

Preliminary Results of a Slow Release Formulation of Imidacloprid for the Control of the Large Pine Weevil (*Hylobius abietis* L.)

MAXIME JACQUET^{1,2}, EEVAMARIA HARALA¹, GUY LEMPERIERE² AND MARJA POTERI^{1*}.

¹ Finnish Forest Research Institute, Juntintie 154, FI-77600 Suonenjoki, FINLAND

² Institut de Recherche pour le Développement (IRD), 2 rue Maxime Rivière, 97490 Sainte Clotilde, La Réunion, FRANCE

*marja.poteri@metla.fi; phone + 358 50 391 4853

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Abstract

The large pine weevil *Hylobius abietis* L. (Coleoptera: Curculionidae) is the most damaging insect for young conifer seedlings in Europe. Laboratory feeding tests were performed to determine the dose effect of the granular formulation of Imidacloprid product 'Suscon Forest®' (Nufarm Ltd.) on the bark consumption of insects and the effect on their physiological conditions. Seedling shoot growth of two-year-old container Norway spruce (*Picea abies*) seedlings was measured at the same time as the seedlings were lifted for feeding tests, which was at 41 days with the nursery field and 61 days with the greenhouse grown seedlings. The shoot growth of the seedlings was not affected by the product. In the feeding tests, untreated seedlings were more damaged compared to treated ones. With the greenhouse seedlings, an average area of 209.7 mm² of bark was consumed on the untreated seedlings versus 29.1 and 17.4 mm² on treated seedlings that had been supplied with 5 and 10 grams of the product respectively. A better control effect against pine weevils was obtained with greenhouse-grown seedlings than with field planted seedlings.

Key words: Curculionidae, *Hylobius abietis*, Imidacloprid, feeding test, *Picea abies*, container seedling.

Introduction

The large pine weevil *Hylobius abietis* L. (Coleoptera: Curculionidae) is a phytophagous insect, and is considered to be one of the major pests of conifer reforestation throughout Europe (Bejer-Petersen et al. 1962, Day et al. 2004). The pine weevil is attracted to clear-cuttings, where it uses the roots of recently dead or cut conifers as a breeding substrate (Eidmann 1974, Nordenhem and Eidmann 1991, Nordlander et al. 1986). The associated silvicultural problem comes from the adult pine weevil, which feeds on the bark of young planted tree seedlings and especially conifers (Christiansen 1971, Eidmann 1974, Orlander and Nilsson 1999, von Sydow 1997). When adults emerge from the pupal chambers, they can easily attack the newly planted seedlings. Therefore, it has been necessary to protect these new seedlings before or at planting time (Nilsson et al. 2010).

In the artificial regeneration area of harvested forestland in Finland, which includes both planting and sowing, Norway spruce (*Picea abies* (L.) Karst.) represented 55 % and Scots pine (*Pinus sylvestris* L.) 42

% of the total planted area in 2010, the remaining 3 % being broad-leaves species, mainly European white birch (*Betula pendula* Roth). In forest planting operations, the proportion of spruce was 70 % of the total planted area (Anon. 2010, p. 139). This high proportion of conifers in Finnish regeneration requires a suitable control method against *H. abietis*.

In Finland, both pine and spruce seedlings are chemically treated in nurseries against *H. abietis* before planting. In 2010 the number of conifer seedlings delivered from Finnish forest nurseries was 177 million (Anon. 2011) and most of them were treated with insecticides (Viiri 2009). Three registered products for the treatment against *Hylobius* weevils have been used in Finland: Deltamethrin (Decis 25 EC, active ingredient (a.i.) 25 g/l, this product was withdrawn 31.12.2010), Imidacloprid (Merit Forest, a.i. 700 g/l) and Lambda-cyhalothrin (Karate Zeon technology, a.i. 100 g/l). Formerly used insecticide Permethrin (in Finland product Gori 920, a.i. Permethrin 250 g/l) was used to control *H. abietis* in Finland and other Nordic countries from the late 1970s until the end of 2003.

In Finland, the chemical control of *Hylobius* has become more challenging as container seedlings have replaced more robust and weevil tolerant bare root seedlings with 99 % of seedling production now being containerised (Anon. 2011). Moreover, earlier chemical treatment for bare root seedlings (3-4 years old) was carried out by dipping seedling bundles into an insecticide solution so that the whole shoot length including the root collar area was treated (Heritage 1997). This method gave an even and adequate coverage for susceptible seedling parts. There were also, however, a number of drawbacks, as work had to be done manually, it was time consuming and it exposed both the workers and the environment to substantial pesticide residues.

