\chi^2 = 8.91$, d.f. = 2, $P = 0.012$). The ambient air temperature averaged over 3 h was positively correlated to the number of weevils in the screen traps in the diel observation cycles ($r = 0.50$, $n = 88$, $P < 0.001$) as well as in the observations during the photophase ($r = 0.59$, $n = 33$, $P < 0.001$).

Considering the total observation period, the number of weevils colonising one tree by crawling as assessed by screen trap averaged at 19.4.

**Limb jarring**

The number of weevils collected by limb jarring peaked on three subsequent sampling dates with 10.3 ± 13.6, 10.7 ± 13.4, and 10.7 ± 9.9 weevils per tree (mean ± SD) on 11/12 March at 253 DD, 14/15 March at 279 DD, and 17/18 March at 303 DD, respectively. The first few weevils were detected on 28 February at 197 DD (Figure 6.4).

The maximum daily temperature was positively correlated with the number of weevils counted during limb jarring ($r = 0.70$, $n = 11$, $P = 0.014$), while mean temperature ($r = 0.53$, $n = 11$, $P = 0.093$), precipitation ($r = -0.25$, $n = 11$, $P = 0.474$), and daily sunshine period ($r = 0.20$, $n = 11$, $P = 0.574$) were not significantly correlated.

**Correlations between the methods**

The methods monitoring the movement of the overwintered weevils towards the tree, the flight trap, and the screen trap yielded captures which were highly and positively correlated with each other ($r = 0.66$, $n = 11$, $P = 0.024$). This also applied when only the critical colonisation period is considered, i.e., the first six of the 11 diel cycles studied ($r = 0.98$, $n = 6$, $P < 0.001$). The two methods monitoring weevils on the tree, the shelter trap and limb jarring, resulted in quantifications that correlated
positively during the critical colonisation period ($r = 0.82$, $n = 6$, $P = 0.047$). No significant correlation was found over the whole spring season ($r = 0.54$, $n = 11$, $P = 0.086$). Captures in shelter traps were significantly and positively correlated with both the captures on flight and in screen traps over the entire season ($r = 0.95$, $n = 11$, $P < 0.001$; $r = 0.67$, $n = 11$, $P = 0.021$, respectively), and also when only the critical colonisation period is considered ($r = 0.96$, $n = 6$, $P = 0.001$; $r = 0.99$, $n = 6$, $P < 0.001$, respectively). Numbers of weevils assessed by limb jarring over the entire season did not correlate with those assessed by flight traps ($r = 0.42$, $n = 11$, $P = 0.197$) but correlated positively with those assessed by screen traps ($r = 0.80$, $n = 11$, $P = 0.002$). Concentrating on the critical colonisation period, no correlation was found in either case ($r = 0.61$, $n = 6$, $P = 0.217$; $r = 0.76$, $n = 6$, $P = 0.087$).
Figure 6.4. Time course of *A. pomorum* captures with progressing season in shelter traps, flight traps, screen traps, and with limb jarring. The values for the different traps are shown as an average number of weevils caught per day and tree.
6.5. Discussion

The newly developed shelter trap caught a high number of *A. pomorum* adults. It exploited the weevils' need for shelter triggered by the temperature fluctuation between day and night, and was otherwise independent of weather conditions. The transparent material of the shelter trap allowed for repeated assessments without disturbing insects and their microclimatic environment. Captures from shelter traps were highly correlated with the results from methods that characterised the time course of colonisation of the tree by flight or by crawling (flight trap, screen trap). In contrast, the results from the limb jarring were not correlated with those methods in the critical colonisation period. Moreover, the flight trap and screen trap proved to be less favourable than the shelter trap. The flight trap resulted in captures that were lower by an average factor of 24. The screen trap, used here for the first time for *A. pomorum*, caught five times fewer weevils than the shelter trap, and captures were dependent on maximum daily temperature.

Our findings using the shelter trap support earlier observations indicating that the proportion of weevils occupying shelters or refuges increased when temperatures dropped to low values (Duan et al. 1996). Temperatures fell below 5°C almost every night in the diel observation cycles. Such low temperatures have been shown to significantly suppress weevil activities such as crawling, feeding and mating (Duan et al. 1996). Microclimate exploitation and the occupation of thermally favourable refuges are particularly important in small species (Willmer et al. 2000) that are forced by their lifecycle adaptation to be active under relatively unfavourable conditions as for example *A. pomorum*. The shelter trap represents such a thermal refuge for the apple blossom weevil. *Anthonomus pomorum* occupies this microhabitat to a maximum degree during the thermally most unfavourable part of the day. Its relatively small body size precludes endothermic regulation by metabolic heat production, which would allow for an independence of body temperature from environmental conditions (Heinrich 1993). The temperature level within the shelter trap was slightly, but significantly, higher than on the trunk. A further experimental increase of this temperature difference by heating the trap did not increase the number of weevils in the shelter. This indicates that the easily
accessible and inexpensive bubble wrap material used for building this trap suffices to
generate the microclimate required by the shelter-seeking adults. Our finding confirms
the hypothesis that insects choose appropriate thermal sites to avoid unfavourable
environmental conditions (Casey 1981).

No consistent effect of trap exposition, that is the proportion of weevils hiding
underneath that part of the shelter oriented towards the north or south, was found in our
study. In any case, a possible effect of trap exposition on captures remains without
consequence on future applications of the shelter trap, as counts will be taken of the
total number of weevils hiding in one shelter trap, and not in a certain part of this trap.
This is feasible as total numbers scarcely exceeded 10 per trap and count.

Trap position, on the trunk below or above the branching point, has no discernable
effect on the number of weevils caught in the present study. Concurrently, the number
of weevils colonising the tree by crawling as assessed by screen traps was in the same
order of magnitude as the number of weevils colonising the tree by flight as assessed by
flight traps and extrapolated to the vertical projection of the tree. With the progression
of the seasons, the average number of weevils hiding decreased in the lower parts of the
tree and increased within the canopy, though these differences were not significant. A
clear movement of the weevil population from low to higher parts of the tree was
reported for the plum curculio (Chouinard et al. 1994). During a 6-day period at the
beginning of tree colonisation, up to 28% of the plum curculios were observed resting
on the ground at the base of the trees. Later in the season the plum curculios were found
in the canopy and no longer on the trunk (Chouinard et al. 1994). A shift from trunk to
canopy is obviously less prominent in A. pomorum. This might be due its higher general
mobility or to a higher proportion of weevils colonising the tree by flight in A. pomorum
as compared to the plum curculio.

