QTL MAPPING POPULATION DEVELOPMENT IN *CORNUS FLORIDA* USING OPEN POLLINATED SEEDLINGS AND SSR MARKERS

Erin Pfarr[1], Josh Honig[1], Jennifer Vaiciunas[1], Christine Kubik[1], John Capik[1], and Thomas J. Molnar[1]

Flowering dogwood (*Cornus florida*) is an important understory tree in deciduous mesic hardwood forests of the Eastern United States, from Massachusetts to Florida and as far west as Texas (Fulcher et al. 2012). In addition, flowering dogwood is a popular tree in managed landscapes thanks to its graceful form and spring blooms with showy bracts. It is honored as the State flower or tree of North Carolina, Virginia, and Missouri. However, since the early 1990s, powdery mildew (PM) (*Erysiphe pulchra*) has been one of the most problematic diseases for this species (Li et al. 2009). Infected leaves can be curled and stunted with unsightly white fungal growth or increased red pigmentation (Li et al. 2009). In the seedling stage, a heavy infection can be fatal (Parikh et al. 2016). For mature trees, the disease negatively affects bloom and repeated severe infections can stunt a tree's growth and reduce its appeal in the landscape (Parikh et al. 2016).

One selection from the Rutgers dogwood breeding program, *C. florida* H4AR15P25, shows excellent resistance to powdery mildew. The resistance holds up in clones across multiple locations and appears to be heritable. The quantitative trait loci (QTL) underlying this resistance will be investigated and mapped in a pseudo F_2 population using genotyping by sequencing (GBS)-derived single nucleotide polymorphism (SNP) markers. To expedite population development, open-pollinated (OP) seeds were harvested from H4AR15P25 grown in a crossing block of limited PM-susceptible male parents. The purpose of this study is to determine the pollen parents of the seedlings using simple sequence repeat (SSR) markers and assemble a full-sibling population of 150 seedlings from the same PM-susceptible pollen parent for the future QTL PM resistance study.

Mature fruit were harvested from *C. florida* H4AR15P25 at Rutgers University New Brunswick Agricultural Experiment Station in October 2017. Seeds were cleaned, stratified, and germinated in the greenhouse, resulting in ~650 healthy seedlings.

Newly expanding leaves were collected from H4AR15P25, 16 possible pollen parents, and a subset of 37 seedlings. DNA was extracted using the Qiagen DNeasy Plant Kit. The samples were genotyped with 11 SSR primer pairs developed by Wang et al. (2008) and Wadl et al. (2008) (table 1) (Wadl et al. 2008, Wang et al. 2008).

Briefly, the primer pairs were synthesized by Integrated DNA Technologies (Coralville, IA) with a 7 bp PIG-tailing sequence added to the reverse primers to aid in scoring true vs. plus A alleles,

Table 1—SSR marker summary statistics for *Cornus florida* H4AR15P25, 16 possible pollen parents, and 37 open pollinated progeny

Locus	k	N	HObs	HExp	PIC	F(Null)
CF020	8	54	0.630	0.620	0.582	-0.002
CF273	8	52	0.808	0.759	0.712	-0.039
CF125	4	54	0.889	0.639	0.566	-0.185
CF701	7	54	0.870	0.814	0.778	-0.043
CF048	5	54	0.667	0.589	0.550	-0.071
CF597	8	54	0.722	0.673	0.615	-0.041
CF1045	3	54	0.333	0.334	0.287	-0.010
CF055	4	54	0.630	0.485	0.413	-0.156
CF646	5	54	0.870	0.765	0.720	-0.067
CK040	3	54	0.222	0.283	0.246	0.114
CK015	4	54	0.556	0.553	0.448	-0.005
Average	5.4	53.8	0.654	0.592	0.538	-0.046

k = the number of alleles for each SSR marker.
N = the number of individuals genotyped at a locus.
HObs = the Observed Heterozygosity.
HExp = the Expected Heterozytosity.
PIC = Polymorphic Information Content.
F(Null) = the null allele frequency estimate.

