



# UNIVERSITA' DEGLI STUDI DI PADOVA

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**SCUOLA DI DOTTORATO DI RICERCA IN  
SCIENZE DELLE PRODUZIONI VEGETALI**  
INDIRIZZO AGRONOMIA AMBIENTALE - CICLO XXIII  
Dipartimento di Agronomia Ambientale e Produzioni Vegetali

## ***EMERGENCE MODELING TO IMPROVE INTEGRATED WEED MANAGEMENT***

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DATA CONSEGNA TESI

31 gennaio 2011

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Donato Loddo

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## Riassunto

La presenza delle piante infestanti rappresenta la principale causa di danno economico per l'agricoltura a livello mondiale, sia in termini di calo di resa delle colture sia come costi sostenuti per il loro controllo. La gestione integrata delle malerbe gioca quindi un ruolo cruciale per il raggiungimento di una maggiore sostenibilità ambientale, sociale ed economica delle produzioni agricole. La modellizzazione del ciclo biologico delle infestanti può essere una componente importante della gestione integrata. In particolare i modelli previsionali della dinamica di emergenza delle malerbe in campo e della loro successiva competizione con le colture possono fornire utili indicazioni sui tempi, sui modi e sulla convenienza economica di un eventuale intervento di controllo. L'obiettivo principale del progetto di ricerca sviluppato nella presente tesi è stato la creazione di uno strumento di supporto alle decisioni (DSS), basato su un modello di previsione delle emergenze (AlertInf) e la sua combinazione con un pre-esistente modello bio-economico (Gestinf), per il controllo in post-emergenza delle infestanti in colture di mais. Tale strumento è stato denominato Gestinf Plus. AlertInf è stato basato sul concetto di Tempo Idrotermico; nell'ambito di tale approccio la dinamica di emergenza viene stimata confrontando le condizioni microclimatiche del suolo (temperatura e potenziale idrico) con dei valori soglia specifici per la germinazione delle infestanti (temperatura e potenziale idrico di base). Tali valori soglia sono stati quindi determinati con prove di laboratorio alcune importanti infestanti (*Abrus precatorius*, *Amaranthus retroflexus*, *Chenopodium album*, *Digitaria sanguinalis*, *Echinochloa crus-galli*, *Setaria pumila*, *Setaria viridis*, *Sorghum halepense*) del mais in Veneto e Toscana. La successiva fase di parametrizzazione di AlertInf è stata effettuata per tre delle suddette specie (*A. theophrasti*, *C. album*, *S. halepense*) utilizzando i dati di emergenza ottenuti in varie prove di campo condotte in località e anni diversi nel Veneto. AlertInf è stato poi validato con dati di emergenza provenienti da ulteriori prove di campo. Il modello creato è stato infine combinato con Gestinf, ottenendo uno strumento (Gestinf Plus) in grado 1) di prevedere il momento migliore per effettuare il rilievo della flora infestante e 2) di definire la convenienza economica dei vari interventi di controllo. Per verificare la trasferibilità a livello europeo di AlertInf, si è poi condotta una ricerca in collaborazione con ricercatori portoghesi e spagnoli per studiare l'effetto del luogo di origine e del luogo di coltivazione sulla temperatura di base per la germinazione di

popolazioni locali di *A. theophrasti* e *Datura stramonium*. Una notevole variabilità è stata evidenziata nel comportamento delle popolazioni di *D. stramonium*. Questo risultato potrebbe ostacolare la trasferibilità del modello per tale specie. Infine si è studiata l'influenza della durata di interrimento invernale dei semi di *A. theophrasti*, *S. viridis* e *S. halepense* sul loro livello di dormienza, individuando per *S. viridis* e *S. halepense* un notevole effetto della stratificazione invernale sulla successiva germinazione ed emergenza in campo. Ciò evidenzia l'opportunità di inserire la dinamica della dormienza dei semi nei modelli di previsione delle emergenze. Tuttavia le conoscenze sul comportamento delle singole specie sono ancora carenti.

## ***Summary***

Weeds represent the main cause of economic losses in agriculture worldwide, both as crop yield reduction and control costs. Integrated Weed Management plays therefore a key role in order to achieve environmental, social and economic sustainability of crop production. Modeling of weed biological cycles may be an important component of IWM. Models able to predict weed emergence dynamics and weed-crop competition may provide useful indications about timing, type and economic convenience of control measures. The main objective of the research project presented in this Thesis is to develop a Decision-Support System (DSS), based on a weed emergence model (AlertInf) and its combination with a pre-existing bio-economic model (Gestinf), to improve weed post-emergence management in maize. This system was called Gestinf Plus. AlertInf was based on a commonly used approach for weed emergence models called Hydrothermal Time concept. According to this approach, weed emergence dynamics are estimated comparing soil microclimate conditions (soil temperature and water potential) with specific threshold values for weed seed germination (base temperature and water potential). These threshold values were estimated with laboratory experiments for some species (*Abutilon theophrasti*, *Amaranthus retroflexus*, *Chenopodium album*, *Digitaria sanguinalis*, *Echinochloa crus-galli*, *Setaria pumila*, *Setaria viridis*, *Sorghum halepense*) considered as important weeds in maize fields of Veneto and Tuscany regions, Italy. Model parameterization for AlertInf was achieved for three weed species (*A. theophrasti*, *C. album*, *S. halepense*) using emergence data from several field trials carried out in different years and locations in Veneto region. Model validation was performed with other emergence data from independent field trials. AlertInf was then combined with Gestinf to obtain an innovative tool (Gestinf Plus) able to 1) predict the best moment for weed sampling and 2) evaluate economic convenience of several control measures. A common experiment was arranged in collaboration with Portuguese and Spanish scientists to assess transferability at European level of AlertInf. The common aim was to evaluate the effect of site of origin and site of cultivation on base temperature for local populations of *A. theophrasti* and *Datura stramonium*. Remarkable variability was found in the behavior of *D. stramonium* populations. This findings may hinder transferability of AlertInf model for this species. The influence of winter burial

length on seed dormancy level was finally studied for *A. theophrasti*, *S. viridis* and *S. halepense*. A significant stimulating effect of winter chilling was identified on *S. viridis* and *S. halepense* germination and field emergence. Including seed dormancy dynamic may consequently represent an important improvement for weed emergence models, however further studies are required to obtain detailed information about specific behaviors for the main weeds.



## GENERAL INTRODUCTION

Weed control undoubtedly represents a key factor to achieve satisfactory field crop yields. In the present world situation weed-crop competition causes economic losses (yield reduction; control cost) equal to about 13% of the maximum potential yields (Zoschke and Quadranti 2002). Weeds have an important impact on human activities in both advanced and developing countries (Akobunudu 1991; Bridges 1994). Oerke (2006) estimated that, if no control measures were applied, at global level the potential yield loss due to weed competition would be approximately 23% for wheat, 37% for rice, 40% for maize and 37% for soybean. For fifty years, weed control in modern agriculture has been based mainly on chemical herbicides, which have certainly permitted notable increases in crop yields. However, during the last two decades public opinion and scientists have expressed increasing concerns about risks for human health and environmental impacts correlated to the use of pesticides in agriculture. Consequently an intense debate has arisen in the political and scientific communities about how to combine economic issues with environmental sustainability. As a result, the European Parliament recently published the Directive 2009/128/EC that identifies as a priority objective to reduce the risks and impacts of pesticide use on human health and the environment (European Parliament 2009) by promoting the use of Integrated Pest Management. Several active ingredients are probably going to be withdrawn from the market due to their negative eco-toxicological profiles. Further studies about weed biology and ecology are therefore required to develop alternative strategies able to guarantee an effective control even with a restricted list of available chemical herbicides (Zanin and Catizone 2003).

In this scenario, studying weed population dynamics and especially seedling emergence may be crucial in order to make estimations about weed-crop competition, ascertain cost effectiveness and control timing (Buhler et al. 2000; Leblanc and Cloutier 2002; Grundy 2003). Weed emergence patterns were initially studied analyzing long-term experiments with an empirical approach (Roberts and Feast 1970; Lawson et al. 1974; Roberts and Potter 1980). Authors then began to focus their efforts on predicting weed germination and emergence according to the effects of environmental factors (such as precipitation, air and soil temperature) and management practices (Satorre et al. 1985; Bewick et al. 1988;

Benech-Arnold et al. 1990; Mohler 1993; Forcella 1998). A commonly-used approach is the Hydrothermal Time concept, which attempts to model seed germination according to the interaction of soil temperature and soil water potential (Gummerson 1986; Alvarado and Bradford 2002; Bradford 2002). This approach has been adopted to model germination of several weeds and crops (Grundy et al. 2000; Roman et al. 2000; Rowse and Finch-Savage 2003; Leguizamon et al. 2004). The first application of the Hydrothermal concept in Italy was by Masin et al. (2005) who created a hydrothermal model (WeedTurf) to predict weed emergence in turf. WeedTurf estimates an accumulation of Hydrothermal Time in relation to the comparison between soil microclimate conditions (soil temperature and water potential) and biological parameters of weeds ( $T_b$ , base temperature and  $\Psi_b$ , base water potential for germination). Hydrothermal time accumulation (HT) is estimated using Archer's WeedCast equation (Archer et al. 2001) modified according to Bradford (2002) which affirmed that the decrease in germination rates at supra-optimal temperature was due to an increase in the  $\Psi_b$  threshold for germination as temperature rose above the optimum ( $T_o$ ). Thus, the  $\Psi_b$  value increased linearly (Fig. 1) until it reached 0 MPa at a temperature defined as the ceiling temperature (CT = maximum threshold temperature at which germination is prevented). Consequently, HT accumulation in a day  $i$  is estimated with the following equation (Masin et al. 2005):

$$HT_i = (n * \max(T_{si} - T_b, 0) + HT_{i-1}) \quad (1)$$

$$T_{si} \leq T_o \quad n = \begin{cases} 0 & \text{if } \Psi_{si} \leq \Psi_b \\ 1 & \text{if } \Psi_{si} > \Psi_b \end{cases}$$

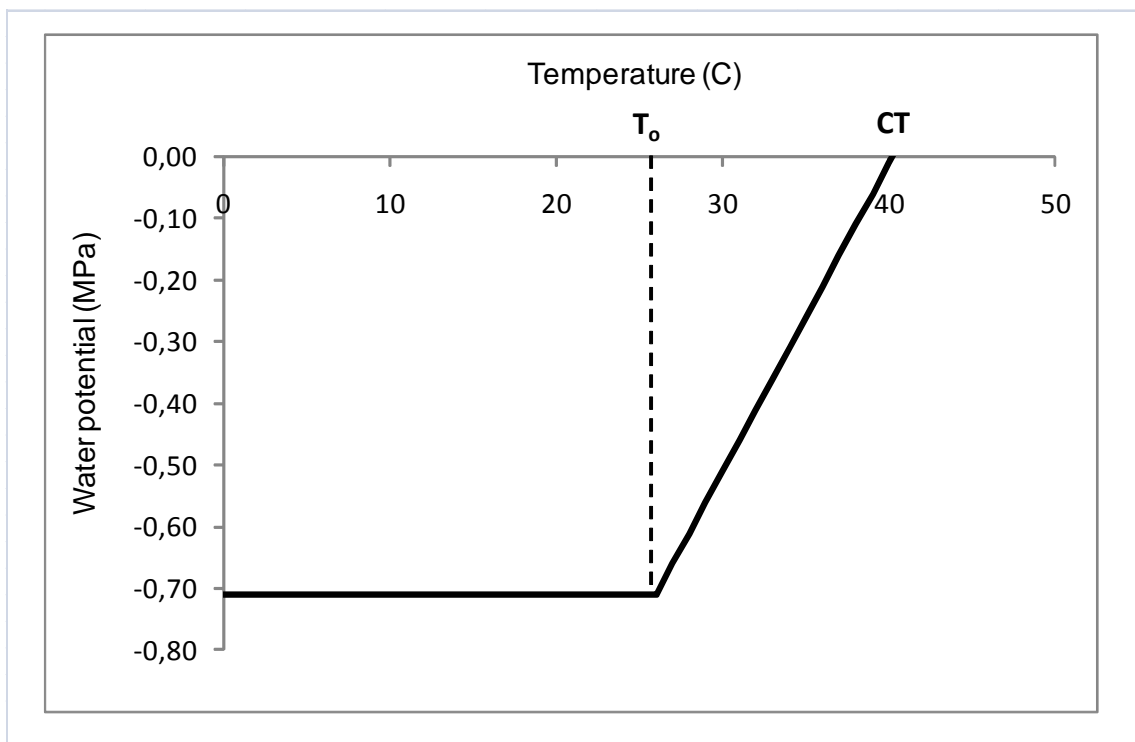
$$T_{si} > T_o \quad n = \begin{cases} 0 & \text{if } \Psi_{si} \leq \Psi_b + Kt(T_{si} - T_o) \\ 1 & \text{if } \Psi_{si} > \Psi_b + Kt(T_{si} - T_o) \end{cases}$$

With  $T_{si}$  = average soil temperature on day  $i$ ;  $\psi_{si}$  = average soil water potential on day  $i$ ;  $T_o$  = optimum temperature for germination and  $Kt$  is the slope of the relationship between  $T_{si}$  and  $\psi_b$  in the supra-optimal temperature range. Parameters  $T_b$ ,  $\psi_b$ ,  $T_o$  and  $Kt$  have to be estimated with laboratory and field experiments. WeedTurf is then able to estimate the

percentage of total weed emergence (Emergence Time) correlated to any given accumulation of HT with the following Gompertz equation (Masin et al. 2005):

$$ET = 100 \exp(-a \exp(-b \text{ SGDD})) \quad (2)$$

where  $a$  represents an HT lag before emergence starts and  $b$  represents the rate of increase of emergence once it is initiated. Both  $a$  and  $b$  are species-specific biological parameters so their determination is required for any weed included in the model.



**Figure 1** - Linear increase of base water potential at temperatures above the optimum ( $T_o$ ) till the ceiling temperature (CT) at which germination is prevented.

WeedTurf was created as a decision support system (DSS) for weed management in turf, but it may easily be transformed in order to predict weed emergence in crop fields. It is just necessary to re-estimate the above-mentioned biological parameters according to the characteristics of the new environment and to change the starting date of accumulation of HT from 1<sup>st</sup> January (as adopted for WeedTurf) to the date of soil cultivation for crop

sowing. However, in Italy there are no available models, based on the Hydrothermal Time concept or any other approach, to predict weed emergence in field crops.

Models like WeedTurf are only able to estimate weed emergence dynamics, i.e. they provide information about the percentage of the final amount of seedlings emerged till a given moment. Instead, they are unable to estimate weed seedling density. As a result, these models could be useful to identify the correct timing for weed control, but they do not give indications about potential yield loss due to weed-crop competition or the cost effectiveness of any weed control operation. This kind of information could be provided by another class of models, which are commonly defined as bio-economic models. A bio-economic model, called Gestinf, is already available to offer economic estimations to support weed management for spring crops in Northern Italy (Berti and Zanin 1997; Berti et al. 2003). Gestinf is able to assess yield loss due to competition from a mixed weed flora, according to its density, its botanical composition and weed size (small/large plants), and to estimate the specific cost effectiveness of several possible control measures (Berti and Zanin 1994; 1997). Gestinf, however, is not able to predict weed population dynamics; therefore its estimations are based on the conditions at the moment of input measurement, which can only be done with a field scouting, which is very time-consuming and expensive. The high cost of scouting represents the main constraint to the acceptance and adoption of bio-economic models like Gestinf as a decision-support system for crop management (Wilkerson et al. 2002). Since the scouting is only feasible once during the crop cycle for economic reasons, it is important that it is done in the right moment. Performing the scouting at the correct time would both provide exhaustive information about weed flora and leave enough time to arrange a possible control operation. An adequately-modified version of WeedTurf model may be used to overcome this problem, because it could estimate the best timing for scouting operations according to environmental trends. The combination of a weed emergence model (WeedTurf modified) and a bio-economic model (Gestinf) could represent an innovative and useful tool for Integrated Weed Management in spring crops in Northern Italy because this could simultaneously provide information about yield loss, timing and cost effectiveness of weed control operations.

As explained above, weed emergence models based on the Hydrothermal Time concept require the determination of several biological parameters for Equations 1 and 2. Since

these determinations are time-consuming and expensive, the transferability of these parameters across different populations may represent one of the main constraints for the development and large-scale use of hydrothermal models (Grundy 2003). Studies reported differences in germination ecology or in the biological parameters for different populations (Del Monte and Tarquis 1997; Christal et al. 1998; Kremer and Lotz 1998; Allen and Meyer 2002; Taab and Andersson 2009) or even for seeds of the same population matured under contrasting environmental conditions (Magyar and Lukacs 2002). However, these findings cannot be considered as a general rule since Grundy et al. (2003) reported evidence of synchrony in emergence timing for three populations of the same species. Different ranges of intra-specific variability in germination ecology may therefore be supposed among different weeds. As a consequence, specific studies analyzing the effect of genetic and environmental differences on germination ecology of different populations of the most important weeds could be necessary for developing generic and transferable emergence models.

Another important research topic for weed population and emergence modeling is represented by the environmental control of seed dormancy (Benech-Arnold et al. 2000), for the reason that the soil seed bank is the main source of seedlings for annual species (Buhler 1999; Grundy and Mead 2000). Dormant seeds have an internal constraint that impedes their germination even if hydric, thermal and gaseous conditions are adequate (Benech-Arnold et al. 2000). Dormancy is not a qualitative (all-or-nothing) property and its level may progressively vary from a minimum to a maximum point according to seasonal dynamics (Batlla et al. 2004). These cyclic changes in seed dormancy status may influence minimum temperature and minimum water potential required for seed germination (Vegis 1964; Christensen et al. 1996; Batlla and Benech-Arnold 2004). Many summer weeds produce seeds that are normally physiologically dormant when they are dispersed from the mother plants in autumn (Taylorson and McWhorter 1969; Van den Born 1971) and this condition is known as primary dormancy (Benech-Arnold et al. 2000; Batlla and Benech-Arnold 2007). During the winter months, dormancy is gradually reduced mostly due to the interaction of low soil temperature and high soil moisture (chilling), so seeds are able to germinate in the following spring when environmental conditions become suitable (Taylorson and McWhorter 1969; Van den Born 1971). Even if including dormancy

dynamics in weed emergence models may notably improve their accuracy (Forcella et al. 2000; Grundy 2003), the complexity of environmental factors influencing dormancy and the difficulty in separating dormancy release and seed germination has hindered the spreading of specific studies (Grundy 2003). Many Hydrothermal weed emergence models therefore do not consider dormancy or remain merely empirical under this aspect (Forcella 1998; Grundy and Mead 2000; Masin et al. 2005). Nor are there universally recognized indications about how to deal with dormancy level of the seeds used for  $T_b$  and  $\Psi_b$  determination. As a consequence, a wide range of different seed storage or chilling treatments are reported by several authors for various weeds (Benvenuti and Macchia 1993; Masin et al. 2005; Kochy and Tielborger 2007; Sartorato and Pignatta 2008; Masin et al. 2010). This methodological uncertainty should be solved because it may lead to different estimations for the same parameters in the same species, with a consequent doubt about the predictive accuracy of models. Winter chilling conditions (temperature, length) might also affect summer weed field emergence, modifying temporal dynamics and magnitude of seedling flushes. For example, Grundy et al. (2003) reported a strong correlation between winter temperature and emergence magnitude for several *Chenopodium album* populations sown in locations with different winter conditions. This aspect should be included in an emergence model in order to maintain its predictive accuracy even in the presence of variable winter conditions in different years or locations.

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## THESIS OBJECTIVES

The general scenario described in the General Introduction was the starting point for the research projects presented in this thesis. The project has been arranged as a series of sub-activities with specific objectives:

- Creation of a predictive model, called AlertInf, based on the WeedTurf structure, for weed emergence in maize fields in Italy. The first phase, described in **Chapter 1**, was the determination with laboratory experiments of  $T_b$  and  $\Psi_b$  for several important weeds (*Abutilon theophrasti*; *Amaranthus retroflexus*; *Chenopodium album*; *Digitaria sanguinalis*; *Echinochloa crus-galli*; *Setaria pumila*; *Setaria viridis*; *Sorghum halepense*). The second phase, reported in **Chapter 2**, was the creation and validation with several field trials of AlertInf for *A. theophrasti*; *C. album* and *S. halepense*.
- Combination of AlertInf with the preexisting Gestinf, presented in **Chapter 3**. This innovative tool, called Gestinf Plus, should be able to predict weed emergence dynamics, identify the correct timing for a scouting or a control operation and provide economic estimations about weed-crop competition.
- Determination of  $T_b$  parameter and study of germination ecology in populations of *A. theophrasti* and *Datura stramonium* with different site of origin and cultivation. The aim of this sub-activity, illustrated in **Chapter 4**, was to assess for the two weeds the variability at European level of  $T_b$  parameter because it is a preliminary indicator of modeling transferability.
- Evaluation of the influence of winter chilling duration on seed germination and seedling emergence for *A. theophrasti*; *S. viridis* and *S. halepense*. The experiments described in **Chapter 5** were conducted to obtain information for each species on the correct seed management for  $T_b$  and  $\Psi_b$  determination and to evaluate the utility of including winter chilling effects in emergence prediction models.

# CHAPTER I

## **Temperature and water potential as parameters for modeling weed emergence in central-northern Italy**

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Published on *Weed Science* (2010) 58:216-222

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## **Abstract**

Predicting weed emergence dynamics can help farmers to plan more effective weed control. The hydrothermal time concept has been used to model emergence as a function of temperature and water potential. Application of this concept is possible if the specific biological thresholds are known. This paper provides a dataset of base temperature and water potential of eight maize weeds (velvetleaf, redroot pigweed, common lambsquarters, large crabgrass, barnyardgrass, yellow foxtail, green foxtail, johnsongrass). For five of these species, two ecotypes from two extreme regions of the predominant maize-growing area in Italy (Veneto and Tuscany) were collected and compared to check possible differences that may arise from using the same thresholds for different populations. Seedling emergence of velvetleaf and johnsongrass were modeled using three different approaches: 1) thermal time calculated assuming 5 C as base temperature for both species; 2) thermal time using the specific estimated base temperatures; 3) hydrothermal time using the specific estimated base temperatures and water potentials. All the species had a base temperature above 10 C, with the exception of velvetleaf (3.9-4.4 C) and common lambsquarters (2.0-2.6 C). All species showed a calculated base water potential equal or up to -1.00 MPa. The thresholds of the two ecotypes were similar for all the studied species, with the exception of redroot pigweed, for which the Veneto ecotype showed a water potential lower than -0.41 MPa, whereas it was -0.62 MPa for the Tuscany ecotype. Similar thresholds have been found to be useful in hydrothermal time models covering two climatic regions where maize is grown in Italy. Furthermore, a comparison between the use of specific estimated and common thresholds for modeling weed emergence showed that for a better determination of weed control timing it is often necessary to estimate the specific thresholds.

## **Nomenclature**

Barnyardgrass, *Echinochloa crus-galli* (L.) Beauv, ECHCG; common lambsquarters, *Chenopodium album* L., CHEAL; green foxtail, *Setaria viridis* (L.) Beauv, SETVI; johnsongrass, *Sorghum halepense* (L.) Pers., SORHA; large crabgrass, *Digitaria sanguinalis* (L.) Scop., DIGSA; redroot pigweed, *Amaranthus retroflexus* L., AMARE; velvetleaf, *Abutilon theophrasti* Medik, ABUTH; yellow foxtail, *Setaria glauca* (L.) Beauv, SETLU.

## **Key Words**

Base temperature, base water potential, emergence prediction, hydrothermal time, models.

## Introduction

The timing of weed emergence has an important effect on crop-weed interactions and crop yield. Numerous studies have been conducted to improve knowledge of weed emergence in the field (e.g. Bradford 2002; Colbach et al. 2002; Forcella et al. 2000). Many of these studies aim to develop prediction models that can provide information to farmers about methods and timings of weed control (Grundy 2003). The first-generation models for predicting weed emergence were based on the thermal time (growing degree days) concept (Bewick et al. 1988; Satorre et al. 1985). In these models, weed emergence dynamics were described considering temperature as the only factor influencing the phase of germination-emergence. Gummerson (1986) introduced the concept of hydrothermal time, which integrated thermal time above a base temperature and hydro time above a base water potential in the same algorithm. The most recent weed emergence models are based on this concept or its modifications (Alvarado and Bradford 2002; Alvarado and Bradford 2005; Ekeleme et al. 2005; Grundy 2003; Larsen et al. 2004; Leguizamon et al. 2005; Masin et al. 2005; Roman et al. 1999).

