Breeding Witchhazel at The Holden Arboretum

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RESEARCH OVERVIEW

Research formally began in 1991 with the addition of staff with advanced training in breeding, genetics, and plant physiology. The centerpiece of research is breeding woody ornamental plants. Complementary work involves: studies related to reproductive biology, genetic diversity, elucidation and utilization of biochemical markers, cytogenetics, propagation, and documentation of the inheritance of important traits.

By formal agreement, The Holden Arboretum acquired the germplasm accumulated by David G. Leach who has been a prodigious breeder of *Rhododendron* for over 50 years. Acquired in 1986, the plant collection is one of the best for cold-hardy *Rhododendron* germplasm. Staff were added in 1992 to help bolster research at this satellite research station of over 20 acres which is located within 30 miles of The Holden Arboretum.

A new building was built at The Holden Arboretum to provide research space and greenhouse/headhouse areas to meet the expanding needs of the organization. Completed in 1994, our Horticulture Science Center provides over 4000 ft^2 in office, herbarium, darkroom, and laboratory space for research. To date, plant breeding at Holden has emphasized work with several genera including: *Aesculus, Cercis, Cornus, Hamamelis, Magnolia,* and *Rhododendron.*

This note provides a progress report on breeding and research activities of *Hamamelis* at The Holden Arboretum.

PLANT ACQUISITION

Currently, the research collection of *Hamamelis* includes 58 cultivars and 13 selections of *H. japonica* (6), *H. mollis* (8), *H. vernalis* (20), and *H.* ×*intermedia* (37). The rationale to acquire this material was to evaluate plants in a common garden. Plant vigor, leaf size, leaf condition (incidence of powdery mildew and fall color), petal size, petal color, bloom period, fertility, calyx color, and fragrance are some of the characteristics that will be evaluated. Cultivars and selections will constitute the backbone of a breeding program for spring-blooming witchhazel.

Considerable effort has been made to acquire wild-collected *H. virginiana.* Seed requests were made to numerous cooperators throughout the U.S. and Canada who collected and sent seed from 65 populations in Fall 1994 and 1995. These populations came from 21 states and one Canadian province including: AL, DE, IL, IO, LA, MA, ME, MI, NC, NH, NJ, NY, OH, OK, ONT, PA, RI, TN, TX, VA, VT, and WI. Eighteen populations were collected from Ohio.

Seed Germination. Seed from wild-collected populations of *H. virginiana* were stratified for 8 weeks (wk) warm followed by 20 wk cold (4C). In general, seed germination was excellent and averaged 67%, with a range of 0% to 100%, and a mode of 72%. Several populations had excellent germination (>95%) including DE, OK, OH, and VA. Seed from other populations germinated poorly (<5%) including

OH, MI, and WI. The extremes in germination may be related to latitudinal differences between populations. We are testing the hypothesis that more northerly populations require a longer cold period during stratification for optimum seed germination.

Other germination experiments included an evaluation of seed harvest date on germinability. Seed collected in August and early September did not germinate. Seed collected in mid September and October, germinated at more than 20% and 40%, respectively. Stratification pretreatments were also tested for effectiveness. Stratification of 8 wk warm followed by 20 wk cold was superior to 8 wk warm followed by either 10 or 15 wk cold. Others have conducted similar work including a comprehensive study by Gaut and Roberts (1984).

POLLINATION AND BREEDING OF *HAMAMELIS*.

Most named cultivars are spring-blooming Asiatic types (either *H. japonica, H. mollis*, or their interspecific hybrid *H. ×intermedia*). Field-grown plants are difficult to work with given they bloom in late February to March when weather is unpredictable and often inclement. In fact, pollinating witchhazel in the field is not recommended if potted plants are available which can be moved to protected quarters for pollination and pollen collections.

Attempts to self pollinate various selections of *Hamamelis* have not yielded mature fruit. Presumably, there is a self-incompatibility system that prevents self-pollen from being successful. Clearly, if witchhazel are self-incompatible, breeding can be conducted without emasculating the flowers. Research has been initiated to verify whether an incompatibility system exists.

The size and proximity of the stigma to the anthers is quite close (1 to 3 mm) and flower manipulation while making controlled crosses is tedious. *Hamamelis* apparently are insect pollinated (though no published studies have been located that document what insects pollinate these interesting flowers). Research is ongoing to develop a system where small groups of *Hamamelis* can be brought together and allowed to naturally cross pollinate. If successful, this approach would eliminate the need to manually pollinate large numbers of flowers. However, such a system would also require a method to determine the pollen parent of each seedling (i.e., whether seedlings were a result of self or cross pollination). To determine the pollen parent, staff have identified three plant genes that are straightforward to evaluate and unequivocal in their mode of inheritance (see the Biochemical Markers section below).