Our study provides, for the first time, empirical data underlining the importance
of tree colonisation by crawling in adult A. pomorum. While flight has been indicated to
be an important mode of movement towards the host tree (e.g., Toepfer et al. 2002),
little information has been available on alternative methods of colonisation. The screen
trap proved a useful tool for assessing the number of weevils crawling up from the base
of the tree. The temporal dynamics of the captures in the screen traps correlated significantly and positively with those on the flight traps, indicating that both modes of movement are readily utilised by migrating *A. pomorum*. This is compatible with a previous release-recapture study, which reported that a substantial proportion of 38 ± 6.5% of the released population remained within a 10 m radius of the release point, while a small proportion of 6 ± 1% dispersed over 40 m, up to a maximum distance of 90 m (Toepfer et al. 1999).

The seasonal course of colonisation is well reflected by the shelter trap captures. This is strongly supported by their correlation with captures on flight traps and in screen traps. These correlations indicate that changes in shelter trap captures reflect changes in the numbers of weevils colonising the tree. The maximum number of captures coincided for all three trapping tools employed. In contrast to the traps tested, sampling by the limb jarring technique yielded information which deviated from the other methods at the very beginning of the season, as well as during the period of highest captures and thereafter. Some weevils were detected by limb jarring before any of the traps caught adults, and captures remained at a very high level for three subsequent sampling dates around the culmination point. Although only useful during dry and sunny weather, limb jarring seems to be a very sensitive method for detecting weevils. However, it depicts peaks of colonisation in a less distinct way than the other methods. This is also reflected by the fact that during the critical colonisation period, i.e., the first six of our 11 diel observation cycles, the numbers of weevils assessed by limb jarring failed to correlate with those assessed by flight traps and by screen traps.

The cumulative heat unit model (Toepfer et al. 2002) predicted an onset of colonisation at 161 ± 27 degree-days (DD). The thermal time totals reached at the time of first weevil detection by limb jarring in our study amounted to 197 DD and thus fits the model. Furthermore, the model forecasted the beginning of the period with highest speed of dispersal (dislocation per day) for the thermal time totals of 210 ± 26 DD (Toepfer et al. 2002). We expected the colonisation of the trees to peak shortly afterwards. Our findings confirm this hypothesis, as all the traps consistently caught most weevils at the thermal time totals of 253 DD, i.e., approximately 4 days after the
thermal time totals of 210 DD predicted for maximal dispersal speed (Toepfer et al. 2002) were reached. The actual timing of the maximal captures could even have been closer to the timing of the maximal speed of colonisation, as our assessments were only made at 3-day intervals.

In conclusion, a number of criteria qualify the newly designed shelter trap as the most promising method for monitoring *A. pomorum* during tree colonisation. The abundance of weevils is high, and the captures are independent of weather conditions and of trap position in the tree. Captures by shelter traps over the spring season correlated with captures by both methods monitoring weevil movement towards the tree, i.e., flight traps and screen traps. Furthermore, the trapped insects are very visible underneath the transparent bubble wrap, and quantification does not require any manipulation of the weevils. If observations are made daily, preferably before 09:00 h, captures by such traps could prove most useful in reflecting tree colonisation by adults. The shelter trap offers a promising tool for timing interventions. The day-degree model developed by Toepfer et al. (2002) can be recommended as an indication of when shelter traps should be installed on the trees. Further studies will have to evaluate the relationship between the colonisation dynamics and the optimal timing of insecticide application, as well as between weevil abundance in shelter traps and the number and rate of blossoms infested by *A. pomorum*. 
7. Significance of shelter traps for spring monitoring of *Anthonomus pomorum* in apple orchards

7.1. Abstract

The significance of the apple blossom weevil, *Anthonomus pomorum* (L.) (Coleoptera: Curculionidae), as a potential threat to apple, *Malus domestica* Borkh., increased during the past two decades in many regions of Europe. The apple blossom weevil immigrates into orchards and colonises apple trees in early spring. Females deposit single eggs into closed blossom buds, which leads to capped blossoms. This study was designed to test the suitability of transparent shelter traps for surveillance of colonisation under different population pressures, and to assess the quantitative relationship between number of weevils caught and: (1) absolute injury, expressed as number of infested buds, as well as (2) relative injury, expressed as percentage of infested buds relative to all buds on the tree. The findings with the new shelter traps were contrasted to results from limb jarring. Numbers of weevils caught with the transparent shelter trap were positively correlated with absolute injury, as well as with relative injury across the five study sites. While, weevil counts with limb jarring were not correlated with absolute injury, but only with relative injury. Our data validate the transparent shelter trap as a method for accurately monitoring the course of spring colonisation of *A. pomorum* and demonstrate its potential for predicting injury by the weevils to blossom buds. In contrast, the significance of limb jarring for monitoring both the time course of colonisation and subsequent injury is limited. The significant linear relationship between the number of weevils caught in shelter traps and the relative injury allows for predictions of potential damage before oviposition takes place, i.e., early enough for an insecticide application where necessary.

7.2. Introduction

The apple blossom weevil, *Anthonomus pomorum* (L.) (Coleoptera: Curculionidae), is a univoltine herbivore and frequently occurs as a pest of apple trees, *Malus domestica* Borkh., in many regions of Europe (Cross et al. 1999). Dwarf apple trees in modern orchards offer few shelters and therefore most adults overwinter in adjacent forests or hedgerows (Toepfer et al. 2000). In early spring, weevils immigrate into orchards and colonise apple trees. After a period of maturation feeding for ovarian development and copulation, females deposit single eggs into the closed blossom buds. Hatched larvae feed inside the buds, eventually leading to capped blossoms and, in many instances, to substantial yield loss (Balachowsky 1963). Over the past few decades, *A. pomorum* populations increased to damaging levels in many regions of Europe. This resurgence is attributed to the selective control of the codling moth and aphids in orchards under organic and integrated crop management (Wildbolz 1992).

