Dormancy-breaking and germination requirements of seeds of four *Lonicera* species (Caprifoliaceae) with underdeveloped spatulate embryos

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Abstract

Dormancy-breaking requirements and types of dormancy were determined for seeds of *Lonicera fragrantissima* Lindl. & Paxt., *L. japonica* Thunb., *L. maackii* (Rupr.) Maxim. and *L. morrowii* A. Gray. Seeds of all four species have underdeveloped spatulate embryos that are about 20–40% fully developed (elongated) when dispersed. Embryos in freshly matured, intact seeds grew better at 25/15°C than at 5°C. Gibberellic acid (GA_3) (tested only in the light) was more effective in breaking dormancy in *L. maackii* and *L. morrowii* than in *L. fragrantissima* and *L. japonica*. Warm- followed by cold stratification was required to break dormancy in seeds of *L. fragrantissima*, whereas seeds of *L. japonica* required cold stratification only. Thus, seeds of *L. fragrantissima* have deep simple morphophysiological dormancy (MPD) and those of *L. japonica* nondeep simple MPD. About 50% of the seeds of *L. maackii* required warm- or cold stratification only to come out of dormancy and 50% of those of *L. morrowii* required warm stratification only, whereas the other 50% did not require stratification to germinate. Thus, about half of the seeds of the two species has nondeep simple MPD, and the other half has morphological dormancy (MD). In these laboratory tests, seeds of *L. japonica*, *L. maackii*, and *L. morrowii* generally germinated to significantly higher percentages in light than in darkness; seeds of *L. fragrantissima* were not tested in darkness. Peaks of germination for seeds of *L. fragrantissima*, *L. japonica*, *L. maackii* and *L. morrowii* sown on a soil surface and covered with *Quercus* leaves under near-natural temperature conditions shortly after seed maturity and dispersal in late June 1997, late November 1997, early November 1996 and late June 1998, respectively, occurred in early March 1998, late February 1998, late March 1997 and early October 1998, respectively. The germination phenologies of seeds of the same species and seed lots buried in soil were similar to those of seeds under leaf litter. High percentages of seeds of all four species germinated both under litter (78–96%) and beneath the soil surface (78–97%). These germination patterns correspond closely with the requirements for embryo growth and dormancy break in the four *Lonicera* species.

Keywords: embryo growth, germination phenology, morphological seed dormancy, morphophysiological seed dormancy, *Lonicera* species, underdeveloped spatulate embryo

Introduction

In many plant species with spatulate embryos, the embryo extends the full length of the seed (Martin, 1946). When tested for germination, freshly matured seeds of these taxa may not germinate under any set of environmental conditions (i.e. they are dormant), only germinate under a limited range of environmental conditions (i.e. they are conditionally dormant), or germinate under a wide range of environmental conditions (i.e. they are nondormant). Thus, seeds with fully developed spatulate embryos either have physiological dormancy or no dormancy at all (Baskin and Baskin, 1998).

In contrast to the fully developed spatulate embryos found in many angiosperm families, those of Caprifoliaceae are underdeveloped (Martin, 1946), i.e. they have distinguishable cotyledons and radicle, but must elongate before germination can occur. One of the purposes of this study was to determine if seeds of the caprifoliaceous genus *Lonicera*, with underdeveloped spatulate embryos (Martin, 1946), have morphological dormancy (MD) and/or morphophysiological dormancy (MPD) and if the latter, which of the eight known type(s) of MPD (see Baskin and Baskin, 1998) they have. Specifically, (1)

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light and temperature requirements for dormancy-break and embryo growth, and (2) effects of gibberellic acid (GA_3) on germination were investigated. If embryo growth is the only pre-germination requirement, seeds of *Lonicera* would have MD (Nikolaeva, 1977; Baskin and Baskin, 1998), whereas if the seeds require (a) treatment(s) such as cold stratification and/or warm stratification to germinate, they would have MPD (Nikolaeva 1969, 1977; Baskin and Baskin, 1998).

In nature, it is conceivable that after dispersal, seeds of *Lonicera* species could become covered with leaf litter or be buried beneath the soil surface. Thus, the second purpose of this study was to monitor the germination phenology of seeds planted on the soil surface beneath oak (*Quercus*) leaf litter and of those buried in soil under near-natural temperature conditions. Information on germination (or lack thereof) of *Lonicera* seeds under these conditions, and especially on germination of seeds buried beneath the soil surface, would provide insight into the potential for these species to form a soil seed bank.

Lonicera species used in this study were *L. fragrantissima* Lindl. & Paxt., *L. japonica* Thunb., *L. maackii* (Rupr.) Maxim. and *L. morrowii* A. Gray. These species were chosen for study because they are members of the Caprifoliaceae, and a large number of seeds of each species could easily be obtained for dormancy/germination studies. *Lonicera japonica* is a perennial vine native to eastern Asia that was introduced to the United States in 1806 as a landscape plant and ornamental and was later used for cattle forage, erosion control and game food (Rehder, 1940; Wyman, 1949). Since its introduction, *L. japonica* has escaped cultivation and has become naturalized in regions with more than 100 cm of annual precipitation and a mean January temperature above 0°C (Leatherman, 1955; Evans, 1984). Amur honeysuckle, *L. maackii*, is a shrub first introduced to the United States from Russia in 1897 for use in soil conservation, to improve habitat for birds, and to serve ornamental functions in landscape plantings (USDA, 1898; Rehder, 1940; Luken and Thieret, 1996). It is one of several exotic bush honeysuckles that are spreading rapidly in the eastern United States and adjacent parts of Canada, less than a century after its introduction (Braun, 1961; Pringle, 1973; Luken, 1988; Trisel, 1997). *Lonicera fragrantissima* was introduced to the United States from eastern China in 1845 as a landscape plant and ornamental (Bailey, 1906; Dirr, 1998) and *L. morrowii* from Japan in 1875 for ornamental purposes (Bailey, 1906). Although both species of shrubs are naturalized in the United States (Radford *et al.*, 1968; Gleason and Cronquist, 1991), we are not aware of any information on invasiveness or on the geographical spread of these two species.