Due to their smaller size and root collar diameter, 1-2 years old container seedlings are more susceptible and thus have a lower recovering ability after a weevil attack (Orlander and Nilsson 1999, Thorsen et al. 2001). Chemical treatment is also more challenging as it has to be carried out by spraying the seedlings in their containers or, as is common nowadays, spraying freezer-stored seedlings inside a cardboard storage package. Container seedling treatments started at the end of the 1980s and spraying was carried out by normal tractor sprayers or backpack sprayers. To improve both spray coverage of container seedlings and operator safety in the chemical control of pine weevil, different technical solutions, including both spraying and dipping, have been tested in Finnish nurseries (Tervo et al. 1994, Tuomainen et al. 1996, Tuomainen et al. 2003, Koponen et al. 2011).

A slow release granular formulation of carbosulfan was used for the control of *H. abietis* damage in the United Kingdom, Poland, Ireland and France (Lemperiere and Julien 2003). This product, based on carbosulfan, is no longer used. The product used here, 'Suscon Forest®', is a slow release formulation of the neonicotinoid Imidacloprid. The granular formulation offers the possibility of a slow release of the active ingredient (a.i.) close to the roots of seedlings and its uptake by the plant. The product can be mixed into the soil or growth media in the nursery or it can be transferred into the planting hole at planting time. This systemic formulation of Imidacloprid is anticipated to protect the seedling from weevil attacks and other phytophagous pests, like *Hylastes* spp. (Lindelöw 1992), threatening it. Imidacloprid granules are used to control e.g. turf-grass and sugarcane grubs (Allsopp 2010) but to our knowledge there are no scientific reports on how conifer seedlings respond to the granules.

A series of tests has been performed on treated seedlings with different doses of Imidacloprid to investigate if there is a dose effect on both plant growth and the level of protection.

Material and methods

Insect collection and maintenance

Approximately 500 adults of *H. abietis* were collected in June 2009 from a sawmill located in Suonenjoki, Finland (°39'51.50"N; 27° 0'45.78"E). Pine weevils were placed and kept in contact with moistened sawdust with Norway spruce branches in ventilated glass jars at +4°C. Weevils of similar weight were selected in all the experiments.

Product

The product is a slow release formulation of the neonicotinoid Imidacloprid (*N*-[1-[(6-Chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl]nitramide; Bayer Cropsciences) produced by Nufarm®. Imidacloprid is designed to be effective through contact or ingestion (Tomlin 2006). It is a systemic insecticide that translocates rapidly through plant tissues after application (Fossen 2006). Mixed with the soil preparation, the seedlings can absorb Imidacloprid. This acts on several types of post-synaptic nicotinic acetylcholine receptors in the nervous system (Buckingham et al. 1997, Matsuda and Sattelle 2005). In insects, these receptors are located only within the central nervous system. Following binding to the nicotinic receptor, nerve impulses are spontaneously discharged at first, followed by failure of the neuron to propagate any signal (Schroeder and Flattum 1984). This blockage leads to the accumulation of acetylcholine, an important neurotransmitter, resulting in the insect's paralysis and eventual death.

Growing the experimental seedlings

The two-year-old container (Plantek® 81F) Norway spruce seedlings used in this experiment had been stored in a freezer over the winter and were defrosted just before planting. All of the seedlings continued their third year of growth during the experiment. In a greenhouse 48 seedlings per treatment were planted into 0.4 L pots and 50 seedlings per treatment were planted into the nursery field. In the greenhouse, the potting mixture was peat amended with sand (2:1) and in the field sandy mineral soil with added peat. The greenhouse experiment started on 24 April and it lasted nine weeks, and the field experiment started on 13 May and lasted six weeks. For the treatments, 5 g (a.i. content 5%) and 10 g (a.i. content 5%) of Suscon Forest® granules were placed into a planting hole prior to potting pot-to-pot in the greenhouse and planting in the field. For control treatments, nothing was put in the pot or planting hole with the seedling. Treatments were randomised into four blocks both in

the greenhouse and in the field. The planting field was located at a nursery where no weevils appeared.

