



## Chapter I: General introduction

### Oilseed rape

Oilseed rape (*Brassica napus* L.) is the most important oilseed crop in Germany, in 2009 covering an acreage of 1.47 million hectares (Eurostat, 2009). Due to its higher yield potential winter oilseed rape is grown much more commonly than to spring rape (Kimber and McGregor, 1995). In 2010 winter oilseed rape yielded in average 39 dt/ha in Germany (Anonymus, 2011). The oil extracted from the harvested seeds is commonly used for human nutrition or for the production of biodiesel (Alford, 2003). The seed meal is used in animal nutrition because of its high content of protein and essential amino acids (Bell, 1995). Today most often cultivars with low content of erucic acid and glucosinolates in the seeds are grown, also known as 'canola quality' or 'double low quality' (Bell, 1995; Alford, 2003). Due to the low content of erucic acid, the oil is suitable for human nutrition. The low level of glucosinolates in the meal increases its suitability for animal nutrition (Bell, 1995; Uppström, 1995).

### Oilseed rape pests in Germany

In Germany oilseed rape is attacked by a wide range of insect pests during the whole growing season (Alford et al., 2003). Economic damage is most often caused by only six insect species: cabbage stem flea beetle (*Psylliodes chrysocephala*), rape stem weevil (*Ceutorhynchus napi*), cabbage stem weevil (*Ceutorhynchus pallidactylus*), pollen beetle (*Meligethes aeneus*), cabbage seed weevil (*Ceutorhynchus obstrictus*) and brassica pod midge (*Dasineura brassicae*) (Williams, 2010).

The cabbage stem flea beetle is a common pest particularly in maritime regions of northern Europe (Williams, 2010). The adult beetles invade the oilseed rape fields during September after emergence of the seedlings (Schulz, 1992). As the ovaries of the females are immature at this stage, they conduct a maturity feeding over a period of two weeks (Williams, 2010). Gravid females lay their eggs into the upper soil layer near the roots of host plants (Kaufmann, 1941; Saringer, 1984). Neonate



larvae bore into petioles and stems of oilseed rape plants where they feed until completion of larval development (Kaufmann, 1941; Godan, 1951). The first larvae start to pupate in soil already in winter but the majority leave their hosts plant in spring (Williams, 2010). New generation beetles emerge from soil in early summer and feed on oilseed rape until they migrate to hibernation sites for summer aestivation (Kaufmann, 1941; Williams & Carden, 1961). In addition to oilseed rape, many other brassicaceous plant species like runch (*Raphanus raphanistrum*), cabbage (*Brassica oleracea*) or turnip rape (*Brassica rapa*) are known to be host plants for cabbage stem flea beetles (Godan, 1951). Major damage results from larval feeding which leads to higher plant losses during winter, weakening of young plants, and facilitating the infestation of oilseed rape by fungal diseases and viruses (Godan, 1950; Bonnemaïson, 1965; Schulz and Daebeler, 1984; Winfield, 1992).

The rape stem weevil starts to invade the oilseed rape fields on sunny days in early spring, when the maximum temperature reaches 10°C (Schmutterer, 1956). After a short maturity feeding, females lay their eggs into the stems of oilseed rape plants close to the shoot tip (Dosse, 1951). Thereby they also excrete substances which cause abnormal growth of the plants and splitting of the stems (Lerin, 1993; Paul, 2003). The feeding wounds promote subsequent plant infestation by fungal pathogens (Broschewitz et al., 1993). The larvae feed inside the stems until they leave the plants to pupate in soil (Dosse, 1951). Adults persist in their pupation cave until the following spring (Günthart, 1949; Alford et al., 2003). The main damage is caused by oviposition and larval feeding inside stems (Lerin, 1995; Williams, 2010).

The cabbage stem weevils usually immigrate into the oilseed rape fields in March and April. They often occur together with the rape stem weevil (Günthart, 1949). After colonisation of their host plant the adults conduct a maturity feeding (Günthart, 1949). Subsequently they start to oviposit into petioles and rarely into stems of oilseed rape plants (Günthart, 1949). Neonate larvae feed inside the petioles before they move into stems (Günthart, 1949; Broschewitz and Daebeler, 1987). Here they complete their development before they leave the plants for pupation in soil (Günthart, 1949). New generation beetles emerge in summer (Günthart, 1949). After a short feeding period, the beetles leave the oilseed rape



fields and migrate to their winter habitats (Günthart, 1949). Here they stay until crop colonisation in the following spring (Günthart, 1949). Economic yield losses are usually caused by larval feeding inside the plants (Kelm and Walczak, 1998; Kelm and Klukowski, 2000). This causes weakening of the plants and increases infestation by fungal diseases (Broschewitz et al., 1993).

Also in spring, when maximum temperature increases above 15°C, pollen beetles invade the oilseed rape fields (Fritzsche, 1957). Adults feed on pollen of a wide range of host plants but for oviposition they rely on brassicaceous species (Free and Williams, 1978). When beetles colonize oilseed rape crops before the plants start to flower, they feed on buds to get access to the pollen (Fritzsche, 1957; Williams and Free, 1978). Thereby they often damage the ovaries and induce abortion of these buds (Fritzsche, 1957; Williams and Free, 1978; Nilsson, 1988). After maturity feeding, females lay their eggs into the buds (Fritzsche, 1957). The larvae develop inside the buds and open flowers (Fritzsche, 1957; Williams and Free, 1978). During this time they feed on pollen without causing damage (Williams and Free, 1978). When the development of larvae is completed, they drop onto the ground to pupate in soil (Fritzsche, 1957). New generation adults emerge in summer and feed on pollen of Brassicaceae and other plants before they migrate to their winter habitats (Fritzsche, 1957). The feeding of adults on buds of oilseed rape often causes high economic losses (Fritzsche, 1957; Nilsson, 1987). Feeding of adults on open flowers or feeding of larvae seldom causes considerable damage (Fritzsche, 1957; Williams and Free, 1978).

The cabbage seed weevil invades the oilseed rape crops in early summer when the maximum temperature reaches 15°C (Edner and Daebeler, 1984). They feed on buds, flowers and pods of oilseed rape plants (Dmoch, 1965a; Williams and Free, 1978). Following maturity feeding females bite holes into young pods and lay one egg into the pod (Dmoch, 1965b; Edner and Daebeler, 1984). These pods are marked by a pheromone to avoid that other females use these pods for oviposition (Ferguson et al., 1999; Ferguson and Williams, 1991). Inside the pods each larva feeds three to five seeds during its development (Dmoch, 1965a; Edner and Daebeler, 1984). The mature larvae bite an exit hole into the wall of the pod and drop onto the soil for pupation (Dmoch, 1965a). New generation adults leave the oilseed rape fields and migrate to their winter habitats after a short period of



feeding (Williams and Free, 1978). The main damage is caused by oviposition and feeding of larval exit holes (Dmoch, 1965a; Williams and Free, 1978; Edner and Daebeler, 1984; Williams, 2010). This can offer ports of entry for fungal pathogens and for oviposition of brassica pod midge (Williams, 2010).

The brassica pod midge usually invades the oilseed rape crops during flowering (Speyer, 1921; Fröhlich, 1956; Buhl, 1960). The midges are not able to fly over long distances, and they mainly infest the margins of fields (Kühne, 1967). For oviposition they are dependent on lesions in the pod wall because they are not able to penetrate older pods on their own (Buhl, 1957). The larvae suck inside the pods, thereby secreting salivary substances which cause deformation of pods and early shedding of the seeds (Speyer, 1921; Paul, 2003). The larvae fall onto the ground and pupate in soil (Fröhlich, 1956; Buhl, 1960). The brassica pod midge has usually three generations per year, but only the first generation most often causes economic yield losses in winter oilseed rape (Fröhlich, 1956; Buhl, 1960; Axelsen, 1992).

### **Pest management strategies**

Today the management of insect pests on oilseed rape strongly relies on chemical insecticides, predominantly of the chemical group of pyrethroids (Williams, 2004). Due to adverse effects of these insecticides on non target insects, there is political pressure to reduce their usage (Thieme et al., 2010; Ulber et al., 2010; Walters et al., 2003). Recently, Bommarco et al. (2011) reported that the parasitism rate of diamond back moth was significantly reduced when insecticides were applied to cabbage crops. Moreover, insecticides in oilseed rape are often applied routinely, even though the pest infestation is below threshold level (Williams, 2004; 2010). In the past much effort has been spent to develop alternative strategies for pest management (Williams, 2004). One approach was to enhance the impact of natural enemies of pest insects (Walters et al., 2003). This can be achieved by reduced tillage after harvest of oilseed rape (Nilsson, 2010), which avoid destruction of larval parasitoids due to deep ploughing (Nitzsche and Ulber, 1998; Nilsson, 2010). The provision of flowering strips is another approach to promote these parasitoids, which then may help to regulate pest population (Büchi, 2002).



