Comparative Seed Germination Biology and Seed Propagation of Eight Intermountain Species of Indian Paintbrush

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Abstract: In chilling experiments with seeds of eight *Castilleja* species, seeds of all species were dormant at harvest and responded positively to moist chilling. Seeds of species from warmer, drier habitats had shorter chilling requirements, germinated more quickly during chilling, and were more likely to lose dormancy in dry storage than those of species from high elevation habitats with longer winters. Seed germination patterns within *Castilleja* were not affected by the hemiparasitic habit, but instead were similar to those of species of *Penstemon* from similar habitats. Seedlings of all eight species were readily produced in container culture, but outplanting success was greatly enhanced by potting the seedlings with a potential host plant for 6 to 8 weeks prior to transplanting outdoors.

Introduction

Indian paintbrush (*Castilleja*: Scrophulariaceae) species are among the most beautiful, well-known, and beloved Western native wildflowers, yet they are very seldom seen in cultivation. Published information on paintbrush propagation and cultural requirements is limited. In this investigation, we attempted to fill this information gap. Our study was carried out with two objectives in mind, namely to learn to propagate paintbrushes from seed as well as to understand something about their establishment ecology in the wild.

The chief obstacle to paintbrush cultivation is often thought to be their hemiparasitic nature. Paintbrushes are not true parasites, in the sense that they have chlorophyll and are capable of making their own food through photosynthesis. But most, if not all, paintbrushes apparently rely on a root connection with a host plant as a source of inorganic and organic nutrients as well as water, especially in more xeric habitats and during the dry season in mesic habitats (Press 1989). Because of this hemiparasitism, there seems to have been the assumption that paintbrush seeds would be difficult to germinate, perhaps requiring a signal from the host as in strict parasites such as *Striga* (Press and Graves 1995). Thus, reports of even the most straightforward chilling experiments with these species are lacking in the literature. In addition, it is not actually known which Intermountain species are hemiparasites, or whether the relationship with a host is an obligate one.

In this study, we use a comparative approach in exploring the relationship between habitat and paintbrush seed germination syndrome. This tactic has been successful for us in work with many Intermountain genera, including *Penstemon*, another large genus in the Scrophulariaceae (for example, Meyer and others 1995). The approach involves making multiple seed collections of related species, and populations within species, from as wide a range of habitats as possible, subjecting seeds to a standard array of laboratory germination tests, and relating their responses to habitat at the site of seed origin. This also provides valuable information for use in propagating these species from seed.

Materials and Methods

Fourteen seed collections representing eight species were made in the summers of 1997 (six species) and 1998 (*C. scabrida* and *C. miniata*; table 1). The collections were cleaned by screening and blowing, and seeds were stored in manila envelopes under laboratory conditions until the initiation of experiments 2 to 4 months later (depending on collection date).

The experiment had a completely randomized design and included six chilling treatments: 0, 4, 8, 12, 16, and 24 weeks of moist chilling at 2 °C in the dark. After chilling, dishes were transferred to a 10/20 °C alternating temperature regime (12 hours:12 hours), with cool white fluorescent light during the period at higher temperature. For each experimental treatment, there were four replications of 25 seeds per collection. Seeds were placed on the surface of two moistened germination blotters in plastic 10- by 100-mm petri dishes, and blotters were remoistened with water as necessary during the course of the experiment. Germinated seeds (that is, with radicles visible) were counted and removed periodically during chilling in the 24-week chilling treatment and weekly during the 4-week postchilling incubation period for all treatments. At the end of postchilling incubation, ungerminated seeds were scored

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Table 1—Collection information for 14 seed collections of *Castilleja* species included in study.

