Performance of cabbage stem flea beetle larvae (*Psylliodes chrysocephala*) in brassicaceous plants and the effect of glucosinolate profiles

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Abstract

The cabbage stem flea beetle (CSFB), *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae), is one of the most important pests in European winter oilseed rape production. Adult beetles feed on young leaves whereas larvae mine within the petioles and stems. Larval infestation can cause significant crop damage. In this study, the host quality for CSFB of four oilseed rape (*Brassica napus* L.) cultivars and seven other brassicaceous species with different glucosinolate (GSL) profiles was assessed under controlled conditions. Larval instar weights and mortality were measured after 14 and 21 days of feeding in the petioles of test plants. To study the impact of GSL on the performance of larvae, the GSL contents in petioles from non-infested and infested plants were analysed before, and 21 days after, the start of larval infestation. Larval performance was not significantly different between the four cultivars of oilseed rape, but differed considerably among the other brassicaceous species tested. In comparison to the weight of larvae in the standard *B. napus* cv. Robust, the larval weight was higher in turnip rape (*Brassica rapa* L. var. *silvestris*) and significantly reduced in white mustard (*Sinapis alba* L.), oil radish (*Raphanus sativa* L. var. *oleiformis*), and cabbage (*Brassica oleracea* L. conv. *capitata* var. *alba*). The duration of larval development increased in white mustard and oilseed radish. The GSL profiles of the petioles showed little difference between non-infested and infested plants of oilseed rape whereas the content of aliphatic GSL increased in the infested turnip rape plants. In contrast, the aliphatic and benzenic GSL decreased in infested Indian rape (*B. rapa* subsp. *dichotoma* Roxb.). Larval weight was not correlated with the total GSL content of plants, neither before infestation nor 21 days after. Larval weight was positively correlated with progoitrin and 4-hydroxyglucobrassicin. White mustard, which provides inferior host quality for larval development, has the potential to introduce insect resistance into high-yielding oilseed rape cultivars in breeding programmes.

Introduction

Oilseed rape (*Brassica napus* L., Brassicaceae) is the most important oil crop in Central and Northern Europe (Eurostat, 2016). It is attacked by a wide range of specialist insect pests during its growing period (Ekbom, 1995; Alford et al., 2003). In humid regions of Northern Europe, cabbage stem flea beetle (CSFB), *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae), is among the most devastating pests of winter oilseed rape (Winfield, 1992; Williams, 2010), causing significant yield losses even at moderate levels of infestation (Nilsson, 1990). During autumn, adult beetles reduce growth by feeding on the leaves of young plants; however, economically important damage is caused mainly by the larvae (Ekbom, 1995). The larvae feed within the petioles and stems of young rapeseed plants, thereby increasing plant mortality during winter (Ekbom, 1995; Williams, 2010). In addition, feeding wounds made by larvae can result in
a higher incidence of fungal pathogens, such as stem canker, *Leptosphaeria maculans* Ces. & de Not. (Newman, 1984). Control of CSFB is usually achieved by insecticidal seed dressing or foliar applications of insecticides, most often using synthetic pyrethroids (Williams, 2004).

Due to high selection pressure, CSFB populations have developed resistance against these insecticides, as recently reported from the UK and Germany (Heimbach & Müller, 2011). The decreasing control efficacy and increasing environmental concern associated with chemical control makes the development of alternative control strategies very important. Moreover, the ban of neonicotinoid seed treatments in oilseed rape increases the need to find alternative solutions (European Commission, 2013). The search for insect-resistant plant cultivars or species may be a promising option, as reported for other specialist pests, such as cabbage seed pod weevil, *Ceutorhynchus obstrictus* Marsham, and cabbage stem weevil, *Ceutorhynchus pallidactylus* Marsham (Carcamo et al., 2007; Eickermann et al., 2011). Such sources of resistance could be integrated into oilseed rape breeding programmes. Planting insect-resistant oilseed rape cultivars would help to reduce the number of insecticide applications and their negative impacts on the environment (Williams, 2010). Knowledge of host plant quality can also be used to improve biological pest control strategies such as trap crops in oilseed rape (Cook et al., 2003; Döring, 2012).

