

Seed characteristics of the invasive alien vine *Vincetoxicum rossicum* are affected by site, harvest date, and storage duration

Antonio DiTommaso, Daniel C. Brainard, and Bradley R. Webster

Abstract: The alien vine *Vincetoxicum rossicum* (Kleopow) Barbar. (Asclepiadaceae) is a major concern in natural areas of the Lower Great Lakes Basin. Reproduction in this perennial occurs largely by polyembryonic seeds that mature from August to November. Many seeds produced in autumn germinate the following spring, but the germinability of seeds produced in late summer is unknown. The influence of parent plant light environment on seed characteristics in this species is also poorly understood. We examined the characteristics of *V. rossicum* seeds harvested in late August and early November 2002 from three habitats in central New York State, USA, differing in light availability. Site had a significant effect on seed weight and germination percentage, but not on the number of embryos germinating per seed or radicle length. Seeds produced in the shaded habitat in August weighed significantly more and had lower germinability than seeds produced in an adjacent open habitat, but this was not observed in a second open habitat. The probability of germination decreased with seed size for seeds ranging from 2 to 8 mg. For the open sites, fresh seeds produced in August had approximately double the germination percentages of those produced in November. However, for all sites, germination following 18 weeks of cold storage was greatest for seeds harvested in November. The probability of multiple embryos germinating was influenced by seed weight. The nature of this relationship varied by site, but not by harvest date or storage duration. Larger seed size resulted in longer total radicle length after 7 d, regardless of site.

Key words: *Cynanchum rossicum*, maternal environment, pale swallowwort, polyembryony, resource allocation, seed dormancy.

Résumé : La vigne adventice *Vincetoxicum rossicum* (Kleopow) Barbar. (Asclepiadaceae) constitue un problème majeur dans les aires naturelles de la région inférieure du bassin des Grands Lacs. La reproduction de cette espèce pérenne se fait à partir de graines poly-embryonnaires, qui mûrissent d'août à novembre. Plusieurs graines produites à l'automne germent le printemps suivant, mais le pouvoir germinatif des graines produites à la fin de l'automne est inconnu. On comprend peu l'influence de l'environnement lumineux reçu par les parents sur les caractéristiques des graines de cette espèce. Les auteurs ont examiné les caractéristiques des graines du *V. rossicum* récoltées à la fin d'août et début de novembre 2002, à partir de trois habitats au centre de l'État de New York, aux É.-U., qui diffèrent par la disponibilité de la lumière. Les sites ont un effet significatif sur le poids des graines et le pourcentage de germination, mais pas sur le nombre d'embryons des graines germées ou la longueur des racines. Les graines produites en août dans un habitat ombragé pèsent significativement plus et ont un pouvoir germinatif moindre que les graines produites dans un habitat adjacent ouvert, mais ceci n'a pas été observé dans un second habitat. La probabilité de germination diminue avec la grosseur des graines, allant de 2 à 8 mg. Pour les sites ouverts, les graines fraîches produites en août ont un pouvoir germinatif du double de celui des graines produites en novembre. Cependant, pour tous les sites, la germination suite à une conservation au froid pendant 18 semaines montre des pourcentages de germination qui doublent ceux des graines produites en novembre. La probabilité de germination d'embryons multiples est influencée par le poids de la graine. La nature de cette relation varie selon le site, mais pas selon la date de récolte ou la durée de la conservation. Les graines les plus grosses produisent les racines les plus longues après 7 jours, indépendamment du site.

Mots clés : *Cynanchum rossicum*, environnement maternel, cynanche pâle, polyembryonie, allocation des ressources, dormance des graines.

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Introduction

It is well recognized that invasive alien plant species pose a serious threat to the stability and diversity of natural ecosystems in North America (Pimentel et al. 2000; Pimentel 2002; Wilcove et al. 2000). Research on invasive species has focused on determining either specific plant attributes that make a given plant species invasive or the characteristics of habitats that make them susceptible to invasion, although the role of each approach in predicting biological invasions remains unclear (Goodwin et al. 1999; Daehler and Carino 2000; Davis et al. 2000; Mack et al. 2000; Lockwood et al. 2001; Heger and Trepl 2003). Nonetheless, obtaining reliable knowledge on the biology of invasive species in their introduced habitat continues to be an essential first step towards a more in-depth understanding of the potential threat posed by alien plants in their new environment (Rejmánek and Richardson 1996; Goodwin et al. 1999).

Maternal growing environment and timing of seed maturation are two factors that can have a significant effect on the number, weight, and germinability of seeds produced in many plant species (Baskin and Baskin 1998; Fenner 1991; Gutterman 2000). Light is one resource that affects several important seed characteristics in plants (Baskin and Baskin 1998). For example, reductions in solar radiation during maturation on the mother plant reduce seed dormancy in *Abutilon theophrasti* (Bello et al. 1995) and *Datura ferox* (Sanchez et al. 1981). In contrast, reductions in the ratio of red to far-red light due to canopy shade during seed maturation are associated with increased seed dormancy in *Amaranthus powellii* (Brainard et al. 2005) and *Piper auritum* (Orozco-Segovia et al. 1993). Reductions in light availability during seed maturation can result in either reductions in seed size (e.g., Willson and Price 1980) or increases in seed size (e.g., Agren 1989). Many invasive plant species are capable of colonizing and growing under different light environments and thus are likely not to produce seeds of equal size or having similar germinabilities in all sites. This, in turn, will likely influence the persistence of seed banks and early competitive hierarchies (Harper 1977). Moreover, the development and maturation of seeds on parent plants earlier in the growing season when resources such as light and nutrients are typically more abundant may result in seeds having different size and germination characteristics than seeds developing and maturing later in the growing season (Cavers and Steel 1984; Kane and Cavers 1992; Nurse and DiTommaso 2005).