Materials and methods

Seed sources

Fruits (berries) of *L. fragrantissima* were collected on 1 June 1997 and on 7 May 1998; those of *L. japonica* on 15 November 1997 in Jessamine County, Kentucky (USA); those of *L. maackii* on 1 November 1996 and on 12 November 1997 in Fayette County, Kentucky; and those of *L. morrowii* on 21 June 1998 in Franklin County, Pennsylvania (USA). At the time of collection, fruits of all four species were ripe, and some of them already had dispersed. When ripe, the fleshy pericarp of the fruit is soft and entirely reddish orange (*L. fragrantissima*), bluish black (*L. japonica*) or red (*L. maackii, L. morrowii*). Seeds were removed from the pulp and dried in the laboratory (approx. 23°C) for 3 to 6 d before studies were initiated. Seeds of *L. fragrantissima* collected in 1997 were used for embryo growth and phenology studies and those collected in 1998 for studies on germination and the effect of GA_3 on dormancy-break. Seeds of *L. maackii* collected in 1996 and in 1997 were used for the germination study and those collected in 1996 for studies on embryo growth and phenology. The 1997 seeds also were used to study the effects of GA_3 on dormancy-break.

Germination studies

Five temperature- and light-controlled incubators and a refrigerator, equipped with a light and a time clock, were used in the studies. The incubators were set at 12/12 h daily thermoperiods of 15/6, 20/10, 25/15, 30/15 and 35/20°C and the refrigerator at a constant 5°C. Thermoperiods in the incubators approximate mean daily maximum and minimum monthly air temperatures in Kentucky and adjacent areas (Wallis, 1977) during the growing season: March and November, 15/6; April and October, 20/10; May, 25/15; June and September, 30/15; and July and August, 35/20°C. Cool white fluorescent tubes, which produced a photon flux density (400–700 nm) at seed level of approx. 40 µmol m^{-2} s^{-1}, were used as the light source for incubation (20 W), warm stratification, and cold stratification (15 W). The daily photoperiod was 14 h in the incubators and in the refrigerator. In the alternating temperature regimes, the photoperiod extended from 1 h before the beginning of the high temperature period to 1 h after the beginning of the low temperature period.

Seeds were placed in 55 mm diameter x 10 mm deep Petri dishes on approx. 20 g of white quartz sand moistened with approx. 5.0 ml of distilled water. All dishes were wrapped with plastic film to reduce water loss during incubation and during stratification, and dishes incubated and stratified in darkness were wrapped additionally with aluminium

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foil. Three replications of 50 seeds per dish were used per treatment.

Radicle protrusion was the criterion for germination. Ungerminated seeds were dissected under a low-power microscope to determine if the embryos were white and firm, indicating viability, or if they were brown and soft, indicating nonviability. A tetrazolium test (Grabe, 1970) confirmed that white embryos were alive and that light brown ones were not.

Dormancy-breaking and germination requirements

Freshly matured seeds of *L. fragrantissima* were incubated in the light, and those of *L. japonica*, *L. maackii* and *L. morrowii* in the light or darkness, at the five alternating thermoperiods for 2 wk.

The 25/15°C thermoperiod was used for warm stratification and 5°C for cold stratification, since they are near-optimal for seeds of many species that require warm or cold temperatures, respectively, for dormancy release (Stokes, 1965; Nikolaeva, 1969). Seeds of *L. fragrantissima* were (1) warm- or cold-stratified in the light for 8 and 12 wk, or (2) warm-stratified in the light for 8 wk followed by cold stratification in the light for 6 wk. Seeds of *L. japonica* were warm-stratified in the light and in darkness for 12 wk and cold-stratified in the light and in darkness for 12 wk. Seeds of *L. maackii* were warm- or cold-stratified in the light and in darkness for 2, 4, 6, 8, 10, and 12 wk, but only data for those stratified for 12 wk are presented. Seeds of *L. morrowii* were warm- or cold-stratified in the light and in darkness for 6 wk. Following all stratification treatments for each species, seeds were incubated in the light and in darkness at the five thermoperiods for 2 wk. Seeds both stratified and incubated in darkness were not checked until the end of the incubation period.

Nonstratified seeds were also incubated in the light at each thermoperiod for 8 to 16 wk. Seeds in these controls were examined every 2 wk, and seedlings that had germinated were counted and removed from the Petri dishes. Water was added to the dishes as needed to keep the sand-substrate at a near-constant moisture level.

Requirements for embryo growth

Embryos were excised from seeds using a razor blade, and their lengths measured under a dissecting microscope equipped with a micrometer. Lengths were determined for embryos in 50 freshly matured seeds of each species that were allowed to imbibe on moist filter paper at room temperature (approx. 23°C) for 24 h. Embryo growth was monitored in seeds of all four species during warm- and cold stratification. Fifty seeds of each species were placed in each of 14 Petri dishes: seven dishes were placed at 25/15°C and the other seven at 5°C. After 2, 4, 6, 8, 10 and 12 wk of warm- or cold stratification in light, one Petri dish of each species was removed from each of the two stratification treatments and embryo lengths determined. If a seed had germinated, embryo length was recorded as the critical length required for germination, i.e. when embryos had grown enough to start splitting the seed coats.

Effect of gibberellic acid (GA_3) on germination

For each of the four species, 50 seeds were placed on two sheets of Whatman No.1 filter paper in each of 12 glass Petri dishes (90 mm diameter × 15 mm deep). The filter paper was moistened with distilled water (control) or with a solution of 10, 100 or 1000 mg l^{-1} GA_3 (potassium salt) dissolved in distilled water. Three replications (Petri dishes) were used for each treatment. Dishes were placed in the light at 25/15°C, and distilled water was added as needed. The 25/15°C thermoperiod was used since it is too high to be effective for cold stratification (Nikolaeva, 1969). Germination was checked after 2, 6 and 12 wk of incubation.