Another non-chemical strategy for management of insect pests in oilseed rape is the use of trap crops (Büchi, 1990; Cook, 2002; Nilsson, 2004; Barari et al., 2005; Büchs, 2009; Carcamo et al., 2007). Hokkannen (1991) defined trap crops as “plant stands that are grown to attract insects or other organisms like nematodes to protect target crops from pest attack. Protection may be achieved by preventing the pests from reaching the crop or by concentrating them in a certain part of the field”. According to this, Shelton and Badenes-Perez (2006) differentiated between “conventional trap cropping” and “dead-end trap cropping”. For conventional trap cropping a highly attractive trap crop is planted next to the targeted crop. Due to its high attractiveness, pest insects are maintained at the trap crop and do not colonize the main crop. In a “dead-end trap cropping” systems, the adult pests also prefer the attractive trap crop but in contrast to conventional trap cropping, the plants are not suitable as host for their offspring. This can either be achieved by insufficient host quality of the trap plant or by application of an insecticide. In order to protect oilseed rape from pest attack, most often turnip rape is used as a trap crop because it is preferred to oilseed rape by most specialist insect pests (Büchi, 1990; Lambdon et al., 1998; Cook et al., 2006; Cook et al., 2007). While the insects are maintained at the turnip rape borders, the main crop is protected against insect damage (Vandermeer, 1989; Hokkanen, 1991; Shelton and Badenes-Perez, 2006). Some studies have shown a positive effect of the trap crop strategy, however, the results of prior studies are inconsistent.

In earlier experiments two different types of experimental designs have been applied. Most often turnip rape was sown as a perimeter strip in order to trap the insect pests when they invade the field (Cook et al., 2004; Barari et al., 2005; Valantin-Morison, 2006; Carcamo et al., 2007). In the second design, a seed mixture of oilseed rape and turnip rape was sown to bound the pests to single turnip rape plants across the whole oilseed rape crop (Büchi, 1990; Nilsson, 2004). In both cases the proportion of the trap crop area in relation to the main crop area had an effect on the efficacy of the system (Hannunnen, 2005).

Previous studies also have shown that volatiles of essential oils emitted from non host plants have potential to distract pollen beetles from their host plants by masking the host plants' volatiles (Mauchline et al., 2005).



Host plant resistance can also provide a tool for integrated pest management. Sources of resistance against cabbage stem weevil and cabbage seed weevil recently were identified in rape cultivars and resyntheses (Eickermann et al., 2010; Tansey et al., 2011).

### **Host selection by herbivorous insects**

Host selection by herbivorous insects is usually divided into three steps (Finch and Collier, 2000; Williams and Cook, 2010). The location of host plants is initiated by long distance orientation, which is commonly driven by olfactory cues (Finch and Collier, 2000). The cabbage seed weevil and other oilseed rape pests are attracted to degradation products of glucosinolates such as isothiocyanates and nitriles as well as non-specific volatiles which are released from plants of various plant families (Bartlet et al., 1993; Bartlet et al., 1997; Cook et al., 2007). The orientation across intermediate distances is driven by visual stimuli such as colour and shape of host plants (Smart et al., 1997; Williams and Cook, 2010). Many specialist insects on oilseed rape are attracted to the yellow colour (Giamoustaris and Mithen, 1996; Smart et al., 1997). When the insect lands on a plants' surface, non-volatile substances are responsible for host plant acceptance (Finch and Collier, 2000). This prefeeding behaviour was investigated in detail in the crucifer flea beetle (*Phyllotreta cruciferae*), an important pest on canola in Canada (Lamb, 1989). When the insects attain a supposed host plant, three stages of behaviour were observed (Henderson et al., 2004). It starts with an acclimation phase, this is followed by stimulation and it ends up with initial feeding (Henderson et al., 2004). The insects perceive the stimuli from the host plant via chemical receptors at their tarsi and antennae and via test bites (Isidoro et al., 1998; Schoonhoven et al., 2005). For specialist insects on Brassicaceae, glucosinolates which are derived from secondary plant metabolism, are known to play a major role for host acceptance (Bartlet et al., 1994; Huang and Renwick, 1994). The feeding of cabbage stem flea beetles on a synthetic agar medium was significantly stimulated by gluconapin, glucotropaeolin, gluconasturtiin and glucobrassicin (Bartlet et al., 1994). Glucosinolates are known to be essential for host acceptance of this insect species (Bartlet and Williams, 1991; Williams and Bartlet, 1993). Further, secondary plant substances such as phenolics and alkaloids are also known to





affect host acceptance and preference of other insect species (Mayer, 2004; Schoonhoven et al., 2005). Earlier studies supposed that cabbage stem flea beetles are also affected by plant chemicals which do not belong to the chemical class of glucosinolates (Bartlet & Williams, 1991). Moreover, sugars and amino acids which are produced in the primary metabolism of plants are known to have an effect on the feeding behaviour of herbivorous insects such as cabbage stem flea beetle (Bartlet et al., 1994; Vanloon & Vaneeuwijk, 1989). Some characteristics of leaf morphology are also known to affect insect-plant interactions. A high trichome density or a thick layer of epicuticular waxes, for example, are known to hamper flea beetles from feeding (Bodnaryk, 1992; Lamb, 1980; Lambdon et al., 1998; Soroka et al., 2011).

## **Objectives**

A major objective of this study was to identify differences in susceptibility or even resistance of brassicaceous species to infestation by specialised insect pests. Furthermore, the mechanisms of resistance were investigated. This knowledge could be used in breeding programs to improve resistance against insect pests. To identify differences in host plant quality, the adult feeding preference and larval performance of cabbage stem flea beetle was assessed on a variety of brassicaceous species under laboratory conditions. Cabbage stem flea beetle was used as test insect in these experiments, because this species is a specialist on Brassicaceae and a major pest in German oilseed rape production. The plant species and lines which were used in these experiments were analysed for substances such as glucosinolates, sugars and phenolics. Trichome density on the leaf surface and dry weight of leaves were also measured. We tried to correlate these host plant characteristics to the feeding preference and larval performance, because these factors were assumed to play a major role in insect-plant interaction (see Schoonhoven, 2005).

Another objective was to assess the suitability of turnip rape as trap crop for control of insect pests on winter oilseed rape under on-farm conditions. Turnip rape was either grown in perimeter strips (Fig. 1) along the edges of oilseed rape fields or in mixtures containing 5% and 10% turnip rape, respectively. Moreover, in

field plot experiments several turnip rape cultivars were tested for their suitability as trap crop. Therefore, cultivars with low erucic acid and low glucosinolate content in the seeds were compared to cultivars containing high contents of both substances in the seeds.

The overall goal was to develop strategies which could help to reduce insecticide use in oilseed rape production.



**Fig 1:** Turnip rape sown as a perimeter strip along the edges of an oilseed rape field; Ebergötzen 2011

## References

- Alford DV (2003) The oilseed rape crop. In: Biocontrol of oilseed rape pests (ed. DV Alford). Blackwell Science Ltd., Oxford, pp. 1-8.
- Alford DV, Nilsson C & Ulber B (2003) Insect pests of oilseed rape crops. In: Biocontrol of oilseed rape pests (ed. DV Alford). Blackwell Science Ltd., Oxford, pp. 9-42.





- Anonymus (2011) Bodennutzung und Ernte. Statistisches Bundesamt Deutschland.
- Axelsen J (1992) The population dynamics and mortalities of the pod gall midge (*Dasyneura brassicae* Winn.) (Dipt., Cecidomyiidae) in winter rape and spring rape (*Brassica napus* L.) in Denmark. *Journal of Applied Entomology* 114: 463-471.
- Barari H, Cook SM, Clark SJ & Williams IH (2005) Effect of a turnip rape (*Brassica rapa*) trap crop on stem-mining pests and their parasitoids in winter oilseed rape (*Brassica napus*). *Biocontrol* 50: 69-86.
- Bartlet E, Blight MM, Hick AJ & Williams IH (1993) The responses of the cabbage seed weevil (*Ceutorhynchus assimilis*) to the odour of oilseed rape (*Brassica napus*) and to some volatile isothiocyanates. *Entomologia Experimentalis et Applicata* 68: 295-302.
- Bartlet E, Blight MM, Lane P & Williams IH (1997) The responses of the cabbage seed weevil *Ceutorhynchus assimilis* to volatile compounds from oilseed rape in a linear track olfactometer. *Entomologia Experimentalis et Applicata* 85: 257-262.
- Bartlet E, Parsons D, Williams IH & Clark SJ (1994) The influence of glucosinolates and sugars on feeding by the cabbage stem flea beetle, *Psylliodes chrysocephala*. *Entomologia Experimentalis et Applicata* 73: 77-83.
- Bartlet E & Williams IH (1991) Factors restricting the feeding of the cabbage stem flea beetle (*Psylliodes chrysocephala*). *Entomologia Experimentalis et Applicata* 60: 233-238.
- Bell JM (1995) Meal and by-product utilization in animal nutrition. In: *Brassica oilseeds - production and utilization* (ed. DS Kimber & DI McGregor). CAB International, Wallingford, pp. 301-338.
- Bodnaryk RP (1992) Leaf epicuticular wax, an antixenotic factor in Brassicaceae that affects the rate and pattern of feeding of flea beetles, *Phyllotreta cruciferae* (Goeze). *Canadian Journal of Plant Science* 72: 1295-1303.



