Effect of *Brassica* genotype on the infestation by cabbage stem weevil *Ceutorhynchus pallidactylus* (Mrsh.) (Col.: Curculionidae) and the parasitism of stem weevil larvae

Dissertation
zur Erlangung des Doktorgrades
der Fakultät für Agrarwissenschaften
der Georg-August-Universität Göttingen

vorgelegt von

Michael Eickermann
geboren zu Göttingen

Göttingen, Dezember 2008
1. Referent: Prof. Dr. Stefan Vidal
2. Korreferent: Prof. Dr. Heiko Becker
„Für euch, Kinder der Wissenschaft und der Weisheit, haben wir dieses geschrieben. Erforschet das Buch und suchet euch unsere Ansicht zusammen, die wir verstreut und an mehreren Orten dargetan haben; was euch an einem Orte verborgen bleibt, das haben wir an einem anderen offen gelegt, damit es fassbar werde für eure Weisheit.“

**Heinrich Cornelius Agrippa von Nettesheim**

*De occulta philosophia* 3, 65

*English translation: “We have written this for you, children of science and wisdom. Explore the sermons and search for our minds we have expounded in many places inside. What is hidden from you in one passage, we have offered you in another, to conceive it for your wisdom“.*
Dedicated to my great-grandmother, Alwine...
# Table of contents

<table>
<thead>
<tr>
<th>Chapter I - General introduction</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host selection of oilseed rape pests</td>
<td>7</td>
</tr>
<tr>
<td>Mechanisms of host plant resistance to oilseed rape pests</td>
<td>8</td>
</tr>
<tr>
<td>Screening methods for host plant resistance</td>
<td>10</td>
</tr>
<tr>
<td>Life cycle and phenology of <em>C. pallidactylus</em></td>
<td>11</td>
</tr>
<tr>
<td>Host plant range and damage potential of <em>C. pallidactylus</em></td>
<td>12</td>
</tr>
<tr>
<td>Strategies for control of <em>C. pallidactylus</em></td>
<td>14</td>
</tr>
<tr>
<td>References</td>
<td>15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter II - Screening of oilseed rape and other brassicaceous genotypes for susceptibility to <em>Ceutorhynchus pallidactylus</em> (Mrsh.)</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter III - Resynthesized lines and cultivars of <em>Brassica napus</em> L. provide sources of resistance to the cabbage stem weevil (<em>Ceutorhynchus pallidactylus</em> (Mrsh.))</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>32</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter IV - Influence of different Brassica host plants on parasitism of ceutorhynchid stem weevils</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>34</td>
</tr>
<tr>
<td>Introduction</td>
<td>34</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>36</td>
</tr>
<tr>
<td>Results</td>
<td>37</td>
</tr>
<tr>
<td>Discussion</td>
<td>45</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>47</td>
</tr>
<tr>
<td>References</td>
<td>48</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter V – General discussion</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening methods</td>
<td>51</td>
</tr>
<tr>
<td>Host plant susceptibility</td>
<td>53</td>
</tr>
<tr>
<td>Influence of glucosinolates</td>
<td>53</td>
</tr>
<tr>
<td>Multitrophic interactions</td>
<td>54</td>
</tr>
<tr>
<td>Conclusion</td>
<td>57</td>
</tr>
<tr>
<td>References</td>
<td>57</td>
</tr>
</tbody>
</table>
Chapter I - General introduction

Oilseed rape (*Brassica napus* L. var. *oleifera* Metzg.) (*Brassicaceae*) is the third most widely grown crop in the European Union (FAO 2005). In Germany the acreage grown in 2008/09 was ca. 1.42 Mill. ha (UFOP 2008). The area of oilseed rape continues to increase due to the increasing demand for plant oils in food (e.g. edible oil) and non-food sectors (e.g. lubrication-purpose, bio energy and bio fuels) (Winter 2004). With the recent extension of the planted area in the last twenty years, damage by diseases and pests of oilseed rape has become more severe (Lamb 1989; Alford *et al.* 2003). One of the most important limiting factors in the production of *Brassica* oilseeds is the complex of insect pests associated with these plants (Ekbom 1995). A succession of pests attacks the crop from its early seedling stage in autumn to the beginning of pod ripening in the following summer. Winfield (1992) has specified two important groups of insect pests infesting in winter oilseed rape in European countries, namely (i) pests attacking the crop in its vegetative phase, i.e. slugs, cabbage root fly, cabbage stem flea beetle, several species of stem miners and wood pigeons, and (ii) pests attacking the flower buds and seed pods, i.e. pollen beetle, cabbage seedpod weevil and brassica pod midge. Damage of each specialist pest species is mostly restricted to a particular plant organ of oilseed rape (Kirk 1992). Among pests infesting the vegetative growth stage of oilseed rape, the cabbage stem weevil, *Ceutorhynchus pallidactylus* (Marsham 1802), syn: *C. quadridens* (Coleoptera: Curculionidae)) is widely distributed on crops of oilseed rape in Central and Northern Europe. In many countries, *C. pallidactylus* is estimated to be an important pest on oilseed rape, e.g. Austria (Berger 1991), Czech Republic (Kazda 1957; Šedevý & Vašák 2002), Finland (Kangas 1976), France (Lerin 1995), Poland (Dmoch 1958; Pałosz 1978; Kelm & Walczak 1998), Slovakian Republic (Holecová *et al.* 2005), Switzerland (Büchi 1990) and the United Kingdom (Winfield 1961a; Graham & Gould 1980; Ferguson *et al.* 2006).

The main objectives of this study are:

1. to develop laboratory screening methods for identification and quantification of host plant quality and resistance in brassicaceous species to cabbage stem weevil (*Ceutorhynchus pallidactylus*).
2. to screen a large assortment of cultivars of oilseed rape, breeding lines, resynthesized oilseed rape lines and other species of Brassicaceae under controlled conditions in growth chambers
3. to validate the resistance of selected genotypes under field conditions
4. to investigate the parasitism of stem-boring ceutorynchid larvae in various cultivars of *Brassica* spp.

The following chapter will present an overview about the state of the art.

**Host selection of oilseed rape pests**

Herbivorous insect species commonly accept only a limited number of plant species as hosts (van Loon *et al.* 1992), and location and selection of host plants is an elementary step in the insects’ life cycle. Many studies were carried out to identify cues involved in host finding of specialist and generalist insects (Singh & Ellis 1993). Schoonhoven *et al.* (1998) have divided the host selection behaviour into five specific phases: searching, selection, acceptance, preference and finally recognition. Two major groups of stimulants are important for host finding: visual and olfactory cues (Hawkes *et al.* 1978; Schoonhoven *et al.* 1998). Visual cues are related to plant morphology, colour, leaf structure, while chemical cues like plant volatiles or phagostimulants can be influenced by various factors, e.g. wounding or physiological status of the plant (Blaakmeer *et al.* 1994; Schoonhoven *et al.* 1998). Plant metabolites are widely considered to be mediators in insect orientation to host plants (Rask *et al.* 2000; Pontoppidan *et al.* 2003), and their acceptance as a resource for feeding or oviposition (Lamb 1989; Schoonhoven *et al.* 1998).

For host finding and acceptance, the specialist insect pests of *Brassica* oilseeds rely on chemical compounds, characteristic for the family *Brassicaceae*: the glucosinolates (GSLs) (Ekborn 1995). Approximately 120 different GSLs have been detected so far (Fahey 2001). They are stored in plant tissue and hydrolyzed particularly by the enzyme myrosinase into breakdown products like isothiocyanate, hydrogen sulphate and D-glucose (Ekborn 1995).

The total amount and composition of GSLs within plants depends on several factors: genetic, environmental, agronomic or on influence of insect attack (Fenwick 1983; Koritsas *et al.* 1991; Bartlet *et al.* 1999a; Wallsgrove *et al.* 1999). The total amount of
GSL is usually not closely related to single GSL compounds or their distribution within plant organs (Fieldsend & Milford 1994; Yungchang et al. 1999). Further, in many studies no correlation was found between the GSL content in the seeds and in the vegetative tissue (Wallsgrove et al. 1999).

The GSL profile within plants can have substantial impact on host plant quality for specialist insects. This highly specific system is generally accepted to be a part of the plant’s defence against generalist pests. GSLs are known as anti-feedants for many polyphagous herbivores. In contrast, insect species, which are adapted on Brassicaceae, use GSLs or their fission products as feeding stimulants or attractants (Lamb 1989; Pontoppidan et al. 2003), which is an effect of co-evolution between host plant and insect (Rask et al. 2000).

Interactions between the content of GSLs in Brassicaceae and the behaviour of pests have been studied extensively in recent years (Rask et al. 2000; Liblikas et al. 2003) with particular emphasis on insect attraction and feeding. In plants injured by the cabbage stem flea beetle, *Psylliodes chrysocephala* L., a significant increase of the indolic GSL glucobrassicin and neoglucobrassicin was demonstrated (Koritsas et al. 1991). Both GSLs have been identified as an attractant for *P. chrysocephala* (Wallsgrove et al. 1999). In addition, GSLs also act as feeding stimulants (Giamoustaris & Mithen 1995). Feeding by *P. chrysocephala* increased with increasing levels of GSLs. The large white butterfly, *Pieris brassicae* L., uses the GSL gluconasturtiin as a recognition cue for location of host plants (Huang & Renwick 1994) and feeding of larvae (Miles et al. 2005). Further, glucobrassicin serves as an attractant for oviposition (Van Loon et al. 1992). GSLs provide also important cues for flea beetles on *Brassica* spp., *Phyllotreta cruciferae* (Goeze) and *P. chrysocephala*, (Lamdon et al. 1998; Liblikas et al. 2003; Henderson et al. 2004), cabbage root fly, *Delia radicum* (L.), (Hardman & Ellis 1978), cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham), (McCaffrey et al. 1999; Ulmer & Dosdall 2006) and brassica pod midge, *Dasineura brassica* Winn., (Bartlett et al. 1999b).

The aim of various studies was to alter the host-herbivore-interaction by modifying the GSL content in host plants (Åhman 1982; Larsen et al. 1983). However, insect feeding responses to cultivars of low- and high-GSL content did not show consistent differences (Weber et al. 1986; Mithen 1992; Giamoustaris & Mithen 1995; Bartlet 1996). In laboratory experiments, pollen beetle, *Meligethes aeneus* (Fabr.), was less attracted to
low-GSL cultivars compared with cultivars containing a high content of GSL (Cook et al. 2003).

