Végéphyl – 24e CONFÉRENCE DU COLUMA
JOURNÉES INTERNATIONALES SUR LA LUTTE CONTRE LES MAUVAISES HERBES
ORLÉANS – 3, 4 et 5 DÉCEMBRE 2019

TEMPERATURE THRESHOLDS, LIGHT REQUIREMENT, AND GROWING-DEGREE-DAY MODEL FOR *GLEBIONIS CORONARIA* L. GERMINATION AND DEVELOPMENT

Z. HADA*[1], H. JENFAOUI[1], A. ROYO-ESNAL [2], J. TORRA[2], T. SOUISSI [1]

[1]: *Département de Santé Végétale et Environnement, Institut National Agronomique de Tunisie Université de Carthage,* 43 Avenue Charles Nicolle, Tunis 1082, *Tunisie*

[2]: *Department d'Hortofructicultura, Botànica i Jardineria, Agrotecnio, Universitat de Lleida,* Alcalde Rovira Roure 191, 25198 Lleida, Spain

*Corresponding author, zeineb.hada@gmail.com

ABSTRACT

Chrysanthemum (*Glebionis coronaria* (L.) Cass. Ex Spach) is a very common annual broadleaf weed in cereal crops in north Tunisia. Recently, it has developed resistance to common ALS inhibiting herbicides. To better understand the biology of this new resistant weed species, three experiments were conducted in order to determine germination and growth requirements. Results showed that seeds could germinate over a wide temperature range (5°C to 30°C), with optimum germination being at 18°C, and the ceiling temperature estimated at 40°C. The predicted base temperature for germination was -1.74°C. The light increased slightly the germination but no significative difference was found between light and darkness. Adopting the estimated base temperature, a model (Y=0.0370 x-0.2085 with R^2=0.97) was adjusted to predict chrysanthemum phenology based on growing degree days (GDD). Under greenhouse conditions, chrysanthemum required 2328.38 GDD from emergence to mature seed production.

Keywords: *Glebionis coronaria* L., base temperature, germination, growing degree days, phenology

RÉSUMÉ

Le chrysanthème (*Glebionis coronaria* (L.) Cass. Ex Spach) est une mauvaise herbe dicotylédone annuelle répandue dans les cultures céréalières du nord de la Tunisie. Récemment, il a développé une résistance aux herbicides inhibiteurs de L'ALS. Afin de mieux comprendre la biologie de cette nouvelle adventice résistante, trois expériences ont été menées afin de déterminer les exigences de germination et de croissance. Les résultats ont montré que les semences pouvaient germer à des températures de 5 à 30°C, avec une germination optimale à 18 °C, la température maximale a été estimée à 40 °C. La température de base est égale à -1,74°C. La lumière a légèrement augmenté le taux de germination, mais aucune différence significative n'a été observée entre lumière et obscurité. A la température de base trouvée, un modèle (Y = 0,0370 x-0,2085 avec R^2 = 0,97) a été ajusté pour prédire la phénologie du chrysanthème en fonction des degrés-jours (JDD). Sous serre, le chrysanthème nécessite 2328,38 JDD de la levée jusqu'à la production de semences.

Mots-clé : *Glebionis coronaria* L., température de base, germination, degrés-Jours (JDD), phénologie.

INTRODUCTION

Chrysanthemum (*Glebionis coronaria* (L.) Cass. Ex Spach) is an heterocarpic annual winter weed belonging to the Asteraceae family. It is usually found as a weed in several crops, along roadsides, on field margins, and on abandoned fields (Cook, 2011). Despite essentially Mediterranean, it was reported as invasive plant species far beyond their native range because of its abundance and persistence (Cook, 2011). In north Tunisia, the chemical control using common ALS inhibiting herbicides is declared ineffective by farmers in last years. This control failure was attributed to the development of herbicide resistance in chrysanthemum as a second case worldwide (Heap, 2019).

For annual weeds, such as chrysanthemum, the timing of germination is critical for its life cycle (Saatkamp et *al*, 2009), with temperature and light as the most important environmental cues for initiating such process (Schutte et *al*, 2014). For seeds buried in the soil, temperature and light are important by providing a sense of their position in the soil profile and the occurrence of soil disturbance (Batlla and Benech-Arnold, 2014).