Germination phenology

Three replications of 300 seeds of each species were sown on soil in 20 width × 30 length × 9 deep cm metal flats and covered with approx. 5 cm of dead *Quercus* leaves to simulate conditions in habitats in which the plants may grow. Seeds of *L. fragrantissima*, *L. japonica*, *L. maackii* and *L. morrowii* were sown on 23 June 1997, 22 November 1997, 21 November 1996 and 27 June 1998, respectively. The soil used was a 3:1 (v/v) mixture of limestone-derived topsoil and river sand. The flats were placed in a greenhouse (no heating or air conditioning, windows open all year). At weekly intervals, the leaves were lifted and the germinated seeds counted and removed from the flats. Thus, seeds of the four species were exposed to light during the germination phenology monitoring period, regardless of whether the microenvironment beneath the *Quercus* leaves was totally dark or not. Temperatures in this greenhouse are near those outdoors in the Lexington, Kentucky, area throughout the year (Baskin and Baskin, 1985). Continuous thermograph records were made inside a weather-house in the greenhouse, and mean maximum and minimum temperatures for each week of the studies calculated. From 1 September to 30 April of each year, the soil was watered to field capacity daily (unless frozen in winter), and during the remainder of the year it was watered once per week. This watering regime approximated soil moisture that might occur in the habitats of the four species, i.e. soil wet in

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autumn, winter and early spring, and alternately wet and dry in late spring and summer.

About 2000 to 3000 seeds of each species were placed in 12 fine-mesh nylon bags and buried 7 cm deep in soil in 15-cm-diameter plastic pots with drainage holes on 11 June 1997 (*L. fragrantissima*), 26 November 1997 (*L. japonica*), 21 November 1996 (*L. maackii*) and 27 June 1998 (*L. morrowii*). The pots were placed under a bench in the greenhouse, and the watering regime was the same as that for seeds under *Quercus* leaf litter. Seeds of *L. fragrantissima*, *L. japonica*, *L. maackii* and *L. morrowii* were exhumed first on 1 September 1997, 1 December 1997, 1 December 1996 and 1 August 1998, respectively. Thereafter, seeds of each species were exhumed at monthly intervals until supplies of buried seeds were exhausted, i.e. most seeds in the bag had germinated. For each exhumation and species, a randomly chosen bag of seeds was removed from the pots, cut open, and both germinated (seedling) and nongerminated (viable) seeds counted.

Statistical analyses

Germination data were transformed to percentages based on the numbers of viable seeds. Means and standard errors were calculated for germination percentages and for embryo lengths. Means of germination percentages were compared by analyses of variances (ANOVAs) and by protected least significant difference tests (PLSDs, $P = 0.05$) (SAS Institute Inc., 1985). The first ANOVA tested the effects and interactions of seed condition (fresh, control, stratified), light regime, and thermoperiod on germination percentages; the second ANOVA tested the effects and interaction of GA_3 concentration and length of incubation. Year was included as a factor in the analysis if germination tests were done on two collection dates. Species was not included as a factor since stratification treatments varied among the four *Lonicera* taxa. Percentages were arcsine-square root transformed in statistical tests.

Results

Seed condition, light regime (not tested for *L. fragrantissima*), thermoperiod, all two-way interactions, and the three-way interaction had significant effects ($P = 0.0001$) on germination of the *Lonicera* species. Since germination responses of *L. maackii* seeds were similar for the 1996 and 1997 collections (year × condition × light × thermoperiod, $P = 0.9771$), the data for only one year (1996) are presented. GA_3 concentration, length of incubation, and their interaction had significant effects ($P = 0.0001$) on germination of all four species.

Lonicera fragrantissima

None of the freshly matured seeds or the nonstratified (control) seeds germinated in the light over the range of thermoperiods during 2 and 16 wk of incubation, respectively (Table 1). Seeds germinated to 0–17% in light over the range of thermoperiods following 8 or 12 wk of warm- or cold stratification in the light. However, seeds germinated to 90–100% in the light over the range of thermoperiods following 8 wk of warm- and 6 wk of cold stratification in light.

Embryos in freshly matured seeds were 0.74 ± 0.02 (mean ± SE) mm long, approximately 23% of the length of the seeds (Fig. 1). They grew to 1.79 ± 0.05 mm during 12 wk of warm stratification in the light but to only 0.89 ± 0.03 mm during 12 wk of cold stratification. Lengths of embryos were approx. 2.42 ± 0.06 mm when they had grown enough to start

Table 1. Effect of stratification on germination (mean ± SE) of *Lonicera fragrantissima* seeds collected in 1998. Following each stratification treatment (warm, cold, warm + cold) in light, seeds were incubated in the light at each thermoperiod for 2 wk. Numbers in parentheses are percentages of germination that occurred during cold stratification. Freshly matured seeds were incubated in the light at each thermoperiod for 2 wk and control (nonstratified) seeds for 16 wk. Values with different uppercase letters within columns or with different lowercase letters within rows are significantly different (PLSD, $P = 0.05$)

Weeks at:		Per cent germination at incubation thermoperiod of:				
25/15°C	5°C	15/6°C	20/10°C	25/15°C	30/15°C	35/20°C
Fresh		0^{Aa}	0^{Aa}	0^{Aa}	0^{Aa}	0^{Aa}
8	0	17 ± 1^{Cb}	13 ± 1^{Cb}	0^{Aa}	0^{Aa}	0^{Aa}
12	0	7 ± 1^{Bc}	1 ± 1^{Bb}	1 ± 1^{Bb}	3 ± 1^{Bb}	0^{Aa}
0	8	0^{Aa}	0^{Aa}	0^{Aa}	0^{Aa}	0^{Aa}
0	12	0^{Aa}	0^{Aa}	0^{Aa}	0^{Aa}	0^{Aa}
8	6	93 ± 1^{Da}	100^{Db}	93 ± 2^{Ca}	91 ± 2^{Ca}	90 ± 1^{Ba}
		(64)	(100)	(61)	(52)	(66)
Control		0^{Aa}	0^{Aa}	0^{Aa}	0^{Aa}	0^{Aa}

