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Efficient Conifer Seedling Protection Against Pine Weevil Damage Using Neonicotinoids

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Abstract

The large pine weevil *Hylobius abietis* (L.) is the most important pest of coniferous plantations in Europe. Two neonicotinoid insecticides, acetamiprid and thiacloprid, have been tested in field conditions to check to what extent they can protect softwood seedlings from injuries caused by this pest. The experiment was organised in a randomized complete block design, included five treatments (two concentrations of 0.5% and 1.0% of each insecticide, and a control) and was performed on four experimental clear-cut sites, three recently-felled, and one of 1-year old, with high populations of large pine weevil. The seedlings were treated by dipping their aerial parts into insecticide emulsion before planting. The tested treatments diminished the proportion of the attacked seedlings only to a moderate extent (by 36.4-89.8%) but greatly reduced the size of the wounds and the mortality of the seedlings, by more than 82.7% and 89.7-100%, respectively. In three of the four experimental sites, no treated seedlings were killed through weevil attack, while 36% of the untreated plants died during the first vegetation season after planting. There were no statistically significant differences between the 0.5% and 1% treatment concentrations of either commercial product, suggesting that under similar conditions the 0.5% concentration could be used to diminish the costs of the treatments. The treatments were highly effective up to the end of the first growing season (5 months after application), even when the density of weevil populations increased through the emergence of new adult beetles from root stumps.

Keywords: Hylobius abietis, Picea abies, seedling protection, neonicotinoids

Introduction

The large pine weevil, Hylobius abietis (L.) (Coleoptera: Curculionidae), is the most important pest of coniferous plantations in Europe (Långström and Day 2004, Evans et al. 2015) and many studies have been conducted to find appropriate ways to prevent the injuries caused by adults feeding on transplanted seedlings. In the last decades considerable progress has been made towards developing an integrated pest management system (Evans et al. 2004, Nordlander et al. 2011, Skrzecz 2017, Willoughby et al. 2017). The published research has focused mainly on biological control of weevil populations using entomopathogenic nematodes and fungi (Armendáriz et al. 2002, Brixey et al. 2006, Dillon et al. 2006, 2008, Ansari and Butt 2012, Skrzecz et al. 2012, Williams et al. 2013a, b, 2015, Martin et al. 2016, Popowska-Nowak et al. 2016, Kapranas et al. 2017a, b), or on improving the resistance to attack by treating the seedlings or the seeds with

jasmonic acid or methyl jasmonate (Heijari et al. 2005, Moreira et al. 2013, Sampedro et al. 2011, Zas et al. 2014, 2017, Berglund et al. 2016, Fedderwitz et al. 2016, Lundborg et al. 2016). Some other measures and means for an integrated pest management, like stump removal or debarking (Skrzecz et al. 2016, Rahman et al. 2018), soil preparation before planting (Petersson et al. 2005, Luoranen and Viiri 2012) and the use of feeding barriers (Nordlander et al. 2009, Härlin and Eriksson 2011, 2014, Eriksson et al. 2017) have also been tested. However, there are still many situations where treating the seedlings with insecticides to prevent damage is inevitable (Willoughby et al. 2017) and synthetic pyrethroids are replaced with neonicotinoids like imidacloprid and acetamiprid, systemic insecticides which are less toxic for aquatic life and with very short persistence in soil, but there are few published data demonstrating the efficacy of these insecticides in field conditions (Nordlander et al. 2009, Härlin and Eriksson 2011, 2014, Wallertz et al. 2016, Eriksson et al. 2017). These and

other substances have yielded promising results under laboratory conditions (Rose et al. 2005, Malinovski 2010, Olenici et al. 2014), but they should also be tested under field conditions, where the action of various environmental factors (solar radiation, precipitation) could significantly reduce their efficiency. Consequently, we present the results of a field experiment, in which spruce seedlings were protected against Hylobius feeding by dipping their aerial parts before planting into an emulsion of acetamiprid or thiacloprid. Imidacloprid has been temporarily banned by the European Commission (2013), because of concerns about the risk it may pose to pollinators and this is why it was not considered for the tests carried out in 2014 and presented in this paper, although all three active substances (acetamiprid, imidacloprid and thiacloprid) do not require a derogation for use in FSC certified forests (FSC 2017).