Various plant species are accepted as hosts by the larvae and adults of CSFB. Most of these belong to the Brassicaceae, but also tropaeolaceous and resedaceous species are used as hosts (Bartlet & Williams, 1991). These plants all have in common that they contain glucosinolates (Bartlet et al., 1994), the predominant group of sulphur-containing secondary metabolites (Mithen, 2001; Schoenhoven et al., 2005). Previous studies have shown that GSL profiles vary widely among brassicaceous genotypes, plant organs, and plant stage (Sang et al., 1984; Brown et al., 2003; Cartea & Velasco, 2008; Burrow, 2016). Based on their chemical structure, GSLs are subdivided into aliphatic, benzenic, and indole compounds (Fahey et al., 2001; Mithen, 2001; Agerbirk & Olsen, 2012). Even small alterations in the side chain structure can have a substantial influence on their biological activity (Mithen, 2001). By acting as feeding stimulants for specialist insects, GSLs play an important role in plant–herbivore interactions (Hoppin et al., 2009). Furthermore, specific GSL structures are assumed to affect the larval performance of several specialized insects on Brassicaceae (Agrawal & Kurashige, 2003; Beekwilder et al., 2008; Mewis et al., 2008; Rohr et al., 2009; Döring, 2012).

This study was carried out to identify brassicaceous species and cultivars that hamper larval development of CSFB and to determine the relationship between larval performance and GSL content in the petioles, the primary feeding site of CSFB larvae. The performance of CSFB larvae was determined after 2 and 3 weeks feeding, and the GSL content and composition in various plant species was analysed before and after 3 weeks feeding.

**Materials and methods**

**Plant material**

The test plants were selected from a wide range of brassicaceous species and rape seed cultivars that differed largely in their GSL profiles. They were chosen based on information from the literature and results of our preliminary experiments. Plant species that showed very high or very low attractiveness to other insect pests of oilseed rape were preferred. Four oilseed rape cultivars were included in the experiments, with Robust, Grizzly, and Campala containing low levels of GSL in their seeds (double low-quality) and Lembkes Normal containing high levels of GSL. Three turnip rape (*Brassica rapa* L. var. *silvestris*) cultivars were selected: Largo (double low quality), and Malwira and Perko, both containing high amounts of GSL in the seeds. Indian rape (*Brassica rapa* subspp. *dichotoma* Roxb.), white mustard (*Sinapis alba* L.) cv. Accent, oilseed radish (*Raphanus sativus* L. var. *oleiferus*) cv. Adagio, and white cabbage (*Brassica oleracea* L. cv. *capitata* var. *alba*) cv. Brunswijker were also included in the experiments. The plants were grown in a 3:1 mixture of potting soil (Fruhstorfer Erde Typ 25; HAWITA Gruppe, Vechta, Germany) and sand under glasshouse conditions. One week before the experiments they were transferred to controlled climate conditions (18 ± 2 °C, 65–85% r.h., L16:D8 photoperiod). Plants were fertilized with essential nutrients once a week by application of 50 ml of a 2 g l⁻¹ HakaPhos Blau® solution (COMPO EXPERT, Münster, Germany).

**Insects**

New-generation adults of CSFB emerging from soil after pupation were collected from the oilseed rape crops in July. They were maintained in plastic boxes (17.5 × 13 × 6 cm) at 15 ± 2 °C and L16:D8 photoperiod during their summer aestivation. To provide food and moisture, oilseed rape leaves (cv. Oase or Mozart) were offered to the beetles and renewed at least once a week. After the end of aestivation, in September, wet filter paper was added to the boxes to stimulate egg laying by the females (Williams et al., 2003). Eggs were collected weekly and stored in plastic boxes on wet sand at 2 ± 1 °C. To induce hatching, 1 week before each experiment the eggs were transferred to 20 ± 2 °C.
Experimental setup
The experiments started 5 weeks after sowing when the plants were in the 6–8 true leaf stage. The oilseed rape cultivar Robust, containing relatively low amounts of glucosinolates in the leaves, was included as a reference plant in all experiments. In each of the five experimental runs larval development on Robust was tested against larval development on two of the test cultivars or species. Of each test genotype 16–20 replicated plants were randomly arranged in a climate chamber, with no contact to adjacent plants, to prevent larval migration between the test plants. Five neonate larvae were transferred to each test plant within 24 h after hatching, using a fine brush. Larvae were released on the petiole base of the fourth youngest leaf closest to the axilla of each plant. To assist larval penetration in the petioles light was turned off for 6 h and the plant surface was gently sprayed with water every hour to avoid drought stressing the larvae; relative humidity in the climate chamber was almost 100%. After 6 h the plants were kept at 18 ± 2°C, 65–85% r.h., and L16:D8 photoperiod. Half of the plants of each genotype were harvested 14 days after inoculation, the other half after 21 days, the petioles and stems were then dissected and the larvae were recovered. The larval fresh weight was recorded using a Sartorius MC 5 microbalance and the head capsule width was measured as an indicator of larval performance. The relative larval weight compares the average larval weight in the test plant with the average larval weight in Robust: 100−[(mean larval weight in test plant/mean larval weight in Robust) × 100].