**Mechanisms of host plant resistance to oilseed rape pests**

Host plant resistance to pest insects has been generally sub-divided in antixenosis and antibiosis (Painter 1951; Kogan & Ortmann 1978). ‘Antixenosis’ refers to the inability of a plant to be an attractant host for herbivorous insects, influencing them to select other plants (Kogan & Ortmann 1978). ‘Antibiosis’ is characterized as plant factors affecting the insect life history and performance negatively (survival, growth or fecundity). Both, antixenosis and antibiosis are mainly caused by morphological or chemical plant parameters (Kogan & Ortmann 1978). In the past, resistant cultivars have been developed in various crop species against various pests, e.g. aphids (Ellis & Farrell 1995; Messina & Bloxham 2004), stem and root borers (Reay-Jones et al. 2003; Murray et al. 2007) and lepidopteran species (Stoner 1990; Sarfraz et al. 2007).

*Brassica* plants have developed different resistant reactions to insect attack, which can be constitutive or induced by the insect itself (Meiners & Hilker 2002). Constitutive defense mechanisms are often caused by plant morphology (Way & Murdie 1965; Schoonhoven et al. 1998; Stoner 1990) or secondary metabolite compounds (Giamoustaris & Mithen 1997; Lambdon et al. 1998). Induced plant resistance is commonly based on different mechanisms. A specific defensive reaction of *Brassica* host plants has been reported with cabbage stem weevil (Pałosz 1980; Nuss 2004). As a response to oviposition into petioles, extensive wound callus tissue grows under the egg batch, thereby pushing the eggs to the surface (Broschewitz 1985). The percentage of egg batches destroyed by callus formation has been reported to vary between 40% (Broschewitz & Daebler 1987) and more than 70% (Pałosz 1980). Kazda (1953) presumed that the production of callus tissue could be increased by oviposition during early growth stages and high humidity conditions. The role of feeding or semiochemicals in activating this physiological reaction is still unknown.

Isothiocyanates have been determined as attractants and feeding stimulants for many brassicaceous pests (Koritsas et al. 1991; Giamoustaris & Mithen 1995). The development of cultivars that produce only low amounts of specific isothiocyanates might change the attraction of specialist insects to their host plants (Cook et al. 2003; Williams 2004). The stem weevils, *C. pallidactylus* and *C. napi*, were attracted to
yellow water traps at significantly higher numbers when 2-phenylethyl isothiocyanate was used as a bait substance (Walczak et al. 1998). Specific parasitoids were attracted by this isothiocyanate as well (Bradburne & Mithen 2000; Ulber & Wedemeyer 2006).

**Screening methods for host plant resistance**

A large number of laboratory screening methods have been developed for testing the susceptibility of different host plants to pest species (Farrell 1977; Harmon & McCaffrey 1997; Ulmer et al. 2001). The basis for the identification of (partial) resistance in plants is the screening of a large assortment of genetically diverse genotypes in bioassays. The identification of resistant genotypes requires simple, rapid and reliable techniques for the evaluation of pest performance (Ellis & Kift 2003). Consequently, the selection of parameters which can be used as indicators for the host quality of the plant is the first step of developing a new screening method (Farrell 1977). Screening methods should allow preferably discrimination between different types of resistance, namely antixenosis and antibiosis.

The standardization of plant material grown under greenhouse conditions rather than the standardization of test insects is of great importance in achieving reliable results (Harmon & McCaffrey 1997). Pests (e.g. aphids or mites) are often reared in the laboratory quite successfully, so the standardization of larval instar or nutritional status is possible. Specialist pests which prefer single plant organs or growth stages for reproduction are often more reluctant to permanent rearing in the laboratory, especially when they are univoltine or rely on winter diapauses (Broshewitz 1985; Barari et al. 2006).

In general, there are two basic methods that can be applied to test host plant quality for herbivorous insects: (i) multi-choice tests and (ii) no-choice tests (Farrell 1977; Ulmer et al. 2001). In no-choice tests, only one individual plant or detached plant part is exposed to the insects, whereas in choice-tests the insects can select between two or more different plant genotypes. Every method has its specifications. Multiple-choice tests allow better comparisons between different genotypes at the same time (Bartlet & Williams 1991), but they do not represent the field situation, where only one cultivar of a crop species is available to colonization by the pest. No-choice tests are often easier to manage (Risch 1985), but the exclusion of choice-factors can deform results in a way, that only extreme responses of the insect can be detected (Farrell 1977). Furthermore,
no-choice test can customize insects to a plant, which may not be a common host. The design of a screening method may have significant effects on results (Risch 1985). For example, screening by using detached leaves or racemes of brassicaceous plant species induces breakdown of GSLs into various products (Blaakmeer et al. 1994), which might act as stimulant cues for specialist pests (Pontoppidan et al. 2003). Generalists are considered to be less affected by the screening design than highly specialist species (Risch 1985). Biochemical assays can apply for detecting alterations of primary or secondary plant metabolites (Farell 1977).

Results from laboratory screening experiments need to be validated under field conditions for confirmation. Chemical cues and stimulants which can influence many phases in host searching and host acceptance, especially when volatile odors are involved, might be inhibited by the screening design in the laboratory.

In the past, various screening methods have been developed for testing plant resistance to pest insects of Brassica spp. in the laboratory (Bartlett & Williams 1991; Harmon & McCaffrey 1997; Sarfraz et al. 2007). However, methods for screening the resistance to cabbage stem weevil, C. pallidactylus, were not available at the beginning of this research work.

**Life cycle and phenology of C. pallidactylus**

*C. pallidactylus* is an univoltine stem miner about 3 mm long and brown to black in colour with a patch of white scales in the centre of the elytra (Fig 1). Adult *C. pallidactylus* migrate from their overwintering habitats (under leaf litter at the edges of woods or shrubs) into crops of oilseed in March/April (Günthart 1949; Alford et al. 2003). First flights commonly occur at temperatures $> 12$ °C and low wind velocity $< 3$m/s (Friesland 1990; Kjær-Pedersen 1992; Johnen & Meier 2000). Females feed on leaves and shoots up to 12 days for ovary maturation (Broschewitz 1985). During March to May they deposit batches of 4-6 translucent eggs (0.4mm by 0.6mm) (Winfield 1992) into small pockets bitten into the parenchyma of petioles and midribs of leaves (Ferguson et al. 2003; Barari et al. 2005).
The total number of eggs laid per female was estimated at 60-70 eggs in the field (Broschewitz 1985) and up to 280 eggs in the laboratory (Körting 1942). Larvae are coloured white to yellowish and have a brown head capsule (Fig 2). During the first and second instar, they feed within the pith of petioles. Third instar larvae bore into the main stems of plants (Broschewitz 1985; Barari et al. 2005).

In June/July, mature third instar larvae leave the stem through exit holes, to pupate 2-3cm deep in the soil (Broschewitz & Daebler 1987; Barari et al. 2005). Depending on weather conditions pupation takes approximately 20-30 days (Broschewitz 1985). Adult weevils emerge from soil at the end of June or beginning of July. After feeding on wild and cultivated Brassicaceae or perennial oilseed rape for four weeks, they migrate to their overwintering sites (Broschewitz & Daebler 1987; Williams 2004) (Fig 3).
Host plant range and damage potential of *C. pallidactylus*

The cabbage stem weevil can exploit a large number of host plants of Brassicaceae (Speyer 1921; Günthart 1949; Tommes 1998). Adults have been reported to feed on cultivated and wild relatives of oilseed rape, *B. napus* L. var. oleifera Metzg., turnip rape, *Brassica rapa* L., cabbage, *Brassica oleracea* L., black mustard, *B. nigra* (L.), Indian mustard, *B. juncea* (L.), radish, *Raphanus* spp., white mustard, *Sinapis alba* (L.), sea-kale, *Crambe maritime* L., and several wild brassicaceous species.

The pest status and plant damage of *C. pallidactylus* has been studied extensively in spring and winter oilseed rape and vegetable *Brassicas* (Günthart 1949; Winfield 1961a; Broschewitz 1985). The damage potential in oilseed rape has been assessed controversially in the literature. Winfield (1961a) and Graham & Gould (1980) supposed that yield losses by *C. pallidactylus* in the UK only occur in spring rape and came to the conclusion, that *C. pallidactylus* is unimportant for winter oilseed rape. Yield reductions by *C. pallidactylus* on winter oilseed rape were also not detected by Lehman (1965). In Poland, economic damage by *C. pallidactylus* only occurred in winter crops of low density with weak and smaller plants (Palosz 1980). In contrast, Broschewitz (1985) found that winter rape crops of high plant densities are more
susceptible and suffer from increased larval infestation, due to thinner main stems and a higher fecundity of females. At low plant density and especially with cultivars producing a high biomass, the infested plants have higher compensation ability (Broschewitz & Daebler 1987). In Slesvig-Holstein, Landschreiber (2005) reported on yield losses up to 20%. Kelm & Walzak (1998) found a positive correlation between the length of the larval feeding tunnel and shedding of pods; they assumed that dry stress of plants might increase damage by C. pallidactylus. Yield effects by feeding of larvae can be compensated by oilseed rape plants at early growth stages (Kelm & Klukowski 2000). Damage by adult cabbage stem weevils feeding on leaves has never been observed (Broschewitz 1985).

While the direct damage of C. pallidactylus larvae by feeding within petioles and stems varies between fields and locations, indirect damage of larvae by causing wounds and ports for entry stem canker (Leptosphaeria maculans (Desm.) Ces. and Not. [anamorph: Phoma lingam (Tode ex Fr. Desm.)]) is more important; it increases early colonization and fast growth of the pathogen within stems (Lücke & Pluschkell 1982; Broschewitz et al. 1993). Pałosz et al. (1994) found a positive correlation between infestation by C. pallidactylus and the severity stem cancer. Insecticide applications for control of cabbage stem weevil reduced the incidence of Leptosphaeria stem cancer and increased yield (Hartleb et al. 1998). Interactions between C. pallidactylus and other pest species using the same ecological niche have been reported for rape stem weevil, Ceutorhynchus napi, (Dechert & Ulber 2004), and cabbage stem flee beetle, Psylliodes chrysocephala (Ferguson et al. 2006).

Strategies for control of C. pallidactylus

Application of insecticides is the most commonly used measure for control of C. pallidactylus as other important pests on oilseed rape (Hartleb et al. 1998; Buntin 1999; Williams 2004; Seta & Wolski 2006). With two insecticide applications in spring, percentage infestation of cabbage stem weevil significantly decreased (Wahmhoff 2000). As the pest invades the oilseed rape crop in irregular succession, exact timing is crucial and single applications are often little effective (Free & Williams 1979). Yellow water traps monitor migration flights of adults in spring (Garbe et al. 1996) and help to detect the ideal date for application. Spraying thresholds are based today on yellow
water trap catches (Williams 2004). Computer-based decision support systems, e.g. PRO_PLANT, are used to forecast the phenology in the crop (Johnen & Meier 2000).

