Mulch Type, Mulch Depth, and Rhizome Planting Depth for Field-grown American Mayapple

Kent E. Cushman¹ and Muhammad Maqbool²

¹North Mississippi Research and Extension Center, 5421 Highway 145 South, Verona, MS 38879
²Experimental Statistics Unit, Mississippi State University, Box 9653, Mississippi State, MS 39762

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Abstract. American mayapple (Podophyllum peltatum L.) is a rhizomatous herbaceous perennial found in wooded areas of eastern North America and is a source of the pharmaceutical compound podophyllotoxin. To explore the possible domestication of this species, this research examined strategies for establishing mayapple in field plantings using organic mulches. Mayapple rhizome segments were harvested from the wild and transplanted to raised beds in northern Mississippi in Fall 2001. Two types of mulch (pine bark or wheat straw), two depths of mulch (7.5 or 15 cm), and two planting depths (0 or 5 cm) of rhizome segments were examined in a factorial arrangement and randomized complete block design. Data were recorded during spring of 2002 and 2003. Shoot number was not affected by mulch depth, but there was a significant three-way interaction between mulch type, rhizome planting depth, and year. During 2002, treatment combinations were not different, but during 2003 rhizome segments planted 0 cm deep and covered with straw mulch produced about 30% fewer shoots compared to any of the other treatment combinations. Number of emerging shoots was also affected by year, with a 33% increase in shoots from 2002 to 2003. Total leaf area and total leaf dry weight were not affected by mulch depth, but there was a significant three-way interaction between mulch type, rhizome planting depth, and year. During 2002, treatment combinations were not different, but during 2003 rhizome segments planted 0 cm deep and covered with straw mulch produced less leaf area and leaf dry weight than any of the other treatment combinations. The ratio of sexual shoots to total shoots was affected by year, with a higher ratio of sexual shoots occurring in 2002 than 2003. Grasses established in bark mulch to a greater extent than in straw mulch in 2002, but weed control was excellent for all treatments in 2003. These results indicate that rhizome segments planted 0 cm deep and covered with straw mulch consistently produced fewer shoots with less leaf area and dry mass compared to any other treatment combination. We preferred bark mulch, but we can recommend either bark or straw mulch for the purpose of establishing field plantings of American mayapple in full sun as long as rhizome planting depth is 5 cm. There was no difference between the two mulching depths used in this study; therefore, a mulch depth of 7.5 cm can be recommended because of its lower cost.

American mayapple (Podophyllum peltatum L.) is a rhizomatous, herbaceous perennial found in wooded areas of eastern North America and is a source of the pharmaceutical compound podophyllotoxin (Goel et al., 1998; Imbert, 1998; Pugh et al., 2001; Rahman et al., 1995). Podophyllotoxin is currently obtained from rhizomes of the Indian mayapple, P. emodi Wall. (syn. P. hexandrum Royle), but due to destructive harvest of the plant and over-exploitation it was reported an endangered species (Foster, 1993; Rai et al., 2000). Leaves of American mayapple contain relatively high levels of podophyllotoxin, and it was reported that they could be used as an alternative—yet renewable—source of the compound if the species was domesticated (Canel et al., 2001; Moraes et al., 2000).

Herbicides are commonly used in commercial horticulture, but few herbicides are labeled for medicinal herbs. Bryson and Croom (1991) evaluated 32 herbicides and herbicide combinations for production of commercial wormwood (Artemisia annua L.) and reported excellent weed control without affecting artemisinin content for several of the treatment combinations. Despite these results, the herbal supplement industry requires herbs to be free of pesticide residues and prefers organically-grown plant material.

Mulches are used to control weeds in a wide variety of plantings, especially perennial plantings, and can be used in place of herbicides. Calkins et al. (1996) compared several types of mulches with 14 herbicides and herbicide combinations for 12 species of herbaceous, field-grown perennials and reported that a 10 to 15 cm layer of wood chips provided the most effective weed control and produced the best quality plants. Skroch et al. (1992) reported that organic mulches reduced total weed counts by 50% compared to control plots, and pine bark mulch was more durable than hardwood bark, cedar chips, longleaf pine needles, or shortleaf pine needles. Pine bark mulch performed as well or better than ground cover fabric in providing excellent in-row weed protection for blueberries (Norden, 1989).

