The Canadian Sweet Chestnut

-Newsletter of the Canadian Chestnut Council-

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http://www.canadianchestnutcouncil.ca

Council Mission - to help restore the American Chestnut to the areas of Canada it once occupied.

Current Priorities

1) Breeding resistance
2) Breaking Isolation / Establishing Gene Nods
3) DNA Analysis
4) Survey of existing Chestnuts in the wild

In this issue:

Breeding Resistance – Propagating seedlings the traditional way for the 2018 resistance program

In the March 2018 issue of our Newsletter, in the article "Trees and Test Tubes", Christie Lovat from McGill University described how she would be using tissue culture techniques to generate about 1/3 of the seedlings for this year's breeding the resistance program.

In this issue, in chronological order, the traditional method of propagating seedlings for planting is described.

October 2017

1) Nuts were harvested from early to late October
   Every nut can be traced back to its heritage and resistance.
2) The collected nuts are husked within 24 hours to prevent penicillium growth (bacterial infection) and placed in labelled plastic bags
3) Nuts are sterilized by immersing the bags in 3% hydrogen peroxide
   Nuts are allowed to drain and allowed to dry and then returned to the bags.

Oct 2017 – Feb. 2018

4) Nuts are stratified for four months between 2 to 4 degrees C in cold room

Cold Storage Room - Agriculture Canada Research Station – Simcoe, ON
Nuts are housed here from Oct. to Feb.

5) Each month the nuts are removed from the cold room and retreated with the hydrogen peroxide solution to prevent mold growth, then dried and re=bagged.

Mid - February 2018

6) Planting begins in mid February. The planting media is very important to ensure proper nutrients and pH (6.4-6.5). The American Chestnut prefers acidic soil.
The preferred media is obtained by blending Sungro Sunshine Mix #3, http://www.sungro.com/professional-product/sunshine-mix-3/
with pine bark mulch in a ratio by volume of 2:1. in a ribbon blender

(Ron Casier preparing planting media utilizing the ribbon blender at the Simcoe Research Station)

7) Coconut fibre pots are filled with the planting media and the nuts are placed 1-2 cm. deep.

(John Hill fills fibre pots with planting media)

(Christine Vey planting nuts prior to three months in the greenhouse)

8) The pots are placed in the greenhouse and watered as needed to ensure they are damp

Mid May to early June

9) Pots are placed out of doors to allow the plants to climatize for one to two weeks prior to planting

Breeding Resistance – How are we doing after 17years?

Breeding American Chestnuts for Canada

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Abstract: *In Canada, we have been breeding blight-resistant American chestnuts to re-establish the species in the Carolinian forests of Canada. We started seventeen years ago with the Canadian Chestnut Council to develop a population of trees with blight tolerance. From a population of 1400 trees, we selected trees from native and hybrid crosses which appeared to have some level of blight tolerance. Now, in our second generation, we have planted over 13,000 trees, some of which should have good tolerance to the blight.*

The Canadian Chestnut Council was formed in 1988 to restore the American chestnut (*Castanea dentata*), after it had been almost eradicated from Canada by Chestnut Blight (*Cryphonectria parasitica*). Their initial efforts were to monitor what was left of the population and to decrease the virulence of the fungus with hypovirulent strains.

The American chestnut is classed as an endangered species which is protected by various Canadian Federal and Provincial laws. The Federal Species at Risk Act (Government of Canada, 2002), classifies the species, and provides legislation for the development of a recovery plan. Ontario's Endangered Species Act (Government of Ontario, 2007) applies to both public and private lands and prohibits various acts, including killing, removal of plant parts, and removal of seeds. However, this only applies to pure American chestnut and not to hybrids. The first step under the Federal legislation was the Recovery plan. This was first prepared in 2004, was revised in 2012, and the Government of Ontario issued a response statement in 2013.

In 1998, Dr Adam Dale was asked by the Canadian Chestnut Council to develop a breeding strategy for breeding blight resistant trees to re-establish the species. This plan was prepared and accepted by the Council in 2000. The goal of the breeding program was to breed blight resistant American chestnuts adapted to Ontario within 20 years. The initial plan, listed seven breeding priorities: 1) to use 3rd and 4th backcrossed American chestnut trees with known intermediate levels of blight resistance, 2) to give blight resistance the highest priority, 3) to use at least 20 Canadian trees in the F1 crosses to limit inbreeding, 4) to use Canadian parental trees as female parents in the first generation to maintain non-nuclear genetic diversity, 5) the first generation crosses were to be between Canadian trees and blight resistant trees from elsewhere, 6) thereafter, selection would be by recurrent selection within progeny from the first generation, and 7) the second generation crosses would be with F1 parents from different Canadian trees.

