Effect of feeding by the western conifer seed bug, *Leptoglossus occidentalis*, on the major storage reserves of developing seeds and on seedling vigor of Douglas-fir

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**Summary**  The impact of feeding by the western conifer seed bug (*Leptoglossus occidentalis* Heidemann) on storage reserves of developing seeds of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) was studied by caging nymphal and adult seed bugs on cones during late-season development, and nymphs on cones during early, mid- and late-season development. Analysis of the major storage reserves of partially damaged seeds revealed that late-season feeding by each life stage and feeding by nymphs at all three stages of cone development significantly reduced the amounts of lipid and buffer-insoluble (crystalloid) protein in seeds at harvest by up to 78 and 97%, respectively. Seeds showing light to moderate damage on radiographs did not exhibit a reduction in the amount of buffer-soluble (matrix) protein. Seeds damaged by feeding during early development compensated in part by continuing to synthesize lipid and crystalloid protein. Light or moderate damage to mature Douglas-fir seeds exposed to *L. occidentalis* in the laboratory reduced seedling emergence by > 80%, but the seedlings that emerged successfully appeared to suffer no adverse effects when grown under standard nursery conditions.

**Keywords:** lipid, protein, *Pseudotsuga menziesii*, seed storage reserves, seedling vigor.

**Introduction**

The western conifer seed bug, *Leptoglossus occidentalis* Heidemann, is a polyphagous pest of conifer seed orchards and is widely distributed throughout western Canada and the USA (Hedlin et al. 1981). Adults and nymphs feed by inserting their proboscises into cones and digesting the contents of developing seeds. At harvest, seeds that have been fed on by seed bugs bear no external signs of damage, but appear empty or partially filled on radiographs. Those that are empty are indistinguishable from seeds that have aborted for environmental or genetic reasons (Schowalter and Sexton 1990). Thus, estimates of seed losses caused by *L. occidentalis* are imprecise, but range from < 5 to 50% in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Schowalter and Sexton 1990, Blatt and Borden 1996, Schowalter 2001).

In Douglas-fir, the major seed storage reserves are lipids and proteins, with the megagametophyte serving as the principal storage organ (Owens et al. 1993). Lipids and proteins are each stored in discrete subcellular bodies (Bewley and Black 1985, Owens et al. 1993). Protein bodies in Douglas-fir have crystalloid insoluble protein inclusions held within an amorphous matrix of water-soluble albumins (Gifford et al. 1982, Green et al. 1991). Little is known about the mechanism by which *L. occidentalis* extracts storage reserves from developing conifer seeds. Based on scanning electron micrographs of mature Douglas-fir seed damaged by *L. occidentalis*, Bates et al. (2000) suggested that the seed bug feeds by the maceration and flushing method as described for some species of plant-feeding Heteroptera (Strong 1970, Miles and Taylor 1994). In this process, various pectinases are secreted into the seed where they degrade the primary cell wall and matrix material. Various other hydrolyzing enzymes are also secreted into the seed, and partially digested cellular contents leak freely through adjacent cell walls without causing mechanical damage. Both lipases and proteases have been identified in *L. occidentalis* saliva (C.G. Lait, S.L. Bates, A.R. Kermode and J.H. Borden, unpublished results); these enzymes presumably hydrolyze the cellular contents before they are taken up by the seed bug. The integrity of the cell walls is reduced, but only when the contents of the seed have been severely depleted do the cell walls begin to collapse, probably because of a lack of structural support. Laboratory experiments using mature, harvested Douglas-fir seed have shown that feeding by *L. occidentalis* can extract almost all of the major seed reserves. Even seeds that sustain only light feeding damage by *L. occidentalis* show a > 55% reduction in both lipid and crystalloid protein storage reserves (Bates et al. 2000a). It was hypothesized that the crystalloid proteins are converted to soluble breakdown products (peptides) which are depleted only after severe feeding damage has occurred. Depending on the extent of reserve depletion, seeds that have been damaged by *L. occidentalis* may be capable of germination. Blatt and Borden (1998) observed that 18% of Douglas-fir seeds fed on by seed bugs in the laboratory germinated. Koerber (1963) and Blatt (1997) suggested that damage by seed bugs may be re-
sponsible for poor germination and reduced seedling vigor in the nursery.