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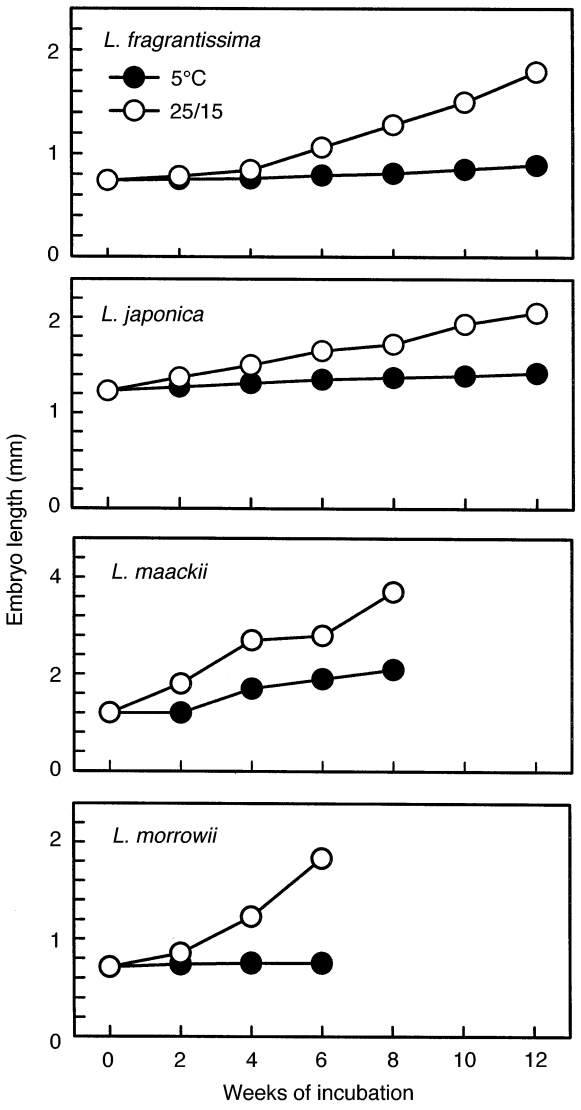

Figure 1. Embryo length (mean ± SE, all SE ≤ 0.07 mm) of freshly matured seeds (0 wk) of four *Lonicera* species and of those following 2–12 wk of warm (25/15°C)- or of cold (5°C) stratification in the light. Mean (± SE) seed length for *L. fragrantissima*, *L. japonica*, *L. maackii* and *L. morrowii* is 3.18 ± 0.05, 2.89 ± 0.04, 4.24 ± 0.05 and 3.03 ± 0.07 mm, respectively.

splitting the seed coat. Thus, embryo length increased about 227% between seed maturity and germination.

Seeds did not germinate during 2 wk of incubation in GA_3 solutions or in distilled water (control), but up to 36% of them germinated during 6 wk (Table 2). During 12 wk of incubation, 26–52% of the seeds germinated in GA_3 solutions, and 17% germinated in the control.

A few seeds sown under leaf litter in the greenhouse on 23 June 1997 germinated in late October and in early November 1997 (Fig. 2). However, peak germination occurred between 1 and 8 March 1998, when mean weekly maximum and minimum temperatures were 14.6 and 6°C, respectively. No additional seeds germinated after 14 March 1998 until the study was ended on 31 May 1999. No seeds buried in soil had germinated when they were exhumed between September 1997 and January 1998 (Fig. 2). However, 26, 36, 60 and 78% of the seeds had germinated in bags exhumed in February, March, April and May 1998, respectively.

Lonicera japonica

Freshly matured seeds germinated to 0–9% in the light over the range of thermoperiods, but none germinated in darkness (Table 3). Seeds germinated to 31–44% in the light over the range of thermoperiods following 12 wk of warm stratification in light, and they germinated to 1–8% in darkness following warm stratification in darkness. However, seeds germinated to 77–99% in the light over the range of thermoperiods following 12 wk of cold stratification in the light, and they germinated to 13–52% in darkness following cold stratification in darkness. During 14 wk of incubation in light, nonstratified (control) seeds germinated ≥31% in all thermoperiods except 35/20°C.

Embryos in freshly matured seeds were 1.23 ± 0.03 mm long, approx. 43% of the length of the seeds (Fig. 1). They grew to 2.05 ± 0.05 mm during 12 wk of warm stratification in the light and to 1.42 ± 0.03 mm during 12 wk of cold stratification. By the end of 12 wk of the warm stratification period, most of the seeds had begun to split the seed coats, and some of them had begun to germinate. Thus, embryo length increased about 67% between seed maturity and germination.

Seeds germinated to 5–14% during 2 wk of incubation in GA_3 solutions and 2% in the distilled water control (Table 2). Up to 55 and 74% of the seeds germinated in GA_3 solutions during 6 and 12 wk of incubation, respectively, but only up to 26 and 30% in the controls.

A few seeds sown under leaf litter in the greenhouse on 21 November 1997 germinated in late January 1998 (Fig. 2). However, peak germination occurred between 15 and 22 February 1998, when mean weekly maximum and minimum temperatures were 12.1 and 6.2°C, respectively. No additional seeds germinated after 30 April 1998, until the study was terminated on 31 May 1999. No seeds buried in soil had germinated when the bags were exhumed between December 1997 and February 1998 (Fig. 2). However, 48, 88 and 96% of the seeds had germinated in bags exhumed in March, April and May 1998, respectively.