To date, insecticide sprays are the only effective means to control the apple blossom weevil irrespective of the management regime. One of the greatest challenges for effective management of the apple blossom weevil in commercial orchards is to determine the need for and timing of these applications, in order to protect blossom buds from injury by adults and avoid unnecessary multiple treatments. To prevent injury to the blossoms, interventions must be implemented in the period between colonisation of the tree and oviposition.

Several candidate trapping methods, including clear Plexiglas flight traps, screen traps, and transparent shelter traps, have been examined for monitoring apple blossom weevils (Hausmann et al. 2004). The most promising trapping system is a transparent shelter trap which is fixed around the trunk of an apple tree as soon as the thermal time totals for the start of dispersal, i.e., 161 ± 27 degree-days (DD, mean ± SD) above 0°C calculated from 1 January, are reached (Toepfer et al. 2002). The numbers of weevils caught with transparent shelter traps have proven to be independent of weather conditions, showing the seasonal course of colonisation by a relatively high number of weevils when examined early in the morning before 09:00 h. In contrast, counts with
the limb jarring technique depicted culmination of colonisation in a less distinct way. Although limb jarring was very sensitive for detecting weevils, its effectiveness varied with weather conditions and it is thus less reliable than shelter traps (Hausmann et al. 2004). Other methods for monitoring the apple blossom weevils, as for example traps using semiochemicals, are not available yet. The orientation of the weevils towards the host tree may include plant-derived chemical cues (Kalinova et al. 2000, Innocenzi et al. 2003), though quantified experimental evidence on the behavioural effect of single or combined compounds is lacking, as is knowledge on pheromones in this species.

An effective method for monitoring *A. pomorum* should not only detect weevil occurrence, but should also allow for conclusions about the level of bud injury to be expected. The present study was carried out to investigate relationships between numbers of *A. pomorum* caught with transparent shelter traps and injury to buds at study sites with different population pressures. The design of the study further allows us to test the reliability of this method for predicting maximum migration irrespective of biotic and abiotic environmental conditions. In order to test whether prediction of damage by the shelter trap depends on exactly encountering the day of maximum catches, the relationships between weevil counts and injury by oviposition were also investigated for a period covering seven days around that day. The results from the shelter traps were contrasted to corresponding data based on the limb jarring method which has been widely used in earlier studies on the seasonal dynamics of *A. pomorum* (e.g., Brown et al. 1993, Toepfer et al. 1999, Toepfer et al. 2002).

7.3. Material and methods

**Study sites**

Experiments were conducted at five different study sites, located in apple orchards under organic or integrated crop management in northern Switzerland. No insecticides were applied during the experimental period. Study sites A, B and C were
located in Niederwil, Aargau (47°23'N, 8°17'E, elevation 440 m, Northern Switzerland). Study sites A and B were each located in the 6 tree rows of the orchards (9,000 m² and 20,000 m² large) adjacent to the forest border and were expected to have high infestation of *A. pomorum*, since generally the abundance of weevils is highest in the tree rows next to a forest (Brown et al. 1993, Toepfer et al. 1999). Study site C was located in a part of an orchard (20,000 m² large) at a distance of approximately 150 m from the forest border and consequently little infestation by *A. pomorum* was expected. Study site A included trees of the variety Retina, Idared, Florina, Liberty, Priam and Spartan (3 m high, canopy volume approximately 7 m³). Study sites B and C included trees of the variety Empire, Maigold, Glockenapfelf, Florina, Renora, Resi, Rewene and Liberty (3 m high, canopy volume approximately 6 m³; 2.5 m high, canopy volume approximately 5.5 m³, respectively). Study site D, with trees of the variety Idared (2 m high, canopy volume approximately 1.5 m³), and study site E, with trees of the variety Gala (2.5 m high, canopy volume approximately 2 m³), were situated in Künten, Aargau (47°24'N, 8°19'E, elevation 437 m, Northern Switzerland) in an orchard (50,000 m² large) bordered on two sides by forest. Sites D and E were located in a part of the orchard at a distance of approximately 100 m from the forest and, accordingly, low to very low infestation by *A. pomorum* was expected.

Climatic data in Niederwil and Künten (air temperature and RH measured 2 m above ground, solar radiation, precipitation) were recorded throughout the study every 10 min with automatic weather stations (site A: Campell Scientific Ltd., Shepshed, Leicestershire, England; site D: Lufft Mess- und Regeltechnik GmbH, Fellbach, Germany).

**Shelter traps**

In each study site, 20 trees were chosen according to randomised plot design and equipped with shelter traps described in detail by Hausmann et al. (2004). In principle, each trap consisted of a 10 cm wide strip of transparent air bubble wrap (bubble diameter 1 cm, Folag AG, Sempach, Switzerland) wound two times around the tree trunk and fixed with sticky tape. A brown plastic band of the same height was attached
to protect the weevils from light. To prevent the weevils from hiding in the bark beneath the trap, the bark was tightly covered with masking tape (Scotch 230, 3M, St. Paul, USA). Shelter traps were installed approximately 1 m above the ground. From mid February on, limb jarring was carried out once a week to determine the start of orchard colonisation. Apple blossom weevils were first detected on 11 March 2003. During the spring colonisation period (from 12 March to 17 April 2003), each trap of all the study sites was examined once every day between 06:00 and 09:00 h in random order. Since the weevils were able to enter and leave the shelter trap freely during the periods of activity, the counts made on the different days did not necessarily assess the same individuals. The traps were examined by removing the brown plastic cover and counting the weevils hiding in the transparent bubble wrap. As the bubble wrap did not have to be removed, the weevils hiding within were not disturbed and the temperature in the trap was not altered by the checks. The trials were conducted throughout the colonisation period until no weevils were found in the shelter traps for three consecutive days.

