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Effects of Light, Temperature, and Soil Depth on the Germination and Emergence of *Conyza canadensis* (L.) Cronq.

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Abstract: Understanding the dynamics of invasive species under global climate change requires knowledge about the effects of environmental factors on germination and emergence. We considered *Conyza canadensis* (L.) Cronq., an invasive species that is quickly invading Southern European agricultural systems, and performed germination assays in growth chambers at eight constant temperatures with alternating light (2.5, 5, 10, 15, 20, 25, 30, and 40 °C, with 12 h/12 h—light/dark), three alternating temperatures in alternating light (12/18, 17/23, and 22/28 °C, with 12 h/12 h—light/dark) and three fixed temperatures (15, 20, and 25 °C) in complete darkness. Furthermore, emergence assays were performed in pots considering four depths (0, 2.5, 5, and 10 mm), three temperatures with alternating light (15, 20, and 25 °C) and un-treated or pre-treated seeds (water imbibition and light for two days). *C. canadensis* was able to germinate in a wide range of temperatures (from 5–10 °C to 30 °C). The highest germination capacity was observed at 15 °C (light/dark); no differences were observed at 17/23 and 22/28 °C with respect to 20 and 25 °C (light/dark), while germinations were significantly reduced at 12/18 °C. The lowest germination time was observed at 25 °C (light/dark) and it was significantly increased at 12/18 °C and in darkness. The highest emergence was from 0 mm depth; pre-treatment significantly increased the emergence from 2.5 mm and 5 mm depth, but not from 10 mm depth. Modeling germination rates as a function of temperature allowed us to determine $T_b = 6.8$ °C (base temperature) and $T_c = 35.8$ °C (ceiling temperature). In light of these results, the potential for *C. canadensis* to spread into new environments and possible new management methods are discussed.

Keywords: climate change 1; invasive species 2; weeds 3; agronomic practices 4; weed biology 5

1. Introduction

The global climate is changing mainly due to increasing levels of greenhouse gases such as CO₂ [1,2]. Consequently, increasing attention is given to possible impacts on agricultural systems and, particularly, on weed population dynamics [1,3,4]. Indeed, an increase in temperature in Mediterranean climates might allow heat tolerant invasive species to spread into regions that were previously too cold, whereas historically indigenous species might simultaneously disappear from their regions of origin due to less favorable conditions [5–7]. Such an increased impact of invasive plant species and consequent change of weed–crop interactions within the agroecosystem [8,9] may require a revision of weed management strategies [10,11].

In regard to invasive species, particular attention has been recently given to the occurrence of *Conyza canadensis* (L.) Cronq., which has considerably increased in European and Italian cropping systems, particularly where minimum or no-tillage is adopted [12–15].

Conyza canadensis is a facultative winter annual species of the *Asteraceae* family, native to North America, which reproduces only by seed and shows the typical eco-physiological requirements of “partially indifferent” species [16]. It shows high phenotypic plasticity, it is autogamous, thermophilic, and shows good tolerance to drought [17]. In general, this species prefers coarse, stony, and well drained soils with neutral or sub-acid reaction [18,19].

Conyza canadensis produces, on average, 60–70 seeds (achenes) per flower head, and the seeds are not dormant at maturity [20,21]. The number of flower heads per plant, and, therefore, total seed production are both proportional to stem height [21], ranging from 2000 seeds per plant (0.4 m stem height) to 230,000 seeds per plant (1.5 m stem height). These values are confirmed by Bhowmik and Bekech [22], who reported that *C. canadensis* produced approximately 200,000 seeds per plant when grown at a density of 10 plants m⁻² in a no-till field without crop. Seed dispersal is mainly driven by the wind and it is favored by the apical positioning of seed and the presence of the pappus. Dauer et al. [23] observed that 99% of *C. canadensis* seed was found within 100 m from the source, while only a small proportion was transported over larger distances (up to 500 m).

Information on *C. canadensis* seed longevity is very scarce. Tsuyuzaki and Kanda [24] reported viable seed of *C. canadensis* in the seedbank of a 20-year-old abandoned pasture, despite the absence of this species in the aboveground vegetation. Under laboratory conditions, *C. canadensis* seed showed a longevity of only 2–3 years [12]. The emergence ability of this seed is influenced by the depth of seed in soil and it is maximal for seed close to the soil surface; indeed, seeds of *C. canadensis* at a depth of 10 mm reduced their emergence by 90%, compared to seeds on the soil surface [25]. Germination and emergence may potentially occur during most of the year, including winter, as long as temperature is above the base level for germination, which was estimated to be around 13°C [19,26]. Usually, seeds germinate best in early fall or spring [16,27]. For facultative winter annuals like *C. canadensis*, germination response to temperature is an important characteristic influencing both fall and spring recruitment timing, duration, and proportion. Regional adaptation of weed species, and in particular facultative winter annuals, may very much be a function of germination response to temperature. In addition, germination response to temperature may impact the flexibility of a given population in relation to variations in climate and adaptation to climate change [19].