- Bommarco R, Miranda F, Bylund H & Bjorkman C (2011) Insecticides suppress natural enemies and increase pest damage in cabbage. *Journal of Economic Entomology* 104: 782-791.
- Bonnemaison L (1965) Insect pests of crucifers and their control. *Annual Review of Entomology* 10: 233-256.
- Broschewitz B & Daebeler F (1987) Beitrag zur Biologie und Schadwirkung des Gefleckten Kohltriebrüblers (*Ceutorhynchus quadridens* Panz.) am Winterraps. *Nachrichtenblatt für den Pflanzenschutzdienst in der DDR* 41: 34-37.
- Broschewitz B, Steinbach P & Goltermann S (1993) Einfluss stengelbewohnender tierischer Schaderreger auf den Befall von Winterraps mit *Phoma lingam* und *Botrytis cinerea*. *Gesunde Pflanzen* 45: 106-110.
- Büchi R (1990) Investigation on the use of turnip rape as a trap plant to control oilseed rape pests. *IOBC/wprs Bulletin* 13 (4): 32-39.
- Büchi R (2002) Mortality of pollen beetle (*Meligethes* spp.) larvae due to predators and parasitoids in rape fields and the effect of conservation strips. *Agriculture, Ecosystems & Environment* 90: 255-263.
- Büchs W (2009) Fangpflanzen (trap crop) als Methode zur Kontrolle tierischer Schädlinge im ökologischen Rapsanbau. *Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie* 17: 241-246.
- Buhl C (1957) Beitrag zur Frage der biologischen Abhängigkeit der Kohlschotenmücke (*Dasyneura brassicae* Winn.) von dem Kohlschotenrübler (*Ceuthorrhynchus assimilis* Payk.). *Zeitschrift für Pflanzenkrankheiten (Pflanzenpathologie) und Pflanzenschutz* 64: 562-568.
- Buhl C (1960) Beobachtungen über vermehrtes Schadauftreten der Kohlschotenmücke (*Dasyneura brassicae* Winn.) an Raps und Rüben in Schleswig-Holstein. *Nachrichtenblatt des deutschen Pflanzenschutzdienstes* 12: 1-6.
- Carcamo HA, Dunn R, Dosdall LM & Olfert O (2007) Managing cabbage seedpod weevil in canola using a trap crop - A commercial field scale study in western Canada. *Crop Protection* 26: 1325-1334.



- Cook SM, L. E. Smart, R. J. P. Potting, E. Bartlet, J. L. Martin, D. A. Murray, N. P. Watts, I. H. Williams (2002) Turnip rape (*Brassica rapa*) as a trap crop to protect oilseed rape (*Brassica napus*) from infestation by insect pests: potential and mechanisms of action. The BCPC Conference: Pests & Diseases 2002 (2): 569-574.
- Cook SM, Rasmussen HB, Birkett MA, Murray DA, Pye BJ, Watts NP & Williams IH (2007) Behavioural and chemical ecology underlying the success of turnip rape (*Brassica rapa*) trap crops in protecting oilseed rape (*Brassica napus*) from the pollen beetle (*Meligethes aeneus*). Arthropod-Plant Interactions 1: 57-67
- Cook SM, Smart LE, Martin JL, Murray DA, Watts NP & Williams IH (2006) Exploitation of host plant preferences in pest management strategies for oilseed rape (*Brassica napus*). Entomologia Experimentalis et Applicata 119: 221-229.
- Cook SM, Watts NP, Hunter F, Smart LE & Williams IH (2004) Effects of a turnip rape trap crop on the spatial distribution of *Meligethes aeneus* and *Ceutorhynchus assimilis* in oilseed rape. IOBC/wprs Bulletin 27 (10): 199-206.
- Dmoch J (1965a) The dynamics of population of the cabbage seedpod weevil (*Ceutorhynchus assimilis* Payk.) and the development of winter rape. Part 1. Ekologia Polska Seria A 8: 249-287.
- Dmoch J (1965b) The dynamics of population of the cabbage seedpod weevil (*Ceutorhynchus assimilis* Payk.) and the development of winter rape. Part 2. Ekologia Polska Seria A 8: 463-489.
- Dosse G (1951) Der große Kohltriebrüssler *Ceutorrhynchus napi* (Gyll.) - Biologie, Schadaufreten und Bekämpfung unter besonderer Berücksichtigung der "Gallbildung" an Kohlpflanzen. Journal of Applied Entomology 32: 489-566.
- Edner B & Daebeler F (1984) Zum Schadgeschehen durch den Kohlschotenrüssler (*Ceutorhynchus assimilis* Payk.). Nachrichtenblatt für den Pflanzenschutz in der DDR 38: 115-117.



- Eickermann M, Ulber B & Vidal S (2010) Resynthesized lines and cultivars of *Brassica napus* L. provide sources of resistance to the cabbage stem weevil (*Ceutorhynchus pallidactylus* (Marsh.)). *Bulletin of Entomological Research*: 1-8.
- Eurostat (2009) <http://epp.eurostat.ec.europa.eu/portal/page/portal/agriculture/data/database>. date: 29.09.2011
- Ferguson AW, Solinas M, Ziesmann J, Isidoro N, Williams IH, Scubla P, Mudd A, Clark SJ & Wadhams LJ (1999) Identification of the gland secreting oviposition-detering pheromone in the cabbage seed weevil, *Ceutorhynchus assimilis*, and the mechanism of pheromone deposition. *Journal of Insect Physiology* 45: 687-699.
- Ferguson AW & Williams IH (1991) Deposition and longevity of oviposition-detering pheromone in the cabbage seed weevil. *Physiological Entomology* 16: 27-33.
- Finch S & Collier RH (2000) Host-plant selection by insects - a theory based on 'appropriate/inappropriate landings' by pest insects of cruciferous plants. *Entomologia Experimentalis et Applicata* 96: 91-102.
- Free JB & Williams IH (1978) The response of the pollen beetle, *Meligethes aeneus*, and the seed weevil, *Ceutorhynchus assimilis*, to oil-seed rape, *Brassica napus*, and other plants. *Journal of Applied Ecology* 15: 761-774
- Fritzsche R (1957) Zur Biologie und Ökologie der Rapsschädlinge aus der Gattung *Meligethes*. *Journal of Applied Entomology* 40: 222-280.
- Fröhlich G (1956) Zur Biologie und Bekämpfung der Kohlschotengallmücke (*Dasyneura brassicae* Winn). *Nachrichtenblatt für den deutschen Pflanzenschutzdienst N.F.* 10: 123-128.
- Giamoustaris A & Mithen R (1996) The effect of flower colour and glucosinolates on the interaction between oilseed rape and pollen beetles. *Entomologia Experimentalis et Applicata* 80: 206-208.
- Godan D (1950) Wann ist der Rapserrdflöhlarven-Befall für den Rapsacker gefährlicher, im Herbst oder im Frühjahr? *Nachrichtenblatt des deutschen Pflanzenschutzdienstes* 2: 149-153.



- Godan D (1951) Über Nahrungs- und Brutpflanzen des Rapserrfloh (*Psylliodes chrysocephala* L.). Anzeiger für Schädlingskunde 24: 81-84.
- Günthart E (1949) Beiträge zur Lebensweise und Bekämpfung von *Ceutorrhynchus quadridens* PANZ. und *Ceutorrhynchus napi* GYLL. Mitteilungen der Schweizerischen Entomologischen Gesellschaft 22: 443-591.
- Hannunnen S (2005) Modelling the interplay between pest movement and the physical design of trap crop systems. Agricultural and Forest Entomology 7: 11-20.
- Henderson AE, Hallett RH & Soroka JJ (2004) Prefeeding behavior of the crucifer flea beetle, *Phyllotreta cruciferae*, on host and nonhost crucifers. Journal of Insect Behavior 17: 17-39.
- Hokkanen HMT (1991) Trap cropping in pest management. Annual Review of Entomology 36: 119-138.
- Huang XP & Renwick JAA (1994) Relative activities of glucosinolates as oviposition stimulants for *Pieris rapae* and *P. napi oleracea*. Journal of Chemical Ecology 20: 1025-1037.
- Isidoro N, Bartlet E, Ziesmann J & Williams IH (1998) Antennal contact chemosensilla in *Psylliodes chrysocephala* responding to cruciferous allelochemicals. Physiological Entomology 23: 131-138.
- Kaufmann O (1941) Zur Biologie des Rapserrfloh (*Psylliodes chrysocephala* L.). Zeitschrift für Pflanzenkrankheiten (Pflanzenpathologie) und Pflanzenschutz 51: 305-324.
- Kelm M & Klukowski Z (2000) The effect of stem weevil (*Ceutorrhynchus pallidactylus* Marsh.) infestation on oilseed rape yield. IOBC/wprs Bulletin 23 (6): 125-130.
- Kelm M & Walczak B (1998) The relationship between the stem weevil (*Ceutorrhynchus pallidactylus* Marsh.) injury and losses of flower buds. IOBC/wprs Bulletin 21 (5): 147-151.





