Growth environment but not seed position on the parent plant affect seed
germination of two *Thlaspi arvense* L. populations

R.W. Gesch^{a*}, A. Royo-Esnal^b, E. Edo-Tena^b, J. Recasens^b, T. Isbell^c, and F. Forcella^a

^aUSDA-ARS-North Central Soil Conservation Research Laboratory, Morris, MN, USA 56267

^bDepartament d'Hortofructicultura, Botànica i Jardineria, Agrotecnio, Universitat de Lleida,
Lleida, Spain 25198

^cUSDA-ARS-National Center for Agriculture Utilization Research, Peoria. IL USA

*Corresponding Author: Russ W. Gesch, Address: USDA-ARS-NCSCRL, 803 Iowa Ave.,
Morris MN 56267, Telephone Number: (320) 589-3411 x-132, FAX Number: (320) 589-3787
E-mail: russ.gesch@ars.usda.gov

ABSTRACT

Thlaspi arvense L. is a common weed found in most temperate regions throughout the world that also shows excellent potential for domestication as an oilseed crop. The complexity of *T. arvense* seed dormancy presently makes it difficult to predict its emergence and manage it as an oilseed crop. Therefore, a better understanding of factors controlling seed dormancy will help develop strategies for managing its weediness and facilitate development of crop genotypes. *T. arvense* has semi-indeterminate growth and seeds developed at the base of the inflorescence (basal) generally mature sooner than those near the top (apical). However, little is known about the maternal influences on the level of dormancy and germination of basal and apical seeds. To study this, two *T. arvense* populations, one from Spain and another from USA, were both grown at field sites in Almenar, Spain and Morris, Minnesota, USA during the same growing season (autumn 2012-summer of 2013). Basal and apical seeds collected at maturity were analyzed for mass, total protein and carbon content, oil content, and fatty acid profiles. Under controlled environment conditions, seed germination was measured at 5, 8, 11, 14, 17, and 20°C and germination rate estimated at 8°C. Seed position on the inflorescence did not affect germination consistently nor were there clear differences in seed attributes between positions. However, seeds of both populations produced at Almenar had lower germination and were consistently larger and contained higher oil and carbon content than seeds produced at Morris. Results indicate that environmental influences at each growth location played a larger role in influencing dormancy and germination of seeds produced than did the seed attributes measured or position of seeds on the inflorescence.

Keywords: *Thlaspi arvense*; Pennycress; Germination; Seed dormancy; Seed oil

1. Introduction

Thlaspi arvense L., often referred to as field pennycress, is widely recognized as a weed in several crops (Warwick et al., 2002). Recently, however, *T. arvense* has gained considerable attention as a winter oilseed cover crop for double cropping systems with soybean [*Glycine max* (L.) Merr.] as the primary crop in the Upper Midwest of the USA (Phippen and Phippen, 2012; Johnson et al., 2015). Moreover, efforts are currently underway to domesticate *T. arvense* as a viable oilseed crop. The genome of *T. arvense* has been sequenced recently (Dorn et al., 2013), and the genomics tools developed are being used in breeding programs to improve its agronomic and end-use characteristics (Sedbrook et al., 2014). Seed oil of *T. arvense* lends itself well to making biodiesel and renewable aviation fuel (Moser et al., 2009; Fan et al., 2013).

T. arvense is a wide spread weed species, commonly found in most temperate regions in the northern and southern hemispheres (Warwick et al., 2002). Its success as a weed is in part due to its ability to withstand climatic extremes of temperature and drought, as well as the complex nature of primary and secondary dormancy of its seeds, and thus, the ability of its seed bank to persist for extended periods (Hazebroek and Metzger, 1990a). Gaining a better understanding of environmental and maternal influences affecting its seed dormancy, and hence, germination characteristics will help to develop strategies for weed management and provide necessary information for developing domestic genotypes lacking dormancy (Sedbrook et al., 2014).

Both growth environment (Saarinen, et al., 2011) and influences on developing seed by the parent plant (Hume, 1994; Donohue et al., 2007) can impart various degrees of seed dormancy and affect germination. With regards to maternal influences, seed traits such as large seed size (Moles et al., 2000) and high oil content (Gardarin et al., 2011) have been linked generally to

greater seed germination. *T. arvense* is semi-indeterminate with respect to flowering and seed set, with seed development initiating from the bottom (basal) and progressing to the top (apical) of the inflorescence similar to the reproductive phenology of *Camelina sativa* L. (Martinelli and Galasso, 2011). Few, however, have studied the differences in physicochemical characteristics between apical and basal seeds of *T. arvense* and whether they affect the degree of dormancy and germination of the seed.

