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TEMPERATURE AND LIGHT REQUIREMENTS FOR GERMINATION AND GROWING-DEGREE-DAY MODEL OF *HYPERICUM TRIQUETRIFOLIUM* TURRA

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ABSTRACT

Hypericum triquetrifolium is a perennial weed that is becoming increasingly problematic in cereal growing systems in northern Tunisia. Chemical control is difficult attributed to its extensive and deep perennial root system. Very little information is available on its life cycle and optimal conditions for germination of its seeds. The phenological development of this weed according to the growing degree-days (GDD) was studied during three years. Germination tests at different temperatures and light regimes were conducted to determine the base temperature of this weed as well as the optimum conditions for germination. Results showed that no germination happened below 10°C, the optimum temperature for germination is 20°C for light and 15°C for darkness. However, no significant difference was found between final percentages of germination in light and in darkness. The predicted base temperature for germination was 8°C. Adopting the found base temperature, a model was adjusted to predict *Hypericum triquetrifolium* phenology based on the growing degree-days (GDD). *H. triquetrifolium* required an average of 6700 GDD from rhizome bud emergence to mature seed production.

Key words: *Hypericum triquetrifolium*, base temperature, development stage, GDD.

RÉSUMÉ

L'*Hypericum triquetrifolium* est une mauvaise herbe pérenne qui devient de plus en plus problématique dans les systèmes de culture céréalière. Très peu d'informations sont disponibles concernant son cycle biologique et les conditions optimales pour la germination de ses semences. Un suivi du cycle biologique de cette adventice en fonction des degrés jours (GDD) a été réalisé pendant trois ans. Des essais de germination en présence de lumière et à l'obscurité et à différentes températures ont été conduits afin de déterminer la température de base de cette adventice ainsi que les conditions optimales de la germination. Les résultats ont montré qu'aucune germination n'a eu lieu au-dessous de 10°C, la température optimale pour la germination était de 20°C pour la lumière et de 15°C pour l'obscurité. Cependant, aucune différence significative n'a été observée entre le pourcentage de germination en lumière et en obscurité. La température de base pour la germination a été estimée à 8°C. En adoptant la température de base trouvée, un modèle a été ajusté pour prédire la phénologie de *Hypericum triquetrifolium* sur la base de degrés jours de développement (GDD). Une moyenne de 6700 GDD est nécessaire à l'*H. triquetrifolium* pour boucler son cycle.

Mots-clés : *Hypericum triquetrifolium*, température de base, cycle de développement, GDD.

INTRODUCTION

Hypericum triquetrifolium (family of *Hypericaceae*) commonly named wavy-leaf St John's wort, is a perennial herb described as a geophyte with shoot bearing roots. The stem is 50 cm high with a dense tangle of thin branches, glabrous but spotted with small black glands. It has deep vertical roots and a shallow rhizome system from which new shoots are produced. Compared with the related *Hypericum perforatum,* its growth and other morphological characters are explained as an adaption to the special edaphic and climatic factors in the semiarid East Mediterranean and Oriental landscapes. In the genus *Hypericum* with many meso-hygrophilous representatives *H. triquetrifolium* may be regarded as a derived xerophilous species (Hagemann and Meusel, 1984).

Hypericum triquetrifolium was listed by Holm et al. (1979) as a principal weed in Tunisia and a common weed in Lebanon. In the Middle East, it is a weed associated with cereals, orchards, and vineyards places. *Hypericum* was established in Australia, where it was listed as a noxious weed and poisonous to livestock (Jouve, 2009). In California, it spreads to over 800.000 ha in 44 years from small infestations in 1900 (Holloway, 1964). Chemical control with herbicides is difficult, because of charges to farmers and the deep perennial root system of this weed.

Control of perennial weeds could be improved if the timing of phenological development stages were better understood. Relative timing of emergence of crops and weeds is an important factor determining crop yield loss due to weeds (Cousens et al 1987). Models that predict weed seed germination and emergence are becoming common, but predictive models for germination and growing of perennial weeds are rare. In fact, there are relatively few studies on germination biology of the genus *Hypericum* (Grime et al. 1981; Campbell 1985; Maya et al. 1988; Keller and Kollmann 1999; Pérez-Fernandez et al. 2006).

