NRC Research Press

ARTICLE

# Exploring the weed biology of two potentially novel oilseed crops: *Euphorbia lagascae* and *Centrapalus pauciflorus*

S. Chakraborty, S.Z.H. Cici, J. Todd, C. Loucks, and R.C. Van Acker



**Abstract:** *Euphorbia lagascae* and *Centrapalus pauciflorus* are natural sources of the plasticizer vernolic acid, and are therefore being considered as potentially new industrial oilseed crops in Canada. Both species show a propensity to grow in undisturbed and unfavourable conditions in their native regions of southern Europe and Africa. Trials were conducted in Ontario between 2013 and 2014 to better understand the biology of these species. The ability of these species to establish, leave a seedbank, and compete with a crop was explored. *C. pauciflorus* emergence in cultivated seedbeds (5.14%–12.15%) was higher than in mowed (0.99%–1.87%) and undisturbed grass (0.00%–0.25%) in spring 2014. *E. lagascae* also emerged at higher rates in cultivated seedbeds (3.07%–4.98%) than mowed (0.88%–1.99%) or undisturbed grass (0.22%–1.00%) in spring 2014, however emergence was higher in mowed grass (6.25%) than seedbeds (4.00%) in fall 2014. The low persistence of seeds in the soil (93%–100% seeds were nonviable) and poor ability to establish a seedbank limit their potential as weeds. Plants that established in unmanaged areas did not produce viable seeds and are therefore unlikely to become weeds. Even though their competitive ability is similar to that of redroot pigweed on a plant per plant basis, they are unlikely to achieve the high densities and persistence of pigweed infestation and are unlikely to threaten farms as weeds.

*Key words:* oilseed, weed biology, overwinter, establishment, interference.

**Résumé :** *Euphorbia lagascae* et *Centrapalus pauciflorus* sont des sources naturelles d'acide vernolique, un plastifiant. Ces plantes pourraient donc constituer de nouveaux oléagineux destinés à la production industrielle, au Canada. Dans les régions du sud de l'Europe et de l'Afrique d'où elles sont originaires, les deux espèces affectionnent les terrains incultes et les conditions difficiles. Entre 2013 et 2014, les chercheurs ont procédé à des essais de culture en Ontario afin de préciser la biologie de ces végétaux. Ainsi, ils ont examiné leur capacité à s'implanter, à créer un réservoir de semences et à concurrencer une culture. Au printemps 2014, la levée de *C. pauciflorus* était plus importante dans les planches de semis (5,14–12,15 %) que dans l'herbe tondue (0,99–1,87 %) et les peuplements intacts de graminées (0,00–0,25 %). *E. lagascae* a aussi mieux levé dans les planches de semis (3,07–4,98 %) que dans l'herbe tondue (0,88–1,99 %) et les peuplements intacts de graminées (0,22–1,00 %) au printemps 2014, cependant, à l'automne de la même année, la levée était plus importante dans l'herbe tondue (6,25 %) que dans les semis (4,00 %). La faible persistance des graines dans le sol (93–100 % des semences n'étaient pas viables) et la piètre capacité à établir un réservoir de semences atténuent le risque que ces plantes se transforment en adventices. Les plants qui s'étaient établis dans des lieux non aménagés n'ont pas engendré de graines viables. Par conséquent, il est peu probable que ces espèces deviennent des mauvaises herbes. Bien que la compétitivité individuelle des plants se rapproche de celle de l'amarante à racine rouge, ces espèces ne devraient pas atteindre une densité de peuplement suffisante ni persister assez longtemps pour donner lieu à des infestations aussi graves que l'amarante et devenir une adventice problématique pour les agriculteurs. [Traduit par la Rédaction]

*Mots-clés :* oléagineux, biologie des mauvaises herbes, survie à l'hiver, établissement, interférence.

## Introduction

When evaluating species as potentially new crops, it is important to consider whether these species have weediness potential and what the risks might be of their unconfined release and cultivation. This concern is especially grievous if the selection of traits in the new

Received 10 September 2015. Accepted 22 January 2016.

**S. Chakraborty, S.Z.H. Cici, J. Todd, C. Loucks, and R.C. Van Acker.** Department of Plant Agriculture, University of Guelph, Guelph, ON N1G 2W1, Canada.

**Corresponding author:** S. Chakraborty (email: sonhita.chakraborty@gmail.com).

**Abbreviations: SHRS**, Simcoe Horticultural Research Station; **GCUOF**, Guelph Centre for Urban Organic Farming; **RB**, relative biomass; **TRB**, total relative biomass.

crop also lead to the inadvertent selection of less desirable weedy traits, as has been shown to be the case in plants grown as a source of biofuel (Buddenhagen et al. 2009). There is an interest in growing *Centrapalus pauciflorus* (Willd.) H. Rob. (Asteraceae) and *Euphorbia lagascae* Spreng. (Euphorbiaceae) as industrial oilseed crops in Ontario, but there have been no specific studies on their weediness potential. *E. lagascae* has been grown for different purposes, such as an oilseed crop and a medicinal or ornamental plant. It also has the capacity to repel rodents (White and Wolff 1968). Both *E. lagascae* and *C. pauciflorus* produce high amounts of natural epoxidized oil (vernolic acid). In comparison with chemically epoxidized oil from soybean and linseed, this oil has low viscosity and very good cold flow properties (Earle 1970).

*Euphorbia lagascae* of the Euphorbia genus and Euphorbiaeae family is an annual herbaceous spurge that is found throughout Spain and Italy (Krewson and Scott 1966; Smith et al. 1997; Turley et al. 2000; Christou et al. 2012). The species produces dehiscent-prone tripartite seeds, the oil content of which varies between 45% to 50%, 60% to 65% of which is vernolic acid (Breemhaar and Bouman 1995; Kleiman et al. 1965; Vogel et al. 1993). An *E. lagascae* plant may reach a height of 1 m (Smith et al. 1997; Christou et al. 2012). Through field studies, Breemhaar and Bouman (1995) have estimated the time for *E. lagascae* to flower (between 63 to 84 d after emergence) and set seed (116 d after emergence) near Lelystad, Netherlands. In its native country of Spain, *E. lagascae* seeds germinate in the autumn, and flower between March and April, producing fruits in April and May. Temperatures for optimum growth, base germination, and vegetative growth for *E. lagascae* have been reported to be 10 °C, 6 °C and 18–22 °C, respectively (Vogel et al. 1993; Angelini et al. 1997; Roseberg and Shuck 2008; Zanetti et al. 2013).

*C. pauciflorus* (previously known as *Vernonia galamensis*) of the Asteracea family is also a herbaceous annual plant from the East African countries of Eritrea, Ethiopia, Sudan, and Kenya (Gilbert 1986). This species also occurs in the southern African countries of Tanzania and Malawi (Gilbert 1986; Mebrahtu et al. 2009). *C. pauciflorus* can grow over a wide range of altitudes (1250–2050 m above sea level) and rainfall conditions (60 to 185 cm) (Perdue et al. 1986; Baye and Becker 2005; Mebrahtu et al. 2009). Fine hairs called pappi (singular pappus) attached to *C. pauciflorus* seeds facilitate seed dispersal (Sheldon and Burrows 1973). The oil content of *C. pauciflorus* is between 35% and 42%, 72%–80% of which is vernolic acid (Thompson et al. 1994; Baye et al. 2001).