**Limb jarring**

On every observation day without rainfall, weevils were sampled with the limb jarring method (Lafleur & Hill 1987) in the afternoon (12.00 – 15.00 h). In each study site, 5 trees without shelter traps were chosen according to randomised plot design. Two white sheets (160 x 220 cm) were laid out on ground close to the tree trunk. Each tree was beaten three times with the top end of a wooden stick (diameter 5 cm, 90 cm long), wrapped in a 3 cm thick layer of rubber foam fastened with a plastic band. The weevils that dropped off these trees were collected from the sheets, counted and then released at the bottom of the trunk. The sampling of 5 trees with the limb jarring method took approximately as much time as did examination of shelter traps on 20 trees.

**Injury scoring**

In May, the total number of capped blooms of every tree that was equipped with a shelter trap was recorded to evaluate whether the number of weevils caught correlated
with absolute bud injury. The absolute injury represents the first important parameter since it is considered to correspond to the abundance of weevils, as females lay their eggs singly into closed apple buds. In addition, the total number of blossoms per tree was counted to calculate the second parameter, the relative injury of the tree, defined as percentage of capped blossoms relative to all blossoms per tree. The relative injury is an important value as it relates counts to the economic threshold.

Data analysis

The numbers of weevils caught with shelter traps and the counts with limb jarring in relation to climatic parameters, i.e., mean temperature, maximum daily temperature, precipitation and daily sunshine period, were analysed with Pearson's correlation coefficient and tested for significance with Fisher's P (Zar 1998).

Pearson's correlation was also applied to analyse the relationships between the number of weevils caught with shelter traps on the day with maximum catches or during the seven-day period of maximum catches (maximum catches ± 3 days) and the absolute injury or the relative injury. These analyses were carried out at the level of study sites, at the level of trees within each single study site, and finally at the level of trees across all study sites. If a significant correlation was found (0.05 level), a linear regression analysis was performed to give the statistics necessary for a prediction of absolute injury and relative injury from the weevil counts.

At the level of study sites, the same correlation and regression analyses were performed for the relationship between the number of weevils counted with limb jarring on the day with maximum catches or during the seven-day period of maximum catches and the absolute injury or the relative injury, as this information is most relevant for determining the need for an application.

The data was analysed with the software StatView 5.0.1 for Apple Macintosh (SAS Institute Inc.).
7.4. Results

Maximum numbers of weevils caught with shelter traps at site A (3.10 ± 6.40 weevils per trap, mean ± SD), site B (3.05 ± 4.36) and site E (0.15 ± 0.49) occurred on 22 March (Figure 7.1; day 81 of the year), at site D (0.1 ± 0.31) on 23 March and in site C (0.25 ± 0.55) on 26 March (Figure 7.1). The maximum counts with limb jarring at site A (14.80 ± 8.70 weevils per tree, mean ± SD) occurred on 25 March (Figure 7.2; day 84 of the year), at site B (16.20 ± 12.83) on 29 March, at site C (8.80 ± 6.98) on 28 March, at site D (3.80 ± 1.92) on 24 March and at site E on 20 March (2.60 ± 2.41) and on 25 March (2.60 ± 2.97) (Figure 7.2).

At all five study sites, the number of weevils hiding in shelter traps was not significantly influenced by maximum ambient air temperature, average ambient air temperature, precipitation, or daily sunshine period (Table 7.1). The weevil numbers counted with limb jarring at sites A, B and C were positively correlated with the maximum ambient air temperature, while the counts with limb jarring at study sites B and C were also correlated with average ambient air temperature (Table 7.1).

Table 7.1. Correlations of the number of A. pomorum hiding in shelter traps and the numbers of weevils counted with limb jarring with the weather parameters: maximum ambient air temperature, average ambient air temperature, precipitation, and daily sunshine period.