Species	Population	County	Habitat	Viability
C. exilis	Calf Creek	Garfield	Desert riparian	91.5
C. exilis	Utah Lake	Utah	Freshwater marsh	95.7
C. linariifolia	North of Boulder	Garfield	Pinyon-juniper woodland	90.0
C. linariifolia	North of Escalante	Garfield	Pinyon-juniper woodland	83.7
C. linariifolia	Boulder Mountain	Garfield	Mountain meadow	99.5
C. chromosa	North of Koosharem	Sevier	Sagebrush steppe	87.0
C. chromosa	Fishlake Pass	Wayne	Sagebrush steppe	92.7
C. chromosa	Santaquin Hill	Utah	Sagebrush steppe	86.7
C. scabrida	East of Zion	Kane	Pinyon-juniper woodland	88.0
C. flava	Soldier Summit	Utah	Mountain brush	96.5
C. miniata	Aspen Grove	Utah	Aspen parkland	87.0
C. applegatei	Bald Mountain Pass	Summit	Alpine meadow	96.0
C. rhexifolia	Albion Basin	Salt Lake	Subalpine meadow	95.8
C. rhexifolia	Mirror Lake	Duchesne	Alpine meadow	93.7

for viability using a cut test (that is, seeds with firm, white embryos were scored as viable). Mean germination percentages for each collection-treatment combination were calculated on the basis of total viable seeds. Viability was generally high (table 1; average 92 percent). Six of the collections included in this study were also part of a later study (Mogensen and others 2001) on the effects of chilling temperature on dormancy loss for species of *Castilleja*, *Penstemon*, and several other genera. These experiments were initiated in April 2000, approximately 3 years after harvest for the 1997 paintbrush collections and 2 years after harvest for the 1998 collections. To examine whether paintbrush seeds lose dormancy in dry storage, we compared our data for recently harvested seeds with the data in Mogensen and others (2001) for seeds laboratory stored for 2 to 3 years. To make the chilling temperatures comparable to our 2 °C chilling data, we averaged the data for the 1 and 3 °C chilling temperatures.

We analyzed the data for each seed collection in the chilling experiment with recently harvested seeds using one-way fixed effects analysis of variance (ANOVA), with chilling duration as the independent variable and germination percentage (proportion) as the dependent variable. Germination proportion was based on the number of viable seeds in each replicate, and was arcsine transformed to improve homogeneity of variance prior to analysis.

Results

Seeds of all eight species of Indian paintbrush were essentially completely dormant without chilling (table 2). Species and populations within species varied greatly in their response, but seeds of all species responded to chilling to at least some degree. In general, populations from warmer, low-elevation sites had shorter chilling requirements and more rapid germination during chilling than populations and species from higher, colder sites. But there were distinctive differences among species superimposed over this general pattern.

Table 2—Mean germination percentages (expressed as percentage of viable seeds) after chilling at 2 °C for 0 to 24 weeks followed by incubation at 10/20 °C for 4 weeks for 14 Utah seed collections belonging to eight Intermountain species of *Castilleja*.

		Chilling duration (weeks)[a]					
Species	Population	0	4	8	12	16	24
C. exilis	Calf Creek	0b	100a	98.8a	99.0a	100a	98.9a
C. exilis	Utah Lake	0b	99.0a	100a	100a	99.0a	100a
C. linariifolia	North of Boulder	3.0b	100a	100a	100a	100a	99.0a
C. linariifolia	North of Escalante	0c	90.6b	93.8b	98.7a	100a	100a
C. linariifolia	Boulder Mountain	0d	17.1c	22.6c	74.3b	100a	100a
C. chromosa	West of Koosharem	0d	6.5c	51.5b	88.3a	81.8a	81.0a
C. chromosa	Fishlake Pass	0c	5.9c	48.7b	83.0a	87.8a	84.9a
C. chromosa	Santaquin Hill	0c	1.3c	32.5b	72.7a	41.0b	66.3a
C. scabrida	East of Zion	6.7d	72.1ab	51.0c	57.2bc	56.6bc	84.1a
C. flava	Soldier Summit	0d	5.7cd	10.3c	56.3b	66.8b	85.9a
C. miniata	Aspen Grove	0b	14.7a	10.3a	11.3a	4.7ab	13.7a
C. applegatei	Bald Mountain Pass	0b	1.1b	5.3b	15.0a	14.3a	18.7a
C. rhexifolia	Albion Basin	0c	3.2c	11.7b	12.4b	10.0b	20.6a
C. rhexifolia	Mirror Lake	0a	0a	1.0a	2.2a	1.0a	6.7a

[a]Within a collection (row), means followed by the same letter are not significantly different according to a means separation test using the Student-Newman Keuls criterion following analysis of variance.