The main research objective was to assess the treatment efficacy in conifer seedling protection against weevil feeding when the seedlings are treated by dipping their aerial parts into insecticide emulsion before planting, but also to assess any possible change of efficacy during the first season after planting. The insecticide emulsions were used both at nominal and half-concentration, to test the possibility to use a lesser amount of insecticide.

Materials and Methods

Site location and description

The experiment was carried out in 2014 in four experimental sites located in the northern part of the Romanian Eastern Carpathian Mountains, two at Demacuşa (DEM1 and DEM2) and two at Baranova (BRN1 and BRN2) (Table 1). The climate in this zone is classified as Dfb by the Köppen-Geiger system (Rubel and Kottek 2010), that means cold continental without dry season and with warm summer. The average annual temperature is 6.7°C and 4.7°C in Demacuşa and Baranova, respectively, while the average precipitation is 684 mm and 804 mm. All experimental sites are situated in the montane

forest belt, the first two ones in the Fagus-Picea-Abies sub-belt, and the other two ones in the *Picea* sub-belt. Clear-cutting of forest over several hectares is a current practice in the region, especially in the spruce sub-belt, and consequently the populations of *H. abietis* are generally high (Olenici et al. 2016).

The harvesting of the forest stands was carried out during January-March 2013 in BRN2, between 15 November and 15 December 2013 in DEM2, and during January-March 2014 in DEM1 and BRN1. Consequently, the first experimental plot was one year old, while the last three were considered 'fresh' clear-fells at the time of the experimental planting.

To ensure that the experimental results were not distorted by the presence of alternative food sources for weevils, the location of the experimental blocks was chosen as having no natural regeneration (present especially in DEM1 area) and no felling debris (excepting the tree stumps – Table 2). In DEM2, where the felling debris had been gathered into heaps at the time of selection of the experimental areas, the experimental blocks were located among the heaps. At BRN1, the fresh and very abundant debris was completely removed from the area of the experimental blocks, but not in the vicinity of them, while at BRN2 the debris was already completely dry and could not be a source of food for the weevils.

Weevil population assessment

To assess the size (relative density) of the large pine weevil populations, one pitfall trap similar to those used by Nordlander (1987), baited with alpha-pinene and ethanol was installed in each experimental block. The traps stayed in the field between April 17 and September 11 at DEM1 and DEM2 and between April 29 and September 16 at BRN1 and BRN2, respectively. They were checked weekly at Demacuşa and at two weeks at Baranova for weevil collection, and the attractants were refreshed every two weeks. The weevils were counted in the laboratory and the relative density of the populations calculated as the mean number of captured weevils per trap during the entire growing season (Table 2).

| | Forest district | | Old star | d characte | ristics | | | |
|-------------------|-----------------|-----------------------------|-----------------------------------|----------------|---------------|---------------------------|-----------------|----------------------|
| Experimental site | and compartment | Geographical coordinates | Composi- tion ¹ (%) | Age (years) | Canop y cover | Soil type ² | Altitude (m) | Aspect/ Slope (°) |
| Demacu§a_1 | Tomnatic, I, | 47º42'19"N | 100Pa | 100 | 0.3 | F0 | 000 | S/11 |
| (DEM1) | 90B | 25°24'30"E | | | | EC | 880 | 5/11 |
| Demacuşa_2 | Tomnatic, I, | 47°42'36"N | 40Pa40Aa | 120 | 0.4 | | 000 | 014//4.0 |
| (DEM2) | 100L | 25°25'43"E | 20Fs | s | | EC | 900 | SW/18 |
| Baranova_1 | Cârlibaba, | 47°42'46"N | 100Pa | 115 | 0.6 | PI | 1230 | S/10 |
| (BRN1) | VII, 49I | 25°04'30"E | IUUPa | 1 15 | 0.0 | PI | 1230 | 5/10 |
| Baranova_2 | Cârlibaba, | 47°42'46"N | 100Pa | 115 | 0.6 | ъ. | 1000 | 0.14.0 |
| (BRN2) | VII, 49I | 25º04'30"E | | | | PI 1230 | | S/10 |

Picea abies; Aa Abies alba. Fagus sylvatica; ²EC - Eutric Cambisol, Pl - Episkeletic Podzol