[1] Rutgers University, Department of Plant Biology, New Brunswick, NJ 08901, (erin.pfarr@rutgers.edu).

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and an 18 bp M13 sequence added to the forward primers for economic PCR florescent labeling (Brownstein et al. 1996, Schuelke 2000).

The following were combined in a 96 well PCR plate with 13 µl reaction volumes: 0.5 pmol forward primer, 1 pmol reverse primer, 1 pmol florescent dye M13 primer (FAM, NED, PET, or VIC), 5 ng DNA, and 10xRamp-Taq PCR buffer (Denville Scientific, Metuchen, NJ), 2 mM MgCl2, and 0.25 mM of each dNTP (Denville Scientific). PCR was performed on GeneAmp 9700 thermalcyclers (Applied Biosystems, Foster City, CA) with the following parameters: initial denaturation of 94 °C for 5 min followed by 30 cycles of 94 °C for 30 s, 55 °C for 45 s, and 72 °C for 45 s, followed by 20 cycles of 94 °C for 30 s, 53 °C for 45 s, and 72 °C for 45 s, followed by a final extension of 72 °C for 10 min. The PCR products were run with LIZ 600 size standard on a capillary electrophoresis genetic analyzer (ABI 3500xl; Applied Biosystems). SSR alleles were binned and assigned with Genemapper 4.0 (Applied Biosystems). Allele frequency analysis and paternity parentage analysis was performed with CERVUS 3.0 (Kalinowski and others 2007), which uses a likelihood-based approach and parentage analysis simulation to determine confidence levels for parentage assignments.

The SSRs were highly polymorphic with 3 to 8 alleles per marker and polymporphic information content (PIC) values between .25 and .78 (table 1.). The SSR marker data confirmed the pedigrees of *C. florida* H4AR17P05 and H4AR17P48, two of the possible pollen parents, as progeny of *C. florida* forma rubra × *C. florida* "Red Beauty", which were also genotyped for this study.

H4AR15P28, the closest tree to H4AR15P25, was identified as the pollen parent of 10 of the seedlings, the most of any other possible parent (fig. 1 and table 2.). H4AR15P28 would be the best susceptible pollen parent for the mapping population because it is the most PM susceptible tree in the breeding block. With slightly over a fourth of genotyped seedlings in this subset assigned to H4AR15P28, we are optimistic that upon screening all 650 OP seedlings, we will find 150 full siblings from this pollen parent for the bi-parental QTL mapping population.

Six plants, H4AR17P05, H4AR15P49, H4AR15P58, forma rubra, H4AR17P48, "Red Beauty", and H4AR15P40, did not have any seedlings in this subset (fig. 1). This may be due to a variety of factors, such as increased distance from and asynchronous flowering with the mother plant, or limited flower set in the case of H4AR15P40 (Rhoades et al. 2011).

Interestingly, 7 seedlings could not be assigned with 80 percent confidence to any of the possible pollen parents. F-003, F-014, and F-106 had one or more alleles that were not present in any of the possible parents. It is unclear where this pollen flow is coming from as this breeding block is separated from the closest *C. florida* trees by 1,100 feet (including a 400 foot-wide river).

The set of 11 markers used here will be paired down to the 8 most informative for more efficient screening. They will be used to screen the rest of the OP seedlings from H4AR15P25 to assemble the 150-individual mapping population. This *C. florida* population will be genotyped using GBS and evaluated for PM response at 2 and 3 years of age. The final goals are to better understand PM resistance inheritance and to subsequently use QTLs for PM resistance to aid in marker assisted selection in the Rutgers dogwood breeding program. This would allow for early culling of PM susceptible trees, maximizing space and resources for a plant with a relatively slow generation time.

