Linköping University Postprint

Comparing after-ripening response and germination requirements of *Conyza canadensis* and *C. bonariensis* (Asteraceae) through logistic functions

L M KARLSSON & P MILBERG

N.B.: When citing this work, cite the original article.

The definitive version is available at www.blackwell-synergy.com:

L M KARLSSON & P MILBERG, Comparing after-ripening response and germination requirements of *Conyza canadensis* and *C. bonariensis* (Asteraceae) through logistic functions, 2007, Weed Research, (47), 433-441.

<http://dx.doi.org/10.1111/j.1365-3180.2007.00576.x>.

Copyright: Blackwell Publishing www.blackwell-synergy.com

Postprint available free at:

Linköping University E-Press: <http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-11881>

Comparing after-ripening response and germination requirements of *Conyza canadensis* and *C. bonariensis* (Asteraceae) through logistic functions

L M KARLSSON & P MILBERG

IFM Division of Ecology, Linköping University, SE-581 83 Linköping, Sweden

Received 26 September 2006

Revised version accepted 9 May 2007

Short running title: Germination of *Conyza canadensis* and *C. bonariensis*

Correspondence: P Milberg, IFM Division of Ecology, Linköping University, SE-581 83 Linköping, Sweden. E-mail: permi@ifm.liu.se. Tel.: +46 13285682. Fax +46 13281399.

Summary

Germination requirements and after-ripening effects during one year of dry storage at 15/5 and 25/15°C (day/night) were compared for *Conyza bonariensis* and *C. canadensis* (Asteraceae). A logistic function was fitted to the results from tests over time in various incubation conditions, using three populations of each species as replicates. Time required for response to dry storage was measured by using a new method; the third derivative of the logistic function. Therefore, a point when major germination was achieved could be detected, without having to rely on maximum germination (which is uncertain), individual data points or any subjectively chosen limit. Fresh seeds of both species were dependent on light for germination and after-ripening was mainly manifested by increasing germination in darkness. Low dormancy status and light requirement might indicate that soil cultivations should rapidly reduce the seed banks of these species, although fecundity and wind dispersal will affect population levels. The species differed in their germination response, with *C. bonariensis* germinating at lower temperatures than *C. canadensis*. This seemingly counter-intuitive result may explain the prevention of fatal germination of *C. canadensis* in cold conditions and its behaviour as a summer annual in northern climates, while *C. bonariensis* is restricted to warmer parts of the world.

Keywords: Dormancy type, fleabane, horseweed, logistic function, response time

Introduction

The two widespread annuals *Conyza canadensis* (L.) Cronquist and *C. bonariensis* (L.) Cronquist, Asteraceae (Cronquist, 1943) seem to have evolved within *Erigeron* on different occasions in northern and southern America, respectively (Noyes, 2000). *Conyza canadensis* occurs in the northern hemisphere between circa 30 and 60°N (Hultén & Fries, 1986), in tropical situations (Acuña, 1974) and in the southern hemisphere (Hultén & Fries, 1986; Wilson *et al.*, 1995; Bromilov, 2001), frequently being a weed in major crops (Holm *et al.*, 1997). *Conyza bonariensis* occurs in tropical to warm temperate regions around the world (Tutin *et al.*, 1976; Foster *et al.*, 1986; Terry & Michieka, 1987; Wilson *et al.*, 1995; Vibrans, 1998; Bromilov, 2001; Kong *et al.*, 2001; Shaukat & Siddiqui, 2004; Whitson *et al.*, 2004). The two species co-occur in parts of the world: in south western USA (Andersen, 1993), Mexico City (Vibrans, 1998), western Mexico (personal observation), north eastern Australia (Wilson *et al.*, 1995), south eastern South Africa (Bromilov, 2001), France (Thébaud & Abbot, 1995), Israel (Zinzolker *et al.*, 1985) and the Czech Republic (Šída, 2003).

Seeds of *C. canadensis* and *C. bonariensis* have fully developed embryos and imbibe water easily. Emergence of *C. canadensis* in the field occurs in both autumn (Regehr & Bazzaz, 1979) and spring (Buhler & Owen, 1997) at about 40-45°N in North America. Seeds of *C. canadensis* that had been stored indoors for at least 60 days germinated to 5% at 10°C, to 50% at 15°C and to nearly 100% at higher temperatures (Hayashi, 1979). Zinzolker *et al.* (1985) reported both species as having "absolute requirement for light" for germination and they also reported germination (fractions not given) between 10 and 25°C. Therefore, the impression is that *C. canadensis* germinates

relatively easily when provided with light and suitable temperatures. However, seed dormancy, defined as a seed character that prevents germination, even when good germination conditions are present, may still play a role. Unless the species are regarded as non-dormant, the dormancy class is assumed to be physiological dormancy (PD) and the level non-deep, according to the classification system suggested by Baskin & Baskin (2004). This kind of dormancy may be subdivided into five different types, depending on the temporal pattern in dormancy alleviation. It is not known what type either of the two species exhibit.