Table 1. Location and the main characteristics of the experimental sites

Table 2. Characteristics of conifer stumps and the relative density of weevil populations within the experimental plots

| Francis: | | Number of | | | |
|----------|-----------|---------------|-----------------|---------------------------|--|
| Experi- | | Surface area | Diameter | | |
| mental | Number/ha | of cut stumps | (cm) | weevils/ trap | |
| site | | (m²/ha) | (mean ± SD) | (mean ± SD) ¹ | |
| DEM1 | 483 | 80.4 | 45.2 ± 9.3 | 249.6 ^b ± 69.6 | |
| DEM2 | 221 | 53.8 | 50.3 ± 24.2 | 418.4° ± 122.9 | |
| BRN1 | 1024 | 92.5 | 32.5 ± 17.0 | 142.5° ± 54.6 | |
| BRN2 | 1052 | 82 7 | 27 6 + 5 1 | 297 Nb + 39 1 | |

 ^{1}The means followed by the same superscript are not statistically different at α = 0.05 (One-way ANOVA test followed by Tukey (HSD))

Design of experiment

Two insecticides were tested, each applied in two concentrations (T1-T4 in Table 3). A polymeric adjuvant, Spodnam DC (main active ingredient cyclohexene, 1-methyl-4-(1-methylethyl)-, dimer, also called Pinolene), was added to the insecticide emulsion in a 1% commercial product concentration. The treatment consisted of dipping the aerial parts of the seedlings into emulsion of insecticide and adjuvant. Approximately 4 L solution is used to treat 1,000 seedlings, but the amount varied depending on the size of the seedlings.

The experiment was organized in a randomized complete block design, each block comprising 5 plots corresponding to the 5 treatments (including untreated seedlings as a control). On each plot (experimental unit) 20 seedlings with the same treatment were planted.

In each of the experimental sites DEM1 and DEM2 there were 5 blocks (replications), totalling 500 seedlings, while in BRN1 and BRN2 there were only 4 blocks, with a total of 400 seedlings. The distance between blocks was at least 30 m in each experimental site, except DEM1 where the blocks were set up closer (15-20 m) to one another.

Seedlings

In the experiment, 3-year-old bare-rooted seedlings of Norway spruce (*Picea abies* (L.) H. Karst) were used, which came from the nurseries of forest districts where the experiment was conducted. In Demacuşa, the seedlings were treated on the 4th of April and planted on the 7th and 8th of April, while in Baranova the treatment took place on 28th of April and the planting on 28th and 29th of April 2014.

Table 3. Experimental treatments and the number of seedlings used in each treatment

| Experimen- | Lance Calaba | A . I . | Concentra- | Number of seedlings/treatments | | | | |
|------------------|-------------------|-------------------------|-------------------------------------|--------------------------------|-------|-------|-------|--|
| tal treatment | Insecticide | Active ingredient | tion of commercial product, % | DEM_1 | DEM_2 | BRN_1 | BRN_2 | |
| T1 | Calypso 480 SC | Thiacloprid 480 g/l | 0.5 | 100 | 100 | 80 | 80 | |
| T2 | Calypso 480 SC | Thiadoprid 480 g/l | 1.0 | 100 | 100 | 80 | 80 | |
| Т3 | Mospilan 20 SG | Acetamiprid 200 g/kg | 0.5 | 100 | 100 | 80 | 80 | |
| T4 | Mospilan 20 SG | Acetamiprid 200 g/kg | 1.0 | 100 | 100 | 80 | 80 | |
| Control | Untreated | - | - | 100 | 100 | 80 | 80 | |

Measurements in the field

Observations of weevil attack were made in July (Demacuşa – 10-15.07.14; Baranova – 7-9.07.2014 and 18.07.14) and then at the end of September (Demacuşa – 24-25 and 29-30.09.2014; Baranova – 17-19.09.2014). On both occasions, each seedling was checked for weevil wounds and the extent of wounds (debarked area) was assessed, measuring the length and width of each piece of wounded bark with a ruler divided in millimetres, the precision being 0.5 mm. Areas with very irregular contours were divided into relatively regular portions to minimize the errors of surface estimation. The dead seedlings were examined to establish the cause of their death (weevil-caused death or death from other causes).