Feeding test

The efficiency of the product was tested with a feeding laboratory test, where pine weevils were allowed to feed on treated and untreated spruce shoots in a Petri dish for seven days. Weevils were placed inside a Petri dish (Ø 15 cm) for 24 hours' fasting before the insertion of treated or untreated Norway spruce stalks. With the greenhouse seedlings the mean weight of males (n=44) and females (n=40) was 117.16 mg (sd=8.518) and 120.03 mg (sd=10.705) respectively and with the field seedlings the mean weight of males (n=39) and females (n=45) was 114.36 mg (sd=10.207) and 115.56 mg (sd=10.778) respectively. The weights between males and females did not differ (for greenhouse seedlings: df=82; F(1, 82)=1.859; p=0.176 and for field seedlings: df=82; F(1, 82)=0.270; p=0.604). Coded dishes were randomized on a table and kept under laboratory conditions: humidity and temperatures were recorded using a Hobo® recorder (Onset Computer., MA, USA). During the feeding experiment, the filter paper on the bottom of the Petri dishes was kept moist with Milli-Q™ water (Millipore).

On 23 June seedling height was measured for both greenhouse and field seedlings and at the same time seedlings were chosen randomly from each of the four blocks for the feeding tests. A 10 cm section of the stem was cut at 1 cm above the soil. Branches and buds were eliminated. Needles were retained to keep the bark undamaged. Field and greenhouse seedlings were collected 41 and 61 days after planting respectively.

The stalks were placed in a Petri dish containing one adult pine weevil. The physiological status of the pine weevils was monitored after the first, third and seventh day, using a specific classification.

In the feeding experiment there were 84 Petri dishes which were divided into 4 blocks of 21 Petri dishes (3 treatments and 7 seedlings per treatment).

The experiment was concluded at the end of Day 7, after the last assessment. Sexing of the pine weevils was carried out. The stems were kept in order to measure the feeding scars on the bark and were placed in individual plastic bags and put in a freezer at -20°C until measured.

Assessments and statistical analysis

The height of the seedlings was measured at the beginning and the end of the growing season (for the greenhouse seedlings 5 May and 23 June and for field seedlings 18 May and 23 June) to determine whether the treatments had an effect on the growth of the seedlings. A one factor analysis of variance (ANOVA) was

carried in order to determine the influence of the different treatments and the different growing conditions on the growth of the seedlings.

Assessments for the condition of weevils in the feeding tests were conducted at the same time each day in order to follow the cycle of the studied insects. At each assessment, individuals were classified as 1= no symptoms, 2= weak, not able to move, lying on its back, 3= not able to eat or control movements, or 4= dead.

The area of feeding scars was drawn on a transparent plastic film placed around the stalk. The feeding scars drawn on films were scanned using the Epson Twain Pro program and edited using Adobe Photoshop 8.0 software. The total measurement of the damaged area was performed using the WINSEEDLE® v2002a program (Regent Instruments).

Statistical analyses were performed using SPSS 16.0 for Windows software (SPSS 2007). The normality of the weight was tested for all the insects used in this test. Normality was also checked for in terms of seedling height and diameter before planting and prior to the feeding experiment.

The total area of bark available on each stalk used for the feeding tests was calculated using the following formula:

$$\rightarrow \text{Area of bark available} = 2 * \pi * \frac{\text{Diameter of the stalk}}{2} * \text{Height of the stalk}$$

A one factor analysis of variance (ANOVA) was carried out in order to determine whether any of the treatments differed in the consumed bark area. A Levene test was used to determine the homogeneity of variances. A Tukey's HSD Test was used to determine which of the treatments differ from each other.

Results

Height growth of the seedlings

Temperatures during the growth of the seedlings fluctuated between 18 and 25°C in the greenhouse and between 0 and 25°C in the field. Significant differences appeared between growth of greenhouse and field seedlings (ANOVA, p-value <0.001). The seedling height growth was equal between the different treatments (Figure 1) and there was no significant difference for this parameter (df=2; F(2, 81)=0.649; p=0.525 for the greenhouse and df=2; F(2, 81)=0.821; p=0.440 for the field seedlings).