Synthetic pyrethroids are the predominant insecticides applied on oilseed rape. However, this group of insecticides is non-selective for non-target insects, such as predators or parasitoids (Ekbom 1995; Williams 2004), being a natural source of potential benefit. Further frequent usage of pyrethroids can result in insecticide resistance. Resistance of pollen beetle against pyrethroids have been reported all over Europe (Hansen 2003; Derron et al. 2004; Heimbach et al. 2006; Wegorek et al. 2006); there are first indications for similar effects on C. pallidactylus. The risk of insecticide resistance places greater emphasis on searching for alternative control methods.

An extensive overview on non-chemical methods of pest management in oilseed rape is given by Williams (2004). Several cultivation techniques can contribute to Integrated Pest Management (IPM) (Williams 2004). A significantly higher reproduction rate of C. pallidactylus was found in conventional farmed, ploughed fields than in organic conservation tillage farming (Büchs & Katzur 2004). Only insignificant reductions of the larval abundance of C. pallidactylus were obtained by using mixed cropping systems of oilseed rape with cereals or legumes (Paulsen et al. 2006). A higher numbers of parasitoids in fields with non-inversion tillage is reported (Nitzsche & Ulber 1998).

Plant architecture and development can influence pest abundance (Büchs & Katzur 2004; Williams 2004). Infestation by cabbage stem weevil increases as plant density decreases because a higher number of leaves and larger leaf size at low plant density increases oviposition (Nuss & Ulber 2007). Generally, hybrid cultivars are considered to have higher compensation ability to pest damage by growing more vigorously (Lamb 1989).

Conservation biological control of C. pallidactylus can provide substantial natural control. The larvae are attacked by five endoparasitoids (Alford et al. 2003). In Germany and other Central European countries, the most common species is Tersilochus obscurator Aubert (Hym.; Ichneumonidae) (Klingenberg & Ulber 1994, Ulber & Nitzsche 2006). Females parasitize host larvae, while these are feeding within petioles. They oviposit through the plant tissue and lay a single egg into pest larvae. The level of parasitism of C. pallidactylus by T. obscurator ranges between 18 and 52% (Nissen 1997, Ulber 2003); it depends on several external factors, e.g. climate (Johnen et al. 2006), stem-base diameter (Ulber & Fischer 2006), plant volatiles (Jönsson et al. 2006).
and crop management practices (Klingenberg & Ulber 1994; Nitzsche & Ulber 1998). Further, predators like Carabidae and Staphylinidae can play an important role (Piper & Williams 2004; Schlein & Büchs 2004; Büchs et al. 2006). They feed on *C. pallidactylus* larvae when these are leaving the plant for pupation in soil. In addition, entomophagous larvae of the muscid fly, *Phaenoia trimaculata* Bouche, feed on the larvae within stems (Fritzsche 1955; Lehmann 1965). Female *P. trimaculata* invades the split stems of oilseed rape to oviposit into the damaged pith. The abundance of stem mining larvae can be reduced up to 55% (Fritzsche 1955).

More recently, the so-called ‘Push-Pull-strategy’ has been developed in the UK as a new component of IPM in oilseed rape (Cook et al. 2003; Williams 2004). It utilises the behavioural responses of pests and parasitoids to plant- and insect-derived semiochemicals (Cook et al. 2007a). Repellent compounds and/or resistant cultivars are used to ‘push’ the pests from the main crops, and attractants or more attractive plants are used to ‘pull’ insects to trap crop areas, e.g. turnip rape (Potting et al. 2005), where they can be killed selectively by insecticides (Barari et al. 2005) or entomopathogenic fungi (Butt et al. 1998). At present, the development of the ‘Push-Pull-strategy’ is in progress (Cook et al. 2007b).

A new approach to IPM is the development of genetically modified organisms. Two types of transgenic insect-resistant *Brassica* have been developed (Earle et al. 2004): expressing *Bacillus thuringiensis* genes (Xue-bao et al. 1995) and the expression of proteinase inhibitor genes (Girard et al. 1998). The impact of these unspecific methods on *C. pallidactylus* has not been evaluated, so far. Additionally, a new method had been established in the laboratory for controlling pollen beetle by transforming oilseed rape with a pea lectin gene (Lehrmann 2007). The usage of transgenic insect-resistant *Brassica* crops as an instrument of IPM is not possible in Europe because of low public acceptance and legal guidelines (Davis 2006).

Host plant resistance to insect pests is an underutilized strategy in IPM (Eigenbrode & Trumble 1994). Classical crop breeding can play an important role for developing oilseed rape cultivars being resistant to insect pests, which could reduce the need for insecticide application (Williams 2004). Resistant lines have been developed so far against important diseases, e.g. *Phoma lingam*, *Alternaria brassicae* and *Sclerotinia sclerotiorum* (Manthey 2005). Screening for resistance in common breeding lines and cultivars has been initiated for various pests in other countries, e.g. aphids, *Lipaphis*

Modern cultivars and breeding lines of *Brassica napus* are characterized by a limited genetic diversity (Seyis *et al.* 2000) and they have little potential to provide sources of resistance for practical exploitation in breeding programs (Ellis 1992). Two genetic resources are of importance to enlarge the genetic variability: wild brassicaceous species and resynthesized oilseed rape (‘resyntheses’).


A large assortment of species belonging to the subtribe Brassicinae has been identified as potential sources for antixenosis, antibiosis or mixtures of both to pest insects, particularly *Brassica incana* Tenore, *B. spinescens* Pomel and *B. fruticulosa* Cyrillo (Cole 1997). However, because of the different genomes within the genus *Brassica*, hybridization between wild brassicaceous species is crucial (Ellis *et al.* 1999; Pink *et al.* 2003).

Recently, the development of resynthesized lines of oilseed rape has become a promising approach to enlarge the genetic divergence in plant breeding (Becker *et al.* 1995; Seyis *et al.* 2000; Becker 2000). The main objective is to re-design the ancestral combination between *Brassica oleracea* x *Brassica rapa* (U 1935) by using biological varieties of both diploid parent species (Becker *et al.* 1995). Resynthesized lines of oilseed rape represent a genetic resource with high diversity for breeding hybrid cultivars (Girke 2002). They provide a source for disease resistance, e.g. against *Phoma lingam* (Crouch *et al.* 1994), *Plasmodiophora brassica* (Diederichsen & Sacristan 1996) and *Verticillium longisporum* (Rygulla *et al.* 2007). Increased levels of resistance to oilseed rape pests can be expected as well (Seyis *et al.* 2000). However, negative
interactions of crossing high yield cultivars with wild brassicaceous species are possible, e.g. yield reduction (Allard 1996).

So far there are very few in-depth studies to evaluate the level of resistance to *C. pallidactylus* in brassicaceous plants. Winfield (1961b) reported on reduced susceptibility of *Sinapis alba* L., and Way & Murdie (1965) found a higher infestation in cultivars of Brussels sprouts without waxy surface on the leaves. In field observations, single low cultivars of oilseed rape (high GSL content, no erucic acid in seeds) were preferred by *C. pallidactylus* (Mrówczyński 1992). Mrówczyński (1998) found significant differences between the infestation levels of modern double low lines (no erucic acid and low glucosinolate content in the seeds) in field trials. An extensive field screening of brassicaceous crops to pests was the EU-Projekt ‘RESGEN’ (Lühs et al. 2003), which tested the susceptibility of approximately 200 *Brassica* genotypes against cabbage stem flea beetle, rape stem weevil, and cabbage stem weevil in a one-year field experiment.

In this study, potential characteristics of resistance to cabbage stem weevil (*Ceutorhynchus pallidactylus*) have been evaluated for a wide spectrum of Brassicaceae (cultivars, breeding lines, varieties, resynthesized rapeseeds, other *Brassica* spp.), for the first time. For the screening of a large assortment with a wide genetic variability, new methods have been developed which enable a rapid and reliable selection of genotypes with (partial) resistance in climate chambers. In field trials, the susceptibility of *Brassica* genotypes to *C. pallidactylus* has to be evaluated to confirm results from laboratory experiments. Finally the study shall be a basis for introduction of oilseed rape with insect resistance in national breeding programs and utilization in agriculture practice.

The following chapters have been published or will be submitted under following titles:


- Eickermann M, Ulber B & S Vidal (2010): Resynthesized lines and cultivars of *Brassica napus* L. provide sources of resistance to the cabbage stem weevil
(Ceutorhynchus pallidactylus (Mrsh.)). Bulletin of Entomological Research. In press. DOI: 10.1017 / S0007485310000489. Published online: 24 November 2010.

Influence of different Brassica host plants to parasitism of ceutorhynchid stem weevils

This study was funded by the German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) and the Board for Support of Private Plant Breeding in Germany e.V. (GFP).

References
Åhman I (1982): A comparison between high and low glucosinolate cultivars of summer oilseed rape (Brassica napus L.) with regard to their levels of infestation by the brassica pod midge (Dasineura brassicae Winn.). Journal of Applied Entomology 94, 103-109.
Barari H, Cook SM, Clark SJ & IH Williams (2005): Effect of a turnip rape (Brassica rapa) trap crop on stem-mining pests and their parasitoids in winter oilseed rape (Brassica napus). Biological Control 50, 69-86.


Cole RA (1997): The relative importance of glucosinolates and amino acids to the development of two aphid pests Brevicoryne brassicae and Myzus persicae on wild and cultivated brassica species. Entomologia Experimentalis et Applicata **85**, 121-133.

Cook SM, Smart LE, Rasmussen HB, Bartlet E, Martin JL, Murray DA, Watts NP and IH Williams (2003): Push-pull strategies to reduce insecticide input to oilseed
rape (Brassica napus): Potential of low alkenyl glucosinolate oilseed rape varieties (push!), and turnip rape (Brassica rapa) trap crops (pull!). Proceedings of the 11th International Rapeseed Congress, Copenhagen, 6-10 July 2003, 3, 1018-1021.


Hanson LM (2003): Insecticide-resistant pollen beetles (Meligethes aeneus F.) found in Danish oilseed rape (Brassica napus L.) fields. Pest Management Science 59, 1057-1059.


Kazda V (1953): Bionomic a hospodarsky vyznam krytonosce ctyrzubeho, Ceutorhynchus quadridens (Panz.), v strednich cechach. Zoologice a entomologice listy 2, 231-240.


Piper R & I Williams (2004): Incidence and feeding activity of epigeic, predatory invertebrates within winter oilseed rape in the UK with comparisons between integrated and conventional crop management. IOBC/wprs Bulletin 27 (10), 281-288.


Schlein O & W Büchs (2004): Approaches to assess the importance of carnivorous beetles as predators of oilseed rape pests. IOBC/wprs Bulletin 27 (10), 289-292.


van Loon JJA, Blaakmeer A, Griepink FC, Van Beek TA, Schoonhoven LM & A de Grot (1992): Leaf surface compound from Brassica oleracea (Cruciferae)
induces oviposition by *Pieris brassicae* (Lepidoptera: Pieridae). Chemocoeology 3, 39-44.


