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Evaluation of resistance mechanisms against *Delia radicum* L. and *Psylliodes chrysocephala* L. in brassicaceous accessions



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Chapter I

General Introduction





Oilseed rape production

Oilseed rape (*Brassica napus* (L.)) is the major oilseed crop in the European Union (EU); a total of 6.7 million hectares of oilseed rape were grown in the EU in 2014, 1.4 million hectares of which were grown in Germany (EUROSTAT 2015). Within the EU, winter oilseed rape is much more commonly grown (> 90% of total production) than spring oilseed rape (RAKOW 2011). Although oilseed rape has high yield potential, pests such as insects can cause significant yield losses and indeed, are the largest challenge in oilseed rape production worldwide (AHUJA *et al.* 2009; WILLIAMS 2010). Over the last decades, six insect pest species have been considered to be of major economic importance for winter oilseed rape: the cabbage stem flea beetle (*Psylliodes chrysocephala* (L.) (Coleoptera: Chrysomelidae)), the rape stem weevil (*Ceutorhynchus napi* (Gyll.) (Coleoptera: Curculionidae)), the cabbage stem weevil (*Ceutorhynchus pallidactylus* (Marsh.) (Coleoptera: Curculionidae)), the pollen beetle (*Brassicogethes aeneus*, syn. *Meligethes aeneus* (Fab.) (Coleoptera: Nitidulidae)), the cabbage seed weevil (*Ceutorhynchus obstrictus* (Marsh.) (Coleoptera: Curculionidae)) and the brassica pod midge (*Dasineura brassicae* (Winn.) (Diptera: Cecidomyiidae)) (WILLIAMS 2010). The cabbage root fly (*Delia radicum* (L.) (Diptera: Anthomyiidae)) has also increasingly become a significant pest in oilseed rape production within the last two decades (ALFORD *et al.* 2003; ERICHSEN & HUNMÖRDER 2005). Due to the damage that can potentially be caused by herbivores, chemical insecticides are commonly applied to protect plants. As a result of the intensive use of broad-spectrum insecticides, mainly pyrethroids, resistant populations of *M. aeneus* have been found in many European countries (SLATER *et al.* 2011); first populations of *P. chrysocephala* and *C. obstrictus* with a reduced sensitivity towards the pyrethroids have also been reported (HEIMBACH & MÜLLER 2013; HØJLAND *et al.* 2015).

Widespread, already in autumn damaging pest species, are *P. chrysocephala* and *D. radicum* (ALFORD *et al.* 2003; CAPINERA 2008). These species immigrate to rape fields shortly after the plants become established. In years with unfavourable growing conditions, the feeding activity of the *P. chrysocephala* and *D. radicum* larvae may considerably reduce plant density, which can lead to substantial economic losses (DOSDALL *et al.* 2000; ALFORD *et al.* 2003). From 2001 to 2013, neonicotinoid insecticides used for seed coating reduced the infestation of *P. chrysocephala* and *D. radicum* in oilseed rape plants in Germany. Moreover, pyrethroids are registered for spray applications that protect against *P. chrysocephala* adults and their larvae. For *D. radicum*, however, there is no chemical



control other than the seed coating effective to reduce the plant damage (ERICHSEN 2006). Since the use of neonicotinoids as a seed treatment in oilseed rape was strongly restricted by the EU in 2013 (BAROSO 2013), there is an urgent need to develop alternative control strategies for both *D. radicum* and *P. chrysocephala*.

Integrated pest management (IPM) may improve the efficiency, profitability and environmental acceptability of oilseed rape production and thereby, contribute to sustainable crop production systems (WILLIAMS 2010). Indeed, IPM “is an ecological approach to managing insect pests, by using different pest control methods, that are aimed at the entire pest complex of a crop ecosystem and finally ensures high quality agricultural production in a suitable, environmentally safe and economically sound manner” (BAJWA & KOGAN 2002). Especially cultivars that are resistant and tolerant to *D. radicum* and *P. chrysocephala* attacks may be an alternative to the problematic chemical approaches that promote the development of pest resistance and adversely affect the environment as well as non-target organisms like polyphagous predators and parasitoids (WILLIAMS 2004).