Since *C. canadensis* and *C. bonariensis* have evolved on different occasions in different regions and their current geographic distributions partly overlap, we wanted to compare their after-ripening pattern and germination preferences, to evaluate if differences are involved in restricting their distribution. A possible way to compare the species could be to establish the type, or types, of non-deep PD. We tested their germination response to different temperature and light regimes at intervals during one year of dry storage conducted at two temperatures. Although there are several methods to describe the pattern of germination over time (e.g. Bauer *et al.* 1998; Grundy *et al.* 2000; Vleeshouwers & Kropff 2000; Bair *et al.* 2006), there seems to be no established method available for measuring the time it takes to achieve full response to a pre-treatment under controlled conditions and consequently, none to objectively compare response times. The methods sometimes used are based on the time it takes to reach 50% germination, or 50% of maximum germination, and have a severe drawback: they ignore what happens after this point in time. Our goal was to measure and compare achieved germination, response strength and response time. Therefore, we summarised germination data by fitting a logistic equation to them and used function parameters to

calculate final achieved germination and magnitude of possible increase over time. To allow response time to a treatment to be measured, we introduced the use of the third derivative of the original logistic function.

Materials and methods

Germination

Achenes (hereafter referred to as seeds) of *C. canadensis* were collected in southern Sweden at Boxholm (58°12'N 15°04'E), at Mjölby (58°19'N 15°06'E) and at Linköping (58°25'N 15°31'E) on the 17th of August, 2001. The three sites are 13, 27 and 35 kilometres apart. All sites were ruderal areas in an agricultural landscape, c. 100 m a.s.l. After being transported to Linköping, the seeds were stored at room temperature (c. 20°C) for five days before the experiments were begun.

Seeds of *C. bonariensis* were collected on the 25th of August, 2001 at Alemaya University (9°N 42°E), west of Harar, in Ethiopia, on the 26th of February, 2002 at Taxco (19°N 99°W) in Mexico, and on the 2nd of November, 2002 at Arhbalou (32°N 8°W), between Marrakech and Ouarzazate, in Morocco. These three sites were between 1500 and 2000 m a.s.l. The site in Ethiopia was a ruderal area in agricultural land, and those in Mexico and Morocco were edges of fields of subsistence farmers. The Ethiopian, Mexican and Moroccan seed batches were transported, at room temperature (c. 20-25°C), to Linköping, and the experiments commenced 13, 7 and 7 days, respectively, after collection.

Four germination incubators (Rubarth Apparatebau, Laatzen, Germany) were used, set at 15/5, 20/10, 25/15 and 30/20°C (day/night temperatures) with 2 h of linear

transference between these temperatures. The light period was 12 h day⁻¹, coinciding with the higher temperatures. Germination tests were done at the four temperatures combined with either light during daytime ("light") or continuous darkness ("darkness").

Seeds were dry-stored in plastic bottles in the 15/5 or 25/15°C incubators. In the germination tests, c. 200 (average 197, SD 47) seeds were distributed in three 52 mm diameter Petri dishes with moist filter paper (two pieces of Munktell 1003, 52 mm diameter, 2.0 mL deionised water) and sealed with parafilm. In the darkness treatments, dishes were individually wrapped in aluminium foil immediately after the seeds were spread on the filter paper. Each germination experiment was terminated after 14 days of incubation (a time period shown to be sufficient for full germination in pilot studies), and seedlings, the un-germinated seeds and apparently dead (soft and/or overgrown with mould) seeds were counted and the dishes discarded. Germination tests were done at the beginning of the study and after seeds had been stored at 15/5 and 25/15°C for 2, 4, 6, 9, 12, 16 and 52 weeks. In addition, the three Swedish batches were tested after 22, 30 and 42 weeks, the Ethiopian after 20, 26 and 38 weeks, the Mexican after 20, 26 and 36 weeks, and the Moroccan after 30 weeks.

Analysis

Germination was calculated as the fraction of seeds germinated during each test and in each treatment, excluding dead seeds (the latter constituted 4.4% (binomial 95% CI: 3.55-5.44) of all seeds tested).

Analysis of variation on initial germination was performed with Statistica (StatSoft, Inc., 2004) on arcsine transformed data. Independent variables were species (three replicates [each seed batch regarded as one replicate]), temperature regime during incubation (four levels) and light regime during incubation (light or darkness).