The depletion of seed storage reserves by seed bugs may vary with the severity of feeding damage, life stage of the seed bug and the stage of seed maturation when feeding occurs. Field caging experiments designed to investigate the effect of *L. occidentalis* on seed production (Bates et al. 2000b) provided seeds that had been fed on by different life stages of the seed bug and during different periods of cone and seed development. To determine how these factors influence the amount of reserves remaining at harvest, we compared the amount of lipid and protein reserves in seeds fed on during development by *L. occidentalis* nymphs, adult females and adult males. We also determined the amount of lipid and protein depletion caused by *L. occidentalis* nymphs feeding during early, mid- and late-season seed development. Because seeds that have sustained only partial depletion of storage reserves may still be viable and capable of germination, we used mature Douglas-fir seeds to investigate how different degrees of feeding damage affected seedling emergence and subsequent vigor in the nursery.

**Methods**

Exposure of seeds to feeding

Seeds for protein and lipid analyses were obtained from field experiments at Mount Newton Seed Orchard, TimberWest Ltd., Saanichton, B.C. in 1997 (Bates et al. 2000b). Groups of cones on individual tree branches of two clones were caged on May 21, 1997, before the emergence of over-wintered *L. occidentalis* adults, and all cones were measured (length and diameter) at the time of caging to ensure uniformity in stage of development. Clone selection was based on cone availability. Cones were caged in white polyester sleeve cages, 50 × 20 cm, with a 1-mm mesh. Seed bugs were introduced into the cages for 2-week periods. Insects were obtained from a laboratory colony that was periodically augmented with seed bugs collected from seed orchards.

In the first experiment, seed bug nymphs, females or males were introduced onto caged cones (Clone 3130) during late-season cone development (August 4–18). The four treatments were: (1) two second or third instar nymphs caged per cone; (2) one adult female caged per cone; (3) one adult male caged per cone; and (4) control, no seed bugs caged.

In a second experiment, second and third instar nymphs were introduced onto cones (Clone 3175) to evaluate feeding damage at different periods of cone development. Nymphs were used to ensure a consistent level of feeding during each period. The four feeding treatments were: (1) early season (June 4–18); (2) mid-season (July 4–18); (3) late-season (August 1–15); and (4) caged control (no seed bugs).

Because there were insufficient seeds from these experiments for both protein and lipid analysis, additional seeds were obtained from the same seed orchard in 1998. Groups of 3–6 cones were caged on individual branches (Clone 3106) on June 14, 1998. Observations in the seed orchard at the time of caging did not reveal the presence of *L. occidentalis*. Feeding damage from *L. occidentalis* before caging was, therefore, assumed to be negligible. Seed bugs were introduced into 11 cages per treatment at similar times and life stages as in 1997, according to the following treatments and densities: (1) three second or third instars per cone at mid-season cone development (June 29–July 14); (2) one female per cone at late-season cone development (August 4–11); (3) three nymphs per cone at late-season development (Aug 4–11); (4) two males per cone at late-season cone development (Aug 4–11); and (5) control, no seed bugs in cage (June 22–Aug 11).

Each cage was inspected after one week, and any dead or missing insects were replaced. At the end of each 2-week period, all seed bugs were removed and the cages resealed until harvest. Early maturation of the cones in 1998 forced the termination of the late-season feeding period one week earlier than anticipated. All cones were harvested on August 11, 1998 or September 5, 1997, air dried at room temperature and the seeds extracted by hand. All seeds were X-rayed using a Model 804 photo machine (Faxitron X-Ray Corp., Wheeling, IL) at the Pacific Forestry Centre, Canadian Forest Service, Victoria, B.C. for 90 s at 19 kV, and sorted into one of four damage categories: light (over 66% of tissue remaining), moderate (33–66% tissue remaining), severe (≤33% of tissue remaining) or extreme (seed empty) (Bates et al. 2000b). It was impossible to determine if empty seeds were due to feeding by *L. occidentalis* or to seed abortion, therefore they were excluded from further analysis. Because of low seed yields in 1998, it was necessary to pool seeds from both years and combine the seeds assigned to the light and moderate damage categories into one light/moderate damage category.

