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Study of germination parameters of summer weeds: transferability of AlertInf model to Croatia

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ABSTRACT

There is no universal definition of "weed" that is accepted by all scientists, but in almost all the definitions and descriptions of weeds the aim is to underline the negative aspects: interference with the crops, which causes damage due to loss of yield, deterioration of the product quality, reduction in the value of the land, etc. The fight against weeds has originated and developed with agriculture and is the most critical point in crop management. Weed-crop competition is the biggest problem for the farmers and must be as lower as possible for a good management and a good remuneration for the market. The weeds most competitive are species with high photosynthetic efficiency C4. In addition, weeds present strong system of survival, such as seed dormancy, that makes seeds to be able to germinate in different moment of the seasons and in the subsequent years. Cultural weed management techniques are important as crop rotations, choice of cultivar, crops establishment, plant spacing. Mechanical weed management is very efficient and versatile in controlling weeds in a variety of cropping systems. The chemical control has been an important success in terms of the additional food production but it presents many negative problems as the environmental persistence. It is possible to improve and combine all these type of managements using models that predict weed emergence. AlertInf is one of these model, it has been developed at Padova University and it is able to predict the emergence of the main weeds in maize for the Veneto region. The information provided by AlertInf is the percentage of weeds that have already emerged on the total number of plants that can potentially emerge until the end of the season. The aim of this thesis is part of a study on the transferability of the model AlertInf from Italy to Croatia through a comparison of germination-emergence characteristics between Italian and Croatian ecotypes. In particular, the parameter estimate in this thesis is the base temperature, the temperature below which the germination process stops. The species studied are: *Abutilon theophrasti*, *Amaranthus retroflexus*, *Chenopodium album* and *Echinochloa crus-galli*. The temperature threshold resulted not statistically different between Croatian and Italian ecotypes of *Abutilon theophrasti* and *Chenopodium album*. Differently for *Echinochloa crus-galli* that showed a lower base temperature in Croatia, confirmed the tendency of this species to develop ecotypes with a thermal response that is related to the climate of origin, the cooler is the climate and the lower is the base temperature of the ecotype. Unfortunately for *Amaranthus retroflexus* the germination data were not sufficient for the statistical analysis. The finding of this thesis are very useful to understand the transferability of the model AlertInf. Some species showed similar thresholds for different ecotypes and it means the possibility to use the base temperature estimated in a location to another without further experiments. Other species, such as *Echinochloa crus-galli*, require necessarily the estimation of the specific thresholds. Knowing the behavior of the species is very useful to decide correctly whether it is necessary to

conduct specific experiments or it is possible to avoid doing them and use the same parameters estimated in other geographical locations, saving time and resources.

RIASSUNTO

Non esiste una definizione universale di "malerba" che sia accettata da tutti gli scienziati, ma in quasi tutte le definizioni e le descrizioni di malerba, l'obiettivo è quello di sottolineare gli aspetti negativi: l'interferenza con le colture, che provoca danni come la perdita di resa, il deterioramento della qualità del prodotto, la riduzione del valore del terreno, ecc. La lotta contro le malerbe si è originata e sviluppata con l'agricoltura stessa ed è il punto più critico nella gestione delle colture. La competizione delle infestanti nei confronti delle colture è il più grande problema per gli agricoltori e deve essere la più bassa possibile per una buona gestione e un buon ritorno economico. Le malerbe più competitive sono le specie ad elevata efficienza fotosintetica C4. Le erbe infestanti presentano efficaci sistemi di sopravvivenza, come la dormienza dei semi, che rende i semi in grado di germinare in diversi momenti delle stagioni e negli anni successivi. Le tecniche agronomiche per la gestione delle infestanti come, la rotazione delle colture, la scelta delle cultivar, la spaziatura delle piante sono importanti per contenere la diffusione delle malerbe. Il controllo meccanico è molto efficiente e versatile contro malerbe infestanti in una ampia varietà di sistemi di coltivazione. Il controllo chimico è stato un successo importante in termini di aumento delle produzioni, ma presenta molti aspetti negativi, come i rischi ambientali. E' possibile migliorare e combinare tutti questi tipi di gestione usando modelli che prevedono l'emergenza delle malerbe. AlertInf è uno di questi modelli, è stato sviluppato presso l'Università di Padova ed è in grado di predire la comparsa delle principali infestanti nel mais per la regione Veneto. L'informazione fornita da AlertInf è la percentuale di malerbe già emerse rispetto al numero totale di piante che possono potenzialmente emergere fino al termine della stagione. Lo scopo di questa tesi è parte di uno studio sulla trasferibilità del modello AlertInf dall'Italia alla Croazia attraverso un confronto tra le caratteristiche di germinazione-emergenza tra ecotipi Italiani e Croati. In particolare, il parametro stimato in questa tesi è la temperatura di base, la temperatura al di sotto della quale si fermano i processi germinativi. Le specie studiate sono: *Abutilon theophrasti*, *Amaranthus retroflexus*, *Chenopodium album* and *Echinochloa crus-galli*. Le temperature di base sono risultate non statisticamente diverse tra ecotipi italiani e croati per *Abutilon theophrasti* e *Chenopodium album*. Il caso è stato diverso per *Echinochloa crus-galli* che invece ha mostrato una temperature di base più bassa in Croazia, confermando la tendenza di questa specie a sviluppare ecotipi con una risposta termica legata alle condizioni climatiche del luogo di origine, più freddo è il clima e più bassa è la temperatura di base dell'ecotipo di quella zona. Sfortunatamente per *Amaranthus retroflexus* i dati di germinazione sono stati non sufficienti per l'analisi statistica. I risultati di questa tesi sono molto utili per valutare la trasferibilità del modello AlertInf. Alcune specie hanno mostrato soglie simili per diversi ecotipi e questo significa che è possibile applicare la temperatura di base calcolata in una località direttamente in un'altra, senza condurre specifici esperimenti. Altre specie, come *Echinochloa crus-galli*, richiedono necessariamente di calcolare le

specifiche soglie. Conoscere il comportamento delle varie specie è utile per decidere correttamente se sia necessario condurre specifici esperimenti o se sia possibile evitarli e usare gli stessi parametri stimati in altre regioni geografiche, con notevoli vantaggi in termini di tempo e denaro.

INDEX

ABSTRACT 1

RIASSUNTO 3

1. INTRODUCTION 7

1.1 Classification for broadleaf and narrow leaf 8

1.2 Classification by biologic groups 8

1.3 Classification by ecophysiological groups 9

1.4 Competition 10

1.5 Dormancy 13

1.6 Germination and emergence 15

1.7 Weed management and control 16

1.7.1. Weed prevention 17

1.7.2 Non-chemical weed management 18

1.7.2.1 Cultural weed management 18

1.7.2.3 Physical methods 20

1.7.2.4 Biological weed management 21

1.7.3 Chemical weed control 22

1.8 Weeds discussed in this thesis 25

1.8.1 *Abutilon theophrasti* Medicus 25

1.8.2 *Amaranthus retroflexus* L. 27

1.8.3 *Chenopodium album* L. 29

1.8.4 *Echinochloa crus-galli* (L.) Palisot e Beauvois 31

1.8.5 Weed diffusion in Croatia 34

1.9 Models 36

1.9.1 Base temperature 36

1.9.2 Thermal time or growing degree days (GDD) 37

1.9.3 Hydrothermal time 37

1.9.4 AlertInf 38

2. OBJECTIVES 39

3. MATERIALS AND METHODS 40

3.1 Seed collection 40

3.2 Cleaning of seeds 42

3.3 Sowing on plates 42

3.4 Preliminary tests ... 42
3.5 Temperature studied and seed germinator setting ... 45
3.6 Base temperature ... 45
4. RESULTS AND DISCUSSION ... 47
4.1 *Abutilon theophrasti* ... 47
4.2 *Chenopodium album* ... 49
4.3 *Echinochloa crus-galli* ... 50
4.4 Base Temperature ... 50
5. CONCLUSIONS ... 54
6. BIBLIOGRAPHY ... 55
7. SITOGRAPHY ... 61

1. INTRODUCTION

In the various cultivations, man has always had to fight against weeds that interferes with the crops, causing damage due to loss of production, deterioration of the products quality, the reduction in the value of the land, the spread of pests, the interference with the management of the water and the increase of the costs of production *(Catizone and Zanin, 2001)*. The definition of weed is perhaps one of the most complex problems of weed science and "there is no universal definition that is accepted by all scientists *(Zimdahl, 2007)*". Old definitions also includes "a plant not valued for its use or beauty" and "a plant whose virtues have yet to be discovered *(Emerson, 1912)*[1]". The Weed Science Society of America (WSSA) has defined a weed as "a plant growing where is not desired *(Buchholtz, 1967)*[1]". There is also another definition from European Weed Research Society (EWRS) which says that a weed is "any plant or vegetation excluding fungi, interfering with the objectives or requirements of people *(EWRS, 1986)*". For example, the grass plants growing in the domestic garden lawn are acceptable and encouraged, but when they spread to the adjacent flowerbed they are considered weeds, similarly, crop seeds which are shed in the field can grow in subsequent crops in following years and contaminate them *(Naylor and Lutman, 2002)*. An ecological definition says: "weeds are pioneers of secondary succession, of which the weedy arable field is a special case *(Bunting, 1960)*[1]". From an environmental point of view "a plant is a weed if, in a specific geographic area, its population grows wholly or predominantly in environments markedly disturbed by man *(Baker, 1965)*". In almost all the definitions and descriptions of weeds the aim is to underline the negative aspects, particularly with reference to their interference with cultivated plants *(Harminder et al., 2006)*. Weeds can be classified in many ways, of which the most important are:

_ classification for broadleaf and narrow leaf;

_ classification by biological groups;

_ classification by ecophysiological groups.

[1] Bibliographic citation not reported.

1.1 Classification for broadleaf and narrow leaf

The broadleaf weeds are considered the dicotyledons. The most common dicotyledons weeds, belongs to the angiosperms, as: amarantus, chamomile, *Chenopodium album*, polygon, vetch, Veronica, bindweed, etc. and the narrow leaf weeds are represented by monocotyledons and in particular by the *Poaceae* family. The *Poaceae* family, is the family with the most weedy species and also the family that includes many of the important crops for human feeding: wheat, rice, barley, millet, oats, rye, corn, sorghum and sugar cane. About two-thirds of the important weeds are broadleaved or dicotyledonous species. Most of rest are grasses, sedges, or ferns *(Zimdahl, 2007)*.

1.2 Classification by biologic groups

According to this classification the plants are divided into groups on the basis of the biological mode with which they pass the disadvantageous period of the year. It can be by seed or by gems, and in this second case we can distinguish different classes on the basis of the position of the gemstone with respect to the ground surface *(Zanin et al., 2001)*.

According to the Raunkiaer's classification *(1905)* can be distinguished:

- propagation by seed only:
  - therophytes (Th) with annual cycle;
  - biennials (H2) with two-year cycle;
- propagation by seed and gems slightly underground:
  - hemicryptophytes (Hr) with multiannual cycle;
- propagation by seed and gems: deeply underground:
  - geophytes (G);
- propagation by seed and gems placed above ground on structures more or less lignified:
  - camefite (Ch);
  - nanofanerofite (NPH);
  - phanerophytes.