BIOCHEMICAL MARKERS OF WITCHHAZEL.

Plant proteins can be evaluated by electrophoresis. Specifically, staff are working to elucidate isozyme differences that are easy to evaluate and simply inherited. Currently, we have identified three genes that control isozyme differences in two enzyme systems of *Hamamelis*. Fortunately, isozyme profiles are not influenced by the environment and are characteristic of the individual. Isozymes are analogous to blood type in humans. While several individuals may have the same genotype, other individuals are different. These differences in plant proteins can be detected by starch gel electrophoresis. Like blood type, isozymes are simply inherited and can be used to help substantiate paternity. Research includes genotyping *Hamamelis* selections for each of three isozyme genes.

Consider a scenario where two different individuals of witchhazel are in close proximity within a garden (perhaps 2 m apart). Assume plant-1 has an "*aa*" genotype and plant-2, a "*bb*" genotype. If the two plants cross pollinate, the resultant seedlings would have an "*ab*" genotype since parent-1 can only contribute an "*a*" allele to its offspring, and parent-2, can only contribute a "*b*" allele. Similarly, if parent-1 self-pollinates, the resultant seedlings can only have an "*aa*" genotype.

Using this logic, a closely spaced grouping of 13 witchhazel is being studied. This planting includes a mixture of cultivars which were established in 1986. The plants regularly set fruit and are spatially close together (individuals are about 2 to 3 m apart). In 1995, seeds were collected from four cultivars which were critically evaluated for isozyme genotypes and rates of cross pollination were estimated. Outcrossing estimates were considerably higher than expected at 71%, 81%, 83%, and 100%. Knowing the genetic composition of the small population of thirteen plants as we do, our data conservatively estimates cross pollination.

Included in this group of 13 witchhazel was a single plant of *H. vernalis* 'Red Imp' surrounded by 12 Asiatic witchhazel. This 'Red Imp' was hybridizing with Asiatics at greater than 80%. This is the first known report that *H. vernalis* freely hybridizes with Asiatic witchhazel.

By knowing the genotypes of the plants of interest, desirable parent plants can be brought together (spatially) to create small breeding populations. By knowing the inheritance of the isozyme markers and genotypes of the selected parents, paternity of the seedlings can be easily determined.

Other research at Holden involves comparing the genetic similarity among cultivars. This work uses DNA analyses and may help to substantiate whether groups of cultivars are closely related or even if two cultivars likely are half-sibs (having one common parent). This analyses suggests that *H. vernalis* is quite dissimilar to the Asiatic witchhazel but that interspecific hybrids likely exist between *H. vernalis* and plants with Asiatic backgrounds (Marquard et al., 1997).

Micropropagation. Traditionally, witchhazel are propagated by grafting with H. virginiana as the rootstock. The major disadvantage of this method is that the rootstock may sucker and out-compete the scion. Cuttings can be rooted successful, however, overwintering survival has been marginal (Dirr and Heuser, 1987). As we acquire sufficient plant material, we will evaluate clonal differences in rooting success and survival associated with various overwintering regimes.

Micropropagation provides an alternative to traditional propagation. Research at Holden has demonstrated that shoot tip explants of *Hamamelis* can be established, will proliferate, and will root in vitro with moderate to excellent success. Staff continue to evaluate basal salt composition on culture initiation; the affect of BA and NAA concentrations on shoot proliferation; the affect of IBA concentration on rooting, and differences in overwintering regimes on plant survival. Equally important will be to compare differences in clonal performance of plantlets grown in culture.

CONCLUSION

Hamamelis research at The Holden Arboretum is proceeding on numerous fronts including: acquisition of cultivars, selections, and wild-collected material; plant evaluations; studies related to genetic similarity and reproductive biology; while developing methods to facilitate the creation and verification of hybrids.

Acknowledgment. *The able assistance of* Charlotte Chan, Eric Davis, and Arthur Richwine in conducting this research is greatly appreciated.

LITERATURE CITED

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PETER DEL TREDICI: Have you crossed *Hamamelis vernalis* by *H. virginiana*?

ROBERT MARQUARD: No, but we are planning on doing it. We have crossed *H. vernalis* by the Asiatic species.

DARREL APPS: Have you seen any disease symptoms, particularly with the *H.* ×*intermedia* cultivars?

ROBERT MARQUARD: Our main stock is growing in containers and we have observed none.

VOICE: Why are you using tissue culture in your work?

ROBERT MARQUARD: The main reason is experimental and we hope to develop a reliable propagation system.