Evaluating the Effectiveness of Two Methods to Control *Leptoglossus occidentalis* Heidemann, 1910 in Black Pine Stands

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Leptoglossus occidentalis (Hemiptera: Coreidae), an invasive pest native to North America, causes significant damage to coniferous seed production worldwide. This study evaluated the effectiveness of contact spray and Arborjet trunk injection methods for controlling *L. occidentalis* in black pine (*Pinus nigra*) seed orchards in Bursa-Karacabey, Türkiye. Abamectin (18 g/L), as insecticide, was applied to plots of 50 trees each in 2025, and 300 cones were harvested in November 2025 for seed fill and empty cone analysis. Statistical analyses included Kruskal-Wallis and Wilcoxon tests with Bonferroni correction. Seed fill percentages were 9.7% (control), 33.9% (spray), and 52.8% (Arborjet trunk injection). The Arborjet method yielded approximately 5.6 times higher seed fill than the control with the lowest coefficient of variation (17.2%). Empty cone ratios differed significantly among groups (Fisher's exact test, $P < 0.001$), with 0% in the Arborjet group *versus* 12% in the spray group. Treatment type explained 63.3% of total variance ($\eta^2 = 0.633$), with very large pairwise effect sizes (Cohen's d = 1.33 to 3.42). These results demonstrate that systemic trunk injection should be prioritized over contact spray in seed orchards and gene conservation areas.

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INTRODUCTION

Leptoglossus occidentalis Heidemann, 1910 (Hemiptera: Coreidae), an invasive pest species native to Western North America, causes significant losses by feeding on the cones and seeds of coniferous forest trees (Hedlin *et al.* 1981; İpekdal *et al*. 2019). The species was first detected in Europe in Italy in 1999. It rapidly spread across the continent and was first recorded in Türkiye in 2009 (Arslangündoğdu and Hızal 2010; Fent and Kment 2011). Currently, *L. occidentalis* is distributed throughout all regions of Türkiye and causes damage to numerous coniferous tree species (İpekdal *et al*. 2019).

Using its piercing-sucking mouthparts, the species inserts its stylet into cone and seed tissues to feed during both the nymph and adult stages. As a result, feeding promotes abscission in young cones, leads to characteristically empty seeds in mature cones, and decreases seed viability for germination (Tamburini *et al.* 2012; Lesieur *et al*. 2014). Therefore, *L. occidentalis* is an economically important pest, as it depletes the potential for both natural and artificial regeneration of trees (İpekdal *et al*. 2019).

According to studies carried out in Türkiye, *L. occidentalis* feeds on various pine species, namely, *Pinus nigra, P. sylvestris, P. pinea, P. brutia*, and *P. radiata* (Arslangündoğdu and Hızal 2010; Hızal 2011; Hızal and İnan 2012; Dursun 2016; Özek and Avcı 2017; Özgen *et al*. 2017; Parlak 2017; Oğuzoğlu and Avcı 2018). It is thought that the significant declines in germination rates regarding *P. nigra* stands might be influenced by the growing pest population. Although the Bursa area faces up to 19% germination loss in *P. nigra* seeds, the proportion of these losses directly induced by *L. occidentalis* has not been sufficiently proven yet (Parlak 2017; İpekdal *et al.* 2019).

Insect biology and phenology are important for the effective timing of control methods. Two generations of *L. occidentalis* were identified in previous studies in the Antalya region; the first flight period was from May to June and the second was from July to August (Oğuzoğlu and Avcı 2020). This means that, taking into account the flight and reproduction period of the pest, the initiation of control applications should be carefully planned.

