Differences Between the Effects of Insecticidal Seed and Foliar Treatments on Pea Leaf Weevils (*Sitona lineatus* L.) in the Field Pea (*Pisum sativum* L.)

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Abstract

Seidenglanz M., Rotrekl J., Smýkalová I., Poslušná J., Kolařík P. (2010): **Differences between the effects of insecticidal seed and foliar treatments on pea leaf weevils (***Sitona lineatus* L.**) in the field pea (***Pisum sativum* L.). Plant Protect. Sci., **46**: 19–27.

Tested seed treatments (two doses of thiamethoxam, thiamethoxam + fludioxonil + metalaxyl-M; two doses of clothianidin + beta-cyfluthrin) showed high effects on pea leaf weevils (*Sitona lineatus* L.) which approved relatively long-lasting and sufficient protection of several bottom nodes of stipules simultaneously. The effects of foliar treatments (chlorpyrifos + cypermethrin; acetamiprid, lambda-cyhalothrin) sometimes were evident only on the node which was determined as the youngest node at the time of spraying. The effects of the compared seed and foliar treatments on the reduction of *S. lineauts* larvae numbers on roots were not proven as positive. It is possible to conclude that the foliar application had no real effect in this sense at all. However, positive significant effects of thiamethoxam and clothianidin + beta-cyfluthrin on root nodulation in general were recorded. Especially higher doses of the seed treatments increased overall nodulation from 43% till 363%.

Keywords: *Sitona lineatus* L.; thiamethoxam; clothianidin; seed-applied insecticides; *Pisum sativum* L.; root nodulation

The pea leaf weevil, *Sitona lineatus* (L.) is a serious pest of the field pea, *Pisum sativum* L., in Europe and in the Pacific Northwest (Landon *et al.* 1995; Williams *et al.* 1995; Wnuk & Wiech 1996; Van de Steene & Vulsteke 1999).

Over-wintered adults migrate to the pea fields in late March to early May where the weevils feed on seedling foliage. Starting always at the edge of the leaf (or stipule) they eat U-shaped notches out of it. These notched leaves are typical of the first signs of weevil presence in pea stand (Van de Steene & Vulsteke 1999). Females scatter eggs singly on the soil surface and in cracks near the seedlings (Schotzko & O'Keeffe 1988). They very soon commence to lay eggs after their appearance in the crop and egg-laying continues until shortly before the death of the parent weevil (Lerin *et al.* 1993). Larvae burrow into the soil and feed exclusively within *Rhizobium* nodules associated with pea roots (Johnson & O'Keeffe 1981). This means that infestation damages a pea crop twice a season. A single effect of *S. lineatus* on seed

Supported by the Ministry of Agriculture of the Czech Republic, Project No. 1B 44008, and by the Ministry of Education, Youth and Sports of the Czech Republic, Projects No. 1M 06030 and No. MSM 2678424601.

yield and yield factors is not easy to determine. According to Williams *et al*. (1995), by pea leaf weevils caused defoliation can reduce the number of pods per plant and pod length although seed yield was never significantly reduced. Experiments carried out by Cantot (1989) show the effect of the larvae on productivity factors in the peas at a threshold of 10 eggs per plant. The effect can be observed by the number of seeds and yield per plant and by the production of total nitrogen per plant. Landon *et al.* (1995) emphasised that leaf weevils are a long-term cause of reduced yields of pea. The control of pea leaf weevils with insecticides is based primarily on foliar applications in Europe with pyrethroids being used for the most part at present. However, foliar sprays with pyrethroids seem to give insufficient control (Vulsteke *et al*. 1994; Dore & Meynard 1995; Van de Steene *et al*. 1999). Seed treatment with systemic insecticides is more effective (Bachmann & Elmsheuser 1986; Salter & Smith 1986; Vulsteke *et al*. 1989; Lee & Upton 1992; Vulsteke *et al*. 1994; Taupin & Janson 1997; Van de Steene *et al*. 1999; Rotrekl & Cejtchaml 2008). Unfortunately, the most frequently tested and recommended seed dressings are rather out of date: imidacloprid, furathiocarb, benfuracarb, carbofuran, phorate. Some of them have also unacceptable chemical properties for environment and were not included in Annex 1: furathiocarb, carbofuran, phorate (EC Directive 91/414). And some relatively newly evolved actives (thiamethoxam and clothianidin) are not registered for the purpose in most European countries. However just the systemic neonicotinoids applied to seeds before sowing could bring an important shift to the control of the insect pest (Rotrekl & Cejtchaml 2008). Residual effects of the new systemic insecticides can also significantly influence occurrences and the seriousness of other insect pests which come into the pea crop later (Rotrekl *et al*. 2007; Rotrekl 2008). In European regions, pea aphids (*Acyrthosiphon pisum* Harris, 1776) and thrips (*Kakothrips pisivorus* Westwood 1880) should mainly be considered.