- Kimber DS & McGregor DI (1995) The species and their origin, cultivation and world production. In: Brassica oilseeds - production and utilization (eds. DS Kimber & DI McGregor). CAB International, Wallingford, pp. 1-8.
- Kühne W (1967) Zur Verteilung der Kohlschotengallmücke *Dasyneura brassicae* Winnertz in großflächigen Rapsbeständen. Beiträge zur Entomologie 17: 287-297.
- Lamb RJ (1980) Hairs protect pods of mustard (*Brassica hirta* Gisilba) from flea beetle feeding damage. Canadian Journal of Plant Science 60: 1439-1440.
- Lamb RJ (1989) Entomology of oilseed *Brassica* crops. Annual Review of Entomology 34: 211-229.
- Lambdon PW, Hassall M & Mithen R (1998) Feeding preferences of woodpigeons and flea-beetles for oilseed rape and turnip rape. Annals of Applied Biology 133: 313-328.
- Lerin J (1993) Influence of the growth rate of oilseed rape on the splitting of the stem after an attack of *Ceutorhyncus napi* Gyll. IOBC/wprs Bulletin 16 (3): 160-163.
- Lerin J (1995) Assessment of yield losses caused by insects in winter oilseed rape, a critical review. IOBC/wprs Bulletin 18 (4): 95-101.
- Mauchline AL, Osborne JL, Martin AP, Poppy GM & Powell W (2005) The effects of non-host plant essential oil volatiles on the behaviour of the pollen beetle *Meligethes aeneus*. Entomologia Experimentalis et Applicata 114: 181-188.
- Mayer AM (2004) Resistance to herbivores and fungal pathogens: Variations on a common theme? A review comparing the effect of secondary metabolites, induced and constitutive, on herbivores and fungal pathogens. Israel Journal of Plant Sciences 52: 279-292.
- Nilsson C (1987) Yield losses in summer rape caused by pollen beetles (*Meligethes* spp.). Swedish Journal of Agricultural Research 17: 105-111.
- Nilsson C (1988) Pollen beetles (*Meligethes aeneus*) and flowering in rape. Swedish Journal of Agricultural Research 18: 113-118.



- Nilsson C (2004) Trap plants to avoid insecticide application against pollen beetles in oilseed rape. IOBC/wprs Bulletin 27 (10): 215-221.
- Nilsson C (2010) Impact of soil tillage on parasitoids of oilseed rape pests. In: Biocontrol-based integrated management of oilseed rape pests (ed. IH Williams). Springer Science+Business Media B.V., Dordrecht, Heidelberg, London, New York, pp. 305-312.
- Nitzsche O & Ulber B (1998) Influence of different tillage treatments following the harvest of oilseed-rape on the mortality of pollen beetle (*Meligethes* spp.) parasitoids. Journal of Plant Diseases and Protection 105: 417-421.
- Paul VH (2003) Raps: Krankheiten, Schädlinge, Schadpflanzen. Verlag Th. Mann, Gelsenkirchen.
- Saringer G (1984) Summer diapause of cabbage stem flea beetle, *Psylliodes chrysocephala* L. (Col. Chrysomelidae). Journal of Applied Entomology 98: 50-54.
- Schmutterer H (1956) Zur Lebensweise und Bekämpfung des Großen Rapsstengelrüsslers *Ceutorhynchus napi* (Gyll.). Journal of Applied Entomology 39: 302-315.
- Schoonhoven LM, van Loon JJA & Dicke M (2005) Insect-plant biology. Oxford University Press, Oxford.
- Schulz R-R (1992) Untersuchung zur Aktivität und zum Zuflugverhalten des Rapserrdflohs (*Psylliodes chrysocephala* L.). Nachrichtenblatt des deutschen Pflanzenschutzdienstes 44: 6-8.
- Schulz R-R & Daebeler F (1984) Zum Schaden durch den Rapserrdfloh (*Psylliodes chrysocephala* L.), insbesondere seiner Imagines. Nachrichtenblatt für den Pflanzenschutz in der DDR 38: 113-115.
- Shelton AM & Badenes-Perez E (2006) Concepts and applications of trap cropping in pest management. Annual Review of Entomology 51: 285-308.
- Smart LE, Blight MM & Hick AJ (1997) Effect of visual cues and a mixture of isothiocyanates on trap capture of cabbage seed weevil, *Ceutorhynchus assimilis*. Journal of Chemical Ecology 23: 889-902.



- Soroka JJ, Holowachuk JM, Gruber MY & Grenkow LF (2011) Feeding by flea beetles (Coleoptera: Chrysomelidae; *Phyllotreta* spp.) is decreased on canola (*Brassica napus*) seedlings with increased trichome density. *Journal of Economic Entomology* 104: 125-136.
- Speyer W (1921) Beitrag zur Biologie der Kohlschotenmücke. Bericht über die Tätigkeit der Biologischen Reichsanstalt für Land- und Forstwirtschaft 16: 208-218.
- Tansey JA, Dossall LM & Keddie A (2011) Incorporation of novel *Ceutorhynchus obstrictus*-resistant canola genotypes into mixed cropping strategies: evidence for associational resistance. *Journal of Insect Behavior* 24: 116-131.
- Thieme T, Heimbach U & Müller A (2010) Chemical control of insect pests and insecticide resistance in oilseed rape. In: *Biocontrol-based integrated management of oilseed rape pests* (ed. IH Williams). Springer Science+Business Media B.V., Dordrecht, Heidelberg, London, New York, pp. 313-336.
- Ulber B, Klukowski Z & Williams IH (2010) Impact of insecticides on parasitoids of oilseed rape pests. In: *Biocontrol-based integrated management of oilseed rape pests* (ed. IH Williams). Springer Science+Business B.V., Dordrecht, Heidelberg, London, New York, pp. 337-356.
- Uppström B (1995) Seed chemistry. In: *Brassica oilseeds production and utilization* (eds. DS Kimber & DI McGregor). CAB International, Wallingford, pp. 217-242.
- Valantin-Morison M, L Quere (2006) Effects of turnip rape trap crops on oilseed rape pests in a network of organic farmers' fields. CD-ROM Proceedings of International Symposium on Integrated Pest Management in Oilseed Rape, 3.-5. April 2006, Göttingen, Germany
- Vandermeer J (1989) *The ecology of intercropping*. Cambridge University Press, Cambridge. pp. 237
- Vanloon JJA & Vaneeuwijk FA (1989) Chemoreception of amino-acids in larvae of 2 species of *Pieris*. *Physiological Entomology* 14: 459-469.



- Walters KFA, Young JEB, Kromb B & Cox PD (2003) Management of oilseed rape pests. In: Biocontrol of oilseed rape pests (ed. DV Alford). Blackwell Science Ltd., Oxford, pp. 43-72.
- Williams IH (2004) Advances in insect pest management of oilseed rape in Europe. In: Insect pest management (eds. AR Horowitz & I Ishaaya). Springer -Verlag, Berlin Heidelberg, pp. 181-208.
- Williams IH (2010) The major insect pests of oilseed rape in Europe and their management: an overview. In: Biocontrol-based integrated management of oilseed rape pests (ed. IH Williams). Springer Science+Business B.V., Dordrecht, Heidelberg, London, New York, pp. 1-43.
- Williams IH & Bartlet E (1993) Host plant factors affecting the feeding of cabbage stem flea beetle (*Psylliodes chrysocephala* L.) on oilseed rape. IOBC Bulletin 16 (9): 202-209.
- Williams IH & Cook SM (2010) Crop location by oilseed rape pests and host location by their parasitoids. In: Biocontrol-based integrated management of oilseed rape pests (ed. IH Williams). Springer Science+Business Media B. V., Dordrecht Heidelberg London New York.
- Williams IH & Free JB (1978) The feeding and mating behaviour of pollen beetles (*Meligethes aeneus* Fab.) and seed weevils (*Ceutorhynchus assimilis* Payk.) on oil-seed rape (*Brassica napus* L.). Journal of Agricultural Science 91: 453-459.
- Williams JJW & Carden PW (1961) Cabbage stem flea beetle in East Anglia. Plant Pathology 10: 85-95.
- Winfield AL (1992) Management of oilseed rape pests in Europe. Agricultural Zoology Reviews 5: 51-95.







## **Chapter II: Performance of cabbage stem flea beetle larvae (*Psylliodes chrysocephala* L.) on brassicaceous host plants with different glucosinolate profile**

### **Abstract**

The cabbage stem flea beetle (CSFB) (*Psylliodes chrysocephala* L.) (Col.: Chrysomelidae) is one of the most important insect pests in European winter oilseed rape production. Adult beetles feed on young leaves while the larvae feed within stems and petioles of various brassicaceous plant species. Larval infestation can result in significant crop damage. In this investigation, the host plant quality of four oilseed rape cultivars and seven other brassicaceous species for CSFB was assessed under controlled climate conditions. Larval weight and larval mortality was measured following 14 or 21 days of feeding inside the petioles of test plants. To determine the impact of secondary plant substances on performance of larvae, the glucosinolate (GSL) content was analysed in petioles of uninfested plants and infested plants 21 days after the start of larval infestation. Larval performance was not significantly different between the four oilseed rape (*Brassica napus* L.) cultivars tested but differed considerably between the other brassicaceous species. The weight of larvae was highest in turnip rape (*Brassica rapa* L. *silvestris*) and lowest in white mustard (*Sinapis alba* L.). The duration of larval development was increased in oilseed radish. The systemic GSL response in petioles to infestation by CSFB varied between different plant species. GSL content of current oilseed rape cultivars showed little difference between non-infested and infested plants while the aliphatic GSL group was increase in infested turnip rape cultivars. In contrast, in indian rape (*Brassica rapa* subsp. *dichotoma* Roxb.) the aliphatic and aromatic GSL decreased in infested plants. Larval weight was positively correlated with progoitrin and 4-hydroxyglucobrassicin. White mustard which provides inferior conditions for larval development has potential to introduce insect resistance into high yielding oilseed rape cultivars in breeding programs.