To date, mechanical, chemical, biotechnical, and biological methods have been explored in combating *L. occidentalis*. Nevertheless, considering the species-specific cryptic feeding behavior and the number of host plants, no method has proven effective and sustainable (Tamburini *et al*. 2012; İpekdal *et al*. 2019). Although the use of pheromone traps, conservation of native enemies, and selective chemical exposure seem like working solutions, they should be evaluated relative to the respective stand type. In its native North American range, *L. occidentalis* is attacked by egg parasitoids — most notably *Gryon pennsylvanicum* (Ashmead) — which provide a degree of natural regulation that is largely absent in the invaded range (Maltese *et al.* 2012). In Europe, only a few native generalist egg parasitoids (*e.g.*, *Anastatus* and *Ooencyrtus* spp.) have been recovered, generally at low parasitism levels (Lesieur and Farinha 2021). Augmentative or classical biological control based on such natural enemies has therefore been proposed as a lower-hazard, complementary option, although it currently remains insufficient as a stand-alone measure. Importantly, the efficacy of chemical control against cone and seed insects is governed not by the choice of active ingredient alone, but also by the applied dose and the mode of delivery, which determine how much of the compound reaches the feeding sites within the cone. Trunk-injection studies have shown that both the injected dose and its seasonal timing can markedly alter control efficacy and residue levels (Coslor *et al.* 2019). Such dose–delivery relationships have not yet been characterized for *L. occidentalis*; accordingly, the present study was designed to contrast a contact and a systemic delivery method at operationally relevant doses rather than to establish a full dose–response relationship.

This study aimed to evaluate the effectiveness of two different methods applied for the control of *L. occidentalis* in *Pinus nigra* stands. The results obtained are expected to contribute to the development of scientifically based and applicable control strategies for pest management in black pine stands.

EXPERIMENTAL

Materials and Methods

The study areas were selected from the black pine (*Pinus nigra*) Seed Orchard located in the Bursa-Karacabey Okçular region. In the experiment, plots consisting of 50 trees each were established and treatments were applied (Fig. 1). The three treatment plots (control, contact spray, and trunk injection) were spatially separated by a buffer of three tree rows between adjacent plots, thereby minimizing spray drift and cross-contamination between treatments. All experimental trees belonged to the same age class (35 years) and showed homogeneous, comparable development across the three plots, ensuring uniform tree architecture and health status among treatments.

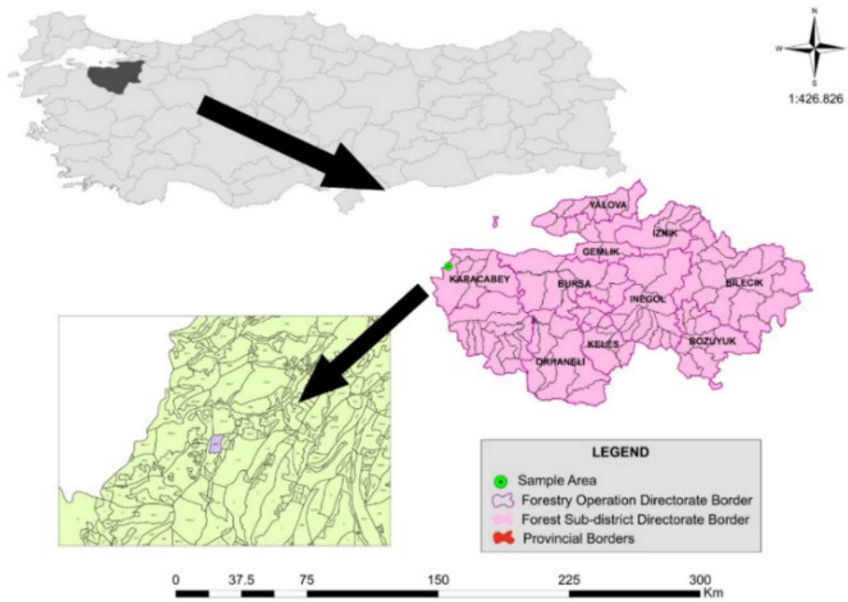

Fig. 1. Study area

Both treatments were applied once, on 16 July 2025, coinciding with the activity period of second-generation *L. occidentalis* adults. Abamectin (18 g/L) was applied as the insecticide in both systemic and contact applications.