<table>
<thead>
<tr>
<th>Site</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>shelter trap (n = 38)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>maximum ambient air temperature</td>
<td>r = 0.16</td>
<td>r = 0.31</td>
<td>r = 0.21</td>
<td>r = -0.26</td>
<td>r = -0.05</td>
</tr>
<tr>
<td>average ambient air temperature</td>
<td>P = 0.352</td>
<td>P = 0.054</td>
<td>P = 0.221</td>
<td>P = 0.351</td>
<td>P = 0.770</td>
</tr>
<tr>
<td>precipitation</td>
<td>r = -0.05</td>
<td>r = 0.11</td>
<td>r = 0.10</td>
<td>r = -0.15</td>
<td>r = -0.045</td>
</tr>
<tr>
<td>daily sunshine period</td>
<td>P = 0.749</td>
<td>P = 0.531</td>
<td>P = 0.539</td>
<td>P = 0.366</td>
<td>P = 0.833</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>limb jarring (n = 36)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>maximum ambient air temperature</td>
<td>r = 0.38</td>
<td>r = 0.34</td>
<td>r = 0.41</td>
<td>r = 0.10</td>
<td>r = 0.23</td>
</tr>
<tr>
<td>average ambient air temperature</td>
<td>P = 0.020</td>
<td>P = 0.042</td>
<td>P = 0.012</td>
<td>P = 0.579</td>
<td>P = 0.188</td>
</tr>
<tr>
<td>daily sunshine period</td>
<td>r = 0.29</td>
<td>r = 0.46</td>
<td>r = 0.42</td>
<td>r = -0.07</td>
<td>r = 0.04</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>maximum ambient air temperature</td>
<td>r = 0.092</td>
<td>P = 0.005</td>
<td>P = 0.010</td>
<td>P = 0.704</td>
<td>P = 0.814</td>
</tr>
<tr>
<td>average ambient air temperature</td>
<td>P = 0.325</td>
<td>P = 0.668</td>
<td>P = 0.391</td>
<td>P = 0.051</td>
<td>P = 0.127</td>
</tr>
</tbody>
</table>
Figure 7.1. Time course of the numbers of A. pomorum caught in shelter traps with progressing season (time period from 8 March to 17 April 2003) at the study sites A, B, C, D and E. The values for the different traps are shown as average number of weevils caught per day and tree (bars). The lines in the lower graph indicate the mean daily temperatures for the different study sites.
Figure 7.2. Time course of A. pomorum counts by limb jarring with progressing season (time period from 8 March to 17 April 2003) at the study sites A, B, C, D and E. The values for the different traps are shown as average number of weevils caught per day and tree (bars). The lines in the lower graph indicate the mean daily temperatures for the different study sites.
On the level of study sites (Figure 7.3), the numbers of weevils caught with shelter traps on the day of maximum catches were significantly correlated with the absolute injury expressed as number of infested buds (Figure 7.3A; $r = 0.96$, $n = 5$, $P = 0.005$; linear regression: $y = 9.41 + 3.33x$, $r^2 = 0.93$, ANOVA, $F_{1,3} = 37.62$, $P = 0.009$) and the relative injury expressed as percentage of infested buds relative to all buds of a tree (Figure 7.3B; $r = 0.98$, $n = 5$, $P = 0.001$; linear regression: $y = 3.49 + 0.68x$, $r^2 = 0.96$, ANOVA, $F_{1,3} = 66.40$, $P = 0.004$). Likewise the numbers of weevils caught with shelter traps during the seven-day period of maximum catches were also significantly correlated with absolute injury ($r = 0.97$, $n = 5$, $P = 0.003$; linear regression: $y = 16.43 + 4.60x$, $r^2 = 0.94$, ANOVA, $F_{1,3} = 47.22$, $P = 0.006$) and relative injury ($r = 0.96$, $n = 5$, $P = 0.005$; linear regression: $y = 5.13 + 0.93x$, $r^2 = 0.95$, ANOVA, $F_{1,3} = 52.95$, $P = 0.005$). In contrast, the number of weevils counted with limb jarring on the day of maximum catches in the different sites was not correlated with the absolute injury (Figure 7.3A; $r = 0.85$, $n = 5$, $P = 0.08$), but it was significantly correlated with the relative injury (Figure 7.3B; $r = 0.95$, $n = 5$, $P = 0.009$; linear regression: $y = -10.34 + 0.71x$, $r^2 = 0.91$, ANOVA, $F_{1,3} = 28.51$, $P = 0.013$). Correspondingly, the number of weevils counted with limb jarring during the seven-day period of maximum counts was not correlated with the absolute injury ($r = 0.77$, $n = 5$, $P = 0.15$) but was significantly correlated with the relative injury ($r = 0.96$, $n = 5$, $P = 0.005$; linear regression: $y = -5.56 + 1.04x$, $r^2 = 0.93$, ANOVA, $F_{1,3} = 38.06$, $P = 0.009$).

On tree level within study sites, the weevil numbers in the shelter traps of single trees on the day of maximum catches were not significantly correlated with the absolute injury or relative injury (Table 7.2). When the seven-day period of maximum catches was considered, there was no significant correlation between the number of weevils in the shelter traps of single trees and the absolute injury (Table 7.2).
Figure 7.3. Relationship between the numbers of weevils counted on the day with maximum counts by the transparent shelter trap (O) and the limb jarring method (□) and the absolute injury (A, number of infested blossoms) and the relative injury (B, percentage of infested blossoms relative to all blossoms on a tree). The solid line indicates the linear regression for the shelter traps (A – absolute injury: $y = 9.41 + 3.33x$; $r^2 = 0.93$; B – relative injury: $y = 3.49 + 0.68x$; $r^2 = 0.96$). For comparison, the linear regression for the limb jarring is indicated by the dashed line (A – absolute injury: $y = -44.03 + 3.15x$; $r^2 = 0.72$, not significant: ANOVA, $F_{13} = 7.80$, $P = 0.068$; B – relative injury: $y = -10.34 + 0.71x$; $r^2 = 0.91$, ANOVA, $F_{13} = 28.51$, $P = 0.013$).
Table 7.2. Absolute injury (the number of infested buds) and relative injury (the percentage of infested buds compared to all buds on a tree) of all five study sites (mean, SD) and their correlation (Pearson's r, analysed within each site) with the numbers of weevils caught with shelter traps on the day of maximum catches and during the seven-day period of maximum catches (20 trees per site).

| Site | Abs. Injury Mean | SD | r | P | Abs. Injury day of maximum catches Mean | SD | r | P | Abs. Injury seven-day period of maximum catches Mean | SD | r | P |
|------|------------------|----|---|---|-----------------------------------------|----|---|---|-----------------------------------------|----|---|---|---|
| A    | 256.3            | 139.3 | 0.18 | 0.448 | 0.21 | 0.387 |
| B    | 171.0            | 122.5 | 0.42 | 0.062 | 0.38 | 0.102 |
| C    | 29.4             | 33.5 | -0.06 | 0.816 | 0.27 | 0.257 |
| D    | 25.0             | 24.3 | -0.01 | 0.954 | -0.01 | 0.954 |
| E    | 7.8              | 6.3 | 0.04 | 0.856 | 0.04 | 0.856 |

| Site | Rel. Injury Mean | SD | r | P | Rel. Injury day of maximum catches Mean | SD | r | P | Rel. Injury seven-day period of maximum catches Mean | SD | r | P |
|------|------------------|----|---|---|-----------------------------------------|----|---|---|-----------------------------------------|----|---|---|---|
| A    | 39.9             | 15.7 | 0.30 | 0.209 | 0.42 | 0.063 |
| B    | 50.6             | 9.5 | -0.04 | 0.886 | -0.06 | 0.755 |
| C    | 8.7              | 9.8 | -0.07 | 0.789 | 0.14 | 0.569 |
| D    | 6.7              | 6.1 | 0.02 | 0.946 | 0.02 | 0.946 |
| E    | 1.9              | 1.9 | -0.14 | 0.576 | -0.14 | 0.576 |