Chapter II - Screening of oilseed rape and other brassicaceous genotypes for susceptibility to cabbage stem weevil (Ceutorhynchus pallidactylus (Mrsh.))

This chapter has been published as:

The abstract is given here for your information. For the full article see:

Abstract
Production of oilseed rape, Brassica napus L., is affected by various insect pests. The cabbage stem weevil, Ceutorhynchus pallidactylus (Mrsh.) (Col.: Curculionidae), is one of the most damaging pests in Northern and Central Europe that requires regular control measures. Host plant resistance is a key factor in integrated pest management systems. To evaluate a large number of genotypes for their susceptibility to infestation by C. pallidactylus, new screening techniques were developed for testing both, the amount of feeding and the number of eggs deposited by adult C. pallidactylus on accessions of Brassicaceae under controlled conditions.
In no-choice screening tests, the leaf area consumed by adult cabbage stem weevil was quantified on a wide spectrum of 107 brassicaceous genotypes (B. napus, B. rapa L. and B. oleracea L. cultivars, breeding lines, resynthesized rapeseed lines and wild Brassicaceae). In comparison to feeding on the standard cultivar ‘Express’, the average leaf area consumed by C. pallidactylus on nine oilseed rape cultivars, four resynthesized rapeseed lines and five other accessions [B. oleracea, Camelina alyssum (Mill.) and Lunaria annua L.] was significantly reduced by 44 – 90%. In dual-choice screening tests for the evaluation of oviposition preferences on 42 genotypes, female C. pallidactylus laid significantly fewer eggs into plants of two oilseed rape cultivars,
five resynthesized rapeseeds and three accessions of *B. oleracea* and *B. fruticulosa* Cyrillo, respectively, than into plants of the standard cv ‘Express’. Results of both laboratory screening test were confirmed by results of additional field testing.

**Keywords:** *Brassica*; cabbage stem weevil; dual-choice oviposition test; no-choice feeding test; oilseed rape; plant resistance
Chapter III - Resynthesized lines and cultivars of *Brassica napus* L. as sources of resistance to cabbage stem weevil (*Ceutorhynchus pallidactylus* (Mrsh.))

This chapter is in press as:


The abstract is given here for your information. For the full article see: [http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=7928439&fulltextType=RA&fileId=S0007485310000489](http://journals.cambridge.org/action/displayAbstract?fromPage=online&aid=7928439&fulltextType=RA&fileId=S0007485310000489)

Abstract

The cabbage stem weevil (*Ceutorhynchus pallidactylus* (Mrsh.)) (Col., Curculionidae) is a serious pest of winter oilseed rape (*Brassica napus* L. var. *oleifera* Metzg.) in central and northern Europe. Although host-plant resistance is a key tool in integrated pest management systems, resistant genotypes are not yet available for this species. Resynthesized rapeseed lines (*B. oleracea* L.×*B. rapa* L.) are broadening the genetic diversity and might have potential as sources of resistance to pest insects. The host quality, of nine resynthesized rapeseed lines and six genotypes of *B. napus* to cabbage stem weevil, was evaluated in laboratory screening tests and in a semi-field experiment. In dual-choice oviposition tests, female *C. pallidactylus* laid significantly fewer eggs on five resyntheses and on swede cv ‘Devon Champion’ than on the moderately susceptible oilseed rape cv ‘Express’, indicating a lower host quality of these genotypes.

Results of laboratory screenings were confirmed in a semi-field experiment, in which twelve genotypes were exposed to *C. pallidactylus* females. The number of larvae was significantly lower in two resyntheses and in cv ‘Devon Champion’ than in oilseed rape cv WVB 9. The total, as well as individual, glucosinolate (GSL) content in the leaves
differed substantially among the genotypes tested. The amount of feeding by larvae of
*C. pallidactylus*, as measured by a stem-injury coefficient, was positively correlated
with the indolyl GSL compounds 3-indolylmethyl and 4-methoxy-3-indolylmethyl, and
with the aromatic GSL 2-phenylethyl, whereas it was negatively correlated with
4-hydroxy-3-indolylmethyl. Thus, the composition and concentration of GSL
compounds within the plant tissue might be a key factor in breeding for pest resistance
in oilseed rape.

**Keywords**: glucosinolates; larval performance; oilseed rape, resynthesis; semi-field
trial; stem injury coefficient
Chapter IV - Influence of different Brassica host plants on parasitism of ceutorynchid stem weevils

Abstract

The rape stem weevil (Ceutorhynchus napi) and the cabbage stem weevil (C. pallidactylus) are major pests of winter oilseed rape (Brassica napus L. var. oleifera Metzg.) in Europe. Their stem-mining larvae are parasitized by the specialist larval endoparasitoids, Tersilochus fulvipes and T. obscurator, respectively. In three-year field experiments, the effect of host plant genotype on the level of parasitism of the ceutorynchid larvae by T. fulvipes and T. obscurator was determined on a large assortment of Brassica genotypes (B. napus, B. rapa, B. oleracea and B. juncea). The species of Brassica plant had a significant effect on larval parasitism. In three cultivars of turnip rape, B. rapa, the level of parasitism of C. napi by T. fulvipes was significantly reduced compared to cultivars of winter oilseed rape, B. napus. Based on data pooled over three years, the rate of parasitism on C. pallidactylus by T. obscurator was significantly reduced in cv ‘Wotan’ in comparison to other accessions, whereas parasitism of larvae on fodder rape ‘Emerald’ (B. napus) was significantly increased in two of three years. The level of parasitism of C. pallidactylus and C. napi by T. obscurator and T. fulvipes, respectively, was not significantly correlated with the number of host larvae/m². The length of main stem and number of racemes/plant had a significantly negative effect on parasitism by T. fulvipes in 2004. However, weak correlations of parasitism rates with plant growth parameters indicate that other cues have major importance for the parasitism of ceutorynchid host larvae.

Introduction

Multitrophic interactions between host plants, pests and parasitoids have been reported several times in literature (Vet & Dicke 1992; Fox et al. 1996; Lill et al. 2002). Host plant quality and genotype have potential to affect the infestation by herbivorous insects as well as the level of biocontrol by natural enemies. Cabbage stem weevil, C. pallidactylus (Mrsh.), and rape stem weevil, C. napi Gyll., (Coleoptera: Curculionidae) are major pests of winter oilseed rape, Brassica napus L. var. oleifera Metzg. in Central and Northern Europe (Alford et al. 2003). Both species are univoltine with three larval instars. Adult stem weevils migrate from their overwintering habitats...
to crops of oilseed rape in March/April. Female \textit{C. napi} lay their eggs singly into the top of the main stem, whereas female \textit{C. pallidactylus} lay egg batches into petioles and mid-ribs of leaves. The larvae of either species mine within the pith of host plants. Mature larvae migrate to soil for pupation. On crops of winter oilseed rape, the univoltine larval endoparasitoids \textit{Tersilochus fulvipes} Gravenhorst and \textit{T. obscurator} Aubert (Hymenoptera: Ichneumonidae) have been identified as key parasitoids of rape stem weevil and cabbage stem weevil, respectively (Klingenberg & Ulber 1994; Ulber 2003; Ulber & Nitzsche 2006). Both parasitoid species are assumed to be host-specific (Ulber 2003). Natural control of host populations by these parasitoid species has been reported from various European countries, e.g. Austria (Kraus & Kromp 2002), Czech Republic (Sedivy 1983), France (Jourdheuil 1960), Germany (Klingenberg & Ulber 1994), Switzerland (Büchi & Roos-Humbel 1991), and the UK (Barari \textit{et al.} 2005).

Female parasitoids lay their eggs singly into host larvae while these are feeding concealed within petioles (\textit{T. obscurator}) or stems (\textit{T. fulvipes}). Parasitoid larvae hatch within hosts; however, development of second and third instar larvae is delayed until the full-grown host larvae have migrated to soil for pupation (koinobiont). The adult wasps diapause inside the pupal cocoon until emergence in the following spring (Ulber 2003). The multitrophic system of host plant (oilseed rape), host larva (ceutorynchid stem weevil) and parasitoid (\textit{Tersilochus} spp.) is affected by various factors, e.g. climate (Johnen \textit{et al.} 2006), plant architecture (Ulber & Fischer 2006), plant volatiles (Jönsson \textit{et al.} 2005), and farming practices, like plant density (Ulber & Fischer 2006), tillage (Klingenberg & Ulber 1994; Nitzsche & Ulber 1998), and insecticide application (Klukowski 2006; Neumann & Ulber 2009, in prep.). The objectives of this research were to investigate influences of host plant species and cultivars on the level of infestation and larval parasitism of \textit{C. napi} and \textit{C. pallidactylus} by \textit{T. fulvipes} and \textit{T. obscurator}, respectively. Three field plot experiments were conducted in 2004, 2005 and 2006 at Goettingen (Northern Germany) to study the level of parasitism in various \textit{Brassica} genotypes and the effect of plant architecture on parasitism by \textit{T. obscurator} and \textit{T. fulvipes}. 
Materials and Methods

Each year, a broad spectrum of *Brassica* species and cultivars was grown in a randomized block design with four replicated plots (12m * 2.5m; 30m² per plot) (Table 1). Selection of cultivars was aimed to cover a broad host plant spectrum for stem weevils.

Table 1: *Brassica* species and cultivars included in field experiments

<table>
<thead>
<tr>
<th>No. of cultivars</th>
<th>Species (Crop type)</th>
<th>Cultivar</th>
<th>Source *</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Brassica napus</em> (fodder rape)</td>
<td>‘Emerald’</td>
<td>F</td>
</tr>
<tr>
<td>1</td>
<td><em>Brassica juncea</em> (Indian mustard)</td>
<td>‘Vitasso’</td>
<td>G</td>
</tr>
</tbody>
</table>

* A: Norddeutsche Pflanzenzucht Hans-Georg Lembke KG (NPZ); B: Deutsche Saatveredelung Lippstadt-Bremen GmbH (DSV); C: Syngenta Seeds GmbH; D: KWS Saat AG; E: Semundo Saatzucht GmbH; F: Raps GbR; G: Lochow-Petkus.

Four cultivars of *B. napus* were examined in all three years (‘Express’, ‘Prince’, ‘Wotan’ and fodder rape ‘Emerald’) while five cultivars were studied in two years and eleven cultivars only in one year. The standard cv ‘Express’ was grown around the experiment to avoid edge effects. Plant densities were monitored at the beginning of March. Plant growth (length of main stem, stem-base diameter, number of leaves and lateral racemes on main stem) was assessed from samples of 20 plants/plot at the end of April.