Conyza canadensis may be found in several crops, in both herbaceous and arboreal farming systems [19,25,28–30]. Furthermore, being a ruderal species, it is also found in uncultivated areas and in non-agricultural land, such as road and railway borders [31]. All over the world, this species is found in orchards, vineyards, corn, soybean, cotton, forages and pastures, fruits and vegetables crops, especially in conservative and no-till cropping systems [32–35]. For instance, *C. canadensis* has been reported as a major weed in more than 40 crops across 70 countries in the world [27]. Due to severe competition for resources, yield losses of up to 68% and 92% over weed-free control have been reported in soybean and cotton, respectively [32,33]. *C. canadensis* has a set of biological features including high seed production, efficient seed dispersal, germination ability under a wide range of environmental conditions, vigorous growth habit, flexible life cycle, and tolerance to harsh climatic conditions, which make it a problematic and invasive weed [19]. Furthermore, in the last few years, the high fruitfulness and the wide dispersion of seed have led to a rapid global spread and to the appearance of new genotypes characterized by particular adaptations, such as the resistance to triazines, paraquat, and glyphosate [35,36]. *C. canadensis* is one of the most difficult-to-manage weed due to multiple reasons, including resilient biological features, successful ecological adaptations, strong interference ability and herbicide resistance. Cultural strategies such as strategic tillage, mulching, crop rotations, solarization, high planting density, narrow row spacing, and allelopathy have proved effective in controlling this weed. However, the degree of control varied with cropping systems and other crop management practices [31].

Considering the above, it is clear that germination and emergence play a fundamental role in the spread of this species to new croplands and new habitats. Unfortunately, little information exists about the effect of multiple environmental factors on *C. canadensis* germination, which would help in estimating and modeling the invasiveness of this species [19,27]. With this respect, it is necessary to note that most weed emergence models are based on threshold temperatures (e.g., base temperature and ceiling temperature), which may depend on biotypes [11,19].

For the above mentioned reasons and while considering the importance of environmental factors on weed dynamics, especially in a period of global climatic changes, the aims of this study were to: (i) evaluate the effects of temperature, light and soil depth, on germination and emergence of *C. canadensis*, (ii) determine the base and ceiling temperatures for seed germination, which can be used to predict emergences in the field, and (iii) use the above information to suggest more efficient management strategies and agronomic techniques for better control of this invasive weed species.

2. Materials and Methods

Conyza canadensis seeds were collected from naturally senescing plants in an experimental field at the University of Perugia (42°57'21" N, 12°22'21" E). Seeds were stored in a screw cap plastic bottle inside a fridge (at temperature of 4 °C) in total darkness, until the assays. Seeds used in the experiments were selected according to the dark brown color of the tegument, which is an indicator of maturity, as shown during preliminary experiments.

2.1. Germination Tests

For these tests, 50 seeds of *C. canadensis* were placed on a single layer of filter paper in plastic Petri dishes with a diameter of 90 mm. The filter paper was moistened with 5 mL of deionized water [27] and Petri dishes were placed inside the growth chamber under eight different constant temperature regimes (2.5, 5, 10, 15, 20, 25, 30, and 40 °C) or three alternating temperature regimes (12/18, 17/23, and 22/28 °C). The photoperiod was always 12:12 h (light:darkness); for the alternating temperatures, while the lowest temperature was during the darkness period. Seed germination was also tested at constant 15, 20, and 25 °C in complete darkness. All treatments were randomly allocated to three replicated Petri dishes.

Seeds were considered as germinated when the radicle protrusion was > 1 mm. Germinated seeds were counted daily and removed from Petri dishes, until no more germinations could be observed for two consecutive days. For assays in complete darkness, counts were made under a green safelight [37].

2.2. Emergence Tests

For these tests, 30 seeds of *C. canadensis* were sown at four different depths (0, 2.5, 5, and 10 mm) in 1 L pots filled with a soil mixture (77% peat, 15.5% loamy soil, and 7.5% gravel). Prior to sowing, seeds were either left un-treated in the dark (NI: no water imbibition and no light exposure), or they were pre-treated by imbibition in water and exposure to light for two days (I: water imbibition and light exposure for two days). After sowing, pots were sub-irrigated as needed to maintain the field capacity of soil and were placed inside growth chambers at three different temperatures (15, 20, 25 °C and 12:12 h light:darkness). Seedlings were considered as emerged when the cotyledons were visible. Emerged seeds were counted daily and removed from pots, until no more emergences could be observed for two consecutive days.

2.3. Statistical Analyses

The data collected in each test and Petri dish/pot were used to parameterize a time-to-event model, assuming a log-logistic distribution of germination times [38], corresponding to the following cumulative distribution function:

$$P(t) = \frac{d}{1 + \exp [b(\log(t) - \log(e))]} \tag{1}$$

where P is the proportion of germinated seeds at time t , d is the proportion of germinated seeds when $t \rightarrow \infty$, b is the slope around the inflection point, and e is the median germination time.

The fitted curves were used to derive, for each Petri dish/pot, the Final Germinated Proportion (FGP) and the time to 30% emergence (T_{30}), which were taken, respectively, as a measure of germination capacity and germination velocity. The FGPs obtained in each dish/pot were analyzed by using a Generalized Linear Model (GLM) with binomial error and logit link [39]. Germination times (T_{30}) obtained in each dish/pot were analyzed by using a GLM, with log-normal error and an identity link [39]. For both GLMs, the combination of temperature and a light regime was included as a factor, with 14 levels. For both cases, means were compared by using a generalized multiple comparison procedure, with multiplicity adjustment [40].