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Table 2. Effect of gibberellic acid on seed germination (mean ± SE) of four species of *Lonicera*. Seeds were incubated in the light with GA_3 solutions at 25/15°C for 2–12 wk. Within each species only, means with different uppercase letters within columns or with different lowercase letters within rows are significantly different (PLSD, $P = 0.05$)

Species	Incubation time (wk)	Per cent germination at GA_3 concentration of:			
		0 mg l^{-1}	10 mg l^{-1}	100 mg l^{-1}	1000 mg l^{-1}
L. fragrantissima	2	0^{Aa}	0^{Aa}	0^{Aa}	0^{Aa}
	6	12 ± 0^{Ba}	11 ± 1^{Ba}	28 ± 3^{Bb}	36 ± 3^{Bb}
	12	17 ± 0^{Ca}	26 ± 1^{Ca}	39 ± 3^{Cb}	52 ± 3^{Cb}
L. japonica	2	2 ± 0^{Aa}	5 ± 1^{Aa}	12 ± 3^{Ab}	14 ± 3^{Ab}
	6	26 ± 3^{Ba}	43 ± 4^{Bb}	53 ± 3^{Bc}	55 ± 5^{Bc}
	12	30 ± 1^{Ba}	56 ± 2^{Cb}	64 ± 2^{Cc}	74 ± 2^{Cd}
L. maackii	2	11 ± 0^{Aa}	6 ± 1^{Ab}	7 ± 3^{Ab}	11 ± 3^{Aa}
	6	53 ± 0^{Ba}	49 ± 1^{Ba}	60 ± 3^{Bb}	72 ± 3^{Bb}
	12	73 ± 0^{Ca}	99 ± 1^{Cb}	99 ± 3^{Cb}	100^{Cb}
L. morrowii	2	35 ± 0^{Aa}	67 ± 1^{Ab}	82 ± 3^{Ac}	100^{Ad}
	6	99 ± 0^{Ba}	100^{Ba}	100^{Ba}	100^{Aa}
	12	100^{Ba}	100^{Ba}	100^{Ba}	100^{Aa}

Lonicera maackii

Freshly matured seeds germinated to 5–33% in the light and in darkness at 15/6–25/15°C, and none of them germinated at 30/15–35/20°C (Table 4). Seeds germinated to ≥87% in the light over the range of thermoperiods following warm or cold stratification in the light. However, seeds germinated to 51–91% and 3% in darkness at 15/6–30/15 and 35/20°C, respectively, following warm stratification in darkness and to 31–58% and 5% following cold stratification. During 14 wk of incubation in light, nonstratified (control) seeds germinated to 58–91% at 15/6–30/15°C and to 34% at 35/20°C.

Embryos in freshly matured seeds were 1.20 ± 0.06 mm long, approximately 35% of the length of the seeds (Fig. 1). They grew to 3.70 ± 0.05 mm during 8 wk of warm stratification in light and to 2.10 ± 0.03 mm during 8 wk of cold stratification. Embryo measurements were discontinued after 8 wk, by which time most of the seeds at 25/15°C had started to split the seed coats, and some of them had begun to germinate. Thus, embryo length increased about 208% between seed maturity and germination.

Seeds germinated to 6–11% during 2 wk of incubation in GA_3 solutions and distilled water (control) (Table 2). However, during 12 wk of incubation, 99–100% of the seeds germinated in GA_3 solutions and 73% germinated in the control.

A few seeds sown under leaf litter in the greenhouse on 21 November 1996 germinated in early February 1997 (Fig. 2). However, peak germination occurred between 15 and 22 March 1997, when mean weekly maximum and minimum temperatures were 17.7 and 8.0°C, respectively. No additional seeds germinated after 31 May 1997, until the study was terminated on 31 May 1999. No seeds buried in soil had germinated when they were exhumed in December 1996. However, 19, 30, 49, 87 and 97% of the seeds had germinated in bags exhumed in January, February, March, April and May 1997, respectively.

Lonicera morrowii

Freshly matured seeds germinated to 3–30% in the light and darkness at 15/6–25/15°C, and none germinated at 30/15–35/20°C (Table 5). Seeds germinated to 98–100% in the light over the range of thermoperiods following 6 wk of warm stratification in the light and 30–51% in darkness following warm stratification in darkness. However, seeds germinated to only 0–8% in the light over the range of thermoperiods following 6 wk of cold stratification in the light, and none germinated in darkness following cold stratification in darkness. During 8 wk of incubation in the light, nonstratified (control) seeds germinated to 0–11% at 15/6 and 35/20°C and to 76–100% at 20/10–30/15°C.

Embryos in freshly matured seeds were 0.71 ± 0.03 mm long, approximately 23% of the length of the seeds (Fig. 1). They grew to 1.83 ± 0.04 mm during 6 wk of warm stratification in the light and to 0.75 ± 0.03 mm during 6 wk of cold stratification. Embryo measurements were discontinued after 6 wk, by which time most of the seeds at 25/15 °C had started to split the seed coats, and some of them had begun to germinate. Thus, embryo length increased about 158% between seed maturity and germination.

Seeds incubated for 2 wk in GA_3 solutions germinated to 67–100% and those in the distilled water control to 35% (Table 2). During 6 and 12 wk of incubation, ≥99% germination was observed in the presence and absence of GA_3.

A few seeds sown under leaf litter in the

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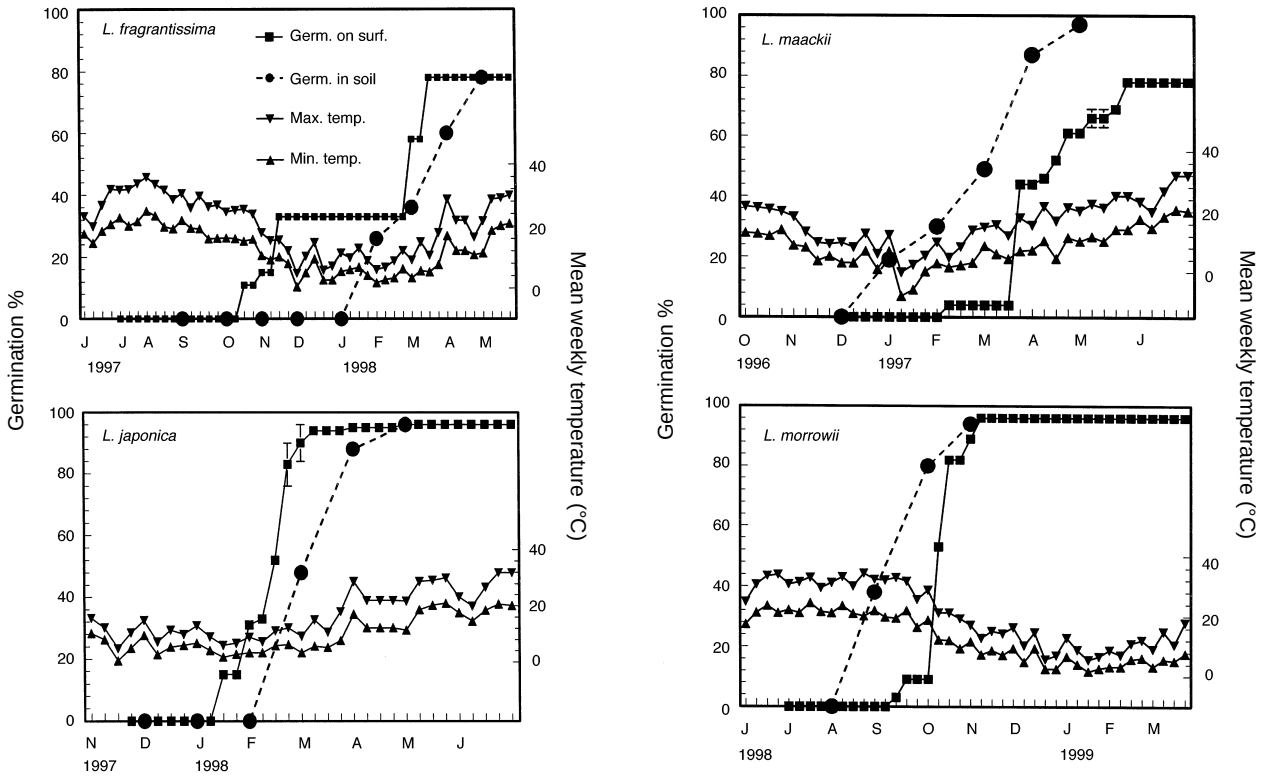