We hypothesized that because of the temporal variation in the development between apical and basal seed on the inflorescences of *T. arvense*, the physicochemical attributes of the seed will be affected and therefore, will influence the level of dormancy and germination between the seed types. To test this hypothesis, two different *T. arvense* populations (i.e., accessions), one originating from northeastern Spain and the other from north-central USA, were both grown at field sites in Almenar, Spain and Morris, Minnesota, USA during the same growing season (autumn 2012 to summer of 2013). Basal and apical seeds were collected from plants at maturity and were analyzed for mass and biochemical attributes. In a separate controlled environment experiment, the germination of these seeds was measured at 5, 8, 11, 14, 17, and 20°C and germination rate estimated at 8°C.

2. Materials and methods

2.1 Cultural practices, site description

T. arvense seeds used for the study were harvested from mature plants between June and July 2012 in Morris, Minnesota USA (45°43'36''N-95°49'17''W) and Camerillas, Teruel, Spain (40°38'39''N-0°48'35''W). Seeds were stored dry under laboratory conditions after harvest

until they were sown for the study in early autumn. Portions of the two seed lots were interchanged and both seed populations were sown for the study at each field site. The field site in Spain was at Almenar (41°46'36''N-0°32'7''E) and in USA was at Morris (same as above). Spring wheat (*Triticum aestivum* L.) was the previous crop at both sites.

Seeds were sown in 1 m² plots in a randomized complete block design with four replicates. The treatment variable was seed origin (Spain or USA). In each plot, 1000 seeds were sown at a depth of 1 cm in four rows (250 seed per row) spaced 25 cm apart to simulate cultivation conditions and to facilitate hand-weeding of the plots. This also helped to identify and remove any volunteer seedlings. Sowing dates were 18 September 2012 for Morris and 4 October 2012 for Almenar.

When plants had fully matured (silicles were dry and brittle and seeds had a dark reddish-brown color), from 10 to 25 randomly chosen plants were harvested from each plot and brought to the laboratory for processing. Plants in Almenar were harvested 3 June 2013 and in Morris they were harvested 9 July 2013. For each plant harvested, five to seven silicles from the bottom (basal seed) and five to seven silicles from the top (apical seed) of the main raceme were sampled, their seeds collected, and the 1000-seed weights determined for each plot.

2.2 Seed chemical analyses

For chemical analyses, because the individual (replicate) seed samples were relatively small, for each population and growth location, all four replicates of basal seed were combined (i.e., pooled) as were those of the apical seed. All chemical analyses of seed were done in triplicate by sub-sampling the pooled seed of apical and basal seeds. Approximately 200 mg of finely ground seed was used to determine total N and C using a Leco CN-2000 combustion analyzer (Leco

Corporation, St. Joseph, MI) and the seed N content was multiplied by 6.25 to convert to estimated protein content (Gesch et al., 2014). Approximately 0.5 to 2.0 g of whole seed were used to determine total oil content non-destructively by pulsed NMR using a Bruker Minispec pc120 (Bruker, The Woodlands, TX). The instrument was calibrated with pure *T. arvense* oil. Additionally, approximately 50 mg of ground seed was extracted with hexane to make fatty acid methyl esters (FAME), which were then used to determine fatty acid profiles of the seed oil by gas chromatography (Hewlett-Packard 5890 Series II, Palo, Alto, CA) equipped with a flame-ionization detector and an auto-sampler/injector using the methods previously described by Forcella et al. (2005).

2.3 Germination experiment

The basal and apical seeds that were collected from the field experiment (2012-2013) were used to conduct germination tests at Lleida, Spain during 2013 and 2014. Seed germination was tested in controlled environment chambers at constant temperatures of 5, 8, 11, 14, 17, and 20°C in the dark. At each temperature, for each pennycress population and seed type (basal and apical), 4 replicates of 25 seeds each were tested. The seeds were placed in Petri dishes on a medium of 14% agar and the number of germinated seeds was counted daily for 21 d under a green light similar to the procedure used by Torra et al. (2015) for five *Ranunculaceae* species. A green light was used for this purpose to avoid red wavelengths of light, which are known to influence dormancy, and hence, germination of seeds (Shinomura, 1997). The germination experiment under all six temperatures was repeated three times for seeds collected from plants grown in Almenar and twice for seeds collected in Morris. The data are presented as mean total percent germination after 21 d, and data collected for the 8°C treatment (found to be generally

most optimal for germination) were used to determine germination rate by analyzing germination as a function of time using a Boltzmann sigmoidal function of the form:

$$Y = A / \{ 1 + \exp[2 * \ln(9) * (G_{50} - GDD) / B] \}$$

where Y is percent seed germination, GDD is accumulated growing degree days, A is percent maximum germination, G_{50} is growing degree days from the start of the experiment to 50% of maximum germination, and B is growing degree days from 10 to 90% of maximum germination (Gesch and Archer, 2005). Previously, a first estimation of the base temperature (T_b) was performed for each seed type, as described by Bradford (2002). This estimation was performed by calculating the inverse of the time (days) needed for 50% of germination at each of the six constant temperatures. The points were then regressed against temperature and intersect with 0 was used as T_b for calculating the GDD for each seed type.