Castilleja exilis

This scarlet-flowered annual species of wetland habitats had a short chilling requirement, with full germination of seeds in postchilling incubation for both populations after only 4 weeks of chilling (table 2). There was no evidence for mechanisms, such as the presence of a chilling-nonresponsive fraction, that would promote seedbank carryover across years. The seeds of this species were very slow to germinate in chilling, however, particularly those of the northern Utah Lake lot (fig. 1). The Utah Lake seeds did not begin to germinate in chilling until 21 weeks, and germinated to less than 20 percent during chilling, while the southern Calf Creek lot began germinating in the cold at 18 weeks and reached about 80 percent by the end of chilling. The chilling periods required for any germination in the cold are much longer than those likely to be encountered by these seeds in their habitat of origin. The remaining seeds of both lots germinated rapidly and completely after transfer to the postchilling incubation regime. Germination during chilling may offer little advantage to this species, which grows in habitats where water is not limited during the growing season.

Castilleja linariifolia

C. linariifolia is a common scarlet-flowered summer species in sagebrush steppe, pinyon-juniper, mountain brush, and lower montane communities. The seeds from the three populations included in the study showed marked differences in germination response to chilling when recently harvested, and these differences were habitat related. The higher elevation Boulder Mountain population showed a pattern of incremental increase in germination percentage as a function of chilling duration up to 16 weeks, while the populations from pinyon-juniper woodland were largely or completely nondormant after only 4 weeks of chilling (table 2). The high-elevation collection also germinated much more slowly in the cold (fig. 1). All three lots germinated completely in the longer chilling regimes, and there was no evidence of any mechanism for seedbank carryover.

When the seeds of the high-elevation *C. linariifolia* collection were subjected to chilling 3 years after harvest, they showed a dramatic change in chilling response (fig. 2). Instead of the incremental increase in germination with chilling duration that was evident for recently harvested seeds, the 3-year-old seeds germinated to 100 percent after only 4 weeks of chilling, a response similar to that seen in recently harvested seeds of lower elevation collections. This change shows that seeds of this species of *Castilleja* can lose dormancy under dry conditions, a process known as dry after-ripening.

Castilleja chromosa

C. chromosa is the common scarlet-flowered spring paintbrush species of warm and cool desert shrublands, sagebrush steppe, and pinyon-juniper woodland in the Intermountain West. The seeds of all three collections of *C. chromosa* in this study were from sagebrush steppe habitats, and showed similar responses to chilling, although