To predict potential germination times, temperature thresholds should be determined, particularly the base temperature (Tb), that is, the temperature below which the process of germination does not occur for a given species (Arnold, 1959).

Base temperature is required also to account for the effect of temperature, as thermal time units, on the germination rate (Trudgill et al., 2005). Expressed in growing degree-days (GDD), thermal time units have been used to describe the timing of biological processes of some winter annual weeds (Ball et al, 2004). Moreover, it has vastly improved the description and prediction of phenological events compared to other approaches such as time of year or number of days (McMaster, 1993).

For chrysanthemum, there have been few studies about the effect of abiotic environmental factors such as temperature and light on seed germination requirements (Bastida et *al*, 2010; Puglia, 2013). Additionally, there is currently no information, as far as we know, on the Tb and cumulative temperature for the germination and the growth of chrysanthemum.

The main objective of this work was to study some biological traits of chrysanthemum, specifically focusing on (1) temperature and light regime requirements for germination, (2) optimum (T_o), ceiling (T_c) and base (T_b) temperature for germination, and finally (3) modeling the phenology of the weed based on the degree days required for every stage.

MATERIALS AND METHODS

Plant material

Mature achenes of a herbicide resistant chrysanthemum population were collected from Fritissa, Mateur, in north Tunisia (37°1'50.91"N, 9°42'26.92"E) in June 2018. Achenes were collected from at least 40 plants randomly chosen from a cereal field which was under cereal monoculture using ALS inhibiting herbicides for more than eight years. Harvested achenes were cleaned, stored in glass bottles at room temperature (20 °C) and used six months later for next experiments.

Experiment 1: Base, ceiling and optimum temperatures requirement for chrysanthemum germination.

A growth chamber experiment was carried out on December 2018. Five replicates of 20 achenes were used for each temperature treatment in a complete randomized design. Scarified achenes were laid out in Petri dishes lined with a single layer of filter paper, and moistened with 8 ml of distillated water. Petri dishes were wrapped with parafilm and placed in growth chambers at six constant temperatures (5, 10, 15, 20, 25, and 30 °C) with light for 12 h a day. The germination percentage was determined twice a day until no more germination occurred. Achenes were considered germinated when the radicle was about 1mm long (Steinmaus et al. 2000). For the T_o and T_c, the relationship between temperature and germination rate was fitted following a Gaussian equation with 3 parameters (Figure1)

$$\text{Equation 1: } GR\ (\%) = GRmax * e^{\left(-0.5*\left(\left(\frac{T-To}{b}\right)^2\right)\right)}$$

Where GR is the germination rate corresponding to a temperature T, GR_{max} is the maximum rate of germination corresponding to the optimum temperature, T_o is the optimum temperature and b is the shape of the curve.

The time to 50% germination (t_{50}) was derived by modelling cumulative germination over time using a nonlinear sigmoidal function with 3 parameters following the equation 2

$$\text{Equation 2: } GPT = \frac{GP\text{final}}{1+e^{\frac{(-(t-t50))}{b}}}$$

Where GP_T is the proportion of germinated achenes up to the sampling time t, t is the time (hours) since seeding in Petri dishes, t_{50} is the time needed (hours) to reach 50% of the final germination proportion and GP_{final} is the maximum proportion of germination and b is the slope, which corresponds to the GR at t_{50}.

For the Tb estimation, the reciprocal time to 50% germination ($1/t_{50}$) was regressed on temperature (Equation 3). From this regression, the Tb was estimated as the x-intercept by a mathematical approach (Steinmaus et *al*, 2000).

$$\text{Equation 3 }: \frac{1}{t50} = y0 + a * t$$

Where a and y_0 are, respectively, the intercept and the slope of the linear regression versus temperature.

Experiment 2: Light requirement for chrysanthemum germination.

A growth chamber experiment was carried out to investigate the effect of light on chrysanthemum germination. Achenes were exposed to two light regimes (a 12-h photoperiod regime and full darkness regime) under three constant temperatures (15, 25 and 30°C temperatures), with five replicates per treatment in a complete randomized design. Each replicate consisted of 20 achenes set on 9 cm Petri dishes with a single filter paper. To achieve the full darkness, five Petri dishes were wrapped with aluminum foil. The number of germinated achenes was assessed twice per day until no germination happened.

