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About Previous Investigations Regarding the Role of Glucosinolates in Controlling Brassica Insect Pests in Slovenia

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Additional information is available at the end of the chapter

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Abstract

The chapter presents previous field and laboratory investigations of cabbage flea beetles (*Phyllotreta* spp.) and cabbage stink bugs (*Eurydema* spp.) interactions with different Brassica crops in Slovenia. The special emphasis is given to an influence of different glucosinolates on injuries caused by mentioned economically important two genera of insect pests. In the study, we found out that the content of glucosinolates differs between different Brassica species, as well as between individual plant organs of the same species. The content of glucosinolates is conditioned also by environmental influences. Among the analysed glucosinolates, glucobrassicin was presented in all plant species. In almost all plant species, it inhibited the feeding of cabbage flea beetles, except in oil rape, where it had stimulative effects. We have established that the influence of individual glucosinolate on *Phyllotreta* spp. and *Eurydema* spp. is not identical as it differs between individual plant species. Because of the variability of glucosinolates as well as different preferences of the studied groups of harmful pests in regard to the plant species, one of the options for diminishing the damage caused by cabbage stink bugs and cabbage flea beetles is the use of mixed Brassica crops for trapping the pests in the growing season. In the future, glucosinolates should be employed to a greater extent in environmentally acceptable ways of food production, one of which is also the use of trap crops in order to reduce harmful effects of cabbage stink bugs.

Keywords: Cabbage, *Eurydema* spp, *Phyllotreta* spp, glucosinolates, Brassica species

1. Introduction

Alternative methods of suppressing harmful organisms are gaining ground, and greater attention is paid to natural resistance of plants, which is conditioned by morphological and

chemical factors. By exploiting natural resistance of plants, we can reduce the use of insecticides and thus avoid the pertaining resistance of harmful insects. Different factors are involved in defence of Brassicas against harmful organisms. It has been established that more solid (more compact heads) cabbage [1] and higher content of epicuticular wax [2] diminish the extent of damage done to cabbage by onion thrips (*Thrips tabaci* Lindeman). Thicker layer of epicuticular wax can negatively influence also the feeding of cabbage flea beetles (*Phyllotreta* spp.) and cabbage stink bugs (*Eurydema* spp.) [2].

In our research, whose results are briefly presented on the following pages, we focused on the study of glucosinolate content in different species of Brassicas and their effects on the target group of harmful organisms, cabbage flea beetles and cabbage stinkbugs.

Before the research, we made the following hypotheses:

- Different concentrations and ratios of glucosinolates in trap crops influence their different susceptibility to cabbage stink bugs and cabbage flea beetles. Thus, we think they are of different suitability for trapping the studied harmful pests.
- We think that glucosinolate content differs in individual organs of the same plant species, which consequently causes different extent of damage due to feeding of the studied groups of harmful pests.
- We think the content of secondary metabolites (glucosinolates) in the studied Brassicas is influenced also by environmental factors, primarily the air temperature.
- We suppose there is a connection between the glucosinolate type and susceptibility/resistance of the plant species to attacks by the studied harmful pests.
- The extent of damage caused by the studied harmful pests depends also on the principles of good agricultural practice and on the application of agrotechnical measures. For this purpose, we will point out the connection between the average index of damage by harmful pests during the growth period and morphological properties of trap crops and cabbage as the main plant species.
- By selecting plant species which differ also in their length of growth period, we want to influence also the bionomy of the studied groups of harmful pests, *Phyllotreta* spp. and *Eurydema* spp. We expect there are differences in susceptibility to damage also between the two cultivars of cabbage, which differ in the length of their growth periods.