In this paper the effects of several seed (thiamethoxam; thiamethoxam + fludioxonil + metalaxyl-M; clothianidin + beta-cyfluthrin) and foliar (chlorpyrifos + cypermethrin; acetamiprid; lambda-cyhalothrin) insecticidal treatments on pea leaf weevils (adults and larvae) are compared. We meant to answer particularly these questions:

– How do compared insecticidal treatments approve themselves on the individual stipules (I. to IV. node) in relation to continual movement of weevils onto the youngest nodes during their crop colonisation?
– How do compared insecticidal treatments affect occurrences of *Sitona* larvae on roots and damage levels of nodules?
– How do compared insecticidal treatments affect root nodulation?

MATERIAL AND METHODS

Exact, small-plot trials (net plot: 1.25 m × 8 m or 2.5 × 10 m; 4 replication; every plot was encompassed by 1.25 m wide, untreated zones on both sides) were sown every year at two different localities (Šumperk 49°58'56.986''N, 17°0'57.191''E: a somewhat coolish region; Troubsko 49°10'12.742''N, 16°30'29.552''E: a somewhat warmer region) during the years 2007 and 2008 in the Czech Republic. Zekon (common semi-leafless variety) was the used variety of pea (*Pisum sativum* L.). After crop emergence the plants were exposed to natural infestations with the pea leaf weevils (*Sitona lineatus* L.).

Seed treatment was performed at least 2 weeks before sowing each year. ROTOSTAT MACHINE No. R 110 (General Engineers Ltd; Watton, Norfolk, UK) was used for the treatment of seeds. Foliar applications were regularly made immediately after finding the first notches on stipules. This was at the time when the majority of plants had their first and second node stipules unfolded (BBCH 11–12). Insecticides were applied with the self-propelling trial sprayer HEGE 32 (HEGE Maschinen Gmbh, Waldenburg, Germany; three separate spraying paths – each of them having six nozzles; spraying span 1.5 m or 3 m, type of nozzles XR TEEJET, No. of nozzle 80015 VS, application pressure 0.3 MPa and flow rate 312.5 l/ha). Tested active substances (and commercial products with their formulations) and their doses are listed in Table 1. The effects of the treatments were evaluated using the criteria described below.

(a) Notches on stipules

Notches on stipules were counted on 10–20 plants per plot usually three times per trial. The first count was made immediately after finding the first notches on the plants in the trial. Usually the majority of plants had their first and second node stipules unfolded (BBCH 12) at that time. Immediately after the assessment the foliar application of insecticides (treatments: 6, 7, 8; Table 1) was made. Other two assessments were carried out with the view of the majority of plants during the second assessment had their second to third node stipules unfolded (BBCH 12–13) and during the third assessment, most of plants had their third to fourth (sixth) node stipules unfolded (BBCH 13–14, 15–16). In Šumperk we recorded the numbers of notches on individual nodes separately on every assessment. In Troubsko the numbers of notches only on the youngest evolved nodes (stipules) were recorded on every assessment.

