Ovicidal Effects of Thiacloprid, Acetamiprid, Lambda-Cyhalothrin and Alpha-Cypermethrin on *Bruchus pisorum* L. (Coleoptera: Chrysomelidae) Eggs

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Abstract

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The effects of two pyrethroid (lambda-cyhalothrin, alpha-cypermethrin) and two neonicotinoid (acetamiprid, thiacloprid) insecticides on *B. pisorum* L. eggs were compared under field conditions in the Czech Republic in 2005–2007. The main objective of the study was to find out what real effects can be expected from the available insecticides registered in Europe when applied at the time of the first egg occurrence on lower pods. In general, the rates of *Bruchus pisorum* egg (+ first instar larvae) survival were significantly lower with all the insecticides compared in the study, in each of the three years (2005, 2006, 2007). The tested insecticides showed some ovicidal effects and also some larvicidal effects. The tested pyrethroids (lambda-cyhalothrin, alpha-cypermethrin) showed somewhat higher effectiveness in comparison with the neonicotinoids (acetamiprid, thiacloprid). Alpha-cypermethrin was the most effective in all three years. In 2006 and in 2007 this insecticide significantly reduced the rates of egg survival in comparison with acetamiprid. In contrast, acetamiprid was the least effective insecticide in each of the three years.

Keywords: *Bruchus pisorum*; pea weevil; pyrethroids; neonicotinoids; ovicidal effects

Bruchus pisorum L. can complete its univoltine life cycle only on the garden and field pea, *Pisum sativum* L. (Burov 1980; Annis 1983; Clement 1992). Reproductively mature females attach eggs to green pods. The eggs are orange-yellow and are 1.5 mm long and 0.6 mm wide (Smith & Hepworth 1992). They can be laid over two to three weeks in a particular locality and the mean numbers of eggs per affected pod can vary from 2.0 to 4.0 under natural conditions. A portion of the eggs is always oviposited in the form of two-egg clusters – then only the bottom egg is attached directly to the pod cover (Seidenglanz *et al*. 2007). The duration of insect egg development is primarily a function of temperature but it can also be affected by other factors (Howe 1967; Smith 1992). Neonate larvae (1st instar) have to penetrate through the pod valve and the seed coat (*testa*) before they get into cotyledons. With regard to the effects of insecticides it is important that the larvae do not occur unsheltered on the pod valve surface after leaving the eggs. They are still protected by the egg coat. Neonate larvae burrow into the pod valve just under the eggs. Development is completed through four larval instars and pupa (Smith & Ward 1995). The management of pea weevil is

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traditionally aimed at controlling adult beetles in a crop before they lay eggs onto pods (Horne & Bailey 1991; Smith 1992; Smith & Hepworth 1992). However, there is a great problem with the timing of spraying. It is not easy and reliable to monitor adults in pea fields (using sweep nets) at the time just before flowering or in the course of flowering (Seidenglanz *et al*. 2006, 2007). It leads to the poor timing of spray applications, which often miss the beetles themselves and the period of egg laying as well. The monitoring of eggs seems to be more useful. Spraying is then carried out at the time the first eggs are seen on the pods (Horne & Bailey 1991). However, there is an absence of information about the effects of available insecticides on eggs or on the first instar larvae. In Europe pyrethroids in particular, and occasionally also neonicotinoids, are used. After treatment pea plants usually evolve new upper nodes (also suitable places for oviposition) and these can be attractive to ovipositing females for quite a long time. Hence the other unresolved problems of available insecticides are systemic effects, repellent activity and residual activity (in relation to the duration of the egg laying period).

In this paper we report on experiments that compared the effects of two pyrethroid (lambda-cyhalothrin, alpha-cypermethrin) and two neonicotinoid (acetamiprid, thiacloprid) insecticides on *B. pisorum* eggs under field conditions. The main objective of this study was to find out what real effects could be expected from the available insecticides, when the times of spraying are derived from the monitoring of egg occurrence on the bottom nodes.