In 2006, the plan was revised, and, although the overall goal remained the same: to breed blight resistant American chestnuts adapted to Ontario within 20 years, two additional objectives were added to the initial one. The three objectives were now: 1) to develop blight resistant trees, 100% *C. dentata,* of Canadian origin, 2) to develop blight resistant trees with resistance from Chinese or Japanese chestnut, which are at least 92% Canadian, and 3) maintain present genetic diversity of existing Canadian *C. dentata* trees. These objectives remain in place today.

The two main pillars of the Canadian Chestnut Council breeding program are: 1) that blight resistance is considered the number one priority, and 2) intercrossing native American chestnuts will produce blight resistant trees as effectively as using other chestnut species.

The first steps towards breeding were taken in 2001 and 2002, when Dragan Galic was hired as the technician for the program. Twenty-one Canadian trees were selected, and Adam and Dragan travelled to Connecticut in the spring to collect pollen from Dr Sandra Anagnostakis' trees. Dragan and Mike Nemoroski travelled all over southern Ontario to use this pollen to pollinate the native trees *in situ.* After that, native pollen was used to pollinate the native trees.

Between 2002 and 2008, 767 back-crossed and 643 Canadian seedlings were planted in no particular order at two sites: Tim Horton's Foundation, Onondaga Farms, St George, Ontario and Riverbend Farms, Aylmer, Ontario.

There were at least three mechanisms to blight resistance, skin or bark resistance, callusing and chemical resistance, from compounds like the phytoalexins. So, in the end the Canadian Chestnut Council decided to inoculate branches of each tree with two strains of the fungus for two years.

Branches were used as everyone thought that the levels of tolerance would be low, and we did not want to lose the trees. An unexpected effect of this was that blight took hold of the orchards and many trees were infected.

So, to identify how the lesions differed between trees, we measured the length and breadth of the lesions twice, once after six weeks and again four weeks later. This was used to calculate the rate of change in length and area. As we had six years of data from different trees, twice in different, not necessarily consecutive, years, we calculated the lesion variability from the site x year mean.

The results were not what we expected. We had assumed that the trees of the Canadian *C. dentata* population were completely susceptible to chestnut blight, and the trees pollinated with the Connecticut backcross pollen would be somewhat tolerant. Instead, we found that both populations varied in their tolerance in the same way. In other words, the two populations did not differ, and most of the trees are still alive.

From this we have hypothesized that the American chestnut has the same gene loci for resistance as the Chinese and Japanese chestnuts, and that over thousands of years there have been mutations at these loci. Some of these mutations give some resistance/tolerance, but they had never been tested by the fungus. Consequently, when the American chestnut was challenged by the blight, these loci were tested and allowed the trees to survive. As a consequence, we are now more interested in our Canadian x Canadian crosses, as we can increase blight resistance and maintain tree stature.

From the first generation, we selected the trees that had the lowest lesion growth in both populations. Each year we selected trees that were thought to be the most tolerant (Table 1), and in 2016 we examined all the data and used standard deviation analysis to choose the best trees. In the second generation we have kept the two populations separate, and from 2009, we crossed the selected trees within each population together. As this is an inter-cross generation, we would expect the trees to react very differently, from very susceptible to good tolerance to the blight. To date, over 13,000 trees have been planted at three locations, and in 2016, we started to inoculate the main trunks.

In the results from the first inoculations on 140 second generation trees, we are seeing the variability we expected. Some trees are dying without reacting to the blight, others have healing cankers. On those with healing cankers we have seen the circumference of the lesion decrease significantly in the second year after the inoculations. As we have a long way to go with our second generation, it remains to be seen what we will find, probably, a lot of dead trees, and some interestingly tolerant trees. (see attached Table 1)

So, after seventeen years, we are seeing tolerance in the trees in the Canadian breeding program. In the future, as we inoculate more of the second-generation trees, we expect to see good resistance to the blight in some of the trees.

Wanted Copies of the Canadian Sweet Chestnut – Doug Fagan

The CCC is looking to post all of the newsletters on the website. We are missing issues number 2, number 4 and number 8. If you have or know of a copy of any of these would you please contact Doug Fagan at d.fagan@sympatico.ca or call me at 519-846-5996. Thank You!

Annual General Meeting – Oct. 20, 2018

*mark your calendar

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