

Overcoming Dormancy of Mayapple Rhizome Segments with Low Temperature Exposure

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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. This research was conducted to determine the optimum duration of low temperature exposure in overcoming dormancy of fall-harvested rhizome segments for subsequent use as propagules in greenhouse plantings. Two types of rhizome segments were harvested from the wild and used in this study: two-node rhizome segments consisting of a terminal node and its adjacent one-year-old node and one-node rhizome segments consisting of a single node, other than a terminal node, of unknown age or rhizome position. For growth cycle I, rhizome segments were exposed to low temperature ($\approx 4^\circ\text{C}$) for 30, 45, 60, 75, or 90 days, planted in pots, and grown in a greenhouse set at 21°C . Shoot emergence, shoots per pot, and plant height were recorded. Leaves were removed from plants when senescence first became evident, and leaf area was recorded. For growth cycle II, rhizome segments remained undisturbed in the original pots and were exposed to low temperatures ($\approx 4^\circ\text{C}$) for 90 days. Pots were again placed in the greenhouse and shoot emergence, shoots per pot, plant height, and leaf area were recorded. Increasing duration of low temperature exposure of rhizome segments up to 75 days appeared to increase percent emergence and plant height and decrease days to emergence, though changes in greenhouse conditions over the study period may have also influenced shoot emergence and plant growth. Two-node rhizome segments exhibited higher percent shoot emergence, shoot longevity, leaf area, and plant height than single-node segments during each growth cycle. Two-node rhizome segments also exhibited fewer days to emergence during growth cycle I. Rhizome segments produced no more than a single shoot in growth cycle I, whereas more than one shoot was produced in growth cycle II. Most of the effects of low temperature exposure during growth cycle I persisted throughout growth cycle II. These results indicate that dormancy of mayapple rhizomes can be overcome with low temperature exposure and shoots can be induced to grow at any time of year.

American mayapple (*Podophyllum peltatum* L.) is a rhizomatous, herbaceous perennial found in wooded areas of eastern North America. The plant is a source of podophyllotoxin, a compound used in the manufacture

of drugs for treatment of cancer, rheumatoid arthritis, genital warts, psoriasis, and multiple sclerosis (Bedir et al., 2002; Beutner, 1996; Lerndal and Svensson, 2000). Because of its podophyllotoxin content, American mayapple is a candidate for domestication and use as a specialty crop in North America. Domestication of American mayapple has the potential of reducing overexploitation of the current source of podophyllotoxin, the Indian mayapple (*P. emodi* Wall), which is an endangered species (Foster, 1993; Rai et al., 2000).

Sexual reproduction in American mayapple is difficult and inefficient (Nautiyal, 1996). Flowers are self-incompatible and do not produce nectar to attract pollinators (Krochmal et al., 1974; Laverty and Plowright, 1988). Fruit set is poor, seeds have a long and persistent dormancy, and seedlings have poor survival rates in the wild (Krochmal

et al., 1974; Rust and Roth, 1981). In addition, plants grown from seed remain juvenile for 4 to 5 years before initiating rhizomes (Foerste, 1884; Holm, 1899). Asexual propagation, using rhizome segments, may be the most practical method of rapid propagation.

In the wild, mature plants with well-developed rhizome systems produce shoots in early spring from buds located at the apex of each rhizome. Shoots produce one or two leaves, depending on their sexual status, and then senesce by late spring or early summer. A bud, located on the node from which the shoot arose, elongates horizontally and forms a new rhizome segment. The new segment continues to elongate throughout summer, and the apex becomes a fully developed terminal bud by fall (Foerste, 1884; Holm, 1899). For the purpose of this report, the location of the terminal bud was designated as the terminal node, Nt (Fig. 1A). During the development of this new rhizome segment, the node from which Nt arises ceases to be a terminal node itself and becomes a 1-year-old node, designated as N1. In this way, and over a period of several years, a rhizome system can have many nodes, with each node being sequentially one year older as they become more distant from the rhizome apex. A 2-year-old node, located two nodes distal to Nt, is therefore designated as N2, and so on. (For our purpose, Nt is the reference point used to describe distal and proximal position.) Nt exerts strong apical dominance over older nodes, and it is the only node to produce a shoot each year. After shoot senescence, Nt can produce more than one new rhizome segment at a time, adding complexity (branching) to the architecture of a single rhizome system. In addition, each node is a complicated structure composed of a highly compressed stem with a terminal bud and many minor buds that remain dormant unless induced to develop into shoots or branches by injury or environment. Roots develop predominantly from rhizome tissue at the base of each node (Foerste, 1884; Holm, 1899).