The invasive alien vine *Vincetoxicum rossicum* (Kleopow) Barbar. (syn. *Cynanchum rossicum*) (Asclepiadaceae) (pale swallowwort) has been a major concern in natural areas of the lower Great Lakes Basin of North America for several decades (DiTommaso et al. 2005; Moore 1959; Pringle 1973; Sheeley and Raynal 1996). This perennial plant was introduced into North America from Ukraine and Russia in the late 1800s (Sheeley and Raynal 1996) and now threatens several unique or rare ecosystems in both Canada and the United States (Bonnano 1999; Ontario Invasive Plants Working Group 2000). Its displacement of native alvar vegetation in New York's Great Lakes Basin threatens 54 rare species of plants, insects, birds, and land snails (Bonnano 1999). Recent work has also focused on the possible deleterious effects of this species on reproduction in the Monarch butterfly (*Danaus plexippus*) (DiTommaso and Losey 2003; Mattila and Otis 2003), on richness and diversity of arthropod communities (Ernst and Cappuccino 2005), and on composition of arbuscular mycorrhizal fungal communities (Greibsson and DiTommaso 2002).

Vincetoxicum rossicum can form extensive monospecific populations in many upland habitats, easily adapting to a wide range of light and moisture conditions, from full sun in open sites to full shade in mature forest understories (Sheeley 1992). It typically requires calcareous soils for optimal growth, and many areas infested with this plant have a history of disturbance. Reproduction occurs primarily by the production of wind-dispersed comose seeds borne in follicles in late summer and autumn. Interestingly, seeds of this species are often polyembryonic, giving rise to two or more genetically identical seedlings (DiTommaso et al. 2005; Cappuccino et al. 2002; Sheeley 1992).

Sheeley (1992) found that seeds of this species collected in early- to mid-October from a central New York open-field site and stored at 4.5 °C for 5 weeks in the dark had relatively high germination percentages under either light (46%) or dark (36%) conditions in a controlled environment chamber when subjected for 7 weeks to fluctuating temperatures of 13–21 °C. In contrast, only 22% of seeds germinated when cold stratified for 28 weeks and placed in a greenhouse in mid-May under natural photoperiod and a temperature range of 25–31 °C. Of seeds germinating in the greenhouse trial, 78% were polyembryonic. Similarly, Cappuccino et al. (2002) reported high germination rates (45%) of freshly collected seeds in early November near Ottawa, Ontario. Seeds collected in mid-October and stored at 4 °C for 12 weeks also had high (45%) germination and polyembryony (55%) levels when grown for about 7 weeks in a greenhouse at a 14 h light : 10 h dark regime (Cappuccino et al. 2002).

Despite these few studies investigating the seed germination characteristics of *V. rossicum*, no research to date has determined the germinability and degree of polyembryony of seeds produced at different times during the growing season or in different light environments (i.e., open versus shaded habitats). These investigations are especially relevant given that seeds of *V. rossicum* can mature and be dispersed from late August to early November in the lower Great Lakes Basin region of North America (Cappuccino et al. 2002; DiTommaso et al. 2005) and that this species can colonize and invade both sunny open sites (e.g., old fields) as well as heavily shaded understories of mature temperate forests (DiTommaso et al. 2005; Lawlor 2000; Sheeley 1992). We also lack information on the effects of cold storage on the germinability of seeds collected at different periods and in open versus shaded habitats. Finally, the vigour of *V. rossicum* seedlings produced from seeds collected at different times during the growing season or from habitats differing in light availability is also not known. Early seedling vigour is expected to have a considerable impact on the ability of plants to successfully compete for essential resources and to eventually reproduce (Cicidiyan and Malloch 1982; Hendrix et al. 1991).

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field and a forest understory; (2) seed harvest date; and (3) seed cold storage duration (0 and 18 weeks) on seed weight, germinability, degree of polyembryony, and seedling vigour in *V. rossicum* from central New York State, USA. An additional goal was to test the relationship between the weight, polyembryonic status, and dormancy of seeds.