Figure 2. Germination percentages (mean ± SE, SE shown if ≥5%) of seeds of four *Lonicera* species placed on a soil surface and covered with leaf litter (cumulative data based on 900 sown seeds) or buried in pots of soil in a nontemperature-controlled greenhouse. Seeds of *L. fragrantissima* were collected on 1 June 1997 and sown on 23 June 1997 or buried on 11 June 1997; *L. japonica* seeds were collected on 15 November 1997 and sown on 22 November 1997 or buried on 26 November 1997; *L. maackii* seeds were collected on 1 November 1996 and sown and buried on 21 November 1996; *L. morrowii* seeds were collected on 21 June 1998 and sown and buried on 27 June 1998. Letters on the *x*-axis represent months of the year, and mean weekly maximum and minimum temperatures are shown for the duration of each study.

greenhouse on 27 June 1998 germinated in late September 1998 (Fig. 2). However, peak germination occurred between 3 and 10 October 1998, when mean weekly maximum and minimum temperatures were 21.2 and 12.2°C, respectively. No seeds germinated after 8 November 1998, until the study was ended on 31 May 1999. No seeds buried in soil had germinated when they were exhumed in August 1998 (Fig. 2). However, 38, 80 and 94% of the seeds had germinated in bags exhumed in September, October and November 1998, respectively.

Discussion

Prior to this study, relatively few data had been collected on the dormancy-breaking and germination requirements of seeds of *Lonicera* species. Swingle (1939) suggested that cold stratification (0–10°C) for 60–90 d was needed to break dormancy in *L. maackii* seeds. However, nonstratified seeds of *L. maackii* germinated to 53–81% in the light and 31–55% in darkness after 88 d at 30/15°C (Luken and Goessling, 1995). Luken and Goessling reported that most seeds of *L. maackii* do not have a well-developed dormancy mechanism and that they germinate easily in warm, moist conditions. Seeds of *L. tatarica* need a cold stratification period to germinate (Heit, 1967; Cram, 1982), and those cold-stratified at 5°C for 30 d germinated to 92% (Cram, 1982). Warm- plus cold stratification is recommended to break dormancy in *L. fragrantissima* seeds (Dirr, 1998). Nichols (1934) reported that there were no differences in germination percentages of cold-stratified and nonstratified seeds of the native North American species *L. canadensis*, *L. dioica* and *L. hirsuta*. Seeds of *L. canadensis*, *L. dioica* and *L. hirsuta* refrigerated for 83 d (presumably at 4–5°C) germinated to 100%, 93% and 43%, and nonstratified seeds germinated to 98%, 93% and 43%, respectively.

In most taxa with spatulate embryos (e.g. members of the families Apocynaceae, Boraginaceae,

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Table 3. Effect of stratification on germination (mean ± SE) of *Lonicera japonica* seeds collected in 1997. Seeds were stratified in the light (L) or in darkness (D) for 12 wk and then incubated in L or D at each thermoperiod for 2 wk. Numbers in parentheses are percentages of germination that occurred during stratification in L. Freshly matured seeds were incubated in L or D at each thermoperiod for 2 wk and control (nonstratified) seeds in L for 14 wk. Values with different uppercase letters within columns or with different lowercase letters within rows are significantly different (PLSD, $P = 0.05$). Strat., stratification; Inc., incubation

Weeks at:		Light regime		Per cent germination at incubation thermoperiod of:				
25/15°C	5°C	Strat.	Inc.	15/6°C	20/10°C	25/15°C	30/15°C	35/20°C
Fresh			L	3 ± 1^{Bb}	9 ± 1^{Bc}	8 ± 1^{Bc}	5 ± 1^{Bb}	0^{Aa}
Fresh			D	0^{Aa}	0^{Aa}	0^{Aa}	0^{Aa}	0^{Aa}
12	0	L	L	31 ± 1^{Ca} (27)	37 ± 1^{Cb} (41)	44 ± 1^{Dc} (40)	43 ± 1^{Dc} (39)	39 ± 1^{Dc} (54)
12	0	D	D	1 ± 1^{Ba}	7 ± 1^{Bb}	7 ± 1^{Bb}	8 ± 2^{Bb}	1 ± 1^{Ba}
0	12	L	L	92 ± 2^{Dc} (47)	99 ± 1^{Ec} (60)	99 ± 1^{Ec} (71)	89 ± 1^{Eb} (69)	77 ± 1^{Ea} (51)
0	12	D	D	52 ± 3^{Cc}	27 ± 2^{Cb}	25 ± 3^{Cb}	17 ± 1^{Ca}	13 ± 1^{Ca}
Control			L	31 ± 2^{Cb}	72 ± 1^{De}	53 ± 1^{Dd}	41 ± 1^{Dc}	5 ± 1^{Ba}