The most interesting group for our study is represented by therophytes. The therophytes are plants that exceed the disadvantageous time at the stage of seed. Therefore they are annual species with sexual reproduction with the seed as the only survival strategy. The therophytes can be divided in:

- summer annual species (Th1): germinate in late winter and do not require vernalization to flower and usually do not pass through the rosette stage;
- winter annual species (Th2): germinate in the fall or winter, have a rosette stadium usually linked to the necessity of vernalization and a possible need of long days to flower;

- indifferent species (Th1-Th2): germinate in all seasons except during periods when the temperature drops below zero, they do not need vernalization or long day to flourish.

From the agronomic point of view, the therophytes are typical of the soils with favorable structure and texture, that are subjected to a complete overturning of the worked layer and in which an intensive rotation is practiced with annual crops but without the insertion of fallow or forage crops. In these situations the average percentage of Th varies from 85 to 100%: 100% is in intensive horticultural systems where the surfaces are subjected to a very tight rotation and where there is a low usage of herbicides *(Zanin et al., 2001)*.

1.3 Classification by ecophysiological groups

The classification proposed by *Montegut* in 1975, is based on the observation that weeds born from seed, either annual or multiannual, emerge in precise periods, in relation to a specific set of ecophysiological requests. This classification distinguishes five ecophysiological groups *(figure 1) (Zanin et al., 2001)*:

- 1) indifferent species (Ind): plants that are able to germinate in all seasons of the year;
- 2) autumn species (A): species that require vernalization in the stage of rosette to flower;
- 3) winter species (W): species with dormant seeds which can germinate only at low temperatures (0-5 °C);
- 4) spring species (SP): species with dormant seeds which requires the permanence at 0-5 °C for 4-6 weeks to exceed the dormancy. To germinate they require a temperature of 10 °C and they can be subdivided into two subgroups:
    - tight spring: they emerge closely in spring;
    - prolonged spring: the emergence is protracted even in early summer;
- 5) summer species (S): the seeds can be dormant but the passing is not always determined by the low temperatures. They can be divided into:
    - subtermofile;
    - thermophilic.

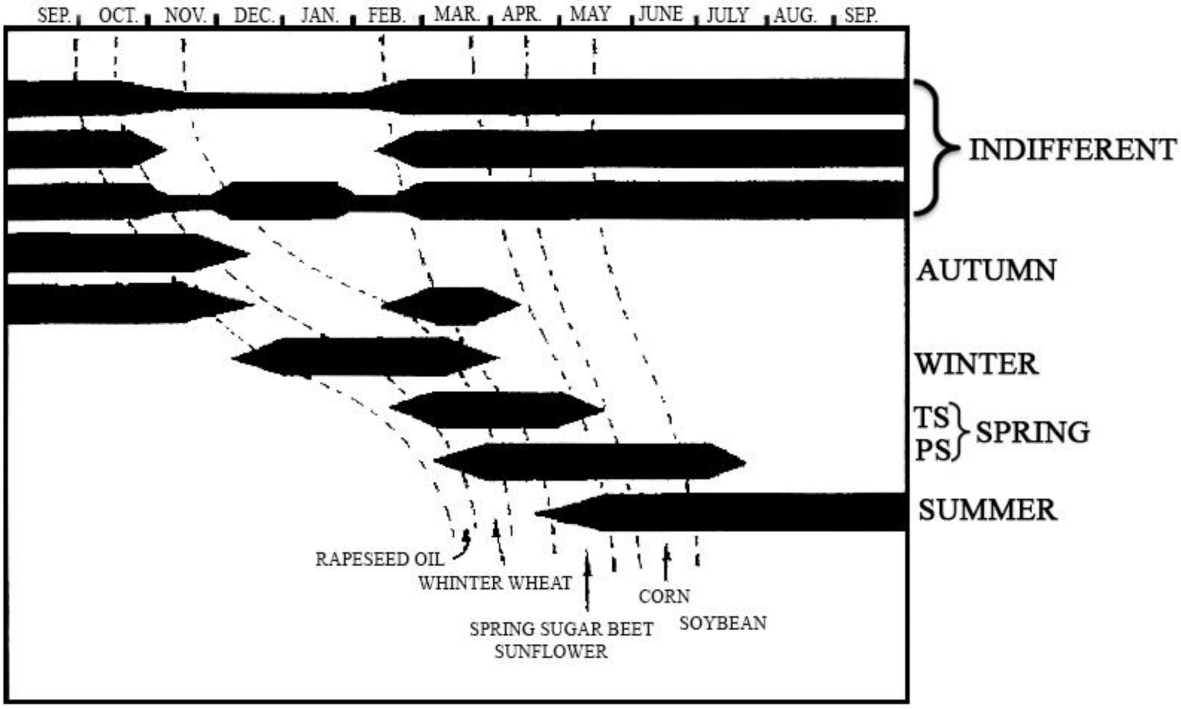

Figure 1. Phenology of emergencies of weeds in relation to the cycle of the main crops *(Zanin et al., 2001).*

1.4 Competition

Why weeds are widespread? Because they have certain characteristics that make them ideal for the proliferation, such as the ability to reproduce more quickly than the crop from the seed to the reproductive phase, the phenotypic plasticity and high heterogeneity in the environment adaptation associated with wild plants *(Baker, 1974)*. Weeds compete with crops for environmental resources available in limited supply, i.e. nutrients, water and light. Competition can be define as "the tendency of neighboring plants to utilize the same quantum of light, ion of mineral nutrient, molecule of water, or volume of space *(Naylor, 2002)"*. As a consequence, the weeds may reduce the crop yield and quality of crops. The production of a crop depends on the interaction between genotype (the characteristics of the species and variety) and the environmental conditions (climate, soil, water and nutrient availability, adversities parasitic, etc.), which can be changed in part through farming techniques *(Sattin and Tei, 2001)*. The damages caused by weeds can be *(Sattin and Tei, 2001)*:

- loss of production due to competition, allelopathic phenomena and parasitism;
- deterioration of product quality;
- damage to the health of the animals;
- reduction in choice of crops and the value of the land;
- spread of parasitic adversity;
- increase in production costs;
- interference with the water management;

- the weeds also creates many problems in non-agricultural areas;
- pollinosis (allergies to humans).

Weeds constitute only 0,1% of the entire plant in the world, but nevertheless they are able to generate enormous economic losses *(Harminder et al., 2006)*. In fact the loss of production due to competition are the major reasons for which farmers acts against the weeds in cultivated fields, but also the deterioration in quality is very important, especially in the case of horticultural products *(Naylor, 2002).*

The competition, as already said, is exercised mainly through the struggle for the supply of energy and functional resources, including first light, water and nutrients, unlike oxygen and carbon dioxide that usually are never limiting factors *(Campagna and Rapparini, 2008)*. Generally an high density of weeds in a crop may lead to a change the surrounding environment, leading to change the growth of the crop, more the culture is able to respond quickly to these changes, more there will be an advantage over weeds *(Sattin and Tei, 2001).* Light is not only one of the main resources for which plants compete intensively in agricultural environments, but also the environmental factor is probably the most used by plants to acquire information on the "state" of the environment *(Cosens and Vince-Prue, 1983; Attridge 1990),* the plants are in fact equipped with receptors similar to biological sensors capable of monitoring the quantity and quality of the light, and the duration of the period of illumination (photoperiod) *(Campagna and Rapparini, 2008)*. To be more precisely we can distinguish two types of competition:

1. indirect: competition for nutrients (light, water, nutrients);
2. direct: when weeds release substances that inhibit seed germination or growth of cultivated plants, as chemicals (allelopathic substances), they increase the ability to claim against weeds of cultivated plants.

Allelopathy is a form of plant interference that occurs when one plant, through living or decaying tissue, interferes with growth of another plant via chemical inhibitor *(Zimdahl, 2007)*. Allelopathy can be produced:

- from a culture and manifested on the same, on another crop or on the weeds;
- from weeds and manifested in other weeds or cultivated species.

If the plants receptor belongs to cultivated species, it poses problems of soil tiredness and others, while if the receptor plants are weeds, you can take advantage of the allelopathy to control them *(Sattin and Tei, 2001)*. For example, the allelopathic effects of *Abutilon theophrasti medicus* and of *Cyperus rotundus* on soybeans and of *Chenopodium album* L*, Cyperus esculentus* L and *Setaria faberii* Herrm. on corn are known *(table 1) (Casini, 2003)*.

Table 1. Negative allelopathic effects in sensitive crops induced from weeds *(Campagna and Rapparini, 2008)*.

Sensibile crops	Weeds with allelopathic propieties
corn	*Chenopodium album, Cyperus esculentus, Rumex crispus, Setaria spp.*
soybean	*Abutilon theophrasti, Cyperus rotundus, Xanthium strumarium*

The weeds which are more competitive, are species with high photosynthetic efficiency like the C4, as Cynodon, Cyperus, Amaranthus, etc., but at the same time are also heliophilous obligated species and therefore they do not tolerate low light intensities where the C3 are tolerant. The shading caused by weeds which generally grow faster than the cultivation, leads to a reduction of the photosynthetic activity with sometimes irreversible damage. A dense vegetation can choke in particular the crop during the germination and the early stages of development *(Campagna and Rapparini, 2008)*.

For what concerns the competition for water, it is particularly important in the areas where the supply of water from the soil and the environment is poor. The competition is determined by the volume of soil explored by the roots of each species *(Catizone and Zanin, 2001)*. Typically species with better water use efficiency and water saving have regulatory mechanisms, such as the ability to close the stoma, are more competitive in drought conditions *(Campagna and Rapparini, 2008)*. For example, the C4 species have a greater water use efficiency compared to C3 species (*table 2*).

Table 2. Water use efficiency expressed in grams of dry matter per kilogram of water *(Zimdahl, 1993; modified by Catizone and Zanin, 2001; Karkanis et al., 2011).*

Specie	WUE g d.m./kg water
Abutilon theophrasti (C3)	0,6-1,1
Amaranthus retroflexus (C4)	3,3-3,8
Chenopodium album (C3)	1,5-2,3
Winter wheat (C3)	1,8-2,5
Corn (C4)	2,7
Soybean (C3)	1,6
Alfalfa (C3)	1,15-1,25

In addition to the WUE, there are many other competitive factors. In the table *3* it is shown the level of competition between some weeds species and some crops.

Table 3. Level of weed-crop competition expressed as percentage of potential yield loss *(Campagna and Rapparini, 2008).*

Weed	corn	sugar beet	soybean
Abutilon theophrasti	46	60	96
Amaranthus retroflexus	51	100	100
Chenopodium album	31	100	88
Echinochloa crus-galli	11	100	100

1.5 Dormancy

Once the seeds are formed and matured on the plant, they will be destined to fall down and to be dispersed in the environment. Once they are deposited in the soil and subjected to the right conditions to germinate, they do not germinate immediately because they are subject to the phenomena of dormancy.