Response to dry storage time was analysed by fitting a logistic regression model (Equation 1), where $f(x)$ is germination, x is storage time, a , b , c and d are constants and e the base of the natural logarithm, to germination data.

$$f(x) = \frac{e^{a+bx} \cdot c}{1 + e^{a+bx}} + d \quad (1)$$

For each seed batch and treatment, one function was calculated for germination results after various times of dry storage, giving a total of 96 specific functions. Calculations were done with Statistica (StatSoft, Inc., 2004), using user-specified regression in the non-linear estimation module. We used the loss function with the absolute difference between observed and predicted values. The weight function, with the pre-selected quasi-Newton estimation, was used in order to base the calculation on every seed used. Start values for constants a , b and c were set to zero, e was approximated to 2.71828, and d was set to the germination of fresh seeds. The starting function was therefore a horizontal line at the level of germination of fresh seeds for each seed batch and incubation condition tested. In the same way, Equation 1 was also fitted to the germination results for each species in each treatment.

Two parameters, final germination ($f(52)$, Equation 1) and change in germination response over time ($f(52)-f(0)$, Equation 1), were analysed with full factorial ANOVA (Statistica, StatSoft, Inc., 2004). Final germination was arcsine transformed before analysis. Independent variables were species (three replicates [one seed batch = one replicate]), temperature during incubation (four levels), light regime during incubation (two regimes) and dry storage temperature (two levels).

The third derivative of $f(x)$ (Equation 1), $f'''(x)$ (Equation 2), was used to calculate after-ripening time.

$$f'''(x) = \frac{b^3 c e^{a+bx} (1 + (e^{a+bx})^6 - 9(e^{a+bx})^4 - 16(e^{a+bx})^3 - 9(e^{a+bx})^2)}{(1 + e^{a+bx})^8} \quad (2)$$

The function from Equation 2 has a maximum where $f(x)$ approaches its higher asymptote (Fig. 1). We defined "after-ripening time" as the time between zero ($x = 0$), which is the beginning of an experiment, and the x value for the second maximum of $f'''(x)$ (Equation 2) (Fig. 1). We used ten percent units of increase between $f(0)$ and $f(52)$ as the lower limit for including a function in calculations of after-ripening time.

Fig. 1 Calculation of time for response to a treatment using the function $f(x) = e^{a+bx} \cdot c / (1 + e^{a+bx}) + d$. The open circle on $f(x)$ shows the point when the major response is considered to be achieved. Response time is calculated as the time between zero (the beginning of an experiment) and the x -value for the corresponding filled circle on the third derivative of $f(x)$.

Results

Initial germination

When provided with light during daytime, fresh seeds of both species reached nearly full germination, within 14 days, at the three highest temperature regimes tested, 20/10, 25/15 and 30/20°C day/night (Fig. 2). There was a difference between the two species

Fig. 2a The temporal pattern in germination of three populations of two species of *Conyza* in response to dry storage at two temperatures and four different incubation environments. Lines describe a logistic function fitted to species-wise data: light during daytime.

Fig. 2b The temporal pattern in germination of three populations of two species of *Conyza* in response to dry storage at two temperatures and four different incubation environments. Lines describe a logistic function fitted to species-wise data: continuous darkness.

in germination response to temperature and to the interaction of temperature and light (Table 1); fresh seeds of *C. bonariensis* but not of *C. canadensis*, germinated substantially at 15/5°C when provided with light (Fig. 2).

Table 1 ANOVA of germination of fresh seeds of *Conyza bonariensis* and *C. canadensis*. Germination was tested by incubation in 15/5, 20/10, 25/15 or 30/20 °C (day/night) with light during daytime or continuous darkness. Germination data were arcsine transformed before analysis. Seeds from three populations were used as replicates of each species

	df	F	p
Intercept	1	1159.78	0.000
Species	1	1.08	0.306
Light	1	970.29	0.000
Incubation temp	3	50.05	0.000
Species*Light	1	0.13	0.724
Species*Incubation temp	3	12.02	0.000
Light*Incubation temp	3	51.34	0.000
Species*Light*Incubation temp	3	7.85	0.001
Error	32		

Final germination

Final germination, i.e. $f(52)$ (Equation 1), was different between the two species over the range of tested environments (Table 2), with *C. bonariensis* germinating to a greater extent at low temperature and in the dark than *C. canadensis* (Fig. 2). The two species differed in their response to incubation temperature (Table 2) and in their response to incubation temperature combined with light regime (Table 2). The different responses to temperature were expressed by *C. bonariensis* germinating at a lower temperature than *C. canadensis* (Fig. 2), and the difference in response to the interaction of temperature and light regime, by *C. canadensis* showing stronger light requirement, in addition to a

preference for higher temperatures, for germination than *C. bonariensis* during the entire experimental period (Fig. 2).