*C. pauciflorus* and *E. lagascae* have been known to grow under conditions that mimic non-farmed areas such as roadsides where the soils are often rich in nitrogen (Baye and Becker 2005). Furthermore, seeds of *C. pauciflorus* acquired from colder regions tend to be more dormant than seeds acquired from warmer regions (Nyamongo et al. 2010). Disturbance cues that mimic conditions found closer to the soil surface, such as alternating temperatures, cold-stratification, longer exposure to light, and exposure to chemicals like gibberellic acid ($GA_3$) and potassium nitrate ($KNO_3$), may break secondary dormancy in *C. pauciflorus* (Nyamongo et al. 2010). There is very little knowledge on the potential weed biology of *E. lagascae* and *C. pauciflorus*. Adequate risk assessment of potentially weedy crops involves evaluating the species in terms of its biology and ecology, competitive ability, and ability to hybridize with related species (Barney and Ditomaso 2010).

Ideally, a weed is able to produce a large number of seeds over a long time, germinate over a wide range of environments, dictate seed germination and dormancy, disperse its seeds over varying distances, and devote resources to competitive structures when competing with a different species (Baker 1974). The purpose of this study was to explore some of the features associated with weediness, namely seedling emergence, overwintering ability, and the ability of *E. lagascae* and *C. pauciflorus* to establish a seed bank under Canadian conditions. Soybean is a commercially important crop in Ontario that faces substantial yield loses from competition with problematic weeds like pigweed (Cowan et al. 1998). Hence, the interspecific competition of *E. lagascae* and *C. pauciflorus* with soybean was also examined and compared with the competition of soybean with a common weed, redroot pigweed [*Amaranthus retroflexus* (L.)]. Information obtained from this study will provide the initial understanding of the potential weediness of *E. lagascae* and *C. pauciflorus*.

## Materials and Methods

### Experiment 1: *E. lagascae* and *C. pauciflorus* seed persistence in the soil

Field experiments were conducted at two sites in Ontario: the Simcoe Horticultural Research Station (SHRS), located in Simcoe (42°51′N, 80°16′W) and the Guelph Centre for Urban Organic Farming (GCUOF) in Guelph (43°32′N, 80°13′W) in 2014. These sites were chosen for their differences in soil type, precipitation, and temperatures, and for the purposes of replication. The experimental site of SHRS was characterized by a loam soil with an organic matter content of 1.2% and a pH of 5.7. The soil at the GCUOF was loam (40% sand, 40% silt, 20% clay) with an organic matter of 4.3 and a pH of 7.4, and a Canadian Land Index of 3.

Experiments had been previously carried out to test the germinability of seeds before they were sowed in the field. Seeds of *E. lagascae* and *C. pauciflorus* underwent one of three treatments: "priming", "gibberellic acid", and "hydrogen peroxide". Imbibition or priming of seeds with water enhances germination by either altering cell structure (Khan 1992) or by prompting protein repair

(Rao et al. 1987). The plant hormone gibberellic acid is known to break dormancy and enhance germination by acting antagonistically with the dormancy inducing plant hormone, abscisic acid (Karssen and Laçka 1986). Hydrogen peroxide also enhances germination either by releasing oxygen required for cellular respiration (Katzman et al. 2001) or by rendering the seed coat more permeable to water (Chen et al. 1993). Seeds were imbibed in distilled water for 0, 12, 24 or 48 h, and 500 or 1000 mM gibberellic acid for 12 or 24 h, rinsed in distilled water, and air dried for 24 h before sowing. As controls, seeds were also soaked in deionized water for 12 h and 24 h prior to being dried at room temperature for 24 h before emergence testing. Seeds were also soaked in hydrogen peroxide (35%) for 10 min and then rinsed and dried for 24 h at room temperature prior to planting. This treatment was compared with seeds that were soaked in deionized water for 10 min before being dried for 24 h at room temperature prior to planting. Emergence tests were conducted in pots containing Pro-Mix® BX manufactured by Premier Tech Horticulture. All treatments were replicated four times with each replicate containing 25 seeds.

The 2013–2014 outdoor seed persistence experiment was conducted as a completely random design and the 2014–2015 experiment at a randomized complete block design. *E. lagascae* seeds collected from a field trial conducted in Oregon in 2010, and *C. pauciflorus* seeds obtained from the USDA germplasm bank (2013) were used for all experiments. A bulk batch of *E. lagascae* seeds were used, which consisted of germplasms E005, E006, and E008 (Roseberg and Shuck 2008). The maternity of the *C. pauciflorus* line could be traced to *V. galamensis* subsp. galamensis var. petitiana (A0399), a day-neutral line. *V. galamensis* subsp. galamensis var. petitiana (A0399), which was crossed with *V. galamensis* subsp. galamensis var. galamensis (A0388 and A0389) to produce the breeding line WCL-VP2 (Dierig et al. 2006) used in this study. The seeds used in these studies were selections that were not yet cultivars. The intension of this study was to consider whether these particular selections (as proxies for potential cultivars) had any weediness potential. Seeds were enclosed in small 8 × 5 cm handmade packets made of insect mesh material, such that 25 seeds were enclosed in each pouch. Due to the small size of the seed pouches, 25 seeds were placed in each packet to ensure that all seeds in a pouch had sufficient contact with the surrounding soil. Treatments were replicated eight times such that there were 200 seeds per treatment. Nails were used to keep seed pouches sown at the surface in place, and from being removed by animals. A preliminary experiment was conducted at the GCUOF on 8 Oct. 2013 to examine the effect of seeding depth (surface vs. 2.5 cm below the surface) on overwintering ability. The experiment was repeated the following year at the SHRS and at the GCUOF, early in the fall (29 Sept. 2014) and late in the fall (23 Oct. 2014). Pouches containing seeds were placed at a depth of either 2.5 cm below the surface or just at the surface in 2 × 2 m subplots that were a part of a larger 8 × 16 m plot at SHRS and GCUOF. Each subplot was subject to a treatment that was replicated eight times, and treatments were randomized within each block during the experimental set up.

In general, seedling emergence increases with increasing depth up to a point, after which it decreases with increasing seeding depth (Grundy et al. 1996; Mohler and Galford 1997). Seeds buried closer to the surface are more likely to dry out or be preyed upon. The weather protected water-laden environment deeper in the soil prevents desiccation while preserving seed viability (Bullied et al. 2012). However, seeds much deeper in the soil are unlikely to emerge due to the lack of appropriate temperature, light, and water conditions (Boyd and Van Acker 2004). A previous study in Manitoba, Canada determined the maximum mean recruitment depth of five annual weeds to be within 4.2 cm (du Croix Sissons et al. 2000). Hence, recruitment at a seeding depth of 2.5 cm was compared with recruitment at the surface to determine if either seeding depths fell in the optimal seeding depth range for seedling emergence for these species.

In the spring of 2014 and 2015, pouches containing seeds were retrieved from the ground and tested for viability. First, freshly dug seeds were tested for their firmness by touch with forceps and sorted from seeds that had either germinated already in the field or were soft to the touch. Firm seeds were washed in a 5% sodium hypochlorite (NaOCl) solution for ten minutes prior to five rinses in deionized water (ISTA 1985). Surface sterilized seeds were germinated in a growth chamber at 12 h photoperiod, 80% humidity, and 25 °C day and 17 °C night temperatures, to determine germinability. The number of germinating seeds was recorded twice a week for a period of three weeks. The ungerminated (but still firm) seeds were bisected longitudinally. The embryos were soaked in 1% tetrazolium chloride (TTC) for 48 h at 30 °C in the dark (ISTA 1985). Pink embryos were scored as alive.