In 1997, additional cones were protected from exposure to *L. occidentalis*. Two cones were picked from Clone 3175 at early and mid-season development stages, and two cones from Clone 3143 at late-season development. The seeds were immediately dissected from the cones, frozen in liquid nitrogen, stored at −80 °C and used for determination of lipid and protein contents in normally developing seeds at each stage of cone development.

**Lipid extraction and quantification**

Three to 12 seeds exposed to feeding by each life stage of *L. occidentalis*, or during each period of cone development, and 15 control seeds were individually analyzed for crude lipid content (chloroform-soluble compounds) according to the method of Halmer et al. (1978). Normally developing seeds dissected from cones during early (*n = 30), mid- (*n = 14) and late-season (*n = 23) development were pooled by stage and also analyzed for lipid content. Seeds were dissected from the seed coat and ground in a Duall ground-glass tissue grinder (3-ml capacity, Kontes Glass Company, Vineland, NJ) in 1 ml of 2-propanol. The resulting homogenate was centrifuged for 20 min at 14,000 g and the supernatant set aside. The pellet was re-extracted for 2 h three times with 2 ml of 2:1 (v/v) chloroform:methanol, centrifuged, and the supernatants added to the supernatant from the first extraction. Solvent was evapo-
rated from the pooled supernatants with a stream of nitrogen. The crude lipids were dissolved in 2 ml of chloroform, filtered through glass wool and transferred to pre-weighed vials. Chloroform was removed with a stream of nitrogen, the vials dried overnight in an oven at 40 °C and the lipid quantity determined gravimetrically.

**Protein extraction and quantification**

Seed coats were removed from 2–15 seeds in each damage category and from 15 control seeds. Twenty, 11 and 22 normally developing seeds from cones picked during early, mid- and late-season development, respectively, were each separated from the seed coat at the time of dissection. Buffer-soluble (matrix) and buffer-insoluble (crystalloid) proteins from individual seeds were separated according to the method of Gifford et al. (1982). Each seed was homogenized in 250 μl of chilled buffer (A) consisting of 0.05 M sodium phosphate and 5 μM leupeptin, pH 7.5. The homogenate was centrifuged at 14,000 g for 30 min and the supernatant, containing matrix proteins, was transferred to an equal volume of buffer (B) containing 62.5 mM Tris-HCl, pH 6.8, 2% SDS (w/v) and 10% glycerol (v/v). The pellet, containing the crystalloid protein fraction, was re-washed three times in buffer A and centrifuged as above, with the supernatant discarded each time. The pellet was resuspended in 250 μl of buffer B, boiled for 5 min and cooled. The suspension was centrifuged at 14,000 g for 15 min and the crystalloid proteins removed with the supernatant. Both matrix and crystalloid proteins were quantified colorimetrically by the Bio-Rad DC Protein Assay (Bio-Rad Laboratories, Inc., Hercules, CA) (Alam 1992).

**Seedling emergence and vigor**

Mature Douglas-fir seeds were obtained from the B.C. Ministry of Forests Tree Seed Centre, Surrey, B.C. Seeds were X-rayed to ensure they were full, and then exposed to feeding by *L. occidentalis* nymphs in the laboratory. After 7 days, the exposed seed was X-rayed again. Seeds that showed signs of partial feeding damage were sorted into light, moderate, and severe damage categories. In seeds sustaining severe damage, the embryo and megagametophyte were damaged beyond recognition or were missing completely; it was assumed that these seeds would not germinate and they were excluded from the experiment. The experiment comprised five treatments: (1) lightly damaged seed; (2) moderately damaged seed; (3) seeds exposed to seed bug feeding but showing no signs of damage on radiographs (full exposed); (4) seeds not exposed to seed bug feeding (full unexposed); and (5) seeds that were not X-rayed but assumed to be full based on a weight of > 8.0 mg (X-ray control).