2.4. Modeling Germination Rates as a Function of Temperature

Seed germination modeling is usually accomplished considering the effect of temperature on Germination Rates (GRs; i.e., the inverse of germination times), based on a few threshold parameters, such as base temperature, optimal temperature and ceiling temperature [41]. GR values are highly variable within a population and, therefore, germination models usually consider the different sub-populations (percentiles). In particular, we used the GRs for the 10th, 20th, 30th, 40th, and 50th percentiles of the whole population (GR_{10} , GR_{20} , GR_{30} , GR_{40} , and GR_{50} , respectively), including the un-germinated fraction. These GR values were derived from the fitted log-logistic curves at constant temperature in alternating light regimes. GRg values were used to fit the following model, taken from Catara et al. [42] and Masin et al. [43]:

$$GR(T)_i = \begin{cases} \frac{T-T_{bi}}{\Theta_{Ti}} \left\{ \frac{1-\exp[k_i(T-T_{ci})]}{1-\exp[k_i(T_{bi}-T_{ci})]} \right\} & \text{if } T_b < T < T_c \\ 0 & \text{if } T \leq T_b \text{ or } T \geq T_c \end{cases} \tag{2}$$

where i is the percentile (10th, 20th, 30th, 40th and 50th percentiles), T is the environmental temperature, T_b is the base temperature, T_c is the ceiling temperature, Θ_T is the thermal time to germination (°C day), and k is a shape parameter. The above model predicts no germinations below T_b and above T_c (threshold parameters); inside this temperature range, for a given g , the GR value increases up to a maximum value and decreases progressively afterwards, dropping down to 0 at the ceiling temperature (T_c). This bell-shaped trend is highly asymmetric, with a slow increase at sub-optimal temperature levels and a steep drop afterwards. In the above model, the four parameters are allowed to assume a different value for each percentile i , so that there is a total of 20 parameters to be estimated.

Based on the observed estimates, we used likelihood ratio tests as a basis to progressively reduce the number of model parameters, by constraining them to either (i) assume the same value across percentiles, or (ii) to be linear functions of the percentile g . In the end, we reached the following equation:

$$GR(g, T) = \begin{cases} \frac{T-T_b}{\Theta_{T(g)}} \left\{ \frac{1-\exp[k(T-T_{c(g)})]}{1-\exp[k(T_b-T_{c(g)})]} \right\} & \text{if } T_b < T < T_c \\ 0 & \text{if } T \leq T_b \text{ or } T \geq T_c \\ \Theta_{T(g)} = \Theta_{T(0)} + b_1g \\ T_{c(g)} = T_{c(0)} + b_2g \end{cases} \tag{3}$$

where g is the percentile (from 10th to 50th), $\Theta_{T(0)}$ and $T_{c(0)}$ are respectively the thermal-time to germination and the ceiling temperature for the quickest seed within the population, while b_1 and b_2 are the slopes, measuring how quickly Θ_T and T_c respectively increase and decrease within the population, passing from the quickest to the lowest germinating seeds. In this final model, the percentile is regarded as a numeric variable, T_b and k are constant for the whole population, while Θ_T and T_c are linearly

related to the percentile g , according to third and fourth equation in the array. In Equation (3) there are only 6 parameters to be estimated, instead of the initial 20 in Equation (2).

Equations (2) and (3) were fitted by using a Box-Cox Transform-Both-Sides technique ($\lambda = 0$), to account for heteroscedasticity [44]. In the end, Equation (3), together with the parameter values as estimated in the previous step, was used to predict the germination times to reach 10%, 20%, 30%, 40%, and 50% seed germination in different temperature regimes. Predictions were compared to observations by using simple graphical methods.

All analyses were made by using the R statistical environment [45], together with the packages 'drc' [46] and 'drcSeedGerm' [47].

3. Results

3.1. Germination Tests

The germination of *C. canadensis* could be adequately described with log-logistic time-to event models at all temperatures and light regimes (Figure 1).

The FGP was zero at a constant temperature of 2.5 °C, it reached the maximum value of 0.7 at 15 °C, and then declined again at increasing temperatures, reaching zero at 40 °C (Figure 1 and Table 1). At the alternating temperature of 12/18 °C, the FGP was 0.49 and it was significantly lower ($p < 0.05$) than at constant 15 °C, but it was not significantly different from those observed at constant 10 and 20 °C (respectively 0.54 and 0.61; Table 1). Considering the alternating temperature treatment 17/23 °C, the FGP (0.67) was not significantly different from those obtained at constant 15, 20 and 25 °C. Finally, at 22/28 °C, the FGP was 0.61 and it was not significantly different from those obtained at constant 20, 25, and 30 °C. On average, it would appear that an alternating temperature regime does not improve germination capacity, compared to a constant daily temperature regime.

In total darkness, FGP values were not significantly different from those obtained in the presence of light at the same temperature (Table 1).

Relating to germination velocity, we considered the germination times for the 30th percentile of the whole population, which roughly corresponds to a half of the maximum germinated fraction (Table 1). Considering the treatments where 30% germination was attained, we can see that T_{30} values ranged between a maximum of 6.92 days to a minimum of 1.49 days (Table 1). The lowest germination time was observed at 25 °C (light/dark) and it was significantly increased at 12/18 °C and in darkness (Table 1). In particular, the germination times in the dark were always significantly higher than in the presence of light at the same temperatures (Table 1). As for the alternating temperatures, compared to constant temperatures with the same light/dark regime, the T_{30} at 12/18 °C was 2.94 days, significantly higher than 2.54 obtained at 15 °C, while it was lower than the 6.92 days obtained at 10 °C. At 17/23 °C, the T_{30} was 1.55 days and it was significantly lower than at 20 °C and not significantly different from 25 °C. At 22/28 °C, the T_{30} was 1.53 days and it was not significantly different from 1.49 obtained at 25 °C, while it was lower than 1.89 and 1.99 days, obtained at 20 and 30 °C, respectively (Table 1).

