STUDIES ON THE REPRODUCTIVE CAPACITY OF AESCULUS PARVIFLORA AND AESCULUS PAVIA: OPPORTUNITIES FOR THEIR IMPROVEMENT THROUGH INTERSPECIFIC HYBRIDIZATION

DISSERTATION

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*****
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ABSTRACT

The genus *Aesculus*, of the family Hippocastanaceae, is comprised of thirteen species, numerous botanic varieties, cultivars, and natural hybrids. All members of this genus can be easily identified by their palmately compound leaves, ornamental flowers and characteristic seeds from which the common name is thought to be derived. Most species of *Aesculus* are propagated from seed and the cultivars by budding or grafting. *Aesculus* are susceptible to two important foliar problems. The most important disease is leaf blotch caused by *Guignardia aesculi*, and the other is physiological leaf scorch. Both of these problems have limited the use of *Aesculus* in the landscape. Sufficient diversity exists within the genus to consider the development of superior horticultural types through controlled hybridization within and/or among species.

To this end, this project focused on the floral, pollen, seed, and reproductive biology of *Aesculus parviflora* and *Aesculus pavia* as the foundation for the development of an *Aesculus* improvement project. *Aesculus parviflora* is a large, rounded, shrub which produces long panicles of white flowers in summer, with foliage resistant to blotch and scorch and good yellow fall color. *Aesculus pavia* is valued for its red flowers and its ability to hybridize with other species.

Both species exhibited andromonecy and most flowers were functionally staminate. The sex ratio for both species was approximately 5.5%. *Aesculus pavia*
panicles contained fewer flowers, 73 on average, with the complete flowers located predominately in the basal portion of the inflorescence. The average *A. parviflora* panicle contained 284 flowers with the complete flowers located predominately in the upper most apical quarter of the panicle. Anthesis for both species progressed base to tip. Complete flowers are present in *A. pavia* from the beginning of anthesis but do not appear in *A. parviflora* until the fifth day of anthesis. Staminate flowers are present throughout anthesis in both species. There appeared to be some plasticity in floral sex expression since mechanical modification increased the number of complete flowers per panicle.

Pollen viability was assessed using five species of *Aesculus* and the interspecific hybrid *A. × carnea* through an in vitro pollen germination method. While fresh pollen germinated at acceptable levels across a broad range of sucrose concentrations and temperatures. The optimal test conditions for maximum germination were determined to be a combination of 20% sucrose and 15°C. Fresh pollen under these conditions had germination rates between 82-93% and all would make suitable male parents. The effect of storage temperature and time were assessed using these optimized conditions. The differences in pollen germination response from pollen stored at –20°C and –80°C was not significant but the duration of the storage time was highly significant. Short periods of storage resulted in only modest declines in pollen germination. However, extended storage (12 months) reduced pollen germination, as measure by these in vitro test conditions, to below the recommended threshold for fruit set.

Seed propagation of *Aesculus* has been limited by the physiological conditions of recalcitrance and dormancy. The high moisture content and metabolism rate of the
seeds make them susceptible to damage during initial seed handling and stratification. While the ability to produce radical growth without the benefit of stratification has been demonstrated for both *A. parviflora* and *A. pavia*, overall the performance was enhanced by a 60 day stratification period at 4°C. The 60 day stratification period improved the uniformity and increased the rate of the germination and emergence while minimizing the losses due to mold and pregermination. Inadequate or extended periods of stratification resulted in deterioration produced by the high rate of metabolism and subsequent mold infection. This chilling period, however, was not able to overcome the expression of epicotyl dormancy observed in most *A. parviflora* seedling, and they require a second period of chilling prior to the resumption of growth. The largest number and highest quality seedlings resulted from the 60 day stratification treatment.

Studies on the floral biology, pollen viability, and seed management provided much of the essential information necessary to initiate a program for the genetic improvement of *A. parviflora* and *A. pavia*. However, successful hybridization programs rely on the accurate selection of superior parental stock. The selection of good maternal parents is based in part upon their ability to initiate and support seed development. One way to evaluate these traits is through the use of a provenance model which measures both the tree’s potential for seed set, seedling efficiency and its realized performance. Realized performance is measured by fruit and seed production. Fruit and seed production in this study was found to be influence significantly by the weather. Most panicles produced one fruit with the maximum number of fruit observed being nine. Most fruit were single seeded and multiple seeds per fruit negatively impacted seed fresh weight. Based in part on the provenance model three *A. parviflora*
and two *A. pavia* were selected as maternal parents. For both *A. parviflora* and *A. pavia*, the frequency of seed maturation from both self pollinations and intraspecific pollinations was equal to or somewhat better than the fruit maturation for naturally occurring open pollinations. Maternal trees differed in their ability to develop fruit. In all cases, the success rate for interspecific pollinations was quite low. It was noted that there were two periods of fruit drop, one in the initial days after pollination and the other toward the end of the reproductive cycle. The provenance model despite its limitations proved to predict which trees would be superior in their fruit development potential and offers a mathematical way to assess breeding potential. Wide interspecific crosses have many challenges but great rewards. The limitation in fruit development will require the utilization of techniques like embryo rescue in conjunction with traditional breeding methodologies.
This work is dedicated
in loving and lasting memory
to my mother
Mary Kathryn Chanon
ACKNOWLEDGEMENT

John Donne the seventeenth century English poet and clergy man wrote "no man is an island" and the same can be said of this doctoral project. It is no exaggeration to say that I would not have been able to undertake and complete this dissertation without the assistance of many people who generously gave of there time and talent, and who deserve special recognition.

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PUBLICATIONS

Hybridization of *Aesculus parviflora* and *Aesculus pavia*. Ornamental Plants: Annual
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FIELDS OF STUDY

Major Field: Ornamental Plant Improvement
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Dedication</td>
<td>vi</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>vii</td>
</tr>
<tr>
<td>Vita</td>
<td>ix</td>
</tr>
<tr>
<td>List of Tables</td>
<td>xiv</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xvi</td>
</tr>
<tr>
<td>Chapters:</td>
<td></td>
</tr>
<tr>
<td>1. The genus <em>Aesculus</em> and opportunities for its improvement.</td>
<td>1</td>
</tr>
<tr>
<td>The distribution and habitats of <em>Aesculus</em></td>
<td>3</td>
</tr>
<tr>
<td>The botanical characteristics of <em>Aesculus</em></td>
<td>6</td>
</tr>
<tr>
<td><em>Aesculus</em> in the landscape</td>
<td>8</td>
</tr>
<tr>
<td>Pests and diseases of <em>Aesculus</em></td>
<td>10</td>
</tr>
<tr>
<td><em>Aesculus</em> as affected by abiotic stresses</td>
<td>16</td>
</tr>
<tr>
<td>Cultural requirements of <em>Aesculus</em></td>
<td>19</td>
</tr>
<tr>
<td>Propagation of <em>Aesculus</em></td>
<td>21</td>
</tr>
<tr>
<td>Seed propagation</td>
<td>21</td>
</tr>
<tr>
<td>Propagation via cuttings, budding and grafting.</td>
<td>25</td>
</tr>
<tr>
<td>Propagation by tissue culture</td>
<td>29</td>
</tr>
<tr>
<td>Commercial sources of <em>Aesculus</em> plants</td>
<td>33</td>
</tr>
<tr>
<td>Additional uses of <em>Aesculus</em> and its products</td>
<td>33</td>
</tr>
<tr>
<td>Ethnobotanical use</td>
<td>33</td>
</tr>
<tr>
<td><em>Aesculus</em> natural products and their pharmacological uses</td>
<td>35</td>
</tr>
<tr>
<td>Saponins and coumarins</td>
<td>35</td>
</tr>
<tr>
<td><em>Aesculus</em> saponins and coumarins</td>
<td>38</td>
</tr>
<tr>
<td>Topic</td>
<td>Page</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Clinical and pharmacologic aspects of <em>Aesculus</em> natural products</td>
<td>40</td>
</tr>
<tr>
<td>The toxicity of <em>Aesculus</em> natural products</td>
<td>45</td>
</tr>
<tr>
<td>Taxonomy and phylogenetic relationship in <em>Aesculus</em></td>
<td>48</td>
</tr>
<tr>
<td>Taxonomy within the order</td>
<td>48</td>
</tr>
<tr>
<td><em>Aesculus</em> centers of origin and diversity</td>
<td>52</td>
</tr>
<tr>
<td>Phylogenetic relationships between <em>Aesculus</em> species</td>
<td>54</td>
</tr>
<tr>
<td>Genetic improvement</td>
<td>56</td>
</tr>
<tr>
<td>Historic improvement efforts</td>
<td>57</td>
</tr>
<tr>
<td>Barriers to breeding</td>
<td>60</td>
</tr>
<tr>
<td>Study goals and objectives</td>
<td>61</td>
</tr>
<tr>
<td>Cited references</td>
<td>92</td>
</tr>
</tbody>
</table>

2. Comparison of inflorescence morphology, anthesis and sex ratio of *Aesculus parviflora* and *Aesculus pavia*  

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>110</td>
</tr>
<tr>
<td>Materials and methods</td>
<td>113</td>
</tr>
<tr>
<td>Plant material</td>
<td>113</td>
</tr>
<tr>
<td><em>Aesculus</em> pollinators</td>
<td>113</td>
</tr>
<tr>
<td>Inflorescence characterization</td>
<td>114</td>
</tr>
<tr>
<td>Spatial pattern of inflorescence anthesis</td>
<td>115</td>
</tr>
<tr>
<td>Inflorescence modification</td>
<td>115</td>
</tr>
<tr>
<td>Data analysis</td>
<td>116</td>
</tr>
<tr>
<td>Results and discussion</td>
<td>117</td>
</tr>
<tr>
<td>Putative <em>Aesculus</em> pollinators</td>
<td>117</td>
</tr>
<tr>
<td>Inflorescence characterizations of <em>Aesculus parviflora</em> and <em>Aesculus pavia</em></td>
<td>118</td>
</tr>
<tr>
<td>Spatial pattern of inflorescence anthesis</td>
<td>124</td>
</tr>
<tr>
<td>Inflorescence modification</td>
<td>126</td>
</tr>
<tr>
<td>Implications for cross pollination</td>
<td>127</td>
</tr>
<tr>
<td>Cited references</td>
<td>136</td>
</tr>
</tbody>
</table>

3. *Aesculus* pollen viability and longevity under storage.            | 139  |

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>139</td>
</tr>
<tr>
<td>Materials and methods</td>
<td>143</td>
</tr>
<tr>
<td>Plant material</td>
<td>143</td>
</tr>
<tr>
<td>Pollen collection and handling</td>
<td>144</td>
</tr>
<tr>
<td>SEM observations of pollen</td>
<td>145</td>
</tr>
<tr>
<td>Optimum test conditions for in vivo germination of pollen</td>
<td>145</td>
</tr>
<tr>
<td>Effects of storage, storage temperature and storage time</td>
<td>146</td>
</tr>
<tr>
<td>Data analysis</td>
<td>147</td>
</tr>
<tr>
<td>Section</td>
<td>Page</td>
</tr>
<tr>
<td>------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Results and discussion.</td>
<td>148</td>
</tr>
<tr>
<td>Optimization of in vitro germination test conditions</td>
<td>148</td>
</tr>
<tr>
<td>Response of fresh pollen to different germination temperatures</td>
<td>150</td>
</tr>
<tr>
<td>Effect of storage temperature and period on pollen germination.</td>
<td>153</td>
</tr>
<tr>
<td>SEM observations of pollen.</td>
<td>155</td>
</tr>
<tr>
<td>Cited References.</td>
<td>169</td>
</tr>
<tr>
<td>4. Stratification factors affecting seed germination and emergence and</td>
<td>173</td>
</tr>
<tr>
<td>seedling efficiency in <em>A. parviflora</em> and <em>A. pavia</em>.</td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>173</td>
</tr>
<tr>
<td>Materials and methods.</td>
<td>178</td>
</tr>
<tr>
<td>Plant materials and seed sample preparation</td>
<td>178</td>
</tr>
<tr>
<td>Stratification</td>
<td>179</td>
</tr>
<tr>
<td>Germination tests</td>
<td>179</td>
</tr>
<tr>
<td>Emergence and seedling growth</td>
<td>180</td>
</tr>
<tr>
<td>Data analysis</td>
<td>181</td>
</tr>
<tr>
<td>Results and discussion.</td>
<td>182</td>
</tr>
<tr>
<td>Initial seed moisture content</td>
<td>182</td>
</tr>
<tr>
<td>Seed germination frequency and rate</td>
<td>182</td>
</tr>
<tr>
<td>Shoot emergence frequency and rate</td>
<td>186</td>
</tr>
<tr>
<td>Seedling production and evaluation</td>
<td>189</td>
</tr>
<tr>
<td>Factors limiting the production of seedlings</td>
<td>192</td>
</tr>
<tr>
<td>Cited References</td>
<td>208</td>
</tr>
<tr>
<td>5. Selection of <em>Aesculus parviflora</em> and <em>Aesculus pavia</em> parents and</td>
<td>212</td>
</tr>
<tr>
<td>their parental efficiency.</td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>212</td>
</tr>
<tr>
<td>Materials and methods</td>
<td>215</td>
</tr>
<tr>
<td>Variability if fruit characteristics and fruiting behavior among species</td>
<td>215</td>
</tr>
<tr>
<td>Provenance formula development and selection of breeding parents</td>
<td>216</td>
</tr>
<tr>
<td>Breeding procedures</td>
<td>217</td>
</tr>
<tr>
<td>Data analysis</td>
<td>219</td>
</tr>
<tr>
<td>Results and discussion</td>
<td>220</td>
</tr>
<tr>
<td>Evaluation of fruit per panicle</td>
<td>220</td>
</tr>
<tr>
<td>Seed per fruit on open pollinated material</td>
<td>222</td>
</tr>
<tr>
<td>Seed fresh weight and other seed characteristics</td>
<td>223</td>
</tr>
<tr>
<td>Provenance evaluation</td>
<td>225</td>
</tr>
<tr>
<td>Effectiveness of controlled pollination</td>
<td>227</td>
</tr>
<tr>
<td>Success of model in predicting fruiting success</td>
<td>228</td>
</tr>
<tr>
<td>Hybridization success</td>
<td>229</td>
</tr>
<tr>
<td>Cited References</td>
<td>242</td>
</tr>
</tbody>
</table>
Appendices:

Appendix A  List of Citations for tables 1.2-1.7, 1.10, and 1.12 .......................... 244
Appendix B  List of Aesculus species, hybrids, and cultivars under commercial production ................................................................. 249
Appendix C  The Names and contact information for producers of Aesculus ................... 251
Appendix D  List of citations for table 1.8 ............................................................... 256
Appendix E  Botanic Gardens and Arboreta with Aesculus collections ............................ 262
Appendix F  Aesculus species used in this research including location and condition .............. 265
Appendix G  Inflorescence characteristics by specimen plant for Aesculus parviflora and Aesculus pavia ......................................................... 268
Appendix H  Percentage of complete flowers within Aesculus panicles ........................... 270
Appendix I  Effect of inflorescence position within the plant on inflorescence characteristics ................................................................. 272
Appendix J  Values for calculating fruit and seed potential ......................................... 274
Appendix K  Values for the fruit and seed realization for the provenance ............................ 276

Biblography ............................................................................................................. 278
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>A list of frequently used common names for various species and botanical</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>varieties of <em>Aesculus</em></td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td>Plant characteristics of <em>Aesculus</em> species and botanical varieties listed</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>by section</td>
<td></td>
</tr>
<tr>
<td>1.3</td>
<td>Leaf characteristics of <em>Aesculus</em> species and botanical varieties listed</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>by section</td>
<td></td>
</tr>
<tr>
<td>1.4</td>
<td>Inflorescence and flower characteristics of <em>Aesculus</em> species and botanical</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>varieties listed by section</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>Fruit and seed characteristics of <em>Aesculus</em> species and botanical</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>varieties listed by section</td>
<td></td>
</tr>
<tr>
<td>1.6</td>
<td>Horticultural characteristics of <em>Aesculus</em> species and botanical</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>varieties listed by section</td>
<td></td>
</tr>
<tr>
<td>1.7</td>
<td>Tolerance/susceptibility to biotic or abiotic stresses exhibited by</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td><em>Aesculus</em> species and botanical varieties</td>
<td></td>
</tr>
<tr>
<td>1.8</td>
<td>Activities, properties or uses of saponin and coumarin natural products</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>from <em>Aesculus</em></td>
<td></td>
</tr>
<tr>
<td>1.9</td>
<td>Synonyms and/or names no longer accepted for species and botanical</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>varieties of <em>Aesculus</em></td>
<td></td>
</tr>
<tr>
<td>1.10</td>
<td>The varieties and known cultivars of <em>Aesculus</em> organized by species</td>
<td>79</td>
</tr>
<tr>
<td>1.11</td>
<td>Putative parentages, synonyms, and possible alternative parentages of</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>recognized interspecific hybrids of <em>Aesculus</em></td>
<td></td>
</tr>
<tr>
<td>1.12</td>
<td>Characteristics of interspecific hybrids of <em>Aesculus</em> and of selections</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>from interspecific hybrids</td>
<td></td>
</tr>
<tr>
<td>Section</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>2.1</td>
<td>Anthesis periods of <em>Aesculus parviflora</em> and <em>Aesculus pavia</em> in central and northeastern Ohio.</td>
<td>130</td>
</tr>
<tr>
<td>2.2</td>
<td>Inflorescence characteristics of <em>Aesculus parviflora</em> and <em>Aesculus pavia</em>.</td>
<td>131</td>
</tr>
<tr>
<td>2.3</td>
<td>The effect of inflorescence modification on inflorescence length, the number of flowers per panicle, frequency of mixed panicles and the number of complete flowers per panicle of <em>Aesculus parviflora</em> and <em>Aesculus pavia</em>.</td>
<td>132</td>
</tr>
<tr>
<td>3.1</td>
<td>The range of temperatures during flowering periods of <em>Aesculus</em> species. Values represent climatological data during the 1997 – 2000 seasons.</td>
<td>157</td>
</tr>
<tr>
<td>3.2</td>
<td>Pollen dimensions of various <em>Aesculus</em> species as found in the current study and reported in the literature.</td>
<td>158</td>
</tr>
<tr>
<td>5.1</td>
<td>Infructescence characteristics of <em>Aesculus parviflora</em> and <em>Aesculus pavia</em>.</td>
<td>232</td>
</tr>
<tr>
<td>5.2</td>
<td>Estimation of maternal potential among specimens of <em>Aesculus parviflora</em> and <em>Aesculus pavia</em> by a provenance formula and a comparison of estimated performance with the level of fruit set achieved during a breeding exercise.</td>
<td>233</td>
</tr>
<tr>
<td>5.3</td>
<td>Performance of <em>Aesculus parviflora</em> and <em>Aesculus pavia</em> specimens involved in open-, self- intraspecific cross- and interspecific cross-pollinations during a breeding exercise.</td>
<td>234</td>
</tr>
<tr>
<td>5.4</td>
<td>Performance of <em>A. parviflora</em> and <em>A. pavia</em> specimens as male parents in a series of intra- or interspecific crosses with <em>A. parviflora</em>.</td>
<td>235</td>
</tr>
<tr>
<td>5.5</td>
<td>Performance of <em>A. parviflora</em> and <em>A. pavia</em> specimens as male parents in a series of intra- or interspecific crosses with <em>A. pavia</em>.</td>
<td>235</td>
</tr>
<tr>
<td>5.6</td>
<td>Seed weights from <em>Aesculus parviflora</em> and <em>Aesculus pavia</em> specimens involved in open-, self- intraspecific cross- and interspecific cross-pollinations during a breeding exercise.</td>
<td>236</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1.1</td>
<td>The <em>Aesculus</em> phylogenetic organizational scheme proposed by Hardin (1957).</td>
<td>88</td>
</tr>
<tr>
<td>1.2</td>
<td>The natural distribution of various <em>Aesculus</em> species. A) <em>A. assmica, A. chinensis, A. indica</em> and <em>A. wilsonii</em> are depicted in orange, yellow, pink and blue, respectively. Overlapping ranges are shown by color mixtures of green and brown; B) <em>A. hippocastanum</em> depicted in green; C) <em>A. turbinata</em> depicted in blue.</td>
<td>89</td>
</tr>
<tr>
<td>1.3</td>
<td>The natural distribution of <em>A. californica, A. flava, A. glabra, A. parryi, A. parviflora,</em> and <em>A. pavia</em> depicted in pink, blue, yellow, mauve, green and red respectively.</td>
<td>90</td>
</tr>
<tr>
<td>1.4</td>
<td>Important natural products derived from <em>Aesculus</em> (Yoshikawa and Yamahara, 1996; Merck, 2001).</td>
<td>91</td>
</tr>
<tr>
<td>2.1</td>
<td>The position of complete flowers within <em>Aesculus</em> panicles. Values represent the percentage of complete flowers in successive quarter panicles of selected <em>Aesculus</em> specimens. Bars with black, white, dark gray and light gray fills indicate basal, second, third and apical quarters of the panicles, respectively.</td>
<td>133</td>
</tr>
<tr>
<td>2.2</td>
<td>The spatial patterns of inflorescence anthesis in <em>Aesculus</em> panicles. Values represent the mean day of anthesis ± S.E. of flowers in ten position increments where flower positions were numbered from the base to the apex of the panicle. a) <em>A. parviflora, n</em> = 20; b) <em>A. pavia, n</em> = 30.</td>
<td>134</td>
</tr>
<tr>
<td>2.3</td>
<td>The rate of floral anthesis for staminate and complete <em>Aesculus</em> flowers. Values represent the mean percentage ± S.E. of the total staminate or complete flowers that had reached anthesis by a given day during panicle flowering. Open and filled circles indicate values for complete and staminate flowers, respectively. a) <em>A. parviflora, n</em> = 20; b) <em>A. pavia, n</em> = 30.</td>
<td>135</td>
</tr>
</tbody>
</table>
3.1 Schematic representation of data sets used in statistical analyses.

3.2 The main effects of media sucrose concentration and germination temperature on pollen germination of various *Aesculus* species. Values represent mean germination percentages and their standard errors averaged across species and pollen storage treatments. Effect means (bars of similar color) with similar superscripts are not significantly different according to SNK tests \((p=0.05)\).

3.3 Interactive effects of *Aesculus* species with test parameters of media sucrose concentration and germination temperature. Values represent means and standard errors for each species X test factor combination. Filled circles, open circles, filled triangles, open triangles, filled squares and open squares correspond to Par, Pav, Fla, Syl, Car, and Hip, respectively.

3.4 The main effects of *Aesculus* species and germination temperature on fresh pollen germination. Values represent mean germination percentages and their standard errors. Effect means (bars of similar color) with similar superscripts are not significantly different according to SNK tests \((p=0.05)\).

3.5 Interactive effects of *Aesculus* species and germination temperature on fresh pollen germination. Values represent means and standard errors for each species X germination temperature combination. Filled circles, open circles, filled triangles, open triangles, filled squares and open squares correspond to Par, Pav, Fla, Syl, Car, and Hip, respectively.

3.6 Interactive effects of *Aesculus* genotypes and germination temperature on pollen germination. Values represent means and their standard errors for each specimen X germination temperature combination. Filled circles correspond to Fla1, Par9 and Pav1, respectively. Open circles correspond to Fla2, Par7 and Pav6, respectively. Filled triangles correspond to Pav3.

3.7 Representative photomicrographs (80X) of *Aesculus* pollen germination at various temperatures 8 hrs. after plating. Figures 3.7 a-f depict pollen of Pav6 germinated at 10, 15, 20, 25, 30 and 35°C, respectively.
3.8 The main effects of *Aesculus* species and storage period on stored pollen germination. Values represent mean germination percentages and their standard errors. Effect means (bars of similar color) with similar superscripts are not significantly different according to SNK tests ($p=0.05$). 166

3.9 Interactive effects of *Aesculus* species and storage period on stored pollen germination. Values represent means and standard errors for each species X germination temperature combination. Filled circles, open circles, filled triangles, open triangles, filled squares and open squares correspond to Par, Pav, Fla, Syl, Car, and Hip, respectively. Pollen with germination percentages $>40\%$ or $<20\%$ were presumed to be adequate or inadequate for pollination and fruit set, respectively. Pollen with germination percentages falling inside the shaded area may or may not effect adequate fruit set. 167

3.10 Interactive effects of *Aesculus* genotypes and storage period on pollen germination. Values represent means and their standard errors for each specimen X storage period combination. Filled circles correspond to Fla1, Par9 and Pav1, respectively. Open circles correspond to Fla2, Par7 and Pav6, respectively. Filled triangles correspond to Pav3. Pollen with germination percentages $>40\%$ or $<20\%$ were presumed to be adequate or inadequate for pollination and fruit set, respectively. Pollen with germination percentages falling inside the shaded area may or may not effect adequate fruit set. 168

4.1 The main effects of stratification period and species on seed germination (radical protrusion) in *A. parviflora* and *A. pavia*. Values represent mean germination percentages and their standard errors. Effect means (bars of similar color) with similar superscripts are not significantly different according to SNK tests ($p=0.05$). 198

4.2 The interactive effects of stratification period and species on seed germination (radical protrusion) in *A. parviflora* and *A. pavia*. Values represent mean germination percentages and their standard errors for each species X stratification period combination. Filled circles and open circles represent correspond to Par and Pav, respectively. 199
4.3 The effects of stratification period on the synchrony of germination (radical protrusion) in *A. parviflora* and *A. pavia*. The left and right sides of the boxes and the left and right whisker lines represent the 25th and 75th percentile and the 10th and 90th percentile of the population’s distribution, respectively. The light and heavy vertical lines within boxes represent the population median and mean, respectively. Values represented by closed circles indicate the day to emergence of individuals that were not within the group enclosed between the 10th and 90th percentiles.

4.4 The main effects of stratification period and species on shoot emergence (epicotyl protrusion) in *A. parviflora* and *A. pavia*. Values represent mean emergence percentages and their standard errors. Effect means (bars of similar color) with similar superscripts are not significantly different according to SNK tests \((p=0.05)\).

4.5 The interactive effects of stratification period and species on seed emergence (epicotyl protrusion) in *A. parviflora* and *A. pavia*. Values represent mean emergence percentages and their standard errors for each species X stratification period combination. Filled circles and open circles represent correspond to Par and Pav, respectively.

4.6 The effects of stratification period on the synchrony of emergence (epicotyl protrusion) in *A. parviflora* and *A. pavia*. The left and right sides of the boxes and the left and right whisker lines represent the 25th and 75th percentile and the 10th and 90th percentile of the population’s distribution, respectively. The light and heavy vertical lines within boxes represent the population median and mean, respectively. Values represented by closed circles indicate the day to emergence of individuals that were not within the group enclosed between the 10th and 90th percentiles.

4.7 The main effects of stratification period and species on the frequency of usable seedling numbers in *A. parviflora* and *A. pavia*. Values represent mean percentages of usable seedlings and their standard errors. Effect means (bars of similar color) with similar superscripts are not significantly different according to SNK tests \((p=0.05)\).

4.8 The interactive effects of stratification period and species on the production of usable seedlings of *A. parviflora* and *A. pavia*. Values represent mean percentages of usable seedlings and their standard errors for each species X stratification period combination. Filled circles and open circles represent correspond to Par and Pav, respectively.
4.9 The effects of stratification period on the frequency of *A. parviflora* and *A. pavia* seedlings within classes. Values represent the proportion of total seedlings within each class. Bars with white, dark gray, black, light gray and hatching indicate frequency of seedlings that were classed as albino, accelerated growth, dead, stunted or useable, respectively.

4.10 The main effects of stratification period and species on seedling height in *A. parviflora* and *A. pavia*. Values represent mean seedling heights and their standard errors. Effect means (bars of similar color) with similar superscripts are not significantly different according to SNK tests (*p*=0.05).

5.1 Estimate of maternal potential in an *Aesculus* breeding program based on fruit set per tree.

5.2 Estimate of maternal potential in an *Aesculus* breeding program based on seed yield per tree.

5.3 The effect of multiseeded fruit on fruit weight in *A. parviflora* and *A. pavia*. Population N for seeds from single-seeded fruits was standardized to that of the seeds from multiseeded fruit by sub sampling single-seeded fruits at random. Values represent the mean seed weights and their standard errors for seeds from single-seeded fruits (black bars) and for seeds from multi-seeded fruits (gray bars).

5.4 Single versus multiple hand pollinations of each flower as a breeding program strategy for obtaining seed from intra- or interspecific crosses of *A. parviflora* and *A. pavia*. Bars with black and gray fills represent the success rate in percent of single and multiple hand pollinations, respectively. Numbers in parentheses represent the total number of flowers pollinated.

5.5 Developing *A. pavia* fruit and seed: a) developing fruit resulting from an intraspecific cross within *A. pavia*; b) developing fruits resulting from an interspecific cross with *A. parviflora*; c and d) putative hybrid seeds.
CHAPTER 1

THE GENUS *AESCULUS* AND OPPORTUNITIES FOR ITS IMPROVEMENT

From the politics of William Henry Harrison to Henry Wadsworth Longfellow's poem, "The Village Blacksmith" and from flower arrangements to medicine, *Aesculus* are an important part of our lives. *Aesculus* artifacts suggesting human use as wooden ware date from as early as before 3000 BC. Currently, they are used predominantly in the landscape as they are among the finest hardy ornamental spring and summer flowering specimens (Chittenden, 1938). *Aesculus* range from medium size shrubs to large trees and are recognized for their bold branch structure, distinctive leaves, early bud break, upright panicles, and attractive blooms. Their emergence is one of the first signs of spring. The white, cream, yellow, pink or red flowers of *Aesculus* are attractive to hummingbirds and a broad array of butterflies. These species are planted for shade in parks and on streets because of their ability to withstand the urban environment (VanDersal, 1942). These trees are commonly planted in parks, arboreta, college campuses, and home landscapes (Dirr, 1990).

*Aesculus* currently has 13 recognized species, plus associated varieties, forms, hybrids, and cultivars spread across the northern hemisphere, mainly in temperate climates (Hardin, 1957 a,b and 1960). According to Hardin’s scheme (1957a, b and 1960).
1960), the species are organized into five sections: Aesculus, Calothyrsus, Macrothyrsus, Parryana, and Pavia (Figure 1.1). Species were grouped into these sections primarily based upon variability in bud, flower, and fruit morphology as characterized from both herbarium specimens and field observations. Hardin also identified numerous interspecific hybrids which were said to result from introgression or hybrid swarms. The botanical and horticultural characteristics of these 13 species and their hybrids are discussed in detail below.

Several explanations have been given for the derivation of the name *Aesculus*. It may have been conferred originally by Pliny after the Latin term *esca*, meaning food or nutrient (Howard, 1945). The name may also be derived from the Latin term *Ischio*, describing a species of oak (*Quercus esculus* L.) sacred to the god Jove. According to Bombardelli et al. (1996) *Aesculus* is Latin for an oak with edible acorns. However, the Romans may have used this term interchangeably to identify oak or any nut-bearing tree. The name *Aesculus* may also be derived from its association with *Aesculapius*, the Roman God of Healing (Furlow, 1991).

The species of *Aesculus* are associated with an abundance of common names; for a given species, the common name may differ regionally or nationally (Table 1.1). In 1924, the American Joint Committee on Horticultural Nomenclature decided that American species of *Aesculus*, were to be considered buckeyes, and all other species of *Aesculus*, horsechestnuts (VanDersal, 1942). The term buckeye “derives from the fanciful resemblance that a fruit, with part of its husk removed to reveal the pale brown scar on the rich brown surface of the seed, as the half-opened eye of a deer” (Everett, 1981). Michaux found so many *A. glabra* trees along the Ohio River during his travels in
1810, that he gave the species its common name of Ohio buckeye and state of Ohio its nickname “The Buckeye State” (Collingwood and Brush, 1964).

The distribution and habitats of Aesculus

The current distribution of Aesculus is spread across the northern hemisphere with seven species found in North America, one species in central Europe, and at least five and potentially up to thirteen species in China and spread across Asia (Fang, 1981).

There have been several discoveries of fossilized Aesculus species. Dating from the early Miocene, Aesculus mioxyla was found in central Japan (Suzuki and Terada, 1996). Onoe (1978), also working in Japan, listed Aesculus majas as one of many tree species found in the Miocene Toyooka formation. Fossilized wood of Aesculus was found in Korea, but it could not be identified to the species level (Jeong et al., 2004). Beck (1945) found fossilized wood of Aesculus dating to the late Miocene at several forest sites in the Vantage region of central Washington. These fossils appeared similar to the wood of Aesculus species currently adapted to Pacific coast. They were found in conjunction with Quercus, Sequoia, Taxodium, Fraxinus, Liquidambar, and other dominant fossilized species at these sites. Prakash and Barghoorn (1961) described Aesculus hankinsii from fossilized wood which was found in the Vantage area of central Washington. The cellular organization of trunk tissues within the fossil bore similarities to that of existing Aesculus, and most closely to that of A. pavia. Pavlyutkin (1999) described a new, Miocene-era Aesculus species found in a river basin of far East Russia. This species, Aesculus iljinskiae, was characterized based on a leaf impression with palmately compound obovate leaflets and serration typical of all Aesculus. The earliest
example of *Aesculus* dated to the late Paleocene period, and was found in North Dakota and Wyoming. *Aesculus hickeyi* was described from both intact leaves and fruit (Manchester, 2001). All this data would seem to support the hypothesis that *Aesculus* evolved by the early Miocene and was distributed over both North America and Asia.

Despite having a cultivated history dating back to 1576, the native range of *A. hippocastanum* was not known for many years. At first, it was mistakenly believed to be native to parts of India, but now it has been established to be native to northern Greece, southern Albania, parts of Bulgaria, and Turkey at elevations of 700-1850 m. Asian members of the Calothyrsus section are found throughout the mountains and valleys of the Himalayas (Figure 1.2). Growing at the highest elevations of 1000-3050 m, *A. indica* is found in the forests and shady valleys across northeastern Afghanistan, Pakistan, Kashmir, northern India, and Nepal (Troup, 1921). *Aesculus assamica* is found in the tropical forests from the northeast states of India, Bhuton, and the northern portions of Myanmar, Thailand, Laos, North Vietnam, and the southwestern part of Yunnan, China at elevations of 100-1400 m. The two best characterized species in China are *A. willsonii*, found in the central Chinese provinces of Yunnan, Hunan, Hubei, Sichuan, Henan and Shaanxi at elevations of 650-3000 m (Everett, 1981; Lee, 1935), and *A. chinensis* native to the provinces of Hebei, Shanxi and Henan of northern China at an altitude of 150-650m. *Aesculus wangii*, one of a large group of recently described species, it was reported to be native to the southern provinces of China. *Aesculus turbinata* is found on the three islands of Hokkaido and in central and northern parts of Honshu and Shikok from sea level to 2000 m, but is more commonly found in the mountain forests (Gorer, 1976).
On the west coast of North America, *A. californica* is found growing in the coastal range at elevations of 20-600 m, where the largest specimens are found, and in the foothills of the Sierra Nevada Mountains at 600-1500 m (Figure 1.3). *Aesculus parryi* has been observed growing in Baja California at elevations up to 700m. *Aesculus parviflora* is the species with the smallest range and is limited to far western Georgia and most of Alabama. It was originally discovered and then rediscovered growing in Aiken County in South Carolina (Wyatt, 1985).

The members of the Pavia section extend over most of the eastern United States in overlapping ranges. *Aesculus flava* is found in a range from Pennsylvania to Georgia through Kentucky and Tennessee and west to Illinois, with the highest concentration found throughout portions of the Appalachian mountain range at elevations of 1220-1525 (Bir, 1992; Little, 1979). The range of *A. glabra* overlaps that of *A. flava* and *A. pavia* and extends along the river bottoms from western Pennsylvania to Southeast Iowa and south to Alabama and Texas (Sargent, 1891). However, *A. glabra* var. *arguta* is localized from eastern Kansas south to central Texas (Little, 1979; Merz, 1957). The southern most species is *A. pavia* and its native range extends from North Carolina west to Missouri, and south from Texas to Florida (Brockman, 1968; Everett, 1981; Little, 1979). *Aesculus pavia* var. *flavescens* is localized to the region of the Edwards Plateau of Texas (Wasowski and Wasowski, 1991). *Aesculus sylvatica* has the smallest range of any member of the Pavia section and is limited to an area from Virginia through South Carolina west to Alabama (Brockman, 1968; Everett, 1981; Little, 1979).
The botanical characteristics of *Aesculus*

*Aesculus* have evolved into a variety of plant forms, from the large forest trees of *A. hippocastanum*, *A. turbinata*, *A. indica*, and *A. flava* to the short and shrubby stature of *A. parryi*, *A. parviflora*, and *A. pavia*. The bark ranges from light gray and smooth to dark gray or reddish brown and broken into large plates or scales (Table 1.2).

The buds of all the members of the sections *Aesculus* and Calothrysus and of the hybrid *A. × carnea* are resinous, while all others are smooth and dry. Mature specimens of *Aesculus* produce mixed terminal buds with well developed leaves and inflorescences. These organs are distinguishable almost immediately after the initiation of seasonal growth. The flower panicles of the summer blooming species, *A. parviflora* and *A. californica*, are less well developed than those of the early spring blooming types, such as *A. glabra*. The largest buds are found on *A. turbinata* (2-3 cm), and the smallest are found *A. parviflora* (about 0.5 cm).

Of all the well described species, only *A. assamica*, found in the subtropics, is thought to be somewhat evergreen, and the rest are deciduous. The leaves in the genus are palmately compound. The leaves are typically composed of five to seven leaflets, but may have as many as eleven leaflets, depending on the species (Table 1.3). Most leaflets are obovate to lanceolate in shape. The depth and regularity of the serration vary between species but can also vary within species. The amount of pubescence on the petioles and abaxial leaf blades is variable and is often used to separate specimens into varieties. *Aesculus turbinata* has the largest leaves in the genera, with widths typically ranging 35-60 cm. Leaves of this species can, however, reach 1 m in width (Phillips, 1978). The leaves of *A. californica* are among the smallest. Both *A. californica* and *A.
*parryi* regularly lose their leaves early in the growing season as a means to avoid drought conditions (Benseler, 1968; Wiggins, 1932). The leaves of *A. glabra* produce an unpleasant odor when crushed, and this is a key identification feature within the Pavia section.

The inflorescences of all *Aesculus* are erect terminal panicles. They vary slightly in shape from pyramidal to cylindrical, and in flower density from tightly compressed to loose (Table 1.4). The flowers of many species are creamy white with distinctive blotches that often turn from yellow to red during the course of anthesis. The color change often coincides with nectar availability for pollinators. The most variable section for flower color is Pavia, ranging from the greenish yellow of *A. glabra* to the brighter shades of yellow in *A. flava* and the variable shades of red with yellow in *A. sylvatica* and *A. pavia*. The flowers are either open and bell-shaped, as exemplified by *A. hippocastanum*, or are tubular, as those of *A. pavia*. The stamens are exserted in most species except for *A. flava* and *A. sylvatica* where they are included. The most distinctive flowers belong to *A. parviflora*, and are comprised of four white petals and five to seven white filaments with pinkish to bright orange anthers that are three to four times as long as the petals. These long exserted stamens give the panicles their "brush-like" appearance and this species its common name (Van Dersal, 1942).

The leathery fruits of all species are subglobose to ovoid to obovoid and most are various shades of brown. The surface features vary from very spiny like those of *A. hippocastanum* to warty or smooth (Table 1.5). Within the Pavia section, fruits of all three types can be observed, and their features are another means to distinguish between species and their hybrids. Despite the fact that the ovaries contain six ovules, rarely are
more than three seeds per fruit produced. The seeds range from light honey-brown, like those of *A. pavia* and *A. parviflora*, to shades of chestnut, mahogany, and the very dark brown (nearly black) of *A. glabra*.

**Aesculus in the landscape**

The first ornamental planting of *A. hippocastanum* can be dated to 1576, when the first seeds were shipped from Constantinople to Clusis in Vienna (Everett, 1981). In 1699, Sir Christopher Wren planted horsechestnuts to create an avenue of trees and to surround a large pool and fountain in Bushey Park. The blooming display was, and is, celebrated with "Chestnut Sunday" (Everett, 1981). Following its importation into colonial America, the flowering of *A. hippocastanum* was recorded and celebrated in letters between Peter Collinson and John Bartram. The streets of Paris obtain part of their beauty in spring from the flowering display of *A. hippocastanum* (Chaney, 1995).

Today, the ornamental value of a plant is subjective and is determined by assessing the aesthetic value of its flowers, fruit, foliage, and overall plant structure. The best plants have what is termed “four season interest,” where in each season, a plant adds to the overall beauty of the landscape (Table 1.6).

The easily recognizable palmately compound leaves of *Aesculus* are among the first to break bud early in the spring, and in many species the newly emerging leaves have pink, bronze, or purple tones (Brickell, 1992). The leaves of *Aesculus* are not typically susceptible to frost damage or sunscald, but some of the variegated cultivars require protection from either early spring frosts or the intense afternoon sun of summer.

As the foliage develops, especially that of *A. turbinata*, *A. indica*, and *A. parviflora*, it
elicits a tropical feeling. *Aesculus parryi* is unique in that it flowers and fruits after losing all its foliage for the growing season. *Aesculus parviflora* has the most consistent bright yellow fall color of any species (Flint, 1966). *Aesculus indica*, *A. turbinata*, and all members of the Pavia section can produce good orange and/or red fall color if the leaves have not been damaged during the summer (Benvie, 2000; Brickell, 1992; Gibson-Watt, 1997; Phillips, 1978; Wright, 1985). Fall color also appears to be highly variable within some species and has been used as a selection tool for developing cultivars of *A. glabra*. *Aesculus* are among the first plants to lose their leaves in the fall, thus revealing the plants branch structure. All *Aesculus* species except for *A. parviflora* have a bold and coarse texture, contrasting with many other landscape plants.

The *Aesculus* feature of most ornamental interest is that of its upright inflorescences, which contain many bell or tube shaped flowers. Panicles differ in length with most species producing inflorescences 10-25 cm long, with *A. parviflora* forma *serotina* 30-45 cm having the longest panicles. The shape of Aesculus panicles varies from the pyramidal of *A. hippocastanum* and *A. flava* to the elongated columnar panicles of *A. californica* and *A. parviflora*. The flowering period begins in early spring with *A. glabra* and concludes in early to midsummer with *A. parviflora*. Most *Aesculus*, including the most widely recognized species *A. hippocastanum* and *A. pavia*, bloom during the middle of spring and have floral displays that last two to three weeks. *Aesculus indica* is valued for both its substantial panicles ranging from 20-40 cm and a flowering period coming four to six weeks after *A. hippocastanum*. The flowers have a variety of shapes from an open bell with large developed petals to the unique flowers of the *A. parviflora* with its exceedingly long stamens and small petals giving the panicles
their characteristic "brush-like" appearance and thus one of its common names. Flowers of *Aesculus* range from the pure white of *A. parviflora* to creamy white to the various shades of red of *A. pavia* and *A. sylvatica*. The creamy white flowers of most species are accented with blotches of color that change from yellow to deep red depending of the flower’s age and adding to their appeal. In *A. sylvatica*, flowers change color from a creamy yellow with golden yellow to orange blotches of the recently opened flowers to shades of light pink with rose to red blotches as the flowers mature just prior to abscission. This species obtained its common name of painted buckeye from having flowers of various colors all clustered on a single panicle. Some of the most valued specimens are the apricot and pink selections of *A. sylvatica*, *A. indica*, and *A. californica*.

Benseler (1968) and Lee (1935) reported that the flowers of *A. californica* and *A. wilsonii* are fragrant. The flowers of many of these species are attractive to butterflies and hummingbirds adding to their appeal. The fruits and seeds are in shades of tan and brown and add little to the ornamental character of the plants. With careful selection it would seem possible obtain *Aesculus* with multi-season interest.

*Pests and diseases of Aesculus*

The extent to which a plant can be utilized in the landscape is often limited by its susceptibility to both pests and diseases. The two major leaf diseases of *Aesculus* are leaf blotch caused by *Guignardia aesculi* and anthracnose caused by *Glomerella cingulata* (Chaney, 1995). Leaf blotch is a fungal disease that occurs frequently throughout the United States and Europe during the summer months of June, July, and August (Table
1.7). The symptoms begin as small irregularly shaped spots that appear water soaked. These spots turn a characteristic reddish brown with a yellow margin (Hartman et al., 2000; Phillips and Burdekin, 1982; Stewart, 1916). Although appearing similar to physiological leaf scorch, leaf blotch can be identified by small black pycnidia of the organism occurring on the upper leaf surface in the center of the lesion (Hartman et al, 2000; Stewart, 1916). Over time the entire leaf may become infected, turn brown, dry out, and abscise (Phillips and Burdekin, 1982). Chaney (1995) considered the disease to be problematic in the nursery where infection can impact the growth rate of young trees once in the landscape, where it drastically impacts plant appearance. However, it has little physiological impact on mature plants in their native habitat. The fungus overwinters on infected leaves, so good sanitation is critical for control of fungal inoculum. Applications of fungicides, at two to four week intervals throughout the summer, can provide a measure of control (Phillips and Burdekin, 1982).