Germination is a critical stage in the life cycle of weeds and crop plants, and often controls population dynamics with major practical implications. Generally, the germination capacity of *Hypericum* species is very low due to seed dormancy, which in *H*. *perforatum* (Campbell, 1985) and H. *aviculariifolium* (Cirak et al, 2007) is caused by a chemical inhibitor in the capsule. For *H. triquetrifolium*, conditions for germination are specific and temperature requirements for optimal germination are still unknown.

The method of degree-days is based on the premise that a plant needs a certain amount of energy, represented by the amount of thermal degrees required to complete any given phenological phase or even the entire cycle. In addition, it enables a linear relationship between temperature increase and plant development (Gramig and Stoltenberg, 2007). Thus, mathematical models and simulation routines based on the concept of degree days can be used. This concept is not different for weeds, but few studies have assessed their development based on accumulated degree-days. According to Ghersa and Holt (1995), the ability to predict different phenological aspects of crops, weeds and other pests with simple thermal equations tends to be an excellent tool to provide practical solutions to crop-related problems.

The main objective of this work was to study the germination ecology of *Hypericum triquetrifolium*, specifically focusing on (1) temperature and light regime requirements for germination, (2) base temperature for germination, and (3) study the phenological development of *Hypericum triquetrifolium* based on growing degree-days (GDD).

MATERIALS AND METHODS

Sample collection

Seeds of Hypericum triquetrifolium were collected in August 2017 from infested areas in northern Tunisia (Siliana). (36°6'30.28''N; 9°14'14.38''E and an altitude of 428 m). The average temperature in Siliana is 16.9 °C. The average annual rainfall is 423 mm. Only mature seeds were collected from at least 20 different plants throughout the field, mixed, placed in a glass jar and stored in at 4°C in the dark prior to the experiments.

Seed Germination study: Temperature and Light Requirements for Germination.

Growth chamber experiments were carried out to determine the base temperature for *Hypericum triquetrifolium* germination. Twenty-five seeds were placed in April 2017 into 9 cm Petri dishes lined with one layer of filter paper. Five mL of distilled water was delivered to each Petri dish. Each Petri dish with its cover was sealed with a piece of parafilm to reduce evaporation and was placed in growth incubators. Six incubators were used to set the temperature at 5, 10, 15, 20, 25, and 30°C with a 16/8 light/dark photoperiod and complete darkness by wrapping the Petri dishes with aluminum foil. Mercury thermometers were placed in each incubator as a check. The light in each cabinet was supplied by two standard fluorescent light tubes that provided at least 40 mmol m^{-2} s^{-1} for 24 h day^{-1}. Four replicates were used for each temperature. Germination was measured as percentage, 21 days after the experiment was initiated. The seeds were checked daily for germination. Germination was defined as the stage when the radicle had extended 1 mm beyond the seed coat (Steinmaus et al. 2000).

The phenological development Study

An outdoor pot trial was set up to study phenological development stages of *Hypericum triquetrifolium* for three consecutive years (2014-2015 and 2016). The study was carried out at the National Institute of Agronomy of Tunisia (36°49'49.01''N; 10°11'02.07''E and an altitude of 23 m). The average annual temperature is 18.1 °C and the average annual rainfall is 448 mm from August 2016 to October 2017. The phenological development stages were evaluated using the extended BBCH scale proposed by Hess et al. (1997). The *Hypericum triquetrifolium* phenological data were adjusted to growing degree days (GDD), through an exponential growth model, y = a*e $^{(b*x)}$, where y refers to the *Hypericum triquetrifolium* development according to the phenological stage (Hess et al., 1997); x refers to the GDD scale used; a and b are the model parameters.

To calculate the daily thermal units (GDD), the Gilmore and Rogers' equation (1958) was used:

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

Where: Tmax is the daily maximum temperature; Tmin is the daily minimum temperature; and Tb refers to basal temperature, evaluated at 8°C (based on the results found in the germination essay). The maximum and minimum daily temperatures were obtained from the meteorological station installed at the National Institute of Agronomy of Tunisia.

Statistics analysis

Most experiments were arranged in a randomized, complete block design with four replicates. The experimental data were subjected to analysis of variance and the treatment means were tested separately for the least significant difference at P = 0.05 or P = 0.01 where appropriate. All Curves fitting were carried out using SigmaPlot 11.0 (Systat Software, San Jose, CA). In order to estimate the base temperature, the reciprocal time to 50% germination (1/T50) was regressed on temperature. From this regression, the base temperature was estimated as the x-intercept by a mathematical approach (Holt and Orcutt 1996; Steinmaus et al.2000). The time to 50% germination (T50) was derived by modelling cumulative germination over time using the sigmoidal and polynomial fitting.