Dormancy is "that state in which a seed or other propagule does not start developing a new individual despite all the environmental conditions are favorable for active growth *(Amen, 1963)*". Dormancy is important because seeds survive for long time in soil and are a continuing source of infestation. It ensures survival for many years, and the aphorism that "one year seeding equals seven years weeding" is reasonably accurate *(Zimdahl, 2007)*. The long survival of dormant seeds of the weed population allows to build a "seed bank" that is a stock formed by seeds of different ages distributed in the soil. The germination of these seeds over the years is a prerequisite for the persistence of annual species *(Håkansson, 2003)*. Soil seed banks are notoriously heterogeneous in both horizontal and vertical planes *(Grundy and Jones, 2002)*. The entity of the seed bank depends on the average seeds per plant production and on the average longevity of seeds in the soil, expressed in years *(table 4).*

Table 4. Average seed/plant production and average longevity of seeds in the soil (years) *(Campagna and Rapparini, 2008)*.

Weed	Average seeds/plant production	Average longevity of seeds in the soil (years)
Abutilon theophrasti	5.000-20.000	/
Amaranthus retroflexus	40.000-100.000 and >100.000*	20-40
Chenopodium album	5.000-20.000 and >100.000*	20-40
Echinochloa crus-galli	1.000-5.000 and 20.000-40.000*	/

Notes: * potential production of seeds in special conditions of fertility and isolation of plants.

According to Harper’s *(1957)*, we can distinguish three type of dormancy:

1. primary or innate dormancy;
2. induced dormancy;
3. enforced dormancy.

The innate dormancy could be caused by a genetic control on the ripened seed when it leaves the plant, there could be a rudimentary or physiologically immature embryo, which is not fully developed when seed is shed. Innate dormancy can also be caused by impermeable or mechanically resistant seed coats, called “hard seed” *(Grundy and Jones, 2002)* as *Abutilon theophrasti*. A third cause is the presence of endogenous chemical inhibitor. The induced dormancy happens when a seed develops dormancy after exposure to specific environmental conditions such as dryness, high carbon dioxide concentration, or high temperature and the acquired dormancy persists after the environmental conditions change *(Grundy and Jones, 2002)*. Enforced dormancy also depends on environmental interaction but it does not persist when conditions change. The *figure 2* shows how common ragweed succeeds as an early successional plant and a weed.

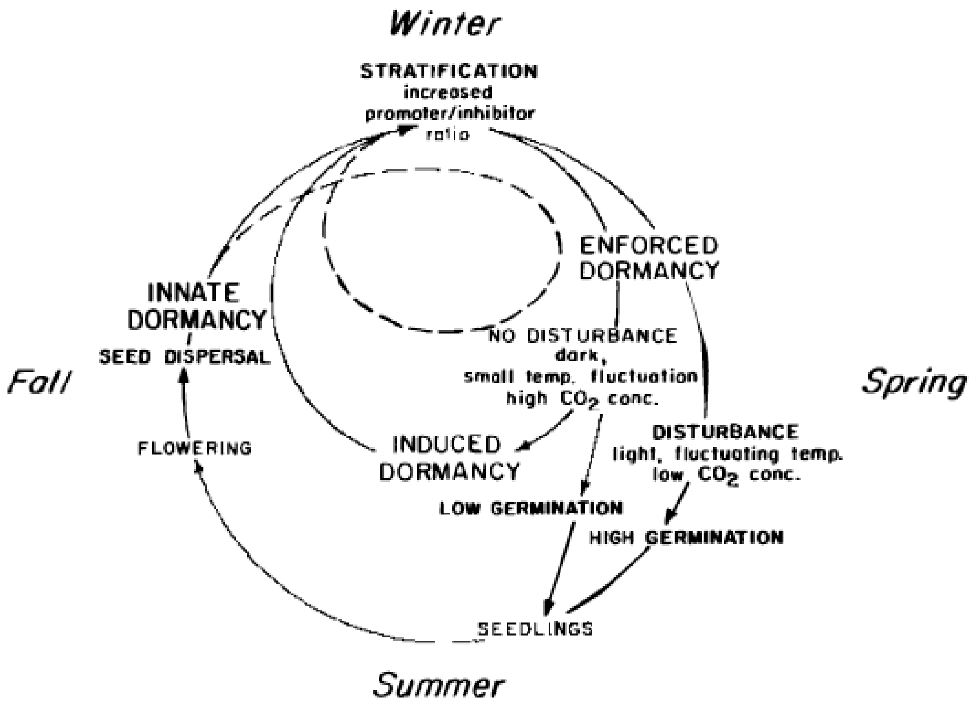

Figure 2. Schematic representation of seed germination in common ragweed, a common colonizer in old field succession and a spring annual weed. The dashed line represents seeds that require more than one stratification cycle to germinate and thus ensure germination and thus germination and establishment across a number of seasons *(Bazzaz, 1979)*.

1.6 Germination and emergence

When the seed comes out of dormancy, the process of germination begins, consisting in the issue of a radicle and then a stem that increases towards the surface of the soil *(Baldoni and Benvenuti, 2001)*.

A definition of germination can be "the end of the quiescent state with the resumption of active growth of the organism *(Baldoni and Benvenuti, 2001)*". There are many factors who influence this process such internal factors as hormones (gibberellins, cytokinins, auxins), or external factors as:

- water content;
- temperature;
- light;
- gas composition (O_2, CO_2, etc);
- other substances of the soil.

The main factor that affects the germination of seeds is certainly water: its absorption is in fact necessary for the activation of the enzyme systems and for transport to the embryo of reserve hydrolyzed substances *(Baldoni and Benvenuti, 2001)*. The initial entry of water into the seed is called imbibition and is a physical process, followed by swelling of the seed that breaks the seed coat encouraging the exchange of gases between it and the atmosphere and leakage of the radicle. The mild temperature typically stimulates the process of germination unlike that excessively high or limited, although typically the temperatures alternating play an important role in stimulating the germination *(table 5) (Campagna and Rapparini, 2008).*

Table 5. Optimum temperature for germination °C *(Campagna and Rapparini, 2008; Leon et al., 2004).*

Weed	Optimum temperature for germination (°C)
Abutilon theophrasti	24
Amaranthus retroflexus	20-32
Chenopodium spp.	5-8
Echinochloa crus-galli	20-30

The influence of light on the germination cannot be considered as quantitative influence but as qualitative, in fact the phytochrome recognizes the wavelength and duration of the light. The phytochrome is a chromoprotein present in two interconvertible forms characterized by different absorption peaks in the red region (P_r, 660 nm wavelength) and in the far red region (P_{fr}, 730 nm) *(Baldoni and Benvenuti, 2001)*. The different quality of the incident light determines a relationship between the two forms defining the "photo balance (ϕ)":

$$\varphi = \frac{Pfr}{Pfr + Pr}$$

In most plants the red light stops the dormant state and promotes germination, while the far red prolongs it *(Baldoni and Benvenuti, 2001)*.

The ratio between oxygen and carbon dioxide in the soil is closely related with the temperature. Oxygen supports germination and carbon dioxide inhibits it *(Campagna and Rapparini, 2008).* The union of all these factors determines the speed of germination. The emergence phase of the weeds, as for crops, is a very delicate phase and it is sensitive to many factors such as frost, asphyxia, etc. Typically the emergence of the weeds is epigeal, i.e. the cotyledons comes out of the ground together with the stem. The exceptions are represented by *Vicia* and *Lathyrus* who have an hypogeal mode of germination (the cotyledons remain in the soil) *(Campagna and Rapparini, 2008)*.

1.7 Weed management and control

The fight against weeds has originated and developed with agriculture itself *(Harminder et al., 2006)* and is the most critical point in the crops management. There are some basic concepts *(Zimdahl, 2007)*:

- invasion prevention is the best strategy to control weeds;
- chemical, non-chemical, and cultural weed control methods have distinct advantages and disadvantages;
- no weed control method has ever been abandoned;
- weed prevention, control, and management are different concepts, and each uses and combines technologies differently.

Some definitions:

- weed prevention: see chapter 1.7.1;
- weed control:
  includes using several techniques to limit weed infestations and minimize competition. These techniques attempt to achieve a balance between cost of control and crop yield loss, but weed control is used only after the problem exits *(Zimdahl, 2007)*;
- weed eradication:
  is the complete elimination of all live weeds, weeds parts, and weed seed. It is 100% or complete control. Is very difficult to achieve and the relative efforts have rarely been completely successful *(Zimdahl, 2007);*
- weed management:
  is the combination of techniques of prevention, eradication, and control to manage weeds in a crop, cropping system, or environment *(Zimdahl, 2007)*.

1.7.1. Weed prevention

Prevention has been a cornerstone of weed management throughout history *(Christoffoleti et al., 2007)*. The most difficult part of weed management is prevention, defined as stopping weds from contaminating an area *(Zimdahl, 2007)*. Preventive management is a very efficient technique for any property size, from a small vegetable crop seedbed to large areas devoted to major field crops and is the most cost-effective approach that grower can take *(Christoffoleti et al., 2007)*. Prevention addresses a potential problem, preventive efforts are harder to observe and measure *(Zimdahl, 2007)* because require awareness of the processes and practices that contribute to species introduction and proliferation *(Christoffoleti et al., 2007)*. Here are a few weed prevention measures *(Zimdahl, 2007)*:

- isolating imported animals for several days;
- not importing weeds or weed seeds in animal feed (buying only clean hay);
- using only clean crop seed that is free of weed seed;
- cleaning equipment between fields and especially between farms;
- preventing weed seed production, especially by new weeds;
- preventing vegetative spread of perennials;
- scouting for new weeds ;
- small patch treatment to prevent patch expansion and large infestations;
- education about weeds (e.g. weed identification).

Weed can be dispersed by human activities as:

- plant introduction;
- crops seeds;
- machinery;
- transportation of plant parts;
- transportation of soil;
- animals and manure;
- water and wind.

In other words, prevention should be implemented at all crop stages, from the acquisition of machinery, seed, water and fertilizers, to crop harvest and processing *(Christoffoleti et al., 2007).*

1.7.2 Non-chemical weed management

Non-chemical methods of weed control integrates together control techniques that do not involve the use of chemical products. Non-chemical management has become increasingly important in Europe due to a lack of herbicides, increasing resistance and a new European Union (EU) requirement for use of Integrated Pest Management (IPM).

1.7.2.1 Cultural weed management

Cultural weed management techniques are particularly important in crops where other weed management options are limited or not available *(Zimdahl, 2007)*. There are some points to consider:

- crop rotation:
  is a cultivation system in which different plant species are grown in succession creating environmental situations and disorders sometimes favoring a completely different parts of weeds not specialized (not of the same species) *(Campagna and Rapparini, 2008);*
- choice of cultivar:
  select cultivars that are weed resistant or that are strongly competitive with weeds;
- crop establishment:
  the use of high-quality seed will ensure rapid and even germination, and improve crop uniformity after emergence. Plants that emerge early in the field are known to have a competitive advantage over those that emerge later *(Bond, 2002)*;
- plant spacing:
  increased seed rate alone has been shown to provide greater weed suppression than narrower spacing *(Bond, 2002)*.

