Scientia Horticulturae 225 (2017) 445–453

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Effect of selenium treated broccoli on herbivory and oviposition preferences of *Delia radicum* and *Phyllotreta* spp.

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ARTICLE INFO

Keywords:
Selenium
Broccoli
Photochemical efficiency
Pest damage
Phyllotreta spp
Delia radicum

ABSTRACT

The effect of selenium on physiological and biochemical characteristics of broccoli (*Brassica oleracea* var *italica*) transplants under controlled conditions and on the plants and some of the brassica pests development under field conditions were studied. For studying the physiological and biochemical response, the transplants were cultivated under controlled conditions and fertilized with various concentrations of selenite or selenate: 2, 5, 10, 20 and 30 mg Se(IV) L^{-1} or 50 mg Se(VI) L^{-1}. For the field trial only the treatment with 50 mg Se(VI) L^{-1} was compared to the untreated control. The addition of high selenium concentrations lowered photochemical efficiency of transplants, while 11 days after the exposure, the photochemical efficiency was not affected. Selenium had a minor impact on the amount of chlorophylls, while it increased the amount of anthocyanins. The content of selenium in plants varied between 0.38 and 5.76 μg g^{-1} (DM) for exposure to Se(IV) and between 2.09 and 45.73 μg g^{-1} (DM) for exposure to Se(VI).

Under field conditions the treatment of broccoli transplants with Se(VI) increased the attractiveness of plants for *Delia radicum* female adults and *Phyllotreta* spp. in the first weeks after the selenium treatment. With high pest pressures increased oviposition of *D. radicum* and increased leaf damage by *Phyllotreta* spp. occurred which retarded the growth of plants. However, under low pest pressure, the selenium treated plants exhibited better initial growth. Despite increased oviposition of *D. radicum* in selenium treated plants the number of pupae recovered at harvest was significantly less than in control plants.

1. Introduction

Selenium (Se) is an essential element for many organisms at low concentrations as an essential component of certain seleno-enzymes and tRNAs (Stadtman, 1990), but it is toxic at elevated concentrations. The chemical similarity of Se to sulphur (S) leads to the nonspecific replacement of S by Se in proteins and other S compounds, causing toxicity (Stadtman, 1990).

It occurs naturally in most soils, rocks and waters. The availability of Se for plants depends on soil properties, including pH, salinity and the content of $CaCO_3$ (Kabata Pendias, 2001).

Although Se has not been proven to be essential for higher plants, plants do take up and accumulate Se. It can increase the tolerance of plants to UV-induced oxidative stress, delay senescence and promote the growth of ageing seedlings (Xue et al., 2001). Se accumulators contain up to 1% Se dry weight (Sors et al., 2005), but do not show negative effects of this element. Several hypotheses have been proposed to explain why some plants evolved over time to hyperaccumulate such extraordinarily high concentrations of metals or metalloids. Hypothesized advantages conveyed by elemental accumulation are elemental allelopathy, drought resistance, elemental tolerance and protection from herbivory or infection (Boyd and Martens, 1992). It was shown that broccoli can accumulate high concentrations of Se (Pedrero et al., 2007; Sugihara et al., 2004).

Previous studies demonstrated that insects do accumulate Se, but the effects of this element on insect growth and survival is quite limited (Trumble et al., 1998). Laboratory studies have shown that selenate incorporated into the diet affects feeding site preferences and host plant selection and acts as antifeedant for larvae of the generalist herbivory beet armyworm *Spodoptera exigua* (Hübner) (Vickerman and Trumble, 1999). *Atriplex* plant lines with high Se accumulation exhibited significantly reduced *S. exigua* growth, development, and survival (Vickerman et al., 2002). Se accumulation in tissues protects plants against caterpillars of cabbage looper *Trichoplusia ni* (Hübner)

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http://dx.doi.org/10.1016/j.scienta.2017.07.032
Received 5 January 2017; Received in revised form 14 July 2017; Accepted 20 July 2017

(Bañuelos et al., 2002), larvae of the cabbage white butterfly *Pieris rapae* (Linnaeus) (Hanson et al., 2003), green peach aphid *Myzus persicae* (Sulzer) (Hanson et al., 2004) and different orthopteran (Freeman et al., 2007).

In the temperate zone of the northern hemisphere more than 20 economically important phytophagous species feed on cole vegetables and related weeds. In Slovenian ecological conditions the damage is most frequently caused by different flea beetles (*Phyllotreta* spp.), cabbage root fly *Delia radicum* (Linnaeus) and cabbage moth *Mamestra brassicae* (Linnaeus). On the increase are the diamondback moth *Plutella xylostella* (Linnaeus), swede midge *Contarinia nasturtii* (Kieffer) and the cabbage whitefly *Aleyrodes proletella* (Linnaeus) (Ugrinović et al., 2013).

Phyllotreta spp. (Coleoptera: Chrysomelidae) are one of the main pests on brassica leaves and their control is economically justified (Trdan et al., 2005). Adults do most of the damage by feeding on the undersides of leaves (Natwick et al., 2010). Large populations can kill or stunt seedlings (Natwick et al., 2010) and young plants (Palaniswamy and Lamb, 1992). Older plants rarely suffer economic damage although their older, lower leaves may be damaged (Natwick et al., 2010).

D. radicum (Diptera: Anthomyiidae) is a serious pest of *Brassicaceae* crops in North America and Europe (Coaker and Finch, 1971; Whistlecraft et al., 1985; Biron et al., 2000) and a major root feeding pest (Finch and Collier, 2000). Larvae damage and destroy root systems of all cole crops. Young plants until about a month after transplanting are most vulnerable; healthy plants attacked after they are well established can usually tolerate moderate infestations (Natwick et al., 2010). Insecticide options for controlling *D. radicum* in brassica crops are now limited and alternatives are needed (Blackshaw et al., 2012; Ferry et al., 2009).

Although much is known about the effects of Se on mature plants and its protective effect against some leaf herbivory insects, minimal information is available on the Se role in protecting seedlings against root herbivory insects and its effects on physiological and biochemical processes in seedlings. Therefore the objective of this study was to investigate the effect of Se on physiological and biochemical parameters of broccoli transplants and to test its potential in protecting the young broccoli plants against its pests in the field.

2. Materials and methods

2.1. Plant material and cultivation

Seeds of *Brassica oleracea* var. *italica* variety Monop were obtained from the seed company Syngenta. The transplants were raised in a greenhouse according to standard agrotechniques: seeding into polypropylene seed trays with 36 mL pots volume filled with peat-based potting substrate (Klasman Podgrond P), regular watering and supplement with nutrients (11N/8P/6K and micronutrents), natural day-light was supplemented with artificial light for a 14 h light period.