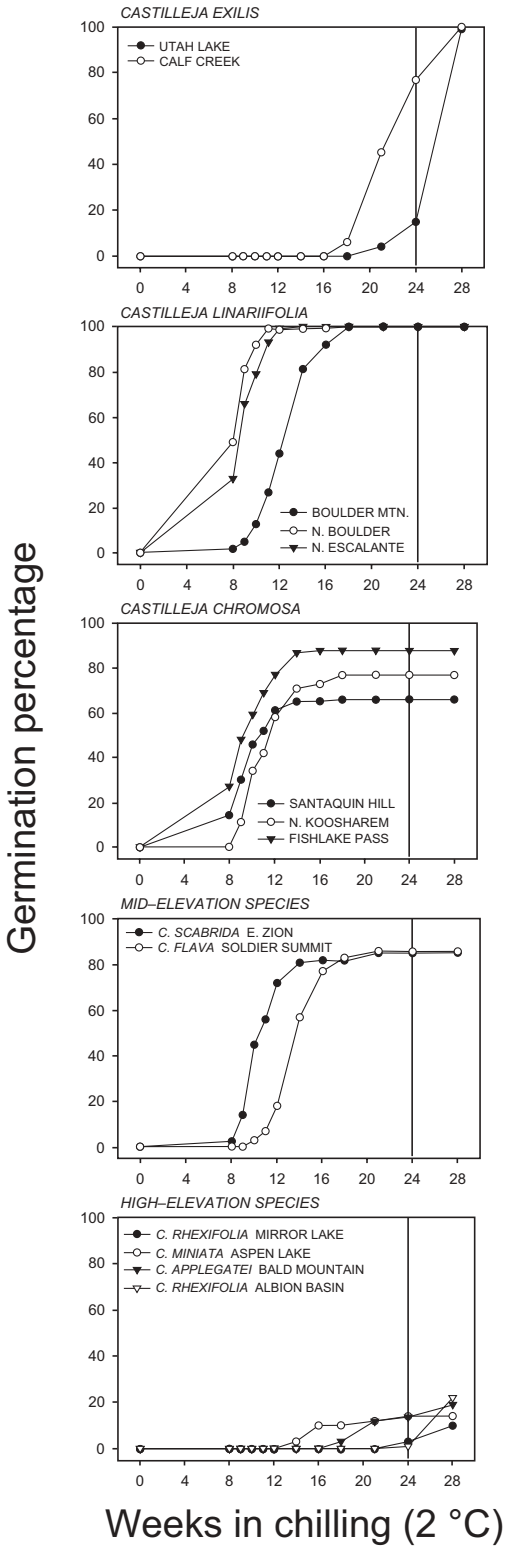

Figure 1—Time courses for germination during 24 weeks of chilling at 2 °C followed by 4 weeks of incubation at 10/20 °C for 14 *Castilleja* seed collections included in the study. Germination percentages are expressed as percentage of viable seeds. The vertical bars represent the point in time (24 weeks) when the seeds were transferred from chilling to postchilling incubation.