MATERIAL AND METHODS

The trials were conducted in 2005–2007 in trial fields in Šumperk (Northern Moravia, Czech Republic); precisely, these were small-plot trials (5 treatments in 4 replications; plots 1.25 × 5.0 m; total trial area of 9.5 × 20.5 m; each plot bordered on both sides with a 1.25 m wide untreated strip of pea plants) sown with pea (*Pisum sativum* L.) variety Zekon (semi-leafless) every year. The plants in the trial were exposed to natural infestation by pea weevil (*B. pisorum* L.) adults. Daily monitoring of pods on bottom nodes for the presence of pea weevil eggs started once the small pods on the first node appeared. Spraying was carried out with a HEGE 32 self-propelled trial sprayer with HEGE 76 implement carrier (HEGE Maschinen GmbH, Waldenburg, Germany; three separate spraying paths – each with six nozzles; spraying span 3 m; type of nozzle XR TEEJET; No. of nozzle 80015 VS; application pressure 0.3 MPa; flow rate 312.5 l ha) after the appearance of the first eggs on pods on two bottom nodes, in accordance with the treatment plan trial 1: control; trial 2: thiacloprid (96 g a.i./ha); trial 3: acetamiprid (36 g a.i./ha); trial 4: lambda-cyhalothrin (7.5 g a.i./ha); trial 5: alpha-cypermethrin (12.5 g a.i./ha) (NB. [1] in Table 1). Immediately after spraying a minimum of 20 pods (1 pod per plant) from two bottom nodes carrying 2–4 eggs (individuals) were chosen on each plot. These chosen pods were marked (tagged with an identification number) and covered with a transparent textile pocket (avoiding any damage to plants and eggs) to stop further eggs being laid on them. The number of eggs on the chosen pods and the portion of eggs (individuals) laid in the form of two-egg clusters were recorded. At the time of ripening the marked pods were picked. The identification of pods was maintained. Then (in the laboratory) the sum of all larval entries to all seeds per pod was determined individually for each pod. Some samples showed more than one entry per individual seed. The entry openings are clearly visible on the round green pulses. Thus the survival of eggs (+ first instar larvae) was determined on the basis of comparing the recorded number of eggs per pod (immediately after treatment) with the number of larval entry openings per pod (after harvest in the laboratory) for each chosen pod individually. The pods with only two seeds (and less) were excluded from the assessment. The obtained data were statistically analysed (one-way ANOVA and subsequently Tukey's test to find out differences between the mean values) using the UNISTAT – Statistical Package, Version 4.53 (Unistat Ltd, London, UK). The effectiveness of the individual treatments was expressed using Abbott's formula (Abbott 1925). It is a simple method of computing the corrected effectiveness of insecticides (NB. [4] in Table 1).

RESULTS

It is obvious from Table 1 (2nd columns) that the choice of pods resulted in a small variability among the mean numbers of eggs per affected pod.

Table 1. The mean numbers of eggs on assessed pods immediately after treatment, the proportions of eggs laid in two-egg clusters and the ovicidal effectiveness of the compared insecticidal active ingredients under field conditions in 2005, 2006 and 2007

Treatment[1]	Mean number of eggs (individuals) per assessed pod[2]	Proportion of eggs (individuals) laid in the form of two eggs clusters (%)	Level of *B. pisorum* eggs survival (%)[3]	Effectiveness of treatment using the Abbott formula (%)[4]
2005 (treatment June 23)				
Control	2.95	63.56	92.73[a]	0.00
Thiacloprid SC (96 g a.i./ha)	2.99	69.46	41.30[b]	55.46
Acetamiprid SP (36 g a.i./ha)	3.03	66.94	47.00[b]	49.31
Lambda-cyhalothrin CS (7,5 g a.i./ha)	2.90	61.21	31.27[b]	66.28
Alpha-cypermethrin SC (12,5 g a.i./ha)	3.05	63.93	26.51[b]	71.41
2006 (treatment June 28)				
Control	3.05	69.67	92.99[a]	0.00
Thiacloprid SC (96 g a.i./ha)	2.91	69.52	46.44[bc]	50.06
Acetamiprid SP (36 g a.i./ha)	3.06	71.83	49.28[b]	47.00
Lambda-cyhalothrin CS (7,5 g a.i./ha)	3.01	67.22	30.45[bc]	67.25
Alpha-cypermethrin SC (12,5 g a.i./ha)	2.97	64.71	23.45[c]	74.78
2007 (treatment June 20)				
Control	2.96	72.57	88.15[a]	0.00
Thiacloprid SC (96 g a.i./ha)	3.09	73.68	45.68[b]	48.18
Acetamiprid SP (36 g a.i./ha)	2.99	69.46	47.67[b]	45.93
Lambda-cyhalothrin CS (7,5 g a.i./ha)	3.14	67.73	30.64[bc]	65.24
Alpha-cypermethrin SC (12,5 g a.i./ha)	3.16	74.31	24.54[c]	72.16

[1]thiacloprid (Calypso 480 SC; 0.2 l/ha); acetamiprid (Mospilan 20 SP; 180 g/ha); lambda-cyhalothrin (Karate Zeon 5 CS; 0.15 l/ha); alpha-cypermethrin (Vaztak 10 SC; 0.125 l/ha)

