Western Conifer Seed Bug

Leptoglossus occidentalis Heidemann
Hemiptera: Coreidae

Bates, S. L.; Lait, C. G.; Borden, J. H.; Kermode, A. R. 2002. Measuring the impact of *Leptoglossus occidentalis* (Heteroptera: Coreidae) on seed production in lodgepole pine using an antibody-based assay. Journal of Economic Entomology 95: 770-777.

Objective: To improve the detection of damage caused by *L. occidentalis* in harvested lodgepole pine seed using an immunoassay.

Abstract: Western conifer seed bug, *Leptoglossus occidentalis* Heidemann, feeds on developing seeds within the cones of lodgepole pine, *Pinus contorta* var. *latifolia* Engelm. This seed bug occurs throughout western North America but appears to be spreading into eastern Canada and the USA. Adult male and female *L. occidentalis* can damage 1.4 and 2.0 seeds per day, respectively, in late cone development. Seed loss due to *L. occidentalis* can be difficult to calculate, as seed damaged by pest feeding cannot be distinguished from aborted seed at harvest unless examined using x-rays. Seed loss estimates are based typically on exclusion studies or caged feeding trials. A new method has been developed using an antibody marker for a salivary protein deposited by *L. occidentalis* while feeding on seeds (Lait et al. 2001). Managers interested in using this immunoassay are strongly encouraged to consult the original publication for detailed information regarding this technique. This method accurately detects seeds damaged by *L. occidentalis*, but traditional radiograph techniques are necessary to estimate the percentage of empty seeds aborted due to other causes.

Sampling Procedure: Collect cones at harvest and store at ≈18°C until processing. Boil cones in water for ≈90 s to remove the resin seal, then bake at 50°C for 8 h. Remove seeds from cones by shaking them vigorously in a container for 90 s. Seeds not dislodged during this procedure are classified as being fused to the cone scales.

Grind individual seeds in 150 μl of a buffer made of 62.5 mM Tris-HCL (pH 6.8), 2% sodium dodecyl sulfate (wt:vol), and 10% glycerol (vol:vol). Load 15 μl of each seed sample on a 15% acrylamide resolving gel and separate the proteins using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions with 2 μl β-mercaptoethanol per sample. Transfer separated proteins onto nitrocellulose with a Western blot. Block overnight in Tris-buffered saline (TBS) made with 5% nonfat milk powder. Incubate Western blots for 2 h at room temperature with affinity-adsorbed primary polyclonal antibody (1:5000) to *L. occidentalis* salivary protein (Lait et al. 2001). Positive binding of the antibody can be demonstrated by using a secondary antibody conjugated to alkaline phosphatase (1:10000) with 5-bromo-4-chloro-3-indoyly phosphate and nitro blue tetrazolium, which react together and form a colored product. Seed samples with this colored product are classified as damaged by *L. occidentalis* feeding. Seeds fused to cone scales should also be considered as being damaged by *L. occidentalis* feeding from early in the season.

Managers must use their experience and the estimated size and value of the expected seed crop to decide if the seed loss determined by this method warrants control measures for *L. occidentalis* the following year.

Notes: The authors did not suggest a minimum number of cones to sample, but they should be collected from a sufficient number of randomly selected trees to adequately assess feeding damage throughout the orchard.

The authors noted that some seeds exposed to *L. occidentalis* early during seed development may be empty when harvested but not test positive for *L. occidentalis* feeding. Also, feeding by *L. occidentalis* on cones early in the season increased the number of seeds fused to the cone scales. The fused seeds, remaining in the cone after the extraction process, are often overlooked as feeding injury on the seed crop. The authors noted that not all fused seeds, when removed from the cones and tested with the immunoassay, produced the colored product indicating *L. occidentalis* feeding damage despite their association with *L. occidentalis*. In both cases, these false-negative results may be due to assimilation of the salivary proteins by the developing ovule or degradation of the proteins over time before harvest. For this reason, the immunoassay is not wholly accurate in detecting *L. occidentalis* damage that may occur early in the season.

Reference:

Lait, C. G.; Bates, S. L.; Kermode, A. R.; Morrissette, K. K.; Borden, J. H. 2001. Specific biochemical marker-based techniques for the identification of damage to Douglas-fir seed resulting from feeding by the western conifer seed bug, *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae). Insect Biochemistry and Molecular Biology 31: 739–746.