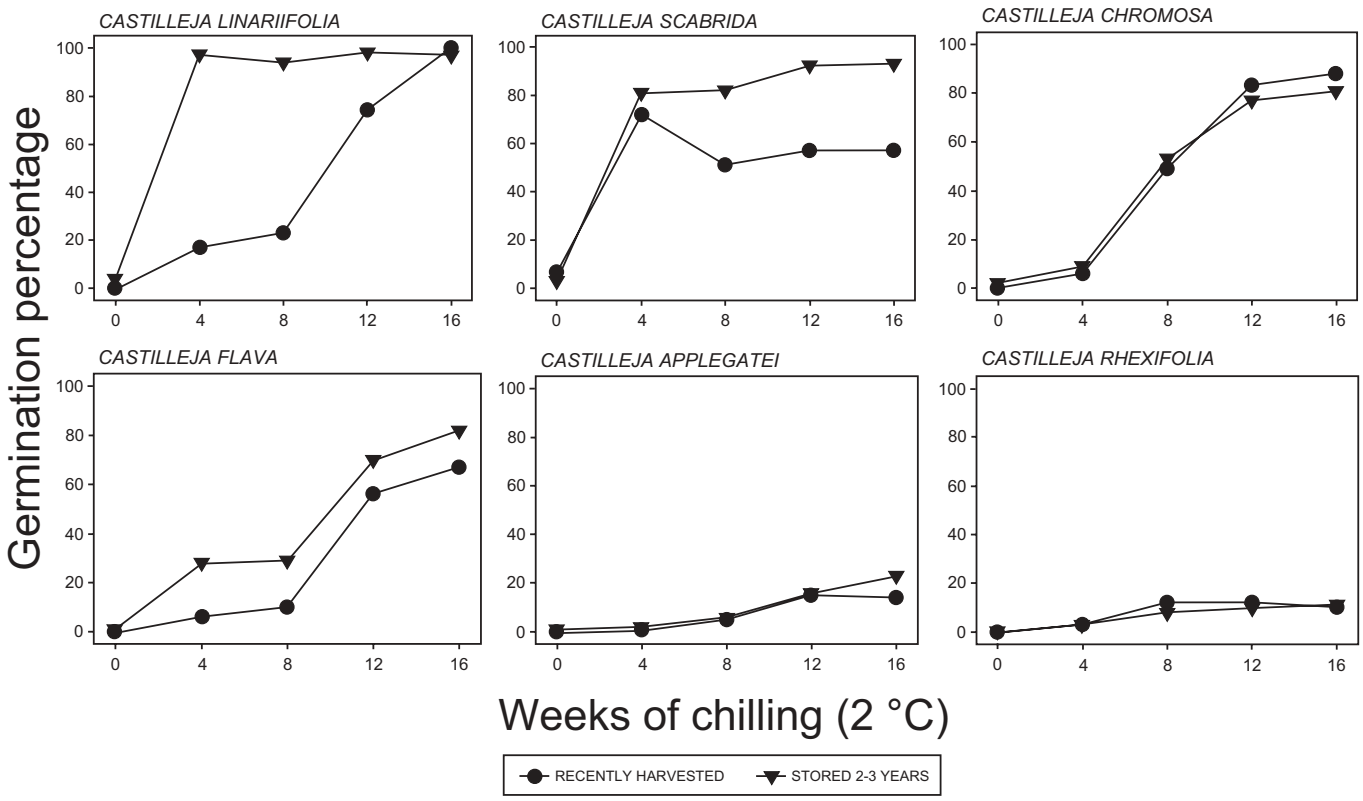

Figure 2—Changes in chilling responsiveness for six *Castilleja* seedlots during 2 to 3 years of laboratory storage. Data for recently harvested seeds are those presented for these lots in table 1, while data for seeds stored 2 to 3 years are from Mogensen and others (2001).

the more northern Santaquin Hill population was somewhat more dormant overall (table 2). The first substantial increase in germination took place in response to 8 weeks of chilling. The germinable fraction for the two southern populations increased with chilling duration up to 12 weeks, then leveled off between 80 and 90 percent. For the northern Santaquin Hill population, there was actually a significant drop in germination percentage between chilling periods of 12 weeks and 16 weeks. The remaining chilling nonresponsive seeds would have the potential to carry over across years as a persistent seedbank. Most of the seeds that became nondormant in chilling germinated in the cold soon after dormancy loss during the period from 8 to 12 weeks (fig. 1). This would place germination in the field in late winter in the sagebrush steppe habitats where these seeds originated.

Seeds from the Fishlake population tested after an additional 3 years in laboratory storage were largely unchanged in their chilling response, suggesting that dry after-ripening plays little role in dormancy regulation, at least for this population (fig. 2).

Castilleja scabrida

Seeds of this scarlet-flowered Colorado Plateau rock crevice specialist were less dormant than those of the closely-related species *C. chromosa*, germinating to over 70 percent after only 4 weeks of chilling (table 2). They showed decreased germination percentages at intermediate chilling periods of 8 to 16 weeks but germinated to a relatively high percentage after 24 weeks of chilling. Chilling durations in *C. scabrida* habitat in the field would never approach 24 weeks, so the information from shorter chilling durations is probably more ecologically relevant. The presence of a large chilling nonresponsive fraction under these intermediate chilling regimes is evidence for a mechanism for seedbank carryover. Germination response under continuous chilling was similar to that for *C. chromosa*, with most of the germination taking place between 8 and 12 weeks.

In contrast to those of *C. chromosa*, the seeds of *C. scabrida* definitely afterripened during dry storage for 2 years (fig. 2). The response to shorter chilling durations was similar for recently harvested and 2-year-old seeds, but the response to intermediate chilling durations was greatly increased for 2-year-old seeds.

Castilleja flava

Seeds of this golden-flowered mid-elevation mountain brush species showed incremental increases in germination percentage as a function of chilling duration up to 24 weeks, with 86 percent germination in response to 24 weeks of chilling (table 2). The fact that not all the seeds germinated even after this prolonged chilling treatment provides some evidence for seedbank carryover. This species showed an

intermediate germination rate in chilling, with most of the seeds germinating between 12 and 16 weeks, soon after dormancy loss (fig. 1).

When the seeds of *C. flava* were retested after 3 years of laboratory storage, they showed evidence of after-ripening in dry storage, with approximately a 20 percent increase in germination percentage over chilling durations from 4 to 16 weeks (fig. 2).

Castilleja miniata

C. miniata is the common crimson-flowered mid-montane species of paintbrush throughout the southern Intermountain region and is found in aspen parkland, montane coniferous forest, and mountain meadow communities. The seeds of the *C. miniata* seedlot in our experiment were highly dormant, with a maximum germination of 15 percent and no incremental increase in response to increasing chilling duration (table 2). It was also relatively slow to germinate in chilling, with most of the seeds germinating between 14 and 16 weeks (fig. 1).