On tree level across all sites, the number of weevils caught with shelter traps on all the trees in all five study sites on the day of maximum catches were significantly correlated with the absolute injury ($r = 0.44$, $n = 100$, $P < 0.001$; linear regression: $y = 77.36 + 15.42x$, $r^2 = 0.19$, ANOVA, $F_{1,98} = 23.6$, $P < 0.001$) and the relative injury ($r = 0.41$, $n = 100$, $P < 0.001$; linear regression: $y = 18.29 + 2.43x$, $r^2 = 0.17$, ANOVA, $F_{1,98} = 19.48$, $P < 0.001$). Likewise, the weevil numbers caught with shelter traps during the seven-day period of maximum catches were also significantly correlated with absolute injury ($r = 0.51$, $n = 100$, $P < 0.001$; linear regression: $y = 71.12 + 30.20x$, $r^2 = 0.26$, ANOVA, $F_{1,98} = 33.98$, $P < 0.001$) and relative injury ($r = 0.51$, $n = 100$, $P < 0.001$; linear regression: $y = 16.97 + 5.14x$, $r^2 = 0.26$, ANOVA, $F_{1,98} = 33.99$, $P < 0.001$).
7.5. Discussion

Results of the present study demonstrate that the numbers of weevil caught with the transparent shelter traps depict weevil occurrence under different environmental conditions, and that these numbers are well suited for predicting oviposition injury by *Anthonomus pomorum* across different apple orchards. Our earlier investigations indicated a high potential of shelter traps for characterising the time course of tree colonisation by *A. pomorum* irrespective of weather conditions (Hausmann et al. 2004), and the present study confirms these findings under both high and low population pressures. Additionally, this study shows that the numbers of weevils caught with shelter trap correlate significantly with subsequent absolute injury, i.e., the number of infested buds, and with relative injury, i.e., the percentage of infested buds relative to all buds on the tree. Although intermediate levels of infestation were not measured with shelter traps in this study, we expect that they fit well into the correlation between weevil counts and injury calculated from five different assessments at five different study sites. In contrast, the number of weevils counted by limb jarring was not correlated with the absolute injury at site level. This corresponds to an earlier study that reports only weak correlation (*r* = 0.42, *P* > 0.05) between the number of weevils counted by limb jarring and the percentage of blossoms infested (Brown et al. 1993).

Similar differences in the effectiveness of monitoring traps were found in another curculionid species, the plum curculio, *Conotrachelus nenuphar* (Herbst.). Numbers of plum curculios caught with baited and unbaited pyramid traps and with clear Plexiglas traps reflected the injury to fruit within unmanaged orchards (Prokopy et al. 2000). However, the same study reports no significant relationship between the numbers of weevils caught with baited or unbaited cylinder traps and plum curculio injury to fruit. In our study, shelter traps, but not limb jarring, fulfil the requirement for predictions of absolute injury, our major criterion for assessing the significance of the monitoring tool. Although results with limb jarring are correlated with relative injury, it is much easier to assess absolute injury. Counting can be confined to the infested buds only, instead of
having to assess in addition the total number of buds on a tree to calculate the ratio between these two values.

Recognition of the maximum colonisation by shelter traps depended on abundance of *A. pomorum* in the investigated orchards. At sites with high population pressure, the day of maximum colonisation was easily detectable in contrast to sites with low abundance of *A. pomorum*. However, even under conditions of low weevil abundance, the numbers of weevils caught with shelter trap allowed for conclusions on subsequent injury, as the numbers both at the day of maximum catches and during the seven-day range of highest catches correlated with the absolute injury. In addition, the detection of a distinct day of maximum colonisation is less critical at low population pressure, as all study sites with low weevil abundance harboured populations clearly below the established economic threshold which is set at 10% relative injury (Reijbroek 1983), or 10 – 15% according to most recent recommendations for Switzerland (Höhn et al. 2003). If no intervention is necessary, the question of proper timing of an insecticide spray becomes irrelevant.

The numbers of weevils caught with the shelter traps fail to reflect absolute injury and relative injury on tree level when analysed within a single study site. This finding could be attributed to the relatively frequent movements of weevils between trees during the colonisation period. Indeed, a mobility study with marked *A. pomorum* demonstrated that only every third weevil (33.5 ± 8%) remained on the tree on which it first settled, while the major part of the population moved over an average distance of 11 meters (Toepfer et al. 1999).

The maximum colonisation as detected with the transparent shelter traps varied over a period of 4 days between the investigated orchards. In contrast, the maximum counts with limb jarring varied over 10 days. Similarly, a 12 day difference was previously found by limb jarring in two orchards which were situated just one kilometre apart (Balachowsky 1963). Indications that shelter traps reflect the temporal pattern of immigration more accurately than limb jarring were also seen in a recent study by Hausmann et al. (2004). In contrast to limb jarring, catches with shelter traps correlate
with the number of weevils colonising the trees by flight and by crawling (Hausmann et al. 2004).

Temperature is a key factor influencing spring colonisation of orchards by *A. pomorum* (Toepfer et al. 2002), since the activity of the weevil requires a certain minimum temperature (Duan 1996). Major fluctuations in temperature are thus expected to be followed by changing colonisation dynamics of an orchard by *A. pomorum* and thus changing numbers of weevils caught in shelter traps. In 2003, the mean daily temperature increased steeply to about 12°C prior to the first peak in colonisation activity of the weevils. Then mean daily temperatures dropped for a few days to values around 3°C, which was followed by a marked decline in number of weevil caught. This finding is compatible with the assumption that such low temperatures prevent further colonisation. A subsequent increase in mean daily temperature was immediately followed by increased number of weevils caught again. For seasons with a period of warm weather in early spring followed by cold days, a prolonged surveillance of *A. pomorum* with shelter traps is recommended. This is especially important if the first peak of numbers of weevils caught indicates a relative injury below the economic threshold, as a subsequent, more intense colonisation phase could follow.

Analysed over the entire colonisation period, numbers of weevils caught with shelter traps proved independent of weather conditions confirming a previous study (Hausmann et al. 2004). In contrast, the counts with limb jarring at study sites A, B and C were all correlated with the daily maximum ambient air temperature, further indicating the limitation of limb jarring as a monitoring tool. The fact that no correlation could be demonstrated between counts with limb jarring at sites D and E and maximal temperature is most likely due to the very low number of weevils collected at these locations. This might have precluded statistically significant correlations.