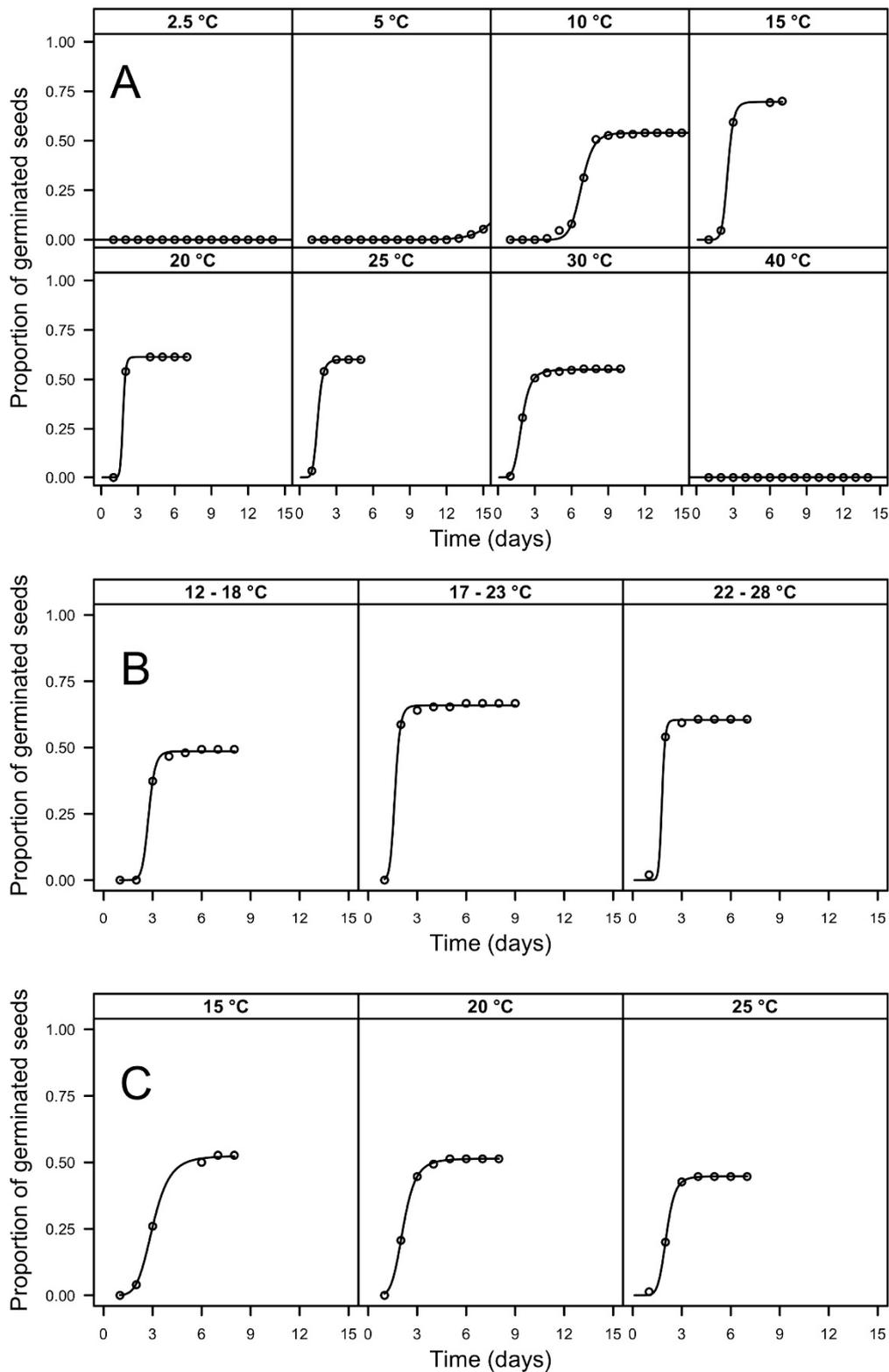


Figure 1. Germination time courses for *Conyza canadensis*, as observed at different temperature and light regimes: (A) = constant temperatures and light/darkness (12 h/12 h) regime; (B) = alternating temperatures and light/darkness (12 h/12 h) regime; (C) = constant temperatures and complete darkness. Symbols show the observed data, while lines show the fitted germination curves, according to Equation (1).

Table 1. Influence of temperature and light regime on maximum proportion of germinated seeds (FGP, Final Germination Proportion) and time to 30% germination (T_{30}) for *C. canadensis*. Standard errors are in parentheses.

Temp.	Light Regime (Light/Dark h.)	FGP		T_{30} (d)	
2.5	12/12	0.00 (0.00)	a	>16	-
5	12/12	0.11 (0.02)	b	>16	-
10	12/12	0.54 (0.04)	cde	6.92 (0.27)	a
15	12/12	0.70 (0.04)	e	2.54 (0.10)	c
20	12/12	0.61 (0.04)	cde	1.89 (0.09)	f
25	12/12	0.60 (0.04)	cde	1.49 (0.06)	g
30	12/12	0.55 (0.04)	cde	1.99 (0.08)	ef
40	12/12	0.00 (0.00)	a	>16	-
12/18	12/12	0.49 (0.04)	cd	2.94 (0.12)	b
17/23	12/12	0.67 (0.04)	de	1.55 (0.06)	g
22/28	12/12	0.61 (0.04)	cde	1.53 (0.07)	g
15	0/24	0.53 (0.04)	cde	3.24 (0.13)	b
20	0/24	0.51 (0.04)	cd	2.26 (0.09)	cd
25	0/24	0.45 (0.04)	c	2.17 (0.11)	de

In each column, values followed by at least one letter in common are not significantly different according to a multiple comparison procedure with a multiplicity adjustment ($p = 0.05$; [40]).

