Elm trees (*Ulmus americana*) are highly valued on the Canadian Prairies and Northern Great Plains, for reasons of aesthetics, longevity, and ecological services. The mature elm population is in decline, accentuating perceived value and need for protection. Red elm bark weevil (REBW), *Magdalis armicollis*, is a potentially serious pest of elm trees in grassland and parkland regions of western Canada and the United States. REBW eggs are laid beneath the bark, and larval and pupal stages develop in situ. Adults emerge from bark the following year, and feed on growing leaves, resulting in typical shothole defoliation. Populations of REBW tend to increase in years with warm summers (M. Booth, unpublished observations at Lethbridge Research Center). During May to August 2006, an experiment was conducted to evaluate the effects of trunk injection of pressurized liquid formulation of acephate (Orthene) on abundance of red elm bark weevil in American elm in Alberta, Canada. Previous tests by the Canadian Forest Service indicated potential effectiveness of the Ecoject injection system developed by BioForest Technologies Inc. (Sault Ste. Marie, ON, Canada). The Ecoject injection tool pressurizes small plastic vials, which inject small quantities of insecticide into the tree when they are inserted into holes drilled into the trunk.

### Experimental Design

#### Randomization and Replication

The experimental units in the trial consisted of 50 mature American elm trees. Treatment was randomly assigned to one tree in each of 25 contiguous pairs, so that each of the 25 randomized blocks of the experiment consisted of two mature American elm trees, standing together on the same side of a paved lane. The lane is on federally owned and managed research property near Highway 3, east of Lethbridge, Alberta, Canada. One reason for using a paired-tree randomized block design was to deter-
mine whether a reduction in REBW numbers could be effected in a situation where injected trees were next to uninjected trees.

**Injection Treatment**

The Pest Management Regulatory Agency (PMRA, Health Canada) granted authorization to utilize a 97% dry flowable (DF) formulation (Arysta LifeScience) for this test, under the national “Risk Reduction Initiative” in support of pest management experimentation and refinement. The target injection application rate was determined according to circumference at breast height (cbh), 0.3 g ai / cm cbh (1.5 g ai / injector / 6 in cbh). Trees were measured and assigned a number of injectors based on tree size: one 8 mL (0.27 fl oz) injector canister per 15 cm (6 in) trunk circumference. The number of injectors selected, determined at the time of treatment by dividing the tree circumference in cm by 15 (cbh in inches divided by six), ranged from 12 to 22 injectors canisters per tree. Injector canisters were pressurized with the Ecoject System and then inserted into holes (one per canister) drilled near the base of the trees, 1.3–1.9 cm (0.5–0.75 in) into the cambium. The injector pressure for this method is approximately 0.379 MPa (55 psi) at the start of the loading stroke, and 0.448 MPa (65 psi) at the end of the stroke. The nozzle size was 5.6 mm (7/32 in). Injectors typically delivered all of the formulated product into the tree within 10 s. The injection procedure was completed on June 6, 2006.

**Sampling**

**Foliage**

Two branches of at least 8 leaves were collected from each tree on two sampling dates: immediately following the day of injection, and 8 weeks later (August 2, 2006) for chlorophyll analysis. Chlorophyll content has been used in previous studies as an indicator of chemical challenge and resulting tissue damage (Knudson et al. 1977), or pest feeding damage (Johnson 1983). In the current REBW study, elm leaves were removed, frozen, and returned to the grassland ecosystem laboratory at the University of Lethbridge (Alberta, Canada). Leaves were kept frozen until being dried and powdered. A sample of 1.0 g from each branch sample was extracted with 80% acetone and water in a liquid grinder/mixer. Absorbance was measured using a Biochrom Ultrospec 3300 pro UV/Visible spectrophotometer, and recorded between 300 and 800 nm for every sample; data at 664, 652, 645, 456, and 432 nm, did not differ between treatments (injected versus noninjected) either at the time of treatment application, or 8 weeks after injection (Figure 1, ANOVA, P > 0.3), for all five measured wavelengths. An example plot of the absorbance spectrum for one sample is shown in Figure 2.

**Insects**

Five yellow “Tanglefoot” sticky traps [10.2 x 15.2 cm (4 x 6 in)] were placed in each tree canopy. A total of 250 traps were placed at the beginning of each sampling period, and then removed and returned to the lab for counting of adult REBW under stereomicroscopes, to estimate changes in abundance. Sampling periods were 0, 2, 4, 6, and 8 weeks following treatment. Relative humidity and ambient temperatures within the canopy and branch tissue were monitored during the experiment with HOBO data recorders (Onset Computer Corp., Bourne, MA) and Oakton temperature recorders in the canopy of one of the trees. General weather data for the site was provided by a permanent weather station within 1 km (0.62 mi), at the Agriculture and Agri-Food Canada Research Centre.