The hydrothermal time concept greatly increased the predictive capability of the models, but also made the algorithm more complicated. Moreover, application of the models to species and environments different from those in which they were created needs further studies to estimate the specific “biological” parameters. These parameters, such as base temperature and water potential, necessary to calculate the hydrothermal time, may change for ecotypes of the same species, and this regulates their germination-emergence in different environments. The necessity to calculate these threshold parameters in different climatic areas is an important limitation in the creation and adoption of weed emergence prediction models. The experiments to estimate the thresholds are very time and resource-consuming, and are usually done every time they are needed for a specific area and for a single or few species of interest.

Given the experimental difficulties in the evaluation of base temperatures and water potentials, there are very few papers that report the threshold parameters for many species. For example, Allen (2003) described the hydrothermal time parameters for 24 wild species,

Kochy and Tielborger (2007) reported the minimum soil moisture for 36 annual species from four sites in Israel, and Steinmaus et al. (2000) estimated the base temperature for nine weed species in California. As an alternative, the base temperature can be obtained empirically by fitting the emergence curves against the thermal or hydrothermal time calculated using different values of base temperature and water potential and selecting that which gives the minimum residual mean square (Del Monte and Tarquis 1997; Grundy et al. 2003). Otherwise the model is created without calculating the specific threshold parameters, using the same base temperature and water potential for all the considered species (e.g. Myers et al. 2004), or obtaining the thresholds from the literature on species of different sites (Leguizamon et al. 2009).

The objectives of this paper are 1) to provide a dataset of base temperature and base water potential for the predominant weed species of maize and 2) compare two ecotypes collected in two sites at the extreme regions of the Italian maize-growing area. This comparison is interesting to verify if it is possible to use the same parameters in the two sites, and consequently throughout the Italian maize-growing area.



## Materials and Methods

The studied species were velvetleaf, redroot pigweed, common lambsquarters, large crabgrass, barnyardgrass, yellow foxtail, green foxtail, and johnsongrass. According to a recent review (Gardarin et al. 2009) (Table 1.1), there are very few references in the CAB Abstracts database about base temperatures and especially base water potentials for these species.

**Table 1.1** - Number of references for base temperature and base water potential for the studied species in the CAB Abstracts database (Gardarin et al. 2009 mod).

Species	Base Temperature	Base Water Potential
Velvetleaf	2	0
Redroot pigweed	4	0
Common lambsquarters	10	0
Large crabgrass	4	0
Barnyardgrass	8	0
<i>Setaria</i> spp.	11	2
Johnsongrass	3	0

### Seed Collection

Seeds of summer annual species common in maize in Italy were collected from September to November 2006 at the Experimental Farm of Padova University in Legnaro (north-eastern Italy, 45°12'N, 11°58'E, 6 m a.s.l.) and at the Experimental Farm of the Agronomy Department of Pisa University (central Italy, 43°84'N, 10°82'E).

The climate of both sites is sub-humid. Padova has an average annual rainfall of about 850 mm fairly uniformly distributed throughout the year. The area is characterized by an average annual temperature of 12.2 C, with temperature increases from January (average minimum value: – 1.5 C) to July (average maximum value: 27.2 C). Pisa has an average annual rainfall of about 930 mm, mainly during the spring and fall. The average annual temperature is 15.0 C, with temperature increases from January (average minimum value: 3.2 C) to July (average maximum value: 28.0 C).

Seeds of mature plants were hand-harvested on warm dry days by shacking into paper bags to ensure that only mature seeds were harvested. For velvetleaf, large mature capsules were collected from mature plants. Seeds were then hand cleaned and those clearly immature were eliminated. Mature seeds were stored in paper bags at room temperature until being used for the trials. The experiments started the next spring.

### **Threshold Parameters Estimation**

Steinmaus et al. (2000) compared several methods for estimating minimum temperature thresholds for seed germination. The most statistically robust and biologically relevant method was the reciprocal time to 50% of germination, which can also be used for other biologically meaningful parameters, such as base water potential. This method is used in this research to estimate both base temperature and base water potential.

Prior to using the seeds in the experiment, the seed coat cover of velvetleaf was scarified with sandpaper to break dormancy due to the seed coat impermeability (Winter 1960), and the glumes of barnyardgrass, johnsongrass and green foxtail were removed.

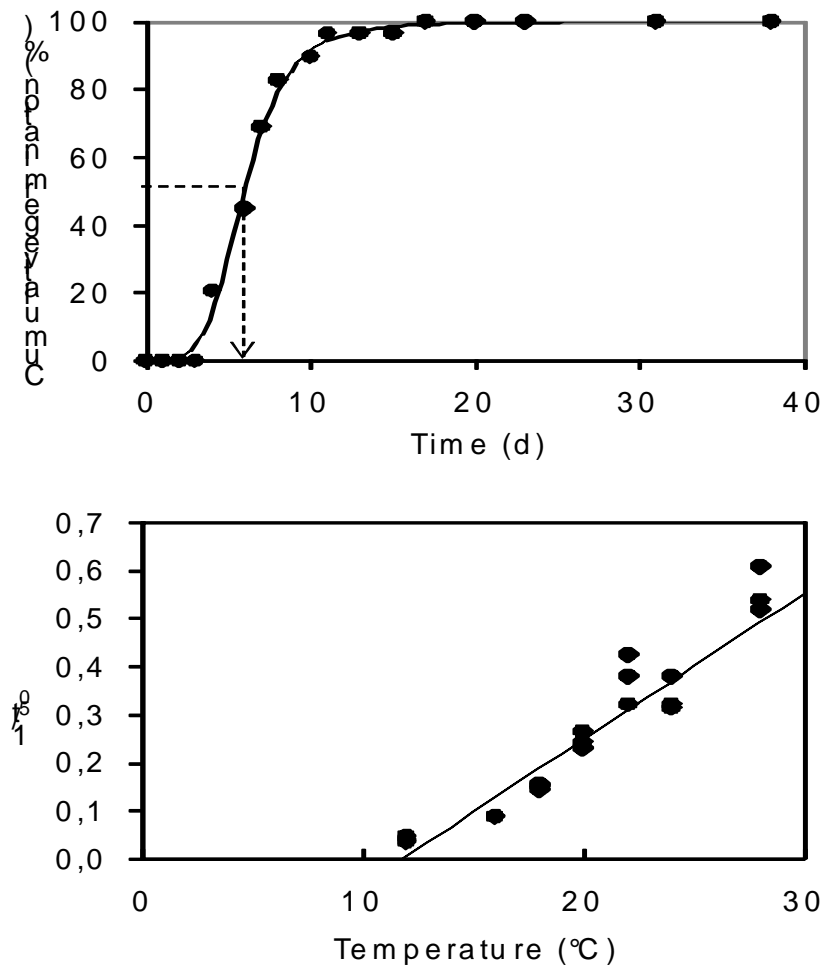
### **Base Temperature Estimation**

Three replicates of 100 seeds for each of the eight species of both ecotypes (Padova and Pisa) were incubated at a set of constant temperatures (6, 8, 12, 16, 18, 20, 22, 24, 28 C) and photoperiod of 12:12 h (light:dark) in 10 cm diameter, 7 cm height transparent plastic containers with 50 ml of deionized water. The seeds were placed on a plastic support covered by filter paper to keep them on the surface of the water but not immersed to prevent the risk of anoxic conditions. Germination was recorded twice daily (higher temperatures) or daily (lower temperatures) until no further germination occurred for 10 days. The seeds were defined as germinated when they had visible radicle emergence of more than 1 mm.

The germination time course was analyzed using a logistic function in the Bioassay97 program (Onofri 2001) as follows:

$$CG = 100 / (1 + \exp(a \cdot (\ln(t + 0.0000001) - \ln(b)))) \quad [1]$$

where CG is the percentage of cumulative germination,  $t$  is the time (days),  $a$  represents the slope of the curve, and  $b$  the inflexion point.



**Figure 1.1** - Estimation of the base temperature of johnsongrass Pisa ecotype using the method of reciprocal time to median germination. In the first graph, the solid line represents the logistic function and the points the observed data of germination. In the second graph, the solid line represents the linear regression line and the points the calculated germination rate ( $1/t_{50}$ ).

The time necessary for germination of half the seeds that had germinated by the end of the experiment ( $b$  of the curve) was estimated. A linear regression provided the best fit of germination rate (reciprocal time to 50%) against incubation temperature. The base temperature was estimated as the intercept of the regression line with the temperature axis

(Fig. 1.1). Statistical confidence intervals for the base temperatures were estimated by the bootstrap method (Efron 1979). Five thousand samples were taken from each species, randomly extracting one of the three replications of each temperature. Five thousand datasets were created and the linear regression was estimated for each of these to determine the base temperature. The bootstrap distribution of the estimated base temperature was used to determine a 95% confidence interval.

### **Base Water Potential Estimation**

Polyethylene glycol (PEG 6000)<sup>1</sup> was used to create solutions with water potentials of 0, -0.05, -0.10, -0.25, -0.375, -0.50, -0.80 and -1.0 MPa, prepared according to Michel and Kaufmann (1973). PEG-solution osmotic concentration was controlled using a Freezing Point Osmometer<sup>2</sup>.

Three replicates of 100 seeds each were placed in 10 cm diameter transparent plastic containers to which 50 ml of water (for water potential of 0 MPa) or one of the 6 PEG solutions was added. A plastic support covered by imbibed filter paper was used to maintain the seeds on the free solution surface to avoid higher concentration of PEG (Bradford 1995). The containers were fitted with tight lids to prevent evaporation and placed in a seed germinator at a constant temperature of 22 C and photoperiod of 12:12 h (light:dark). Germinated seeds were counted and removed twice daily and the experiment was stopped after 10 days with no germination. Germination was recorded when there was a visible radicle of more than 1 mm.

The germination time course was modeled using the same logistic function (equation 1) used to estimate the base temperature, and the time of 50% germination (b of the curve) was estimated. Base water potential was calculated by regressing time to 50% germination against water potential using the bootstrap method (Efron 1979). The base water potential was estimated as the intercept of the regression line with the water potential axis.

### **Field Experiments**

In order to monitor emergence of the studied weed species, an experiment was conducted in 2007 and repeated in 2008 at the sites where seeds had been collected (Padova and Pisa). In autumn-winter 2006 and 2007, polypropylene pipes (10 cm diameter and 25 cm in length)

were buried vertically in the soil and filled with sandy loam soil sifted through a 1-mm screen. Two hundred seeds of velvetleaf and johnsongrass were placed at depths of 2 cm. In both experiments two pipes were monitored by temperature probes<sup>3</sup>, one per pipe, at a depth of 2 cm and two other pipes were monitored by moisture probes<sup>4</sup> at a depth of 5 cm, connected to an external data logger<sup>5</sup>. In order to obtain accurate measurement of soil moisture, Kennedy et al. (2003) recommended not installing an in-situ sensor any deeper. Air temperature (daily average, maximum, minimum) and rainfall were recorded at the on-farm weather station. From spring onwards, emerged weed seedlings were counted and eliminated twice weekly.

Three approaches were used to model weed emergence. Thermal time was either calculated assuming 5 C as base temperature for all the species or estimated with specific base temperatures for each species. Hydrothermal time was estimated with specific base temperatures and water potentials. Thermal and hydrothermal times were measured from 1 March in both years. The cumulative emergence of weeds was then modeled with Gompertz functions (Onofri 2001) in which the independent variables were the two thermal times and the hydrothermal time as follows:

$$CE = 100 \cdot \exp(-\exp(a \cdot (\ln(THT + 0.0000001) - \ln(b)))) \quad [2]$$

where CE is the cumulative emergence, THT is the thermal or hydrothermal time,  $a$  represents the slope of the curve, and  $b$  is the inflection point.

The goodness of fit was evaluated with the model efficiency index (EF) (Loague and Green 1991). The model EF is calculated as:

$$EF = \frac{\sum_{i=1}^n (O_i - \bar{O})^2 - \sum_{i=1}^n (P_i - O_i)^2}{\sum_{i=1}^n (O_i - \bar{O})^2} \quad [3]$$

where  $P_i$  is the predicted value,  $O_i$  the observed value, and  $\bar{O}$  the mean of observed values. The value of EF can range from  $-\infty$  to 1. For an ideal fit, EF value equals 1.

## Results and Discussion

### Base Temperature Estimation.

Base temperatures of the ecotypes from the two locations (Padova and Pisa) were very similar for all the species (Table 1.2). Velvetleaf and common lambsquarters showed the lowest base temperatures among the studied species, 3.9-4.4 C and 2.0-2.6 C, respectively. The low base temperature of common lambsquarters was confirmed by Vleeshouwers and Kropff (2000) who estimated a base temperature of 2.0 C from seeds collected in Wageningen, The Netherlands. Different values have been reported for velvetleaf. Dorado et al. (2009) in Central Spain found a base temperature of 6.8 C in non-chilled seeds and 7.2 C in chilled seeds buried in soil. All the other species had a base temperature higher than 10 C (Table 1.2). Wide variability is known to exist in base temperatures for germination of the species used in this study. For example, Steinmaus et al. (2000), using the same method of reciprocal time to 50% of germination, showed higher base temperatures than those found in this experiment for seeds of large crabgrass collected in California (13.65 C). In the same study, Steinmaus et al. (2000) found a base temperature of 13.85 C for barnyardgrass, whereas Martinkova et al. (2006) found a threshold of 11.7 C for this species. Base temperature of yellow foxtail estimated by Steinmaus et al. (2000) resulted as very similar to the value found in this study (9.79 C). Forcella et al. (2000) reported base temperatures for green foxtail of between 3.5 C and 11.5 C in seeds collected from Iowa, Kansas, Minnesota, Nebraska and South Dakota (USA). Benech-Arnold et al. (1990a, b) estimated a base temperature of 8.5 C for johnsongrass, whereas Holt and Orcutt (1996) found a base temperature of 12 C for this species.

**Table 1.2** - Base temperatures (Tb) estimated with the bootstrap method, 95% confidence interval (c. int.), and coefficient of determination ( $r^2$ ).

Species	Padova			Pisa		
	Tb (C)	± c. int.	$r^2$	Tb (C)	± c. int.	$r^2$
Velvetleaf	3.9	0.59	0.93	4.4	0.43	0.88
Redroot pigweed	12.3	1.12	0.92	12.1	0.43	0.95
Common lambsquarters	2.6	0.77	0.84	2.0	0.80	0.93
Large crabgrass	10.3	0.92	0.97	11.8	0.28	0.95
Barnyardgrass	11.7	0.28	0.89	-	-	-
Yellow foxtail	-	-	-	10.4	0.95	0.97
Green foxtail	12.5	0.34	0.79	-	-	-
Johnsongrass	11.8	0.47	0.89	11.8	0.54	0.92

Dashes indicate data not determined.

#### **Base Water Potential Estimation.**

All species showed high sensitivity to water stress, with a calculated base water potential of up to  $-1.04$  MPa (Table 1.3). The water potential threshold levels were not significantly different between ecotypes, based on 95% bootstrap confidence intervals. The only exception to this was redroot pigweed, the Padova ecotype of which did not germinate if the water potential was less than  $-0.41$  MPa, whereas the Pisa ecotype showed a base water potential of  $-0.62$  MPa. This difference may be explained by the tendency of this species to develop local ecotypes adapted to the environmental conditions of the site, which differ by various characteristics that include phenology, growth and development, morphology, and biochemistry (Sibony and Rubin 2003; Wassom and Tranel 2005).

Although there are few papers (Gardarin et al. 2010; Masin et al. 2005) that report base water potential parameters, based on this scarce bibliography, they appear to be highly variable among ecotypes. Base water potentials are used by WeedCast (Archer et al. 2001), a weed emergence prediction model that has been applied within the north-central region of the United States and neighbouring Canada. The model uses a higher base water potential than that estimated in the present study for velvetleaf ( $-0.15$  MPa) and barnyardgrass ( $-0.1$

MPa), about the same value for redroot pigweed (−0.5 MPa), and much lower values for common lambsquarters (−4.0 MPa), and both green foxtail and yellow foxtail (−5.0 MPa) (WeedCast Version 4.0).

**Table 1.3** - Base water potentials ( $\Psi_b$ ) estimated with the bootstrap method, 95% confidence interval (c. int.), and coefficient of determination ( $r^2$ ).

Species	Padova			Pisa		
	$\Psi_b$ (MPa)	$\pm$ c. int.	$r^2$	$\Psi_b$ (MPa)	$\pm$ c. int.	$r^2$
Velvetleaf	-0.78	0.11	0.88	-0.82	0.06	0.80
Redroot pigweed	-0.41	0.07	0.92	-0.62	0.07	0.96
Common lambsquarters	-0.96	0.10	0.84	-1.04	0.06	0.99
Large crabgrass	-0.74	0.07	0.93	-0.86	0.16	0.93
Barnyardgrass	-0.97	0.04	0.95	-	-	-
Yellow foxtail	-	-	-	-0.93	0.11	0.85
Green foxtail	-0.91	0.24	0.65	-	-	-
Johnsongrass	-0.78	0.19	0.88	-0.80	0.16	0.77

Dashes indicate data not determined.

### Field Experiments.

During the five months of emergence in 2007 and 2008 rainfall was higher in Padova. The driest months were April in 2007 (both sites) and July in Pisa in both years.

The simulations of emergence dynamics in 2007 and 2008 in both sites showed high EF ranging between 0.87-0.90 for velvetleaf and 0.97-0.99 for johnsongrass (Fig. 1.2). Therefore the use of TT or HT in the simulation does not seem to be relevant in terms of EF. Although the improvement of the efficiency of the simulation using HT instead of TT in velvetleaf is limited, it is interesting to note that the pauses in emergence, observable using TT at about 60% and 80% of cumulative emergence (Fig. 1.2), are eliminated using HT. These pauses correspond to dry periods, when the water potential decreases and emergence ceases until the next rainfall or irrigation event. Padova ecotypes of velvetleaf and johnsongrass have the same base water potentials, nevertheless the emergence pauses,

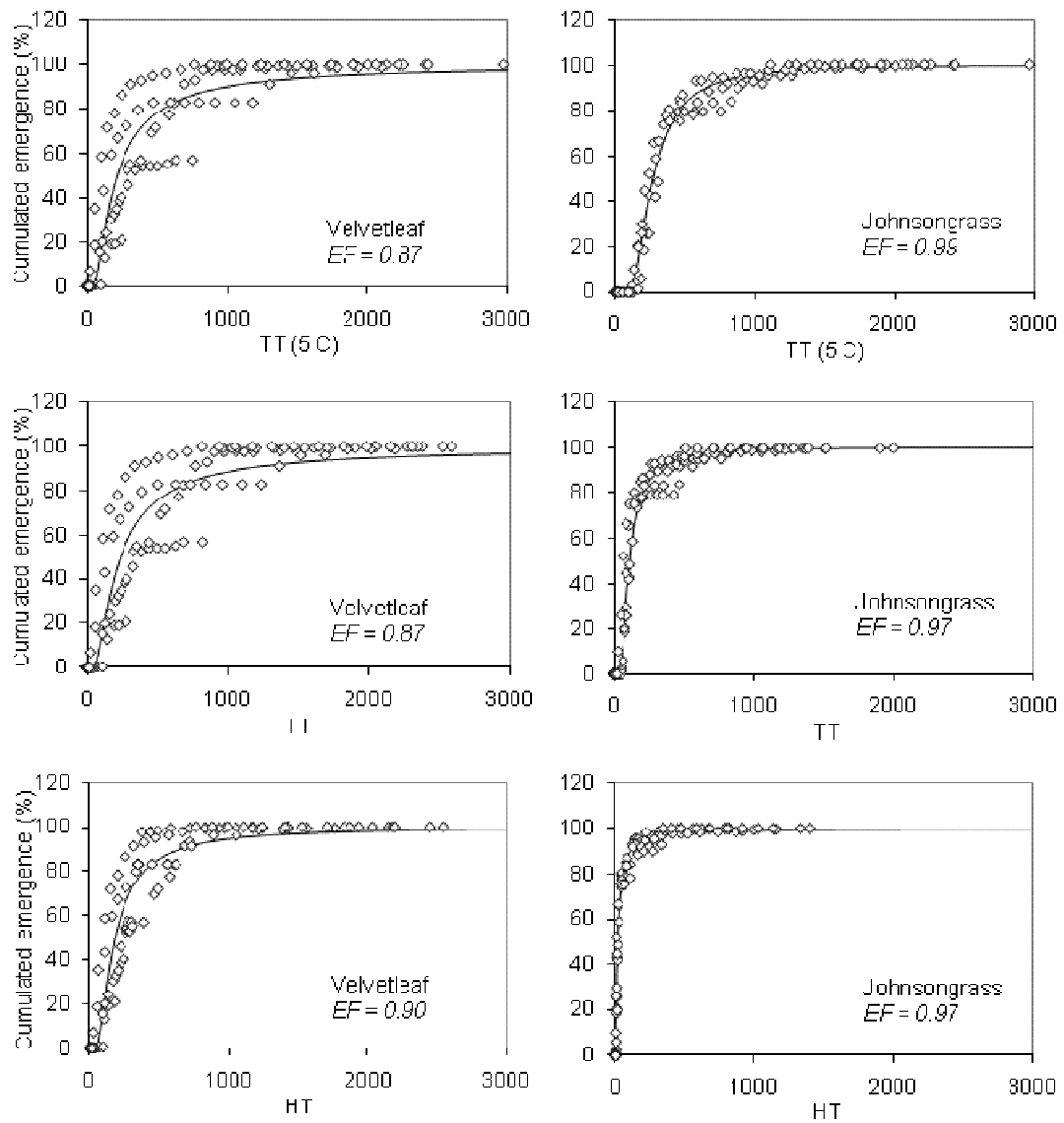


particularly evident in 2007, when April rainfall was very scarce (1.4 mm in 2007 against 107.8 mm in 2008), do not appear for johnsongrass. The reason is that johnsongrass emerged later than velvetleaf so was not influenced by the dry periods in April 2007.

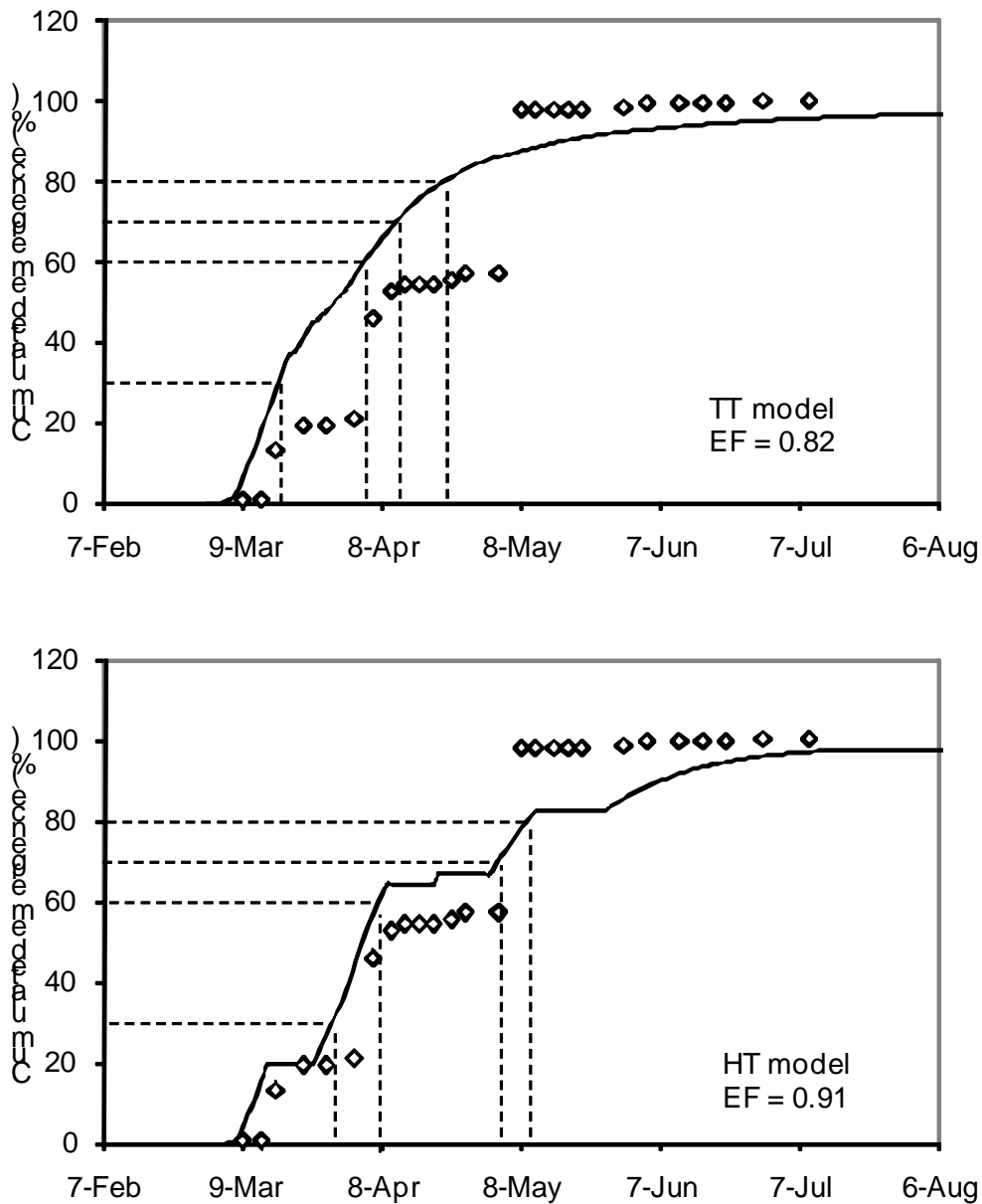
The benefit of using HT instead of TT is due to its capacity to predict the emergence pauses caused by low soil water potential, which is important to reduce the error in practical applications of the emergence model.

