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Source: The Journal of the Torrey Botanical Society, 138(3):298-307. 2011.
Published By: Torrey Botanical Society
DOI: 10.3159/TORREY-D-11-00021.1
URL: http://www.bioone.org/doi/full/10.3159/TORREY-D-11-00021.1

Journal of the Torrey Botanical Society 138(3), 2011, pp. 298–307

Seed ecology of *Lindera melissifolia* (Lauraceae) as it relates to rarity of the species[1]

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Hawkins, T. S. (USDA Forest Service, Center for Bottomland Hardwoods Research, Box 9681, Mississippi State, MS 39762), J. L. Walck, and S. N. Hidayati (Department of Biology, P.O. Box 60, Middle Tennessee State University, Murfreesboro, TN 37132). Seed ecology of *Lindera melissifolia* as it relates to rarity of the species. J. Torrey Bot. Soc. 138: 298–307. 2011.—The seed ecology of the federally endangered shrub, *Lindera melissifolia* was investigated to determine if this aspect of the species life history contributes to the rarity of the species. *Lindera melissifolia* has the capacity to form a short-lived (two growing seasons) persistent soil seed bank if fruit pulp remains on seeds following dispersal and subsequent winter flooding occurs. Seeds, both with or without pulp (i.e., mesocarp and exocarp), exhibited tolerance to submergence, but were not hydrochorous. Following 6–12 weeks cold stratification (5° or 5/1° C) or submersion in cold water (5/1° C) for 12 weeks, ≥ 63% of seeds germinated when incubated in light and at temperatures of 35/20° and 30/20° C. When incubated in darkness, 100% of seeds germinated following 6 weeks of cold stratification. Giberrelic acid was moderately effective in breaking dormancy. Collectively, our results indicate that seeds of *L. melissifolia* have nondeep physiological dormancy. Aspects of the seed ecology of *L. melissifolia* that may contribute to continued rarity of the species include absence of a long-term persistent soil seed bank, no obvious mechanism of long-distance dispersal, and late season germination that prevents seedling growth to a sufficient size for survival prior to cold temperatures and flooding.

Key words: endangered species, germination, nondeep physiological dormancy, pondberry.

The leading cause of species imperilment in the United States is habitat loss (Wilson 1992, Wilcove et al. 1998, Edwards and Weakley 2001). In turn, persistence of a species within disturbed and often fragmented habitats is dependent on how well its life history traits are adapted to this environment. Two of these traits, which are typical of understory species, are a perennial life form (Edwards and Weakley 2001) and clonal propagation as the predominant form of reproduction (Neufeld and Young 2003). Both provide an interspecific competitive advantage in terms of light capture and spatial presence. However, they may also offer only short-term advantages. For example, primary use of clonal propagation may preclude long-range emigration. Indeed, seed production provides a mechanism for rare species to migrate and establish beyond the immediate environment (Kunin and Shmida 1997, Walck et al. 1999, Murray et al. 2002). Additionally, lack of investment in sexual reproduction or unsuccessful seed germination and seedling recruitment may be more damaging for rare species (Jolls 2003) if it results in a genetic bottleneck which threatens long-term persistence (Young et al. 1996).

Beyond maintaining genetic diversity, sexual reproduction is a key element in the ecological success of a rare plant population. For plant species growing in wetlands or periodically flooded habitats, several attributes associated with sexual reproduction have been noted. Population persistence may be strongly influenced by the species' capacity to form a persistent soil seed bank (Levin 1990, Jensen 2004). This adaptation allows a seed cohort to germinate for more than one year, minimizing the risk of all seeds germinating in a single year when conditions may be less than optimum for seedling survival. Further, the capacity to form a persistent soil seed bank often is inherently linked to a seed's dormancy cycle (Walck et al. 2005, Hawkins et al. 2007).

[1] The authors thank Drs. Claudia Jolls and Tom Dell for review of an earlier draft of this manuscript, the Arkansas Game and Fish Commission for collection permits and help with fruit harvest at St. Frances Sunken Lands NWR, and the U. S. Army Corps of Engineers for their contribution to pondberry research.

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Received for correspondence March 17, 2011, and in revised form July 11, 2011.