Glucosinolate analysis
The GSL content was analysed from petioles of both non-infested and infested plants. Samples of petioles were collected from non-infested plants at the start of the experiment. These plants were not used for testing larval performance. Samples of petioles from infested plants were collected 21 days after the release of larvae. The third and fourth youngest petioles of five plants from each genotype were pooled into one sample. The plant material was snap frozen at −80°C, immediately followed by freeze drying and grinding. The GSLs were extracted using methanol. Glucotropinolin was added as an internal standard in the analyses. The samples were treated with sulfatase and, after incubation, the desulphoglucosinolates were analysed by HPLC, as described by Cleemput & Becker (2012).

Statistical analysis
To compare the mean weight of the larvae between test genotypes and the reference cultivar Robust the Student t-test was used. Plants which did not yield any larvae were excluded from statistical analysis. Correlations (Pearson r) between the GSL content in the petioles (initial and 21 days after infestation) and the relative larval weights were analysed. Differences between the GSL contents and profiles in the tested genotypes were not analysed statistically because no replicates were available. The number of larvae recovered from plants was subjected to ANOVA followed by Tukey’s honestly significant difference (HSD) test. STATISTICA v.9.1 (StatSoft) was used for all statistical analyses.

Results
The performance of the larvae differed widely between the plant genotypes tested (Figure 1). Generally, these differences were more obvious at 14 than at 21 days of larval development. Larval weight did not differ significantly between Robust and the other cultivars of oilseed rape tested (Grizzly, Campala, Lembkes Normal), neither at 14 nor at 21 days of development within the plants. After 14 days of feeding, the weights of larvae in the turnip rape and Indian rape were 10–30% higher than in Robust. In contrast, they were significantly smaller in white mustard, oilseed radish, or white cabbage than in Robust. Only in white mustard was the number of larvae recovered from plants after 14 days significantly smaller, and the larval mortality higher, as compared to Robust (Table 1).

At 21 days after release of the larvae a high proportion of larvae from Robust and other genotypes had already migrated to the soil for pupation. Only in oilseed radish was the number of larvae remaining in the petioles significantly higher than in Robust (Table 1). After 21 days, larval feeding in oilseed radish were no longer significantly lighter than larvae grown in Robust, whereas the weight of larvae feeding in white mustard and cabbage was still significantly lower than that of larvae in Robust (Figure 1B). In white mustard only few larvae had not reached the final third instar. At 14 days after release of the neonate larvae the percentages of third instars in white mustard and oilseed radish were substantially lower than in Robust. Even at 21 days after release, 75% of larvae in white mustard had not developed to the third instar (Table 1).

In the non-infested plants, the total content of GSL in the petioles of the double low-quality oilseed rape cultivars Robust, Grizzly, and Campala was lower than in Lembkes Normal and in any other brassicaceous genotype tested (Table 2), and this was mainly represented in the aliphatic fraction. In petioles of Lembkes Normal the total content of GSL was 4.7–11× higher than in the double low-quality cultivars of oilseed rape. The aliphatic GSL fraction, particularly glucobrassicanapin, was also predominant in the cultivars of turnip rape tested but, in contrast to oilseed rape, the benzenic GSL gluconasturtiin was on a higher level in the petioles of turnip rape. Furthermore, a high
initial content of indole GSL was found, particularly in Malwira. Indian rape and white mustard showed a high content of the benzenic compound sinalbin whereas the indole compounds were at a low level in both species. In contrast to all other plant genotypes tested, in oilseed radish the contents of the indole GSL glucobrassicin and the benzenic GSL gluconasturtiin were relatively high. In petioles of white cabbage, the total content of GSL was low and this was mainly represented by aliphatic compounds. Almost no correlation was detected between the relative larval weight and the GSL content per genotype of any of the chemical classes of GSL in the non-infested or infested plants. Positive correlations were only found between the relative larval weights at 14 days after release and the initial content of progoitrin ($r = 0.65, \text{d.f.} = 10, P = 0.03$) and 4-hydroxyglucobrassicin ($r = 0.63, \text{d.f.} = 10, P = 0.04$) in the petioles.