The mechanical weed management represent the oldest system of weed control *(Casini and Ferrero, 2001a)*. The appearance of herbicides in the mid-20th century contributed to a decreased reliance on mechanical weeders on farms. Nevertheless, these implements have continued to evolve and are very efficient and versatile in controlling weeds in a variety of cropping systems *(Cloutier et al,. 2007)*. To be successful with mechanical control, farmers must rely more on skill and planning to get the timing right and to select the proper mechanical tool *(Kurstjens et al., 2004)* than is required if they use chemical control *(Zimdahl, 2007)*. There are different types of interventions and can be divided according to the presence of the crop or less:

- Interventions in the absence of the crop:
  - plowing and plowing-subsoiler
  - subsoiler and scarifying
  - weeding and harrowing

    - minimum tillage
    - no tillage (direct seeding, sod-seeding)
    - tillage in darkness
    - stale seedbed and false seedbed
- Interventions in the presence of the crop:
    - harrowing
    - disking
    - brush weeders
    - mowing

Tillage remains the most important technique for mechanical weed management, however tillage alone should not be relied on as a sole weed control technique but instead it should be part of an overall cropping management strategy *(Cloutier et al., 2007)*. According to the American Society of Agricultural Engineers *(ASAE, 2005)*, tillage generally refers to the changing of soil conditions for the enhancement of crop production. Tillage turns under crop residue, conditions soil, and facilitates drainage. It controls weeds by burying them, separating shoots from roots, stimulating germination of dormant seeds and buds (to be controlled by another tillage), desiccating shoots, and exhausting carbohydrate reserves of perennial weeds *(Zimdahl, 2004; Zimdahl, 2007)*. Tillage can be dived into three categories *(Whicks et al., 1995; ASAE, 2004)*:

1. primary tillage:
   is the first soil-working operation in soil-inversion based cropping systems. Its objective is to prepare the soil for planting by reducing soil strength covering plant material, and by rearranging aggregates *(ASAE, 2005)*;
2. secondary tillage:
   the soil is not worked as aggressively or as deeply as in primary tillage *(Cloutier et al., 2007)*. The purpose of secondary tillage is to further pulverize the soil, mix various materials such as fertilizer, lime, manure and pesticides into the soil, level and firm the soil, close air pockets, and control weeds *(ASAE, 2004)*;
3. cultivating tillage:
   previously referred to as tertiary tillage, cultivating tillage is the term suggested by the ASAE *(2004)*. Cultivating tillage equipment is used after crop planting to carry out shallow tillage to loosen the soil and to control weeds *(Cloutier and Leblanc, 2001)*. These implements are commonly called cultivators *(Cloutier et al., 2007)*.

Successful weed control with tillage is determined by biological factors *(Zimdahl, 2007)*:

1. how closely weeds resemble the crop. Weeds that have a growth habit and time of emergence similar to the crop may be the most difficult to control with tillage, especially when they grow in crop rows. Weeds that emerge earlier or later than the crop are often easier to control;
2. if a weed have a short, specific period of germination of seeds, it is easier to control by tillage contrarily to that whose seeds germinate over a long time;
3. perennial weeds that reproduce vegetatively are particularly difficult to control with tillage alone.

In any case, tillage is a cultural practice and therefore, by definition, it requires cultural knowledge. It requires the mind of a good farmer who knows the land *(Zimdahl, 2004)*.

1.7.2.3 Physical methods

The physical methods are:

- heat:
  - flaming technology:
    many plant processes are susceptible to high-temperature disruption attributed to coagulation and denaturation of protein, increasing membrane permeability, and enzyme inactivation *(Zimdahl, 2007)*. Commercial flame weeders use LPG (propane-butane mixture) as fuel. Propane flames generate temperatures up to 1900 °C *(Ascard et al., 2007)*;
  - solarization and heat:
    solarization uses plastic sheets placed on soil moistened to field capacity and thus heats soil by trapping solar radiation just as a greenhouse does *(Horowits et al., 1983)*. Weed seed germination is suppressed by high soil temperatures and seedlings are killed but its effectiveness for weed control is dependent on a warm, moist climate and intense radiation with long days to raise soil temperature enough to kill weed seeds and seedlings *(Zimdahl, 2007)*;
  - steam:
    steam under pressure is applied beneath metal pans forced down onto freshly formed beds for periods of 3-8 min. The steam raises the soil temperature to 70-100 °C, killing most weeds seeds to a depth of at least 10 cm *(Bond, 2002)*;
  - hot water:
    weed control can be achieved by applying hot water either as a foliar spray, on the soil surface and/or by injection into the soil followed immediately by cultivation. Many of the affected weeds may regenerate since the roots are not sufficiently damaged, making repeated applications necessary *(Ascard et al., 2007)*;
- mulching:

weed-seed germination and seedling emergence can be suppressed by covering or mulching the soil surface to exclude light. Mulches are generally less effective against established perennial weeds that have sufficient food reserves to emerge through all but the most impenetrable materials *(Bond, 2002)*. Mulches in addition to control weeds, increase soil temperature and may promote better plant growth *(Zimdahl, 2007)*;

- sound and electricity:
  ultra-high-frequency (UHF) fields are selectively toxic to plants and seeds. There is a linear and positive correlation between seed water content and susceptibility to electromagnetic energy *(Zimdahl, 2007)*. The equipment for electrical energy weed control consists of a generator, a transformer, one or more electrodes, and rolling coulters *(Vigneault, 2002)*. Because of the plant's resistance to electrical current, electrical energy is converted to heat, volatilizes cellular water and other volatiles, and ruptures cells, causing plant death *(Ascard et al., 2007);*
- infrared radiation:
  produced by heating ceramic or metal surfaces, is used to induce thermal injury to weed tissues *(Ascard et al., 2007)*;
- ultraviolet radiation:
  high levels of UV radiation (1-100 GJ/ha range) have been shown to control weeds *(Andreasen et al., 1999)*. Weeds are damaged due to heating of the foliage following the absorption of UV radiation by plant tissues *(Ascard et al., 2007)*;
- microwave radiation:
  absorption of microwaves causes water molecules within tissues to oscillate, thereby converting electromagnetic energy into heat *(Ascard et al., 2007)*;
- lasers:
  lasers can be used to cut weed stems. Light absorption from CO_2 lasers by water molecules heats tissue contents and causes their explosive boiling *(Langerholc, 1979)*;
- freezing:
  weed control with freezing systems can be achieved with liquid nitrogen, which at atmospheric pressure reaches a temperature of -196 °C. In this way it causes the mechanical destruction of the cell membrane as a result of the formation of ice crystals in the protoplasm *(Sakai and Larcher, 1987)*.

1.7.2.4 Biological weed management

Biological weed control is the action of parasites, predators, or pathogens to maintain another organism's population at a lower average density than would occur in their absence *(Zimdahl, 2007)*. The theoretical assumption of this method is based on the paradigm that the more a biocenosis is complex, more it is stable *(Casini and Ferrero, 2001b)*. In its classical or idealized form, biological weed control can be permanent weed management because once an organism is released, it may be self-perpetuating, and control will continue without further human intervention *(Zimdahl, 2007)*. The main limitations of biological control are related to the high investments needed to identify the biological agent and the precise evaluation of its specific target in the release. Moreover, the effects are not always rapid and can therefore bad reconciled with the need to act in the critical period of competition, as in the case of the control of weeds in crop *(Casini and Ferrero, 2001b)*.

There are four methods of applying biological control agents *(Bond, 2002)*:

1. classical or inoculative:
   it describes the introduction of host-specific, exotic natural enemies to manage alien weeds;
2. augmentative or inundative:
   it involves the mass production and release of (usually) native, natural enemies against (usually) native weeds;
3. conservative:
   it is an indirect method whereby the natural levels of the pests and diseases that attack the native biomanagement agents that feed on the target weed are reduced to a low level. This is a long-term strategy that requires a detailed knowledge of the ecology of all the organisms involved;
4. broad-spectrum or total vegetation management:
   rarely relates to a single weed and often refers to modification of a whole habitat.

1.7.3 Chemical weed control

According to the definition given by the Encyclopedia of Agricultural Science *(1994)*[2] with the term herbicide refers to any synthetic chemical that is used for the control of weeds. The definition accepted by the Weed Science Society of America *(Vencill, 2002, p.459)* is that an herbicide is "a chemical substance or cultured organism used to kill or suppress the growth of plants". In effect, a herbicide disrupts the physiology of a plant over a long enough period to kill it or severely limit its growth *(Zimdahl, 2007)*. The development of chemical weed control has been an important success in terms of the additional food production that has resulted. We can classify chemicals by usage in terms of *(Naylor and Lutman, 2002)*:

- where they are applied (e. g. to the foliage or to soil);
- when they are applied (pre-sowing or pre-planting, pre-emergence of seedlings or post-emergence);
- the extent of the application (overall, directed away from the crop or in a band along the crop row);
- their mode of action (total or selective).

[2] Bibliographic citation not reported.

Herbicides have several advantages and disadvantages all of which should be considered prior to use. Some advantages of herbicides are *(Zimdahl, 2007)*:

- they permit selective weed control in ochards;
- proper herbicide selection maintains plant cover and reduces or eliminates the need for tillage that encourages soil erosion;
- they can reduce fertilizer and irrigation requirements by eliminating competing weeds;
- they reduce harvest costs by eliminating interfering weeds.

As already mentioned, there are many disadvantages in the utilization of herbicides, like:

- cost:
  it is often suggested that herbicides reduce crop production costs *(Zimdahl, 2007)* but we need to consider the cost of the machine and of the operator for the distribution, the timeliness of the intervention and other details;
- mammalian toxicity:
  all have some toxicity to humans and other plant and animal species;
- environmental persistence:
  none persist forever, but all have a measurable environmental life;
- weed resistance to herbicides:
  herbicide resistance in weeds is defined as the decreased response of a species population to an herbicide *(LeBaron and Gressel, 1982)*. It is "survival of a segment of the population of a plant species and herbicide dose lethal to the normal population" *(Penner, 1994)*;
- monoculture:
  monoculture is ideal for use of selective herbicides but monoculture can facilitate the appearance of weed resistance.

One important classification of herbicides is according to "site of action" purposed by the Herbicide Resistance Action Committee (HRAC) which is an international body founded by the agrochemical industry to supporting a cooperative approach to the management of herbicide resistance. The herbicides are classified alphabetically according to their target sites, sites of action, similarity of induced symptoms or chemical classes *(table 6)*. If different herbicide groups share the same site of action only one letter is used. In the case of photosynthesis inhibitors subclasses C1, C2 and C3 indicate different binding behavior at the binding protein D1 or different classes. Bleaching can be caused by different ways. Herbicides with unknown sites of action are classified in group Z as "unknown" until they can be grouped exactly *(HRAC, 2013)*.

Table 6. HRAC groups and site of action *(HRAC, 2013)*.