In general, autumn dispersed seeds of temperate wetland species require a period of cold stratification for dormancy break, and subsequent germination is favored by light, fluctuating temperatures (Schütz 1999, Jensen 2004), and in some cases, production of ethylene in inundated soil (Baskin et al. 2003). Emigration is often achieved by hydrochorous seeds (Schneider and Sharitz 1988, Danvind and Nilsson 1997), and seeds that maintain viability while submerged (van der Pijl 1982).

To further elucidate the role of sexual reproduction in persistence and population expansion of rare species, we investigated the seed ecology of *Lindera melissifolia* (Walt.) Blume, a federally endangered dioecious shrub endemic to the southeastern United States. Within the classification system developed by Rabinowitz (1981), the species displays a form of rarity whereby, populations have a wide geographic distribution, are restricted in habitat specificity, but some extant populations may be large in number. Disjunct *L. melissifolia* populations grow in periodically flooded bottomland hardwood forests in the Lower Mississippi Alluvial Valley (LMAV) (Hawkins et al. 2009a, 2010) and along the edges of limestone sinks, ponds, or other depressional wetlands in the southeastern Coastal Plain (Aleric and Kirkman 2005). Adult *L. melissifolia* appear to be well-adapted to inundation, as they flower and leaf out in early spring during flooded conditions (Hawkins et al. 2010). Female *L. melissifolia* may invest heavily in sexual reproduction (Connor et al. 2007) and produce bright red drupes that are dispersed from late autumn to early winter. However, seedlings are rarely observed in the natural habitat (Wright 1990, 1994, Devall et al. 2001), and asexual propagation by rhizomes appears to be the primary means of reproduction (Wright 1990, 1994). Reliance on asexual reproduction is evidenced by *L. melissifolia* population demography. Extant populations are composed of numerous spatially segregated single-sex colonies made up of 20 to >1000 stems. *Lindera melissifolia* populations in the LMAV are strongly male-biased, with male colony to female colony ratios ranging from 7:1 (Wright 1994) to 19:1 (Hawkins et al. 2009b).

Field ecology studies in the LMAV (Devall et al. 2001, Hawkins et al. 2009a, 2010) and in the Coastal Plain (Aleric and Kirkman 2005) suggest that many existing *Lindera melissifolia* populations are stable, and we can presume that this is due in part to clonal propagation. However, long-term persistence and expansion of extant populations relies on successful seed germination and subsequent seedling survival. To determine if seed ecology plays a role in the rarity of *L. melissifolia*, we investigated the species' potential to form a long-term persistent soil seed bank, its seed dormancy breaking and germination requirements, and if hydrochory is a possible mechanism for long-range seed dispersal.

Materials and Methods. GENERAL. Mature fruits were harvested in October 2003 or in September 2006 from *Lindera melissifolia* plants growing in a bottomland hardwood forest at St. Francis Sunken Lands National Wildlife Refuge, Craighead County, Arkansas (see Hawkins et al. 2009a for site description), or on 19 October 2009 from plants growing at a United States Forest Service flooding research facility in Sharkey County, Mississippi (see Lockhart et al. 2006 for site description). Following harvest, fruits were brought to the laboratory and either the fruit pulp (mesocarp + exocarp) was gently peeled away from the seed (hereafter seeds) or it remained (hereafter fruit). Both seeds and fruits were kept on a bench in the laboratory (22–24° C) for approximately 36 h before they were used in experiments.

Six incubators set at 12 h/12 h daily alternating temperature regimes of 15/6°, 20/10°, 25/15°, 30/20° (or 30/15°), and 35/20° C were used for incubation, and two incubators were at 5° C or at 5/1° C for cold stratification. These temperature regimes approximate the mean maximum and minimum air temperatures in the Yazoo Basin of Mississippi (Rogers 1958, Scott and Carter 1962) and adjacent sites in the Lower Mississippi Alluvial Valley (Fielder et al. 1978, Ferguson 1979, Graves 1983): December and January, 5° C (or 5/1°); February and November, 15/6°; March, April, and October, 20/10°; May and September, 25/15°; and June, July, and August, 30/20° (or 30/15°) and 35/20°C. Emergence of the radicle was the criterion for germination. With the exception of the germination phenology component, all ungerminated seeds were checked for viability upon completion of an experiment. Firm,

white embryos were considered viable and soft, brown to gray embryos were considered non-viable.