After 21 days of CSFB infestation, the composition of GSLs in the double low-quality oilseed rape cultivars showed only minor changes from the composition in the non-infested plants. In contrast, the GSL content in Lembkes Normal was nearly doubled at 21 days after larval infestation, mainly resulting from an increase in the aliphatic GSL progoitrin. Similarly, all turnip rape cultivars showed a strong increase in the aliphatic GSL contents in the infested plants. In contrast, in Indian rape and white mustard the total GSL decreased following infestation by larvae. Particularly the benzenic GSL sinalbin decreased in both species whereas aliphatic compounds decreased in Indian rape and increased in white mustard. Oilseed radish showed an increase in both glucobrassicin and gluconasturtiin. In contrast, the GSL content or composition in the white cabbage cultivar Brunswijker was very little affected by larval infestation.

Discussion

The larval performance tests demonstrated that the host quality of the tested genotypes for CSFB larvae differed significantly between brassicaceous species. Whereas the
turnip rape cultivars proved to be most suitable hosts, larval weight and mortality in white mustard clearly showed antibiosis resistance to the larvae. Larval mortality was not affected in oilseed radish; however, the high number of larvae remaining in the petioles of oilseed radish after 21 days indicated that larval development was prolonged in this host compared to the oilseed rape. This was also confirmed by the small number of third instars formed after 14 days compared to the proportion of third instars in Robust. The low number of larvae recovered after 21 days from all other genotypes, compared to the number of larvae obtained after 14 days, was probably due to a high proportion of larvae having already completed their development and left the plants for pupation in soil. The number of larvae recovered from the reference cultivar Robust showed differences between consecutive runs of the larval performance test. Factors responsible for the different mortalities of the larvae were not clear. Environmental and pre-treatment factors, such as duration of storage of the eggs, may have affected the vitality and survival of the larvae. Due to the overall low number of larvae per plant it was very unlikely that the larvae in plants with higher infestations suffered from competition.

Earlier studies dealing with performance of insects specialized on brassicaceous plants have also shown considerable differences between larval development in host genotypes. Similar to our experiment, larvae of *Phaedon cochlearia* (Fabricius) were heaviest on *B. rapa*, lightest on *S. alba*, and intermediate on *R. sativa* (Uddin et al., 2009). This order has also been recorded for *Mamestra configurata* Walker (McCloskey & Isman, 1993). Sarfraz et al. (2010) found that swede (*B. napus* subsp. *rapifera*) was a better host for larval development of turnip root fly, *Delia floralis* Fall. A similar difference between *B. napus* and *B. oleracea* was observed for CSFB in our experiments. The inferior host quality of *S. alba* has also been reported for cabbage seedpod weevil, *C. obstrictus* Marsham: when seed weevil larvae were reared in pods of oilseed rape and white mustard, the larval weight was significantly reduced on the white mustard (Ulmer & Dosdall, 2006; Tansey et al., 2010).

Factors responsible for the different host qualities are not yet fully understood. Secondary plant metabolites such as GSL were found to act as feeding stimulants for specialist insects on brassicaceous plants, like adults of CSFB or crucifer flea beetle, *Phyllotreta cruciferae* (Goeze) (Feeny et al., 1970; Bartlet & Williams, 1991; Bartlet et al., 1994). However, the effect of GSL on larval performance was found to differ between herbivore species. Generally, the GSL content has a negative effect on larval development of generalist insects (Hopkins et al., 2009). Contradictory results have been reported on the effect of GSL on larval