Of all the *Aesculus* species evaluated, *A. hippocastanum* and all of its cultivars, including ‘Baumanni’, ‘Incisa’, ‘Luteovariegata’, and ‘Pyramidalis’, have been consistently rated the most susceptible to leaf blotch (Chaney, 1995; Hartman et al., 2000; Neely, 1971; Neely and Himelick, 1963; Walker, 1988). The susceptibility of many of the rarer Asian species has not been assessed. However, of the species that have been studied, susceptibility appears to be highly variable. The susceptibility of *A. chinensis* to leaf blotch ranges from none to severe, but this inconsistency could be associated with the plant being misidentified in many collections. *Aesculus parviflora* and *Aesculus parviflora* forma *serotina* do not appear to be susceptible to leaf blotch (Carter 1975; Neely, 1971; Neely and Himelick, 1963). *Aesculus glabra* appears to be moderately to
highly susceptible to leaf blotch, but *A. glabra* var. *arguta* does not appear susceptible (Neely, 1971; Neely and Himelick, 1963). Neely and Himelick (1963) also observed that *A. pavia* appeared to be susceptible, but the degree of severity appeared to be dependant on the specimen. *Aesculus flava* and *A. sylvatica* appear to be only moderately susceptible; however, once again it appeared to be specimen specific (Neely, 1971; Neely and Himelick, 1963). However, Carter (1975) found individual specimens of *A. glabra*, *A. pavia*, and *A. sylvatica* to be highly resistant to leaf blotch. This variability in the expression of resistance needs to be examined further.

The initial symptoms of anthracnose are browning of the petioles, midribs, and leaves. As the disease progresses, leaves eventually abscise (Pierce and Hartley, 1916). The location of the infection along the veins is one of the critical identification features in separating this disease from either leaf blotch or leaf scorch. The severity of the disease can be lessened by pruning the canopy to increase air circulation and by removing the infected leaves to reduce the amount of fungus in the environment (Chaney, 1995). It is not possible to eliminate the disease during the growing season once, it is established, but it is possible to control infection through the use of fungicides. Fungicides need to be applied beginning early spring just after bud break and then at two week intervals to avoid the infection (Chaney, 1995; Tattar, 1978).

There are several other fungi that cause a variety of leaf spot, (*Septoria hippocastani*, *Septoria glabra*, and *Cercospora aesculina*), powdery mildew, (*Uncinula flexuosa*), and rust (*Puccinia andropogonis*) diseases, but none of these are considered very serious or limiting in the landscape (Hartman et al., 2000; Hepting, 1971; Phillips and Burdekin, 1982). Wei and Katumoto (1998) identified and named a new and
somewhat serious leaf ring spot fungus, *Mycodidymella aesculi*, which attacks *A. turbinata* in its native habitat. There has been a report of horsechestnut mosaic virus or yellow mosaic virus in Europe. This disease causes the leaves to become bright yellow and then die; over time there is a loss of vigor and diminished blooming. Transmission of this virus can occur by budding or grafting, although the causal organism(s) have not been identified (Cooper, 1993). Various verticillium wilts and bleeding canker (*Phytophthora cactorum*), can occasionally affect *Aesculus* but are not serious (Chaney, 1995; Hepting, 1971). There are also several wood rot fungi, *Nectria cinnabarina*, *Collybia velutipes*, *Fomes applanatus* and *Ganoderms* spp., that while usually saprophytic, can cause wood rot when a tree is injured and death if severely infected (Chaney, 1995; Hartman et al., 2000; Hepting, 1971; Phillips and Burdekin, 1982).

In the United States, most insect pests of *Aesculus* were considered of minor importance and typically did not require control unless there was an infestation on young trees. However, two recently introduced pests into Europe are significantly impacting the health of *Aesculus*. *Pulvinaria regalis*, the horsechestnut scale, was introduced from the United States to the United Kingdom in the late 1960s but is now spread throughout Belgium, France, Germany and Switzerland (Harris, 1968; Speight et al., 1998). *Pulvinaria regalis* has a wide host range of at least 65 species, but preferentially feeds upon *Acer*, *Tilia*, and *Aesculus*, especially *A. hippocastanum* and *A. × carneum* (Sengonca and Faber, 1996). After hatching, the crawlers move to the underneath side of the leaves, where they feed throughout the summer. In the fall, the nymphs move to twigs to overwinter and the adult females move to the main stems (Hippe and Frey, 1999). The feeding results in a loss of vigor and decreased dry weight accumulation (Speight, 1991).
The problem appears worse on plants in urban areas. Control is limited to washing or spraying the trees while dormant to remove young scales and dead females with egg sacs (Alford, 1995).

_Cameraria ohridella_, horsechestnut leaf miner, was first discovered in Macedonia in 1984 but has now become widely distributed throughout Austria, Hungary, Czech Republic, Slovakia, Germany, and France (Augustin et al., 2004; Tomiczek and Krehan, 1998). The origin of the horsechestnut leaf miner moth is not clear. Researchers have speculated that it originated in another part of the world and was latter introduced to Europe but upon close examination this species seems quite different from all known leaf miners. _Aesculus hippocastanum_ is its preferred host and the larvae damage leaves by eating tunnels through the palisade parenchyma layer, with severe damage resulting in defoliation by late July (Tomiczek and Krechan, 1998). Tree decline as a result of repeated defoliation may occur. It appears as though _A. × carnea_ is not as susceptible to feeding as _A. hippocastanum_. Currently, the treatment is to spray with either a topical, or systemic, insecticide to kill the larvae (Tomiczek and Krechan, 1998).

In addition to these insect pests, the larvae of _Orgyia leucostima_ (white-marked tussock moth), _Lymantria dispar_ (gypsy moth), _Hyphantria cunea_ (fall webworm), and adult _Popillia japonica_ (Japanese beetle), and _Alebra wahlbergi_, and _Aguriahana stellulata_ (leafhopper) either consume, or damage foliage. Overall they have not resulted in long term injury (Alford, 1995; Chaney, 1995; Hartman et al., 2000; Johnson and Lyon, 1991). _Pseudococcus comstocki_ (comstock mealybug), _Quadraspidiotus juglansregiae_ (walnut scale), _Eulecanium tiliae_ (nut scale), _Lepidosaphes ulmi_ (oystershell scale), and _Eulecanium cerasorum_ (calico scale) are difficult insects to
control, and infestations weaken the trees causing reduced growth, and over time can be fatal (Alford, 1995; Hartman et al., 2000; Johnson and Lyon, 1991). *Zeuzera pyrina* (Leopard moth) was introduced into the United States from Europe. It bores into the petiole, destroys the leaf, and then feeds on the wood of twigs and branches (Alford, 1995). A serious pest, introduced from Asia into the United States in 1996, is *Anoplophora glabripennis* (starry sky beetle). The larvae bore into the vascular system of living trees creating large galleries and ultimately death. The only control is to remove and destroy all the infected trees and to quarantine the area (Becker, 1998).

The top growth of *Aesculus* is not typically damaged by wildlife. Some *Aesculus*, those in the Pavia section and *A. parviflora*, provide nectar for hummingbirds during their migration north and through the early summer (Bertin, 1980 and DePamphilis, 1988). Seed consumption by wildlife can significantly reduce a species’ population density over time. According to deWit (1967) and Pokorny (1974), *A. hippocastanum* seeds were an important food source for European wildlife, especially red and roe deer. Burns and Honkala, (1990) reported that *A. glabra* provided between two to five percent of the food consumed by fox and gray squirrels during the autumn and winter months, but this seed loss was not a limiting factor in the tree’s reproduction. This is not the case in the southern provinces of China, where the consumption of seed by wildlife, coupled with habitat destruction by human activity, has endangered the survival of *A. wangii* (Li-Kuo, 1992). Hoshizaki et al. (1999) studied the relationship between *A. turbinata* and the indigenous wildlife. The 'scatter hoarding' behavior of rodents, specifically *Apodemus speciosus* (wood mice), resulted in more than 95% of all sound seed being removed from their original location within three weeks and, on average, were moved more than 40 m
(Hoshizaki et al, 1997; Hoshizaki et al, 1999). Although more than 90% of all seeds in the study were lost to presumed predation, the redistribution of the remaining seeds resulted in a larger 'seed shadow.' Therefore, a greater number of seeds were germinated under more conducive growing conditions away from the mature tree (Hoshizaki et al., 1999).

*Aesculus as affected by abiotic stresses*

Along with leaf blotch, leaf scorch, a physiological stress factor, limits the widespread planting of *Aesculus*. The symptoms of these leaf disorders are similar. Starting from the tips and leaflet margins, browning and curling begin and in a short period of time the entire leaf can dry. Although it may be observed at anytime in the growing season, including wet periods, leaf scorch is most common in July and August (Everett, 1981; Hartman et al., 2000). The cause has been attributed to the plant’s inability to absorb and translocate a sufficient amount of water from the root system to the leaves to compensate for the loss of moisture through transpiration (midday wilt). The problem can be intensified by high winds, high temperatures, drought, girdling roots, limited root area, reflected heat from pavement, or compacted soil, or by any combination of these factors. In urban areas, these problems may be antagonized by air pollution. The best solution to this problem is locating *Aesculus* on deep moist well-drained soil. Leaf scorch severity can be lessened by pruning to reduce the amount of leaf surface and deep watering during dry periods. There seems to be variability in the extent to which trees are susceptible to this condition and, although unsightly, it is not typically responsible for plant mortality (Everett, 1981; Hartman et al., 2000).
Other forms of abiotic stress in the urban environment include soil compaction, drought, pollution, soil contamination by salts and heavy metals, mineral deficiencies, or lack of mineral availability due to high pH. The two major types of pollution are air pollution from both automobile traffic and manufacturing plants and soil pollution from de-icing salts, especially NaCl and CaCl2. *Aesculus* have been recommended for the urban landscape because of their tolerance to both types of pollution.

Velagic-Habul et al. (1991) reported that *A. hippocastanum* ranked in the top three species tested for its tolerance to SO$_2$ and had a higher total sulfur concentration in its leaves. It has been a recommended species for planting throughout Eastern Europe because of its tolerance to high SO$_2$ and H$_2$S. In a study on the effects of sulfur dioxide, fluorides, and other pollutants on the forest ecosystem in the Ohio river valley, the population of *A. flava* appeared stable and tolerant of air pollution (McClenahen, 1978). *Aesculus glabra* was found to be intermediate in its ability to assimilate ozone (Townsend, 1974). Walker (1988) noted that *A. × carnea* 'Broitii' showed a degree of tolerance to pollution and was recommended for planting in towns with high pollution. Sufficient irrigation and good mineral nutrition tended to enhance *Aesculus'* ability to tolerate pollution.

There are three common sources of salt: naturally saline soils, sprays or inundation from the oceans, and especially in the northern metropolitan areas, the largest source, road deicing products. All sources of salt damage plant tissue in a similar manner; the overall result of which can be decline of vigor and death. Salt damage to the leaves was characterized by a browning of the margins and was linked with an increased concentration of chloride in the leaf tissue (Meyer and Hoster, 1980). It reduces
secondary growth and thus reduces the capacity to move water (Petersen et al., 1982). Deicing salt can damage plants from road spray or from salt that leaches into the soil. Road spray reaches beyond road or highway shoulders and can travel up to 75 m through the air, relative to traffic speed and type. *Aesculus californica*, *A. hippocastanum*, and *A. pavia* have all been recommended for seaside gardens (Villis, 1975; Wymann, 1955). *A. hippocastanum* is reported to have the highest degree of aerial salt tolerance as well as tolerance to soil salt (Chaney, 1995; Dirr, 1976; Wymann, 1955). In a study of damage from the 1938 New England hurricane, Moss (1940) reported that horsechestnuts, even those plants adjacent to the shore, showed no salt damage, while other hardwood species were severely damaged. There is anecdotal evidence that *A. pavia* grows on the barrier islands and outerbanks of Georgia and North Carolina and is tolerant to both aerial and well as edaphic salt concentrations. *Aesculus flava* has also been listed as moderately tolerant to salt (The Morton Arboretum, 2002).

*Aesculus* showed an overall better water balance and did not change its osmotic effects due to the salt. However, damage was induced by chloride ion toxicity (Mekdaschi et al., 1988; Spirig, 1981). Balder (1990), Leh (1990), and Mekdaschi et al. (1988) reported cessation of road salting resulted in the quick recovery of minimally damaged *A. hippocastanum* in usually two to three years. More severely damaged trees took from five to ten years to recover, and their recovery was enhanced by the addition of gypsum. Chloride levels in the soil quickly returned to normal, but sodium levels decreased more slowly. Salt tolerance appears to be a heritable trait. Mother trees that appeared vigorous under saline conditions produced a higher number of seedlings also capable of growing under high salt conditions (Fostad and Pedersen, 1998).
Cultural requirements of Aesculus

The success of *Aesculus* in the landscape is dependent upon choosing plant species or clone best suited to the prevailing cultural conditions (i.e., climatic and edaphic characteristics). With the exceptions of *A. parryi*, *A. californica* and *A. glabra* var. *arguta*, all *Aesculus* require a moist, well, drained soil. As stated above, dry soil can contribute to leaf scorch, and premature defoliation can occur in the case of drought. *Aesculus glabra*, *A. flava*, and *A. pavia* are moderately tolerant to wet sites and can withstand short periods of flooding. Cold hardiness is species specific, but most are hardy from zones 5-9. Adapted to zone 3, *A. glabra* and *A. hippocastanum* are the most cold tolerant. The semi-tropical *A. assamica* is limited to zone 8, and *A. californica* and *A. indica* grow only in zones 7-9. The range of *A. indica* is further limited by its sensitivity to temperatures in excess of 35°C and its need for a steady supply of moisture. This makes *A. indica* the most climatically sensitive species of all *Aesculus* characterized to date, and outside of its native range can only be successfully planted in England and the Pacific coastal region of North America.

Soil preferences are species specific, with *A. californica* and *A. parryi* flourishing on hot, rocky, sandy soils. *Aesculus glabra* and *A. pavia* are tolerant of gravelly soils, and *A. hippocastanum* and *A. glabra* tolerant of clay soils. According to Wright (1985), *Aesculus* seem to do best in soils with a pH of 6.0 to 7.0 but are adaptable. *Aesculus hippocastanum, A. glabra, A. indica, and A. pavia* are specifically described as tolerant of alkaline soils with *A. glabra* having the widest acceptable pH range of 6.1 to 7.5 (Hightshoe, 1988; Hillier, 1991; Sternberg and Wilson, 1995). The possible exception to this is *A. parviflora* which seems to prefer a slightly more acidic soil of pH 6.0-6.5, but
even this species is listed as pH adaptable (Poor and Brewster, 1996; and Hottes, 1959). Very little is known about *A. wangii*, but Li-Kuo (1992) reports that this species requires a soil pH of 4.6-5.5.

The light requirements of *Aesculus* reflect their place in the forest canopy. *Aesculus californica* and *A. parryi* do best in a full sunny exposure. The large tree species, *A. chinessis, A. flava, A. hippocastanum, A. indica*, and *A. turbinata*, will grow in either full sun or light partial shade. This shade tolerance is especially true of their seedlings. The understory species, *A. glabra, A. pavia*, and *A. sylvatica* grow best in partial shade where their root systems remain cool. *Aesculus parviflora* does well in sun or partial shade, but thrives in full shade. The minimum light requirements for *Aesculus* is not known.

Most *Aesculus* require only minor pruning to repair injuries caused by storm damage, either by high winds or ice, to maintain the specimen's shape, for cosmetic reasons, or to open the canopy for greater air flow (Chaney, 1995). More and White (2002) and Wymann (1955) describe *Aesculus* as “messy” to reflect the common shedding of older brittle branches, but other authorities do not consider this to be a significant problem. *Aesculus parviflora* can be rejuvenated by pruning the plant to the ground, but this is usually not necessary. Pruning should be done in early spring when problems are most visible. *Aesculus* in general, but especially *A. × carnea*, can suffer from frost cracks along the trunk and large branches either during severe winters or when the specimens are planted in the northern-most part of their ranges (Chaney, 1995).

*Aesculus* can be difficult to transplant and establish because their course, fleshy root system, and deep tap roots (Poor, 1984). The best method is to transplant the balled-
and-burlapped specimens in either early spring or in the fall when the plants are dormant to help minimize transplant shock (Hightshoe, 1988; Phillips, 1978). *Aesculus parviflora* can also be transplanted from container-produced material. More and White (2002) state that *Aesculus* should be planted at least 30m or more from any building to reduce the possibility of damage that might be caused by the plants root system.

*Propagation of Aesculus*

*Seed propagation*. Propagation can occur sexually through the production and subsequent germination of seeds, or asexually through the manipulation of somatic tissues. In their native habitat, seeds are shed in early autumn and secondarily dispersed either through the activity of small mammals or flood waters. The seeds become buried which protects their viability until germination the following spring.

In the nursery industry, seed propagation is the most frequently used method to reproduce *Aesculus* (Browse, 1970; Wright, 1985). It requires only simple inexpensive procedures. Normally, seeds are readily available. However, to ensure an adequate supply of seeds, nursery propagators should identify good provenances and monitor prospective seed sources as the time of seed maturity approaches. MacDonald (1986) observed that uniformly sized seeds germinated more evenly. Bhagat et al. (1993) found large seeds of *A. indica* germinated with greater frequency and produced more robust seedlings with greater survivability than small seeds.

Special collection and handling techniques are necessary to maximize the success rate in managing these large recalcitrant seeds that store the majority of their energy reserves as carbohydrates and fats (MacDonald, 1986). *Aesculus* seeds should be
collected as soon as they fall from the tree. Unprotected seeds desiccate at a rate proportional to their exposure to air, resulting in reduced viability. If a seed becomes severely dehydrated (wrinkled), it loses its ability to imbibe and to metabolize stored food reserves, even if it is planted under ideal conditions. MacDonald (1986) and May (1963) reported that germination of improperly stored seed was quite low and that dried and/or wrinkled seed often failed to germinate. *Aesculus* seed should be handled and stored in small lots to avoid excessive heat resulting from seed metabolism. Under crowded conditions, fresh seeds begin to “sweat” leading to dehydration and increased fungal infection. Seeds should also be cleaned of any husk material, inspected, and any damaged seeds discarded prior to storage. Browse (1970) suggests that if seeds have been shipped that they should be soaked for 24 hours before planting.

The optimum duration and storage conditions for *Aesculus* seed varies with species, but, according to experiential evidence, most require or benefit from stratification. Suggested controlled stratification conditions for *A. flava*, *A. glabra*, *A. hippocastanum*, *A. sylvatica*, and *A. turbinata*, range from 1.0-4.5°C for periods of 3-5 months (Bir, 1992; Browse, 1970; Fordham, 1960; Furlow, 1991; Rudolf, 1974; Villis, 1975; Widmoyer and Moore, 1968;). *Aesculus* seeds exhibit a short period of viability (Wright, 1985), and therefore, cannot be stored for prolonged periods, even under optimum storage/stratification conditions. Furlow (1991) suggests that stored seeds of *A. flava*, *A. glabra*, and *A. hippocastanum* exhibit decreased germination rates after 7-8 months of storage. According to Widmoyer and Moore (1968), *A. hippocastanum* seeds began germinating in storage after one year. Seed viability was greatly reduced despite showing no outward signs of deterioration. Likewise, storage temperatures higher than
optimum resulted in substantial losses in viability and increased decay (Widmoyer and Moore, 1968; Wright, 1985). Storing seeds under refrigeration eliminates environmental fluctuations, but proper conditions must be monitored and maintained to insure seed quality. The cleaned seeds should be divided into small lots and placed into plastic bags before the seed has the opportunity to dessicate.

Although several researchers have studied Aesculus seed germination empirically, few have conducted controlled experiments exploring the physiology of seed dormancy in this genus. May (1963) provided preliminary data indicating that storing A. hippocastanum seed at -1.1°C for up to six months was optimal. These conditions decreased germination in storage and seed decay; thus allowing for more efficient germination after removal from storage. More recently, Pritchard and coworkers (Pritchard et al., 1996; Pritchard et al., 1999) published detailed studies of A. hippocastanum seed germination as affected by temperature and by prior stratification treatments. Stratification treatments at 6°C prior to germination at warmer temperatures increased the proportion of seeds that germinated and the rate at which they germinated. Germination percentage and stratification time were related linearly. Conversely, when germination temperature was held constant at 16°C, the seed germination percentage and the speed at which it occurred was increased as stratification temperatures decreased to 2°C.

Fall planting immediately after harvest may be a suitable method for satisfying the stratification requirements of A. flava, A. hippocastanum, A. sylvatica, A. turbinata, A. indica, A. chinensis, A. wilsonii, and A. × carnea seeds under natural conditions (Bhagat et al., 1993; Bir, 1992; Wright, 1985). Ideally, planting should occur within a
few days of seed collection. Seed is sown directly in the planting bed at a spacing of 10-12 cm between seeds and covered by 5 cm of soil (Browse, 1970). MacDonald (1986) stressed the necessity for hand planting so that each seed could be placed with the hilum pointing down. Orienting the seed in this position improves seedling quality by encouraging the growth of straight stems and eliminated the possibility of shoot-root crossovers. Seed beds should be covered with hardware cloth or some other material to exclude squirrels and other mammals for disturbing the seeds. Germination will not become evident until the following spring. First, the radical emerges developing into a carrot-like tap root, and then the shoot emerges above ground, immediately producing true leaves. First-year, seedling shoots can grow as much as 30 cm (Browse, 1970; Mitchell, 1987). The cotyledons, kept within the seed coat, remain attached to the seedling, providing nutrients during the first growing season (Mitchell, 1987). The success of the direct sowing technique may be hampered by cold temperatures or excessive soil moisture which may foster seed decomposition (Gibson-Watt, 1997). Moreover, if not protected, newly emerged seedlings can be damaged by spring frosts (Browse, 1970).

Seeds of species such as *A. californica*, or *A. pavia*, native to warmer climates, do not require stratification (Bhagat et al., 1993; Dirr and Heuser, 1987; Furlow, 1991). According to several authors, seeds of *A. parviflora* also exhibit little or no dormancy and must be sown as soon as they are ripe (Bir, 1992; Flint, 1996; Fordham, 1987; Furlow, 1991). Fordham (1987) described the germinating *A. parviflora* seed as developing a fleshy root and an epicotyl that became dormant after cotyledon reserves were exhausted. He also found that seeds failed to germinate when left exposed (i.e., unburied) for as little
as 16 days. When he stored ripe seeds at 4.5°C, they underwent partial germination and then decomposed. On the other hand, exposing *A. parviflora* seed to a minimal cool storage period of 30 days seemed beneficial to Bir (1992). MacDonald (1986) portrayed *A. parviflora* as exhibiting epicotyl dormancy; under this condition, radical emergence occurs within the first year after sowing, but shoot emergence is conditioned only by an intervening cold period. MacDonald (1986) also suggested *A. hippocastanum* seed to be free of dormancy or stratification requirements, although experimental evidence described above suggests his supposition to be in error.

*Propagation via cuttings, budding and grafting.* Vegetative propagation techniques allow for the production of large numbers of plants and the maintenance of specific combinations of morphological or physiological traits. There are no reports of natural shoot regeneration in either *A. glabra* or *A. flava*. Merz (1957) and Carmean (1958) suggest that if shoot regeneration were to occur in these species, it would be more likely to occur in younger specimens. Conversely, *Aesculus indica* produces a large number of shoots from roots, especially if the roots are naturally or culturally pruned (Troup, 1921).

Commercial methods of asexual propagation are typically centered upon cuttings of all types or on budding and grafting. The propagation of *Aesculus* by stem cuttings has enjoyed limited success. The ease of rooting stem (softwood or hardwood) cuttings varies from specimen to specimen, and a plant’s ability to form adventitious roots can only be ascertained by attempting to root it (Bir, 1992). Bergmann et al. (1988) found that the degree of success in rooting stem cuttings was limited by both the stage of development of the cutting and the age and condition of the specimen being propagated.
In their experience, stem cuttings from the spring growth flush of young specimen plants were most likely to root well. Furthermore, they found that sucker growth from the base of a mature *A. × arnoldiana* ‘Autumn Splendor’ rooted, whereas stem cuttings taken from the crown did not. However, commercially acceptable levels of rooting were not achieved, even with sucker-derived cuttings. Chapmann and Hoover (1981) found *A. hippocastanum* stem cuttings to root at commercially acceptable levels (≈ 75%) when newly-emerged shoots were used as propagules. Stem cuttings from late-season growth failed to root. Finally, Bir (1992) found that fair rooting percentages could be obtained through the use of mist and 1000 ppm IBA. Concentrations of IBA below this level were ineffective and those above this level were toxic.

Stem cuttings have been widely studied in *A. parviflora* because of its poor seed set and its shrubby suckering plant habit. Flint (1966) reported that *A. parviflora* could be propagated by layers made in June. Working with *A. parviflora*, the form *serotina* and the cultivar ‘Rogers’, Dirr and Burd (1977) and King (1993), achieved rooting percentages of approximately 50-80% using softwood cuttings from shoots, sucker growth, or crown tissue. Rooting was enhanced using IBA at rates of 1000-5000 ppm. Bir and Barnes (1994) successfully rooted 88% of *A. parviflora* cuttings using propagules harvested in the first six weeks following bud break. They also reported 2500 ppm IBA dissolved in alcohol to be the most effective rooting promoter.

The traditional method of propagation for *A. parviflora* is by root cuttings. *Aesculus parviflora* is the only *Aesculus* species that can be propagated by this method (MacDonald, 1986). According to both Browse (1970) and MacDonald (1986), root cuttings are best harvested in late winter or early spring. Young healthy roots are
removed from the ground and cleaned prior to being cut into 7 to 12 cm long pieces, dipped in fungicide, and then stuck in deep rooting flats. During the sticking process, it is critical to maintain the correct polarity of the cuttings (i.e., the proximal end of the root should point up). Moreover, the medium-water balance should be managed carefully as the cuttings are susceptible to various forms of rot. Browse (1970) reported that roots nearest to the crown had the highest rates of regeneration. The plants produced from these cuttings were of sufficient size to be moved at the end of the growing season. McDaniel (1972) suggested that the clone ‘Rogers’ could best be commercially propagated by this method using larger roots near the crown.

There are a number of grafting and budding techniques that have been used for the clonal propagation of *Aesculus*. Grafting techniques most commonly used are variations of the basic whip graft. Leiss (1967) successfully used the splice graft, a variant of the whip and tongue graft, for various scion and rootstock combinations. He further suggested bark grafting over methods of budding for the propagation of *A. hippocastanum*, *A. × plantienersis*, *A. × carneaa*, and *A. parviflora* or their cultivars. MacDonald (1986) advocated the use of the side whip or basal whip graft technique for bench grafting onto seedling rootstock. Side grafting was also recommended for the propagation of *A. × arnoldiana* ‘Autumn Splendor’ and *A. × carneaa* ‘Broitii’ (Bergmann et al., 1989; MacDonald, 1986). The wedge graft method has been used to propagate *A. × neglecta* ‘Erythroblastos’ (Villis, 1975). In the field, top grafting has been practiced to establish weeping cultivars onto 2 m high standards (MacDonald, 1986). These grafts can be made in the early spring. However, top grafts made from mid-September to mid-
October allow rootstock and scion to knit prior to winter, resulting in greater scion growth the following season.

For scions of *A. hippocastanum* and *A. × carnea* cultivars, the rootstock most often used in grafting procedures is seedling *A. hippocastanum* (MacDonald, 1986). Wright (1985) also recommended *A. hippocastanum* rootstocks for the propagation of larger hybrids, but supposed that *A. flava* and *A. glabra* might make better rootstocks for the North American species. Actively growing *A. glabra* was used successfully as the understock for the propagation of *A. × arnoldiana* ‘Autumn Splendor’ (Bergmann et al., 1989). Villis (1975) maintained that seedlings of *A. hippocastanum* or *A. flava* performed well as rootstocks if produced following traditional methods. MacDonald (1986) stressed that *A. indica* ‘Sydney Pearce’ should be grafted onto *A. indica*.

Although grafting techniques can be used successfully to propagate *Aesculus*, they present several disadvantages. Browse (1970) did not favor the use of grafting because success rates were poor and graft unions were often “unsightly.” MacDonald (1986) mentioned that grafts involving *A. hippocastanum* sometimes produced overgrowths at the union. More and White (2002) found that grafted cultivars of *A. hippocastanum* lacked vigor. Furthermore, grafted wood tends to be brittle, and top grafts of pendulous cultivars have the propensity to break. Collectively, their observations are indicative of graft incompatibilities.

Browse (1970) preferred budding selected *Aesculus* onto seedling horsechestnut over the use of grafting procedures. When practiced in late summer on well-developed root stocks, T-budding and inverted T-budding techniques were highly successful methods of propagation (i.e., 60-80% bud growth). To enhance success rates, Browse
(1970) recommended the harvest of medium-sized buds from the middle of the plant; large terminal buds were difficult to attach tightly and small buds taken at the base of stem were deeply dormant, resulting in erratic bud break the following year. Propagation by budding may be most limited by the scarcity of suitable budwood.

Propagation by tissue culture. The science of tissue culture, encompassing various techniques of micropropagation, embryogenesis and cryopreservation, is based upon the cell property of totipotency, where each cell maintains the ability to reproduce the entire organism. Both micropropagation and somatic embryogenesis have become common techniques to reproduce “difficult-to-propagate” plants or high-value species such as Kalmia latifolia (Kamenicka and Rypak, 1989). Cryopreservation has provided an alternative method for germplasm preservation in recalcitrant species which do not survive under conventional long-term storage conditions. Tissue culture has several unique advantages over other propagation/preservation methods: large numbers of plants can be produced in a relatively short period of time; virus-free materials can be developed; and there is a possibility of long-term storage of valuable germplasm. It also has several disadvantages: the costs of production are high; the techniques are exacting; the specific conditions for optimum success vary for each species and sometimes each genotype; plant conversion from culture is sometimes difficult; and tissue cultured plants often require specialized care in the nursery.

From early in the development of tissue culture science, the endosperm of unripe seeds has been an important source of biologically active materials. Shantz and Steward (1968) reported that a liquid extract from immature Aesculus was comparable to coconut milk for encouraging tissue culture growth. Endosperm of unripe Aesculus seed was later
shown to increase growth and enhance the total number of protocorms of *Cymbidium* orchids (Mandy and Jambor-Benczur, 1986).

Shoot multiplication though organogeneis is perceived as the most clonally faithful process. There are only a few reports of organogenesis in *Aesculus*. Masubuchi (1991) demonstrated that shoot tips of a 15 year old *A. × carnea* were capable of shoot proliferation when initially cultured on modified MS (Murshige-Skoog) medium with 5 µM BAP and 0.1 µM IBA. Shoot multiplication occurred with normal leaf development on MS with 1 µM BAP and 53% of the shoots rooted within 60 days on WPM (woody plant medium). *Aesculus hippocastanum* was successfully propagated from terminal buds (Kamenicka and Rypak, 1989). The key factor in success was the growth stage of the initial tissue. If terminal buds were acquired during periods of active growth, then regeneration occurred normally; if bud tissue was deeply dormant when harvested, then only callus was formed in culture.

Trippi (1963) demonstrated that both juvenile and adult tissue could proliferate callus, but there are no reports of adventitious shoot formation from undifferentiated *Aesculus* tissue. Likewise, *Aesculus* protoplasts have not been studied as explant sources for either adventitious shoot formation or somatic embryogenesis.

Somatic embryogenesis could be a useful technique to propagate mature elite genotypes of *Aesculus* as well as an important source of material for germplasm cryopreservation, and genetic improvement through plant transformation. Embryogenesis in *Aesculus* has been most extensively studied using tissues derived from *A. hippocastanum* (Bisio et al., 1996; Chalupa, 1987, 1990; Gastaldo et al., 1996; Kamenicka and Rypak, 1989; Profumo et al., 1994). Embryo formation from tissue-
cultured explants of *A. × arnoldiana* ‘Autumn Splendor’, *A. glabra*, and *A. parviflora* has also been attempted, with different degrees of success being achieved (Bergmann et al., 1996; Radojevic, 1991; Trick and Finer, 1999).

Many different tissues types have been explored as explant sources, immature and mature embryos, sections of mature cotyledons, leaves from seedling and mature trees, pith, cambial tissue, stems, internode segments from mature plant crowns, bark, and flower filaments. Most somatic embryogenesis protocols require the use of 2,4-D along with other plant growth regulators in the imitation phase. Embryoids can form directly from the explant, or as is more often the case, indirectly from callus that becomes embryogenic (Bisio et al., 1996; Gastaldo et al., 1996; Kamenicka and Rypak, 1989; Radojevic, 1991). Once embryoids have been initiated, the tissue is transferred to media with either reduced hormone concentrations or to a hormone free media for further embryo development. The greatest impediment to this process is generating morphologically and physiologically “normal embryos” that are capable of germinating and growing into plantlets. Many techniques including dark treatments, cold and desiccation have been investigated as procedures to increase the conversion rate.

Androgenic (haploid) embryos have been produced through anther culture or, more directly, through microspore culture. Radojevic (1978) was the first to develop an anther culture procedure for *A. hippocastanum* and *A. × carnea*. Upon karyotypic analysis, several of the plantlets derived from this procedure proved to be haploids (n = 20). Various protocols and their modifications have been developed to improve both the quantity and quality of embryos created (Marinkovic and Radojevic, 1992). The percentage of haploids among regenerated plantlets was improved using a microspore
culture technique (Calic et al., 2003/2004). In this system, somatic tissues are not initially cultured. Unfortunately, anther culture of *A. parviflora* only led to the production of callus tissue without subsequent formation of androgenic embryos (Radojevic, 1991).

Since *Aesculus* have large recalcitrant seeds with a high moisture content, long term germplasm storage using conventional systems and standard environmental controls is not possible. In an attempt to alleviate this problem, Jorgensen (1990) assessed the potential for freezing *A. hippocastanum* somatic embryos at the globular stage using various cryoprotectant solutions and techniques. Under the best combination of conditions, the embryos remained alive but did not develop further. Jekkel et al. (1998) offered a procedure for improved embryo survival. First, the embryos were preconditioned on a medium containing 0.75 µM ABA for four days. Then, they were dried for four hours in a laminar flow cabinet before being frozen in liquid nitrogen. Research continues on ways to improve the conversion rate of somatic embryos to plants and techniques for the quicker recovery and regeneration following cryopreservation.

There have been two successful reports of the tranformation of *Aesculus*. Trick and Finer (1999) were able to transform somatic embryo of *A. glabra* using the sonication-assisted *Arbogacterium*-mediated technique (SAAT). Androgenic embryos of *A. hippocastanum* were transformed using *Argobacterium rhizogenes* (Zdravkovic-Korac et al., 2004). Stable transformation was confirmed using Southern hybridization for both transformation techniques.
Commercial sources of Aesculus plants

Members of the Ohio Nursery and Landscape Association listed the following reasons why Aesculus have limited availability: difficulties in propagation, slow growth rates, long production times, and lack of demand. The list of Aesculus under commercial production in Ohio in the 2005 nursery stock survey is limited to five species and five cultivars, three of which are cultivars of A. × carnea. The most popular species to produce is A. parviflora with 24 nurseries producing it throughout Ohio. In an industry survey conducted in 2000, the two most popular cultivars of A. × carnea were 'Briotii' and 'Fort McNair'. Whereas, 'Homestead', A. parviflora f. serotina 'Rogers' and the best two selected cultivars of A. glabra are propagated only by a few producers (Appendix B). Although there is an extensive list of cultivars and selected hybrids, most have not been made commercially available and accessibility to this germplasm would appear limited. Even the recently released A. californica 'Canyon Pink' seems destined to remain the domain of arboretum collections due to the lack of commercial interest.

Additional uses of Aesculus and its products

Ethnobotanical use

Specific ethnobotanical uses of Aesculus reflect the diversity within the genus and in the societies that used them. One of the first postulated uses of Aesculus dates to the Turks and Greeks who ground the seeds of A. hippocastanum and fed them to their horses as a cure for overexertion or coughs (Bombardelli et al., 1996; Mitchell, 1987). This species may have acquired the common name, horsechestnut, based on its equine use
(Furlow, 1991; Howard, 1945). Centuries later, Europeans used *A. hippocastanum* for multiple purposes. For example, its wood was harvested for fuel; its bark and seed leachates were used as a febrifuge, an anti-pyretic, or as a substitute for quinine. Yellowish extracts from seed (perhaps gallic and or tannic acid) were also employed as dyeing agents and used in the tanning and processing of leather (Bombardelli et al., 1996; Radojevic, 1991).

*Aesculus* throughout Asia have been used in multiple ways. *Aesculus chinensis* is sacred to both the Buddhists and Taoists and is cultivated in temple grounds and homes (Wang, 1939). Wooden artifacts made from *A. turbinata* have been found dating from 6000 to 3000 BC (Noshiro and Suzuki, 1989). The seeds of *A. wilsonii* have been and are used in Chinese medicine. The bark *A. indica* has been used as a tonic because of its astringent properties (Hooker, 1859). The seeds of *A. indica* have also been eaten during periods of famine after the removal of toxins (Hooker, 1859).

Native Americans from both the Southeast and the Southwest (USA) used or created various products from their native buckeyes. Seeds of *A. glabra, A. flava, and A. californica* were tossed into small bodies of water to tranquilize fish to make them easier to catch (Collingwood and Brush, 1964; Tantaquidgeon, 1942). Once the toxic components had been eliminated from the seeds; they could be roasted or ground into flour for food (Standley, 1926; Sternberg and Wilson, 1995; Sudworth, 1908). They were also used for their medicinal properties. Extracts of the seeds were used to treat earaches, sores, colic, sprains and chest pains (Hamel and Chiltoskey, 1975; Herrick, 1977; Tantaquidgeon, 1942). Powdered bark was sometimes used to alleviate toothaches and ulcer pain (Bocek, 1984). According to an old folk remedy, carrying a buckeye in
your pocket could ward off rheumatism and bring good luck (Benvie, 2000; Bir, 1992; Peattie, 1991; Tantaquidgeon, 1942). Extracts from bark were used by the early pioneers to treat brain and nervous system disorders. Native Americans used the wood of *A. californica*, *A. flava*, and *A. glabra* to make bows, ceremonial masks, and household items (Goodrich and Lawson, 1980 and Zigmond, 1981). The wood of *A. flava* and *A. glabra* was carved to make troughs and cradles and the wood shavings were used for summer hats (Hamel and Chiltoskey, 1975; Peattie, 1991). They used the roots and seeds to create a paste which was then used as a form of laundry soap. The seeds were also used as a soap substitute during World War I (deWit, 1967).

**Aesculus natural products and their pharmacological uses**

*Saponins and coumarins.* The ethnobotanical uses of *Aesculus* are based upon the species’ ability to produce a variety of biologically and/or chemically active secondary products, present in the plant predominantly as glycosides. The most important of these compounds are classed as either saponins or coumarins (Hart et al., 2000; Russell et al., 1997, University of Georgia, 2002).

The general structure of saponins (i.e., cyclic triterpene, steroid or steroidal alkaloid aglycones esterified to sugar or uronic acid moieties) renders them amphiphilic (Hostettmann and Marston, 1995). Because they are highly surface-active, many saponins form stable foams in aqueous solutions that emulsify in a manner similar to detergents (Hostettman and Marston, 1995, Walthelm et al., 2001). Although not all saponins form foams, this trait has historically characterized the class and has been used to indicate the presence or absence saponin components and to estimate their quantity in a
given plant part. In fact, the class name is derived from the Latin word *sapo*, meaning soap. Typically, saponins are bitter substances, but some are sweet-tasting. Others have been shown to inhibit human perception of the sweetness of sucrose (Hostettman and Marston, 1995).

Coumarins (simple coumarins, furanocoumarins, pyranocoumarins and substituted coumarins) are highly aromatic, highly oxygenated heterocyclic lactones; coumarin, the parent compound of the family is a benzopyrone (i.e. an $\alpha$-pyrone joined to a benzene ring) (Keating and O’Kennedy, 1997). Coumarins vary widely in molecular weight, in the number of and type of carbon and heterocyclic rings present in their structures, and in the number and types of oxygenated side groups they contain. They are related closely to anthocyanins and other flavonoids via shared biosynthetic pathways (Keating and O’Kennedy, 1997; Weinmann, 1997). Coumarins are primarily derived from plant sources (most notably from the Rutaceae and Apiaceae) but some are produced by fungi (e.g., Aflatoxin B$_1$) and animals. Perhaps the most recognized compounds in this class are dicumarol and warfarin, used extensively as anticoagulants (Mabry and Ulubelen, 1980).

Members of both classes have long been considered to act as phytoanticipins, plant protective compounds synthesized *a priori* that are effective against infection or predation. As examples, the oat saponin avenacin inhibits the growth of various strains of *Gaeumannomyces graminis* pathogenic to oats and wheat (Vidhyasekaran, 1997), whereas simple coumarins display antibacterial activity against a variety of plant and animal bacteria, including *Pseudomonas* (Kayser and Kolodziej, 1999). Plant saponins and coumarins also act as toxins and feeding deterrents to a variety of mites and insects.
Saponins bind to dietary sterols in the insect gut, thus preventing sterol uptake and the synthesis of steroidal molting hormones. Comarins and furanocoumarins are effective oxicides, and also act as photosensitizing agents that bind to pyrimidine bases of DNA in the presence of UV light (Sadasivam and Thayumanavan, 2003). In addition, coumarins, furanocoumarins and dihydropyranocoumarins from several plant sources have been classed as phytoalexins, or defense compounds synthesized de novo in response to fungal attack (Vidhyasekaran, 1997). Coumarins may also act as kairomones (host recognition compounds) for co-evolved insects (Sadasivam and Thayumanavan, 2003).

Historically, human culture has taken advantage of the biological and pharmacological activities of saponins and coumarins as agents in herbal remedies and for other ethnobotanical uses. The herbal properties of saponins were first chronicled in Kofler’s *Die Saponine*, published in 1927 (Hostettmann and Marston, 1995), whereas those of coumarins were mentioned in the German Pharmacopoeia, published in 1926 (Weinmann, 1997). Subsequent decades of research have uncovered a myriad of bioactive characteristics associated with specific compounds or compound groups within these classes (Hostettman and Marston, 1995; O’Kennedy and Thornes, 1997; Waller and Yamaski, 1996). In general, these compounds have been found to sustain or improve cardiovascular health as fibrinolytic or anti-clotting agents, factors that improve blood vessel function or strength, or as compounds that stabilize heart rhythms. They have also been shown to reduce tissue edema associated with poor circulation or physical trauma. Their potential role as anticarcinogens and immunomodulators has been documented. In addition, some saponins or coumarins have analgesic, anti-inflammatory, antipyretic, or antihistamine-like properties. As antibiotics, they have been used as antibacterial,
antifungal, antiviral, anthelminitic (antifilarial) or antiprotozoan agents and as
ichthyotoxins, molluscicides, insecticides and rodenticides. They have also been shown
to negatively affect human and animal fertility in a variety of ways.

*Aesculus saponins and coumarins.* Several natural products of *Aesculus* have
been shown to be clinically active, and the most notable among these products are the
saponin(s), escin, and the coumarin, esculin, and their related compounds (Figure 1.4).
Escin (alternate names: aescin, aescine, aescusan, reparil) is not a single compound, but is
utilized as mixture of closely related triterpene saponins of the oleanane (β-amyrin) type
isolated from the seed of *Aesculus hippocastanum* (Hostettmann and Marston, 1995;
Merck, 2001). The two main glycosides are composed of an aglycone (protoescigenin)
glycosidically-linked to a molecule of glucuronic acid and subsequently to two molecules
of glucose (Figure 1.4a). Both of these structures are acylated at the C-22 position with
acidic acid, but differ at their C-21 positions which are acylated with either tiglic or
angelic acid (i.e. trans- or cis- 2,3-dimethylacrylic acid, respectively) (Merck, 2001).
Yoshikawa and Yamahara (1996) describe these compounds (which they designated as
“escin Ia” and “escin Ib”) and three others differing in their substitution patterns at C-21
or in their sugar moieties (Figure 1.4a) as the major active saponin constituents of
horsechestnut seed extracts. Confusingly, the term “escin” can also refer to several
commercially-prepared ethanol extracts of horsechestnut seeds containing complex
mixtures of over 30 distinct triterpene ester saponins (Hostettman and Marston 1995;
Sirtori, 2001). Subcomponents of commercial “escin” include: β-escin, α-escin and
kryptoescin, which contain unique ester mixes that differ in their melting points, specific
rotation, hemolytic index, and aqueous solubilities (Hostettman and Marston 1995;
As reported by Bombardelli et al. (1996), saponins represent between 24-28% of the total dry weight of *A. hippocastanum* seeds; whereas 3 to 6% of the seed dry weight was reported to be escin (Hostettmann and Marston, 1995). These materials are typically extracted from mature dry seeds using 80% ethanol. Khan et al. (1995) described the commercial extraction of escin from *A. indica* seed using a mixture of ethanol and water followed by acidification to a pH of 1 after which the escin precipitated out of solution. Marton and Bakan (1995) developed a “waste-free” system to extract β-escin from horsechestnut seeds. With their scheme, approximately 1% of the seed powder was isolated as β-escin at purities of 80 to 90%, and approximately 75 to 85% of the ethanol solvent was recycled through the system.