(b) Roots

When the plants were in flower (during full-flowering stage in Troubsko and at the end of the flowering stage in Šumperk) the roots with surrounding soil were sampled from the individual plots. Each sample consisted of five roots from the plants growing side-by-side on the row and the soil immediately surrounding them (depth: 150 mm; four samples per plot). Aerial parts of the plants were removed. In the laboratory, after rinsing the samples using sieves, the following points were assessed:

(1) The total number of nodules per five roots;
(2) The number of *S. lineatus* larvae-damaged nodules per five roots;
(3) The number of still active nodules per five roots (only in Šumperk);
(4) The number of *S. lineatus* larvae per five roots (only in Troubsko).

The obtained results were statistically analysed. One-factorial 'ANOVA' and subsequently the Tukey test were performed in order to distinguish differences among the mean values. For analysis the software Statistica version 8 and UPAV GEP Version 1.6 (official statistical software used and recommended by State Phytosanitary Administration of the Czech Republic for evaluating the trials aimed at new registrations of pesticides) were used.

RESULTS

Notches on stipules

Šumperk 2007 (Table 2): Significant differences were recorded among damage levels of stipules from the first node ($F_{1.node;\ 1.term}$ = 55,6732) and second node ($F_{2.node;\ 1.term}$ = 91,664) immediately before foliar applications (treatments 6, 7 and 8). One week after foliar application the differences in the damage levels of second node stipules remained significant in relation to the comparison of seed and foliar treatments ($F_{2.node;\ 2.term}$ = 83,641). At time of application the damage levels of third node

Table 1. The list of used active ingredients and their doses on both localities (Šumperk, Troubsko, 2007, 2008)

Treatment	Used active substance(s)	Doses (g of a.i/ha)	Formulation	Used product(s)	Locality	Target of application
1	Untreated control (UN)	–	–	–	Šumperk, Troubsko	–
2	Thiamethoxam (T LD)	87.5	FS	Cruiser 350 FS	Šumperk, Troubsko	seeds
3	Thiamethoxam (T HD)	175	FS	Cruiser 350 FS	Šumperk, Troubsko	seeds
4	Clothianidin + betacyfluthrin (C+B LD)	60 + 12	FS	Elado 480 FS	Šumperk, Troubsko	seeds
5	Clothianidin + betacyfluthrin (C + B HD)	120 + 24	FS	Elado 480 FS	Šumperk, Troubsko	seeds
6	Chlorpyrifos + cypermethrin (Ch + Cy)	300 + 30	EC	Nurelle D	Šumperk, Troubsko	stipules
7	Acetamiprid (Ac)	36	SP	Mospilan 20 SP	Šumperk only	stipules
8	Lambda-cyhalothrin (L-Cyh)	7.5	CS	Karate ZEON 5 CS with ZEON technology	Šumperk only	stipules
9	thiamethoxam + fludioxonil + metalaxyl-M (TLD + F + M)	87.5 + 5.75 + 10	FS	Cruiser 350 FS + Maxim XL 035 FS	Šumperk only	seeds

Table 2. Numbers of notches comparison. The results are derived from the consecutive assessments (Šumperk, 2007)