3.2. Emergence Tests

The emergence of *C. canadensis* could also be adequately described with log-logistic time-to event models (Figure 2).

The FGP was at its maximum value for seeds at 0 mm depth and decreased dramatically with increasing depth, for all temperatures, regardless of pre-treatments (Table 2). In the trials without pre-treatment, no seedlings emerged from burial depths of 5 mm and deeper. Seeds with pre-treatment generally had a higher capacity of emergence from 2.5 mm and 5 mm depth, with respect to not pre-treated seeds, while no significant differences were observed at 10 mm depth (Table 2). Considering seeds at 0 mm depth, no significant differences emerged between temperature levels and pre-treatments.

The time to 30% emergence could only be calculated for seeds close to the soil surface, as the proportion of emerged seeds from higher depths never reached 30%. For those seeds, the T_{30} was significantly lower at 20 and 25°C, especially with pre-treatment (Table 2).

Table 2. Influence of sowing depth, temperature and seeds pre-treatment (I: water imbibition and light exposure for two days; NI: no water imbibition and no light exposure) on maximum proportion of emerged plants (FGP, Final Germination Proportion) and time to 30% emergence (T_{30}) for *C. canadensis*. Standard errors are in parentheses.

Depth (mm)	Temperature (°C)	Pre-Treatment	FGP		T_{30} (d)	
0	15	NI	0.34 (0.03)	a	11.7 (2.58)	a
2.5	15	NI	0.02 (0.01)	cde	>16	
5	15	NI	0.00 (0.00)	c	>16	
10	15	NI	0.00 (0.00)	c	>16	
0	20	NI	0.36 (0.03)	ab	8.4 (1.48)	a
2.5	20	NI	0.04 (0.01)	def	>16	
5	20	NI	0.00 (0.00)	c	>16	
10	20	NI	0.00 (0.00)	c	>16	
0	25	NI	0.40 (0.03)	a	4.7 (1.04)	ab
2.5	25	NI	0.07 (0.01)	dfa	>16	
5	25	NI	0.00 (0.00)	c	>16	
10	25	NI	0.00 (0.00)	c	>16	

Table 2. Cont.

Depth (mm)	Temperature (°C)	Pre-Treatment	FGP			T ₃₀ (d)		
0	15	I	0.25	(0.02)	bh	>16		
2.5	15	I	0.13	(0.02)	g	>16		
5	15	I	0.05	(0.01)	def	>16		
10	15	I	0.00	(0.00)	c	>16		
0	20	I	0.48	(0.03)	a	2.5	(0.47)	b
2.5	20	I	0.16	(0.02)	gh	>16		
5	20	I	0.08	(0.01)	dfg	>16		
10	20	I	0.00	(0.00)	c	>16		
0	25	I	0.47	(0.03)	a	1.8	(0.56)	b
2.5	25	I	0.10	(0.02)	fg	>16		
5	25	I	0.09	(0.02)	fg	>16		
10	25	I	0.01	(0.01)	ce	>16		

In each column, values followed by at least one letter in common are not significantly different according to a multiple comparison procedure with multiplicity adjustment ($p = 0.05$; [40]).