The percentage of nonviable, viable, and dormant seeds at different stages of the experiment were calculated according to the equations given below:

$$\mathrm{Seed_{nonviable}}\,\% = (\mathrm{A_1} + \mathrm{A_2}/\mathrm{Seed_{total}}) \times 100 \tag{1}$$

$$\mathrm{Seed_{viable}}\,\% = (\mathrm{B_1} + \mathrm{B_2}/\mathrm{Seed_{total}}) \times 100 \tag{2}$$

$$\mathrm{Seed_{dormant}}\,\% = (\mathrm{C_1}/\mathrm{Seed_{total}}) \times 100 \tag{3}$$

where $A_1$ is the number of soft dead seeds that did not survive the winter in the field, $A_2$ is the number of seeds that tested negative for the TTC test, $B_1$ is the number of seeds that germinated in the field at some point during the overwintering process, $B_2$ is the number of seeds that germinated in petri dishes, and $C_1$ is the number of seeds that tested positive for the TTC test.

### Experiment 2: *E. lagascae* and *C. pauciflorus* establishment in different environments

The experiment was conducted in spring 2014, fall 2014, and spring 2015, in a perennial rye grass [*Lolium perenne* (L.)] stand at the SHRS, located in Simcoe, Canada (42°51′N; 80°16′W) and in heterogeneous swards of quackgrass [*Elymus repens* (L.) Gould], sheep fescue [*Festuca rubra* (L.)], orchard grass [*Dactylis glomerata* (L.)], and broadleaves including dandelion [*Taraxacum officinale* (Weber in Wiggers)], smooth bromegrass [*Bromus inermis* (Leyss.)], fringed brome [*Bromus ciliatus* (L.)], Canada thistle [*Cirsium arvense* (L.) Scop.], late goldenrod [*Solidago altissima* (L.)], Canada goldenrod [*Solidago canadensis* (L.)], and panicled aster [*Symphyotrichum lanceolatum* (Willd.) G.L. Nesom.] at the GCUOF in Guelph, Canada (43°32′N; 80°13′W). The grass swards at both locations were chosen for their likeness to roadside conditions and the ease with which they could be mowed and manipulated into seedbeds. *E. lagascae* seeds collected from a 2010 Oregon trial and *C. pauciflorus* seeds collected from the USDA germplasm bank in 2013, were used for the spring and fall trials of 2014. Newer seeds from a 2014 trial at Simcoe, Ontario were used for the fall 2015 trial.

The experiment utilized a randomized complete block design. The experiment was conducted over three seasons (spring 2014, fall 2014, and spring 2015) and two sites: SHRS and GCUOF for both *E. lagascae* and *C. pauciflorus*. In Simcoe, establishment trials commenced on 12 June 2014, 29 Sept. 2014, and 17 June 2015. In Guelph, establishment trials commenced on 18 June 2014, 2 Oct. 2014, and 17 June 2015. To examine how temperature and soil conditions influence the periodic emergence rates of *E. lagascae* and *C. pauciflorus*, the experiment was initiated in the spring and fall seasons (Stoller and Wax 1973). Disturbance treatments included an undisturbed grassy sward environment (control), mowed sward (disturbance), and a well-tilled seed bed. Each treatment was replicated four times. Seeds of *E. lagascae* and *C. pauciflorus* were dispersed by hand at a seeding rate of 300 seeds per half meter square over each experimental unit ($0.5 \times 0.5$ m subplots) within the entire experimental area ($3 \times 11.5$ m). For the tilled plots, the grass sward was dug up using spades to a depth of approximately 10 cm and this material was then raked using a hand rake to create a smooth seed bed. This was done prior to sowing. Mowed plots were mowed to a sward height of approximately 3–5 cm every 7 d using pruning shears and large scissors. The first mowing was done just prior to sowing.

The number of emerging seedlings at the SHRS and the GCUOF was observed weekly over a period of twelve weeks in the spring and fall of 2014 and spring of 2015. Emerged plants were tagged weekly using different coloured paper clips to distinguish them from seedlings that had emerged previously. At the end of the study, a final count on the number of plants per half meter square subplot was recorded as a measure of establishment potential. Plots were monitored past the twelve weeks of data collection for observations on flowering and seed production of the emerged plants.

### Experiment 3: *E. lagascae* and *C. pauciflorus* competition with soybean

Two runs of the competition experiment were conducted consecutively in a growth room at the University of Guelph at a photoperiod of 16 h (16 h of light, 8 h of darkness), 70% relative humidity, light levels of 350 μE $m^{-2}$ $s^{-1}$, and day temperatures of 25 °C and night temperatures of 20 °C. In the first run, three gallon pots were used for each subplot while one gallon pots were used in the second run. Plants were grown in pots containing Sun Gro® Horticulture's Sunshine Mix 4 Aggregate Plus, which were watered every two days or as needed.

The experiment was set up as a replacement series. Replacement series are widely used as a method of initial evaluation of relative competitiveness of species when no prior information is available (Jolliffe 2000). In this method, yield per unit area is used as a measure of competitive ability. A minimum of two different species are grown in pure stands (species in question is grown with members of its own species) and mixed stands (species in question is grown with a different species) at fixed combined densities (Jolliffe 2000).

Each of *E. lagascae*, *C. pauciflorus*, and common pigweed were grown in pots with soybeans at five different species ratios: 4:0, 3:1, 2:2, 1:3, and 0:4. The total number of plants in each pot was four. Seeds of *E. lagascae*, *C. pauciflorus*, common pigweed, and soybean were germinated in trays. Seedlings of *E. lagascae*, *C. pauciflorus*, and common pigweed with similar emergence timings were transplanted into pots with members of their own species or soybean according to the various species ratios. To minimize edge effects in the experiment, border rows were set up at the periphery of the experiment. Pots within each block and whole blocks were randomized every other week in the growth room.

Plants were grown for six wks, at which time the soybeans reached anthesis (R1). This has been identified to be the critical period of weed control in soybean (Van Acker et al. 1993). At three wks after emergence, data on plant height (cm) was recorded for each plant. At the end of the six wk study, final plant height (cm) was recorded again prior to harvesting above ground plant material for each plant. Individual aboveground plant dry mass was weighed and recorded after plant matter was dried in an oven at 80 °C for 48 h.

## Statistical Analysis

### Experiment 1: *E. lagascae* and *C. pauciflorus* seed persistence in the soil

Status of *C. pauciflorus* and *E. lagascae* seeds (viable, non-viable, and dormant) after over-wintering was analyzed using PROC GLIMMIX (SAS version 9.3). The 2013–2014 experiment at GCUOF was arranged as a completely random design. Separate analyses were conducted for each of the two species due to the uneven number of data points. For the 2013–2014 experiment, seeding depth (surface and deep) was set as the fixed effect. A log link function and a Poisson distribution of errors were used for the 2013–2014 analysis. When residual variances were found to be heterogeneous between seeding depths, the generalized chi-square value normalized by the degrees of freedom was used to confirm the benefit of using a heterogeneous error model. The effect of seeding depth was considered significant at $P \leq 0.05$. Because there were no dormant seeds observed after over-wintering for *C. pauciflorus* in the 2013–2014 trial, this data was not analyzed but is reported in the results. The seed pouches that were not recovered the subsequent spring were counted as missing data.

The 2014–2015 experiment at SHRS and GCUOF was arranged as a randomized complete block design and two separate analyses were conducted for each of the two species. Seeding depth (surface and deep) and time of the planting (early and late) were included as fixed effects and block was considered to be a random effect. For these trials, an identity link and Gaussian distribution of errors were used for all analyses in GCUOF as well as for viable seeds of species *C. pauciflorus* and dormant seeds of species *E. lagascae* in SHRS. In all other cases, a log link function and a poisson distribution of errors were used. The effects of seeding depth and planting time were considered significant at $P \leq 0.05$. The total percentage of *C. pauciflorus* seeds that were nonviable, viable or dormant at SHRS were not analysed as all the seeds seemed to be nonviable. Predation of seeds at GCUOF lead to missing data.