Table 2 ANOVA of final germination and change in germination over time for seeds of *Conyza bonariensis* and *C. canadensis* stored under dry conditions for 52 weeks at two temperature regimes and tested for germination nine to eleven times at four temperatures and two light regimes. The function $f(x)=e^{a+bx} \cdot c/(1+e^{a+bx})+d$ was fitted to germination data. Final germination ($f(52)$) was arcsine transformed before analysis, and change in germination was analyzed as $f(52)-f(0)$. Results including the factor "species" are in bold

Factor	df	Final germination		Change in germination	
		F	p	F	p
Intercept	1	1440.89	0.000	32.46	0.000
{S}Species¹	1	18.01	0.000	2.03	0.159
{D}Dry storage ²	1	2.00	0.162	2.13	0.149
{L}Light ³	1	679.62	0.000	12.31	0.001
{I}Incubation temp ⁴	3	52.09	0.000	2.59	0.061
S*D	1	0.99	0.324	0.40	0.528
S*L	1	0.82	0.368	0.82	0.369
S*I	3	18.72	0.000	1.74	0.169
D*L	1	0.15	0.702	0.20	0.659
D*I	3	0.34	0.797	0.55	0.649
L*I	3	44.19	0.000	0.71	0.547
S*D*L	1	0.03	0.859	0.05	0.815
S*D*I	3	0.13	0.945	0.05	0.984
S*L*I	3	3.47	0.021	4.31	0.008
D*L*I	3	0.10	0.961	0.04	0.990
S*D*L*I	3	0.20	0.894	0.41	0.750
Error	64				

¹⁾ *Conyza bonariensis* or *C. canadensis*, three populations of each species used as replicates.

²⁾ Dry storage at either 15/5 or 25/15°C day/night.

³⁾ Germination test in either light during daytime or continuous darkness.

⁴⁾ Germination test at either 15/5, 20/10, 25/15 or 30/20°C day/night.

Change in germination response

Of the 96 specific functions derived from Equation 1, 77 increased over time or had no change within the study period. The regression function was negative in 19 cases; of these, eight had a difference between $f(0)$ and $f(52)$ larger than two percent units.

The germination percentage of the two species increased in darkness during storage (Fig. 2); in the ANOVA, light condition explained the major part of the variation (Table 2). The only other significant explanatory variable to change in germination was the interaction of species, light and incubation temperature (Table 2). This was due to the germination in light by only *C. bonariensis*, and different responses by the two species to temperature; increased germination in darkness mostly at lower and higher temperatures for *C. bonariensis* and *C. canadensis*, respectively (Fig. 2).

After-ripening time

Ten combinations of seed batch and incubation treatment showed more than ten percent units of change in germination over time when stored at both 15/5 and 25/15°C. Calculated after-ripening times for these cases were used to compare after-ripening times at the two storage temperatures (Fig. 3). A paired t-test showed no consistent difference between the two storage temperatures tested ($t_{(9)}=1.603$, $p=0.143$). The average after-ripening time was 9.3 (SD 5.2) weeks.

Discussion

Seed dormancy and after-ripening

Seed dormancy has been investigated in numerous studies. Several results were summarised by Baskin & Baskin, (1998), who also recently suggested a classification system to be generally used (Baskin & Baskin, 2004). Unfortunately, different definitions of dormancy are used within the literature. We regard seed dormancy to be an innate attribute of the seed not related to the particular environment the seed happens

to be in (similar to the suggestions by Vleeshouwers *et al.* (1995) and Baskin & Baskin (1998)).

Fig. 3. Calculated after-ripening times for seeds of *Conyza bonariensis* and *C. canadensis* when three seed batches of each species (*C. bonariensis*: Ethiopia, Mexico and Morocco, *C. canadensis*: Boxholm, Linköping and Mjölby in Sweden) were stored at 25/15 or 15/5°C for one year and tested for germination nine to eleven times at different temperatures and light regimes. Only incubation treatments where the change in germination was at least ten percent units at both storage conditions are shown. There was no consistent difference between the two storage temperatures; paired *t*-test: $t_{(9)}=1.603$, $p=0.143$.

Depending on species, dormancy level may be reduced by dry storage at temperatures around room temperature, a phenomenon for which we use the term after-ripening. After-ripening is reported in many species and is common within Asteraceae (Schütz, 1999). Storage temperature may affect dormancy reduction during after-ripening; for some species from warm regions, high temperature has been shown to increase the after-ripening response (Baskin & Baskin, 1998; Pérez-Fernández *et al.*, 2000), but this pattern was not found by Schütz *et al.*, (2002). The seed batch's capacity to widen germination preferences when dormancy is reduced is the basis for differentiating between five types of non-deep PD (Baskin & Baskin, 2004).