HRAC Group	**Site of Action**
A	Inhibition of acetyl CoA carboxylase (ACCase)
B	Inhibition of acetolactate synthase ALS (acetohydroxyacid synthase AHAS)
C1	Inhibition of photosynthesis at photosystem II
C2	Inhibition of photosynthesis at photosystem II
C3	Inhibition of photosynthesis at photosystem II
D	Photosystem-I-electron diversion
E	Inhibition of protoporphyrinogen oxidase (PPO)
F1	Bleaching: Inhibition of carotenoid biosynthesis at the phytoene desaturase step (PDS)
F2	Bleaching: Inhibition of 4-hydroxyphenyl-pyruvate-dioxygenase (4-HPPD)
F3	Bleaching: Inhibition of carotenoid biosynthesis (unknown target)
G	Inhibition of EPSP synthase
H	Inhibition of glutamine synthetase
I	Inhibition of DHP (dihydropteroate) synthase
K1	Microtubule assembly inhibition
K2	Inhibition of mitosis / microtubule organisation
K3	Inhibition of VLCFAs (see Remarks) (Inhibition of cell division)
L	Inhibition of cell wall (cellulose) synthesis
M	Uncoupling (Membrane disruption)
N	Inhibition of lipid synthesis - not ACCase inhibition
O	Action like indole acetic acid (synthetic auxins)
P	Inhibition of auxin transport
Z	Unknown Note: While the site of action of herbicides in Group Z is unknown it is likely that they differ in site of action between themselves and from other groups.

Notes: for the full table, including chemical family and active ingredient please see the HRAC webpage.

1.8 Weeds discussed in this thesis

1.8.1 *Abutilon theophrasti* Medicus

The name Abutilon derives from Arabic and it means “Indian mallow”. The species’ name derives from Greek philosopher and naturalist Teofrasto. The synonym avicennae is dedicated to the Arabic doctor and philosopher Avicenna *(Simonetti and Watschinger, 2001)*. It is a plant belonging to the *Malvacee* family *(table 7)* and it is an annual weed; its origin is Chinese-Tibetan where it is just cultivated as textile and officinal plant. It was introduced accidentally in Italy with seeds and feeds then it was naturalized and spread at first in the Southern Lombardy and in Veneto and later in Emilia-Romagna *(Campagna and Rapparini, 2008)*.

Table 7. Summary of the main characteristics of *Abutilon theophrasti*.

International nomenclature	ABUTH
Family	Malvacee
Biological group	Therophytes (Th)
Ecophysiological group	Prolonged spring
Common name	Velvetleaf

Main morphologic characters

The cotyledons are large until 3 cm, with heart form with an hollow apex and covered by short and fine hairs *(figure 3) (Campagna and Rapparini, 2008)*. The surface of the stem is smooth with short velvety hairs *(CABI, 2011a)*. The heart shape leaves have a fine dentate edge and they are all similar among their selves, included the first one that develops to the complete extension of the cotyledons differing from the others only for shorter dimensions *(Campagna and Rapparini, 2008)*. The width of the leaf blade is 7-20 cm and the leaf area ranges from 300 to 470 cm² *(CABI, 2011a)*. The flower *(figure 4)* is deep yellow, with 2 cm of diameter maturing into button-shaped capsules which split lengthwise to release the seeds *(Campagna and Rapparini, 2008)*. The blooming is from June/July to August *(Hanf, 1982; Simonetti and Watschinger, 2001)*.
The fruits *(figure 4)* are semi-globose capsules, about 2 cm in diameter and 1,2 cm in length, with 15-20 scabrous mericarps bearing two long awns at the apex arranged at crown as in all the family *(Campagna and Rapparini, 2008)*. The seeds are purplish-brown, kidney-shaped, notched, flattened, 1 mm thick and 2-3 mm long *(CABI, 2011a)*. Generally the plant is not branched and it has a rising bearing until a height of 50-250 cm (until to 400 cm) with a wooden trunk great to 2-3 cm. It can superimpose the stage of vegetative growth with reproductive stage and it highlights considerable phenotypic plasticity to answer to the different environmental conditions *(Campagna and Rapparini, 2008)*.

Figure 3. Evolution of cotyledonar stadium *(photos: Dario Magosso).*

Figure 4. Abutilon theophrasti plant, flower, fruit and seeds *(photos Marzorati Attilio)*.

Main bio-ecological aspects

Every plant produces at least 100-200 seeds (until to 12000) that remain vital in the soil for a period higher than 5 years, thanks to the high number of hard seeds (tegumentary dormancy) *(Campagna and Rapparini, 2008)*. Seeds emerge in soil between 1 and 5 cm depth *(CABI, 2011a)*. They germinate layering in spring with great adaptability to the medium-high temperatures, in every case higher than 10 °C *(Campagna and Rapparini, 2008)* and with an optimal toward 24-30 °C *(Campagna and Rapparini, 2008; CABI, 2011a)*.

It is a Nitrophic species, it lies in all the soils preferring those rich, damp and well-watered, very rich in organic substance and humus, manured and sewaged. It is diffused in north Italy, infesting all the crops with spring-summer cycle, with which it is very competitive and dangerous *(Campagna and Rapparini, 2008)*.

1.8.2 *Amaranthus retroflexus* L.

It is an annual weed *(table 8)*, originary from the warm areas of America, actually diffused in all over the world, in fact it is the most diffuse species especially in the warmest period *(Campagna and Rapparini, 2008)*. *A. retroflexus* has the C4 pathway of photosynthesis, typical 'Kranz' leaf anatomy, with a low carbon dioxide compensation point and high water use efficiency *(Weaver and MacWilliams, 1980; Tremmel and Patterson, 1993)*.

Table 8. Summary of the main characteristics of *Amaranthus retroflexus*.

International nomenclature	AMARE
Family	Amarantacee
Biological group	Therophytes (Th)
Ecophysiological group	Summer, thermophilic
Common name	Redroot pigweed

Main morphologic characters

It has elliptical-lengthened cotyledons (10-15 mm length) carried by short stalks on a purple hypocotyl axis of 3-4 cm long, with red-purple ribs on the lower leaf *(Campagna and Rapparini, 2008)*. The leaves are alternate, egg-shaped or rhombic-ovate, cuneate at base, up to 10 cm long, margins somewhat wavy, with prominent veins on underside, apex may be sharp *(figure 5)* (*CABI, 2011b)*. The flowers are unisex, without petals, with 5 greenish tepals and prickly bracts *(figure 5) (Campagna and Rapparini, 2008)*. The blooming is from May/Jun to October *(Hanf, 1982; Simonetti and Watschinger, 2001)*. The fruit is a utricle, membranous, flattened, 1,5 to 2 mm long, dehiscing by a transverse line at the middle, wrinkled upper part falling away (*CABI, 2011b).* The seeds are black, shining and with a form of lens and are very little (they weight about 0,6-0,7 mg) *(Campagna and Rapparini, 2008)*. The adult plant is erected and branched from the base and it take the form of a bush which reaches an high of 80-100 cm (until to 2 m in the most rich soils, but also few centimeters in unfavorable conditions). At the cotyledonar stadium and sometimes also at the first phenological phases, it can be confused with *Solanum nigrum* and with *polygonacee (Campagna and Rapparini, 2008)*.

Main bio-ecological aspects

A plant can produce until to 1 million of seeds that remain vital in the soil for almost 10 years and generally they germinate on the surface to a depth of 0,5-3 cm *(Campagna and Rapparini, 2008)*.

Research suggests that germination is stimulated by light and high temperatures *(Gallagher and Cardina, 1997; Oryokot et al., 1997)* although when too high (>40 °C), it represses germination *(Campagna and Rapparini, 2008)*. Generally the overcoming of the high dormancy of these seeds is favored by alternated temperature. *A. retroflexus* is a nitrophilic plant and is found often in manured and sewaged soils *(Campagna and Rapparini, 2008)*. It prefers warm-humid climates, it infests weeded crops, vineyards and orchards, becoming very competitive *(Hanf, 1982)*. It gives hospitality to the nematode *Heterodera schachtii*, the virus of the spotted withering of tomato, of the yellowish of the sugar beet, etc. *(Campagna and Rapparini, 2008).*

Figure 5. Amaranthus retroflexus plants and flower *(photos: Dario Magosso)*.

Management

To contain this very competitive species, it is important to control it before the ripening of the seeds, that the plant produces in short times and in abundance. The agronomic adjustments are important, and it is indicated to clean the operation machines, to avoid to draw water (well-watered) from infested sources, to exclude the ripening plants, etc. The false sowing can be used only with crops to very late sowing or transplantation, because it is a specie with a late germination. To obtain better results in the weeded crops it is advisable to combine the ridging to the weeding especially where the crops grow tall *(Campagna and Rapparini, 2008)*.

1.8.3 *Chenopodium album* L.

It is an annual weed *(table 9)* of European origin, now diffused almost all over the world (subcosmopolite). It is one of the most dangerous species for cultural crops *(Campagna and Rapparini, 2008)*. In certain countries the seeds are still used as a food crop *(Hanf, 1982; Campagna and Rapparini, 2008)*. The specific name refers to the surface of the leaves and stem covered with whitish bloom, with farinaceous appearance *(Simonetti and Watschinger, 2001)*.

Table 9. Summary of the main characteristics of *Chenopodium album*.

International nomenclature	CHEAL
Family	Chenopodiacee
Biological group	Therophytes (Th)
Ecophysiological group	Partially prolonged spring and summer subthermophilic
Common name	Common lambsquarters

Main morphologic characters

Cotyledons petioled, lanceolate-linear, mealy, bluish-grey with a reddish tinge beneath, 6-12 mm long and 1,5-4 mm broad *(figure 6) (Korsmo et al., 1981)*. The first two leaves are oval with lower leaf of edge purple colored. The second pair of leaves has a rhomboidal and dentate form with a long stalk similar to subsequent ones *(Campagna and Rapparini, 2008)*. The blooming is from June/July to October *(Hanf, 1982; Simonetti and Watschinger, 2001)*. The flowers are little, hermaphrodite, without petals and with 5 greenish tepals; they are assemble in glomerules displayed in panicle on the armpit of the leaves *(Hanf, 1982; Campagna and Rapparini, 2008)*. The fruits are utricles with little lenticular black seeds of 1-2 mm section. They are characterized by a different tegument which gives them a different degree of dormancy *(Campagna and Rapparini, 2008)*. Seeds are nearly circular in outline, oval in cross section, sides convex, beak shaped, glossy, black, mean size 1.5 mm x 1.4 mm in diameter, with a weight 1,2 mg per seed (*CABI, 2011c)*.

Figure 6. *Chenopodium album* from cotyledonar stadium to flowering stadium *(photos: Dario Magosso and missouriplants(A), 2013a)*.

The adult plant, often branched, has an erect angular and reddish stem *(figure 6)*. It can develop till 2 m in rich soils *(CABI, 2011c ; Campagna and Rapparini, 2008)*, whereas in unfavorable conditions it can bear fruit to 10 cm height. It can be confused with other species of *Chenopodium*, as *C. fulcifolium* and *C. giganteum*, which presents certainly greater dimensions (reaching a height up to 3 m) and red-purple shades on the leaves and on the stem. *Chenopodium* presents great phenotypic variability of the morphologic characters depending on the environmental conditions, and this makes difficult the botanical classification of the species. At the cotyledonar stadium it can be confused with other *Chenopodiacee*, and also with other species belonging to *Amarantaceae* and *Polygonaceae*. At the first phenological plant stadium it can be distinguishes from *Chenopodium opulifolium* by leaves, where in *C. album* are longer than large *(Campagna and Rapparini, 2008)*.