### Experiment 2: *E. lagascae* and *C. pauciflorus* establishment in different environments

Seedling emergence of *C. pauciflorus* and *E. lagascae* seeds under different establishment environments was analyzed using PROC GLIMMIX (SAS version 9.3). The experiment was arranged as a randomized complete block design. Analyses were conducted for each species in six different scenarios including two sites (SHRS and GCUOF) and three planting dates (spring and fall 2014 and spring 2015). The effect of establishment environment (mowed, seedbed, and undisturbed) was considered fixed and the effect of block was considered random. In spring 2014, across all sites, a log link function and a Poisson distribution of errors were used for the analysis. In fall 2014, many zero values precluded statistical treatment. For fall 2014, statistical analysis was only conducted for species *E. lagascae* at SHRS in which a Gaussian distribution of errors and a heterogeneous error model for establishment environment was used. The generalized chi-square value normalized by the degrees of freedom was used to confirm the benefit of using a heterogeneous error model. In spring 2015, a log link function and Poisson error distribution were used for the analysis, except for species *E. lagascae* in SHRS where an identity link and Gaussian distribution of errors were used. The effect of establishment environment was considered significant at $P \leq 0.05$ and Tukey's HSD test was used to test significance between establishment environments.

### Experiment 3: *E. lagascae* and *C. pauciflorus* competition with soybean

For competitive ability analysis, relative aboveground dry biomass (RB) was used as a relative measure of competitive ability and was calculated using the following equation:

$$\begin{aligned} \mathrm{RB} = {} & \text{Dry biomass of species A in a treatment}/ \\ & \text{Dry biomass of species A in a monoculture} \end{aligned} \tag{4}$$

Total relative biomass (TRB), the sum of the total RB of all plants in a given pot, was used to assess how plants were behaving within pots. If TRB equals one, then both plant species are using the same resources optimally. A TRB value of greater than one would signify synergy as the two plant species are not competing for resources and instead were tapping into resources differentially. Likewise, a TRB value of less than one would signify competition for resources or antagonism between the two species in the pot (Harper 1977).

$$\mathrm{TRB} = \mathrm{RB}_{\text{Species A}} + \mathrm{RB}_{\text{Species B}} \tag{5}$$

RB and TRB were graphed using a scatter plot to visualize how the different species were competing for resources. The two runs of the experiment were plotted separately but compared against each other to examine if pot size (three gallon pots in the first run and one gallon pots in the second run) had an effect on competition for resources.

## Results

### Examining the ability of *E. lagascae* and *C. pauciflorus* seeds to persist in the soil and leave a seedbank

Application of various chemical agents at different concentrations over different periods of exposure did not significantly enhance emergence levels in either *E. lagascae* or *C. pauciflorus* in a previous trial (data not shown). The data of the 2013–2014 trial is represented as the mean percentage (±standard error) of viable,

**Table 1.** Non-viable, viable, and dormant seeds[a] as affected by species and burial depth after overwintering in 2013–2014 at the Guelph Center for Urban Organic Farming (GCUOF), Guelph ON. Standard error (±S.E.) of the mean is indicated in parentheses.

| | *C. pauciflorus* | | | *E. lagascae* | | |
|---|---|---|---|---|---|---|
| Seeding depth | Nonviable | Viable | Dormant | Nonviable | Viable | Dormant |
| Surface | 98.5a[b] (1.05) | 1.5b (1.05) | 0.0 (0.00) | 94.3a (1.24) | 0.6a (0.57) | 5.1a (1.14) |
| Deep | 89.6a (3.92) | 10.4a (3.92) | 0.0 (0.00) | 98.4a (1.50) | 0.8a (0.80) | 0.8a (0.80) |

**Note:** No letter groupings were assigned to 0 values due to the lack of retrieval of dormant seeds after overwintering.
[a]Proportion represented as total percentage (%).
[b]Means within a column with the same letter are not significantly different at $P = 0.05$.



non-viable, and dormant seeds observed after overwintering in Table 1. An overwhelming percentage of *C. pauciflorus* that were placed at or just below the surface did not overwinter as most (the great majority) were not viable when they were retrieved in the spring of 2014. From the 2013–2014 trial at Guelph, the percentage of nonviable *C. pauciflorus* seeds was higher when seeds were placed just at the surface (98.5%) versus when they were placed deeper in the soil (89.6%), but this difference was not significant (Table 1). Seeds at the surface were more likely to desiccate due to the ephemeral moisture conditions at the soil surface (Harper 1977) and these seeds would be exposed to much more extreme winter temperatures. Deeper sown seeds were also significantly more viable (10.4%) than their surface sown counterparts (1.5%) (Table 1). Regardless of seeding depth, *C. pauciflorus* were either viable or non-viable and never found to be dormant in this experiment (Table 1).

Most *E. lagascae* seeds did not survive the winter of 2013–2014 in the Guelph Centre for Urban Organic Farming, Guelph ON. A very small percentage of seeds germinated in the field or in petri dishes. Seeding depth had no effect on the percentage of nonviable or viable seeds (Table 1). 94.3% of the *E. lagascae* seeds were nonviable when sown at the surface or sown 2.5 cm below the surface. Likewise, 0.6% and 0.8% of *E. lagascae* seeds were viable when they were sown at the surface and 2.5 cm below the surface, respectively (Table 1). A small percentage (0.7%–1.0%) of *E. lagascae* seeds that were firm and had not germinated in the petri dishes did stain red when exposed to TTC. The number of seeds that were dormant was greater if they were sown at the surface (5.1%) than if they were sown 2.5 cm below the surface (0.8%), but the difference was not significant (Table 1). *E. lagascae* seedlings emerged in June 2015 at the Guelph Centre for Urban Organic Farming, Guelph ON in simulated seedbeds upon which *E. lagascae* seeds had been dispersed in October 2014 for a different experiment (personal observation).

The data of the 2014–2015 trial is represented as the mean percentage (±standard error) of viable, non-viable, and dormant seeds observed after over-wintering in Table 2. For *C. pauciflorus*, results from the experiments in the 2014–2015 experiment were very similar to those from the 2013–2014 seasons. All seeds at Simcoe were nonviable regardless of the time they were sown and the depth they were sown at. A very small percentage of the seeds sown at Guelph did prove to be viable as they emerged either in the field or in petri dishes (Table 2). The percentage of nonviable seeds was significantly higher when seeds were placed at the surface versus when they were placed deeper in the soil (Table 2). Seeds sown later in the fall were also significantly more nonviable than when they were sown earlier in the season (Table 2). Based on visual observations, none of the seeds showed a positive reaction to TTC (Table 2). For *E. lagascae*, the effect of seeding depth or time of seeding was more difficult to decipher for the 2014–2015 trial. There was no significant difference within the total percentage of nonviable seeds, viable seeds or dormant seeds at the Simcoe site due to a different seeding depth or time of sowing (Table 2). Furthermore, predation of *E. lagascae* seed that was in pouches at the surface of the soil at the Guelph site resulted in missing data. Deeper sown *E. lagascae* seeds at the Guelph site were mostly nonviable, with a small percent viable and a very small percent that was too small to report with statistical confidence, dormant (Table 2). Some of the deeper sown seed packets were not recovered the following spring, probably due to their displacement under the soil from fluctuating moisture conditions. These packets were subsequently not counted or included in statistical analyses.