EMBRYO GROWTH. Embryos were excised from 30 fresh 2009-collected seeds, and embryo lengths along with seed lengths were measured using a dissecting microscope equipped with an ocular micrometer. Five Petri dishes each with 20 seeds on moist sand were cold stratified for 12 wk at 5/1°C. Following cold stratification, embryos were measured in the 20 seeds of one Petri dish (day 0), and the other dishes were placed in the 30/20° C incubator. Thereafter, one Petri dish each was removed from incubation on days 3, 6, and 9 and embryos were measured for growth. Embryos in seeds of the fifth Petri dish were not measured due to seed germination on day nine.

GERMINATION PHENOLOGY. The germination phenology study was conducted on the grounds of the Center for Bottomland Hardwoods Research in Stoneville, Mississippi. Six replicates of 100 seeds and six replicates of 100 fruits, both collected in 2003, were sown on the surface of potting soil in 31 cm (height) × 35 cm (diameter) plastic pots and covered with approximately 5 cm of dead oak leaves. Pots were placed in two 379 L aquaculture tanks. One tank contained six pots (three replicates of seeds and three replicates of fruits) randomly chosen for the non-flooding treatment and had four 1 cm holes near the bottom to prevent flooding of pots during rainfall. The other tank contained six pots (3 replicates of seeds and 3 replicates of fruits) randomly chosen for the flooding treatment. Pots in both treatments remained outdoors throughout the study with 50% neutral shade cloth suspended approximately 1.5 m above them during late spring and summer to simulate canopy cover. In the the non-flooding treatment, soil in the pots was watered throughout the year as needed to maintain field capacity. In the flooding treatment, soil in the pots was maintained at field capacity, except from January 2 to March 31 in 2004, in 2005, and in 2006, when the water level in the tank was raised to approximately 3 cm above the leaf litter. At 2-wk intervals between April 1 and September 30 of each year, leaves were lifted from the soil in pots of both treatments and germinated seeds were counted and discarded. Leaves were then placed back on the soil. Mean maximum and minimum temperatures for the duration of the study were calculated from temperatures recorded by an outdoor thermometer data logger.

EFFECTS OF COLD STRATIFICATION. Seeds and fruits collected in 2006 and 2009 were placed in Petri dishes on moist sand. Three replicates of 25 seeds (2006 collection) or 50 seeds or 50 fruits (2009 collection) per dish were used in each treatment. Seeds were cold stratified at 5° or 5/1° C for 0, 2, 6, or 12 weeks in light, and fruits were stratified for 12 wks. Fruits were depulped prior to incubation to avoid mold growth. Following cold stratification, incubation occurred over the range of temperature regimes for 6 wks (2006 seeds) or for 12 wks (2009 seeds) in light with germination being scored at 2-week intervals.

EFFECTS OF LIGHT. Seeds collected in 2009 were placed in Petri dishes on moist sand. Three replicates of 50 seeds were used in the treatments and in the control. Seeds received 0 or 6 wks of cold stratification (5/1° C) in either light or darkness, followed by 2 wks of incubation in either light or darkness at 15/6, 20/10, 25/15, and 30/20° C. Seeds in the controls were incubated in light for 8 wks with germination being monitored at 2-week intervals.

EFFECTS OF GIBBERELLIC ACID. Seeds collected in 2009 were placed in Petri dishes on filter paper moistened with either water (control), or a solution of 10, 100, or 1000 mg L^{-1} gibberellic acid (GA_3). Three replicates of 25 seeds per dish were used in the control and in each treatment. Seeds were incubated in light at 25/15° C for 12 wks. To examine whether GA_3 can substitute for cold stratification, a temperature regime of 25/15° C was used because it is too high to be effective for cold stratification (Stokes 1965). Germination was monitored at 2-week intervals.

EFFECTS OF FLOODING. Seeds and fruits collected in 2009 were placed in ten 0.47 L clear plastic containers (150 fruits or seeds in each container) in deionized water. Both seeds and fruits immediately sank to the bottom of the container, and remained submerged throughout the flooding treatment. The top of the container was covered with aluminum foil, and the containers were placed at 5/1° C. After 12 wks of submergence, seeds and fruits were removed from the water and the pulp was

gently removed by hand from fruits. Afterwards, seeds and fruits (now depulped) were placed in Petri dishes on moist sand, with 3 replicates of 50 seeds each. Incubation was at 15/6, 20/10, 25/15, and 30/20°C for 12 wks with germination being monitored at 2-week intervals.