For the assessment of biological and biochemical parameters the seeds were sown on March 14 and May 20, 2014, since the experiment was repeated twice. Plants were arranged into 6 different groups, each group containing 24 plants. When plants had 4 true leaves, Na selenite or selenate in various concentrations was added to the substrate. Five groups were treated with 0.5 mL of Se solution per plant in concentrations 2, 5, 10, 20 and 30 mg Se(IV) L^{-1} or 50 mg Se(VI) L^{-1}. Group 6 was used as a control. Physiological and biochemical parameters were measured five times during the experiment.

Field experiments were conducted in 2013 and 2014. Sowing was performed in March 2013 and 2014. In the 4–5 leaf stage half of the transplants was fertilized once with 0.5 mL 50 mg Se(VI) L^{-1}, while the other half was left untreated (control). Three days after the treatment (April 26, 2013 and April 17, 2014) the transplants were planted in the field. The plots with Se treated and control transplants were designed in randomized blocks in three repetitions in 2013 and in four repetitions in 2014. Plots were 4 rows wide (70 cm between rows) and 6.4 m long (40 cm between plants) and consisted of 64 plants. The trials were carried out at the experimental field of Agricultural Institute of Slovenia in Jablje near Ljubljana (46.14 N latitude,14.56 E longitude). Field management followed standard production procedures. Before transplanting, the field was fertilized with 30 kg N in the form of NH_3, 96 kg P_2O_5 and 384 kg K_2O per hectare. Three weeks after transplanting the plants were supplied with 54 kg of N per hectare and additional 46 kg N per hectare was supplied 3 weeks later, both times 50% in the form of nitrate and 50% in the form of ammoniac. Mechanical weeding was performed simultaneously with fertilizer incorporation at both side dressings. Insecticides and other plant protection products were not applied. The major pests (*Phyllotreta* spp., *D. radicum*, *P. xylostella* and *C. nasturtii*) were monitored on the same field where the trial was set. Additionally in 2014 the oviposition of *D. radicum* was monitored on separated plots of control and Se treated plants arranged in 2 repetitions. The monitoring plots also, were 4 rows wide but 3.2 m long and consisted of 32 plants. The trial was harvested between July 2 and 9 2013 and on June 17, 2014. The harvest was adjusted to the development of the curds/florets. During the vegetation period, the growth of plants and damage caused by the pests were observed. One month after the transplanting the first set of parameters (number of leaves per plant, the plant height and the percentage of leaf surface damaged by *Phyllotreta* spp.) was assessed. Second set of parameters (plant weight, curd weight and number of *D. radicum* pupae) was assessed at harvest.

2.2. Assessments of physiological and biochemical parameters

2.2.1. Photochemical efficiency

Chlorophyll fluorescence was measured *in situ* on four plants from each group using a fluorometer (PAM 2100 Chlorophyll Fluorometer, Heinz Walz GmbH, Germany). Measurements of chlorophyll fluorescence were made after 10 min of darkness, provided by dark-adaptation clips. Fluorescence was excited with a saturating beam of “white light” (PPFD = 8000 µmol m^{-2} s^{-1}, 0.8 s).

2.2.2. Photosynthetic pigments and anthocyanins

To assess the content of chlorophylls *a* and *b* and carotenoids, leaves of two plants from each group were measured. The amount of pigments was determined as described by Lichtenthaler and Buschmann (2001a, 2001b). The total anthocyanin content was measured as described by Drumm and Mohr (1978).

2.3. Field trial

2.3.1. Delia radicum monitoring

The oviposition of *D. radicum* was monitored on the 8 central plants (4 plants in each of the 2 central rows) of each monitoring plot. Felt traps were set around the broccoli plant stems 2 days after transplanting. Until the harvest the traps were regularly inspected every 3–5 days and the eggs inside were counted and removed.

2.3.2. Growth of plants and damage by pests

All the assessments of plants in the field trial were made on the 20 central plants (10 plants in each of the 2 central rows) of each plot. The number of leaves per plant was scored and the height of plant measured one month after transplanting for each of the 20 plants per plot.

Damage by *Phyllotreta* spp. was assessed as percentage of damaged leaf area according to the scale proposed by OEPP/EPPO (2002) for each of the 20 plants per plot one month after transplanting.

The weight of plants and the weight of curd of each plant were determined for each of the 20 plants per plot at the harvest. Plants with the curds that reached technological maturity were cut above the ground and weighed. Later the curd was cut off the plant and weighed separately.

The number of *Delia radicum* pupae was determined for the group of 5 successive plants together (4 groups of 5 plants per plot). The roots and surrounding soil (in a 10 cm radius around the plant) were sampled just after the harvest. The 5 roots were than inspected for the presence of pupae and larvae. The results are expressed as a mean number of *Delia radicum* pupae found per root.

2.4. Selenium

2.4.1. Selenium measurements

All selenium measurements were carried out in the Analytical Chemistry Laboratory, in Embrapa Clima Temperado, Brazil, using atomic absorption spectrometer with longitudinally-heated graphite atomizer (AA240Z from Varian) with Zeeman-effect background correction and equipped with an autosampler (PSD120, also from Varian) and ultrAA hollow cathode lamp (Agilent Technologies, Australia).

The following operating conditions were adopted: wavelength, 196.0 nm; slit-width, 1.0 nm; current, 10.0 mA. Argon with a purity of 99.996% (Air Liquide, São Paulo, Brazil) was used as a protective gas, at a pressure of 400 kPa. Peak area was used for signal evaluation. Samples aliquots were weighed using an analytical scale AY220 (Shimadzu, Japan).

All chemicals used were at least of analytical grade and the solutions were prepared using high-purity water with a resistivity of 18.2 MΩ cm, obtained from a purification system (Puritech, Permution, Brazil). Sixty-five % nitric acid (Merck, Germany) was used.

A reference solution of 100 μg L^{-1} for Se was prepared daily by appropriate sequential dilution of 1000 mg L^{-1} inorganic analyte stock solution (Titrisol 1000 mg Se in 6.3% nitric acid, Merck, Germany). Daily verification of sensitivity was performed by using the same reference solution of 100.0 μg L^{-1}. The Pd-Mg modifier was prepared by mixing equal volumes of solutions containing 10000 mg L^{-1} of Pd and 10000 mg L^{-1} of Mg, both as nitrate (Merck, Germany). A 5 μL aliquot of this mixed solution was added to the measurement solution in the graphite tube for each determination, according to Welz et al. (1992).