Treatment	Mean number of notches per stipule (SE)					
	1. node; 1. term	2. node; 1. term	2. node; 2. term	3. node; 2. term	3. node; 3. term	4. node; 3. term
1 (UN)	4.84^{b} (0.41)	7.38^{b} (0.40)	12.66^{a} (0.44)	11.94^{a} (0.48)	15.25^{a} (0.57)	10.05^{a} (0.71)
2 (T LD)	1.01^{cd} (0.14)	1.70^{d} (0.20)	4.75^{e} (0.35)	3.03^{e} (0.32)	5.75^{d} (0.47)	2.88^{e} (0.38)
3 (T HD)	0.81^{d} (0.14)	1.10^{d} (0.17)	3.13^{e} (0.34)	1.98^{e} (0.27)	2.95^{e} (0.40)	1.74^{e} (0.31)
4 (C + B LD)	2.21^{c} (0.24)	3.61^{c} (0.34)	10.14^{bc} (0.39)	9.08^{b} (0.47)	10.81^{bc} (0.58)	7.95^{ab} (0.63)
5 (C + B HD)	1.30^{cd} (0.19)	2.05^{d} (0.21)	7.50^{d} (0.38)	5.84^{d} (0.43)	8.49^{c} (0.54)	5.44^{cd} (0.56)
6 (Ch + Cy)	7.04^{a} (0.49)	9.15^{a} (0.49)	11.26^{ab} (0.56)	8.13^{bc} (0.59)	11.46^{b} (0.74)	5.89^{bc} (0.74)
7 (Ac)	4.68^{b} (0.35)	7.06^{b} (0.40)	12.08^{a} (0.46)	9.33^{b} (0.49)	11.63^{b} (0.58)	5.60^{bcd} (0.57)
8 (L-Cyh)	5.04^{b} (0.41)	7.15^{b} (0.42)	9.40^{c} (0.41)	7.04^{cd} (0.49)	9.39^{bc} (0.61)	3.34^{de} (0.44)
9 (TLD + F + M)	1.09^{cd} (0.15)	1.21^{d} (0.17)	3.28^{e} (0.29)	2.03^{e} (0.25)	3.36^{de} (0.37)	1.95^{e} (0.27)

1. term (= term of foliar application): 26. 4. 2007, prevailing growth stage BBCH 12; 2. term: 3. 5. 2007, BBCH 12–13; 3. term: 11. 5. 2007, BBCH 14–16

The mean values followed by different letters in one column are significantly different (Tukey test; $P < 0.05$)

N = 80 stipules per treatment (20 per repetiton)

were very low across the trial because the stipules were still not completely unfolded (BBCH 12). Even though the sprays were applied in time, the effect of insecticidal sprays did not outperform the effect of seed treatments even on the third node ($F_{3.node;\,2.term}$ = 65,766). Two weeks after spraying (3. term) the differences in levels of damage on the third node became more pronounced between seed and foliar treatments ($F_{3.node;\,3.term}$ = 55,163). This tendency is also apparent from the results recorded on stipules on the fourth node ($F_{4.node;\,3.term}$ = 27,3276).

Troubsko 2007 (Table 3). Immediately before foliar application (19.4.; treatment 6) the damage levels of plants from seed treatments were significantly lower in comparison with plants from the control plots ($F_{19.4.07}$ = 28,769). The positive effect of foliar application (treatment 6) was evi-

Table 3. Numbers of notches comparison. The results are derived from the consecutive assessments (Troubsko, 2007, 2008)

Treatment	Mean number of notches per youngest unfolded stipule				
	19. 04. 2007	23. 04. 2007	02. 05. 2007	29. 04. 2008	05. 05. 2008
1 (UN)	13.6^{a}	15.63^{a}	14.9^{a}	1.13^{ab}	1.25^{a}
2 (T LD)	2.2^{bc}	8.88^{b}	7.5^{b}	0.33^{b}	0.18^{c}
3 (T HD)	1.15^{c}	3.1^{c}	4.2^{c}	0.28^{b}	0.18^{c}
4 (C + B LD)	3.78^{b}	8.34^{b}	6.9^{b}	0.73^{b}	0.15^{c}
5 (C + B HD)	1.65^{bc}	5.43^{bc}	4.5^{c}	0.38^{b}	0.1^{c}
6 (Ch + Cy)	13.75^{a}	9.1^{b}	4.6^{c}	1.2^{a}	0.5^{b}

Season 2007: 19. 4. 2007 assessment made immediately before foliar application (treatment 6), prevailing growth stage BBCH 12; 23. 4. 2007, prevailing growth stage BBCH 12–13; 2. 5. 2007 , prevailing growth stage BBCH 14–15

Season 2008 (low occurrence of *Sitona* imagoes in trial after crop emergence): 29. 4. 2008 assessment made immediately before foliar application (treatment 6), prevailing growth stage BBCH 14; 5. 5. 2008, prevailing growth stage BBCH 16