STATISTICAL ANALYSES. Means and standard errors were calculated for germination percentages based on number of seeds sown (phenology study) or on viability (laboratory study); all ungerminated seeds in each laboratory experiment contained a viable embryo. The square root of percentages was arcsine transformed before analyses, but actual values are used for presentation. Means of percentages were compared by analyses of variances (ANOVAs, $P = 0.05$) followed by protected least significant difference tests (PLSD, $P = 0.05$). Three-way ANOVAs were used to test the effects and interactions of the following factors on germination; (1) length of cold stratification and of incubation, incubation temperature, and/or the presence/absence of fruit pulp, and incubation temperature in the stratification experiments; (2) light regime, condition of seeds (fresh vs. cold stratified), and incubation temperature in the light experiment; and (3) presence/absence of fruit pulp, incubation temperature, and length of incubation in the flooding experiment. A two-way ANOVA tested the effects and interaction of GA_3 concentration and length of incubation on germination. The SAS procedure GLM was used to perform all statistical analyses (SAS Institute 2002).

Results. EMBRYO GROWTH. Mean (± SE) length of seeds was 6.65 ± 0.08 mm, and that of embryos in fresh seeds was 1.4 ± 0.04 mm, yielding an embryo to seed ratio of approximately 1:4. Embryos in fresh seeds were differentiated and the radicle and shoot were easily discernable. Embryos did not grow during cold stratification. When seeds were moved to 30/20° C, the radicle emerged and the seed coat split simultaneously. Extension of the shoot and emergence of the epicotyl followed germination, and germination was hypogeous.

GERMINATION PHENOLOGY. Neither seeds nor fruits floated at any time during the flooding treatment, and seeds did not germinate while submerged. Throughout the study,

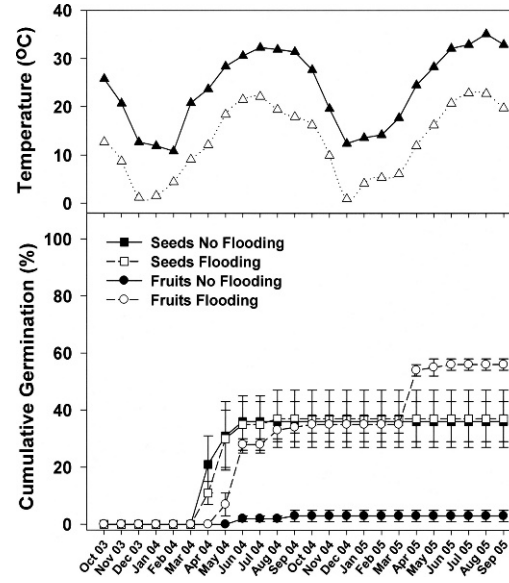

FIG. 1. Mean monthly minimum (△) and maximum (▲) temperatures for Stoneville, Mississippi, USA, and germination (mean ± SE) for seeds and fruits of *Lindera melissifolia*. Seeds and fruits receiving the flooding treatment were inundated from 2 January to 31 March of each year.

germination occurred when mean maximum monthly temperatures ranged from 23.7–30.6° C and mean minimum temperatures ranged from 12.1–21.5° C (Fig. 1). Thirty-five percent of seeds in both the flooding and non-flooding treatments germinated in the first spring and early summer (April–June 2004) following sowing (Fig. 1). Fruits in the non-flooding treatment germinated to only 3% in 2004 and they did so from June through September. When flooding ceased on 31 March 2004, fruits in the flooding treatment were intact and the pulp showed no breakdown or decay following the 3 months of submergence. Fruits in the flooding treatment germinated to 35% during May–October 2004, and an additional 21% germinated in April–June 2005 (Fig. 1). Although the study continued until October 2006, seeds in both treatments and fruits in the non-flooding treatment did not germinate beyond the first year (2004) after sowing, and fruits receiving the flooding treatment did not germinate beyond the second year (2005) after sowing.

EFFECTS OF COLD STRATIFICATION. Lengths of stratification and of incubation, incubation temperature, and the interaction of these

Table 1. Results of ANOVAs showing the effects and interactions of length of cold stratification, length of incubation, and incubation temperature on germination of *Lindera melissifolia* seeds harvested in September 2006 and October 2009.