Materials and methods

Seed collection and study sites

Mature seeds of *V. rossicum* were collected on 29 August and 4 November 2002 from three invaded sites in central New York State, USA. For each of the harvest dates, at least 500 seeds were collected from recently opened follicles of 25–30 randomly selected plants at each site. Two of the sites were located within the Great Gully Nature Preserve in Cayuga County, New York, USA (42°48'N, 76°40'W). The first site, Great Gully forest, was a mature mixed deciduous forest (25 ha) comprised primarily of *Acer saccharum*, *Quercus rubra*, *Carya cordiformis*, *Tsuga canadensis*, *Pinus strobus*, and *Fraxinus americana*. The forest understory vegetation was dominated by *V. rossicum* (117 shoots·m⁻²) (Table 2). Plants for this study were collected from a 0.25-ha sample area within this site. The second site, Great Gully old field, was a 0.2-ha section of old field located approximately 20 m from the Great Gully forest edge in the northeastern section of the preserve. This section of old field had not been mowed for at least 5 years and was dominated by herbaceous grasses and herbs including *Poa* spp., *Festuca* spp., *Rubus* spp., *Cirsium arvense*, and *Solidago* spp. Plants of *V. rossicum* were present at low densities (15 shoots·m⁻²) in the old field and were most common near the forest edge. Anecdotal information from landowners and personal observations of the senior author suggests that *V. rossicum* plants were spreading from the edge of the forest towards both the understory and the old field. The third site, Aurora old field, was located at the Robert Musgrave Research Farm of Cornell University in Aurora, Cayuga County, New York (42°73' N, 76°65'W) about 10 km from the Great Gully forest and old-field sites. This 0.2-ha open site was located near a pond embankment and included high density stands of *V. rossicum* (130 shoots·m⁻²). The site had not been mowed for at least 8 years. Other abundant plant species at this site included *Dactylis glomerata*, *Poa* spp., *Arctium minus*, *Pastinaca sativa*, and *Phalaris arundinacea*.

Soil characteristics and *V. rossicum* population attributes for each of the three sample sites are shown in Table 2. Soil and vegetation data for the sites were collected in early May and 20 June 2002, respectively. Soil samples were collected using a standard 2.5-cm-diameter soil corer to a depth of 15 cm and were submitted to the Cornell Nutrient Analysis Laboratory for chemical analysis. Most of the *V. rossicum* root system is located within this 15-cm depth (A. Di-Tommaso, personal observation). Available concentrations of phosphorus and potassium were determined using the Morgan extractable method with sodium acetate buffered at a pH of 4.8 (Morgan 1941). At each site, the shoot density and a visual estimate of percentage cover of *V. rossicum* were recorded in five randomly placed 0.25-m² (50 cm × 50 cm) quadrats. Mean height and the proportion of shoots in flower

were estimated from 20 randomly selected shoots in each plot.

Germination testing

Seeds were initially screened to remove any obviously nonviable seeds (i.e., having no embryo). Seeds were tested for germination either immediately after harvest, or following 18 weeks of dry storage at 4 °C in the dark. At each testing date, either 90 seeds (freshly collected seeds from 29 August) or 100 seeds (4 November) were randomly subsampled, individually weighed, and randomly divided into either three replicates of 30 seeds (29 August testing) or five replicates of 20 seeds (4 November testing). Seeds were then sandwiched between two sheets of 25 cm × 37.5 cm (No. 38) Anchor® (Anchor Paper, St. Paul, Minnesota) germination paper (20 or 30 seeds per sheet) moistened with distilled water. Each set of seeds was subsequently rolled into 3-cm-diameter cylinders and placed in a 1000-mL beaker. To maintain moisture on the germination paper, the beaker was filled with 2.5 cm of distilled water, and covered with aluminum foil. Beakers were placed in a growth chamber set at 25 °C day : 17 °C night temperatures, a 14-h photoperiod, and photosynthetic photon flux density (PPFD) of 100 μmol·m⁻²·s⁻¹. The same growth chamber was used at each testing date. Seeds were examined daily for germination for 4 weeks, and the number of germinating embryos (radicle at least 2 mm in length) was recorded. Following germination of the first embryo, seeds were monitored for 7 d. On day 7, the length of all radicles from each seed was recorded.

Statistical analyses

Soil and *V. rossicum* data were compared between sites by one-way ANOVA followed by a Student–Newman–Keuls (SNK) multiple range test at the $p < 0.05$ level of significance (SAS Institute Inc. 2001). Proportions were arcsine square root transformed to stabilize variance.

The effect of site, harvest date, and storage time on seed weight, the number of germinated embryos per seed, and the percentage of seeds with at least one germinated embryo were analyzed using the general linear model procedures of SAS (SAS Institute Inc. 2001). Germination percentage data were arcsine square root transformed, and the number of germinated embryos was log-transformed prior to statistical analysis to improve assumptions of normality and equal variance of population distributions. The relationship between individual seed weight and the probability of germination was estimated using Logistic regression procedures in SAS (Proc Logistic). Predictor variables in the logistic model included seed weight and seed weight squared and dummy variables for site, harvest date, and storage time class variables. The probability of more than one embryo germinating (versus only one embryo germinating) was tested with a nonquadratic logistic regression using the same set of predictors. Linear regression (PROC GLM) was used to estimate the effect of seed weight on both the number of days to germination, and the total seedling length (sum of lengths of all germinating embryos) 7 d after germination of the first embryo with dummy variables for site, harvest date, and storage time. In all regression analyses, nonsignificant

Table 1. Mean temperature and total precipitation (and 30-year averages) for the months of April–November 2002 at Aurora, N.Y.; also shown are average temperatures and total precipitation for the entire 8-month period.