Greenhouse seedlings

No significant differences were found for bark feeding between males and females (df=1; F(1, 82)=0.002; p=0.969) for the greenhouse seedlings. The mean area of bark consumed for the untreated seedlings was more than seven times higher than the area

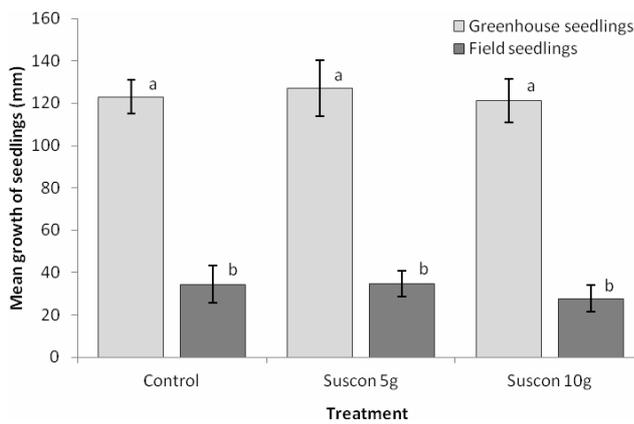


Figure 1. Height growth (mean + SEM) of greenhouse and field grown Norway spruce container seedlings in different treatments during the growing period 07.05.09 to 22.06.09 for greenhouse seedlings and 18.05.09 to 24.06.09 for field seedlings. Bars capped by different letters indicate significant differences between treatments among greenhouse and field grown seedlings. (p-value <0.05, ANOVA)

of bark consumed from the treated ones. An average of 209.7 mm² of bark was consumed on the control and 29.1 and 17.4 mm² for the seedlings treated with 5 g and 10 g of Suscon Forest® respectively (Figure 2). The ANOVA results gave significant differences between the treatments (df = 2; F(2, 81) = 58.967; p=0.0001). The bark area consumed by the insects differed significantly between the control and treated seedlings but not between the two doses (Tukey’s HSD Test). The estimates of the surface of bark available for each insect gave an average of 15.9 % of the area of bark consumed for the control seedlings versus 2.1 and 1.9 % for the seedlings treated with 5 g and 10 g of Suscon Forest® respectively.

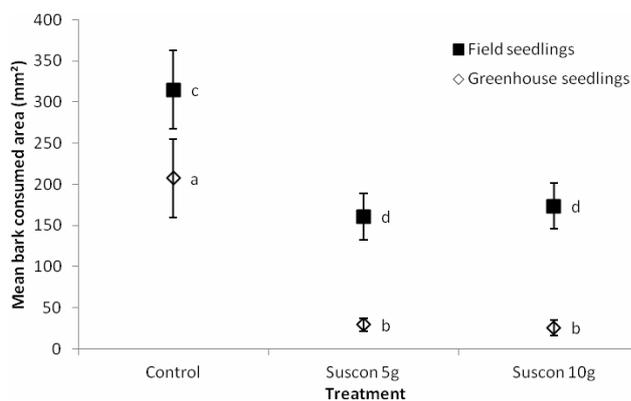


Figure 2. Bark area consumed (mean + SEM) (mm²) by *H. abietis* according to the treatment for the greenhouse and field grown Norway spruce container seedlings in a Petri dish feeding experiment. Significant differences between the treatments among field and greenhouse-grown seedlings are shown using different letters. (p-value<0.05, ANOVA)

In the Petri dish, the feeding activity of the pine weevil was affected when in contact with the treated greenhouse seedlings. All the insects which consumed control seedlings were active until the end of the test. With those that consumed the treated seedlings, six insects died for each treatment and five and eight were, at least once, in “health condition 2” (insect can’t move) for the 5 g and 10 g Suscon Forest® treatments respectively.

Field seedlings

With field seedlings the feeding behavior did not either differ between males and females (df=1; F(1, 82)=1.477; p=0.228). The differences between treated and untreated seedlings were not as obvious as they were in the greenhouse-grown seedlings. The mean area of bark consumed for the control seedlings was the double of the area of bark consumed for the treated plants. An average of 322.9 mm² for the control versus 159.4 and 172.8 mm² for the seedlings treated with 5 g and 10 g of Suscon Forest® respectively was observed (Figure 2). The ANOVA results gave significant differences between the treatments (df= 2; F(2, 81)= 28.433; p=0.0001), and the bark area consumed by the insects differed significantly between the control and the treated plants, but not between the two doses (Tukey’s HSD Test). The estimates of the surface of bark available for each insect gave an average of 32.8 % of the area of bark for control seedlings versus respectively 15.9 and 17.4 % for the seedlings treated with 5 g and 10 g of Suscon Forest®.

