ADULT HOST SELECTION TRIALS FOR SPRUCE BUDWORM RESISTANCE IN DOUGLAS-FIR: A CAUTIONARY TALE

Ward Strong[1]

Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, is a major commercial forestry species in British Columbia, Canada. Its range includes wet coastal areas and dryer Interior regions (Hermann and Lavender 1990) (fig. 1). In the interior range, it is subject to attack by the western spruce budworm, *Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae). The larvae of this moth can cause up to 100 percent foliage loss, and tree death can occur after successive years of defoliation (Maclauchlan et al. 2006). During the latest outbreak, which peaked in 2007, over 800 000 ha were infested (British Columbia Forest Service 2007). Current means of controlling *C. occidentalis* include aerial sprays of *Bacillus thuringiensis*, a bacterium toxic to Lepidoptera larvae. In 2012, 116 012 ha were sprayed to attempt control of this insect (British Columbia Forest Service 2012), at an estimated cost of CDN $2.51M (Personal communication. 2018. Lorraine Maclauchlan, BC Ministry of Forests, Lands, Natural Resource Operations,

Figure 1—Range of Douglas-fir (*Pseudotsuga menziesii*) in British Columbia. (courtesy photo by Province of British Columbia)

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[1] BC Ministry of Forests, Lands, Natural Resource Operations, and Rural Development, Vernon, British Columbia, CANADA V1B 2C7, (ward.strong@gov.bc.ca).

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and Rural Development, Kamloops, BC Canada). A more cost-effective means of control might be to develop genetically-based resistance to *C. occidentalis*.

The British Columbia Forest Service has been conducting genetics studies and breeding programs for commercially important trees since the 1960s (e.g., Roche 1969). Selective traits of importance have historically been volume, tree form, and wood quality, and more recently pest resistance has become a focus. One region of BC, where we have an advanced breeding program, is from the Thompson-Okanagan region, which suffered widespread *C. occidentalis* infestation during the 2007 outbreak. This breeding program is composed of 78 parent genotypes, and clones of these parents are held in a breeding arboretum in Vernon, BC, with 3 ramets per clone. We decided to examine these trees for *C. occidentalis* resistance, with the objective of incorporating resistance into our breeding program, for eventual deployment across the landscape if gains can be made for this trait.

We started in 2012 by purchasing *C. occidentalis* pupae from an insectary (NRCan Insect Production services, Sault Ste-Marie, Ontario), derived from British Columbia stock. These were reared at 22 °C and 40 percent RH until eclosion. Moths were allowed to mate and females oviposited on wax paper. Paper tabs supporting 10 eggs were cut with scissors and affixed with a paper clip to new shoots of Douglas-fir, shortly after budburst. One shoot on each ramet of each parent genotype was thus infested. Over each shoot a 53 x 63 cm Organza mesh bag was installed (Creative Bag, Toronto, Canada) to keep *C. occidentalis* in and predators or parasites out. In late June the bags were removed, remaining moths, pupae or larvae within counted, and foliage rated for proportion consumed.

There was a wide range in variation in feeding performance (fig. 2), ranging from 0 percent of foliage consumed to over 90 percent. No larvae remained, but the number of pupae and moths that came out also varied, with generally the lowest numbers coming from the least palatable parents, and the highest numbers from the most palatable parents. This is a result typical of other studies (Cates 1983, McDonald 1981), though no other studies specifically measured the influence of genotype. However, the usefulness of these results might be limited, because while the larvae cause the damage, it is their parents, which are strong