## Introduction

Oilseed (*Brassica napus* L.) rape is the most important oil crop in central and northern Europe (Eurostat, 2009). During the whole growing period it is attacked by various specialist pest insects (Alford et al., 2003; Ekbom, 1995). In the humid regions of northern Europe, the cabbage stem flea beetle (CSFB) (*Psylliodes chrysocephala* L.) (Col.: Chrysomelidae) is among the most devastating pests of winter oilseed rape (Williams, 2010; Winfield, 1992). In some years it causes significant yield losses even at moderate levels of infestation (Nilsson, 1990). During autumn adult beetles feed externally on the leaves of young plants but economically important damage is caused by the larvae (Ekbom, 1995). Larvae feed within petioles and stems of young rapeseed plants, thereby increasing plant losses during winter (Ekbom, 1995; Williams, 2010). In addition, their feeding wounds can result in a higher incidence of fungal pathogens, such as stem canker (*Leptosphaeria maculans* Ces. & de Not.) (Schulz & Daebeler, 1984). Control of CSFB is usually achieved by insecticidal seed dressing or spray application of insecticides, most often by using synthetic pyrethroids (Williams, 2004). Due to the high selection pressure CSFB populations may develop resistance against this group of insecticides, as recently has been reported from northern Germany (Heimbach & Müller, 2011). Problems related to chemical control makes the research for alternative control strategies more important. The search for insect resistant cultivars or plant species may be a promising option, as already known for cabbage seed weevil (*Ceutorhynchus obstrictus* Mrsh.) and root fly (*Delia floralis* L.) which are also specialist pests of Brassicaceae (Birch, 1988; Carcamo et al., 2007; Eickermann et al., 2010). Such sources of resistance could be integrated in oilseed rape breeding programs. Insect resistant oilseed rape cultivars could also help to reduce the number of insecticide applications and their negative impact on the environment (Williams, 2010).

Many plant species are accepted as host plants by larvae and adults of CSFB. Most of these species belong to the family of Brassicaceae, but also tropaeolaceous and resedaceous plant species are accepted as host (Bartlet & Williams, 1991). All these plants have in common that they contain glucosinolates (GSL) (Bartlet et al., 1994), the predominant group of sulphur containing



secondary plant substances in Brassicaceae (Mithen, 2001; Schoonhoven et al., 2005). Former studies have shown that the GSL profile varies between different brassicaceous species and plant organs (Sang et al., 1984). Due to their chemical similarities GSL can be subdivided in aliphatic, aromatic and indole compounds (Fahey et al., 2001; Hopkins et al., 2009). Even small changes in the side chain structure can have substantial influence on their biological activity (Mithen, 2001). By acting as feeding stimulants for specialist insects of the orders Coleoptera, Diptera and Lepidoptera GSL are known to play an important role in plant herbivore interactions (Hopkins et al., 2009). Furthermore, GSL are assumed to affect the larval performance of several insects specialized on Brassicaceae (Agrawal & Kurashige, 2003; Beekwilder et al., 2008; Mewis et al., 2008; Rohr et al., 2009). The aim of this investigation was to identify brassicaceous plant species and cultivars which hamper larval development of CSFB and to analyse the relationship between the GSL content in petioles and larval performance. Moreover, the effect of infestation by CSFB larvae on systemically induced changes of the GSL content and composition in different plant species was investigated. The GSL content was measured in petioles, the most important feeding site of CSFB larvae.

## Materials and Methods

### *Plant Material*

The test plants were selected to cover a wide range of different brassicaceous species and oilseed rape cultivars which differed largely in their glucosinolate profiles. Cultivars were chosen according to information from the literature or results of preliminary experiments. Four oilseed rape (*Brassica napus* L.) cultivars were included in the experiments. 'Robust', 'Grizzly' and 'Campala' contain low levels of GSL and erucic acid in their seeds (double low-quality) while seeds of 'Lembkes Normal' contain high levels of GSL and erucic acid. Three turnip rape (*Brassica rapa* L. *silvestris*) cultivars were selected: 'Largo' has a double low quality, whereas 'Malwira' and 'Perko' both contain high amounts of erucic acid and GSL in the seeds. Indian rape (*Brassica rapa* subsp. *dichotoma* Roxb.), white mustard (*Sinapis alba* L.) cultivar 'Accent', oilseed radish (*Raphanus sativa* L. var.



*oleiformis* Pers.) cultivar 'Adagio' and white cabbage (*Brassica oleracea* L. convar. *capitata* var. *alba*) cultivar 'Brunswijker' were also tested in the experiments. The plants were grown in a 3:1 mixture of potting soil (Fruhstorfer Erde Typ 25) and sand under glasshouse conditions. They were transferred to controlled climate conditions ( $18^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , 65-85% rH and a day/night period of L16:D8 hours) one week before the experiments started. Plants were fertilized once a week with all essential nutrients by application of 50ml of a 2g/l "HakaPhos Blau®" solution.

### *Insects*

New generation adults of CSFB emerging from soil after pupation were collected from crops of oilseed rape in July. They were maintained in plastic boxes (17,5cm \* 13cm \* 6cm) at  $15^{\circ}\text{C}$  during their summer aestivation. To provide food and moisture one oilseed rape leaf (cv Oase or Mozart) was offered to the beetles at least once a week. Following the end of aestivation in September, wet filter paper was placed into the boxes to stimulate egg laying of females (Williams et al., 2003). Eggs were collected weekly and stored in plastic boxes on wet sand at  $2^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for several months. To induce hatching of larvae the eggs were transferred to  $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$  one week before each experiment

### *Experimental setup*

The experiments started five weeks after sowing when the plants were in the six to eight leaf stage. In all experiments the oilseed rape cultivar 'Robust' was chosen as a reference because of the low glucosinolate content in the leaves. In each experiment larval development on the reference cultivar was tested against the development on two other cultivars. Sixteen to twenty plants of each cultivar were randomly arranged in a climate chamber without contact to adjacent plants, to prevent larval migration between test plants. Neonate larvae were released on test plants 24 hours after hatching by using a fine brush. Five larvae were released on the petiole base of the fourth youngest leaf near the axilla of each plant. After inoculation the light was turned off for six hours. Plants were sprayed with water every hour to avoid drought stress of the larvae; relative humidity was near to 100%. The inoculated plants were kept at  $18^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , 65-85% rH and L16:D8 hours. Half of the plants was harvested 14 days and 21 days, respectively, after



inoculation. The petioles and stems were dissected and larvae were recovered. Larval fresh weight was recorded using a microbalance (Sartorius MC 5) and head capsule width was measured as indicators of larval performance.

### *Glucosinolate analysis*

The GSL content was analysed from petioles of both infested and uninfested plants. Samples of petioles were collected from five uninfested plants at the start of the experiment and from five infested plants 21 days after release of CSFB larvae. The third and fourth youngest petiole of five plants of each treatment was pooled. The plant material was shock frozen at -80 °C immediately followed by freeze drying and grinding. The GSL were extracted by methanol; glucotropolin was added as an internal standard in the analyses. The samples were treated with sulfatase and after incubation desulphoglucosinolates were analysed by HPLC as described by Cleemput & Becker (2011).

### *Statistical analysis*

To compare mean weight of larvae in test plants and in the reference cultivar 'Robust' students t-test was used. Plants which did not contain any larvae were excluded from statistical analysis of weight and head capsule width. Correlations between the initial GSL content in the petioles and relative larval weight were analysed. No statistical analysis of the GSL content was conducted because no replicates were available. To compare the number of larvae recovered from plants, ANOVA was conducted followed by a post-hoc Tukey HSD test. For all statistical analyses Statistica 9.1 (StatSoft Inc.) was used.

