Propagule Type and Planting Time for Field-established Mayapple

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INTRODUCTION

The American mayapple (*Podophyllum peltatum* L.; Berberidaceae) is a rhizomatous perennial species found throughout eastern North America. The species has received attention recently due to pharmaceutical compounds found in its leaves. Podophyllotoxin, along with α-peltatin, and β-peltatin, are aryltetralin lignans known to have biological activity such as anti-cancer, anti-fungal, anti-viral, anti-mitotic, and immunostimulatory properties (Rahman et al. 1995; Goel et al. 1998; Imbert 1998; Pugh et al. 2001). Podophyllotoxin is also used as a precursor in the semi-synthesis of etoposide, teniposide, and etopophos, three drugs used in chemotherapy (Stähelin and von Wartburg 1991; Giri and Narasu 2000).

Podophyllotoxin is currently available from the Indian mayapple ([*Podophyllum emodi* Wall., Berberidaceae (syn. *P. hexandrum* Royle)], a species native to specific regions of the Himalayas. Rhizomes and roots of the Indian mayapple contain up to 53.5 mg·g⁻¹ podophyllotoxin on a dry weight basis compared to 14.7 mg·g⁻¹ for rhizomes of the American mayapple. However, leaves of the American mayapple contain up to 55.9 mg·g⁻¹ podophyllotoxin on a dry weight basis (Canel et al. 2001; Moraes et al. 2001). Because leaves are a renewable resource, it may be possible to establish commercial plantings of this species. Rhizomes could be left undisturbed while leaves could be harvested annually. In addition, commercial plantings could reduce harvest pressure on the Indian species, which was declared endangered due to over-exploitation of wild populations (Foster 1993; Nautiyal 1996; Giri and Narasu 2000; Rai et al. 2000).

It has been shown that podophyllotoxin content in leaves of American mayapple is highly variable, and some wild populations have very low content. Wildcrafting (that is, harvesting of plant materials from wild populations) would lead to inconsistent results and possibly poor drug yield (Bastos et al. 1996; Moraes et al. 2001). Commercial plantings, in contrast, would rely on selection and propagation of clonal populations with high podophyllotoxin content. Successful propagation of American mayapple using in vitro techniques has been reported (Sadowska et al. 1997; Moraes-Cerdeira et al. 1998), but most reports of vegetative propagation of *Podophyllum* species have been limited to the Indian mayapple. Results have been highly variable using rhizome cuttings. From 34% to 98% of rhizome cuttings 2.0 to 2.5 cm in length and planted from May to July in India were successful in establishing plants (Badhwar and Sharma 1963; Choudhary et al. 1998). Nadeem et al. (2000) found 70% of rhizome cuttings treated with 100 µM IBA produced roots compared to only 30% for untreated cuttings. Troup (1915) estimated that plants grown from rhizome cuttings would require 12 years to attain marketable size.

Rhizome structure of American mayapple is distinctly different from that of the Indian mayapple. Reports about vegetative propagation of American mayapple were not found; therefore, the purpose of research presented in this report was to identify types of rhizome cuttings suitable for establishing plantings of American mayapple under field conditions. In addition, the purpose was to compare fall versus spring planting.

METHODOLOGY

Mayapple rhizomes of known podophyllotoxin content were harvested from a native population in Oxford, Mississippi on Oct. 12, 2000 and Feb. 20, 2001. At each harvest, rhizomes were divided into segments to create three types of propagules: two-node segments comprised of a single terminal node, an adjacent dormant node, and several cm of rhizome tissue (T+D); one-node segments comprised of a single terminal node with 3 to 5 cm of rhizome tissue (T); and one-node segments comprised of a single dormant node with 3 to 5 cm of rhizome tissue extending from each side of the node (D). Only well-rooted propagules were used, though T segments generally had shorter and fewer roots than T+D and D segments.

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Propagules were transplanted to raised beds located in Verona, Mississippi, soil type Ora fine sandy loam (fine-loamy, siliceous, thermic, typic Fragiudults), one day after harvest. Raised beds were prepared in fall 2000 with a press-pan-type bed shaper and spaced 1.8 m apart, center to center. Beds were formed 15 cm high and 0.8 m wide across the top, and drip irrigation tubing was installed in the center of the bed. Each experimental unit consisted of 30 propagules of a single type arranged in two parallel rows spaced 30 cm apart and 1.5 m in length. Propagules were placed 10 cm apart and 4–5 cm deep within each row and covered with soil. Care was taken to orient propagules with roots down and nodes up. Beds were then mulched with a 10 cm layer of wheat straw. Irrigation was seldom needed during the spring of 2001 due to timely and frequent precipitation. Plantings were side dressed once with ammonium nitrate fertilizer applied at the rate of 22 kg·ha⁻¹ N in May. Weeds that emerged through the mulch during growth and senescence of the mayapple crop were removed by hand.

A 2×3 factorial arrangement of treatments (three levels of propagule type and two levels of planting time) was used in a randomized complete block design with four replications. Date of shoot emergence, days to senescence, and plant height of the mature mayapple plants were recorded during the spring of 2001. Leaves were removed from the plants to measure leaf area after plants began to senesce. Leaf area was measured using a LI-3100 Area Meter (LI-COR, Lincoln, Nebraska). Data were analyzed with Statistical Analysis System for PC using the General Linear Model procedure (SAS Institute Inc., Cary, North Carolina). The Mixed procedure was used for the height data because of an unequal number of subsamples.