If the 2007 dataset of velvetleaf in Padova is isolated from the other datasets and the two simulations with TT and HT are compared, it is evident that HT is able to explain the pauses in emergence, improving the simulation efficiency (EF rises from 0.82 to 0.91) (Fig. 1.3). In this case, HT permits the timing of weed control to be chosen better in practical applications. For example, if a farmer decides to control weeds with a post-emergence herbicide he/she has to wait until most of the weeds have emerged but are not above the range of seedling heights for optimum herbicide control. The farmer may therefore decide to treat when cumulative emergence reaches 70-80% (WeedCast Version 4.0 Documentation). In the example to control velvetleaf in 2007 (Fig. 1.3), the first model (using TT) suggests that the farmer sprays from 11 to 21 April, when in reality the observed cumulative emergence is 54%. The second model (using HT) suggests treating in the period 3-9 May, when the observed emergence ranges from about 65 to 97%. In the first case the model suggests spraying too early with the consequence that a later second treatment will be necessary.

Another example of how HT permits better timing of weed control in practice can be made following the indication of Oriade and Forcella (1999) for maximizing weed control using rotary hoeing or first inter-row cultivation. They observed that the efficacy appeared more consistent if the mechanical control was timed following the emergence percentage: rotary hoeing at 30% and first inter-row cultivation at 60% emergence of green foxtail (the species higher density in their experiments). If the same indications (30% of emergence for rotary hoeing and 60% for inter-row cultivation) are applied to velvetleaf (Fig. 1.3), using the first model (TT) the rotary hoeing should be done on 13 March when the observed emergence was about 13%, with the second model (HT) on 29 March (observed emergence about 20%), while in reality the date corresponding to 30% in the field was 3 April.



**Figure 1.2** - Emergence dynamics of velvetleaf and johnsongrass. Points indicate experimental data from Padova and Pisa in 2007 and 2008. Solid lines represent interpolation with the Gompertz model. Thermal time (TT 5 C) is calculated using a base temperature of 5 C. Thermal time (TT) is calculated using the estimated base temperature. Hydrothermal time (HT) is calculated using the estimated base temperature and base water potential.



**Figure 1.3** - Predictive models for velvetleaf in Padova in 2007. Points indicate the experimental data and solid lines interpolation with the Gompertz model. Dashed lines represent the timing for maximizing weed emergence control following the indications of Oriade and Forcella (1999) for rotary hoeing (30% emergence) and first inter-row cultivation (60% emergence) or the indications when post-emergence herbicides should be used to avoid a second treatment (in this example when cumulative emergence reaches 70-80%).

Following the first model the control is performed 22 days before the correct date, with the second the anticipation is reduced to 5 days. The first inter-row cultivation following the first model should be timed for 4 April when the observed emergence is between 20% (2 April) and 46% (6 April), with the second model the control should be performed on 8 April at about 50% of the observed emergence. On 13 April the observed emergence had reached 55%.

The use of hydrothermal time, with the ability to predict the pauses in emergence due to low soil water potential, reduces the errors of the model, improving its practical application for weed control, especially in dry conditions. The use of HT instead of TT in emergence dynamics prediction could be useful for providing more accurate estimates for timing of weed control applications.

## Sources of Materials

- <sup>1</sup> Polyethylene glycol 6000, Sigma-Aldrich Chemie GmbH, Steinheim, Germany.
- <sup>2</sup> Hermann Roebling, Automatic micro-osmometer, MeBtechnik 1 Berlin 38, Katteweg 32.
- <sup>3</sup> Pendant data logger HOBO UA-001-08, Onset Computer Corporation, Bourne, MA.
- <sup>4</sup> 253-L Watermark Soil Matric Potential, Campbell Scientific Inc., Shepshed, UK.
- <sup>5</sup> External data logger HOBO 4-Channel U12-008, Onset Computer Corporation, Bourne, MA.

## Acknowledgements

The research was financed by a PRIN 2006 project entitled: “*Predicting weed emergence as a tool for improving weed management in maize*”. The research was also partly funded by the Fondazione Raimondo Franceschetti e Di Cola Dott. Giovanni e Famiglie.

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## Chapter II

### Modelling weed emergence in maize fields

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## **Abstract**

Models for prediction of weed emergence may facilitate well-timed and efficient post-emergence herbicide applications. An hydrothermal time model was developed to simulate field emergence for three weed species in maize (*Abutilon theophrasti*, *Chenopodium album*, and *Sorghum halepense*). The model, called AlertInf, was created monitoring seedling emergence from 2002 to 2008 in field experiments at three sites located in Veneto (region in northeastern Italy). The hydrothermal time was calculated using threshold parameters of temperature and water potential for germination estimated in previous laboratory studies with seeds of population collected in Veneto. AlertInf was validated with datasets from independent field experiments carried out in Veneto (the same region of creation) and in Tuscany (region in the central western Italy). Results of model validation were satisfactory in both sites, with EF values ranging from 0.96 - 0.99. AlertInf, based on parameters estimated in a single region, was able to predict the timing of emergence in several sites with different environmental conditions located at the two extremes parts of the maize growing area. These findings support the hypothesis that a single general model may be adopted to predict weed emergence in maize in Italy

## **Nomenclature**

Velvetleaf, *Abutilon theophrasti* Medik., ABUTH; common lambsquarters, *Chenopodium album* L., CHEAL; johnsongrass, *Sorghum halepense* (L.) Pers, SORHA

## **Keywords**

hydrothermal time, emergence prediction, modelling, weed control

## Introduction

The European Commission recently published Directive 2009/128/EC, which provides requirements for the implementation of Integrated Pest Management (IPM) in EU member states before 2014. Integrated Weed Management (IWM), a basic component of IPM, has the objective of developing effective weed control systems and efficient use of herbicides. Although pre-emergence herbicides are often considered fundamental in weed management, as they are often applied without regard for the density and botanical composition of weed communities (Lemieux et al. 2003), some of their applications may not be necessary (Swanton and Weise 1991). A possible alternative is a post-emergence weed management that entails to wait until weeds have emerged, evaluate their density and competitiveness, predict the crop yield loss that they could cause, and then decide if a chemical or mechanical control is required. Systematic pre-emergence applications can be therefore replaced by a conditional control in post-emergence (Lemieux et al. 2003). Anyhow timing is particularly important for a successful post-emergence weed management . The major cause of poor post-emergence weed control is indeed the improper application timing, which can be both too early or too late. In the first case the flushes of emergence that take place after the application are not affected by the herbicide action, while in the second case weeds become less sensible to herbicide due to their larger size. Predictive weed emergence models may be a tool to achieve well-timed and efficient post-emergence applications because these models may estimate in a given moment of crop cycle the percentage of weeds that have already emerged and the successive seedling emergence dynamic The agronomic importance of knowing weed emergence patterns has been recognized since many years (Buhler et al. 2000; Forcella et al. 2000; Leblanc and Cloutier 2002) and several studies have been carried out on weed emergence dynamics with various approaches (Grundy 2003). Significant progress has been recently made in the development of predictive models (Leguizamon et al. 2005; Colbach et al. 2007; Dorado et al. 2009). Both mechanistic and empirical approaches have been used to forecast weed emergence and both present advantages and disadvantages (Grundy 2003). There is no universal best approach to create a accurate model, since it depends on many factors, such as application area/areas, local climatic characteristics, cultivation practices and uses of the

model (scientific or practical). A commonly used approach is represented by the hydrothermal time concept (Gummerson 1986; Alvarado and Bradford 2002), based on the idea that seeds need a certain amount of hydrothermal time to germinate. The hydrothermal time is accumulated according to a comparison between daily soil conditions (temperature and water potential) and specific biological thresholds for seed germination (base temperature and water potential).

In the Italian maize-growing area crops are not always irrigated and there are periods of water deficiency which may affect weed seed germination. In these conditions hydrothermal time models, which consider both soil temperature and water potential, seem to be the most adequate to predict emergence with a certain accuracy (Masin et al. 2010). The objectives of this study were consequently to construct and evaluate a hydrothermal time model to predict the emergence of three important weeds in Italian maize fields: *Abutilon theophrasti* Medik., *Chenopodium album* L. and *Sorghum halepense* (L.) Pers. The validation of the model was carried out in two regions: in the same region of creation of the model (Veneto) and in a region (Tuscany) at the other extreme of the main area where maize is grown in Italy. This process was done to evaluate the possibility to extend the model, created using datasets from a single region, to all the regions of the Italian maize-growing area without recalibration. This hypothesis was proposed because Masin et al. (2010) reported for the three species homogeneous values of base temperature and water potential for local populations present in the two regions.

## **Materials and Methods**

### **Experimental sites**

Eight field experiments were conducted from 2002 to 2008 in three localities in the northeastern Po Valley (northeast Italy): at Montemerlo (2002; 2003 and 2005) in silty clay loam soil, at Carbonara (2006 and 2007) in silty clay loam soil and at Legnaro (2006, 2007 and 2008) in loam soil (creation dataset). All experimental sites were less than 50 km apart and had the same climatic conditions, whereas micrometeorological parameters, especially

rainfall, differed slightly. Seedbed preparation was done according to local practices: primary tillage consisted of fall moldboard plowing and spring harrowing. Maize was sown in late winter (March) in some experiments and later in mid-spring (traditional sowing) in other. Rows were spaced 0.75 m apart. Crops were irrigated if required. Weed emergence were monitored in 33 fixed sampling areas (0.3 x 0.3 m) placed on the soil in the inter-row. Weed seedlings in these sampling areas were counted, classified and removed weekly. At the end of the crop growing season weed emergence were cumulated and used to create a emergence predictive model. The emergence data obtained from these five experiments were used to create the model (creation dataset). Other three experiments were conducted following the same method during 2010 at Legnaro and from 2007 to 2008 at Pisa (Tuscany). This site was chosen because located in Tuscany region at the other extreme of the Italian maize-growing area. The emergence data obtained from these three experiments were used to validate the model (validation dataset).

### **Weather monitoring**

Average daily precipitation and air temperature were collected during the experiments from ARPA meteorological stations located to less than 5 km from the experimental sites. Average daily air temperature and precipitation were used to determine soil temperature and soil water potential at a depth of 5 cm by the STM<sup>2</sup> model (Soil Temperature and Moisture model) (Spokas et al. 2007). This model has been used successfully to predict the soil microclimate used as input for weed emergence modeling (Spokas and Forcella 2009) and other applications. Soil temperature and water potential were monitored beginning on sowing date at Carbonara in 2009 (with a proper experiment, not repeated at Montemerlo because the soil is the same of Carbonara), at Pisa in both experimental years 2007-2008 and in all years at Legnaro,. Temperature was measured using four mini loggers HOBO<sup>1</sup> buried 5 and 10 cm deep. Soil water potential was monitored using water moisture probes<sup>2</sup> buried at a depth of 10 cm (to obtain accurate measurement of soil moisture it was decided to bury the sensor only at the deeper depth) and connected to an external data logger<sup>3</sup>. The data loggers took readings of soil temperature and water potential every 2 hours. The real recorded values were used to test the STM<sup>2</sup> model simulation and to calibrate the model for the simulation of temperature and water potential where they were not directly measured.

STM<sup>2</sup> simulated the soil environment with a certain accuracy, in fact measured daily average soil temperatures and soil water potentials were satisfactory correlated with daily average values estimated with STM<sup>2</sup> ( $r > 0.94$  for temperature,  $r$  ranges from 0.65 to 0.82 for water potential,  $P < 0.001$ ).

### Hydrothermal time and model creation

The model developed in this study is based on hydrothermal time concept (Gummerson 1986; Alvarado and Bradford 2002). According to this approach, all species accumulate hydrothermal time in proportion to soil temperature only when soil water potential is above a base value. This base value of water potential increases linearly as temperature rose above the optimum temperature until it reached 0 MPa at a temperature defined as the ceiling temperature (Bradford 2002). The hydrothermal time ( $HT_i$ ) is calculated as a combination of soil temperature and soil water potential as follows:

$$HT_i = n * \max (Ts_i - T_b, 0) + HT_{i-1} \quad [1]$$

when  $Ts_i < T_o$ :  $n = 0$  when  $\Psi_{s_i} \leq \Psi_b$ ,  $n = 1$  when  $\Psi_{s_i} > \Psi_b$ ; and when  $Ts_i > T_o$ :  $n = 0$  when  $\Psi_{s_i} \leq \Psi_b + K_t (Ts_i - T_o)$ ,  $n = 1$  when  $\Psi_{s_i} > \Psi_b + K_t (Ts_i - T_o)$ ;  $Ts_i$  and  $\Psi_{s_i}$  are the average daily soil temperature and water potential at 5 cm depth,  $T_b$  and  $\Psi_b$  are the base temperature and the base water potential,  $T_o$  is the optimum temperature and  $K_t$  is the slope of the relationship between  $\Psi_b$  and  $Ts_i$  in the supra-optimal temperature range. Base thresholds of the three species were calculated with previous laboratory experiments (for details see Masin et al. 2010) (Table 2.1). Accumulation of HT starts from the sowing date.

Cumulative emergence (CE) is expressed by a Gompertz function, as follows:

$$CE = 100 \exp(-a \exp(-b HT)) \quad [2]$$

where  $a$  is related to a HT lag before emergence starts, and  $b$  is related to the slope of the curve.

The values of  $T_o$  and  $K_t$  were estimated systematically varying in an iterative method until the best simulations were obtained for each species. Initially, the hydrothermal time was

recalculate for different values of  $T_o$  and with  $K_t = 0$ , then  $K_t$  was varied incrementally to find the combination between the values of  $K_t$  and  $T_o$  giving the best fit least-squares. The data used to estimate  $K_t$ ,  $T_o$  and the coefficients  $a$  and  $b$  of the Gompertz function were soil temperature and soil water potential at 5 cm estimated by  $STM^2$ , and weed emergence recorded at Montemerlo (2002; 2003 and 2005), at Carbonara (2006 and 2007) and at Legnaro (2006, 2007 and 2008). The created model henceforth will be called “AlertInf.”

AlertInf performance in predicting weed emergence was evaluated with an efficiency index (EF) (Loague and Green 1991) calculated as:

$$EF = \frac{\sum_{i=1}^n (O_i - \bar{O})^2 - \sum_{i=1}^n (P_i - O_i)^2}{\sum_{i=1}^n (O_i - \bar{O})^2} \quad (4)$$

where  $P_i$  is the predicted value,  $O_i$  the observed value, and  $\bar{O}$  the mean of observed values. The value of EF can range from 1 downwards. An EF value of 1 would mean that the model produced exact predictions.

### **Model validation with independent dataset**

In order to validate the model, datasets of weed emergence collected in experiments carried out at Legnaro in 2010 and at Pisa in 2007 (only velvetleaf) and 2008 (common lambsquarters and johnsongrass) were used. The daily average values of soil temperature and soil water potential were those estimated by  $STM^2$  at a depth of 5 cm. The model was also validated at Pisa to verify its transferability to a region with a different climate without recalibrating it. In this way, it was verified if the same biological parameters ( $T_b$ ,  $\Psi_b$ ,  $K_t$ ,  $T_o$ ) and Gompertz coefficients ( $a$  and  $b$ ) estimated in Veneto were usable in another region at the other extreme of the Italian maize-growing area. Simulated emergence from AlertInf was compared with observed emergence data obtained at Legnaro and at Pisa using the model efficiency index (EF).

## Results and Discussion

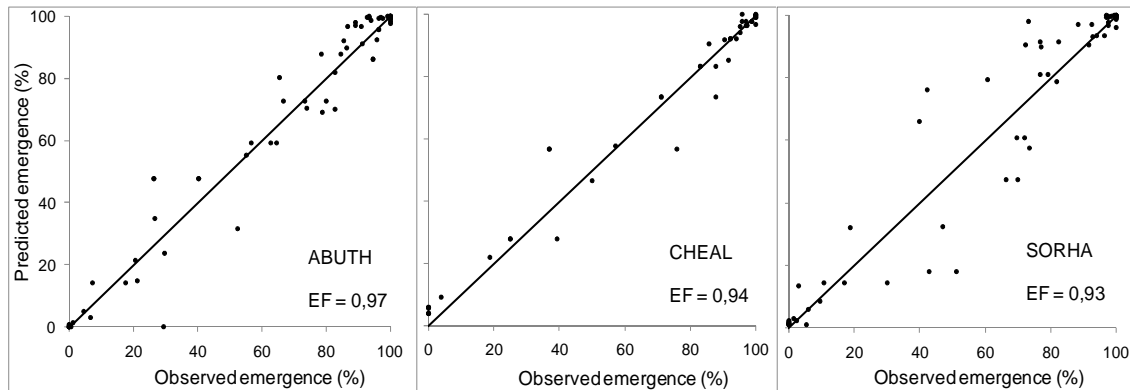
### Model creation

Parameters  $a$  and  $b$  of the Gompertz function and the input variables  $T_o$  and  $K_t$  are shown on Table 2.1 together with base temperatures and base water potentials determined by Masin et al. (2010) which were also used for AlertInf parameterization. The resulting optimum temperatures ( $T_o$ ) of the three species were from 23 to 25 C. These values were essentially in agreement with those reported in bibliography for these species (Bouwmeester and Karssen 1993; Roman et al. 1999; Leon et al. 2004). The model adequately described the cumulated emergence in the experiments used for the creation as confirmed by the high EF values (from 0.93 to 0.97) (Fig. 2.1).

**Table 2.1** - Biological parameters for the calculation of the hydrothermal time and  $a$  and  $b$  coefficients of the Gompertz function used for modelling cumulated emergence.  $T_b$  and  $\Psi_b$  estimated by Masin et al. (2010).

<b>Species</b>	<b><math>T_b</math></b>	<b><math>\Psi_b</math></b>	<b><math>T_o</math></b>	<b><math>K_t</math></b>	<b><math>a</math></b>	<b><math>b</math></b>
	<b>(C)</b>	<b>(MPa)</b>	<b>(C)</b>			
ABUTH	3.9	-0.78	25	0.10	10.28	0.02
CHEAL	2.6	-0.96	23	0.20	3.56	0.01
SORHA	11.8	-0.78	24	0.30	4.72	0.03





**Figure 2.1-** Observed vs. predicted cumulated weed emergence (creation datasets) for the three species and relative EF values.

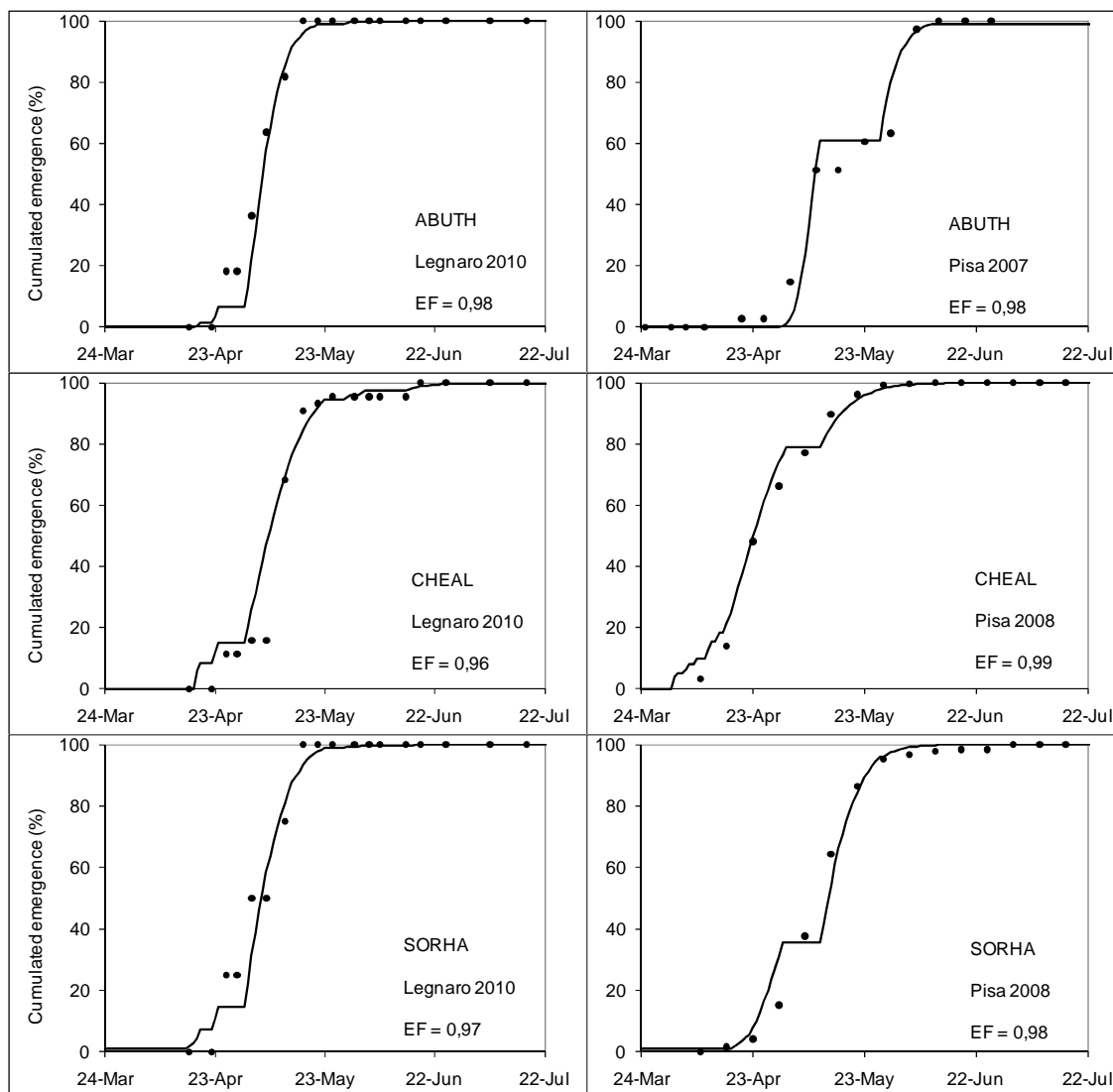
### **Model validation with independent datasets**

Model simulations of emergence in 2010 at Legnaro and 2007-2008 at Pisa (validation dataset) resulted in EF values ranging from 0.96 - 0.99 (Fig. 2.2) , AlertInf prediction showed one pause of emergence at the end of April at Legnaro for all species. In correspondence of this pause, the model underestimated the percentage of cumulated emergence in velvetleaf and johnsongrass, and overestimated it in common lambsquarters. The second pause between 3<sup>rd</sup> and 7<sup>th</sup> May present in the real emergence pattern of common lambsquarters and johnsongrass was not predicted by the model. This incorrect estimation is difficult to explain given that the analysis of the soil temperature and soil water potential in those five days showed that those two parameters were not under the threshold (not only the estimated but even the measured values). Hydrothermal time was therefore accumulated during this period and consequently emergence percentage was supposed to increase, as for velvetleaf. Anyhow even if the estimation did not predict the second pause, all the simulations were satisfactory both statistically (high EF) and practically (for practical applications of the model).