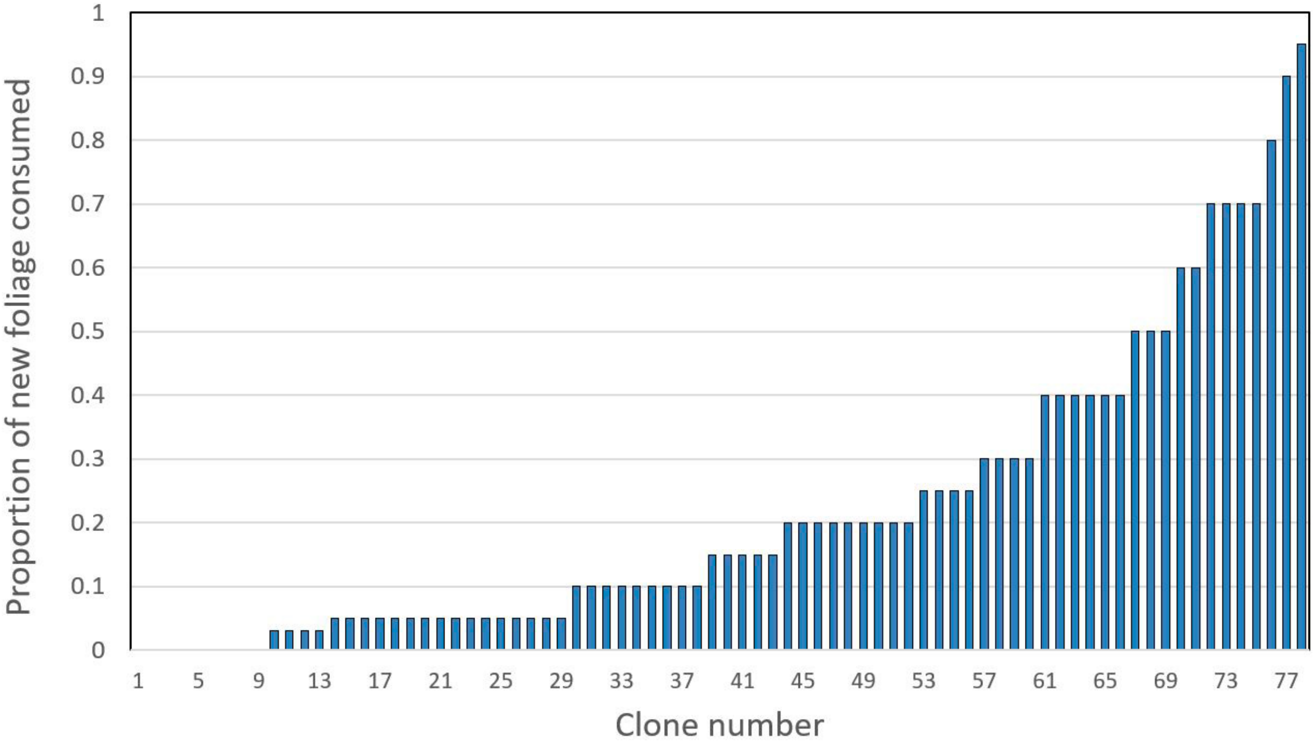

Figure 2—Proportion of new foliage consumed by captive spruce budworm larvae on 78 different Douglas-fir genotypes.

flyers that choose the host tree that the larvae must then feed upon. We therefore decided to investigate adult host selection of *C. occidentalis* on Douglas-fir.

Surprisingly, only two papers were found on adult budworm host selection: Städler (1974) described host plant characteristics, presumably under genetic control, that influence oviposition choice in eastern spruce budworm, *C. fumiferana*; and Leyva et al. (2000) investigated adult host preference of *C. occidentalis* among 3 host species, without reference to genotype within species. Another similar system in which adult host preference has been investigated is in fruit trees with other members of the same moth family, Tortricidae. Oriental fruit moth *Grapholyta molesta* females show oviposition preference between fruit tree species (Myers et al. 2006a), while obliquebanded leafroller *Choristoneura rosaceana* adult females prefer some host species over others (Carrier et al. 1995), both again without reference to genotype within species. Only one paper was found investigating within-species host preference: Myers et al. (2006b) found that *C. rosaceana* oviposited preferentially on certain apple cultivars. Discussion with Spruce Budworm researchers in Canada revealed no knowledge of adult host selection, within host species, in any *Choristoneura* in Canada. This is apparently a wide-open field.