2.4 Statistical analysis

Data of percent germination and chemical composition of seed were analyzed by 2-way ANOVA for each of the two pennycress populations studied using the General Linear Model Procedure of SAS (SAS for Windows 9.1, SAS Inst., Cary, NC). The main effects and their interaction used in the model were location (i.e., Almenar and Morris) where the seed was produced and position of seed (i.e., basal and apical) on the plant. When effects were significant at the $P \leq 0.05$ level, mean comparisons were made using Fisher's LSD test. Germination rate was estimated using JMPPro11 (SAS Inst., Cary, NC).

3. Results

3.1 Climate

Between sowing and seed collection of *T. arvense*, the overall average air temperature at the Morris location was 3.8°C compared to 10.0°C at Almenar for the same period (Table 1). During the winter months (November to March), the temperature averaged 14°C lower in Morris compared to Almenar. The number of accumulated GDD (using a base temperature of 4°C) from sowing to harvest was 1198°C d at Morris and 1387°C d at Almenar. Although the total amount of precipitation received at both sites was not greatly different, the pattern of accumulation was (Table 1). For instance, only 16% of total precipitation at Morris fell between September and January, and 84% fell between February and July. In contrast, total precipitation at Almenar was divided evenly between the two periods of October through January and February through June (Table 1).

3.2. Seed germination and germination rate

No clear pattern of total germination distinguished apical from basal seeds of the Spanish population (Fig. 1 A and C). Apical seed of the Spanish population grown at Almenar had higher germination than basal seed at 5, 8, and 17°C, but when grown at Morris, their basal seed had higher germination than apical seed at 17 and 20°C. For the USA population, there was a tendency for apical seed to have greater germination than basal seed (Fig. 1 B and D). However, for this population grown at Almenar, differences between apical and basal seeds were not significant at any temperature, whereas apical seed germination was higher than basal seed at 5, 11, and 14°C from plants grown at Morris.

One clear pattern that developed, was that seeds from plants of both populations grown at Morris always had higher germination rates than those from plants grown at Almenar at all

temperatures except for the apical seeds of the USA population at 5°C (Fig. 1 A-D). Also, for plants grown at Morris, across all temperatures tested, the seeds of the Spanish population tended to have higher germination rates than the USA population (Fig. 1 C and D; $P \leq 0.05$), which may reflect seed size differences (see below).

In general, the rate of germination at 8°C, as determined by the parameters G_{50} (i.e., accumulated GDD from initiation to 50% of maximum germination) and B (i.e., GDD from 10 to 90% of maximum germination) of the Boltzmann sigmoid function, were similar between apical and basal seeds of both populations regardless of growing location (Table 2). However, for the Spanish population grown in Morris, apical seed did have a lower G_{50} (69 GDD) than basal seed (85 GDD) indicating a more rapid initiation of germination, although the rate of germination following initiation, as determined by B , did not differ (Table 2). As expected, there were differences in parameter A (i.e., maximum germination) in several instances between seed position and populations, which are also reflected in the results shown in Figure 1. In comparison between growth locations, when averaged across both populations and seed position, seeds produced in Morris generally had lower B (43 GDD) and G_{50} (79 GDD) values than those produced in Almenar (B 59 and G_{50} 88), indicating a slightly faster germination rate for the seeds produced in Morris regardless of seed origin.

3.3 Seed physicochemical attributes

Seed mass was not different between apical and basal seed of the Spanish population, but it was different for the USA population where basal seed was heavier at both locations grown (Table 3). For both populations, seed mass was generally greater for seeds produced in Almenar Spain. In comparison, the Spanish-origin seeds tended to be heavier than seed of the USA

population regardless of location grown (Table 3). Averaged across growth locations, the Spanish seeds were 1.25 g per 1000-seeds, whereas the USA seeds weighed 0.97 g per 1000-seeds ($P \leq 0.05$).

Seed oil content varied between apical and basal seed of the Spanish population at Morris and Almenar but was consistently higher in basal seed for the USA population (Table 3). Seed oil content of both populations was greater for seeds produced in Almenar, consistently $> 32\%$ (Table 3). When grown in Morris, however, seed oil dropped to 27% for Spanish seeds and 31% for USA seeds. When averaged across growing locations, the USA seed had higher oil content (31.5%) than the Spanish seed (29.4%; $P \leq 0.05$).