Cabbage stem flea beetle

The cabbage stem flea beetle (*Psylliodes chrysocephala* (L.)) (Coleoptera: Chrysomelidae) is located throughout the maritime regions of northern Europe (ALFORD *et al.* 2003). In late August to early September, shortly after seedling emergence the adult beetles invade into the oilseed rape fields (ALFORD *et al.* 2003) (Fig. 1).

The females feed for approximately two weeks (maturity feeding) before they begin to deposit their eggs in the soil (NUSS 2004). Egg-laying may continue throughout autumn and winter if weather conditions are favourable (high humidity and 4-16°C) (SCHULZ 1985). A developmental threshold of around 5°C has been determined for life stages (MATHIASSEN 2015). The neonate larvae bore into the petioles of plants close to the nodality and burrow into the pith tissue (GODAN 1950). When the weather conditions are mild, the larvae continue to feed throughout the winter and during their development they move to younger leaves and later, into the stems (SCHULZ 1985). Mature thrid instar larvae leave the plant to pupate in the soil (DOBSON 1960). New generation adults emerge in late spring (May-July) and, before they begin to aestivate (summer diapause), they feed on the leaves of ripening oilseed rape plants, thereby causing no economic feeding damage. The cabbage stem flea beetle is univoltine (ALFORD *et al.* 2003).

Feeding of post-aestivation adults on the emerging winter oilseed rape causes characteristic holes in cotyledons and young leaves. Severe feeding by the adults may lead to the death of seedlings (WILLIAMS 2010). The feeding of larvae, however, is considered to be more dangerous and may result in growth reduction and wilting; plant losses may occur if the vegetation point is damaged or if water penetrates the plants via injuries caused by the larvae and freezes during the winter, subsequently causing the plant tissue to burst (SCHULZ & DAEBLER 1984). Injuries may also act as entry points for fungal phytopathogens such as *Leptosphaeria maculans* (Desm.) (anamorph *Phoma lingam*) (BROSCHWITZ *et al.* 1993). The host plants of the oliophagous cabbage stem flea beetle include glucosinolate-containing plants, like *B. napus*, *Bassica rapa* (L.) and *Brassica oleracea* (L.), as well as resedaceous (*Reseda alba* (L.)) and tropaeolaceous (*Tropaeolum majus* (L.)) plants (BARTLET & WILLIAMS 1991).



Figure 1: Adult *P. chrysocephala* beetle (upper left); damage caused by beetle feeding (upper right); damage pattern of larvae (lower left); *P. chrysocephala* larvae feeding in petiole (lower right).



Cabbage root fly

The cabbage root fly (*Delia radicum* (L.) syn. *D. brassicae*) (Diptera: Anthomyiidae) feeds on brassicaceous crops throughout the northern hemisphere; plant damage is caused by *D. radicum* larvae feeding on the roots (CAPINERA 2008) (Fig. 2). Three to four generations occur in Germany annually, with the third generation being the most damaging to winter oilseed rape crops, as it coincides with a highly vulnerable developmental stage of the plants. Commonly, oviposition of the third generation begins during the second week of September (ERICHSEN 2006). After eclosion, the flies feed nonspecifically on nectar and pollen. The gravid females deposit batches of eggs (up to 10 eggs per batch) beneath the soil surface, near the base of the brassicaceous host plants (CAPINERA 2008). The eggs may even stick to the plants' hypocotyls (ZOHREN 1968). Each female lays 300-400 eggs during its life span (CAPINERA 2008). First instar larvae feed on the root hairs, whereas second and third instar larvae (maggots) feed on the tissue of the taproot (MCDONALD & SEARS 1992). Extensive damage to the lateral root system may limit the plant's water and nutrient uptake and if the taproot is detached, the plants may die (MCDONALD & SEARS 1992). Aboveground symptoms of larvae feeding on the plant roots include purpling and wilting of leaves (CAPINERA 2008). Furthermore, injured roots are more vulnerable to fungal pathogens like *Verticillium longisporum* (KEUNECKE 2009) and *Fusarium spp.* (GRIFFITHS 1986). While winter oilseed rape can tolerate a moderate infestation level of the third and fourth *D. radicum* generation, a high larval infestation in autumn combined with suboptimal growth conditions (e.g. drought) may result in significant economic yield losses (ERICHSEN & HUNMÖRDER 2005). Thus far, economic thresholds for the cabbage root fly are not available and it is evident that more research is needed to quantify the economic impact of different levels of cabbage root fly infestation.