2.4.2. Sample preparation

Samples were freeze dried (freeze dryer LIO 5P LT, Kambič, Slovenia) before slurry preparation. For the pot experiment the whole young plants (transplants) were used, while the concentration of Se from the field experiment was measured in the edible part of the plants (curds). The optimization of sample preparation was done with a two levels factorial design, based on the works of Oliveira et al. (2016) and Torres et al. (2016). In this context, 0.2000–0.2500 g of the freeze-dried sample was weighed directly in the PP tubes. After that, 1.0–1.2 mL of nitric acid was added. Sulfuric acid was also added for some tests, according to further discussion. Samples were submitted to an ultrasound step for 1–2 h at room temperature or at 80 °C, and then the volume was made up to 15.0 mL with deionized water.

Beyond the factorial design investigations, the heating program of the graphite furnace was optimized, according to the Supplement 1. For this study, 5 μL of the Pd-Mg mixture were used as chemical modifiers, in solution. The standard solution presented a more effective interaction with the modifiers which resulted in larger thermal stability, especially for the pyrolysis curve. Nevertheless, despite the fact the sample was slurry, which represents one of the possibilities for solid sampling, similar thermal behaviour could be achieved for standard solution and the slurry of broccoli sample. After the investigation, the adopted temperatures were 600 °C for pyrolysis and 2100 °C for atomization.

The employed conditions for Se determination were: 20 μL of the sample or standard solution; 5 μL of the Pd-Mg mixture as chemical modifiers in solution. The pyrolysis and atomization temperatures were optimized and the adopted conditions were 600/2100 °C. The calibration standard solutions were prepared in 6.67% v/v nitric acid medium and the calibration range was from 10.0 to 100.0 μg L^{-1}. The limit of detection and limit of quantification were calculated as three and ten times the standard deviation of ten measurements of the blank, divided by the slope of the calibration curve. The detection limit in the sample was 0.06 μg g^{-1}, and the quantification limit in the sample was 0.21 μg g^{-1}.

2.4.3. Factorial design

In order to optimize sample preparation of the broccoli parts as acidic slurries for the determination of Se by GF AAS, a 2^{5-2} factorial design was applied. In this sense, the five experimental variables and their levels were: sample mass (0.2000 or 0.2500 g), nitric acid volume (1.0 or 1.2 mL), sulfuric acid volume (0 or 0.2 mL), ultrasound time (60 or 120 min), and heating of ultrasound bath (none or ~80 °C). The sample volume was made up to 15.0 mL with deionized water. The fractional factorial design is a valuable tool to evaluate the parameters involved in an analytical method, which can, for example, simplify the analytical procedure, reduce the time of a specific step or save reagents. The design was performed with duplicate samples. This choice provides enough degrees of freedom to determine experimental error and evaluate of the design results via Pareto chart. Supplement 2 shows the Pareto Charts obtained from this study. From this graph it is possible to observe that none of the evaluated factors presented significant influence, meaning that we can freely choose the level of the evaluated factors for our best convenience. That means, a sample mass of 0.2500 g (for higher sensitivity), 1 mL of nitric acid (less reagent), exclusion of sulfuric acid, 60 min of ultrasound under no heating. Regarding the last two factors, although there was no statistical significance for the tested levels, the method benefits best from longer times in the ultrasound bath under heating, which produces a clearer slurry, a desired quality when dealing with slurry technique. Then, we adopted 120 min under heated ultrasound bath. From the evaluation of seedlings of broccoli plants exposed to Se as well as the pupae, the concentration of Se varied from below the LOQ to 46 μg g^{-1} DM.

To sum up the factorial design optimization tool, acidic slurries of broccoli plant parts obtained after ultrasound exposure has shown to be a reliable and simple way for sample preparation aiming at the measurement of Se by GF AAS, which was greatly facilitated by applying a 2^{5-2} fractional factorial design in order to optimize the levels of the method parameters. In this context, 0.2500 g of the freeze-dried sample was weighed directly in the PP tubes. After that, 1.0 mL of nitric acid was added. Samples were submitted to an ultrasound step for 2 h at 80 °C, and then the volume was made up to 15.0 mL with deionized water.

2.5. Statistical analysis

The results were statistically analysed using the Statgraphics Centurion XVI (Statgraphics Centurion XVI, 2009). The significance of the differences between the treatments was determined by the analysis of variance (ANOVA). In case of physiological and biochemical parameters a one-way ANOVA was performemed while in case of field trials a two-way ANOVA with treatments and repetitions as factors was performed. The least significant difference (LSD) test was used to check which means are significantly different from each other. Differences at $p \leq 0.05$ were considered as statistically significant. For details on ANOVA see the Supplement 3.

3. Results

3.1. Indoor experiment

3.1.1. Physiological and biochemical measurements

The potential photochemical efficiency of PSII is presented in Fig. 1. The addition of higher Se(IV) concentrations the day after treatment statistically lowered photochemical efficiency, while Se(VI) did not affect photochemical efficiency in any of Se concentrations with the