Main bio-ecological aspects

Korsmo et al. *(1981)* reported average seed production varying between 3000 and 20,000 seeds/plant, whilst Mandal *(1990)* reported production from as 50,000 to 70,000 seeds by individual plants; Campagna and Rapparini *(2008)* says that is also up to 176000. They remain vital in the soil for about 20 years, but, if buried deep, they remain vital also till 40 years *(Toole and Brown, 1946)*. They germinate in the layer between 0,5 and 3 cm and in dry soils and also ventilated up to 8 cm *(Hanf, 1982)*. In Italy, the germination occurs between March and April after a light stimulus *(Hanf, 1982; Campagna and Rapparini, 2008)*, with an average temperature of the soil of almost 8-10 °C (*Campagna and Rapparini, 2008)*. The initial high dormancy level depends mainly on the very hard seed coats that do not inhibit either after a period of rest in the water. Under certain conditions of growth the young plants can be red-violet color that contrasts with the typical dark green color. *C. album* prefers very rich soils, well manured and sewaged, it is a nitrophilic adapted to all types of soil, it is a weed very competitive for nutrients. It can colonize the new cultivated soils and it is present in all the crops where it becomes particularly dangerous especially in the spring and summer crops, as cucumber, corn and oats. It hosts the nematode *Heterodera schachtii* and numerous viruses of crops and beets, in addition to vectors such as the nematode *Xiphinema index* that causes the infective degeneration of the vine and a vector fungus of Rhizomania *(Campagna and Rapparini, 2008)*.

1.8.4 *Echinochloa crus-galli* (L.) Palisot e Beauvois

It is a Gramineae annual cycle *(table 10)* of tropical origin at summer development, cosmopolitan for the strong adaptability to different environments due to its polymorphism created by hybridization between different subspecies. The genus *Echinochloa*, at which belongs few species, is very similar to *Panicum (Campagna and Rapparini, 2008)*.

Table 10. Summary of the main characteristics of *Echinochloa crus-galli.*

International nomenclature	ECHCR
Family	Graminacee
Biological group	Therophytes (Th)
Ecophysiological group	Summer thermophilic
Common name	Barnyardgrass

Main morphologic characters

The pre-leaf is convoluted and a little flattened *(figure 7)*. The first leaves are long and narrow with slightly wavy and rippled edge *(Medri, 2013)*. The next leaves, with a clear midrib white, are similar but larger (up to 50 cm long and 1-2 cm wide) and rough, if you rub up and down. The leaves are hairless and light green, while the leaf sheath, flattened laterally, have a reddish-purple colouring *(Campagna and Rapparini, 2008)*. The best criterion for recognition is a feature hardened zone instead of the ligule and auricles *(figure 8) (Campagna and Rapparini, 2008)*.

Figure 7. *Echinochloa crus-galli* early stages of growth *(photos: Dario Magosso)*.

Figure 8. *Echinochloa crus-galli* plant, leaf and inflorescence *(missouriplants, 2013b)*.

From late May/June to October (*Simonetti and Watschinger, 2001; Medri, 2013)* it emits the inflorescence raceme or panicle, consisting of spikelets dorsally humped of 2-4 mm, placed on one side of the racemes arranged on the main spine in order to simulate a foot of cock rather bristly *(Campagna and Rapparini, 2008)*. The spikelets biflore, of which only one flower is fertile, with or without awns, green or red-purple, and polymorphic in different ecotypes. The seeds are 1,5-2 mm long (*CABI, 2011d)*, kernels dressed and they were also used for food purposes, such as *E. frumentacea*, which is still used in some parts of India *(Campagna and Rapparini, 2008)*. The adult plant is light green with shades of red-purple on the sleeves and can be up to 150 cm high (*CABI, 2011d)* with a tuft bearing. The culms are robust and flattened produced in number of about 15 for each plant, they are erected, but also half-prostrated on the soil. Close to the nodes tufts of hairs can be also present. At the cotyledonal stage, but also in the adult phases, it may be confused with other species of *Echinochloa* growing in Italy as: *E. phyllopogon*, present in rice crops that is hairy on the collar of the leaves, *E. cruciformis* presents in the Centre and South; *E. crus-pavonis, E. erecta, E. hostii, E. frumentacea* and *E. colonum* are occasionally located in moist soil. *E. crus-galli* is distinguished from other summer grasses for general morphological characteristics typical of the genus, including the absence of ligule and auricles. It is often confused with *Setaria, Panicum, Paspalum* and also *Digitaria* and *Sorghum (Campagna and Rapparini, 2008)*.

Main bio-ecological aspects

E. crus-galli shows great plasticity depending on the level of competition, soil fertility, soil moisture and daylength. In favourable conditions, it is capable to produce a large, competitive plant with a large number of panicles (*CABI, 2011d)*. In competition with maize and sorghum, it was reported to produce less than 3500 seeds per plant, but over 80,000 seeds per plant in low-competitive crops *(Norris et al., 1996)*. *E. crus-galli* reproduces only by seed and a high capacity for seed production allows large populations to rapidly establish. Data collected from 45 sites in Italy showed soil seed content was highly variable with on average 5500 seeds per m^2 being found at more than 50% of the sites *(Zanin et al., 1992)*. Under dry-seeded rice conditions, the growth of rice and E. crus-galli was greatest at a seeding depth of 3 cm and 40% soil moisture *(Park et al., 1997)*. The seeds exhibit a high degree of primary dormancy at the time of ripening, which is partially and gradually lost after weeks of storage *(Benvenuti et al., 1997)*. They can maintain their vitality in the ground up to 8-10 years *(Campagna and Rapparini, 2008)*. Due to the demands of the warm season species, germination is scalar with abundant soil moisture, starting from late spring (April-May) when temperatures reach at least 13-15 °C in the first centimeters of the soil, with optimal development on about 20-30 °C, up to 40 °C. Only 10-15% of the seeds germinate on the first year, in the case they have fallen in depth and compact soils, they can prolong the permanence in the soil. This is due to the dormancy of at least 3-4 months after ripening, especially if produced at the beginning of the season. The seeds can emerge from a depth of 10 cm if the soil is non-compacted and dry, while when it is flooded they emerge from a maximum depth of 2 cm. It is a hygrophylous species and better adapted to warm climate, with a high photosynthetic efficiency C4, it prefers soft soil, fertile, rich in humus, adapted to all types of soils, particularly wet or irrigated soils. It differs from all other species for their ability to live in the absence of water on the land. Developing in the summer, this species is one of the most common weeds of all row crops with spring sowing *(Campagna and Rapparini, 2008)*.

Management

Prevention is based mainly on agronomic methods for reducing the dissemination.

1.8.5 Weed diffusion in Croatia

Weed diffusion in Croatia was studied using the dataset collected in field trials for herbicide registration in row crops conducted by Professor Ostojić *(2011)*. From these experiments, every five years, from 1969 to 2009, the percentage of the presence of 21 different weeds was evaluated. Analysing these dataset shown in *table 12*, it is possible to make some considerations about weed diffusion in Croatia throughout the years. In 1969 there were ten main weed species, including *E. crus-galli* at the 1st place of presences in the fields and *C. album* at the 5th place. During the following years some new weeds appeared in the fields as *Sorghum halepense* and *Cirsium arvens. E. crus-galli* and *C. album* during the years were always placed between the 1st and the 4th place. *A. theophrasti* appeared in 1999 at the 18th place spreading really fast until to reach the 11th place in 2009. In these years it is also possible to notice the decreasing of the presence of some weeds as *Raphanus raphanistrum, Sinapis arvensis* (in 2009)*, Equisetum arvense, Centaurea cyanus* and *Sonchus arvensis.* Similarly, in winter cereals *(table 11) Agrostema githago, Centaurea cyanus, Raphanus raphanistrum, Sinapis arvensis, Vicia spp, Lathyrus spp, Lithospermum arvense, Lepidium draba,* etc. are completely lost. In contrast, as dominant species are *Galium aparine, Stellaria media, Matricaria chamomilla, Polygonum spp, Apera spica-venti, Convolvulus arvensis, Anthemis arvensis (Ostojić, 2011)*.

Table 11. Order of the most important weed species in cereals according dominance in field trials on 232 locations from 1985 till 2000 *(Ostojić, 2011)*.

No.	Weed	Number of locations
1	GALAP	125
2	STEME	100
3	MATCH	82
4	POLPE	77
5	APESV	70
6	CHEAL	68
7	CONAR	65
8	ANTAR	62
9	AMBEL	54
10	CIRAR	50
11	POLAV	42
12	VIOAR	40
13	MYOAR	35

Table 12. The most important weeds of row crops in Croatia based on the results of field trials (165) set up for scientific investigation and official herbicide registration purposes conducted every five years from 1969 to 2009 *(Ostojić, 2013)*.

Year	1969	OD	1974	OD	1979	OD	1984	OD	1989	OD	1994	OD	1999	OD	2004	OD	2009	OD
Weeds/ Number of trials	**11**		**8**		**13**		**7**		**16**		**15**		**63**		**23**		**9**	
ABUTH													7,9	18	17,4	13	14,3	11
AMARE	81,8	4	62,5	7	69,2	6	42,8	10	12,5	23	80	3	31,7	6	52,2	5	57,1	5
AMBEL					15,4	24	57,1	6	100	1	40	7	69,8	2	73,9	3	71,4	2
ANAAR	45,5	13	62,5	6	61,5	7	28,6	16	25	14	26,7	10	9,5	14	13	19		
CALSE					23,1	20	28,6	18	12,5	22	13,3	20	1,6	39	8,7	18		
CHEAL	81,8	5	75	4	100	2	85,7	4	93,75	2	100	1	69,8	3	82,6	1	71,4	3
CHEPO					76,9	5	57,1	5	37,5	8	60	5	47,6	5	47,8	6	28,6	8
CIRAR			75	5	23,1	21	14,3	27	18,8	20	26,7	8	3,2	33	26,1	12		
CONAR	90,9	3	62,5	8	53,8	9	42,8	9	18,8	21	53,3	6	30,2	7	34,8	9		
CYNDA							14,3	21					3,2	31	13	17		
ECHCG	100	1	100	1	100	1	100	1	87,5	4	93,3	2	87,3	1	73,9	2	85,7	1
HIBTR	9,1	41	25	24	38,5	15	42,8	8			6,7	27	15,9	10	13	20	14,3	11
POLAV	54,5	15	62,5	10	38,5	15	14,3	31	25	17	20	15	4,8	25	30,4	10		
POLPE	100	2	100	2	92,3	3	85,7	3	87,5	3	73,3	4	60,3	4	52,2	4	57,1	4
SETGL	72,7	7	75	3	84,6	4					26,7	11	12,7	12	43,5	7	42,9	6
SETVI					23,1	19	85,7	2	31,3	10	13,3	18	26,9	8	13	16	28,6	7
SOLNI	45,5	12	37,5	20	38,5	11	28,6	14			20	13	15,9	11	17,4	15		
SONAR	0	0	62,5	9	15,4	32	28,6	17	18,8	20	6,7	28	4,8	28	17,4	14		
SORHA							14,3	20	6,3	35	6,7	22	7,9	16	13	18	14,3	10
XANST																	28,6	9

Note: OD-order of detection

1.9 Models

Forecasting when weeds emerge and the length of the emergence period, it makes possible to optimize herbicide application timing and rate. In particular, the prediction of weed emergence can contribute to timing of chemical and non-chemical post-emergence applications to control weeds in crops, but it could be used also for timing pre-emergence herbicide applications to control annual weeds in turf *(Masin et al., 2005)*. The prediction of weed emergence also can help to detect very early and very late emerging plants that sometimes are not well controlled by post-emergence control and may contribute to competition with the crop, that causes yield loss, or to seed return *(Grundy, 2002)*. Therefore, development of accurate models to describe weed emergence dynamics is useful for planning an efficient weed management program *(Masin et al., 2005)*. Lundkvist *(1997)* categorized models of weed competition as:

- research models (to develop an understanding of processes):
  that attempt to quantify the effects of the density of one species, on crop yield or biomass of a competing weed species *(Lundkvist, 1997; Radosevich, 1987)*;
- practical models (decision-aid or weed management tools):
  that incorporate scouting or economic thresholds and purport to be decision aids for weed management *(Wilkerson et al., 2002)*.