2. Field and laboratory investigations of interactions of cabbage flea beetles (*Phyllotreta* spp.) and cabbage stink bugs (*Eurydema* spp.) with cabbage and selected trap Brassica crops

2.1. Association between glucosinolate concentration and injuries caused by cabbage stink bugs, *Eurydema* spp. (Heteroptera: Pentatomidae), on different Brassicas

In 2010, our group was involved in determining [3] the content of glucosinolates in different *Brassica* species in order to determine their impact on feeding of cabbage stink bugs (*Euryde-*

ma spp.) and the consequent extent of damage caused by feeding. We confirmed that the level of glucosinolates depends on plant species, plant part and the time of sampling. In these samples, aliphatic glucosinolates prevailed. Glucobrassicin, an important indolic glucosinolate, was detected in all tested Brassica species. Its content was the highest in oil radish samples during the first assessment (30 DAS), $8.84 \pm 0.65 \mu\text{mol g}^{-1} \text{ ds}$, while the oilseed rape samples displayed lowest concentration during the last assessment (134 DAS), $4.30 \pm 0.80 \mu\text{mol g}^{-1} \text{ ds}$. Based on these results, we cannot confirm the stimulative activity or negative influence of a specific glucosinolate on feeding of *Eurydema* spp. Based on the results of our research, we can conclude that oil rape was the most adequate trap crop used to allure cabbage stink bugs. In future, glucosinolates should be employed to a greater extent in environmentally acceptable ways of food production, one of which is also the use of trap crops in order to reduce harmful effects of cabbage stinkbugs.

2.2. Glucosinolates as arsenal for defending Brassicas against cabbage flea beetles attack

Feeding of cabbage flea beetles (Figure 1) on various *Brassica* species can reduce the plant's productivity. While progressing towards the goal of reducing the use of synthetic pesticides and promotion of environmental protection, we wish to exploit plants' natural resilience [4]. The results of our study carried out in 2010 show that glucosinolate contents vary with plant species, plant organs and period of growth. Among the indole glucosinolates, all *Brassica* species displayed the presence of glucobrassicin, whose influence on cabbage flea beetles varied according to the plant species.



Figure 1. Injuries caused by cabbage flea beetles (*Phyllotreta* spp.) on oil radish (photo: T. Bohinc)

We established that progoitrin ($r = 0.51$), sinalbin ($r = 0.61$) and gluconapin ($r = 0.67$) stimulate the feeding of flea beetles on oil rape, while the gluconasturtiin content in oil rape negatively ($r = -0.99$) influenced the feeding of flea beetles. The oil rape displayed the significantly highest damage done by the said harmful pest, but no significant influence of gluconasturtin and glucoiberin influence of flea beetles was detected in this species. Oil radish thus proved to be the most suitable species as a trap crop for flea beetles. We maintain that the protection of the Brassica family against flea beetles can efficiently depend on glucosinolates content in combination with other agrotechnical measures.

2.3. Environmental factors affecting the glucosinolate content in Brassicaceae

This study describes the effects of environmental factors, the average and highest daily temperatures, the average relative air humidity and the duration of the daily solar radiation on the glucosinolate content in Brassicaceae [4]. The results of our study indicate that the content of indole glucosinolate, glucobrassicin, is influenced by the average daily and highest air temperature. Indole glucosinolates were much more susceptible to environmental factors than aliphatic or aromatic glucosinolates. Although the impact of the environment on the groups of aliphatic and aromatic glucosinolates was variable, there was a significant impact of the environment on specific aliphatic or aromatic glucosinolates. We conclude that climatic conditions cannot be neglected in the future planning of cropping systems, as our results showed significant effect of environmental factors on the glucosinolate content in Brassicaceae.

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2.4. Trap crops for reducing damage caused by stink bugs (*Eurydema* spp.) and flea beetles (*Phyllotreta* spp.) on white cabbage: Fact of fantasy?