Table 4. Effect of stratification on germination (mean ± SE) of *Lonicera maackii* seeds collected in 1996. Seeds were stratified in the light (L) or in darkness (D) for 12 wk and then incubated in L or D at each thermoperiod for 2 wk. Numbers in parentheses are percentages of germination that occurred during stratification in L. Freshly matured seeds were incubated in L or D at each thermoperiod for 2 wk and control (nonstratified) seeds in L for 14 wk. Values with different uppercase letters within columns or with different lowercase letters within rows are significantly different (PLSD, $P = 0.05$). Strat., stratification; Inc., incubation

Weeks at:		Light regime		Per cent germination at incubation thermoperiod of:				
25/15°C	5°C	Strat.	Inc.	15/6°C	20/10°C	25/15°C	30/15°C	35/20°C
Fresh			L	5 ± 1^{Ab}	25 ± 4^{Ac}	33 ± 5^{Ac}	0^{Aa}	0^{Aa}
Fresh			D	6 ± 2^{Ab}	21 ± 1^{Ac}	26 ± 3^{Ac}	0^{Aa}	0^{Aa}
12	0	L	L	92 ± 2^{Db} (84)	99 ± 2^{Dc} (78)	96 ± 4^{Ec} (76)	91 ± 2^{Eb} (81)	90 ± 2^{Da} (83)
12	0	D	D	75 ± 8^{Cc}	91 ± 6^{Dd}	77 ± 2^{Cc}	51 ± 5^{Cb}	3 ± 1^{Ba}
0	12	L	L	95 ± 2^{Dc} (84)	99 ± 1^{Dc} (77)	93 ± 0^{Eb} (76)	90 ± 1^{Eb} (81)	87 ± 1^{Da} (83)
0	12	D	D	58 ± 1^{Bc}	57 ± 1^{Bc}	43 ± 3^{Bb}	31 ± 6^{Bb}	5 ± 1^{Ba}
Control			L	58 ± 4^{Bb}	87 ± 1^{Cd}	91 ± 3^{Dd}	74 ± 3^{Dc}	34 ± 4^{Ca}

Cornaceae, Polemoniaceae, Rosaceae, Urticaceae and Verbenaceae) the embryo is fully elongated at seed maturity (see Martin, 1946). Thus, seeds in these and other families with fully elongated spatulate embryos either have physiological dormancy or no dormancy at all (Baskin and Baskin, 1998). In contrast, seeds of three *Sambucus* species (Hidayati *et al.*, 2000) and seeds of the four *Lonicera* species investigated in the present study have underdeveloped spatulate embryos that must grow (elongate) before germination can occur. Rates of embryo growth in all four species were higher at warm than at cold temperatures (Fig. 1). However, the amount of time required to reach the critical length for germination differed among the species.

Fresh seeds of *L. fragrantissima* failed to germinate, and those of *L. japonica* germinated to very low percentages in the light or in darkness in 30 d; thus, they have MPD (*sensu* Baskin and Baskin, 1998). Further, the seeds required warm plus cold stratification (*L. fragrantissima*) or cold stratification only (*L. japonica*) for dormancy break (Table 6). In contrast, fresh seeds of *L. maackii* and *L. morrowii* germinated to 48–52% in the light and to 27–31% in darkness after 4 wk at 15/6–25/15°C (Hidayati, 2000). However, warm- or cold stratification treatment of seeds of these two species increased the temperature range for germination. Seeds given warm or cold stratification only (*L. maackii*) or warm stratification only (*L. morrowii*) germinated to high percentages over the entire range of temperatures (15/6–35/20°C), while nonstratified seeds germinated to high percentages in a narrower range of temperatures (20/10–30/15°C) (Tables 4 and 5). Some seeds of *L. maackii* and of *L. morrowii* appear to have MD only.

There is no clear distinction between MD and MPD in the length of incubation time needed for fresh seeds to germinate. Following Baskin and Baskin

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Table 5. Effect of stratification on germination (mean ± SE) of *Lonicera morrowii* seeds collected in 1998. Seeds were stratified in the light (L) or in darkness (D) for 6 wk and then incubated in L or D at each thermoperiod for 2 wk. Numbers in parentheses are percentages of germination that occurred during stratification in L. Freshly matured seeds were incubated in L or D at each thermoperiod for 2 wk and control (nonstratified) seeds in L for 8 wk. Values with different uppercase letters within columns or with different lowercase letters within rows are significantly different (PLSD, $P = 0.05$). Strat., stratification; Inc., incubation

Weeks at:		Light regime		Per cent germination at incubation thermoperiod of:				
25/15°C	5°C	Strat.	Inc.	15/6°C	20/10°C	25/15°C	30/15°C	35/20°C
Fresh			L	3 ± 1^{Bb}	30 ± 5^{Cd}	13 ± 2^{Cc}	0^{Aa}	0^{Aa}
Fresh			D	3 ± 1^{Bb}	5 ± 1^{Bb}	11 ± 1^{Cc}	0^{Aa}	0^{Aa}
6	0	L	L	98 ± 2^{Ea} (93)	99 ± 1^{Fa} (92)	100^{Ea} (100)	100^{Da} (94)	100^{Ca} (100)
6	0	D	D	51 ± 4^{Dc}	42 ± 3^{Dbc}	36 ± 1^{Dab}	30 ± 2^{Ba}	30 ± 4^{Ba}
0	6	L	L	5 ± 1^{Bb} (0)	8 ± 1^{Bb} (0)	8 ± 1^{Bb} (0)	0^{Aa} (0)	0^{Aa} (0)
0	6	D	D	0^{Aa}	0^{Aa}	0^{Aa}	0^{Aa}	0^{Aa}
Control			L	11 ± 1^{Cb}	76 ± 2^{Ec}	100^{Ee}	96 ± 2^{Cd}	0^{Aa}

Table 6. Summary of seed dormancy-breaking requirements of the four *Lonicera* species

	Temperature required:[a]		
Species	to break dormancy	for embryo growth	Type of MPD[b]
L. fragrantissima	W + C	W	Deep simple
L. japonica	C	W	Nondeep simple
L. maackii	W or C	W	Nondeep simple[c]
L. morrowii	W	W	Nondeep simple[d]

[a] W, warm stratification; C, cold stratification.
[b] Morphophysiological dormancy.
[c] About 50% of the seeds of *L. maackii* have morphological dormancy.
[d] About 50% of the seeds of *L. morrowii* have morphological dormancy.