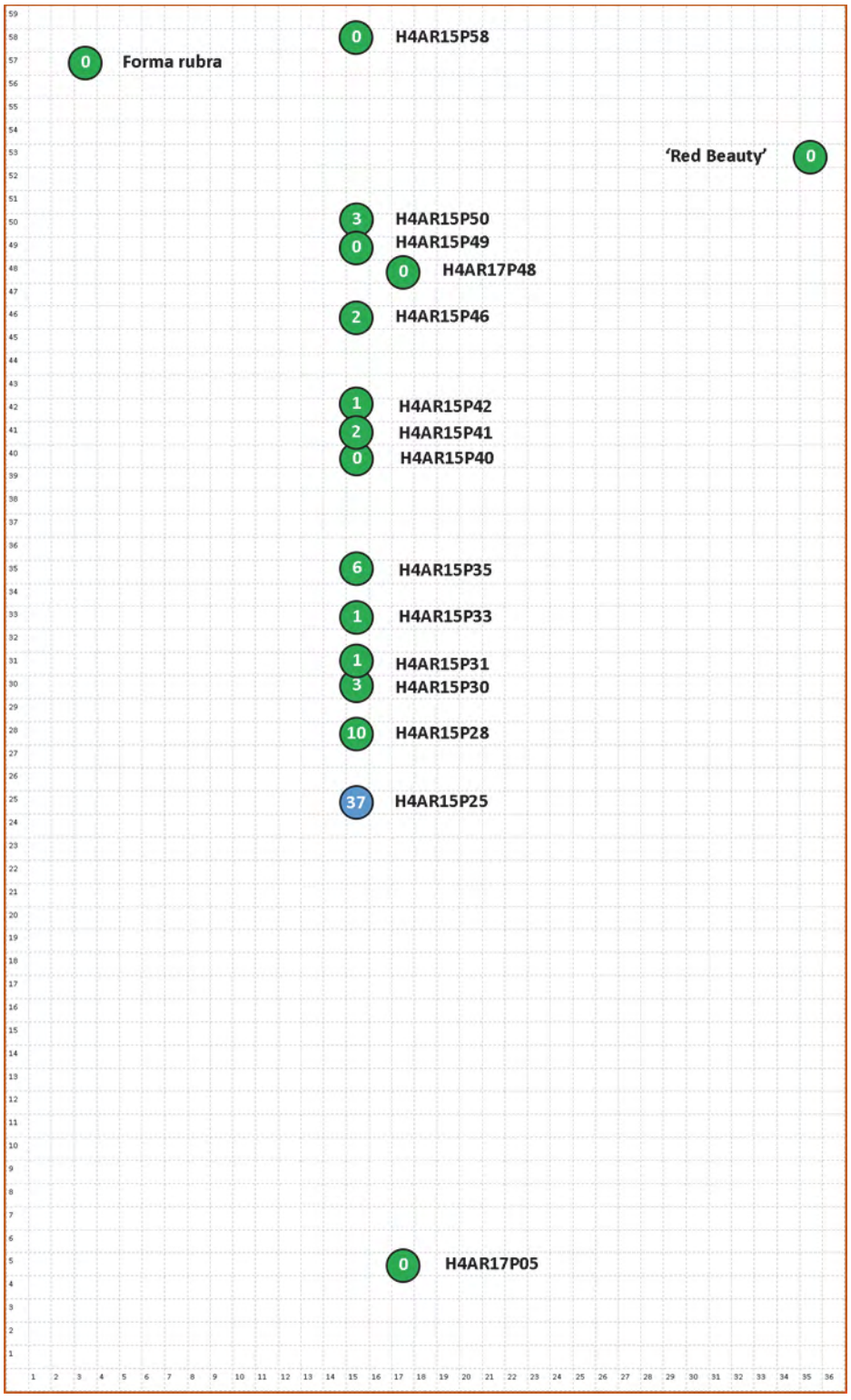

Figure 1—Approximate spatial relationships of *C. florida* H4AR15P25 (blue circle) and the possible pollen parents (green circles). Numbers inside of the circles represent number of progeny assigned to trees with 95% trio confidence or greater by CERVUS.