Statistical analyses

The null hypothesis is that the treatments have no effect on weevil feeding and do not influence the proportion of seedlings that suffer from debarking, or the extent of the wounds. The alternative hypothesis is that the treatments affect the weevil feeding, reducing the proportion of seedlings suffering wounds, and also the size of debarked area of each wounded seedling.

Because the experimental unit was represented by the group of 20 seedlings in each plot, to avoid pseudoreplication in statistical analyses (Hurlbert 1984), the sum of debarked areas for all 20 seedlings and the proportion of wounded or dead seedlings from the group was used, rather than individual values recorded for each seedling. For statistical analyses, data concerning the debarked area were square-root- or logarithmic-transformed (x' = sqrt(x) or x' = log(x+1)), and those concerning the proportions were arcsine transformed (x' = arcsin(sqrt(x)), to normalize the distributions and to ensure the homogeneity of variances (Zar 2010). The normality of distributions and equality of variances were checked using the Shapiro test and Levene test, respectively. When assumptions of distribution normality and homoscedasticity were fulfilled, an analysis of variance was performed using a one-way ANOVA test, or the Kruskal-Wallis test otherwise. Using multiple comparison post hoc analysis to compare the tested groups, it was found that in most cases there were no statistically significant differences between the treatments T1-T4; in some in-

stances, the tests did not identify significant differences between any of treatments T1-T4 and the control, although ANOVA or Kruskal-Wallis tests indicated the existence of such differences. Consequently, the pair-wise comparisons were limited only to those between T1-T4 on the one side, and the control on the other side, using the Dunnett and Steel procedures after the ANOVA test and Kruskal-Wallis test, respectively.

To compare the mean debarked area and the proportion of damaged seedlings in July with that in September, the two-sided paired t-test was used, and for the treatments T1-T4 the observed values were pooled. For all statistical analysis, the level of significance was set at 5%.

For situations, where all distributions became normal after data transformation, the mean, or geometric mean, when logarithmic transformation was used (Zar 2010), is given as an indicator of central tendency, while for nonnormal distributions the median value is given. The presented results are on the original scale, the values being obtained by backward transformation of mean or median of the analysed data. Because the standard deviation of back-transformed data is meaningless, the limits of 95% confidence interval (CI) of the mean are given. For nonnormal distribution, the mean of ranks is given too, because the Kruskal-Wallis test compares the sum of ranks, not the means or the medians of analysed data.

Finally, the efficacy of treatments was assessed comparing the average area of wounds and the proportion of damaged seedlings in T1-T4, with the same parameters computed for the control (untreated) seedlings.

Results

Debarked area in treated and untreated seedlings

At the first assessment of the attack characteristics in July, the level of injuries suffered by insecticide treated seedlings was much lower (18.4-191.9 mm²/20 seedlings) than that of untreated seedlings (Table 4), but statistically significant differences between treatments and control, at p = 0.05, were found only in the first two experimental areas (DEM1 and DEM2). In September, the differences become more obvious and were statistically significant for all experimental sites (Table 5). Between July and September observations the debarked area increased significantly both in treated and untreated seedlings (Figures 1-2) and the ratio of increase was about the same (1.5-3.0 times) in treated and untreated seedling from all experimental sites, excepting BRN2, where treated seedlings had a debarked area 3.7 times and untreated seedlings 15.5 times larger than in July. All the data suggest that the protection efficacy of the treatment did not decline in the second part of the season.

Proportion of damaged seedlings

The proportion of seedlings injured by weevils varied between experimental sites and from July to September (Table 6-7), but generally the treated seedlings were

| N2 |
|----------------------------|
| |
| Ranks mean ³ |
| 5.6 |
| 9.1 |
| 10.6 |
| 11.8 15.4 |
| |

¹The means followed by * significantly differ from the control at α = 0.05 (One-Way ANOVA test and Dunnett procedure)