In conclusion, the transparent shelter trap qualifies as an adequate method for accurately monitoring the course of spring colonisation of *A. pomorum* and its injury to blossom buds. The significant linear relationship between the number of weevils caught in shelter traps and the subsequent injury allows predictions to be made about a
potential injury before oviposition takes place, i.e., at a time when an intervention with an insecticide is still possible. Daily examination of 20 shelter traps in an orchard takes about 15 to 20 min for a moderately experienced person. A sample size of 20 trees equipped with shelter traps seems adequate for detecting *A. pomorum* at both low- and high-population densities. We expect a potential insecticide application to be optimally timed if carried out shortly after the maximum catches are reached, and trials relating such interventions with yield assessment at harvest are recommended.
8. General Discussion

The present thesis aimed at investigating the visual and thermal orientation of overwintered *Anthonomus pomorum* in relation with their host plant and combining the findings to develop a tool for surveillance of the species.

Concerning visual cues, the colour and contrast preferences of the apple blossom weevil were investigated in the laboratory (chapter 4). The results indicate a trichromatic visual orientation of the weevils. Positive phototaxis and a preference for blue colour suggest that weevils are able to use visual cues among other cues during host plant location in the field. Females show a more discriminating response in the visual test arena than males. This finding may reflect a greater visual acuity in females due to a possibly higher affinity for the host plant.

Further investigations in the laboratory concentrated on the thermal orientation of the weevil during photophase and during scotophase (chapter 5). The results of these experiments demonstrate that overwintered apple blossom weevils perform active behavioural thermoregulation by selection of warm microhabitats. However, during scotophase the thermal preference disappears in females 6 days after termination of diapause. This finding suggests that the females' behavioural priority changes between thermal orientation and oviposition-site selection in response to feeding status and degree of egg maturation.

In a field study, tree colonisation and hiding behaviour of the weevils were investigated using different trap types (chapter 6). Tree colonisation by flight and by crawling appeared in almost equal frequencies and occurred mainly during photophase. The temporal pattern of hiding demonstrated that the majority of the weevils hide early in the morning, during the coldest part of the day. The newly developed shelter trap was the most efficient and reliable monitoring tool of the methods tested.

In a last phase the shelter trap was validated under different environmental conditions (chapter 7). With this trap the time of maximum colonisation can be assessed and the infestation can be predicted irrespective of weather conditions.
This study shows how a monitoring tool can successfully be developed based solely on the use of physical stimuli. Furthermore, it demonstrates the importance of knowledge on the environmental physiology of a target species as well as on its orientation behaviour towards physical stimuli.

The mechanism underlying the shelter trap

The small body size of the apple blossom weevil precludes endothermic regulation of its body temperature (Heinrich 1993) and makes it highly sensitive to changes in ambient temperatures. Mobile insect stages are capable of avoiding harsh ambient conditions by exploiting more favourable microhabitats (Willmer 1982). Occupation of thermally favourable refuges is particularly important for the apple blossom weevil after termination of diapause early in spring. During this time the adults are forced to be active under relatively unfavourable conditions during the seasonal cycle because they use apple blossom buds in an early stage for ovarian maturation and for oviposition. The apple blossom weevil can benefit from higher body temperature by a higher activity level during immigration, for mating and for oviposition and from faster development (Willmer 1982). Indeed the results from the present laboratory study prove that overwintered apple blossom weevils perform active behavioural thermoregulation by selection of warm microhabitats (chapter 5). In addition, previous behavioural studies demonstrated that ambient temperatures influence activities of the weevil, such as crawling, feeding and mating (Duan et al. 1996, Toepfer et al. 2002), and that low temperature values increase the frequency by which weevils occupy shelters or refuges (Duan et al. 1996).

The present approach in the development of a monitoring tool makes use of the weevils' expected need for a thermal refuge. The artificial shelter mimics relatively warm hiding sites. Hiding behaviour of the apple blossom weevils in the shelter traps is greatest during the coldest periods of the diurnal cycle (chapter 6) and therefore the hypothesis is supported that this trap exploits the weevil’s affinity for warmer sites and is triggered by the temperature fluctuation between day and night. The temperature level within the shelter trap is slightly, but significantly, higher than on the trunk (chapter 6).
This indicates that this shelter trap can generate the microclimate required by the 
shelter-seeking adults. Disappearance of a thermal preference in females 6 days after 
termination of diapause (chapter 5) corresponds with data from our field study whereby 
weevils were found to hide in shelter traps only on the first days after colonisation 
(chapter 6). Reasons for this could be the changing degree of egg maturation which is 
not yet completed after termination of diapause (Ctvrtecka & Zdarek 1992). Also, the 
feeding status of the female weevils may play a role in these behavioural changes. 
Females might prefer to stay near the buds, used for feeding and as oviposition sites. In 
fact, behavioural priorities of female insects towards oviposition sites often correlates 
with conditions of the ovaries (Browne 1993).

The perspectives for using the results from the visual orientation for monitoring

The first approach towards development of a monitoring tool for the apple 
blossom weevil concentrated on the thermal orientation of the weevil. The results of 
these investigations led to the development of a transparent shelter trap. This trap 
proved to be very effective, reliable and easy to use. An integration of visual cues into 
the shelter trap is not reasonable, since the majority of the weevils hid in the shelter trap 
early in the morning when the ambient temperatures were low. During this part of the 
day, visual cues play a minor role in insect orientation.

In other monitoring approaches, however, visual stimuli are often used in 
combination with chemical stimuli, enhancing the efficacy of a method (Pedigo 1996). 
For example baited multiple-funnel traps for Dendroctonus frontalis Hopkins 
(Coleoptera: Scolytidae) caught 75% more beetles in blue traps and 86% less beetles in 
yellow traps compared to captures in black traps (Strom & Goyer 2001). Since our 
laboratory results demonstrate that the apple blossom weevils orientate positively 
towards transmissive colours and show preferences between different colours, an 
important role of colour would be expected in a possible pheromone trap for the weevil. 
Blue or dark tones would first be recommend as trap colours, because A. pomorum was 
demonstrated to prefer the colour blue when offered versus green (chapter 4). 
Furthermore, the weevils' response to silhouettes of different achromatic contrasts was
influenced by the background colour. In front of green, the colour of the vegetation surrounding the dark apple trees, weevils preferred a higher contrast (chapter 4).