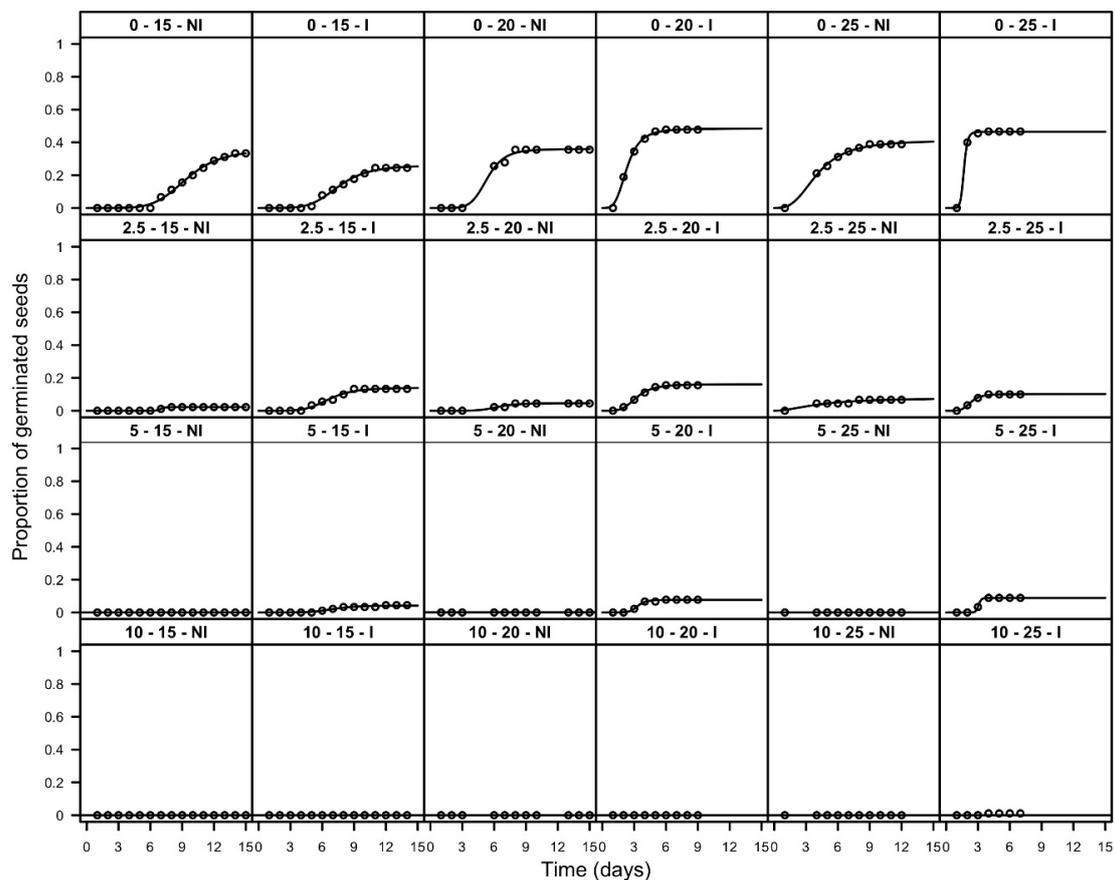


Figure 2. Emergence trends in assays conducted at different burial depth (0; 2.5; 5; 10 mm), temperatures (15, 20 and 25 °C) and pre-treatments (I: water imbibition and light exposure for two days; NI: no water imbibition and no light exposure). The heading of each panel plot represents the ‘depth - temperature - pre-treatment’ combination. The assays were conducted under a photoperiod of 12 h light and 12 h darkness.

3.3. Modeling Germination Rates as a Function of Temperature

The germination rates from the 10th to 50th percentiles were used to parameterize Equation (2), by regarding the percentile g as a factor and allowing different parameters for different percentiles,

so that we had a total of 20 parameters. Consistently with literature [41,48], first of all we tested the hypothesis that T_b and k were common to the whole population, which proved to be acceptable ($p = 0.455$). Refitting Equation (2) with common T_b and k yielded the estimates in Table 3.

Table 3. Influence of temperature on the germination rates for *Conyza canadensis* for the different percentiles: parameter estimates for Equation (2). T_b : base temperature, Θ_T : thermal time for seed germination, k : shape parameter, T_c : ceiling temperature. Standard errors are in parentheses.

Percentile	T_b (°C)		Θ_T (°C Day)		k		T_c (°C)	
10 th	6.67	(0.53)	20.24	(1.57)	0.23	(0.06)	33.90	(0.82)
20 th	6.67	(0.53)	21.69	(1.81)	0.23	(0.06)	33.33	(0.70)
30 th	6.67	(0.53)	22.82	(2.00)	0.23	(0.06)	32.95	(0.62)
40 th	6.67	(0.53)	24.03	(2.22)	0.23	(0.06)	32.55	(0.54)
50 th	6.67	(0.53)	24.32	(2.72)	0.23	(0.06)	30.71	(0.22)

Equation (2) predicts the GR_s for the 10th, 20th, 30th, 40th, and 50th percentiles, but it does not allow for any extrapolation to other subpopulations (e.g., the GR_{15} cannot be predicted). However, Table 3 shows that Θ_T increases approximately linearly with the percentile. Likewise, the ceiling temperature (T_c) appears to decrease linearly. We coded this result into Equation (3), which did not prove to fit significantly worse than Equation (2), in spite of a much lower number of parameters ($p = 0.136$; Figure 3). The estimated parameters for Equation (3) are shown in Table 4.

The base temperature (T_b) is equal to 6.8 °C (± 0.54) and it is constant throughout the population. The ceiling temperature (T_c) is 35.8 °C and it decreases from the 10th to the 50th percentile, so that the germination of the slowest seeds tends to have lowest T_c values (Figure 3, Table 4).

The thermal-times to germination are equal to 19.6 degrees-days for the fastest seeds (10th percentile), and increase progressively within the population for higher percentiles.

Thermal-time parameters in Table 4 are relevant in themselves, as they can be used in one of the available germination models to predict the emergences of *C. canadensis* in the field [49,50]. However, Equation (3) can also be used to predict the germination times to reach a certain proportion of germinated seeds, according to the temperature regime. These predictions are very good for the constant temperature regime with 12 h light and 12 h dark (Figure 4, left), which was expected, as the model was parameterized by using the dataset obtained in these conditions. However, predictions are also good for the dishes at alternating temperatures, consistently with the result that germination behavior for this species is not affected by daily temperature fluctuations (Figure 4, middle). On the contrary, the model underestimates the time to germination for dishes in dark conditions, which we had already mentioned before.

Table 4. Influence of temperature on the germination rates for *Conyza canadensis*: parameters estimates for Equation (3). T_b : base temperature, $\Theta_{T(0)}$: thermal time for seed germination of the quickest seed in the lot, b_1 : slope for Θ_T as a function of the percentile, k : shape parameter, $T_{c(0)}$: ceiling temperature for the quickest seed in the lot, b_2 : slope for T_c as a function of the percentile.