Dormancy in American mayapple appears to be the result of two influences. First, nodes distal to Nt are dormant as a result of apical dominance. Second, all nodes during fall and winter are dormant. It has been reported that older nodes are released from apical dominance and produce shoots when injury to the rhizome system causes separation from Nt (de Kroon et al., 1991). Dormant rhizome segments harvested in fall, winter, or early spring have been used effectively as propagules in field plantings (Maqbool et al., 2002).

Other plants with a longer history of horticultural manipulation than mayapple also exhibit similar processes of growth, rhizome elongation, and dormancy, and these species may serve as models for mayapple production. Myoga (*Zingiber mioga* Roscoe) is a herbaceous plant that produces thickened and fleshy rhizomes. Rhizome segments exposed to low temperatures produced twice the number of shoots as those not exposed (Follett, 1991). In another study, uniformity of emergence of myoga shoots was improved by exposure to 4°C for 2 to 4 weeks and rate of emergence was hastened by increasing the

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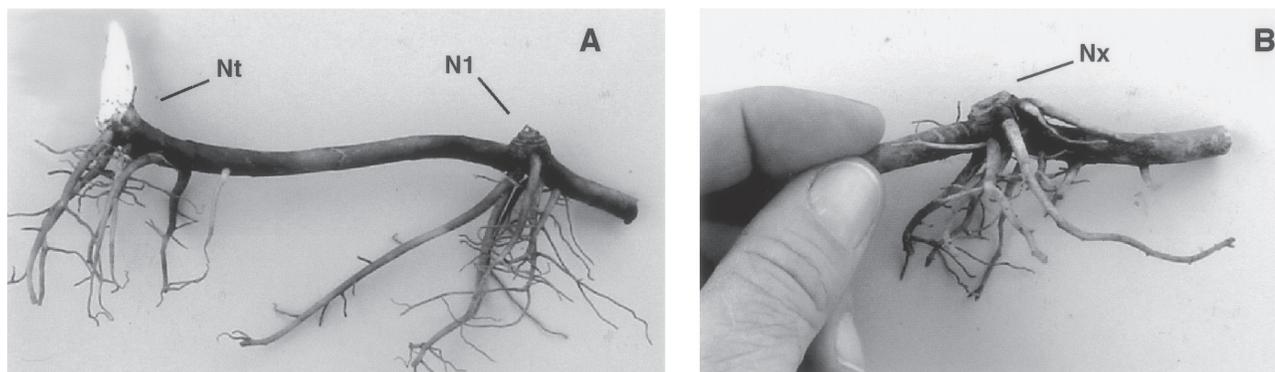


Fig. 1. (A) Two-node rhizome segment of American mayapple, designated as Nt+N1, consisting of a terminal node (Nt) and adjacent 1-year-old node (N1). (B) One-node rhizome segment consisting of a single node of unknown age or rhizome position, designated as Nx.

duration of low temperature exposure (Gracie et al., 2000). Similarly, low temperature exposures increased sprout emergence and mean number of shoots per propagule of *Paeonia* L. cultivars and emergence rate was hastened by increasing the duration of low temperature exposure (Fulton et al., 2001). Lily-of-the-valley (*Convallaria majalis* L.) is another rhizomatous, herbaceous plant that, like mayapple, is often found in wooded areas, emerges in early spring, senesces by early summer, and acquires dormancy during summer and fall. To break dormancy of lily-of-the-valley dormant rhizomes require 1 week at 1 °C or 3 weeks at 5 °C (Wareing and Phillips, 1981).

The objective of this research was to investigate duration of low temperature exposure in overcoming dormancy of fall-harvested rhizome segments for subsequent use as propagules in greenhouse plantings.