Conyza bonariensis and *C. canadensis* have weak dormancy, so reduction was mainly manifested in darkness where few fresh seeds germinated but where germination increased with length of dry storage (Fig. 2). Because of the extensive germination in fresh seeds (Fig. 2) and the relatively short after-ripening time, we conclude that the two species, as predicted, have non-deep PD according to Baskin & Baskin (2004). The classification of non-deep PD types for these two species could be type 1 and type 2 for *C. canadensis* and *C. bonariensis*, respectively. Type 1 refers to seeds that first germinate only at low temperature, but during dormancy reduction gain the capability to germinate also at intermediate and high temperatures, and type 2 to seeds that first germinate only at high temperature, and gain the capability to germinate also at intermediate and low temperatures (Baskin & Baskin 2004). *Conyza canadensis* gained the ability to germinate in darkness at the higher temperatures tested, but not at the lowest temperature. *Conyza bonariensis* gained the ability to germinate at the lowest temperatures tested, but not in darkness at the highest temperature. Another view could be that both species germinate to the highest degree at the most preferable temperature, which seems to be around 20/10°C and 25/15°C for *C. bonariensis* and *C. canadensis*, respectively (Fig. 2). After-ripening reduces dormancy, and germination increased at all temperatures tested; reaching the highest level in the best test conditions. Following this reasoning, both species may be regarded as type 3 (increasing germination was observed both at lower and higher temperatures). Thus, from the present data of these two species (based on c. 100,000 seeds per species), type classification could not be used for comparison of seed dormancy and germination patterns.

Germination timing

Regardless of storage time, storage temperature or light condition during incubation, only *C. bonariensis* germinated at the lowest test temperature (15/5°C; Fig. 2). Thus, *C. bonariensis* germinates at lower temperatures than *C. canadensis* (Fig. 2), but *C. bonariensis* and *C. canadensis* occurs in warmer and colder climates, respectively (e.g. Tutin *et al.*, 1976).

Because of the weak dormancy of both species, the range of temperatures that promotes germination becomes critical for determining the time of germination in the field. At ca 40°N *C. canadensis* mainly occur as a winter annual (Regehr & Bazzaz, 1979, Illinois; Thébaud *et al.*, 1996, France), and seedlings that have not developed rosettes of sufficient size before winter risk winter mortality (Regehr & Bazzaz, 1979; Buhler & Owen, 1997). Because the species does not germinate at low temperatures (Fig. 2), it is restricted from germinating too late in autumn. Following winter survival, plants can develop and set seeds well before next autumn; thus, newly dispersed seeds would be able to germinate before temperatures are too low. Thus, germination temperature preferences would maintain the winter annual pattern. In colder climates, as at 58°N in Sweden, *C. canadensis* apparently performs as a summer annual (personal observation). That could be explained by little time from dispersal until autumn temperatures are too low for germination, and thus that seeds remain ungerminated over winter and germinate when spring temperatures are high enough. This germination pattern leads to late seed dispersal, which gives reduced possibility to germinate in autumn. Thus, the germination temperature preferences would maintain the summer annual pattern. In contrast, the germination preferences of *C. bonariensis* suggest a scenario where the non-specific temperature preferences for germination will allow

virtually all seeds to germinate during autumn, irrespective of climate zone. Thus, even if seedlings of *C. bonariensis*, as those of *C. canadensis* (Regehr & Bazzaz, 1979; Buhler & Owen, 1997), can survive winter in relatively cold climates if of sufficient size, there is a high possibility for germination resulting in small seedling that succumb to freezing. This would be especially pronounced if seeds are dispersed to areas in late spring, when they would germinate and grow without problem, which would lead to late seed dispersal and the following risk of late germination and winter mortality.

Hence, germination biology may *per se* be an important factor for determining geographical distribution. In cold climates, *C. bonariensis*, which germinates at low temperatures (Fig. 2), cannot act as winter annual as it emerges too late in autumn to survive winter. In contrast, *C. canadensis* can, to a high extent, over winter as seeds. *Conyza bonariensis* may also be restricted from being a summer annual in cold climates: even if seeds stay ungerminated in autumn, for example if buried; early germination, at low temperatures, in spring leads to high risk for frost.

In a pilot study (data not shown) with sown seeds, there was high mortality of seedlings of both species during a Swedish winter, better survival of spring-germinated than autumn-germinated seedlings, much more spring-germination in *C. canadensis*, and many more flowering plants of *C. canadensis* than of *C. bonariensis*. Although these preliminary observations need confirmation, they give the impression that *C. canadensis* is mainly a summer annual in Sweden, and that *C. bonariensis* seems unlikely to become established in colder climates than it already occupies. The latter suggestion, however, rests on the assumption that the germination attributes have not evolved during the dispersal of *C. bonariensis* over the globe. Our data, in fact, suggests that the studied attributes are evolutionarily conservative, as populations from

geographically separated populations (Mexico, NW and E Africa) were relatively similar (Fig. 2).