Month	Temperature (°C)		Precipitation (mm)	
	2002	30-year average	2002	30-year average
April	8.9	7.4	83.8	83.3
May	11.5	14.2	126.0	80.5
June	19.3	19.3	115.1	103.9
July	22.3	21.8	20.6	84.1
August	22.0	20.9	38.6	91.7
September	18.9	16.7	125.0	106.9
October	9.6	10.5	79.0	81.3
November	4.0	4.7	73.7	85.3
Average	14.6	14.4	661.8	717.0

higher order interactions were removed following a backwards step-wise procedure.

Results

Climate and site data

Mean temperatures during the April–November period in 2002 (14.6 °C) were similar to the 30-year average temperatures (14.4 °C) (Table 1). However, total precipitation in 2002 (661.8 mm) was about 8% below the 30-year average (717 mm) for these months. The below-average rainfall in 2002 was due largely to the exceptionally dry conditions experienced in July and August (i.e., precipitation levels were only 24% and 42% of long-term averages, respectively).

Soils at the Aurora old-field site had a greater ($p < 0.05$) pH and organic matter content than the Great Gully forest and old-field sites (Table 2). Concentrations of plant available phosphorus and potassium were greater at the two old-field sites than the forest site.

Shoot density and percentage cover of *V. rossicum* plants were substantially greater at the Great Gully forest and Aurora old-field sites than the Great Gully old-field site (Table 2). However, plants in the old-field sites were taller, and a greater proportion of them were in flower compared with plants in the forest site.

Site effects

Site had a significant effect on seed weight and seed germination percentage, but not on the number of embryos germinating or radicle length (Table 3). Seeds collected on 29 August from the Great Gully forest site were significantly heavier (Fig. 1A) and had significantly lower germination percentage (Figs. 1B and 1C) than those from the neighbouring Great Gully old-field site. However, the weight and germination percentage of seeds harvested on 4 November were not significantly different between these adjacent sites. Seeds harvested on 4 November from the Aurora old-field site were significantly heavier and more dormant than those from the other sites.

Table 2. Summary (mean \pm SE) of soil chemical characteristics and *Vincetoxicum rossicum* population attributes for three field sites in central New York State.

Site	Great Gully forest	Great Gully old field	Aurora old field
Soil parameters^a			
pH	7.2 \pm 0.2b ^b	6.3 \pm 0.1c	8.1 \pm 0.0a
Organic matter (%)	3.6 \pm 0.1b	2.8 \pm 0.1c	6.7 \pm 0.3a
Available P (mg·kg ⁻¹)	0.5 \pm 0.1b	3.0 \pm 0.5a	3.2 \pm 0.2a
Available K (mg·kg ⁻¹)	52.0 \pm 3.2b	88.3 \pm 6.7a	76.0 \pm 4.4a
<i>V. rossicum</i> parameters^c			
Shoot density (m ²)	117 \pm 9a ^d	15 \pm 2b	130 \pm 30a
Cover (%)	70 \pm 7a	10 \pm 9b	54 \pm 7a
Height (cm)	85 \pm 8b	139 \pm 6a	146 \pm 4a
Shoots in flower (%)	58 \pm 9b	100 \pm 0a	100 \pm 0a

^aValues are means of $n = 3$ samples collected in May 2002.

^bSoil parameter means with the same letter are not significantly different ($p < 0.05$) between sites.

^cValues are means of $n = 5$ samples collected in late June in 0.25-m² (50 cm \times 50 cm) quadrats.

^dMeans for *Vincetoxicum rossicum* attributes with the same letter are not significantly different ($p < 0.05$) between sites.

Harvest date effects

The date of harvest significantly influenced seed weight and radicle length, but not the number of germinated embryos (Table 3). The effect of harvest date on seed weight and germination varied by site. Seeds harvested in November from the two old-field sites were heavier (Fig. 1A) and had more dormant fresh seed (Fig. 1B) than those harvested in August. However, the weight and germinability of fresh seeds harvested from the forested site did not vary significantly by harvest date. For all sites, seeds harvested in November had significantly higher germination than those harvested in August following 18 weeks of cold storage (Fig. 1C).

Storage effects

The length of dry seed storage at 4 °C (0 versus 18 weeks) significantly influenced the percentage of seeds germinating (Table 3). The effect of storage on germination percentage varied according to harvest date, but was consistent across sites. For seeds harvested on 29 August, cold storage reduced germination percentages by between 50% and 60% (Figs. 1B and 1C). However, for seeds harvested on 4 November, cold storage had little effect on germination percentages.