Esculin is composed of a simple coumarin aglycone (6,7-dihydroxycoumarin, esculetin, cichorigenin) bound to glucose at the C-6 position (Figures 1.4b and 1.4c). It is soluble in water and various polar organic solvents (Merck, 2001). Esculetin, the aglycone is moderately soluble in hot alcohol or glacial acetic acid but not soluble in boiling water. Esculin can be commercially extracted from the leaves and bark as well as the seeds of horsechestnuts. However, unlike its saponin counterparts, esculin is not unique to *Aesculus*. Other plant sources, such as the bark of *Fraxinus ornus*, may be richer, more commercially-extractable sources of this compound (Bogan et al., 1997; Sirtori, 2001).

Although commercial production of escin and esculin can be accomplished using materials harvested from natural stands, alternative methods of production hold some promise. Specifically, these compounds could be synthesized in and extracted from in vitro-cultured *Aesculus* plant tissues. Dameri et al. (1986) developed methodology to
obtain both callus and embryoids from leaf explants of *A. hippocastanum*. Both of the
techniques proved useful for selecting cell lines that produced high levels of escin. In a
later study, leaf-derived somatic embryos synthesized escin in concentrations that were
comparable to or greater than those found in ripe cotyledons (Profumo et al., 1991).
Moreover, callus culture of cotyledonary tissues produced 31-47% escin on a dry weight
basis whereas the dry weight of mature harvested cotyledons was composed of 11% escin
(Profumo et al., 1994). Similarly, Gastaldo et al. (1996) developed a system to produce
esculin and esculitin via somatic embryos derived from bark explants. Collectively, these
authors described the advantages of an in vitro production system for obtaining
pharmacologically important compounds of *Aesculus*. Tissue culture systems are
independent of climate and season, and are not limited by temporal availability of seeds
or other plant organs. In vitro production does not deplete natural stores of seed for
regeneration nor does it repeatedly damage trees. The desired compounds can be
produced in high concentrations from uniform plant sources in a limited space. Finally,
extractions procedures may be less expensive and laborious.

*Clinical and pharmacologic aspects of Aesculus natural products*. The medicinal
properties of *Aesculus* natural products have been utilized for many centuries but the
chemical and pharmacological investigation into these properties did not begin until the
1800s. *Aesculus* is currently used in homeopathic medicine to treat a wide variety of
conditions as diverse as hemorrhoids and baldness (Dean, 2000). Horsechestnut seed
extracts (HCSE) are used in Europe as a remedy for coughs and fevers and as an anti-
inflammatory to reduce pain and swelling from sprains or inflammation from arthritis and
rheumatism. An ointment containing horsechestnut extract was suggested to protect
exposed skin from sunburn (Vines, 1960). The most widely suggested herbal use seems to be as a treatment for chronic venous insufficiency and edema.

Investigations since the mid 20th Century have uncovered a variety of potential clinical uses for the natural products of *Aesculus* (Table 1.8). Almost all of these studies were conducted with crude extracts, mixtures or pure compounds obtained from *Aesculus hippocastanum*. Alcohoholate HCSE (i.e., commercial escin) was used extensively in these trials, but some studies measured the effects of specific escin fractions (e.g., β-escin) or specific compounds (e.g., escin I or escin II).

HCSE has been administered most often as therapeutic agent for the treatment of the physiologically-linked abnormalities associated with disorders of blood and lymph circulatory system, edema and/or tissue inflammation. HCSE has been used to treat chronic peripheral vascular insufficiency (CVI) (Pittler and Ernst, 1998; Tiffany et al., 2002). Diehm et al. (1996) treated 240 patients with CVI by administering 50 mg HCSE, placebo or via the use of compression stockings (i.e., the standard clinical treatment) in a 12 week study. Patients receiving placebo suffered increased leg volume (approximately 10 ml) whereas the leg volume of those medicated with HCSE was reduced by over 40 ml. In their study, treatment with HCSE was not statistically different than the compression stocking control. Bombardelli et al. (1996) and Tiffany et al. (2002) reviewed several other clinical trials where similar levels of effectiveness were reported for HCSE-treated CVI.

The effectiveness of escin against CVI and other edemas may be conditioned by several factors. Treatment with escin has been shown to decrease lymphatic flow and to alter the permeability of cell membranes, which allows fluid to be reabsorbed. Moreover,
escin increases blood flow and the veinotonic (elastic, contractile) properties of human saphenous veins without increasing blood pressure (Sirtori, 2001), and improves capillary strength resulting in increased resistance to physical rupture, the reduction of capillary exudates and edema. As cited in Bombardelli et al. (1996), Lorenz and Marek described, the anti-edematic power of escin to be very strong and long lasting. Escin has been used in dermatology for the treatment of venous stasis and its most serious complication, cellulitis (Cristoni and diPierro, 1998; Hostettman and Marston, 1995), where it increased capillary blood flow and reduced edema. In induced trauma studies with guinea pigs, escin significantly reduced capillary hyperpermeability, capillary compromise and the collection of blood beneath the skin (Bombardelli et al., 1996).

Beta-escin also has positive effects on arterial health. For instance, Dworschak et al. (1996) reported in a preliminary animal study that a 1% HCSE reduced elevated blood cholesterol from 6 mmol/l to 2 mmol/l in three weeks with no short-term side effects.

The potential of β-escin for ameliorating the tissue damage associated with cerebrovascular incidents (strokes) has been recently studied (Hu and Zeng, 2004; Hu et al., 2004 a and b). Laboratory rats were pretreated with 15-60 mg/kg of the saponin mixture for 7 days prior to the induction of a transient focal cerebral ischemic attack. The resulting infarct volume, water content and post-trauma neurological damage were significantly reduced in treated rats over those of the control group. Beta escin also decreased the concentration of I-Cam and E-selection enzymes in anoxic, ischemic parenchyma cells, associated with the onset of edema. Furthermore, β-escin treatment inhibits the production of caspases and oxidizing activity of cytochrome c, potent
promoters of cell apoptosis (planned cell death), thus, reducing the loss of surrounding tissues.

Escin is also a strong anti-inflammatory agent (Leach, 2001; Matsuda et al., 1997). HCSE was investigated as a treatment for cerebral swelling following fractures and traumas and intracranial aneurysms, subdural hematomas, encephalitis, meningitis and cerebral abscesses by inhibiting inflammation (Bombardelli et al., 1996; Leach, 2001; Sirtori, 2001). HCSE has been used in treating phlebitis (vein inflammation) with few side effects. Using human volunteers, Calabrese and Preston (1993) reduced the soreness of injection sites with one application of a topically-applied gel containing 2% escin. They suggested that these findings might extend to other injuries including impact hematomas. Escin also has dental applications, reducing swelling, bleeding, and pain associated with teeth cleaning and gingivitis (Bombardelli et al., 1996). It has also been evaluated as a treatment for multiple sclerosis because of its anti-inflammatory and anti-edema properties.

Early studies summarized by Hostettman and Marston (1995) suggested escin’s anti-inflammatory properties to be associated with its ability to decrease capillary leakage. Escin may also act as an anti-histaminic or anti-serotoninergic agent during the early exudative stages of the inflammation process (Matusda et al., 1997). It may also function by stimulating the pituitary gland to release the adrenocorticotropic hormone, ACTH, which, in turn, promotes the release of corticosteroids from the adrenal glands (Leach, 2001). Matsuda et al. (1997) uncovered differences in anti-inflammatory activity among purified escins, indicating the importance of the C-21 and C-22 acylated groups for anti-inflammatory response.
The antioxidant properties of escins and their potential as anticarcinogens have been documented. (Hostettman and Marston, 1995; Konoshima and Lee, 1986). Escins, may also have potential for the treatment of pre AIDS patients. Yang et al. (1999) assessed the inhibitory activity of eight different forms of escin from *A. chinensis* individually and in combinations for their effect on HIV-1 protease activity. All compounds had inhibitory activities; the most effective combination consisted of a 2:1 ratio escin I and escin II, which inhibited 86.1% of the HIV-1 protease activity.

Although it shares some pharmacological characteristics with escin (Table 1.8), the therapeutic potential of esculin has received less attention. Esculin has positive effects on the cardiovascular system, as an anti-clotting agent and by improving blood vessel function (Bombardelli et al., 1996). It has been reported to relieve the symptoms of edema, to have analgesic, antipyretic and anti-inflammatory properties, and to have sedative effect at low dosages (Bombardelli et al., 1996; Stefanova et al., 1995). Perhaps the most noteworthy pharmacologic applications of esculin and related compounds are related to dermal health and skin care. Esculin treatments limited the formation of dermal tumors in hamsters treated with the potent carcinogen, BOP [(N-niorosobis (2-oxopropl)-amine] (Kaneko et al., 2004). Moreover, esculin treatment reduced age-related skin damage by improving microcirculation and reducing oxidative stress (Bombardelli et al., 1996; Masaki et al., 1995). Lazarova et al. (1993) found that esculetin had an equally protective effect as that of p-aminobenzoic acid (PABA) and that esculetin might be useful as a sun screen. Along with other compounds, esculin has been used effectively to reduce alopecia (hair loss), increase microcirculation to the scalp and hair vitality, along with other compounds (Bombardelli et al., 1996).
The toxicity of Aesculus natural products

Although they have practical and pharmacological uses, many of the bio-active compounds of *Aesculus* are considered to be highly toxic (Hosttetmann and Marston 1995; Sirtori, 2001; Weinmann, 1997) when ingested/used at supraoptimal doses. All *Aesculus* species and all portions of the plant including the seeds, seedlings, leaves, bark, flowers and honey from *Aesculus* flowers have been reported to be poisonous (Neher, 2004). Williams and Olsen (1984) reported crude glycosidic extracts of horsechestnut to be nearly eight-fold more toxic than those of *A. flava* and *A. glabra* based on their LD$_{50}$s in hamsters and chicks.

*Aesculus* poisoning can be mild to severe depending on the type and quantity of material consumed and the animal consuming it. Symptoms include the following: depression, hyperexcitability, hyperesthesia (skin tightness, capillary hardening), inflammation of mucus membranes, pyrexia (fever), gastroenteritis, vomiting, abdominal tenderness or pain, diarrhea, obstruction of the gastrointestinal tract, thirst, anorexia, weight loss, trembling, muscle weakness, myoclonus (muscle spasms), dilated pupils, strabismus (loss of stereo vision), ataxia (staggering), difficulty breathing, recumbancy (loss of ability to stand), paralysis, tonoclonic spasms (seizures), coma and death (Campbell, 1998; Hart et al., 2000; Magnusson et al., 1983; Williams and Olsen, 1984).

In some farming communities in the Midwest, buckeyes were eliminated to prevent livestock, especially cattle, from grazing on them and becoming ill (Merz, 1957). Cattle and mountain goats have been known to become intoxicated or poisoned after consuming seeds of *A. glabra* (Casteel et al., 1992; Mayer et al., 1986; Merz, 1957). Howard (1992) reported *A. californica* to pose a toxic threat to cattle and wildlife within
its range, causing hemolysis of red blood cells and depression of the central nervous system. Further, it was purported to induce abortion in cattle (Chestnut, 1902). Ingestion of *A. hippocastanum* seeds caused electrolytic imbalances in cattle and horses (Campbell, 1998). Thirty-three range cattle suspected of consuming yellow buckeye (*A. flava*) nuts were treated for a variety of symptoms (Magnusson et al., 1983). Post-mortem examination of three fatal cases revealed partially digested nuts in the fore-stomachs and gross to moderate lesions and congestion associated with renal and hepatic tissues. Subsequent experimentation on calves dosed with yellow buckeye seeds at 0.5 - 1.0% of their weight developed symptoms similar to those in the field. Cattle were reported to be most at risk from *A. pavia* (red buckeye) toxins when plant materials were abundant such as in an infrequently used pasture, or when other feed was unavailable (Hart et al., 2000). However, according to Hedrick (1951) the browse of *A. californica* is palatable to both cattle and black-tailed deer, but less so to other wildlife.

Household pets are also at risk if exposed to the toxic constituents of *Aesculus* (ASPCA, 2004; Campbell, 1998). Small animals that have ingested *Aesculus* seeds may be treated by emesis or gastric lavage within two hours of exposure. In some small animal cases, laproscopic surgery may be necessary to remove gastrointestinal obstructions of impacted seed material or the animal may be ventilated to relieve respiratory stress.

The pollen and nectar of California buckeye was thought to be toxic to bees (Benseler, 1968; Howard, 1992), but no similar claims were reported for other *Aesculus* species. Alternatively, deWit (1967) and Haragsim (1977) found bees to be important pollinators of horsechestnut and members of the Pavia section. Likewise hummingbirds,
often pollinators of red buckeye and other members of the Pavia section, do not appear to be adversely affected by *Aesculus* nectar (Bertin, 1980; DePamphilis, 1988). Interestingly, an aqueous extract of California buckeye flowers significantly inhibited the development of mosquito larvae in preliminary studies (Haas, 2002). Similar extracts might be useful as an alternative agent for mosquito abatement programs.

The US Food and Drug Administration listed *Aesculus* as unsafe for human consumption and are not appropriate for use around children and people with dementia (DOH, 2003). Symptoms of exposure are similar to those in animals, and commonly include the following: flush skin, pruritis (itching), gastroenteritis, gastrointestinal disturbances, weakness, dehydration, enlarged pupils, drowsiness, loss of coordination, nausea, unusual bleeding and vomiting (Nutritionfocus, 2003; University of Georgia, 2002). As its toxicity is well-known and well-publicized, *Aesculus* poisoning is, admittedly, rare in modern society. However, *A hippocastanum* was reported as one of seven common poisonous plants most frequently reported in incidences of human poisonings in Utah (Williams, 1985). There have been reports of humans falling ill after eating the honey of bees frequenting *A. californica* blossoms (Howard, 1992), but other *Aesculus*-based honeys do not appear to be toxic. According to Fuller and McClintock (1986), ingestion of *A. californica* seeds has resulted in human illness, but not death. However, fatalities have been reported following ingestion of horsechestnut seeds, especially when eaten by children and are listed as potentially fatal by the US FDA (DOH, 2003; Williams and Olsen, 1984).
Taxonomy and phylogenetic relationship in Aesculus

Taxonomy within the order

The genus *Aesculus* was classified by Linnaeus in 1737. It, along with its most closely-related species *Billia*, compose the family Hippocastanaceae in the order Sapandales. Sapandales contains five families spread throughout both hemispheres, and representative species are found throughout both tropical and temperate regions. Hippocastanaceae is closely related to Aceraceae and Sapindaceae. Aceraceae is composed of three genera, primarily from the temperate zone of the Northern hemisphere, and the largest genus within this family is Acer, with 200 species (Mehra et al., 1972). Sapindaceae contains approximately 2000 species divided into 150 woody genera with a pantropical distribution, with a few extensions into the warmer temperate areas. As discussed by Forest et al. (2001) the family Hippocastanaceae as determined by Pax in 1896 has traditionally consisted of the 13 species of *Aesculus* and two species of *Billia*. Later, some botanical authorities including Wang (1939), did not deem Hippocastanaceae to be distinct from Sapindaceae and combined the two families. Hardin’s (1957a, b; 1960) morphological observations supported the maintenance of Hippocastanaceae as a separate family. According to Singh (2004), Hutchinson, Takhtajan, Cronquist and Dahlgren continue to maintain Hippocastanaceae as a separate family, and this method of taxonomic classification is most widely accepted. However, this family arrangement is, once again, under review. The rational for this reorganization is based on shared similarities in morphology and *rbcL* sequences among members within these closely related families. Therefore, Thorne, Judd, and APGII/(APweb) unite
them (Singh, 2004). This places the genera of the Aceraceae and Hippocastanaceae as sister taxa within the Sapindaceae (Savolainen et al., 2000).

The characteristics common to genera of Hippocastanaceae are opposite leaves, flowers with four or five petals, and leathery fruits with large seeds. *Billia* is composed of two species, *Billia columbiana* (Planchor) and *Billia hippocastanum* (Peyritsch) and is sometimes mistakenly referred to as *Aesculus mexicana* (Bentham and Hooker). *Billia* is considered to be more phylogenetically primitive than *Aesculus*. Both species are evergreen and can be distinguished by their entire margins and large conspicuous veins (Heywood, 1982). The inflorescences are arranged as terminal panicles with perfect flowers scattered throughout. *Billia columbiana* has white flowers and golden tomentosum, and it can be found from Guatemala to Columbia (Standley, 1926). The leaves of *Billia hippocastanum* are lanceolate, 7-20 cm long and are glabrous and the flowers are deep red. Its native range is southern Mexico. Another species that is often confused with the genus *Aesculus* is *Ungnaadia speciosa*, whose common name is Mexican Buckeye. This species is a member of the Sapindaceae and has alternate, pinnately compound leaves (Makins, 1936).

The number of reported species within *Aesculus* varies from as few as 12 (Krußmann, 1976) to as many as 20 or more (Brockman, 1968; Sargent, 1891, 1902, 1924). These differences are due at least in part to the fact that plants within a given species express a high degree of variability and seem to hybridize freely. This resulted in many species, variants, and hybrids being described as new species.

The International Code of Botanic Nomenclature has developed and periodically revises a set of rules and recommendations for the classification of plants and the
relationships between them. There are a number of classifications below the species level including: subspecies, variety, subvariety, group, and form. These are terms used to describe the extent of naturally occurring variation from the species. A subspecies possess significant consistent morphological differences and are typically associated with separate geographical regions. A botanical variety is a distinct subgroup that retains most of the characteristics of the species but also possesses minor morphologically distinct characteristics that can be observed and maintained in the wild population, often through geographic isolation. While having little botanic value, the divergent morphological characteristics of these plants maybe of interest for plant improvement. The term variety is further confounded because of its incorrect use as a synonym for cultivar or clone. The term cultivar refers to an individual specimen or group of individuals that can be distinguished from the species by morphological, physiological, chemical, or cytological characteristics and that is maintained through human manipulation. A form, *(forma)*, occurs among sporadic individuals and diverges minimally from the species with its variation typically being observed in the color of leaves, flowers, or fruit and often have only of horticultural interest. Thus, many *Aesculus* plants that are referred to as varieties are not varieties in a botanic sense. From author to author, reviews or descriptions of this genus are likely to contain inconsistent designations for *Aesculus* entities, and no two sources are likely to be in full agreement on botanical nomenclature of the genus. This confusion arises directly from changes in the botanical nomenclature code that have occurred over many years, or from a lack of adherence to the codes by plant explorers and other enthusiasts (Table 1.9) (Johnson, 1939; VanDersal, 1942). The oldest accepted name has precedence over all others, and as members of the genus were
renamed, the former genus names are replaced with the oldest accepted name *Aesculus*. Formerly, the generic synonym of *Pavia* was used for species with smooth fruits and four-petaled flowers in contrast with *Aesculus* which was supposed to have prickly fruits and five-petaled flowers (Wright, 1985).

Hardin (1957 a, b and 1960) tried to bring order to the genus through his taxonomic examination of both herbarium specimens and field observations. Using a broad species concept, he reclassified the genus as having 13 species in five sections refer to (Figure 1.1). Hardin characterized each of the thirteen species based on its morphological characteristics, but also observed and described numerous examples of interspecific hybrids and species variants. He grouped these specimens with the species and concluded that these expressions of variability were the result of introgression or hybrid swarms.

There is also confusion in regards to the speciation of *A. chinensis* and *A. wilsonii* because they are very similar in appearance to each other and because there are a number of specimens that are intermediate in form between the two species. It is unclear whether *A. chinensis* and *A. wilsonii* are separate species that are hybridizing to produce these intermediate forms, or if there is just one species expressing a high degree of variability for a number of characteristics Hardin (1960). Fang (1981) lists eight additional *Aesculus* species from China *A. polyneura, A. lantsangensis, A. tsiangii, A. chuniana, A. megaphylla, A. kwangsiensis, A. chingsiensis, A. chinpinensis*, along with *A. chinensis* var. *chekiangensis* and *A. wangii* var. *rupicola* all within the section Calothyrsus. Only minimal information is currently available on these “new species.” Reports seem to indicate that these plants are quite similar to the previously described species, and their
recognition remains questionable. The origins of these genotypes may result from hybridization and be similar to that of the diversity observed in the North American *Aesculus*. Continued observation of trueness to type, the ability to set seed, and genetic analysis will assist in determining if these are truly separate species, hybrids between existing species or expressions of genetic variation that exists within the known species.

**Aesculus centers of origin and diversity**

There have been four proposed centers of origin for *Aesculus*. Hardin (1957a) hypothesized, based on morphology, that *Aesculus* diverged from an ancestral species in Central or South America during the Tertiary period and migrated northward eventually diverging into two distinct lines. One line, migrated to the southeast, became the species that developed into the Pavia section. The other ancestral line went through several periods of divergence: first to form the species *A. parryi* (section Parryanae) and then later developed into the material that would become the other three sections. *Aesculus parviflora* (section Macrothyrsus) developed possibly in the highlands of southern Mexico and then moved northeastward into the United States. Likewise, *Aesculus californica* remained in the west coast of United States becoming a relic of the Calothyrsus section. Then, other progenitors of the Calothyrsus and Aesculus sections crossed the land bridge and developing into the species of Asia. *Aesculus hippocastanum* also developed out of this ancestral species and migrated further west into Europe where it underwent additional speciation. Hardin thought that the current species arrangement was merely the remnant of a once greater distribution. Using the fossil record, Raven and
Axelrod (1974, 1978) proposed that *Aesculus* originated in North America and then became distributed throughout the world.

In contrast, Xiang et al. (1998) using an analysis of the molecular marker ITS (Internal Transcribed Spacer) of nuclear ribosomal RNA and the fossil record concluded that the original ancestor of *Aesculus* was a plant that evolved during the interval between the late Cretaceous to the early Tertiary period and originated in the north temperate area of eastern Asia. They hypothesized that the initial *Aesculus* ancestor diverged to form two separate lines, during in the early Eocene period. The one line moved southward into China and the Himalayan region and diverged into Calothyrsus. The other group expanded east and west, and then split during in the middle of the Eocene to form the *Aesculus* section and the other that would become North American species. During the late Eocene or early Oligocene, the North American line further separated into the ancestor of Pavia and Parriyana sections and the ancestor of *A. californica* and *A. parviflora*. Finally the Pavia section diverged from Parriyana, and *A. parviflora* diverged from *A. californica*. Xiang et al (1998) concluded that the distribution of *Aesculus* species was the result of climate changes.

The most recent assessment of the information by Forest et al. (2001) proffers another interpretation. Their analysis of the regional variability of the various species supports Hardin’s theory of a North American origin of *Aesculus* from a Billia-like ancestor. They suggest that *Billia* could have moved into its present distribution during the period of the “Tertiary cooling and Great Interchange.” Forest et al. (2001) agree with Xiang et al. (1998) assessment that *Aesculus* diverged at high latitudes in the late Cretaceous or early Tertiary period and became widely distributed on both sides of the
land bridge throughout the “boreotropical flora.” However, Forest et al. (2001) differs in
the conclusion on the center of origin, supporting the North American over the Asian
origin. Furthermore, they state that *A. parryi* diverged from the primary ancestor. The
changes in the environment were postulated to have propelled the division between
Calothyrsus and Aesculus from the Macrothyrsus and Pavia. Aesculus then separated
from Calothyrsus as it spread into Europe and Japan. Climate changes were also thought
to have impacted the redistribution of Pavia to the southeastern part of the United States
where species development occurred.

**Phylogenetic relationships between Aesculus species**

Understanding the phylogenetic relationships between species may reveal
similarities not readily apparent, and may indicate possible opportunities for successful
interspecific hybridization. These relationships have traditionally been examined through
the comparison morphological and chemical characteristics. There are several obstacles
in developing and characterizing these genetic relationships. The first challenge is the
complex nomenclature of the genus where plants of the same species maybe listed under
several different names. The second is the extensive number of hybrids, and plants of
uncertain origin. Currently, molecular tools are being employed to reexamine these
characterizations and Forest et al. (2001) using morphological classification attempted to
reconsider organization of the genus.

Xiang et al. (1998) attempted to confirm Hardin’s species classification through
the use of chloroplast gene *matK* and ITS (internal transcribed spacer) sequences. The
data from the two sequences were analyzed separately to create independent parsimonious trees, and small differences between the trees were observed. The phylogenetic relationships expressed in these trees corresponded to the distribution of the species and supports all the botanic sections as described by Hardin except for Calothrysus. In both cases, the Calothrysus section was sister to the rest of the genus and with the \textit{matK} sequence the Aesculus section was nested inside Calothrysus. The ITS analysis grouped all the species into the same sections as Hardin’s morphological analysis with the exception of \textit{A. californica} which grouped more closely to \textit{A. parviflora} than to the other members of the Calothrysus section. Hardin initially grouped \textit{A. californica} in Calothrysus because of its smooth fruit and resinous bud scales. Additionally, the molecular evidence indicates that the species within the \textit{Pavia} section diverged relatively recently and that the relationships between the species are poorly resolved (Xiang et al., 1998).

Kobayashi and Yamada (2002) using both RFLP and RAPD data from chloroplasts reported that the genus could be separated into two major groups, one comprising the species from the southeastern United States, and the other the species of Eurasia. Based upon the single most parsimonious RFLP tree, both specimens of \textit{A. turbinata} grouped together but \textit{A. pavia} and \textit{A. splendens} (now recognized as another name for \textit{A. pavia}) did not. With the inclusion of interspecific hybrids and cultivars, the interpretation of the phylogeneic relationships becomes more complex. \textit{A. \times worlitzensis}, a putative hybrid did not group with or between the parental species. Additionally, the cultivars ‘Baumannii’ and ‘Digitata’ of \textit{A. hippocastanum} that were studied did not cluster with the species. They interpreted the position of these cultivars within the
phylogeneic trees to indicate that the cultivars were of hybrid origin. The most surprising result from the RFLP data was the clustering of *A. parviflora* with *A. glabra* indicating the same progenitor.

The phylogenetic analysis by Forest et al. (2001) bares the most similarity to that of Hardin. The only difference is in the placement of the section Aesculus within the section Calothyrsus. This finding, based on morphology, matches that of the *matK* sequence found by Xiang et al. (1998). Here, *A. parviflora* is in a section unto itself. Forest et al. (2001) state that there are limitations when making comparisons between different analyses due to differences in specimen sampling.

The discrepancies in the various parsimonious trees have been interpreted as a consequence of using plant material of hybrid origin. To a significant extent, the work of Forest et al. (2001) and Kobayashi and Yamada (2002); Xiang et al. (1998) support Hardin’s original organization of the genus and all the agreement that *Aesculus* evolved from a common ancestor. Questions still remain about the relationship of *A. parviflora* to the rest of the members of the genus and the lack of clarity between the members of the Pavia section.

*Genetic improvement*

Botanic gardens and arboreta, including Chicago Botanic Garden, Morton Arboretum, Morris Arboretum, Holden Arboretum, and Dawes Arboretum, often have collections of both exotic germplasm and selected cultivars which can be utilized as sources of variability for plant breeding (Appendix E). Usually, they are willing to share
these resources through budwood, scions, or pollen with other researchers. Alternatively, germplasm could still be obtained from wild populations of trees throughout the ranges of the North American species, and selected materials and unimproved germplasm could be obtained from Asia.

*Historic improvement efforts*

There have been no continuous concerted efforts to improve *Aesculus* through a controlled crossing methodology. The selections available are presumed hybrids based on morphology without genetic verification. Some selections have been made through academic or public institutions with materials obtained from arboreta and botanic gardens, *A. californica* ‘Canyon Pink’, *A. parviflora* ‘Rogers’, *A. × arnoldiana* ‘Autumn Splendor’, and *A. ×* ‘Homestead’. The Holden Arboretum had initiated an *Aesculus* breeding project that resulted in the creation of a large number of seedlings. However, the evaluation of this material and the future of this research is uncertain. The majority of selections were the result of careful observations by nursery professionals or plant collectors. Most selections have been made for either flowering or fall color characteristics. These unique phenotypes are typically propagated without regard to pedigree. Of special interest are the selections: *A. hippocastanum* ‘Baumanii’ which has double flowers, *A. pavia* ‘Biltmore’, which has especially good flower color, and the late flowering forms of *A. parviflora* called *serotina* and the cultivar ‘Rogers’, which extend the flowering period by three to four weeks (Table 1.10). There are also selections for various expressions of leaf variegation or shape within species noted for both *A.*
hippocastanum and A. pavia. Variable-leafed phenotypes are likely to be present in all species, and could be found if desired.

Hybridization and introgression between members of the Pavia section in the wild has been well characterized (Hardin, 1957 c,d). Johnson (1939) listed 11 hybrids between the various Aesculus species, but several of these have now been combined. There are currently seven hybrids recognized by the U.S.D.A. along with several others (USDA, NRCS, 2004) (Table 1.11). All possible cross combinations among the four species in this section have been identified somewhere within the various overlapping ranges. There are also believed to be several tri-parental hybrids. However, this degree of interspecific hybridization makes species identification difficult (DePamphilis, 1988). Aesculus pavia has clearly been the most widely recognized species in most of these crosses. DePamphilis (1988) reported outcrossing rates of at least 80% and detected high levels of gene flow. Molecular data has indicated that the zones of hybridization are asymmetrical in a northerly direction (Xiang et al., 1998). Burns and Honkala (1990) reported that in a region where A. flava was not native or naturalized, its germplasm was present in a population of A. glabra.

This high degree of interspecific hybridization is believed to be due at least in part to the feeding behavior of hummingbirds (Bertin, 1980; DePamphilis, 1988). These “natural hybrids” within the population represent multiple independent hybridization events occurring over both the species range and the course of time. This has resulted in some hybrid combinations being named more than once. Out of these hybridization events have come a number of noteworthy plants. Aesculus × arnoldiana ‘Autumn Splendor’ and A. × ‘Homestead’ are two selections that were made from putative tri-
parental crosses that have been made for their fall foliage effects as well as good overall plant appearance (Table 1. 12). *Aesculus × neglecta* ‘Erythroblastos’ represent an extreme in variability that can be found. The leaves of this plant exhibit a variety of colors throughout the growth period changing from rosy pink and shades of peach in early spring to pale green in summer to warm orange in autumn.

In addition to the events occurring in the wild populations, there have also been chance garden hybrids. The most ornamental of these are *A. × carnea* and *A. × plantierensis*. *Aesculus × carnea* is a hybrid between *A. hippocastanum* and *A. pavia*, and the most popular cultivars arise from this hybrid. These cultivars have been selected for their flower color and, to a lesser extent, overall plant appearance and tolerance to the urban landscape. The most widely planted cultivar is *A. × carnea* ‘Briotii’. *Aesculus × plantierensis* is a backcross between *A. hippocastanum* and *A. × carnea*. Some authorities have listed *A. hippocastanum* as the seed parent but this is uncertain. This resulting plant is a sterile triploid. This enhances its landscape value having the beauty of the flowers without the mess of seeds. Another plant of particular interest is *A. × dallimorei*. This plant is believed to be either an example of a “graft hybrid”, where a periclinal chimera formed between *A. hippocastanum* understock and *A. flava* scion, or a true interspecific hybrid between the two species (Sealy, 1956). Its buds are dry like *A. flava*, and its leaf morphology is intermediate between the two species. The inflorescences are shaped like *A. hippocastanum*, but flowers of both types can be observed on the tree. A true interspecific hybrid between these two species would represent the second time that an intersectional-cross had been achieved, but this requires further study and documentation.
Aesculus remains largely unimproved, but there have been two studies utilizing A. hippocastanum that demonstrate that gains can be made through selection. Fostad and Pederson (1998), using half-sib families, demonstrated that there was a significant correlation between seedling response to salt stress and the phenotypic condition of the mother tree. The offspring from highly vigorous mother trees showed less leaf damage and greater overall growth than those produced from poorly adapted trees when placed under the selection pressure. This research suggests that it might be possible to create trees with greater urban tolerance could be developed through careful selection and breeding. The production of secondary plant products including escin was also variable between genotypes and under strong genetic control. Selections with improved levels (either increased or decreased) of these compounds could be attained through selective breeding (Ocokoljic et al., 1996/1997).

**Barriers to breeding**

All known Aesculus are diploid, and chromosomes counts of most of the species have been completed with n=20 throughout the genus with the exception of the hybrid A. × carnea n=40 (allopolyplod) and A. × plantierensis n=30 (triploid) (Benseler, 1968; Hoar, 1927; Mehra et al., 1972, Skovsted, 1929; Upcott, 1936; Wang, 1939). The cytological differences between the species of Aesculus were slight, and chromosomes are small, uniform, and typically rod shaped (Upcott, 1936; Wang, 1939). Hoar (1927) noted the presence of lagging chromosomes and suggested that this was indicative of the hybrid nature of some specimens, but no frequencies of irregularities were presented between the putative hybrids and their pure species. Furthermore, lagging chromosomes
were also observed in *A. hippocastanum*, which he believed to be a pure species. Upcott (1936) examined chromosome paring and found mostly bivalents with occasional quadrivalents and a few lagging chromosomes and a low frequency of chiasma. The estimated DNA content of *A. hippocastanum* is 4c=0.5 pg compared to 4c=1.3 pg for *Acer pseudoplatanus* and 0.2 pg in *Arabidopsis thaliana* (Bennett et al., 1982).

Benseler (1968) and Hoar (1927) stated that apomixes was not observed in *Aesculus*. There are no indications of self-incompatibility or other reproductive barriers, based on experience, but no formal investigations of stigmatic receptivity or pollen/pistal interactions have been conducted.

The long term goal of this research is to produce interspecific hybrids with the hope of creating plants which have improved characteristics for the landscape, including better disease and scorch resistance, fall color, extended flowering period, and flower color. In order to complete this goal, a better understanding of a species floral, pollination, and seed biology is necessary for the development of a breeding program. An investigation into the floral biology *Aesculus parviflora* might lead to a clearer understanding of its naturally low seed-set. By studying the interspecific and intraspecific compatibility and breeding behavior of the red and bottlebrush buckeye, it might be possible to gain insights that will enhance our ability to successfully produce hybrid seed.

**Study goals and objectives**

To compile a review of the status of *Aesculus* research and improvement

To characterize the floral biology of *A. parviflora* and *A. pavia*
To examine the pollen viability and storage for various Aesculus species

To describe the seed biology and seedling development of A. parviflora and A. pavia

To investigate the factors affecting pollination and fruit-set between Aesculus crosses.
Table 1.1. A list of frequently used common names for various species and botanical varieties of *Aesculus*.

<table>
<thead>
<tr>
<th>Section, species and varieties</th>
<th>Common Names&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aesculus</strong></td>
<td></td>
</tr>
<tr>
<td><em>A. hippocastanum</em> (L.)</td>
<td>Common horsechestnut</td>
</tr>
<tr>
<td></td>
<td>European horsechestnut</td>
</tr>
<tr>
<td></td>
<td>Horsechestnut</td>
</tr>
<tr>
<td></td>
<td><em>A. turbinata</em> (Blume)</td>
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<tr>
<td><strong>Calothyrsus</strong></td>
<td></td>
</tr>
<tr>
<td><em>A. assamica</em> (Griffith)</td>
<td></td>
</tr>
<tr>
<td><em>A. californica</em> (Nuttall)</td>
<td>California buckeye</td>
</tr>
<tr>
<td><em>A. chinensis</em> (Bunge)</td>
<td>Chinese horsechestnut</td>
</tr>
<tr>
<td><em>A. indica</em> (Colebrook)</td>
<td>Indian horsechestnut</td>
</tr>
<tr>
<td><em>A. wilsonii</em> (Rehder)</td>
<td>Wilson's horsechestnut</td>
</tr>
<tr>
<td><strong>Macrothyrsus</strong></td>
<td></td>
</tr>
<tr>
<td><em>A. parviflora</em> (Walter)</td>
<td>Bottlebrush buckeye</td>
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<tr>
<td></td>
<td>Dwarf buckeye</td>
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<tr>
<td><strong>Parryana</strong></td>
<td></td>
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<tr>
<td><em>A. parryi</em> (Gray)</td>
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<tr>
<td><strong>Pavia</strong></td>
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<tr>
<td><em>A. flava</em> (Solander)</td>
<td>Yellow buckeye</td>
</tr>
<tr>
<td></td>
<td>Sweet buckeye</td>
</tr>
<tr>
<td></td>
<td>Big buckeye</td>
</tr>
<tr>
<td><em>A. glabra</em> (Willdenow)</td>
<td>Ohio buckeye</td>
</tr>
<tr>
<td></td>
<td>Stinking buckeye</td>
</tr>
<tr>
<td></td>
<td>Fetid buckeye</td>
</tr>
<tr>
<td><strong>var. arguta</strong> (Robinson)</td>
<td>Texas buckeye</td>
</tr>
<tr>
<td>*<em>A. pavia</em> (L.)</td>
<td>Red buckeye</td>
</tr>
<tr>
<td></td>
<td>Scarlet buckeye</td>
</tr>
<tr>
<td></td>
<td>Red-flowering buckeye</td>
</tr>
<tr>
<td></td>
<td>Woolly buckeye</td>
</tr>
<tr>
<td><strong>var. flavescens</strong> (Sargent)</td>
<td>Fire-cracker plant</td>
</tr>
<tr>
<td></td>
<td>Yellow buckeye</td>
</tr>
<tr>
<td><strong>A. sylvatica</strong> (Bartram)</td>
<td>Painted buckeye</td>
</tr>
<tr>
<td></td>
<td>Georgia buckeye</td>
</tr>
</tbody>
</table>

<sup>2</sup> List of common names includes names that are associated with particular phenotypes or regions.
<table>
<thead>
<tr>
<th>Section, species and varieties</th>
<th>Height (m)</th>
<th>Spread (m)</th>
<th>Shoot characteristics</th>
<th>Bark characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aesculus</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>A. hippocastanum</em> (L.)</td>
<td>15-25 to 35</td>
<td>to 15</td>
<td>Stout; coarse texture; reddish brown</td>
<td>Smooth to platy; reddish or grayish brown</td>
</tr>
<tr>
<td><em>A. turbinata</em> (Blume)</td>
<td>30-40</td>
<td>15</td>
<td>Stout; orange-brown cuticle</td>
<td>Smooth to platy; gray (39)</td>
</tr>
<tr>
<td><strong>Calothyrsus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. assamica</em> (Griffith)</td>
<td>25</td>
<td>Spreading</td>
<td>—</td>
<td>Smooth; light gray</td>
</tr>
<tr>
<td><em>A. californica</em> (Nuttall)</td>
<td>3-5 to 10</td>
<td>5</td>
<td>Glabrous; red to grayish brown (11)</td>
<td>Smooth; light silver gray to white</td>
</tr>
<tr>
<td><em>A. chinensis</em> (Bunge)</td>
<td>25-30</td>
<td>10</td>
<td>Slightly pubescent to glabrous; prominent lenticels</td>
<td>Rough with age; grayish brown</td>
</tr>
<tr>
<td><em>A. indica</em> (Colebrook)</td>
<td>10-30</td>
<td>15</td>
<td>Low to ground; stiff; glabrous</td>
<td>Smooth peels with age; grayish green to reddish gray (20, 33)</td>
</tr>
<tr>
<td><em>A. wangii</em> (Hu)</td>
<td>15-20</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>A. wilsonii</em> (Rehder)</td>
<td>25</td>
<td>15</td>
<td>Brown; with lenticels and gray cuticle (11)</td>
<td>Flaky; light gray (30)</td>
</tr>
<tr>
<td><strong>Macrothyrsus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. parviflora</em> (Walter)</td>
<td>2-4</td>
<td>4-5</td>
<td>Slender; gray brown; light brown lenticels</td>
<td>Smooth; light gray</td>
</tr>
<tr>
<td><strong>Parryana</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. parryi</em> (Gray)</td>
<td>1-6</td>
<td>—</td>
<td>Glabrous; light gray</td>
<td>Very smooth; light gray to white</td>
</tr>
<tr>
<td><strong>Pavia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. flava</em> (Solander)</td>
<td>20-30</td>
<td>15-20</td>
<td>Reddish brown to pale brown</td>
<td>Large smooth plates and scales; light gray brown (46)</td>
</tr>
<tr>
<td><em>A. glabra</em> (Willdenow)</td>
<td>10-20</td>
<td>6-7</td>
<td>Stout, reddish brown; orange lenticles</td>
<td>Very rough and scaly; dark gray (39)</td>
</tr>
<tr>
<td>var. <em>arguta</em> (Robinson)</td>
<td>4-6</td>
<td>—</td>
<td>Arching and pubescent (17)</td>
<td>—</td>
</tr>
<tr>
<td><em>A. pavia</em> (L.)</td>
<td>5-8 to 10</td>
<td>3-4</td>
<td>Slender olive brown; brown lenticels (31)</td>
<td>Smooth; light gray to grayish brown</td>
</tr>
<tr>
<td>var. <em>flavescens</em> (Sargent)</td>
<td>3-4</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>A. sylvatica</em> (Bartram)</td>
<td>2-6</td>
<td>—</td>
<td>Stout; glabrous; light grayish brown; (8)</td>
<td>Scaly; grayish brown (8)</td>
</tr>
</tbody>
</table>

* Numbers in table refer to citations that can be found in Appendix A.