It is very interesting that the simulation at Pisa, a different site from those used to create the model, was satisfactory as showed by the high EF values (0.98-0.99). The simulated emergence was in delay in comparison with the real dynamic at the beginning of emergence for velvetleaf in 2007, and in advance of some days at the beginning of emergence in common lambsquarters and johnsongrass in 2008. Anyway, for practical purposes (i.e. for

timing stale seed bed preparation), errors of estimation of a few days at the beginning of emergence could be acceptable. The more relevant error was in the simulation of johnsongrass emergence. AlertInf anticipated and overestimated the initial flush of emergence and then reported a pause in correspondence of 36% of emergence that was not present or maybe began later and lasted less in the real emergence pattern of this species. This incorrect prediction causes an error of estimation of more than 20% of cumulated emergence, i.e. on 30<sup>th</sup> Apr the real emergence was 15% but the model estimated a very higher value (36%). A similar pause is evident also in the simulation of the emergence pattern of common lambsquarters. However, this pause was not confirmed in the real emergence dynamic due to lack of data in that period for this species.

In conclusion, even if some errors of simulation were present, the predicted emergences of all three species showed high EF values in both sites considered for the validation. The prediction resulted accurate not only statistically, but also under a practical point of view. In general, AlertInf showed difficulties to accurately forecast the onset of emergence, which is a critical period only for implementing weed control practices such as stale seed bed preparation, but not for the use of post-emergence control which is applied later in the growing season. In fact, farmers are usually suggested to apply post-emergence herbicides when most of the weeds have emerged (70-80% of emergence) (WeedCast Version 4.0 Documentation). In all the model validations (species and sites), the dates corresponding to this percentage of emergence were accurately estimated (the maximum difference was 2 days, an acceptable error for practical use). Another example can be made analyzing the predicted percentage requested for optimizing weed control using rotary hoeing or first inter-row cultivation according to Oriade and Forcella (1999) indications. They observed that the more consistent efficacy of rotary hoeing could be obtained at 30% emergence of the species with higher density (in their experiments green foxtail, *Setaria viridis* L.) and first inter-row cultivation at 60%. AlertInf was able to predict accurately also these dates for optimizing those practices in all species and both sites. The more relevant error of the model was in predicting the date when johnsongrass reached the 30% of emergence (for an hypothetical rotary hoeing) at Pisa. In this case the prediction was in advance of 5 days compared to the real date (the model estimated 30<sup>th</sup> Apr and the real 30% was reached 4<sup>th</sup> May).



**Figure 2.2** - Cumulated emergence predicted using AlertInf (line), and observed (black circles) in three experiments conducted in 2010 at Legnaro and in 2007 and 2008 at Pisa (validation datasets).

These satisfactory results obtained with the model validation at Pisa and Legnaro lead to the conclusion that AlertInf created using a dataset collected in Veneto can be used to predict velvetleaf, common lambsquarters and johnsongrass emergence not only in this region but also in the whole Italian maize-growing area.

Since 2008, a simplified version of AlertInf is available on the web site of the ARPAV Agrobiometeorology Unit ([www.arpa.veneto.it](http://www.arpa.veneto.it)). That version uses daily soil temperature

and daily rainfall to calculate the hydrothermal time. The species included in that version are six important weeds in maize: *Abutilon theophrasti* Medik., *Amaranthus retroflexus* L., *Chenopodium album* L., *Polygonum persicaria* L., *Solanum nigrum* L., *Sorghum halepense* (L.) Pers. The response of the users was considered as positive due to the high number of recorded visits of the AlertInf webpage throughout the growing season. This denotes a certain interest. Also in USA (Archer et al. 2002) and Australia (Walsh et al. 2002) similar predictive models for weed emergence in arable fields accessible through interactive computer software are being used by farmers and crop advisors with positive feedbacks. The improved version of AlertInf showed in this article has demonstrated the ability to predict emergence of the three studied species with good accuracy. The current objective is to improve this new version of the model adding other species and then make it accessible to farmers and advisors through interactive computer software or information in bulletin distributed by extension services

## Source of materials

<sup>1</sup> Pendant data logger HOBO UA-001-08, Onset Computer Corporation, Bourne, MA.

<sup>2</sup> 253-L Watermark Soil Matric Potential, Campbell Scientific Inc., Shepshed, UK.

<sup>3</sup> External data logger HOBO 4-Channel U12-008, Onset Computer Corporation, Bourne, MA.

## Acknowledgements

The research was financed by a PRIN 2006 project entitled: “*Predicting weed emergence as a tool for improving weed management in maize*”.

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## CHAPTER III

### **A single time survey method to predict the daily weed density for weed control decision-making**

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Accepted by *Weed Science* on 30<sup>th</sup> December 2010, in press

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## **Abstract**

Decision-making processes must give indications on “if”, “how”, and “when” weed control should be practiced. So far, Decision Support Systems (DSSs) for weed control to prevent crop yield losses can guide decisions about the first two questions. Experience shows that farmers need a DSS that can also guide the decision on “when” to treat but this can only be obtained if the actual weed density observed in the field is known during the crop cycle. Emergence models allow the prediction of daily density, but precision depends on the survey date. This study focuses on the estimation of the date of the survey for the best prediction of the daily density throughout the crop cycle. The predicted daily density of each species can be used by DSSs without any further survey, saving time and money and improving the use of the DSSs. Results showed that the best date is when the actual density of each weed reaches or exceeds 50% emergence, and this is earlier than the Critical Point date, supporting the validity of the date estimation method. The possibility to provide specific advice for farmers considering a proper mortality rate of weed seedlings is then discussed. This study can improve the reliability of decision-making tools for Integrated Weed Management (IWM), in agreement with the European Union goal of the sustainable use of pesticides and more environmentally sustainable cropping systems through the use of Integrated Pest Management.

## **Nomenclature**

Common lambsquarters, *Chenopodium album* L., CHEAL; johnsongrass, *Sorghum halepense* (L.) Pers., SORHA; ladysthumb, *Polygonum persicaria* L., POLPE; redroot pigweed, *Amaranthus retroflexus* L., AMARE; velvetleaf, *Abutilon theophrasti* Medik, ABUTH; corn, *Zea mays* L.

## **Key Words**

Weed density, weed sampling, Decision Support Systems, emergence prediction, hydrothermal time.

## **Introduction**

The European Union has recently published the Directive 2009/128/EC on the sustainable use of pesticides, with the objective of reducing the risks and impacts of pesticide use on human health and the environment by promoting the use of Integrated Pest Management (IPM) and alternative approaches or techniques (European Parliament 2009). The Directive implies the need for innovative tools to be introduced and applied to IPM strategies aiming mainly at pesticide use reduction. The quali-quantitative knowledge of the weeds in the field is essential in order to apply Integrated Weed Management (IWM), a basic component of IPM, and enable a decision-making process to be developed that will determine “if”, “how”, and “when” weed control should be done.

Decision Support Systems (DSSs) can guide farmers in making decisions about the first two questions: “if” and “how” to treat, and field surveys can provide the information needed (Berti et al. 1992). However, DSSs are still not broadly accepted and used by farmers (Swanton et al. 2008; Wilkerson et al. 2002; Berti et al. 2003) even if some have demonstrated the potential for reducing herbicide inputs by up to 40-50% at national level (Rydahl et al. 2009). There are many reasons for this non-use, i.e., the relatively low cost of routine herbicide treatments (Rydahl et al. 2009), the additional costs for surveys and the fact that DSSs are unable to give information about the correct timing of application. At the moment, the survey is done when the farmer, based on his own experience, decides that it is time to treat. The output of the system is therefore related to that specific survey time, while what may happen later during the crop cycle is unknown without another survey. Thus, farmers consider the DSSs as tools giving partial information since they do not advise “when to treat” because this can only be provided if the daily weed density is known. The prediction of weed density dynamics could be obtained by combining a weed emergence model, which estimates the dynamics of seedling emergence throughout the crop cycle, with the actual weed density, i.e., the density measured on the field at a given date.

Numerous studies have been done to develop emergence prediction models (Grundy 2003; Forcella et al. 2000; Masin et al. 2010). The information provided by such models is the percentage of emerged weeds out of the total number of plants that may potentially emerge

during the cropping cycle. This information is useful for efficient timing of either chemical or mechanical weed control based on weed phenological traits but does not consider economic aspects. Indeed, the treatment is usually applied when the predicted percentage of emergence is high (more than 80%, if the weed phenological stage allows it), so that many weeds are controlled and only a few emerge later. In this way a second treatment is avoided. Nevertheless, treating at high emergence percentage might not be the best control strategy as weeds that emerged with the crop may have already caused a yield loss. Unlike DSSs, which identify if a treatment is necessary or not, and list the best solutions, the information provided by weed emergence models is not advice to be followed, but has instead to be interpreted by the farmer. In order to provide complete information to farmers, emergence models lack the capacity to predict the actual weed density. The survey process of DSSs could be used to relate the actual weed density with a specific emergence percentage and predict the daily density pattern with a simple proportion. The precision of the prediction depends on the survey date, and theoretically the highest precision can be obtained only with various consecutive surveys during the weed-crop competitive period. Therefore, if the emergence models are able to predict the daily density of weeds during the crop cycle with a single survey, they can provide the necessary input on density of each species for DSSs without further surveys, saving time and money and providing specific and practical advice to farmers.

This combination of bio-economic DSSs and emergence models can help in optimizing the timing of weed control operations, improving efficacy of control strategies and reducing herbicide use through implementation of IWM (Buhler et al. 2000). Within this framework and specifically for Italian corn fields this study analyses the possibility of using emergence predictive models to predict the daily weed density throughout the crop cycle and focuses on the estimation of the date of the survey in order to obtain the best prediction.

## Materials and Methods

### Experimental Site, Design and Surveys

Seven experiments were conducted from 2006 to 2009 in the Po Valley (northeast Italy): four at Carbonara (indicated as CA) and three at the Experimental Farm of Padova University in Legnaro (indicated as PD). All soils were silty clay loams and soil preparation was conducted according to local cropping practices for corn: primary tillage consisted of fall moldboard plowing and spring harrowing. Corn was sown at all the experimental sites in rows 0.75 m apart and spaced 0.18 m on the row. Sowing dates ranged from March 7 to March 27. The basic experimental design of these weed-crop competition studies was a randomized complete block with three replicates. In this study only the data of the season-long weed-infested plots were used. In all experiments, 12 rectangles (0.50 m x 0.10 m) were fixed on the soil between rows within plots (five rows wide by 5 m long) and left untreated during the entire crop cycle. Weed seedlings in each rectangle were counted, classified and removed weekly (for a total of 8-15 surveys per experiment) to determine weed densities and emergence dynamics. All experiments in CA were conducted with natural infestations, whereas in PD (2008 and 2009) the species of interest (common lambsquarters, ladythumb, johnsongrass, redroot pigweed and velvetleaf) were sown in the fall of the year prior to corn sowing to enrich the seedbank.

### Weed Emergence Model

AlertInf is the model used for predicting emergence of the principal weeds in corn, using the Hydrothermal Time (HT) concept (Masin et al. 2010):

$$HT = \sum (n \cdot (T_{smi} - T_b) + HT_{i-1}) \quad (1)$$

Where  $T_{smi}$  (C) is the soil temperature given by the average of the daily temperatures at 0 and -10 cm,  $T_b$  (C) is the base temperature.  $n = 0$  if the total rainfall in the past  $x$  days ( $x$  depends on the species) is lower than the rainfall limit ( $R_{limit}$  depends on the species) and  $n = 1$  if the total rainfall in the past  $x$  days is higher than  $R_{limit}$ . The parameters ( $T_b$ ,  $x$  days,

$R_{limit}$ ) are reported on the AlertInf website page ([www.arpa.veneto.it/upload\\_teolo/agrometeo/infestanti.htm](http://www.arpa.veneto.it/upload_teolo/agrometeo/infestanti.htm)).

The input data required by the model were obtained from soil temperature and daily rainfall data measured at ARPAV (Regional Environmental Protection Agency of Veneto) weather stations located about 5 km from each experimental site. The accumulation of hydrothermal time starts from the corn sowing date. When hydrothermal time has been calculated, the cumulated emergence percentage ( $E_i$ ) is determined with a Gompertz equation:

$$E_i = 100 \cdot \exp(-a \cdot \exp(-b \cdot HT)) \quad (2)$$

where  $a$  represents the HT lag before emergence starts, and  $b$  the rate of increase of emergence once it has begun.  $a$  and  $b$  depend on the species and are reported on the AlertInf website page.

The species currently included in the model are: the spring emerging common lambsquarters, ladythumb and velvetleaf, and the summer emerging johnsongrass and redroot pigweed.

### **Daily Density Prediction**

The weed emergence model was used to predict the daily density of each species and the total density (sum of the five species' densities predicted by AlertInf) starting from the actual density on different dates.

The daily density of each species ( $D_{ti}$ ) on a specific  $i$ -th date was predicted as:

$$D_{ti} = D_{ts} \cdot E_{ti} / E_{ts} \quad (3)$$

where  $D_{ts}$  is the actual density measured with a survey,  $E_{ts}$  is the emergence percentage predicted by AlertInf at the time of the survey, and  $E_{ti}$  is the percentage predicted by AlertInf on  $i$ -th day.

### **Selection of the Best Date for the Survey**

The evaluation of the date for survey for the best prediction of daily density during the crop cycle (hereafter: best date) was done by grouping the surveys into four ranges of emergence percentage predicted by AlertInf. This grouping was needed to have enough data to yield statistical analysis and the groups were: 10-30%, 30-50%, 50-70% and 70-90% of the cumulated emergence. Redroot pigweed data were insufficient for a specific analysis and were only considered in the prediction of the total weed infestation. Model performance to predict daily density was evaluated with an efficiency index (EF) calculated as:

$$EF = \frac{\sum_{i=1}^n (O_i - \bar{O})^2 - \sum_{i=1}^n (P_i - O_i)^2}{\sum_{i=1}^n (O_i - \bar{O})^2} \quad (4)$$

where  $P_i$  is the predicted value,  $O_i$  the observed value, and  $\bar{O}$  the mean of observed values. The value of EF can range from 1 downwards. An EF value of 1 would mean that the model produced exact predictions. The lowest emergence percentage group with a prediction of the daily density with an  $EF > 0.85$  was set as the best date for the survey.

The actual density on the selected date was then used to predict the daily density of each species and the total infestation (sum of 5 species' densities predicted by AlertInf) by relating the actual density to the daily percentage of emergence predicted by AlertInf. The model performance in predicting daily density was also evaluated using the EF.

### **Comparison between Best Date for Survey and Critical Period**

The proposed method can be used by the farmer if the best date of the survey occurs earlier than the CP (Critical Point, sensu Otto et al. 2009), which is the point of intersection between DTC (duration of tolerated competition) and WFP (weed-free period) curves, and is approximately in the middle of the CPWC (Critical Period of Weed Control). This period is an interval in the crop cycle when the crop must be kept weed free to prevent a given yield loss. Its length is dependent on yield economic value, weed control cost, and the selected value of acceptable yield loss. The CP is not market-dependent and is determined only by the competition between weeds and crops. Thus, if the best date is some days prior

to the CP, it means that there is still time to perform an effective weed control to prevent unacceptable yield losses.

To verify if the best date occurs earlier than the CP, the CP calculated from four weed-corn competition experiments were used. The experiments were conducted during 2002, 2003 and 2005 at Montemerlo (indicated as MT), Po Valley, northeast Italy. Corn was sown in early March in all the experiments, except in 2005 when the crop was sown on two dates, the first in March, early sowing (MT05e), and the second in April, traditional sowing (MT05t). For details of these experiments see Otto et al. (2009). The best date of the survey was identified with the method described above.

## Results and Discussion

### Selection of the Best Date for the Survey

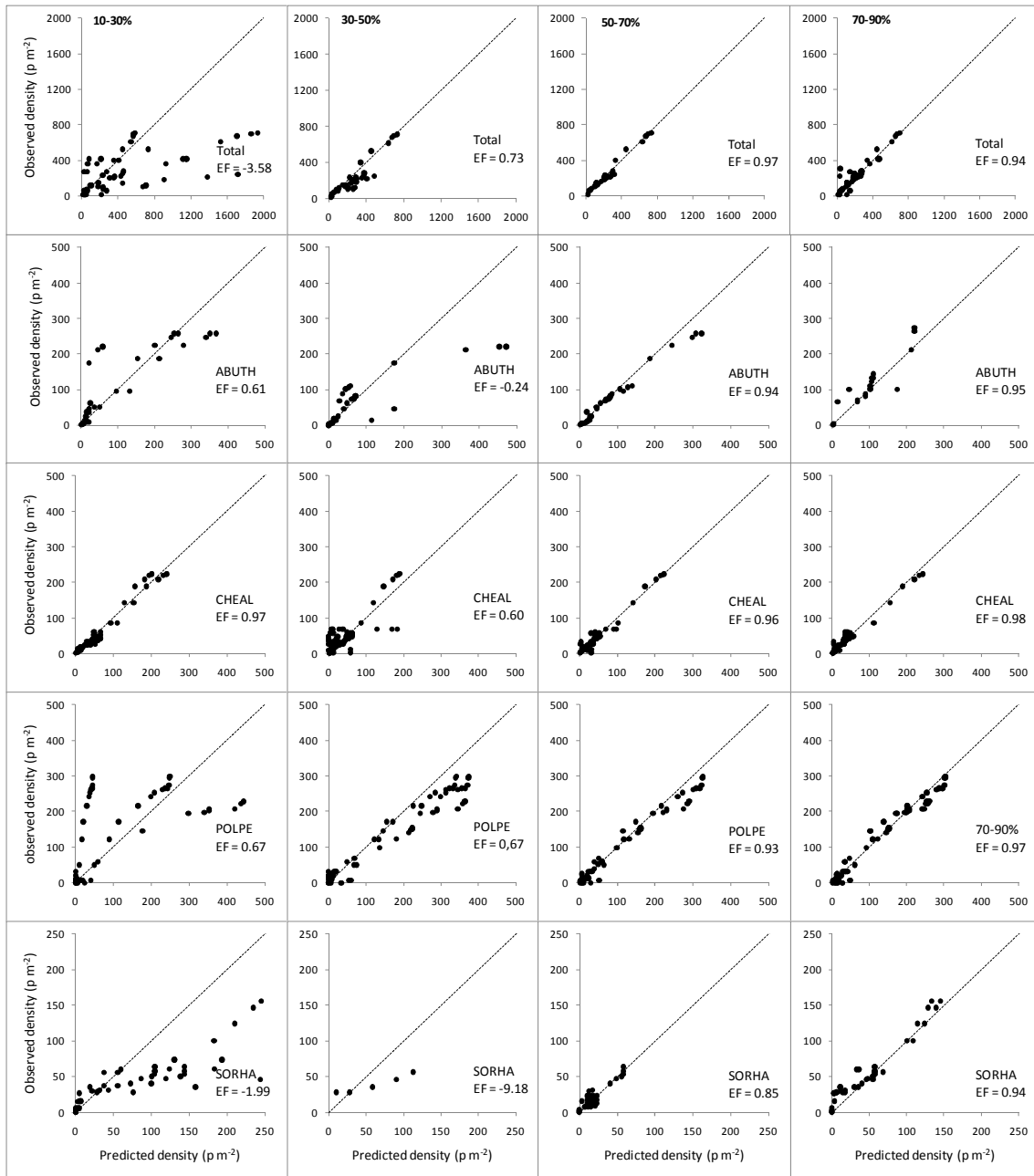
The observed vs. predicted daily density showed that the lowest emergence percentage group with a prediction of the daily density with an  $EF > 0.85$  was that obtained using as input the actual density measured after 50% of emergence (50-70% group), for which the EF ranges from 0.85 to 0.97 (Figure 3.1). Accordingly, the best date of the single survey for the prediction of the daily density was defined as the first date when each species observed in the field reached or exceeded 50% emergence.

Using the actual density in the first survey after the best date (Table 3.1), the cumulated density was estimated (Figure 3.2). For the seven experiments the model slightly underestimated the final density in four cases (CA06, PD07, PD08 and PD09), overestimated it in two cases (CA07 and CA09), whereas a very good estimation was obtained in the case of CA08.

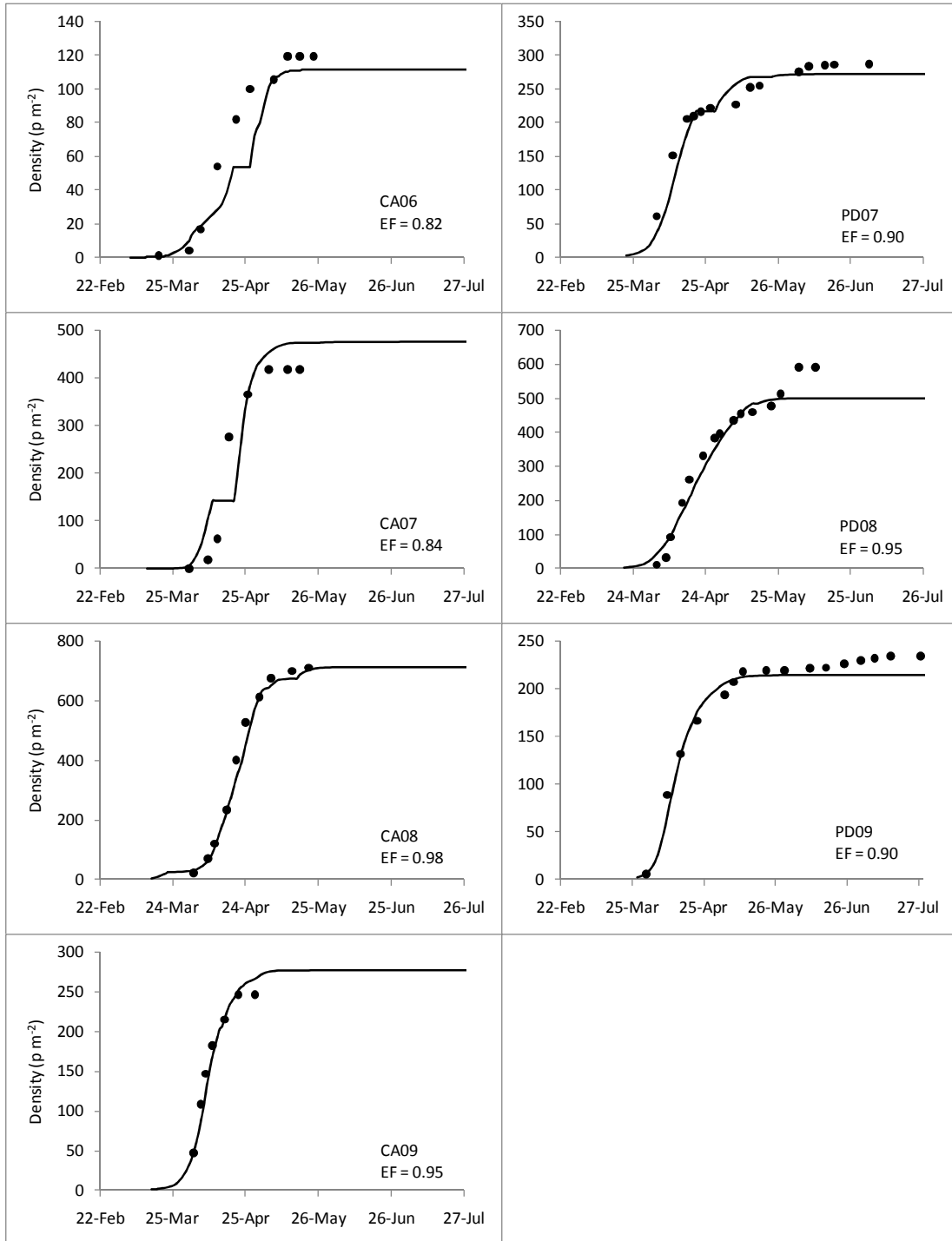
**Table 3.1** - Dates of first surveys in the field (per species) after 50% of predicted weed emergence, and best date (when all five species reached 50% of emergence).