Larval development requires about four weeks and subsequent pupation in the soil lasts roughly two weeks, unless it is interrupted by cold temperatures during the winter or heat in the summer (COAKER & FINCH 1971). For the majority of life stages, a developmental threshold of approximately 6°C has been ascertained (CAPINERA 2008). The pupae of the third or fourth generation remain dormant during the winter and emerge the following spring. The feeding of the first and second generation larvae in spring and early summer does not damage the winter oilseed rape plants, as the root system is sufficiently developed at these times. The host plant range of *D. radicum* comprises vegetable *Brassicacae*, brassicaceous weeds and *B. rapa* as well as *B. napus* (CAPINERA 2008).

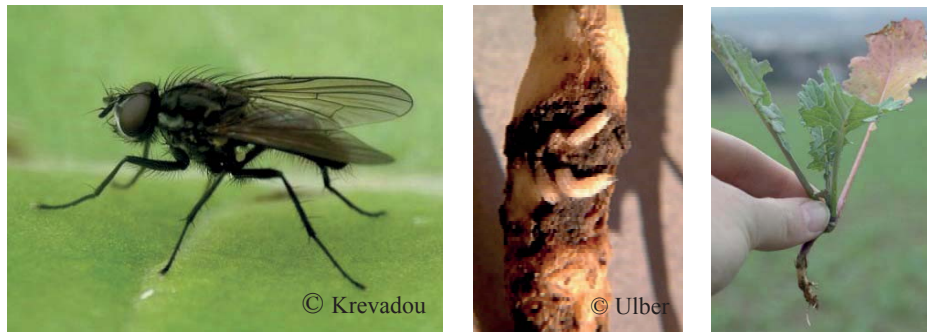


Figure 2: *D. radicum* adult (left); larvae feeding on roots (middle); oilseed rape plant with damage symptoms (right).

Plant resistance to insects

The aforementioned problems of pest control have shifted the focus to alternative measures for IPM and as a result, the use of cultivars that are resistant to pests and diseases should be a central part of any integrated crop management strategy (COOK *et al.* 2006), because they may reduce the current reliance on chemical insecticides (DOSDALL *et al.* 2000). Thus far, although cultivars of oilseed rape have been developed that are resistant to important fungal diseases such as *Plasmodiophora brassicae* (DIEDERICHSEN *et al.* 2009; FRAUEN 2011), few attempts have been made to breed cultivars that are resistant to insects (FRAUEN 2011). Moreover, these breeding programs often struggle to stay ahead of insect pest evolution (GOULD 1998). Host plant resistance to insect pests is generally based on reducing the host plant's attractiveness for colonization, feeding and egg deposition (antixenosis) and on the direct defence-responses and inhibitory effects of specific phytonutrients (antibiosis) (SCHOONHOVEN *et al.* 2005). Antixenosis is defined as “plant properties [that] evoke negative responses or total avoidance by insects” (SCHOONHOVEN *et al.* 2005), whereas antibiosis is characterized by reductions in the fecundity, longevity and development or by an increase in mortality of the pests and refers to plant properties that negatively affect the physiology of the herbivore (SCHOONHOVEN *et al.* 2005). Furthermore, a third defence mechanism is tolerance, which is described as the ability of a plant to support an insect infestation without loss of vigour and reduction of yield as compared to a susceptible plant (DENT 2000). Plants generally become resistant to insect pests through a combination of the defence-mechanism types (antixenosis, antibiosis and tolerance. Moreover, it is rare that the achieved degree of resistance to specific insect herbivores is complete; partial resistance is a more common phenomenon (DENT 2000;



SCHOONHOVEN *et al.* 2005). In contrast to complete resistance, partial resistance is expected to exert a lower selection pressure on insect populations and may therefore, more effectively counter the persistent adaptability of insects (SCHOONHOVEN *et al.* 2005).