The activity of the insects did not seem to be affected by feeding on the seedlings from the field. All of the insects were still active and were feeding on the plants at the end of the feeding experiment.

Discussion

Height growth of the seedlings

Suscon Forest® did not have any significant impact on the growth of the greenhouse and field seedlings, although vegetable and field crops have showed enhanced growth, especially under sub-optimal conditions (Gonias et al. 2008, Wallace et al. 2000, Thielert 2006).

However, the growth of the greenhouse seedlings was significantly higher than the growth of the field seedlings. Daily watering and higher temperatures in the greenhouse gave better growth conditions for Norway spruce seedlings than in the field where there was drought and an average temperature that was 7.8°C colder. Besides the environment, growth substrate also differed (peat versus mineral soil) which could have an effect on the uptake of Imidacloprid and

explain the differences of the bark area consumed by pine weevil between greenhouse and field seedlings. As this setup was one of the first trials to study the uptake of Imidacloprid with conifer seedlings, further research is needed to evaluate the uptake of Imidacloprid and effective doses with conifer seedlings.

Experimental conditions

The amount of consumed bark did not differ between males and females, even though a few females laid eggs in the Petri dish. This reproductive behaviour did not have an impact on bark consumption, contrary to previous findings in the literature (Bylund et al. 2004, Wainhouse 2004a, Wainhouse et al. 2004b).

For the feeding tests, temperatures fluctuated between 22 and 27°C and relative humidity between 34 and 46 %. According to the literature, recorded temperatures in these Petri dish tests can be regarded as optimum for the feeding of pine weevils (Christiansen 1971, Christiansen and Bakke 1968).

Feeding test

There were distinct differences in feeding behavior between treated and untreated seedlings. The results showed a significant difference in bark consumption between controls and treated seedlings. However, differences appeared between tests with seedlings from the field or from the greenhouse.

Seedlings from the greenhouse were treated three weeks before those from the field. Growing conditions were better for seedlings in the greenhouse, with its regulated temperature and daily watering, and thus the uptake and metabolisation of Imidacloprid was enhanced for greenhouse seedlings when compared to those grown in the field.

For greenhouse and field, seedlings treated with 5 g and 10 g of product did not show any significant difference in the area of consumed bark by pine weevils. The maximum amount of insecticide which was effective for the pine weevil was already reached with doses of 5 g for both greenhouse and field seedlings. For the greenhouse seedlings, the dose of 5 g, when applied at planting time, gave protection against the insects by killing them or by reducing their attacks by a factor of between two and seven. The same number of insects were killed in both treatments. In this experiment, for greenhouse seedlings with 5 g of product the seedling was protected and, moreover, there were no advantages in using the higher dose, for example for the stimulation of growth.

The results of the field seedlings raised the issue of the delay in the sufficient uptake of the product into the plant under certain hygrometric and temperature conditions. In our tests, the insects fed more on treat-

ed seedlings from the field than treated seedlings from the greenhouse, with no impact on their activity. Results showed a significant difference in bark consumption between treated and control seedlings, but the field seedlings could be adversely affected even with Imidacloprid exposure, as treated field seedlings were eaten as much as control greenhouse seedlings.

Silvicultural applications

In silviculture, weevils have traditionally been controlled with insecticides but over the last two decades alternatives for chemicals have been investigated, too. With regard to the demand for non-chemical methods one promising product is a Swedish Coniflex, which is based on a flexible sand coating (Nordlander et al. 2009). It has also been suggested that in mineral soil sites, proper soil scarification where humus is not mixed with mineral soil may reduce *Hylobius* damage to acceptable levels (Nordlander et al. 2011). There are, however, regeneration sites, e.g. peatlands where mineral soil covered planting spots cannot easily be obtained and where *Hylobius* damages can be high (von Sydow 1997, Luoranen and Viiri 2012). Another obstacle with insecticide pretreated seedlings has been the persistence of the products as they are gradually degraded at a forest site, while at the same time the radial growth of a seedling also decreases the proportion of the treated surface. According to preliminary results from an unscarified field experiment, Imidacloprid granules transferred into a planting hole may extend protection time to also cover the season after planting (unpublished results).