#### Examining the ability of *E. lagascae* and *C. pauciflorus* to establish in different environments

The data on the 2014–2015 seedling establishment study is represented as mean percentage in Table 3. Both *C. pauciflorus* and *E. lagascae* were able to establish at either site and under all treatments, demonstrating the ability of both species to establish from seed with or without anthropogenic manipulation. *C. pauciflorus* emerged at significantly higher levels at Simcoe than at

**Table 2.** Non-viable, viable, and dormant seeds[a] as affected by species and burial depth after overwintering in 2013–2014 and 2014–2015 at the Simcoe Horticultural Research Station (SHRS*), Simcoe ON and the Guelph Center for Urban Organic Farming (GCUOF), Guelph ON. Standard error (±S.E.) of the mean is indicated in parentheses.

| | | SHRS* | | | GCUOF** | | |
|---|---|---|---|---|---|---|---|
| Fall 2014 | Seeding depth | Nonviable | Viable | Dormant | Nonviable | Viable | Dormant |
| *C. pauciflorus* | | | | | | | |
| Early | Surface | 100.0[b] (0.00) | 0.0 (0.00) | 0.0 (0.00) | 98.5ab (0.63) | 1.5ab (0.63) | 0.0 (0.00) |
| | Deep | 100.0 (0.00) | 0.0 (0.00) | 0.0 (0.00) | 92.2b (1.64) | 7.8a (1.66) | 0.0 (0.00) |
| Late | Surface | 100.0 (0.00) | 0.0 (0.00) | 0.0 (0.00) | 99.7a (0.87) | 0.3b (0.87) | 0.0 (0.00) |
| | Deep | 100.0 (0.00) | 0.0 (0.00) | 0.0 (0.00) | 96.8ab (0.86) | 3.2ab (0.87) | 0.0 (0.00) |
| *E. lagascae* | | | | | | | |
| Early | Surface | 96.6a[b] (1.84) | 3.3a (1.58) | 0.1a (0.50) | P | P | P |
| | Deep | 96.6a (1.70) | 2.8a (1.36) | 0.6a (0.46) | 93.7a (2.22) | 6.0a (2.41) | × |
| Late | Surface | 97.5a (1.60) | 1.5a (0.92) | 1.0a (0.43) | P | P | P |
| | Deep | 93.0a (1.56) | 7.0a (2.02) | 0.0a (0.43) | 96.6a (2.42) | 0.1a (2.96) | × |

**Note:** P = Missing data due to predation of seed packets; × = Incomplete analysis due to small sample size.
[a]Proportion represented as total percentage (%).
[b]Means within a column with the same letter are not significantly different at $P = 0.05$.

**Table 3.** Proportion of seedling emergence[a] from seed of *C. pauciflorus* and *E. lagascae* in swards of grass either mowed, or tilled (seedbed) or undisturbed from seeds sown in either spring 2014, fall 2014, and spring 2015 at sites at the Simcoe Horticultural Research Station (SHRS*), Simcoe ON and Guelph Centre for Urban Organic Farming (GCUOF**), Guelph ON.

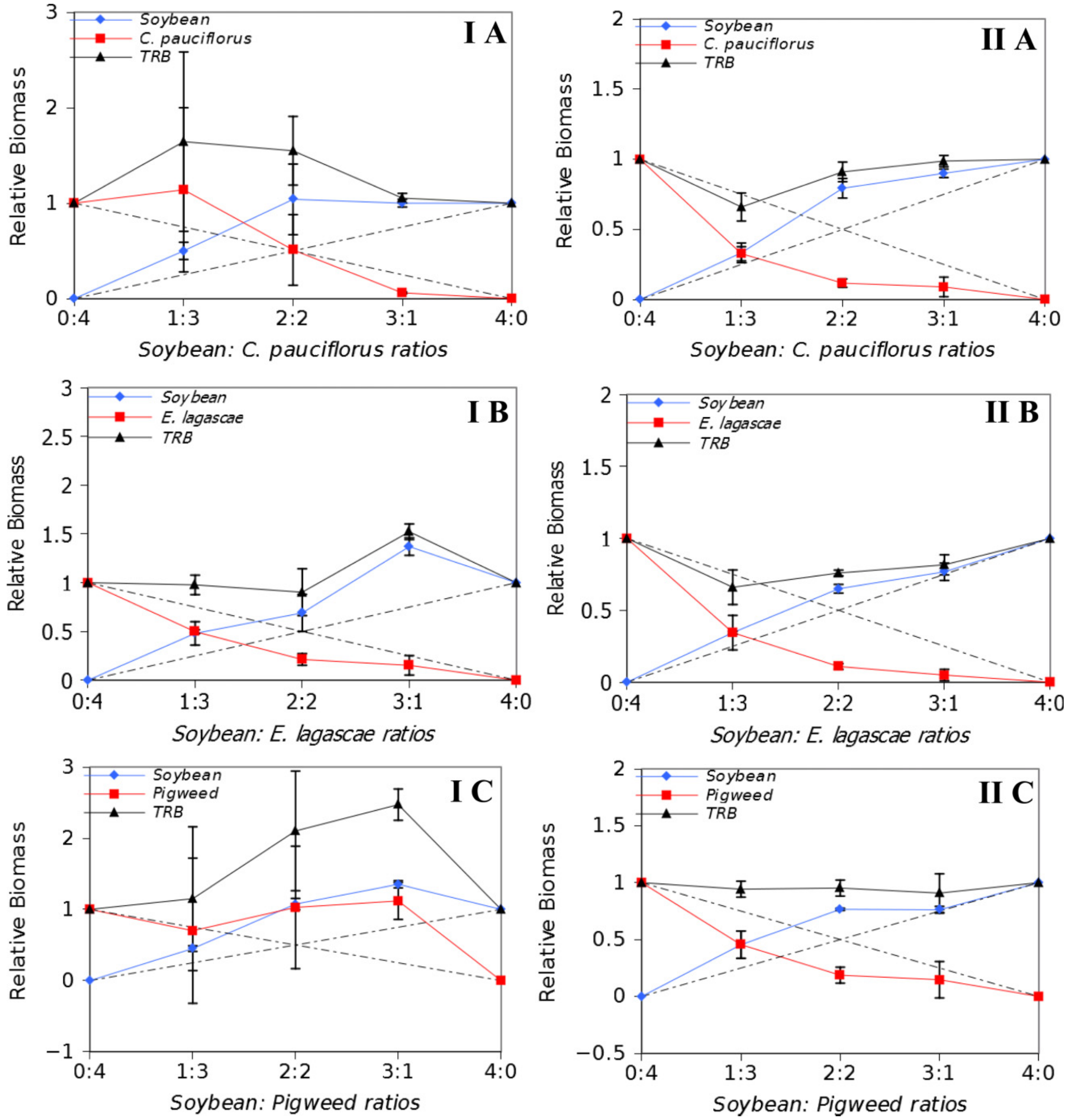
| | SHRS* | | | GCUOF** | | |
|---|---|---|---|---|---|---|
| | Spring 2014 | Fall 2014 | Spring 2015 | Spring 2014 | Fall 2014 | Spring 2015 |
| *C. pauciflorus* | | | | | | |
| Mowed | 0.99b[b] | 0.00 | 0.25b | 1.87b | 0.00 | 0.75b |
| Seedbed | 12.15a | 0.75 | 4.25a | 5.14a | 0.00 | 3.50a |
| Undisturbed | 0.25b | 0.00 | 0.00b | 0.23b | 0.00 | 0.00 |
| *E. lagascae* | | | | | | |
| Mowed | 0.88b[b] | 6.25a | 2.00b | 1.99ab | 0.00 | 5.15a |
| Seedbed | 3.07a | 4.00a | 9.75a | 4.98a | 0.00 | 4.67a |
| Undisturbed | 0.22b | 4.25a | 0.50b | 1.00b | 0.00 | 3.44a |

**Note:** No letter groupings were assigned to Fall 2014 data due to the lack of seedling emergence.
[a]Proportion represented as total percentage (%).
[b]Means within a column with the same letter are not significantly different at $P = 0.05$.