Materials and Methods

Mayapple rhizome segments were harvested from one location in the wild, encompassing an area of ≈900 m², near Oxford, Miss., on 4 Dec. 2000. Two types of rhizome segments were used. Two-node rhizome segments consisted of a terminal node and its adjacent 1-year-old node and were designated as Nt+N1 (Fig. 1A). One-node rhizome segments consisted of a single node, other than Nt, and were of varying ages and rhizome positions from N1 to about N5. These one-node rhizome segments were designated as Nx (Fig. 1B). Rhizome segments were harvested with roots intact and 4 to 6 cm of subtending rhizome tissue attached to each node. Rhizome segments were rinsed and treated with 1.32 mL·L⁻¹ copper hydroxide fungicide (Kocide DF, Griffin, Valdosta, Ga.) for 30 min to prevent fungal growth during cold storage. Rhizome segments were spread on paper towels overnight to remove excess moisture, placed in plastic bags, and then placed in a dark controlled-temperature chamber at 4 ± 2 °C measured with a temperature recording datalogger (model WD-35710-00, Oakton Instrument, Vernon, Ill.). Rhizome segments were removed from cold storage after 30, 45, 60, 75, or 90 d.

Growth cycle I. After low temperature treatment, rhizome segments were placed in pots (15

cm in diameter and 11 cm deep; volume 1.5 L), one per pot, and placed in a greenhouse under natural light conditions. Average daylength was 10.1, 11.0, 12.0, 13.0, 14.0, and 14.4 h for months Jan. to June 2001, respectively. The greenhouse temperature was set at 21 °C with two stages of heating and four stages of cooling separated by 1.5 °C increments. A commercial potting mix consisting of shredded peat moss and vermiculite was used (Jiffy Mix Plus, Jiffy Products, Batavia, Ill.). Fifteen pots per replication were arranged in a greenhouse in a split plot design, with rhizome segment as main plot and duration of exposure as subplot. Plants were watered when the surface of the potting media showed dryness. Plants were fertilized with a slow release 14N–6.12P–11.62K fertilizer (113 g per pot Osmocote 14–14–14, Scotts Co., Marysville, Ohio) soon after emergence, when plants reached a height of 7 to 10 cm. One month after emergence, soluble 20N–8.74P–16.6K fertilizer (Peters 20–20–20 Peatlite Special, Scotts Co.) was applied at the rate of 100 mg·L⁻¹ N with each watering during shoot growth. Every 7 d thereafter, water was applied to remove any build up of excessive salts in the potting media. Shoot emergence, leaf area, and shoot height were recorded. Leaves were removed at the first indication of senescence and measured using a LI-3100 area meter (LI-COR, Lincoln, Nebr.). Final harvest of leaves was 11 June 2001. Pots remained in

the greenhouse for an additional 5 weeks to ensure proper development of new rhizome segments and terminal buds. Pots were kept moist during this time as described above.

Growth cycle II. Pots used in growth cycle I were moistened and again placed in a dark controlled-temperature chamber at 4 ± 2 °C on 18 July 2001, and all pots (rhizome segments), regardless of the initial duration of low temperature, received low temperature exposure for 90 d. Pots were then placed in the greenhouse on 15 Oct. 2001 using the identical experimental design and greenhouse conditions as growth cycle I. Daily maximum and minimum greenhouse temperatures for growth cycle II were 30 °C and 17 °C, respectively, as recorded with a max/min thermometer (model 5458, Taylor Precision Products, Oak Brook, Ill.). Average daylength was 11.2, 10.4, 10.1, 10.1, and 11.0 h for months Oct. 2001 to Feb. 2002, respectively. Plants were watered when the surface of the potting media showed dryness, and soluble 20N–8.74P–16.6K fertilizer (Peters 20–20–20 Peatlite Special, Scotts Co., Marysville, Ohio) was applied at the rate of 100 mg·L⁻¹ N at each watering beginning at shoot emergence. Every 7 d, water was applied to remove any build up of excessive salts in the potting media. Shoot emergence, leaf area, plant height, and shoots per pot were recorded. Leaves were harvested as they senesced and were measured using an area meter as described

Table 1. Percent emergence of shoots arising from mayapple rhizome segments harvested from the wild and exposed to low temperatures.

Low temperature exposure (days)	Growth cycle I ^a		Growth cycle II		
	Emergence (%)		Emergence (%)		
	Nt+N1	Nx	Nt+N1	Nx	
30	0 c A ^x	13 a A	90	73 a A	80 a A
45	40 b A	20 a A	90	93 a A	67 a A
60	100 a A	27 a B	90	100 a A	73 a B
75	100 a A	47 a B	90	93 a A	73 a A
90	100 a A	40 a B	90	87 a A	60 a A

^aGrowth cycle I: Low temperature exposure (≈4 °C) for 30, 45, 60, 75, or 90 d prior to growth in pots in a greenhouse. Growth cycle II: Following senescence, pots were given low temperature exposure (≈4 °C) for 90 d and again placed in a greenhouse. Values are means of 15 replications.