An optimal or a long period of growth is required to give the seedlings time to get enough Imidacloprid to protect it. Thus, further research is required to understand the minimum time of uptake of the product for the plant before exposing it to the attacks of the pine weevils when seedlings are planted in a clear-cut field, for example. In Nordic countries, good growth results have been obtained if spruce seedlings are planted on mineral soil covered mounds, which have been shown to create a better environment for emerging new roots, due to a higher temperature and moisture content inside the mound when compared with unscarified soil (e.g. Orlander et al. 1990, Nordborg et al. 2003, Heiskanen and Rikala 2004). To control pine weevils with Imidacloprid granules, the uptake of the product by the spruce seedling is most likely enhanced to some extent in these kinds of planting spots, but this needs further investigation.

It could be useful to treat seedlings in the nursery with a dose of Suscon Forest® and add more product at planting time in the forest. There is also a demand for easier and safer application methods where

mechanical planting may make it possible to adopt some new approaches in controlling *Hylobius* damages. It is predicted that mechanical planting, the proportion of which in Finland is estimated to be currently about 5 %, will increase at least in northern Europe (Nilsson et al. 2010). Control measures of *Hylobius* may be incorporated in planting machines and for the application, granules can constitute a better formulation than liquid-based products.

However, the different levels of damage between field and greenhouse-grown seedlings demonstrated that the field seedlings remained without protection in the first weeks because of less suitable growing conditions due to e.g. climatic and edaphic factors. In the Finnish climate, harsh environmental conditions may limit efficient uptake, even during the entire first growing season. If an attack occurred during this time, treated seedlings could be damaged to the same degree as untreated ones. A preventive treatment before forest planting could be an option for the efficient use of this product.

Conclusion

Laboratory feeding tests have demonstrated that the root uptake of a 5 g dose of Suscon Forest® could protect Norway spruce seedlings against pine weevil damage and that the a.i. had no effect on the growth of two-year-old Norway spruce seedlings. Growing seedlings with Suscon Forest® in a greenhouse before planting them in the field appeared to be a better method of protecting seedlings than planting them directly in the field with granules added into the planting hole. Using this procedure, the seedling could be protected from the first day of planting to the end of its vulnerability against pine weevils.

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МЕДЛЕННОДЕЙСТВУЮЩАЯ ФОРМА ИМИДАКЛОПИДА ДЛЯ БОРЬБЫ С БОЛЬШИМ СОСНОВЫМ ДОЛГОНОСИКОМ (*HYLOBIUS ABIETIS* L.): ОПТИМАЛЬНАЯ ЭФФЕКТИВНАЯ ДОЗА И ВЛИЯНИЕ НА РОСТ САЖЕНЦЕВ ЕЛИ ОБЫКНОВЕННОЙ (*PICEA ABIES* L.)

M. Jacquet, E. Harala, G. Lemperiere, M. Poteri

Резюме

Большой сосновый долгоносик *Hylobius abietis* L. (Coleoptera: Curculionidae) является наиболее опасным вредителем молодых хвойных насаждений в Европе. Цель проведенных лабораторных экспериментов заключалась в определении дозы воздействия гранулированной формы имidakлопридного препарата Suscon Forest® (производство Nufarm Ltd.) на поедание коры насекомыми и влияния на условия их жизнедеятельности. Рост двухлетних молодых саженцев ели обыкновенной (*Picea abies*) определялся при выкапывании саженцев для лабораторных опытов, которые проводились на 41-й день для саженцев полевого питомника и на 61-й день для саженцев, выращенных в теплице. Препарат не оказал влияния на рост молодых деревьев. Испытания показали, что необработанные саженцы были повреждены в большей степени, чем обработанные. На выращенных в теплице необработанных препаратом саженцах было повреждено в среднем 209,7 мм² площади коры, в то время как на саженцах, которые были обработаны 5 и 10 граммами препарата – 29,1 и 17,4 мм² площади коры соответственно. Препарат был более эффективным по отношению к саженцам, выращенным в теплице, нежели к саженцам, выращенным в полевых условиях.

Ключевые слова: Curculionidae, *Hylobius abietis*, имidakлопид, тест на питательную среду, *Picea abies*, кассетная рассада