Protein and carbon content did not differ between apical and basal seed of the Spanish population at either growing location but both were greater in basal compared to apical seed of the USA population (Table 3). Overall, protein content of the Spanish population was greater for seeds produced in Morris but there was no difference between locations for the USA population. In comparison of the two populations, when averaged across locations, seeds of the Spanish population generally had greater protein and lower carbon content than seeds of the USA population ($P \leq 0.05$).

The fatty acid (FA) profile of seed oil differed slightly between apical and basal seed of the Spanish population (Table 4). More intriguing, however, were differences in FA distribution for seeds produced between locations. For the Spanish population, seeds produced in Almenar contained a higher degree of FA unsaturation (i.e., C18:2 and C18:3) than seeds produced in Morris. Conversely, the Morris-grown seeds had greater saturation of FAs (i.e., C16:0, C18:1, C20:1, and C22:1) than those from Almenar (Table 4). There were no differences in FA distribution between apical and basal seed for the USA population grown at either location.

However, there was again a difference in saturation in FAs between the two locations with the seed produced in Morris having a greater level of saturation (Fig. 2).

When averaged across both growing locations, the two different populations tended to have relatively similar FA profiles (data not shown). However, the Spanish population had a significantly greater ($P < 0.05$) erucic acid (C22:1) content (35.7%) than the USA population (33.2%). This came at a cost of its C18:1 and C18:2 FA content, which were about 1 to 2% lower than that of the USA population seed.

4. Discussion

In the present study, seed position on the stem did not have a clear effect on seed germination for either of the *T. arvense* populations regardless of growth location. This disproved our hypothesis that basal and apical seeds would differ in their degree of germination due to differences in the accumulation of seed reserves resulting from temporal differences in seed development on the inflorescence. Nevertheless, there was a clear pattern of substantially higher seed germination at all temperatures tested for both populations grown in Morris compared to seeds produced in Almenar. Furthermore, seeds of both populations produced in Morris generally had faster germination rates than those from Almenar. Lower total germination and slower germination rate are indicative of dormancy in weed seeds (Colbach et al., 2006; Gardarin et al., 2011), and our results indicate that seeds from Almenar developed a greater degree of dormancy. The question then becomes how and why?

With regard to the relationship between germination and physicochemical attributes of the seed tested, certain associations were found. Seed mass, oil content, and total carbon content

were always greater in the seeds produced at Almenar. Seed size and shape can influence germination and dormancy among weed species (Moles et al., 2000) and within a species (Baloch et al., 2001). Studying a relatively wide range of seed size (< 6 to > 11 mg) from a single population of *Abutilon theophrasti* Medik., Baloch et al. (2001) found that germination was greatest for seed that ranged from 8.0 to 9.9 mg and became lower with seeds of higher or lower mass.

Lipids have a higher energy content than other primary storage reserves found in seeds, such as protein and sugars, and they have been linked to variations in germination and dormancy of a wide range of weed species (Bretagnolle et al., 2015; Gardarin et al., 2011; Linder, 2000). High seed oil content typically has been associated with greater germination and germination rate (Gardarin et al., 2011), although its potential involvement in dormancy is not fully clear (Gardarin and Colbach 2015; Okagami and Terui, 1996). In the case of *T. arvense*, our results showed that higher oil content was associated with low germination and greater dormancy. Also intriguing was the difference that we found in the saturation of oil between seeds produced in Almenar and Morris. Differences in the metabolism of various fatty acids during the germination process may affect germination rate and even total germination (Okagami and Terui, 1996). Linder (2000) proposed that the lower melting point of unsaturated compared to saturated fatty acids, allows seeds with high unsaturated FA levels to germinate earlier and grow faster at low temperatures. Results from the present study, however, do not appear to fit this theory, as the seeds of both *T. arvense* populations produced in Almenar had higher unsaturation of FA levels in their seeds [i.e., $(C18:2 + C18:3)/(C16:0 + C18:1 + C20:1 + C22:1)$] than those produced in Morris, but also had lower and slower germination. However, it should also be noted that

saturated fatty acids (i.e., C16:0, C18:0, and C20:0) in the seed oil of both populations regardless of growth location were generally low, only making up 3 to 4% of the total oil.

The differences observed in germination and dormancy of *T. arvense* from Almenar and Morris were far more likely due to environmental conditions experienced (Gulden et al., 2004; Saarinen et al., 2011), or maternal influence (Hume, 1994; Donohue et al., 2007), during maturation and seed development. Environmental conditions were generally drier and cooler during seed development at Almenar, which might have impacted their germination. Both temperature and dry conditions have been shown to influence dormancy in *T. arvense* seed (Hazebroek and Metzger, 1990a; Hazebroek and Metzger, 1990b). Plants grown at Almenar, most of which emerged in autumn (Royo-Esnal et al., 2015), flowered in April, and their seed matured in May, while at Morris, most plants emerged in the spring, began flowering in late May, and their seeds matured in June. During May and June, plants at Morris received much more precipitation (240 mm) than did plants at Almenar (92 mm) during April and May when they were flowering and setting seeds (Table 1). Also, temperatures were warmer at Morris during seed development than Almenar (Table 1). Lastly, daylength during seed maturation in Almenar (May) and Morris (June) averaged 14.3 and 15.4 hr, respectively (Table 1), and may have influenced oil contents and FA profiles (Seiler, 1986).