Source of Variation	2006				2009			
	df	MS	*F*	*P*	df	MS	*F*	*P*
Length of stratification (LS)	2	2576.60	58.37	<0.0001	2	13185.28	397.75	<0.0001
Length of incubation (LI)	3	3253.95	73.72	<0.0001	5	572.48	17.27	<0.0001
Incubation temperature (T)	4	4837.04	109.58	<0.0001	3	17093.03	515.64	<0.0001
LS × LI	4	401.91	9.11	<0.0001	10	127.20	3.84	0.0001
LS × T	8	671.24	15.21	<0.0001	6	3257.94	98.28	<0.0001
LI × T	12	629.61	14.26	<0.0001	15	126.83	3.83	<0.0001
LS × LI × T	16	103.90	2.35	0.0052	30	70.25	2.12	0.0018
Error	100	44.14			145	33.15		

variables had significant effects on germination of the 2006-collected and 2009-collected seeds (Table 1). Following 0 wk of cold stratification, germination was highest (57%) at 35/20° C; germination for 2006 seeds at other temperatures and that for 2009 seeds at all temperatures was < 8% (Fig. 2). Seeds gained the ability to germinate ≥ 63% at 30/20° and 35/20° C with 6 wks of cold stratification, and ≥ 29% at 25/15°, 30/15°, 30/20°, and 35/20° C with 12 wks of cold stratification. Seeds did not germinate at 15/6°C with or without cold stratification (Fig. 2).

Presence of fruit pulp during stratification ($F = 93.50$, df = 1, $P < 0.0001$), length of incubation ($F = 22.30$, df = 3, $P < 0.0001$), incubation temperature ($F = 425.91$, df = 3, $P < 0.0001$), and the interactions of these variables ($P \leq 0.0015$) significantly affected fruit germination relative to that of seeds. Following 12 wks of cold stratification (pulp on), mean (± SE) cumulative germination percentages for fruits (incubated with pulp off) were 0, 1 ± 1%, 9 ± 3%, and 51 ± 6%, at temperatures of 15/6°, 20/10°, 25/15°, and 30/20° C, respectively (data not shown). With the exception of no germination at 15/6°C, these percentages were significantly lower ($P \leq 0.0176$; one-way ANOVA) than germination percentages for seeds receiving the same pretreatment (see Fig. 2).

EFFECTS OF LIGHT. Light regime ($F = 132.11$, df = 3, $P < 0.0001$), cold stratification ($F = 409.74$, df = 1, $P < 0.0001$), incubation temperature ($F = 603.52$, df = 3, $P < 0.0001$), and interaction among these variables ($F =$

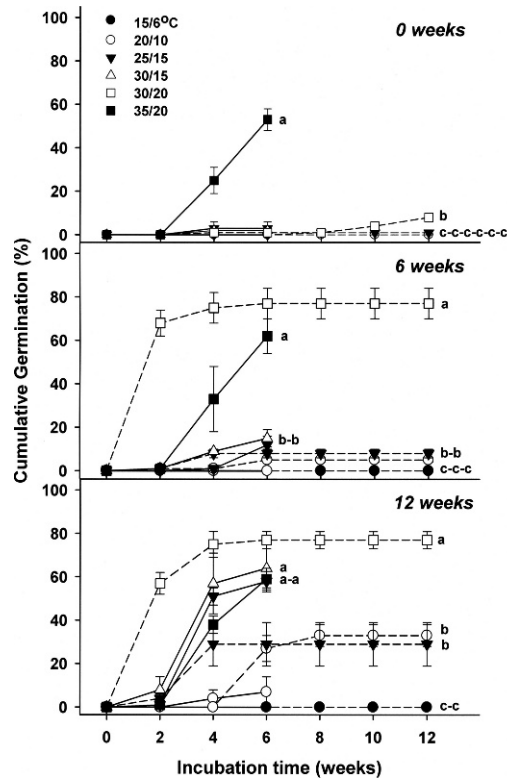

FIG. 2. Mean (± SE) germination percentages for 2006-collected seeds of *Lindera melissifolia* incubated for 6 weeks following 0–12 weeks cold stratification at 5° C (solid lines), and for 2009-collected seeds incubated for 12 weeks following 0–12 weeks cold stratification at 5/1° C (dashed lines). In the top two panels, data points for 20/10° C are superimposed on 15/6° C and therefore are not seen at all scorings. Means with dissimilar letters are significantly different (PLSD, $P = 0.05$); where data points are overlaid, letters representing each point are separated by dashes.