In the systemic treatment, the insecticide was delivered directly into the trunk using the Arborjet injection system with its Arborplug closed-injection components. Because the product is delivered directly into the tree, this approach limits exposure of the applicator, bystanders, and non-target organisms compared with spray or soil applications. Holes were drilled into the trunk with a cordless drill according to tree diameter and bark thickness, and an average of 10 Arborplugs (range 6 to 10, adjusted to trunk diameter) were installed per tree. The Arborplugs form a tight seal within the trunk that maintains injection pressure and prevents product leakage or blow-back. Through each plug, 5 mL of solution — prepared as a 1:1 mixture of the insecticide and a carrier solvent — was injected, yielding an average of approximately 50 mL of solution per tree. The carrier solvents consisted of surfactant (31.20%), organic solvent 1 (24.40%), organic solvent 2 (24.40%), and water (20.00%). The injection dose was based on the standard Arborjet application protocol, which specifies a fixed volume per plug (5 mL) and varies the number of plugs based on trunk diameter, thus correlating the amount of product applied to the tree size.

Contact sprays made use of a Spray Team Mist blower that had a tank of 400 to 450 litres and could reach heights between 30 and 35 metres. These mist blowers delivered approximately 7 litres of the diluted spray per minute while atomizing it to produce droplets with a size range of 18 to 20 micrometres. The contact spray was prepared and applied at the rate described within the manufacturer's label and instructions for the formulated product.

Cone Collection and Seed Counting

The cones were harvested in November 2025, one year after the treatments were applied. Of each tree, two cones were randomly selected, for a total of 300 cones. The cones were allowed to dry in a lab setting at 45 °C, for 24 to 48 hours, after which they were split open and their seeds removed. The number of seeds in each cone that were both filled and empty was recorded as a separate number. Seed fullness was determined by a cutting test; this method distinguishes filled from empty seeds but does not, by itself, confirm seed viability or germination capacity.

Statistical Analyses

The conformity of data to normal distribution was evaluated using the Shapiro-Wilk test, and homogeneity of variances was assessed using the Bartlett test. Due to failure to meet the normal distribution assumption ($P < 0.05$), the non-parametric Kruskal-Wallis test was used for group comparisons. Pairwise comparisons were evaluated based on the Wilcoxon test with Bonferroni correction. Fisher exact test was carried out to compare the ratio of empty cones between groups, and the Chi-square test was conducted for total seed counts. The effect size was estimated for the general comparison as eta-squared and for pairwise comparisons as Cohen d values. The result consistency was examined with the coefficient of variation (CV). All data processing was carried out in the R software (version 4.3.3, R Core Team, 2024) and the results were considered statistically significant at $P <$ 0.05.

RESULTS AND DISCUSSION

In total, thirteen of the 300 cones were not included in the ratio analyses, as the cones had no seeds, although empty cone ratios were analyzed as a distinct group. The empty cone ratios appeared to differ significantly among the groups (Fisher exact test, $P =$ 0.000033). Empty cone ratios were 1.0% (1/100) in the control group, 0.0% (0/100) in the Arborjet group, and 12.0% (12/100) in the spray treatment group.

In the control group, of 1012 total seeds, 98 were filled (9.68%) and 914 were empty. Of 832 seeds obtained from spray-treated trees, 282 were filled and 550 were empty, with a seed fill percentage of 33.89%. Of 1200 seeds obtained from trees treated with Arborjet trunk injection, 634 were filled and 566 were empty, with a seed fill percentage of 52.83% (Table 1).

Table 1. Total Seed Counts and Seed Fill by Treatment Group

Treatment Type	Total Seeds	Filled Seeds	Empty Seeds	Seed Fill (%)
Control (Untreated)	1012	98	914	9.68
Spray Treatment	832	282	550	33.89
Arborjet (Trunk Injection)	1200	634	566	52.83

When evaluated on a per-cone basis, the mean seed fill percentage was calculated as 11.71 ± 14.84% in the control group, 31.93 ± 15.68% in the spray group, and 53.98 ±

9.26% in the Arborjet group (Table 2). All pairwise comparisons were found to be statistically significant (Bonferroni-corrected Wilcoxon test, $P < 0.001$; Table 3).