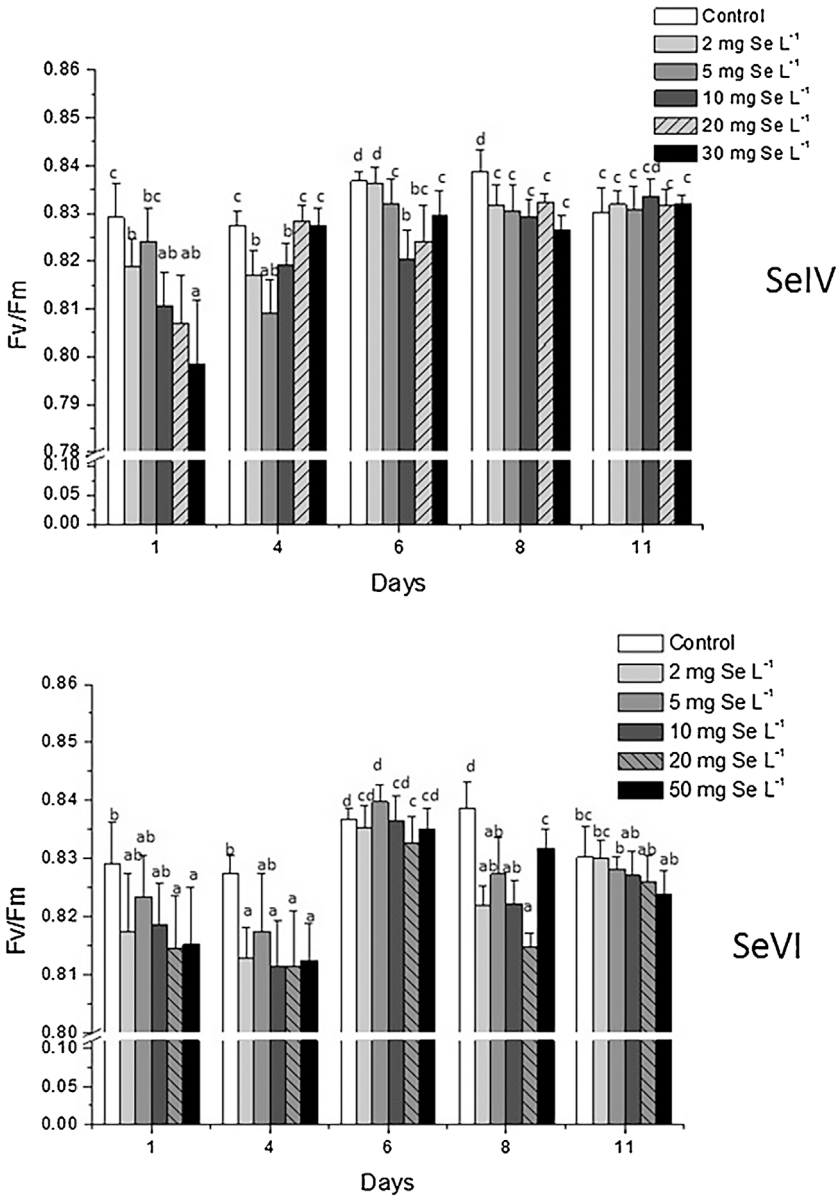

Fig 1. The values of photochemical efficiency in broccoli transplants, exposed to Se (means ± SD, n = 8). Values with different letters were statistically different from each other.

exception of 20 and 50 mg Se(VI) L^{-1} (Fig. 1). Four days after treatment the values of photochemical efficiency were negatively affected by 2, 5 and 10 mg Se(IV) L^{-1}. The values of photochemical efficiency in plants, treated with Se, were the highest in day 6, later on Se addition again lowered the values of photochemical efficiency. After 11 days there was no effect of Se on photosynthesis observed.

Photosynthetic pigments and anthocyanin level in broccoli transplants treated with various Se concentrations are shown in Fig. 2 and 3. Se increased the amount of chlorophyll *a* in 2, 5 and 30 mg Se(IV) L^{-1} 4 days after treatment, but only 5 mg Se(IV) L^{-1} was statistically different from the control. A statistically significant decrease in content of chlorophyll *a* from day 1 to day 6 was observed at 10 mg Se(IV) L^{-1} (Fig. 2). The amount of chlorophyll *b* was increased in day 1 after treatment with high concentrations of Se(IV) in comparison to the control (Fig. 2). Addition of 10 mg Se(IV) L^{-1} decreased the content of chlorophyll *b* 6 days after treatment. The amount of carotenoids was increased 4 days after treatment with Se(IV) compared to the control, but there was no statistically significant differences (Fig. 2). Towards the end of an experiment the amount of carotenoids decreased. The amount of anthocyanins increased from day 6 towards day 11 in plants exposed to Se(IV) (Fig. 2). The highest amount of anthocyanins was measured in plants, exposed to 5 mg Se(IV) L^{-1} on day 6 (Fig. 2).

Addition of Se(VI) significantly increased the content of chlorophyll *a* in day 4 in 20 mg Se(VI) L^{-1} and day 8 in 5 mg Se(VI) L^{-1} treatment (Fig. 3). The decrease in content of chlorophyll *a* was observed 6 days after treatment with 5 mg Se(VI) L^{-1} compared to the control and 11 days after treatment with 2, 20 and 30 mg Se(VI) L^{-1}.

There was an effect on the amount of chlorophyll *b* with 2, 20 and 50 mg Se(VI) L^{-1} the day after treatment, after 4 and 6 days with 20 mg Se(VI) L^{-1} treatment and after 8 days with 5 mg Se(VI) L^{-1} treatment (Fig. 3).

Statistically increased content of carotenoids was observed in day 4 after treatment with 20 mg Se(VI) L^{-1} and day 8 after treatment with 5 mg Se(VI) L^{-1} (Fig. 3). The trend of decreasing content of carotenoids with longer exposure to Se(VI) was observed. The amount of anthocyanins in broccoli plants, exposed to Se(VI) increased in the 20 mg Se (VI) L^{-1} treatment 8 days after treatment and 11 days after treatment with all Se(VI) concentration except 20 mg Se(VI) L^{-1} (Fig. 3).

3.1.2. Selenium

In Table 1 the content of Se in young broccoli plants 11 days after the treatment is presented. Plants took up Se regardless of the form,