Approximately 50 seeds from each treatment were moist chilled for 21 days at 4 °C and then planted in Styroblocl tree seedling containers (11.7 × 4.2 cm cells, 364 cells m⁻²) on February 4, 1998 at Green Timbers Nursery, Surrey, B.C. Nine to 14 seeds from each treatment were randomly distributed within each multi-cavity Styroblocl and a buffer of non-experimental seeds was planted in outside cavities surrounding the experimental seeds to eliminate edge effects. Seedlings were maintained under standard irrigation and fertilizer regimes in the nursery. The number of seedlings that emerged in each treatment was recorded daily, and biweekly height measurements were recorded for the first 20 weeks. Seedlings were lifted on November 3, and height and root collar diameter were measured. Six to 18 randomly selected seedlings from each treatment were dried for 12 days at about 40 °C, severed at the root collar, and weighed to determine aboveground biomass.

**Statistical analysis**

Lipid and protein data were log or square root transformed as necessary to improve homoscedasticity and normality (Zar 1984). The amounts of lipid and protein per seed in each treatment for each damage category were analyzed by the general linear model procedure (SAS 1988). Means between treatments were compared with the Ryan-Einot-Gabriel-Welsh test (REGW), α = 0.05 (Day and Quinn 1989). In 10 out of 13 cases in which data for storage reserves of exposed seed were pooled between clones or years or both, there was no significant difference in the mean percentage of reserve material remaining, ANOVA (n = 3) or t-test (n = 2), P > 0.05. In the other three cases, there were significant differences in the mean percentage of reserve material remaining (x ± SE) between years. Case 1: lipids—late-season exposure to nymphs, light/moderate damage category, 1997 percentage = 80.7 ± 4.9, n = 5, and 1998 percentage = 55.8 ± 3.5, n = 4, t = 2.3646, P = 0.0005; Case 2: matrix protein—late-season exposure to males, severe damage category, 1997 percentage = 49.7 ± 6.0, n = 4, and 1998 percentage = 85.0 ± 8.9, n = 4, t = 2.4648, P = 0.0165; and Case 3: matrix protein—mid-season exposure to nymphs, severe damage category, 1997 percentage = 13.6 ± 3.8, n = 5, and 1998 percentage = 28.3 ± 4.4, n = 5, t = 2.3060, P = 0.0360.

Percent emergence of seedlings across treatments was analyzed by a χ² test for multiple proportions (Conover 1980). Days to emerge and dry biomass were analyzed by the general linear model procedure (SAS 1988) and the means compared by the REGW test for multiple proportions (Day and Quinn 1989). In all cases, α = 0.05.

**Results and discussion**

**Protein and lipid depletion**

Feeding by each life stage significantly reduced the amount of crude lipid reserves (F₆,₅⁹ = 20.91, P < 0.0001), and severely damaged seeds had significantly less lipid than seeds sustaining light/moderate damage (Figure 1A). The amount of lipid extracted from seeds in each damage category was similar irrespective of whether damage was caused by nymphs, females or males, indicating that lipid extraction did not vary with life stage or sex of the seed bug.

Feeding during each period of cone development resulted in a significant decrease in lipid reserves compared with the control value (F₆,₅⁰ = 39.15, P < 0.0001) (Figure 1B). Despite a
similar appearance on radiographs, seeds subject to light/moderate or severe damage during early cone development contained substantially less crude lipid than seeds damaged to the same extent during mid- and late-season cone development.

At fertilization in early June (Allen and Owens 1972), immature seeds become separated from the cone scale (Owens et al. 1993), indicating that most of the precursors for storage products in the seed are present before the majority of *L. occidentalis* feeding damage occurs. Lipid analysis of normally developing seeds showed that < 2% of the total lipid at harvest was synthesized during early season development (Table 1). By harvest, however, the amount of lipid in seeds damaged during early cone development had risen to 13% of the control value for seeds in the severe damage category and to 15% for seeds in the light/moderate damage category (Figure 1B). Presumably, seeds were capable of resuming lipid synthesis following early and mid-season feeding by the seed bug; whereas late-season feeding occurred when about 100% of the seed lipid reserves had been synthesized (Table 1), and the synthetic capacity of the seed was reduced to an extent that prevented the resumption of lipid synthesis.