The mean values followed by different letters in one column are significantly different (Tukey test; $P < 0.05$)

N = 40 stipules per treatment (10 per repetiton) dent four days after spraying in the numbers of notches on third node stipules especially ($F_{23.4.07}$ = 8,172). The damage levels of fourth – fifth node stipules were significantly lower for the all seed and foliar treatments in comparison with the control, even 13 days after foliar application. However, lower doses of seed-applied thiamethoxam and clothianidin + beta-cyfluthrin were markedly less effective in comparison with their higher doses and with foliar application on the fourth – fifth node stipules ($F_{2.5.07}$ = 11,449).

Šumperk 2008 (Table 4). Immediately before foliar applications (12. 5.; treatments 6, 7 and 8) the plants had two nodes evolved. From the results it is clear that stipules on the first resp. second nodes were significantly less damaged on the plants from seed treatments at that time ($F_{1.node;\ 1.term}$ = 81,184 resp. $F_{2.node;\ 1.term}$ = 216,77). Differences in damage levels between the seed and foliar treatments were still apparent on the first resp. second nodes even in the next two terms of assessment: 17.5.; 23.5. ($F_{1.node;\ 2.term}$ = 132,126; $F_{1.\ node;\ 3.term}$ = 95,538 resp. $F_{2.node;\ 2.term}$ = 172,059; $F_{2.node;\ 3.term}$ = 154,056). Foliar applications were ineffective on the two bottom nodes because the eating activity of pea weevils moved onto upper nodes as soon as third node stipules appeared on plants. At the time of foliar application third node stipules were not yet unfolded. Therefore, the areas of third stipules were not fully covered by the insecticide sprays (treatments 6, 7 and 8). This resulted in the effects of foliar applications being significantly lower in comparison with the seed treatments on the third node 5 resp. 11 days after spraying ($F_{3.node;\ 2.term}$ = 149,682 resp. $F_{3.node;\ 3.term}$ = 122,481).

Troubsko 2008 (Table 3). A very low occurrence of *S. lineatus* imagoes was recorded in trial after crop emergence in that year. The first notches occurred when the plants had their fourth node stipules unfolded (29.4.). Plants originated from treated seeds were almost entirely intact at that time ($F_{29.4.08}$ = 4,212). Even 1 week after spraying (5.5.) the damage levels of sixth node stipules on seed treatments stayed markedly lower in comparison with untreated control and foliar treatment (treatment 6) ($F_{5.5.08}$ = 4,047).

Roots

Šumperk 2007 (Table 5). Significant differences among the mean numbers of nodules per five roots at the end of flowering were recorded (F = 181,789). In particular, roots originating from seeds treated with thiamethoxam (with or without fludioxonil and metalyxyl-M) were markedly more nodulated than the others. In addition, the roots from seeds treated with clothianidin and beta-cyfluthrin were also significantly more nodulated than roots from the control and foliar treatments. From the assessment of damaged nodule numbers it is obvious that seed treatments did not result in lower numbers of damaged nodules. On the contrary total numbers of nodules damaged by *S. lineatus* larvae were higher in seed treatments in comparison with the control (F = 7,1537). The highest numbers of still active nodules at the end of flowering were found on the roots that originated from treated seeds (F = 83,8140). Thiamethoxam (with or without fludioxonil + metalaxyl-M) showed a significantly positive effect on the prolongation of nodule activity on roots.

Troubsko 2007 (Table 6). Plants from seed treatments displayed significantly more numbers of undamaged nodules on their roots than plants from the control and sprayed plots (F = 7,101). Seed treatment in general had no effect on the reduction of damaged nodules numbers (F = 1,512) but was evident in lower mean portions of damaged nodules on root systems. The effects of seed treatments on the reduction of larvae numbers on roots proved non-significant after comparison to the control in 2007 (F = 3,136).