The effect of host plant genotype on the abundance of host larvae and the level of parasitism was determined from samples of 20 plants/plot collected in mid May, before larvae started to migrate to soil for pupation. Stems and lateral racemes were cut longitudinal to collect larvae of stem weevils. They were stored in 70% Ethanol and
later dissected under a microscope (sample size: 40 – 250 larvae/genotype). The identity of *T. fulvipes* and *T. obscurator* was confirmed by rearing out adult parasitoids from subsamples of host larvae. Full-grown host larvae were transferred to boxes containing moist soil substrate; after ten weeks, parasitoid cocoons were isolated from soil and adult parasitoids identified. All data were subjected to ANOVA; differences between means (p ≤ 0.05) were evaluated by Tukey-HSD test. Percentages were transformed by arc-sin-transformation. Regression analyses were conducted by using Sigma Plot Version 10.

**Results**

*Abundance of stem weevils and overall level of parasitism in 2004-2006*

In all years, the number of stem weevil larvae per plant was relatively high, particularly in 2005 (*C. napi*) and in 2006 (*C. pallidactylus* and *C. napi*) (Table 2). The level of parasitism of *C. pallidactylus* larvae was higher than parasitism of *C. napi* and increased over years. Only specimen of *T. fulvipes* and *T. obscurator* were obtained by rearing adult parasitoids from host larvae.

Table 2. Mean number (± SEM) of ceutorynchid larvae and mean percentage (± SEM) of parasitism in field experiments at Goettingen in 2004 – 2006

<table>
<thead>
<tr>
<th></th>
<th><em>C. pallidactylus</em></th>
<th><em>C. napi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Larvae/plant</td>
<td>% parasitism</td>
</tr>
<tr>
<td>2004</td>
<td>3.4 ± 0.5</td>
<td>17.3 ± 1.9</td>
</tr>
<tr>
<td>2005</td>
<td>3.6 ± 0.3</td>
<td>20.8 ± 1.5</td>
</tr>
<tr>
<td>2006</td>
<td>11.5 ± 2.8</td>
<td>27.6 ± 2.5</td>
</tr>
</tbody>
</table>
_Larval abundance and parasitism of C. pallidactylus_

Significant differences were detected between the number of host larvae on 16 different cultivars tested in 2004 (Fig 1). Plant infestation by _C. pallidactylus_ was significantly reduced in the accession of _B. oleracea_ ‘Markola’ and ‘Gruener Angeliter’ compared to _B. rapa_ cultivars ‘Malvira’ and ‘Perko’. No significant difference was found between the number of larvae in 10 cultivars of _B. napus_. Parasitism of _C. pallidactylus_ larvae by _T. obscurator_ was not significantly affected by _Brassica_ genotypes in 2004 (Fig 1).

![Fig 1: Mean level of infestation (+ SEM) by _C. pallidactylus_ and mean level of parasitism (+ SEM) on different _Brassica_ genotypes in 2004. Larvae/plant: ANOVA; Tukey-HSD; n = 64; F = 4.72; p ≤ 0.0001. Parasitism: ANOVA; Tukey-HSD; p = 0.9555](image)

In 2005 no significant difference occurred between the level of infestation by cabbage stem weevil in 12 tested genotypes (Fig 2). However, numbers of larvae in the hybrid cultvar ‘Extra’ and the turnip rape ‘Salut’ were very low. The level of parasitism by _T. obscurator_ differed significantly between the _B. napus_ cultivars ‘Wotan’ (10%) versus ‘Express’, ‘Smart’, ‘Viking’ and ‘Extra’ (25-27%). In all other genotypes, the level of parasitism ranged between 15 and 23%.
In 2006 only five cultivars were included in the field experiment (Fig 3). The abundance of *C. pallidactylus* was significantly reduced in fodder rape ‘Emerald’ compared to the open pollinating oilseed rape cultivars ‘Prince’ und ‘Wotan’. Parasitism of *C. pallidactylus* in ‘Wotan’ and turnip rape ‘Salut’ was significantly lower than in ‘Express’. Pooled data of the years 2004, 2005 and 2006 showed that the level of parasitism in ‘Wotan’ was relatively low, although but not statistically significant. In contrast, a relative high level of parasitism by *T. obscurator* was observed in all years in the accessions ‘Express’, ‘Prince’ and fodder rape ‘Emerald’ (only 2005 and 2006).
Fig 3: Mean level of infestation (+ SEM) by *C. pallidactylus* and mean level of parasitism (+ SEM) on different *Brassica* genotypes in 2006. Larvae/plant: ANOVA; Tukey-HSD; n = 20; F = 5.47; p = 0.0064. Parasitism: ANOVA; Tukey-HSD; n = 20; F = 4.37; p = 0.0153
Larval abundance and parasitism of *C. napi*

In 2004, the abundance of *C. napi* larvae was significantly reduced in *B. juncea* ‘Vitasso’ and *B. oleracea* ‘Markola’ in comparison to *B. napus* fodder rape ‘Emerald’ (Fig 4). No significant difference occurred between cultivars of oilseed rape and turnip rape. No parasitized host larvae were found in *B. rapa* cv ‘Malvira’ (Fig 4), and a significantly lower rate of parasitism was determined in *B. rapa* cv ‘Perko’ in comparison to *B. oleracea* ‘Markola’.

![Graph showing larval abundance and parasitism](image)

Fig 4: Mean level of infestation (+ SEM) by *C. napi* and mean level of parasitism (+ SEM) on different *Brassica* genotypes in 2004. Larvae/plant: ANOVA; Tukey-HSD; n = 64; F = 2.67; p ≤ 0.005. Parasitism: ANOVA; Tukey-HSD; n = 64; F = 4.31; p ≤ 0.0001

In 2005 the level of infestation by *C. napi* was significantly lower on ‘Salut’, ‘Extra’ and ‘Viking’ compared to ‘Wotan’, fodder rape ‘Emerald’ and WRH 242 (Fig 5). The level of parasitism of *C. napi* by *T. fulvipes* on *B. rapa* cv ‘Salut’ (4%) was significantly lower than on all other accession except ‘Emerald’. ‘Idol’ showed an exceptionally high rate of parasitism in 2005 (27%).
Fig 5: Mean level of infestation (+ SEM) by *C. napi* and mean level of parasitism (+ SEM) on different *Brassica* genotypes in 2005. Larvae/plant: ANOVA; Tukey-HSD; n = 48; F = 8.19; p ≤ 0.0001. Parasitism: ANOVA; Tukey-HSD; n = 48; F = 10.12; p ≤ 0.0001

In 2006 the abundance of *C. napi* larvae was significantly lower in *B. napus* cv ‘Express’, ‘Prince’ and *B. rapa* cv ‘Salut’ than in cv ‘Wotan’ and fodder rape cv ‘Emerald’ (Fig 6). Parasitism of *C. napi* in *B. rapa* cv ‘Salut’ was significantly lower than in *B. napus* cv ‘Prince’.
Relationship between density of ceutorynchid host larvae and parasitism

In all years, the number of *T. obscurator* per m$^2$ was significantly correlated to the number of *C. pallidactylus* larvae per m$^2$ (Table 3). Similarly, the number of *T. fulvipes* per m$^2$ was significantly correlated to the number of *C. napi* per m$^2$ (Table 3). However, percentage parasitism of *C. napi* and *C. pallidactylus* by *T. fulvipes* and *T. obscurator*, respectively, showed no significant relationship to host abundance in all years (Table 4).

Table 3. Effect of abundance of ceutorynchid host larvae per m$^2$ on density of *T. obscurator* and *T. fulvipes* per m$^2$ on different *Brassica* species and cultivars at Goettingen in 2004 – 2006

<table>
<thead>
<tr>
<th>Year</th>
<th>n</th>
<th>F</th>
<th>r$^2$</th>
<th>y</th>
<th>p ≤ 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. pallidactylus</em></td>
<td>2004</td>
<td>64</td>
<td>25.97</td>
<td>0.295</td>
<td>3.44 + 0.14 x</td>
</tr>
<tr>
<td><em>C. pallidactylus</em></td>
<td>2005</td>
<td>48</td>
<td>100.34</td>
<td>0.686</td>
<td>-2.43 + 0.23 x</td>
</tr>
<tr>
<td><em>C. pallidactylus</em></td>
<td>2006</td>
<td>20</td>
<td>74.69</td>
<td>0.806</td>
<td>1.78 + 0.26 x</td>
</tr>
<tr>
<td><em>C. napi</em></td>
<td>2004</td>
<td>64</td>
<td>28.08</td>
<td>0.312</td>
<td>-13.18 + 0.13 x</td>
</tr>
<tr>
<td><em>C. napi</em></td>
<td>2005</td>
<td>48</td>
<td>17.42</td>
<td>0.275</td>
<td>15.99 + 0.11 x</td>
</tr>
<tr>
<td><em>C. napi</em></td>
<td>2006</td>
<td>20</td>
<td>6.95</td>
<td>0.279</td>
<td>5.44 + 0.08 x</td>
</tr>
</tbody>
</table>

* - p ≤ 0.05
Table 4. Effect of abundance of ceutorychid host larvae per m\(^2\) on level of parasitism (\%) of *T. obscurator* and *T. fulvipes* on different *Brassica* species and cultivars at Goettingen in 2004 – 2006

<table>
<thead>
<tr>
<th>Year</th>
<th>Plant parameter</th>
<th>F</th>
<th>r(^2)</th>
<th>y</th>
<th>p ≤ 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. pallidactylus</em></td>
<td>2004</td>
<td>64</td>
<td>0.61</td>
<td>22.30 – 0.02 x</td>
<td>ns</td>
</tr>
<tr>
<td><em>C. pallidactylus</em></td>
<td>2005</td>
<td>48</td>
<td>1.96</td>
<td>24.59 + 0.02 x</td>
<td>ns</td>
</tr>
<tr>
<td><em>C. pallidactylus</em></td>
<td>2006</td>
<td>20</td>
<td>0.29</td>
<td>32.39 + 0.01 x</td>
<td>ns</td>
</tr>
<tr>
<td><em>C. napi</em></td>
<td>2004</td>
<td>64</td>
<td>2.01</td>
<td>18.86 - 0.01 x</td>
<td>ns</td>
</tr>
<tr>
<td><em>C. napi</em></td>
<td>2005</td>
<td>48</td>
<td>0.02</td>
<td>21.82 + 0.00 x</td>
<td>ns</td>
</tr>
<tr>
<td><em>C. napi</em></td>
<td>2006</td>
<td>20</td>
<td>1.86</td>
<td>25.57 – 0.04 x</td>
<td>ns</td>
</tr>
</tbody>
</table>

ns - not significant

Effect of plant architecture on parasitism

The relationship between plant growth parameters (diameter and length of main stem, number of leaves and number of lateral racemes per main stem) and the rate of parasitism was analysed in order to estimate the role of plant architecture on host finding and host accession by the parasitoids *T. fulvipes* and *T. obscurator*.