During the years 2009 and 2010, a field experiment was carried out to determine the effect of three trap crops (oil radish, oil rape and white mustard) as a protection method against cabbage stink bug (*Eurydema* spp.) and flea beetle (*Phyllotreta* spp.) attacks on two hybrids of white cabbage. The experiment was designed as a randomised complete block with four treatments, each replicated four times. The damage caused by cabbage stinkbugs and flea beetles was estimated in 10-day intervals, considering main cash crop and trap crops. Based on statistical analysis, we can conclude that oil rape was the most effective trap crop against cabbage stink bugs. Flea beetles have shown specific preference to oil radish as a trap crop in 2010, while

they did not show specific preference to any of the trap crop tested in 2009. The damage caused by cabbage stink bugs on cabbage started to increase by the beginning of July, while that caused by flea beetles started to increase at the end of May.



Figure 2. The field experiment at the laboratory field of the Biotechnical Faculty in Ljubljana (photo: T. Bohinc).



Figure 3. Mating cabbage stink bugs (*Eurydema oleracea*) on a blossom of white mustard (photo: T. Bohinc).

2.5. Sowing mixture of Brassica trap crops is recommended to reduce *Phyllotreta* beetles injury to cabbage

We studied the extent of damage caused by cabbage flea beetles on four different *Brassica* species in a 2-year field experiment (2009–2010) at two locations in Slovenia. The entire experiment was based on testing oilseed rape, white mustard and oil radish as potential trap crops to protect cabbage from cabbage flea beetles [5,6]. A significant influence of the *Brassica* species on the feeding by the flea beetles was confirmed at both the locations. The damage index on oil radish was the highest throughout most of the growth period, whereas oilseed rape and white mustard were preferred only during a certain growth period. The initial damage caused by the cabbage flea beetles occurred in the first half of May, whereas the heaviest damage occurred at the beginning of July. This research shows that the onset of cabbage flea beetle feeding can be controlled in a medium–late cabbage cultivar using trap cropping. However, since none of the tested trap crops strongly attracted the flea beetles throughout the entire growing period of the crop, we recommend sowing mixtures of crops for cabbage production such that each of three *Brassica* species would attract phytophagous insect during a particular part of the cabbage growing season.

3. Results and discussion

Different authors [7,8] reported that glucosinolate content differs in different plant species, this was confirmed also by our research. In the samples of the studied species of Brassicas we established the presence of only one glucosinolate, namely glucobrassicin, it was noticed in all plant species. We found out that the content of glucobrassicin in the samples of oilseed radish negatively influence the studied group of harmful organisms, both cabbage flea beetles and cabbage stink bugs. Despite the fact that the connection between the content of glucobrassicin and the extent of damage by cabbage flea beetles ($r = -0.30$) and cabbage stink bugs ($r = -0.32$) was negative, we cannot talk about identical influence throughout the growth period. This is consequently conditioned by the fact that glucosinolate content in Brassicas varied both during the growth period and between individual plant organs. It is also interesting that we found more glucobrassicin in the blossoms of oilseed radish than in the leaves.

Research has established [9] higher contents of five different glucosinolates in blossoms of black mustard (*Brassica nigra*) than in leaves of the said plant species. In our research this statement holds true for glucobrassicin in glucoraphanin in the samples of oilseed radish and for sinalbin and epiprogoitrin in the samples of white mustard. Based on research done by [10], glucosinolate content can vary also within individual genotypes of the same plant species, this was confirmed also in our research – between the selected hybrids of cabbage we discovered significant differences in the contents of progoitrin and glucobrassicin. In the samples of the hybrid 'Hinova' we discovered more progoitrin, while a higher share of glucobrassicin was present in the hybrid 'Tucana'. Of the nine glucosinolates which were analysed in the main crops, we confirmed the presence of four (sinalbin, gluconasturtiin, glucoraphanin and progoitrin) in both hybrids of cabbage, yet only in traces (below detection threshold).

High concentrations of aliphatic glucosinolates in Brassicas can be an important factor of resistance for the said plants against harmful organisms [11]. We also found out that progoitrin in oilseed radish and white mustard was below detection threshold, which enables wider spectrum of usefulness of these plant species, as the said aliphatic glucosinolate can cause negative effects for feeding of animals [12].