RESULTS AND DISCUSSION
Mayapple shoots emerged in March, grew in April and May, and senesced in June (Fig. 1). Propagule type had a greater effect on shoot emergence than planting time. Propagules T+D and T exhibited higher percentages of emergence than propagule D regardless of planting time (Table 1). However, a significant interaction occurred between propagule type and planting time. Spring-planted T+D exhibited a higher percentage of emergence than fall-planted T+D while spring-planted D exhibited a lower percentage compared fall-planted D. Propagule T was not affected by planting time.

Propagule type and planting time affected leaf size (cm² per leaf). Propagules T+D and D consistently produced leaves about twice the size of propagule T, and propagules planted in the spring produced leaves about twice the size of those planted in the fall (Table 1).

Propagule type and planting time also affected leaf area (cm²·m⁻²). The combined effect of greater emergence and larger leaf size resulted in propagule T+D exhibiting significantly greater leaf area than propagules T or D. In addition, spring-planted T+D exhibited significantly greater leaf area than fall-planted T+D (Table 1). Spring-planted T also exhibited significantly greater leaf area than fall-planted T, and this was due to the larger leaf size of spring-planted compared to fall-planted T. Small leaf size of fall-planted T and low percent-
age of emergence of spring-planted D resulted in lower leaf area for each of these treatment combinations and contributed toward the significant interaction effect for leaf area.

Planting time had a small but significant effect on plant height. Spring-planted propagules were slightly taller than fall-planted propagules.

Overall, spring-planted T+D propagules produced the greatest leaf area and appeared to offer greater potential yields of leaf tissue compared to the other treatment combinations. T+D propagules were larger than the other propagules, which resulted in greater growth. Propagule mass, however, was not the only factor influencing leaf area. Spring-planted T produced slightly more leaf area than fall-planted T+D. Propagules T+D and T appeared to benefit from having well developed terminal buds that were able to emerge in the spring in greater numbers than propagule D. Despite the greater leaf size of D, a lower percentage of emergence resulted in lower leaf area.

Physiological factors characteristic of mayapple rhizomes may have influenced the low percentage of emergence of propagule D. First, mayapple rhizomes exhibit strong apical dominance. Regardless of the number of nodes along the length of a rhizome system, only the terminal node produces a shoot in most cases. When a node is severed from the rhizome system by insect, animal, or human activity, the node is released from apical dominance and produces a shoot. It is not known, however, how long apical dominance persists in rhizome segments after being severed from the rhizome system. The results of this study show that spring-planted D exhibited a lower percentage of emergence than fall-planted D and this suggests that apical dominance was more persistent in the spring-planted propagules than in the fall-planted material. Second, mayapple rhizomes in the wild are dormant during the winter months and we have found that exposure to low temperature is required to break this dormancy (unpubl. data). Propagules used in this study were exposed to naturally occurring cold temperatures either after they were transplanted, i.e. fall-planted propagules that overwintered in raised beds, or before they were harvested, i.e. spring-planted propagules that overwintered in the wild. We have observed that dormant node segments appear to require more chilling than segments with a terminal node. Chilling may have influenced fall-planted propagules, which received chilling after being severed from the parent rhizome, differently than spring-planted propagules, which received chilling while remaining attached to the parent rhizome.

Table 1. Shoot emergence, leaf area, leaf size, and plant height of three types of mayapple (P. peltatum) propagules planted in fall 2000 or spring 2001. Propagules were two-node rhizome segments consisting of a terminal node and a dormant node (T+D), one-node rhizome segments consisting of either a single terminal node (T), or a single dormant node (D). About 5 cm of subtending rhizome material remained attached to each node.

<table>
<thead>
<tr>
<th>Propagule type</th>
<th>Shoot emergence (%)</th>
<th>Leaf area (cm²·m⁻²)</th>
<th>Leaf size (cm²)</th>
<th>Plant height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fall</td>
<td>Spring</td>
<td>Mean</td>
<td>Fall</td>
</tr>
<tr>
<td>T+D</td>
<td></td>
<td></td>
<td></td>
<td>679</td>
</tr>
<tr>
<td>T</td>
<td>76</td>
<td>88</td>
<td>82</td>
<td>275</td>
</tr>
<tr>
<td>D</td>
<td>54</td>
<td>18</td>
<td>36</td>
<td>467</td>
</tr>
<tr>
<td>Mean</td>
<td>72</td>
<td>69</td>
<td>93</td>
<td>474</td>
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</table>

<table>
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<tr>
<th>Significance</th>
<th>Propagule type</th>
<th>0.0001</th>
<th>0.0001</th>
<th>0.0059</th>
<th>0.1485</th>
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<tbody>
<tr>
<td>Planting time</td>
<td>0.5081</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0114</td>
<td></td>
</tr>
<tr>
<td>Type × time</td>
<td>0.0001</td>
<td>0.0003</td>
<td>0.8167</td>
<td>0.1458</td>
<td></td>
</tr>
</tbody>
</table>

Values are means of four replications with 30 propagules in each replication. Not all propagules emerged.

More than one shoot can arise from each propagule. Thus, values greater than 100% are possible.

Leaf size calculated from leaf area divided by number of emerged shoots.

Plant height measured from soil surface to highest point of the plant.

P values for propagule type and planting time effects.
Reports have shown that rhizome diameter is correlated with plant size such that larger rhizomes produce larger plants. Propagules used in this study were not sorted by rhizome diameter but were assigned randomly to each experimental unit. Differences in leaf size in this study may be an indication of the effect of propagule vigor. Spring-planted T+D and D exhibited significantly greater leaf size than the other treatment combinations. In a similar manner, spring-planted T+D and T exhibited slightly greater plant height than the other treatment combinations. These measures of plant growth may be indirect indications of the superior vigor of these propagules and of the optimum time of harvest and planting. The American mayapple is a slow-growing plant and we report here first-year observations from an ongoing study.

REFERENCES