Table 1.2. Plant characteristics of *Aesculus* species and botanical varieties listed by section.
<table>
<thead>
<tr>
<th>Section species and varieties</th>
<th>Leaflet No.</th>
<th>Size</th>
<th>Leaflet shape and serration</th>
<th>Color</th>
<th>Surface features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aesculus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. hippocastanum</em> (L.)</td>
<td>7 (5)</td>
<td>PL 7 6-12; LtL 10-25; LtW 10</td>
<td>Obovate; double serrate</td>
<td>Dull medium green</td>
<td>Rusty brown, abaxial pbsc. (2)</td>
</tr>
<tr>
<td><em>A. turbinata</em> (Blume)</td>
<td>5-7</td>
<td>PL 15-25; LtL 20-35; LtW 5-15</td>
<td>Obovate to lanceolate; finely/evenly toothed crenate</td>
<td>Medium green</td>
<td>Orange pbsc in abaxial vein axils (39)</td>
</tr>
<tr>
<td><strong>Calothyrsus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. assamica</em> (Griffith)</td>
<td>5 (7)</td>
<td>PL 9-20; LtL 25; LtW 8</td>
<td>Lanceolate to oblong; finely serrulate to crenulate</td>
<td>Lustrous dark green</td>
<td>Leathery</td>
</tr>
<tr>
<td><em>A. californica</em> (Nuttall)</td>
<td>5-7 (9)</td>
<td>PL 8-10; LtL 6-15; LtW 3-6</td>
<td>Lancelate, elliptical or oblong; finely serrate</td>
<td>Bright green gray green abaxial</td>
<td>Slight adaxial &amp; dense abaxial pbsc</td>
</tr>
<tr>
<td><em>A. chinensis</em> (Bunge)</td>
<td>5-7</td>
<td>PL 10-15; LtL 10-20; LtW 5-9</td>
<td>Narrow oblong to obvate; finely serrate</td>
<td>Medium green; glossy</td>
<td>Pbsc along vein</td>
</tr>
<tr>
<td><em>A. indica</em> (Colebrook)</td>
<td>7 (9)</td>
<td>PL 8-20; LtL 20-25; LtW 5-8</td>
<td>Obovate to lanceolate; finely serrate</td>
<td>Petiole red; leaf metallic green (6)</td>
<td>Glabrous</td>
</tr>
<tr>
<td><em>A. wilsonii</em> (Rehder)</td>
<td>5-7</td>
<td>PL 8-15; LtL 17-26; LtW 3-6</td>
<td>Oval to oblong to obvate; finely serrate</td>
<td>Dark green adaxial &amp; gray green abaxial</td>
<td>Gray pbsc on petioles and abaxial leaflets</td>
</tr>
<tr>
<td><strong>Macrothyrsus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. parviflora</em> (Walter)</td>
<td>5-7</td>
<td>PL 7-11; LtL 8-20; LtW 5-10</td>
<td>Elliptical, oval or obvate; smooth to finely serrate (55)</td>
<td>Petiole wine-colored; adaxial dark green</td>
<td>Gray abaxial pbsc</td>
</tr>
<tr>
<td><strong>Parryana</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. parryi</em> (Gray)</td>
<td>5-7</td>
<td>PL 1-9; LtL 3-12; LtW 2-4</td>
<td>Obovate to oblong-obovate</td>
<td>—</td>
<td>Abaxial pbsc (34)</td>
</tr>
<tr>
<td><strong>Pavia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. flava</em> (Solander)</td>
<td>5 (7)</td>
<td>PL 8-19; LtL 15-20; LtW 3-6</td>
<td>Oblong, obovate or narrowly elliptical; finely/evenly serrate (40)</td>
<td>Dark green adaxial; yellow green abaxial</td>
<td>Glabrous to slight pbsc (46)</td>
</tr>
<tr>
<td><em>A. glabra</em> (Willdenow)</td>
<td>5 (7)</td>
<td>PL 7-15; LtL 13-15; LtW 3-5</td>
<td>Obovate to ovate; finely serrate</td>
<td>Dull medium green</td>
<td>Glabrous to pbsc</td>
</tr>
</tbody>
</table>

Table 1.3 Leaf characteristics of *Aesculus* species and varieties listed by section.
Table 1.3 (continued)

<table>
<thead>
<tr>
<th>Section species and varieties</th>
<th>Leaflet No</th>
<th>Size</th>
<th>Leaflet shape and serration</th>
<th>Color</th>
<th>Surface features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pavia</strong> (continued)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. glabra</em> (continued)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>var. <em>arguta</em> (Robinson)</td>
<td>7-9</td>
<td>LtW 2-5</td>
<td>Lanceolate, tapering at tip (38, 39); deeply/ coarsely serrate (22)</td>
<td>-</td>
<td>Slight abaxial pubescence (spring)</td>
</tr>
<tr>
<td>(continued)</td>
<td>(11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. pavia</em> (L.)</td>
<td>5-7</td>
<td>LtL 8-17; LtW 3-6</td>
<td>Oblong to obovate; irregularly doubly serrate (5, 8, 33)</td>
<td>Dark green</td>
<td>Glabrous adaxial; slight abaxial pubescence</td>
</tr>
<tr>
<td>var. <em>flavescens</em> (Sargent)</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>Glossy green</td>
<td>-</td>
</tr>
<tr>
<td><em>A. sylvatica</em> (Bartram)</td>
<td>5</td>
<td>LtL 10-15; LtW 5</td>
<td>Oblong-obovate; finely/doubly serrate (18)</td>
<td>Bright green (22)</td>
<td>Glaucous or abaxial pubescence (17, 18)</td>
</tr>
</tbody>
</table>

Abbreviations PL, LtL, LtW are petiole length, leaflet length, and leaflet width respectively.
Abbreviation for pubescence = pubescence.
Numbers in table refer to citations that can be found in Appendix A.
<table>
<thead>
<tr>
<th>Section, species and varieties</th>
<th>Inflorescence</th>
<th>Flowers</th>
<th>Color and characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aesculus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. hippocastanum</em> (L.)</td>
<td>L 20-30; W 12</td>
<td>Open upright-pyramidal</td>
<td>5 petals fold back; stamens extruded</td>
</tr>
<tr>
<td><em>A. turbinata</em> (Blume)</td>
<td>L 15-25; W 6-8.5</td>
<td>Cylindrical</td>
<td>Dia. 2 cm; smaller than Hip; 5 petals; excerted stamens (42)</td>
</tr>
<tr>
<td><strong>Calothyrsus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. assamica</em> (Griffith)</td>
<td>L 30-40</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td><em>A. californica</em> (Nuttall)</td>
<td>L 10-25</td>
<td>Dense narrow cylindrical</td>
<td>Dia. 2.5-3 cm; 4 equal petals; long excerted stamens (38)</td>
</tr>
<tr>
<td><em>A. chinensis</em> (Bunge)</td>
<td>L 20-30; W 7</td>
<td>Slender and cylindrical</td>
<td>Small numerous flowers; stamens sl. longer than petals (17, 14)</td>
</tr>
<tr>
<td><em>A. indica</em> (Colebrook)</td>
<td>L 20-40; W 13</td>
<td>More abundant than on Hip cylindrical</td>
<td>Four petals narrower than Hip; greater no. flowers are produced; stamens sl. excerted (23)</td>
</tr>
<tr>
<td><em>A. wangii</em> (Hu)</td>
<td>L 30-40</td>
<td>—</td>
<td>Dia. 1 cm; stamens longer than petals; style sl. curved</td>
</tr>
<tr>
<td><em>A. wilsonii</em> (Rehder)</td>
<td>L 15-35</td>
<td>Cylindrical</td>
<td>Larger than <em>A. chinensis</em>; 4 petals; stamens very long (30)</td>
</tr>
<tr>
<td><strong>Macrothyrsus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. parviflora</em> (Walter)</td>
<td>L 20-30</td>
<td>Columnar</td>
<td>Four petals with 5-7 stamens exerted 3-4 times longer than petals</td>
</tr>
<tr>
<td><strong>Parryana</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. parryi</em> (Gray)</td>
<td>L 8-20; W 5</td>
<td>Erect columnar smaller than <em>A. californica</em></td>
<td>Petals are uneven with stamens longer than petals</td>
</tr>
</tbody>
</table>

Table 1.4. Inflorescence and flower characteristics of *Aesculus* species and botanical varieties listed by section.
Table 1.4 (continued)

<table>
<thead>
<tr>
<th>Section, species and varieties</th>
<th>Inflorescence</th>
<th>Flowers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length</td>
<td>Characteristics</td>
</tr>
<tr>
<td><strong>Pavia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. flava</em> (Solander)</td>
<td>L 10-18; W 5-10</td>
<td>Pyramidal</td>
</tr>
<tr>
<td><em>A. glabra</em> (Willdenow)</td>
<td>L 10-20; W 5-8</td>
<td>Conical</td>
</tr>
<tr>
<td>var. <em>arguta</em> (Robinson)</td>
<td>like species</td>
<td>like species</td>
</tr>
<tr>
<td><em>A. pavia</em> (L.)</td>
<td>L 10-25</td>
<td>Loose</td>
</tr>
<tr>
<td>var. <em>flavescens</em> (Sargent)</td>
<td></td>
<td>Campanulate with included stamens</td>
</tr>
<tr>
<td><em>A. sylvatica</em> (Bartram)</td>
<td>L 12-15; W 7 at the base</td>
<td>Bell shaped</td>
</tr>
</tbody>
</table>

* Numbers in table refer to citations that can be found in Appendix A.
<table>
<thead>
<tr>
<th>Section, species and varieties</th>
<th>Size (cm)</th>
<th>Shape</th>
<th>Color</th>
<th>Features</th>
<th>Seed characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aesculus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. hippocastanum</em> (L.)</td>
<td>5-6</td>
<td>Subglobose</td>
<td>Dark brown</td>
<td>Dense hard spines; 1cm length</td>
<td>1-3 dark chestnut brown seeds; hilum covers 1/3 to 1/2 of seed coat</td>
</tr>
<tr>
<td><em>A. turbinata</em> (Blume)</td>
<td>5</td>
<td>Large ovoid</td>
<td>Gray</td>
<td>Rough and warty but not spiny</td>
<td>3 dark brown seeds; hilum covers ⅓ of seed coat</td>
</tr>
<tr>
<td>Calothyrsus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. assamica</em> (Griffith)</td>
<td>6</td>
<td>Ovoid (17)°</td>
<td>Brown</td>
<td>Smooth</td>
<td>-</td>
</tr>
<tr>
<td><em>A. californica</em> (Nuttall)</td>
<td>6-10</td>
<td>Ovoid (38)</td>
<td>Pale greenish brown</td>
<td>Develops June-Nov.; rough leathery but not spiny</td>
<td>1 rarely 2 glossy orange brown; 5 cm diameter mass up to 49 g</td>
</tr>
<tr>
<td><em>A. chinensis</em> (Bunge)</td>
<td>2-5</td>
<td>truncate or indented at top</td>
<td>Yellow brown</td>
<td>Rough warty but not spiny</td>
<td>2 chestnut brown seeds; 2-2.5 cm diameter</td>
</tr>
<tr>
<td><em>A. indica</em> (Colebrook)</td>
<td>3-5</td>
<td>Ovoid (49)</td>
<td>Reddish brown</td>
<td>Smooth to lightly roughened</td>
<td>1-3 chestnut brown seeds; 2-3 cm in diameter (48)</td>
</tr>
<tr>
<td><em>A. wangii</em> (Hu)</td>
<td>6-7.5</td>
<td>-</td>
<td>Dark brown</td>
<td>-</td>
<td>Usually 1 seed subglobose; 6 cm diameter; chocolate brown with hilum covering 1/2 the seed coat</td>
</tr>
<tr>
<td><em>A. wilsonii</em> (Rehder)</td>
<td>3.5</td>
<td>Globular to ovoid</td>
<td>Yellow brown</td>
<td>Smooth</td>
<td>Chestnut brown; 3.5 cm in diameter.; hilum covering 1/3 of seed coat</td>
</tr>
<tr>
<td>Macrothyrsus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. parviflora</em> (Walter)</td>
<td>2.5-4</td>
<td>Oval to ovoid</td>
<td>Light greenish brown</td>
<td>Smooth</td>
<td>1-3 honey brown seeds; 1.5-2 cm diameter</td>
</tr>
</tbody>
</table>

Table 1.5. Fruit and seed characteristics of *Aesculus* species and botanical varieties listed by sections.
Table 1.5 (continued)

<table>
<thead>
<tr>
<th>Section, species and varieties</th>
<th>Fruit characteristics</th>
<th>Seed characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Size (cm)</td>
<td>Shape</td>
</tr>
<tr>
<td>Parryana</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. parryi (Gray)</td>
<td>Small</td>
<td>Light brown</td>
</tr>
<tr>
<td>Pavia</td>
<td>5-7</td>
<td>Round-oblique to sub-globose</td>
</tr>
<tr>
<td>A. flava (Solander)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. glabra (Willdenow)</td>
<td>2.5-5</td>
<td>Ovoid to obovoid</td>
</tr>
<tr>
<td>var. arguta (Robinson)</td>
<td>like species</td>
<td>Like species</td>
</tr>
<tr>
<td>A. pavia (L.)</td>
<td>2.5-7.5</td>
<td>Obovoide</td>
</tr>
<tr>
<td>var. flavescens (Sargent)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A. sylvatica (Bartrum)</td>
<td>3-5</td>
<td>Subglobose to obovoid</td>
</tr>
</tbody>
</table>

Numbers in table refer to citations that can be found in Appendix A
### Table 1.6. Horticultural characteristics of *Aesculus* species and botanical varieties listed by section

<table>
<thead>
<tr>
<th>Section, species and varieties</th>
<th>Climatic adaptation</th>
<th>Habit</th>
<th>Bloom period</th>
<th>Fall color</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aesculus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. hippocastanum</em> (L.)</td>
<td>Zones 4-7B*: full sun; deep moist, well-drained soil</td>
<td>Pyramidal-oval to round tree</td>
<td>Spring</td>
<td>Golden yellow, amber or soft orange; leaves often damaged by disease</td>
</tr>
<tr>
<td><em>A. turbinata</em> (Blume)</td>
<td>Zones 5-8; moist, well-drained soil</td>
<td>Narrow upright to round tree</td>
<td>Late spring; 2-3 wks after Hip (39)*</td>
<td>Bright orange or brown (39)</td>
</tr>
<tr>
<td><em>Calothyrsus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. assamica</em> (Griffith)</td>
<td>Zone 8; limited hardiness (17)</td>
<td>—</td>
<td>Feb/Mar in S.Asia; July in England (49)</td>
<td>Evergreen in the tropics</td>
</tr>
<tr>
<td><em>A. californica</em> (Nuttall)</td>
<td>Zone 8-10, [-10C]; full sun; rich, well-drained soils (?)</td>
<td>Broad, round bush or small tree</td>
<td>May/Jun in CA; duration = 1 mo</td>
<td>Leaves shed in summer as arid land adaptation</td>
</tr>
<tr>
<td><em>A. chinensis</em> (Bunge)</td>
<td>Zone 6-8, [-15C]; full sun or partial shade; moist, well-drained soil (5)</td>
<td>Rounded to spreading tree</td>
<td>Apr/May where native; midsummer (5)</td>
<td>—</td>
</tr>
<tr>
<td><em>A. indica</em> (Colebrook)</td>
<td>Zone 7-9, [-10 to 35C]; adapted to moderate climate of England and Pac. NW; moist well-drained soil (49)</td>
<td>Oval to rounded crown tree</td>
<td>Ju/Jul in Suffolk; 4 wks after Hip (23)</td>
<td>Yellow, amber or orange</td>
</tr>
<tr>
<td><em>A. wilsonii</em> (Rehder)</td>
<td>Zone 6; partial shade; moist, well-drained soil (17)</td>
<td>Tree; shrubby occasionally</td>
<td>Jun in England</td>
<td>—</td>
</tr>
<tr>
<td><em>Macrothyrsus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. parviflora</em> (Walter)</td>
<td>Zone 4-9A; full sun or shade; acidic, moist, well-drained soil (35)</td>
<td>Multi-stemmed; usually wider than high; specimen shrub</td>
<td>Early/mid-summer; duration = 3 wks</td>
<td>Bright, clear yellow</td>
</tr>
<tr>
<td><em>Parryana</em></td>
<td>Hot, dry climates; rocky sandy soil on open hillsides (34)</td>
<td>Shrub or small tree</td>
<td>Apr in Baja CA; after leaf abscission occurs</td>
<td>None</td>
</tr>
<tr>
<td><em>A. parryi</em> (Gray)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pavia</em></td>
<td>Zone 3-8; full sun; deep, moist, well-drained, slightly acidic [pH 6-7] humus soil (40)</td>
<td>Upright, oval to spreading tree</td>
<td>Apr/May where native; 7-10 days after Pav</td>
<td>Golden yellow, pumpkin orange, or brilliant scarlet</td>
</tr>
<tr>
<td><em>A. glabra</em> (Willdenow)</td>
<td>Zone 3-7A; sun to partial shade; deep, moist soil; requires wind protection (35)</td>
<td>Large, round shrub or tree</td>
<td>Early spring; among the first trees to bloom</td>
<td>Yellow, yellow-orange, red, brown (22, 35)</td>
</tr>
</tbody>
</table>

Continued
<table>
<thead>
<tr>
<th>Section, species and varieties</th>
<th>Climatic adaptation</th>
<th>Habit</th>
<th>Bloom period</th>
<th>Fall color</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pavia</strong> (continued)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. glabra</em> (continued)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>var. <em>arguta</em> (Robinson)</td>
<td>Zone 5; adapted to hot, dry conditions (47)</td>
<td>Shrubby tree</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. pavia</em> (L.)</td>
<td>Zone 5-9A; sun to partial shade; deep, cool, moist, well-drained soil conditions (35, 47)</td>
<td>Dense, round large shrub to small tree</td>
<td>Spring</td>
<td>Red (39)</td>
</tr>
<tr>
<td>var. <em>flavescens</em> (Sargent)</td>
<td>Zone 5-9A</td>
<td>Shrub</td>
<td>After Pav where native</td>
<td>—</td>
</tr>
<tr>
<td><em>A. sylvatica</em> (Bartram)</td>
<td>Zone 5-9A; partial shade; moist humus soil (17)</td>
<td>Round, shrub to small tree</td>
<td>Apr where native; spring</td>
<td>Rich red, variable</td>
</tr>
</tbody>
</table>

*Zone refers to designations on the USDA Hardiness Zone Map.

1Hip and Pav are abbreviations for *A. hippocastanum* and *A. pavia*, respectively.

2Numbers in parentheses refer to citations that can be found in Appendix A.
### Table 1.7 Tolerance/susceptibility to biotic or abiotic stresses exhibited by *Aesculus* species and botanical varieties.

<table>
<thead>
<tr>
<th>Section, species and varieties</th>
<th>Biotic stress response</th>
<th>Abiotic stress response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tolerance</td>
<td>Susceptibility</td>
</tr>
<tr>
<td><strong>Aesculus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. hippocastanum</em> (L.)</td>
<td>—</td>
<td>Powdery mildew, anthracnose, leaf blotch [+]</td>
</tr>
<tr>
<td><em>A. turbinata</em> (Blume)</td>
<td>—</td>
<td>Leaf blotch [-]</td>
</tr>
<tr>
<td><strong>Calothyrsus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. assamica</em> (Griffith)</td>
<td>—</td>
<td>Leaf blotch [-]</td>
</tr>
<tr>
<td><em>A. californica</em> (Nuttall)</td>
<td>—</td>
<td>Leaf blotch [-]</td>
</tr>
<tr>
<td><em>A. chinensis</em> (Bunge)</td>
<td>Leaf blotch [some genotypes]</td>
<td>Leaf blotch [- to ++]</td>
</tr>
<tr>
<td><em>A. indica</em> (Colebrook)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>A. wilsonii</em> (Rehder)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Macrothyrsus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. parviflora</em> (Walter)</td>
<td>Leaf blotch; foliar diseases; insects (36, 37)</td>
<td>—</td>
</tr>
<tr>
<td><strong>Parryana</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. parryi</em> (Gray)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Pavia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. flava</em> (Solander)</td>
<td>—</td>
<td>Leaf blotch; foliar diseases (36)</td>
</tr>
<tr>
<td><em>A. glabra</em> (Willdenow)</td>
<td>—</td>
<td>Leaf blotch [+,++]; powdery mildew; anthracnose; wood rot; canker; Japanese beetles; tussock moth; walnut scale (37)</td>
</tr>
<tr>
<td>var. <em>arguta</em> (Robinson)</td>
<td>Leaf blotch (36, 37)</td>
<td>—</td>
</tr>
<tr>
<td><em>A. pavia</em> (L.)</td>
<td>—</td>
<td>Leaf blotch [+,++, and variable among genotypes]; mildew (37)</td>
</tr>
<tr>
<td>var. <em>flavescens</em> (Sargent)</td>
<td>See <em>A. pavia</em> (L.)</td>
<td>See <em>A. pavia</em> (L.)</td>
</tr>
<tr>
<td><em>A. sylvatica</em> (Bartram)</td>
<td>—</td>
<td>Leaf blotch [++] (37)</td>
</tr>
</tbody>
</table>

*[, +] and [++] indicates weak, strong or very strong susceptibility or tolerance to the corresponding biotic or abiotic factor.

Numbers in parentheses refer to citations that can be found in Appendix A.

CEFCLS = complex environmental factors causing leaf scorch.
### Table 1.8. Activities, properties or uses of saponin and coumarin natural products from *Aesculus*

<table>
<thead>
<tr>
<th>Activity, property or use</th>
<th>Escin and related compounds</th>
<th>Esculin and related compounds</th>
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<tbody>
<tr>
<td><strong>Pharmacological properties</strong></td>
<td></td>
<td></td>
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<tr>
<td>Cardiovascular effects</td>
<td></td>
<td></td>
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<tr>
<td>Fibrinolytic or anti-clotting agents</td>
<td>1, 14, 51</td>
<td>3, 21</td>
</tr>
<tr>
<td>Improved blood vessel function</td>
<td>1, 3, 8, 9, 14, 19, 20, 25, 29, 31, 37, 39</td>
<td>3</td>
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<tr>
<td>Anti-ischemic agent</td>
<td>26, 27, 28</td>
<td></td>
</tr>
<tr>
<td>Suppression of arteriosclerotic advance</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Cholesterol reduction</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td><strong>Effects on lymph and tissue systems</strong></td>
<td></td>
<td></td>
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<tr>
<td>Anti-edematic activity</td>
<td>1, 3, 9, 14, 20, 25, 37</td>
<td>38</td>
</tr>
<tr>
<td>Hematoma reduction and wound healing</td>
<td>5, 14</td>
<td>3</td>
</tr>
<tr>
<td><strong>Effects on mucosa</strong></td>
<td></td>
<td></td>
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<tr>
<td>Diuretic agent</td>
<td>32</td>
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<tr>
<td>Anti-ulcer agent</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Anti-hemorrhoidal agent</td>
<td>14, 19, 24, 30</td>
<td>3</td>
</tr>
<tr>
<td>Cell antiproliferative agent</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Anticarcinogenic (cytotoxic) agent</td>
<td>14, 16</td>
<td>15</td>
</tr>
<tr>
<td>Immunomodulating agent</td>
<td>4</td>
<td></td>
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<tr>
<td>Anti-inflammatory agent</td>
<td>1, 3, 4, 14, 19, 25, 26, 29, 37, 41</td>
<td>2, 3, 38</td>
</tr>
<tr>
<td>Anti-rheumatic activity</td>
<td>32</td>
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<tr>
<td>Analgesic agent</td>
<td>3</td>
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Table 1.8 (continued)

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<th>Activity, property or use</th>
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<th>Esculin and related compounds</th>
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<tr>
<td>Pharmacological properties (continued)</td>
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<td></td>
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<tr>
<td>Antipyretic agent</td>
<td>3, 14</td>
<td>3</td>
</tr>
<tr>
<td>Anti-allergenic (anti-asthmatic) agent</td>
<td>3, 25</td>
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<tr>
<td>Suppression of alcohol absorption</td>
<td>43, 44</td>
<td></td>
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<tr>
<td>Blood sugar stabilization</td>
<td>19, 43</td>
<td></td>
</tr>
<tr>
<td>Antioxidant activity</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Adiposity (cellulite) management</td>
<td>3, 6, 14</td>
<td></td>
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<tr>
<td>Skin tone/anti-aging activity</td>
<td></td>
<td>3, 23</td>
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<tr>
<td>Alopecia control</td>
<td>3</td>
<td></td>
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<tr>
<td>Sun screen activity</td>
<td></td>
<td>18</td>
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<tr>
<td>Antibiosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibacterial agents</td>
<td>22</td>
<td>10, 17</td>
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<tr>
<td>Antifungal agents</td>
<td>13, 14</td>
<td></td>
</tr>
<tr>
<td>Antiviral agents</td>
<td>34, 42</td>
<td></td>
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<tr>
<td>Other uses</td>
<td></td>
<td></td>
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<tr>
<td>Cosmetic formulations</td>
<td></td>
<td>33</td>
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<tr>
<td>Reactants for chemical analyses</td>
<td></td>
<td>3, 7, 12</td>
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<tr>
<td>Phyto-depressants</td>
<td></td>
<td>35, 42</td>
</tr>
</tbody>
</table>

*Including references to hemolytic, anti-thrombosis, anticoagulant activities.
*Numbers refer to reference identities listed in Appendix D.
*Including references to treatment of chronic vascular insufficiency and issues of vasomodulation and capillary strengthening and blood flow.
### Section, species and varieties of Aesculus

- **Aesculus**
  - *A. hippocastanum* (L.)
  - *A. turbinata* (Blume)
  - *A. sinensis* (Hort) ex Garden Chronical
  - *A. dissimilis* (Bloom)
  - *A. chinensis* (Hort) ex Bean
  - *A. japonica* (Hort) ex Bean
  - *A. turbinate var. pubescens* (Rehder)

- **Calothyrsus**
  - *A. assamica* (Griffith)
  - *A. californica* [Spach][Nuttall]
  - *A. chinensis* (Bunge)
  - *A. indica* (Colebrook)
  - *A. wilsonii* (Rehder)

- **Macrothyrsus**
  - *A. parviflora* (Walter)
  - *A. macrostachya* (Michaux)
  - *Pavia alba* (Poiret)
  - *Pavia edulis* (Poiteau and Turpin)
  - *A. odorata* (Dietrich)
  - *Pavia macrostachya* (Loiseleur)
  - *A. monostachya* (Eaton)
  - *Macrothyrsus discolor* (Spach)
  - *Macrothyrsus odorata* (Rafineque-Schmaltz)
  - *A. stolonifera* (Bartram)
  - *A. parviflora forma serotina* (Rehder)

- **Parryana**
  - *A. parryi* (Gray)

- **Pavia**
  - *A. flava* (Solander)
  - *A. octandra* (Marshall)
  - *A. lutea* (Wangenheim)
  - *Pavia flava* (Moench)
  - *Pavia lutea* (Poiret)
  - *Paviana flava* (Rafineque-Schmaltz)
  - *Pavia reticulata* (Rafineque-Schmaltz)
  - *A. flava forma vestita* (Sargent)
  - *A. flava forma virginica* (Sargent)
  - *A. flava var. sanguinea* (Hort)
  - *A. flava var. rosea* (Hort)
  - *A. flava var. purpurea* (Hort)

Continued

Table 1.9. Synonyms and/or names no longer accepted for species and botanical varieties of *Aesculus.*
Table 1.9 (continued)

<table>
<thead>
<tr>
<th>Section, species and varieties</th>
<th>Synonyms and no longer accepted names</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. glabra (Willdenow)</td>
<td>A. ohioensis (De Candolle)</td>
</tr>
<tr>
<td></td>
<td>A. pallida (Willdenow)</td>
</tr>
<tr>
<td></td>
<td>Pavia ohioensis (Michaux)</td>
</tr>
<tr>
<td></td>
<td>A. echinata (Muehlenburg)</td>
</tr>
<tr>
<td></td>
<td>Pavia axillata (Rafineque-Schmaltz)</td>
</tr>
<tr>
<td></td>
<td>Pavia glabra (Spach)</td>
</tr>
<tr>
<td></td>
<td>Pavia pallida (Spach)</td>
</tr>
<tr>
<td></td>
<td>A. muricata (Rafineque-Schmaltz)</td>
</tr>
<tr>
<td></td>
<td>A. ochroleuca (Rafineque-Schmaltz)</td>
</tr>
<tr>
<td></td>
<td>A. verrucosa (Rafineque-Schmaltz)</td>
</tr>
<tr>
<td></td>
<td>Pavia bicolor (Rafineque-Schmaltz)</td>
</tr>
<tr>
<td></td>
<td>A. glabra var. buckleyi (Sargent)</td>
</tr>
<tr>
<td></td>
<td>A. glabra var. leucoedermis (Sargent)</td>
</tr>
<tr>
<td></td>
<td>A. glabra var. mycrantha (Sargent)</td>
</tr>
<tr>
<td></td>
<td>A. glabra var. monticola (Sargent)</td>
</tr>
<tr>
<td></td>
<td>A. glabra var. nana</td>
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<tr>
<td></td>
<td>A. glabra var. pallida (Kirchner)</td>
</tr>
<tr>
<td></td>
<td>A. glabra forma pallida [Willdenow]</td>
</tr>
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<td></td>
<td>(Schelle)</td>
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<td></td>
<td>A. glabra var. Sargentii (Rehder)</td>
</tr>
<tr>
<td>var. arguta (Buckley)</td>
<td>A. arguta (Buckley)</td>
</tr>
<tr>
<td></td>
<td>A. glabra var. sargentii (Rehder)</td>
</tr>
<tr>
<td>A. pavia (L.)</td>
<td>A. discolor (Pursh)</td>
</tr>
<tr>
<td></td>
<td>A. discolor var. Koehnei (Rehder)</td>
</tr>
<tr>
<td></td>
<td>A. pavia var. humilis [Loddiges ex Lindley] Mouillefert</td>
</tr>
<tr>
<td></td>
<td>A. splendens (Sargent)</td>
</tr>
<tr>
<td></td>
<td>A. austrina (Small)</td>
</tr>
<tr>
<td></td>
<td>Pavia rubra [Poirot] ex Lamarck</td>
</tr>
<tr>
<td></td>
<td>Pavia michauxii (Spach)</td>
</tr>
<tr>
<td></td>
<td>Pavia atropurpurea (Spach)</td>
</tr>
<tr>
<td></td>
<td>A. humilis [Loddiges] ex (Lindley)</td>
</tr>
<tr>
<td></td>
<td>A. rubra (of no botanical standing)</td>
</tr>
<tr>
<td></td>
<td>A. pavia var. discolor (Torrey and Gray)</td>
</tr>
<tr>
<td></td>
<td>A. pavia var. Koehnei (Rehder)</td>
</tr>
<tr>
<td></td>
<td>A. discolor (Pursh)</td>
</tr>
<tr>
<td></td>
<td>A. pavia var. humilis (Lindley)</td>
</tr>
<tr>
<td></td>
<td>A. rubra var. humilis (Loudon)</td>
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<tr>
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<td>A. humilis (Loddiges)</td>
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<tr>
<td></td>
<td>A. pavia var. nana (Dippel)</td>
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<td></td>
<td>Pavia rubra var. humilis (Loudon)</td>
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<tr>
<td></td>
<td>A. pavia var. pendula (Hort.)</td>
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<tr>
<td></td>
<td>A. humilis Koehne (not Lindley)</td>
</tr>
<tr>
<td></td>
<td>A. pavia var. humilis (Voss) partly</td>
</tr>
<tr>
<td></td>
<td>A. humilis (Loddiges)</td>
</tr>
<tr>
<td></td>
<td>A. pavia var. mollis (Rafinesque-Schmaltz)</td>
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<tr>
<td></td>
<td>A. mollis (Small)</td>
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<tr>
<td></td>
<td>A. austrina (Sargent)</td>
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<tr>
<td></td>
<td>A. pavia var. splendens (Sargent)</td>
</tr>
<tr>
<td></td>
<td>A. pavia var. sublaciniata (Watson)</td>
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<td>Pavia atropurpurea (Spach)</td>
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Continued
Table 1.9 (continued)

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<tr>
<th>Section, species and varieties</th>
<th>Synonyms and no longer accepted names</th>
</tr>
</thead>
<tbody>
<tr>
<td>var. <em>flavescens</em> (Sargent)</td>
<td>A. discolor var. flavescens (Sargent)</td>
</tr>
<tr>
<td><em>A. sylvatica</em> (Bartram)</td>
<td>A. georgiana (Sargent) sometimes listed as a var</td>
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<tr>
<td></td>
<td>A. glaucescens (Sargent)</td>
</tr>
<tr>
<td></td>
<td>A. neglecta (Lindley)</td>
</tr>
<tr>
<td></td>
<td>A. neglecta var. georgiana [Sargent]</td>
</tr>
<tr>
<td></td>
<td>[Sargent] (Sargent)</td>
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<td></td>
<td>A. neglecta var. pubescens [Sargent]</td>
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<td>[Sargent] (Sargent)</td>
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<td>A. neglecta var. tomentosa (Sargent)</td>
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<td></td>
<td>A. virginica (Bartram)</td>
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<td>Pavia neglecta (Spach)</td>
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<td>Pavia wilddenowiana (Spach)</td>
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<td>Pavia punctata (Rafineque-Schmaltz)</td>
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<td>Pavia fulva (Rafineque-Schmaltz)</td>
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<td>A. microcarpa (Ashe)</td>
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<td>A. michauxii (Hort.)</td>
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<td></td>
<td>A. discolor (Hort. Non Pursh)</td>
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<tr>
<td></td>
<td>A. rubra canea superba (Hort.)</td>
</tr>
<tr>
<td>Species varieties and cultivars</td>
<td>Origin, date and location</td>
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<tr>
<td>--------------------------------</td>
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<tr>
<td><em>A. hippocastanum</em></td>
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<tr>
<td>Alba</td>
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<td>Baumannii</td>
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<td>Crispa</td>
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<td>Hampton Court Gold</td>
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<tr>
<td>Henkelii</td>
<td>1904; UK (2)</td>
</tr>
<tr>
<td>Honiton Gold</td>
<td>2002; UK</td>
</tr>
<tr>
<td>Incisa</td>
<td>1838; Germany; Booth (2)</td>
</tr>
<tr>
<td>Laciniata</td>
<td>1844; France (2)</td>
</tr>
<tr>
<td>Luteovariegata</td>
<td>Unknown</td>
</tr>
<tr>
<td>Memmingeri</td>
<td>1855</td>
</tr>
<tr>
<td>Monstrosa</td>
<td>Unknown</td>
</tr>
<tr>
<td>Nigra</td>
<td>Unknown</td>
</tr>
<tr>
<td>Pendula</td>
<td>1800; France</td>
</tr>
</tbody>
</table>

Table 1.10. The varieties and known cultivars of *Aesculus* organized by species.
Table 1.10 (continued)

<table>
<thead>
<tr>
<th>Species varieties and cultivars</th>
<th>Origin, date and location</th>
<th>Notable characteristics</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A hippocastanum</strong> (continued)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Praecox</td>
<td>1838; Germany</td>
<td>FP &amp; LP – 10-14 days before species (2)</td>
<td>Not for locations with late frosts (2)</td>
</tr>
<tr>
<td>Pumila</td>
<td>Unknown</td>
<td>L – deeply cut; GH – dwarf (3, 24)</td>
<td>AD – Digitata (3)</td>
</tr>
<tr>
<td>Pyramidalis</td>
<td>1895; UK</td>
<td>GH – narrow, pyramidal, branch angle = 45° (2, 24, 45)</td>
<td>AD – Fastigiata; pollution [tol+] ; leaf blotch [sus++]; not grown (37, 45)</td>
</tr>
<tr>
<td>Rosea</td>
<td>Unknown</td>
<td>F – pinked toned (24, 26)</td>
<td></td>
</tr>
<tr>
<td>Schirnhofeir</td>
<td>Austria</td>
<td>F – yellowish red (29)</td>
<td></td>
</tr>
<tr>
<td>Tortuosa</td>
<td>Unknown</td>
<td>GH – branches twisted and pendulous (29)</td>
<td></td>
</tr>
<tr>
<td>Umbraculifera</td>
<td>1884</td>
<td>GH – dwarf, dense, compact, rounded (2, 45)</td>
<td>Best grafted high on standard for umbrella shape</td>
</tr>
<tr>
<td>Wisselink</td>
<td>Germany</td>
<td>L – white becoming variegated with lime green veins</td>
<td>Unusual plant for collectors only</td>
</tr>
<tr>
<td><strong>A. turbinata</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>var. pubescens* (Rehder)</td>
<td>1911</td>
<td>L – densely tomentose</td>
<td></td>
</tr>
<tr>
<td><strong>A. californica</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canyon Pink</td>
<td>1987; USA</td>
<td>F – light to blush pink</td>
<td>Introduced by the Santa Barbara Botanic garden from wild collected seed; not commercially available</td>
</tr>
<tr>
<td>Grant's Ruby</td>
<td></td>
<td>F – dark red</td>
<td></td>
</tr>
<tr>
<td><strong>A. indica</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sydney Pearce</td>
<td>1928; UK; R. C. Coats</td>
<td>F – pink with pinkish orange blotch, panicles 18-30 cm; densely flowering, flowers 2.5 cm dia; L – dark olive; GH – upright, symmetric (22)</td>
<td>Budded midsummer (3)</td>
</tr>
<tr>
<td><strong>A. parviflora</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forma serotina</td>
<td>1928; USA; Rehder</td>
<td>F – panicle 30-45 cm; FP – 14-21 days after species; L – blueish green, glaucescent (44)</td>
<td>AD – late bottle brush buckeye; leaf blotch [tol++] (36)</td>
</tr>
<tr>
<td>Rogers</td>
<td>USA; J. C. McDaniel</td>
<td>F – panicle 35-65 cm; FP – later than species or forma serotina</td>
<td>Selected from forma serotina</td>
</tr>
</tbody>
</table>

Continued
Table 1.10 (continued)

<table>
<thead>
<tr>
<th>Species varieties and cultivars</th>
<th>Origin, date and location</th>
<th>Notable characteristics</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. flava</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forma vestita (Sargent)</td>
<td>1915; USA</td>
<td>L – densely tomentose (24)</td>
<td>Leaf blotch [sus++]; fall color variable between trees (37)</td>
</tr>
<tr>
<td>Forma virginica (Sargent)</td>
<td>USA</td>
<td>F – pink or apricot</td>
<td>Possible hybrid of <em>A. pavia</em></td>
</tr>
<tr>
<td><em>A. glabra</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>var. Buckleyi (Sargent)</td>
<td>USA</td>
<td>L – fine abaxial pubescence; L1 – 7</td>
<td>Often confounded with <em>A. glabra</em> var. arguta; native to western part of the species’ range</td>
</tr>
<tr>
<td>var. luecodermis (Sargent)</td>
<td>1901; USA</td>
<td>FP – later than species; L – white pubescence; B – nearly white or sometimes striped (52)</td>
<td>Very similar to <em>A. glabra</em> var. Buckleyi;</td>
</tr>
<tr>
<td>var. micrantha (Sargent)</td>
<td>USA</td>
<td>F – 1 cm in length; L – more pubescent than species; GH – shrubby</td>
<td></td>
</tr>
<tr>
<td>var. monticola (Sargent)</td>
<td>1922; USA</td>
<td>F – smaller than species; L1 – 5-7, doubly serrate; GH – dwarf; ht to 2 m (47)</td>
<td></td>
</tr>
<tr>
<td>var. nana (Hort.)</td>
<td>USA</td>
<td>Frt – small, very spiny; Sd – nearly black; GH – round, dwarf; GR – 2.5 m in 18 years</td>
<td>AD – <em>A. glabra</em> var. <em>monticola</em>, perhaps; True breeding from seed*</td>
</tr>
<tr>
<td>var. pallida (Kirchner)</td>
<td>1809; USA</td>
<td>L &amp; B – thick, persistent pubescence; GH – erect branches; ht to 16 m (22, 52)</td>
<td></td>
</tr>
<tr>
<td>var. sargentii (Rehder)</td>
<td>USA</td>
<td>L1 – 7+; narrow, tapering; finely pubescent (52)</td>
<td></td>
</tr>
<tr>
<td><em>Fall Red</em></td>
<td></td>
<td>F – typical of species; L – good red fall color; GH – typical of species</td>
<td></td>
</tr>
<tr>
<td><em>Klein's Weeping</em></td>
<td>USA; Klein</td>
<td>GH – weeping</td>
<td>Discovered in Indiana; grafted on <em>A. hippocastanum</em> or <em>A. pavia</em> seedlings</td>
</tr>
<tr>
<td><em>October Red</em></td>
<td>Unknown</td>
<td>L – copper in spring; red and yellow in fall</td>
<td></td>
</tr>
</tbody>
</table>

Continued
<table>
<thead>
<tr>
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<th>Origin, date and location</th>
<th>Notable characteristics</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. pavia</td>
<td>1812; USA</td>
<td>F – red calyx, 1 cm long, petals yellow flushed with red and unequal; L – leaves dark green, serrate, white abaxial pubescence; GH – vase-shaped, ht to 10 m (47)</td>
<td></td>
</tr>
<tr>
<td>var. discolor (Torrey and Gray)</td>
<td>1905; USA</td>
<td>F – long panicles, deep scarlet to bright red flowers; L – dense, even abaxial pubescence; GH – shrub, ht to 6 m (21, 52)</td>
<td>AD – flame buckeye; occasionally listed as a cultivar</td>
</tr>
<tr>
<td>var. mollis (Rafinesque-Schmaltz)</td>
<td>1913 USA</td>
<td>F – bright red tubular, 3.5 - 4 cm. petals deep scarlet red, narrow panicles, 20-30 cm, L1 – lanceolate and finely serrate</td>
<td></td>
</tr>
<tr>
<td>var. splendens</td>
<td>USA</td>
<td>F – very dark red; L – long narrow and deeply serrated (50, 52)</td>
<td></td>
</tr>
<tr>
<td>var. sublanciniata (Watson)</td>
<td>USA</td>
<td>F – dark crimson red, panicles slender; GH – ht to 3-5 m (28, 52)</td>
<td>AD – red wine buckeye; listed as cultivar or variety; asexually propagated; leaf blotch [sus++]</td>
</tr>
<tr>
<td>Atrosanguinea (Kirchner)</td>
<td>USA</td>
<td>F – bright fire-engine red, 15-30 cm panicles; GH – small tree; ht 3-7 m</td>
<td>Original plant found at the Biltmore House in Asheville, NC</td>
</tr>
<tr>
<td>Biltmore</td>
<td>USA</td>
<td>F – bright red, panicles smaller than species; L – more pubescent than species; GH – prostrate or weeping, dwarf, ht 0.5-1.5 m (50)</td>
<td>AD – A. pavía ‘Pendula’ or prostrate red buckeye; listed as cultivar or variety; can be grafted on standard</td>
</tr>
<tr>
<td>Humilis</td>
<td>1826; USA; Lindley</td>
<td>F – rose pink to red, panicles &lt;10 cm; L1 – small GH – dwarf shrub, A. pavía var. discolor-like; GR – slow (52)</td>
<td>Confused with A. pavía Humilis; formerly Rosea Nana (52)</td>
</tr>
<tr>
<td>Koehnei</td>
<td>Before 1893; Rehder</td>
<td>F – purplish new growth in spring</td>
<td>Slow growing with rose-red flowers</td>
</tr>
<tr>
<td>Spring Purple</td>
<td>USA; Pavia Nursery</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Continued
### Table 1.10 (continued)

<table>
<thead>
<tr>
<th>Species varieties and cultivars</th>
<th>Origin, date and location</th>
<th>Notable characteristics</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. sylvatica var. georgiana (Sargent)</td>
<td>USA</td>
<td>F – petals red and yellow, panicles 10-15 cm wide, denser than species; GH – ht 2 m or less (15, 22)</td>
<td></td>
</tr>
<tr>
<td>var. lanceolata (Sargent)</td>
<td>1917; USA</td>
<td>F – bright rosy pink</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lt – lanceolate, elongated, little or pubescence (45)</td>
<td></td>
</tr>
<tr>
<td>var. pubescens (Sargent)</td>
<td>1905; USA</td>
<td>F – red and yellow; L – dense, straight, abaxial pubescence</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GH – ht to 10 m</td>
<td></td>
</tr>
<tr>
<td>var. tomentosa (Sargent)</td>
<td>1880; USA</td>
<td>F – bright red; Lt – dense, curly, abaxial pubescence, appearing gray</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations used for notable characteristics and comments are as follows: AD = alternate designation, B = branches, F = flowers or inflorescences, FP = flower phenology, Frt = fruit, GH = growth habit, GR = growth rate, L = leaves, Lt = leaflets, LP = leaf phenology, Sd = seeds, [sus++], [tol+] = highly susceptible to and moderately tolerant of, respectively.

*Numbers in table refer to citation information located in Appendix A.

*For the sake of clarity, the notation var. in this table denotes plants from the wild with a specific phenotype.

*From personal communication with Robert McCartney, propagator for Woodlanders, Inc.
<table>
<thead>
<tr>
<th>Hybrids</th>
<th>Putative parents</th>
<th>Alternate names</th>
<th>Alternate parentages</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. × arnoldiana</td>
<td>[Gla’ X (FlaX Pav)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. × bushii</td>
<td>Gla X Pav</td>
<td>A. × mississippiensis</td>
<td>[Gla X (Gla X Pav)] or Gla X Syl</td>
</tr>
<tr>
<td>A. × carnea</td>
<td>Hip X Pav</td>
<td>A. × rubicunda</td>
<td></td>
</tr>
<tr>
<td>A. × hybrida</td>
<td>Fla X Pav</td>
<td>A. × discolor,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A. lyonii (Hort);</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A. versicolor,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A. whiteyi</td>
<td></td>
</tr>
<tr>
<td>A. × ‘Homestead’</td>
<td>FlaX Gla</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. × marylandica</td>
<td>FlaX Gla</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. × mutabilis</td>
<td>Pav X Syl</td>
<td>A. × harbisonii</td>
<td></td>
</tr>
<tr>
<td>A. × neglecta</td>
<td>Fla X Syl</td>
<td>A. × glaucescens</td>
<td></td>
</tr>
<tr>
<td>A. × plantierensis</td>
<td>[(Hip X Pav) X Hip]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. × woerlitzensis</td>
<td>[Fla X (Pav X Syl)]</td>
<td>A duportii</td>
<td>Hyb X Syl or Pav X Gla or PavX Syl</td>
</tr>
</tbody>
</table>

Car, Fla, Gla, Hip Hyb, Pav, and Syl are abbreviations for A. × carnea, A. flava, A. glabra, A. hippocastanum, A. × hybrida, A. pavia, and A. sylvatica respectively.