Despite previously proven nematicide effects of sinigrin [13], in our experiment this glucosinolate in the mid-late hybrid of cabbage stimulated the feeding of cabbage stink bugs, thus causing greater extent of damage. Because the content of progoitrin in the samples of oilseed rape was highest on the date of assessment (31st August), the authors maintain that this substance can potentially negatively influence cattle if oilseed rape is used as fodder [12]. Marked preference of different harmful pests for oilseed rape (in our case this holds true for both years of the experiment) is often caused by the fact that farmers do not choose to grow the said plant species [14].

On the basis of the results of our research, we can conclude that the content of progoitrin in the hybrids of cabbage could in future represent an important factor when selecting hybrids for cabbage production in our conditions. A research carried out in the Netherlands [15] found out that the content of sinigrin and progoitrin influences the taste of Brussels sprouts. Consequently, great efforts were made to reduce the content of the said glucosinolates or improve plants [15]. Despite the reports about negative influence of progoitrin in fodder for cattle, we cannot talk about negative influence of the said glucosinolate and other glucosinolates on human diet [16]. In the past, there were suspicions also about negative effects of glucosinolates on human diet [17], but they were later rejected [16, 18].

Potential negative influence of glucobrassicin on feeding of cabbage flea beetles was detected in all plant species except in oilseed rape. On one hand this glucosinolate has negative influence on the species from the genus *Phyllotreta*, on the other hand it has positive (anticarcinogenic) influence on human health [16]. These are thus »good« properties of this secondary metabolite. Despite this, glucobrassicin proved to be very susceptible to environmental factors, which will be dealt with later in our discussion.

We confirmed that glucoraphanin and sinalbin were significantly most present among aliphatic glucosinolates in the samples of oilseed radish, while among indole glucosinolates glucobrassicin was present. The content of sinalbin was among the studied plant species of Brassicas highest in the samples of white mustard ($30.12 \pm 5.52 \mu\text{mol/g}$ mass of dry seed) and oilseed rape ($11.16 \pm 6.50 \mu\text{mol/g}$ mass of dry seed). On the basis of the above finding that glucosinolate content differs between individual species of Brassicas, we believe that there are also differences between species of Brassicas in regard to their suitability for feeding of cabbage flea beetles and cabbage stink bugs. While in the hybrids 'Tucana' and 'Hinova' and in white mustard and oilseed radish, we noticed negative influence of glucobrassicin on the extent of damage by cabbage stink bugs, in the samples of oilseed rape we noticed positive influence of glucobrassicin on the extent of damage. Gluconapin is also one of the glucosinolates which in our research stimulated feeding of the species from the *Eurydema* spp.

The content of secondary metabolites [19] in plants is influenced also by environmental factors, which was noted by [20], this fact was confirmed also in our research. The knowledge about the influence of individual parameters of the environment on glucosinolate content in plants is thus gradually building up. Authors [21] report about positive influence of extreme temperatures on the content of the said secondary metabolites. Influence of temperatures is supposed to be more pronounced in indole glucosinolates [22], this was confirmed also in our research in which we found out that the content of glucobrassicin was conditioned primarily by the average daily and the highest daily temperature of air.

Yet very little is known about the influence of individual environmental factors on the content of aliphatic and aromatic glucosinolates. Our research has established that the influence of environment on individual glucosinolates from the said two groups differs greatly. Thus we cannot speak about some uniform effects of environment on types of glucosinolates. Experts suppose that the reason for different responses of glucosinolates to the environment's activity can be the fact that the genesis of specific glucosinolates involves specific enzymes, which differ from the enzymes which are required to produce other glucosinolates [23].