Table 2. Effects of light regime on mean (± SE) germination percentages of *Lindera melissifolia* seeds. Seeds were cold stratified at 5/1° C in light (L) or in darkness (D) and then incubated in L or in D at each temperature regime for 2 wks. Non-stratified seeds were incubated in L or in D for 2 wks (fresh) or for 8 wk (control). Means with dissimilar uppercase letters within columns or lowercase letters within rows are significantly different (PLSD, $P = 0.05$).

Stratification	Light Regime		Incubation temperatures (°C)			
Wk	Strat.	†Inc.	15/6	20/10	25/15	30/20
0 (Fresh)		L	0^{Aa}	0^{Aa}	0^{Aa}	0^{Aa}
0 (Fresh)		D	0^{Aa}	0^{Aa}	0^{Aa}	29 ± 5^{Bb}
6	L	L	0^{Aa}	1 ± 1^{Aa}	1 ± 3^{Aa}	68 ± 6^{Cb}
6	D	D	0^{Aa}	0^{Aa}	50 ± 4^{Bb}	100 ± 0^{Dc}
6	L	D	0^{Aa}	1 ± 1^{Aa}	56 ± 4^{Bb}	100 ± 0^{Dc}
6	D	L	0^{Aa}	0^{Aa}	2 ± 1^{Aa}	52 ± 2^{Cb}
0 (Control)		L	0^{Aa}	0^{Aa}	0^{Aa}	0^{Aa}

† Inc. = Incubation

25.37, df = 3, $P < 0.0001$) had significant effects on seed germination. Germination of fresh and control seeds was ≤ 29% regardless of the light regime or incubation temperature (Table 2). Highest germination percentages (100%) occurred for seeds incubated in darkness at 30/20° C, regardless of light regime during cold stratification. In contrast, seeds incubated in light at this temperature germinated to 52% when stratified in darkness and to 68% when stratified in light.

Effects of GA_3. Concentration of GA_3 ($F = 70.19$, df = 3, $P < 0.0001$), length of incubation ($F = 15.93$, df = 5, $P < 0.0001$), and their interaction ($F = 2.86$, df = 15, $P = 0.003$) had a significant effect on seed germination. Seeds began germinating at 2, 4, and 6 wks of incubation in 1000, 100, and 10 mg L^{-1} GA_3 solutions, respectively (Fig. 3). At 12 wks of incubation, mean cumulative germination percentage was highest for seeds in 1000 mg L^{-1} GA_3, and was significantly greater than that for seeds in the 10 mg L^{-1} solution. Seeds incubated in distilled water germinated to only 5% at 12 wks of incubation.

Effects of Flooding. Germination percentages for seeds and fruits were affected by the presence or absence of fruit pulp during submergence ($F = 29.34$, df = 1, $P < 0.0001$), incubation temperature ($F = 178.52$, df = 3, $P < 0.0001$), length of incubation ($F = 20.43$, df = 5, $P < 0.0001$) and the interaction of presence or absence of fruit pulp and incubation temperature ($F = 3.71$, df = 2, $P = 0.0287$). In general, fruits germinated to higher percentages than seeds at 20/10, 25/15, and 30/20°C but the presence of fruit pulp during submergence had a significant effect on germination only at 30/20°C (Fig. 4). Germination for both fruits and seeds decreased with decreasing incubation temperatures, and no germination occurred during submergence or at 15/6°C.

Discussion. At the time of fruit (drupe) dispersal, embryos in seeds of *Lindera melissifolia* are small relative to seed length, but are differentiated into radicle and shoot. The embryo does not grow prior to seed germination. A moderate percentage (59%) of seeds without pretreatment germinated when incubated in light at 35/20° C for 6 weeks. Although, some seeds (29%) germinated when incubated in darkness for 2 wks at 30/20° C, a significantly higher percentage of seeds (100%) germinated when incubated at 25/15° and

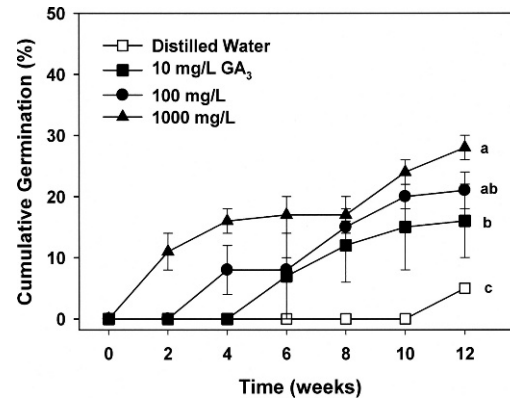

Fig. 3. Mean (± SE) cumulative germination for seeds of *Lindera melissifolia* receiving no cold stratification and being incubated for 12 weeks at 25/15° C in distilled water (control) or a solution of 10, 100, and 1000 mg L^{-1} gibberellic acid (GA_3). Means at week 12 with dissimilar letters are significantly different (PLSD, $P = 0.05$).