Experiment 3: Chrysanthemum phenology using growing degree days

Growth and development experiment was carried out in a greenhouse located in the city of Tunis (36°49'49.01''N; 10°11'02.07''E, and an altitude of 23 m), from April to July 2018. Scarified achenes were used for the entire experiment. One achene of chrysanthemum was put to germinate in each cell of 0.7 mm thick plastic trays filled with commercial substrate inside the greenhouse. Uniform seedlings (2 leaf stage) were selected for transplanting each to a single pot; transplantation was done nine days after sowing (DAS). Chrysanthemum plants were grown under natural photoperiod, and a range of temperature between 18 and 28°C. The temperature was registered every hour using a data logger recorder. Every 10 days ten plants were harvested. The date of every stage was considered when at least 80 % of the plants reached the same stage. The phenological stages were determined through the BBCH weed phenological scale (Hess et *al*, 1997). Ten replicates (one plant per pot) were used in a complete randomized design. The cumulative GDD were calculated following the next equation

$$Equation\ 4: GDD = \left(\frac{Tmax+Tmin}{2}\right) - Tb$$

Where Tmax is the daily maximum temperature; Tmin is the daily minimum temperature and Tb refers to base temperature.

Statistical Analysis

All Curves fitting and the ANOVA for the effect of light regime on chrysanthemum germination were carried out using SigmaPlot 11.0 (Systat Software, San Jose, CA), the R^2 values were calculated as (sum of error squares) ⁄ (total corrected sum of squares).

RESULTS

1. **Temperatures thresholds.**

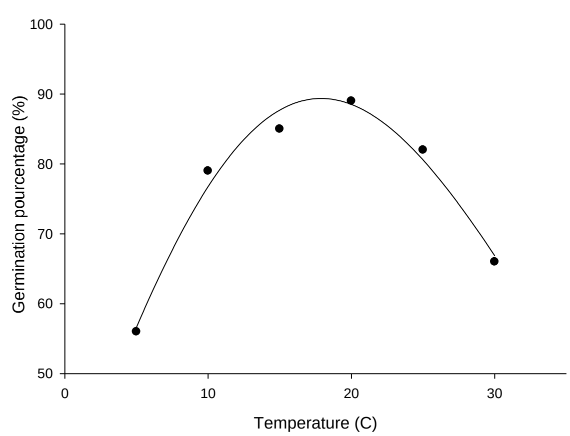
In order to estimate the optimum and the maximum temperature for the germination of chrysanthemum, a three parameter Gaussian model was successfully fitted, which represents the relationship between the GR and temperature (Fig 1).

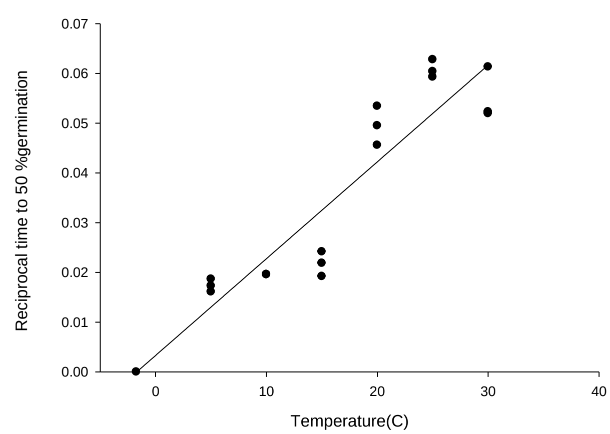
Figure. 1. Temperature (°C) effect on the germination rate (%) of *Glebionis coronaria* L. The curve is fitted as a Gaussian equation with 3 parameters.

Figure. 1. Effet de la température (°C) sur le taux de germination (%) de *Glebionis coronaria* L. La courbe est ajustée selon une équation de Gauss à 3 paramètres.

Figure. 2 Reciprocal time to 50% germination regressed on temperature for *Glebionis coronaria* L. Only the data points derived statistically for the time to 50% germination were plotted as observations (Symbols).

Figure. 2. Droite de régression de la durée 50% de germination sur la température pour Glebionis coronaria L. Seuls les points de données dérivés statistiquement pour la germination à 50% ont été reportés sous forme d'observations (Symboles).