^bTwo-node rhizome segment with one terminal and an adjacent 1-year-old node (Nt+N1). One-node rhizome segment, not a terminal node, of unknown age or rhizome position (Nx).

^xCochran's test used to separate means in columns and rows. Values in columns or rows followed by the same lowercase or uppercase letters, respectively, were not significantly different at *P* ≤ 0.05.

above. Final harvest of leaves was completed on 26 Feb. 2002.

Data other than shoot emergence results were analyzed using the MIXED procedure of SAS (SAS Institute, Cary, N.C.). Random effects for this split-block design were block and block by rhizome segment interaction. Least square means were used to compare combinations of rhizome segment and duration of low temperature exposure in the event of significant interaction. When interactions were not significant, least square means were compared for main effects of rhizome segment and duration of low temperature exposure. Shoot emergence results were treated differently because it consisted of binary response data (0 = no emergence and 1 = emerged) for each subplot unit. Hence, Cochran's test was used to perform multiple comparisons in a pairwise fashion for percent shoot emergence means (Lehmann, 1975).

Results and Discussion

Growth cycle I. Duration of low temperature exposure and type of rhizome segment affected shoot emergence (Table 1). As duration of low temperature exposure increased from 30 to 60 d, emergence of shoots from Nt+N1 rhizome segments increased from 0% to 100%. Durations >60 d also resulted in 100% emergence, indicating that low temperature exposure of 60 d or more appeared to be sufficient to overcome dormancy of Nt+N1 segments. Emergence of shoots from Nx rhizome segments was not affected by duration of low temperature exposure. These data indicate that low temperature exposure appeared to be more effective in overcoming dormancy of Nt+N1 segments compared to Nx segments. The number of shoots arising from rhizome segments receiving 30 d of low temperature exposure was not sufficient to measure growth characteristics, such as days to emergence, leaf area, and plant height. Therefore, Tables 2 to 6 do not contain data for 30 d of exposure during growth cycle I.

There was a significant interaction between duration of low temperature exposure and type of rhizome segment for average d to shoot emergence (Table 2). As duration of low temperature exposure increased from 45 to 75 d, shoots arising from either Nt+N1 or Nx rhizome segments required half as many d to emerge. Days to emergence for 90 d of exposure were similar to that of 75 d. When given 90 d of exposure, however, days to shoot emergence of Nx rhizome segments increased significantly, to a level equivalent to that of 60 d of exposure. In a similar manner to that of percent shoot emergence, it can be concluded that durations of low temperature exposure of 60 to 75 d appeared to be most effective in overcoming dormancy of mayapple rhizome segments.

There was a significant interaction between duration of low temperature exposure and type of rhizome segment for shoot longevity (Table 3). As duration of low temperature exposure increased from 45 to 75 d, longevity of shoots arising from Nt+N1 rhizome segments

Table 2. Days to emergence of shoots arising from mayapple rhizome segments harvested from the wild and exposed to low temperatures.

Growth cycle I ^a			Growth cycle II		
Low temperature exposure (days)	Emergence (days)		Low temperature exposure (days)	Emergence (days)	
	Rhizome segment ^b			Rhizome segment	
	Nt+N1	Nx		Nt+N1	Nx
30	---	---	90	11 c A	11 c A
45	27 a B ^w	37 a A	90	13 c A	16 ab A
60	19 b B	24 b A	90	21 a A	13 a-c B
75	13 c B	18 c A	90	19 ab A	17 a A
90	10 c B	27 b A	90	21 a A	17 a A
<i>Significance</i>					
Exposure (E)	<0.0001				0.0001
Rhizome (R)	<0.0001				0.0691
E × R	0.0001				0.0055

^aGrowth cycle I: Low temperature exposure (≈4°C) for 30, 45, 60, 75, or 90 d prior to growth in pots in a greenhouse. Growth cycle II: Following senescence, pots were given low temperature exposure (≈4°C) for 90 days and again placed in a greenhouse. Values are means of 15 replications.