Depletion of buffer-insoluble (crystalloid) protein reserves was predominantly influenced by the severity of feeding damage (Figure 2). All damaged seeds showed a significant reduction in the amount of crystalloid protein remaining at harvest compared with the control, regardless of the life stage of the seed bug causing the damage ($F_{6,47} = 64.98$, $P < 0.0001$) or the period of cone development ($F_{6,47} = 37.60$, $P < 0.0001$) when damage occurred. There were no differences between the life stages in either damage category (Figure 2). Unlike the results for lipid, there were no differences between early, mid- and late-season feeding within each damage category (Figure 2B). Seeds subjected to light/moderate damage during early season development (Figure 2B) contained more crystallloid protein at harvest (35% of control) than at the time of feeding (5%) (Table 1), suggesting that synthesis of storage protein also occurred subsequent to feeding.

Buffer-soluble (matrix) protein was depleted only in severely damaged seeds that were fed on by females ($F_{6,47} = 64.98$, $P < 0.0001$) or during the early and mid-season periods of cone development ($F_{6,47} = 21.00$, $P < 0.0001$) (Figure 3). The apparent lack of soluble protein depletion in seeds with light/moderate damage may reflect changes in the solubility of hydrolysis products caused by *L. occidentalis* feeding. Bates et al. (2000a) showed that feeding on mature Douglas-fir seeds by *L. occidentalis* nymphs and males increased the amount of buffer-soluble protein in lightly damaged seeds by about 62% compared with the control. This increase was at-

Table 1. Amounts of lipid and crystalloid and matrix protein reserves in developing Douglas-fir seeds dissected from cones picked during early, mid- and late-season development, compared with reserves in mature seeds at harvest.

<table>
<thead>
<tr>
<th>Reserve material</th>
<th>Amount of reserve (mg) (mean ± SE) in control seeds at harvest ($n = 15$)</th>
<th>Stage of cone development</th>
<th>No. of seeds sampled and pooled</th>
<th>% Reserve in developing seed/control seed at harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid</td>
<td>3.72 ± 0.21</td>
<td>Early</td>
<td>30</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mid</td>
<td>14</td>
<td>20.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late</td>
<td>23</td>
<td>104.4</td>
</tr>
<tr>
<td>Crystalloid protein</td>
<td>0.72 ± 0.03</td>
<td>Early</td>
<td>20</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mid</td>
<td>11</td>
<td>40.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late</td>
<td>22</td>
<td>98.1</td>
</tr>
<tr>
<td>Matrix protein</td>
<td>0.90 ± 0.07</td>
<td>Early</td>
<td>20</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mid</td>
<td>11</td>
<td>23.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late</td>
<td>22</td>
<td>47.7</td>
</tr>
</tbody>
</table>
tributed to the hydrolysis of crystalloid protein to soluble peptide components, which were depleted only as feeding progressed. The significant reduction in matrix protein in severely damaged seeds fed on by females compared with controls (Figure 3A) suggests that females have a greater capacity than males or nymphs to extract buffer-soluble protein reserves, including both matrix proteins and crystalloid degradation products. In agreement with this interpretation, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of mature Douglas-fir seeds fed on by nymphs, females and males indicated that females cause the greatest amount of damage to both crystalloid and matrix protein reserves (C.G. Lait, S.L. Bates, A.R. Kermode and J.H. Borden, unpublished results). High efficiency in seed protein extraction would help females of reproductive status meet the high nitrogen requirements of egg production.

Normally developing seeds analyzed during late-season development contained only half as much buffer-soluble (matrix) protein as control seeds at harvest (Table 1). Because the extraction of buffer-soluble protein was not specific for matrix protein that only has a storage role, it is possible that the increase in buffer-soluble protein between late season development and harvest was a result of the accumulation of several proteins important for late maturation. For example, synthesis of late embryogenesis abundant proteins (LEAs), which may have a role in the acquisition of desiccation tolerance of seeds, increases during late seed maturation in numerous plant species (Kermode 1995, Dure 1997). Genes encoding proteins similar to the LEAs have been identified in seeds of loblolly pine, *Pinus taeda* L. (Kermode 1995), white spruce, *Picea glauca* (Moench) Voss (Leal and Misra 1993) and Douglas-fir (Jarvis et al. 1996).