Castilleja applegatei

C. applegatei is a scarlet-flowered species found in rocky subalpine and alpine meadows. In our experiment, its seeds showed an incremental increase in germination percentage with increasing chilling duration, but the maximum germination was less than 20 percent (table 2). Most of the germination in the 24-week chilling treatment took place in the cold between 18 and 21 weeks (fig. 1). When this seedlot was retested 3 years later, its chilling response was largely unchanged (fig. 2).

Castilleja rhexifolia

C. rhexifolia is a pale yellow to crimson or purple-flowered species that is closely related to *C. miniata* and is found in similar habitats, but is more northerly in its distribution and ranges up into subalpine and even alpine communities. The two populations in our study were both from the upper elevational limits for the species and had highly dormant seeds. The Albion Basin collection germinated to a maximum of 20 percent, while the Mirror Lake population had a maximum germination of 7 percent (table 2). Both seedlots tended to show incremental increases in germination percentage with increasing chilling, but this increase was significant only for the Albion Basin collection. Neither collection germinated during chilling; the germination that did occur took place during postchilling incubation (fig. 1). These results, coupled with the fact that snow cover commonly lasts 8 to 9 months in high mountain habitats, suggest that our longest chilling duration was not long enough to obtain a maximum chilling response. This statement is also true for the two preceding species (*C. miniata* and *C. applegatei*). Longer chilling durations could be effective for these species.

Retesting the Albion Basin seed collection after 3 years of storage gave a chilling response essentially identical to the one for recently harvested seeds (fig. 2). It is therefore unlikely that dry after-ripening plays much of a role in dormancy regulation for this species.

Discussion

Comparative Germination Ecology

It is clear from our experiments that seed germination regulation in Intermountain species of *Castilleja* is not particularly impacted by the potentially hemiparasitic nature of these plants. The seeds are relatively easy to germinate, responding to moist chilling in a manner essentially similar to that of many other species of the Intermountain West. In fact, there are a great many parallels between the results reported here for *Castilleja* and those we have previously published for the related, nonhemiparasitic genus *Penstemon* (for example, Meyer and others 1995). The generally obligate requirement for some chilling, even in after-ripened seeds, is related to the fact that species of both genera have spring-emerging seedlings.

Variation in laboratory chilling responses can be tied to mechanisms for timing germination to occur appropriately in early spring in different habitats. If germination is delayed too long, the seedbed may dry out before the seedlings have a chance to become established, whereas if germination occurs too early, long before snowmelt, the newly germinated seedlings are at risk from pathogens that operate at low temperatures. This is why seeds of populations from warmer, drier, low-elevation habitats tend to respond to shorter chilling periods and to germinate more quickly in chilling than seeds of populations from high-elevation habitats with long winters.

These habitat-correlated differences are seen both within species with wide ecological amplitude, such as *C. linariifolia* in this study, and between species. For example, the three high-elevation species in this study had very long chilling requirements, so that even a 6-month chilling period resulted in relatively low germination percentages. This is not surprising given that chilling conditions under snow cover often last much longer than 6 months in these habitats. In contrast, species and populations from southern pinyon-juniper woodland, where long periods of chilling conditions are unlikely, respond to chilling periods as short as 4 weeks. Mid-elevation sagebrush steppe and mountain brush species and populations have intermediate chilling requirements that correspond to the chilling periods likely to be encountered in those habitats.

Most of the species of paintbrush in our study did not germinate fully even after 24 weeks of chilling. Seedlots of several of the mid-elevation species contained a sizeable chilling nonresponsive fraction that would not germinate even after prolonged chilling, in spite of the fact that the chilling responsive fraction became nondormant after much shorter chilling periods. The function of such a chilling nonresponsive fraction is probably to permit carryover of seeds across years, even in years when winter conditions result in longer than average periods of snow cover.

Although most are perennial, many paintbrush species have populations that turn over fairly rapidly, especially during cycles of drought. There are often scenarios, particularly at lower elevations, where establishment from a persistent seedbank is necessary for population survival. The chilling nonresponsive fraction makes this possible. The mechanism that renders these seeds chilling responsive in later years may be related to the dry after-ripening we

observed in some of these seedlots. We also have some preliminary evidence that drying after inadequate chilling may render the seeds more responsive to chilling the following winter.