Table 2—CERVUS program parentage assignments for 37 open pollinated *Cornus florida* seedlings

Offspring ID	Loci typed	Candidate mother ID	Pair loci mismatch	Candidate father ID	Trio loci mismatch	Trio LOD score	Trio Delta	Trio confidence
F-006	11	H4AR15P25	0	H4AR15P28	1	4.23	4.23	*
F-011	11	H4AR15P25	0	H4AR15P28	1	5.12	5.12	*
F-015	11	H4AR15P25	0	H4AR15P28	1	4.78	4.78	*
F-038	11	H4AR15P25	0	H4AR15P28	0	9.72	6.51	*
F-047	11	H4AR15P25	0	H4AR15P28	1	4.52	4.52	*
F-073	11	H4AR15P25	0	H4AR15P28	1	4.74	4.74	*
F-075	11	H4AR15P25	0	H4AR15P28	0	10.5	10.5	*
F-096	11	H4AR15P25	0	H4AR15P28	0	9.73	6.51	*
F-104	11	H4AR15P25	0	H4AR15P28	1	4.52	4.52	*
F-129	11	H4AR15P25	0	H4AR15P28	1	4.51	4.51	*
F-020	11	H4AR15P25	0	H4AR15P30	0	12.4	12.4	*
F-024	11	H4AR15P25	0	H4AR15P30	1	5.33	5.33	*
F-029	11	H4AR15P25	0	H4AR15P30	0	10	10	*
F-018	11	H4AR15P25	0	H4AR15P31	0	14.1	14.1	*
F-004	11	H4AR15P25	0	H4AR15P33	0	9.69	9.69	*
F-016	11	H4AR15P25	0	H4AR15P35	0	8.15	8.15	*
F-021	11	H4AR15P25	0	H4AR15P35	1	3.29	3.29	*
F-026	11	H4AR15P25	0	H4AR15P35	1	5.25	5.25	*
F-042	11	H4AR15P25	0	H4AR15P35	0	2.54	2.54	*
F-101	11	H4AR15P25	0	H4AR15P35	0	8.47	8.47	*
F-109	10	H4AR15P25	0	H4AR15P35	0	1.16	1.16	*
F-098	11	H4AR15P25	0	H4AR15P41	0	4.26	1.51	*
F-110	11	H4AR15P25	0	H4AR15P41	0	6.39	6.39	*
F-007	11	H4AR15P25	0	H4AR15P42	0	8.24	8.24	*
F-113	11	H4AR15P25	0	H4AR15P46	1	0.79	0.79	*
F-128	11	H4AR15P25	0	H4AR15P46	0	2.48	2.48	*
F-009	11	H4AR15P25	0	H4AR15P50	0	7.37	4.89	*
F-017	11	H4AR15P25	0	H4AR15P50	0	7.08	7.08	*
F-095	11	H4AR15P25	0	H4AR15P50	0	7.88	7.88	*
F-022	11	H4AR15P25	0	H4AR15P28	1	5.37	0.16	+
F-071	11	H4AR15P25	0	H4AR15P28	2	#######	0	
F-106	10	H4AR15P25	0	H4AR15P41	2	-0.167	0	
F-003	11	H4AR15P25	0	H4AR15P46	5	#######	0	
F-013	11	H4AR15P25	0	H4AR15P46	1	-0.11	0	
F-005	11	H4AR15P25	0	H4AR15P50	3	#######	0	
F-014	11	H4AR15P25	0	H4AR15P50	5	#######	0	
F-136	11	H4AR15P25	0	H4AR15P50	2	#######	0	

Summary statistics for CERVUS program parentage assignments.
The most likely pollen parent ID is listed for each seedling.
For trio confidence levels * = 95 percent; + = 80 percent.

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