| Table 1 | Mean number of *Psylliodes chrysocephala* larvae recovered from brassicaceous cultivars 14 and 21 days after start of the experiments, and the percentages of larvae recovered, dead larvae, and third instars. Three cultivars were included in five consecutive runs, Robust was included as a reference plant |
|---|---|---|---|---|---|
| Run | Cultivar | After 14 days | After 21 days | | |
| | | No. larvae/plant | % larvae recovered | % dead larvae | % third instar | No. larvae/plant | % larvae recovered | % dead larvae | % third instar |
| 1 | Robust | 4.4 | 88 | 0 | 89 | 1.5 | 30 | 6.2 | 93 |
| | Grizzly | 4.5 | 90 | 0 | 93 | 1.4 | 28 | 0 | 100 |
| | Campala | 4.0 | 80 | 7.5 | 90 | 2.0 | 40 | 0 | 100 |
| 2 | Robust | 2.1 | 42.5 | 0 | 75 | 1.5 | 30 | 0 | 100 |
| | Perko | 2.9 | 57.5 | 0 | 61 | 1.1 | 22.5 | 0 | 100 |
| | Largo | 2.5 | 50 | 0 | 60 | 2.3 | 45 | 0 | 100 |
| 3 | Robust | 4.3 | 86 | 0 | 93 | 0.7 | 14 | 0 | 100 |
| | Malwira | 4.3 | 86 | 0 | 95 | 1.1 | 22 | 0 | 100 |
| | Indian rape | 4.2 | 84 | 0 | 98 | 1.0 | 20 | 0 | 100 |
| 4 | Robust | 4.1a | 82 | 2.4 | 98 | 1.1a | 22 | 0 | 91 |
| | Mustard | 1.8b | 36 | 53.8 | 36 | 0.3a | 6 | 10 | 25 |
| | Radish | 4.4a | 88 | 0 | 53 | 4.0b | 80 | 2.4 | 100 |
| 5 | Robust | 2.9 | 58 | 3.3 | 90 | 0.6ab | 12 | 25 | 100 |
| | Lembkes Normal | 2.0 | 40 | 9.1 | 62 | 0.2a | 4 | 66.7 | 67 |
| | Cabbage | 2.7 | 54 | 3.6 | 82 | 1.6b | 32 | 11.1 | 100 |