The summer annual wetland species *C. exilis* had a unique germination response pattern in our study. It had an obligate requirement for chilling that would function to prevent autumn emergence, but its chilling requirement was short, only 4 weeks or less. Once the seeds were rendered nondormant in chilling, they remained ungerminated even after several months in the cold. This is in contrast to the other species with relatively nondormant seeds, whose seeds germinated in chilling soon after they lost dormancy. *C. exilis* is a plant of relatively warm low-elevation habitats where the length of field chilling would be short, thus necessitating a short chilling requirement. But unlike low-elevation upland habitats, the wetland habitat does not pose the problem of a seedbed that dries rapidly in spring. There would therefore be no advantage to germinating during chilling for a wetland species, so it is not unexpected that nondormant *C. exilis* seeds do not germinate in the cold.

Seed Propagation

The first steps of seed propagation for species of *Castilleja* are essentially similar to those for any native species whose seeds require moist chilling. The individual seeds within a lot often have different chilling requirements, and because the seeds tend to germinate in chilling soon after losing dormancy, it is difficult to obtain uniform emergence when ungerminated chilled seeds are planted. By the time most of the seeds are nondormant, a large fraction has already germinated, and germinated seeds that remain in the cold tend to become stringy and difficult to plant successfully. On the other hand, if the seeds are planted as soon as the first few begin to germinate, most of the seeds are still dormant and poor stands are obtained. We can solve this problem on a small scale by planting the seeds as they germinate in the cold, while the radicles are still very short, and returning the remaining seeds to chilling. This results in seedlings of staggered age, which could be a problem in commercial production. If seeds are not in short supply, it may be possible to chill enough so that large batches of seedlings of each age can be obtained. The germinants are planted carefully in shallow depressions and covered with a thin (2 to 4 mm) layer of soil. Paintbrush seeds and germinants are very small, so it is important not to plant them too deeply. Poor emergence of newly germinated seedlings usually results either from planting too deeply or from allowing the surface to dry out during emergence. The germinants should be planted into well-watered potting medium, and the surface should be misted daily, especially if conditions are sunny, until emergence is complete. At this point it is best to water less often but more thoroughly, making sure that the soil is wetted to its full depth each time. Young paintbrush plants in containers are not as drought hardy as those of most native species, and it is important to pay careful attention to the watering. Paintbrushes, perhaps because of their hemiparasitic nature, tend to have relatively little root biomass in relation to their shoot biomass. Plants that have wilted from lack of water sometimes recover by resprouting from the base, but the resulting plants are not as sturdy as plants that have been watered consistently.

We prefer a relatively fast-draining potting medium for *Castilleja* propagation. Planting containers that produce a long, narrow root mass, such as root trainers (Spencer-Lemaire, Edmonton, Alberta) or Ray Leach Conetainers (Steuwe and Sons, Corvallis, OR), work best for paintbrushes, as they do for most other native herbaceous perennials. The plants can be grown in these containers for 3 to 4 months, depending on growing conditions and container volume. At this point, when other plants would normally be hardened for outplanting or potted up individually into larger containers, our propagation procedure deviates from the usual pattern.

While we do not have quantitative data to substantiate our recommendations, we have found through experience that small paintbrush stock planted out on its own usually exhibits poor survival, even when planted early in the spring and even in a garden setting. We have much higher success rates if we pot the paintbrush seedling up with a potential host plant and let the two plants grow together for 6 to 8 weeks, then harden them off and outplant them as a unit. Paintbrush species are not host specific, and we have had success using shrubs, grasses, and other perennial herbs as host plants. We particularly like aesthetically pleasing and ecologically appropriate combinations, such as planting *C. chromosa* with various species of sagebrush, the plants with which it is most often associated in the wild. Paintbrushes planted out with big sagebrush often live for many years in gardens and freely self-seed, so that as the shrub begins to overgrow the paintbrush, more small paintbrushes are produced around its periphery.

In a naturalized garden or restoration project, most species of paintbrush are easily obtained through direct seeding as long as the seed source used is adapted to the planting site. If sufficient seed is available, this is clearly the simplest and least labor-intensive way to establish these plants. Obviously, autumn planting will be necessary in order to ensure that the seeds receive the chilling they require in order to germinate and establish the following spring.

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