As we have already emphasised, the importance of alternative methods in plant protection is increasing, and in connection with this we are glad to report that also the trap crop method studied in our dissertation was successful at both locations of the field experiment. The trap crops were much more susceptible to damage by cabbage stink bugs (*Eurydema* spp.) and cabbage flea beetles (*Phyllotreta* spp.) than the cabbage. In Gorenjska, oilseed rape was most susceptible to feeding by cabbage stink bugs, preference of cabbage flea beetles was at the same location detected in oilseed radish. In Ljubljana in 2009, we established the significantly highest extent of damage by the species from the genus *Eurydema* on oilseed rape, while in 2010, we noticed no difference between the trap crops. We have nevertheless established that susceptibility of different Brassicas to attacks by the studied harmful insects varies during the growth period.

In the field in Gorenjska, we noticed in 2009 the highest extent of damage due to cabbage stink bugs on oilseed rape (the average index of damage 3.38 ± 0.05), in the second year of the experiment, the most susceptible to damage by cabbage stink bugs was oilseed rape (3.58 ± 0.02). In white mustard in the first (2.72 ± 0.04) and the second (2.56 ± 0.05) year of the experiment, we recorded the significantly lowest index of damage by cabbage stink bugs. In the second year of the experiment, we recorded the highest susceptibility to damage by cabbage flea beetles in oilseed radish (3.5 ± 2.82), while the extent of damage on white mustard was significantly lowest (2.82 ± 0.02).

Authors [15] report that glucosinolate content in Brassicas is conditioned also by feeding of harmful organisms. With their feeding – by damaging the cellular structure – they regulate glucosinolate content. In our research, we established that the connection between glucosinolate content and the content of damage by the species from the genera *Phyllotreta* or *Eurydema* differs between different glucosinolates, as well as that individual glucosinolates differently influence the extent of damage.

The plant species which were used as trap crops in our research are in Slovenia usually used as catch crops [24]. Oilseed rape is among the studied trap crops considered a plant species

which is very susceptible to attacks by harmful organisms and is consequently less popular in ecological production [14]. If we compare indexes of damage by cabbage stink bugs on different species of Brassicas, we see that these were the highest on the said plant species. From the point of view of possible application of Brassicas as trap crop systems in the production of cabbage, white mustard represents the least suitable species, as it has been confirmed as the least susceptible to feeding of cabbage flea beetles. White mustard is in the research carried out by [6] and [25] mentioned as a plant species with very high natural resistance to attacks by flea beetles. It has been established that epidermis, thickly covered with trichomes, is the main reason for the resistance of plants, which is confirmed also by the research carried out by [26].

By seeding trap crops among the main plant species, the latter (cabbage) was protected against early attacks by cabbage flea beetles and cabbage stink bugs. It is known that cabbage flea beetles which appear in the beginning of May are very harmful for young plants of Brassicas, which have at that time small surface of leaves [27]. The age of plants is among more important factors which influence the extent of damage by the species from the genus *Phyllotreta*. Authors [28] in their research report successful reduction of the extent of damage on cabbage done by the species from the genus *Phyllotreta* by different terms of seeding.

We have found out that there is a negative correlation between the plants of trap crops and the extent of damage by cabbage flea beetles in the beginning of the developmental stage in which leaves are developed (BBCH 12-14). On the basis of the results of our research, we can conclude that adult specimens of cabbage stink bugs in spring appear in the second half of May or in the beginning of June. In the said period we noticed the first damage interval [6]. Damage was first detected on the trap crops, only later on the main crops. This was the case at both locations in the experiment.

The hybrid cabbage 'Hinova' in all comparisons proved to be much more susceptible to damage by cabbage stink bugs and cabbage flea beetles [29] – in comparison with the hybrid 'Tucana'. We have nevertheless found out that susceptibility of Brassicas in individual parts of the growth period varies between genotypes of cabbage, in some parts of the growth period we thus noticed pronounced damage also on the hybrid 'Tucana'. The damage by cabbage flea beetles on the cultivar 'Tucana' were thus more extensive in the beginning of the growth period, in the phase when plants were developing leaves and opened from 4 to 7 proper leaves. Greater extent of damage was later recorded on the cultivar 'Hinova'. The mid-late hybrid cabbage was not so susceptible to damage by cabbage stink bugs. This finding was established already in the research by [30]. The connection can be found in the interaction between the length of the growth period of the hybrid and bionomy of the studied harmful pest. At the time when the species *Eurydema ventrale* and *Eurydema oleracea* only just began to appear massively, the crops of the early hybrid were already collected.