According to Singh et al. (1991), weeds caused a 40% reduction in herbaceous and oil yield in three perennial aromatic grasses. Application of organic mulch (waste pulp of citrus) at 3 t·ha⁻¹ provided weed control equal to that of the hand-weeded weed-free check and similar to that of several herbicide treatments. For American ginseng, oak or poplar bark–sawdust mixes used as mulches consistently produced larger roots and higher yields than wheat straw or hardwood leaf mulches (Konsler, 1982; Konsler and Shelton, 1984). Organic mulches not only control weeds but also modify the soil environment by reducing loss of soil moisture, modifying soil temperatures, decreasing soil compaction, decreasing erosion, and altering root and shoot growth (Bennett, 1982; Borland, 1990; Goldman, 1979; Meyer, 1997; Munn, 1992; Skroch et al., 1992).

Some disadvantages of organic mulches have also been reported. Davis (1994) reported bacterial soft rot (Erwinia spp.) increased when sweet and bush basil were grown with wheat straw mulch. Weed control, however, was reported as acceptable for all treatments: black polyethylene, wheat straw, hardwood bark, or mixed wood chips. Svenson and Witte (1989) reported chlorosis, leaf scorch, and plant mortality caused by sour mulch; that is, mulch that had produced toxic substances such as methanol, acetic acid, ammonia gas, or hydrogen sulfide gas. Too much moisture, trapped under mulches, can encourage infestations of slugs and development of fungal diseases (Bennett, 1982).

The purpose of the research reported here was to 1) explore methods of establishing American mayapple under field conditions, and 2) produce weed-free plantings using organic mulches while using few, if any, herbicides. This research was part of a larger effort to explore the domestication of mayapple for use by growers of specialty crops serving the pharmaceutical industry (Cushman et al., 2005; Cushman and Maqbool, 2005; Moraes et al., 2000).

Materials and Methods

Mayapple rhizomes were harvested from the wild at a location near Holly Springs, Miss., on 31 Oct. 2001 (34.821° N, 89.438° W, elevation 167 m). The location of the wild population was in full sun and situated between a highly eroded drainage area and agricultural land used for the annual production of row crops. Mayapple and red buckeye (Aesculus pavia L.) dominated the site during spring and kudzu (Pueraria montana [Lour.] Merr. var. lobata
(Willd.) Maesen & S.M. Almeida] dominated during summer and fall. Rhizome segments were separated into four groups based on size for the purpose of blocking: extra large, large, medium, and small. Length of rhizome segments ranged from 5 to 7.5 cm for small-sized rhizomes to 18 to 20 cm for extra-large-sized rhizomes. Segments were then transplanted into raised beds at the Horticulture Research and Education Unit in Verona, Miss., on 1 Nov. 2001. The soil at Verona is a Quitman fine sandy loam (fine-loamy, siliceous, thermic, Aquic Paleudult) and was pH 6.5.

Treatments consisted of two mulch types (pine bark or wheat straw), two mulch depths (7.5 or 15 cm), and two planting depths of rhizome segments (0 or 5 cm). A 2 × 2 × 2 factorial arrangement of treatments was used in a randomized complete block design with four blocks. Blocking was arranged according to size of rhizome segments, with the largest segments placed in block one and the smallest segments in block four. Each experimental unit (plot) consisted of 12 rhizome segments, three per row arranged in four parallel rows spaced 15 cm apart on top of raised beds. Raised beds were prepared with a press-pan-type bed shaper and spaced 1.8 m apart, center to center. Beds were formed 15 cm high and 75 cm wide across the top. Drip irrigation tubing was installed in the middle of each bed. Rhizomes were planted 15 cm apart within each parallel row. Rhizomes were centered in plots that were 1.2 m long. Mulch treatments were applied to the entire 1.2-m plot immediately after transplant of rhizome segments. Plots were watered after transplant.

During Spring 2002, it was observed that the finely ground bark mulch used for bark mulch treatments (particle size 0 to 1.0 cm) was too easily washed away by rain. Therefore, on 31 Oct. 2002 the fine mulch was replaced with a more coarsely ground pine bark mulch (particle size 1.0 to 2.5 cm). Also at this time, the 7.5 and 15 cm depths of bark and straw mulch were reestablished to compensate for loss of mulch depth (setting) that had occurred over a period of about 1 year. The pine bark mulch was obtained from a commercial source and consisted of bark and wood chips from southern yellow pine species.