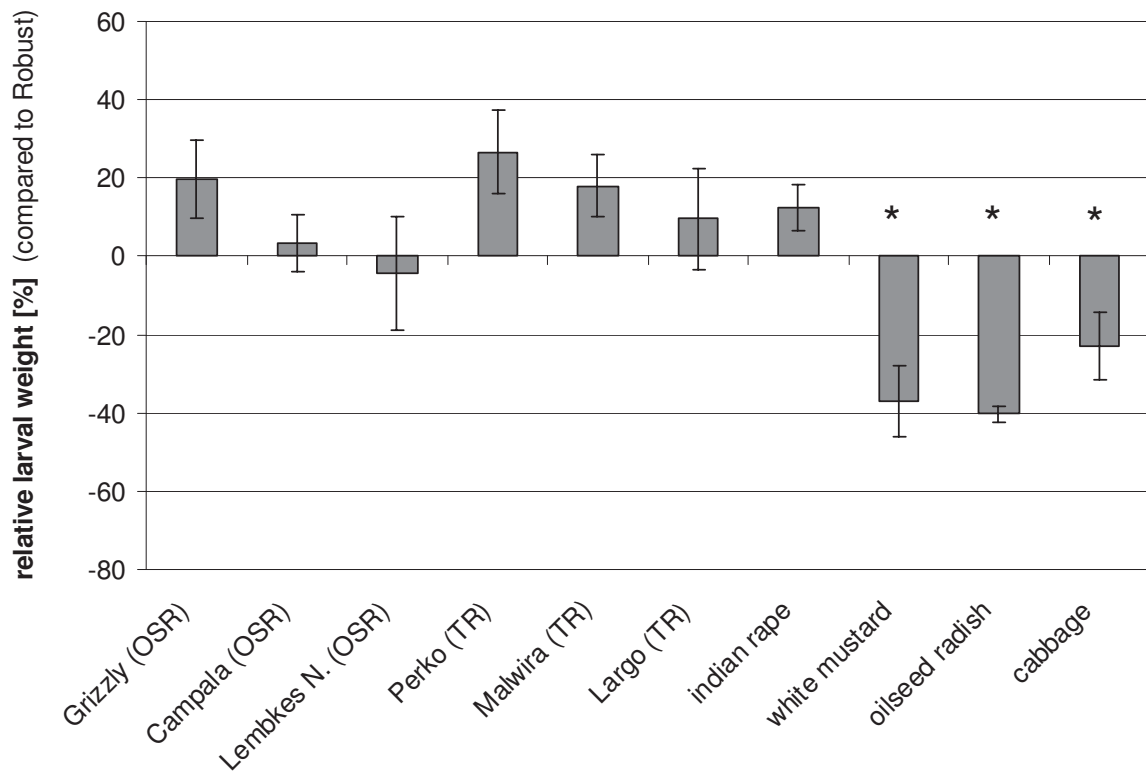
## **Results**

The development of larvae differed widely between the host plants tested (Fig. 1 & 2). Generally, the differences were more obvious at 14 days than at 21 days after infestation. Larval weight did not differ significantly between 'Robust' and the other oilseed rape cultivars, neither at 14 nor at 21 days of development within the plants. In turnip rape and indian rape the larval weight after 14 days was increased



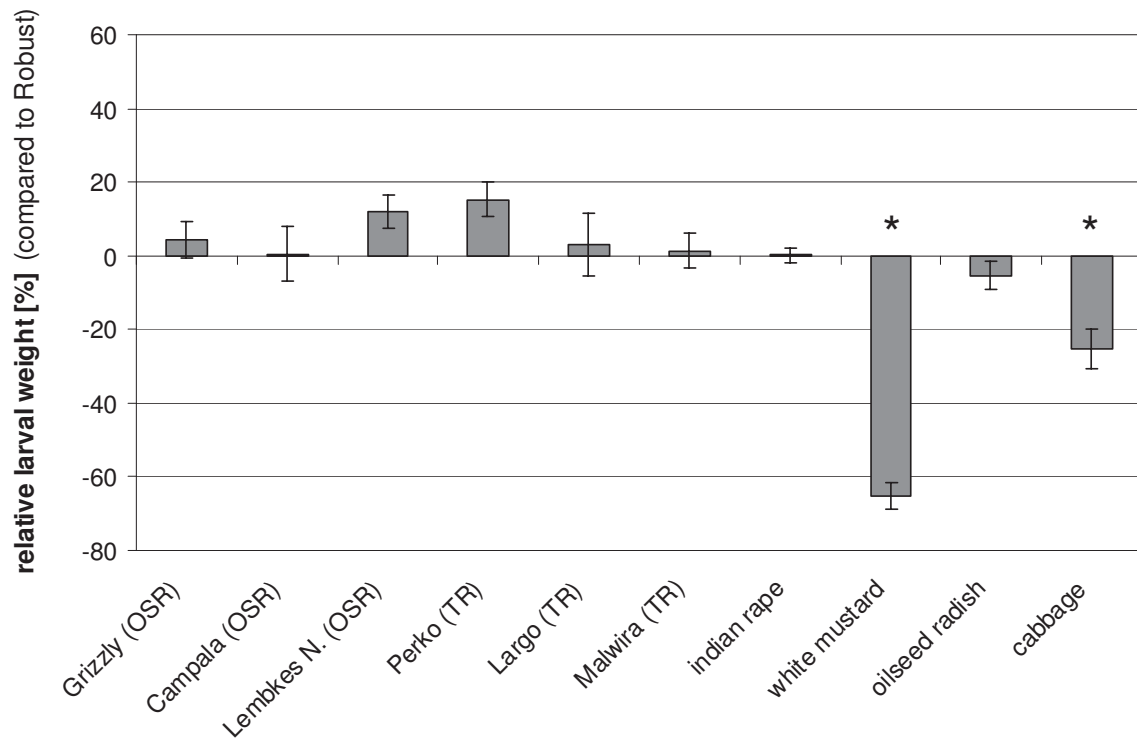


by 10 - 30% compared to 'Robust', however, these differences were not significant and were no longer present at 21 days after the start of experiment. After 14 days of feeding, the larval weight was significantly smaller on white mustard, oilseed radish or white cabbage than on 'Robust'.. The number of larvae recovered from plants after 14 days was significantly smaller and the larval mortality was higher only in white mustard as compared to 'Robust' (Tab. 1).



**Fig. 1:** Relative larval weight of *Psylliodes chrysocephala* reared in 10 test plants compared to larvae reared in the oilseed rape cultivar 'Robust' 14 days after release of larvae to plants. OSR - oilseed rape, TR - turnip rape; mean $\pm$ SE; asterisks indicate significant differences to reference cultivar  $\alpha \leq 0.05$ ; after 14 days larval fresh weight in 'Robust' varied from 1.7 to 2.4mg.

After 21 days significantly more larvae were found in oilseed radish because on 'Robust' and other plants tested a high proportion of larvae already had migrated to soil for pupation (Tab. 1). At 21 days after release the weight of larvae in oilseed radish was no longer smaller than the weight of larvae grown in 'Robust' while larvae feeding in white mustard and cabbage were still significantly lighter than larvae in 'Robust'.



**Fig. 2:** Relative larval weight of *Psylliodes chrysocephala* reared in 10 test plants compared to larvae reared in the oilseed rape cultivar 'Robust' 21 days after release of larvae to plants. OSR - oilseed rape, TR - turnip rape; mean $\pm$ SE; asterisks indicate significant differences to reference cultivar  $\alpha \leq 0.05$ ; after 21 days larval fresh weight in 'Robust' varied from 4.5 to 5.3mg.

At 14 days after release the percentage of 3<sup>rd</sup> instar larvae was substantially lower in white mustard and oilseed radish than in 'Robust. Even at 21 days after release, 75% of larvae in white mustard had not developed to the 3rd larval instar (Tab. 1).

In plants not infested by larvae, the GSL content in petioles of the double low oilseed rape cultivars 'Robust', 'Grizzly' and 'Campala' was lower compared to 'Lembkes Normal' and other tested brassicaceous species (Tab. 2). In these three oilseed rape cultivars the GSL were mainly represented by the aliphatic fraction. The GSL in petioles of the old cultivar 'Lembkes Normal' was also dominated by the aliphatic fraction but the total content of GSL was 4.7 to 11 times higher than in the double low cultivars of oilseed rape. The aliphatic GSL were also predominant in the tested turnip rape cultivars but in contrast to oilseed rape in all turnip rape cultivars the aromatic GSL were on a higher level in the petioles.



**Tab. 1:** Number of larvae recovered 14 and 21 days after start of the experiments (dpi), proportion of dead larvae and percentage of larvae in third larval stage; different letters indicate significant differences between three cultivars included in each experimental run. Tukey HSD test  $\alpha \leq 0.05$

	14 dpi			21 dpi		
	larvae/plant	% dead	% 3 <sup>rd</sup> instar	larvae/plant	% dead	% 3 <sup>rd</sup> instar
<b>Robust</b>	4.4 a	0	89	1.5 a	6.2	93
<b>Grizzly</b>	4.5 a	0	93	1.4 a	0	100
<b>Campala</b>	4.0 a	7.5	90	2.0 a	0	100
<b>Robust</b>	2.1 a	0	75	1.5 a	0	100
<b>Perko</b>	2.9 a	0	61	1.1 a	0	100
<b>Largo</b>	2.5 a	0	60	2.3 a	0	100
<b>Robust</b>	4.3 a	0	93	0.7 a	0	100
<b>Malwira</b>	4.3 a	0	95	1.1 a	0	100
<b>Ind. rape</b>	4.2 a	0	98	1.0 a	0	100
<b>Robust</b>	4.1 a	2.4	98	1.1 a	0	91
<b>Mustard</b>	1.8 b	53.8	36	0.3 a	10	25
<b>Radish</b>	4.4 a	0	53	4.0 b	2.4	100
<b>Robust</b>	2.9 a	3.3	90	0.6 ab	25	100
<b>Lembkes N.</b>	2.0 a	9.1	62	0.2 a	66.7	67
<b>Cabbage</b>	2.7 a	3.6	82	1.6 b	11.1	100

Further, a high initial content of indole GSL was found particularly in 'Malwira'. Indian rape and white mustard both showed a high content of aromatic compounds whereas indole compounds were on a low level in both species. In contrast to all other plant species tested, the content of indole GSL and aromatic GSL in oilseed radish was on a relatively high level.

In petioles of white cabbage the total content of GSL was low and mainly represented by aliphatic compounds. While no correlation was detected between individual chemical groups of GSL and relative larval weight, positive correlations were found between relative larval weight after 14 days and initial content of progoitrin (df = 10; r = 0.65; p = 0.03) and 4-hydroxyglucobrassicin (df = 10; r = 0.63; p = 0.04) in petioles of host plants.