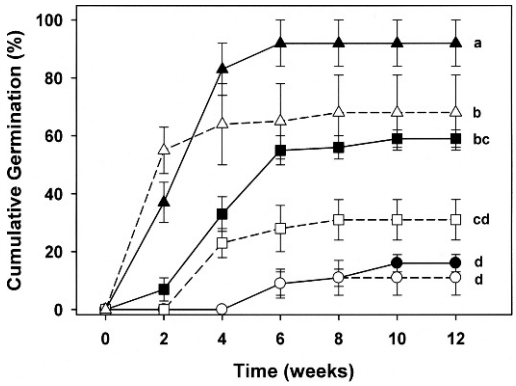

FIG. 4. Mean (±SE) cumulative germination percentages for seeds (dashed lines) and fruits (solid lines) of *Lindera melissifolia* incubated at 20/10 (○, ●), 25/15 (□, ■), and 30/20° C (△, ▲) following 12 weeks of submergence in cold (5/1°C) deionized water. Fruits were submerged with pulp present and were incubated with pulp removed. Means with dissimilar letters are significantly different (PLSD, $P = 0.05$). Neither seeds nor fruits germinated at 15/6° C (not shown).

30/20° C in darkness following 6 wks of cold stratification, regardless of whether stratification occurred in light or darkness. The highest germination percentages across a wide range of temperatures (≥ 20/10° C) occurred after seeds were cold stratified for 12 wks. Collectively, these results suggest that while germination is enhanced by high incubation temperatures and darkness, seed dormancy break requires a period (≥ 6 wks) of cold stratification. Since *L. melissifolia* seeds have fully-developed embryos and require no greater than 12 wks of cold stratification for dormancy break, they are classified as having nondeep physiological dormancy. Additionally, seed exposure to GA_3 was moderately successful in breaking dormancy, which is further indication of nondeep physiological dormancy (Baskin and Baskin 2004).

Nondeep physiological dormancy has been reported for seeds of *Neolitsea acuminatissima* (Hayata) Kanchira & Sasaki, a member of Lauraceae that is endemic to Taiwan (Chen et al. 2006). We can infer that other species of *Neolitsia* have seeds that are physiologically dormant (Holmes 1954, Goo 1976, Lin 1996); however, the level (nondeep, intermediate, or deep) of physiological dormancy cannot be concluded based on the methods of those studies. In *Lindera benzoin* (L.) Blume, a North American congener of *Lindera melissifolia*, cold stratified seeds germinate to higher percentages than those receiving no pretreatment (Schroeder 1935, Hoss 2006), indicating seeds of this species may also have some level of physiological dormancy. To date, physiological dormancy has been found in species phylogenetically placed in sister clades of the Laureae tribe and include genera with either Asian or amphi-Pacific distributions (Chanderbali et al. 2001). However, the level of physiological dormancy has yet to be determined for many species within these genera, and hypotheses regarding the evolution of seed dormancy within the Laureae tribe would be difficult, if not impossible, at this time.

The influence of the fruit pulp (mesocarp and exocarp) on dormancy break in seeds of *Lindera melissifolia* was largely dependent on experimental conditions following fruit dispersal. In the germination phenology experiment, when fruits received a flooding treatment, seeds germinated in the first and second years following sowing, and thus formed a short-lived persistent soil seed bank (sensu Walck et al. 2005). Seeds with intact fruit pulp, that were not exposed to flooding, germinated to < 5% in the first growing season and no germination occurred in subsequent years. In the laboratory, when seeds were cold stratified with fruit pulp on, germination was significantly lower than when seeds were cold stratified without pulp. On the other hand, when fruits and seeds were submerged in water for 12 wks, subsequent germination percentages at incubation temperatures of 25/15 and 30/20° C were significantly higher for submerged fruits than for submerged seeds. This latter observation may be attributed to increased levels of ethylene derived from decay of the fruit pulp during submergence. Baskin et al. (2003) found ethylene to be a germination cue for seeds of *Schoenoplectus hallii* (A. Gray) S. G. Sm. (Cyperaceae), a rare summer annual that grows in occasionally flooded sites in the eastern United States.

