Canadian Agri-Science Cluster for Horticulture 3

Update to Industry

Final Report – 2018 – 2023

Activity title:
Development of All-Male Asparagus Hybrids with Improved Traits

Name of Lead Researcher:
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Activity Objectives:

(A) Breeding

- Identify all-male asparagus hybrids superior to Guelph Millennium, Eclipse and Equinox for yield, quality, and/or disease resistance.

(B) Purple spot (Stemphyllium) pathology

- To determine the relative resistance of asparagus cultivars and breeding lines to both purple spot, caused by infection of the asparagus spears, and Stemphylium leaf blight on the asparagus ferns.
- To determine if resistance to both stages of the disease is related.
- To determine the mechanisms of resistance to Stemphylium infection.
- To determine the role of environmental factors in disease development on spears and ferns. Environmental factors will include mechanical damage, temperature, leaf wetness and relative humidity.

(C) Winterhardiness – Seedling de-acclimation

- Develop a seedling screen that recapitulates cultivar difference for spring deacclimation field traits to assist in selection of new germplasm for winterhardiness.

(D) Purple spot (Stemphyllium) resistance mapping

- Conduct mapping analysis on purple spot resistance and rate of disease progression traits to determine genetic architecture.

(E) Winterhardiness - transcriptomics

- Conduct transcriptomics analysis to identify genes and pathways related to winter hardiness, acclimation and de-acclimation to facilitate selection.

(F) Planting depth and density study

- Determine planting depth and density parameters to optimize yields and spear size in new hybrids.

(G) QTL mapping- breeding traits

- Determine genetic architecture for important breeding traits in asparagus such as yield, quality, disease resistance and longevity to facilitate future marker assisted selection.

Research Progress & Results:

(A) Breeding

During the five-year tenure of the grant, 375 new hybrid combinations were generated for testing in (preliminary) P-trials, 318 germplasm crosses were planted to identify new female, male and supermale selections, and small seed quantities of 25 advanced hybrids were made for additional testing. From current and past P-trial, over 20 hybrids that appeared superior to Guelph Millennium for marketable yield (20-40%) and/or percent marketable yield (10% or greater) were advanced to Y-trials (advanced replicated yield trials); for those where data have been collected, UG028, 31, 33, 34 and 38 are candidates for pre-commercial trials. UG028 is most promising currently, as it has shown good performance in multiple trials. As P-trials are planted annually and five are currently under evaluation, additional new, superior hybrids can be identified and advanced to Y-trials in the coming years.

(B) Purple spot (Stemphyllium) pathology

Development of a growth chamber assay for identifying genetic resistance to purple spot in the spear, which correlates with natural field infection, would be beneficial to an asparagus breeding program. Significant differences were detected among four cultivars in the field: Guelph Millennium, Guelph Eclipse, Jersey Giant and Gijnlim. Initial growth chamber experiments, where cut spears from the field were inoculated, placed in standing water, and incubated at 100% humidity, successfully induced infection but results were not correlated with those from the field (r=0.3). Since cut spears respire and endogenous sucrose levels may be decreasing compared to those from the field, and the physiological state of spears may be important for infection, a growth chamber experiment was conducted where inoculated spears were placed standing in 0, 5 or 10% sucrose. The 5% sucrose treatment resulted in infection levels that were highly correlated with field results (r=0.9). For the 0% treatment endogenous sucrose levels decreased faster for cultivars Guelph Eclipse and Jersey Giant compared to the other two hybrids. For 5 and 10% sucrose treatments Jersey Giant and Guelph Eclipse had greater sucrose uptake than Guelph Millennium and Gijnlim, and increased lesion numbers. Overall, results indicate that high losses of endogenous sucrose for Jersey Giant and Guelph Eclipse, when inoculated spears were incubated in a growth chamber without sucrose, diminished the levels of purple spot infection and was a contributing factor to the low correlation between field and growth chamber results. When high infection levels were restored to these cultivars upon incubation of spears with sucrose, high correlations were observed between the field and growth chamber for the four cultivars.

(C) Winterhardiness – Seedling de-acclimation

The MSC student on this project suffered from mental health that was exacerbated during the pandemic. During multiple episodes the student withdrew without contacting anyone and neglected plants in growth chambers which ultimately died. Given extra time to complete the degree, the student continued to have issues and ultimately withdrew from the program without completing experiments. Thus, there are no results to report.

(D) Purple spot (Stemphyllium) resistance mapping

Mapping of quantitative trait loci (QTL) for purple spot resistance was conducted for spear lesions in the spring and foliar infection in the fall. A QTL identifies a location on a chromosome linked to a molecular marker that affects increased/decreased resistance. Four QTL were identified for number of purple spot lesions in the spear. The one stable locus, found consistently over years, accounted for 23% of the phenotypic variation while the others, found only for one year of observation, accounted collectively for 23%. Five QTL were identified for levels of foliar infections; one was stable, accounting for 35% of the variation, and four, identified in only one year, together accounted for 34%. Two overlapping minor quantitative trait loci (QTL) for fern and spear disease severity were identified on chromosome 9, suggesting a common factor for resistance, or separate, closely linked genes. Overall, identifying stable and potential QTLs for resistance in the spear and fern provided valuable information for breeding programs. These QTLs can be used to develop molecular markers for selecting resistant plant varieties, which can help increase yield and enhance crop productivity.