Depending on environmental conditions, *T. arvense* can act as a winter or spring annual (Baskin and Baskin, 1989; Warwick et al., 2002). Saarinen et al. (2011) found that *T. arvense* plants that emerged in the autumn and experienced a period of warming during the winter tended to impart a greater degree of dormancy on seeds that they produced compared to seeds produced by plants that emerged in the spring. Similar results were observed in *Papaver rhoeas* L., where seeds from plants germinated in autumn-winter had greater dormancy than those seeds from

plants germinated in spring (April) (Torra and Recasens, 2008). This to a large degree may explain the results for germination that we observed for seeds produced from plants in Almenar and Morris. At Almenar, the proportion of the USA population that emerged in autumn was 87% with 13% emerging in the spring, while for the same population in Morris, only 9% emerged in autumn and 91% in the spring (Royo-Esnal et al., 2015), and results were nearly identical for the Spanish population.

Also, the length of time for plants to mature may have played a role in imparting dormancy. Others have also shown that the maternal environment can affect growth of plants and the dormancy of their offspring (Hume, 1994; Donohue et al., 2007). For instance, Hume (1994) found that seeds produced by a late-flowering strain of *T. arvense* germinated faster than seeds collected from an early-flowering strain. In the present study, both *T. arvense* populations grown at Almenar matured over a longer period, flowered earlier, and accumulated more GDD (Table 1) than plants at Morris, in part due to Almenar's milder winter. This is the most likely reason why Almenar-grown seeds were generally much larger and contained more oil and total carbon content than Morris-grown seeds. Our results corroborate those of Saarinen et al. (2011), who also found that seeds produced by autumn-emerging plants were generally larger than those from plants that emerged in spring, and also had lower germination.

Moreover, some authors confirm for other species that seeds produced in locations with more daylight hours present lower dormancy than those produced in locations with less daylight hours (Baskin and Baskin, 1998). Daylength was longer in Morris (15.4 h in June) than Almenar (14.3 h in May) when seeds were maturing on plants, which emphasizes this theory.

5. Conclusions

The germination and degree of dormancy of seeds produced from two *T. arvense* populations, one from Teruel, Spain and the other from Minnesota, USA, which were both grown at each field site in Almenar, Spain and Morris, Minnesota, appear to have been affected more greatly by growth environment and maternal influence during development than by physicochemical attributes of the seeds themselves. Furthermore, we did not see a clear relationship in differences in germination amount or rate between apical and basal seeds, which temporally differ in their maturity on the plant stem. These results help gain a better understanding of conditions affecting the degree of dormancy imparted on *T. arvense* seed during their development. This will help to make better predictions of seedling emergence dynamics from native seed populations, and perhaps help target traits to modify to reduce or eliminate dormancy in efforts to domesticate *T. arvense* as a commercial oilseed crop.

Acknowledgements

The authors would like to thank Mr. Joe Boots for helping to maintain plots in Morris, Minnesota and collect and process seeds. The authors also thank Ms. Linda Manthey of the USDA-ARS National Center for Agriculture Utilization Lab in Peoria, IL for analyzing oil content and fatty acid profiles of the seed oil. The experiment carried out in Almenar, Spain was supported by the Spanish project AGL2010-22084-C02-01 and by a grant from the Sociedad Española de Malherbologia (SEMh) in 2013. We would also like to thank Inés Olalla for the germination experiment assistance and Ana Isábal and Joel Torra for the field assistance during 2012-13.