Relationship between seed weight and germination

The probability of germination was significantly influenced by seed weight (Table 4). The nature of this relationship did not vary with site ($p > 0.10$ for all terms containing site), but did vary with harvest date and storage time. For seeds ranging from 2 to 8 mg (93% of all seeds), the probability of germination decreased significantly with seed size (Fig. 2). For example, seeds weighing between 3 and 4 mg had approximately double the probability of germinating as those weighing between 5 and 6 mg. For seeds harvested on 29 August, cold storage for 18 weeks significantly reduced

Table 3. Analysis of variance for effect of site, harvest date, storage duration, and interactions on *Vincetoxicum rossicum* seed and seedling characteristics.

	df	Significance			
		Seed weight	(%) germination	No. of embryos ^a	Length of radicles ^b
Site	2	***	***	NS	NS
Harvest date ^c	1	***	NS	NS	**
Storage duration ^d	1	NS	**	NS	NS
Site × harvest date	2	***	*	NS	NS
Site × storage duration	2	NS	NS	NS	NS
Harvest × storage duration	1	NS	***	NS	NS
Site × harvest date × storage duration	2	NS	NS	NS	NS
Error	33				

Note: *, statistical significance at $p < 0.05$; **, statistical significance at $p < 0.01$; ***, statistical significance at $p < 0.001$.

^aNumber of embryos that germinated.

^bTotal length of all germinated embryos 7 d after germination of first embryo.

^cHarvest date (29 August or 4 November).

^dStorage duration (0 or 18 weeks).

the probability of germination for a given seed weight. However, for seeds harvested in November, cold storage had little effect on the relationship between seed weight and germination. The timing of germination (number of days to germinate) was not significantly related to seed weight (linear regression, $p = 0.8511$), although seeds from the largest seed weight class (>7 mg) germinated approximately 3 d earlier than those from the smallest seed weight class (<2 mg) (Fig. 3A).

Relationship between seed weight and probability of more than one embryo germinating

The probability of multiple embryos germinating was significantly influenced by seed weight. Overall, the mean number of germinated embryos increased with seed weight from about 1.2 for the <2 mg weight class to 1.6 for the >7 mg weight class (Fig. 3B). The nature of this relationship varied by site (Fig. 4), but not by harvest date or storage duration ($p > 0.10$ for all terms containing harvest date or storage time). At the Great Gully forest site, the probability of more than one embryo germinating from a seed increased significantly with seed weight ($p = 0.014$). For example, a seed weighing between 4 and 5 mg had a 25% probability of more than one germinating embryo, while a seed weighing more than 6 mg had an 80% chance of more than one germinating embryo. The probability of polyembryony was not significantly related to seed weight at the two other sites.

Relationship between seed weight and total mean seedling length

Total mean seedling length 7 d following germination was significantly related to seed weight (Fig. 3C). The nature of this relationship did not vary with site ($p > 0.10$ for all terms containing site), but did vary with harvest date and storage time. Larger seed size resulted in longer total embryo length after 7 d for seeds harvested in November and for fresh seeds harvested in August (slopes were significantly different from zero at 0.05 level and ranged from 0.23 to 0.61 cm·mg⁻¹). However, for seeds harvested in August, and

stored for 18 weeks, there was no significant relationship between seed weight and radicle length ($p = 0.586$).

Discussion

Several seed characteristics varied considerably according to the site from which they were collected. For example, we found that seeds from the shaded forest site were either larger (August harvest date) or the same size (November harvest date) as those from the less shaded old-field site (Fig. 1A). Without a common garden experiment, it is impossible to know whether such differences have a genetic or environmental basis. However, given the proximity of the two Great Gully sites, it seems likely that seeds from these sites are genetically similar, and that observed differences can be attributable to differences in environmental conditions between the two sites. In particular, lower light availability in the forested site may have altered resource allocation or plant phenology and thus directly or indirectly altered seed characteristics. In contrast with our results, Willson and Price (1980) found that for *Asclepias syriaca*, a close relative of *V. rossicum*, shade resulted in a 44% reduction in seed size. Evolutionary theory predicts that large seed size is especially advantageous under closed communities, because it increases the ability of seedlings to obtain light or nutrients (Kidson and Westoby 2000; Salisbury 1942; Tilman 1988). On the other hand, large seeds of *V. rossicum* cannot disperse as far (Cappuccino et al. 2002), which may limit their ability to reach and colonize more open habitats such as forest edges, old fields, or pastures. The growth and fecundity of *V. rossicum* plants have been shown to be substantially greater in open, sunny sites or forest gaps than in the understory of dense forests (Lawlor 2000; Sheeley 1992).

Larger seeds in our study had a lower probability of germinating (Fig. 2). Such a positive correlation between seed size and dormancy has been observed in other species including *Pogogyne abramsii* (Zammit and Zedler 1990) and *Abutilon theophrasti* (Baloch et al. 2001; Nurse and DiTommaso 2005). In contrast, no relationship or a negative

Fig. 1. Mean (\pm SE) seed weight (A), germination percentage of fresh seed (B), and germination percentage of seed stored dry at 4 °C for 18 weeks (C) for seeds of *Vincetoxicum rossicum* harvested on either 29 August or 4 November from three contrasting sites.