Šumperk 2008 (Table 5). Early insecticidal sprays had no positive effect on the total nodulation of roots but the seed treatments (especially their higher doses) showed significant effects (F = 85,481). As in 2007 both seed and foliar insecticidal treatments had no positive effect on the reduction of damaged nodules numbers (F = 15,5664) and only seed treatments had a significantly positive effect on the prolongation of nodule activity on roots. In contrast to 2007 such distinct differences between the effects of thiamethoxam and clothianidin + beta-cyfluthrin were not present in 2008 (F = 59,0053).

Troubsko 2008 (Table 6). Once again, significantly more undamaged nodules were recorded on roots originating from the treated seeds (F = 23,697). Furthermore, there were markedly fewer damaged nodules on the roots of the seed treatments in comparison with untreated control and sprayed treatment (F = 12,283). *S. lineatus* larvae

Table 4. Numbers of notches comparison. The results are derived from the consecutive assessments (Šumperk, 2008)

Treatment	Mean number of notches per stipule (SE)								
	1. node; 1. term	1. node; 2. term	1. node; 3. term	2. node; 1. term	2. node; 2. term	2. node; 3. term	3. node; 2. term	3. node; 3. term	4. node; 3. term
1 (UN)	8.78^{a} (0.40)	11.50^{a} (0.36)	11.58^{a} (0.37)	9.51^{ab} (030)	13.58^{a} (0.35)	14.21^{a} (0.26)	10.85^{a} (0.34)	13.16^{a} (0.42)	7.41^{a} (0.49)
2 (T LD)	0.89^{b} (0.13)	2.69^{d} (0.29)	3.18^{de} (0.32)	0.84^{d} (0.14)	3.26^{de} (0.30)	5.95^{cd} (0.27)	1.13^{ef} (0.19)	3.85^{e} (0.26)	1.34^{de} (0.22)
3 (T HD)	1.01^{b} (0.17)	2.39^{d} (0.22)	2.30^{e} (0.24)	0.86^{d} (0.14)	2.18^{e} (0.24)	3.06^{e} (0.26)	0.91^{f} (0.17)	1.59^{f} (0.19)	0.36^{e} (0.11)
4 (C + B LD)	2.41^{b} (0.25)	5.50^{c} (0.45)	6.16^{c} (0.39)	2.99^{c} (0.32)	6.96^{c} (0.47)	9.23^{b} (0.42)	4.54^{d} (0.37)	7.70^{c} (0.47)	4.00^{c} (0.45)
5 (C + B HD)	1.93^{b} (0.20)	3.11^{d} (0.31)	4.50^{d} (0.34)	1.90^{cd} (0.21)	3.96^{d} (0.33)	7.04^{c} (0.36)	2.34^{e} (0.32)	5.50^{d} (0.39)	2.49cd (0.36)
6 (Ch + Cy)	9.01^{a} (0.95)	10.66^{ab} (0.36)	9.83^{b} (0.39)	9.83^{a} (0.36)	11.94^{b} (0.45)	12.80^{a} (0.37)	8.63^{bc} (0.37)	9.54^{b} (0.46)	2.35d (0.34)
7 (Ac)	7.68^{a} (0.37)	9.46^{b} (0.40)	9.41^{b} (0.41)	8.43^{b} (0.41)	11.69^{b} (0.31)	13.45^{a} (0.31)	8.95^{b} (0.37)	12.45^{a} (0.35)	5.63^{b} (0.50)
8 (L - Cyh)	7.84^{a} (0.36)	10.65^{ab} (0.43)	9.64^{b} (0.37)	9.63^{ab} (0.38)	11.58^{b} (0.41)	12.83^{a} (0.32)	7.40^{c} (0.48)	9.69^{b} (0.41)	2.41d (0.34)
9 (TLD + F + M)	1.13^{b} (0.15)	2.48^{d} (0.24)	3.60^{de} (0.31)	0.89^{d} (0.14)	2.65^{de} (0.27)	5.51^{d} (0.38)	0.78^{f} (0.17)	2.95^{ef} (0.36)	1.25de (0.24)