Implication for weed management

Our data suggest that seeds of both species should have the ability to remain ungerminated for some time in the soil because of the initial requirement for light for germination. This assumption, and also the fact that seeds survive for several years, is confirmed for *C. canadensis* by its inclusion in several soil seed bank studies (e.g. Salzmänn, 1954; Bigwood & Inouye, 1988; Zanin *et al.*, 1989; Leck & Leck, 1998). We are not aware of any such studies reporting *C. bonariensis*, but the scarcity is probably due to a strong bias in geographical distribution of such studies, not to the species' inability to accumulate in soil.

Because of the weak dormancy and the germination preferences of these two species (Fig. 2), one can assume that most seeds occurring in soil seed banks are non-dormant but remain ungerminated because of a lack of light. Therefore, soil cultivation will probably allow the portion of the seeds exposed to light during or after the cultivation to germinate (Milberg *et al.*, 1996), as long as temperature and soil moisture are adequate. In fact, in a field study of different fallow practices in New South Wales, there was little *Conyza* sp. present where soil cultivation had been used, but otherwise it was among the most common species during the middle of summer (Felton *et al.*, 1994). In theory, it should then be relatively easy to get rid of these two species by repeated soil cultivation and allowing germination in between. This suggestion is important, especially in the light of the documented evolution of herbicide resistance in

the two species (Smisek *et al.*, 1998; Amsellem *et al.*, 1993), and most notably with respect to glyphosate (*C. canadensis*: Main *et al.*, 2004, *C. bonariensis*: Urbano *et al.*, 2005). In practice, however, their control on arable land is complicated by the very high fecundity of plants (Thébaud & Abbott, 1995) and achenes that are well suited to wind-dispersal (Andersen, 1993). Therefore, adjacent areas should be taken into account when planning weed control.

Generality of results

Despite the fact that seeds of *C. bonariensis* were collected from different parts of the world and on different occasions and that those of *C. canadensis* were collected on the same day from a relatively restricted geographic area, all *C. bonariensis* seed batches germinated to a higher extent than all *C. canadensis* seed batches at low temperatures. Further, the largest difference between seed batches was found in *C. canadensis* (Fig. 2). Thus, we think that the results may cover a substantial part of the within-species variation of both species. Although it cannot be concluded that the Swedish *C. canadensis* populations tested are representative of the species worldwide, the same general pattern with no or little germination at low temperatures (Fig. 2) has also been reported from Britain (Trudgill *et al.*, 2000), North Carolina (Shontz & Oosting, 1970) and Japan (Hayashi, 1979).

Considerations regarding design and analysis

Inference and the level of replication Allowing a single seed batch to represent a species (or other experimental unit) severely limits the range of appropriate inferences. In this study, we sampled seeds from three populations per species and pooled

germination data from three pseudo-replicated dishes. Thus we were able to analyse the results with three replicates per species without involving pseudo-replication.

The method used here allows an analysis of the general germination patterns, not highlighting individual points in the data or variation unrelated to the trend in time. The function used seemed to be rather robust for describing the temporal pattern in germination, at least when, as in this study, using a minimum of nine points in time.

An underlying assumption when using a logistic function is that if a change occurs over time, it is a one-way change; i.e. this function cannot be used to describe both increasing and decreasing germination as a continuum. Nor, of course, can the function be used for extrapolation.

Fitting a function and estimating response time To evaluate and compare germination responses over time we suggest, instead of comparing single points in time, fitting functions to germination data and using function parameters for the analysis of variance. These estimates for statistical evaluation have at least two advantages. First, they eliminate the binomial properties of germination data in favour of parameters that can be assumed to be normally distributed. Secondly, they eliminate a large proportion of the "random" variation and help to focus on the more large-scale pattern and response.

The logistic function we used allows, by the four constants included, the shape and placement of the entire curve to be determined by the germination data, and not to be dependent on any assumptions. Further, the function was, at the start of the iterations, a horizontal line at the level of germination of fresh seeds. Therefore, the fitting of the function to data was determined by the changes over time in each environment and not by initially different germination responses in different environments.

We regard it as an advantage to be able to calculate after-ripening times (Fig. 1) and not to be dependent on subjectively deciding the time only from the different points in time that were tested. The selected point for measuring time should allow comparisons between species and treatments, should be present in all possible comparisons involving a completed positive response to time, should not be strongly influenced by the maximum slope of $f(x)$ and should correspond to the response level when a major response is apparent from visual observation. The use of the right maximum of the third derivative of $f(x)$ (Fig. 1) meets those requirements by being a point close to the asymptotic level of $f(x)$, whilst not including the final portion of the germination response. Thus it does not depend on whether a minor fraction germinates early or late during the test. Therefore, the measured response time is not strongly dependent on the natural, unexplained, variation between data points around the level of a completed response, but includes the major response to the treatment.