Table 1.11. Putative parentages, synonyms, and possible alternate parentages of recognized interspecific hybrids of Aesculus.
<table>
<thead>
<tr>
<th>Hybrids and selections</th>
<th>Origin</th>
<th>Plant traits</th>
<th>Flowers, fruit and seed</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. × arnoldiana</em> (Sargent)</td>
<td>1900; Garden hybrid; selected at Arnold Arboretum</td>
<td>L – glabrous; FC – individuals good fall color; GH – ht to 25m (48)</td>
<td>C – yellow tinged red; P – dense, 9-15 cm; Frt – Short spines, <em>A. glabra</em>-like; Sd – Gla-like (48)</td>
<td></td>
</tr>
<tr>
<td>Autumn Splendor</td>
<td>1989; U.SA Bergmann et al.; selected at the MN Landscape Arboretum;</td>
<td>L – glossy, dark green; FC – brilliant red or maroon; GH – ht 15 m; Leaf scorch [tol+];</td>
<td>C – yellow with an orange-red blotch; P– 20 cm</td>
<td></td>
</tr>
<tr>
<td><em>A. × bushii</em> (Schneid)</td>
<td>1901; Natural hybrid; hybrid has been named twice</td>
<td>L – new foliage is silvery and pubescent; GH – low spreading habit; Leaf blotch [sus to sus+++]; (37, 51, 55)</td>
<td>FP – blooms in June; C – yellow, pink or red within inflorescence; P – 8-10 cm; St – longer than petals (45, 47, 55)</td>
<td>Often confused with <em>A. × hybridia</em>;</td>
</tr>
<tr>
<td><em>A. × carnea</em> (Hayne)</td>
<td>1812; Garden hybrid</td>
<td>L – shiny dark green and crinkled; Bds – slightly sticky; GH – ht 8-15 m Urban conditions [tol+] (55)</td>
<td>C – Rose pink to red with yellow blotch; P ≤ 20 cm; Frt – slightly prickly (55)</td>
<td>Conflicting reports about coming true from seed</td>
</tr>
<tr>
<td>Aureo-marginata</td>
<td>Unknown</td>
<td>L – yellow margined (3)</td>
<td></td>
<td>AD – Aurea Marginata</td>
</tr>
<tr>
<td>Aureo-maculata</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Big Boy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Briotii</td>
<td>1858; France (53)</td>
<td>L – crinkled and dark green; GH – dense, rounded, compact, branches bending close to ground, ht 10-25 m GR –strong (9)</td>
<td>P – bright pink rosy to red color , 15-25 cm (9)</td>
<td>Same cultural requirements and limits as <em>A × carnea</em></td>
</tr>
<tr>
<td>Fort McNair</td>
<td>1991; USA (27)</td>
<td>L – remain dark green until drop; GH – round, dense, ht 12-16 m; GR – 30-50% faster than Car; Foliar diseases [tol+]</td>
<td>C – dark pink to red, less yellow than some clones; P – 15-20 cm (27)</td>
<td></td>
</tr>
<tr>
<td>Marginata</td>
<td></td>
<td>L – variegated, center light green with a dark green margin separated by a yellow band (29)</td>
<td></td>
<td>AD – Foliis Marginatis (55)</td>
</tr>
</tbody>
</table>

Table 1.12. Characteristics of interspecific hybrids of *Aesculus* and of selections from interspecific hybrids.
<table>
<thead>
<tr>
<th>Hybrids and selections</th>
<th>Origin</th>
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<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>O'Neil's Red</td>
<td>1979, USA (27)</td>
<td>L – glossy green FC – none GH – ht 12-25 m</td>
<td>C – deep red, less pink tints; P – large, 25-30 cm</td>
<td>AD – O’Neil Red, O’Neill; incorrectly listed as double (27)</td>
</tr>
<tr>
<td>Owen's Red</td>
<td>Unknown</td>
<td></td>
<td>C – rose red and yellow, more uniformly colored than 'Fort McNair'</td>
<td></td>
</tr>
<tr>
<td>Pendula</td>
<td>1902; UK Garden variety</td>
<td>GH – semi-weeping (45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosca</td>
<td>Unknown</td>
<td>C – rich, rose pink with a contrasting blotch that changes from golden to scarlet (25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. + dallimorei (Sealy)</td>
<td>UK</td>
<td>L – broadly elliptic, dark green</td>
<td>F – 2 types; C – white blotched with maroon or yellow blotched with ochre; Frt – none observed (48, 55)</td>
<td>Possibly a graft chimera</td>
</tr>
<tr>
<td>A. × 'Homestead'</td>
<td>1987; USA; Dr. Evers; selected for cold hardness; putative parents are Fla and Gla</td>
<td>FC – dark red with brunt orange highlights; Resistance to leaf blotch and powdery mildew resembles Fla Hardy to Zone 4, perhaps 3 resembling Gla</td>
<td>C – creamy white; Frt – typically fruitless</td>
<td></td>
</tr>
<tr>
<td>A. × hybrida (Sargent)</td>
<td>1815; Natural hybrid of the Alleghany mountains; probably a group of hybrids</td>
<td>L – glossy green; downy on abaxial surface; FC – good yellow; coarser than yellow buckeye (45)</td>
<td>FP – blooms later C – red or yellow tinged with red; P – loose, 10-18 cm e panicles; blooms latter; Frt – smooth</td>
<td>AD purple sweet buckeye or yellow buckeye variant; similar to A. × mutabilis (55, 22, 47)</td>
</tr>
<tr>
<td>A. × marylandica (Booth)</td>
<td>1861 Garden hybrid, backcross and hybrid intermediates</td>
<td>L – lustrous adaxial, rusty pubescence abaxial (48)</td>
<td>C – light yellow (48)</td>
<td></td>
</tr>
<tr>
<td>A. × mutabilis (Spath)</td>
<td>1834</td>
<td>L – abaxial pubescence</td>
<td>C – yellow flushed with pink to red (48, 55)</td>
<td></td>
</tr>
<tr>
<td>Harbisonii</td>
<td>1905; USA; Open-pollinated selection of A. sylvatica made at Arnold Arboretum</td>
<td>L – glaucous; GH large shrub, ht 5 m; leaves glaucose Leaf blotch [sus++] (37);</td>
<td>FP – blooms later than A. pavia C – rich red; P - 15-20 cm (17, 29)</td>
<td>Originated from an open pollinated seed;</td>
</tr>
</tbody>
</table>

Continued
## Table 1.12 (continued)

<table>
<thead>
<tr>
<th>Hybrids and selections</th>
<th>Origin</th>
<th>Plant traits</th>
<th>Flowers, fruit and seed</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induta</td>
<td>1905; Germany; Hesse-Weener; could be tri-parental hybrid with Pav, Fla, and Syl (16)</td>
<td>L – bronze (spring), blue green (summer), densely pubescent and glaucescent; GH – shrub, 3 X 2 m; GR – slow; Leaf blotch [sus++]; (25, 37, 55)</td>
<td>FP – Blooms in late spring or early summer; C – pinkish apricot with yellow blotches; P – abundant, 20 cm (37, 55)</td>
<td></td>
</tr>
<tr>
<td>Penduliflora</td>
<td>Before 1902; Presumed parents Pav and Fla, with Sly influences</td>
<td>L – lanceolate, new growth pubescent GH – large shrub or small multi-stemmed tree, ht 8 m</td>
<td>C – creamy yellow and rose, over 2.5 cm long; P – 14-16 cm</td>
<td></td>
</tr>
<tr>
<td>A. × neglecta (Lindl.)</td>
<td>1826; Natural hybrid</td>
<td>L – entire or serrate; medium green, abaxial veins downy; FC – rich orange and golden yellow; GH – ht 8 m (4, 48)</td>
<td>FP – blooms in May and June; C – pale yellow or yellow-flushed red; P – 15 cm; Frt – smooth</td>
<td></td>
</tr>
<tr>
<td>Autumn Fire</td>
<td>1902; (29)</td>
<td>L – spring foliage coppery; FC – apricot orange</td>
<td>C – creamy yellow, typical</td>
<td></td>
</tr>
<tr>
<td>Erythroblastos</td>
<td>1902; (29)</td>
<td>L – spring foliage brilliant rose to shrimp pink to creamy peach to lime green; FC – warm orange; GR – extremely slow; Hardy to Zone 5 but easily damaged by frost, requires shelter (55, 57)</td>
<td>FP – rarely blooms; C – creamy yellow of little interest</td>
<td>AD – Roseo-variegata; extremely rare in USA (5, 51, 57)</td>
</tr>
<tr>
<td>A. × plantierensis (Andre)</td>
<td>1890; France; Garden hybrid, a triploid backcross hybrid, hip seed parent; (18, 22, 45, 55)</td>
<td>Lt – Hip but crinkled and wavy like Car</td>
<td>C – soft pink; P – like Hip in size and shape; Frt – none</td>
<td></td>
</tr>
<tr>
<td>A. × worlitzensis (Kochne)</td>
<td>after 1820; Garden hybrid</td>
<td>L – obovate, dark green adaxial, yellow green abaxial; GH – ht 6 m (48)</td>
<td>C – red St – as long as petals; Calyx tubular and narrow; P – 8-12 cm (48)</td>
<td></td>
</tr>
<tr>
<td>Ellerwangeri</td>
<td>1901</td>
<td>FC – golden brown with hints of red; GH – small 5-7m (1);</td>
<td>Intense red flower color;</td>
<td>AD – A. aurosanguinea and A. whitleyi from European sources; rare in cultivation; good selection for landscape (1, 29)</td>
</tr>
</tbody>
</table>

*Abbreviations used for notable characteristics and comments are as follows: AD = alternate designation, Bds = buds, C = flower color, F = flowers or inflorescences, FC = fall color, FP = flower phenology, Frt = fruit, GH = growth habit, GR = growth rate, L = leaves, Lt = leaflets, Sd = seeds St = stamens; [sus++] = highly susceptible to and moderately tolerant of, respectively.

Numbers in table refer to citation information located in Appendix A.

Abbreviation in the table Car Fla, Gla, Hip, and Pav are A × carnea, A. flava, A. glabra, A. hippocastanum, and A. pavia, respectively.
Figure 1.1. The *Aesculus* phylogenetic organizational scheme proposed by Hardin (1957).
Figure 1.2. The natural distribution of various *Aesculus* species. A) *A. assmica*, *A. chinensis*, *A. indica* and *A. wilsonii* are depicted in orange, yellow, pink and blue, respectively. Overlapping ranges are shown by overlapping color mixtures; B) *A. hippocastanum* depicted in green; C) *A. turbinata* depicted in violet.
Figure 1.3. The natural distribution of *A. californica*, *A. flava*, *A. glabra*, *A. parryi*, *A. parviflora*, and *A. pavia* depicted in pink, blue, yellow, mauve, green and red respectively.
Figure 1.4. Important natural products derived from *Aesculus* (Yoshikawa and Yamahara, 1996; Merck, 2001)

**a. Major components of escin**

**b. Eculetin**

**c. Esclulin**

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**escin Ia (20):**
- **R<sup>1</sup>:** tigloyl
- **R<sup>2</sup>:** Ac
- **R<sup>3</sup>:** CH₂OH

**escin Ib (21):**
- **R<sup>1</sup>:** tigloyl
- **R<sup>2</sup>:** Ac
- **R<sup>3</sup>:** CH₂OH

**escin IIa (22):**
- **R<sup>1</sup>:** tigloyl
- **R<sup>2</sup>:** Ac
- **R<sup>3</sup>:** H

**escin IIb (23):**
- **R<sup>1</sup>:** tigloyl
- **R<sup>2</sup>:** Ac
- **R<sup>3</sup>:** H

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**escin IIIa**
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CHAPTER 2

COMPARISON OF INFLORESCENCE MORPHOLOGY, ANTHESIS AND SEX RATIO OF AESCULUS PARVIFLORA AND AESCULUS PAVIA

INTRODUCTION

Aesculus, commonly known as buckeyes and horsechestnuts, are trees or shrubs cultivated as ornamentals, or to a limited extent for commercial forestry. The genus consists of thirteen species divided into five sections (Hardin, 1957a). Aesculus are among the first plants to leaf out in the spring, having mixed buds containing both leaves and inflorescences. The inflorescences are composed of a central axis called a rachis and lateral branches off the rachis called cincinni (Hardin, 1956). The genus Aesculus has an unusual reproductive strategy including a large portion of functionally staminate flowers, and a high microspore to mature seed ratio. Hardin (1956) concluded that the term andromonecious best characterized the flowering biology of the genus since both functionally staminate flowers and at least a few complete flowers could be found within individual plants. The functionally staminate flowers have a vestigial gynoecium with an undeveloped ovary and stunted style. Complete flowers have fully developed androecium and gynoecium (Hardin, 1956).
Aesculus parviflora, bottlebrush buckeye, is native to the southeastern United States, in northern Georgia, Alabama, and South Carolina, but it is cultivated as far north as Chicago. In natural settings, it is most frequently found along waterways and at the bases of slopes as a few scattered individuals (Wyatt, 1985). Bottlebrush buckeye was first collected by Thomas Walter in 1788 and was placed in the genus Macrothryrsus. It was reclassified several times before being placed in the genus Aesculus and is the sole member of the Macrothryrsus section indicating its phylogenetic separation (Hardin, 1957a). Bottlebrush buckeye is a multistemmed plant with a low, broad, rounded crown and a horizontal branching pattern. It can reach 4 m in height and an equal spread (Clark, 1982). The white flowers of bottlebrush buckeye are borne on panicles and have long stamens and styles that extend beyond the corolla, giving the inflorescences a brush-like appearance and, thus, its common name. Aesculus parviflora inflorescences typically reach anthesis in early summer in Ohio. Aesculus parviflora forma serotina was described for its larger inflorescences and late blooming habit, but it is really of horticultural and not botanical significance (Clark, 1982; Dirr and Burd, 1977). All have vibrant yellow fall color and resistance to physiological leaf scorch and leaf blotch caused by Guignardia aesculi.

Aesculus pavia, red buckeye, is a red flowered species native to the southeastern United States. It was the first of the North American Aesculus species to be described by Plukenet in 1696 (Taylor, 1982). It is a small tree or large multi-stemmed shrub with an oval or pyramidal habit often with branches to the ground, and it ranges in height from 3 to 8 m. The four members of the Pavia section are A. pavia, A. flava, A. sylvatica, and A. glabra. All are closely related species, and natural interspecific
hybridization occurs easily in their overlapping native ranges (DePamphilis, 1988; Hardin, 1957b; Hardin, 1957c). *Aesculus pavia* has the ability to hybridize successfully with other species both within and between its section, which makes it a good potential breeding parent. The plant is relatively rare in cultivation; it is difficult to propagate clonally and exhibits a slow growth rate in the landscape (Dirr and Heuser, 1987).

Most of the research, on *Aesculus*, has focused on either nursery production or somatic embryogenesis for the production of secondary plant compounds of medicinal interest. *Aesculus* breeding and improvement efforts have been sporadic and have primarily focused upon selecting from naturally occurring hybrids for form and fall color. Although they are uncommon as landscape plants, *A. parviflora* and *A. pavia* have many desirable horticultural characteristics. Bottlebrush buckeye is resistant to leaf diseases and flowers in summer when few other landscape plants are blooming, whereas the most outstanding feature of red buckeye is its flower color. Hybridizing these two species could create a plant of outstanding horticultural merit. However, a breeding program for the improvement of *Aesculus* would benefit from a fundamental understanding of the species floral and reproductive biology. To this end, this investigation characterized the inflorescences of both *A. parviflora* and *A. pavia* including panicle length, flower production, and the frequency and distribution of complete flowers. Variation in the distribution and frequency of complete flowers was assessed with respect to genotype, year, and crown position. Anthesis patterns within the inflorescence with respect to both rachis position and flower type were described. Mechanical modification (inflorescence mutilation) is a means to increase complete flower production and balance its distribution along the rachis was examined. The
information generated through this investigation is also of botanical significance as it
documents the floral biology of *A. parviflora* (previously minimally described) and
further explores the degree of consistency of andromonecy within and between
individual plants and the species.

MATERIALS AND METHODS

*Plant material*

The plants used for this project were located in central or northeastern Ohio. By
using plants in two locations 150 miles apart, it was possible to extend the blooming
season and to explore the effects of climatic conditions. Plants of various ages and sizes
were used to characterize the inflorescences of *A. parviflora*, specimens designated as
Par1 to Par14, and *A. pavia*, specimens designated as Pav1 to Pav10, (Appendix F). All
specimens were grown in cultivated settings and appeared healthy. Par1, Par6, and
Par14 were grown in full sun. Par10 and Par12 were grown in partial shade, and the
balance were grown in full shade under the canopy of mostly large oak trees. Some
*A. parviflora* specimens were single plants; others could best be described as stands.
All the *A. pavia* studied had exposed crowns and had single trunks.

*Aesculus pollinators*

No formal studies of natural pollinators were conducted during the project.
However, during routine field observations, the types of pollinators as well as their
behavior were noted. Pollinators were identified using the Field Guide to the Birds of
North America (National Geographic Society, 1999) and the National Audubon Society

Inflorescence characterization

The inflorescences were monitored weekly from emergence until the first sign of bud swelling and color change, indicating anthesis was about to begin. Then, observations were made daily until the end of anthesis. The number of inflorescences studied varied with the specimen, for large plants n=25 or more and for smaller plants n=10. Inflorescences were selected from all sides of the plant and all parts of the crown except where noted. Panicles were tagged with tree-marking tape and assigned a number prior to anthesis to avoid any bias in selection. For each panicle, the length, the total number of flowers and the number, and location of the complete flowers were recorded. The percentage of mixed panicles was calculated for each plant. The percentage of complete flowers per panicle was calculated along with the floral distribution. This procedure was repeated for two to four years when possible to determine if the frequency and distribution of complete flowers remained consistent year to year for an individual genotype. Separate panicles from five A. parviflora genotypes were used to ascertain the effect of inflorescence position within the plant upon the frequency or distribution of male and complete flowers. Plants were divided visually into thirds (ground level to 1.5 m, 1.5 m – 3.0 m, and >3.0 m, respectively) and 13 to 25 panicles were sampled from each third.
Spatial pattern of inflorescence anthesis

The pattern of floral anthesis along the rachis over time was studied during the 1998 and 1999 seasons. Prior to the beginning of anthesis, five panicles of Par1 and Par7, ten panicles of Par8, and 30 panicles of Pav2 were randomly selected. Inflorescences were tagged and visually divided into four sections using pieces of floss to facilitate observation. The first day of anthesis for a panicle was established as the day when the first flower had fully expanded and had one dehisced anther. For each flower, its date of anthesis, position on both the rachis and cincinnus, and sex were recorded, after which the flower was removed to avoid confusion. At the end of anthesis, all the panicles were measured for length. Genotypes within species responded similarly and were grouped together prior to analysis. For graphic clarity, anthesis data were combined over years and presented as the mean of every ten flowers with of their position on the rachis as noted.

Inflorescence modification

A panicle modification study was carried out in an attempt to elicit an increase in the number of female competent flowers based on the observations made by Bertin (1980) and Benseler (1968) that damaged panicles seemed to have more complete flowers. Par7, Par8, Pav1 and Pav2 were used for this experiment, because they had been shown to have consistent complete flower production. Pairs of panicles located nearly adjacent to each other, often on the same branch, and for *A. parviflora* occupying all parts of the crown (to diminish either directional or crown effects) were selected for
this study. In early April, for red buckeye, when the panicles were approximately 5-7 cm long and in late May, for bottlebrush buckeye, when the panicles were approximately 8-15 cm long, the rachis of a randomly selected member of each pair was severed in half, and the other was left intact to serve as a control. All inflorescences were allowed to develop. Just prior to anthesis each pair of panicles was diagramed with the cincinni numbered consecutively from the base of the rachis. When anthesis began, each pair of panicles was examined daily. The sex of each flower was recorded on the diagram and then removed to avoid confusion. At the conclusion of anthesis, the panicles were measured. The data from the severed and intact panicles (combined over two years of study) were analyzed and compared for the number, frequency and distribution of complete flowers.

**Data analysis**

Data were analyzed using software and procedures (PROC GLM and/or PROC MEANS) in accordance with the SAS Institute (1990). All field experiments reported herein were organized using a completely random experimental design. Treatment means were described using standard errors of the mean or compared using the Student-Newman-Keuls tests at $\alpha = 0.05$. 
RESULTS AND DISCUSSION

*Putative Aesculus pollinators*

The most common insect visitors to bottlebrush buckeye were as follows: bumblebees (*Bombus* spp.); honeybees (*Apis mellifera*); metallic bees (Hymenoptera: Halictidae); tiger swallowtail butterflies (*Pterourus glaucus*); spicebush swallowtail butterflies (*Pterourus troilus*); red admiral butterflies (*Vanessa atalanta*); buckeye butterflies (*Junonia coenia*); and hummingbird moths (Lepidoptera: Sphingidae). Also, ruby-throated hummingbirds (*Archilochus colubris*) were frequently observed feeding on the uppermost panicles. During peak bloom, the wings of the butterflies would become visibly orange with pollen. The butterflies would begin feeding at the top of the inflorescence and would move downward visiting both buds ready to open and open flowers. Thrips (Thysanoptera: Thripidae) were also observed crawling over the flowers. Both honeybees and bumblebees have been commonly reported gathering nectar and pollen in *Aesculus* species (Benseler, 1968; deWit, 1967; Knuth, 1908; Newell, 1893). Brizicky (1963) stated that bumblebees were considered to be the main pollinators of *Aesculus hippocastanum*. Benseler (1968) developed an extensive list of 43 different insect species including a number of butterflies that visited *Aesculus californica*. Bertin (1980) reported the presence of bumblebees, honeybees, and Ruby-throated hummingbirds feeding on red buckeye. He noted no differences between the visitation rates to staminate or complete flowers. Both bumblebees and hummingbirds tended to visit the outward facing flowers first; the bumblebees tended to visit flowers
at the base of the panicle first, while hummingbirds tended to visit the panicle apex first (Bertin, 1980).

*Inflorescence characterizations of* Aesculus parviflora *and Aesculus pavia*

Inflorescences of both *A. parviflora* and *A. pavia* were formed at the tips of branches. Although it breaks winter dormancy with the other *Aesculus*, bottlebrush buckeye is the only summer blooming *Aesculus* hardy in Ohio. The inflorescences of *A. parviflora* become visible shortly after bud break and are covered with tiny undeveloped flower buds. This differs from *A. pavia* inflorescences, which emerge with more developed cincinni and flower buds. Flowering of bottlebrush buckeye occurs about eight to ten weeks after bud break with the *A. parviflora* forma *serotina* blooming ten days to two weeks after *A. parviflora* (Table 2.1). The spring-blooming *A. pavia* breaks dormancy in Columbus around 5 April and flowers within three to four weeks of bud break.

Eight hundred eighty-eight inflorescences on twelve specimens of bottlebrush buckeye and 718 inflorescences on nine specimens of red buckeye were used to evaluate various panicle traits. Mean inflorescence lengths varied among and within species (Table 2.1, and Appendix G). Inflorescences of *A. parviflora* ranged in length from 26.4–45.8 cm and inflorescences of *A. pavia* ranged from 11.3–17.2 cm. Similar to our data for red buckeye, Coker and Totten (1937) reported mean *A. pavia* panicle lengths of 12–15 cm. The mean number of cincinni per panicle was greater in *A. parviflora* than in *A. pavia* (114.9 ± 3.2 and 24.4 ± 0.5, respectively). However, the average *A. pavia* cincinnus held more flowers (3.6 ± 0.06) than did those of
A. *parviflora* (2.3 ± 0.06). In bottlebrush and red buckeye, each cincinnus held one to three flowers or one to five flowers, respectively. In both species the number of flowers per cincinnus was in part determined by the position of the cincinnus on the rachis. Typically, cincinni in the basal two thirds of the panicle had multiple flowers, and cincinni in the apical third had two flowers except for the tip, where a few cincinni held single flowers. Cincinnus density also varied independently of panicle length. For example, Pav1 and Pav2 have the same average panicle length, but Pav1 has nearly twice as many flowers as Pav2 (Appendix G). The average number of flowers per unit length (floral density) was greater in bottlebrush buckeye than in red buckeye (7.9 flowers/cm and 5.5 flowers/cm, respectively), supporting Coker and Totten’s (1937) assertion that red buckeye inflorescences were less compact than other Aesculus

In *A. parviflora*, the total number of flowers per panicle varied as much as 57% among specimens. All bottlebrush buckeye specimens produced both staminate but only some complete flowers. No genotype produced exclusively staminate panicles, and no functionally pistillate flowers were observed. Pistillate inflorescences were found infrequently in *A. hippocastanum* and in *A. pavia*, where two plants out of many hundreds had only functionally females flowers (Bertin, 1980). The percentage of mixed panicles and the number of complete flowers per panicle varied greatly between specimens. Par9 had the lowest ratio of mixed panicles, (41.4%) where Par2 had the highest, (95.4%). Typically, complete flowers represented a small fraction of the total number of flowers on each panicle (low sex ratio); some specimens produced in excess of 90% staminate flowers. Of the mixed panicles observed, 32.0% of them had 1-9 complete flowers; only 7.1% of the panicles had more than 50 complete flowers. There
was no strong relationship between the percentage of mixed panicles per plant and the
number of complete flowers per panicle. This differs from *A. californica* (California
buckeye) where plants with a high percentage of mixed panicles also had a high
frequency of complete flowers per panicle (Benseler, 1968).

In *A. pavia*, the total flowers per panicle varied as much as 250% from tree to
tree. Sixty-two percent of the inflorescences observed produced at least one complete
flower. As with bottlebrush buckeye, *A. pavia* specimens in this study varied
substantially with respect to the number of mixed inflorescences and the mean number
of complete flowers per inflorescence. Pav10 displayed the greatest mean number of
complete flowers per panicle (16.1); Pav9 exhibited a mean of only 1.4 complete
flowers per panicle. Six specimens averaged fewer than five complete flowers per
panicle. Bertin (1980) found considerable variation between red buckeye
inflorescences; the number of complete flowers ranged from 4-17. Sex ratios within
*A. pavia* panicles were also low (Table 2.2), but not as low as that estimated in a
previous study (1.1%) by Bertin (1980). Of the mixed panicles, 50.1% produced just 1-4
complete flowers and only 4.4% produced more than 20 complete flowers per panicle.
In contrast to Bertin (1980), our red buckeye specimens produced no functionally
pistillate flowers or inflorescences.

In both species, panicle shape, bud volume or flower size could not be used to
predetermine which panicles would be completely staminate from those that were
mixed. Bottlebrush and red buckeye staminate inflorescences were significantly shorter
than their mixed inflorescences by an average of 2.8 cm and 1.3 cm, respectively.
However, because panicle lengths vary widely, this characteristic was not useful in
identifying mixed panicles prior to anthesis. In bottlebrush buckeye, the total number of flowers was similar in both panicle types, but the number of functionally staminate flowers was reduced in the mixed panicles. In contrast, red buckeye mixed inflorescences had, on average, 12 more flowers than did staminate inflorescences, six of which were staminate and six of which were complete. In previous studies, flower buds of both California and red buckeye were dissected at various stages throughout their growth. Differences in gynoecium development were apparent only in buds that were a week or less away from blooming (Benseler, 1968; Bertin, 1980). In the specimens we studied, both types of flowers were also likely formed from similar floral primordia and then developed their sexual functionality late in ontogeny as buds matured.

There are a number of factors, both genetic and environmental, which can influence the number of complete flowers produced. In bottlebrush buckeye, both flower types seemed to be maintained in some proportion on every plant in every year. However, no consistent year-to-year trends in sex ratio were found (Appendix H). The number of complete flowers per plant was similar from year to year in Par3 and Par8. However, in Par11 and Par 14, a high frequency of complete flowers one year tended to be followed by a low frequency of complete flowers the next year. In red buckeye, Pav1, Pav4, Pav6, Pav8, and Pav9 were consistent in their annual production of complete flowers, and in all but Pav1 (sex ratio > 9%), the complete flower production was quite low. In Pav2 and Pav3, the trend in complete flower production was downward over time. Pav7 was unique; in 1998, it produced on average nine complete flowers per panicle, but in 1999, no complete flowers were found in the panicles
sampled. Upon further observation of all of its panicles, no complete flowers were found on the plant. It was the only genotype that produced entirely staminate panicles at any time. Further observations in 2000 were not possible because the plant had been removed the previous autumn. Pav10, which had the highest complete flower ratio, was also removed that year.

In woody species, skewed sex ratios within genotypes have been attributed to plant vigor and/or the amount of resources that can be used for reproduction (Matsui, 1995). Environmental factors affecting floral sex expression in woody species include moisture and nutrient availability, light levels, temperature extremes and predation (Benseler, 1968; Bertin, 1980; Charnov and Bull, 1977; Shifriss, 1956; Wolfe and Drapalik, 1999). Benseler (1968) found that temperature and soil moisture altered the number of complete flowers in California buckeye with plants grown in more moderate and moist climates producing more complete flowers per panicle than those grown in hot and dry climates.

According to Bertin (1980), high light levels favored a higher production of complete flowers in red buckeye. Suo et al. (1995), using *Aesculus turbinata*, reported that panicles from the upper crown (presumably receiving optimum photosynthetically active radiation) were longer and had a greater number of flowers per panicle and a higher sex ratio than those from lower portions of the crown. In this study, five *A. parviflora* genotypes were used to assess the effect of crown position. Inflorescences from the upper third of the plant were more frequently of mixed floral expression, longer, contained a greater number of flowers, and had a higher floral density than those observed in other areas of the plant (Appendix I). However, the greatest number of
complete flowers per panicle was observed in panicles from the middle third of the plant. Benseler (1968) stated that influence of crown position in California buckeye had little effect upon the number of complete flowers within a panicle. Bertin (1980) also found no effect of crown position (height from the ground) or cardinal direction on the frequency of complete flowers, but plants in open habitats had more complete flowers than those in wooded habitats.

Bertin (1980) also stated that considerable genetic variation in sex expression might exist because plants growing near each other often had different frequencies of complete flowers. Additional research over several more years is needed to clearly demonstrate what portion of year-to-year variability in *Aesculus* spp. can be attributed to genetic control and what portion is the result of environmental factors. However, from the year-to-year variation in sex ratio demonstrated by some specimens in our study, it is clear that environment plays a role in the *A. parviflora* and *A. pavia* floral sex expression.

The distribution of complete flowers in mixed inflorescences may influence pollinator strategies in nature and affect the temporal availability of female flowers for controlled plant improvement. Complete flowers were observed in all portions of the *A. parviflora* inflorescence (Figure 2.1a), but within a specimen, rarely were they equally distributed throughout the panicle. Overall, 42.3% of the complete flowers were found in the apical quarter of the panicle, and only 14.3% were found in the basal quarter. Within specimens, complete flower position in the inflorescence was fairly consistent from year-to-year (data not shown). No *A. parviflora* inflorescences observed had complete flowers restricted to the basal cincinni. These results are similar to California
buckeye where 55% of the panicles studied produced their complete flowers in the top third and 21.4% produced them along their entire length (Benseler, 1968).

In contrast, 62% of all the complete flowers of red buckeye were found in the basal quarter of the panicle (Figure 2.1b). The apical quarter had only 3.9% of the complete flowers. Only Pav1, Pav2, and Pav10 produced any complete flowers in the upper half of their panicles. The distribution of complete flowers in panicles of these specimens was consistent with that reported in other studies of red buckeye (Bertin, 1980), *A. turbinata* (Suo et al., 1995), *A. sylvatica* (Coker and Totten, 1937) and *A. hippocastanum* (Newell, 1893), where complete flowers appeared mainly in the basal portions of the panicles. Bertin (1980) reported that the distribution of complete flowers was uneven. More complete flowers were clustered toward the central axis of the rachis and on the outward facing side of the panicle rather than toward the trunk of the tree. He suggested that, the complete flowers in this position would be more likely to receive pollen from another plant based on the feeding habits of bumble bees. Also, large and heavy fruits would receive greater support in a basal position on the inflorescence.

*Spatial pattern of inflorescence anthesis*

The inflorescences of *A. parviflora* and *A. pavia* underwent floral anthesis over a period of six to eleven days, and in both species, inflorescence anthesis progressed basipetally to acropetally (Figure 2.2a and b). The pattern of acropetal blooming substantiated the descriptions of anthesis reported earlier (Bertin, 1980; Coker and Totten, 1937; Hardin, 1956; Newell, 1893; Suo et al., 1995) for other *Aesculus* species.
This differs from the anthesis pattern within *A. californica* where flowering advanced either acropetally or from both ends towards the center (Benseler, 1968). Within a given cincinnus, the flower bud closest to the rachis was the first to mature and subsequent development proceeded outward. In bottlebrush buckeye, flowers at the base, middle and top portion of the inflorescence bloomed within the first three, five and seven days of first flower anthesis, respectively (Figure 2.2a). Anthesis rates were similar in red buckeye, where flowers in the lower third of the panicle open in the first four days of anthesis, and flowers in the upper third open day six or later (Figure 2.2b).

In both species, functionally staminate flowers opened at a steady rate for the first seven days of anthesis and then slowed toward the end of blooming (Figure 2.3a, and b). However, the rate of complete flower anthesis was different. In bottlebrush buckeye, complete flowers were not observed until day five (Figure 2.3a). Thereafter, the number of complete flowers increased sharply before opening at a consistent rate through the remainder of the blooming period. In contrast, both types of red buckeye flowers were presented on the first day of anthesis (Figure 2.3b). More than 30% of the available complete flowers were open on the first day of anthesis, but ceased to be available for pollination after day 5. Staminate and complete flowers of other *Aesculus* species seem to open at slightly different times (Benseler, 1968; Brizicky, 1963; Newell, 1893). Our work confirms Bertin's (1980) observation that the peak of complete flower production occurred before the peak of the staminate flower production in red buckeye. This blooming sequence has implications for self-compatibility and outcrossing.
Inflorescence modification

The ability to manipulate the number of complete flowers would be useful to increase the number of possible pollinations. Panicles of *A. californica* that had been naturally damaged had a much higher ratio of complete flowers than undamaged panicles (Benseler, 1968). Bertin (1980) was able to increase the frequency of complete flowers within red buckeye panicles that had been severed intentionally from 1.5% to 6.7%. This phenomenon was further supported with some preliminary observations of bottlebrush buckeye where damaged panicles seemed to have a higher frequency of complete flowers than undamaged panicles.

Inflorescence modification proved effective in reducing the length of severed panicles to about one-half of their intact counterparts as well as reducing their total number of flowers at maturity (Table 2.3). Both specimens exhibited an increase in the number of complete flowers. In Par7, all 14 of the severed and 12 of the intact inflorescences produced mixed panicles. The increase in complete flowers was significant for Par7, where the average number of complete flowers more than doubled. Similar results from Par8 were not significant because the number of complete flowers was highly variable among panicles within treatments. The severing treatment seemed to increase the number of mixed panicles for both genotypes. The distribution of complete flowers in the intact panicles was similar to that observed earlier with other inflorescences, with the majority of the complete flowers being found in the top quarter of the panicle. The severed panicles had the majority of their complete flowers in the upper portion of the panicles for both genotypes.
Panicles of *A. pavia* were also severed in an attempt to increase the number of complete flowers. For both specimens, the overall length of the inflorescences was reduced by half or more along with a reduction in the total number of flowers (Table 2.3). The severing treatment did not increase the number of complete flowers for Pav1, a plant that averaged 15 complete flowers per inflorescence over the course of the study. The severing treatment increased the average number of complete flowers from 3.1 to 9 for Pav2. The distribution of complete flowers for intact panicles was similar to that of the inflorescences studied previously. The distribution of the complete flowers was not changed by the treatment. The complete flowers were more frequently found in the basal portion of the panicle. Our results indicate that this technique might prove to be beneficial in increasing the number of possible pollinations in both species.

**Implications for cross pollination**

A primary objective of our research program is focused upon optimizing methods of hybridization within the genus *Aesculus* in order to develop plants of superior horticultural quality. The timing of complete flower anthesis and the distribution of complete flowers along the panicle are important factors to consider for hybridization success. The distribution of complete flowers was quite different between the two species studied. Bottlebrush buckeye produced 42.3% of its complete flowers in the apical quarter of its inflorescence. Complete flowers were not observed until the fifth day and were often the last flowers to open on the panicle. In contrast, red buckeye produced 62.2% of its complete flowers in the basal quarter, and complete flowers were present on the first day of anthesis and were unavailable by the sixth day.
Both intra- and interspecific cross pollinations among and between these species and with other *Aesculus* may be aided by the knowledge of specific temporal and spatial patterns in complete flower anthesis developed in this study. Functionally staminate flowers proceeded through anthesis at almost the same rate for both species despite differences in the environment, suggesting the ample availability of pollen for intra-specific crosses.

More importantly, this study indicated the availability of complete flowers to be the predominant factor limiting opportunities for controlled cross-pollination between *A. parviflora* and *A. pavia*. Both species had similar sex ratios of approximately 5.5%, and were similar to those reported for other *Aesculus* species [i.e., *A. turbinata*, 1.3 to 9.3% (Satio et al., 1990; Suo et al., 1995); *A. pavia*, 1.1% (Bertin, 1980); *A. californica*, <5% (Benseler, 1968); and *A. sylvatica*, 7.0% (Coker and Totten, 1937)].

The ability to modify sex ratios of plants has been an important tool in plant improvement. Floral development is controlled by hormonal and nutritional levels within floral tissues which are influenced by genetic expression and environmental triggers (Bernier et al., 1981, Chailakhyan and Khrianin, 1987). While the results of the addition of plant growth regulators and changes in temperature are well characterized for herbaceous species, these effects are less well known in woody ornamentals. Using *A. turbinata*, Yoshino (1996) found only a combination of both uniconazole-p and stem girdling produced an increase in the formation of inflorescences as well as the ratio of complete flowers on those inflorescences. Benseler (1968) unsuccessfully attempted to alter the frequency and distribution of complete flowers by using exogenous IAA, NAA or a synthetic kinetin on young panicles. The presence of specific hormones and their
ratio within floral tissues likely alters the frequency of complete flowers in *Aesculus*. However, as Benseler (1968) stated, experiments elucidating hormonal influences are hard to conduct on large established plants in the field due to a lack of clonal material, differences in plant age and weather conditions. The application of exogenous plant growth regulators, especially auxin, was beyond the scope of this study but offers an interesting avenue of research in the future.

Physical modification of inflorescences, presumably altering hormonal levels, may offer another avenue to alter sex ratio. Benseler (1968) observed that naturally damaged panicles of California buckeye had a higher frequency of complete flowers. Of the 227 naturally mutilated inflorescences observed, 84% had complete flowers, compared to 47% of the undamaged panicles. However, he was not able to induce an increase in the number of complete flowers under controlled experimental conditions. Bertin (1980) was able to induce an increase in the frequency of complete flowers from 1.5% to 6.7% in *A. pavia*. In this study, panicle modification increased the frequency of complete flowers for both specimens of *A. parviflora* and one of *A. pavia*. It may have also increased the percentage of mixed panicles for the more recalcitrant *A. parviflora* f. *serotina*, Par8. In addition to increasing the number of female competent flowers, severing the panicles in half had another plant breeding benefit. It reduced the number of functionally staminate flowers that must be removed, thereby reducing the possibilities for pollen contamination and the amount of time needed to work on each panicle.
<table>
<thead>
<tr>
<th>Species and region</th>
<th>Mean date of first flower anthesis ± S.E.</th>
<th>Mean date of last flower anthesis ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aesculus parviflora</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Ohio&lt;sup&gt;z&lt;/sup&gt;</td>
<td>15 June ± 1 day</td>
<td>3 July ± 1 day</td>
</tr>
<tr>
<td>Northeastern Ohio&lt;sup&gt;y&lt;/sup&gt;</td>
<td>3 July ± 2 days</td>
<td>20 July ± 2 days</td>
</tr>
<tr>
<td><strong>Aesculus parviflora forma serotina</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northeastern Ohio&lt;sup&gt;x&lt;/sup&gt;</td>
<td>22 July ± 4 days</td>
<td>12 August ± 1 day</td>
</tr>
<tr>
<td><strong>Aesculus pavia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Ohio&lt;sup&gt;w&lt;/sup&gt;</td>
<td>28 April ± 2 days</td>
<td>20 May ± 3 days</td>
</tr>
<tr>
<td>Northeastern Ohio&lt;sup&gt;v&lt;/sup&gt;</td>
<td>9 May ± 3 days</td>
<td>30 May ± 4 days</td>
</tr>
</tbody>
</table>

<sup>z</sup>Specimens examined were Par3, Par9, Par10, Par11 and Par13
<sup>y</sup>Specimens examined were Par1, Par2, Par6, Par7 and Par14.
<sup>x</sup>Specimens examined were Par8 and Par12.
<sup>w</sup>Specimens examined were Pav1 and Pav4.
<sup>v</sup>Specimens examine were Pav2, Pav3, Pav7 and Pav8.

Table 2.1. Anthesis periods of *Aesculus parviflora* and *Aesculus pavia* in central and northeastern Ohio.
Table 2.2. Inflorescence characteristics of *Aesculus parviflora* and *Aesculus pavia*.
<table>
<thead>
<tr>
<th>Specimens</th>
<th>Treatment</th>
<th>Panicle length (cm)</th>
<th>Flowers per panicle</th>
<th>Frequency of mixed panicles (%)</th>
<th>Complete flowers per panicle</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. parviflora</em></td>
<td>Par7&lt;sup&gt;z&lt;/sup&gt;</td>
<td>Severed</td>
<td>14.3 b&lt;sup&gt;y&lt;/sup&gt;</td>
<td>143.1 b</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Intact</td>
<td>28.7 a</td>
<td>262.9 a</td>
<td>85.7</td>
<td>20.0 b</td>
</tr>
<tr>
<td></td>
<td>Par8&lt;sup&gt;x&lt;/sup&gt;</td>
<td>Severed</td>
<td>13.9 b</td>
<td>113.6 b</td>
<td>81.8</td>
</tr>
<tr>
<td></td>
<td>Intact</td>
<td>26.1 a</td>
<td>213.3 a</td>
<td>45.5</td>
<td>2.5</td>
</tr>
<tr>
<td><em>A. pavia</em></td>
<td>Pav1&lt;sup&gt;w&lt;/sup&gt;</td>
<td>Severed</td>
<td>6.3 b</td>
<td>58.7 b</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Intact</td>
<td>15.4 a</td>
<td>110.8 a</td>
<td>100.0</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>Pav2&lt;sup&gt;v&lt;/sup&gt;</td>
<td>Severed</td>
<td>5.8 b</td>
<td>39.6 b</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Intact</td>
<td>12.2 a</td>
<td>66.2 a</td>
<td>90.0</td>
<td>3.1 b</td>
</tr>
</tbody>
</table>

<sup>z</sup>Number of treatment pairs = 14.
<sup>y</sup>Means within treatment pairs lacking postscripts are not statistically different at the $\alpha = 0.001$ level as determined by the analysis of variance.
<sup>x</sup>Number of treatment pairs = 11.
<sup>w</sup>Number of treatment pairs = 25.
<sup>v</sup>Number of treatment pairs = 10.

Table 2.3. The effect of inflorescence modification on inflorescence length, the number of flowers per panicle, frequency of mixed panicles and the number of complete flowers per panicle for *Aesculus parviflora* and *Aesculus pavia*. 

132
Figure 2.1. The position of complete flowers within *Aesculus* panicles. Values represent the percentage of complete flowers in successive quarter panicles of selected *Aesculus* specimens. Bars with black, white, dark gray and light gray fills indicate basal, second, third and apical quarters of the panicles, respectively.
Figure 2.2. The spatial patterns of inflorescence anthesis in *Aesculus* panicles. Values represent the mean day of anthesis ± S.E. of flowers in ten position increments where flower positions were numbered from the base to the apex of the panicle. a) *A. parviflora*, n = 20; b) *A. pavia*, n = 30.
Figure 2.3. The rate of floral anthesis for staminate and complete *Aesculus* flowers. Values represent the mean percentage ± S.E. of the total staminate or complete flowers that had reached anthesis by a given day during panicle flowering. Open and filled circles indicate values for complete and staminate flowers, respectively. a) *A. parviflora*, n = 20; b) *A. pavia*, n = 30.


CHAPTER 3

AESCULUS POLLEN VIABILITY AND LONGEVITY UNDER STORAGE

INTRODUCTION

*Aesculus* (commonly referred to as the genus of buckeyes and/or horsechestnuts) is found in the northern hemisphere in both the new and old worlds. It is composed of thirteen species and their interspecific hybrids, including *Aesculus × carnea* (Hardin, 1957; Upcott, 1936). *Aesculus flava, Aesculus glabra, Aesculus pavia* and *Aesculus sylvatica* are found in overlapping ranges in the eastern United States and bloom in spring. *Aesculus parviflora* is also native to the southeastern United States. However, it is isolated by its summer flowering period. Through the use of allozymes, DePamphillis (1988) confirmed the formation of natural hybrid complexes between *A. pavia* and *A. flava, A. glabra,* and *A. sylvatica* and detected high levels of gene flow wherever the range of two or more species overlap.

Phenotypic selections have been made from natural hybrids and genetic potential appears to exist for the production of useful landscape plants *Aesculus 'Autumn Splendor'* (Bergmann et al., 1989) and *Aesculus × 'Homestead'* (N. Evers, personal communication). *Aesculus parviflora* possess many desirable horticultural
characteristics including: summer bloom, distinctive panicles of flowers with their extended stamens and styles, vibrant yellow fall color, and resistance to physiological leaf scorch and leaf blotch caused by *Guignardia aesculi*. Cappiello (1999) describes *A. flava* as having fall foliage which develops a distinctive orange color reliably each autumn, and *A. sylvatica* as producing flowers with an unusual coloration pattern. The outstanding red flowers and good plant habit of *A. pavia*, along its ability to hybridize successfully with other members of the genus make it a good potential parent in a breeding program. Hybridizing these species could create plants of outstanding horticultural merit with showy floral displays. However, an *Aesculus* breeding program with these highly heterozygous woody plants with divergent flowering periods would benefit from the development of a method for evaluating pollen viability among potential male parents and a means to preserve that pollen.

Viability can be measured in four ways. The most direct measure of pollen viability is in vivo pollen germination on stigmatic surfaces and ultimately seed production. While this method is the only true measure of whether or not a particular pollen source is capable of fertilization, it also has limitations. Factors such as a lack of suitable flowers to pollinate, issues of receptivity and/or compatibility, and extended seed development period may affect the accuracy of this assessment. Therefore, many researchers employ staining or in vitro pollen germination techniques to estimate pollen viability in vivo. Non-vital staining with I$_2$KI, aceto-carmine, aniline (cotton) blue and the like can be used to evaluate if a pollen grain contains cytoplasm and has a normal morphological shape. Vital stains
like TTC (2,3,5-triphenol tetrazolium chloride), MTT [3(4,5-dimethyl thiazolyl-2)-
2,5-diphenyl tetrazolium bromide] FDA (fluorescein diacetate) or peroxidase gauge
viability by the presence or absence of metabolic activity. Staining methods have
been found to be unreliable since they may overestimate actual pollen germination
(Oberle and Watson, 1953; Pearson and Harney, 1984; and Werner and Chang,
1981). The utilization of an in vitro pollen germination method either on a defined
agar medium or in a hanging drop is considered the most consistent method to
estimate the viability for both fresh and stored pollen (Werner and Chang, 1981).
However, the optimal conditions for in vitro pollen germination vary species to
species.

The factors that can influence in vitro germination on a solidified medium are
pH, agar, inorganic nutrients such as $\text{H}_2\text{BO}_3$, $\text{Ca(NO}_3)\text{2}$, $\text{Mg(SO}_4)\text{2}$, and $\text{KNO}_3$, and
carbohydrate source and concentrations. The environmental factors that influence
germination are relative humidity and temperature. A neutral pH is favored by most
species and this was true for *Aesculus turbinata* (Suo, et al., 1995) and *Corylus
avellana* (Kim et al., 1985). Sucrose is the most commonly used carbohydrate source
although glucose and fructose and lactose have been tested for some species (Kim et
al., 1985; Maguire and Sedgley, 1997). However, the concentration of sucrose varies
widely from 5% to 55% (Adhikari and Campbell, 1998; Honda et al., 2002; Hughes
et al, 1991; Purewal and Randhawa, 1945). Boric acid has been shown both to
promote pollen germination and pollen tube elongation and is most often used in a
concentration range of 10-100 mg L$^{-1}$ (Gershoy and Gabriel, 1961; Visser et al.,
1977;). Brewbaker and Kwack (1963) and Thompson and Batjer (1950) reported that
the addition of calcium enhanced pollen germination and the growth and stability of
the pollen tube. The temperature under which pollen is germinated also impacts its
viability and for most species maximum germination and tube growth is achieved
between 20°C to 25°C. Below 5°C pollen germinates slowly, if at all, and above
35°C the pollen tubes fail to elongate properly and burst (Johri and Vasil, 1961).