1. term: 12. 5. 2008, prevailing growth stage BBCH 12; 2. term: 17. 5. 2008, prevailing growth stage BBCH 13; 3. term: 23. 5. 2008, prevailing growth stage BBCH 14–16
The mean values followed by different letters in one column are significantly different (Tukey test; $P < 0.05$); $N = 80$ stipules per treatment (20 per repetiton)

Table 5. Results of assessments of roots (Šumperk, 2007, 2008)

Treatment	Season 2007				Season 2008			
	mean No. of nodules per 5 roots (SE)	mean No. of damaged nodules per 5 roots (SE)	mean portion of damaged nodules per 5 roots (%)	mean No. of active nodules per 5 roots (SE)	mean No. of nodules per 5 roots (SE)	mean No. of damaged nodules per 5 roots (SE)	mean portion of damaged nodules per 5 roots (%)	mean No. of active nodules per 5 roots (SE)
1 (UN)	21.00^{a} (1.25)	7.50^{cd} (0.93)	35.71	0.63^{a} (0.22)	24.44^{a} (2.63)	12.94^{cd}(1.69)	52.95	2.50^{a} (0.52)
2 (T LD)	74.00^{c} (2.13)	15.50^{abc} (0.76)	20.95	22.50^{c} (1.78)	88.75^{c} (4.50)	28.50^{bc} (1.95)	32.11	22.69^{c} (2.02)
3 (T HD)	77.44^{c} (2.62)	15.13^{abc} (0.75)	19.54	26.31^{cd} (1.40)	113.25^{d} (4.78)	57.19^{a} (10.21)	50.50	30.56^{d} (1.56)
4 (C + B LD)	37.19^{b} (1.80)	8.94b^{cd} (1.11)	24.04	9.56^{b} (0.80)	57.75^{b} (3.55)	21.13b^{cd} (2.17)	36.59	15.56^{b} (1.61)
5 (C + B HD)	43.81^{b} (1.61)	17.31^{ab} (5.45)	39.51	11.88^{b} (0.97)	101.38^{cd} (5.26)	32.56^{b} (2.34)	32.12	24.94^{cd} (1.87)
6 (Ch + Cy)	25.31^{a} (2.61)	6.31^{d} (1.14)	24.93	3.06^{a} (1.09)	21.88^{a} (2.07)	10.13^{d} (1.15)	46.30	0.81^{a} (0.26)
7 (Ac)	21.81^{a} (1.24)	6.31^{d} (0.89)	28.93	0.88^{a} (0.29)	31.25^{a} (2.64)	13.25^{cd} (1.00)	42.40	4.69^{a} (0.91)
8 (L-Cyh)	22.81^{a} (1.36)	6.88^{cd} (0.47)	30.16	1.50^{a} (0.43)	33.63^{a} (2.03)	12.19^{cd} (1.24)	36.25	4.00^{a} (0.85)
9 (TLD + F + M)	90.31^{d} (2.84)	19.81^{a} (0.95)	21.94	28.19^{d} (2.36)	92.38^{c} (5.40)	24.06^{bcd} (1.78)	26.05	23.50^{c} (2.36)

The mean values followed by different letters in one column are significantly different (Tukey test; $P < 0.05$); $N = 16$ samples per treatment (4 per repetiton) occurred significantly more on the roots of the control and foliar treatment (F = 3,585).

DISCUSSION

Notches on stipules

Foliar applications sometimes showed their efficacy only on the node which was determined as the youngest node (either fully or almost fully unfolded) at the time of spraying. The damage levels of older nodes were often not substantially affected by the sprays because the stipules (unfolded earlier) had already become unattractive to the pea weevils at the time of application. Such applications were made too late with regards to the protection of older stipules. The numbers of notches grew up only slowly on the earlier unfolded nodes even on the control plants after the appearance of upper, new node (Table 4). It conforms to previous findings of Landon *et al.* (1995) that the pest prefers to attack new, tender leaves. On the other hand, the stipules which had not yet unfolded (or had only partly unfolded) at the time of spraying, were also later insufficiently protected. Regarding the stipules, we can say that applications were made too early (Tables 2–4). It can also have connection with other complicating factor: adult weevil arrives in the field in waves, so spraying too early means the later waves might be missed. Spraying too late means the early wave of females might have damaged plants and laid their eggs before being controlled (Barker 2007). From pyrethroids applied on leaves (stipules) it is not possible to expect any systemic effects (Vulsteke *et al.* 1994; Taupin & Janson 1997; Van de Steene *et al.* 1999). Neither the spraying with systemic neonicotinoid acetamiprid nor non-systemic organophosphorus chlorpyrifos did not resulted in better effects (Tables 2–4).