The model had a good fit (R^2 =0.97) with $P_{model} =$ 0.0045<0.01. Both normality (P = 0.3295) and homogeneity (P = 0.0600) tests were passed. The results showed that achenes could germinate across a wide range of temperatures from 5°C to 30 °C. The germination percentages recorded were high for all temperature treatments 56 % and 89 % for 5°C and 20°C, respectively (Fig 1). It was required nearly 1.7 and 1 days for 50% germination at 15°C and 20°C, respectively. Our results suggested that T_b and

T_c are out of the range of the tested temperatures, although T_o can be detected directly from the fitting model, which was about 18 °C. Using the Gaussian equation, the maximum temperature for which no germination may occur was estimated at 39.5 °C. We expected that chrysanthemum germination should be entirely inhibited around 40°C.
Sigmoidal curves with three parameters were fitted to the germination percentages vs temperature (data not showed) in order to estimate the t_{50} for each experimental unit according to Steinmaus et *al*. (2000). The linear regression of $1/t_{50}$ over temperatures was highly significant (P_{model} <0.0001) and the normality (P = 0.2229) and homogeneity (P = 0.1097) tests were passed (fig 2). The Tb of chrysanthemum was estimated to be -1.74ºC almost -2°C, (Fig. 2).

2. Chrysanthemum light requirement

Germination rates were similar at all temperatures under complete darkness or 12/12 h light/dark. The light and the dark did not show any significant difference on seed germination. Nevertheless, we observed slightly higher germination percentages in complete darkness than under 12/12h of light/dark regime. Unlike, for the high temperature (30 °C), we observed that seeds required a little bit more light to reach higher GR (table I).

Table I. Germination percentage of *Glebionis coronaria* L. under two light regimes (12 h light, and full darkness), and three constant temperatures (15 °C, 25 °C, 30°C).

Tableau I. Pourcentage de germination de *Glebionis coronaria* L. sous deux régimes de lumière (12 h de lumière et obscurité totale) et trois températures constantes (15 ° C, 25 ° C, 30 ° C).

	Light regime		**Statistical differences**
	Light	Darkness	
Temperature °C			
15	85±10	87±9	NS
25	82±9	89±8	NS
30	65±14	61±10	NS

3. Growing-degree-day model for chrysanthemum development

Chrysanthemum emergence began four days after sowing. The model prediction for the beginning of the emergence was 103 GDD. Leaf development, side shoots formation and stem elongation required predicted 217, 673 and 812 GDD, respectively. Chrysanthemum began to flower 47 days after emergence (1238 GDD). 99% of flowering period was predicted to occur at 1793 GDD. Chrysanthemum closed its cycle by 2328 GDD (Table II).

The phenological development of chrysanthemum was fitted to the accumulated thermal units (GDD), the linear model equation was yielded with a high coefficient of determination (97 %) (Fig 3).

Figure 3: Phenological development of *Glebnions coronaria* L. as related to growing degree days (GDD) Figure 3: Développement phénologique de *Glebnions coronaria* L. par rapport aux degrés-jours (JDD).

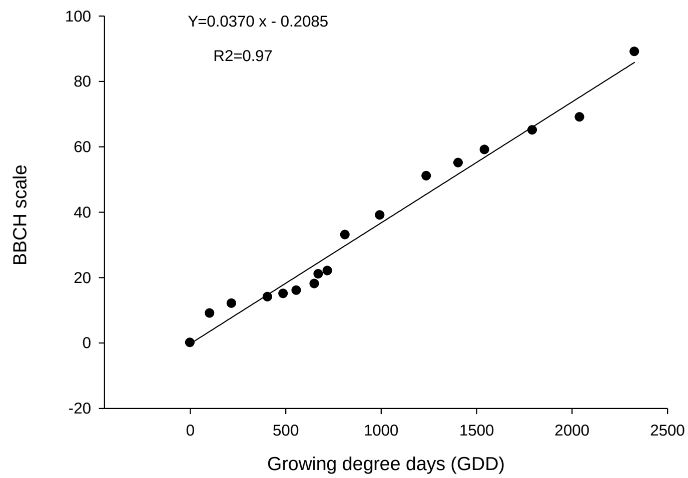


Table II - Principal growth stages of *Glebnions coronaria* L. (BBCH scale) in function of the evaluation period (in days after sowing – DAS and days after emergence – DAE and the GDD accumulated)