Pollen storage is an important tool for plant breeders when hybridizing plants
that are isolated by either location or flowering time. It is routine with other species
to store pollen both within seasons, to bridge the gap between early and late
flowering cultivars, and between bloom seasons. Both storage temperature and
duration affect the longevity of stored pollen. Pollen has been stored under various
temperature regimes: room temperature, under refrigeration at 4°C, frozen at 0°C,
–20°C, –40°C or –80°C, or cryopreserved in liquid nitrogen at –196°C. Although
storage conditions vary species to species for most woody plants, storing pollen
under low temperature and low humidity seemed to conserve pollen viability (Johri
and Vasil, 1961). For example, pollen of Hydrangea spp. stored at room temperature
lost viability in five days. Under refrigeration viability declined over a five month
period of time but under –20°C retained germinability for up to 11 months (Kudo
and Niimi, 1999). Craddock et al. (2000); Honda et al. (2002); Koopowitz et al.
(1984); and Sato et al. (1998) found that pollen stored at or below –20°C tended to
retain its viability longer. Cryopreservation often resulted in the best retention of
viability over periods of up to ten years without significant loss of viability (Parfitt
and Almehdi, 1984). In most studies, the loss of germination capacity was a function
of time in storage with extended storage periods resulting in diminished to nonexistent germination.

Studies evaluating in vitro pollen germination, pollen quality and preservation were undertaken as part of a larger investigation of *Aesculus* reproductive biology. Given the limited number of female competent flowers present in *Aesculus* and other reproductive limitations, a method to assess pollen viability and optimally maintain that viability in storage would be helpful prior to undertaking breeding activities and genetic experimentation. The objectives of this study were as follows: 1) to identify in vitro assay conditions that would result in an adequate estimate of the viability of fresh or stored pollen among and within a diverse group of *Aesculus* species; 2) to determine the array of temperatures at which fresh pollen of spring blooming and summer blooming species are capable of germinating; and 3) to study the effects of storage temperature and storage duration on the retention of pollen viability as an aid for hybridization between plants with asynchronous flowering periods. In addition, the pollen of various *A. parviflora*, *A. pavia*, *A. × carnea*, and *A. × plantierensis* were examined by electron microscopy for physical characteristics (dimensions and regularity of shape) as an alternative means to assess viability.

**MATERIALS AND METHODS**

*Plant material*

Pollen was collected from fifteen genotypes representing five species, *A. flava*, *A. hippocastanum*, *A. parviflora*, *A. pavia*, *A. sylvatica*, and interspecific
hybrids, *A. × carneae* and *A. × plantierensis*. The healthy, mature specimens of *Aesculus* used for this project were located at three sites in central and northeastern Ohio (Appendix F).

Pollen collection and handling

For each genotype, pollen was collected from freshly dehisced anthers from functionally staminate or complete flowers between 07:00 and 12:00 hrs. Freshly dehisced anthers were recognized by an abundance of bright orange pollen on their surfaces. Pollen was gathered in bulk into a 60 X 15 mm Falcon petri plate (Becton Dickinson & Co., Lincoln Park, NJ) after rubbing the anthers over a sheer nylon mesh to remove debris. Pollen was then divided into approximately 5 mg aliquots and placed into No. 00 gelatin capsules (Eli Lilly & Co., Indianapolis, IN). Aliquots of fresh pollen were examined for their ability to germinate within two hours of their collection. For storage treatments, capsules of pollen were placed into polypropylene vials (13 mm diameter, Wheaton Omni-Vial, Daigger®, Inc. Vernon Hills, IL) containing a desiccant (CaCO₃). Vials were capped, labeled and placed in a 705 ml snap-lid food storage container (Rubbermaid Inc., Wooster, OH) with additional desiccant and then stored at –20°C or at –80°C in freezers for periods of one, three, six or twelve months. Gelatin capsules containing pollen for scanning electron microscopy (SEM) studies were held under refrigeration at 4°C for a period not longer than 10 days.
SEM observations of pollen

Pollen grains of nine genotypes from *A. parviflora*, *A. pavia*, *A. × carnea* or *A. × plantierensis* were applied to brass stubs and sputter coated with gold for ten minutes using Polaron E-5100 Sputter Coater. Scanning electron micrographs were taken with a Hitachi S-3500N variable pressure Scanning Electron Microscope at various magnifications. The polar axis lengths and equatorial widths of 100 pollen grains for each genotype were measured in µm from micrographs. As an assessment of pollen viability, five fields of pollen per genotype were inspected for the presence of normal, unusually large or misshapen pollen grains.

Optimum test conditions for in vitro germination of pollen

To optimize in vitro assay procedures, germination of both fresh and stored pollen from ten genotypes from *A. flava*, *A. hippocastanum*, *A. parviflora*, *A. pavia*, *A. sylvatica* and *A. × carnea* was tested using media formulations modified from Brewbaker and Kwack (1963). To prepare the media, 100 mg L$^{-1}$ H$_2$BO$_3$, 150 mg L$^{-1}$ Ca(NO$_3$)$_2$, were dissolved in double distilled water and 10 g L$^{-1}$ Bacto-agar (Difco Laboratories, Detroit, MI) was added along with varying concentrations of sucrose (AR grade, Fisher Scientific, Pittsburgh, PA). Solutions were adjusted to pH 7.0, then autoclaved for 15 min following the procedures of Kim et al. (1985) and Suo et al. (1995). Test variables examined included factorial combinations of sucrose concentration (0, 5, 10, 15, 20, 25, 30, and 35%) and incubation temperatures (10, 15, 20, 25, 30, and 35°C). After autoclaving, each media formulation was distributed in 10 ml aliquots into test plates under a laminar flow
hood. Petri plates were covered with their lids and media was allowed to solidify in the hood, and then used immediately or stored under refrigeration (4°C) until used. If refrigerated, media were allowed to equilibrate to room temperature before pollen was applied.

Pollen was applied uniformly to surfaces of the various media with sable hair artist’s brushes (Nos. 1-4, Yasutamo Co., Japan). Plates were then recovered, sealed with Parafilm (Amer. Natl. Can, Neenah, WI) and placed into germination chambers varying in temperature as described above. Pollen was incubated in the dark for eight hours and then stained with a 1% solution of aniline blue (Allied Chemical Co., Rochester, NY) in lactophenol to aid in observation (Darlington and LaCour, 1976). Pollen was examined under the dissecting microscope at 80X magnification. Three fields were examined per replicate plate and a pollen grain was considered germinated if it had a pollen tube at least as long as the diameter of the pollen grain. The three fields were averaged to produce a replicate value. Three replicate plates for each combination of sucrose concentration and germination temperature for each genotype were observed.

**Effects of storage, storage temperature and storage time**

Fresh pollen or pollen stored at –20°C or –80°C for 1, 3, 6, and 12 months after collection was evaluated for germinability under optimized test conditions using pollen sources and techniques described above. At the end of a storage period, three replicate samples of pollen for each storage time X storage temperature X specimen combination were sampled for germination. Pollen was allowed to warm
to room temperature prior to being applied to the various media. Unused portions of pollen samples were discarded after plating. For all storage experiments, pollen was incubated for 24 hours in the dark prior to staining and was evaluated as outlined above. Plates not immediately examined were stained and stored under refrigeration (4°C) until counted.

**Data analysis**

All statistical analyses were carried out using software and procedures in accordance with the SAS Institute (1990). The inverse sine transformation (arcsine-square root of X) was performed on all replicate values of percent pollen germination prior to statistical analysis (Steele and Torrie, 1980). When species was considered as an independent variable in any analysis, weight coefficients were applied to individual species in accordance with the number of genotypes studied. Where appropriate, treatments were compared by orthogonal contrasts. Means were described using standard errors of the mean or compared using the Student-Newman-Keuls tests at

\[ \alpha = 0.05 \]

To determine the optimum in vitro test conditions for pollen germination, the full data set containing results from all germination temperature, media sucrose concentration, storage treatment, and specimen combinations was analyzed as a 6 X 8 X 9 X 11 factorial design in three replications. For this analysis, the variable “storage treatment” was created to represent fresh pollen germination and germination of pollen stored at various times and temperatures as nine separate
treatments. The effects of germination temperature on fresh pollen and the effects of storage period and temperature on stored pollen were determined using appropriate data subsets containing test entries representing optimum temperatures and/or optimized sucrose concentrations (Figure 3.1).

The study comparing pollen dimensions among specimens was analyzed as a completely random design. Comparisons were made both within and among species. The predicted viability from the SEM observations was compared with actual pollen germination under optimal conditions.

RESULTS AND DISCUSSION

In a preliminary experiment, pollen germination was assessed for functionally staminate and complete flowers from two specimens of *A. parviflora* and two of *A. pavia*. There were no differences in germination percent between the flower type (84.3% and 83.8% for *A. parviflora* and 82.0% and 80.8% for *A. pavia* respectively) and with an overall $p = 0.8976$. Therefore, pollen from both flowers types was bulked for all other experiments. Similarly, no differences in pollen germination were observed between pollen collected from pin or thrum flowers of *Fagopyrum esculentum* (Adhikari and Campbell, 1998).

Optimization of in vitro germination test conditions

Polito and Luza (1988) argued for the necessity of using both fresh and stored pollen in developing optimized in vitro tests for pollen viability. When species and
test factors (germination temperature and sucrose concentration) were analyzed across all storage treatment levels, all main effects were highly significant, 

\[ p < 0.0001 \] (Figure 3.2). Germination was observed at all test sucrose concentrations and germination temperatures, but when the sucrose concentration was 0% or exceeded 25% or when temperatures exceeded 30°C, test conditions appeared to be unfavorable for pollen germination. Main effect means for sucrose concentrations and for germination temperature were highest at 20% and 15°C, but these means were not significantly different from those at 15% sucrose or 20°C germination temperature, respectively. Interactions between species and sucrose concentration and between species and germination temperatures were also statistically significant. However, much of this interactivity appeared to be associated with the extremes in test conditions (Figure 3.3). At the midrange of test conditions, all species followed similar response patterns with the test conditions of 20% sucrose and 15°C germination temperature appearing to optimize estimates of pollen viability in most cases. Therefore, we adopted these conditions as being optimum for further studies of pollen viability.

In *Aesculus chinensis*, the highest percentage of pollen germination and greatest pollen tube length was observed on medium containing 20% sucrose over both fresh and stored pollen samples (Ostrolucka and Bencat, 1987). When no sucrose was added to the medium few pollen grains germinated and the pollen tubes were short compared to a concentration of 20%. Similarly, when no sucrose was added to the germination medium used for fresh pollen of *Aesculus turbinata* less than 85% of the pollen grains germinated, whereas the highest germination rate
(95%) was achieved on medium containing a 10% sucrose concentration (Suo et al., 1995). Bessonova (1992) observed a germination rate of 75.5% for *A. hippocastanum* pollen on medium without salts containing 10% sucrose, and incubated at 25°C. *Aesculus* in general were similar to *Cornus florida*, *Pistacia vera*, *Corylus avellana*, *Carya illinoensis*, and *Populus maximowiczii* which all expressed optimal pollen germination using 20% sucrose (Craddock et al., 2000; Kim et al., 1985; Polito and Luza, 1988; Rajora and Zsuffa, 1986; Wetzstein and Sparks, 1985).

In a series of studies, pollen of *Pyrus communis*, of *Corylus avellana*, of *Prunus dulcis*, and of six species of *Delphinium*, was found to germinate optimally at 15°C (Godini et al., 1987; Honda et al., 2002; Kim et al., 1985; and Vasilakakis and Porlingis, 1985). Kim et al. (1985) additionally found that when the temperature was increased from 15°C to 30°C that pollen germination decreased and that, cultivars responded differently to changes in temperature.

The viability of fresh pollen was found to be significantly different than stored pollen (*p = <0.0001*) by orthogonal contrasts derived from the analysis of a data set containing entries from optimized test conditions. Therefore, the behavior of fresh pollen is discussed separately from that of stored pollen. Since all species and specimens studied germinated greater than 80%, they would all make suitable pollen parents for the breeding projects.

*Response of fresh pollen to different germination temperatures*

Pollen from each species was analyzed for its response to temperature using a data set optimized for sucrose concentration. Both main effects of species and
temperature were highly significant ($p < 0.0001$). Fresh pollen germinated well in excess of 80% for all the species with the exception of $A. \times plantierensis$, a reported triploid (Upcott, 1936). Pollen of the latter species did not germinate in excess of 5% under any conditions and was excluded from further analyses. Pollen viability differed significantly among species, but the extremes within the range were separated by only 8.8% (Figure 3.4). Within species, germination rates remained steady over the 10°C to 30°C range. The only adverse temperature to the germination of fresh pollen was 35°C for all species. There was also a significant interaction between species and germination temperature ($p = 0.0074$), but no biologically relevant pattern could be discerned from an array of the interactive means (Figure 3.5). Orthogonal contrasts exploring patterns of germination temperature and pollen germination percentage were not significant for spring flowering vs. summer flowering species ($p = 0.12$), or between members of the Pavia section vs. other spring flowering species ($p = 0.9099$). Genotypes within species varied in response to germination temperature as much or more than species within $Aesculus$ (Figure 3.6). In $A. turbinata$, the highest germination rate in excess of 90% was achieved using 20°C to 25°C.

The relationship between in vitro and in vivo pollen germination and subsequent growth has been explored for a number of species. Vasilakakis and Porlingis (1985) demonstrated that in vitro pollen germination and tube growth of two cultivars of $Pyrus communis$ at test temperatures from 10° to 23°C simulated that observed in vivo over the same range of ambient temperatures. However, the rate of in vitro pollen germination and that observed in the field were both reduced at
lower temperatures. In *Juglans*, a consistent relationship between the minimum temperature requirement for pollen germination and the earliness of bloom was identified (Luza et al., 1987). Using the pollen of early and late blooming cultivars *Prunus dulcis*, Godini et al. (1987) found a relationship between the rate of pollen germination and flowering period. The pollen of the late blooming cultivars germinated more rapidly at warmer temperatures. Pollen germination and pollen tube growth rates of *Betula pendula* were consistent under different temperature regimes in both in vitro and in vivo and evidence was found for genotype X environment interactions in pollen tube growth (Pasonen et al., 2000). The highest rate of pollen germination occurred at 20°C for *Zingiber officinale*, but germination was not significantly different over the range of 14° to 26°C. Likewise, in vivo germination occurred over this same range of temperatures with varying rates of pollen tube elongation with the highest rates occurring at 17° to 20°C (Adaniya, 2001). The wide range of temperatures over which fresh pollen of *Aesculus* germinated in vitro is similar to range of temperatures to which pollen would be exposed in its natural environment during the flowering period in Central Ohio (Table 3.1). Having this broad range of temperatures under which pollen germination and tube growth can occur may convey an adaptive advantage for successful fruit set throughout the species native ranges.

Although pollen germination percentages were adequate across a wide range of temperatures, temperature did appear to alter the rate of pollen tube elongation. A representative series of photomicrographs from in vitro test involving Pav6 is shown in Figure 3.7. From visual observation, the maximum pollen tube length at 8 hrs
after plating was observed when tests were conducted at 15°C and 20°C, whereas only minimal pollen tube growth was observed at 35°C. These results were similar to those reported for *A. turbinata*, where the optimal temperature for fresh pollen germination and pollen tube growth was 20°C (Suo et al., 1995). Vasilakakis and Porlingis (1985) also observed different rates of pollen tube growth at various temperatures for two cultivars of pear.

*Effects of storage temperature and period on pollen germination*

There was no significant difference in the measurable pollen viability between −20°C, or −80°C (*p* = 0.7146) by orthogonal contrast. Therefore, data from both storage temperatures was combined for further analyses. Martinez-Gomez et al. (2002) also found no advantage to almond (*Prunus dulcis*) pollen storage at −80°C; almond pollen stored at −20°C was equally viable as that stored at the lower temperature.

Species retained varying amounts of pollen viability over the storage period (Figure 3.8). The effect of the storage period on in vitro pollen germination was also found to be highly significant. Pollen viability for all species was comparable to that of fresh pollen for the first month in storage. At three months, pollen viability was still approximately 50%. However at six months, and to an even greater extent, at 12 months, pollen viability declined sharply. At the 12-month storage period, only 24% of the pollen germinated.

Species differed significantly with respect to their viability at 3 and 6 months of storage, but not at one or twelve months of storage (Figure 3.9). For *A. flava*,
A. pavia and A. × carnea, differences in pollen viability were observed after just three months in storage and declined steadily thereafter. Other species maintained viability through the first six months in storage. These results were similar to those by Ostrolucka and Bencat (1987) for A. chinensis which showed that pollen stored for three months maintained nearly the same germination rate as that of fresh pollen but then germination dropped to zero at six months of storage.

Although there were differences among specimens within a given species, as to the rate and degree of decline in pollen viability over time, all followed a similar trend to that of the genus as a whole (Figure 3.10). After twelve months of storage, all the specimens of A. flava, A. parviflora and A. pavia had pollen viability estimates below the threshold set for effective in vivo fruit set.

Freezing had been shown to extend pollen longevity of a number of woody species over that of storage at room temperature or refrigeration (Craddock et al., 2000; Kudo and Niimi, 1999). However, differences in the rate of decline of in vitro germination have also been observed among other samples of frozen pollen. For example, Hydrangea macrophylla retained 36% germination after 11 months storage at –20°C while Hydrangea arborescens failed to germinate (Kudo and Niimi, 1999). In Delphinium, only four of species tested at six months of storage at –30°C maintained pollen viability above 50% (Honda et al., 2002). The longevity of frozen stored pollen of Pistacia vera was quite variable with three genotypes having less than 20% germinability after four months of storage while the fourth retained greater than 80% germination until twelve months (Polito and Luza, 1988).
Maintaining a high rate of pollen viability after storage is essential if the pollen is going to be used for hybrid production. Johri and Vasil (1961) established the following relationship between in vitro pollen germination percentage and fruit set in field-grown apple (*Malus × domestica*) and pear (*Pyrus communis*): less than 20% – nil to poor; from 20% to 40% – poor to moderate; from 40% to 60% – moderate to normal; and over 60% – normal. Using their criteria, storage of pollen for six months at –20°C or –80°C may be detrimental to the effectiveness of *A. flava*, *A. pavia* and *A. × carnea* as male parents in a breeding program, whereas storage for twelve months at these conditions is inadvisable for any of the *Aesculus* species studied.

**SEM observation of pollen**

The polar axis length and equatorial width were measured for *A. parviflora*, *A. pavia*, and *A. × carnea*. In overall size, *Aesculus parviflora* pollen grains were the smallest and *A. × carnea* were the largest. There were no significant differences between the two specimens of *A. parviflora* and *A. parviflora* forma *serotina*. This study reports similar results to those of other published studies (Table 3.2).

The presence of unusually large (possibly unreduced) pollen grains was not observed but misshapen, and/or abnormal pollen grains were present. A comparison was made between the percentage of “morphologically normal” grains and the pollen germinated under optimal conditions. In all cases, viability could not be predicted based on the appearance of the pollen grains. It seems that many normal looking pollen grains were unable to germinate under optimal conditions.
The pollen biology of *Aesculus* appears conducive to controlled intraspecific and interspecific hybridization. When analyzed across all species and storage treatments, the optimal test for in vitro pollen germination consisted of a medium containing 100 mg L\(^{-1}\) H\(_2\)BO\(_3\), 150 mgL\(^{-1}\) Ca(NO\(_3\))\(_2\), 1% agar and a sucrose concentration of 20% and incubation temperature of 15°C. Fresh pollen germinated in excess of 80% for all species. Within the temperature range used, the only temperature which adversely affected germination was 35°C. The rate of pollen tube elongation was influenced by the incubation temperature, with 15 to 20°C resulting in the maximum growth. Therefore, pollen fertility as assessed by in vitro germination does not seem to be a limiting factor in the creation of hybrids. Storing pollen at –80°C did not confer extended pollen longevity over that stored at –20°C as indicated by pollen germination tests. *Aesculus* pollen can be stored at –20°C for three months and in some species up to six months to aid in interspecific hybridization, but the length of time in storage negatively impacted viability. Therefore, pollen should be stored as briefly as possible and used within the same bloom season. An alternative pollen preservation method such as cryopreservation should be investigated for its effectiveness for long-term storage of *Aesculus* pollen.
<table>
<thead>
<tr>
<th>Species</th>
<th>Flowering period</th>
<th>Average temperature ranges during flowering periods (°C)</th>
<th>Minimum (°C)</th>
<th>Maximum (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. flava</td>
<td>28 April – 17 May</td>
<td>9.3 – 22.7</td>
<td>1.0</td>
<td>32.2</td>
</tr>
<tr>
<td>A. hippocastanum</td>
<td>26 April – 18 May</td>
<td>9.0 – 22.6</td>
<td>1.0</td>
<td>32.2</td>
</tr>
<tr>
<td>A. parviflora</td>
<td>15 June – 3 July</td>
<td>17.0 – 28.9</td>
<td>8.3</td>
<td>34.4</td>
</tr>
<tr>
<td>A. pavia</td>
<td>28 April – 20 May</td>
<td>9.6 – 22.1</td>
<td>1.0</td>
<td>32.2</td>
</tr>
<tr>
<td>A. sylvatica</td>
<td>3 May – 20 May</td>
<td>10.5 – 24.0</td>
<td>1.2</td>
<td>32.2</td>
</tr>
<tr>
<td>A. × carnea</td>
<td>1 May – 18 May</td>
<td>10.1 – 23.5</td>
<td>1.2</td>
<td>32.2</td>
</tr>
</tbody>
</table>

Values represent mean minimum and maximum temperatures during flowering periods averaged over four years.

Values represent extreme temperature events within the four flowering periods of 1997-2000.

Table 3.1. The range of temperatures during flowering periods of *Aesculus* species. Values represent climatological data during the 1997 – 2000 seasons.
<table>
<thead>
<tr>
<th>Species and data source</th>
<th>P(^z) (µm)</th>
<th>E(^z) (µm)</th>
<th>P/E(^z)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. parviflora</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current study(^y)</td>
<td>25 (24 – 26)</td>
<td>15 (13 – 16)</td>
<td>1.71 (1.55 – 1.87)</td>
</tr>
<tr>
<td>Pozhidaev (1995)</td>
<td>NR (^x) (24 – 27)</td>
<td>NR (17 – 19)</td>
<td>NR</td>
</tr>
<tr>
<td>Kim et al. (1997)</td>
<td>25 (24 – 26)</td>
<td>13 (11 – 15)</td>
<td>1.90 (1.67 – 2.18)</td>
</tr>
<tr>
<td><strong>A. parviflora f. serotina</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current study(^w)</td>
<td>25 (23 – 27)</td>
<td>15 (14 – 16)</td>
<td>1.71 (1.55 – 1.91)</td>
</tr>
<tr>
<td>Kim et al. (1997)</td>
<td>21 (18 – 24)</td>
<td>15 (14 – 19)</td>
<td>1.40 (1.27 – 1.68)</td>
</tr>
<tr>
<td><strong>A. pavia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current study(^y)</td>
<td>32 (28 – 35)</td>
<td>22 (20 – 24)</td>
<td>1.46 (1.31 – 1.58)</td>
</tr>
<tr>
<td>Pozhidaev (1995)</td>
<td>NR (30 – 37)</td>
<td>NR (22 – 26)</td>
<td>NR</td>
</tr>
<tr>
<td>Kim et al. (1997)</td>
<td>30 (29 – 32)</td>
<td>20 (19 – 22)</td>
<td>1.49 (1.32 – 1.63)</td>
</tr>
<tr>
<td><strong>A. × carnea</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current study(^y)</td>
<td>36 (34 – 38)</td>
<td>22 (18 – 23)</td>
<td>1.78 (1.50 – 1.94)</td>
</tr>
<tr>
<td>Heath (1984)</td>
<td>32 (29 – 36)</td>
<td>22 (18 – 26)</td>
<td>1.45 (1.39 – 1.50)</td>
</tr>
</tbody>
</table>

\(^z\)Polar axis length (P), equatorial width (E) and their ratio (P/E). Values represent means with ranges reported in parentheses.

\(^y\)n = 200.

\(^x\)NR = not reported.

\(^w\)n = 100.

\(^\prime\)n = 300.

Table 3.2. Pollen dimensions of various *Aesculus* species as found in the current study and reported in the literature.
Full Data Set (14,256 entries)
6 Species (11 genotypes)
9 Storage trts. (fresh + 4 durations X 2 storage temps.)
6 Germination temps.
8 Media sucrose concs.
3 Reps per trt. combination

- Weight genotype data to equalize effects among species
- Remove data from sub-optimum media sucrose concs.
- Remove data from sub-optimum germination temps.

Partial Data Set A (162 entries)
6 Species
9 Storage trts. (fresh + 4 durations X 2 storage temps.)
3 Reps per trt. combination

- Remove data from stored pollen trts.

Partial Data Set B (18 entries)
6 Species

- Add data from fresh pollen at all germination temps.

Partial Data Set C (108 entries)
6 Species
6 Germination temps.
3 Reps per trt. combination

- Remove data from fresh pollen trts.

Partial Data Set D (144 entries)
6 Species
8 Storage trts. (4 durations X 2 storage temps.)
3 Reps per trt. combination

- Determining optimal in-vitro test
- Comparing fresh vs. stored pollen viability
- Comparing fresh pollen viability among species
- Assessing range of temps. over which pollen of each species is capable of germinating
- Determining the effects of pollen storage temp. and duration on pollen viability

Figure 3.1. Schematic representation of data sets used in statistical analyses.
Figure 3.2. The main effects of media sucrose concentration and germination temperature on pollen germination of various *Aesculus* species. Values represent mean germination percentages and their standard errors averaged across species and pollen storage treatments. Effect means (bars of similar color) with similar superscripts are not significantly different according to SNK tests ($p=0.05$).
Figure 3.3. Interactive effects of *Aesculus* species with test parameters of media sucrose concentration and germination temperature. Values represent means and standard errors for each species X test factor combination. Filled circles, open circles, filled triangles, open triangles, filled squares and open squares correspond to Par, Pav, Fla, Syl, Car, and Hip, respectively.
Figure 3.4. The main effects of *Aesculus* species and germination temperature on fresh pollen germination. Values represent mean germination percentages and their standard errors. Effect means (bars of similar color) with similar superscripts are not significantly different according to SNK tests ($p=0.05$).
Figure 3.5. Interactive effects of *Aesculus* species and germination temperature on fresh pollen germination. Values represent means and standard errors for each species X germination temperature combination. Filled circles, open circles, filled triangles, open triangles, filled squares and open squares correspond to Par, Pav, Fla, Syl, Car, and Hip, respectively.
Figure 3.6. Interactive effects of *Aesculus* genotypes and germination temperature on pollen germination. Values represent means and their standard errors for each specimen X germination temperature combination. Filled circles correspond to Fla1, Par9 and Pav1, respectively. Open circles correspond to Fla2, Par7 and Pav6, respectively. Filled triangles correspond to Pav3.
Figure 3.7. Representative photomicrographs (80X) of *Aesculus* pollen germination at various temperatures 8 hrs. after plating. Figures 3.7 a-f depict pollen of Pav6 germinated at 10, 15, 20, 25, 30 and 35°C, respectively.
Figure 3.8. The main effects of *Aesculus* species and storage period on stored pollen germination. Values represent mean germination percentages and their standard errors. Effect means (bars of similar color) with similar superscripts are not significantly different according to SNK tests (*p*=0.05).
Figure 3.9. Interactive effects of *Aesculus* species and storage period on stored pollen germination. Values represent means and standard errors for each species X germination temperature combination. Filled circles, open circles, filled triangles, open triangles, filled squares and open squares correspond to Par, Pav, Fla, Syl, Car, and Hip, respectively. Pollen with germination percentages >40% or <20% were presumed to be adequate or inadequate for pollination and fruit set, respectively. Pollen with germination percentages falling inside the shaded area may or may not effect adequate fruit set.
Figure 3.10. Interactive effects of *Aesculus* genotypes and storage period on pollen germination. Values represent means and their standard errors for each specimen X storage period combination. Filled circles correspond to Fla1, Par9 and Pav1, respectively. Open circles correspond to Fla2, Par7 and Pav6, respectively. Filled triangles correspond to Pav3. Pollen with germination percentages >40% or <20% were presumed to be adequate or inadequate for pollination and fruit set, respectively. Pollen with germination percentages falling inside the shaded area may or may not effect adequate fruit set.
CITED REFERENCES


INTRODUCTION

Propagators and seed producers seek ways to enhance germination and emergence potential and the successful production of vigorous seedlings. Biotic factors affecting seed quality include: the genetics of the seed itself, the vitality of the maternal tree supporting seed production, competition for resources between developing fruits/seeds on the plant, the number of seeds within a fruit and the frequency of insect predation (Bertin, 1980). Abiotic factors such as light interception, water availability and quality, climatic patterns and edaphic factors also influence seed quality (Daws et al., 2004; Delouche, 1980). The production of useable seedlings is further limited by impediments to germination and emergence, the presence of abnormalities and poor seedling vigor. Current recommended procedures for maximizing the germination and emergence of Aesculus seeds are largely based on experience and not on systematic
studies, whereas scientifically-based information on *Aesculus* seedling development is limited.

Seeds of *Aesculus*, along with *Castanea* and some forms of *Acer* and *Quercus* are classified as recalcitrant (Connor and Bonner, 2001); when desiccated beyond a critical point, recalcitrant seeds loose their ability to imbibe water and to metabolize stored food reserves. They often fail to germinate, even when planted under favorable circumstances (May, 1963; MacDonald, 1986). Due to their high metabolic activity, recalcitrant seeds have limited life spans that are not significantly prolonged by storage at optimal conditions (Pammenter et al., 1994). Therefore, exacting collection and handling techniques are necessary to maximize the success rate in managing recalcitrant seeds. Unprotected *Aesculus* seeds desiccate at a rate proportional to their exposure to air, and when exposed to air for as little as 16 days, *A. parviflora* seeds failed to germinate (Fordham, 1987; MacDonald, 1986). *Aesculus flava* exhibited a 50% loss of viability when exposed to low humidity and room temperature under laboratory conditions (Levy, 1984). Connor and Bonner (2001) reported *A. pavia* seed that had been allowed to desiccated prior to storage exhibited a reduction in viability within three months of storage at 4°C and that properly hydrated seeds did not survive more than one year. Further, *Aesculus* seed should be handled and stored in small lots to avoid excessive accumulation of metabolic heat and subsequent dehydration (“sweating”) and limit fungal deterioration (Browse, 1982). Recalcitrance in *Aesculus* significantly impacts propagation options, as seed must be planted in a timely manner to avoid desiccation.
Seed dormancy, or the lack of it, also influences propagation strategies for *Aesculus*. *Aesculus* species exhibit varying forms and degrees of dormancy, but, according to experiential evidence, most require or benefit from stratification. Suggested controlled stratification conditions for *A. flava*, *A. glabra*, *A. hippocastanum*, *A. indica*, *A. sylvatica*, and *A. turbinata*, range from 1.0-4.5°C for periods of 3 to 5 months (Bhagat et al., 1989; Bir, 1992; Browse, 1970; Fordham, 1960; Furlow, 1991; Rudolf, 1974; Widmoyer and Moore, 1968). Stratification success is time dependent, and within a seed lot may be limited by several factors: loss of vigor due to depletion of stored reserves, deterioration and subsequent mold proliferation and pre-germination of seeds during the chilling period. Furlow (1991) suggests that stored seeds of *A. flava*, *A. glabra* and *A. hippocastanum* exhibit decreased germination rates after 7 to 8 months of storage. In a study by Widmoyer and Moore (1968), the germination rate of intact *A. hippocastanum* seeds was greatly reduced after one year of chilling, despite the fact that the seed showed no outward signs of deterioration. These authors also noted the presence of pregerminated seeds during the stratification period. In general, seeds that germinate while in storage produce weak seedlings and are often discarded by propagators. Likewise, storage temperatures higher than optimum resulted in substantial losses in viability and increased decay (Widmoyer and Moore, 1968; Wright, 1985). With an incremental series of storage treatments, May (1963) outlined direct relationships between temperature and time and seed losses due to mold. When *A. hippocastanum* seed was stored at 7.5°C, more than 44% of the seed lot was lost; but at −1°C, losses were limited to 2-5 % after storage for 180 days.
Pritchard and his coworkers (Daws et al., 2004; Pritchard et al., 1999) found that variability among *A. hippocastanum* seed sources, as influenced by the environment and/or maternal genotype moderated the need for stratification. In some instances, little or no stratification was required for adequate germination. *Aesculus indica* seeds required stratification to break dormancy, but the chilling period was minimal (Maithani et al., 1990). In this species, germination percentages were as high as 60% after only 15 days of treatment and rose to 79% after treatment for 30 days. Dirr and Heuser (1987) recommended a 30 day stratification period for *A. pavia* seed lots. In contrast, Bir (1992) and Rudolf (1974) stated that *A. pavia* had no dormancy requirement and would germinate without stratification. Bir (1992) concluded that recently-ripened *A. pavia* seeds will germinate immediately when planted out of doors in early autumn.

According to several authors, (Bir, 1992; Dirr and Heuser, 1987; Flint, 1966; Fordham, 1987) *A. parviflora* will germinate without pretreatment, but Birr (1992) suggested a minimal stratification period of 30 days to be potentially beneficial for seed germination in this species. Seeds of species such as *A. californica*, native to warmer regions of the world, do not require stratification (Dirr and Heuser, 1987; Furlow, 1991).

Following controlled stratification, *Aesculus* seeds are typically sown in early spring, or container-produced under controlled conditions until weather permits planting outdoors. Alternately, the seeds may be sown in seedling production fields immediately after harvest. Fall planting may be suitable for satisfying the stratification requirements of *A. flava, A. hippocastanum, A. sylvatica, A. turbinata, A. indica, A. chinensis, A. wilsonii* and *A. × carnea* seeds under natural conditions (Bir, 1992; Bhagat et al., 1993; Wright, 1985), while minimizing seed moisture losses (Browse, 1970; MacDonald
1986). Under the fall-planting scheme, germination and emergence may not become evident until the following spring (Browse, 1970). In the direct sowing technique, the timing of germination, emergence and exposure of the epicotyl to the environment is of critical concern. Ideally, seeds would be fully stratified and become germination-competent in spring. The success of this technique can be limited by cold temperatures or excessive soil moisture which may foster seed decomposition (Gibson-Watt, 1997). Moreover, if not protected, newly emerged seedlings can be damaged by spring frosts (Browse, 1970). Likewise, seedlings with precocious epicotyl expansion in the late fall may be lost to winter injury. *A. parviflora* seeds produce a radical in the fall, but the plumule is believed to express epicotyl dormancy (Fordham, 1987; MacDonald, 1986). Under this condition, shoot emergence is conditioned only by an intervening cold period, which might be adaptable to the fall-planting system.

*Aesculus parviflora* and *A. pavia* are species valued for their aesthetic contribution to the landscape but still have limited availability, linked at least in part to difficulties in propagation (Bir, 1995). Successful plant production centers on the propagator’s ability to manage the seed-related difficulties of high moisture content, recalcitrance and varying dormancy requirements. Unfortunately, optimum management strategies are not well-characterized. The objective herein was to study the effect of stratification period on the following: 1] germination competency (quantity and uniformity of radical development); 2] emergence competency (quantity and uniformity of epicotyl development); 3] frequency of seed loss due to mold; 4] types and frequency of abnormalities (albinism, various shoot development problems); and 5] seedling efficiency (the production of usable seedlings) within seed lots of *A. parviflora*.
and *A. pavia*. This information may, in turn, be employed by propagators to increase the availability of these colorful but yet infrequently produced species.

**MATERIALS AND METHODS**

*Plant materials and seed sample preparation*

Fruit of two specimens of *A. parviflora* (Par2 and Par7) and four specimens of *A. pavia* (Pav1, Pav3, Pav4 and Pav 9) were monitored during development and harvested when pericarps began to dry and split (Appendix F). Seeds were removed from the fruits manually. Each seed was cleaned and observed for damaged. Seeds from each specimen were individually placed into a graduate cylinder containing a 250 to 500 ml volume of water to determine their density. Density was used as a measure of seed maturity (fill); the fraction of seeds that floated consisted largely of seed coat tissues. Unfilled seeds were discarded and the remaining seeds were towel-dried. For each specimen, intact seeds were surface-disinfested using a cloth saturated with a 10% (0.6% sodium hypochlorite) Chlorox® (Chlorox Company; Oakland, CA) bleach solution. The seeds were allowed to air dry, mixed thoroughly and randomly divided in lots of 12 or 25 seeds each as supplies allowed. Random 25 seed samples of each species were dried to a constant weight in a forced-air tissue dryer (approximately 50°C) for moisture analysis. Each seed lot to be stratified was placed into 1 qt., heavy-duty sealable plastic bags (Ziplock®, S. C. Johnson and son. Inc., Racine, WI), and seed lots to be stratified were placed under refrigeration at 4°C.
*Stratification*

The experiment was conducted over two seasons, employing eight treatments (stratification periods of 0, 30, 60, 90, 120, 180, 240 and 360 days) and two replications per treatment, per specimen within species, per year. The seed lots were arranged randomly during the stratification and germination phases of the experiment. Seedlings were handled as individual plants.

During stratification, seed lots were monitored for the appearance of mold at approximately 21 day intervals. When a moldy seed was encountered, it was removed and recorded. The remaining seeds within the lot were disinfested again as described above, and then returned to storage in a new plastic bag. Pregerminated seeds (i.e., those that germinated during stratification) were retained with the seed lot for the remainder of the treatment period.

*Germination tests*

Germination tests were conducted using standardized seed testing techniques (where seed germination was measured using favorable conditions of nearly sterile medium, high humidity, and a temperature controlled environment) to estimate the maximum plant producing ability. A sheet of seed germination paper (Anchor Paper Co., Eau Claire, WI) was folded to fit into a (15 X 30) cm clear plastic food grade re-sealable clam-shell tray and then moistened with approximately 40 ml of distilled water. Excess water was eliminated. Following the stratification period, each seed lot was removed from refrigeration, opened and examined for moldy or pre-germinated seeds. The frequency of soft and moldy seeds was recorded and the decayed seeds were then
discarded. Intact seeds were wiped to remove condensation or the clear, sticky exudates that accumulated on the seed surfaces. Seeds were placed in the clam-shell trays with the hilium facing down. The trays were sealed and held at ambient room temperature (23 ± 3°C) with indirect light (16 hour photoperiod). Each tray was examined every three days for the presence of germinated or moldy seeds. Again, when a moldy seed was encountered, it was removed and recorded. The remaining seeds within the lot were disinfested using a towel saturated Chlorox solution as described above, and then placed in a new tray lined with moistened germination paper. A seed was considered germinated if its radical was equal to the diameter of the seed. The germination date of each seed was recorded.

Seeds that developed a radical during storage were held in germination trays with their original seed lots for 24 hrs to verify their viability. Thereafter, viable pre-germinated seeds were handled similarly to intact seeds; their date of germination was recorded as Day 0.

Emergence and seedling growth

Germinated or pre-germinated seeds were removed from the tray and placed into a 1500 ml square black plastic container (Classic 250 sqr, Nursery Supply Company Inc., Fairless Hills, PA) with a mixture of 50% coir air soil-less medium (Sun Gro Horticulture, Bellevue, WA) and 50% perlite (Therm-O-Rock, East, Inc., New Eagle, PA). Seeds were planted by inserting the radical into the medium to insure good root soil contact while leaving the seed exposed. Containers were placed in a greenhouse under moderately-controlled conditions (25 ± 3°C). Containers were checked for
seedling emergence at approximately 3 day intervals. A seedling was considered to have emerged when the epicotyl was first observed protruding from its seed coat. An emergence date was recorded for each seed. Seedlings were observed periodically for abnormal development, primarily albinism or continuous growth (i.e., the lack of epicotyl dormancy). Seedling evaluations (e.g., height, vigor, etc.) were made at the end of the first growing season. For this study, a useable seedling was described as one with a well developed root system and a shoot of at least 5 cm and a well developed bud and would be likely to survive until the next growing season.

Data analysis

Statistical analyses were performed using SAS software and procedures (SAS Institute, 1990). Prior to analysis, all data expressed as percentages were transformed using the inverse sine (i.e., the arcsine-square root of X) procedure (Steele and Torrie, 1980). Analyses of variance were conducted for transformed data with PROC GLM using a factorial model in two species and eight chilling treatments. Main effect means were compared using Student-Newman-Keuls tests at $\alpha = 0.05$. Interactive means were presented with their respective standard errors of the mean. Box plots (SigmaPlot, Systat Software Inc., Point Richmond, CA) were used to illustrate the variability (i.e., synchrony) among individuals within each population for rates of seed germination and emergence for each species treatment combination.
RESULTS AND DISCUSSION

Initial seed moisture content

Unlike orthodox seeds, recalcitrant seeds do not undergo a desiccation process prior to abscission from the plant. The moisture content of a seed has been used as a measure of its physiological condition and maturation (Farrant and Walters, 1998; Pritchard et al., 1996; Uniyal and Nautiyal, 1996). In *A. hippocastanum*, seeds drop from a moisture content of 75% at 95 days after anthesis to approximately 50% at abscission (Tompsett and Pritchard, 1993). Upon further investigation, a range of moisture contents (49-57%) was observed over a number of species, locations and years (Pritchard et al., 1996; Suszka, 1980; Uniyal and Nautiyal, 1996). The moisture content of *A. parviflora* and *A. pavia* were 63.6% and 65.1% respectively. This value for *A. pavia* was similar to that reported by Connor and Bonner (2001) of 64.4%, but was higher than the 56% Young and Young (1992) and could be indicative of either differences in development or in handling procedures.

Seed germination frequency and rate

The germination of *A. pavia* was significantly higher than *A. parviflora* across all stratification periods (Figure 4.1). In general, seed quality was consistent within a species but not between species, with *A. pavia* being larger in size, and thus, superior in quality to that of *A. parviflora*. Bhagat et al. (1993) reported a similar finding with *A.
indica; medium and large seed classes (14.5 - 29.0 g) had a germination of 71% compared to 51% for the small seed class (7.0 - 14.5 g). The relationship between climate, seed size and germination has been established (Aizen and Woodcock, 1996; Daws et al., 2004).

Across species, the stratification treatments from 0 - 120 days all resulted in germination greater than 80% (Figure 4.1). However, species responded differently to stratification treatments (Figure 4.2). The highest percentage of germination in A. parviflora was observed in the no-chill treatment, but germination frequency remained fairly constant through 90 days of stratification. At 120 days of stratification, the frequency of mold development increased to approximately 20%, either during chilling or after setting the seeds in the germination trays. All A. parviflora seeds stratified for 180 or more days became soft and moldy and did not germinate.

Aesculus pavia seeds also performed well (83% germination) without chilling. Connor and Bonner (2001) also reported excellent germination of A. pavia seeds without stratification. These results with A. pavia, were substantially higher than for untreated A. flava seeds which had an average germination frequency of 31% under room temperature germination conditions (Levy, 1984). However, a brief stratification period has been recommended for maximizing seed germination in this species (Dirr and Heuser, 1987). Supporting Dirr and Heuser (1987), germination percentages increased with minimal to moderate chilling, and rose to a maximum germination of 95% at 60 days of treatment. The germination of A. pavia seeds remained more consistent across stratification treatments than that of A. parviflora, with commercially acceptable levels of germination (above 80%) being maintained through 180 days of
stratification. A decline in germination with a corresponding increase in the number of moldy seeds was observed at the 240 and 360 stratification periods.

In both species, physiological barriers to successful germination increased over time, most significantly due to an increased susceptibility to mold and a greater tendency towards pregermination. Losses due to mold rose in both $A.\ parviflora$ specimens from a low of approximately 8% at 90 days of chilling to 100% in seed lots chilled for 180 days of chilling or more. With extended chilling, the seeds darkened in color and began to produce a clear sticky exudate. Even the seeds that had not softened with 180 days of stratification were completely covered in mold with 72 hours of being placed in the germination tray. An increase in the number of seeds lost to mold was also observed for $A.\ pavia$. Approximately 10% of these seeds became moldy after 120 days stratification but this percentage rose to nearly 90% at 360 days of stratification. Similarly, between 14-25% of all $A.\ hippocastanum$ seed chilled for 6 months were lost to mold (May, 1963). Benseler (1968) reported seeds of $A.\ californica$ that were stored for more than 90 days deteriorated due to mold.