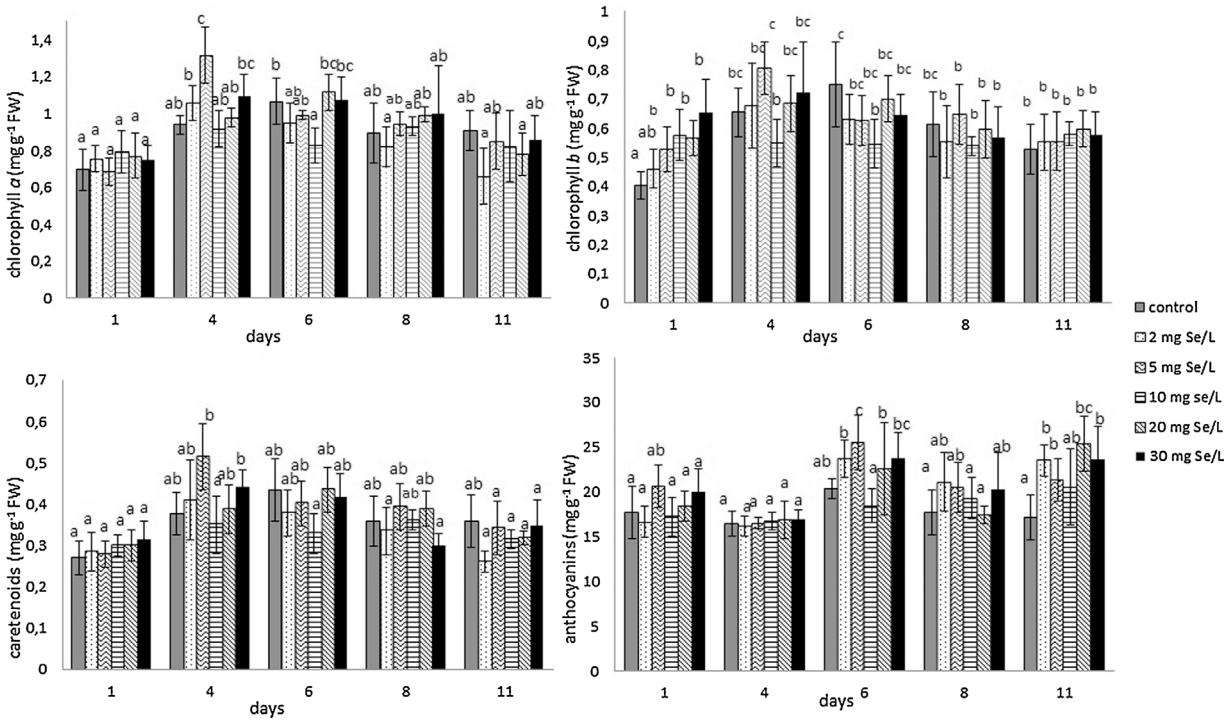

Fig. 2. Pigment contents in plants, exposed to Se(IV). Results are presented as means ± SD (n = 4). Values with different letters were statistically different from each other.

although the uptake was greater for Se(VI). Plants, exposed to 50 mg L^{-1}, contained 46 μg g^{-1} DM.

3.2. Field trial

The cold and rainy spring in 2013 delayed transferring the broccoli transplants to the field and their development after transplanting. The occurrence of major pests was delayed as well. In 2014 the beginning of spring was relatively warm and the transplanting and initial development of broccoli plants was average. Also, the occurrence of major pests was earlier than the year before. The first *Phyllotreta* spp. were recorded at the beginning of May 2013 but the population did not increase until the middle of June and reached its first peak at the end of June. In 2014 the first *Phyllotreta* spp. were caught in the middle of April, the population started to increase in the middle of May and reached its first peak at the end of May and a strong second peak at the end of June. First adult males of *P. xylostella* were caught in the first days of May 2013 and in the last days of April 2014. The population of

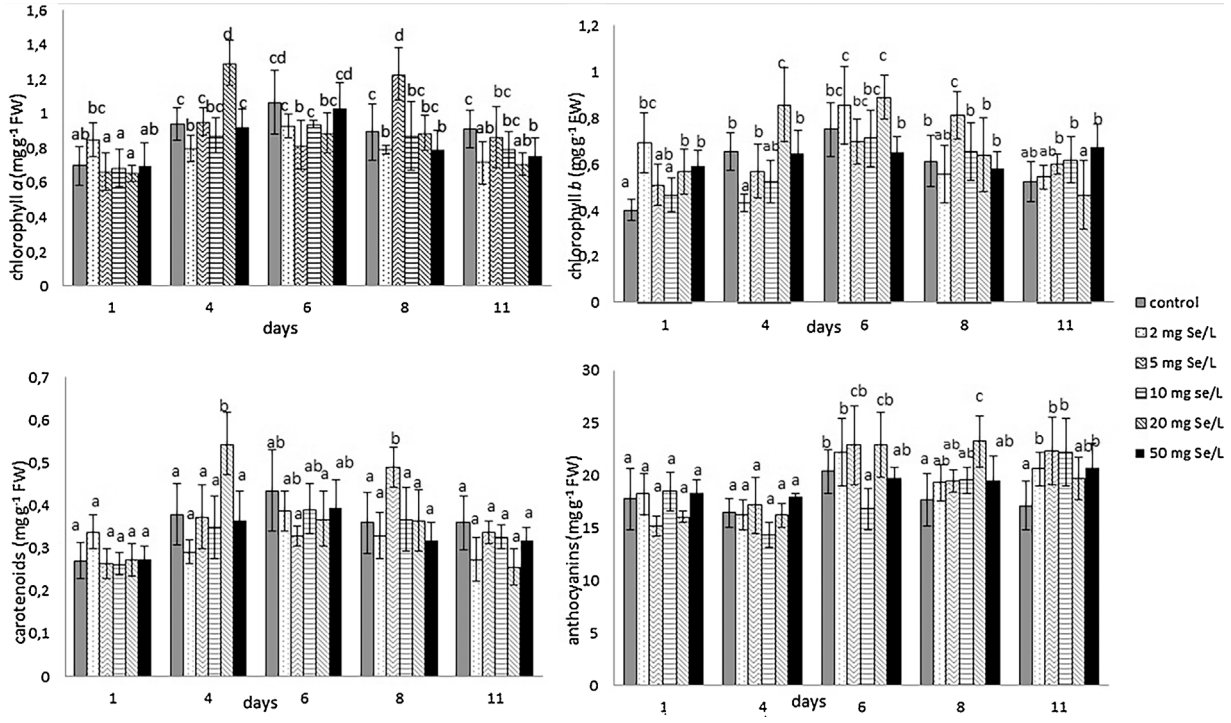

Fig. 3. Pigment contents in plants, exposed to Se(VI). Results are presented as means ± SD (n = 4). Values with different letters were statistically different from each other.

Table 1
Concentration of total Se in young broccoli plants (measured by GF AAS).

Treatment	Se (μg g^{-1})	Se (μg g^{-1})
2 mg L^{-1}	0.38 ± 0.11	2.09 ± 0.11
5 mg L^{-1}	0.52 ± 0.03	2.71 ± 0.68
10 mg L^{-1}	1.58 ± 0.32	8.61 ± 0.69
20 mg L^{-1}	3.12 ± 0.16	18.35 ± 0.37
30/50 mg L^{-1}*	5.76 ± 0.46	45.73 ± 0.46

Means ± SD, n = 2, results are presented on a dry matter (DM) basis.
* 30 mg L^{-1} treatment for Se(IV) and 50 mg L^{-1} treatment for Se(VI).

first generation in both years stayed relatively slim and almost did not cause any damage. The first males of *C. nasturtii* were caught at the end of May 2013 and 2014, the population continuously increased until the beginning of June 2014 and the middle of June 2013 and then slowly decreased. The first generation of this pest was relatively small and did not cause major damage. The first eggs of *D. radicum* were recorded in the middle of May 2013. The oviposition of the first generation reached its peak at the end of May and was completed at the beginning of June. On average 10 eggs per plant in the whole period of oviposition of the first generation were laid. The second generation started to lay eggs at the very end of June, just before the harvest, and additional 10 eggs per plant were laid. In 2014 the first eggs of *D. radicum* were recorded one month earlier than a year before, in the middle of April. The oviposition of the first generation lasted until the middle of May. On monitoring plots, 14 eggs per control plant and 20 eggs per Se(VI) treated plant were laid on average in this period. The second generation started to lay eggs on about June 10 around one week before the harvest and around 15 eggs per plant were laid on the control and Se(VI) treated plants (Fig. 4). The number of eggs laid to control and Se(VI) treated plants did not differ significantly for either fly generation. Comparison of both treatments at single monitoring periods/dates reveals that during the fly's first generation at all monitoring dates, with the exception of the counting on April 30, more eggs were laid to the Se(VI) treated plants, but the differences between the two treatments were statistically significant only on April 28 and May 5.