^bTwo-node rhizome segment with one terminal and an adjacent one-year-old node (Nt+N1). One-node rhizome segment, not a terminal node, of unknown age or rhizome position (Nx).

^wShoots did not emerge, or did not emerge in sufficient numbers, for analysis when given 30 d of exposure during growth cycle I.

^xValues in columns or rows followed by the same lowercase or uppercase letters, respectively, were not significantly different at $P \leq 0.05$.

decreased 12%. Shoot longevity for 90 d of exposure increased slightly compared to the 75-d exposure, but this was not different than that of all other durations. Shoots arising from Nx rhizome segments also exhibited fewer d of shoot longevity as low temperature exposure increased from 45 to 75 d. Shoot longevity for the 90-d exposure was not different than that of the 75-d exposure. Shoot longevity of mayapple was reported to be influenced by plant vigor, with larger and more vigorous rhizome systems producing longer-lived shoots than smaller and less vigorous rhizome systems (Watson and Lu, 1999). Duration of low temperature exposure may influence rhizome vigor, which in turn may affect shoot longevity. Shoot longevity also may have been affected by greenhouse conditions, with shoots arising from rhizome segments receiving 90 d of low temperature exposure experiencing slightly warmer greenhouse temperatures and slightly

longer d than shoots arising from rhizomes segments receiving 75 d or fewer of low temperature exposure.

Duration of low temperature exposure did not affect shoot leaf area (Table 4). However, shoots arising from Nt+N1 segments produced three times more leaf area than shoots arising from Nx, indicating that Nt+N1 produced larger leaves than that of Nx. Of the rhizome segments that produced shoots, only one shoot per propagule emerged (Table 5).

Duration of low temperature exposure and type of rhizome segment affected shoot height (Table 6). Increasing duration of low temperature exposure from 45 to 90 d increased plant height by ≈37%. Segment type had a slightly greater effect on plant height than duration of low temperature exposure, with Nt+N1 shoots being 44% taller than Nx shoots. As mentioned above, however, greenhouse conditions also may have affected shoot height.

Table 3. Longevity of shoots arising from mayapple rhizome segments harvested from the wild and exposed to low temperatures.

Growth cycle I ^a			Growth cycle II		
Low temperature exposure (days)	Shoot longevity (days)		Low temperature exposure (days)	Shoot longevity (days)	
	Rhizome segment ^b			Rhizome segment	
	Nt+N1	Nx		Nt+N1	Nx
30	---	---	90	85 bc A	92 ab A
45	122 a A ^w	127 a A	90	77 c A	84 ab A
60	117 ab A	107 b B	90	99 ab A	81 b B
75	107 b A	92 c B	90	99 ab A	96 a A
90	113 ab A	84 c B	90	105 a A	78 b B
<i>Significance</i>					
Exposure (E)	<0.0001				0.0181
Rhizome (R)	0.0042				0.0374
E × R	0.0172				0.0010

^aGrowth cycle I: Low temperature exposure (≈4°C) for 30, 45, 60, 75, or 90 d prior to growth in pots in a greenhouse. Growth cycle II: Following senescence, pots were given low temperature exposure (≈4°C) for 90 d and again placed in a greenhouse. Values are means of 15 replications.

^bTwo-node rhizome segment with one terminal and an adjacent 1-year-old node (Nt+N1). One-node rhizome segment, not a terminal node, of unknown age or rhizome position (Nx).

^wShoots did not emerge, or did not emerge in sufficient numbers, for analysis when given 30 d of exposure during growth cycle I.

^xValues in columns or rows followed by the same lowercase or uppercase letters, respectively, were not significantly different at $P \leq 0.05$.

Table 4. Total leaf area of shoots arising from mayapple rhizome segments harvested from the wild and exposed to low temperatures.

Growth cycle I ^a				Growth cycle II			
Low temperature exposure (days)	Total leaf area (cm ²)			Low temperature exposure (days)	Total leaf area (cm ²)		
	Rhizome segment ^b				Rhizome segment		
	Nt+N1	Nx	Mean		Nt+N1	Nx	
30	---	---	---	90	44 b A ^w	88 a A	
45	128	36	89	90	142 b A	86 a A	
60	101	37	69	90	258 a A	76 a B	
75	108	43	75	90	286 a A	124 a B	
90	115	27	70	90	314 a A	61 a B	
Mean	113 A	36 B					
<i>Significance</i>							
Exposure (E)	0.8070						<0.0001
Rhizome (R)	<0.0001						<0.0001
E × R	0.5146						<0.0001

^aGrowth cycle I: Low temperature exposure (≈4 °C) for 30, 45, 60, 75, or 90 d prior to growth in pots in a greenhouse. Growth cycle II: Following senescence, pots were given low temperature exposure (≈4 °C) for 90 d and again placed in a greenhouse. Values are means of 15 replications.