Seeds that initiated the germination process while undergoing stratification (i.e., pregerminated seeds) were more susceptible to damage during handling and planting, had reduced vigor when removed from chilling and were more susceptible to pathogens than seeds that remain quiescent. The phenomenon of pregermination occurred in $A.\ pavia$, and was first observed in one specimen after 60 days of stratification. Thereafter, the frequency of this problem increased, reaching an average of $20 \pm 3\%$ across four specimens at the 120 day stratification period. The frequency of pregermination appeared of decrease for stratification periods exceeding 120 days.
However, this trend may be an artifact, as the increased seed losses to mold after prolonged stratification treatment resulted in reduced germination percentages of all seeds. In this study, pregermination was not observed in *A. parviflora* while the seeds were being stratified. This result differs from Fordham (1987) who observed germination and subsequent decay of *A. parviflora* seeds when placed in a plastic bag and stratified at 4 °C. May (1963) and Widmoyer and Moore (1968) also observed the pregermination of *A. hippocastanum* seed after 6 or 12 months of chilling. According to Pritchard and his coworkers (Daws et al., 2004; Pritchard et al., 1996; Pritchard et al., 1999; Steadman et al., 2003), the germination base (cardinal) temperature of seed lots (i.e. the threshold temperature at which seeds are physiologically competent to germinate) decreases over time under stratification conditions. Moreover, pregermination events occur more rapidly as stratification temperatures are reduced or as time in storage increases because the magnitude of base temperature reduction is highly influenced by these factors. This condition was further influenced by the seed source and environmental and physiological conditions during seed development. Seeds produced in a warmer climate had a lower stratification requirement and became germination-competent more rapidly than seeds produced under colder conditions, but Pritchard and his coworkers could not determine if this was strictly environmentally induced or if genetics also played a role (Daws et al., 2004).

Additionally, stratification enhanced the uniformity of germination in both species. In the sample population of *A. parviflora* that was not chilled, 90% of the seeds germinating within approximately 45 days (Figure 4.3a). However, with each increment of chilling, up to 90 days, the period necessary for 90% germination of
sample population members was decreased by approximately 10 days. The positive effect of stratification on germination synchrony was even more evident in *A. pavia* (Figure 4.3b). With increased chilling, the germination period for 90% of the seeds decreased from over 100 days for seeds without stratification to approximately 10 days or less for populations from the 90 or the 120 day stratification periods. This reduction in germination time was comparable for all *A. pavia* specimens tested (data not shown). In early studies, the frequency of germination for untreated *A. glabra* and *A. hippocastanum* seeds was quite low (less than 25%); seeds that did germinate within these populations did so over an extended period greater than 230 days (USDA Forest Service, 1948). Sixty days of stratification reduced the time necessary for *A. californica* seeds to germinate to 10 days as compared to the 53 days required for untreated seeds to germinate (Benseler, 1968). Maithani et al. (1990) further demonstrated the positive effect of a brief stratification period on *A. indica* seeds; upon stratification, the germination frequency rose from 12% to 79% and the germination period dropped from an average of 83.3 days to 14.5 days. In *Chamaecyparis thyoides*, a member of the Cupressaceae, both the seed germination frequency and rate improved with chilling (Jull et al., 1999), indicating the benefits of minimal stratification to be widespread among higher plants.

*Shoot emergency frequency and rate*

While the frequency and rate of germination have been examined to some extent in *Aesculus*, there are few reports documenting frequency and rate of shoot emergence or the production of useable seedlings. Approximately 75% of all *A. pavia* seeds in this
study produced a shoot (Figure 4.4). *Aesculus parviflora* seeds exhibited an emergence rate which was approximately 11% lower than that of its counterpart. The frequency of emergence was greater than 70% for all stratification periods of 0-120 days, but then declined to less than 15% for 240 and 360 days of stratification. The effect of stratification on emergence differed among species (Figure 4.5). Emergence frequency in untreated *A. parviflora* seeds was nearly 90%; light or moderate stratification did not increase emergence capacity, as the percentage emergence remained constant through the 90 day chilling treatment. However, by 120 days of stratification, the total emergence declined to less than half of the initial population.

Among untreated seed lots, the mean frequency of shoot emergence in *A. pavia* was approximately 15% less than that of *A. parviflora*. Chilling treatments did, however, improve shoot emergence in this species as emergence frequency rose with stratification and was maximized at 60 days of chilling to just over 90%. However, about 4% of the population receiving either no chilling or inadequate chilling did not express dormancy of any type. Shoot emergence and growth among these variants were accelerated, with shoot development patterns that were similar to those emerging from adequately chilled seeds. This supports Bir’s (1992) observation that a portion of *A. pavia* seed planted in warm soil would germinate immediately, and subsequently be subjected to winter injury. Prolonged stratification periods were detrimental to the emergence of *A pavia* seeds. Shoot emergence percentage declined only when the stratification period was 180 days or more.

Overall, the patterns shoot emergence among and within species and stratification treatments (Figures 4.4 and 4.5) were remarkably similar to those
exhibited for germination percentage (Figures 4.1 and 4.2, respectively). Overall, the germination and emergence performance of *A. pavia* seeds were superior to those of *A. parviflora*, which appeared to be linked to initial seed quality. In this study, approximately 10.6 and 10.8% of the seeds of *A. parviflora* and *A. pavia*, respectively, had a fresh weight of 5.0 g or less, but in *A. parviflora* only 27.3% of the seeds produced were more than 10.0 g compared to 63.5% of the *A. pavia* seed. Shoots of *A. hippocastanum* also exhibited a response to chilling, with the epicotyls expanding within 15 days of germination; the rate of shoot development was influenced by the germination temperature (Suszka, 1980).

The effect of stratification on synchrony of emergence was less uniform in *A. parviflora* than in *A. pavia* (Figure 4.6). In *A. parviflora*, 60 days of stratification compressed the time necessary for 90% emergence to approximately 30 days. Stratification periods less than or more than 60 days increased 90% emergence rates as much as two-fold. Seedlings treated for extended periods of time may be weakened, and therefore, require an extended period of time to elongate an epicotyl. Chilling enhanced emergence uniformity in *A. pavia* (Figure 4.6b). Seed lots chilled between 60 and 180 days showed 90% shoot emergence in 57 days or less, improving the emergence performance by as much as 20 days over that of untreated seed.

Although chilling resulted in a greater degree of uniformity of germination and emergence periods in both species, *A. parviflora* seedlings expressed epicotyl dormancy whereas those of *A. pavia* did not. In this condition, the newly-formed shoots expanded to approximately 1.0-3.0 cm long, but then no further growth was observed. Seedlings expressing epicotyl dormancy desiccated in a normal greenhouse environment.
However, when these plants received a second six- to eight-week period of chilling in a minimally heated poly-house, and were then returned to permissive conditions, the shoots elongated normally. All in all, stratification did not replace or alleviate the need for a second chilling treatment for the resumption of shoot growth in most *A. parviflora* seedling in this study.

*Aesculus parviflora* produced a second class of seedlings (designated herein as accelerated growth) that expressed no epicotyl dormancy. The shoots of accelerated growth seedlings elongated quickly and expanded two or more pairs of leaves. The frequency of this seedling class was between 2-4.5% in both specimens and was observed in four of the stratification treatments. In other studies, this phenomenon was observed in seeds which were directly sown outdoors in seed beds (Fordham, 1987). The precocious growth of these seedlings did not have sufficient time to acclimatize to the harsh winter conditions and these seedlings were lost. In their rate of development, accelerated growth seedlings resembled the no-chill variants of *A. pavia*.

*Seedling production and evaluation*

The production of useable seedlings is the end point in a long series of developmental processes and maximizing this fraction of the population is the fundamental goal for nurseries. The frequency of usable seedlings was similar for both *A. parviflora* and *A. pavia* and was approximately 40% (Figure 4.7). The 60 day stratification period resulted in the greatest number of useable seedlings. In the 0, 30, and 90 day treatments, approximately half of the initial seeds developing into seedlings, but less than 5% of the initial seeds produced plants in the two longest stratification
periods. The effect of stratification treatment upon the number of usable seedlings also differed among species (Figure 4.8). In *A. parviflora*, the production of usable seedlings was adequate in the 0, 30, 60 and 90 day chilling treatments, but decreased significantly at the 120 day period. *Aesculus pavia* produced the maximum number of seedlings in the 60 day chilling treatment. The extended time needed to both germinate a radical and produce a shoot may have contributed to the smaller number of useable seedlings observed in the 0 and 30 day treatments. The percentage of viable seedlings declined gradually over the 90 to 360 periods, with less than 10% of the initial seeds becoming usable plants at either the 240 or 360 day chilling periods.

The impact of stratification on the growth of useable seedlings was measured for both *A. parviflora* and *A. pavia* (Figure 4.9). The duration of the chilling treatments influenced seedling height and total dry weight (both shoot and root) accumulation similarly in both species. *Aesculus parviflora* seedlings were taller averaging 7.3 cm and accumulating more dry weight than the 4.0 cm *A. pavia* seedlings. Seedlings from the 0, 30, 60, and 90 treatments were approximately 5.5-6.6 cm tall, whereas the 120 and 180 seedlings were only one third to one half as tall as the seedlings from the other stratification periods.

The distribution of seedlings within five growth classes was established for each of the stratification periods (Figure 4.10). *Aesculus parviflora* consistently produced approximately 10% albino seedlings in both specimens tested. Albino plants typically expanded to a height of 5-8 cm and produced one or occasionally two pairs of true leaves. They survived for several weeks before exhausting the stored reserves and then deteriorated and died. Albinism has been noted previously in other *A. parviflora*
populations at a frequency of 16% (Fordham, 1987). It has been postulated that the expression of this lethal mutation is detected as the result of inbreeding among isolated individuals. In contrast, only two mutants lacking chlorophyll were observed in *A. pavia*. The one mutant was a typical albino but the other produced pink leaves; neither survived one month following shoot growth.

In both species, the frequency of seedlings that had died or that were stunted increased when the stratification period was not optimal. Given that recalcitrant seeds typically have high metabolic rates, conditions that extend the period between harvest and germination and/or emergence, such as inadequate chilling or prolonged stratification periods may reduce the cotyledonary reserves and limit the amount of energy available for seedling growth and development. Hoshizaki et al. (1997) studied the role of the cotyledons as an energy reserves in natural stands of *A. turbinata* seedlings damaged by predation. Seedlings which had their hypogeal cotyledons removed by predation had a lower survival rate and re-growth potential than those in which the hypogeal cotyledons remained intact.

In this study, stunted seedlings developed slender epicotyls that ceased elongation at the first node, produced leaves that were subject to marginal leaf necrosis, and set a terminal bud. Both leaves and terminal buds of stunted seedlings were approximately half the size of those found on usable seedlings. Seedlings that did not survive also produced true leaves, but lacked a viable terminal bud and exhibited symptoms of desiccation over time. Plant losses appeared to be a consequence of the seedling’s inability to tolerate environmental stress and pathogenic attack. Aizen and
Woodcoock (1996) also reported a positive relationship between seed size [cotyledon mass] and seedling survival under stressful conditions for *Quercus rubra*.

Factors limiting the production of seedlings

The frequency and quality of *Aesculus* seedlings appears to be directly related to initial seed quality. In turn, initial seed quality is controlled by the conditions on and within mother tree, and the environment. One measure of seed quality is maturity which is reflected in part by seed weight or degree of filling. Browse (1982) and MacDonald (1986) and recommended grading seeds by size and discarding small or light seeds prior to planting in order to obtain more uniform stands of seedlings. Work with *A. hippocastanum* has shown that seed weight is directly related to the length of the growing season in a given area (Daws et al., 2004; Farrant and Walters, 1998). Seeds from less favorable environments are likely to be smaller and may not have reached physiological maturity (point of maximum dry weight accumulation) prior to shedding. Development of these seeds may be halted as the result of a combination of many factors including decreasing day length, changes in temperature (either day, night or both), or changes in edaphic moisture. *Quercus rubra* acorns from more northern latitudes amassed less fresh weight than those of more southern environments (Aizen and Woodcock, 1996). Browse (1982), working in England with a variety of *Aesculus* species, found cool wet summers to result in poor seed quality, if seeds were set at all. Problems with *A. parviflora* seed quality were the consequence of an insufficient number of “warm days after flowering for the seeds to ripen” (Bir, 1995). The growing season in Northeastern Ohio may not be long enough to fully mature *Aesculus* seed
crops, especially those of *A. parviflora*, whose fruit typically begin to abscise after two months of development.

The environment, in which a seed develops, seems to establish two crucial parameters which alter the seed’s ability to germinate and grow: 1) the total amount of energy the seed has for its vital processes and 2) the cardinal temperatures at which these processes will take place. Seeds that develop under less than optimum conditions develop poor, weak and/or immature embryos and accrue few reserves in their cotyledons which might sustain them both prior to and during germination. These seeds are typically produced on plants from colder climates and have more stringent dormancy requirements which require longer periods of stratification, which exacerbates the energy deficit.

Degradation in seed integrity and viability are unidirectional and irreversible, and the two physiological conditions that limit *Aesculus* seed germinability and subsequent growth are recalcitrance and dormancy. Recalcitrance was first characterized by Roberts (1973). The characteristics of recalcitrant seeds are a short live span even if properly stored, typically larger seed size compared to orthodox seed, possessing a high moisture content that can not be reduced below 30% without creating injury and loss of viability, and a high rate of metabolism, (Connor and Bonner, 2001; Copeland and McDonald, 1995). This condition is complex, and its impact on various biochemical and physiological processes is poorly characterized. Recalcitrant seeds are reported to be metabolically active and to have the hydration and cellular organization typical of a germinating seed. They do not undergo the typical maturation processes observed in orthodox seeds. The survival strategy proposed for most recalcitrant species
from the tropics is to initiate germination process and become established immediately after abscission (Farrant and Walters, 1998). An extreme example of this would be vivipary, a phenomenon which has been reported in A. californica, but not in other Aesculus species (Benseler, 1968). Recalcitrant seeds continue their developmental progress toward germination through the consumption of stored energy reserves by the metabolic processes, for increased protein synthesis, production of endoplasmic reticulum, and cell division (Pammenter et al., 1994).

Many of these attributes of recalcitrance have been observed in Aesculus. Aesculus typically abscise from the trees with moisture content in excess of 45% (Pritchard et al., 1996; Suszka, 1980; Uniyal and Nautiyal, 1996). Uniyal and Nautiyal (1996) observed that seeds of A. indica remained metabolically active at abscission. Aesculus hippocastanum seed is composed primarily of 37-38% starch, 8-10% protein, and 7.5-8% fatty oil, and the bulk of energy is stored in the cotyledonary tissues (Bombardelli et al., 1996).

The metabolic processes require both an energy source (like starch or lipids) and water. These metabolic processes in and of themselves can cause dehydration even in a seed that was initially fully hydrated. These changes can result unregulated metabolism leading to lipid peroxidation and the production of free radicals. Some authors have associated the loss of seed viability with this process (Hendry et al., 1992). While others suggest that the accumulation of free radicals is not the source of the reduced viability (Connor and Bonner, 2001; Finch-Savage et al., 1994; Greggains et al., 2000). It is likely that membrane damage occurs as part of these detrimental metabolic processes and can be monitored through the detection of cellular leakage. For the first
60 days in storage, *A. indica* seeds initially retained their cellular organized without significant disruption. However, after 120 days in storage there had been a significant weight loss with the starch and protein contents of the seed reduced by half. Significant cellular deterioration and *Aspergillus* and *Penicillium* species were observed by day 240, and by day 480 the seed constituents were “lumpy, broken, and disorganized” Khan et al (1993). Pores became apparent on the seed coat as evidence of progressive deterioration and diminishing reserves. This work documents that as time in storage increased, degradation also increased. This type of cellular damage and membrane leakage could explain the presence of the exudates observed in this study. This (potentially nutrient rich) exudate along with the high moisture content could have served as an ideal environment for fungi, which were commonly observed on seeds of *A. parviflora* stored for more than 120 days and in *A. pavia* stored for over 180 days. The presence of the mold in turn could further promote seed mortality. The smaller seeds would seem to be more susceptible to these problems because they contain both less stored energy to support the metabolic process and less moisture.

The function of dormancy is to inhibit growth until the environmental conditions are favorable. For recalcitrant species, this combination of conditional dormancy and a high rate of metabolism posses certain challenges. Since the seed is in the same metabolic state as if in active growth, it seems reasonable that as soon as the dormancy requirements are completed that germination would occur even if the seed was held under less than ideal conditions. In this study, initially no germination in storage was observed, but when seeds of *A. pavia* were planted directly asynchronous germination and emergence occurred because chilling requirement had not been satisfied. The rate
of germination while in storage (prergermination) in *A. pavia* nearly doubled for each storage period up to 120 day. This compromises the seeds integrity and makes it vulnerable to damage in handling and pathogenic attack and weakens the potential seedling. This detrimental phenomenon was observed in *A. hippocastanum* by May (1963); Widmoyer and Moore (1968); Suszka (1980). It was also seen in recalcitrant-seeded species of *Quercus* and *Castanea* (Greggains et al., 2000). There is some limited evidence that suggested reduced temperature maintained germinability longer perhaps by slowing the metabolic rate and reducing the microorganism activity when seeds were maintained at –1.1°C (Widmoyer and Moore, 1968).

All things being equal, a larger seed with its greater energy reserves would be better able to cope it constraints of a high metabolic rate and the limitations of dormancy. Seeds that have minimal dormancy requirements which can be satisfied quickly and that can be immediately placed under permissive growing conditions should result fast germination and emergence and ultimately result in the largest and most robust seedlings.

Propagators must therefore, learn how to manage the biology of *Aesculus* seed to obtain the highest levels of germination efficiency. These recommendations are only a guideline and are developed based on experience with seed collected in northeastern Ohio. Caution must be exercised in generalizing these methods between sources of the same species due to potential difference in seed development and depth of dormancy. Larger and potentially more mature seed may respond quite differently in the germination process. Good germination, emergence and ultimately useable seedling are contingent upon high quality seed that is properly handled. Seed lots had to be carefully
examined for small or poorly developed seed which needed to be discarded prior to stratification. The optimum stratification treatment that maximized both the germination and emergence time while minimizing losses due to mold and pregermination for both *A. parviflora* and *A. pavia* was 60 days of chilling. Since *A. parviflora* expressed epicotyl dormancy after the initial shoot elongation phase, seedlings required an additional period of chilling to promote normal growth. Any aspect of the process which extended the period of time from seed shed to active seedling growth (either extended germination and emergence time as the result of insufficient chilling or prolonged time in storage) had a negative impact on whether or not a seed would ever germinate and then on the shoots emergence and ultimate performance.
Figure 4.1. The main effects of stratification period and species on seed germination (radical protrusion) in *A. parviflora* and *A. pavia*. Values represent mean germination percentages and their standard errors. Effect means (bars of similar color) with similar superscripts are not significantly different according to SNK tests ($p=0.05$).

**ANOVA**

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Figure 4.2. The interactive effects of stratification period and species on seed germination (radical protrusion) in *A. parviflora* and *A. pavia*. Values represent mean germination percentages and their standard errors for each species X stratification period combination. Filled circles and open circles represent correspond to Par and Pav, respectively.
Figure 4.3. The effects of stratification period on the synchrony of germination (radical protrusion) in *A. parviflora* and *A. pavia*. The left and right sides of the boxes and the left and right whisker lines represent the 25th and 75th percentile and the 10th and 90th percentile of the population’s distribution, respectively. The light and heavy vertical lines within boxes represent the population median and mean, respectively. Values represented by closed circles indicate the day to emergence of individuals that were not within the group enclosed between the 10th and 90th percentiles. Numbers in parenthesis indicate the total number of germinated seeds.
Figure 4.4. The main effects of stratification period and species on shoot emergence (epicotyl protrusion) in *A. parviflora* and *A. pavia*. Values represent mean emergence percentages and their standard errors. Effect means (bars of similar color) with similar superscripts are not significantly different according to SNK tests (*p*=0.05).
Figure 4.5. The interactive effects of stratification period and species on seed emergence (epicotyl protrusion) in *A. parviflora* and *A. pavia*. Values represent mean emergence percentages and their standard errors fore each species X stratification period combination. Filled circles and open circles represent correspond to Par and Pav, respectively.
Figure 4.6. The effects of stratification period on the synchrony of emergence (epicotyl protrusion) in *A. parviflora* and *A. pavia*. The left and right sides of the boxes and the left and right whisker lines represent the 25th and 75th percentile and the 10th and 90th percentile of the population’s distribution, respectively. The light and heavy vertical lines within boxes represent the population median and mean, respectively. Values represented by closed circles indicate the day to emergence of individuals that were not within the group enclosed between the 10th and 90th percentiles. Numbers in parenthesis indicate the total number of seeds which emerged a shoot.
ANOVA

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Figure 4.7. The main effects of stratification period and species on the frequency of usable seedling numbers in *A. parviflora* and *A. pavia*. Values represent mean percentages of usable seedlings and their standard errors. Effect means (bars of similar color) with similar superscripts are not significantly different according to SNK tests ($p=0.05$).
Figure 4.8. The interactive effects of stratification period and species on the production of usable seedlings of *A. parviflora* and *A. pavia*. Values represent mean percentages of usable seedlings and their standard errors for each species X stratification period combination. Filled circles and open circles represent correspond to Par and Pav, respectively.
Figure 4.9. The effects of stratification period on the frequency of *A. parviflora* and *A. pavia* seedlings within classes. Values represent the proportion of total seedlings within each class. Bars with white, dark gray, black, light gray and hatching indicate frequency of seedlings that were classed as albino, accelerated growth, dead, stunted or useable, respectively.
ANOVA

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Figure 4.10. The main effects of stratification period and species on seedling height in *A. parviflora* and *A. pavia*. Values represent mean seedling heights and their standard errors. Effect means (bars of similar color) with similar superscripts are not significantly different according to SNK tests ($p=0.05$).
CITED REFERENCES


CHAPTER 5

SELECTION OF AESCULUS PARVIFLORA AND AESCULUS PAVIA
PARENTS AND THEIR PARENTAL EFFICIENCY

INTRODUCTION

_Aesculus_ are most valued for their spring ornamental floral display. Specimens of _A. flava_, _A. glabra_, and _A. sylvatica_ have also been noted for their fall color (Hillier, 1991; Morton Arboretum, 2002; Phillips, 1978). _Aesculus parviflora_ is especially valued for its summer blooming period, consistently healthy summer foliage, and bright yellow fall color. The red flower color, its small size, and in some individuals, its fall color make _A. pavia_ an excellent choice for the landscape. There appears to be intra-species variation for many horticulturally important traits.

These species also show some adaptability to the urban or suburban environment including to stresses resulting from air pollution, salinity, high pH or disturbed soils. The level of adaptability exhibited depends upon the species and the stress in question, with _A. flava_, _A. glabra_, _A. hippocastanum_, _A. pavia_ showing the greatest variability in tolerance among individuals (Bridwell and Zuk, 1997; Hightshoe, 1988; Hudak, 1980; McClenahen, 1978; Morton Arboretum, 2002; Townsend, 1974; Velagic-Habul et al., 1991; Wasowski, and Wasowski, 1991). Capturing stress
tolerance variability in combination with other horticultural traits and incorporating them into *Aesculus* genotypes uniquely suited to the landscape may be possible through judicious intra- and/or interspecific hybridization. Limited breeding efforts have already enjoyed modest levels of success. For instance, Fostad and Penderson (1998) using half-sib families of *A. hippocastanum* were able to make selections for improved salt tolerance in seedlings based on the phenotypic performance of the mother trees.

Successful breeding is founded on a thorough understanding of many aspects of reproductive biology and how to manage them. *Aesculus* are andromonecious, and a breeder must understand when and where the complete flowers will be located, the difference in sex ratio of various individuals, and how to manipulate that ratio. The number of complete flowers sets the threshold of the number of possible pollinations. The gap between blooming periods has been bridged with long-term low temperature pollen storage in a number of woody species. This technique has been demonstrated to be successful at least for short period of time for the *Aesculus* in this study. The seeds of *Aesculus* are reported to be recalcitrant and to have varying degrees of dormancy. A brief stratification period enhances the capacity and rate of germination and emergence without increasing losses due to deterioration by mold. The short stratification period resulted in plants with the optimum first year growth. Detailed analysis of each of these aspects of biology can be found in Chapters 2, 3, and 4.

The initial selection of suitable parents for the hybridization process is paramount if breeding progress is to be made in a timely manner. One potential method for the evaluation for various mother trees is to develop a provenance formula. These types of instruments have been utilized to evaluate trees in seed orchard production.
facilities. One such tool, the Southern Pine Cone Analysis, was developed to evaluate seed production efficiency and to determine at what stages in the seed production system losses occur (Bramlett et al., 1977). It details the stages of ovule abortion, failures in seed filling, and problems during development through germination. Similar, often privileged, methods have been adapted by breeders to assess the quality of their parental materials. These measures of parental “breeding values” are based on estimates of the heritability among useful traits and often consider multiple selection criteria.

As outlined in Chapter 4, the production of large quantities of seed that develop into vigorous seedlings is important to the commercial production of *Aesculus*. Likewise, as seed yield in many *Aesculus* specimens is typically quite low, the lack of high quality seed production per tree and the resulting insufficiency of viable seedlings often limit the potential for gain through selection. Limitations in seed and seedling production suggest the importance of seed set as a driver of provenance values. Each *Aesculus* fruit has the potential to produce as many as six seeds. However, this potential is rarely reached. Hardin (1955) found averages among six species ranging from 1.0 to 2.6 seeds per fruit. Multi-seeded fruit in Hardin’s study were reported in greater frequency than in Benseler’s study (1968) where fruits of *A. californica* were typically single seeded.

Based on previous flowering and fruiting data, a provenance model could be developed for evaluating specific *A. parviflora* and *A. pavia* seed parents on the basis of their potential seed yields. If data were available, these could be further expanded to include aspects of seedling growth or other characteristics of breeding interest. The
model could be tested by comparing the calculated value of percent seed set with the actualized (realized) percentage seed set resulting from a number of successful open pollinations and/or controlled pollinations using a variety of pollen sources and crossing techniques.

The objectives of this study were as follows: 1) to characterize the variability in fruit and seed behavior among and within specimens of *A. parviflora* and *A. pavia*; 2) to develop a mathematical model to predict seed parent potential; 3) to predict seed parent potential of available specimens using the model and information from the inflorescence (see Chapter 2) and infructescence studies; 4) to initiate *Aesculus* improvement programs using the best available *A. parviflora* and *A. pavia* parents in a series of intra and interspecific crosses; 5) to compare the actual breeding efficiency of female parents with the estimate of efficiency calculated by the provenance formula.

MATERIALS AND METHODS

*Variability in fruit characteristics and fruiting behavior among species*

Infructescence characteristics including number of fruits per panicle, location of the fruit on the panicle, number of seeds per fruit, the frequency of multi-seeded fruit and variability in seed fresh weight were studied for three consecutive years using specimens of *A. parviflora* and *A. pavia* grown at one of two locations. Specimens of *A. parviflora* (Par1 through Par9 from Appendix F) were used to determine the prevalence of multiple seeded fruit and its impact on seed size. Due to insufficient fruit
numbers only a subset of Par1, Par2, and Par7 were used to characterize the average number of fruit per panicle, seed per fruit, distribution of that fruit and seed fresh weight. *Aesculus pavia* specimens, Pav1, Pav3, Pav4, Pav6 and Par9 were used for both studies to describe the frequency of multi-seededness and the infructescence characteristics.

Fruit development of naturally occurring open pollinated seed was monitored from the end of anthesis until the pericarps began to dry and change color (from green to yellow green in *A. parviflora* or tan in *A. pavia*). The specimens were then monitored every other day until the fruit capsules were observed to begin to split. Infructescences were harvested with fruit intact and allowed to dry slightly in a cool and well-ventilated area until all fruits had naturally split and the seed could be removed from the pericarp without damage. The number of fruits per panicle and the number of seeds per fruit were recorded. The position of the fully developed seed was observed. Each seed was then observed for malformation and/or damage. A string and ruler were used to measure the circumference around the middle of the seed. The volume was obtained by displacement and the mass was measured. A comparison of the seed weight was made between multi-seeded and single seeded fruit.

**Provenance formula development and selection of breeding parents**

Utilizing the data collected on the number of panicles per tree, frequency of panicles with complete flowers, number of flowers per panicle, and the frequency of complete flowers, a value was calculated for each tree’s potential fruit and seed bearing capacity (Figures 5.1 and 5.2). The number of panicles that produced fruit, average
number of fruits per panicle, and the average number of seeds per fruit were used to
determine the realized fruit and seed set. These two pairs of values, realized fruit / fruit
potential or realized seed / seed potential, were then used to obtain the frequency of the
actualized potential and to identify suitable parents for the initiation of the breeding
program. The predicted values were later compared with the results from the breeding
study to assess the validity of the model for choosing maternal parents.

Breeding procedures

Two *A. pavia*, Pav1 and Pav2, and three large *A. parviflora* specimens, Par6, Par7, and Par8 were used as female parents. The plants were of various ages and sizes but all were in good to excellent condition. The pollen parents included Pav1, Pav2, Pav3, Pav4, Pav6, Pav9, and Pav10 and Par1, Par3, Par6, Par7, Par8, Par9, along with additional pollen from Car1, Car2, Fla1, Syl1 and Hip1. The specimens used to evaluate the provenance model were Par6, Par7 and Par8 and Pav1.

Panicles were examined weekly from emergence until the buds at the base of the panicle reached approximately 5 mm in length, and examined daily to monitor the rate of development. Panicles were marked and bagged with the breathable Delnet® pollinating bags, (Delstar Technologies Inc., Middletown, DE) once the buds on the lower most portion of the panicle were between 7.5 and 10 mm and had started to acquire their characteristic flower color. Since initial observations revealed no clear morphological indicators that any given flower was going to be functionally staminate or complete, all buds were initially treated as if complete. Just as the petals began to expand beyond the calyx and could be separated, each flower was opened using fine
tipped forcepts to view the internal structures. This was referred to as the crown stage because the anthers sit immediately adjacent to each other in a ring. If a developed gynoecium was present, the flower was emasculated, and if functionally staminate, the flower was removed. The pollination bag was then placed over the inflorescence to exclude any possible pollinators from visiting the panicle.

Preliminary observations indicated no visible changes in the stigmatic region, (e.g., color change, expansion, changes in the surface, or exudate) to denote changes in receptivity. Therefore, style length expansion was adopted as a measure of gynoecium development and potential receptivity. Because stigmatic receptivity could not be ascertained visually, a comparison of two pollination procedures – one consisting of a single pollination during the presumed receptive period and the other involving a series of pollinations to maximize the probability of capturing the true window of receptivity – was undertaken.

The multiple (repeated) pollination technique was initiated when the styles had reached approximately 2.5 cm. The flowers were pollinated daily until the tip of the style began to wither. Each flower was typically pollinated four to seven times. On average five to seven flowers were pollinated per panicle each as its development warranted. Each bag was removed and the tip of the style was coated with either freshly collected pollen from another panicle (for the self pollinations) or freshly thawed previously collected pollen (for the intraspecific or interspecific pollinations). All the flowers within one panicle were pollinated with the one pollen source.

Flowers to be singly pollinated were allowed to develop until the style was perceived to be fully expanded, approximately 3.0 cm. At the time of pollination, the
flower was marked with a piece of colored floss to designate that it had been pollinated. The remaining complete flowers on that panicle were then handled in a similar manor. Another pollination method (cut style technique) was also employed where the style was cut off approximately 5 mm above the ovary, and then the cut surface was coated with pollen.

Pollination bags were left in place for three weeks after anthesis to retain any initially developing fruit that might abscise during the initial course of development. Afterwords, the pollination bags were removed and replaced with a nylon mesh bag to protect the developing fruit. Fruit development was observed approximately every three weeks until the fruits began to change color. The fruits were harvested when the pericarps began to split. The seed circumference, volume, and mass were measured as described previously.

Data analysis

Data for the infructescence study were analyzed using software and procedures (PROC GLM and/or PROC MEANS) in accordance with the SAS Institute (1990). Treatment means were described using standard errors of the mean. A correlation was conducted between seed circumference, volume and mass. The seeds from the multi-seeded fruit were compared to a randomly selected subsample of seeds from single seeded fruit, equivalent in size to the sample of seeds from the multi-seeded fruit for the effect of seed number on weight.
RESULTS AND DISCUSSION

The production of useable seedlings is the end point in a long series of events in the reproductive process that begins with pollination at anthesis. Most _Aesculus_ panicles contain at least a small frequency of complete flowers. This frequency of complete flowers represents the potential number of fruits that could be produced (see Chapter 2). Benseler (1968) and Hardin (1955) both reported that most of the panicles that contain female flowers, fail to produce any mature fruits. Hardin (1955) suggested that fruit production was limited by effective pollination, fertilization, and fruit development on the tree. This lack of fruit maturation negatively impacts the success of breeding program by limiting the number of hybrid seed realized from any given cross.

An understanding of the natural biology of fruit location (which flowers might be best to pollinate), number of fruit on a panicle (how many fruits are normally mature) and number and size of seeds (what are the average characteristics of a seed) might enhance the possibilities for controlling factors that influence successful hybridization.

_Evaluations of fruit per panicle_

There was considerable variation between the two species and between the two years for all fruit and seed characteristics observed (Table 5.1). The three specimens of _A. parviflora_ (Par 1, Par2, and Par7) averaged between 2.1 and 3.1 fruit per panicle with
the highest number of fruits on a single panicle observed on Par7 being nine (Table 5.1). Unfortunately some *A. parviflora* trees initially considered for the infructescence study set insufficient fruit numbers for inclusion in the data set (> 10 fruit/plant).

Five specimens of *A. pavia* (Pav1, Pav3, Pav4, Pav6, and Pav9) produced on average 1.3 fruits per panicle for both years studied, with the maximum number of fruits being five on Pav1 in 1999. These results were similar to those observed in *A. californica* where 87.1% of all panicles produced a single fruit and panicles with three or more fruits were rare. Bertin (1980) also found that the percentage of mature fruits differed between years and locations from a high of 11.5% to a low of 1.2%.

The number of fruit per panicle varied widely between specimens and between years. The variable weather conditions of 1998 and 1999 between the two locations seemed to have influenced the number of fruits that reached maturity in both species. The lack of sufficient rainfall in northeastern Ohio during the *A. parviflora* fruit setting and seed filling periods of 1998 may have resulted in moisture levels that were limiting, and, thus reducing in fruit set. Fruit remaining on these plants developed seeds that were 20% greater in mass than those produced in 1999, perhaps because the trees held fewer sinks than usual. In contrast, fruit production on Pav1 appeared to be increased in 1999, when supplemental irrigation and mineral nutrition were provided to alleviate an environmentally-stressful situation precipitated by extenuating circumstances outside of this study. In addition to greater seed mass, the horticultural care given to this tree resulted in a high number of fruit matured in 1999. The superior performance of this tree in 1999 increased the overall fruit per tree average for this species by 59% (i.e., from 85.2 ± 32.6 in 1998 to 143.2 ± 56.8 in 1999).
Seed per fruit on open pollinated material

Each *Aesculus* fruit has the potential to produce six seeds, two in each of three locules (Hardin, 1955). Hardin (1955) reported that 100% of the 28 fruits of *A. parviflora* examined were single seeded, and that of the 16 fruits of *A. discolor* studied (another name for *A. pavia*), none were single seeded. Fruit of the latter species typically contained 2-4 seeds, but never more than four seeds. Unlike previous studies, Hardin did not conclude that single seededness was the most common level of seed production in *Aesculus*. In his study multi-seededness was most common in *A. pavia* with an average of more than two seeds per fruit. However, like *A. parviflora*, Benseler (1968) found that 96.1% of all fruits of *A. californica* were single seeded and only 0.2% were triple seeded.

In this study, 1306 fruits from eight different *A. parviflora* specimens and 1617 fruits from nine *A. pavia* specimens were husked to determine the average number of seeds per fruit. *Aesculus pavia* had a higher average number of seeds per fruit in both years than did *A. parviflora*. There were species differences in the frequency of multi-seeded fruit. Six specimens of *A. parviflora* were completely single seeded. *Aesculus parviflora* produced 94% single seeded fruit, and out of the 1306 fruits studied there was only 1 three-seeded fruit, and no fruits with four or more seeds were observed. All specimens of *A. pavia* produced at least some multi-seeded fruit. Approximately, 58% of all fruit were single seeded and another 27.6% were double seeded. Although they occurred at low frequency four, five and one six seeded fruit were observed. Fresh seed weight was influenced by seed frequency. In both species, multi-seededness negatively
impacted the weight of each seed within the fruit (Figure 4.3). However, both classes of
*A. pavia* seeds were larger than the seeds produced by single seeded *A. parviflora* fruit.
Many of the seeds in a multi-seeded fruit were small, light and floated when volumetric
measurements were taken. A sub sample of these seeds was cut in half to determine
their quality. Most of these seeds consisted of a seed coat without a viable embryo axis
or cotyledonary tissue. The effect of ovule position on the frequency of seed set and
subsequent development could not be determined in either species due to locule
distortion and collapse caused by developing seeds within the fruit.

*Seed fresh weight and other seed characteristics*

The seeds of all *Aesculus* appear to be similar in shape but differ in color and
size. The correlation between volume and mass was grater than *r* = +0.98 for both *A.
*parviflora* and *A. pavia*. Circumference had a lower correlation to fresh weight *r* =
+0.886 and *r* = +0.895 for *A. parviflora* and *A. pavia* respectively. This could be
explained by two factors: a fraction of seeds that were hollow though nearly normal in
size and to a lesser extent by irregularities in seed shape in multi-seeded fruit. Fresh
weight seemed to be the most accurate measurement, and since there was a high degree
of correlation between weight and volume only fresh weight was reported herein.

The mean fresh seed weight differed for both species and years. Again these
differences were most likely the result of varying environmental conditions and/or
varying number of seeds produced per panicle or per tree. Although the largest seeds of
both *A. parviflora* (24.2 g) and *A. pavia* (29.5 g) were similar, the average seed size of
*A. parviflora* was 10-35% smaller than the seeds of *A. pavia*. In this study, the fresh
weight *A. parviflora* was approximately half the size (50 ± 10 seeds /kg) of what was characterized by Browse (1982). However, the average *A. pavia* seed weight was very similar to those reported by Browse (1982) and Rudolf (1974) (100 ± 20 seeds /kg) but less than the 16.9 g reported by Bertin (1980). The small seededness of the *A. parviflora* in this study may be indicative of seed not reaching full maturity prior to abscission, resulting from an insufficient number of days after anthesis for seed development. Fruit development takes place over a two month period for *A. parviflora* and a four month period for *A. pavia* in northeastern Ohio, compared to five to six months for *A. pavia* in the southern part of its range (Rudolf, 1974). In comparison, *A. californica* fruit develop over five months and other members of the Pavia section mature in three to six months depending on species and location (Benesler, 1968; Rudolf, 1974).

Seed size within both species differed among years. Hoshizaki et al. (1997) reported *A. turbinata* seed size to be highly influenced by seed number per tree. Seeds tended to be larger in years where seed set was low than in years where seed yield was abundant. In this study, seed fresh weight appeared to be inversely related to fruit per panicle in *A. parviflora*, but not in *A. pavia* (Table 5.1). In *A. pavia*, the 1999 seed fresh weight represented a 16.8% increase over that associated with 1998 seed, even though the number of fruit per panicle was similar in both years. The high mean seed weight enjoyed in 1999 most likely resulted from seeds collected from Pav1, the specimen receiving supplemental horticultural inputs as described above. In other words environmental influences likely mediated (overrode) the seed set-seed weight relationship in this instance.
Benseler (1968) stated that *A. californica* seeds were among the largest in the genus, with an average diameter 5 cm and an average fresh weight of 57.2 g, but had a range of 13.1-153.2 g. *Aesculus turbinata* have an average fresh weight of 18.8 g (Hoshizaki et al., 1999). In this species, seed quality, and specifically seed weight, was reported to have a direct impact on seedling development and survival (Hoshizaki et al., 1997). Larger seed tend to produce seedlings with higher survival rates. Seed size is the culmination of many related factors, including embryo and endosperm development, competition for resource both within and between fruit and other plant functions.

*Provenance evaluation*

The partitioning of resources among biological functions, for increases in primary and secondary growth, secondary plant products, and reproductive structures and seeds is mainly under genetic control. Because resource allocation patterns differ among individuals, each tree expresses a different breeding potential. Breeders routinely evaluate their parental materials for their effectiveness as parents both in terms of their genetic impact on the traits of interest and on their ability to provide support for the developing offspring. In forest trees the parental performance is usually calculated by measuring the number and quality of the resulting seeds, seedlings, whips or marketable materials. These populations from seed orchard studies were developed using a large population of elite trees over the course of a number of reproductive cycles.
Following these examples, a model was developed to assess the reproductive capacity of individual *Aesculus* specimens. The model attempted to account for the impact of andromonecy on the fruiting potential and the overall diminished seed set observed in the genus. This formula provides only an initial estimate of maternal plant quality. The model’s effectiveness is potentially limited by number of specimens and years over which it was developed, and the technical limitations associated with estimating parameters (e.g., panicles per tree, in large canopies with dense foliage; number of panicles bearing fruit, the effects of predation on the seed crop). This formula could also be expanded to include other factors, like the fraction of seed weighing less than 5 g, the number of seed that are lost in the germination and emergence process, and the frequency of abnormal seedlings that occur in the seedling population.

The fruit potential was highly variable for both species, and ranged for *A. parviflora* from a low of 393 fruit to a staggering 61,265 (Table 5.2). *Aesculus pavia* showed similar results with a wide spread in fruiting potential from 279 to a high of 11,955 for Pav1. The two most significant factors in determining this potential were the overall abundance of inflorescences on the plant and the sex ratio for that individual. Two examples of the impact of these factors were observed: in *A. parviflora*, Par11 which was a small specimen had nearly the same breeding potential as Par3 with nearly four times the number of panicles and five times the spread, and in *A. pavia* where Pav2 with its average of 52 panicles had a nearly equivalent breeding potential as Pav9 which had an average of 700 panicles. The seed potentials were calculated to be six times that of the fruiting potential. However, this is potential that is largely unrealized since
Aesculus produce largely single seeded fruit. The predicted percentage of fruit set was quite low and ranged from 0.1% to 11% in *A. parviflora* and from 1.6% to 21.1% for *A. pavia*. Due to the limited number of panicles, Par2 could not be used in the estimation of the realized fruit set model and was excluded. Par11, which was used for the estimate of fruit potential, failed to mature any seed so it also was eliminated from further development of the model. The plants used for the breeding study were chosen in part based on their actualized fruit set.

**Effectiveness of controlled pollination**

Barriers to interspecific hybridization include temporal and spatial barriers and physiological barriers associated with pollen stigma/style compatibility, fertilization both for the formation of the embryo and the endosperm. Spatial and temporal barriers have been most often managed through the use of stored pollen for woody species. As *Aesculus* pollen retains its germination capacity for short periods (3 months or less; see Chapter 3), controlled crosses were possible between species with disparate flowering phenologies, such as *A. parviflora* and *A. pavia*.

A series of pollination experiments was conducted to address the following uncertainties: the effectiveness of controlled versus natural pollination; the difference in fruit set between self and intraspecific pollination; and evidence for or against self incompatibility. These experiments also substantiated the efficacy of various male and female parents for their potential as breeding stock in a successful intraspecific hybridization program.
In this study, none of the 426 emasculated but unpollinated flowers set fruit, indicating the effectiveness of the bags at preventing unintended pollinations and the lack of apomixis within these two species. Benseler (1968) also found no evidence for this phenomenon. Overall both the seed set and maturation was low for both species for all types of crosses. The number of fruits matured from the controlled self pollinations was generally equal to or slightly better than that of natural (open) pollination with an average of 13.1% for open pollination and 17.1% of self pollinations (Table 5.3). Maternal parents differed in their ability to develop fruit, with Par6 consistently exhibiting the poorest performance. The rate of fruit maturity between the self pollinations and intraspecific crosses was comparable and there was no evidence indicating self incompatibility. *Aesculus californica* responded similarly to pollination, with success rates of 5 to 10 % for natural pollinations, 6.7% for self pollinations and a 4.7% for intraspecific crosses (Benseler, 1968). Bertin(1980) reported rates of 1.1% and 2.1% for self and intraspecific pollinations respectively. Based on the in vitro germination of fresh pollen it would seem unlikely that the lack of seed production could be result of a lack of viable pollen.

*Success of model in predicting fruiting success*

The estimates derived from this formula were reasonably accurate in predicting the success of pollination. Plants that were predicted to be poor parent based on the model demonstrated a lower frequency of both natural as well as the controlled pollination success. The trends were consistent between the model’s prediction and the fruiting response of observed in the (monitored) open pollinated seed and that of the
controlled self and intraspecific crosses. The model predicted that Par8 should be approximately five times better than Par6 in setting fruit, and the observed open pollinated were 4.2 times better and the controlled crosses were 4.0 times. Similar results were observed when comparing the breeding potentials between Par6 and Par7. Conclusion about the effectiveness of the model for *A. pavia* require further study, but the fruit data from open pollination for Pav1 was similar to the predicted.

**Hybridization success**

Both *A. parviflora* and *A. pavia* matured more than 20% of the intraspecific pollinations compared to less than 6% of the Par X Pav or Pav X Par crosses. The success rate of these interspecific crosses was similar to that observed in other species (Van Creij et al., 1997; Van Tuyl et al., 1988; Zhou et al, 1999). A small number of other types of interspecific crosses were also made using several pollen sources of *A. × carnea*, *A. flava*, *A. hippocastanum*, and *A. sylvatica*. The number of fruit resulting from these crosses was minuscule. Only six fruit resulted from these 557 crosses with *A. parviflora* as the female parent. The number of interspecific crosses with *A. pavia* as the female parent was too small to be accurately compared.