Means within a run and within a column followed by different letters are significantly different (Tukey’s HSD test: P≤0.05).
|                | Robust n-Inf | Robust Inf | Grizzly n-Inf | Grizzly Inf | Campala n-Inf | Campala Inf | Lembkes n-Inf | Lembkes Inf | Perko n-Inf | Perko Inf | Malwira n-Inf | Malwira Inf | Largo n-Inf | Largo Inf | Ind. Rape n-Inf | Ind. Rape Inf | Accent n-Inf | Accent Inf | Adagio n-Inf | Adagio Inf | Brunswijker n-Inf | Brunswijker Inf |
|----------------|--------------|------------|---------------|-------------|---------------|-------------|---------------|-------------|------------|-----------|---------------|-------------|--------------|-----------|----------------|---------------|--------------|-----------|--------------|-----------|----------------|----------------|
| Progoitrin     | 0.7          | 0.5        | 0.2           | 0.4         | 0.3           | 1.0         | 0.2           | 7.1         | 0.8        | 1.4       | 1.1           | 1.4         | 0.5          | 2.0       | 0.4            | 0.3           | 0.2         | 0.2       | 0.1          | 0.4        | 0.2                       | 0.1                   |
| Sinigrin       | –            | –          | 0.1           | –           | –             | 3.5         | –             | –           | –          | –         | 0.1           | –           | 0.3          | –         | 0.1            | 0.3           | –           | –         | –            | –          | –                         | –                       |
| Gluconapoleiferin | –             | –          | –             | 0.1         | –             | –           | –             | 0.1         | –          | –         | 0.1           | –           | 1.0          | –         | –              | –             | –           | –         | –            | –          | –                         | –                       |
| Gluconaphanin  | –            | –          | –             | –           | –             | –           | –             | –           | –          | –         | –             | –           | –            | –         | –              | –             | –           | –         | –            | –          | –                         | –                       |
| Gluconapin     | 0.3          | 0.2        | –             | –           | 0.3           | 1.0         | 1.1           | 1.9         | 2.4        | –         | 0.2           | 3.8         | 0.1          | 1.0       | 0.1            | 1.6           | 0.3         | –         | –            | –          | 0.9                       | 1.0                   |
| Glucobrassicinap | –             | 0.2        | 0.1           | 0.1         | 1.3           | 2.5         | 5.0           | 8.0         | 2.6        | 7.5       | 4.8           | 18.2        | 0.1          | 0.4       | –              | –             | –           | –         | –            | –          | –                         | –                       |
| Glucoerucin    | –            | –          | –             | –           | –             | –           | –             | –           | –          | –         | –             | –           | –            | –         | 0.1            | –             | –           | –         | –            | –          | –                         | –                       |
| Total aliphatic GSL | 0.9        | 0.9        | 0.4           | 0.4         | 1.3           | 6.0         | 10.8          | 7.6         | 11.8       | 3.7       | 9.3           | 9.1         | 27.7         | 1.7       | 0.9            | 1.9           | 0.4         | 0.4       | 1.1          | 1.1        | –                         | –                       |
| 4-Hydroxyglucobrassicin | 0.2   | –          | 0.1           | 0.1         | 0.1           | 0.4         | 0.5           | 0.2         | 0.2        | 0.4       | 1.3           | –           | –            | –         | –              | –             | –           | –         | –            | –          | –                         | –                       |
| Glucobrassicin | –            | 0.1        | 0.1           | 0.1         | 0.2           | 0.3         | 0.2           | 0.7         | 0.1        | 0.1       | 0.3           | 0.2         | –            | 0.1       | –              | 3.6           | 4.9         | 0.7       | 0.5          | 0.5        | –                         | –                       |
| 4-Methoxyglucobrassicin | –       | 0.1       | –             | –           | 0.2           | 0.6         | 0.2           | –           | 0.1        | 0.1       | 0.1           | –           | –            | 0.1       | –              | 0.1           | 0.1         | –         | –            | –          | –                         | –                       |
| Neoglucobrassicin | –           | –          | –             | –           | 0.2           | 0.4         | 0.3           | 0.4         | 0.3        | 0.1       | 0.4           | –           | –            | –         | –              | –             | –           | –         | –            | –          | –                         | –                       |
| Total indole GSL | 0.2       | 0.2        | 0.2           | 0.1         | 0.3           | 0.2         | 0.9           | 0.7         | 1.0        | 1.9       | 0.8           | 0.6         | 2.0          | 0.3       | 0.2            | 0.1           | 0.0         | 3.7       | 5.0          | 0.8        | 0.6                       | –                       |
| Sinalbin       | –            | –          | –             | –           | –             | –           | 0.3           | 0.8         | –          | –         | 15.3          | 11.1        | 6.6          | 4.3       | 0.1            | –             | –           | –         | 2.0          | –          | –                         | –                       |
| Gluconasturtiin | 0.3         | –          | 0.2           | 0.3         | 0.4           | 0.6         | 2.5           | 2.5         | 0.4        | 0.3       | 1.1           | 2.0         | 1.0          | 0.6       | 0.2            | 1.9           | 3.8         | 0.3       | 0.4          | 0.4        | –                         | –                       |
| Total benzenic GSL | 0.3      | 0.0        | 0.2           | 0.0         | 0.3           | 0.4         | 0.6           | 2.5         | 2.5        | 0.7       | 1.1           | 1.1         | 2.0          | 16.3      | 11.7           | 6.6           | 4.5         | 2.0       | 3.8          | 0.3        | 0.4                       | –                       |
| Total GSL      | 1.4          | 1.1        | 0.6           | 0.7         | 0.5           | 1.9         | 6.6           | 12.3        | 10.8       | 15.3      | 6.3           | 11.2        | 10.8         | 18.3      | 12.8           | 7.0           | 6.4         | 6.1       | 9.2          | 2.2        | 2.1                       | –                       |
growth for specialist insects on brassicas (Hopkins et al., 2009). Some authors found significant correlations between larval growth parameters and the GSL content in the plant tissue whereas others did not. Ulmer & Dosdall (2006) suggested that a high content of the benzenic GSL sinalbin might be responsible for the extended larval development time and the reduced larval weight of cabbage seedpod weevils in S. alba. However, in recent studies, Tansey et al. (2010) found a positive correlation between the larval weights of cabbage seedpod weevil and the content of gluconasturtiin in pods. The authors also reported a negative correlation between larval weight and the content of neoglucobrassicin in the pods. Oviposition of *Celtotrychus napi* Gyllenhal into stems of oilseed rape was found to be positively correlated to contents of glucosinolates, gluconasturtiin, glucobrassicinapin, glucobrassicanapin, and neoglucobrassicin (Schafer-Koesterke et al., 2017). In experiments using *Arabidopsis thaliana* (L.) Heynh. mutants, the larval performance of the two specialist insects, *Pieris rapae* L., and *Pieris brassicae* (L.), was not affected by a strong reduction of aliphatic or indole GSL in the leaves (Mewis et al., 2008). No correlation between GSL content and larval performance was found in studies with cabbage stem weevil (Eickermann et al., 2011) or cabbage root fly (Birch et al., 1992). Bodnaryk (1997) concluded that resistance of white mustard against *P. cruciferae* is independent of the GSL content in the cotyledons. Our results suggest that GSL may play a role in host quality even for specialist insects such as CSFB. However, as the effect of a single GSL on various insects is very variable (Hopkins et al., 2009), further research is needed on this topic. Recent studies showed that adult CSFBs have developed different pathways to overcome the GSL myrosinase defence of their host plants (Beran et al., 2018). It can be assumed that this is also true for CSFB larvae and that different GSL profiles or contents in the brassicaceous plants tested may exert different effects and toxicities on CSFB larvae, thereby impacting larval development. Artificial diets or genetically modified host plants with altered GSL profiles may offer suitable tools for further experiments.