^bTwo-node rhizome segment with one terminal and an adjacent 1-year-old node (Nt+N1). One-node rhizome segment, not a terminal node, of unknown age or rhizome position (Nx).

^wShoots did not emerge, or did not emerge in sufficient numbers, for analysis when given 30 d of exposure during growth cycle I.

^vValues in columns or rows followed by the same lowercase or uppercase letters, respectively, were not significantly different at $P \leq 0.05$.

Growth cycle II. Emergence of shoots from Nt+N1 and Nx rhizome segments following a second low temperature exposure, this time of 90 d, ranged from 60% to 100% (Table 1). Segments that did not produce shoots, or produced few shoots, during growth cycle I when given 30 d of initial low temperature exposure emerged at much greater percentages during growth cycle II. Apparently, mayapple rhizomes can endure long periods of dormancy and still produce shoots when exposed to durations of low temperature sufficient to break that dormancy.

There was a significant interaction between initial duration of low temperature exposure and type of rhizome segment for average d to shoot emergence (Table 2). As the initial duration of low temperature exposure given during growth cycle I increased from 30 to

90 d, shoots arising from Nt+N1 rhizome segments during growth cycle II required about twice as many days to emerge. Shoots arising from Nx rhizome segments required ≈50% more days to emerge as duration of low temperature exposure increased from 30 to 90 d. Type of rhizome segment did not affect days to shoot emergence, except at 60 d of initial low temperature exposure, for which Nt+N1 required more days to emerge than Nx. The overall trend of these data are opposite of that observed during growth cycle I, where increasing durations of low temperature exposure generally decreased days to shoot emergence. Greenhouse conditions during growth cycle II were generally opposite of those during growth cycle I, with slightly decreasing temperature and daylength compared to growth cycle II.

Table 5. Number of shoots arising from mayapple rhizome segments harvested from the wild and exposed to low temperatures.

Growth cycle I ^a			Growth cycle II			
Low temperature exposure (days)	Shoots/pot		Low temperature exposure (days)	Shoots/pot		
	Rhizome segment ^b			Rhizome segment		
	Nt+N1	Nx		Nt+N1	Nx	Mean
30	---	---	90	1.8	1.6	1.7
45	1.0	1.0	90	1.8	1.6	1.7
60	1.0	1.0	90	2.5	1.4	2.0
75	1.0	1.0	90	2.2	1.4	1.8
90	1.0	1.0	90	2.4	1.3	1.9
Mean				2.2 A ^w	1.5 B	
<i>Significance</i>						
Exposure (E)				0.6843		
Rhizome (R)				0.0001		
E × R				0.0521		

^aGrowth cycle I: Low temperature exposure (≈4 °C) for 30, 45, 60, 75, or 90 d prior to growth in pots in a greenhouse. Growth cycle II: Following senescence, pots were given low temperature exposure (≈4 °C) for 90 d and again placed in a greenhouse. Values are means of 15 replications. Data reported for growth cycle I did not exhibit any variation. Therefore, these data were not statistically analyzed.

^bTwo-node rhizome segment with one terminal and an adjacent 1-year-old node (Nt+N1). One-node rhizome segment, not a terminal node, of unknown age or rhizome position (Nx).

^wShoots did not emerge, or did not emerge in sufficient numbers, for analysis when given 30 d of exposure during growth cycle I.

^vMeans significantly different at $P \leq 0.05$.

There was a significant interaction between duration of low temperature exposure and type of rhizome segment for days of shoot longevity (Table 3). As the initial duration of low temperature exposure during growth cycle I increased from 30 to 90 d, d of shoot longevity of shoots arising from Nt+N1 rhizome segments during growth cycle II increased about 25%. In contrast, shoot longevity of shoots arising from Nx segments exhibited no clear trend, fluctuating as much as 20% as a result of initial duration of exposure. Type of rhizome segment affected only shoots exposed to 60 and 90 d of low temperature, with days of shoot longevity for Nx segments being significantly less than that of Nt+N1 segments.