The parasitism of *T. obscurator* was not significantly affected by plant architecture during the experimental years. For *T. fulvipes* an effect of plant parameters was only found in 2004 (Table 5). Regression analysis of parasitism rates of *C. napi* showed significant relationships to plant growth parameters only in one of three experimental years. In 2004 both, stem length and the number of racemes per main stem were significantly negative related to parasitism. The low coefficients of determination (r\(^2\)) indicate that other factors than plant architecture may have higher impact on the level of parasitism within plants.

Table 5. Relationship between plant architecture and level of parasitism of *C. napi* by *T. fulvipes* at Goettingen in 2004

<table>
<thead>
<tr>
<th>Year</th>
<th>Plant parameter</th>
<th>F</th>
<th>r(^2)</th>
<th>y</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>Stem length</td>
<td>22.87</td>
<td>0.27</td>
<td>31.94 - 0.29 x</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>n = 64</td>
<td>Number of racemes</td>
<td>5.59</td>
<td>0.08</td>
<td>23.73 – 1.31 x</td>
<td>0.021</td>
</tr>
</tbody>
</table>
Discussion

In this study the relationship between the *Brassica* host plant and the rate of larval parasitism of the ceutorynchid stem weevils *C. napi* and *C. pallidactylus* by the parasitoids *T. fulvipes* and *T. obscurator*, respectively, was investigated.

The level of infestation by ceutorynchid host larvae was significantly affected by *Brassica* host plant. *C. napi* and *C. pallidactylus* have a wide range of brassicaceous host plants (Alford *et al.* 2003). Different susceptibilities of oilseed rape cultivars, turnip rape and brassicaceous species were reported by Eickermann & Ulber (2009a).

The overall abundance level of *C. pallidactylus* increased from 2004 and 2005 (3 larvae/plant) to 2006 (12 larvae/plant), whereas peak abundance of *C. napi* occurred in 2005 (10 larvae/plant). There was a slight tendency of increasing levels of parasitism with increasing host density, but parasitism did not show a significant density-dependant response in all years.

During three experimental years, the mean level of parasitism of *C. pallidactylus* by *T. obscurator* was higher than parasitism of *C. napi* by *T. fulvipes*. This is in agreement with reports in literature for this region (Ulber 2003): Parasitism of *C. pallidactylus* was found to vary between 14% and 52% at Goettingen (Ulber 2003; Ulber & Fischer 2006) and between 40% and 70% in Austria (Kraus & Kromp 2002). However, a comparison between the years is biased by the change of cultivars.

The results of this study showed that the three-trophic-level system can be significantly affected by the *Brassica* host plant. Similar effects have been found for other specialist or generalist pest species and their parasitoids (Vet & Dicke 1992; Fox *et al.* 1996).

The species of *Brassica* host plant had a significant effect on larval parasitism of stem weevils. For *T. obscurator* high levels of parasitism were found on oilseed rape cultivars ‘Express’, ‘Prince’, fodder rape ‘Emerald’ and in 2004 in cultivars of *B. oleracea* and *B. juncea*. In contrast, low levels of parasitism were detected in the oilseed rape cv ‘Wotan’ and the turnip rape cv ‘Salut’. Similarly, cultivars of turnip rape showed low rates of parasitism by *T. fulvipes*. There was no consistent difference between the parasitism rates of *C. pallidactylus* and *C. napi* in open pollinating and hybrid cultivars of oilseed rape.

Our study for the first time has determined a multitrophic interaction between *Brassica* host plant, stem-boring pests and parasitoids on crops of oilseed rape. Close interactions within multitrophic systems of *Brassica* crops have been shown for the leaf feeding
diamondback moth, *Plutella xylostella* (L.), and its parasitoid *Diadegma insulare* (Cresson) (Sarfraz *et al.* 2008). The parasitism by larval endoparasitoid, *Phradis morionellus* Holmgr., of pollen beetle, *Meligethes aeneus* (Fabr.), was not significantly different between cultivars of *Sinapis alba* and *B. napus* (Billqvist & Ekbom 2001a). In contrast, parasitism of pollen beetle, by the parasitoid *Diospilus capito* Nees, was significantly increased on host plants of *S. alba* compared to *B. napus* (Billqvist & Ekbom 2001b).

Host location and host acceptance of parasitoids on *Brassica* are mainly affected by two cues: plant architecture and plant volatiles (Schoonhoven *et al.* 1998). In our study several factors of plant architecture were assessed for possible effects on parasitism: A significantly negative influence of stem length and number of lateral racemes on parasitism by *T. fulvipes* was only determined in one year. On larger stems and increased number of racemes, host location by *T. fulvipes* might have biased due to larger areas of stem that females have when searching for host larvae. Additionally, host larvae can use more space and refuges for dispersal within the pith of larger stems. No significant effect of plant architecture on *T. obscurator* was detected. Plant architecture can vary widely between cultivars (Eickermann & Ulber 2009b) and is influenced by environmental conditions. This might be the reason why parasitism rate was not significantly related to parameters of plant growth in all years. A close interaction between plant development, host larval abundance and migration of Tersilochinae to crops of oilseed rape had been shown by Ulber & Nitzsche (2006).

In our study a high level of parasitism by *T. obscurator* and *T. fulvipes* was detected in *B. juncea* and in cultivars of *B. oleracea*, which began to flower three weeks later than cultivars of oilseed rape. Late flowering plants might have attracted adults of Tersilochinae much longer for feeding on pollen. A high density of food source is assumed to retain parasitoids and increase parasitism (Lewis *et al.* 1998). In contrast, very low levels of parasitism on the three cultivars of turnip rape in all years might have resulted from the very early flowering time of turnip rape. In this case, flowering was completed too early to provide pollen for the attraction of adult parasitoids. In trap crop experiments, *T. obscurator* was more abundant in field plots of oilseed rape than in plots of turnip rape (Barari *et al.* 2005). Additionally, distribution of parasitoids within the field can be affected by external factors like wind direction (Williams *et al.* 2007) and inter-specific interaction (Ferguson *et al.* 2006).
An impact of secondary plant chemicals to herbivores and their natural enemies has been reported several times (Pickett et al. 1999; Sznajder & Harvey 2003; Gols et al. 2007). The most important secondary plant metabolites of Brassica species, the glucosinolates and their breakdown products, play an important role in mediating the plant-pest-interaction (Rask et al. 2000). Significant effects of glucosinolates in tritrophic systems have been shown in field trials with the parasitoid Diaeretiella rapae (McIntosh) of Brevicoryne brassicae (L.) on B. oleracea cultivars (Bradburne & Mithen 2000). Enhanced production of but-3-enyl isothiocyanate within plants increased attraction of the parasitoid.

Further, orientation of D. rapae to the aphid species, Lipaphis erysimi (Kalt.), was increased by but-3-enyl isothiocyanate as well (Blande et al. 2007). This indicates that host location of the parasitoid is mainly mediated by plant volatiles, namely the isothiocyanates. T. obscurator and T. fulvipes have been shown to be strongly attracted by 2-phenylethyl isothiocyanate (Ulber & Wedemeyer 2006). Adults C. pallidactylus and C. napi were also attracted by this isothiocyanate (Walczak et al. 1998). The release of volatiles from plants of Brassica can be affected by the phenological stage, thereby influencing host finding of parasitoids (Jönsson et al. 2005). In our study, observations were focused only on the effect of host plant genotype on the level of parasitism, isothiocyanates and other plant volatiles have not been analyzed. Other influences of host plants on the performance, sex ratio and biomass of parasitoids have been shown in other tritrophic systems, e.g. for the parasitoid Diadegma semiclausum Hellen of P. xylostella on B. oleracea and S. alba (Gols et al. 2007) or for the predator Chrysoperla carnea (Stephens) on different accessions of B. oleracea (Reddy et al. 2004).

Further studies are needed to determine the mechanisms, how host plant traits affect parasitism of stem-mining weevils in crops of Brassica. The manipulation of plant attributes to promote the level of parasitism, in combination with plant resistance to pests, could play an important role in integrated pest management strategies.

**Acknowledgements**

We are grateful to breeding companies NPZ, DSV, KWS, Syngenta, Lochow–Petkus, Raps GbR and Semundo for supplying the seed of tested genotypes and D Mennerich for technical assistance.
The study was funded by German Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) and the Board for Support of Private Plant Breeding in Germany (GFP).

References


Eickermann M & B Ulber (2009a): Screening of oilseed rape and other brassicaceous genotypes for susceptibility to cabbage stem weevil (*Ceutorhynchus pallidactylus* (Marsh.)). Submitted.


Jourdheuil P (1960): Influence de quelques facteurs écologiques sur les fluctuations de population d’une biocénose parasitaire: étude relative à quelques Hyménoptères


Kraus P & B Kromp (2002): Parasitization rate of the oil seed rape pests Ceutorhynchus napi, Ceutorhynchus pallidactylus (Coleoptera, Curculionidae) and Meligethes aeneus (Coleoptera, Nitidulidae) by Ichneumonids in several localities of Eastern Austria. IOBC/wprs Bulletin 25 (2), 117-122.


Chapter V - General discussion

The cabbage stem weevil, *Ceutorhynchus pallidactylus* (Mrsh.) (Coleoptera: Curculionidae), is a major pest of oilseed rape in Central Europe (Alford *et al.* 2003). It is adapted to a wide range of wild and cultivated brassicaceous plant species, such as *B. napus* (Broschewitz 1985; Mrówczyński 1998), *B. rapa* (Büchi 1990), *B. nigra* (L.), Indian mustard, *B. juncea* (L.), radish, *Raphanus* spp., white mustard, *Sinapis alba* (L.) (Winfield 1961), sea-kale, *Crambe maritime* L. and field penny-cress, *Thlaspi arvense* L. (Speyer 1921; Günthart 1949). Resistant cultivars of oilseed rape are highly demanded for minimizing the number of insecticide applications in integrated pest management systems (Williams 2004). The development of plant resistance to insect pests in winter oilseed rape (*Brassica napus* var. *oleifera* Metzg.) is still in the early stages. So far, only a few studies have been conducted to identify sources of resistance against *C. pallidactylus* in brassicaceous plants (Winfield 1961; Mrówczyński 1998; Lühs *et al.* 2003; Mrówczyński *et al.* 2006), showing only minor differences of susceptibility. In addition, the potential mechanisms of resistance are still unknown.