[2]choice pattern resulted in a small variability of the results of pod sampling (ANOVA: $F = 0.710$; df = 4, 12; $F_{tab} = 3.259$; $P = 0.05$); $n = 80$ (pods per treatment)

[3]numbers marked with the diverse letters are significantly different [ANOVA and subsequently Tukey's test; transformation: $x = \arcsin\sqrt{(x/100)}$]

[4]effectiveness 0.00 is always assigned to the control; corrected effectiveness (%) = [1 – (mean number of larval entries to seeds per pod in sprayed treatment/mean number of larval entries to seeds per pod in control) × 100]

It is possible to say that the starting situation was always well-balanced in the trials.

The proportion of eggs (individuals) laid in the form of two-egg clusters varied slightly for the individual treatments in each of the years (2005–2007; Table 1). We did not analyse these values statistically due to the low number of data entries ($n_{\text{per treatment}} = 4$) and owing to the purely supplemental character of the assessment.

In 2005 the used insecticides had a highly significant influence on the outcomes of the trial (ANOVA: $F = 26.176$; df = 4, 12; $F_{tab} = 5.412$; $P < 0.01$). Significantly higher rates of *Bruchus pisorum* egg survival (%) were recorded in the control than in the other treatments (Tukey's test, $P < 0.01$). However, no significant differences were found among the effects of individually compared insecticides on the pea weevil eggs (Tukey's test, $P = 0.05$) (Table 1). The comparison of the mean pea weevil egg survival, which was expressed separately for the group of pyrethroid insecticides (grouped results of lambda-cyhalothrin and alpha-cypermethrin) and for the group of neonicotinoid insecticides (grouped results of thiacloprid and acetamiprid), revealed no significant differences between the two groups (ANOVA: $F = 2.181$; df = 3, 9; $F_{tab} =$

Table 2. Comparison of insecticidal effects on *Bruchus pisorum* L. eggs in 2005–2007

Treatment	2005		2006		2007	
	level of *B. pisorum* eggs survival (%)[1]	effectiveness of treatment in according to Abbott (%)[2]	level of *B. pisorum* eggs survival (%)[1]	effectiveness of treatment in according to Abbott (%)[2]	level of *B. pisorum* eggs survival (%)[1]	effectiveness of treatment using the Abbott formula (%)[2]
Neonicotinoid group[3]	44.15	52.39	47.86	48.53	46.67	47.05
Pyrethroid group[4]	28.89	68.85	26.95	71.02	27.59	68.70

[1]only in 2007 were significant differences found between the effects of the two compared insecticidal groups on *Bruchus pisorum* eggs mortality (ANOVA: $F = 6.158$; df = 3, 9; $F_{0.05} = 3.860$; $F_{0.01} = 6.990$; $P < 0,05$; Transformation: $x = \arcsin\sqrt{(x/100)}$)

[2]effectiveness 0.00 is always asigned to Control

[3]grouped results for acetamiprid and thiacloprid ($n = 160$)

[4]grouped results for lambda-cyhalothrin and alpha-cypermethrin ($n = 160$)

3.860; $P = 0.05$) in 2005 (Table 2). However, the effectiveness of pyrethroid insecticides (especially of alpha-cypermethrin) seems to be relatively higher (Tables 1 and 2).

In 2006 the insecticidal treatments also had a highly significant influence on the outcomes of the trial (ANOVA: $F = 27.172$; df = 4, 12; $F_{tab} = 5.412$; $P < 0.01$). On the basis of Tukey's test ($P < 0.01$) there were highly significant differences between the mean rate of *B. pisorum* egg survival in the untreated control and in all the treated variants in general (Tukey's test, $P < 0.01$). Significant differences were also found between the effects of alpha-cypermethrin and acetamiprid (Tukey's test; $P < 0.05$) (Table 1). The comparison of the mean pea weevil egg survival, expressed separately for the group of pyrethroid and neonicotinoid insecticides, did not prove a significant difference between the two groups (ANOVA: $F = 3.662$; df = 3, 9; $F_{tab} = 3.860$; $P = 0.05$) in 2006 (Table 2). The alpha-cypermethrin treatment was once again the most effective for that year (the effectiveness expressed according to Abbott exceeded 70%) (Table 1).