	Site						
	CA06	CA07	CA08	CA09	PD07e	PD08e	PD09e
ABUTH	-	Apr 26	Apr 20	Apr 8	Apr 11	Apr 23	-
AMARE	May 7	Apr 26	Apr 30	Apr 16	Apr 20	May 9	Apr 22
CHEAL	Apr 13	Apr 26	Apr 20	Apr 11	Apr 17	Apr 17	Apr 15
POLPE	Apr 13	Apr 26	Apr 16	Apr 11	Apr 17	Apr 23	Apr 15
SORHA	May 7	Apr 26	Apr 30	Apr 16	-	May 6	-
Best date	May 7	Apr 26	Apr 30	Apr 16	Apr 20	May 9	Apr 22





**Figure 3.1** - Observed vs. predicted daily density for the total density (five species) and per species at 10-30%, 30-50%, 50-70% and 70-90% of emergence predicted by AlertInf (data are averaged over years and locations). Redroot pigweed data were only considered in the total density calculation



**Figure 3.2** - Cumulative total daily density (five species) predicted using the actual density in the first survey after the best date (line), and observed (black circles) in seven experiments conducted from 2006 to 2009 at Carbonara (indicated as CA) and Legnaro (PD) (northeast Italy).

The prediction of the daily density dynamic, more important than the final density for guiding weed control decisions, was in general accurate and the EF values for all cases were high, exceeding 0.82. In CA06 and CA07, AlertInf prediction showed a pause of emergence not present in the real emergence pattern. This may be due to rainfall variability in space, as the weather station located about 5 km from the experimental sites perhaps provided rainfall data that did not correspond accurately to the real on-site conditions. In the other five cases the prediction of the daily density dynamic was satisfactory until approximately 80-90% of the final density, whereas, in particular in Padova, the model showed poor prediction of the latest weed emergence flush. However, it is important to stress that the late emergence flushes are less competitive than the earlier ones and have less impact on crop yield.

### **Comparison between Best Date of Survey and Critical Period**

The estimated best dates of the survey were compared with the date of the CP to verify if the surveys could be done some days prior to the CP and thus used to make efficient decisions about weed control.

In the CPWC experiments of Otto et al. (2009), the CP was calculated based on the total density given by the sum of all the species present in the field (Table 3.2). However, the best dates of the survey (Table 3.3) were estimated considering only the 50% emergence of the five species predicted by AlertInf, which represented about 70-80% of the total density, except for MT03e where their density was very low and only represented 5%.

The best date for the survey (when all five species reached 50% of emergence) occurred earlier than the CP in all the experiments, verifying the validity of this method. The latest best date for the survey was May 4 in MT03t, nine days before the CP, which is enough time for a weed control decision. These results are just an indication, as the selection of the survey date is based on just five species, whereas the CP is determined by all species present in the field. When AlertInf is updated with more species, it will be possible to predict a more realistic emergence pattern. However, this also shows that using Alertinf to predict emergence of these specific weed species of great importance in corn could provide a reliable indication for the best date of the single survey before CP and eventually more timing-efficient weed management decisions to prevent corn yield losses.

**Table 3.2** - Characteristics of the weed flora in the four experiments conducted during 2002, 2003 and 2005 at Montemerlo (MT), northeast Italy.

Site	Species (n)	Total density (all species m <sup>-2</sup> )	Proportion of the five selected species*	
			(% of the total density)	
MT02		13	207.8	83
MT03e		20	316.3	5
MT03t		19	98.5	68
MT05		15	690.3	78

\* common lambsquarters, johnsongrass, ladysthumb, redroot pigweed, velvetleaf.

**Table 3.3** - Estimated best date for the survey (when all five species reached 50% of emergence) and Critical Point date (sensu Otto et al. 2009), which is approximately in the middle of the Critical Period of Weed Control.

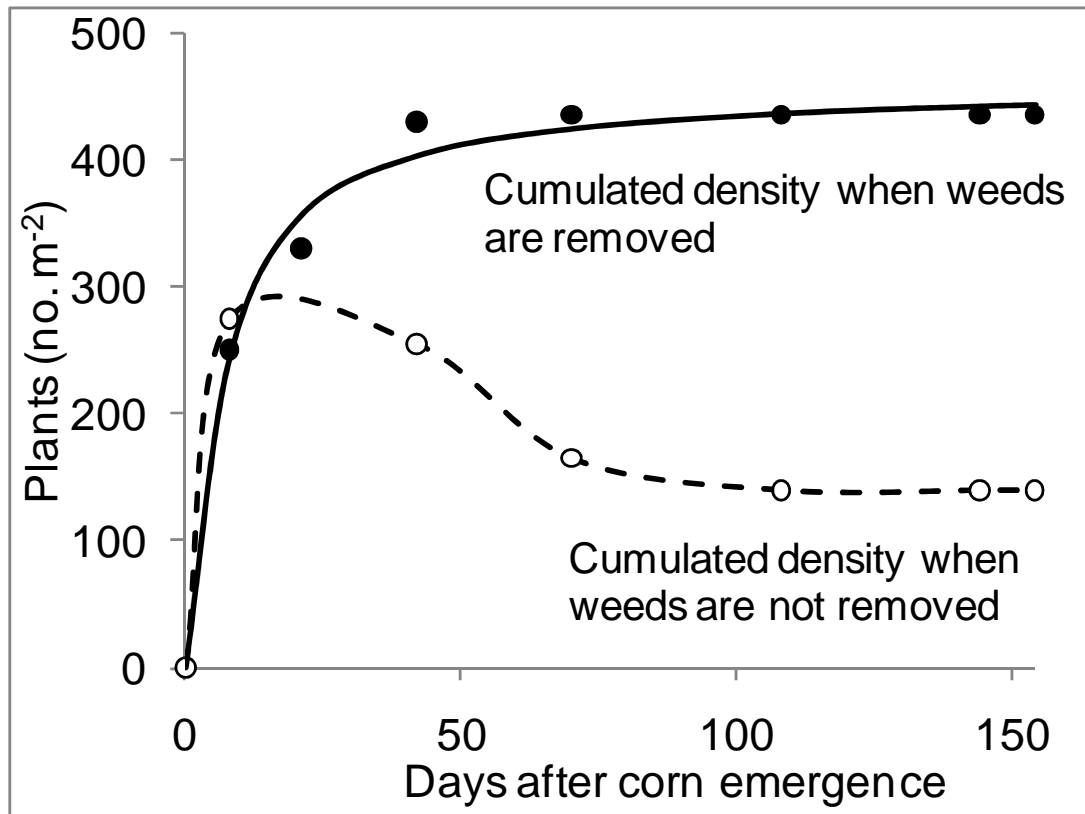
Site	Estimated best date for the survey	Critical Point date
MT02	Apr 26	May 29
MT03e	Apr 21	May 9
MT03t	May 4	May 13
MT05	Apr 30	May 14

## **Prospects and Challenges for DSSs**

Fernandez-Quintanilla et al. (2008) consider the necessity to create novel weed monitoring techniques and analytical tools to improve the applicability of the principles of IWM. The results of this study suggest that the described method to predict daily density may contribute to reach this objective, improving DSSs precision. One of the most difficult aspects to consider in a DSS is the both temporal and spatial non-uniformity of weed infestation (Slaughter et al. 2008). The possibility to predict the daily density of different weeds during the crop cycle allows the temporal variability of weeds to be integrated into the practical decisions. On the other hand, the survey method and calculation algorithm of a bio-economic DSS, GESTINF (Berti and Zanin 1997), validated in corn, take into account spatial distribution of weeds, by estimating the yield loss at field level not on average density of weeds but on average of yield losses for every sampling area (Berti et al. 1992; Zanin et al. 1998).

The method based on the prediction of daily density can be improved when the natural weed seedling mortality during the crop cycle is considered. Indeed, in the absence of weed control, not all the weeds that emerge survive until crop harvest (Zanin and Sattin 1988). The causes are numerous: fungal and insect attacks, unfavorable climatic conditions (frost, drought, etc.), competition and self-thinning. Plants may respond to increasing density in a different way. Some species use phenotypic plasticity, which is the modification of some morphologic traits (ramification, height, etc.) and cohabit, whereas others compete and die. According to Donald (1981), the former species are defined as “communal plants” and the latter as “competitive plants”. There are both types in a weed community, which means that when there is a high density of weeds, it is expected that some individuals die (Harper 1977). In sampled areas where weeds were left to emerge, counted and removed, the density is maintained low and the density reduction due to mortality is likely low, while when weeds are not removed the resulting higher density enhances weed mortality. Anderson (2008) found that 37% of emerged weed seedlings in a soybean crop survived to produce seeds. Similarly, Mohler and Calloway (1992) reported that, in sweet corn, the survival of redroot pigweed and common lambsquarters in the absence of treatment ranged from 17% to 46% for the earlier emergence cohorts, while later cohorts emerged in the shade of the earlier ones and their survival was limited by the competition. Zanin and Berti

(2001) observed that, with a medium weed density (about 450 plants  $m^{-2}$ ), a 67% weed density reduction was obtained in untreated corn. This density reduction started 2-3 weeks after crop emergence and lasted 40-50 days, while the density remained stable after the phenological phase of corn canopy closure (Figure 3.3).



**Figure 3.3** - Cumulative total weed density in sampled areas where weeds are left to emerge, counted and removed (filled circles) and modeled with a Gompertz function (solid line), and in sampled areas where weeds are not removed (empty circles and dashed line). Graph from Zanin and Berti (2001).

A survey can only measure the density at that particular moment, but if mortality is not considered this density may be lower than that estimated by the emergence model until that time. Some studies on the relation between weed mortality and density make attempts at modeling the density reduction. Rainbolt et al. (2004), in order to predict the natural seedling mortality, used an exponential equation where seedling mortality increases as

weed seedling density increases. Colbach et al. (2007), in ALOMYSIS, a model on the effects of the cropping system on blackgrass (*Alopecurus myosuroides* Huds.) lifecycle, determined the probability of plant mortality due to intra- and inter-specific competition as a function of the cumulated density of emerged plants over the crop cycle and the maximum surviving adult density in the infested crop. Many other mortality models have been suggested (Cousens and Mortimer 1995; Jones and Medd 2005; Watkinson 1980), showing that modeling the mortality rate is complex but possible. It is therefore possible to include the density reduction in the emergence model, to better relate the actual density at the best date to the daily density throughout the crop cycle, which are all the data needed to run the DSS and give specific and practical advice to the farmers. This will help to reduce pesticide loads, in agreement with the European Union goal of the sustainable use of pesticides and more environmentally sustainable cropping systems through the use of IPM.

## Acknowledgements

The research was financed by a PRIN 2006 project entitled: “*Predicting weed emergence as a tool for improving weed management in maize*”. The authors thank ARPAV (Regional Environmental Protection Agency of Veneto) for providing the weather data.

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## **CHAPTER IV**

### **Combined effects of origin and growth environment on base temperature of jimsonweed and velvetleaf**

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## **Abstract**

Hydrothermal models for weed emergence prediction represent useful tools to improve IPM, allowing more environmentally sustainable and efficient crop protection. Hydrothermal models require biological parameters, such as base temperature and base water potential for germination, whose determination is time- and money-consuming. Transferability of these parameters across different populations may represent a constraint for the development and practical use of hydrothermal models. Local weed populations may present specific germination ecology due to several factors, such as the presence of genetic diversity among distant populations or the maternal effects related to local climatic differences during seed ripening. A collaborative project has been carried out to analyze the interactive effect of site of origin (genetic factor) and site of cultivation (environmental factor) on germination ecology of three local European populations of *Abutilon theophrasti* Medik. and *Datura stramonium* L. Seeds were collected from each spontaneous population and sown at three different sites of cultivation. Nine different populations (3 sites of origin x 3 sites of cultivation) were obtained for both species. Germination tests at constant temperatures were performed to assess germination patterns as a function of temperature and to estimate specific base temperature for germination in each population. Relevant differences were found in the behavior of the two species. Germination performances of *A. theophrasti* were slightly influenced by site of origin, site of cultivation or incubation temperature and similar values of base temperature were estimated for all *A. theophrasti* populations. Site of origin, site of cultivation and incubating temperature strongly affected *D. stramonium* germination responses. Site of origin also affected base temperatures of *D. stramonium* seed lots. The results of the present study suggest contrasting indications about transferability at European level of base temperature parameters for the two species.

## **Nomenclature**

Velvetleaf, *Abutilon theophrasti* Medik, ABUTH; jimsonweed, *Datura stramonium* L., DATST

## **Key Words:**

Germination, models, base temperature, population diversity

## **Introduction**

The European Union has recently published the Directive 2009/128/EC on the sustainable use of pesticides, with the objective of reducing the risks and impacts of pesticide use on human health and the environment by promoting the use of Integrated Pest Management (IPM) and alternative approaches or techniques (European Parliament 2009). Regarding Integrated Weed Management, weed emergence models may represent innovative tools to aid in achieving these ambitious objectives without reducing crop yields (Grundy 2003). Indeed, weed emergence models could provide indications about the correct timing for the application of post-emergence herbicide (Masin et al. 2005) which are normally characterized by better eco-toxicological profiles than pre-emergence herbicide due to the low application doses, short persistence and low toxicity (Sbriscia Fioretti et al. 1998; FOOTPRINT 2007). These models may also improve strategies of mechanical weed control, helping to coordinate seedbed preparation with cultivation (Leblanc and Cloutier 2002). Many weed emergence models used for applicative purpose are developed according to the hydrothermal approach (Gummerson 1986; Forcella 1998; Bradford 2002; Dorado et al. 2009b) which requires the estimation of laboratory-derived biological parameters, such as base temperature and base water potential for germination, for each species included in the model. Since these laboratory determinations are time- and money-consuming, the transferability of these parameters across different populations may represent one of the main constraints for the development and use at large scale of hydrothermal models (Grundy 2003). Several studies reported differences in germination ecology and in the biological parameters, for different populations (Del Monte and Tarquis 1997; Christal et al. 1998; Kremer and Lotz 1998; Allen and Meyer 2002; Taab and Andersson 2009) and even for seeds of the same population matured under contrasting environmental conditions (Magyar and Lukacs 2002) of the same population. However, these findings cannot be considered as a general rule since Grundy et al. (2003) reported evidence of synchrony in emergence timing for three populations of the same species. Therefore, different range of intra-specific variability in germination ecology may be supposed among different weed species. As a consequence, specific studies analyzing the effect of genetic and

environmental differences on germination ecology of different populations of the main weeds would be necessary for developing robust and transferable emergence models.

*Abutilon theophrasti* Medik (velvetleaf) and *Datura stramonium* L. (jimsonweed) are common and competitive annual weeds for summer crops worldwide. Their agronomic and economic impact on crop production in Europe has led to notable scientific interest on studying germination ecology of these species in order to develop sustainable management strategies. Velvetleaf seeds are characterized by a physical dormancy (Baskin and Baskin 1989) due to an impermeable seed coat that prevents imbibition and consequently germination (Winter 1960). When the seed coat becomes permeable and soil conditions are adequate, germination can proceed (Horowitz and Taylorson 1984). Jimsonweed seeds acquire physiological dormancy in the later ripening stages on mother plants (Benvenuti and Macchia 1997). Buried jimsonweed seeds show cyclic dormancy (Reisman-Berman et al. 1991). Jimsonweed germination is controlled by deep-sensing mechanism; germination is in fact promoted under certain condition of temperature, light and soil atmosphere which may be normally found only in the shallowest soil layers (Benvenuti and Macchia 1998). Several scientific works have been published about modeling of germination and emergence for the two species, focusing on determination of base temperature and base water potential for germination (Benvenuti and Macchia 1993; Sartorato and Pignatta, 2008; Dorado et al. 2009a; Masin et al. 2010a) as on field emergence modeling (Dorado et al. 2009b; Masin et al. 2010b). However, most of these studies have analyzed just one or two populations and few information are available about how intra-specific genetic variability or environmental differences of the site of cultivation could influence germination ecology of velvetleaf and jimsonweed. As a consequence, those developed models and estimated parameters should not be considered *a priori* as transferable to other environments with different conditions from the ones of the area of study.

In order to reduce these constrains a collaborative project has been carried out among three groups of researchers working in maize in different European countries (Italy, Portugal and Spain). The main objective of this study was to analyze the interactive effect of site of origin (genetic factor) and site of cultivation (environmental factor) on germination ecology of local populations of velvetleaf and jimsonweed. In particular, experiments were

performed to assess germination patterns as a function of temperature and to estimate specific base temperature for germination in each population.

## **Materials and Methods**

### **Initial seed collection**

Seeds were collected in summer 2008 from spontaneous populations in maize fields at three different locations (collection sites) in Italy, Portugal and Spain. As a consequence three initial seed lots were obtained for both species. Fruits were gently beaten in order to collect only fully ripened seeds which fell easily from plants.

Main environmental and agronomic characteristics of each collection site are described below.

Italy: (Legnaro, Padua 45° 20' N, 11° 58' E) The climate of Italian site is sub-humid. Legnaro has a mean annual rainfall of about 850 mm fairly uniformly distributed throughout the year. The area is characterized by 15.6 C of yearly mean temperatures, with temperature increases from January (average minimum value: 1.2 C) to August (average maximum value: 32.6 C). The soil is a loam soil (fulvi-calcaric Cambisol, FAO 2006). Maize cropping technique is quite uniform in this area: sowing takes place from mid March to mid April and harvest operations are normally concentrated between mid September to mid October. Consequently crop cycle lasts about 180 days and the adopted maize hybrids belong to FAO 500 or 600 classes.

Portugal: (Tapada da Ajuda, Lisbon 38°42'N, 9°11'W). The climate of Tapada is Mesomediterranean, with a mean annual rainfall of about 700 mm distributed among spring, autumn and winter with dry summers. The area is characterized by 16.6 C of yearly mean temperatures, with temperature increases from January (average minimum value: 8.0 C) to August (average maximum value: 28.0 C). The soil in the Portuguese site is a clay loam soil (Cambisol, FAO 2006). Maize sowing takes usually place from mid April to early

May, while harvest is carried out from mid September to early October, with an average crop cycle of 150-180 days. Farmers normally use FAO 600-700 class hybrids.

Spain: (Arganda del Rey, Madrid 40°19'N, 3°29'W). The climate of the Spanish site is Mediterranean Continental climate with cold winters, hot summers and very scant precipitation (about 400 mm). The mean annual temperature is 14.1 C; July is the hottest month (average maximum value: 33.0 C) while January is the coldest (average minimum value: 0.3 C). The local soil is a sandy loam soil (Eutric Fluvisol, FAO 2006). Maize is sown in the first half of April and is harvested in late October-early November, with an average crop cycle length of 200 days. The commonly adopted hybrids belong to FAO 700 class.

### **Seed production**

The three initial seed lots for both species were sown at all collection sites in autumn 2008. Thus, three different original populations of the same species were simultaneously grown at each cultivation site. These three populations were kept spatially separated to minimize cross- hybridization. After seedling emergence in spring 2009, plants were thinned to reduce competition. Plant management was similar among all collection sites: irrigation and fertilizer application were arranged in order to provide to plants environmental conditions comparable to the maize field situation.

Collections of the second seed lots (experimental seed lots) were carried out in summer-autumn 2009 with the above mentioned procedure (fruit beating).

Nine (3 initial seed lots × 3 cultivation sites) different experimental populations were obtained for both species. These populations were labeled with the code site of origin (the first three letters) - site of cultivation (the first letter), so the following combinations were obtained for both species: Ita-I (originally collected in Italy and then cultivated in Italy), Ita-P, Ita-S, Por-I, Por-P, Por-S, Spa-I, Spa-P, Spa-S. These denominations will be used in the proceeding of the text.

Air temperature and rainfall were monitored using data from the nearest local weather stations throughout the growing cycle at each cultivation site. Local monthly average minimum, medium and maximum temperatures and monthly rainfall were calculated for



the entire duration of the experiment in order to identify differences among cultivation sites related to environmental factors.

### **Germination test**

All the experimental populations were included in the germination test. Seeds were checked with an unimbibed seed crush test (Sawma and Mohler 2002) to assess their viability. Seeds considered as non viable were not included in germination tests.

Mechanical scarification treatment, called pricking, was performed on seeds for both species before germination test to remove physical barriers for seed imbibition without damaging seed embryos. This technique has been already adopted with good results for velvetleaf and for *Datura ferox* L. (Dorado et al. 2009a). Seeds were also surface sterilized by immersion in a 1% v/v sodium hypochlorite solution for 5 minutes. Preliminary studies demonstrated that this process prevents fungi contamination without affecting seed germination.

Seeds were placed on filter paper imbibed with deionized water into plastic Petri dishes sealed with parafilm. Petri dishes were then incubated in germination chambers in which constant temperature conditions were monitored every 20 min by using temperature sensors linked to data loggers<sup>1</sup>. Data were considered acceptable if the recorded temperature remains within the prescribed limit of  $\pm 0.5^{\circ}\text{C}$ . The Petri dishes were randomly placed within incubators, each incubator containing one complete set of treatments (i.e., 3 sites of origin  $\times$  3 sites of cultivation) with three replicates, i.e. three Petri dishes with 50 seeds for each treatment. Randomness was an important element of the experimental design since the statistical analyses used are based on the assumptions that the data were normally distributed and that the test was free from bias. Each species was studied with a specific set of germination tests according to their specific requirements of light and temperature. Velvetleaf seeds were incubated at a range of constant temperatures (8-10-13-15-18-22 C) and darkness, since this species does not require light to germinate (LaCroix and Staniforth 1964). Jimsonweed seeds were incubated at a different range on constant temperatures (10-12-14-16-18-20-24 C) with a photoperiod of 12/12 h (light/darkness). Benvenuti and Macchia (1993) reported that these conditions are suitable for jimsonweed germination.

Germination was recorded twice a day (velvetleaf at higher temperatures) or daily (velvetleaf at lower temperatures and jimsonweed) and germinated seeds were counted and removed. Seeds were defined as germinated when an emerging radicle was visible ( $> 1$  mm). Tests were stopped when no further germination occurred for 10 days. At the end of the experiment un-germinated seeds were checked with an imbibed seed crush test (Borza et al. 2007) and with a visual examination to assess their viability. Seeds considered as non viable were not taken into consideration during data analyses.

### **Data analysis and estimation of base temperature**

At the end of the experiment, average percentages of germinated seeds were calculated for each factor (site of origin; site of cultivation; incubating temperature) and their interactions. Percentage data were transformed by the arcsine of square root transformation to reduce non-normality of the dataset distribution (Gomez and Gomez 1984). This result was confirmed by data distribution analyses.

Factorial Anova ( $p=0.05$ ) was performed using General Linear Models module of Statistica 7.1<sup>2</sup> (StatSoft Inc. 2005) to analyze the effects of the above mentioned factors and their interactions on transformed germination data.

Post-hoc multiple comparisons were performed using Duncan's test ( $p=0.05$ ) to identify statically significant differences among means.

Base temperature determination was performed according to the approach by Rochè et al. (1997) with following modification by Masin et al. (2010a). The germination dynamic of each replicate was analyzed using a logistic function in the Bioassay97 program (Onofri 2001) as follows:

$$CG = 100 / (1 + \exp(a \cdot (\ln(t + 0.0000001) - \ln(b)))) \quad [1]$$

where  $CG$  is the percentage of cumulative germination,  $t$  is the time (days),  $a$  represents the slope of the curve, and  $b$  the inflexion point.

The germination rate was estimated as the reciprocal of the time necessary for the germination of half the germinated seeds ( $t_{50}$ ) by the end of the experiment. The  $t_{50}$  in the above equation corresponds to the inflexion point ( $b$ ).