We have found out that usefulness of the selected alternative methods in plant protection is conditioned by the selection of an appropriate main crop and appropriate hybrid. The average crop of cabbage was influenced by the selection of a cultivar, while we did not notice any pronounced differences in the average crop between individual treatments (species of trap crops). We also compared the damage by the species from the genera *Phyllotreta* and *Eurydema* on the main crop according to the distance from the trap crop. We found out that cabbage

stink bugs prefer to appear on plants of cabbage which are farthest from the trap crop, while the influence of a plant's distance on damage by cabbage flea beetles was not established. 'Hinova' in our conditions of production proved to be the hybrid which yielded a bigger average crop, the selection of the early hybrid 'Tucana' proved as unsuitable. According to the results of our research we can say this is congruent with the findings of the authors [31–33], who report that alternative methods (intermediate crops, green covers) did not reach the desired goals.

By more intensive care for the main crop (watering during the growth period, dressing with fertilisers...) we could provide bigger crops. It is well known that the trap crop method can be carried out in several ways. One of these is to collect the trap crops in the middle of the growth period where we carried out the experiment [34]. At the same time we would risk that the studied group of harmful pests massively moved to the main crop [34]. We could treat the trap crop with insecticides and thus provided the so-called dead-end trap crop. Yet by using insecticides we would bring more damage than benefits [35]. The species which were used as trap crops were for a part of their growth period also blossoming and thus attracting useful organisms. The reasons for different susceptibility in the studied species of Brassicas can be found in natural resistance of plants. Glucosinolates, which are considered by some as an important factor of plants' resistance against harmful organisms, and by others as negligible in this regard, can in the protection of plants act stimulatory or deterring. While gluconasturtiin in oilseed rape acts as a deterrent ($r = -0.99$) for feeding by cabbage flea beetles, we cannot attribute the said glucosinolate the same property in case of white mustard and oilseed radish. According to the research, we can conclude that the effects of these secondary metabolites on harmful insects are very complex. We thus cannot talk about some universal influence of the three groups of glucosinolates on harmful organisms, and the influence of each glucosinolate should be analysed separately.

Gluconasturtiin in oilseed rape acts negatively on feeding by cabbage flea beetles ($r = -0.99$) and cabbage stink bugs ($r = -0.98$). Feeding by cabbage flea beetles ($r = -0.80$) and cabbage stink bugs ($r = -0.99$) on the said plant species is strongly influenced by epiprogoitrin content. Glucoiberin content in the samples of oilseed rape negatively influenced the feeding by cabbage flea beetles ($r = -1$) and cabbage stink bugs ($r = -1$), progoitrin in the samples of oilseed rape stimulated the feeding by cabbage stink bugs ($r = 0.51$) and cabbage flea beetles ($r = 0.51$), while the activity in the samples of 'Tucana' negatively influenced the feeding by cabbage stink bugs ($r = -1.0$). Gluconapiin is the only glucosinolate which in the plants oilseed rape acts stimulatory on the feeding by cabbage stink bugs ($r = 0.64$) and cabbage flea beetles ($r = 0.67$). We detected no pronounced influence of the studied glucosinolates on feeding by the species from the genus *Phyllotreta* in the samples of oilseed radish. We can conclude that the barely perceptible presence of gluconasturtiin, glucoiberin and gluconapiin most probably contributed to the higher index of damage by cabbage flea beetles on the studied species of Brassicas.

Because of the thus far collected findings that glucosinolate content changes during the growth period and depends on the plant species, we wanted to compare the extent of damage by cabbage flea beetles also in regard to the location of the experiments.