The effects of insect feeding on GSL composition in plants have been repeatedly reported in the literature (Koritsas et al., 1989, 1991; Birch et al., 1992; Bartlet et al., 1999; Textor & Gershenzon, 2009; Beran et al., 2018), but the results were not consistent. In our experiments, infestation by CSFB larvae had only a minor effect on indole GSL whereas aliphatic GSL increased in most of the genotypes tested. The aliphatic GSL glucobrassicin and glucobrassicanapin were particularly on a high level in the three cultivars of turnip rape which showed positive, but non-significant effects on larval performance. Gluconapin stimulated feeding of adult CSFB when added to agar (Bartlet et al., 1994). In former studies feeding adult CSFB on leaves of oilseed rape induced a systemic increase in indole GSL in young leaves after 7 days of feeding, whereas the content of other GSL classes did not change significantly (Bartlet et al., 1999). Similar results were reported for non-systemic responses of oilseed rape to infestation by CSFB larvae. In petioles infested by larvae the content of indole GSL was higher, whereas the content of aliphatic GSL was lower, compared to the non-infested plants (Koritsas et al., 1989, 1991). When petioles of oilseed rape were mechanically damaged the GSLs showed similar changes. In kale, indole as well as aliphatic GSL increased after mechanical damage whereas, in white mustard, only benzenic GSL increased (Koritsas et al., 1991). Feeding by crucifer flea beetles, *P. cruciferae* on the leaves of black mustard [*Brassica nigra* (L.) Koch] had no effect on the systemic induction of sinigrin and glucobrassicin (Traw & Dawson, 2002a,b).

Bartlet et al. (1999) suggested that the modified GSL profile in oilseed rape may have the potential to protect the plants against generalist insect pests and diseases, as feeding by adult CSFB was not affected. An increased GSL content may attract more specialist insects, because the degradation products of aliphatic and benzenic GSLs, and isothiocyanates, are used for finding hosts (Bartlet et al., 1992). Furthermore, stimulating effects of the aliphatic GSL allylglucosinolate on the feeding behaviour of *Phyllostreta nemorum* (L.) on transgenic *A. thaliana* were reported by Nielsen et al. (2001). For future studies Lemibkes Normal and Largo are promising cultivars because, in our experiments, they responded strongly to larval feeding by changing their GSL contents compared to the other brassicaceous species. Beside GSL, other plant secondary metabolites should also be taken into consideration as defence compounds. The content of phenolic substances, for example, is known to affect insect feeding behaviour and to vary widely between brassicaceous species (Onyilagha et al., 2003; Schoonhoven et al., 2005). Other studies showed that sugar and amino acids stimulate feeding of many insect species (Chapman, 2003), such as feeding of CSFB on agar substrate (Bartlet et al., 1994) or feeding pollen beetles on buds of oilseed rape (Hervé et al., 2014). Furthermore, field experiments are needed in order to evaluate the performance of the larvae of CSFB on different host plant species under natural conditions. In the field, slower larval development may promote a higher parasitisation rate, due to longer exposure of the larvae to their enemies (Singh & Singh, 2005). Furthermore, as well as plant secondary chemicals, the fibre and sugar contents in the petioles of the various host plants should also be considered, as larval performance might be affected by the...
digestibility of the plant material ingested (Waldbauer, 1964; Hopkins et al., 1999).

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