Plant traits used for host selection and acceptance

The ability of an insect to detect a host plant relies on both biochemical and morphological plant traits (AHUJA *et al.* 2009). Concerning the biochemical plant traits in brassicaceous plants, volatiles and other secondary compounds such as glucosinolates, alkaloids and phenolics are discussed to influence the resistance of plants. Moreover, the effects of primary metabolites, like sugars are investigated. Additionally, morphological resistance traits like leaf colour, trichome density and epicuticular waxes may play a role in the insect-plant interaction (GATEHOUSE 2002; FÜRSTENBERG-HÄGG *et al.* 2013; HERVÉ 2014).

In addition to the direct defence of plants (plants produce physical barriers against herbivores or compounds that are repellent, antinutritive or toxic to herbivores (antixenosis and antibiosis mechanisms)), indirect defence mechanisms may protect plants against herbivory by attracting predators or parasitoids (FÜRSTENBERG-HÄGG *et al.* 2013). The defence traits employed by plants act as both constitutive “static” mechanisms through direct impairment (e.g. toxification or lower digestibility of plant tissue) and as inducible “active” mechanisms, which are accumulated in response to tissue damage by herbivores (GATEHOUSE 2002; AHUJA *et al.* 2009). In this study, we screen the direct antixenosis and antibiosis mechanisms of plants against the oilseed rape pests *P. chrysocephala* and *D. radicum*.

Biochemical plant traits: Numerous studies on insect-plant interactions have investigated the important role of the glucosinolates and their metabolites (AHUJA *et al.* 2009). Glucosinolates are a relevant group of secondary plant substances, which are only present in a limited group of plants. The most important family of plants containing glucosinolates are the *Brassicacea* (MITHEN *et al.* 2000). Approximately 30 different glucosinolates have been documented in brassicaceous species (FAHEY *et al.* 2001). All glucosinolates have a common chemical structure and consist of a beta-thioglucose moiety, a sulphonated oxime moiety and a variable side chain derived from an amino acid. Glucosinolates can be subdivided into three major classes according to their biosynthesis, i.e. aliphatic (derived from the amino acid methionine), indolic (derived from tryptophan)

and aromatic (derived from phenylalanin) (FEENY 1977). Different side chains result in differences in the biological activity of glucosinolates and their breakdown products, respectively (MITHEN 2001). Glucosinolates are stored in the cytoplasm of plant cells and damage to plant tissue (e.g. by herbivory) results in the enzymatic hydrolysis of glucosinolate molecules (via myrosinase) into glucose, aglucones and elementary sulphur. Depending on abiotic factors, like pH values and temperature, aglucones are further metabolized into isothiocyanates, nitriles and thiocyanates (MITHEN *et al.* 2000). The concentration and composition of glucosinolates within the plant tissue are controlled by various factors such as plant species and variety, plant organ, plant growth stage and environmental conditions (like sulphur and nitrogen availability) (MITHEN 2001; HOPKINS *et al.* 2009).