References

- Baloch, H.A., Di Tommaso, A., Watson, A.K., 2001. Intrapopulation variation in *Abutilon theophrasti* seed mass and its relationship to seed germinability. *Seed Sci. Res.* 11, 335-343.
- Baskin, J.M., Baskin, C.C., 1989. Role of temperature in regulating timing of germination in soil seed reserves of *Thlaspi arvense* L. *Weed Res.* 29, 317-326.
- Baskin, J.M., Baskin, C.C., 1998. *Seeds: Ecology, biogeography, and evolution of dormancy and germination*. Academic Press, San Diego, CA, USA, 666 pp.
- Bradford, K., 2002. Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Sci.* 50, 248-260.
- Bretagnolle, F., Matejcek, A., Gregoire, S., Reboud, X., Gaba, S., 2015. Determination of fatty acids content, global antioxidant activity and energy value of weed seeds from agricultural fields in France. *Weed Res.* 56, 78-95.
- Colbach, N., Dürr, C., Roger-Estrade, J., Chauvel, B., Caneill, J., 2006. ALOMYSYS: Modelling black-grass (*Alopecurus myosuroides* Huds.) germination and emergence, in interaction with seed characteristics, tillage and soil climate I. Construction. *Europ. J. Agron.* 24, 95-112.
- Donohue, K., Heschel, M.S., Chiang, G.C.K., Butler, C.M., Barua, D., 2007. Phytochrome mediates germination responses to multiple seasonal cues. *Plant Cell Environ.* 30, 202-212.
- Dorn, K.M., Fankhauser, J.D., Wyse, D.L., Marks, M.D., 2013. De novo assembly of the pennycress (*Thlaspi arvense*) transcriptome provides tools for the development of a winter cover crop and biodiesel feedstock. *Plant J.* 75, 1028-1036.
- Fan, J., Shonnard, D.R., Kalnes, T.M., Johnsen, P.B., Rao, S., 2013. A life cycle assessment of pennycress (*Thlaspi arvense* L.)-derived jet fuel and diesel. *Biomass Bioenerg.* 55, 87-100.

- 364 Forcella, F., Gesch, R.W., Isbell, T.A., 2005. Seed yield, oil, and fatty acids of cuphea in the
365 northwestern Corn Belt. Crop Sci. 45, 2195-2202.
- 366 Gardarin, A., Dürr, C., Colbach, N., 2011. Prediction of germination rates of weed species:
367 relationships between germination speed parameters and species traits. Ecol. Model. 222,
368 626-636.
- 369 Gardarin, A., Colbach, N., 2015. How much of seed dormancy in weeds can be related to seed
370 traits? Weed Res. 55, 14-25.
- 371 Gesch, R.W., Archer, D.W., 2005. Influence of sowing date on emergence characteristics of
372 maize seed coated with a temperature-activated polymer. Agron. J. 97, 1543-1550.
- 373 Gesch, R.W., Archer, D.W., Berti, B.T., 2014. Dual cropping winter camelina with soybean in
374 the Northern Corn Belt. Agron. J. 106, 1735-1745.
- 375 Gulden, R.H., Thomas, A.G., Shirliffe, S.J., 2004. Secondary dormancy, temperature, and burial
376 depth regulate seedbank dynamics in canola. Weed Sci. 52, 382-388.
- 377 Hazebroek, J.P., Metzger, J.D., 1990a. Environmental control of seed germination in *Thlaspi*
378 *arvense* (Cruciferae). Am. J. Bot. 77, 945-953.
- 379 Hazebroek, J.P., Metzger, J.D., 1990b. Seasonal pattern of seedling emergence, survival, and
380 reproductive behavior in *Thlaspi arvense* (Cruciferae). Am. J. Bot. 77, 954-962.
- 381 Hume, L., 1994. Maternal environment effects on plant growth and germination of two strains of
382 *Thlaspi arvense* L. Int. J. Plant Sci. 155, 180-186.
- 383 Johnson, G.A., Kantar, M.B., Betts, K.J., Wyse, D.L., 2015. Field pennycress production and
384 weed control in a double crop system with soybean in Minnesota. Agron. J. 107, 532-540.
- 385 Linder, C.R., 2000. Adaptive evolution of seed oils in plants: Accounting for the biogeographic
386 distribution of saturated and unsaturated fatty acids in seed oils. Am. Nat. 156, 442-458.

- 387 Martinelli, T., Galasso, I., 2011. Phenological growth stages of *Camelina sativa* according to the
388 extended BBCH scale. *Ann. Appl. Biol.* 158, 87-94.
- 389 Moles, A.T., Hodson, D.W., Webb, C.J., 2000. Seed size and shape and persistence in the soil in
390 the New Zealand flora. *Oikos* 89, 541-545.
- 391 Moser, B.R., Knothe, G., Vaughn, S.F., Isbell, T.A., 2009. Production and evaluation of
392 biodiesel from field pennycress (*Thlaspi arvense* L.) oil. *Energ. Fuels* 23, 4149-4155.
- 393 Okagami, N., Terui, K., 1996. Differences in the rates of metabolism of various triacylglycerols
394 during seed germination and the subsequent growth of seedlings of *Dioscorea tokoro*, a
395 perennial herb. *Plant Cell Physiol.* 37, 273-277.
- 396 Phippen, W.B., Phippen, M.E., 2012. Soybean seed yield and quality as response to field
397 pennycress residue. *Crop Sci.* 52, 2767-2773.
- 398 Royo-Esnal, A., Necajeva, J., Torra, J., Recasens, J., Gesch, R.W., 2015. Emergence of field
399 pennycress (*Thlaspi arvense* L.): comparison of two accessions and modelling. *Ind. Crops*
400 *Prod.* 66, 161-169.
- 401 Saarinen, T., Lundell, R., Åström, H., Hänninen, H., 2011. Parental overwintering history affects
402 the responses of *Thlaspi arvense* to warming winters in the north. *Environ. Exp. Bot.* 72,
403 409-414.
- 404 Sedbrook, J.C., Phippen, W.B., Marks, M.D., 2014. New approaches to facilitate rapid
405 domestication of a wild plant to an oilseed crop: Example pennycress (*Thlaspi arvense* L.).
406 *Plant Sci.* 227, 122-132.
- 407 Seiler, G.J., 1986. Analysis of the relationships of environmental factors with seed oil and fatty
408 acid concentrations of wild annual sunflower. *Field Crops Res.* 15, 57-72.
- 409 Shinomura, T., 1997. Phytochrome regulation of seed germination. *J. Plant Res.* 110, 151-161.