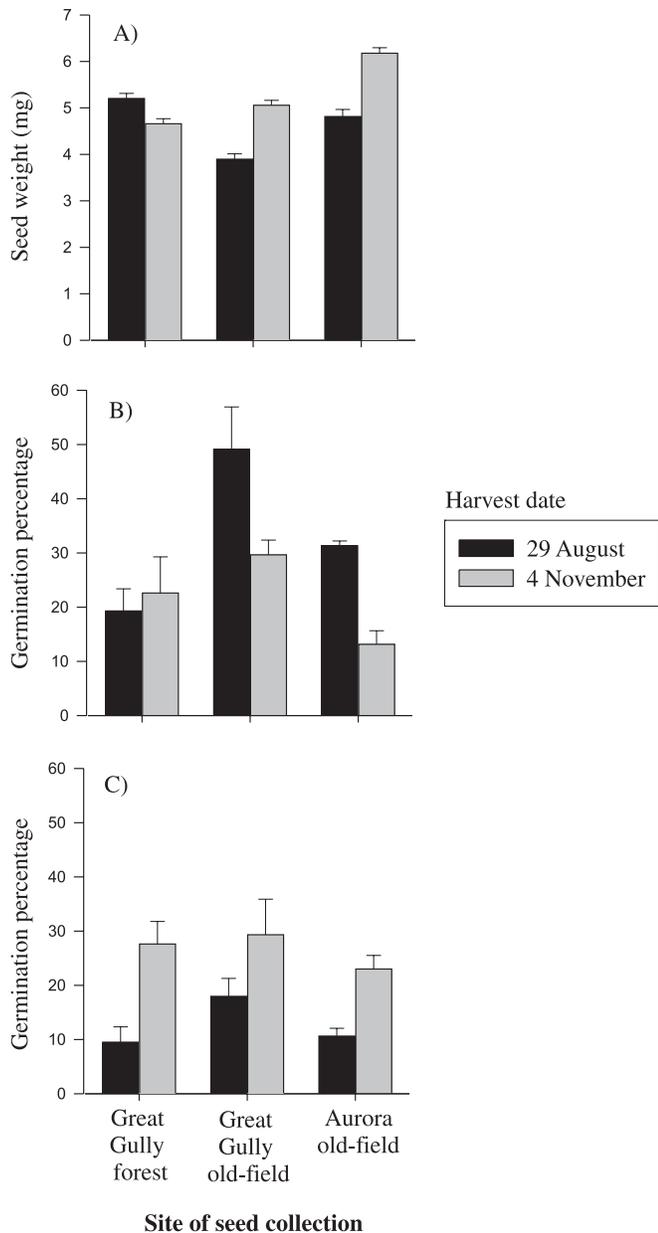


Table 4. Logistic regression parameter estimates for relationship between probability of germination and seed weight in *Vincetoxicum rossicum*.

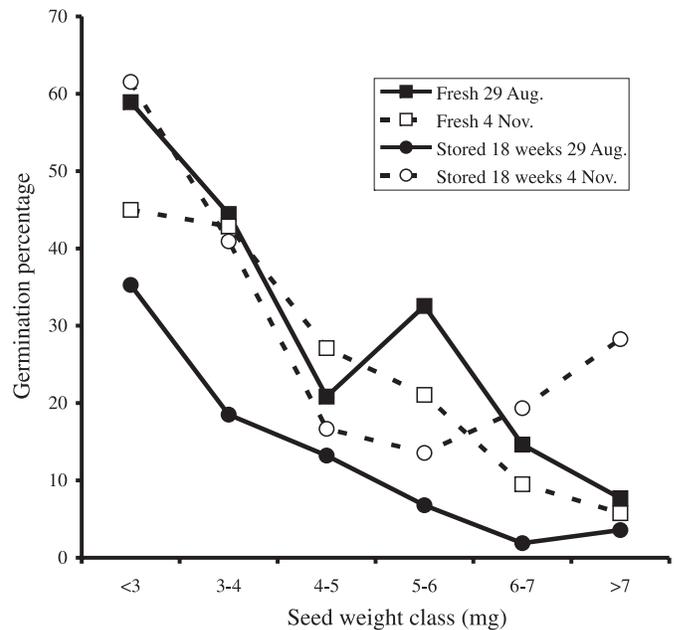
Treatment	Parameter estimates ^a		
	B_0	B_1	B_2
29 August seed			
Fresh	-0.848NS	0.669*	-0.130**
18 weeks	-0.258NS	-0.373***	0.000 ^b
4 November seed			
Fresh	1.005*	-0.445***	0.000 ^b
18 weeks	3.394***	-1.578***	0.125**

Note: *, statistical significance at $p < 0.05$; **, statistical significance at $p < 0.01$; ***, statistical significance at $p < 0.001$; NS, not statistically significant.

^aRelationship between probability of germination (G) and seed weight (W) is modelled: $G = 1/[1 + \exp(B_0 + B_1 + B_2W^2)]$.

^bQuadratic term not included in model.

Fig. 2. Germination percentage by seed weight class for seeds of *Vincetoxicum rossicum* harvested on 29 August (solid lines) or 4 November (broken lines) and tested immediately following harvest (squares) or after 18 weeks of dry storage at 4 °C (circles).