RESULTS

Seed Germination study: Temperature and Light Requirements for Germination.

Principal factors, date (D) and temperature (T), were highly significant (Table I). Two-way interactions between factors were significant except for light-darkness treatment (LD). The three-way interactions D by LD by T were not significant. No significant difference was recorded for the two light regimes.

Table I. Effect of date (D), light/darkness treatment (LD), temperature (T) on the germination of *Hypericum triquetrifolium*.

Tableau I. Effet de la date(D), du régime de lumière (LD) et de la température (T) sur la germination de *Hypericum triquetrifolium*

Treatment	df	P
D	20	***
LD	1	NS
T	4	***
D*LD	20	NS
D*T	80	***
LD*T	4	NS
D*LD*T	80	NS

Abbreviation: NS, not significant, *** P<0.001.

The relationship between the accumulated percentage germination of *Hypericum triquetrifolium* and the germination temperature was fitted to a three parameter Gaussian model, y=a*exp(-0.5*((x-

$x_0)/b)^2$) (P < 0.01, Figure 1). No seed germination was observed in both light and darkness for 5 and 10°c indicating that germination is very susceptible to low temperature and suggesting that 10°C is close to the base temperatures for seed germination. Seed germination increased gradually as temperature increased and peaked at 20°C to 40% of germination for light and 15°C to 38% of germination for darkness. Seed germination declined at temperature above 30°c for two light regimes. According to the fitted nonlinear Gaussian model, the optimum temperature for germination was estimated at 19.11°C in 16/8 light/dark photoperiod and 19.68°C in darkness.

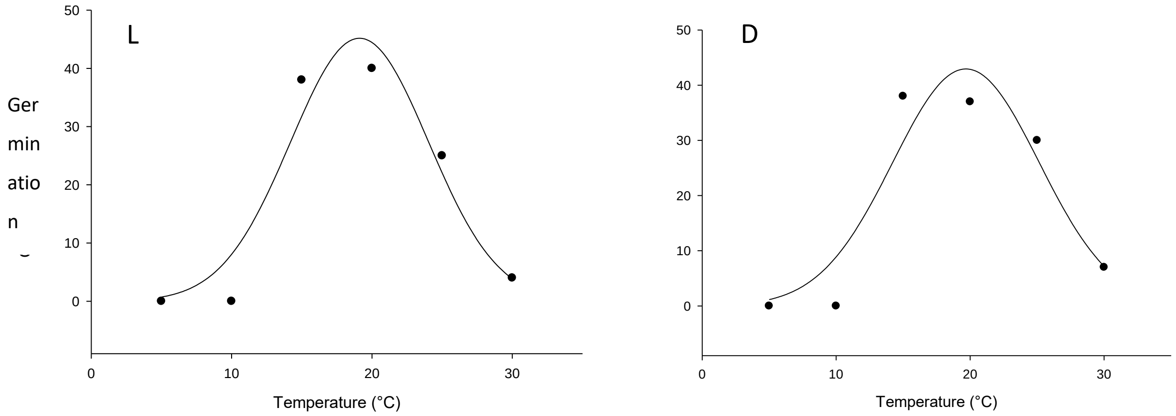

Figure 1. Effects of constant temperatures of 5, 10, 15, 20, 25, and 35°C on the accumulated germination (%) of *Hypericum triquetrifolium*. The line is a fitted nonlinear Gaussian model $y=45.17*\exp(-0.5*((x-19.11)/4.91)^2)$ in light(L) and $y=42.94*\exp(-0.5*((x-19.68)/5.45)^2)$in darkness(D).
Figure 1. Effet de la température (5, 10, 15, 20, 25, et 35°C) sur la germination finale (%) de *Hypericum triquetrifolium* à la lumière (L) et à l'obscurité(D). La ligne a été ajustée suivant une régression non linéaire de la forme $y=a*\exp(-0.5*((x-x_0)/b)^2)$.

In 16/8 light/dark photoperiod, the relationship between the accumulated percentage germination of *Hypericum triquetrifolium* over time (Figure 2) was fitted to a polynomial quadratic model ($y=y_0+a*x + b*x^2$) for 10, 15 and 30°C. For 20°C the accumulated percentage germination over time in 16/8 light/dark photoperiod was fitted to the sigmoidal model ($y= a/(1 + \exp(-(x-x_0)/b))$ as in darkness for all tested temperatures. All the parameters were highly significant (P<0.001). *Hypericum triquetrifolium* had higher germination at 20°C in 16/8 light/dark photoperiod and at 15°C in complete darkness.