**RESULTS AND DISCUSSION**

**Tree Measurements**

Circumference at breast height of the 50 trees used in the experiment ranged between 135 and 361 cm (53–142 in), mean = 96.8 cm (38.1 in), SEM = 2.73 cm (6.9 in) (good fit to a normal distribution; G.O.F. Shapiro-Wilk Test, W = 0.9808, P > 0.5, SAS Institute, 2007). The 25 trees assigned to the control did not differ in average size from the 25 trees assigned to the treatment (t = 0.304, P > 0.5; this confirmed there was no initial bias in trunk size).

**Chlorophyll Content**

Chlorophyll content (and related variables affecting leaf color), as represented by spectrophotometric absorbance at 664, 652, 645, 456, or 432 nm, did not differ between treatments (injected versus noninjected) either at the time of treatment application, or 8 weeks after injection (Figure 1, ANOVA, P > 0.5; this confirmed there was no initial bias in trunk size).

**Insect Sampling and Population Estimates**

Of the total 500 sticky traps that were attached in the canopies of the 50 trees for the first two sampling periods, 18 and 55 (respectively) were lost because of wind, but losses were approximately equal for treated and untreated trees. The resulting counts ranged typically between 0 and 5 adult beetles per sticky trap card. The counts were log-transformed before statistical analysis: ln (REBW+0.5). Tree size was not a significant covariate; G.O.F. Shapiro-Wilk Test, W = 0.9808, P > 0.5.

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On later dates, the populations of all blocks and both treatments declined, resulting in more frequent “zero” counts and treatment differences were no longer measurable after that time. The sampling scheme in our experiment did not distinguish between mortality and possible consequence of repellency.

Relative Population Changes
The more rapid drop in the REBW counts on the treated trees (T), with respect to that of the untreated control trees (C), during the first two weeks after injection, resulted in a decline in abundance of 36.7% on the treated trees, adjusted against the change on the control trees. This is an adjusted value (Abbott 1925), calculated as 100 * (1 - odds ratio), where the odds ratio is \((T_2 * C_1) / (T_1 * C_2)\). This measure has been shown to be a reliable indicator of adjusted changes in pest abundance (often interpreted as mortality), applicable to randomized complete block designs sampled over time in field studies, such as this, and confirmed in Monte Carlo simulation studies (Schaalje et al. 1986).

Table 1. Numbers of valid (not blown down by wind) sticky traps, and mean numbers of REBW per trap, during the first two weeks following injection.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>a. At the time of injection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>treated</td>
<td>116</td>
<td>0.3103</td>
<td>0.05254</td>
</tr>
<tr>
<td>control</td>
<td>116</td>
<td>0.4138</td>
<td>0.06825</td>
</tr>
<tr>
<td><strong>b. Two weeks post-treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>treated</td>
<td>102</td>
<td>0.2451</td>
<td>0.05107</td>
</tr>
<tr>
<td>control</td>
<td>93</td>
<td>0.5161</td>
<td>0.08596</td>
</tr>
</tbody>
</table>

**CONCLUSION**
Although the declines of red elm bark weevil populations later in the season precluded a longer record of the effects of tree injection, the method was effective in delivering sufficient active ingredient to the target to reduce REBW numbers in the elm trees. The result was detectable even though treated and untreated trees were contiguous, typically on the same side of a paved lane. No phytotoxic effects were apparent. Future studies could be conducted to determine the dynamics of active ingredient concentrations in leaf and wood tissue, to compare the results of a range of rates, and to determine rates that would be required for economical and effective operational-scale control.

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**LITERATURE CITED**
SAS Institute, 2007. SAS Ver. 9.1 and JMP Ver. 5.1

Figure 2. Typical absorbance spectrum (Biochrom Ultrospec 3300 pro UV/Visible spectrophotometer) for one elm leaf sample (tree 7, date 2).
Tucker, E.S. 1907. Concerning some insects collected and bred from dead and dying elm. Transactions of the Kansas Academy of Science 21:158–162.


Dan Johnson
University of Lethbridge
4401 University Drive
Lethbridge, AB, Canada
T1K 3M4
dan.johnson@uleth.ca

Michael Booth (corresponding author)
Agriculture and Agri-Food Canada
Lethbridge Research Centre, 5403 – 1 Avenue S.
Lethbridge, AB, Canada
T1J 4R1
BoothM@agr.gc.ca