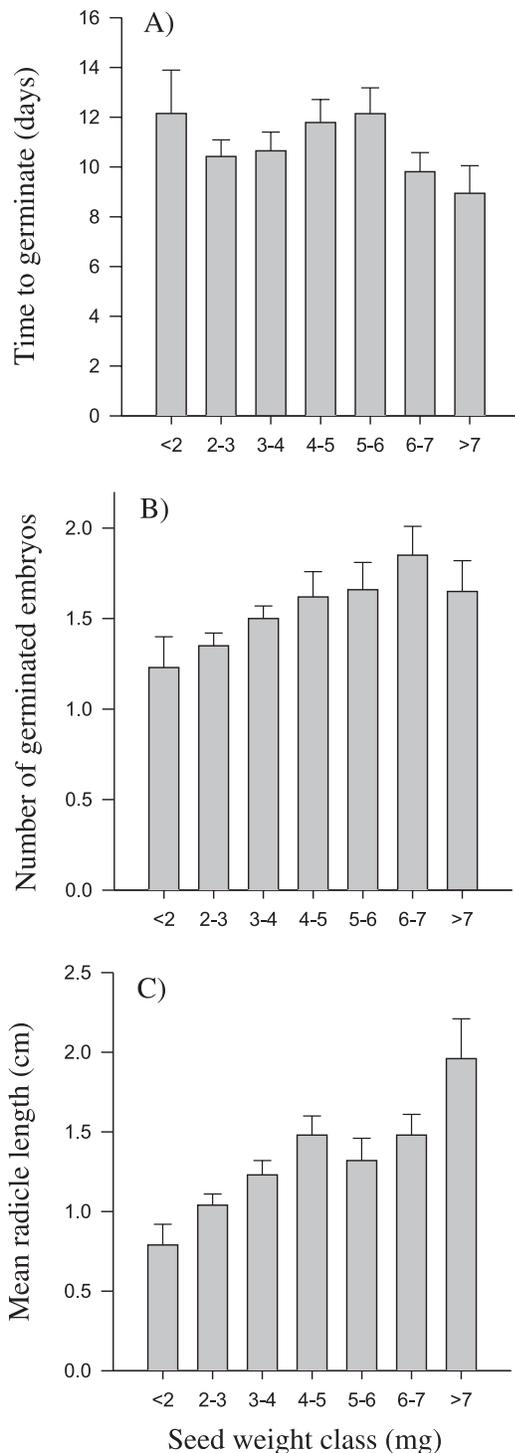


relationship between seed size and dormancy has been found in a *V. rossicum* population growing near Ottawa, Ontario (Cappuccino et al. 2002), and other species including *Convallaria majalis* (Eriksson 1999), *Erodium branchycarpum* (Stamp 1990), and *Amaranthus powellii* (D.C. Brainard, unpublished observation). The inconsistent relationship found between seed size and germinability for the Ottawa, Ontario, and New York State *V. rossicum* populations might be due to genetic differences between the populations. Larger seed size may be associated with larger embryo or endosperm or thicker seed coats (Lacey et al. 1997), both of which may influence seed dormancy (Baskin and Baskin 1998). The

greater length of radicles among larger seeds in our study (Fig. 3C) suggests that larger seeds had larger embryos or endosperm. Although no information is available regarding the mechanism of dormancy in *V. rossicum*, our results are consistent with the hypothesis that larger seeds may have had thicker seed coats (in addition to larger embryo or endosperms) that promoted dormancy.

From an evolutionary perspective, large seeds might be expected to be less dormant, because their seedlings can draw on a larger energy reserve and therefore establish under less favourable environmental conditions (Rees 1996). On the other hand, a negative correlation is also expected between seed dormancy and the ability to spatially disperse, since dispersal reduces the probability that all seeds will be exposed to unfavourable conditions (Venable and Brown

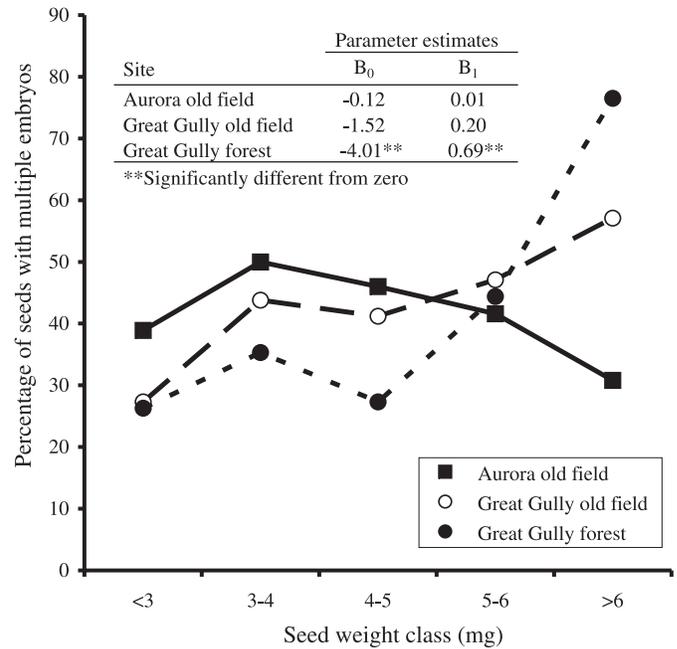
Fig. 3. The relationship between seed weight class and mean (\pm SE) days to germinate (A), embryo number (B), and radicle length (C) for seeds of *Vincetoxicum rossicum*. Data were averaged over all sites, harvest dates, and storage intervals.