Glucosinolates may have a repellent effect on polyphagous pests (MOENS *et al.* 1992), but can act as kairomones for specialized insect herbivores and can trigger both feeding and oviposition (BARTLET *et al.* 1999a; BRUCE 2014). For specialised herbivores, glucosinolates are important host plant stimuli that, e.g. act as attractants to *P. chrysocephala* and *Phyllotreta spp.* (Coleoptera: Chrysomelidae) (LIBLIKAS *et al.* 2003; HENDERSON *et al.* 2004), as well as to *D. radicum* (ROESSINGH *et al.* 1992; FELKL *et al.* 2005), *C. obstrictus* (ULMER & DOSDALL 2006) and *D. brassicae* (BARTLET *et al.* 1999b). The role of glucosinolates as resistance factors has been documented numerous times (AHMAN 1993; GIAMOUSTARIS & MITHEN 1995; BARTLET 1996; UDDIN *et al.* 2009) and the negative effects of single glucosinolates have been demonstrated, e.g. on *C. obstrictus* (EICKERMANN *et al.* 2011), *Pieris rapae* (L.) (Lepidoptera: Pieridae) (AGRAWAL & KURASHIGE 2003) and *Phyllotreta spp.* (BODNARYK 1991). Nevertheless, the plant-insect interactions are evidently less influenced by the total amount of single glucosinolates than by their composition, e.g. the indolyl-aliphatic ratio. As the drastic reduction of the glucosinolate content in oilseed rape seeds of the 00-quality cultivars, however, did not change their attractiveness for specialized herbivores (WILLIAMS 1989; GIAMOUSTARIS & MITHEN 1995; BARTLET 1996).

In addition to glucosinolates, other secondary metabolites like several flavonoids have been found to act as feeding deterrents to insects (TREUTER 2006), for instance the flavonoid kampferol has been shown to reduce the larval performance of *C. obstrictus* (LEE *et al.* 2014). Moreover, phytoalexins (produced from flavonoids) have been documented to influence host plant selection of gravid *D. radicum* (BAUR *et al.* 1998).



Additionally, females have been shown to be strongly stimulated by a particular chemical surface compound (a complex tetracyclic carboxylic acid) (ROESSINGH *et al.* 1997) of *Brassica oleracea* leaves. Beside secondary metabolites, primary metabolites such as amino acids and sugars have been shown to affect host plant selection and utilization of insects (BERENBAUM 1995; HERVÉ *et al.* 2014). Sugars are especially phagostimulating to adults of *M. aeneus* (HERVÉ *et al.* 2014), *D. radicum* (HOPKINS *et al.* 1999) and *P. chrysocephala* (BARTLET *et al.* 1994). Moreover, the nutrient status of plants, particularly the nitrogen and sulphur content, may affect the host plant choice of insect pests (MARAZZI & STÄDLER 2005; RUSCH *et al.* 2013).

Morphological plant traits: Morphological plant traits such as leaf colour (SOUTHWOOD 1986; TANSEY *et al.* 2010a), wax layer (BODNARYK 1992; LAMBTON *et al.* 1998) or the pubescence of the leaves (SOROKA *et al.* 2011) have been shown to affect a plant's resistance to insects. The toughness of the plant tissue of below- and above ground parts may act as a physical barrier to chewing and sucking insects (FÜRSTENBERG-HÄGG *et al.* 2013). This morphological trait has been widely disregarded until recently. The compensatory regrowth of plant tissue of infested plants also influences the host plant-insect interaction (as reviewed in FÜRSTENBERG-HÄGG *et al.* 2013). Phenological factors, such as the growth stage of the plant, influences host plant selection (MCDONALD & SEARS 1992; SCHOONHOVEN *et al.* 2005), e.g. the infestation of *C. pallidactylus* on oilseed rape increases as the number of leaves per plant increases (EICKERMANN *et al.* 2011) and females of *D. radicum* were found to prefer larger plants for oviposition (MCDONALD & SEARS 1992;).

Current status of insect resistance breeding

In the continuous efforts to develop high-yielding oilseed rape varieties, the diversity related to defensive traits and resistance levels to herbivores might have been lost (SCHOONHOVEN *et al.* 2005). Rapeseed originated from the spontaneous hybridization of cabbage (*B. oleracea*) and turnip rape (*B. rapa*) and the breeding material currently in use might derive from only a small number of centuries-old interspecific hybrid plants (BECKER *et al.* 1995). While there is a relatively limited gene pool for *B. napus* (BROWN *et al.* 1997), the use of various resistance and quality traits from the progenitors and related plant species is a promising approach for breeding programs (GIRKE 2002). Differences in the susceptibility of brassicaceous species to insect attack are known and intensively studied for *D. radicum* (ELLIS *et al.* 1999; DOSDALL *et al.* 2000; JENSEN *et al.* 2002).