The effect of pollen source was examined using six male parents and five female parents. The pollen sources of Par3 and Par9 were consistent in their ability to set fruit on *A. parviflora* between 6 to 40% depending on the female parent. Crosses using Par9 on *A. pavia* failed matured any fruit, and Par1 and Par7 produced only 1.2 to 7.5% fruit set on *A. pavia* (Table 5.4 and 5.5). When either Pav1 or Pav6 pollen was used on any of the *A. parviflora* specimens fruit set ranged from 0 to 9.9%. However, these same *A.
pavia pollen sources resulted in 10.6 to 37.0% fruit set when used on A. pavia seed parents. When pairs of interspecific reciprocal crosses were compared, no clear benefit was conferred by having one species as the maternal parent over the other. It appears as though the pollen source had less effect on fruit set than did the seed parent.

Since the timing of receptivity could not be determined easily, a multiple pollination technique was compared to one using a single pollination event. In general, the multiple pollination technique seemed somewhat more successful than the one time pollination for the intraspecific pollinations (Figure 5.4. Again, interspecific pollinations were far less successful at setting fruit than intraspecific pollinations. It was not clear whether the greater success of the single pollination technique for the interspecific pollinations was an artifact based from the overall very poor fruit or if this technique or the small sample size or if it was actually better. Self pollinations and intraspecific and interspecific cross pollinations using the cut style technique (Van Tuyl et al., 1988) were universally unsuccessful (data not shown).

The interval between anthesis and 21 days post pollination saw a substantial fruit drop for both the natural and controlled pollinations. A second period of fruit drop was observed toward the end of development where fruit that initially grew withered and then abscised off. The resulting seeds were small, soft and had a dark sunken in appearance. When the seeds were dissected, an immature poorly developed embryo was observed (Figure 5.5). Taniguchi et al (2003) in studying A. turbinata, reported that 70-80% of all initially developing fruits abscised within the first month following pollination. Additional fruit losses were observed throughout development. Benseler (1968) reported that only 5-10% of all complete flowers of A. californica set seed.
The frequency of multi-seeded fruit was not higher under controlled pollination conditions than under natural conditions. Greater than 90% of all *A. parviflora* fruit were single seeded where *A. pavia* average 1.9 ± 0.2 seed per fruit. Seeds from the open pollinated, self pollinated, and intraspecific crosses all had similar fresh weights, but the interspecific seeds in some cases were smaller (Table 5.5). This could perhaps indicate problems with embryo, endosperm development or both.

Further studies are needed to more fully address the success if the direction of the hybrid cross would improve fruit set along with the time of day that crossing is most successful. The use of other species for the incorporation of flower color traits should be reexamined based on the phylogenetic relationship between species, especially the use of the newly released *A. californica* ‘Canyon Pink’ and ‘Grant’s Ruby’ for flower color in *A. parviflora*. Detailed studies on the natural development of *A. parviflora* and *A. pavia* embryos and endosperm would be beneficial in preparation off studies utilizing embryo rescue.
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Aesculus parviflora</th>
<th>Aesculus pavia</th>
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<tbody>
<tr>
<td></td>
<td>1998 population</td>
<td>1999 population</td>
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<tr>
<td></td>
<td>mean ± S.E^a</td>
<td>mean ± S.E^b</td>
</tr>
<tr>
<td>Fruit per panicle</td>
<td>2.1 ± 0.10</td>
<td>3.1 ± 0.20</td>
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<tr>
<td>Seeds per fruit</td>
<td>1.1 ± 0.02</td>
<td>1.1 ± 0.20</td>
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<td>Distribution of fruit within panicles</td>
<td>acropetal</td>
<td>acropetal</td>
</tr>
<tr>
<td>Seed fresh weight (g)</td>
<td>9.6 ± 0.16</td>
<td>8.1 ± 0.12</td>
</tr>
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^aStatistics derived from the combined observation of 161 panicles from 3 specimens in 1998.
^bStatistics derived from the combined observation of 159 panicles from 3 specimens in 1999.
^cStatistics derived from the combined observation of 290 panicles from 5 specimens in 1998.
^dStatistics derived from the combined observation of 263 panicles from 5 specimens in 1999.

Table 5.1. Infructescence characteristics of *Aesculus parviflora* and *Aesculus pavia*. 
<table>
<thead>
<tr>
<th>Species and specimens as female parents</th>
<th>Maternal potential&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Observed performance of maternal parents in a breeding exercise</th>
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<tr>
<td></td>
<td>Fruit potential (X 1000)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Realized Fruit&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>A. parviflora</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Par1</td>
<td>39.6</td>
<td>41.2</td>
</tr>
<tr>
<td>Par2</td>
<td>61.3</td>
<td>109.7</td>
</tr>
<tr>
<td>Par3</td>
<td>0.4</td>
<td>8.3</td>
</tr>
<tr>
<td>Par6</td>
<td>1.5</td>
<td>35.1</td>
</tr>
<tr>
<td>Par7</td>
<td>6.2</td>
<td>609.6</td>
</tr>
<tr>
<td>Par8</td>
<td>5.3</td>
<td>584.4</td>
</tr>
<tr>
<td>Par9</td>
<td>6.8</td>
<td>40.8</td>
</tr>
<tr>
<td>Par11</td>
<td>0.4</td>
<td>0</td>
</tr>
<tr>
<td>A. pavia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pav1</td>
<td>12.0</td>
<td>324.4</td>
</tr>
<tr>
<td>Pav3</td>
<td>68.8</td>
<td>253.4</td>
</tr>
<tr>
<td>Pav4</td>
<td>13.6</td>
<td>61.6</td>
</tr>
<tr>
<td>Pav6</td>
<td>13.4</td>
<td>20.9</td>
</tr>
<tr>
<td>Pav9</td>
<td>0.3</td>
<td>73.7</td>
</tr>
</tbody>
</table>

<sup>a</sup>Fruit potential is based on the number of panicles per individual, the frequency of mixed panicles, the average number of flowers per panicle and the frequency of complete flowers (See Figure 5.1).

<sup>b</sup>Realized fruit is based on the number of panicles that set fruit and the average number of fruit per panicle.

<sup>c</sup>The actualized fruit is the ratio of realized fruit set to fruit potential expressed as a percentage.

<sup>d</sup>Specimens of <i>Aesculus parviflora</i> and <i>Aesculus pavia</i> are described in Appendix F.

Table 5.2. Estimation of maternal potential among specimens of <i>Aesculus parviflora</i> and <i>Aesculus pavia</i> by a provenance formula and a comparison of estimated performance with the level of fruit set achieved during a breeding exercise.
### Table 5.3

<table>
<thead>
<tr>
<th>Species and specimens as female parents</th>
<th>Open pollinations</th>
<th>Self pollinations</th>
<th>Intraspecific pollinations</th>
<th>Interspecific pollinations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N$^4$</td>
<td>Fruit set (%)</td>
<td>N</td>
<td>Fruit set (%)</td>
</tr>
<tr>
<td><strong>A. parviflora</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Par6$^y$</td>
<td>304</td>
<td>4.2</td>
<td>120</td>
<td>9.2</td>
</tr>
<tr>
<td>Par7</td>
<td>176</td>
<td>23.3</td>
<td>76</td>
<td>18.4</td>
</tr>
<tr>
<td>Par8</td>
<td>191</td>
<td>17.8</td>
<td>84</td>
<td>27.4</td>
</tr>
<tr>
<td><strong>A. pavia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pav1</td>
<td>217</td>
<td>6.5</td>
<td>66</td>
<td>10.6</td>
</tr>
<tr>
<td>Pav2</td>
<td>—</td>
<td>—</td>
<td>21</td>
<td>9.5</td>
</tr>
</tbody>
</table>

$^4$N = number of open-, self-, intraspecific and interspecific crossing events initiated.

$^y$Specimens of *A. parviflora* and *A. pavia* are described in Appendix F.

Table 5.3. Performance of *Aesculus parviflora* and *Aesculus pavia* specimens involved in open-, self-, intraspecific cross- and interspecific cross-pollinations during a breeding exercise.
Table 5.4. Performance of *A. parviflora* and *A. pavia* specimens as male parents in a series of intra- or interspecific crosses with *A. parviflora*.

<table>
<thead>
<tr>
<th>Species and specimens as male parents</th>
<th><em>A. parviflora</em> specimens as female parents</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Par6</em></td>
<td><em>Par7</em></td>
</tr>
<tr>
<td></td>
<td>N⁷  Fruit set (%)</td>
<td>N      Fruit set (%)</td>
</tr>
<tr>
<td><em>A. parviflora</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Par3</td>
<td>59 11.9</td>
<td>92 21.7</td>
</tr>
<tr>
<td>Par9</td>
<td>119 5.9</td>
<td>143 16.1</td>
</tr>
<tr>
<td><em>A. pavia</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pav1</td>
<td>67 0</td>
<td>103 3.0</td>
</tr>
<tr>
<td>Pav6</td>
<td>124 0.8</td>
<td>122 2.5</td>
</tr>
</tbody>
</table>

*Specimens of *A. parviflora* and *A. pavia* are described in Appendix F.

*N = number of intraspecific or interspecific crossing events initiated.*

Table 5.5. Performance of *A. parviflora* and *A. pavia* specimens as male parents in a series of intra- or interspecific crosses with *A. pavia*.

<table>
<thead>
<tr>
<th>Species and specimens as male parents</th>
<th><em>A. pavia</em> specimens as female parents</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Pav1</em></td>
<td><em>Pav2</em></td>
</tr>
<tr>
<td></td>
<td>N⁷  Fruit set (%)</td>
<td>N      Fruit set (%)</td>
</tr>
<tr>
<td><em>A. parviflora</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Par1</td>
<td>81 1.2</td>
<td>40 7.5</td>
</tr>
<tr>
<td>Par7</td>
<td>67 0</td>
<td>46 2.2</td>
</tr>
<tr>
<td>Par9</td>
<td>62 0</td>
<td>57 0</td>
</tr>
<tr>
<td><em>A. pavia</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pav1</td>
<td>66 10.6</td>
<td>16 25.0</td>
</tr>
<tr>
<td>Pav6</td>
<td>27 37.0</td>
<td>23 26.1</td>
</tr>
</tbody>
</table>

*Specimens of *A. parviflora* and *A. pavia* are described in Appendix F.

*N = number of intraspecific or interspecific crossing events initiated.*
### Table 5.6

Seed weights from *Aesculus parviflora* and *Aesculus pavia* specimens involved in open-, self-, intraspecific cross- and interspecific cross-pollinations during a breeding exercise.

<table>
<thead>
<tr>
<th>Species and specimens as female parents</th>
<th>Open pollinations</th>
<th>Self pollinations</th>
<th>Intraspecific pollinations</th>
<th>Interspecific pollinations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N&lt;sup&gt;z&lt;/sup&gt;</td>
<td>Wt ± S.E. (g)</td>
<td>N</td>
<td>Wt ± S.E. (g)</td>
</tr>
<tr>
<td><em>A. parviflora</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Par6&lt;sup&gt;y&lt;/sup&gt;</td>
<td>15</td>
<td>9.1 ± 0.7</td>
<td>5</td>
<td>9.0 ± 1.4</td>
</tr>
<tr>
<td>Par7</td>
<td>59</td>
<td>7.9 ± 0.3</td>
<td>26</td>
<td>9.5 ± 0.5</td>
</tr>
<tr>
<td>Par8</td>
<td>39</td>
<td>6.4 ± 0.5</td>
<td>31</td>
<td>4.2 ± 0.5</td>
</tr>
<tr>
<td><em>A. pavia</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pav1</td>
<td>17</td>
<td>15.4 ± 1.2</td>
<td>24</td>
<td>12.7 ± 1.0</td>
</tr>
<tr>
<td>Pav2</td>
<td>—</td>
<td>—</td>
<td>4</td>
<td>8.2 ± 1.0</td>
</tr>
</tbody>
</table>

<sup>z</sup>N = number of open-, self-, intraspecific and interspecific crossing events initiated.

<sup>y</sup>Specimens of *A. parviflora* and *A. pavia* are described in Appendix F.
Figure 5.1. Estimate of maternal potential in an *Aesculus* breeding program based on fruit per tree.
Figure 5.2. Estimate of maternal potential in an *Aesculus* breeding program based on seeds per tree.
Figure 5.3. The effect of multiseeded fruit on fruit weight in A. parviflora and A. pavia. Population N for seeds from single-seeded fruits was standardized to that of the seeds from multiseeded fruit by sub sampling single-seeded fruits at random. Values represent the mean seed weights and their standard errors for seeds from single-seeded fruits (black bars) and for seeds from multi-seeded fruits (gray bars).
Figure 5.4. Single versus multiple hand pollinations of each flower as a breeding program strategy for obtaining seed from intra- or interspecific crosses of *A. parviflora* and *A. pavia*. Bars with black and gray fills represent the success rate in percent of single and multiple hand pollinations, respectively. Numbers in parentheses represent the total number of flowers pollinated.
Figure 5.5. Developing *A. pavia* fruit and seed: a) developing fruit resulting from an intraspecific cross within *A. pavia*; b) developing fruits resulting from an interspecific cross with *A. parviflora*; c and d) putative hybrid seeds.
CITED REFERENCES


APPENDIX A

LIST OF CITATIONS FOR TABLES 1.2-1.7, 1.10, AND 1.12


245


33. Makins, F.K. 1936. The identification of trees and shrubs: how to name without previous knowledge of botany any wild or garden tree or shrub likely to be met with in the British isles. J. M. Dent & Sons, London.


APPENDIX B

LIST OF *AESCULUS* SPECIES, HYBRIDS, AND CULTIVARS UNDER COMMERCIAL PRODUCTION
Various species, varieties and cultivars of *Aesculus* under production.

**Table:**

<table>
<thead>
<tr>
<th>Species</th>
<th>Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. hippocastanum</em></td>
<td>1, 6, 16, 18, 26, 37, 45-46, 48, 50, 52, 54-55, 59, 62, 67-68, 78-79, 82</td>
</tr>
<tr>
<td>'Baumannii'</td>
<td>1, 6, 16, 23, 32, 38, 44, 46, 50, 55, 62, 72-73, 75, 82, 85, 92-93</td>
</tr>
<tr>
<td>'Lanciata'</td>
<td>6, 11, 32</td>
</tr>
<tr>
<td>'Pyramidalis'</td>
<td>6</td>
</tr>
<tr>
<td>'Unbractillata'</td>
<td>6, 48</td>
</tr>
<tr>
<td>'Wisselink'</td>
<td>11</td>
</tr>
<tr>
<td><em>A. turbinata</em></td>
<td>6, 32, 42, 48</td>
</tr>
<tr>
<td>var. <em>pubescens</em></td>
<td>6, 11</td>
</tr>
<tr>
<td><em>A. californica</em></td>
<td>32</td>
</tr>
<tr>
<td><em>A. chinensis</em></td>
<td>6</td>
</tr>
<tr>
<td><em>A. indica</em></td>
<td>'Sydney Pearce'</td>
</tr>
<tr>
<td><em>A. wilsonii</em></td>
<td>6</td>
</tr>
<tr>
<td><em>A. parviflora</em></td>
<td>1, 2, 4-5, 10-11, 13, 16, 19, 24, 26-29, 32, 34-36, 38-42, 45-48, 50, 53, 57, 60, 62, 64-65, 67-68, 74, 77-78, 81, 84-87, 90-94</td>
</tr>
<tr>
<td>f. <em>serotina</em></td>
<td>32</td>
</tr>
<tr>
<td>'Roger'</td>
<td>80</td>
</tr>
<tr>
<td><em>A. flava</em></td>
<td>1-3, 6, 10, 13, 16, 23, 25-27, 32, 38, 42, 46, 50, 53, 60-62, 64, 67, 72, 78, 87</td>
</tr>
<tr>
<td>var. <em>arguta</em></td>
<td>6</td>
</tr>
<tr>
<td>'Fall Red'</td>
<td>32</td>
</tr>
<tr>
<td>'October Red'</td>
<td>11</td>
</tr>
<tr>
<td><em>A. pavia</em></td>
<td>2, 6-7, 13-14, 16-17, 20, 26-29, 31-32, 36-38, 41-42, 46, 48, 50-52, 54-56, 59-61, 63, 66-67, 70, 82, 86, 88-89, 91, 94</td>
</tr>
<tr>
<td>'Atrosanguinea'</td>
<td>11</td>
</tr>
<tr>
<td>'Biltmore Selection'</td>
<td>29, 91</td>
</tr>
<tr>
<td>'Humilis'</td>
<td>11</td>
</tr>
<tr>
<td>'Koehnei'</td>
<td>11</td>
</tr>
<tr>
<td>'Spring Purple'</td>
<td>11</td>
</tr>
<tr>
<td>'Splendens'</td>
<td>11, 48</td>
</tr>
<tr>
<td><em>A. sylvatica</em></td>
<td>6</td>
</tr>
<tr>
<td>var. <em>pubescens</em></td>
<td>6, 48</td>
</tr>
<tr>
<td>var. <em>tomentosa</em></td>
<td>6, 48</td>
</tr>
<tr>
<td><em>A. × arnoldiana</em></td>
<td>6, 42,</td>
</tr>
<tr>
<td>'Autumn Splendor'</td>
<td>6, 13, 32, 38, 46</td>
</tr>
<tr>
<td><em>A. × bushii</em></td>
<td>48</td>
</tr>
<tr>
<td><em>A. × carnea</em></td>
<td>6, 12, 81</td>
</tr>
<tr>
<td>'Fort McNair'</td>
<td>1-2, 4, 9, 13, 16, 19, 21-23, 27, 32-33, 38, 46, 49, 54, 58, 61, 65-66, 68-69, 71, 74, 75, 82, 84, 86, 91</td>
</tr>
<tr>
<td>'O'Neill Red'</td>
<td>6, 13, 32, 38, 49, 54, 56, 63</td>
</tr>
<tr>
<td>*A. × 'Homestead'</td>
<td>78</td>
</tr>
<tr>
<td><em>A. × hybrida</em></td>
<td>48</td>
</tr>
<tr>
<td><em>A. × mutabilis</em></td>
<td>6, 42</td>
</tr>
<tr>
<td>'Induta'</td>
<td>32</td>
</tr>
<tr>
<td>'Penduliflora'</td>
<td>11</td>
</tr>
<tr>
<td><em>A. × neglecta</em></td>
<td>6</td>
</tr>
<tr>
<td>'Autumn Fire'</td>
<td>11</td>
</tr>
<tr>
<td>'Erythroblastos'</td>
<td>6, 11, 32</td>
</tr>
<tr>
<td><em>A. × plantierensis</em></td>
<td>32</td>
</tr>
<tr>
<td><em>A. × woerlitzensis</em></td>
<td>48</td>
</tr>
<tr>
<td>'Ellwangeri'</td>
<td>6, 48</td>
</tr>
</tbody>
</table>

* Numbers in this table refer to specific producers found in Appendix C.
APPENDIX C

THE NAMES AND CONTACT INFORMATION FOR PRODUCERS OF _AESCULUS_
The names and contact information for producers of various *Aesculus* species.

Numbers of producers correspond to numbers found next to various *Aesculus* listed on Appendix B.
Appendix C (continued)

25. Dykes & Son Nursery
   825 Maude Etter Rd.
   McMinnville TN 37110
   931-668-8833

26. Earthscapes, Inc.
   10403 State Rt 48
   Loveland, OH 45140
   513-683-0144
   pjwhite1@aol.com
   earthscapesinc.com

27. Eastside Nursery Inc.
   6723 Lithopolis Rd.
   Groveport OH 43125
   614-836-9800
   david.grff@east sidenursery.com
   eastsidenursery.com

28. E. F. Pouly Co
   9088 Back Orrville Rd.
   Orrville, OH 44667
   330-683-2037
   pouly@sssnet.com

29. Elk Mountain
   P.O. Box 599
   Asheville, NC 28802
   828-683-9330

30. Fairway Nursery
    12568 SE 162nd
    Clackamas OR 97015
    503-658-2520

31. Fisher Nursery
    8485 SE 282nd Ave.
    Gresham, OR 97080
    503-663-0680

32. Forestfarm
    990 Tetherow Rd.
    Williams, OR 97544-9599
    541-846-7269
    FAX 541-846-6963
    plants@forestfarm.com

33. Fullmer's Landscaping, Inc.
    9547 West 3rd Street
    Dayton, OH 45427
    937-835-5642
    kent@fullmerslandscaping.com
    fullmerslandscaping.com

34. Goowin's Nurseries & Trees
    2158 Henderson Ave.
    Washington, PA 15301
    724-228-6238

35. Greenbrier Nurseries
    HC65 Box 31
    Talcott, WV 24981
    304-466-2660

36. Harkridge Farms Inc.
    P.O. Box 3349
    Hickory, NC 28603
    919-562-0114

37. Heritage Seedlings Inc.
    4199 75th Ave.
    Salem, OR 97301
    503-585-9835

38. Herman Losely & Son Inc.
    3410 Shepard Rd
    Perry, OH 44081
    440-259-2725
    bryan@losely.com
    losely.com

39. Highlandbrook Nursery
    1720 Allensville Rd.
    Elkton, KY 42220
    502-265-0020

40. Hillcrest Nursery
    998 Red House Road
    Richmond, KY 40475
    859-623-9394

41. Hobby Nursery
    570 TR2152
    Loudonville, OH 44642
    419-368-3314,

42. Indiana Propagation Co.
    #3 Lyon Block
    Salem, IN 47167
    812-883-4500

43. J. C. Bakker & Sons Limited
    1209 Third St RR#3
    St. Catharines, Ontario Canada
    905-935-4533

44. J. Frank Schmidt & Son Co.
    9500 S.E. 327th Ave.
    P.O. Box 189
    Boring, OR 97009
    800-825-8202

45. King Wholesale Nurseries
    Rd # 14 RT 130E
    Greensburg, PA 15601
    724-834-8930

46. Klyn Nurseries Inc.
    3322 South Ridge Rd.
    Perry, OH 44081
    440-259-3811
    bhendricks@alltel.net
    klynnurseries.com

47. Lake County Nursery, Inc.
    P.O. Box 122, Rt. 84
    Perry, OH 44081
    440-259-5571
    maryjos@lakecountynursery.com
    lakecountynursery.com

48. Lawyer Nursery Inc.
    950 Highway 200 West,
    Plains, MT 59859
    406-826-3881
    FAX 406-826-5700
    trees@lawyernursery.com

Continued
49. Limbs & Leaves Tree Farm  
10944 State Route 316, W  
Williamsport, OH 43164  
740-869-3898  
gliffarm@msohio.net

50. Listerman & Associates, Inc.  
1236 Freeman Drive  
Beavercreek, OH 45434  
937-426-6301  
adam@listermanassoc.com  
listermanassoc.com

51. McRury-Tebbe Tree Farm  
6260 Havens Road  
Blacklick, OH 43004  
614-855-9545

52. Meadow Lake Nursery  
3500 NE Hawn Green Rd.  
McMinnville, OR 97128  
503-435-2000

53. Mentor Heights Nursery, Ltd.  
7343 Chillicothe Road  
Mentor, OH 44060  
440-255-2421

54. Miller Landscape Nursery  
838 Ankeny Hill Rd.  
Jefferson, OR 97352  
503-399-1599

55. Mineral Springs Ornamentals  
1150NW McBride Cemetery Rd.  
Cariton, OR 97111  
503-852-6129

56. Mobjack Nurseries  
HC 75 Box 7965  
Mobjack, VA 23056  
800-729-6625

57. Monrovia  
13455 S.E. Lafayette Hwy  
Dayton, OR 97114  
503-868-7941

58. Moore's Nursery  
493 Wilson Mill Rd.  
New Wilmington, PA 16142  
412-946-3581

59. Mori Nurseries  
RR#2 Niagara-on-the-Lake  
ON, Canada L0S1JO  
905-468-3217

60. Muskingum Valley Nursery  
P.O. Box 351  
Dresden, OH 43821  
740-754-6214

61. Musser Forest  
P.O. Box 340  
Indiana, PA 15701  
412-465-5685

62. North Branch Nursery, Inc.  
P.O. Box 353  
Pemberville, OH 43450  
419-287-4679  
barb@northbranchnursery.com  
northbranchnursery.com

63. Northside Tree Farm, Inc.  
1195 Fairview Road  
Zanesville, OH 43701  
740-452-7452  
northside@y-city.net

64. Oakland Nursery Inc.  
1156 Oakland Park Avenue  
Columbus, OH 43224  
614-268-3511  
paul@oaklandnursery.com  
oaklandnursery.com

65. Oldham County Nursery  
3918 Glenarm Rd.  
Crestwood, KY 40014  
502-241-6025

66. Paradise Tree Farm, Inc.  
4136 Paradise Road  
Seville, OH 44273  
330-723-0478

67. Pickens Tree Farms  
10501 Cochran Road  
Williamsport, OH 43164  
740-869-2888  
rpickens@qam.net  
pickensstreefarm.com

68. The Poruban Nursery  
38029 Detroit Rd.  
Avon, OH 44011-2162  
440-934-6221  
fporuban@eriecoast.com

69. R & J Farms, Inc.  
9800 W. Pleasant Home Road  
West Salem, OH 44287  
419-846-3179  
rjfarms@bright.net

70. Riverfarm Nursery  
P.O. Box 56  
Goshen, KY 44026  
502-228-5408

71. Roemer Nursery Inc.  
2310 Green Rd.  
Madison, OH 44057  
440-428-5178

72. Rusty Oak Nursery Ltd.  
P.O. Box 436  
Valley City, OH 44280  
330-225-7704  
rustyoak@aol.com

Continued
### Appendix C (continued)

<table>
<thead>
<tr>
<th>No.</th>
<th>Nursery Name</th>
<th>Address Details</th>
<th>Phone Numbers</th>
<th>Email/Website</th>
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<tr>
<td>73</td>
<td>Scarff's Nursery Inc.</td>
<td>411 N. State Route 235, New Carlisle, OH 45344</td>
<td>937-845-3821</td>
<td><a href="mailto:scarffnurs@aol.com">scarffnurs@aol.com</a> scarffs.com</td>
</tr>
<tr>
<td>74</td>
<td>Scioto Gardens</td>
<td>3351 State Route 37, W Delaware, OH 43015</td>
<td>740-363-8264</td>
<td><a href="mailto:sales@sciotogardens.com">sales@sciotogardens.com</a> sciotogardens.com</td>
</tr>
<tr>
<td>75</td>
<td>Schichtel's Nursery</td>
<td>7420 Peters Rd., Springsville, NY 14141</td>
<td>716-592-9383</td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>Shade Tree's Unlimited</td>
<td>8260 S 800 E92, Ft. Wayne, IN 46804</td>
<td>219-625-3269</td>
<td></td>
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<tr>
<td>77</td>
<td>Shemin Nurseries, Inc.</td>
<td>4877 Vulcan Avenue, Columbus, OH 43228</td>
<td>614-876-1193</td>
<td><a href="mailto:ahevezi@sheminnurseries.com">ahevezi@sheminnurseries.com</a> shemin.com</td>
</tr>
<tr>
<td>78</td>
<td>Sheridan Nurseries</td>
<td>R.R. #4 12302 10th Line, Georgetown, ON L7G457</td>
<td>416-798-7970</td>
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<tr>
<td>79</td>
<td>Sherman Nursery</td>
<td>1300 Grove Street, Charles City, IO 50616</td>
<td>515-288-1124</td>
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<tr>
<td>80</td>
<td>Simpson Nursery Company</td>
<td>1504 Wheatland Rd., Vincennes, IN 47591</td>
<td>812-882-2441</td>
<td></td>
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<tr>
<td>81</td>
<td>Smith's Gardens, Inc.</td>
<td>7520 Home Road, Delaware, OH 43015</td>
<td>740-881-6147</td>
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<tr>
<td>82</td>
<td>Spaargaren W. J. b.v.</td>
<td>P.O. Box 18, Boskoop Holland 2770 AA, The Netherlands</td>
<td>+31-172-218058</td>
<td></td>
</tr>
<tr>
<td>83</td>
<td>Split Rail Nursery</td>
<td>26629 Kingston Pike, Circleville, OH 43113</td>
<td>740-474-2028</td>
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<tr>
<td>84</td>
<td>Studebaker Nurseries Inc.</td>
<td>11140 Milton-Carlisle Rd., New Carlisle, OH 45344</td>
<td>937-845-3816</td>
<td><a href="mailto:salessmi@bizwoh.rr.com">salessmi@bizwoh.rr.com</a> studebakenurseries.com</td>
</tr>
<tr>
<td>85</td>
<td>Sunleaf Nursery</td>
<td>5900 North Ridge Road, Madison, OH 44057</td>
<td>440-428-4108</td>
<td><a href="mailto:tim@sunleaf.com">tim@sunleaf.com</a> sunleaf.com</td>
</tr>
<tr>
<td>86</td>
<td>The Homestead Nurseries</td>
<td>Biezen 105, 2771 CT Boskoop, The Netherlands</td>
<td>+31-172-218021</td>
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<tr>
<td>87</td>
<td>The Siebenthaler Co.</td>
<td>3001 Catalpa Dr., Dayton, OH 45405</td>
<td>937-274-1154</td>
<td><a href="mailto:siebenthaler@erinet.com">siebenthaler@erinet.com</a> siebenthaler.com</td>
</tr>
<tr>
<td>88</td>
<td>The Tree Fann</td>
<td>3072 RT 303, Richfield, OH 44266</td>
<td>330-659-3669</td>
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<tr>
<td>89</td>
<td>Tree Tyme Nursery</td>
<td>1919 State Route 307 East, P.O. Box 92, Austinburg, OH</td>
<td>440-275-3332</td>
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<tr>
<td>90</td>
<td>W. A. Natorp Corp.</td>
<td>8601 Snider Road, Mason, OH 45040</td>
<td>513-398-4634</td>
<td><a href="mailto:psmommer@natorp.com">psmommer@natorp.com</a> natorp.com</td>
</tr>
<tr>
<td>91</td>
<td>Warner Kingwood Nurseries</td>
<td>37611 Pleasant Valley Rd., Willoughby, OH 44094</td>
<td>440-946-0880</td>
<td><a href="mailto:warnerkingwood@aol.com">warnerkingwood@aol.com</a></td>
</tr>
<tr>
<td>92</td>
<td>We-Du Nurseries</td>
<td>2055 Polly Spout Road, Marion, NC 28752</td>
<td>828-738-8300</td>
<td><a href="mailto:info@we-du.com">info@we-du.com</a></td>
</tr>
<tr>
<td>93</td>
<td>Willoway Nurseries Inc.</td>
<td>4534 Center Rd., P.O. Box 299, Avon, OH 44011</td>
<td>440-934-4335</td>
<td><a href="mailto:danny@willowaynurseries.com">danny@willowaynurseries.com</a> willownurseries.com</td>
</tr>
<tr>
<td>94</td>
<td>Wilson Nurseries Inc.</td>
<td>3690 East-West Connector, Frankfort, KY 40601</td>
<td>502-223-1488</td>
<td></td>
</tr>
<tr>
<td>95</td>
<td>Yadkin Valley Nursery Co.</td>
<td>1132 Coniner Ridge Dr., Yadkinville, NC 27055</td>
<td>336-463-2181</td>
<td></td>
</tr>
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</table>
APPENDIX D

LIST OF CITATIONS FOR TABLE 1.8
Cited in references table 1.8


APPENDIX E

BOTANIC GARDENS AND ARBORETA WITH *AESCULUS* COLLECTIONS
A list of Botanic Gardens and Arboreta that have collections of *Aesculus*. 

Continued
<table>
<thead>
<tr>
<th>Name</th>
<th>Address</th>
<th>Phone</th>
</tr>
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<tbody>
<tr>
<td>The Morton Arboretum</td>
<td>4100 Illinois Rte. 53, Lisle, IL 60552</td>
<td>(630) 968-0074</td>
</tr>
<tr>
<td>Royal Botanic Garden, Kew</td>
<td>Kew, Surrey TW9 3AB, UK</td>
<td>Tel. +44/181/332-5000</td>
</tr>
<tr>
<td>Winterthur Museum and Gardens</td>
<td>Winterthur, DE 19735</td>
<td></td>
</tr>
<tr>
<td>Nebraska Statewide Arboretum</td>
<td>University of Nebraska</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P.O. Box 830715, Lincoln, NE 68583</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(402) 472-2971</td>
<td></td>
</tr>
<tr>
<td>Royal Botanic Garden</td>
<td>1212 Mission Canyon Road, Santa Barbara, CA 93105</td>
<td>(805) 682-4726</td>
</tr>
<tr>
<td>The University of Wisconsin- Madison</td>
<td>1207 Seminole Highway, Madison, WI 53711</td>
<td>(608) 262-2746</td>
</tr>
<tr>
<td>The New York Botanical Garden</td>
<td>200th Street &amp; Kazimiroff Blvd., Bronx, NY 10458</td>
<td>(718) 817-8700</td>
</tr>
<tr>
<td>Secrest Arboretum</td>
<td>Agricultural Research &amp; Development Center, The Ohio State University, 1680 Madison Ave., Wooster, OH 44691</td>
<td>(330) 263-3761</td>
</tr>
<tr>
<td>Palmenergarten</td>
<td>Siesmayerstrasse 61, 6000 Frankfurt, Germany</td>
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</tr>
<tr>
<td>Spring Grove Cemetery</td>
<td>4521 Spring Grove Avenue, Cincinnati, OH 45232</td>
<td></td>
</tr>
<tr>
<td>Planting Fields Arboretum</td>
<td>Planting Fields Rd., Oyster Bay, NY 11771</td>
<td>(516) 922-9200</td>
</tr>
<tr>
<td>The State Botanical Garden of Georgia</td>
<td>University of Georgia, 2450 South Milledge Avenue, Athens, GA 30605</td>
<td>(706) 542-1244</td>
</tr>
<tr>
<td>Rancho Santa Ana Botanic Garden</td>
<td>1500 North College Avenue, Claremont, CA 91711</td>
<td>(909) 625-8767</td>
</tr>
<tr>
<td>The U.S. National Arboretum</td>
<td>3501 New York Avenue NE, Washington, DC 20002</td>
<td>(202) 245-4523</td>
</tr>
<tr>
<td>J.C. Raulston Arboretum</td>
<td>North Carolina State University, Box 7609, Raleigh, NC 27695</td>
<td>(919) 515-3189</td>
</tr>
<tr>
<td>Washington Park Arboretum</td>
<td>University of Washington, Box 358010, Seattle, WA 98195</td>
<td>(206) 543-8800</td>
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APPENDIX F

AESCULUS SPECIES USED IN THIS RESEARCH INCLUDING

LOCATION AND CONDITION
<table>
<thead>
<tr>
<th>Specimen</th>
<th>Location of Specimen $^c$</th>
<th>Origin of plant material</th>
<th>Plant establishment</th>
<th>Plant condition $^b$</th>
<th>Height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Par1</td>
<td>(NE) Holden Arboretum, Kirtland, OH</td>
<td>Wayside Garden, Hodges, SC</td>
<td>1975</td>
<td>E</td>
<td>3.7</td>
</tr>
<tr>
<td>Par2</td>
<td>(NE) Holden Arboretum, Kirtland, OH</td>
<td>Unknown</td>
<td>1968</td>
<td>E</td>
<td>4.9</td>
</tr>
<tr>
<td>Par3</td>
<td>(C) Private residence, Columbus, OH</td>
<td>Unknown</td>
<td>Unknown</td>
<td>G</td>
<td>2.5</td>
</tr>
<tr>
<td>Par6</td>
<td>(NE) Holden Arboretum, Kirtland, OH</td>
<td>Wayside Garden, Hodges, SC</td>
<td>1970</td>
<td>G</td>
<td>2.1</td>
</tr>
<tr>
<td>Par7</td>
<td>(NE) Holden Arboretum, Kirtland, OH</td>
<td>Arnold Arboretum, Boston, MA</td>
<td>1969</td>
<td>E</td>
<td>4.9</td>
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<tr>
<td>Par8</td>
<td>(NE) Holden Arboretum, Kirtland, OH</td>
<td>Morton Arboretum, Chicago, IL</td>
<td>1969</td>
<td>E</td>
<td>4.9</td>
</tr>
<tr>
<td>Par9</td>
<td>(C) Jeffrey Mansion, Bexley, OH</td>
<td>Unknown</td>
<td>1963</td>
<td>E</td>
<td>4.5</td>
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<tr>
<td>Par10</td>
<td>(C) Ohio State Univ., Columbus, OH</td>
<td>Unknown</td>
<td>Unknown</td>
<td>VG</td>
<td>2.5</td>
</tr>
<tr>
<td>Par11</td>
<td>(C) Chadwick Arboretum, Columbus, OH</td>
<td>Unknown</td>
<td>Unknown</td>
<td>VG</td>
<td>1.4</td>
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<tr>
<td>Par12</td>
<td>(NE) Holden Arboretum, Kirtland, OH</td>
<td>Klehm Nursery, Avalon, WI $^a$</td>
<td>1991</td>
<td>E</td>
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<tr>
<td>Par13</td>
<td>(C) Franklin Park, Columbus, OH</td>
<td>Unknown</td>
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<tr>
<td>Par14</td>
<td>(NE) Holden Arboretum, Kirtland, OH</td>
<td>Klyn Nursery, Perry, OH</td>
<td>1997</td>
<td>F</td>
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</tbody>
</table>

**A. parviflora**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Location of Specimen $^c$</th>
<th>Origin of plant material</th>
<th>Plant establishment</th>
<th>Plant condition $^b$</th>
<th>Height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pav1</td>
<td>(C) Ohio State Univ., Columbus, OH</td>
<td>Unknown</td>
<td>1969</td>
<td>E</td>
<td>6.1</td>
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<tr>
<td>Pav2</td>
<td>(NE) Private residence, Madison, OH</td>
<td>Falconscape Gardens, Medina, OH</td>
<td>1979</td>
<td>VG</td>
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<tr>
<td>Pav3</td>
<td>(NE) Holden Arboretum, Kirtland, OH</td>
<td>Leonard Hanna Estate, Kirtland Hills, OH</td>
<td>1957</td>
<td>E</td>
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<tr>
<td>Pav4</td>
<td>(C) Dawes Arboretum, Newark, OH</td>
<td>Hillier &amp; Sons, Hampshire, England</td>
<td>1948</td>
<td>G</td>
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<tr>
<td>Pav6</td>
<td>(C) Dawes Arboretum, Newark, OH</td>
<td>Hillier &amp; Sons, Hampshire, England</td>
<td>1948</td>
<td>VG</td>
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<td>G</td>
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Continued

The identification, location, age, origin and condition of *Aesculus* genotypes examined for their inflorescence, pollen, fruit, seed, and breeding characteristics.
Appendix F (continued)

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Location of Specimen</th>
<th>Origin of plant material</th>
<th>Plant establishment</th>
<th>Plant condition$^y$</th>
<th>Height (m)</th>
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<tbody>
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<td>Pav9</td>
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<td>Unknown</td>
<td>1949</td>
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<td>Unknown</td>
<td>Unknown</td>
<td>VG</td>
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<tr>
<td>A. flava</td>
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<td>Fla1</td>
<td>(C) Ohio State Univ., Columbus, OH</td>
<td>Unknown</td>
<td>Unknown</td>
<td>E</td>
<td>26.0</td>
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<td>Unknown</td>
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<td>A. hippocastanum</td>
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<td>Hip1</td>
<td>(NE) Holden Arboretum, Kirtland, OH</td>
<td>Kohankie and Sons, Painesville, OH</td>
<td>1964</td>
<td>E</td>
<td>9.5</td>
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<td>A. sylvatica</td>
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<td>Sy11</td>
<td>(C) Dawes Arboretum, Newark, OH</td>
<td>U. S. National Arboretum</td>
<td>1985</td>
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<td>A. × carnea</td>
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<td>Car1</td>
<td>(C) Dawes Arboretum, Newark, OH</td>
<td>Cole Nursery, Circleville, OH</td>
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<td>Car2</td>
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<td>Mentor Heights Nursery, Mentor, OH</td>
<td>1965</td>
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<tr>
<td>A. × plantierensis</td>
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<td>(C) Dawes Arboretum, Newark, OH</td>
<td>Hillier &amp; Sons, Hampshire, England</td>
<td>1947</td>
<td>E</td>
<td>12.0</td>
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</table>

$^x$ (Area in Ohio) institution, city, state; (NE) = northeastern Ohio, (C) = central Ohio.

$^y$ Condition determined by visual inspection; E = excellent, VG = very good, G = good, F = fair.

$^z$ Now incorporated as Song Sparrow Perennial Farms.
APPENDIX G

INFLORESCENCE CHARACTERISTICS BY SPECIMEN PLANT FOR

AESCUIUS PARVIFLORA AND AESCULUS PAVIA
The mean length, number of total flowers, and complete flowers per panicle for various *Aesculus parviflora* and *Aesculus pavia.*
APPENDIX H

PERCENTAGE OF COMPLETE FLOWERS WITHIN AESCULUS PANICLES
The percentage of complete flowers within *Aesculus* panicles. Values represent the mean percentage ± S.E. of complete flowers for various years. Bars with black, light gray, dark gray and hatched represent 1997, 1998, 1999, and 2000 respectively.
APPENDIX I

EFFECT OF INFLORESCENCE POSITION WITHIN THE PLANT ON INFLORESCENCE CHARACTERISTICS
The effect of inflorescence position within the plant on inflorescence length, the number of flowers per panicle and the number of complete flowers per panicle for *Aesculus parviflora*.

<table>
<thead>
<tr>
<th>Crown position</th>
<th>Panicle length (cm)</th>
<th>Flowers per panicle</th>
<th>Frequency of mixed panicles (%)</th>
<th>Complete flowers per panicle</th>
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</thead>
<tbody>
<tr>
<td>Upper</td>
<td>39.5 a*</td>
<td>321.8 a</td>
<td>85.0</td>
<td>20.5 b</td>
</tr>
<tr>
<td>Middle</td>
<td>37.8 b</td>
<td>294.0 b</td>
<td>71.3</td>
<td>28.2 a</td>
</tr>
<tr>
<td>Lower</td>
<td>36.4 b</td>
<td>264.6 c</td>
<td>78.0</td>
<td>24.7 ab</td>
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*Means with similar postscripts are not statistically different at the $\alpha = 0.01$ level as determined by the SNK means test.
APPENDIX J

VALUES FOR CALCULATING FRUIT AND SEED POTENTIAL
<table>
<thead>
<tr>
<th>Specimen</th>
<th>Average number of panicles per plant</th>
<th>Sex ratio</th>
<th>Fruit potential</th>
<th>Seed potential</th>
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<tbody>
<tr>
<td>Par1</td>
<td>1170.5</td>
<td>0.11</td>
<td>39647.8</td>
<td>237887.1</td>
</tr>
<tr>
<td>Par2</td>
<td>1997.5</td>
<td>0.11</td>
<td>61265.2</td>
<td>367591.0</td>
</tr>
<tr>
<td>Par3</td>
<td>189.0</td>
<td>0.02</td>
<td>411.6</td>
<td>2469.9</td>
</tr>
<tr>
<td>Par6</td>
<td>244.5</td>
<td>0.08</td>
<td>1575.9</td>
<td>9455.6</td>
</tr>
<tr>
<td>Par7</td>
<td>505.5</td>
<td>0.06</td>
<td>6025.6</td>
<td>36153.8</td>
</tr>
<tr>
<td>Par8</td>
<td>476.5</td>
<td>0.07</td>
<td>5273.2</td>
<td>31639.1</td>
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<tr>
<td>Par9</td>
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<td>0.05</td>
<td>6781.6</td>
<td>40689.4</td>
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<td>46.0</td>
<td>0.06</td>
<td>393.8</td>
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<table>
<thead>
<tr>
<th>Specimen</th>
<th>Average number of panicles per plant</th>
<th>Sex ratio</th>
<th>Fruit potential</th>
<th>Seed potential</th>
</tr>
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<tbody>
<tr>
<td>Pav1</td>
<td>997.0</td>
<td>0.12</td>
<td>11955.4</td>
<td>71732.5</td>
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<td>Pav2</td>
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<td>0.11</td>
<td>279.7</td>
<td>1678.1</td>
</tr>
<tr>
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<td>1974.0</td>
<td>0.07</td>
<td>6876.8</td>
<td>41260.6</td>
</tr>
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<td>Pav4</td>
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<td>1359.0</td>
<td>8153.9</td>
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The values for calculating the fruit and seed potential for the provenance study.
APPENDIX K

VALUES FOR THE FRUIT AND SEED REALIZATION FOR THE PROVENANCE
<table>
<thead>
<tr>
<th>Specimen</th>
<th>Average number of panicles with seed</th>
<th>Average number of fruit per panicle</th>
<th>Average number of fruit per panicle</th>
<th>Realized fruit set</th>
<th>Realized fruit set</th>
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</thead>
<tbody>
<tr>
<td>A. parviflora</td>
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<td></td>
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</tr>
<tr>
<td>Par1</td>
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</tr>
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<td>584</td>
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<td>A. pavia</td>
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The values for calculating the realized fruit and seed set for the provenance study.
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