1988). Since seed size is negatively correlated with dispersal ability in *V. rossicum* (Cappuccino et al. 2002), the latter hypothesis implies a positive correlation between seed size and dormancy.

The highest germination rates observed in our study (30%–50%) occurred among fresh seeds collected in August

Fig. 4. Percentage of seeds with more than one embryo germinating by seed weight class and site. Logistic regression parameter estimates are provided for relationship between seed weight and the probability of more than one embryo germinating for each site.



from the open, old-field sites (Fig. 1B). Such high germination rates are consistent with field observations in central New York of a flush of fall germination beginning in early September (A. DiTommaso, personal observation). Freshly collected seeds of *V. rossicum* buried at shallow depths in a Syracuse, New York, garden showed a bimodal germination pattern peaking both in autumn and the following spring (F.M. Lawlor, unpublished data). Seedlings of *V. rossicum* emerging in early autumn under nonshaded conditions may attain sufficient size prior to the onset of cold temperatures to survive the winter. Lumer and Yost (1995) working with the conspecific *Vincetoxicum nigrum* suggested that seeds from early-maturing fruits of this species in New York State also likely germinated and became established before the first frost, whereas seeds from late-maturing fruits overwintered and germinated in the spring. Lower germination (10%–30%) among seeds harvested late in the autumn may be an adaptive mechanism to avoid emergence too late for successful autumn establishment. For the shaded forested site, lack of solar radiation may prevent successful establishment and overwintering of autumn germinating seeds. This may explain why germination levels of fresh seeds from the forest site were relatively low at both harvest dates. Previous studies assessing *V. rossicum* germination have used only seeds collected in mid- to late autumn (Cappuccino et al. 2002; Sheeley 1992). Our results suggest that germination percentages from those studies may underestimate germination likely to occur in the field in late summer and early autumn. The ability of a significant proportion of *V. rossicum* seeds produced in August and early September to quickly germinate suggests that the period of favourable climatic conditions may be long enough for seedlings to become well

established and to overwinter successfully. These seedlings would thus have a competitive advantage over spring germinating seedlings of *V. rossicum* and, more importantly, of other plants including threatened native species.

Cold storage for 18 weeks significantly reduced the germination percentage of seeds harvested in August, but had no effect on seeds harvested in November (Figs. 1B and 1C). Cold temperatures may induce dormancy in *V. rossicum* and prevent germination late in the autumn. However, it is important to recognize that the impact of cold storage in this study may not reflect changes likely to occur under field conditions, where moisture and other environmental factors may interact with temperature to determine germination behaviour (Baskin and Baskin 1998). In fact, cold wet storage for 10 d can significantly increase germination of some seed lots of *V. rossicum* (unpublished data).

The number of embryos germinating from each seed was not significantly influenced by site, harvest date, or storage time (Table 1). Overall, among seeds that germinated, 42% had more than one embryo germinate. This is somewhat lower than the levels of polyembryony found in this species in previous studies. For example, Cappuccino et al. (2002) reported that of seeds collected near Ottawa, Ontario, in October and germinating under glasshouse conditions, 55% produced two or more seedlings. Similarly, Sheeley (1992) found that 78% of seeds collected near Syracuse, New York, and germinating under glasshouse conditions were polyembryonic. The somewhat lower levels of polyembryony in our study probably reflect the fact that seeds were monitored for only 1 week following germination of the first embryo compared with 7–8 weeks of monitoring in previous studies. Longer monitoring may have resulted in a greater number of embryos germinating. In addition, if embryo number and dormancy are related, then the number of germinating embryos may not reflect the overall degree of polyembryony. We found a positive relationship between polyembryony and seed weight at the Great Gully forest site, but not at the other sites (Fig. 4). In contrast, Cappuccino et al. (2002) found no relationship between polyembryony and seed weight for *V. rossicum* seeds collected from plants growing along the edge of a mowed field in Ottawa, Ontario.

Polyembryony may be an adaptive response to pollen shortage, although this species is capable of producing most seeds via selfing (St. Denis and Cappuccino 2004), or a mechanism that increases the probability of high-density patches of *V. rossicum*, which may increase fitness in this species via an Allee effect (Cappuccino 2004). Moreover, Cappuccino et al. (2002) reported that polyembryony was most advantageous (i.e., weight of seedlings were greater for polyembryonic seeds than for seeds producing a single seedling) in the absence of grass competition than when seedlings were grown in the presence of grasses. These findings suggest that polyembryony may be beneficial in habitats that have been disturbed or where the resident vegetation has been removed, but this benefit is lost when polyembryonic seeds are dispersed into habitats where the resident vegetation is intact. Field-testing of this hypothesis warrants further study. It is possible that factors influencing the relative costs and benefits of polyembryony (e.g., prevalence of pollinators, neighbour types) may not have varied sufficiently across our sites to alter the degree of polyembryony.

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