Guelph, with much more emergence for spring versus fall sowed seeds (Table 3). No *C. pauciflorus* emerged at the Guelph site in the fall of 2014 and at the Simcoe site too, *C. pauciflorus* emergence levels were nominal (if not negligible) for the fall 2014 sown seeds (0.75%). For the spring 2014 and 2015 sowing, *C. pauciflorus* emergence was significantly affected by treatment, with emergence being highest under the seedbed (tilled) treatments followed by the mowed and then the undisturbed treatment (Table 3). Contrary to our expectations, *C. pauciflorus* establishment levels in spring 2015 were lower than that of spring 2014 even though the seeds used in the 2015 trial were obtained from a 2014 field trial (Table 3).

For *E. lagascae*, establishment was greater for the spring versus the fall sown seeds at the Guelph site (Table 3). No emergence was observed for *E. lagascae* at the Guelph site for the fall sowing. However, at the Simcoe site, emergence was significantly greater for the fall 2014 versus the spring 2014 sowing. For the fall sowed *E. lagascae*, emergence was significantly greater in the mowed treatment (6.25%) than in the seedbed (tilled) treatment (4.00%) which in turn, was comparable to the emergence level in the undisturbed treatment (4.25%) (Table 3). In contrast, the highest emergence levels were observed for the seedbed (tilled) treatment for the spring sowing at both sites. Even though similar establishment trends were observed in the spring of 2014 and 2015, the

**Fig. 1.** Relative aboveground dry biomass of soybean and (A) *C. pauciflorus*, (B) *E. lagascae*, and (C) pigweed at six weeks after emergence, at 0:4, 1:3, 2:2, 3:1, and 4:0 soybean: *C. pauciflorus*, *E. lagascae*, and pigweed proportions in the (I) first run and (II) second run of a replacement series experiment conducted in a growth room at the University of Guelph, Guelph ON. Figure appears in colour on the Web.






establishment levels were much higher in spring 2015 from the seeds from a 2014 trial (Table 3). Some of the *C. pauciflorus* and *E. lagascae* plants that emerged from spring sowed seeds were killed by frost before seeds could mature. Frost killed the fall-sown *C. pauciflorus* plants before they could finish flowering.

#### Examining the ability of *E. lagascae* and *C. pauciflorus* to compete with another crop

In the first run of this experiment, TRBs were greater than one for many of the ratio treatments for all three species (Fig. 1. I A–C). In this first run, the RB of soybean assumed a convex curve with increasing soybean density and the weed species occupying the same pot exhibited either a slight concave shape or a convex shape as their density in the pot decreased. The lack of concave curvature of the weed RB with decreasing density resulted in a TRB of greater than one. However, when the same species (*C. pauciflorus*, *E. lagascae*, and pigweed) were grown with soybean at the same five ratios (0:4, 1:3, 2:2, 3:1, and 4:0) in pots of smaller size and volume, the results were quite different (Fig. 1. II A–C). Pots including *C. pauciflorus* and *E. lagascae* in the second run showed signs of resource competition, as evidenced by the dip in TRB below a value of one. A TRB of less than one was indicative of antagonistic competition. TRB in the pigweed-soybean treatments dipped only very slightly below one (Fig. 1. II C), indicating no niche overlap between the two species. In this run, while the RB of soybean assumed a convex curve with increasing soybean density, the weed species (*C. pauciflorus*, *E. lagascae* or *A. retroflexus*) occupying the same pot assumed a more characteristic concave shape at their density in the pot decreases. The opposing RBs of the soybean and the weed species lead to a TRB of less than one.

## Discussion

The results from the experiments examining seed persistence give no indication that *C. pauciflorus* seed exhibits dormancy after overwintering. Dormancy in *C. pauciflorus* has been discussed in the literature and one study stated that seeds of this species were more likely to exhibit dormancy if they originated from cooler regions (Nyamongo et al. 2010). The lack of dormancy being reported for *C. pauciflorus* in our experiments might be due to the fact that the seed embryos are small and thin, making longitudinal dissections of the embryo challenging and observing colour change to the TTC test challenging. This could result in us under reporting the proportion of dormant seeds. TTC is a convenient way to gauge the status of cellular respiration in seeds within a short period of time. However, it might not reliably test viability in seeds where a structural barrier in the form of a seed coat causes dormancy, or when the seed is damaged from disease. Additionally, a negative TTC test might simply indicate the low metabolic activity of physiologically dormant seeds, which need to be stratified for longer periods to unblock the corresponding chemical pathway (Vankus 1997). Use of the pouch method without addition of supplementary soil might have also lead to an overestimation of nonviable seeds if the seeds were not constantly in contact with surrounding soil. While the seedling emergence method (Ter Heerdt et al. 1996) might be a more accurate way to estimate seed bank composition, there are few *in situ* alternative ways to evaluate seed banks in a realistic environmental setting. Hence, the pouch method was used to gain insight into the overwintering ability of *E. lagascae* and *C. pauciflorus* seeds.

Based on this, the results of these experiments suggest that *E. lagascae* seeds might exhibit dormancy. Given that there is no information about the dormancy of *E. lagascae* in current scientific literature, the findings of this experiment provides some insights that this species shows extremely low levels of potential dormancy and suggests that this species is unlikely to leave a viable seedbank. In general, this observation indicates that *E. lagascae* seed is capable of overwintering and potentially forming a modest seed bank. These results support the anecdotal observation that *E. lagascae* seeds that were sown in summer of 2014 were emerging in late winter/early spring 2015 at Woodstock, ON (Dr. J. Todd, personal communications). Missing data owing to predation of seed packets that were pinned to the surface of the soil might have led to an underestimation of the ability of *E. lagascae* to leave a persistent seedbank.

The results from the experiments examining the ability of seeds to establish under different conditions suggest that *C. pauciflorus* establishes better from seed dispersed in the spring versus the fall. This makes sense given that *C. pauciflorus* is an annual and emergence may be driven by accumulated growing degree days and perhaps to some extent by a cue of rising temperatures, which is common for summer annuals (Van Acker et al. 2000). The ideal soil temperatures for germinating *C. pauciflorus* are not known. However, this finding would also relate to the warm climate experienced by this species at the time of dispersal in its native Africa. In contrast, the results of this study suggest that *E. lagascae* seedlings may emerge well either from spring or fall sown seed, in spite of its 6 °C base germination temperature (Zanetti et al. 2013). The highest emergence level achieved by *C. pauciflorus* in our study was 12.15% and 9.75% for *E. lagascae*. Some of the *C. pauciflorus* and *E. lagascae* plants that were sown in the spring did go on to flower. However, frost killed these plants before they could produce mature seeds. The fall-sown *C. pauciflorus* plants were killed by frost before they could even finish flowering and they did not regrow the following spring. A very small number of *E. lagascae* seedlings emerged in the spring from seeds that had not emerged in the previous fall. Using seeds from a newer source also enhanced *E. lagascae* emergence levels across all treatments in spring 2015. The opposite was true for *C. pauciflorus*, with fewer seeds emerging from newer seeds in spring 2015 than from older seeds in spring 2014.

The critical period of weed control in soybean has been identified to be the first flowering stage (R1). Weeds need to be controlled before the R1 stage to prevent permanent yield loss to soybean (Van Acker et al. 1993). These experiments were run until soybeans reached this development stage. The non-competitive commensalism exhibited in the first run of the experiment is not uncommon as a form of interaction between weeds and crops (Radosevich 1987) if resources (especially space) are not limiting. While a replacement series set up is a convenient method to explore relative competitiveness of species when no prior information is available, this method tests interference in a one-dimensional plane using two species densities (Jolliffe 2000). It is often difficult to identify if results are due to inter and intra-specific competition and a small sample size can lead to skewed results (Jolliffe 2000). Large outdoor multidimensional experiments that are more realistic should be the next step to studying crop-weed competition among these species in the future (Radosevich 1987).