Tableau II - Principaux stades de croissance de *Glebnions coronaria* L. (échelle BBCH) en fonction de la période d'évaluation (en jours après le semis - DAS et en jours après l'émergence – DAE et en DJ accumulés)

Date	**BBCH**	**Development Stage**	**DAS**	**DAE**	**GDD**
17/04	0	Germination	0	-	0
25/04	1	Leaf development (main shoot)	9	4	217
16/05	2	Formation of side shoots	31	25	673
22/05	3	Stem elongation	37	31	812
7/06	5	Inflorescence emergence (main shoot	53	47	1238
28/06	6	Flowering (main shoot)	74	68	1793
17/07	8	Ripening or maturity of fruit and seed	93	87	2328

DISCUSSION

Glebionis coronaria L. appears to be widespread in the Mediterranean region (Turland,2004), characterized by hot, dry summers and mild, wet winters, where the minimum temperature is comprised between 5°C and 10 °C. This could explain why chrysanthemum is abundant in a region like North Africa and southern Europe but not in north Europe; where winters are colder and temperatures drop below the Tb estimated in this study. The Tb estimated suggests that chrysanthemum requires a low temperature for its germination, and may explain the high germination rate found previously for low temperatures (5°C and 10°C). This Tb is typical for winter annual plants, Torra et *al*. (2016) estimated under growth chamber conditions that values of Tb ≤0 °C for three Mediterranean weeds, *Hypecoum pendulum* (T_b = −2.6), *Roemeria hybrida* (T_b = −0.8 C) and *Papaver argemone* (0 ºC). For annual Asteraceae weeds such as *Crassocephalum crepidioides*, *Conyza canadensis*, and *Ageratum conyzoides*, it was reported that the maximum germination occurred at temperature range of 15–30˚C (Yuan and Wen, 2018) and it was highest at the average expected winter temperature, which is around 15°C (Schutz et *al*., 2002). These results are in agreement with what we found in this study for chrysanthemum.

In Tunisia, chrysanthemum germination occurs all the year starting from September (early autumn) to June (early summer) but there is no germination and emergence from June to September where the temperature can reach more than 35°C. Using the Gaussian fitting from our experience, the T_c for chrysanthemum germination was around 40°C. It seems to be that summer coincides well with the primary dormancy period of chrysanthemum. According to Bell et *al*. (1993), maintenance of dormancy under high temperatures is vital for species in Mediterranean-climate since occasional rainfall in summer don't provides sufficient moisture for germination.

In general, for annual Asteraceae under a Mediterranean climate, germination is greater in darkness than in light (Bell, 1993). For chrysanthemum no significant differences were found between light and darkness, with slight higher germination percentage at the T_o range. For the favorable period of chrysanthemum germination, seeds don't need light as an obligate cue to start germination. These results are the same found in previous works on *Glebionis coronaria* (Chiang et *al*, 1994; Puglia 2013). According to Bastida (2004), chrysanthemum emergence occurs in autumn and seedlings develop into overwinter rosettes that bolt in January and February. Flowering and fruiting start in late February or March and continue to early summer. These stages depend on the starting of the rainy season, the emergence period can start in autumn up to early winter (November, December) and the achene release starts probably in early June (Bastida et *al*, 2010). Our results provide a GDD characterization of the main stages of chrysanthemum development, which allows a better prediction of every stage. Nevertheless, a confirmation of the model fitted based on field data is required.

These results could partially explain the high competition of chrysanthemum observed in wheat fields. The low Tb, the rapid germination, the high germination rates under a wide range of temperatures, and the comparative life cycle behavior confer to this weed a strong competitiveness against winter wheat.

CONCLUSION

With this study, we target the most important environmental factors for the germination establishment of chrysanthemum. Light and temperature requirements were determined. Likewise, the base temperature was elaborated using the reciprocal time for 50% of germination method, this base temperature lead to predict and modeling the crucial stages of chrysanthemum development *versus* the GDD accumulated during the whole life cycle of the weed. Such study is likely to be useful for a first understanding of this weed's biology, which may help to more effectively manage this new threat in winter cereal fields.