A datalogger (LI-1000; LI-COR, Lincoln, Nebr.) was installed on 21 Feb. 2002 and 6 Feb. 2003 to record soil temperatures for each treatment during each growing season. Thermocouples were placed in the center of the plot at a depth equal to the original depth of the rhizome segment, either 0 or 5 cm below the soil surface depending on treatment. The data logger was equipped with a capacity to record a maximum of five channels of temperature data. Therefore, soil temperature was monitored for only four treatment combinations (Fig. 1). The fifth thermocouple was placed 15 cm above ground level to record ambient air temperature.

During the 2002 growing season, weeds that had established during winter were killed with glyphosate (4.5 kg·ha⁻¹) applied 17 Feb. before mayapple shoots had emerged. Weeds that had established during spring were not controlled as long as mayapple plants were emerging, growing, or senescing. Instead, these weeds were killed with glyphosate applied 8 July after all mayapple shoots had senesced. In 2003, the same weed control strategy was applied on 28 Feb. and again on 25 June. To control damage caused by black cutworm (Agrotis ipsilon Hufnagel), beds were sprayed with diazinon (2.2 kg·ha⁻¹) on 12 and 25 Mar. Rodenticide (0.005% rodent bait [d-CON; Reckitt & Colman Inc., Wayne, N.J.]) was used once in 2002 to control voles (Microtus sp.). Voles were not a problem in 2003.

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covered with bark mulch experienced slightly less variation in temperature than segments covered with straw mulch. Regardless of mulch type, rhizome segments planted 5 cm deep and covered to a depth of 15 cm of mulch appeared to have experienced less variation in temperature than rhizome segments planted 0 cm deep and covered to a depth of 7.5 cm of mulch.

Number of emerging shoots was not affected by mulch depth, but there was a significant interaction between mulch type and rhizome planting depth (Table 1). Rhizome segments planted 0 cm deep and covered with straw mulch produced about 30% fewer shoots than any of the other treatment combinations (Table 2). Numbers of shoots were also significantly affected by year (Table 1) and increased by 33% from 2002 to 2003 (Table 2).

Table 1. Probability table for effects of mulch type, mulch depth, rhizome planting depth, and year on number of shoots, total leaf area, total dry weight, shoot height, and ratio of sexual shoots to total shoots of American mayapple. Study was conducted in 2002 and 2003.

| Factor                        | Shoots (no/plot) | Total leaf area (cm²) | Total leaf dry wt (g) | Shoot height (cm) | Ratio | Transformed Ratio |
|-------------------------------|------------------|-----------------------|-----------------------|-------------------|-------|-------------------|
| Mulch type (mt)               | 0.0162           | 0.0112                | 0.0055                | 0.0017            | 0.2066| 0.2259            |
| Mulch depth (md)              | 0.3178           | 0.0746                | 0.1443                | 0.0248            | 0.5808| 0.3791            |
| Planting depth (pd)           | 0.4350           | 0.4012                | 0.4364                | 0.0007            | 0.6449| 0.8355            |
| mt x pd                       | 0.0082           | 0.1203                | 0.0785                | 0.0013            | 0.9598| 0.5046            |
| Year (yr)                     | 0.00001          | <0.0001               | <0.0001               | <0.0001           | 0.0003| 0.0003            |
| yr x mt                       | 0.0517           | 0.0076                | 0.0126                | 0.0001            | 0.3993| 0.3869            |
| yr x md                       | 0.3255           | 0.3468                | 0.2198                | 0.0618            | 0.7531| 0.8396            |
| yr x mt x pd                  | 0.9683           | 0.8174                | 0.6650                | 0.0382            | 0.5695| 0.8336            |
| yr x pd                       | 0.0713           | 0.6966                | 0.5854                | 0.0920            | 0.5506| 0.4713            |
| yr x mt x pd                  | 0.1977           | 0.0106                | 0.0156                | 0.1079            | 0.6979| 0.8025            |
| yr x md x pd                  | 0.6065           | 0.9366                | 0.9099                | 0.7898            | 0.5810| 0.5853            |
| yr x mt x md x pd             | 0.4530           | 0.3959                | 0.4307                | 0.8874            | 0.3660| 0.2852            |

Values are means of four replications. Values in columns followed by the same lowercase letters were not significantly different at $P \leq 0.05$.