Parameters	Estimate	SE
T_b	6.81	0.54
$\Theta_{T(0)}$	19.64	1.48
b_1	0.073	0.037
k	0.201	0.054
$T_{c(0)}$	35.78	1.16
b_2	-0.094	0.021

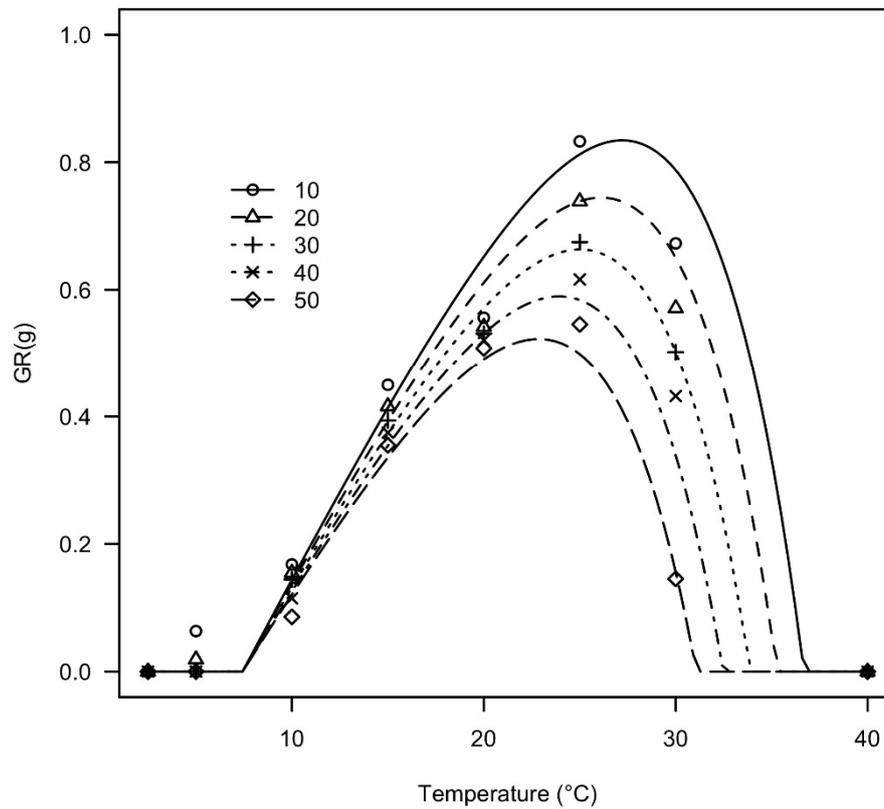


Figure 3. Relationship between germination rates for different percentiles (g) and temperatures. Symbols show the observed data, while lines show the fitted curves (Equation (3), with the parameters in Table 4).

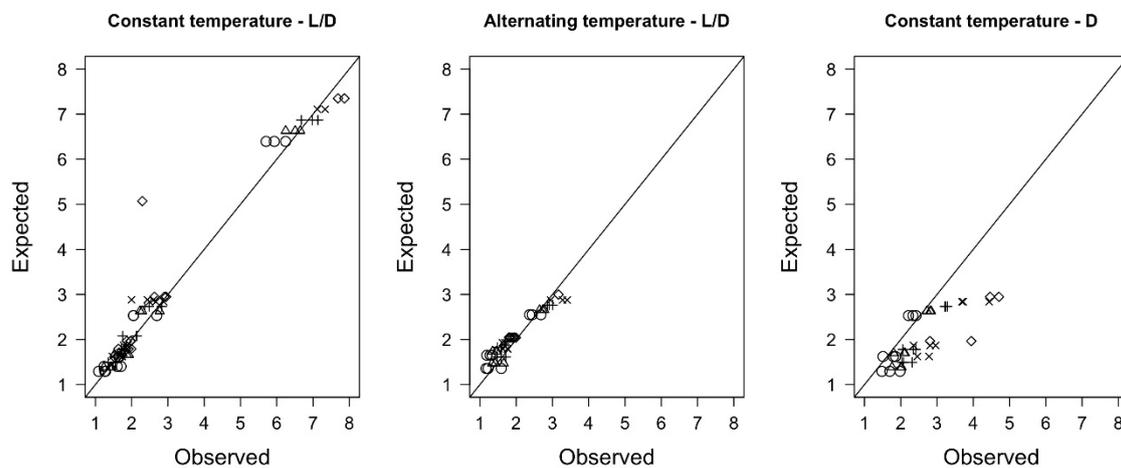


Figure 4. Germination times for the 10th (circle), 20th (triangle), 30th (cross), 40th (multiplication sign) and 50th (diamond) percentiles. Observed values in all dishes and predictions with Equation (3) and the parameters in Table 4 at different temperature (constant, alternating) and light (alternating light/dark, L/D and complete darkness, D) regimes.

4. Discussion

The above results show that *C. canadensis* has the ability to germinate under a broad range of temperatures (from 5–10 °C to 30 °C), thus it can be classified as a facultative winter annual weed germinating species [16,51]. Assuming adequate soil moisture, this trait gives *C. canadensis* the possibility of emerging almost throughout the year, with the exception of very cold winters

when the temperature falls below the base temperature level. Furthermore, this species showed no particular requirement in terms of daily temperature fluctuations. These traits should ensure a high potential to spread into habitats with a wide range of thermal conditions. On the other hand, germination is limited by temperatures outside of the above mentioned range (from 5 °C to 30 °C), which could represent an adaptive strategy for this species to survive in un-favorable conditions. Within this strategy, seed dormancy could play a role, as observed by Karlsson and Milberg [51], who defined *C. canadensis* as a species with “non-deep physiological dormancy type 1”. Others have classified *C. canadensis* as non-dormant, with potential for a small fraction of seeds to have “non-deep physiological dormancy” [12,19,27].