REFERENCES

Arnold C.Y., 1959-The determination and significance of the base temperature in a linear heat unit system. Am. Soc. Horticult. Sci, 74, 430–445.

Ball D.-A.,Frost S.-M., Gitelman A.-I., 2004-Predicting timing of downy brome (*Bromus tectorum*) seed production using growing degree days Weed Science, 52,518–524.

Bastida F., Menendez J., 2004-Germination requirements and dispersal timing in two heterocarpic weedy Asteraceae. Communications in Agricultural and Applied Biological Sciences, 69, 67–76.

Batlla D., Benech-Arnold R.-L., 2014-Weed seed germination and the light environment: implications for weed management. Weed Biol Manag, 14, 77–87.

Bell D.-T., 1993-Germination responses to variations in light quality of eight species from sandy habitats in Western Australia. Australian Journal of Botany, 41, 321-326.

Bell D.-A., Plummer J.-A., Taylor S.-K., 1993-Seed germination ecology in southwestern Western Australia. The Botanical Review, 59, 24-73.

Chiang, M.-H., Park, K.-W., 1994-Effects of temperature, light and mechanical treatment on seed germination of Chrysanthemum coronarium L. Journal of Korean Society Horticultural Sciences 35,6, 534-539.

Cook R., Talley T.-S., Dayton P., Wang M., Nguyen, K., 2011-The effect of invasive crown daisy (*Chrysanthemum coronarium*) on a coastal sage scrub arthropod community in Southern California. California Invasive Plant Council Symposium, 63-65.

Bastida F., Gonzalez-Andujar J.-L., Monteagudo F.-J., Menendez J., 2010- Aerial seed bank dynamics and seedling emergence patterns in two annual Mediterranean Asteraceae. Journal of Vegetation Science, 21,541–550.

Hess M., Barralis G., Bleiholder H., Buhr L., Eggers T.-H., Hack H., Stauss R., 1997-Use of the extendend BBCH escale – general for descriptions of the growth stages of mono-and dicotyledonous weed species. Weed Res., 37, 6,433-441.

Heap I., 2019. The International Survey of Herbicide Resistant Weeds.www.weedscience.org. Accessed 25 September 2019.

McMaster G.-S., 1993- Another wheat (Triticum spp.) model Progress and applications in crop modeling. Rivista di Agronomia, 27, 264-272.

Puglia G.-D., 2013- Physiological and molecular mechanisms of seed germination in weed species.

Saatkamp A., Affre L., Dutoit T., Poschlod P., 2009-The seed bank longevity index revisited: limited reliability evident from a burial experiment and database analyses. Ann Bot (Lond), 104,715–724.

Schutte B.-J., Tomasek B.-J., Davis A.-S., Andersson L., Benoit D.-L., Cirujeda A., Dekker J., Forcella F., Gonzalez-Andujar J.-L., Graziani F., Murdoch A.-J., Neve P., Rasmussen I.-A., Sera B., Salonen J., Tei F., Torresen K.-S., Urbano J.-M., 2014- An investigation to enhance understanding of the stimulation of weed seedling emergence by soil disturbance. Weed Res, 54, 1–12.

Schutz W., MILBERG P., LAMONT B.-B., 2002-Seed Dormancy, After-ripening and Light Requirements of Four Annual Asteraceae in South-western Australia. Annals of Botany, 90, 707-714.

Steinmaus S.-J., Prather T.-S., Holt J.-S., 2000-Estimation of base temperatures for nine weed species. J. Exp. Bot, 51, 275–286.

Torra J.,Royo-Esnal A., Recasens J.,2016-Temperature and Light Requirements for Germination and Emergence of Three Arable Papaveraceae Species. Weed Science, 64,248–260.

Trudgill D.- L., HONEK A., LI D., VAN STRAALEN N.- M., 2005-Thermal time – concepts and utility,Annals of Applied Biology,146,1–14.

Turland N.-J., 2004-Proposal to conserve the name Chrysanthemum coronarium (Compositae) with a conserved type. Taxon, 53, 4, 1072-1074.

Yuan X., Wen B., 2018-Seed germination response to high temperature and water stress in three invasive Asteraceae weeds from Xishuangbanna, SW China. PLoS ONE. 13, 1, e0191710.