Table 4. The average debarked area (mm2) at the plot level (20 seedlings) in treated (T1-T4) and untreated (control) seedlings in July

| | DEM1 | | DEM2 | | | BRN1 | BRN2 | | |
|-----------|--------|----------------------------|--------|---------------|-------------------|---------------|--------|---------------|--|
| Treatment | Median | Ranks mean ¹ | | 95% CI | Mean ³ | 95% CI | | | |
| T1 | 288 | 14.4* | 244.7* | 70.0/525.4 | 266.2* | 97.3/518.3 | 69.9* | 0.0/364.5 | |
| T2 | 105 | 8.8* | 144.9* | 44.7/302.5 | 213.9* | 18.4/622.8 | 128.0* | 22.0/321.8 | |
| T3 | 106 | 9.7* | 425.3* | 238.3/665.9 | 179.3* | 95.1/290.0 | 59.3* | 0.0/283.3 | |
| T4 | 92 | 9.1* | 155.0* | 54.4/307.2 | 162.2* | 99.2/240.7 | 31.5* | 0.0/184.1 | |
| Control | 1438 | 23.0 | 3408.7 | 1470.2/6150.5 | 3325.9 | 1250.9/6395.6 | 6540.9 | 3928.7/9815.4 | |

 $^{^{\}scriptscriptstyle 1}$ The means followed by * significantly differ from the control at α = 0.05 (Kruskal-Wallis test and Steel

Table 5. The average debarked area (mm²) at the plot level (20 seedlings) in treated (T1-T4) and untreated (control) seedlings in September

²The Kruskal-Wallis test indicates that there would be at least a significant difference at α = 0.05, but the Steel procedure did not identify any significant difference between treatments T1-T4 and control.

There are no significant differences between the treatments T1-T4 and control at $\alpha = 0.05$ (Kruskal-Wallis test)

²The means followed by * significantly differ from the control at $\alpha = 0.05$ (Welch's ANOVA test and Dunnett procedure);

³The means followed by * significantly differ from the control at $\alpha = 0.05$ (One-way ANOVA test and Dunnett procedure)

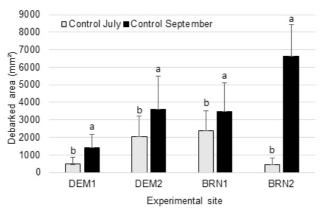
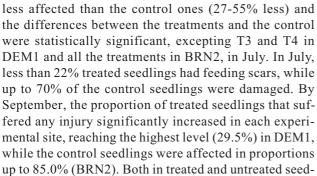
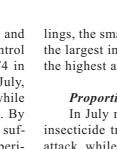


Figure 1. Comparison between debarked area at the plot level (20 seedlings) in untreated seedlings (control) in July and September. For each site, means with the same letter are not significantly different from each other at $\alpha = 0.05$ (two-tailed *t*-test for two paired samples). Error lines represent standard deviation of the mean





| 500 - 450 - | | a DT | 1-T4 July ■T1 | -T4 September |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|------------------|---------------|---------------|
| Deparked area (mm _s) = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = 0.00 = | b b | b | a T | a J |
| Ü | DEM1 | DEM2 Experime | BRN1 | BRN2 |

Figure 2. Comparison between debarked area at the plot level (20 seedlings) in treated seedlings (T1-T4) in July and September. For each site, means with the same letter are not significantly different from each other at $\alpha = 0.05$ (two-tailed *t*-test for two paired samples). Error lines represent standard deviation of the mean

lings, the smallest increase was recorded in BRN1, and the largest in BRN2, where in July there were attained the highest and the lowest proportions, respectively.

Proportions of seedlings killed by weevils

In July no dead seedlings were recorded from the insecticide treated seedlings due to large pine weevil attack, while 9% of the control seedlings at DEM2 and 3.8% at BRN1 and BRN2 were dead. In September, just a few treated seedlings were found dead due to weevil debarking, up to a mean of 3.8% in T1 at BRN1, but the

| | DEM1 | | DEM2 | | BRN1 | | BRN2 | |
|-----------|-------------------|-----------|--------|----------------------------|-------------------|-----------|--------|----------------|
| Treatment | Mean ¹ | 95% CI | Median | Ranks mean ² | Mean ¹ | 95% CI | Median | Ranks mean³ |
| T1 | 6.3* | 0.4/18.5 | 15 | 10.5* | 22.0* | 5.5/45.8 | 5.0 | 10.4 |
| T2 | 10.3* | 0.6/29.8 | 5 | 5.3* | 13.0* | 0.0/61.8 | 7.5 | 11.9 |
| T3 | 17.6 | 2.3/35.1 | 30 | 14.8* | 10.8* | 4.2/20.2 | 0.0 | 6.1 |
| T4 | 15.2 | 8.5/24.3 | 20 | 11.5* | 20.9* | 3.5/47.7 | 2.5 | 8.8 |
| Control | 33.1 | 11.9/58.7 | 60 | 22.9 | 70.0 | 39.1/93.2 | 35.0 | 15.4 |