Evidently, pot size had an impact on competition in these experiments as has been shown to be the case in other indoor weed competition experiments (Poorter et al. 2012). A TRB of less than one observed in the second run of the experiment conducted in smaller pots is indicative of antagonistic competition. Additionally, the concave shape of the *C. pauciflorus* and *E. lagascae* RB curves is reminiscent of Model IIa proposed by Radosevich (1987), in which the more competitive species (soybean in this case) "contributes more than expected to the total yield, while the other contributes less than expected. This is the model for competition. In each combination, one curve is always concave while the other is always convex, indicating that the interaction between species is for a common resource(s) and that one species gains more than the other" (Radosevich 1987). According to this model, the two species are competing for resources where soybean is acquiring more of the shared resources than the other species. Soybean and pigweed RBs in the second run also appeared as convex and concave curves respectively. However, since their TRB was closer to one, resources were being shared rather than being competed for. These are characteristic of Model IIa proposed by Radosevich (1987). The similar performance of *C. pauciflorus* and *E. lagascae* to pigweed when faced with a competitor shows that *C. pauciflorus* and *E. lagascae* perform similarly to pigweed in terms of relative competitiveness with soybean on a per plant basis. However, at low densities, neither *C. pauciflorus*, *E. lagascae*, nor pigweed may be competitive with soybean. Based on a replacement series examining soybean-pigweed completion, it was suggested that soybean yields are likely to be affected if more than a quarter of the plants in a mixed stand of soybean and pigweed are pigweed (Chivinge and Schwappenhauser 1995). When soybeans were grown with velvetleaf in a similar replacement series set up, competition became more apparent 59 d after planting, when TRB started to dip below one as velvetleaf started interfering with soybean growth by increasing its RB relative to soybean (Akey et al. 1991).

A weed escape is likely to cause farmers problems if it produces a large number of seeds that are persistent and live long in the field (Van Acker 2009). For example, a single pigweed can produce between 5000 to 100,000 seeds, which is considered to be very high even among weeds. These seeds in turn are also highly dormant, being able to persist in the soil for 10–40 y (Van Acker 2009). Density also plays an important role in any plant-plant competition scenario. Pigweed as a very competitive weed (Cowen et al. 1998) caused up to 45% yield loss when present at high densities with soybean in an additive experiment (Dieleman et al. 1995). Furthermore, competition with soybean is more fierce (12.3% soybean yield loss) when the time of pigweed emergence coincides with the time of emergence of soybean (Dieleman et al. 1995).

## Conclusion

Even if *E. lagascae* or *C. pauciflorus* were to escape as new weed species into the field, neither species satisfies the criteria of a problematic weed (such as pigweed). It is quite unlikely that either *E. lagascae* or *C. pauciflorus* could produce massive amounts of seeds in order to achieve the high densities that pigweed can. Although the competitive ability of *E. lagascae* and *C. pauciflorus* is possibly comparable to that of pigweed on a per plant basis, the inability of the two species to produce high density pigweed-like infestations reduces their threat as weeds. Viable *C. pauciflorus* seeds were not dormant. Although *E. lagascae* produced some dormant seeds, the percentage was so small that is it highly unlikely that the seeds of this species will produce a large persistent seedbank in the field. The ability of *E. lagascae* and *C. pauciflorus* to establish in competitive environments and produce very small stands also limits the threat posed by either species as weeds, especially if they cannot produce mature seeds before they are killed off by frost. The results from this study suggest that the potential weediness of *E. lagascae* and *C. pauciflorus* is low due to their low seed production capability, low potential for producing a persistent seedbank, and poor overwintering ability. However, since these studies were carried out in the context of exploring the potential weediness of these species as potential crops, seed selections that were on their way to becoming cultivars were used instead of a collection of wild types. At this point, it is difficult to discern if the results of this study can be extrapolated to *E. lagascae* and *C. pauciflorus* plants of different genotypes found in the wild.

## Acknowledgements

We thank OMAFRA for the Highly Qualified Personnel (HQP) Scholarship, and Rob Grohs and Rachel Riddle for their technical assistance.

## References

Akey, W.C., Jurik, T.W., and Dekker, J. 1991. A replacement series evaluation of competition between velvetleaf (*Abutilon theophrasti*) and soybean (*Glycine max*). Weed Res. **31**: 63–72. doi:10.1111/wre.1991.31.issue-2.

Angelini, L.G., Moscheni, E., Colonna, G., Belloni, P., and Bonari, E. 1997. Variation in agronomic characteristics and seed oil composition of new oilseed crops in central Italy. Ind. Crops Prod. **6**: 313–323. doi:10.1016/S0926-6690(97)00022-8.

Baker, H.G. 1974. The evolution of weeds. Ann. Rev. Ecol. Syst. **5**: 1–24. doi:10.1146/annurev.es.05.110174.000245.

Barney, J.N., and DiTomaso, J.M. 2010. Invasive species biology, ecology, management and risk assessment: Evaluating and mitigating the invasion risk of biofuel crops. Pages 263–284 in Plant biotechnology for sustainable production of energy and co-products. Springer, Berlin Heidelberg.

Baye, T., and Becker, H.C. 2005. Exploration of *Vernonia galamensis* in Ethiopia, and variation in fatty acid composition of seed oil. Genet. Resour. Crop Ev. **52**: 805–811. doi:10.1007/s10722-003-6086-5.

Baye, T., Kebede, H., and Belete, K. 2001. Agronomic evaluation of *Vernonia galamensis* germplasm collected from Eastern

Ethiopia. Ind. Crops Prod. **14**: 179–190. doi:10.1016/S0926-6690(01)00082-6.

Boyd, N.S., and Van Acker, R.C. 2004. Seed and microsite limitations to emergence of four annual weed species. Weed Sci. **52**: 571–577. doi:10.1614/WS-03-118R.

Breemhaar, H.G., and Bouman, A. 1995. Harvesting and cleaning *Euphorbia lagascae*, a new arable oilseed crop for industrial application. Ind. Crops Prod. **4**: 173–178. doi:10.1016/0926-6690(95)00029-C.

Bullied, W.J., Van Acker, R.C., and Bullock, P.R. 2012. Review: Microsite characteristics influencing weed seedling recruitment and implications for recruitment modeling. Can. J. Plant Sci. **92**: 627–650. doi:10.4141/cjps2011-281.

Buddenhagen, C.E., Chimera, C., and Clifford, P. 2009. Assessing biofuel crop invasiveness: A case study. PLoS One, **4**: e5261. doi:10.1371/journal.pone.0005261. PMID:19384412.

Chen, Z., Silva, H., and Klessig, D.F. 1993. Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science, **262**: 1883–1886. doi:10.1126/science.8266079. PMID:8266079.

Christou, M., Alexopoulou, E., Pages, X., Alfos, C., Monti, A., and Nissen, L. 2012. Non-food crops-to-industry schemes in EU27 WP1. Non-food crops. [Online]. Available: http://www.crops2industry.eu/images/pdf/members_area/winschoten/1.%20MYRSINI.pdf [17 Dec. 2013].

Chivinge, O.A., and Schwappenhauser, M.A. 1995. Competition of soybean with blackjack (*Bidens pilosa* L.) and pigweed (*Amaranthus hybridus* L.). Afr. Crop Sci. J. **3**: 73–82.

Cowan, P., Weaver, S.E., and Swanton, C.J. 1998. Interference between pigweed (*Amaranthus* spp.), barnyardgrass (*Echinochloa crus-galli*), and soybean (*Glycine max*). Weed Sci. **48**: 533–539.

Dierig, D.A., Thompson, A.E., Ray, D.T., and Coffelt, T.A. 2006. Registration of three day-neutral germplasms. Crop Sci. **46**: 2335–2336. doi:10.2135/cropsci2006.04.0222.