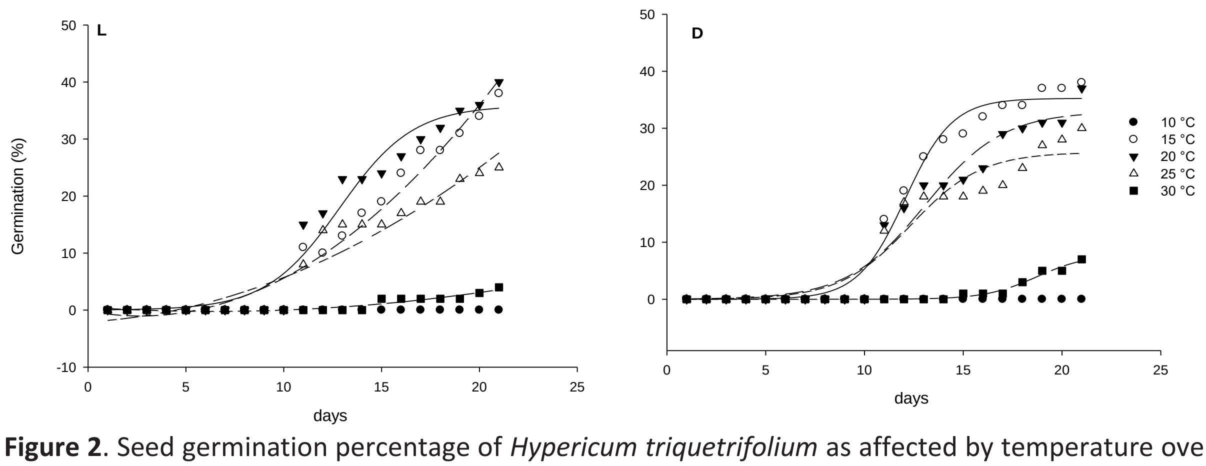

Figure 2. Seed germination percentage of *Hypericum triquetrifolium* as affected by temperature over time.
Figure 2. Le pourcentage de germination de *Hypericum triquetrifolium* pour chaque température en fonction du temps.

The inverse Gaussian distribution curve was fitted to the germination counts and the estimated T50 was calculated for each experimental unit according to Steinmaus et al. (2000). When the 1/T50 was regressed on temperature, the base temperature for *H. triquetrifolium* was estimated at 8°C (Figure3). This agrees with the observation that no germination occurred between 5 and 10°C.

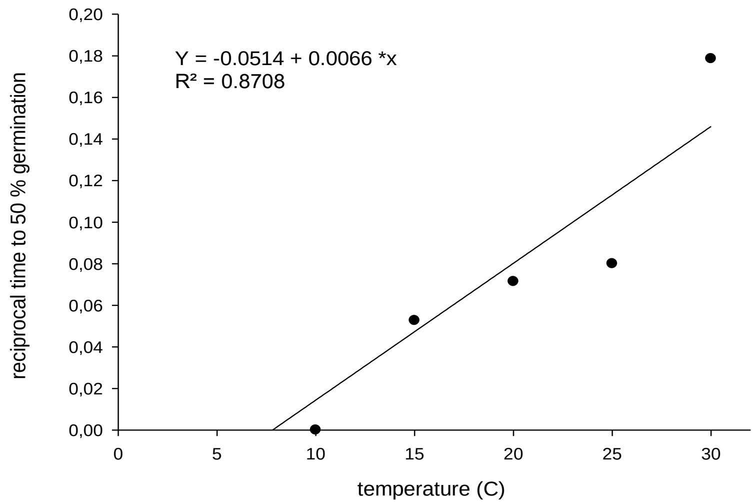

Figure 3. Base temperature estimation basing on reciprocal time to 50% germination regressed on temperature (10, 15, 20, 25 and 25°C) for *Hypericum triquetrifolium*

Figure 3. Estimation de la température de base en se basant sur l'inverse du temps nécessaire à la germination de 50% des semences d'*Hypericum triquetrifolium* en fonction des températures testées.