In general, we regard the use of Equation 1 and Equation 2 as a way to study and compare general patterns that change over time, and time for response to a treatment, as meaningful not only for after-ripening, but also when studying dormancy reduction during the stratification of seeds.

Acknowledgements

We would like to thank Tamado Tana for supplying us with seeds from Ethiopia. The study was funded by Formas (the Swedish council for Environment, Agricultural Sciences and Spatial Planning).

References

- ACUÑA J (1974) *Plantas indeseables en los cultivos cubanos*. Havana: Academica de Ciencias de Cuba.
- AMSELLEM Z, JANSEN MAK, DRIESENAAR ARJ & GRESSEL J (1993) Developmental variability of photooxidative stress tolerance in paraquat-resistant *Conyza*. *Plant Physiology* **103**, 1097-1106.
- ANDERSEN MC (1993) Diaspore morphology and seed dispersal in several wind-dispersed Asteraceae. *American Journal of Botany* **80**, 487-492.
- BAIR NB, MEYER SE & ALLEN PS (2006) A hydrothermal after-ripening time model for seed dormancy loss in *Bromus tectorum* L. *Seed Science Research* **16**, 17-28.
- BAUER MC, MEYER SE & ALLEN, PH (1998) A simulation model to predict seed dormancy loss in the field for *Bromus tectorum* L. *Journal of Experimental Botany* **49**, 1235-1244.
- BASKIN CC & BASKIN JM (1998) *Seeds: Ecology, biogeography, and evolution of dormancy and germination*. San Diego, Academic Press.
- BASKIN JM & BASKIN CC (2004) A classification system for seed dormancy. *Seed Science Research* **14**, 1-16.
- BIGWOOD DW & INOUE DW (1988) Spatial pattern analysis of seed banks: an improved method and optimized sampling. *Ecology* **69**, 497-507.
- BROMILOV C (2001) *Problem plants of South Africa*. Pretoria: Briza Publications CC.
- BUHLER DD & OWEN MDK (1997) Emergence and survival of horseweed (*Conyza canadensis*). *Weed Science* **45**, 98-101.

- CRONQUIST A (1943) The separation of *Erigeron* from *Conyza*. *Bulletin of the Torrey Botanical Club* **70**, 629-632.
- FELTON WL, WICKS GA & WELSBY SM (1994) A survey of fallow practices and weed floras in wheat and grain sorghum in northern New South Wales. *Australian Journal of Experimental Agriculture* **34**, 229-236.
- FOSTER RB, ARCE JB & WACHTER TS (1986) Dispersal and the sequential plant communities in Amazonian Peru floodplain. In: *Frugivores and Seed Dispersal*. (eds A Estrada & TH Fleming), 357-370. Dr W. Junk Publishers, Dordrecht.
- GRUNDY AC, PHELPS K, READER RJ & BURSTON S (2000) Modelling the germination of *Stellaria media* using the concept of hydrothermal time. *New Phytologist* **148**, 433-444.
- HAYASHI I (1979) Secondary succession of herbaceous communities in Japan: seed germination and shade tolerance of seedlings of the dominants. *Bulletin of the Yokohama Phytosociological Society* **16**, 407-414.
- HOLM L, DOLL J, HOLM E, PANCHO J & HERBERGER J (1997) *World Weeds: Natural Histories and Distribution*. John Wiley & Sons, Inc., New York.
- HULTÉN E & FRIES M (1986) *Atlas of North European vascular plants*. Königstein: Koeltz Scientific Books.
- KONG LD, ABLIZ Z, ZHOU CX, LI LJ, CHENG CHK & TAN RX (2001) Glycosides and xanthine oxidase inhibitors from *Conyza bonariensis*. *Phytochemistry* **58**, 645-651.
- LECK MA & LECK CF (1998) A ten-year seed bank study of old field succession in New Jersey. *Journal of the Torrey Botanical Society* **125**, 11-32.

- MAIN CL, MUELLER TC, HAYES RM & WILKERSON JB (2004) Response of selected horseweed (*Conyza canadensis* (L.) Cronq.) populations to glyphosate. *Journal of Agricultural and Food Chemistry* **52**, 879-883.
- MILBERG P ANDERSSON L & NORONHA A (1996) Seed germination after short-duration light exposure: implications for the photo-control of weeds. *Journal of Applied Ecology* **33**, 1469-1478.
- NOYES RD (2000) Biogeographical and evolutionary insights on *Erigeron* and allies (Asteraceae) from ITS sequence data. *Plant Systematics & Evolution* **220**, 93-114.
- PEREZ-FERNÁNDEZ MA, LAMONT BB, MARWICK AL & LAMONT WG (2000) Germination of seven exotic weeds and seven native species in south-western Australia under steady and fluctuating water supply. *Acta Oecologica* **21**, 323-336.
- REGELER DL & BAZZAZ FA (1979) The population dynamics of *Erigeron canadensis*, a successional winter annual. *Journal of Ecology* **67**, 923-933.
- SALZMANN R (1954) Untersuchungen über die Lebensdauer von Unkrautsamen im Boden. *Mitteilungen der Schweizerische Landwirtschaft* **10**, 170-176.
- SCHÜTZ W (1999) Some ecological and biogeographical aspects of achene dormancy and afterripening in Asteraceae. In: *Results of Worldwide Ecological Studies. Proceedings of the First Symposium by the A.F.W. Schimper-Foundation, Hohenheim*, October 1998. (eds S-W Breckle, B Schweizer & U Arndt), 153-168., Verlag Günter Heimbach, Stuttgart, Germany.