Dieleman, A., Hamill, A.S., Weise, S.F., and Swanton, C.J. 1995. Empirical models of pigweed (*Amaranthus* spp.) interference in soybean (*Glycine max*). Weed Sci. **43**: 612–618.

du Croix Sissons, M.J., Van Acker, R.C., Derksen, D.A., and Thomas, A.G. 2000. Depth of seedling recruitment of five weed species measured in situ in conventional- and zero-tillage fields. Weed Sci. **48**: 327–332. doi:10.1614/0043-1745(2000)048[0327:DOSROF]2.0.CO;2.

Earle, F.R. 1970. Epoxy oils from plant seeds. J. Amer. Oil Chem. Soc. **47**: 510–513. doi:10.1007/BF02639239.

Gilbert, M.G. 1986. Notes on East African Vernonieae (Compositae) a revision of the *Vernonia galamensis* complex: Notes on East African Vernonieae (Compositae) 4. Kew Bull. **41**: 19–35. doi:10.2307/4103021.

Grundy, A.C., Mead, A., and Bond, W. 1996. Modelling the effect of weed seed distribution in the soil profile on seedling emergence. Weed Res. **36**: 375–384. doi:10.1111/wre.1996.36.issue-5.

Harper, J.L. 1977. Population biology of plants. Academic Press, London, UK.

[ISTA] International Seeding Testing Association. 1985. International rules for seed testing. Seed Sci. Technol. **13**: 299–513.

Jolliffe, P.A. 2000. The replacement series. J. Ecol. **88**: 371–385. doi:10.1046/j.1365-2745.2000.00470.x.

Karssen, C.M., and Laçka, E. 1986. A revision of the hormone balance theory of seed dormancy: Studies on gibberellin and (or) abscisic acid-deficient mutants of *Arabidopsis thaliana*. Pages 315–325 in Plant growth substances 1985. Springer, Berlin Heidelberg.

Katzman, L.S., Taylor, A.G., and Langhans, R.W. 2001. Seed enhancements to improve spinach germination. Hort. Sci. **36**: 979–981.

Khan, A.A. 1992. Preplant physiological seed conditioning. Hortic. Rev. **13**: 131–181.

Kleiman, R., Smith, C.R., Jr., Yates, S.G., and Jones, Q. 1965. Search for new industrial oils. XII. Fifty-eight euphorbiaceae oils, including one rich in vernolic acid. J. Amer. Oil Chem. Soc. **42**: 169–172. doi:10.1007/BF02541123.

Krewson, C.F., and Scott, W.E. 1966. *Euphorbia lagascae* Spreng., an abundant source of epoxyoleic acid; seed extraction and oil composition. J. Amer. Oil Chem. Soc. **43**: 171–174. doi:10.1007/BF02646296.

Mebrahtu, T., Gebremariam, T., Kidane, A., and Araia, W. 2009. Performance of *Vernonia galamensis* as a potential and viable industrial oil plant in Eritrea: Yield and oil content-1. Afr. J. Ecol. **8**: 635–640.

Mohler, C.L., and Galford, A.E. 1997. Weed seedling emergence and seed survival: Separating the effects of seed position and soil modification by tillage. Weed Res. **37**: 147–155. doi:10.1046/j.1365-3180.1997.d01-21.x.

Nyamongo, D.O., Daws, M.I., Nyabundi, J.O., Hay, F.R., and Ayiecho, P.A. 2010. Onset of dormancy, dormancy levels, and appropriate seed production environment for two subspecies of *Vernonia galamensis* (Cass.) Less. J. New Seeds, **11**: 16–27. doi:10.1080/15228860903518141.

Perdue, R.E., Carlson, K.D., and Gilbert, M.G. 1986. *Vernonia galamensis*, potential new crop source of epoxy acid. Econ. Bot. **40**: 54–68. doi:10.1007/BF02858947.

Poorter, H., Bühler, J., van Dusschoten, D., Climent, J., and Postma, J.A. 2012. Pot size matters: A meta-analysis of the effects of rooting volume on plant growth. Funct. Plant Biol. **39**: 839–850. doi:10.1071/FP12049.

Radosevich, S.R. 1987. Methods to study interactions among crops and weeds. Weed Technol. **1**: 190–198.

Rao, S.C., Akers, S.W., and Ahring, R.M. 1987. Priming *Brassica* seed to improve emergence under different temperatures and soil moisture conditions. Crop Sci. **27**: 1050–1053. doi:10.2135/cropsci1987.0011183X002700050045x.

Roseberg, R.J., and Shuck, R.A. 2008. Agronomic requirements of *Euphorbia lagascae*: A potential new drought-tolerant crop for semi-arid Oregon. Pages 1–23 in Agronomic Research in the Klamath Basin 2008 Annual Report.

Sheldon, J.C., and Burrows, F.M. 1973. The dispersal effectiveness of the achene–pappus units of selected Compositae in steady winds with convection. New Phytol. **72**: 665–675. doi:10.1111/nph.1973.72.issue-3.

Smith, N.O., Maclean, I., Miller, F.A., and Carruthers, S.P. 1997. Crops for industry and energy in Europe. Office for Official Publication of the European Communities, University of Reading, Luxembourg, 72 pp.

Stoller, E.W., and Wax, L.M. 1973. Periodicity of germination and emergence of some annual weeds. Weed Sci. **6**: 574–580.

Ter Heerdt, G.N.J., Verweij, G.L., Bekker, R.M., and Bakker, J.P. 1996. An improved method for seed-bank analysis: Seedling emergence after removing the soil by sieving. Funct. Ecol. **10**: 144–151. doi:10.2307/2390273.

Thompson, A.E., Dierig, D.A., and Kleiman, R. 1994. Characterization of *Vernonia galamensis* germplasm for seed oil content, fatty acid composition, seed weight, and chromosome number. Ind. Crops Prod. **2**: 299–305. doi:10.1016/0926-6690(94)90121-X.

Turley, D., Froment, M., and Cook, S. 2000. Development of *Euphorbia lagascae* as a new industrial oil crop. European Community Concerted Action (FAIR-CT98/4460) Handbook. ADAS Woodthorne, Wolverhampton, UK. 38 pp.

White, G.A., and Wolff, I.A. 1968. From wild plants to new crops in USA. World Crops, **6**: 70–76.

Van Acker, R.C. 2009. Weed biology serves practical weed management. Weed Res. **49**: 1–5. doi:10.1111/wre.2009.49.issue-1.

Van Acker, R.C., Swanton, C.J., and Weise, S.F. 1993. The critical period of weed control in soybean [*Glycine max* (L.) Merr.]. Weed Sci. **41**: 194–200.
Van Acker, R.C., Thomas, A.G., Leeson, J.Y., Knezevic, S.Z., and Frick, B.L. 2000. Comparison of weed communities in Manitoba ecoregions and crops. Can. J. Plant Sci. **80**: 963–972. doi:10.4141/P99-175.
Vankus, V. 1997. The tetrazolium estimated viability test for seeds of native plants. Pages 57–62 in T.D. Landis and J.R. Thompson, tech. cords. National Proceedings, Forest and Conservation Nursery Associations, Gen. Tech. Rep. PNW-GTR-419. U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, OR.
Vogel, R., Pascual-Villalobos, M.J., and Röbbelen, G. 1993. Seed oils for new chemical applications. 1. Vernolic acid produced by *Euphorbia lagascae*. Angew. Bot. **67**: 31–41.
Zanetti, F., Monti, A., and Berti, M.T. 2013. Challenges and opportunities for new industrial oilseed crops in EU-27: A review. Ind. Crop Prod. **50**: 580–595. doi:10.1016/j.indcrop.2013.08.030.