Before testing traps with different colours in field trials, laboratory trials are recommended to investigate the response of the weevils towards reflective colours. In the present study preferences of crawling weevils were analysed. Therefore, prior to combining colours with pheromone traps that attract flying weevils, the response of weevils flying towards different colours should be investigated.

Implementation of the monitoring tool developed

A trap for monitoring *A. pomorum* should attract and withhold the dispersing weevils in such a way that they can be counted to assess qualitative information. Furthermore, the numbers of weevils caught during early spring should indicate when maximum colonisation of trees by *A. pomorum* occurs. For risk assessment, the number of weevils caught with the monitoring tool at the time of maximum colonisation should predict the probable infestation of the blossom buds and allow for a decision on the need for an intervention. With the transparent shelter trap, the seasonal course and the maximum of tree colonisation by *A. pomorum* can be assessed accurately. In particular, there is a correlation between the catches with shelter traps and catches in traps which directly relate to tree colonisation by flight, i.e., the flight trap, and by crawling, i.e., the screen trap (chapter 6). Shelter traps also fulfil the requirement for predictions of injury before oviposition takes place, i.e., early enough for an insecticide application where necessary, the other major criterion for assessing the significance of the monitoring tool. This is verified by the significant linear relationship between the numbers of weevils caught in shelter traps at the time of maximum colonisation and the subsequent injury in five different study sites (chapter 7). Weevils trapped are well visible underneath the transparent bubble wrap, and quantification does not require any manipulation of the weevils. Furthermore, *A. pomorum* will be the only weevil species hiding in the traps so early in the season. The relatively simple use of the shelter traps makes them ideal even for untrained hands. The easy handling of this tool is particularly important as the apple blossom weevil must be monitored on an individual orchard level. Regional
surveillance programs may not reflect the local situation adequately. Infestation can differ substantially in level and timing in different orchards in a single region (chapter 7). The installation of the traps should take place before the thermal time totals for the start of dispersal (Toepfer et al. 2002) are reached. The traps should then be examined every day or every second day in the morning before 09:00 h. The information thus acquired is only valuable for the current season, as the abundance of the apple blossom weevil can vary substantially between seasons (Brown et al. 1993).

In conclusion, the newly designed shelter trap is a very promising monitoring tool, because the abundance of weevils in the traps is high, the time of maximum colonisation can be assessed, the injury to the buds can be predicted, and finally the captures are independent of weather conditions or of trap position in the tree. The results of the present thesis indicate a potential insecticide application to be optimally timed if carried out shortly after the maximum colonisation of the orchards and before oviposition starts. However, the optimal timing for an intervention must be investigated in further field trials. The relationships we found between the number of weevils caught in shelter traps and the relative injury allows for predictions of potential damage. In order to ascertain these predictions more investigations are needed because we cannot exclude possible shifts in the correlations between numbers of weevils caught and numbers of infested buds due to very different weather conditions during the oviposition period. The data of several seasons would allow the calculation of a precise economic threshold. With the transparent shelter trap the aim of the thesis to develop a reliable tool for monitoring spring activity of A. pomorum based solely on physical cues was achieved.
9. References


10. Acknowledgements

I wish to thank everyone who contributed to this research project:

Prof. Dr. Silvia Dorn as the head of the group 'Applied Entomology' for supporting me both scientifically and personally throughout the study. She initiated this exciting project. I also thank her for challenging questions and fruitful discussions.

Dr. Jörg Samietz who worked out the project together with Silvia Dorn and guided me during the whole time. I also thank him for encouraging discussions and for his scientific support in trial design, statistics and the preparation of publications.

Dr. Pierre-Joseph Charmillot of the Swiss Federal Research Station for Plant Production of Changins who kindly accepted to be co-examiner.

Dr. Hainan Gu, Dr. Anja Rott and Dr. Kathrin Tschudi-Rein for useful suggestions on earlier stages of the manuscripts.

Dr. Stefan Töpfer for stimulating and critical discussion about the project.

Dr. Claude Fornallaz for technical support and for organising equipment for experiments.

Michelle Aymon, Marco Hinnen, Slavica Katulic and Sibylle Stöckli for their perseverance and reliability with the field and laboratory experiments.

The farmers Franz Peterhans and Hans Vock for providing the orchards for my field experiments. They were very friendly and helpful.
The actual and previous members of the group including scientific assistants, PhD students, Diploma students and laboratory assistants for their cooperativeness at work, the relaxed conversations and our coffee-breaks. I appreciate very much the beautiful time we spent together.

Ute Kührt for her friendship. We did not only share the office but also all the pleasures and sorrows during our PhD studies.

Last but not least, my family, my loved ones and my friends for their personal support.

I dedicate this thesis to my mother. In spite of a severe disease she supported me in every way possible for as long as she could. She died on 23 February 2003.
11. Curriculum vitae

Claudia Hausmann

born 23 December 1970, Cham, Switzerland

2001 - 2004 PhD studies 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 and Dr. Jörg Samietz. Assistant in entomology courses and excursions.


1995 - 1999 Studies in Biology (Systematic Biology and Zoology) at the Swiss Federal Institute of Technology (ETH) in Zurich. Diploma thesis at the Institute of Plant Sciences, Applied Entomology under the guidance of Prof. Dr. Silvia Dorn and Dr. Letizia Mattiacci. Thesis title: Influences of host feeding-niche and host refuges on the habitat preference of *Hyssopus pallidus*, a larval parasitoid of *Cydia pomonella*.

1991 - 1995 Academic high school in Zurich. AKAD Matura type C.


1991 Language studies in Montreal and Vancouver (Canada).