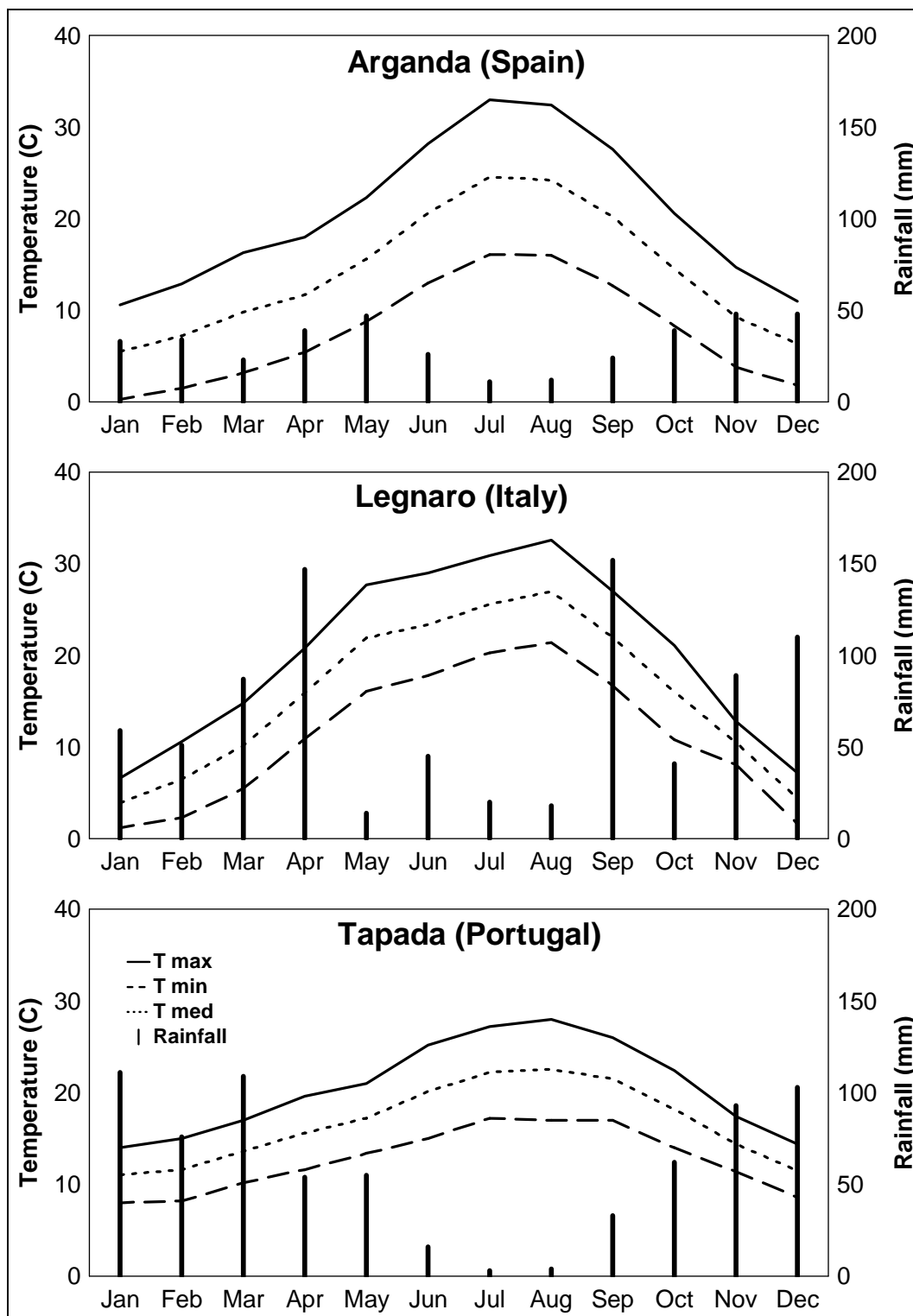
A linear regression was performed with germination rates of the three replicates against incubation temperature for each experimental population (site of origin  $\times$  site of cultivation). The base temperature of the experimental population was estimated as the intercept of the specific regression line with the temperature axis. Statistical confidence intervals for the base temperatures were estimated by a bootstrap method (Efron 1979) using an artificial re-sampling procedure. Five thousand artificial samples were taken from each population extracting randomly one of the three replication of each temperature. Five thousand datasets were created and the linear regression was estimated for each of these datasets determining the base temperature. The bootstrap distribution of the estimated base temperature was used to determine a 95% confidence interval.

As a consequence, nine different base temperatures, deriving from nine different experimental populations (3 sites of origin  $\times$  3 sites of cultivation), were estimated for both species. These values were then compared among themselves according to the criteria that if their respective confidence intervals are not overlapping, they will be considered as different

## **Results and Discussion**

### **Climatic conditions at the growing sites**

The comparison of climatic patterns at the three cultivation sites showed some interesting differences (Fig. 4.1). The Spanish cultivation site at Arganda was characterized by the largest thermal fluctuation, since it achieved the lowest average temperatures among the three sites during spring months and high temperatures during the summer.. Arganda presented also the largest difference between maximum and minimum monthly temperatures during all the growing cycle. On the contrary, the Portuguese site at Tapada da Ajuda was characterized by the most constant thermal pattern, with mild temperatures in spring and summer. The Italian site at Legnaro experienced temperature conditions more similar to those found in the Spanish site. However, Legnaro was characterized by the largest rainfall during the growing period (524 mm from March to October) while Arganda was the driest site (221 mm).



**Figure 4.1** - Average maximum temperature (T max), average temperature (T med), average minimum temperature (T min) and rainfall (Rain) at the three cultivation sites. Temperatures are expressed as C while rainfall as mm.

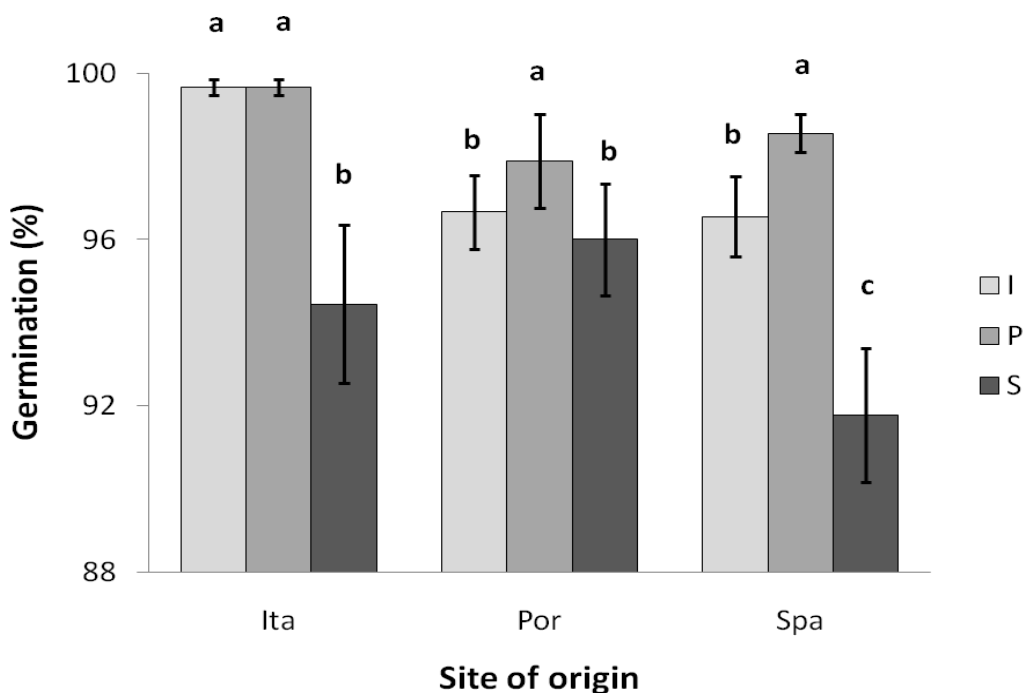
### Germination test

Factorial Anova ( $p=0.05$ ) identified significant effects of each factor (site of origin, site of cultivation, incubating temperature) and their interactions on germination of both species. All experimental populations of velvetleaf showed similar and high (above 90%) mean percentage of germination. The site of origin (genetic effect) was a factor that significantly affected mean germination percentages (Table 4.1). Indeed, seeds from Italy reached the highest germination values while seeds from Spain the lowest, with seeds from Portugal showing intermediate values. A similar behavior was found for site of cultivation (environmental effect) (Table 4.1), with populations cultivated in Spain reaching the lowest mean germination percentage while seeds cultivated in Portugal showed the highest germination values, with intermediate values observed in seed lots cultivated in Italy. Figure 4.2 shows the germination of velvetleaf from different combinations of site of origin and site of cultivation, with the highest values in seed lots Ita-I and Ita-P and the lowest values in Spa-S.

**Table 4.1** - Mean germination percentages (with standard errors) of velvetleaf (ABUTH) and jimsonweed (DATST) populations with different sites of origin and cultivation.

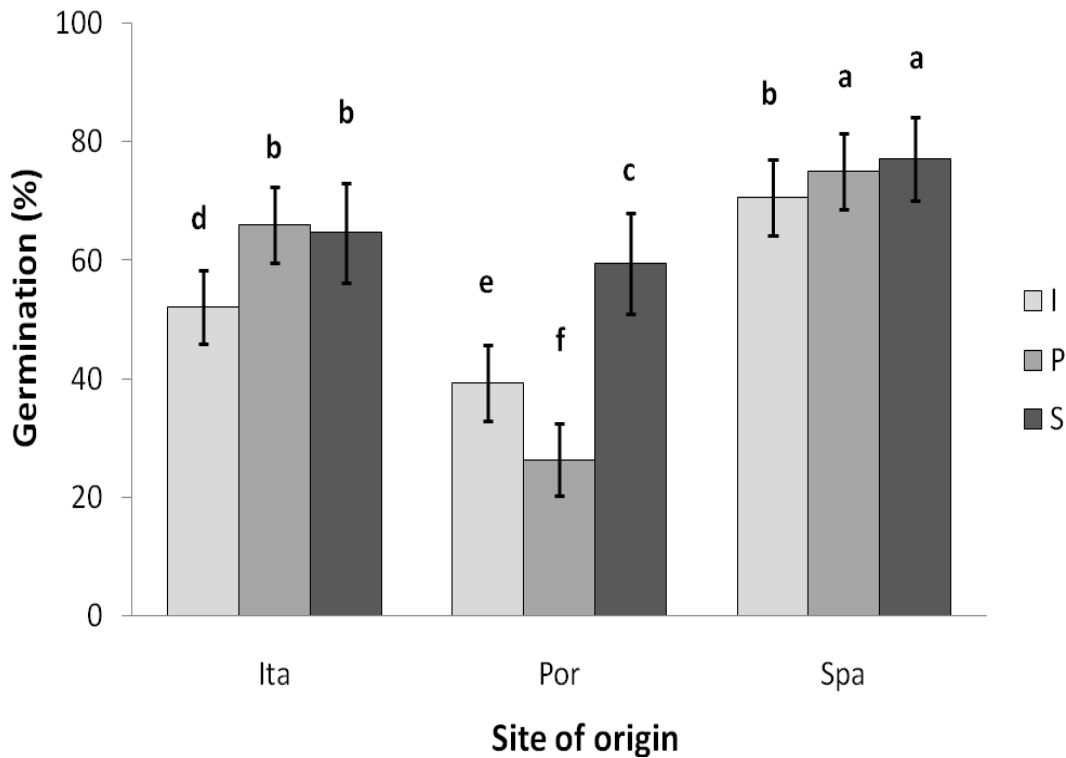
	<b>Legnaro</b>	<b>Tapada</b>	<b>Arganda</b>
	<b>(Italy)</b>	<b>(Portugal)</b>	<b>(Spain)</b>
<b>ABUTH</b>			
<b>Site of origin</b>	<b>97.9 ± 0.7a</b>	<b>96.9 ± 0.7 b</b>	<b>95.6 ± 0.7 c</b>
<b>Site of cultivation</b>	<b>97.6 ± 0.5 b</b>	<b>98.7 ± 0.4 a</b>	<b>94.1 ± 1.0 c</b>
<b>DATST</b>			
<b>Site of origin</b>	<b>60.9 ± 4.1 b</b>	<b>41.7 ± 4.3 c</b>	<b>74.3 ± 3.8 a</b>
<b>Site of cultivation</b>	<b>54.0 ± 3.9 b</b>	<b>55.8 ± 4.4 b</b>	<b>67.1 ± 4.6 a</b>

Letters identify significant differences among values of the same line according to Duncan's test ( $p=0.05$ )



**Figure 4.2** - Effect of the interaction site of origin (Ita, Por and Spa) \* site of cultivation (I, P and S) on mean germination percentages for velvetleaf. Vertical bars represent standard errors while letters identify significant differences according to Duncan’s test (p=0.05)

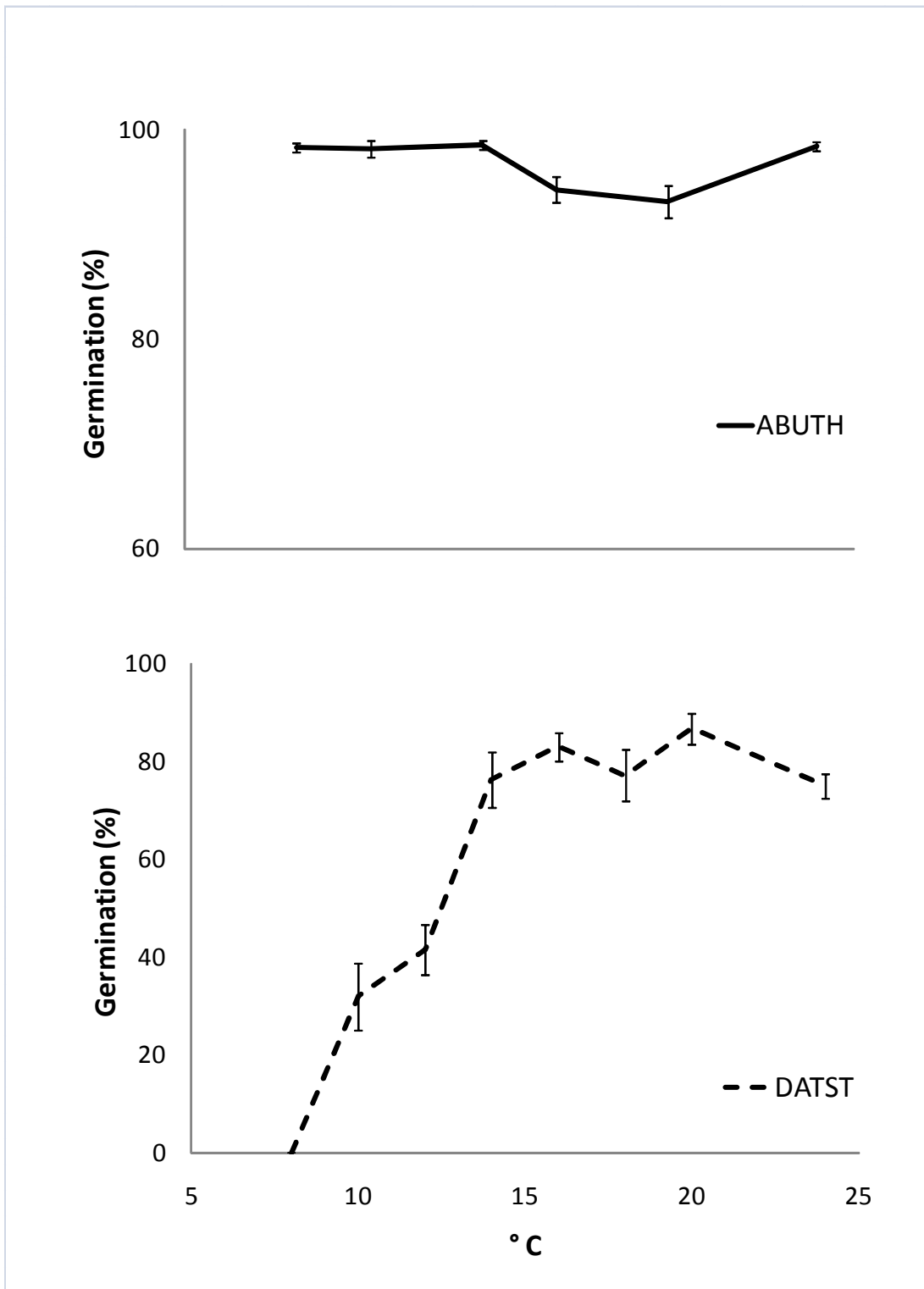
Significant differences were found in mean germination percentage of experimental populations of jimsonweed. As for velvetleaf, the site of origin significantly affected germination of jimsonweed (Table 4.1). Experimental populations with origin in Spain reached the highest mean germination percentage while the ones with origin in Portugal obtained the lowest value. Experimental populations with origin in Italy obtained an intermediate mean germination percentage. A narrower range of germination response was found for jimsonweed populations cultivated at different sites (Table 4.1): experimental populations cultivated in Spain obtained the highest mean germination percentage, with significant differences according to Duncan’s test (p=0.05) from the ones cultivated in Italy and Portugal, which achieved similar results. Figure 4.3 illustrates the germination of jimsonweed from different combinations of site of origin and site of cultivation, with population Spa-S showing the highest values while population Por-P the lowest.



**Figure 4.3** - Effect of the interaction site of origin (Ita, Por and Spa) \* site of cultivation (I, P and S) on mean germination percentages for jimsonweed. Vertical bars represent standard errors while letters identify significant differences according to Duncan's test ( $p=0.05$ )

Incubating temperature affected germination responses of the two species in a completely different way, being its effect significant for both species. Indeed, velvetleaf germination percentage was only slightly influenced by incubating temperatures (Fig. 4.4) and all experimental populations achieved values above 90% at all the temperatures. The lowest germination percentages were reached with the tests at 15 and 18 C, with a mean value above 90%.

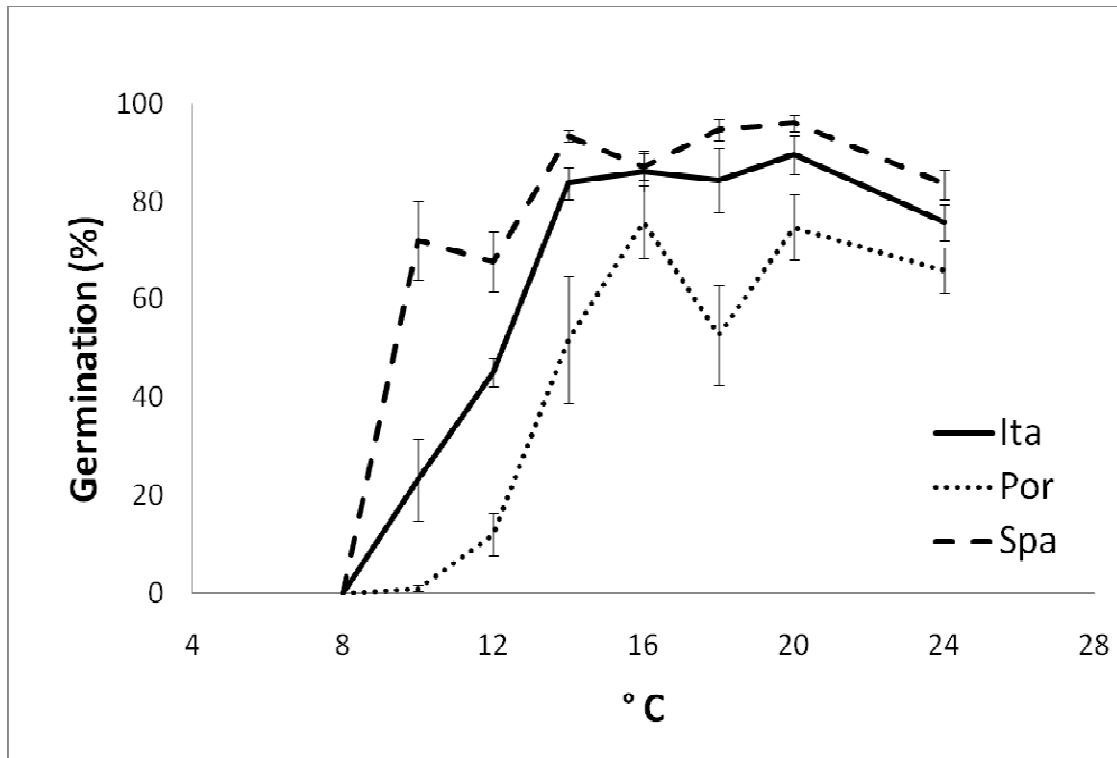
In contrast, jimsonweed germination percentage reached average values around 80% at high temperatures (16; 18; 20 and 24 C) but it was significantly reduced with incubating temperatures below 14 C and no germination occurred at 8C (Fig. 4.4).



**Figure 4.4** - Effect of incubating temperatures on mean germination percentages of velvetleaf and jimsonweed. Values represent mean of all populations. Vertical bars represent standard errors



Differences among germination percentage of different experimental populations were notably large at low temperatures (14; 12 and 10 C) (Fig. 4.5). According to the Factorial Anova ( $p=0.05$ ) performed, most of this variability was due to different behavior of experimental seed lots with different sites of origin.

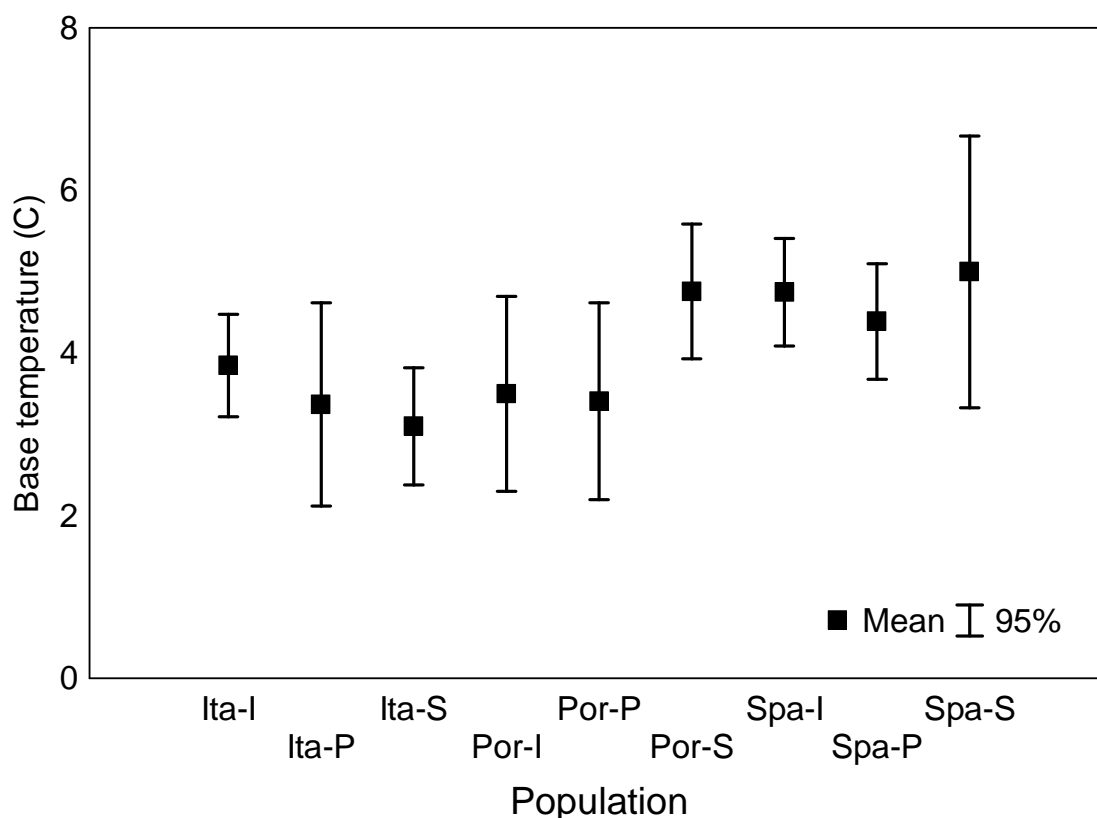


**Figure 4.5** - Effect of the interaction site of origin \* incubating temperature on jimsonweed germination percentages. Vertical bars represent standard errors.

At low incubating temperatures (14; 12 and 10 C), indeed, experimental populations with site of origin in Portugal achieved significantly lower germination percentages than the other ones (Fig. 4.5), while populations with site of origin in Spain showed the highest germination performances. The interaction between site of cultivation and incubating temperature was found significant by the performed Factorial ANOVA but no clear tendencies could have been identified due to large data variability (data not shown).