- SCHÜTZ W, MILBERG P & LAMONT BB (2002) Seed dormancy, after-ripening and light requirement of four annual Asteraceae in south-western Australia. *Annals of Botany* **90**, 707-714.
- SHAUKAT SS & SIDDIQUI IA (2004) Spatial pattern analysis of seeds of an arable soil seed bank and its relationship with above-ground vegetation in an arid region. *Journal of Arid Environments* **57**, 311-327.
- SHONTZ JP & OOSTING HJ (1970) Factors affecting interaction and distribution of *Haplopappus divaricatus* and *Conyza canadensis* in North Carolina old fields. *Ecology* **51**, 780-793.
- ŠÍDA O (2003) *Conyza triloba*, new to Europe, and *Conyza bonariensis*, new to the Czech Republic. *Preslia (Praha)* **75**, 249-254.
- SMISEK A, DOUCET C, JONES M & WEAVER S (1998) Paraquat resistance in horseweed (*Conyza canadensis*) and Virginia pepperweed (*Lepidium virginicum*) from Essex County, Ontario. *Weed Science* **46**, 200-204.
- STATSOFT INC. (2004) STATISTICA (data analysis software system), version 7.
www.statsoft.com.
- TERRY PJ & MICHIEKA RW (1987) *Common Weeds of East Africa*. Food and Agriculture Organization of the United Nations, Rome.
- THÉBAUD C & ABBOT RJ (1995) Characterization of invasive *Conyza* species (Asteraceae) in Europe: quantitative trait and isozyme analysis. *American Journal of Botany* **82**, 360-368.
- THÉBAUD C, FINZI AC, AFRE L, DEBUSSCHE M & ESCARRE J (1996) Assessing why two introduced *Conyza* differ in their ability to invade Mediterranean old fields. *Ecology* **77**, 791-804.

- TRUDGILL DL, SQUIRE GR & THOMPSON K (2000) A thermal basis for comparing the germination of some British herbaceous plants. *New Phytologist* **145**, 107-114.
- TUTIN TG, HEYWOOD VH, BURGESS NA, MOORE DA, VALENTINE DH, WALTERS SM & WEBB DA (1976) *Flora Europea* 4. Cambridge University Press.
- URBANO JM, BORREGO A, TORRES V, CALDERON S & BARNES J (2005) Screening for biotypes of *Conyza bonariensis* resistant to glyphosate in southern Spain. In: *Proceedings of the 13th EWRS Symposium, Bari 19-23 June 2005*. European Weed Research Society (eds P Bàrberi *et al.*).
- VIBRANS H (1998) Urban weeds of Mexico City. Floristic composition and important families. *Anales del Instituto Biología, Universidad Nacional Autónoma de México, Serie Botánica* **69**, 37-69.
- VLEESHOUWERS LM & KROPFF MJ (2000) Modelling field emergence patterns in arable weeds. *New Phytologist* **148**, 445-457.
- VLEESHOUWERS LM, BOUWMEESTER HJ & KARSEN CM (1995) Redefining seed dormancy: an attempt to integrate physiology and ecology. *Journal of Ecology* **83**, 1031-1037.
- WHITSON TD, BURRILL LC, DEWEY SA, CUDNEY DW, NELSON BE, LEE RD & PARKER R (2004) *Weeds of the West, 9th Edition*. Western Society of Weed Science. Newark.
- WILSON BJ, HAWTON D & DUFF AA (1995) *Crop Weeds of Northern Australia*. Department of Primary Industries, Brisbane.
- ZANIN G, BERTI A & ZUIN MC (1989) Estimation of the seedbank in cultivated and direct drilled systems. *Weed Research* **29**, 407-417.

ZINZOLKER A, KIGEL J & RUBIN B (1985) Effects of environmental factors on the germination and flowering of *Conyza albida*, *C. bonariensis* and *C. canadensis*. Abstract of papers presented at the 9th conference of the weed science society of Israel. *Phytoparasitica* **13**, 229-230.