Table 2. Number of shoots of American mayapple as affected by year and the interaction of mulch depth and planting depth during 2002 and 2003.

| Mulch type | Planting depth (cm) | Year     | Total shoots (no/plot) |
|------------|---------------------|----------|------------------------|
| Bark       | 0                   | 2002     | 13.5 a                 |
|            | 5                   | 2002     | 13.8 a                 |
|            | 0                   | 2003     | 9.3 b                  |
|            | 5                   | 2003     | 13.8 a                 |
| Straw      | 0                   | 2002     | 7.5                    |
|            | 5                   | 2002     | 15.0                   |
|            | 0                   | 2002     | 10.8 b                 |
|            | 5                   | 2002     | 14.4 a                 |

Values are means of four replications. Values in column followed by the same lowercase letters were not significantly different at $P \leq 0.05$.

Table 3. Leaf area and leaf dry weight of American mayapple as affected by mulch depth and the interaction of mulch type, planting depth and year during 2002 and 2003.

| Mulch type | Planting depth (cm) | Year | Leaf area (cm²) | Leaf dry wt (g) |
|------------|---------------------|------|----------------|----------------|
| Bark       | 0                   | 2002 | 1410 b          | 6.9 b          |
|            | 5                   | 2002 | 1510 b          | 7.2 b          |
|            | 0                   | 2003 | 3660 a          | 17.6 a         |
|            | 5                   | 2003 | 3010 a          | 14.7 a         |
| Straw      | 0                   | 2002 | 1070 b          | 4.7 b          |
|            | 5                   | 2002 | 1520 b          | 7.1 b          |
|            | 0                   | 2003 | 3010 a          | 14.6 a         |
|            | 5                   | 2003 | 2300            | 10.8           |
|            | 15.0                |      | 1890            | 9.2            |

Values are means of four replications. Values in columns followed by the same lowercase letters were not significantly different at $P \leq 0.05$.
respectively, of bark mulch remained intact (Table 6) (Cushman et al., 2003). In 2003, seven months after reestablishing the original mulch depths, 97% and 70% of the restored 7.5 and 15 cm depths, respectively, of wheat straw remained intact (data not shown). In contrast, 97% and 88% of the restored 7.5 and 15 cm depths, respectively, of bark mulch remained intact (data not shown).

Some additional aspects of mayapple growth were observed but not measured. Cutworm damage was present only in the bark mulch plots. Cutworms appeared to prefer bark mulch to straw mulch. It was observed that during early spring, when light frosts occurred, stems of mayapple shoots arising from straw mulch were slightly damaged while those in bark were not. Stems became discolored only in a small area where the stem came in contact with the uppermost layer of the straw mulch. Straw mulch also had a tendency to hinder the emergence of sexual shoots. Sexual shoots have two leaves and a forked stem. The forked stem would sometimes become entangled in the straw mulch during emergence. Shoots from all plots, regardless of treatment, experienced early-season wind damage, especially during 2002 when windbreaks were absent.

### Table 5. Ratio of number of sexual shoot to total shoots of American mayapple as in lowercase letters were not significantly different at \( P \leq 0.05 \).

| Mulch type | Mulch depth (cm) | Planting depth (cm) | Year | Ratio of number of sexual shoot to total shoots of American mayapple as in lowercase letters were not significantly different at \( P \leq 0.05 \) |
|------------|------------------|---------------------|------|-------------------------------------------------------------------|
| Bark       | 7.5              | 0                   | 2002 | 16.1 a                                                            |
|            | 15.0             | 0                   | 2003 | 15.4ab                                                            |
| Straw      | 7.5              | 0                   | 2002 | 11.5 e                                                            |
|            | 15.0             | 0                   | 2003 | 13.1d                                                             |
| Bark       | 7.5              | 0                   | 2002 | 15.1 bc                                                           |
|            | 15.0             | 5                   | 2003 | 14.7bc                                                            |
| Straw      | 7.5              | 0                   | 2002 | 11.8 e                                                            |
|            | 15.0             | 5                   | 2003 | 15.0bc                                                            |

Values are means of four replications. Values in column followed by the same lowercase letters were not significantly different at \( P \leq 0.05 \).