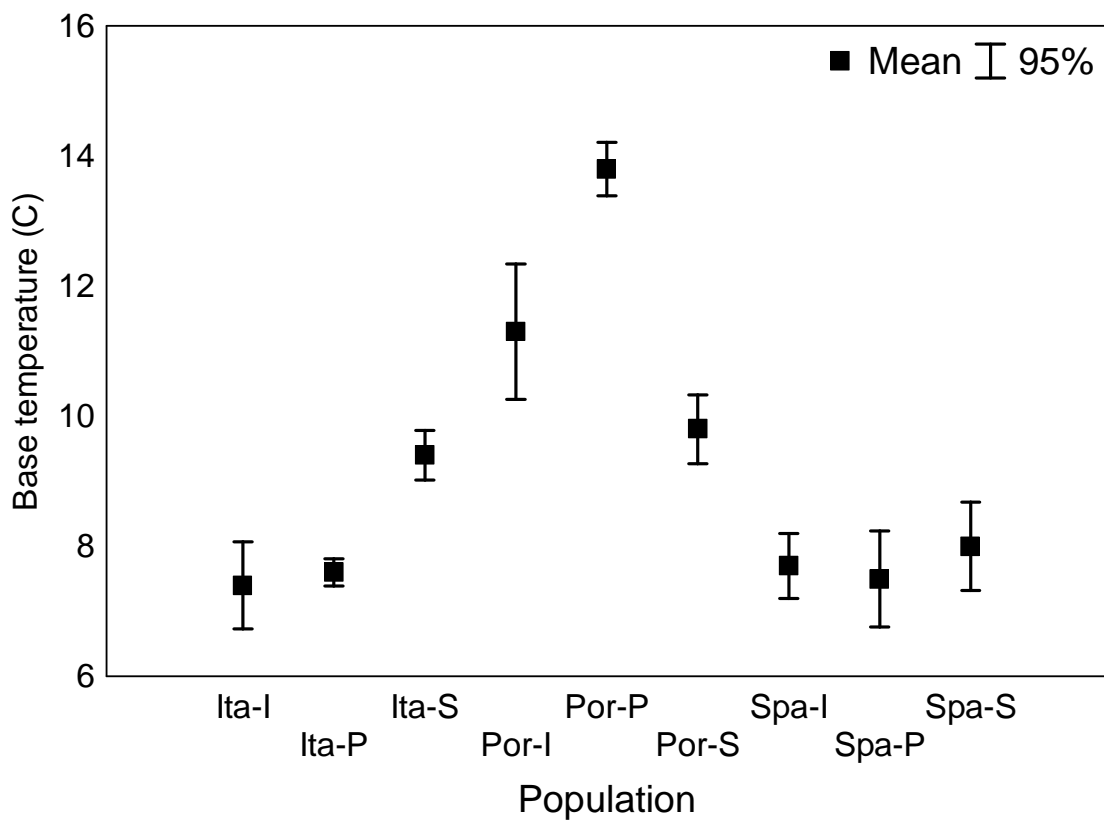
### Determination of base temperature

Estimated base temperatures of velvetleaf populations varied from  $3.1 \pm 0.7$  C for population Ita-S to  $5.0 \pm 1.7$  C for the Spa-S one (Fig. 4.6). The population with origin in Spain was characterized by highest base temperatures at all the cultivation sites, but these differences were non-significant according to the criteria of the overlap of confidence intervals (Fig. 4.6). Thus, no clear patterns could be identified among different sites of cultivation.



**Figure 4.6** - Base temperatures of velvetleaf experimental populations. Vertical bars represent confidence intervals (0.95)

On the contrary, significant differences in base temperature of jimsonweed were found as a function of the site of origin (Fig. 4.7). Experimental population of the Portuguese population (Por-I, Por-P, Por-S) showed significantly higher base temperature than all the other population apart from the Ita-S one. The experimental population Ita-I presented the lowest base temperature ( $7.4 \pm 0.7$  C), while the highest one ( $13.8 \pm 0.4$  C) was achieved by the experimental population Por-P. Similar to velvetleaf, no notable tendencies were found based on site of cultivation.



**Figure 4.7** - Base temperatures of jimsonweed populations. Vertical bars represent confidence intervals (0.95)

Germination response of velvetleaf seeds were found significantly different, as a function of site of origin and site of cultivation (genetic and environmental factor, respectively). Nevertheless, these differences could be considered as not relevant under a practical point of view, since base temperatures did not significantly vary among the populations with different site of origin or site of cultivation. The threshold values estimated in this study were also comparable with the ones determined by Masin et al (2010) for two other Italian populations. This finding is interesting because velvetleaf is known to exhibit polyploidy and high levels of population differentiation (Warwick 1990; Warwick and Black 1986). In addition, several authors reported variability for shade-avoidance responses (Weinig 2000), seed size and dormancy (Nurse and DiTommaso 2005) among populations grown under contrasting environmental conditions. Besides, different field emergence patterns were reported for the Portuguese and Spanish velvetleaf populations studied in this experiment (Dorado et al 2009b). This different behavior seems to be more related to the climatic differences during seed development and ripening (maternal effect) or during winter seed burial (dormancy loss) than to pre-existing genetic differences among the populations, since similar base temperatures were estimated for them.

On the contrary, jimsonweed germination behavior was strongly affected by site of origin of populations (genetic factor). Portuguese populations, even if grown at different sites of cultivation, showed lower germination percentages specially at temperatures below 14 °C. As a consequence, base temperatures for the seed lots with Portuguese origin were notably higher than the others. It should be underlined that the Portuguese site of origin is characterized by warmer temperature than the Spanish and Italian ones during autumn months when seed ripening takes place and during spring months when field germination occurs (Fig. 4.1). Therefore, a correlation could be hypothesized between base temperature of different jimsonweed populations and temperatures of their environments of origin. Similar trend was recently reported for *Chenopodium album* (Murdoch et al 2010): populations grown at southern latitudes in warmer environments were found more dormant than other populations from northern latitudes characterized by colder temperatures.

Finally, environmental differences of the site of cultivation (environmental factor) seemed to not affect germination responses and base temperatures of both species with clear, constant patterns. It is not ruled out that this result is due to the necessity of these species of

a period longer than one growing season to adapt their germination ecology to a new environment.

According to the results of the present study, the same value of base temperature for germination could be adopted for the velvetleaf populations without reducing predictive accuracy of applicative emergence models. Research efforts might be directed toward comparing other ecological or phenological aspects of the different local populations. Environmental fluctuations during plant growing cycle and seed ripening seem to not affect velvetleaf base temperature. As a result, this parameter, once estimated, could be maintained to model germination-emergence of seeds produced in years with different climatic conditions.

On the other hand, the relevant differences among the base temperature estimated for the three jimsonweed populations stressed the necessity to determine specific biological parameters for a correct modeling of the Portuguese population.

Analyzing the possible correlation between the thermal conditions of the site of origin and the base temperature of the local jimsonweed population could represent a central issue in order to find a general rule useful for developing more transferable models for this species. As such, further detailed studies are required to investigate this phenomenon.

## Sources of materials

- <sup>1</sup> Pendant data logger HOBO UA-001-08, Onset Computer Corporation, Bourne, MA.  
<sup>2</sup> StatSoft Inc., 2300 East 14th Street, Tulsa, OK 74104. <http://www.statsoft.com>.

## Acknowledgements

Donato Loddo thanks the Foundation “Ingenere Aldo Gini” for founding his stay at the Instituto de Ciencias Agrarias of the CSIC, Madrid (Spain)

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## **CHAPTER V**

### **Effects of natural chilling on germination and emergence of green foxtail, johnsongrass and velvetleaf**

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## **Abstract**

Seed dormancy represents a crucial point of seed bank dynamics for weeds. Dormancy may influence timing and magnitude of weed seedling emergence in the field. Understanding seed dormancy may thus improve the estimation ability of a weed emergence model. Exposure to low winter soil temperatures (chilling) is a driving factor for dormancy release of summer weeds. Laboratory and field experiments were conducted to analyze the effect of chilling on seed germination and seedling emergence for green foxtail, johnsongrass and velvetleaf. Weed seeds were buried in the soil on three different dates to expose them to chilling treatment of different duration. The germination experiment involved several tests at constant temperatures, while seedling emergences were monitored with field trials. Significant differences and contrasting trends were found among germination responses of different species and seed treatments. Duration of chilling treatment influenced seedling emergence of the three species differently. Seedling percentage of velvetleaf was reduced by the long chilling treatment, while emergence dynamic was unaffected. Green foxtail and johnsongrass emergence was promoted by long chilling treatment. The findings of this study show the importance of including winter chilling effect in emergence models for green foxtail and johnsongrass. Whereas, studying how burial conditions affect velvetleaf seed decay, and consequently magnitude of seedling emergence, could be the central issue to improve emergence models for this species.

## **Nomenclature**

Green foxtail, *Setaria viridis* (L.) Beauv, SETVI; johnsongrass, *Sorghum halepense* (L.) Pers., SORHA; velvetleaf, *Abutilon theophrasti* Medik, ABUTH

## **Key Words**

dormancy, germination, emergence prediction, models

## **Introduction**

Seed dormancy is crucial for weed population dynamics in fields (Benech-Arnold et al. 2000), mainly because soil seed bank is the principal source of seedlings for weed species in temperate regions (Buhler 1999; Grundy and Mead 2000). Dormant seeds have an internal constraint that impedes their germination even if hydric, thermal and gaseous conditions are adequate (Benech-Arnold et al. 2000). Dormancy is not a qualitative (all-or-nothing) seed property and seed dormancy level may progressively vary from a minimum to a maximum point according to seasonal dynamics (Batlla et al. 2004). The level of seed dormancy influences the range of environmental conditions suitable for germination: seeds with a low level of dormancy are able to germinate under a wider range of environmental conditions than seeds with a high level of dormancy. For example, in many weed species, dormancy status influences minimum temperature and minimum water potential required for seed germination (Vegis 1964; Christensen et al. 1996; Batlla and Benech-Arnold 2004). In addition, dormancy affects seed sensitivity to environmental factors that may promote germination, such as light (Derkx and Karssen 1993), nitrate (Hillhorst 1990) and fluctuating temperatures (Benech-Arnold et al. 1990). Weed seedlings normally emerge when seed dormancy level is at its minimum (Probert 1992) and if soil temperature and water potential are above specific threshold values for germination (Batlla and Benech-Arnold 2007).

Understanding dormancy dynamics of a seed population is crucial in order to estimate field emergence timing and magnitude of annual weeds (Forcella et al. 2000; Grundy 2003). However, the complexity of environmental factors influencing dormancy and the difficulty in separating dormancy release and seed germination has hindered the spreading of specific studies (Grundy 2003). As a result, most weed emergence models do not consider dormancy or remain merely empirical under this aspect (Forcella 1998; Grundy and Mead 2000; Masin et al. 2005). Only a few emergence models include dormancy in their estimation process (Vleeshouwers and Kropff 2000; Colbach et al. 2002; Colbach et al. 2006). However, these models are focused on single or few species and require not easily

found inputs. Consequently, they are useful tools for scientific research, but are not suitable as weed management decision support systems for growers or advisors.

Weed emergence models used for applied purposes are often based on the thermal or hydrothermal time concept (Bradford 1995; Bradford 2002; Forcella 1998; Gummerson 1986). Hydrothermal models are based on laboratory-derived biological parameters, such as base temperature and base water potential for germination, therefore, even if the dormancy process is not considered by the model, it is crucial to investigate the influence of seed dormancy level on those parameters. However, there are no universally recognized indications about how to deal with dormancy level of the materials used for those laboratory determinations. Consequently, some authors used seeds stored in cold and wet conditions for laboratory experiments (Benvenuti and Macchia 1993; Kochy and Tielborger 2007) in order to relieve seed dormancy through a chilling treatment, while other authors stored at room temperature in dry conditions (Sartorato and Pignatta 2008; Masin et al. 2005; Masin et al. 2010). These differences in experimental protocol may lead to the estimation of different values for the same parameters in the same species, with a consequent reduction in the predictive accuracy of models.

Several studies are available on the qualitative effects of dormancy dynamics on seed germination and weed emergence (Stoller and Wax 1974; Baskin and Baskin 1990; Baskin et al. 1996; Cardina and Sparrow 1997; Mennan 2003; Taab and Andersson 2009a, b) but only a few of them tried to quantitatively analyze this phenomenon (Christensen et al. 1996; Batlla and Benech-Arnold 2003; Batlla and Benech-Arnold 2004; Dorado et al. 2009). Even for important summer weeds, such as green foxtail (*Setaria viridis* (L.) Beauv), johnsongrass (*Sorghum halepense* (L.) Pers) and velvetleaf (*Abutilon theophrasti* Medik), few data exist about the effect of dormancy level on seed germination (Cardina and Sparrow 1997; Dorado et al. 2009) or on timing and magnitude of field seedling emergence. Green foxtail and johnsongrass seeds are normally physiologically dormant when they are dispersed from the mother plants in autumn (Van den Born 1971; Taylorson and McWhorter 1969) and this condition is known as primary dormancy (Benech-Arnold et al. 2000; Batlla and Benech-Arnold 2007). During the winter months, dormancy is gradually reduced mostly due to the interaction of low soil temperature and high soil moisture (chilling), so seeds are able to germinate in the following spring when

environmental conditions become suitable (Van den Born 1971; Taylorson and McWhorter 1969). Anyway, in laboratory tests a good germination percentage has been achieved even with non-chilled seeds for both species by mechanically removing seed glumes (Masin et al. 2010). Velvetleaf is characterized by a physical dormancy (Baskin and Baskin 1989) due to an impermeable seed coat that prevents imbibition and consequently germination (Winter 1960). When the seed coat becomes permeable and soil conditions are adequate, germination can proceed (Horowitz and Taylorson 1984).

A better knowledge of dormancy relief process for green foxtail, johnsongrass and velvetleaf could indicate the suitable seed management for the determination of biological parameters (base temperature and base water potential for germination). This would facilitate the creation of a robust prediction model for seedling emergence of these species. Winter chilling conditions (temperature, length) might also affect green foxtail, johnsongrass and velvetleaf seed germination and field emergence differently, modifying temporal dynamics and magnitude of seedling flushes. For example, Grundy et al. (2003) reported a strong correlation between winter temperature and emergence magnitude for several *Chenopodium album* populations cultivated in locations with different winter conditions. This aspect should be included in an emergence model in order to maintain its predictive accuracy even in the presence of variable winter conditions in different years or locations.

Experiments were therefore conducted to investigate the effect of periods of natural chilling with different durations and seed treatments on i) green foxtail, johnsongrass and velvetleaf seedling emergence in the field (percentage of emerged seedlings and emergence dynamics) and ii) their seed germination (percentage of germinated seeds) at different temperatures. The final aim is to obtain information for each species on the correct seed management for the determination of base temperature and to use this information to evaluate the utility of including winter chilling effects in emergence prediction models.

## **Materials and Methods**

### **Seed collection**

Weed seeds were collected for all the species in autumn 2008 from spontaneous populations in maize fields at the Experimental Farm of Padova University in Legnaro (northeastern Italy, 45°12'N, 11°58'E, 6 m a.s.l.).

The inflorescences of mature plants were gently beaten in order to collect only mature seeds, which were then cleaned and checked to remove immature or damaged ones. Seeds were stored in dark paper bags at room temperature until they were used for experiment trials.

### **Field emergence experiment**

A field experiment was conducted to study the effect of chilling periods of different lengths on green foxtail, johnsongrass and velvetleaf seedling emergence. Seeds were sown at the Experimental Farm of Padova University in 5 cm-deep furrows. They were mixed with the substrate used to fill the furrows to give them a random vertical distribution in the top 5 cm of the soil profile. The substrate used was just local soil, collected from the surface layers. A preliminary test confirmed that no seeds of the studied species were naturally present in the substrate before use. Three different sowing dates were adopted in order to apply three chilling treatments of different length: 19<sup>th</sup> November 2008 (long chilling, T1), 29<sup>th</sup> January 2009 (short chilling, T2) and 3<sup>rd</sup> March 2009 (no-chilling, T3). The experimental layout was a randomized design with three 100-seed replicates for each treatment.

Mini data loggers<sup>1</sup> were installed in some furrows at a depth of 5 cm in order to monitor soil temperature. Daily rainfall was monitored at the ARPA (Regional Environmental Protection Agency) weather station located 500 meters from the experimental site.

From spring onwards, weed emergences were monitored, and seedlings counted and eliminated twice weekly. At the end of the experiment, emergence dynamics of each replicate was modeled using the logistic function (1) in the Bioassay97 program (Onofri 2001) from which the time of 50% relative emergence ( $t_{50}$ ) was estimated.  $t_{50}$  was expressed as number of days after 1<sup>st</sup> January 2009.

$$CE = 100 / (1 + \exp(a * (\ln(t + 0,0000001) - \ln(b)))) \quad (1)$$

where CE is the percentage of cumulated emergence, t is the time (days), a represents the slope of the curve, and b the inflexion point.

Average percentages of germinated seeds were calculated for each species, chilling treatment and their combinations. Factorial ANOVA ( $p=0.05$ ) was performed using General Linear Models module of Statistica 7.1 (StatSoft Inc. 2005) to analyze the effect of species, chilling duration and their interaction on percentage and  $t_{50}$  of total emerged seedlings. Post-hoc multiple comparisons were performed using Duncan's test ( $p=0.05$ ) for mean separation.

### **Germination experiment**

A germination experiment was arranged with four different seed treatments: long chilling period (C1); short chilling period (C2); no-chilling (C3) and mechanical scarification (Sca). For the chilling treatments seeds were placed in bags of metallic mesh and buried in the soil. The burial dates were the same as the autumn and winter sowing of the emergence experiment, so for treatment C1 seeds were buried on 19<sup>th</sup> November 2008, for C2 on 29<sup>th</sup> January 2009, and for C3 seeds were not buried. Bags were exhumed during the first week of March 2009 and chilled seeds were kept for one week at room temperature to allow them to dry before being used for the germination trials. Non-chilled seeds (C3) were stored in paper bags at room temperature until March 2009 when the germination trials started. For the mechanical scarification treatment (Sca) seeds were managed as for the C3 treatment until the day before the trials when already tested procedures were used to remove physical barriers to seed imbibition without damaging the seed embryos: velvetleaf seeds were rubbed with sandpaper to eliminate seed coat waxes (Leon and Owen 2003; Leon et al. 2004; Masin et al. 2010), while seed glumes of green foxtail and johnsongrass were manually removed (Masin et al. 2010).

Experiments were arranged according to a fully randomized design with three replicates of 100 seeds per treatment. The seeds were placed in Petri dishes on a plastic support covered by wet filter paper so that they were on the surface of the water but not immersed to prevent the risk of anoxic conditions (Masin et al. 2005). Petri dishes were lined with 50 ml of



deionized water and then incubated at a range of constant temperatures (9-12-15-18-21-24 C) and photoperiod of 12:12 h (light:dark) in germination chambers in which two mini data loggers<sup>1</sup> were placed to verify the actual incubating temperatures. Germination was recorded twice daily (higher temperatures) or daily (lower temperatures). Tests were considered complete when no further germination occurred for 10 days. The seeds were defined as germinated at the time of visible radicle emergence of more than 1 mm.

Average percentages of germinated seeds were calculated for each species, seed treatment, incubating temperature and their combination. Percentage data were transformed by the arcsine of square root transformation to reduce non-normality of the dataset distribution (Gomez and Gomez 1984). This result was confirmed by distribution analyses.

Factorial ANOVA ( $p=0.05$ ) was performed using the General Linear Models module of Statistica 7.1 (StatSoft Inc. 2005) to analyze the effects of species, seed treatments, incubation temperatures and their interactions on transformed germination data. Post-hoc multiple comparisons were performed using Duncan's test ( $p=0.05$ ) to identify statistically significant differences among means.

## Results and Discussions

### Climatic conditions

January was the coldest month (Table 5.1) with an average daily soil temperature of 3.2 C (min 0.4 C; max 7.3 C), while July was the hottest month during the experiment with an average daily soil temperature of 28.7 C (min 24.2 C; max 31.7 C). Total rainfall (Nov-Jul) was 736 mm and the rainiest period was autumnal (Nov-Dec around 200 mm) while the driest period was May with 25.8 mm of rain.

**Table 5.1** - Monthly minimum, average and maximum soil temperature and rainfall

Period	Mean daily soil temperature (C)			Rain (mm)
	Minimum	Average	Maximum	
Nov-Dec	0.4	4.6	8.4	199.6
Jan	0.4	3.2	7.3	57.2
Feb	2.2	5.3	10.0	57.2
Mar	7.2	9.7	13.0	104.6
Apr	12.6	16.0	19.0	126.0
May	17.5	23.6	29.9	25.8
Jun	20.8	25.8	30.5	86.6
Jul	24.2	28.7	31.7	79.4

### Field emergence experiment

Factorial ANOVA identified significant effects of species, chilling duration and their interaction on percentage and  $t_{50}$  of total emerged seedlings.

*Emergence percentage.* Regarding the variable “species”, velvetleaf had the highest percentage of emerged seedlings among species, while johnsongrass showed the lowest one (Table 5.2). Regarding the variable “chilling duration”, the short chilling treatment (T2) achieved the largest mean percentage of emerged seedlings and the non-chilled treatment the smallest (Table 5.2). Anyway, each species showed a different behavior, as clearly reported in Fig. 5.1.

**Table 5.2** - Mean emergence percentage and  $t_{50}$  (expressed as days after 1<sup>st</sup> January) in relation to different length of chilling period.

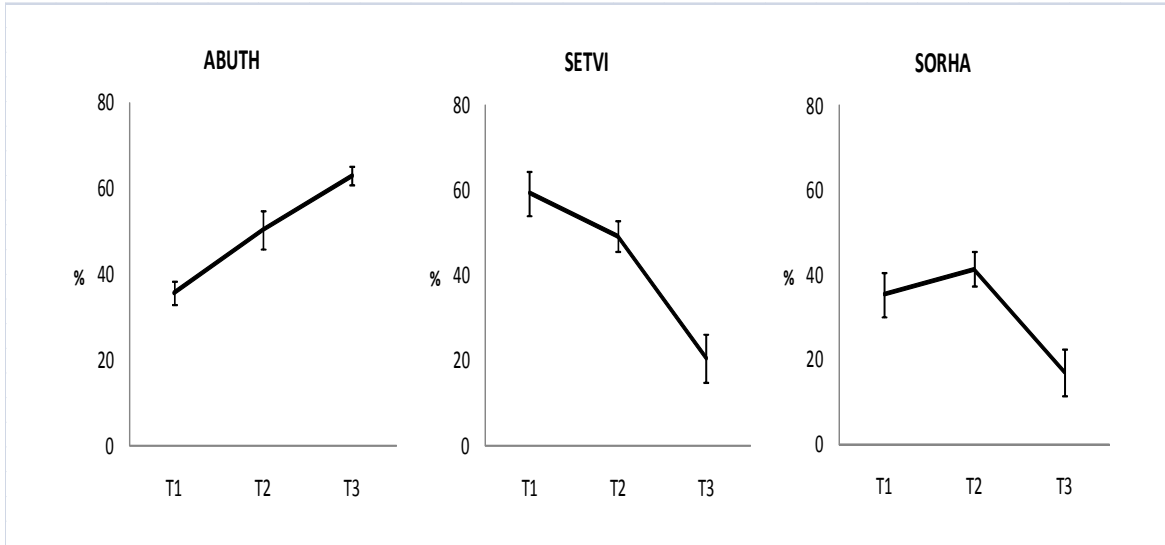
Main Factor	% Emerged seedling	$t_{50}$ (days)
<b>"Species"</b>		
<b>ABUTH</b>	<b>49.7 a</b>	<b>90.7 c</b>
<b>SETVI</b>	<b>43.1 a</b>	<b>118.4 a</b>
<b>SORHA</b>	<b>31.2 b</b>	<b>106.8 b</b>
<b>"Chilling duration"</b>		
<b>T1</b>	<b>43.4 a</b>	<b>96.3 a</b>
<b>T2</b>	<b>47.0 a</b>	<b>101.5 b</b>
<b>T3</b>	<b>33.6 b</b>	<b>118.2 c</b>

Letters identify significant differences according to Duncan's test ( $p=0.05$ ) among means of different treatments of the two main factors.