The composition of the GSL in the double low-quality oilseed rape cultivars did not show induced changes after 21 days of CSFB infestation. In contrast, the high



initial content of 'Lembkes Normal' nearly doubled 21 days after larval infestation, resulting mostly from an increase in aliphatic GSL. All turnip rape cultivars showed a clear response to insect feeding with a strong increase of total GSL content. In contrast, in indian rape and white mustard the total GSL decreased following infestation by CSFB larvae.

Aromatic GSL compounds decreased in both species whereas aliphatics decreased in indian rape and increased in white mustard. Oilseed radish showed an increase in both aliphatic and aromatic GSL. In contrast the infestation by CSFB larvae had no effect on GSL content or composition in white cabbage cultivar 'Brunswijker'.

## Discussion

Our larval performance tests demonstrated that host plant quality of brassicaceous species for CSFB larvae is significantly different. While turnip rape cultivars were most suitable host plants white mustard clearly showed antibiosis resistance to the larvae. The high number of larvae present in oilseed radish after 21 days indicates that larval development was hampered in this host plant compared to oilseed rape. This was also confirmed by the low proportion of 3<sup>rd</sup> instar larvae after 14 days compared to larvae grown in 'Robust'. In contrast to white mustard larval mortality was not increased in oilseed radish. The low number of larvae recovered from most test plants after 21 days compared to the number of larvae obtained after 14 days is due to the fact that a high proportion of the larvae had already completed their development and had left the plants for pupation in soil. Even though the larvae were treated the same way before each experimental run, the number of larvae found in the reference cultivar 'Robust' showed substantial differences between experimental runs. The factors responsible for the different mortality of larvae are not clear. Environmental and pre-treatment factors such as duration of storage of the eggs may have affected survival of larvae.



**Tab. 2:** Glucosinolate (GSL) content [ $\mu\text{mol} \cdot \text{g DM}^{-1}$ ] in petioles of uninfested (-) and infested (+) plants 21 days after inoculation; <sup>1</sup> progoitrin, sinigrin, gluconapoleiferin, glucoraphanin, gluconapin, glucobrassicinapin, glucoerucin; <sup>2</sup> 4-hydroxyglucobrassicin, glucoerucin, glucobrassicin, 4-methoxyglucobrassicin, neoglucobrassicin; <sup>3</sup> sinalbin, gluconasturtiin

	Robust	Grizzly	Campala	Lembkes	Perko	Malwira	Largo	ind. rape	mustard	radish	cabbage											
	-	+	-	+	-	+	-	+	-	+	-											
Aliphatic GSL <sup>1</sup>	0.9	0.4	0.4	1.3	6.0	10.8	7.6	11.8	3.7	9.3	9.1	27.7	1.7	0.9	0.3	1.9	0.4	0.4	1.1	1.1		
Indole GSL <sup>2</sup>	0.2	0.2	0.1	0.1	0.1	0.3	0.2	0.9	0.7	1.0	1.9	0.8	0.6	2.0	0.3	0.1	0	3.7	5.0	0.8	0.6	
Aromatic GSL <sup>3</sup>	0.3	0	0	0.2	0	0.3	0.4	0.6	2.5	2.5	0.7	1.1	1.1	2.0	16.3	11.7	6.6	4.5	2.0	3.8	0.3	0.4
<b>total GSL</b>	<b>1.4</b>	<b>1.1</b>	<b>0.6</b>	<b>0.7</b>	<b>0.5</b>	<b>1.9</b>	<b>6.6</b>	<b>12.3</b>	<b>10.8</b>	<b>15.3</b>	<b>6.3</b>	<b>11.2</b>	<b>10.8</b>	<b>31.7</b>	<b>18.3</b>	<b>12.8</b>	<b>7.0</b>	<b>6.4</b>	<b>6.1</b>	<b>9.2</b>	<b>2.2</b>	<b>2.1</b>



Earlier studies dealing with the performance of insects specialized on brassicaceous plants also have shown considerable differences in the host plant quality for larval development. For example, larvae of *Phaedon cochlearia* (F.) were heaviest on *Brassica rapa* and lightest on *Sinapis alba*, while the weight of larvae reared on *Raphanus sativus* was intermediate (Uddin et al., 2009). The ranking of host plant quality was similar to CSFB in our experiments; it has also been recorded for *Mamestra configurata* (McCloskey & Isman, 1993). Sarfraz et al. (2010) compared the host plant quality of swede (*Brassica napus* subsp. *rapifera*) and kale (*Brassica oleracea* var. *acephala*) for turnip root fly (*Delia floralis* Fallén) larvae and found that swede was a better host for larval growth. A similar difference between *B. napus* and *B. oleracea* was observed for CSFB in our experiments. An inferior host plant quality of *Sinapis alba* has been reported for cabbage seed weevil (*Ceutorhynchus obstrictus* Marsham). When seed weevil larvae were reared on pods of oilseed rape and white mustard, a significant reduction of larval weight was observed on white mustard (Dosdall & Kott, 2006; Tansey et al., 2010; Ulmer & Dosdall, 2006).

The factors which are responsible for the different host plant quality are not yet fully understood. For specialist insects on brassicaceous plants, like cabbage stem flea beetle or crucifer flea beetle (*Phyllotreta cruciferae* (Goeze)), secondary plant metabolites such as GSL can act as feeding stimulants (Bartlett & Williams, 1991; Feeny et al., 1970). The effect of GSL on larval performance was found to differ between insect species. The GSL content has a negative effect on larval development of generalist insects. For specialist insects, contradictory results on the effect of GSL on larval growth have been reported (Hopkins et al., 2009). Some authors found significant correlations between larval growth parameters and GSL content in plant tissue whereas others did not. Ulmer & Dosdall (2006) suggested that the aromatic GSL gluconasturtiin might be responsible for the delayed larval development and reduced larval weight of cabbage seedpod weevil in some brassicaceous species. However, in recent studies Tansey et al. (2010) found a positive correlation between larval weight of cabbage seed weevil and the content of gluconasturtiin in pods. The authors also reported a negative correlation between larval weight and the content of neoglucobrassicin in the pods. In





experiments using *Arabidopsis thaliana* mutants, the larval performance of the two specialist insects *Pieris rapae* and *Pieris brassicae* was not affected by a drastic reduction of aliphatic or indole GSL in leaves (Mewis et al., 2008). No correlation between the GSL content and larval performance was found in studies on cabbage stem weevil (*Ceutorhynchus pallidactylus* Marsh.) and cabbage root fly (Birch et al., 1992; Eickermann et al., 2010). Bodnaryk (1997) concluded that resistance of white mustard against *Phyllotreta cruciferae* is independent from GSL content in the cotyledons. Our results suggest that the GSL are playing a role for host plant quality even for specialist insects such as CSFB. However, as the effect of single GSL on various insects was very heterogenous, further research is needed on this topic. Artificial diets or genetically manipulated host plants with modified GSL profiles may offer suitable tools for further experiments.

The effects of insect feeding on the GSL composition in plants have been repeatedly reported in literature (Koritsas et al., 1989; Koritsas et al., 1991; Birch et al., 1992; Bartlet et al., 1999), but results were not consistent. In our experiments the infestation by CSFB larvae had only little effect on indole GSL while aliphatic GSL increased in most test plants. In former studies feeding of adult CSFB on leaves of oilseed rape induced a systemic increase of indole GSL in young leaves seven days after feeding whereas the content of other GSL classes did not change significantly (Bartlet et al., 1999). Similar results were reported for non systemic responses of oilseed rape to infestation by CSFB larvae. Petioles infested by larvae showed a higher content of indole GSL and reduced content of aliphatic GSL compared to uninfested plants (Koritsas et al., 1989; 1991). When petioles of oilseed rape were mechanically damaged the GSL showed similar changes. In kale, indole as well as aliphatic GSL increased after mechanically damage whereas in white mustard only aromatic GSL increased (Koritsas et al., 1991). Contrasting observations were reported following infestation of swede, kale and oilseed rape by larvae of turnip root fly. Larvae feeding on the roots induced systemic changes of the GSL composition in aerial plant parts. The level of aliphatic GSL increased while indole GSL decreased in the leaves of these plants (Birch et al., 1992). Feeding by crucifer flea beetle had no effect on systemic induction of sinigrin and glucobrassicin (Traw & Dawson, 2002a; b).



Bartlet et al. (1999) suggested that a modified GSL profile may protect the plant against generalist insects and diseases while feeding by adult CSFB is not affected. A higher GSL content may attract higher numbers of specialist insects, because degradation products of GSL, isothiocyanates, are used for host finding (Bartlet, 1992). For future experiments on this topic 'Lembkes Normal' and 'Largo' are promising cultivars because of their very strong response in their GSL content compared to other brassicaceous species in our experiments. Field experiments need to be conducted to evaluate the performance of larvae of cabbage stem flea beetle on different host plant species under natural conditions. In the field the extended larval development may also increase the parasitism rate because larvae are exposed to their enemies for a longer time (Singh & Singh, 2005). Plants which hamper the larval development of cabbage stem flea beetle larvae may also increase efficacy of natural control mechanisms (Singh & Singh, 2005). Furthermore, other plant substances such as the fiber content in the petioles of different host plants have to be considered, due to the fact that the digestibility of plant material affects the larval performance (Waldbauer, 1964).