In 2007 the insecticidal treatments also had a highly significant influence on the outcomes of the trial (ANOVA: $F = 34.765$; df = 4, 12; $F_{tab} = 5.412$; $P < 0.01$). Significantly higher rates of egg survival (%) were recorded in the control than on the treated plots. (Tukey's test, $P < 0.01$). The trials also revealed a significantly higher effect of alpha-cypermethrin in comparison with acetamiprid and thiacloprid on the pea weevil eggs in 2007 (Tukey's test; $P < 0.05$) (Table 1). From the comparison of the mean pea weevil egg survival, expressed separately for the group of pyrethroid and neonicotinoid insecticides, the pyrethroid group emerged as significantly more effective (ANOVA: $F = 6.158$; df = 3, 9; $F_{0.05} = 3.860$; $F_{0.01} = 6.990$; $P < 0.05$) (Table 2). Alpha-cypermethrin was the most effective that year again (the effectiveness exceeded 70% again) (Table 1).

DISCUSSION

We recorded that pea weevil females laid somewhat more clusters of two eggs than single eggs. The proportions of eggs (individuals) laid in the form of two-eggs clusters (%) ranged from 60% to 75%. This means that most of the newly hatched larvae should come from egg clusters. Maybe the uppermost eggs serve as a shield contributing to the lower mortality of eggs (and subsequently larvae) located just on the pod cover (Seidenglanz *et al.* 2006). The results in Table 1 document that the effects of neonicotinoid insecticides were lower in the years when the higher proportions of two-egg clusters on pods were recorded. The effects of pyrethroids were more stable in this aspect. At the time of writing the manuscript the authors had no information about studies evaluating how the type of egg influences insecticidal effects.

The ovicidal effect of the individual insecticides can be influenced by temperature conditions, crop density, position of eggs on the pod in relation to the direction of spraying, the form of the eggs, pea variety and also the prevailing morphological stage of eggs at the time of spraying (Smith 1992). In the trials (2005, 2006, 2007) the eggs on bottom pods were either at stage 1 or mostly at stage 2 (according to the scale described in Smith (1992) during the spraying. Black-spotted eggs (stage 3) were not observed at those times.

The assessments of pod infestation were based on two initial presumptions: each larva which gets into the pod cavity is able to make only one point of entry into the seed (clearly visible on green and round seeds as a small rusted spot). Each larva makes its own entry into the seed.

It is evident from the assessment method that it was not possible to distinguish exactly what portion of the recorded *B. pisorum* mortality is a consequence of the direct effect of insecticide on eggs and what is the consequence of larvicidal effects. A certain proportion of hatched larvae can die in the course of coming through the pod valve. Such individuals could be negatively affected at the egg stage and their death in pod tissues is a consequence of the previous insecticidal effect on the eggs. However, the death of larvae can also be caused by direct effects of insecticide in pod tissues (larvicidal effect). At the time of writing the manuscript, the authors did not have any information about studies comparing ovicidal and larvicidal effects of pyrethroids or neonicotinoids on *Bruchus pisorum*. We were able to record only differences among the starting status (number of individuals = eggs/pod) and the final status (number of successful individuals = first instar larvae/pod) at the individually assessed pods. Hence we were not able to trace if there were any differences in ovicidal and larvicidal effects among the compared insecticides.

The trial results could not be influenced by eggs which were laid after the spraying. Hence neither residual effects nor repellent effects (on females) of insecticides were assessed. The compared insecticides significantly decreased the survival of eggs (+ first instar larvae) in all three years (2005, 2006, 2007) (Tables 1 and 2). The insecticides showed some ovicidal and maybe also some larvicidal effects under field conditions. The effectiveness levels of tested pyrethroids (lambda-cyhalothrin, alpha-cypermethrin) seem to be somewhat higher in comparison with the neonicotinoid (acetamiprid, thiacloprid) effects.

Though the future of pea weevil control will probably be connected with the use of genetic technologies (bean α-amylase inhibitors in transgenic peas) (Schroeder *et al.* 1995; Sousa-Majer *et al.* 2004; Gatehouse 2008 and many others), European growers are still fully dependent on the use of insecticides (especially pyrethroids) and they cannot usually afford more than 1 application aimed at this particular pest. In addition, the demanding infestation limits for pea seeds strictly required from growers in Europe complicate the difficulties with this pest. With regard to the achieved insecticidal effectiveness in the trials (which ranged from 46% to 56% for neonicotinoids and from 65% to 75% for pyrethroids) and to the fact that the ovipositing activity of *B. pisorum* females can take about two to three weeks and can shift onto the upper nodes, it is also necessary to draw this conclusion: The control of pea weevils based on one application of the tested insecticides does not always achieve satisfactory results when the spray timing is derived from the first eggs occurring on pods.

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