Analyzing the interaction of "species \* chilling duration" on the percentage of emerged seedlings for the three species, interesting differences were found (Fig. 5.1). Velvetleaf emerged seedling percentage diminished progressively from the non-chilled T3 to the long-chilled T1 while, on the contrary, green foxtail emerged seedling percentage clearly increased from the non-chilled T3 to the long-chilled T1. Johnsongrass followed a similar pattern to green foxtail, with a higher percentage of emerged seedlings for the chilled treatments T1 and T2 than for the non-chilled T3.

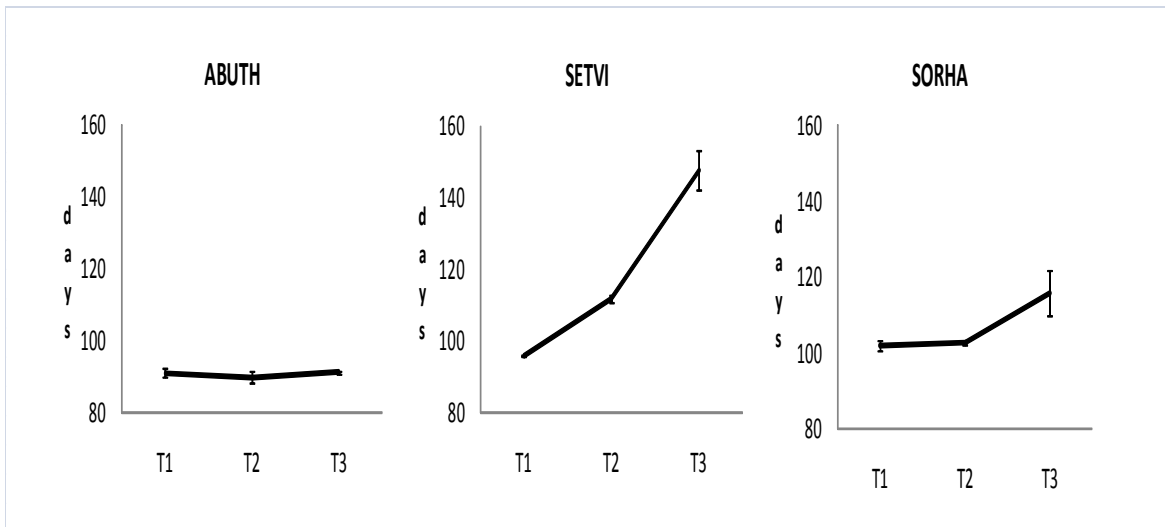
*Emergence  $t_{50}$ .* Regarding the variable "species", velvetleaf was the earliest emerging species while green foxtail was the latest one (Table 5.2). Regarding the variable "chilling duration", long chilling treatment (T1) achieved the lowest mean value at around 7-8<sup>th</sup> April (Table 5.2). On the contrary, non-chilled treatment (T3) showed the highest mean value, with a  $t_{50}$  around 30<sup>th</sup> April.

The different behavior between velvetleaf and the other two species was also observed in the interaction of "species \* chilling duration" on emergence dynamics (Fig 5.2). Winter chilling did not affect velvetleaf emergence dynamics and  $t_{50}$  was similar for the three treatments, taking place about 1<sup>st</sup>-2<sup>nd</sup> April. In contrast, green foxtail emergence dynamics was notably influenced by winter chilling duration, with about two weeks of delay between



**Figure 5.1** - Interaction “species \* chilling duration” on mean percentage of emerged seedlings. Vertical bars represent standard errors.

$t_{50}$  of T1 (6<sup>th</sup> April) and T2 (22<sup>nd</sup> April) and more than one month between the latter and T3 (29<sup>th</sup> May). Johnsongrass showed an intermediate behavior, with similar dates for  $t_{50}$  for the chilled treatments (T1 on 13<sup>th</sup> April, T2 on 14<sup>th</sup> April), while the non-chilled T3 was later ( $t_{50}$  on 27<sup>th</sup> April).



**Figure 5.2** - Interaction “species \* chilling duration” on emergence  $t_{50}$ . Vertical bars represent standard errors.

### Germination experiment

Factorial ANOVA identified a highly significant effect of species, seed treatment, incubation temperature and their interaction on transformed data of germination percentage. Regarding the variable “species”, green foxtail obtained the highest germination response, while johnsongrass showed the lowest (Table 5.3). Regarding the variable “seed treatment”, Sca treatment (scarified seeds) achieved the highest mean percentage of germinated seeds, while the non-chilled treatment (C3) was characterized by the lowest one (Table 5.3).

Analyzing the interaction of “species \* seed treatment”, it was noticed that the species showed different behaviors (Table 5.3). The lowest total mean percentage of germinated seeds was achieved with the treatment C3 (no-chilling) for all the species (less than 4%). On the contrary, the highest mean percentage was obtained with the Sca treatment for velvetleaf and Johnsongrass (even if the percentage was in any case very low for the latter species) and with the treatment C1 for green foxtail. Treatment C2 gave intermediate results for all the species, anyway, johnsongrass mean germination percentage remained around 3%.

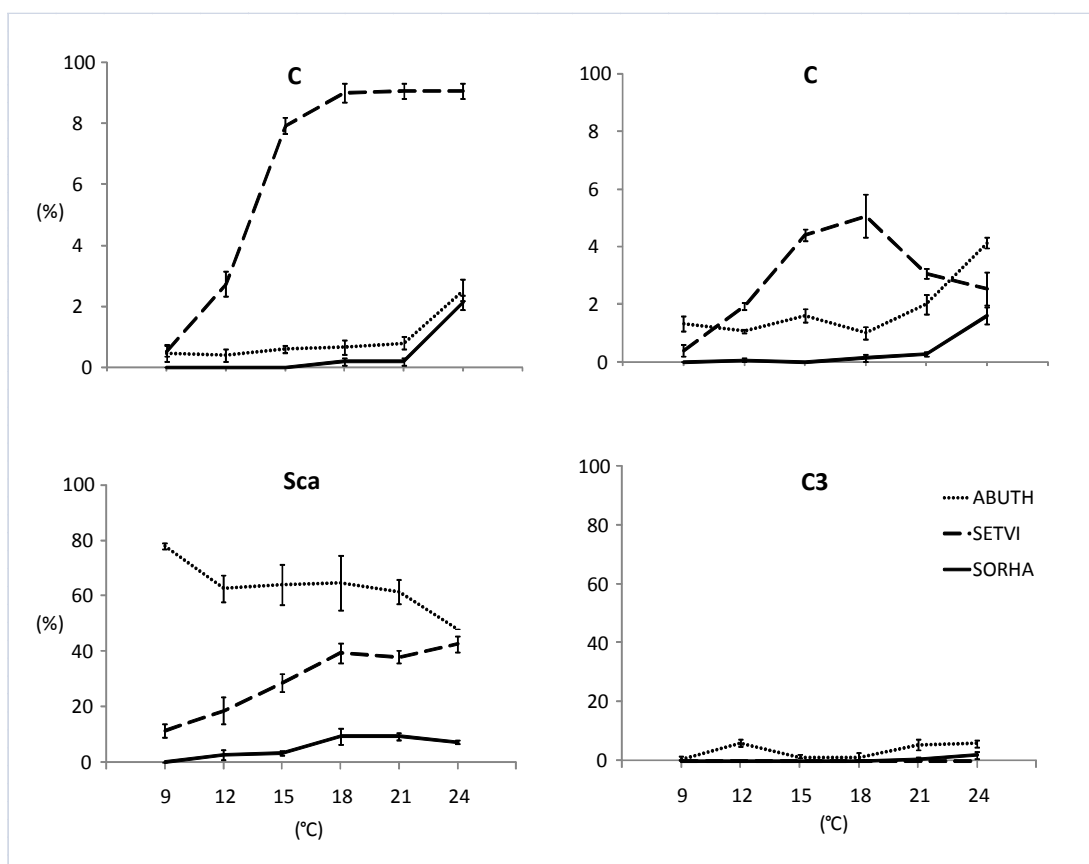
**Table 5.3** - Germination percentage of different seed treatments and species as a mean of the different incubating temperatures.

	<b>ABUTH</b>	<b>SETVI</b>	<b>SORHA</b>	<b>Mean</b>
<b>C1</b>	9.1	63.9	4.2	<b>25.7 b</b>
<b>C2</b>	18.6	29.0	3.4	<b>17.0 c</b>
<b>C3</b>	3.4	0.0	0.4	<b>1.3 d</b>
<b>Sca</b>	63.1	29.8	5.3	<b>32.7 a</b>
<b>Mean</b>	<b>23.6 b</b>	<b>30.7 a</b>	<b>3.4 c</b>	

Letters identify significant differences according to Duncan’s test (p=0.05) among means of different species or seed treatments.

Analyzing the interaction of “species \* seed treatment \* incubation temperature” some interesting patterns can be detected (Fig. 5.3). For velvetleaf high temperatures increased seed germination percentages for treatments C2 and C1, which reached their maximum at 24 C (41.3 and 25.3%, respectively). On the contrary high temperatures reduced

germination of scarified seeds (Sca treatment), which achieved their highest germination percentage at 9 C (78.0%). Green foxtail germination was promoted by temperatures above 12 C for treatment C1 (with a maximum of 90.7% at 21 and 24 C) and for treatment Sca (with a maximum of 42.6% at 24 C), while treatment C2 achieved the maximum percentage of germinated seeds at 18 C (50.6%) and then decreased at higher temperatures. Johnsongrass germination was significantly higher at 24 C for treatments C1 and C2 (21.3 and 16.0%, respectively), while the Sca treatment showed the maximum percentage of germinated seeds at 18 and 21 C (9.3% for both). All the remaining johnsongrass treatments achieved lower germination percentage. For treatment C3 germination percentages were so low for all three species (less than 6%) that no clear relations with incubation temperatures could be identified.

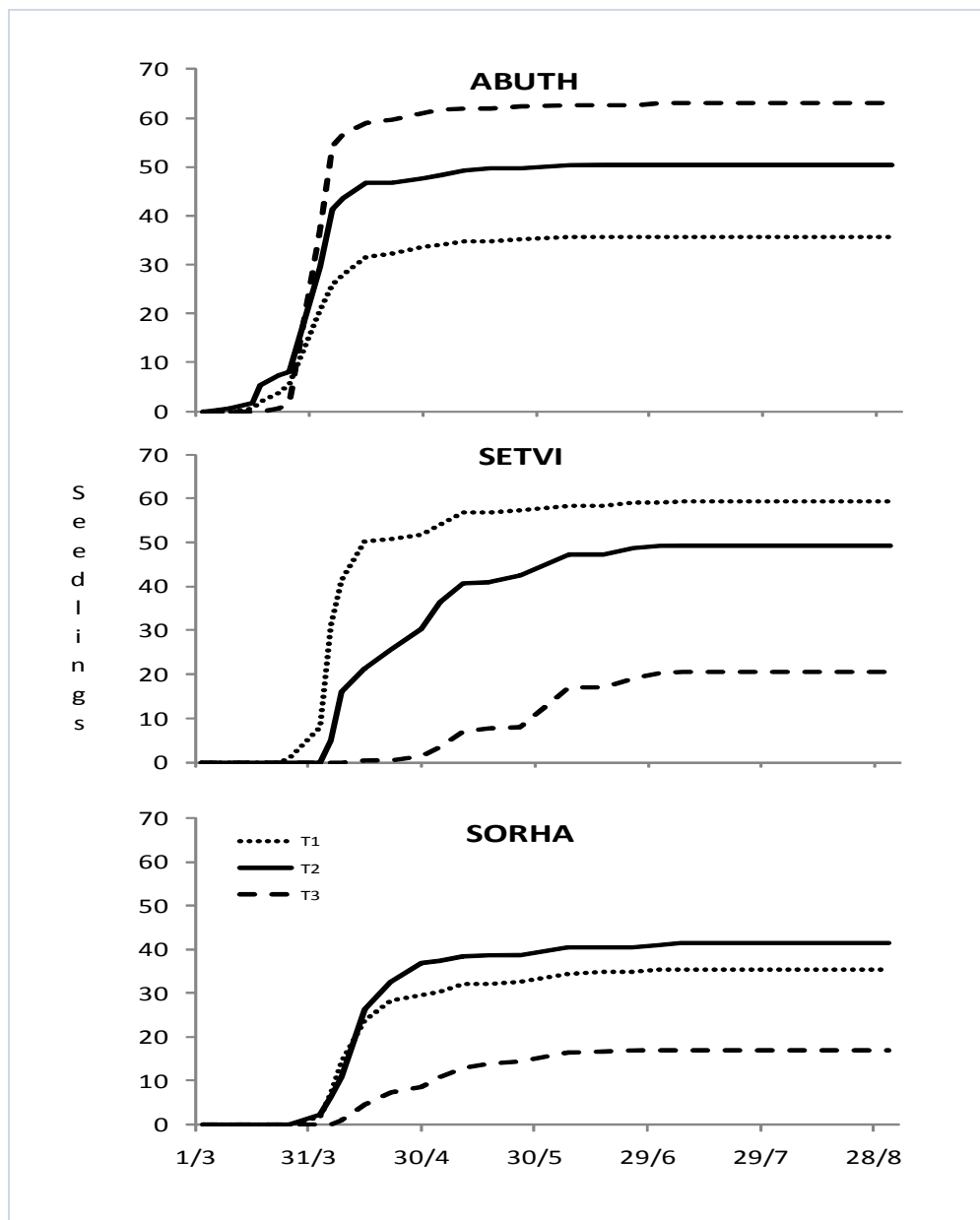


**Figure 5.3** – Mean germination percentage of different seed treatments at different incubation temperatures. Vertical bars represent standard errors.

Comparing results from the two experiments, clear differences may be identified between the effect of winter chilling on velvetleaf and on the other two species. Winter chilling reduced velvetleaf emerged seedling percentage (Fig. 5.4), probably because winter soil conditions and the presence of plant pathogens caused a certain percentage of seed decay and death. In support of this hypothesis, Davis and Renner (2007) reported the influence of *Pythium ultimum*, a soilborne pathogen, on velvetleaf fatal germination and seed death in soil during winter. Secondary embryo dormancy has never been reported for velvetleaf, and Cardina and Sparrow (1997) found that seeds exposed to different chilling periods were able to achieve close to 100% germination after a mechanical scarification. On the contrary, different chilling treatments did not affect velvetleaf timing of emergence (Fig. 5.4), consequently chilling does not seem to modify the range of temperature and water potential suitable for seed germination, as already stated by Dorado et al. (2009). Physical dormancy breaking required few days, since even the spring sown treatment achieved a good emergence percentage within just one month after sowing. It may be supposed that the decomposing biochemical reactions, which affect seed coat due to the contact with soil during seed burial, benefit from warm and wet soil conditions. This theory could be confirmed by the findings of Cardina and Sparrow (1997), who reported a more rapid decrease of velvetleaf seed dormancy during autumnal burial than winter. This might also explain the poor germination percentages shown during laboratory experiments by chilled treatments (C1 and C2) whose seeds, which were buried in bags of metallic mesh, had less contact with soil particles. This situation could have hindered biochemical and physical reactions responsible for velvetleaf seed coat breaking and the consequent seed dormancy relief.

Winter chilling had a notable effect on green foxtail, modifying timing and magnitude of seedling emergence (Fig. 5.4), with the longer chilling period (treatment T1) determining earlier and higher emergence. This finding is confirmed by the results of Masin et al. (2006) who reported a high level of germinability for green foxtail seeds exposed to chilling by winter soil burial for between 100 and 200 days. Long-chilled seeds were also found to be more sensitive to temperature increase, as revealed by C1 performances during the germination tests. Non-chilled treatments achieved a certain percentage of field emergence but their germination percentages were very low during laboratory tests

probably because seed glumes prevented seed imbibition. Indeed, non-chilled scarified seeds (Sca treatment) were able to germinate, as reported by Masin et al.(2010), although their germination was slower than the chilled ones. In the field emergence experiment, non-chilled seed (T3) glumes might be degraded during spring burial due to soil microbial action.



**Figure 5.4** – Mean emergence percentages and dynamics of velvetleaf, green foxtail and johnsongrass with different chilling treatments



Johnsongrass emergence was affected by winter chilling in a similar way to green foxtail, even if some differences can be identified (Fig. 5.4). For this species, emergence timing and magnitude of both chilled treatments (T2 and T1) were quite similar, so probably short chilling periods, such as T2 treatment, are sufficient to promote johnsongrass germination under field conditions. On the other hand, all seed treatments showed less than 10% germination at constant temperatures during laboratory tests, with the exception of the two tests at 24 °C for the chilled treatments C1 and C2 (21.3 and 16.0%, respectively). Benech-Arnold et al. (1990) reported that johnsongrass seeds, even when primary dormancy is relieved, required fluctuating temperatures to germinate. The findings of the present study stressed that chilled johnsongrass seeds still show a partial dormancy that hinders germination at constant temperatures lower than 24 °C. This behavior could be in part a consequence of the particular chilling conditions that seeds experienced. In fact, being enclosed in a metallic mesh bag, seeds had less contact with the surrounding soil. This could have limited the microbial and chemical degradation of johnsongrass seed glumes which act as a physical barrier for seed imbibition and germination. This theory seems to be supported by the fact that scarified non-chilled seeds reached higher germination percentage than the other treatments at most of the incubation temperatures in the laboratory experiment and by the findings of Masin et al. (2010), who achieved a certain level of germination at constant temperatures with scarified seeds. Therefore, johnsongrass seed dormancy seems to be relieved by the interaction of many factors, such as exposure to low temperatures or seed coat degradation due to soil microbial activity. However, variability in dormancy level and dormancy relief requirements among populations or years, due to genetic or environmental differences, cannot be excluded.

This study underlines the strong influence of winter chilling duration on green foxtail and johnsongrass field germination and consequently on timing and magnitude of their seedling emergence (Fig. 5.4). These results raise very important questions for emergence model development. Hydrothermal time models frequently use threshold parameters (base temperature and base water potential) estimated from laboratory experiments. Considering the results obtained in this study, it is important to wonder whether seed dormancy could lead to estimating incorrect threshold values and whether, in order to obtain correct estimations, seeds should be exposed to chilling treatment before being used in laboratory

tests. This observation is in agreement with the findings of Dorado et al. (2009) in their study on *Datura ferox*, which showed a different germination pattern and a different base water potential between chilled and non-chilled seeds.

Another important question should be introduced about weed emergence prediction. The question is Whether the inclusion of winter chilling effect in predictive models could improve the accuracy of the simulation and their transferability to environments characterized by different climatic conditions. Chilling effect on green foxtail and johnsongrass dormancy relief could be quantified and modeled using a hydrothermal approach as proposed by Bradford (2002) and as already successfully used to model dormancy loss in *Bromus tectorum* (Bauer et al. 1998) and *Elymus elymoides* (Meyer et al. 2000). Other authors instead decided to adopt a thermal approach to model dormancy changes in soil seed bank (Vleeshouwers and Kropff 2000; Vleeshouwers and Bouwmeester 2001; Batlla and Benech-Arnold 2003). Both these approaches are based on the accumulation of degree days, which Batlla and Benech-Arnold (2007) defined as stratification thermal time, below a specific threshold temperature. This threshold represents the higher limit below which seed dormancy is lost. The hydrothermal models also take into account soil moisture, i.e. the accumulation of degree days takes place only if soil water potential is above a specific threshold, which could be defined as base water potential for dormancy relief. As a consequence, further studies are required to estimate specific threshold values (minimum/maximum temperature and base water potential for dormancy relief) for green foxtail and johnsongrass in order to proceed with the consequent seed dormancy relief modeling.

On the contrary, winter chilling duration has no effect on velvetleaf dormancy nor on base temperature for germination, so dry storage at room temperature and mechanical scarification seems to be the best seed management for laboratory threshold determination. Winter chilling duration has no effect on velvetleaf emergence timing but percentage of emerged seedlings was clearly reduced by the long chilling treatment (Fig. 5.4). Therefore, studying how winter burial conditions (soil temperature and moisture; burial depth) affect seed decay, and consequently magnitude of seedling emergence, could be the central issue to improve velvetleaf emergence models.

## Sources of materials

<sup>3</sup> Pendant data logger HOBO UA-001-08, Onset Computer Corporation, Bourne, MA.

<sup>4</sup> StatSoft Inc., 2300 East 14th Street, Tulsa, OK 74104. <http://www.statsoft.com>.

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## GENERAL CONCLUSIONS

Analyzing the findings of the experiments presented in the different chapters of this thesis, some general conclusions may be summarized:

AlertInf can be considered an easy, user-friendly model for weed emergence prediction in maize fields; it requires inputs that are easy to measure (or even to estimate) and provides clear, immediately usable outputs. It is also a robust model, since the parameterization and validation process was carried out with several field trials in different years and locations, under various environmental conditions. AlertInf is a transferable, adaptable model: weed populations in the two extreme Italian maize cropping areas (Veneto and Tuscany regions) presented similar biological parameters ( $T_b$  and  $\Psi_b$ ). As a consequence, the model created with datasets from Veneto region demonstrated a very good predictive accuracy even when it was applied in Tuscany. These findings support the hypothesis that a single general model may be adopted to predict emergence of the studied species (common lambsquarters, johnsongrass and velvetleaf) in maize fields in Italy, while further studies are required to evaluate model transferability at European level or for other weed species. AlertInf could be improved by increasing the number of weed species included or by calibrating the model on other spring crops (sugar beet, soybean, sunflower).

The AlertInf/Gestinf combination, called Gestinf Plus, may be a useful, versatile tool for Integrated Weed Management in maize. Indeed, Gestinf Plus is able to estimate weed emergence dynamics and weed-crop competition according to environmental trends, so it could provide information about timing and cost effectiveness of weed control measures. Consequently, more efficient and sustainable weed control strategies could be adopted in order to obtain lower yield losses due to weed competition, a more rational herbicide use and reduced environmental impact of maize cropping system.

Local European populations of some weeds may have different base temperatures for germination. In the studied species (jimsonweed) this behavior seems to be connected to genetic differences among populations. This could be a consequence of adaptation processes to the local environmental conditions, such as average soil temperature in spring. Specific studies are therefore required to evaluate transferability at European level of



biological parameters and predictive models among environments with contrasting characteristics.

Environmental control of seed dormancy is a key factor for seed bank dynamics and seedling emergence for many weeds. However, the introduction of dormancy dynamics in weed emergence models is hindered by the variable effects of environmental factors on weed germination and emergence. For example, winter chilling duration promotes higher and earlier seedling emergence for some spring-summer weeds (such as green foxtail and johnsongrass), but shows restrictive effects for other species (such as velvetleaf). Including this aspect in weed emergence models could improve their predictive accuracy but further experiments are necessary to investigate specific weed behaviors.

## **RINGRAZIAMENTI**

Giunto alla fine del dottorato di ricerca mi sembra doveroso tracciare un bilancio di questo periodo appena concluso: dopo tre anni di mia permanenza nel nebbioso Veneto mi ritrovo con un insperato bagaglio di esperienze, conoscenze e ricordi ma soprattutto con un grande debito di riconoscenza nei confronti delle molte persone che ho avuto la fortuna di incontrare lungo il cammino. È con molto piacere quindi che esprimo i miei profondi ringraziamenti e il mio affetto a tutti loro:

Al professor Giuseppe Zanin e alla dottoressa Roberta Masin, per avermi guidato, incoraggiato e sopportato in questi tre anni trascorsi lavorando insieme.

Ai colleghi del Imaf-CNR di Legnaro per aver condiviso con me tanti momenti di lavoro, di riflessione e di vita.

Al professor Cesar Fernández-Quintanilla, al dottor Jose Dorado e agli altri colleghi del CSIC di Madrid per avermi accolto nel loro laboratorio con calore e amicizia.

Al dottor Stefano Benvenuti dell'Università di Pisa per avermi introdotto nel “magico mondo” della ricerca scientifica.

A tutto il personale del Dipartimento di Agronomia Ambientale e Produzioni Vegetali dell'Università di Padova e dell'Azienda agraria “L. Toniolo” per avermi aiutato nel mio lavoro.

Infine alla mia famiglia e tutti i miei cari per il loro appoggio e affetto. Dedico in particolare questa mia tesi a mio nonno Giorgio e mio zio Claudio che hanno fatto nascere in me la passione per il bellissimo mondo dell'agricoltura.

Grazie a tutti!