Table 2. Descriptive Statistics of Cone-based Seed Fill Percentages by Treatment Group

Treatment	n	Mean (%)	SD	Median	Min	Max	CV (%)
Control	99	11.71	14.84	6.25	0.00	66.67	126.71
Spray	88	31.93	15.68	33.33	0.00	53.33	49.09
Arborjet	100	53.98	9.26	52.40	35.71	100.00	17.15

In terms of result consistency, the Arborjet group had the lowest coefficient of variation (CV = 17.15%). This value was 49.09% in the spray group and 126.71% in the control group. These findings indicate that the Arborjet application not only provided the highest seed fill percentage but also yielded the most consistent results (Table 2).

Table 3. Pairwise Comparison Results between Groups

Comparison	Difference (%)	Cohen d	P-value
Arborjet - Control	+42.26	3.42	< 0.001
Spray - Control	+20.22	1.33	< 0.001
Arborjet - Spray	+22.04	1.74	< 0.001

The effect of treatment type on seed fill was found to be very high (eta-squared = 0.633). This value indicates that 63.3% of the total variance was attributable to treatment type. In pairwise comparisons, Cohen d was calculated as 3.42 for Arborjet-Control, 1.33 for Spray-Control, and 1.74 for Arborjet-spray, with effect sizes found to be very large for all comparisons (Table 3).

In this study, seed fill and empty cone formation were evaluated to determine the efficiency of two different application methods used for controlling *Leptoglossus occidentalis* in black pine (*Pinus nigra*) stands. The results reveal a clear difference by application method and suggest that the systemic treatment performed better.

The fact that the seed fill percentage was as low as 9.68% in the control group serves to illustrate the devastating effect of *L. occidentalis* on black pine seed production. Additionally, the control group had an empty cone ratio of 1.0%, whereas the spray treatment group exhibited a markedly higher ratio of 12.0%. This counterintuitive increase—where the treated group showed more empty cones than the untreated control—suggests that the high-pressure contact application *via* the mistblower may have induced localized mechanical stress or mild phytotoxicity in young, developing cones, potentially triggering early seed abortion. In contrast, the 0% empty cone ratio in the Arborjet group indicates that systemic delivery protected cone tissues throughout all developmental stages without the secondary canopy disturbance associated with high-power spraying.

Arborjet trunk injection demonstrated the highest performance in the seed fill experiment, at 54.0%. This was 5.6 times higher than the control and 1.7 times higher than the spray treatment. Concerning Cohen d effect size values, it can also be observed that the systemic treatment was considerably more effective. Although both the Arborjet-control and Spray-control comparisons showed very large effect sizes, the former was much larger (d = 3.42 and d = 1.33, respectively).

Sarıkaya *et al.* (2024) reported that in *Pinus pinea* stands the seed fill in control trees ranged from 32% to 48%, whereas after trunk injection it ranged from 63% to 71%.

The control value obtained for black pine in the present study (9.68%) is significantly lower than that for stone pine. This could mean that either black pine is more susceptible to *L. occidentalis* feeding in general, or the pest population was higher in the study area.

The spray treatment, while providing a significant increase in the seed fill compared to the control group (31.9%), still yielded an economically inadequate level. The obtained results are consistent with findings reported by Summers and Ruth (1987) and Ogden (2013), indicating that contact-effect application of insecticides is insufficient to suppress the pest within the cones. The main reasons for this phenomenon are the inability of insecticide to penetrate effectively between cone scales, the pest living in protected sites within the cone, and weather conditions that reduce the effectiveness of the application.