A survey study of Wilkerson et al. *(2002)* shows that some models are too simple because they do not include all factors that influence weed competition or all issues a grower considers when decides how to manage weeds, others models are too complex because many users do not have time or possibilities to obtain and enter the required information, or again are not necessary because growers use a zero threshold or because skilled decision makers can make better and quicker recommendations.

1.9.1 Base temperature

In this paragraph is given the definition of the most important parameter used in predictive models for improving weed control: the base temperature (T_b). This thermal parameter is the temperature below which the rate of germination becomes nil, generally estimated as the x-intercept of a linear regression of the germination rate with temperature *(Gummerson, 1986)*. This parameter is fundamental to calculate the two most important independent variables driving growth and development processes, such as germination and emergence: thermal time and hydrothermal time.

1.9.2 Thermal time or growing degree days (GDD)

Thermal time provides “a measure of physiological time” *(Trudgill et al., 2005)* and can be expressed as a sum of efficient temperatures, accumulated each day above a base temperature *(Washitani and Takenaka, 1984)*. Thermal time is calculated by summing the everyday difference between the average temperature of the day minus the base temperature, with the following equation:

$$GDD = \frac{Tmax + Tmin}{2} - Tb$$

Where Tmax and Tmin are the maximum and the minimum daily temperatures, respectively.

1.9.3 Hydrothermal time

Hydrothermal time defines the interaction of water potential above a threshold level, temperature above a threshold level, plus time in a single function *(Bradford, 1995; Gummerson, 1986)*. Another definition of hydrothermal time is “a combination of thermal time above a base temperature and hydrotime above a base water potential *(Grundy and Jones, 2002)*”. 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) *(Masin et al., 2012)*. The hydrothermal time concept greatly increased the predictive capability of the models, but also made necessary to estimate more ‘‘biological’’ parameters for each species, both base temperature and base water potential, parameters that can be different among ecotypes *(Masin et al., 2010)*. The modeling approach was based on concepts developed by Forcella *(1998)*. This model, called WeedCast *(Archer et al., 2001)*, predicts the rate of weed emergence in arable soil. The basic concept of the WeedCast model is that seeds of all species accumulate hydrothermal time according to the soil temperature only when the soil water potential is above a base value *(Masin et al., 2005)*. Soil Growing Degree Days ($SGDD_i$) are a combination of soil temperature and soil water potential and are calculated as:

$$SGDD_i = \eta * \max(Ts_i - T_b, 0) + SGDD_{i-1}$$

where $\eta = 0$ when $\psi_{si} \leq \psi_b$, $\eta = 1$ when $\psi_{si} > \psi_b$, Ts_i is the average daily soil temperature at 2,5 cm depth, T_b and ψ_b are the base temperature and water potential thresholds for each weed species, and ψ_{si} is the average daily soil water potential at 5 cm depth. Models that predict weed germination in arable soils usually accumulate temperature from the date of soil cultivation *(Masin et al., 2005)*.

1.9.4 AlertInf

Knowing the dynamic of weed emergence means being able to estimate how many plants can be eliminated with an operation performed at a precise moment and how many will survive to this treatment. Knowledge of the emergence dynamic can then indicate whether it is necessary to control immediately or if it is more appropriate to wait *(Masin et al., 2008)*. There are numerous studies on the dynamics of emergency that have as their purpose the creation of models capable of predicting germination, most of these models are based on growing degree days (GDD) or hydrothermal time. One of the main limitations in the diffusion of these models is that they require parameters depending on the ecotype, therefore to transfer a model to an environment different from that of creation, it requires a study in loco for the recalibration of the parameters *(Masin et al., 2008)*. In the main maize-growing area in Italy, a study was conducted to create a model suitable to that environment and that management systems. The resulted model was *AlertInf (figure 9)*, a model that predicts emergence of the main weeds in maize. AlertInf was adopted in 2008 and made available to the users through an interactive web-service for the Veneto region. The model realization required laboratory tests for calculating the base temperature according to the method of Masin et al. *(2005)* and field tests conducted from 2002 to 2006 for the study of the dynamics of emergence and for the validation. The information provided by AlertInf is the percentage of weeds that have already emerged on the total number of plants that can potentially emerge until the end of season. This information is not advice to follow, but a fact which must be interpreted by the farmer *(Masin et al., 2008)*.

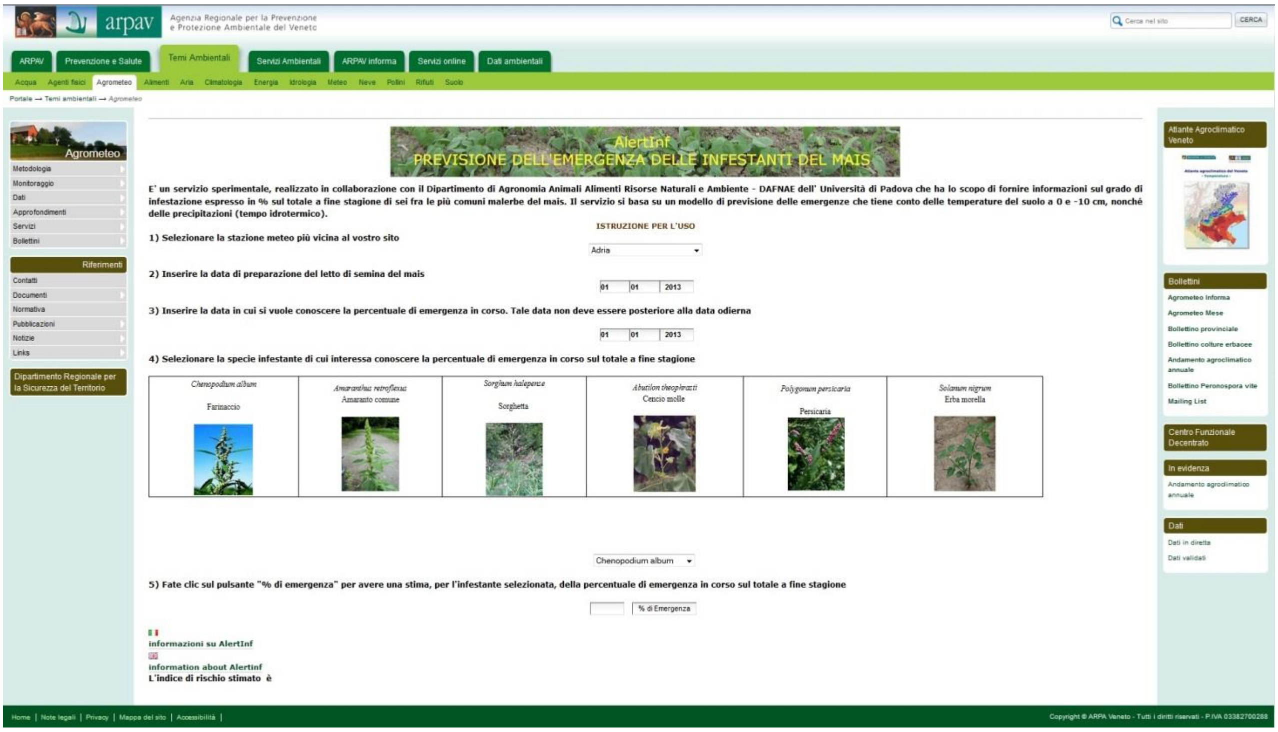

Figure 9. AlertInf screen from the Arpav's website.

2. OBJECTIVES

The aim of this thesis is part of a study on the transferability of the existing weed emergence model AlertInf, useful for weed control in maize, from Italy to Croatia. This is possible through a comparison of germination-emergence characteristics between Italian and Croatian ecotypes, in particular base threshold parameters, such as base temperature and base water potential, and emergence dynamics. In this thesis the study concerns the estimation of base temperature of Croatian ecotypes of four main weed species in maize: *Abutilon theophrasti*, *Amaranthus retroflexus*, *Chenopodium album* and *Echinochloa crus-galli*. The comparison of the temperature thresholds estimated in Croatian ecotypes with Italian ecotypes of the same species is the first main step to evaluate the possibility to transfer the model.

3. MATERIALS AND METHODS

3.1 Seed collection

The seeds of *A. theophrasti* and *A. retroflexus* were collected from populations growing in the Agronomski Fakultet fields located in Zagreb. *C. album (figure 10)* and *E. crus-galli* seeds were collected from a farm near Zagreb, in October 2012. The seeds were collected by hand and taken to the laboratory.

Figure 10. On the left: *C. album* plants harvested and on the right the seeds uncleaned *(photos: Dario Magosso)*.

Analyzing the average trend of the temperature, from October 2011 to May 2012 compared with the average of ten years (from 1996 to 2005) *(figure 11)* it is possible to notice that October and November were colder than the pluriannual average while December and January were warmer. February has reached the lowest average temperature of the period with -1,9 °C instead of 2,7 of average for the same month. The temperature in March was a little bit higher than the 10-years average while in April, May and June was around the pluriannual average. The total precipitation for this period was 468,5 mm , much lower than the average of 586,2 mm for the same period. The most significant differences *(figure 12)* were recorded in November, with no precipitation, in January, when the precipitation was a half of the 10-years average, in March, with 4,5 mm instead of 40,7 mm, and in June, with 128 mm. Regarding October, December, February, April and May, the average of 2011/2012 was similar to the pluriannual average.