Within an ecological context, several seed traits appear to be well-suited to the habitat of extant *Lindera melissifolia* populations. Fruits are dispersed from fall to early winter and receive either a period of cold stratification (on soil) or submergence (Hawkins et al. 2010). Seeds, with or without fruit pulp, are flood tolerant for a period of up to at least 12 wks, and either submersion in cold water or cold stratification is effective for dormancy break.

In years when flooding does not occur, *L. melissifolia* seeds at or near the soil surface from late spring through summer, should be exposed to soil temperatures sufficient for germination (Londo et al. 1999). In years when winter flooding occurs, soil temperatures warm as flood waters recede in spring and ethylene may accumulate in the anaerobic waterlogged soil (Smith and Restall 1971, Arshad and Frankenberger 2002) reaching concentration levels that promote germination (Beaudoin et al. 2000). Therefore, an ethylene cue for germination may provide compensation for lower than optimum germination temperatures in spring.

There are also several traits associated with *Lindera melissifolia* seeds that may either directly or indirectly contribute to the continued rarity of the species. Although seeds have the capacity to form a short-lived persistent soil seed bank (two growing seasons), this was indicated only when fruits were exposed to flooding (germination phenology experiment). Therefore, in years when winter flooding events do not occur, seed viability may be lost after the first growing season. Additionally, the darkness requirement for germination and a relatively large seed size further substantiates that this species has limited capacity to form a long-lived seed bank.

Neither seeds nor fruits floated at anytime during the germination phenology experiment, nor while submerged in the laboratory experiment. This suggests that hydrochory is not a method of seed dispersal. Long-range dispersal of seeds in their natural environment by movement of sediment is also unlikely. In these bottomland hardwood forests, change in topography is minimal, and thus generates little to no current velocity as flood waters slowly recede. The high density of woody plant stems and other emergent substrates such as fallen trees and tree limbs would further serve to impede sediment movement over long distances (Schneider and Sharitz 1988). Smith et al. (2004) suggested hermit thrush (*Catharus guttatus*) as possible dispersers of *Lindera melissifolia* seeds. However, the number of observations of ingestion was very few relative to the fruit set, and these birds move only short distances during winter and are unlikely to carry seeds across open spaces to other forest patches (Brown et al. 2000). In general, if a species lacks the capacity to form a long-term persistent soil seed bank, it is not likely that the species can naturally re-establish (Bakker 1996), and seed dispersal limited to local scales may contribute to rarity of a species (Burmeier and Jensen 2008).

For many plant species growing in wetlands and periodically flooded habitats, early germination and growth are important for successful seedling establishment and tolerance to submersion. Weisner and Ekstam (1993) showed that survival of *Phragmites australis* (Cav.) Trin. ex Steud. juveniles was related to plant size achieved during the first growing season. Early season germinants gained more biomass than late-season germinants and had a higher flood tolerance. Simlarly, early germinants of *Acer rubrum* L., *Carpinus caroliniana* Walter, and *Platanus occidentalis* L. growing in floodplain forests may grow an order of magnitude larger than that of late season germinants (Jones and Sharitz 1989). As evidenced by the germination phenology study, *Lindera melissifolia* seeds germinate from April through October suggesting that there is potential for late season germinants in the natural habitat. *Lindera melissifolia* seeds do not germinate while submerged and temperatures required for germination are relatively high which would further encourage late-season germination. These attributes, coupled with the low relative growth rate of seedlings (Hawkins, unpublished) and flood-intolerance of metabolically active juvenile plants (Hawkins et al. 2009b), may well explain the lack of seedlings in naturally occurring populations.

From an ecological perspective, *Lindera melissifolia* plants do not possess the capacity to form long-lived persistent soil seed banks, nor is there an apparent mechanism of long-range seed dispersal. The absence of these two attributes in the life cycle of *L. melissifolia* may have a direct effect on long-term persistence of the species. Indirectly, there is strong potential for late season germinants with little chance of survival beyond the initial growing season, and this too could contribute to the continued rarity of the species.

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