In 2013, we collected shoots from each of the 78 parent genotypes, in early July when moths emerge, fly, and oviposit in the wild. We installed these shoots in water troughs in a small greenhouse, arranged in a randomized complete block design with 5 replicates. Insect mesh was installed inside the glass to contain adult moths. Into this enclosure we introduced 500 *C. occidentalis* pupae, again purchased from the Canadian insectary, spread among 10 petri dishes. By the next morning, all pupae had been consumed by mice. Because the insectary had no more pupae available, we were shut down for the year.

In 2014, we tried again with a similar experimental design and physical facilities, but with a mouse-proof release device. We also purchased an extra 500 pupae as a backup. The moths emerged within 5 days and immediately flew to the ground and laid their eggs on the concrete floor. The backup pupae produced moths that behaved in exactly the same manner. Our moths were highly confused.

In discussion with several budworm experts in Canada, we identified three potential issues.

- The cuttings were removed from the parent trees, they were not a part of the natural tree. In other systems, as soon as a shoot is removed from the parent plant, its volatiles change dramatically, potentially losing attractiveness, or even repelling adult *C. occidentalis*.
- The greenhouse environment might confuse the moths, by altering the light, temperature, humidity, or wind flow characteristics that moths outdoors use to detect potential hosts.
- The *C. occidentalis* colony might have a highly restricted genetic makeup, having been selected through several generations for freedom from disease and good performance in a laboratory colony on artificial diet, ovipositing on wax paper. Their normal host-finding systems may have been affected, and even their ability to fly may have been impacted.

We decided to deal with these issues by grafting scion from each of the 78 parents onto rootstock growing in an outdoors compound. Four hundred and fifty Douglas-fir seedlings of random genetic origin were planted in 2015. Growth was not as expected by 2016, so we waited until 2017 to collect scion and make the grafts. Grafts were arranged in a 13 x 6 alpha-design (Fu et al. 1998) with five linearized replicates. In 2018, grafts were growing well, so one-half the rootstock foliage was removed. The objective is to remove all the rootstock by 2019 so that the only foliage remaining is from the parent genotype.

In 2019, a screenhouse will be constructed over the outdoor trees. This light structure, covered with a large-mesh insect screen, will provide very little shade or other light disturbance, and allow maximum airflow through the grafts, but contain the moths once they have emerged.

We plan to release wild-collected pupae. Wild-collected pupae might be more adapted to host-finding in their native habitat and will have a broader genetic background. Though the greater genetic variation of the budworm pupae might add experimental error, any inferences will be more broadly applicable.

Though we have no data yet in the 4th year of our *C. occidentalis* adult host selection study, we have answered one question: why this has not been done before. It's hard! This sort of study is not amenable to a graduate student program, so it remains to government researchers like our group to conduct it. We hope to have some results by the end of 2019.

Addendum, April 2020. Results from the year 2019. We constructed a screenhouse as planned over the grafted outdoor trees. We collected about 1,800 pupae from the wild in late June. These were placed in brass soil screens (20.3 cm diameter, 5 cm rim, 8–12 mesh) suspended by wire from the screenhouse structure. Soil screens were used so that rain would not accumulate and drown the pupae; they were suspended by wires, and not allowed to touch any trees, in order to prevent ants from discovering and preying upon the pupae. Pupal emergence was only 13.8 percent, and the moths that emerged died within 1 or 2 days of emergence, without laying any eggs. We determined later that the brass soil screens heated in the sun to temperatures in excess of 45 ºC, which likely killed most of the pupae. Those that did emerge encountered very dry conditions with no dew formation or wildflowers to provide moisture, so moths likely died of dehydration. We will try one last kick at the can in 2020, modifying the release devices to provide shade (so they do not heat up in the sun) and misting the plants daily to provide moisture. Maybe after 7 years of trying we will finally get some results.

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