The main objectives of this study were as follows:

1. to develop laboratory screening methods for identification and quantification of resistance to cabbage stem weevil (*Ceutorhynchus pallidactylus*) in brassicaceous species
2. to screen a large assortment of cultivars of oilseed rape, breeding lines, resynthesized rapeseed lines and other species of Brassicaceae under controlled conditions in growth chambers
3. to validate the resistance of selected genotypes under field conditions
4. to evaluate the influence of host plant genotype on parasitism of ceutorynchid stem weevils

Screening methods

Screening tests based on leaf feeding by herbivorous insect species have been applied successfully for testing the susceptibility of plants to various pests (Risch 1985), e.g. for bean leaf beetle, *Cerotoma trifurcate* (Forster) (Bradshaw *et al.* 2007), *Helicoverpa armigera* (Hübner) (Sharma *et al.* 2005), *Mamestra configurata* Walker (Ulmer *et al.* 2001) and cabbage stem flea beetle, *Psylliodes chrysocephala* L. (Bartlet & Williams
For quantification of the leaf area removed by herbivores, techniques like computer scanning or computer-aided video image analysis can be used (O’Neal et al. 2002).

Screening methods based on oviposition in dual-choice tests are able to detect host finding and host acceptance of adult pest species and performance of their larvae as well. They have been applied successfully for testing the susceptibility of plants to root maggots, *Delia* spp. (Dosdall et al. 1994), pollen beetle, *Meligethes aeneus* (Fabr.) (Ekboth & Borg 1996) and diamondback moth, *Plutella xylostella* (L.) (Sarfracz et al. 2007). The screening method and the way the test plants are offered to the insects can have significant effects on the results. Generalists are less attracted by specific volatiles emitted from plants than specialist herbivores (Risch 1985). Using detached leaves or wounded plants in screening tests can affect the cues responsible for attraction of specialist species (Farrell 1977). While no-choice tests are only able to discriminate between plants of remarkably differing host plant quality (Risch 1985), multi-choice tests which include cues relevant for host location and selection are more favourable for detecting minor differences between plant susceptibility (Bartlet & Williams 1991).

Two new screening methods have been developed in this study which can be used to quantify the amount of leaf feeding of *C. pallidactylus* in no-choice tests and the oviposition preference in dual-choice tests, respectively. Both methods are suitable for rapid screening of a large assortment of genotypes and for assessment of significant differences between host suitability. A comparison between the results of both screenings methods showed a significant positive relationship. However, both methods address only the antixenosis effect of plant genotypes. To characterize antibiosis effects, parameters of larval performance such as larval mortality or larval weight need to be determined in greater detail.

Validation of the results of laboratory screening methods under field conditions showed significant correlations. The coefficient of determination differed between both screening methods. The results of the dual-choice oviposition screening test were more consistent with results of field studies ($r = 66\%$), as this test method is probably better adapted to the process of host finding and host selection in the field. Relevant cues like plant morphology or plant volatiles are included. Thus, the oviposition preference test appears to be superior to the feeding test for estimating the level of host suitability.
Host plant susceptibility

Research on host plant resistance in plant genotypes relies mainly on laboratory assays, with adult stages of pest insects. Screening commonly includes at first commercial varieties of the crop plant, followed by breeding lines, land races and wild genotypes (Ellis & Kift 2003). A wide spectrum of brassicaceous plant species was tested in this study for susceptibility to cabbage stem weevil. In dual-choice and no-choice tests various cultivars or lines of oilseed rape, resynthesized rapeseed lines, cultivars of *B. oleracea* and wild species of Brassicaceae showed significantly less susceptibility to *C. pallidactylus*. Considerable differences were detected not only between the host quality of genotypes of oilseed rape vs. other species and genera of Brassicaceae, but also between different cultivars of oilseed rape, which have only a low genetic variability (Becker *et al.* 1995). In studies comparing the leaf feeding of pests on modern lines of oilseed rape significant differences between these lines were also observed with cabbage flea beetle, *Phyllorelata cruciferae* (Goeze) (Bodnaryk & Palaniswamy 1990) and diamondback moth (Sarfraz *et al.* 2007). The leaf area consumed by *C. pallidactylus* on different genotypes of *B. oleracea* tested showed significant differences. Similarly, the host of aphids for various accessions of *B. oleracea* differed significantly (Singh & Ellis 1993; Cole 1997).

Among the other species of *Brassica* tested for their susceptibility to *C. pallidactylus* in the laboratory, only three genotypes of *Brassica fruticulosa* Cyrillo showed a significant reduction of oviposition compared to ‘Express’. *B. fruticulosa* had been selected as a source of resistance to cabbage aphid, *Brevicoryne brassicae* L., cabbage root fly, *Delia radicum* (L.) and other pest species of brassicaceous plants (Cole 1997; Jensen *et al.* 2002; Pink *et al.* 2003; Felk* et al.* 2005). So far, no resistance loci have been identified, but a polygenic resistance is assumed (Pink *et al.* 2003). *Crambe abyssinica* Hochst. ex R.E. Frees was found to be little attractive to pollen beetle, *Meligethes aeneus* (Fabr.) (Ekbo* & Borg 1996), cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Kalischuk & Dosdall 2004; Cárcamo *et al.* 2007) and cabbage flea beetles, *Phyllorelata* spp. (Henderson *et al.* 2004), respectively. The factors causing this non-preference trait have not been yet determined. For pollen beetle the form of flower buds is supposed to effect oviposition preferences (Ekbo* & Borg 1996). Chemical cues (Palaniswamy & Lamb 1992) and plant morphology (Kalischuk & Dosdall 2004) are known to affect the host preference of flea beetles and cabbage
seedpod weevil, respectively. Plant architecture is significantly positive correlated with oviposition of *C. pallidactylus*, i.e. length of main stem (Ferguson *et al.* 2003) and the number of lateral racemes and leaves on main stems (Eickermann & Ulber 2009, in prep.). However, plant architecture appears not to be the most important factor in the plant-weevil-interaction, and other cues like plant volatiles may have higher impact.

The gene pool utilized for elite oilseed rape breeding has been eroded as major emphasis was given to specific oil and seed quality, but not traits like resistance (Seyis *et al.* 2000). As a consequence, genetic variability within modern cultivars is highly reduced. Resynthesized oilseed rape has potential to enhance the genetic diversity significantly (Becker *et al.* 1995); they were found to provide new sources of resistance to plant diseases (Diederichsen & Sacristan 1996; Rygulla *et al.* 2007) and possibly to pest species as well (Seyis *et al.* 2000). In our study, we have proved for the first time that some resyntheses exhibit a significantly lower susceptibility to *C. pallidactylus* in laboratory and field experiments. Characteristics of resyntheses are based on their progenitors (Girke 2002). Different cultivars of Chinese cabbage, *B. rapa* ssp. *pekinensis*, were used as progenitor of resyntheses H 226, L 16 and L 122, which were significantly less susceptible to cabbage stem weevil. However, further experiments are required to determine potential resistance factors of *B. rapa* ssp. *pekinensis* in detail.

**Influence of glucosinolates**

Plant species belonging to the family Brassicaceae are able to biosynthesize glucosinolates (GSLs). GSLs and their breakdown products are considered to play an important role in plant-insect-interactions (Mithen 1992; Wallsgrove *et al.* 1999). The content and profile of GSLs within plants depend on several factors, such as genetic, environmental, agronomic and attack by herbivores or diseases (Bartlet *et al.* 1999; Wallsgrove *et al.* 1999; Textor & Gershenzon 2009). GSLs are subdivided into three different major chemical classes: aliphatic, aromatic and indolic. Their relative concentration can differ significantly between plant species, cultivars and even ecotypes and also interact differently with herbivore species (Textor & Gershenzon 2009). The reduction of the total GSL content in seeds by breeding selection often resulted in a reduction of aliphatic GSLs, whereas the level of indolic GSLs remained approximately constant (Weber *et al.* 1984; Mithen 1992). In general, the majority of specialist pests on oilseed rape were found to be attracted more strongly by indolic GSLs. Only
C. obstrictus responded more clearly to aliphatic GSLs of host plants, e.g. gluconapin and glucobrassicanapin (McCaffrey et al. 1999; Ulmer & Dosdall 2006). Additionally, the aromatic GSL sinalbin is assumed to have an antixenotic effect on the larvae of C. obstrictus (Dosdall & Kott 2006). In recent studies (Cárcamo et al. 2007), an antibiotic effect of aliphatic GSL sinigrin on larvae of cabbage seedpod weevil was detected as well.

In our semi-field experiments, a significant effect of single GSL compounds in Brassica cultivars and resynthesized rapeseed lines on the stem injury coefficient by C. pallidactylus was found. In resyntheses that were significantly less infested by C. pallidactylus larvae the GSL 4-methoxyglucobrassicin was completely missing, whereas the aromatic GSL gluconasturtiin and the indolic GSL glucobrassicin were found only at low or medium concentrations compared to other genotypes tested. Glucobrassicin is the dominant GSL in the leaves during early vegetative growth stages of oilseed rape (Rothe et al. 2004). The indolic GSL 4-hydroxyglucobrassicin occurred at a medium concentration in resyntheses L 16, L 122 and H 226. This indicates that host preference of C. pallidactylus is affected by a certain balance between single GSL compounds, rather than individual GSLs.

Genotypes of Brassicaceae were found to be significantly less susceptible to C. pallidactylus in the laboratory screenings, e.g. honesty, Lunaria annua L., false flax, Camelina alyssum (L.) and B. fruticulosa Cyrillo. These species produce only very low amounts of glucobrassicin and neoglucobrassicin (Schraudolf 1968; Cole 1997). However, L. annua also contains alkaloids and saponins which have been described as feeding deterrents for cabbage flea beetle, Phyllotreta nemorum L. (Nielsen 1989). The leaves of C. alyssum contain the flavonoid quercetin (Onyilagha et al. 2003), but the role of these phytochemicals in host finding and host acception of C. pallidactylus has not been examined in our experiment. In addition to secondary plant metabolites like GSLs, proteins and carbohydrates can affect host plant quality as well (Gols et al. 2007). The specific relation between these plant substances needs to be determined in further experiments.

**Multitrophic interactions**

The promotion of the parasitism of pests by host plant cues has been discussed in literature (Bottrell et al. 1998; Gols & Harvey 2009). So far, this strategy has failed
because knowledge on such highly specific interactions is insufficient. However, various cues have been identified to assist host finding of parasitoids like plant architecture (Wang et al. 1997), plant growth stages (Gols et al. 2007) and allelochemicals (Reddy et al. 2004). A significant effect of host plant species on parasitism had been detected so far only with parasitism of pollen beetle by *Diospilus capito* Nees on oilseed rape (Billqvist & Ekbom 2001). *C. pallidactylus* can be attacked by five larval endoparasitoids in Europe (Alford et al. 2003) all belonging to the family Ichneumonidae. *Tersilochus obscurator* Aubert is the most important parasitoid in Germany (Ulber 2003, Ulber & Nitzsche 2006). The level of parasitism depends on several external factors such as weather conditions (Johnen et al. 2006), plant architecture (Ulber & Fischer 2006), plant volatiles (Jönsson et al. 2005) and crop management (Nitzsche & Ulber 1998; Ulber & Fischer 2006). In three-years field experiments, the level of parasitism of *C. napi* by *T. fulvipes* was significantly lower in cultivars of *B. rapa* than in cultivars of oilseed rape. Further, parasitism of cabbage stem weevil by *T. obscurator* was significantly reduced in oilseed rape cv ‘Wotan’. The late flowering period of *B. oleracea* and *B. juncea* cv ‘Vitasso’ compared to oilseed rape might have provided food resources to adult Tersilochinae and increased parasitism of larvae. Late flowering period can affect contents and profiles of GSLs in this specific growth stage that can influence host recognition of parasitoids (Jönsson et al. 2005; Gols et al. 2007). Interaction between host larvae and parasitoids can differ widely between cultivated and wild Brassicaceae (Kahuthia-Gathu et al. 2008).