- 410 Torra, J. Royo-Esnal, A., Recasens, J., 2015. Germination ecology of five arable *Ranunculaceae*
411 species. *Weed Res.* 55, 503-513.
- 412 Torra, J., Recasens, J., 2008. Demography of corn poppy (*Papaver rhoeas*) in relation to
413 emergence time and crop competition. *Weed Sci.* 56, 826-833.
- 414 Warwick, S.I., Francis, A., Susko, D.J., 2002. The biology of Canadian weeds. 9. *Thlaspi*
415 *arvense* L. (updated). *Can. J. Plant Sci.* 82, 803-823.
- 416
- 417

Figure Captions

Figure 1. Total percent germination of apical and basal seeds at six different temperatures for seeds of Teruel Spain (A and C) and Minnesota USA (B and D) *Thlaspi arvense* populations grown at Almenar Spain (A and B) and Morris, Minnesota USA (C and D). Bars at each temperature for a given graph followed by a different letter are significantly different at the $P \leq 0.05$ level. Values are means $\pm$ SE.

Figure 2. Differences in the primary fatty acids as a percent of total seed oil for seeds of the Minnesota USA *Thlaspi arvense* population produced at Morris, Minnesota USA (black bars) and Almenar Spain (gray bars). For each fatty acid, bars followed by different letters are significantly different at the $P \leq 0.05$ level. Values are means across both apical and basal seeds $\pm$ SE.

431 Table 1. Monthly climate data for Almenar Spain and Morris, Minnesota USA during propagation of *Thlaspi arvense* plants from sowing to
 432 harvest during 2012-2013.

Month	†Morris, Minnesota USA				Almenar Spain			
	Mean air temperature (°C)	Accumulated GDD (°C d)	Accumulated precipitation (mm)	Mean daylength (h)	Mean air temperature (°C)	Accumulated GDD (°C d)	Accumulated precipitation (mm)	Mean daylength (h)
Sept	12.3	106	0	12.3	-	-	-	-
Oct	7.0	112	29	10.6	15.2	325	92	10.8
Nov	0.2	15	16	9.3	9.2	166	30	9.6
Dec	-8.3	2	5	8.6	5.6	73	7	9.1
Jan	-10.8	0	11	9.0	4.6	35	31	9.4
Feb	-10.3	0	0	10.1	5.4	60	8	10.4
March	-6.8	0	33	11.7	9.2	169	68	11.7
April	1.8	38	16	13.3	11.7	237	78	13.1
May	13.1	292	61	14.7	13.0	284	14	14.3
Jun	19.3	456	179	15.4	16.3	38	0	15.0
Jul	24.1	177	21	15.1	-	-	-	-
Total		1198	371			1387	328	

† Seeds were sown on 18 September 2012 at Morris and 4 October 2012 at Almenar and were harvested 9 July 2013 at Morris

and 3 June 2013 at Almenar.

Table 2. Estimated T_b for the calculation of GDD and Boltzmann function parameters for seed germination rates of the Spain and USA *T. arvense* populations grown in Almenar, Spain, and Morris, USA germinated at 8°C. Values are means $\pm$ SE.

Different letters denote significances at $P < 0.05$. The first lower case letters denote differences between apical and basal seeds within a population and growth location; upper case letters denote differences between the same seed position of different populations at the same growth location; and the second lower case letters denote differences between the same seed position of the same population grown at different locations.