¹The means followed by * significantly differ from the control at α = 0.05 (One-Way ANOVA test and Dunnett procedure);

³There are no significant differences between the treatments T1-T4 and the control at $\alpha = 0.05$ (Kruskal-Wallis test)

| | DEM1 | | DEM2 | | BRN1 | | BRN2 | |
|-----------|--------|----------------------------|-------------------|-----------|-------------------|-----------|-------------------|-----------|
| Treatment | Median | Ranks mean ¹ | Mean ² | 95% CI | Mean ² | 95% CI | Mean ² | 95% CI |
| T1 | 25.0 | 8.4 [*] | 31.3 [*] | 14.8/50.9 | 29.8 [*] | 12.0/51.6 | 15.9 [*] | 1.7/40.8 |
| T2 | 30.0 | 8.9* | 21.2* | 9.1/36.6 | 32.4* | 6.7/66.1 | 25.9* | 13.3/41,0 |
| T3 | 35.0 | 14.4* | 36.9* | 30.0/44.1 | 20.8* | 6.2/41.0 | 14.4* | 0.0/54.4 |
| T4 | 35.0 | 10.7 [*] | 25.9 [*] | 10.3/45.5 | 25.2 [*] | 6.7/50.4 | 8.7 [*] | 0.0/41.1 |
| Control | 55.0 | 22.6 | 72.9 | 56.3/86.6 | 84.1 | 37.2/100 | 85.0 | 34.1/100 |

¹The means followed by * significantly differ from the control at $\alpha = 0.05$ (Kruskal-Wallis test and Steel procedure);

Table 6. Proportion (%) of seedlings attacked by large pine weevil when observing during July

Table 7. Proportion (%) of seedlings attacked by large pine weevil when observing during September

²The means followed by * significantly differ from the control at α = 0.05 (Kruskal-Wallis test and Steel procedure);

²The means followed by * significantly differ from the control at $\alpha = 0.05$ (One-way ANOVA and Dunnett procedure)

proportion of dead seedlings increased up to about 36% for the untreated ones (Table 8). The highest mortality was in BRN2 and BRN1, and the lowest in DEM1.

Table 8. Proportion (%) of seedlings killed by large pine weevil when observing during September

damaged seedlings and the size of debarked area), very likely due to the variable density of beetle populations and to alternative food sources. However, even at BRN2

| | DEM1 | | DEM2 | | BRN1 | | BRN2 | |
|-----------|------|---------|------|----------|--------|----------------------------|------|----------|
| Treatment | Mean | 95% CI | Mean | 95% CI | Median | Ranks mean ¹ | Mean | 95% CI |
| T1 | 0.0 | - | 0.0 | - | 0.0 | 9.3 | 0.0 | - |
| T2 | 0.0 | - | 0.0 | - | 0.0 | 8.9 | 0.0 | - |
| T3 | 0.0 | - | 0.0 | - | 0.0 | 8.9 | 0.0 | - |
| T4 | 0.0 | - | 0.0 | - | 0.0 | 7.0 [*] | 0.0 | _ |
| Control | 2.4 | 0.0/6.0 | 15.5 | 6.4/27.8 | 34.9 | 18.5 | 35.7 | 9.8/67.4 |

The means followed by * significantly differ from the control at $\alpha = 0.05$ (Kruskal-Wallis test and Steel

Efficacy of the treatments

During the first half of the growing season, the treatment applied before planting reduced the proportion of seedlings damaged by more than 46.8% (Table 6), the debarked area by more than 82.9% (Table 4), and prevented any mortality caused by this pest.

Estimated treatment efficiency based on autumn observations led to similar values, namely a reduction in the proportion of damaged seedlings by 36.4-89.8% (Table 7), size of debarked area by more than 82.7%, and mortality by 89.7 -100%.