Plant growth and development parameters one month after transplanting to the field and the extent of damage, due to *Phyllotreta* spp., on leaves are shown in Table 2. In 2013 plants treated with Se(VI) just before transplanting exhibited better initial growth – they had statistically significant more leaves and were higher than control plants. In 2014 the results were just opposite – the control plants had more leaves and were statistically higher than Se(VI) treated plants. *Phyllotreta* spp. did not cause major damage in 2013, on the average 1–2% of the leaf area was affected, and the differences between Se(VI) treated and control plants were not significant. In 2014 flea beetles caused considerably more damage than the year before and Se treated plants were significantly ($p \leq 0.05$) more damaged than the control plants (respectively, 9 and 5% of affected leaf surface).

Table 2
The number of leaves, the plant height and the degree of leaf damage by *Phyllotreta* spp. of broccoli plants one month after the transplanting.

Year	Treatment	No. of leaves	Plant height (cm)	*Phyllotreta* spp. damage (%)
2013	Control	7.5 ± 1.1[a]	13.7 ± 2.5[a]	1.7 ± 0.8
	Se(VI)	7.9 ± 1.0[b]	15.1 ± 2.6[b]	1.5 ± 0.6
2014	Control	8.6 ± 1.0	18.0 ± 3.1[B]	5.0 ± 4.0[A]
	Se(VI)	8.5 ± 1.4	15.8 ± 2.7[A]	9.0 ± 6.7[B]

Means ± SD (n = 60 in 2013 and n = 80 in 2014). Within each year, means followed by different letters are significantly different at $p \leq 0.05$.

The plant weight, curd weight and number of *D. radicum* pupae recorded at the harvest are presented in Table 3. The plants treated with Se(VI) did not significantly differ from the control plants in the weight of the part above ground level in either of experiment. The curd weight of Se(VI) treated plants in 2013 was equal to the curd weight of control plants while in 2014 it was significantly ($p \leq 0.05$) lower than at the control. The concentration of Se in the curds was below the detection limit (results not shown). The average number of *D. radicum* pupae found per single plant in 2013 was relatively low, on average 1–2 pupae per plant were recorded, while in 2014 more (2–5) pupae per plant were found. In both years more pupae were found at the control than at Se (VI) treated plants, but the difference was statistically significant only in 2014. Few larvae of the fly's second generation were also recorded. Since, at the time of harvest, the oviposition of the second generation was at its very beginning, only the number of pupae of the first generation is presented. Se concentration in the pupae found in the Se(VI) treated plants was 3.18 and 0.79 μg g^{-1} in year 2013 and 2014, respectively. The pupae from Se treated plants had higher Se concentration in comparison to pupae from control plants (Table 3).

4. Discussion

4.1. Physiological and biochemical measurements

The addition of Se(VI) did not affect the values of photochemical efficiency the day after Se treatment, except the addition of highest concentrations. A decrease in higher Se(IV) treatment was observed (Fig. 1). The highest values of photochemical efficiency were observed in the middle of the experiment. We presume that Se temporary acted similar as antioxidants do (Hartikainen et al., 2000) therefore the rate of photochemical efficiency was unaffected. The increase in photochemical efficiency due to Se(VI) addition was also observed in soybeans, exposed to 50 mg Se L^{-1} (Djanaguiraman et al., 2005). From the middle of the experiment on the Se(IV) did not affect photochemical efficiency, while Se(VI) had still some effect. In the end plants may adapted to Se addition and no effect was observed.

The unaffected photochemical efficiency in plants, exposed to Se

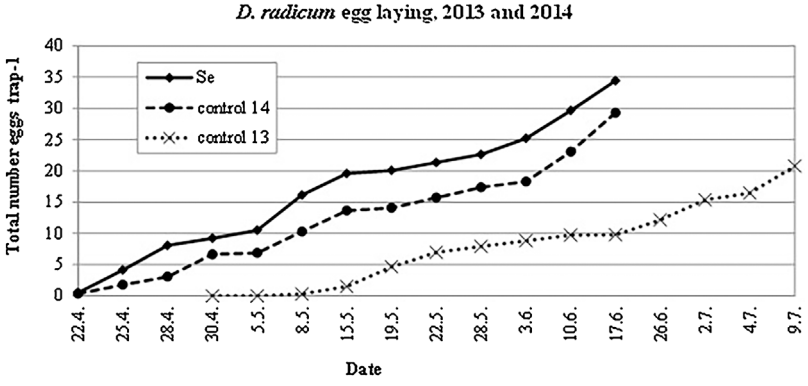

Fig. 4. The average number of *Delia radicum* eggs laid per felt trap on broccoli plants for the control plants in 2013 and for the control and 50 mg Se(VI) L^{-1} treated plants in 2014.

Table 3
The plant and the curd weight and the number of *Delia radicum* pupae per plant at harvest and the concentration of Se in pupae.

Year	Treatment	Plant weight (g)	Curd weight (g)	*D. radicum* pupae (No. per plant)	Se concentration in pupae (μg g^{-1})*
2013	Control	373 ± 148	83 ± 56	1.2 ± 1.1	> 0.21
	Se(VI)	415 ± 173	82 ± 61	1.0 ± 0.9	3.18 ± 0.17
2014	Control	517 ± 217	115 ± 74^{A}	4.9 ± 2.0^{B}	0.44 ± 0.30
	Se(VI)	467 ± 203	91 ± 66^{B}	2.2 ± 0.9^{A}	0.79 ± 0.41

Means ± SD (n = 60 in 2013 and n = 80 in 2014 for plant and curd weight and n = 15 in 2013 and n = 20 in 2014 for *D. radicum* pupae). Within each year, means followed by different letters are significantly different at $p \leq 0.05$.