The phenological development Study

Phenological development of *Hypericum triquetrifolium* was observed for three consecutive years. An average of these three observed years was represented in figure 4. This phenological development was fitted to the accumulated thermal units following an exponential growth model, $y=a*e^{(b*x)}$. Accuracy of the function was high (R^2= 0.93). Bud emergence was adopted as the beginning of the life cycle of *H. triquetrifolium*, vegetative winter dormancy was observed at an average of 874 GDD. *H. triquetrifolium* resumes development after accumulating an average of 2584 GDD. *H. triquetrifolium* flowering and full flowering started at an average of 4819 and 5491 GDD respectively. *H. triquetrifolium* closes its development at an average of 6700 GDD.

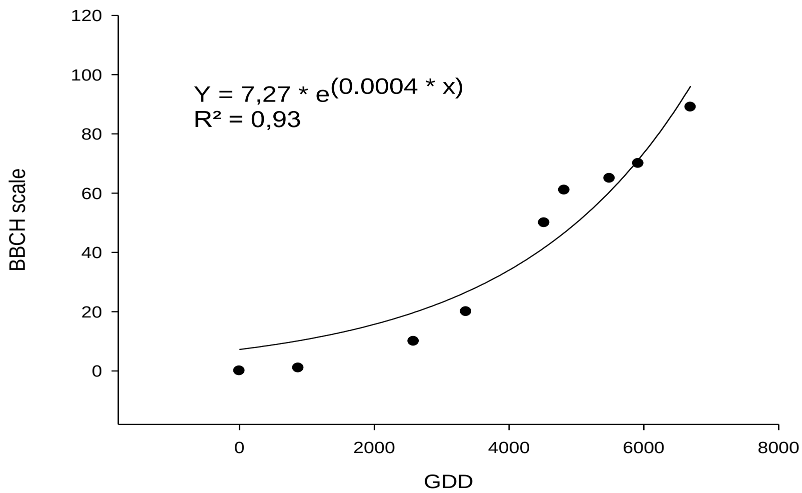

Figure 4. Phenological development of *H. triquetrifolium* fitted to growing degree-days, with base temperature of 8°C.

Figure 4. Cycle de développement phénologique de *H. triquetrifolium* en fonction des dégrée-jours en adoptant une température de base de 8 °C.

DISCUSSION

Light has been recognized as a germination-controlling factor and it was frequently found to be a requirement in plant species native to arid lands (Baskin, 2004). In general, absence of light has a negative effect on germination in several *Hypericum* species such as *Hypericum perforatum* (Campbell,

1985) and *Hypericum avicularrifolium* (Cirak et al, 2007). Crockettet and Robson, 2011 affirmed that conditions and temperature requirements for optimal germination are closely related to *Hypericum* species. In our study, no significant difference was recorded for the two light regimes.

Optimum seed germination temperature was estimated approximately at 19°C in 16/8 light/dark photoperiod and darkness. This agrees with similar study of Cirak et al, 2006, which reported a predicted optimum temperature for germination of *H. perforatum, H. bupleuroides, H. pruinatum, H. nummularioides* between 17 and 22°C.

The base temperature of the *Hypericum* species has not been estimated by subsequent studies. For other perennial weeds, the lowest temperature threshold has been determined as 6, 11 and 12 °C for Yellow nutsedge (*Cyperus esculentus*), purple nutsedge (*Cyperus rotundus* L.), and johnsongrass (*Sorghum halepense* L.) respectively (Holt and Orcutt, 1996).

Rhizome bud sprouting emergence was adopted as the beginning of the life cycle of *H. triquetrifolium*, it occurs in autumn. Vegetative winter dormancy was observed when temperature has dropped below10°C. *Hypericum triquetrifolium* starts winter dormancy while maintaining the stems developed at a vegetative stage. In spring, these stems restart growing and develop side shoots. Further studies published by Weed Science Society of America reported that *Hypericum triquetrifolium* Stems die in the autumn before regenerating from crowns and rhizomes in early spring. This is in disagreement with what we observed. Stems develop flowers and fruits between July and September which coincides with longest photoperiod (18h light).

These informations have valuable practical application because they ensure that, after seeds germination and rhizome bud sprouting, there is an interval of at least 8 months available to use the management practices before flowering occurs depending on installed crops.

CONCLUSION

Our results provide practical knowledge about the biology of *Hypericum triquetrifolium.* Base, optimum temperature and light determined allowed us to better understand the winter dormancy period of *H. triquetrifolium.* In fact, no development was registered below 10°C which coincide well with the temperature recorded in Tunisian winter. The phenological stages predicted may aid to develop adequate timing and practices to manage this invasive weed.

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