In Slovenia, cabbage flea beetles are most massively present in July [36], this was confirmed also in our research. The feeding of cabbage flea beetles is influenced also by the average daily temperature [37], which was also found out in our research; we noticed several such cases during the growth period in both years of the experiment. In 2010 we thus in the last ten days of May on the laboratory field of the Biotechnical Faculty in Ljubljana noticed higher average daily temperatures than on the location of the experiment in Gorenjska.

Indexes of damage due to both groups of harmful pests were in the second year of the experiment markedly higher, which confirms the fact that using crop rotation is also one of the principles of successful agricultural practice [38, 39, 40, 41, 42]. We thus in both years of the experiment detected a higher extent of damage at the laboratory field of the Biotechnical Faculty and in the field in Gorenjska. The importance of crop rotation was confirmed especially in Gorenjska, where some years before our experiment in the vicinity there were no larger field with Brassicas, so the attacks of both groups of harmful pests in the first year of the experiment were relatively weak. At the laboratory field of the Biotechnical Faculty, Brassicas have been grown every year for quite some time, consequently, the attacks of cabbage flea beetles and cabbage stink bugs were strong already in the first year of the experiment. The finding that a considerably lesser extent of damage by harmful pests is influenced also by crop rotation [42, 43] is confirmed also in our research.

The average daily temperature 17.4 °C at the laboratory field of the Biotechnical Faculty in Ljubljana thus stimulatory influenced the group of the studied harmful pests, while the average daily temperature in the same period in Gorenjska was 16.1 °C.

We have found out that white mustard was the first to reach the phase of maturing (BBCH 80–89) [25], at the same time the susceptibility of this plant species to attacks by the studied harmful pests was reduced. The low susceptibility of white mustard to damage by the species from the genus *Phyllotreta* in the beginning of the growth period was still very pronounced in 2009 in Gorenjska. We can thus sum up that the developmental phase of plants is one of the more important factors of feeding by cabbage flea beetles and cabbage stink bugs. In Gorenjska, the trap crops for cabbage flea beetles were most susceptible at the time of blossoming (BBCH 60–67), at that time the cabbage was entering the phase of developing vegetative parts of plants suitable for crops (forming heads). Feeding by cabbage flea beetles at the plants' blossoms was recorded already in other studies [44].

4. Conclusions

In the years 2009–2010, we were in two field experiments establishing the efficiency of trap crop methods for reducing the extent of damage by cabbage flea beetles (*Phyllotreta* spp.) and cabbage stink bugs (*Eurydema* spp.) on the main plant species, cabbage. Because the extent of damage was significantly higher on the trap crops and because the damage was first noticed on the trap crops, we can say that the chosen alternative method of plant protection proved as efficient.

The first appearance of cabbage flea beetles was in our research detected in the beginning of May, while cabbage stink bugs began appearing in the second half of May. The said findings are congruent with the results of bionomy of the studied groups of harmful pests in the central part of Europe. Feeding of cabbage flea beetles and cabbage stink bugs was noticed on all selected plant species. On the basis of the collected data, we have found out that cabbage stink bugs display particular preference to feeding on oilseed rape, while cabbage flea beetles were feeding mostly on oilseed radish, which proved the most attractive host for them.

The trap crop method that we studied in our research will be feasible especially in the systems of mid-late cabbage production. The average mass of cabbage was influenced primarily by the selection of a hybrid. Mid-late hybrid 'Hinova' in our research proved to be more productive and consequently more suitable for production in our growth conditions.

On the basis of our research results, which were obtained at two different locations, we can conclude that air temperature has an important effect on the extent of damage by cabbage flea beetles on Brassicas. Rising average daily temperature of air caused higher extent of damage by cabbage flea beetles on the studied species of Brassicas.