* Means ± SD, n = 2, results are presented on a dry matter (DM) basis.

(VI) was observed in cabbage and red cabbage (Mechora et al., 2011, 2014), soybean (Mechora and Germ, 2010) and *Hordeum vulgare* (Valkama et al., 2003).

Higher Se doses (30/50 mg Se L^{-1}) with longer exposure did not significantly decrease the photochemical efficiency (Fig. 1), although the values were low. This suggests that plants were not in stress, since values of photochemical efficiency for a variety of unstressed plants ranges from 0.80 to 0.83 (Schreiber et al., 1995).

In the beginning a positive effect of low Se concentrations on chlorophyll *a* was observed (Fig. 2 and 3). Selenite at a concentration of 10 mg L^{-1} had the most negative effect on the amount of chlorophylls (statistically decreased values in day 6), which resulted in low photochemical efficiency. Both added Se forms increased the content of chlorophyll *b* in day 1 (Fig. 2 and 3). From day 1–6 the 20 mg Se(VI) L^{-1} increased values of this pigment, later on, the content lowered comparing to control. After 6–8 days of exposure to Se(VI) the content of chlorophyll *b* was increased compared to samplings before. The increased chlorophyll content in Se treated plants might be attributed to efficient scavenging of reactive oxygen species by glutathione peroxidase or otherwise they would have destroyed the chlorophyll pigments (Thomas et al., 2001). Changes in chlorophyll *a* content affect the process of photosynthesis. Increased synthesis of chlorophylls 4 days after treatment together with higher carotenoid levels could possibly later on maintain the level of photochemical efficiency unchanged for several days.

In cabbage and red cabbage, exposed to Se(VI) the amounts of chlorophyll were unchanged (Mechora et al., 2011, 2014). On the contrary Se lowered the amount of chlorophylls in barley (Akbulut and Çakir, 2010), maize (Hawrylak-Nowak, 2008) and ryegrass (Hartikainen et al., 2000). Reduction of plant growth and decrease of chlorophyll content are common symptoms for plant growth under stress conditions (Hawrylak-Nowak, 2008), therefore we presume, that Se did not severely affect chlorophyll synthesis in broccoli transplants.

The amount of carotenoids increasing from day 1–6 and slowly decreasing towards the end of the experiment (Fig. 2 and 3). An increase or constant level of carotenoids is a defence strategy of the plant to reduce metal stress (Fargašová, 1998), since carotenoids are non-enzymatic antioxidants that play important roles in the protection of chlorophyll under stress. Carotenoid levels were increased in the same treatments as chlorophylls (5 mg Se(IV) L^{-1}, 5 and 20 mg Se(VI) L^{-1}). This could suggest that Se affects these pigments in the same way. Increased carotenoid contents could show that stress was present. On general, the carotenoids were increased 4 days after the treatment and then slowly decreased towards the contents observed 1 day after the treatment. This together with the unchanged levels of chlorophyll *a* and the unaffected photochemical efficiency shows on current adaptation of broccoli transplants to Se(VI) exposure. The decrease in content of carotenoids is in agreement with findings in *Brassica napus*, exposed to 14, 36, 71, 107 mg Se L^{-1} (Molnárová and Fargašová, 2009).

In our study the amount of anthocyanins increased from day 1 to day 11 (Fig. 2 and 3). Increased amounts of anthocyanins could be the sign of stress in the plant (Winkel-Shirley, 2002). Since there were increases in the amounts of anthocyanins we concluded that plants were in minor stress conditions. When carotenoids were higher (day 4), the anthocyanins were in the range of control. When carotenoids decreased, the increase in the amount of anthocyanins was observed. In maize, Se(VI) treatments at concentrations between 0.4-3.9 mg Se L^{-1} had no effect on anthocyanin accumulation, but at 7.9 mg Se L^{-1} increased the content of anthocyanins (Hawrylak-Nowak, 2008).

The trend of first increased and later on decreased content of chlorophylls exposed to Se(IV) was observed. Higher Se additions increased content of chlorophyll *b* in the beginning. The positive effect of 20 mg Se(VI) L^{-1} on the content of chlorophyll *b* was observed. The same trends in protective pigments were observed in both inorganic Se forms: carotenoids increased in the beginning, while anthocyanins increased later on when carotenoids were already decreased. In the end of the experiment all Se concentrations increased the amount of anthocyanins. Overall the lower values of carotenoids and higher values of anthocyanins were measured with Se(IV) additions.

4.2. Selenium

Broccoli transplants took up a great amount of Se. Plants, exposed to 2 and 5 mg Se(IV) L^{-1} contained around 0.5 μg Se g^{-1}, while exposure to Se(VI) amounts to four times greater levels of Se in the 2 and 5 mg Se (VI) L^{-1} treated plants (Table 1). Broccoli treated with 30 mg Se(IV) L^{-1} and 50 mg Se(VI) L^{-1} contained 6 μg Se g^{-1} and 46 μg Se g^{-1}, respectively. The amount of Se in the plants increased with the treatment concentrations. The dose dependent Se concentration was also observed in aquatic plants (Zayed et al., 1998; Mechora et al., 2015). It has to be emphasized that increases of added Se above 5 mg Se L^{-1} resulted in sharper increases of Se levels in the plants, which is clearly observed in Se(VI) treatment (Table 1).

In another study, cabbage, exposed to 20 mg Se(VI) L^{-1} contained 4.77 μg Se g^{-1} (Mechora et al., 2014) which is lower amount than broccoli, exposed to the same concentration in the present study. Broccoli exposed to 3 mg Se kg^{-1} contained 155 μg Se g^{-1} (Hamilton, 1964), while broccoli exposed to 20 and 50 mg Se L^{-1} contained 9 and 22 μg Se g^{-1}, respectively (Adhikari, 2012).

From the experiments the direct comparison of uptake of inorganic Se forms can be observed. Broccoli transplant more efficiently absorbed Se in the form of selenite (Table 1) without any substantial damage to the plants. These can be supported with unaffected photochemical efficiency and chlorophylls 11 days after Se(VI) addition, lowered carotenoids levels and on the other hand increased anthocyanins, which have a protective role. Broccoli plants, exposed to Se(IV) also had increased values of anthocyanins, which may point to stressful conditions already at lower content of Se compared to plants, exposed to Se(VI).