Discussion and Conclusions

Our results confirmed the hypothesis that the treatments affect the weevil feeding by reducing the proportion of seedlings suffering wounds, and also the size of debarked area of each wounded seedling. As demonstrated in laboratory experiments (Olenici et al. 2014), the treatment of seedlings with neonicotinoids did not ensure their total protection against injuries by weevils, even if these substances also have a repellent effect (Rose et al. 2005), so that the proportion of treated seedlings that were injured by insects reached about 37%. However, this proportion was lower than in laboratory conditions, possibly due to a smaller number of weevils per seedling, as well as to the attraction of weevils to volatiles released from seedlings already attacked (Tilles et al. 1986, Nordlander 1991, Zagatti et al. 1997). Alternatively, they may have found alternative food sources at the field sites (Örlander et al. 2001, Nordlander et al. 2003), especially in the first three experimental areas. On the other hand, the size of the debarked area was considerably lower than that of untreated seedlings, which resulted in no dead seedlings due to weevil injuries among the treated ones (except at BRN1), while the proportion of seedlings killed was up to 36% among the untreated plants.

There were large differences between experimental sites regarding the attack characteristics (proportion of where the alternative food resources were very low and the pressure on the seedlings must have been higher, both from the mature weevils, which fed more intensely during the first part of the season, and especially from newly emerging beetles during the second part of the season, the treatment applied to the seedlings was effective and no mortality occurred.

The dynamics of the injuries observed in the four experimental areas correspond to those found in other studies (Örlander et al. 1997, Olenici and Olenici 2003) and revealed that the treatment applied to the seedlings before planting can be effective throughout the growing season, even when the intensity of the attack increases towards the end of the season (autumn injuries), after the seedlings have been exposed to environmental factors (solar radiation, rainfall, etc.).

The lack of statistically significant differences between T1-T4 treatments suggests that, under similar conditions, emulsions of 0.5% concentration can be used instead of those of 1%, as currently used in Romania for Mospilan 20 SG insecticide, thus reducing the cost of treatments. However, under more severe conditions, such as larger weevil populations, higher concentrations may be needed to ensure complete treatment efficiency, as is the case in Sweden and the UK (Härlin and Eriksson 2011, 2014, Eriksson et al. 2017, Willoughby et al. 2017).

Calypso 480 SC and Mospilan 20 SG treatments applied to seedlings prior to planting are highly efficient. In addition, the treatment application in the nursery minimizes the impact of insecticides on the environment and on pollinating insects which are not attracted to seedlings because they do not produce flowers. Additionally, acetamiprid and thiacloprid have lower contact honey bee toxicity than other nicotinoids like imidacloprid, clothianidin, thiametoxam, dinotefuran and nitenpyram (Iwasa 2004, Laurino et al. 2011). Consequently, the use of tested products and this type of treatment could be an economically and ecologically viable solution where there is a high risk of attack and where other more environmentally sensitive solutions cannot be applied.

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The tested neonicotinoids could also be used for treating seedlings in the field after planting, as is the case in the UK (Willoughby et al. 2017). In such situations, there is some risk that the insecticide will also reach non-target vegetation near the seedlings, including flowering plants which could be visited by pollinating insects. In order to reduce the potential impact on pollinators, under such circumstances, consideration should be given to cutting the herbaceous vegetation around the seedlings prior to application, especially if the treatment is to be carried out shortly before or during flowering. Clearing of herbaceous vegetation typically also prevents it from overgrowing the seedlings and facilitates effective insecticide application by fully exposing the stems.

The neonicotinoids acetamiprid and thiacloprid, in emulsions with a concentration of 0.5-1% of the commercial product (Mospilan 20 SG and Calypso 480 SC), ensure good protection of spruce seedlings during their first growing season, when applied by dipping the aerial parts of the seedlings prior to planting.

Under the test conditions, there were no statistically significant differences between treatments of 0.5% and 1% of commercial product, suggesting that in similar conditions the 0.5% concentration could be used, reducing the costs of the treatments.

The treatment also remained effective until the end of the first growing season, approximately 5 months after application, even in situations where the density of beetle populations increases considerably (e.g. as a result of the emergence of new adult beetles from stumps).

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