The most important advantage of the usage of systemic seed treatments over foliar treatments is a relatively long-lasting and sufficient protection of several bottom nodes simultaneously. According to Wnuk and Wiech (1996) pea plants up to 4-leaf stage are the most vulnerable to *S. lineatus* caused damage. When the dosage of thiamethoxam resp. clothianidin is correct the plants should be out of danger during the most vulnerable period (Rotrekl & Cejtchaml 2008). There is not any risk that the treatment would be made too early or too late.

Roots

The effects of the compared seed and foliar treatments on the reduction of *S. lineatus* larvae numbers on roots were not proven as positive. It is possible to conclude that the foliar application had no real effect in this sense at all – it is in agreement with some previous studies (Vulsteke *et al.* 1994; Taupin & Janson 1997; Van de Steene *et al.* 1999). Seed treatments were clearly effective only in 2008 (Table 6). Significantly lower mean numbers of damaged nodules on treated alternates were only apparent at Troubsko in 2008. There was recorded significant effect of seed treatments in that year but otherwise the mean number of damaged nodules was not affected by insecticides (especially foliar applications) or was even higher (sometimes significantly) for the seed treated specimens (Tables 5 and 6). Some authors (Bachmann & Elmsheuser 1986; Salter & Smith 1986; Vulsteke *et al.* 1994; Taupin & Janson 1997; Van de Steene *et al.* 1999) advert to very good effects of several older insecticides used as a seed treatment (especially furathiocarb) on *Sitona* larvae. Unfortunately, there are not any studies aimed at evaluation of thiamethoxam resp. clothianidin effects on *S. lineatus* larvae among the recent available literature sources.

Regardless of the somewhat ambiguous insecticidal effects on *S. lineatus* larvae, the positive effects of thiamethoxam and clothianidin on the nodulation of roots were clearly proven at both localities and in both years. The total nodulation of roots originating from treated seeds was markedly higher compared to the nodulation levels of roots from untreated controls and foliar treatments (Tables 5 and 6). Furthermore, the positive effect of the seed treatments on the prolongation of nodule activity was also shown to be significant (Table 5). It is well known that some neonicotinoids offer growers additional benefits beyond broad-spectrum insect protection. The effects are evidenced (among others) by increased root mass of treated plants (Nauen *et al.* 2003; Cox *et al.* 2007; Tansey *et al.* 2008). Unfortunately, we did not find any available study which evaluates effects of seed-applied neonicotinoids on the root nodulatin of pea (*Pisum sativum*). In general, the effects of various pesticides on *Rhizobium* spp. nodulation and nitrogen fixation in leguminous plants have been studied for a long time (Pareek & Gaur 1970; Aggarwal *et al.* 1986; Ahmad *et al*. 2006). As legumes grow in symbiosis with soil bacteria, *Rhizobium* spp., the compatibility of fungicide or insecticide treatments with the bacteria and with the establishment of symbiosis can be crucial for the plant growth (Ahmad *et al*. 2006). The positive growth effect of thiamethoxam and clothianidin + beta-cyfluthrin on root nodulation showed in the study could be the major factor which markedly distinguishes seed and foliar treatments from one another in their implications for the development of pea crops. The effects could present a great advantage to farmers even in those years with low occurrences of pea weevils.

Acknowledgements. The authors would like to thank to Darren Eve for revision of the manuscript.

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Received for publication August 4, 2009
Accepted after corrections November 6, 2009

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