4.3. Outdoor experiment

The addition of 0.5 mL of 50 mg Se(VI) L^{-1} to the plants three days before transplanting to the open field did not exhibit any visible effects on plants at the time of transplanting. In 2013 the pests did numerously appear only about one month after transplanting, while in 2014 the pressure of *D. radicum* and *Phyllotreta* spp. started already at the

beginning of the field experiment. In 2014 in a period of 2 weeks after the transplanting the cabbage root fly laid more eggs to the Se(VI) treated than to control plants (Fig. 4). Additionally, one month after the transplanting, Se(VI) treated plants were significantly more damaged by flea beetles than the control plants (Table 2). It is known that oviposition by *D. radicum* is governed to a large extent by chemicals present on the leaf surface of the host plant *Brassica oleracea* (Roessingh et al., 1992) and that olfactory and contact chemosensory stimuli mediate host acceptance by *Phylotretta* spp. (Henderson et al., 2004). Selenium was observed to increase glucosinolates in general and sulforaphane in particular, when applied up to a certain doses, above which it decreased glucosinolate and phenolic acids production in broccoli plants (Robbins et al., 2005). Glucosinolates have been shown to induce oviposition by the cabbage root fly (Roessingh et al., 1992). On the other hand, the volatile Se compounds (dimethylselenide and dimethyldiselenide) emitted from plants supplied with Se (Terry et al., 2000) are believed to have deterring effect for different above ground herbivores (Hanson et al., 2003, Hanson et al., 2004, Freeman et al., 2007, Hladun et al., 2013). Anyhow, some papers do report on unchanged honeybee visitation of flowers of Se treated radish plants (Hladun et al., 2013) and on increased attraction of Se treated leaves for snails *Mesodon ferrissi* (Hanson et al., 2003).

Our observations suggest that chemical changes provoked by Se(VI) addition to broccoli transplants increased attractiveness of plants for *D. radicum* female adults and *Phyllotreta* spp. in the first weeks after the Se (VI) treatment. In 2014, when both pests were present already at the time of transplanting, when the concentration of Se in Se treated plants was around 45 μg Se g^{-1} dry matter (Table 1), this resulted in higher oviposition of *D. radicum* and greater extent of leaf damage by *Phyllotreta* spp. on Se(VI) treated plants. In 2013, when both pests appeared more massively only about one month after the transplanting, the differences in leaf damage by *Phyllotreta* spp. were not observed. We assume that the concentrations of Se in Se(VI) treated plants one month after the transplanting (the time of pest appearance in 2013) were lower than few days after the transplanting (the time of pest appearance in 2014). Our results from another study in 2015 do suggest so − 28 days after the transplanting, plants treated with the same amount of Se(VI) at the same developmental stage contained between 1 and 2 μg Se g^{-1} dry matter.

However, the number of *D. radicum* pupae recovered in the root zone just after the harvest was lower for Se(VI) treated then for control plants in both years, but the difference was significant only in 2014 (Table 2). Interestingly, in 2014 in spite of the higher number of eggs laid to Se(VI) treated than to control plants, the number of pupae recovered at harvest was higher for control plants. In percentages, for the control plants around 30%, while for the Se(VI) treated plants only around 11% of the eggs reached the pupae stage. These results suggest that Se accumulated in the roots of broccoli plants acted as antifeedant for *D. radicum* larvae. Previous studies have demonstrated that Se accumulated in tissues can protect plants from different insect pests (Vickerman and Trumble, 1999; Bañuelos et al., 2002; Hanson et al., 2003; , 2004). Se concentration in the pupae of a root herbivore from the field was low. When feeding caterpillars with leaves containing 1600 μg Se g^{-1}, they contained around 90 μg Se g^{-1} (Hanson et al., 2003). Cabbage loopers contained 2960 μg Se g^{-1} when feeding on 465 μg Se g^{-1} (Bañuelos et al., 2002). But there is important difference: i) the plants in both studies contained higher amount of Se and ii) leaf herbivores were collected and measured for Se concentration, while in our study Se in the root herbivory insect was measured.

The damage on leaves caused by *Phyllotreta* spp. affected plant growth, therefore one month after the transplanting, the Se(VI) treated plants in 2014 were shorter than the control plants (which had significantly less damaged leaves). It is well known that large populations of *Phyllotreta* spp. can stunt the growth of young plants (Palaniswamy and Lamb, 1992). The plants in our experiment did not compensate for this handicap until the harvest, when the Se(VI) treated plants were still slightly, but not significantly, lighter and had significantly lighter curds than the control plants. The situation was quite different in 2013, when leaves of young plants were only slightly damaged by *Phyllotreta* spp. and there was no difference between the Se(VI) treated and control plants in the percentage of leaf area affected. In these circumstances, one month after the transplanting, the Se(VI) treated plants had more leaves and were higher than the control plants. This effect later subsided and at the harvest the Se(VI) treated plants were only slightly, but no more significantly, heavier than control plants and there was no difference in the curd weight between the Se(VI) treated and control plants. The positive effect of Se(VI) on height of the plants was observed in red cabbage (Mechora et al., 2011).

5. Conclusion

To conclude, the photochemical efficiency was negatively affected by high Se addition, but plants adapted to Se and in the end of an experiment no effect of Se was observed. The amount of chlorophylls, carotenoids and anthocyanins was the mostly affected by 20 mg Se(VI) L^{-1}. We observed that increased concentration in chlorophyll *a* resulted in increased photochemical efficiency. The amount of protective anthocyanin pigments increased towards the end of an experiment, which shows the presence of stress. The values of photochemical efficiency were lowest in high Se doses. A dose relationship between uptake of Se and internal content was observed. Plants took up greater amounts of Se when exposed to Se(VI) compared to Se(IV) exposure, which shows greater availability of Se(VI) for the plants.

Anyhow the treatment of broccoli transplants with 0.5 mL of 50 mg Se(VI) L^{-1} did not cause any visible effect to the plants that were transplanted to the field although it must have provoked chemical changes that increased the attractiveness of plants for *D. radicum* female adults and *Phyllotreta* spp. in the first weeks after the Se(VI) treatment. In the situation of high pest pressure this resulted in increased oviposition of *D. radicum* and increased leaf damage by *Phyllotreta* spp. which retarded the growth of plants. However, in circumstances of low pest pressure, the Se(VI) treated plants exhibited better initial growth. Despite increased oviposition of *D. radicum* in Se(VI) treated plants, the number of pupae recovered at harvest was significantly less than in control plants what leads us to the conclusion that Se accumulated in the roots of broccoli plants affected the survival of *D. radicum* larvae.

Acknowledgement

The research leading to these results has received partial funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under the grant agreement no. FP7-265865.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.scienta.2017.07.032.

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