A significant influence of single GSL compounds, especially indolic and aromatic GSLs, on interaction between host plant and specialist pests have been shown in our study. Similarly, single GSLs seem to play also an important role as cues for host finding of parasitoids as well. 3-butenyl had a direct effect on parasitism of aphids by *Diaeretiella rapae* (McIntosh) (Bradburne & Mithen 2000). Gols et al. (2007) found only a weak effect of the GSL content within leaves of *S. alba* and *B. oleracea* on parasitism of *Plutella xylostella* L. by *Diadegma semiclausum* Hellen. The authors concluded that a complex of factors is influencing the tritrophic system. Further studies are needed to determine the role of GSL compounds for the parasitism of ceutorynchid stem miners in oilseed rape in greater detail.
Conclusion

More information is required to determine the potential of genotypes showing low susceptibility to cabbage stem weevil for breeding of modern cultivars of oilseed rape. Because species of the genus *Brassica* have different genomes, hybridization between wild species and *B. napus* is difficult (Ellis *et al.* 1999; Pink *et al.* 2003) and requires specific techniques like embryo rescue (Srivastava *et al.* 2004). Negative effects of crossing high yield cultivars with wild species on yield and quality traits are possible (Allard 1996). Whereas accessions of *B. fruticulosa* are genetically distant from modern oilseed rape, resyntheses promise to be an important source of resistance that can be more easily used for breeding of resistant oilseed rape in near future. Host plant resistance to pest insects is an underutilized strategy in IPM in oilseed rape. A better understanding of plant-insect interaction and the factors affecting host location and host acceptance are major points in breeding of (partly) resistant cultivars to cabbage stem weevil and other pest species of oilseed rape for the future.

References


Bartlet E & IH Williams (1991): Factors restricting the feeding of the cabbage stem flea beetle (*Psylliodes chrysocephala*). *Entomologia Experimentalis et Applicata* 60, 233-238.


Billqvist A & B Ekborn (2001): Effects of host plant species on the interaction between the parasitic wasp *Diospilus capito* and pollen beetle (*Meligethes* spp.). *Agricultural and Forest Entomology* 3, 147-152.


Bradshaw JD, Rice MW & JH Hill (2007): No-choice preference of Cerotoma trifurcata (Coleoptera: Chrysomelidae) to potential host plants of Bean pod mottle virus (Comoviridae) in Iowa. Journal of Economic Entomology 100, 804-814.


Cole RA (1997): The relative importance of glucosinolates and amino acids to the development of two aphid pests Brevicoryne brassicae and Myzus persicae on wild and cultivated Brassica species. Entomologia Experimentalis et Applicata 85, 121-133.


Eickermann M & B Ulber (2009): Impact of Brassica genotypes on infestation and larval abundance of ceutoryngid stem miners (Ceutorhynchus pallidactylus (Mrsh.) and Ceutorhynchus napi Gyll.). In prep.


Farrell JAK (1977): Plant resistance to insects and the selection of resistant lines. The New Zealand Entomologist 6, 244-261.


Summary

Effect of *Brassica* genotype on the infestation by cabbage stem weevil *Ceutorhynchus pallidactylus* (Mrsh.) (Col.: Curculionidae) and the parasitism of stem weevil larvae

The search for plant resistance to insect pests in oilseed rape (*Brassica napus* L. var. *oleifera* Metzg.) is still in an early stage of development. In this study, a wide spectrum of *Brassicaceae* representing a broad genetic variability (*B. napus* and *B. rapa* cultivars, breeding lines, resynthesized rapeseed lines, other *Brassica* spp.) was evaluated for resistance characteristics to cabbage stem weevil (*Ceutorhynchus pallidactylus*) for the first time. Two new methods were developed for the screening of host plant quality of a large assortment under controlled conditions in climate chambers to allow a rapid and reliable selection of genotypes with partial or full resistance.

For the quantification of leaf feeding by adult beetles, 106 genotypes were tested in no-choice screening tests for their susceptibility. In comparison to the standard cultivar ‘Express’, the average leaf area consumed by *C. pallidactylus* was reduced significantly by more than 40% on ten oilseed rape cultivars, four resynthesized rapeseed lines and five other accessions. In dual-choice screening tests for the evaluation of oviposition preferences, female *C. pallidactylus* laid significantly fewer eggs into plants of two oilseed rape cultivars, five resynthesized rapeseed lines and eight accessions of *B. oleracea* and *B. fruticulosa*, respectively, than into plants of the standard cultivar ‘Express’.

Within three years, field experiments were carried out to evaluate the susceptibility of 42 *Brassica* genotypes to infestation by *C. pallidactylus* under field conditions. A significant correlation was found between the results obtained by laboratory screening tests and by field experiments, thereby confirming the reliability of the new laboratory tests for predicting the susceptibility of *Brassica* genotypes to *C. pallidactylus* under field conditions.

In a semi-field trial, no significant relationship was found between the total leaf glucosinolate content and the intensity of attack by *C. pallidactylus* in 12 genotypes tested. However, the stem injury coefficient was significantly correlated with single glucosinolate compounds, particularly aromatic glucosinolate gluconasturtiin and the indolic glucosinolates glucobrassicin, 4-methoxybrassicin and 4-hydroxybrassicin.
The larval parasitism of *C. pallidactylus* and *C. napi* by *Tersilochus obscurator* and *T. fulvipes* (Hym.: Ichneumonidae), respectively, was found to be significantly different between host plant genotypes. In three cultivars of turnip rape, *B. rapa*, the level of parasitism of *C. napi* by *T. fulvipes* was significantly reduced compared to cultivars of winter oilseed rape, *B. napus*. The level of parasitism was also significantly affected by host plant architecture.

In summary, the results present a new method useful in studying essential fundamentals for the development of oilseed rape cultivars with partial resistance to cabbage stem weevil and provide evidence for the impact of host plant genotype on multitrophic interactions between stem-boring insect pests and specialised parasitoids.
Zusammenfassung

Effect of Brassica genotype on the infestation by cabbage stem weevil Ceutorhynchus pallidactylus (Mrsh.) (Col.: Curculionidae) and the parasitism of stem weevil larvae


In Zwangsversuchen wurden 106 Brassica-Genotypen geprüft, um durch Erfassung der durch den Schädling konsumierten Blattfläche Hinweise auf die Anfälligkeit der Prüfglieder gegenüber dem Gefleckten Kohltriebrüssler zu erlangen. Im Vergleich zur Kontroll-Sorte ‘Express’, die Standardsorte in allen Versuchen fungierte, war die von C. pallidactylus konsumierte Blattfläche bei 10 kommerziellen Raps-Sorten, 4 Raps-Resynthesen und 5 weiteren Genotypen um mehr als 40% reduziert.

In Wahlversuchen fanden sich an insgesamt 2 kommerziellen Raps-Sorten, 5 Raps-Resynthesen und 8 Genotypen von B. oleracea und B. fruticulosa signifikant weniger Eigelege der Weibchen von C. pallidactylus als an der Kontroll-Sorte ‘Express’.


In einem Halbfreilandversuch fanden sich keine signifikanten Zusammenhänge zwischen dem Gesamtgehalt an Glucosinolaten und der Befallsstärke von
C. pallidactylus in 12 Brassica-Genotypen. Allerdings zeigte sich ein signifikanter Einfluss einzelner Glucosinolate auf den so genannten “stem injury coefficient”: das aromatische Glucosinolat Gluconasturtiin und die indolischen Glucosinolate Glucobrassicin, 4-Methoxybrassicin und 4-Hydroxybrassicin.


Acknowledgements

I would like to thank many people for giving me a helping hand in so many situations during these last few years. I would like to thank Prof Dr Stefan Vidal, who did the reviewing of this thesis, and allowed me to work in his Entomological Section. Many thanks also go to Prof Dr Heiko Becker for the co-reviewing of my thesis. I am especially grateful to Dr Bernd Ulber for making me interested in the topic, supporting me in my experiments and answering my questions so many times. Finally, he gave me the chance to explore the world of entomology. A lot of thanks additionally go to Dr Roland Rohringer, who advised me in the digital image analysis, and developed the macro for measuring leaf damage by cabbage stem weevil.

I am also grateful to: Dr Frauen (Norddeutsche Pflanzenzucht) and Dr Stelling (Deutsche Pflanzenveredelung), providing seeds of rare cultivars and supporting me in my work. Additional thanks go to: Mr Mäntz (KWS), Dr Detering (Raps GbR), Dr Meyer zu Beerentrupp (W von Borries Eckendorf), Dr Reinhold (LimagrainNickerson) and Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany. I would like to express thanks to Prof Dr Becker (Georg-August-Universität, Göttingen), Dr Diederichsen (FU Berlin) and Dr Luehs (IFZ Gießen) for allocating me seeds of resynthesized rapeseed and more ‘exotic’ Brassica genotypes.

It gives me great pleasure in thanking Dr Udelhoven (CRP-Gabriel Lippmann, Luxembourg) for supporting me in statistical analyses. I would also like to express my gratitude to Mrs Mennerich, Mrs Metje, Mrs Neumann, Mrs Trittermann, Mr Hodyl, Mr Gremmes, Mr Reintke and Mr Wedemeyer for helping me in the field, in the greenhouse and in the laboratory. Additional thanks go to Mr Torsten Balz for his collection of delicious booze, and Mr Charles Napier-Kristiansson for revamping my English. Last but not least, I am very grateful to my parents!

Finally to my alma mater: vivat, crescat, floreat Georgia Augusta in aeternum.

*Extra Gottingam non est vita, si est vita non est ita!*
Curriculum vitae

14th March, 1973  born at Göttingen, Germany

1992  Matriculation at Grammar school “Max-Planck-Gymnasium”, Göttingen

1992 – 1994  Apprenticeship as a gardener at a tree nursery, Göttingen

1994 – 1999  Study of horticultural sciences at the University of Hannover, Germany. Final examination: “Diplom-Agraringenieur”


2003 – 2006  PhD-study at Georg-August-University, Göttingen, Department of Crop Sciences, Entomological Section

since 2007  Senior Researcher at the “Centre de Recherche Public – Gabriel Lippmann”, Belvaux, G-D of Luxembourg