**Seedling emergence and vigor**

Both light and moderate feeding damage to mature seeds reduced seedling emergence by > 80% compared with the controls ($\chi^2 = 117.8, df = 8, P < 0.0001$) (Figure 4A). About 14% of seeds in both the light and moderate damage categories germinated and the seedlings had similar germination and growth characteristics to seedlings that developed from undamaged seeds. Thus, the length of time between sowing and seedling emergence was similar ($F_{4,126} = 0.81, P = 0.5197$) (Figure 4B) and seedlings had comparable shoot biomass after lifting (at 34 weeks) as seedlings from undamaged seeds ($F_{4,56} = 1.29, P = 0.2842$) (Figure 4C). The growth rates of seedlings in each treatment were also similar (Figure 5), and by 20 weeks post-emergence the mean height of seedlings in each treatment was similar ($F_{4,126} = 0.68, P = 0.61$). Also, there was no difference between treatments with respect to the percentage of seedlings that met nursery production height ($F_{4,126} = 0.80$, 

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**Figure 2.** Amount of buffer-insoluble (crystalloid) protein (mg) remaining in Douglas-fir seeds fed on by *L. occidentalis*. Seeds were assigned to damage categories by radiography. A. Seeds damaged by nymphs, females and males during late-season cone development, and B. seeds damaged by nymphs during early, mid- or late-season development. Numbers of replicates (seeds) for each treatment are beneath the bars. Bars with the same letter are not significantly different (REGW test, $P < 0.05$).

**Figure 3.** Amount of buffer-soluble (matrix) protein (mg) remaining in Douglas-fir seeds fed on by *L. occidentalis*. Seeds were assigned to damage categories by radiography. A. Seeds damaged by nymphs, females and males during late-season cone development, and B. seeds damaged by nymphs during early, mid- or late-season development. Numbers of replicates (seeds) for each treatment are beneath the bars. Bars with the same letter are not significantly different (REGW test, $P < 0.05$).
P = 0.5253) or root collar diameter (F_4,126 = 0.02, P = 0.9988) specifications (18 cm and 3 mm, respectively) when the seedlings were lifted in the fall. The only significant impact of _L. occidentalis_ feeding was a severe reduction in seedling emergence. During normal nursery production, a varying percentage of the Douglas-fir seed sown does not germinate or emerge. It is possible that _L. occidentalis_ damage contributes to this variation. Lightly and moderately damaged seeds from the field experiments had mean biomass values of 9.6 and 7.3 mg, respectively; these weights were within the range (6.5–17.5 mg) of full seeds that had not been exposed to seed bug feeding (Bates et al. 2000a). St. Clair and Adams (1991) found that seed biomass was only weakly correlated to successful emergence in Douglas-fir. Presumably, if the embryo is intact and sufficient storage reserves remain in the seed to allow germination and emergence, the resulting seedling becomes photosynthetically active and is able to grow and successfully compete in a nutrient-rich nursery environment.

We note that these conclusions are based on the assumption that mature seeds fed on by seed bugs in the laboratory behave in the same way as seeds fed on during their development in the field. It is possible that feeding at different stages of seed development, which differentially affects the storage reserves of seeds at harvest, may influence subsequent germination and seedling vigor. For example, seeds with moderate/light damage that were fed on during early season development had significantly fewer lipid reserves than seeds showing similar damage fed on late in the season (Figure 1B), an effect that could influence germination success and seedling vigor.

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**Figure 4.** Emergence and subsequent performance of Douglas-fir seedlings grown from seeds sustaining light and moderate damage from feeding by _L. occidentalis_ compared with full seeds that had been X-rayed and then exposed or not exposed to seed bugs, and full seeds not X-rayed. A. Percentage of seeds emerging, B. length of time between sowing and seedling emergence, and C. dry biomass of seedlings after 40 weeks. Numbers within bars indicate the number of seeds (A) or seedlings (B, C). Bars with the same letter are not significantly different, χ² test for multiple proportions (percent germination) and REGW test for multiple comparisons (duration to emergence and shoot biomass), P < 0.05.

**Figure 5.** Growth of Douglas-fir seedlings emerging from seeds sustaining light and moderate damage after feeding by _L. occidentalis_ compared with growth of seedlings in three control groups: full exposed, full unexposed and X-ray control.
References