There was a significant interaction between duration of low temperature exposure and type of rhizome segment for total leaf area (Table 4). As the initial duration of low temperature exposure during growth cycle I increased from 30 to 90 d, shoots arising from Nt+N1 rhizome segments during growth cycle II increased leaf area by about seven-fold. In contrast, shoots arising from Nx segments during growth cycle II appeared unaffected by the initial low temperature exposures.

Rhizome segment affected the number of shoots produced by each propagule (Table 5). Nt+N1 and Nx rhizome segments produced more shoots during growth cycle II compared to growth cycle I. In addition, during growth cycle II Nt+N1 segments produced ≈50% more shoots than Nx segments. These data show that when given repeated low temperature exposures, rhizome segments with two nodes (Nt+N1) were able to produce more shoots per propagule than those with only one node (Nx).

There was a significant interaction between duration of low temperature exposure and type of rhizome segment for plant height (Table 6). As the initial duration of low temperature exposure during growth cycle I increased from 30 to 90 d, plant height of shoots arising from Nt+N1 rhizome segments during growth cycle II more than doubled. In contrast, and as with leaf area, shoots arising from Nx segments during growth cycle II appeared unaffected by the initial low temperature exposures.

Conclusions

Overall, rhizome segments exposed initially to 60, 75, or 90 d of low temperature emerged in greater numbers and grew better than those exposed to 30 or 45 d. The threshold duration of low temperature to overcome dormancy of mayapple rhizomes appears to be 60 d, with segments receiving < 60 d emerging in fewer numbers and producing smaller plants than segments receiving 60 d or more. With 60 d being the threshold, the optimum duration of exposure may be 75 d. These results are similar to those reported for overcoming the dormancy of myoga (Follett, 1991; Gracie et al., 2000) and lily-of-the-valley (Wareing and Phillips, 1981) rhizome segments, where increasing durations of low temperature exposure improved shoot emergence and growth. Compared to lily-of-the-valley, however, may-

Table 6. Plant height of shoots arising from mayapple rhizome segments harvested from the wild and exposed to low temperatures.

Low temperature exposure (days)	Growth cycle I ^z			Growth cycle II		
	Plant height (cm)			Plant height (cm)		
	Rhizome segment ^y			Rhizome segment		
	Nt+N1	Nx	Mean		Nt+N1	Nx
30	---	---	---	90	6 c B ^w	9 a A
45	24	14	19 b	90	9 b A	9 a A
60	26	19	22 ab	90	13 a A	10 a B
75	24	19	22 ab	90	14 a A	11 a B
90	29	22	26 a	90	15 a A	9 a B
Mean	26 A	18 B				
<i>Significance</i>						
Exposure (E)	0.0135			<0.0001		
Rhizome (R)	0.0004			0.0012		
E × R	0.7928			<0.0001		

^zGrowth cycle I: Low temperature exposure ($\approx 4^\circ\text{C}$) for 30, 45, 60, 75, or 90 d prior to growth in pots in a greenhouse. Growth cycle II: Following senescence, pots were given low temperature exposure ($\approx 4^\circ\text{C}$) for 90 d and again placed in a greenhouse. Values are means of 15 replications.

^yTwo-node rhizome segment with one terminal and an adjacent 1-year-old node (Nt+N1). One-node rhizome segment, not a terminal node, of unknown age or rhizome position (Nx).

^xShoots did not emerge, or did not emerge in sufficient numbers, for analysis when given 30 d of exposure during growth cycle I.

^wValues in columns or rows followed by the same lowercase or uppercase letters, respectively, were not significantly different at $P \leq 0.05$.

apple requires about three times the duration of low temperature exposure at $\approx 4^\circ\text{C}$ to break dormancy. Some effects of low temperature exposure of mayapple during growth cycle I persisted to growth cycle II.

Rhizome segments Nt+N1 performed better during each growth cycle than Nx for most aspects of growth and development. The Nt+N1 segments generally exhibited equal or greater percent shoot emergence, shoot longevity, leaf area, plant height, and shoots per propagule than Nx. The Nt+N1 segments also required fewer d to emergence than Nx during growth cycle I.

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