### Table 4. Shoot height of American mayapple as affected by two three-way interactions during 2002 and 2003.

| Mulch type | Mulch depth (cm) | Planting depth (cm) | Year | Shoot height (cm) |
|------------|------------------|---------------------|------|-------------------|
| Bark       | 7.5              | 0                   | 2002 | 16.1 a            |
|            | 15.0             | 0                   | 2003 | 15.4 b            |
| Straw      | 7.5              | 0                   | 2002 | 11.5 e            |
|            | 15.0             | 0                   | 2003 | 13.1 d            |

Values are means of four replications. Values in column followed by the same lowercase letters were not significantly different at \( P \leq 0.05 \).

### Table 6. Number of grass and sedge weeds, weed dry weight, and final mulch depth of American mayapple in 2002. Two types of mulch (bark mulch or wheat straw), two mulch depths (7.5 cm or 15 cm) and two planting depths (0 cm or 5 cm) were used.

| Mulch type | Mulch depth (cm) | Planting depth (cm) | Grasses (no/plot) | Sedges (no/plot) | Weed dry wt (g/plot) | Final mulch depth (cm) |
|------------|------------------|---------------------|-------------------|------------------|----------------------|------------------------|
| Bark       | 7.5              | 0                   | 3.9               | 0.3              | 2.1                  | 4.3 ± 0.3              |
|            | 15.0             | 0                   | 2.8               | 0.3              | 0.1                  | 12.9 ± 0.7             |
|            | 7.5              | 5                   | 4.3 a             | 0.3              | 0.1                  | 8.3 ± 0.8              |
| Bark       | 7.5              | 0                   | 9.4 a             | 0.3              | 1.7                  | 6.0 ± 0.4              |
|            | 15.0             | 0                   | 3.1               | 1.9              | 0.5                  | 12.9 ± 0.7             |
| Straw      | 7.5              | 0                   | 3.3               | 2.2              | 1.7                  | 8.0 ± 0.9              |
|            | 15.0             | 0                   | 3.1               | 1.9              | 0.5                  | 7.7 ± 1.0              |

Values are means of four replications. Values in column followed by the same lowercase letters were not significantly different at \( P \leq 0.05 \). NS not significant.
mulch tended to produce shorter shoots than bark mulch. This was due to straw mulch being less dense than bark mulch, and shoots arising from straw mulch were exposed to light before reaching the uppermost surface of the mulch. As a result, shoot height was probably shortened compared to bark mulch.

Year was an important factor in our study. Shoot number increased from 2002 to 2003, indicating that rhizomes used in this study had established and were producing increasing numbers of growing points. In contrast, the ratio of sexual shoots to total shoots decreased from 2002 to 2003, indicating that these rhizomes had declined in reproductive vigor. The ratio of sexual shoots to total number of shoots in a given area may be an indication of the overall vigor of mayapple plantings. Vigorous mayapple colonies in the wild appear denser, with more shoots per unit area, and more sexually productive, with a higher ratio of sexual shoots in the population, than colonies that appear weak (unpublished data). During the two years of this study, mayapple rhizomes increased in vegetative growth at the expense of sexual vigor.

One of the many purposes of organic mulches, and one of the main purposes of this research, is to minimize or prevent weed growth. In 2002, straw mulch performed better than bark mulch for control of grass weeds and for retaining mulch depth over time. However, when the finely ground bark mulch used in 2002 was replaced with coarsely ground bark mulch in 2003, there were no differences between the straw and bark mulches for weed control or retention of mulch depth. Mulches were not fumigated before use in this study; therefore, the mulches themselves may have been a source of weed seeds.

In conclusion, we recommend bark or straw mulch in combination with a rhizome planting depth of 5 cm for the purpose of establishing field plantings of American mayapple in full sun. Bark mulch should be a coarsely-ground material. Mulching depth of 7.5 cm was adequate for weed control when used in combination with glyphosate applications before mayapple emergence to eliminate established winter weeds and after senescence to eliminate established spring and summer weeds. Mulching depth of 7.5 cm was also adequate for retention of mulch depth over time and providing for successful establishment and growth of mayapple rhizome propagules. Under our growing conditions, we preferred the bark mulch to the straw mulch but, regardless of the type, we recommend using no less than a 7.5 cm layer. We do not recommend a rhizome planting depth of 0 cm.

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