(1998), we used 30 d to distinguish MD and MPD: if the seeds germinate within 30 d they have MD, whereas if they require a longer period to germinate they have MPD. Embryos with MD need only a period of time to elongate to the critical length for germination. There is no delay in growth of an MD embryo due to a (unknown) physiological inhibiting mechanism (*sensu* Nikolaeva, 1969, 1977), and after the embryo is fully developed, it will germinate over a wide range of temperatures without any stratification treatment. If the seeds do not germinate within 30 d, they have MPD. Delay in germination may be caused both by (1) a physiological inhibiting mechanism that is overcome by dry storage, warm stratification, cold stratification, or warm plus cold stratification, and (2) a time requirement for the embryo to elongate (Baskin and Baskin, 1998). The seeds of neither *L. maackii* nor *L. morrowii* germinated over a wide range of temperatures without a stratification treatment. Thus, about 50% of *L. maackii* and *L. morrowii* have MD only and about 50% have MPD.

GA_3 has been used in attempts to promote germination of seeds with MPD, and its effects vary with the type of MPD. For seeds of *Lonicera* species that required either warm plus cold stratification (*L. fragrantissima*) or cold stratification only (*L. japonica*) for germination, GA_3 was successfully substituted for cold stratification (Table 2). These results are similar to those reported by Baskin and Baskin (1984) for seeds of *Stylophorum diphyllum*, by Walck *et al.* (1999) for seeds of *Thalictrum mirabile*, and by Hidayati *et al.* (2000) for seeds of *Sambucus racemosa*. Moreover, GA_3 overcame dormancy in a portion of *L. maackii* seeds and of *L. morrowii* seeds that have MPD. This pattern was found in seeds of *Chaerophyllum tainturieri* (Baskin and Baskin, 1990) and in those of *Sambucus canadensis* and *S. pubens* (Hidayati *et al.*, 2000).

Eight types of MPD have been distinguished based on (1) temperatures required to break

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dormancy, (2) temperatures at the time of embryo growth, and (3) whether GA_3 overcomes dormancy (Baskin and Baskin, 1998). These types of MPD can be divided into two categories: (1) relatively high temperatures (≥15°C) are required for embryo growth in seeds with simple MPD, and (2) low temperatures (0–10°C) are required for growth in those with complex MPD. The MPD types can be subdivided further depending on the level of physiological dormancy: nondeep, intermediate, and deep. Since in seeds of *L. fragrantissima* (1) warm plus cold stratification was required to break dormancy, (2) embryos grew better at a warm- than at cold temperature, and (3) GA_3 overcame dormancy, they have deep simple MPD. In contrast, both cold stratification only and GA_3 overcame dormancy in seeds of *L. japonica* and *L. maackii*, and both warm stratification only and GA_3 overcame dormancy in seeds of *L. maackii* and *L. morrowii*. Further, embryos of *L. japonica*, *L. maackii* and *L. morrowii* grew better at warm than at cold stratification; thus, seeds of these three species have nondeep simple MPD.

Seeds of *L. fragrantissima* are dispersed in late May to early June and those of *L. morrowii* in June to August (Bailey, 1906; Wyman, 1969; Brinkman, 1974; Dirr, 1998). Thus, seeds of these two species are exposed to warm temperatures after they are dispersed. However, seeds of *L. japonica* are dispersed in late October to December and those of *L. maackii* in September to November (Bailey, 1906; Wyman, 1969; Brinkman, 1974; Dirr, 1998). Consequently, most seeds of *L. japonica* probably are not exposed to a period of warm temperatures between dispersal and germination, whereas those of *L. maackii* that are dispersed in September may be exposed to several weeks of warm temperatures before the onset of cold weather. Like seeds of *L. japonica*, those of *L. maackii* that are dispersed later in the seed dispersal season would be exposed to cold temperatures only before the spring germination season. The dispersal patterns of the four *Lonicera* species correspond well to their dormancy-breaking/germination patterns. *Lonicera fragrantissima* seeds require warm- plus cold stratification to break dormancy, *L. japonica* seeds need cold stratification only, *L. morrowii* seeds warm stratification only, and *L. maackii* seeds, either warm- or cold stratification only (Tables 1, 3, 4 and 5).

From an ecological perspective, seeds of *L. fragrantissima*, *L. japonica*, and about 50% of those of *L. maackii* are prevented from germinating in autumn because they are dormant. Physiological dormancy in these species is broken under natural temperatures between November and February, but the embryos do not grow until temperatures increase in late winter/early spring. Seeds of these three species sown in the greenhouse in June, November and October, respectively, germinated primarily in late February or March (Fig. 2). In contrast, seeds of *L. morrowii* can germinate in the autumn, after the embryos are exposed to warm temperatures, and they have enough time to grow following dispersal in the summer, before they are exposed to cold winter temperatures. More than 90% of the seeds of *L. morrowii* buried in the soil in late June 1998 had germinated when they were exhumed in November 1998. Peak germination for seeds of this species sown in June 1998 under *Quercus* leaf litter in the greenhouse was in October 1998 (Fig. 2), and a high percentage of the seedlings survived over winter (Hidayati, personal observations). In laboratory tests, seeds of *L. japonica*, *L. maackii* and *L. morrowii* germinated to higher percentages in the light than in darkness (seeds of *L. fragrantissima* were not tested in darkness). Further, the four *Lonicera* species can germinate to high percentages under leaf litter or while buried in soil. Thus, none of the four species appear to have the potential to form a persistent seed bank, i.e. to live until the second (spring) germination season.

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