The modeled base temperature level for our Italian population was 6.8°C, which was lower than the values of 13 °C and 12/18 °C, which were found respectively for Californian [26] and Mississippian [27] populations. Likewise, in our experiments, the highest germination capacity (FGP) was observed at 15 °C, while Nandula et al. [27] reported 24/20 °C as the optimal temperature levels for best germination capacity. It would appear that our central Italian population is more highly adapted to colder climates, with respect to the populations from the Southern part of the US. Indeed, California and Mississippi are characterized by lower latitudes with respect to central Italy (33°–34° N vs. 43° N) and higher yearly average minimum temperatures (14–12 °C vs. 7 °C; see www.usclimatedata.com and www.climatedata.eu). Similar adaptation phenomena were also noted by Tozzi et al. [19] for different populations of *C. canadensis* from several habitats with contrasting climates.

It is therefore confirmed that *C. canadensis*, similarly to other invasive species, is very plastic in terms of adaptation to temperature variations. This is in accordance with Davidson et al. [52], who showed that invasive species demonstrate significantly higher plasticity than non-invasive species. Furthermore, although we considered only one population, our findings support the idea that studying local weed populations is essential to be able to predict the impact of weeds in agro-ecosystems. A high plasticity is usually associated to high adaptability to climate changes. This trait, in addition to increased resistance to glyphosate and ability of producing seeds with elevated ozone concentrations [53], seems to confirm that this species holds a high potential to show exponential population growth in the near future [1,9].

Another important trait is the ability of weed species to emergence and establish when seeds are buried into the soil or when the soil surface is covered by a dense canopy of competing plant species. In this respect, our experiments showed that germination capacity (FGP) and velocity (T_{30}) in the dark were often lower than in the presence of light, confirming the results obtained by Nandula et al. [27]. This finding is consistent with the observed decrease in emergence ability when seed was buried at 2.5 mm and 5 mm depth and with the inability of emerging from 10 mm depth. Such findings could be explained by considering the low amount of food reserves in seed and by the absence of the light stimulus in deeper soil layers. This was confirmed by the result that the ability of emerging from 2.5 and 5 mm depths was improved by a pre-treatment with water imbibition and exposure to light.

These characteristics, combined with wind dispersal, high seed production, non-deep dormancy and rapid germination and emergence from the soil surface may represent key factors for the spread of this weed species in the case of conservation tillage or no-tillage [31,54]. Moreover, our findings could help explain why the emergence of *C. canadensis* has been found to be decreased by the presence of crop residues [22,55], which can reduce the light stimulus.

From a modeling perspective, we have used our data to fit a model describing the variation of germination rates, depending on the environmental temperature. This model showed that base temperature is common to all seeds in the population, while ceiling temperature changes from seed to seed, so that the slowest germinating seeds tend to have the lowest ceiling temperatures values. A similar result was observed, for example, in Rowse and Finch-Savage [48], working with carrot and onion. These estimated threshold values can be used to predict the germination and emergence of *C. canadensis* in the field, by using one of the available models [49,50]. We have also proposed that Equation (3) can be used as a predictive model, although our dataset is too small to draw any reliable

conclusion about the predictive value of our modeling approach. Future studies should shed more light on this aspect.

Considering the above results, useful information can be derived to improve the control of *C. canadensis*. First of all, a good prevention strategy should be based on careful control on already infested fields, field margins, and uncultivated areas in order to reduce the spread of this weed to adjacent fields [28]. The prevention is fundamental to reduce the risks of expanding infestation when considering the high potential of seed dispersal shown by *C. canadensis* [23]. The use of the false seedbed technique should be considered to promote the emergence of seeds close to the soil surface and, subsequently, control the plants prior to crop sowing, which might help minimize competitive effects in the early crop stages [56]. The wide range of temperatures for germination and emergence showed by *C. canadensis* confirms the crucial role of preventive agronomic practices to manage effectively this weed species. Literature data in soybean show that, without the false seedbed technique, there was a dramatic increase of many early germination summer annual weed species, including *C. canadensis* [57]. The introduction of cover crops in the rotations and the use of dead mulching could be good strategies for the management of this weed due to the effects of cover crop competitiveness and mulching action on weed suppression [22]. In particular, Pittman et al. [58] found a sensible reduction in the density of *C. canadensis* (88% to 96%, with respect to the untreated check) when cereal (rye) or legume (crimson clover and hairy vetch) cover crops were used. Likewise, Campiglia et al. [59] found that hairy vetch and barley straw were the most suppressive dead mulching for controlling *C. canadensis* in strip-tilled tomato.

We have already mentioned that *C. canadensis* may become particularly dense in cropping systems based on minimum-tillage or no-tillage. In these systems, the repeated use of glyphosate may pose additional problems, relating to the risk of increased frequency of glyphosate resistant biotypes. Since the emergence is mainly from seeds in shallow soil layers, as found in our research, and by seeds endowed of low longevity [12], it is suggested that occasional plough tillage could be applied after the main peaks of seed dispersal [32]. In this way, seeds can be buried deep into the soil, which should increase their mortality and decrease the soil seed bank [7,60]. In fact, in a field study of different fallow practices in New South Wales, there was little *Conyza* spp. present where soil cultivation had been used [51]. Bhowmik and Bekech [22] found that spring tillage in corn caused a reduction in the density of *C. canadensis*, by controlling plants in the early vegetative stages.

Future research on the management of *C. canadensis* should be directed towards the adoption of integrated approaches [61], including the improvement of crop competitiveness, by way of improved genotypes, appropriate sowing techniques, and intercropping.

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