A significant advantage is the consistency of the results, and the Arborjet group with the lowest coefficient of variation has this characteristic (CV = 17.15%). Thus, CV was 126.71% in the control group and 49.09% in the spray group. The low value of the CV indicates that the treatment was characterized by a stable and practically non-random result in different trees. This is particularly important in areas such as seed orchards where each tree has high genetic value.

In addition to the high efficiency of chemical insecticides used in the control of *L. occidentalis*, such protection techniques should be given environmental and residual hazard justification. The method of chemical protection by trunk injection minimally affects non-target organisms, since the product is supplied directly to tree tissues. According to the insecticide residue analysis in pine products by Jano *et al*. (2025), pesticide exposure is low under appropriate dose and application methods. Trunk injections can be considered as an environmentally favorable control method since they demonstrate high biological efficiency without imposing a serious environmental risk.

Limitations and Future Work

A limitation of this study is that total cone production per tree was not quantified. Because stand-level seed output depends on both per-cone seed fill and the number of cones reaching maturity, the present results should be interpreted as reflecting per-cone protection rather than total seed yield.

Several aspects fell outside the scope of the present study and warrant further investigation. First, dose–response relationships for both delivery methods should be examined, as the efficacy and cost-effectiveness of contact and systemic applications may vary substantially with the amount of active ingredient applied. Second, future research should document the number of cones produced by each tree and the proportion of seeds filled within the cones as that will contribute to the total production of seeds for the stand (*i.e*., both the amount of mature cones and the amount of filled seeds per cone are important in determining total seed production for the stand). Third, future research will need to directly measure the viability of recovered seeds, *e.g*., *via* germination or tetrazolium testing, because the cutting test employed in this study can distinguish between filled and empty seeds but does not demonstrate whether the filled seed will germinate or not. Fourth, as an additional area of research, the use of natural enemies of *L. occidentalis* from their homeland (*i.e*. biological control) should be investigated as a potential complementary approach to reduce the risk associated with other management strategies. Finally, by expanding these comparisons to include additional host species, varied pest populations, multi-year operational data, and economic and cost-benefit evaluations, it would result in the development of a sound and transferable management strategy.

CONCLUSIONS

1. This study demonstrated that the method of application of the insecticide is a critical factor in determining the protection of black pine (*Pinus nigra*) seed crops from *Leptoglossus occidentalis*. The type of treatment accounted for 63.3% of the variance in seed fill ($\eta^2 = 0.633$), which suggests that the way in which the active ingredient is applied to the cone is more important than simply having it present within the stand.
2. Systemic trunk injection (Arborjet) produced the greatest and most consistent protection of the treatments tested, by increasing the average percent of filled seeds to 54.0%, about 5.6 times greater than that of the control, and produced the lowest variation (CV = 17.15%) per tree. In a seed orchard where each tree has high genetic value, the stability of results between trees (*i.e.*, stable yield from tree-to-tree) is nearly as important as mean yield (*i.e.*, higher average yield).
3. The complete absence of empty cones (0%) in the injected group indicates that systemic delivery protects developing cone tissues continuously across all phenological stages. By contrast, contact spraying achieved both a lower seed fill percentage (31.9%) and the highest empty cone ratio (12.0%), exceeding even the untreated control. This counterintuitive outcome suggests that high-pressure canopy application may itself impose mechanical or phytotoxic stress on young cones — a secondary risk that systemic injection avoids.
4. Collectively, these results demonstrate that trunk injection should be made a higher priority than contact spraying as a tool to manage *L. occidentalis*, especially in seed orchards and in areas where seed is being grown to conserve genetic resources or to provide natural regeneration. Seed quality and genetic integrity in these situations can be critical. Because of its minimal effect on non-target organisms, it will also be an appropriate strategy for use in ecologically sensitive areas.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Use of Generative AI

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) as AI-assisted language tools for improving English grammar, clarity, and readability of the text, including the abstract and introduction sections. These tools were also used to assist with formatting and organizing the reference list. All AI-generated suggestions were critically reviewed, verified, and edited by the authors, who take full responsibility for the content and accuracy of the manuscript.

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