The different preferences of the studied groups of harmful pests are conditioned also by natural resistance of plants. One of the factors of natural resistance in Brassicas is also glucosinolate content. Glucosinolates in our research proved to be an important, yet variable factor of natural resistance of Brassicas to attacks by cabbage flea beetles and cabbage stink bugs. We found out that variability of glucosinolates was conditioned by plant species, and the content of these substances differs considerably also between different organs of the same plant species. Our research also revealed variability in the content of glucosinolates between individual genotypes of the same plant species, i.e., between individual genotypes of cabbage. In the samples of oilseed radish, we detected the largest amount of glucoraphanin ($8.66 \pm 1.81 \mu\text{mol/g}$ mass of dry seed); sinalbin was present in the significantly largest amount in the samples of oilseed rape and white mustard. Although the analysis confirmed sinalbin as the most frequent glucosinolate in the samples of white mustard, the further analysis of the data revealed weak correlation ($r = 0.36$) between its content versus Brassicas and the content of damage by cabbage stink bugs on them.

Sampling of different plant parts showed that the content of certain glucosinolates, for example glucobrassicin and glucoraphanin in blossoms of oilseed radish or sinalbin and epiprogoitrin in blossoms of white mustard, is much higher than in the leaves of the studied plant species. We found out that cabbage flea beetles were at the time of blossoming very intensively feeding on plants of white mustard and oilseed radish.

We have found out that glucosinolate content in plants is influenced primarily by temperature extremes. The results of past studies concerning activities of these secondary metabolites support the notion that they act in a manner that is specific for an individual group of these secondary metabolites. However, we found out that there are differences in their activities also within individual groups of glucosinolates. Glucobrassicin, the only detectable glucosinolate in all studied plant species, belongs to indole glucosinolates. We have found out that this glucosinolate in oilseed radish affects negatively the extent of damage by the species from the

genera *Phyllotreta* and *Eurydema*. The said substance belongs to the group of those which were most significantly influenced by environmental parameters, primarily the average daily and highest daily air temperature. Gluconasturtiin and epiprogoitrin in oilseed rape had on both studied groups of harmful pests negative effects, sinalbin had negative effects on the feeding of cabbage stink bugs in the samples of oilseed rape, while it stimulated cabbage flea beetles.

The obtained data at the two different locations showed that the extent of damage by cabbage flea beetles can be successfully controlled by using the mixed crops of Brassicas, which were used in our experiment. The said combination of plant species would be in view of our findings suitable for both locations at which we were carrying out the experiment as well as for other Slovenian regions in which cabbage is produced and where producers encounter the harmful pests studied in our research. By seeding plants trap crops before the main crops in both years of the experiment, we considerably influenced the fact that damage by the species from the genera *Phyllotreta* and *Eurydema* first appeared on the trap crops.

The selected trap crop method successfully controlled the appearance of the species from the *Phyllotreta* spp. and *Eurydema* spp. on cabbage. Since little or no synthetic preparations are available for suppressing cabbage flea beetles or cabbage stink bugs, alternative methods is a potential option. Since agricultural land intended for production of Brassicas has been in recent years expanding, new methods for protecting crops represent new possibilities for environmentally acceptable production of cabbage. The said method is useful primarily in ecological and integral production. Commercial consumption teaches us that we increasingly trust the locally grown food, if possible without insecticides. It is true that our experiment did not produce the extent of crops which would be of commercial interest, yet we produced cabbage without using insecticides, and this will enable us to further arouse the interest of consumers, who want healthy food. In order to achieve reduction in the population of the species from the genera *Phyllotreta* and *Eurydema* on cabbage, the use of mixed crops of Brassicas seem quite realistic. As our research shows, the method is more efficient in systems of production where mid-late genotypes of cabbage are grown. As we found out, due to the spectrum of different factors (among others also glucosinolate content [45,46]), susceptibility of plants to damage by harmful organisms during the growth period varies. By using mixed crops as a means to reduce damage on the main crops, the said method could be used in different regions of Slovenia and thus override the influence of environmental factors on trap crops as well as the bionomy of the said harmful species itself [47]. Other species of Brassicas can be used as trap crops, but that is the subject of another research.

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