## References

- Agrawal AA & Kurashige NS (2003) A role for isothiocyanates in plant resistance against the specialist herbivore *Pieris rapae*. *Journal of Chemical Ecology* 29: 1403-1415.
- Alford DV, Nilsson C & Ulber B (2003) Insect pests of oilseed rape crops: Biocontrol of oilseed rape pests (ed. DV Alford) Blackwell Science Ltd., Oxford, pp. 9-42.
- Bartlet E, I. H. Williams, M. M. Blight, A. J. Hick (1992) Response of the oilseed rape pests, *Ceutorhynchus assimilis* and *Psylliodes chrysocephala*, to a mixture of isothiocyanates. *Proceedings of the 8th International Symposium on Insect-Plant Relationships*: 103-104.
- Bartlet E, Kiddle G, Williams I & Wallsgrove R (1999) Wound-induced increases in the glucosinolate content of oilseed rape and their effect on subsequent herbivory by a crucifer specialist. *Entomologia Experimentalis et Applicata* 91: 163-167.



- Bartlet E, Parsons D, Williams IH & Clark SJ (1994) The influence of glucosinolates and sugars on feeding by the cabbage stem flea beetle, *Psylliodes chrysocephala*. *Entomologia Experimentalis et Applicata* 73: 77-83.
- Bartlet E & Williams IH (1991) Factors restricting the feeding of the cabbage stem flea beetle (*Psylliodes chrysocephala*). *Entomologia Experimentalis et Applicata* 60: 233-238.
- Beekwilder J, van Leeuwen W, van Dam NM, Bertossi M, Grandi V, Mizzi L, Soloviev M, Szabados L, Molthoff JW, Schipper B, Verbocht H, de Vos RCH, Morandini P, Aarts MGM & Bovy A (2008) The impact of the absence of aliphatic glucosinolates on insect herbivory in *Arabidopsis*. *Plos One* 3: 12.
- Birch ANE (1988) Field and glasshouse studies on the components of resistance to root fly attack in swedes. *Annals of Applied Biology* 113: 89-100.
- Birch ANE, Griffiths DW, Hopkins RJ, Smith WHM & McKinlay RG (1992) Glucosinolate responses of swede, kale, forage and oilseed rape to root damage by turnip root fly (*Delia floralis*) larvae. *Journal of the Science of Food and Agriculture* 60: 1-9.
- Bodnaryk RP (1997) Will low-glucosinolate cultivars of the mustards *Brassica juncea* and *Sinapis alba* be vulnerable to insect pests? *Canadian Journal of Plant Science* 77: 283-287.
- Carcamo H, Olfert O, Dossdall L, Herle C, Beres B & Soroka J (2007) Resistance to cabbage seedpod weevil among selected Brassicaceae germplasm. *Canadian Entomologist* 139: 658-669.
- Cleemput S & Becker HC (2011) Genetic variation in leaf and stem glucosinolates in resynthesized lines of winter rapeseed (*Brassica napus* L.). *Genetic Resources and Crop Evolution*. doi:10.1007/s10722-011-9701-x.
- Dossdall LM & Kott LS (2006) Introgression of resistance to cabbage seedpod weevil to canola from yellow mustard. *Crop Science* 46: 2437-2445.
- Eickermann M, Ulber B & Vidal S (2010) Resynthesized lines and cultivars of *Brassica napus* L. provide sources of resistance to the cabbage stem



- weevil (*Ceutorhynchus pallidactylus* (Marsh.)). Bulletin of Entomological Research 110: 1-8.
- Ekbom B (1995) Insect pests: Brassica oilseeds - production and utilization (ed. by DS Kimber & DI McGregor) CAB International, Wallingford, pp. 141-152.
- Eurostat (2009) <http://epp.eurostat.ec.europa.eu/portal/page/portal/agriculture/data/database>. date: 29.09.2011
- Fahey JW, Zalcmann AT & Talalay P (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 56: 5-51.
- Feeny P, Paauwe KL & Demong NJ (1970) Flea beetles and mustard oils: host plant specificity of *Phyllotreta cruciferae* and *P. striolata* adults (Coleoptera: Chrysomelidae). *Annals of the Entomological Society of America* 63: 832-841.
- Heimbach U & Müller A (2011) Pyrethroid resistance of oilseed rape pest insects from 2005-2010 and German insecticide resistance management strategy: 13th International Rapeseed Congress, Prague, pp. 1278-1281.
- Hopkins RJ, van Dam NM & van Loon JJA (2009) Role of glucosinolates in insect-plant relationships and multitrophic interactions. *Annual Review of Entomology* 54: 57-83.
- Koritsas VM, Lewis JA & Fenwick GR (1989) Accumulation of indole glucosinolates in *Psylliodes chrysocephala* L. infested, or *Psylliodes chrysocephala* L. damaged tissues of oilseed rape (*Brassica napus* L.). *Experientia* 45: 493-495.
- Koritsas VM, Lewis JA & Fenwick GR (1991) Glucosinolate response of oilseed rape, mustard and kale to mechanical wounding and infestation by cabbage stem beetle (*Psylliodes chrysocephala*). *Annals of Applied Biology* 118: 209-221.
- McCloskey C & Isman MB (1993) Influence of foliar glucosinolates in oilseed rape and mustard on feeding and growth of bertha armyworm, *Mamestra configurata* Walker. *Journal of Chemical Ecology* 19: 249-266.



- Mewis I, Rohr F, Tokuhiya JG & Ulrichs C (2008) Auswirkung einer Modifizierung des Glucosinolatprofils von *Arabidopsis thaliana* (L.) auf verschieden spezialisierte Lepidoptera. Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie 16: 167-169.
- Mithen R (2001) Glucosinolates - biochemistry, genetics and biological activity. Plant Growth Regulation 34: 91-103.
- Nilsson C (1990) Yield losses in winter rape caused by cabbage stem flea beetle larvae (*Psylliodes chrysocephala* (L.)). IOBC/wprs Bulletin 13 (4): 53-55.
- Rohr F, Ulrichs C & Mewis I (2009) Variability of aliphatic glucosinolates in *Arabidopsis thaliana* (L.) - Impact on glucosinolate profile and insect resistance. Journal of Applied Botany and Food Quality 82: 131-135.
- Sang JP, Minchinton IR, Johnstone PK & Truscott RJW (1984) Glucosinolate profiles in the seed, root and leaf tissue of cabbage, mustard, rapeseed, radish and swede. Canadian Journal of Plant Science 64: 77-93.
- Sarfraz RM, Dosedall LM & Keddie BA (2010) Performance of the specialist herbivore *Plutella xylostella* (Lepidoptera: Plutellidae) on Brassicaceae and non-Brassicaceae species. Canadian Entomologist 142: 24-35.
- Schoonhoven LM, van Loon JJA & Dicke M (2005) Insect-plant biology. Oxford University Press, Oxford.
- Schulz R-R & Daebeler F (1984) Zum Schaden durch den Rapserrdfloh (*Psylliodes chrysocephala* L.), insbesondere seiner Imagines. Nachrichtenblatt für den Pflanzenschutz in der DDR 38: 113-115.
- Singh DP & Singh A (2005) Disease and pest resistance in plants. Science Publishers, Enfield.
- Tansey JA, Dosedall LM, Keddie A, Fletcher RS & Kott LS (2010) Antixenosis and antibiosis resistance to *Ceutorhynchus obstrictus* in novel germplasm derived from *Sinapis alba* x *Brassica napus*. Canadian Entomologist 142: 212-221.
- Traw MB & Dawson TE (2002a) Differential induction of trichomes by three herbivores of black mustard. Oecologia 131: 526-532.



- Traw MB & Dawson TE (2002b) Reduced performance of two specialist herbivores (Lepidoptera: Pieridae, Coleoptera: Chrysomelidae) on new leaves of damaged black mustard plants. *Environmental Entomology* 31: 714-722.
- Uddin MM, Ulrichs C, Tokuhisa JG & Mewis I (2009) Impact of glucosinolate structure on the performance of the crucifer pest *Phaedon cochleariae* (F.). *Journal of Applied Botany and Food Quality* 82: 108-113.
- Ulmer BJ & Dossall LM (2006) Glucosinolate profile and oviposition behavior in relation to the susceptibilities of Brassicaceae to the cabbage seedpod weevil. *Entomologia Experimentalis et Applicata* 121: 203-213.
- Waldbauer GP (1964) The consumption, digestion and utilization of solanaceous and non-solanaceous plants by larvae of the tobacco hornworm, *Protoparce sexta* (Johan.) (Lepidoptera: Sphingidae). *Entomologia Experimentalis et Applicata* 7: 253-269.
- Williams IH (2004) Advances in insect pest management of oilseed rape in Europe. In: *Insect pest management* (eds. AR Horowitz & I Ishaaya). Springer-Verlag, Berlin Heidelberg, pp. 181-208.
- Williams IH (2010) The major insect pests of oilseed rape in Europe and their management: an overview. In: *Biocontrol-based integrated management of oilseed rape pests* (ed. IH Williams). Springer Science+Business B.V., Dordrecht, Heidelberg, London, New York, pp. 1-43.
- Williams IH, Büchi R & Ulber B (2003) Sampling, trapping and rearing oilseed rape pests and their parasitoids. In: *Biocontrol of oilseed rape pests* (ed. DV Alford). Blackwell Science Ltd, Oxford, pp. 145-160.
- Winfield AL (1992) Management of oilseed rape pests in Europe. *Agricultural Zoology Reviews* 5: 51-95.