Location grown	Population	Seed position	T_b (°C)	<i>A</i>	<i>B</i>	G_{50}	Model $P < 0.01$
Almenar	Spain	Apical	+3.0	4.3 ± 0.2 -Ab	44 ± 13 -Aa	83 ± 3 -Aa	**
	Spain	Basal	+2.4	-	-	-	n.a.†
	USA	Apical	+1.7	2.7 ± 0.2 aBb	74 ± 19 aAa	88 ± 5 aAa	**
	USA	Basal	+2.0	2.1 ± 0.3 a-b	60 ± 27 a-a	93 ± 8 a-a	**
Morris	Spain	Apical	+1.3	21.1 ± 0.2 bAa	38 ± 3 aAa	69 ± 1 bAb	**
	Spain	Basal	+1.6	22.3 ± 0.4 aA-	42 ± 4 aA-	85 ± 1 aA-	**
	USA	Apical	+2.5	14.9 ± 0.4 aBa	49 ± 9 aAa	82 ± 2 aAa	**
	USA	Basal	+2.1	12.5 ± 0.4 bBa	43 ± 7 aAa	78 ± 2 aAb	**

†Abbreviation: n.a., not applicable.

Table 3. Oil, crude protein, and carbon content of apical and basal seeds for the Spain and USA *Thlaspi arvense* populations that were grown in Almenar and Morris during 2013. Comparisons in the upper half of the table are between apical and basal seeds for each growing location.

Comparisons in the lower half of the table are for location grown averaged across seed position and seed position averaged across locations grown for a given attribute. Values are means, and the SE is included for apical and basal seed for each growth location. Comparisons within columns followed by a different letter are significant at the $P \leq 0.05$ level.

Location Grown	Seed position	<i>T. arvense</i> (Spain population)				<i>T. arvense</i> (USA population)			
		Seed mass (g/1000 seed)	Oil (%)	Protein (g kg ⁻¹)	Carbon (g kg ⁻¹)	Seed mass (g/1000 seed)	Oil (%)	Protein (g kg ⁻¹)	Carbon (g kg ⁻¹)
Morris	Apical	1.01 ± 0.04 a	26.9 ± 0.04 a	286 ± 1 a	564 ± 2 a	0.74 ± 0.01 b	30.5 ± 0.03 b	262 ± 3 b	578 ± 1 b
	Basal	1.12 ± 0.03 a	26.4 ± 0.09 b	287 ± 7 a	562 ± 4 a	0.88 ± 0.01 a	30.8 ± 0.08 a	272 ± 3 a	584 ± 2 a
Almenar	Apical	1.44 ± 0.02 a	32.1 ± 0.02 b	267 ± 3 a	587 ± 2 a	1.14 ± 0.02 b	32.2 ± 0.04 b	266 ± 1 b	588 ± 1 b
	Basal	1.40 ± 0.03 a	32.3 ± 0.04 a	267 ± 2 a	585 ± 2 a	1.28 ± 0.04 a	32.6 ± 0.04 a	278 ± 3 a	594 ± 2 a
Comparison by									
Location Grown	Morris	1.07 b	26.7 b	287 a	563 b	0.81 b	30.6 b	267 a	581 b
	Almenar	1.42 a	32.2 a	267 b	586 a	1.12 a	32.4 a	272 a	591 a
Seed position	Apical	1.28 a	29.5 a	277 a	575 a	0.96 b	31.3 b	264 b	583 b
	Basal	1.29 a	29.3 b	276 a	574 a	1.10 a	31.7 a	275 a	589 a

Table 4. Fatty acid distribution of seeds for the Spain *Thlaspi arvense* population that was grown in Morris, MN USA and Almenar, Spain during the 2012-2013 growing season. Values are means $\pm$ SE. For seed position and location grown within a column, values followed by a different letter are significantly different at the $P \leq 0.05$ level.

Seed Position	Seed fatty acids					
	C16:0	C18:1	C18:2	C18:3	C20:1	C22:1
Apical	3.0 $\pm$ 0.16 a	11.2 $\pm$ 0.26 b	20.4 $\pm$ 0.29 a	13.6 $\pm$ 0.33 a	9.4 $\pm$ 0.03 a	35.5 $\pm$ 0.28 b
Basal	2.8 $\pm$ 0.16 b	11.8 $\pm$ 0.30 a	20.1 $\pm$ 0.20 a	13.3 $\pm$ 0.25 b	9.2 $\pm$ 0.14 b	35.9 $\pm$ 0.07 a
Location Grown						
	C16:0	C18:1	C18:2	C18:3	C20:1	C22:1
Morris	3.3 $\pm$ 0.03 a	12.1 $\pm$ 0.16 a	19.8 $\pm$ 0.09 b	12.8 $\pm$ 0.07 b	9.4 $\pm$ 0.05 a	36.0 $\pm$ 0.08 a
Almenar	2.6 $\pm$ 0.02 b	10.9 $\pm$ 0.14 b	20.6 $\pm$ 0.25 a	14.1 $\pm$ 0.14 a	9.1 $\pm$ 0.11 b	35.3 $\pm$ 0.24 b

Figure 1

Figure 2