(E) Winterhardiness - transcriptomics

During the fall season, as soil temperatures decreased, freezing tolerance of cultivars increased (LT50 values decreased) and more genes were downregulated or turned off in response to cold temperatures than those turned on, or upregulated. Guelph Millennium (GM) had a higher freezing tolerance than UC157 (UC) at the second and third harvest dates which suggested that GM acclimated to cold earlier in the season than UC. This difference in freezing tolerance was reflected in the gene expression, more genes were differentially expressed in GM than UC. However, both cultivars achieved similar levels of freezing tolerance by the third harvest and an equal number of genes were differentially expressed in both cultivars. In the spring, as temperatures increased, freezing tolerance decreased for both cultivars and more genes were upregulated, than downregulated, as plants came out of dormancy. Genes involved in carbohydrate metabolic process, response to oxidative stress, cell wall modification, cell cycle and division, integral component of membrane, for example, were downregulated during the fall and upregulated during the spring, in both the buds and rhizomes of both cultivars. Genes upregulated in the fall and/or downregulated in the spring included those involved in carbohydrate processes, circadian rhythm, and phloem development. Additionally, pathways related to "starch and sucrose metabolism" and "plant hormone signal transduction" were also enriched among the downregulated genes in the fall and upregulated genes in the spring, suggesting hormones and carbohydrates may play crucial roles in cold signaling processes, and genes involved in cell membrane stabilization could be important for acclimation to cold.

(F) Planting depth and density study

A total of 18 treatment combinations were tested in a split-plot design: two cultivars, Guelph Millennium and Guelph Eclipse; three spacings within rows, 10, 17.5, and 25 cm (4, 7 and 10 inches); and three depths, 10, 17.5, and 25 cm (4, 7 and 10 inches). One experiment was planted in 2018 and an identical replicate experiment was planted in 2019. During 2022, the second and first full harvests were conducted for the 2018 and 2019 experiments, respectively.

For pooled data over two harvest years in the 2018 trial, consistent trends were noted: increasing density improved spear number, increasing depth decreased number, and density and depth generally did not affect yield. Guelph Eclipse appeared to have thicker spears compared to Guelph Millennium for the greatest depth and lowest density treatment combination. Comparing first harvest year data for the 2018 and 2019 replicate trials, similar effects of density and depth were observed for the two cultivars, although significance among some treatment means were not always consistent between years. In a perennial such as asparagus, with an underground rhizome that expands with each growing season, density and depth studies often require many harvest seasons to determine long-term effects of treatments.

(G) QTL mapping- breeding traits

Two mapping populations were developed to identify the genetic architecture of several traits: spear number and diameter (yield proxy), height of first branch from the ground (quality proxy), and foliar disease. Significant variation was found for all attributes, suggesting major genetic regulators could be identified and ultimately used to enhance selection in the future.

DNA was extracted from 420 individuals and genotyping-by sequencing was conducted, revealing 3000 molecular marker loci in each population. Mapping of the markers within the two populations has been difficult and genetic maps could not be produced using standard programming tools. The MSC student is working currently with the computational biologist to develop a solution. In November 2022, he left for a job before the project was completed, however, he is continuing as a part-time student and we anticipate a thesis defense in the summer of 2023.

Key Message(s):

- New hybrids with commercial potential are advancing thorough the program to benefit growers in the future.
- A growth chamber screen for purple spot in spears has been developed to advance the selection and breeding of resistant cultivars.
- Quantitative trait loci for purple spot resistance in spears and fern have been identified and can advance future breeding efforts. An additional study to be completed this summer can identify loci important for yield, quality and rust resistance.
- Expression analysis has identified genes and biochemical/physiological pathways important for winterhardiness which can be explored further for improvement of the trait.

- Density and depth analysis provided preliminary information for planting recommendations of two Guelph hybrids.

Overall benefit to industry:

The main objectives of this project were to continue the development of improved asparagus hybrids to benefit growers in the near future, and conduct genetic and physiological analyses leading to new information that can be incorporated into the breeding program to hasten advancements in the long-term. These latter studies have identified genes/chromosomal locations important for purple spot resistance in spears and fern, and winterhardiness, a component of longevity. Before fall 2023 we should have additional information for yield, quality and rust resistance.

High marketable yields are important for the profitability and sustainability of the industry. This trait is complex and difficult to select due to high environmental effects. The work conducted to support breeding, identifying gene/chromosomal locations for yield per se, or contributors to the trait such as disease resistance and winterhardiness, can facilitate the future utilization of molecular techniques that accelerate crop improvement. Enhanced yields per acre or hectare, diminished spray costs through foliar disease resistance, and extended longevity, reducing the need to remove and plant new fields, will all benefit the asparagus industry in Canada.

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