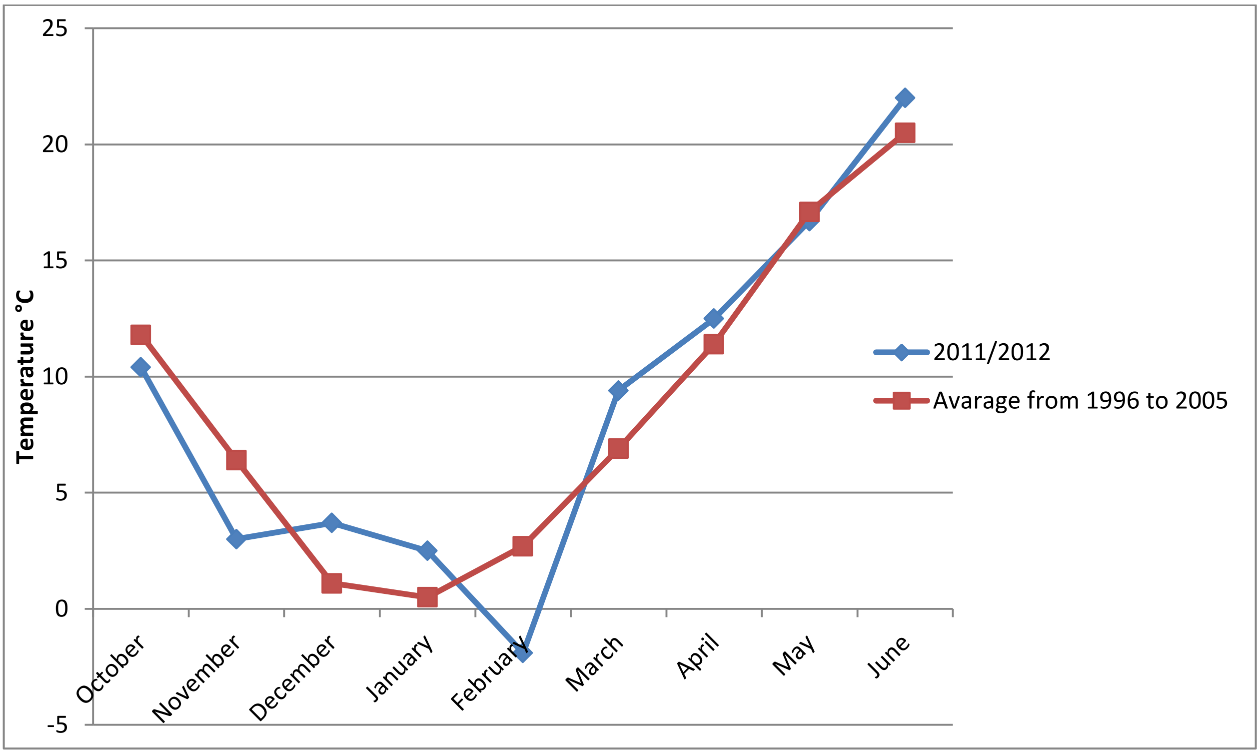

Figure 11. Trend of average temperatures from October 2011 to June 2012 compared with the average from 1996 to 2005.

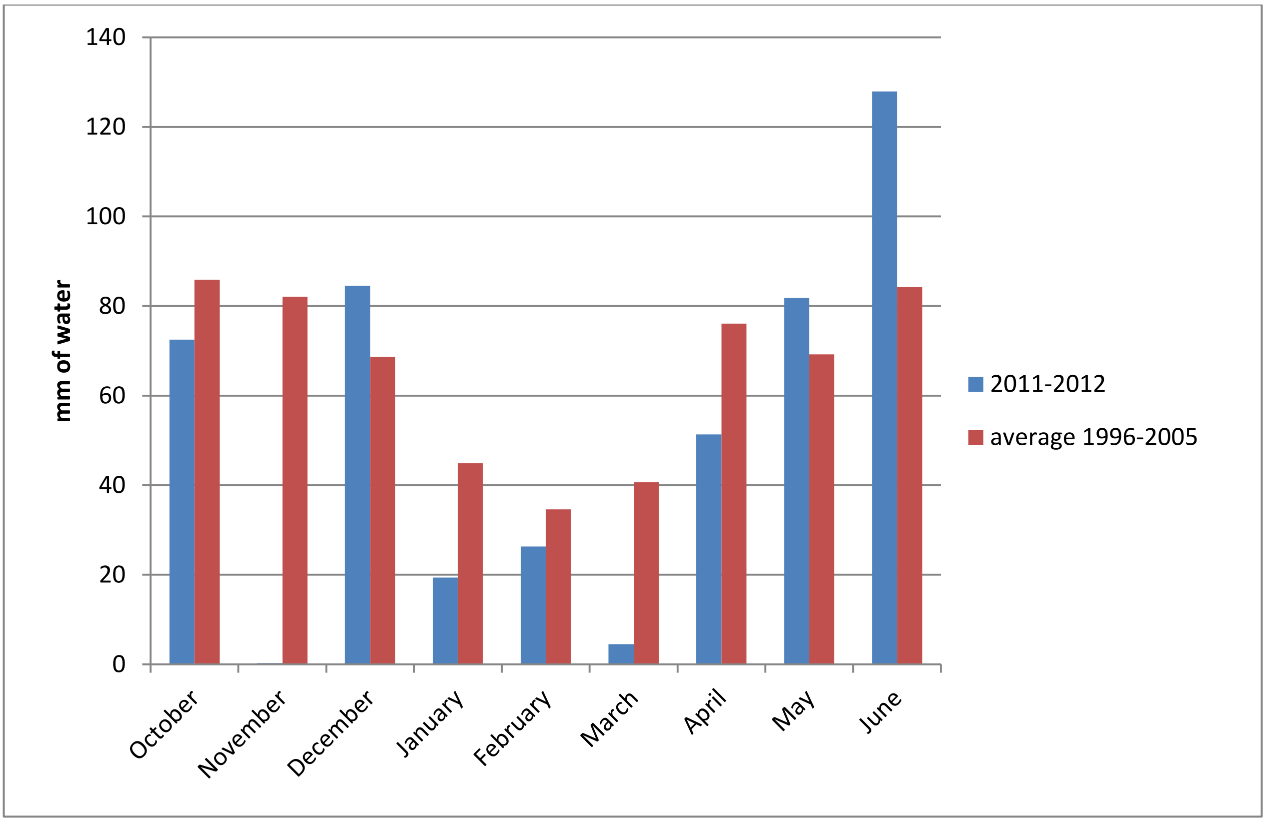

Figure 12. Trend of average precipitation from October 2011 to June 2012 compared with the average from 1996 to 2005.

3.2 Cleaning of seeds

Mature seeds of *A. theophrasti* and *A. retroflexus* were hand-picked from the plants, sieved and stored in plastic containers. The seeds of *C. album* and *E. crus-galli* have been removed from inflorescences and cleaned from their glumes. The cleaning from the glumes was made by hand-rubbing the seeds and then using a hair dryer.

3.3 Sowing on plates

The seeds of *A. retroflexus*, *C. album* and *E. crus-galli* were placed on glass Petri dishes with an internal diameter at the base of 14 cm with a lid of 15 cm. The dishes are 2.2 cm high while the cover is 1,7 cm. *A. theophrasti*, because of the seed size, was sown in Petri dishes larger than the others: base diameter 19 cm, height 2,7 cm, with lid of diameter 20 cm. For each small plate (14 cm) were placed inside 5 ml of distilled water together with the filter paper with a diameter of 13,5 cm which serves as the basis of sowing while for the bigger dishes (19 cm) were putted 10 ml of distilled water together with the filter paper. The seeds were sown using a pre-printed sheet with 100 black points to help to place the seeds in the Petri dishes *(figure 13)*. The plates and the relative filter papers, before sowing, have been disinfected with 70% ethyl alcohol. Petri dishes were then closed with “parafilm” and placed in a seed germinator.

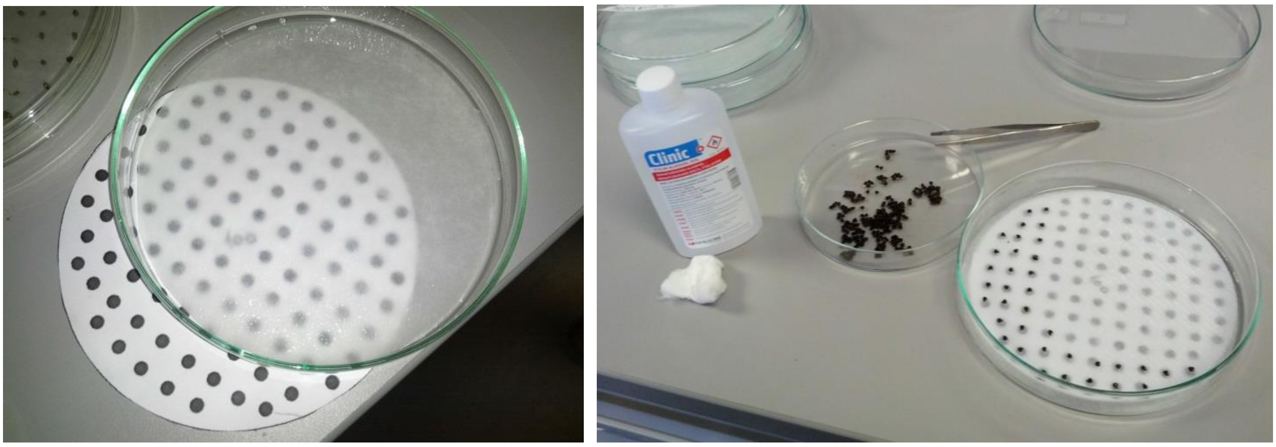

Figure 13. Sowing of *A. theophrasti* on petri dishes using a pre-printed sheet with 100 black points printed *(photos: Dario Magosso)*.

3.4 Preliminary tests

Before starting the experiments in the germinator, some preliminary tests were carried out to verify the germinability of the seeds. In collaboration with a thesis project of three Croatian students concerning the systems to break the dormancy in weeds, tests were conducted for selecting the best method to treat the seeds in order to improve germination of the studied weed species . *A. theophrasti* shows a dormancy due to seed coat, it means that the dormancy is linked to the structure of the seed. Normally the scarification of the seeds of this species is performed manually using sandpaper and scratching the seeds on it. Therefore the preliminary test for *A. theophrasti* was performed to find a more precise and standardized solution. 300 seeds (equivalent to the amount required for 3 replicates) were placed in small plastic containers lined with sandpaper. Different watermarks of papers were tested and each container was placed in an oscillator for 5 hours. The results, compared with seeds not scarified, have shown that the "standardized scarification" does not allow to obtain good percentage of germination. The choice was, therefore, to scarify the seeds by hand for all subsequent experiments. In addition to these tests, further experiments were performed by Obajgor Tihana *(2013)* for studying other seed treatments for braking the dormancy. The treatments were:

1. vernalization (4 ˚C/7 days);
2. vernalization (4 ˚C/14 days);
3. vernalization (4 ˚C/7 days) + KNO_3;
4. vernalization (4 ˚C/14 days) + KNO_3;
5. KNO_3 (0,2%) / room temperature (21 °C);
6. Scarification + H_2SO_4 (77%) for 5min;
7. Scarification + H_2SO_4 (77%) for 15 min;
8. Immersion of seeds in H_2O at 70 ˚C for 1h;
9. Immersion of seeds in H_2O at 70 ˚C for 2h;
10. Mechanical (manual) seed scarification;
11. Control (untreated seed).

The results of these treatments are shown in *figure 14*.

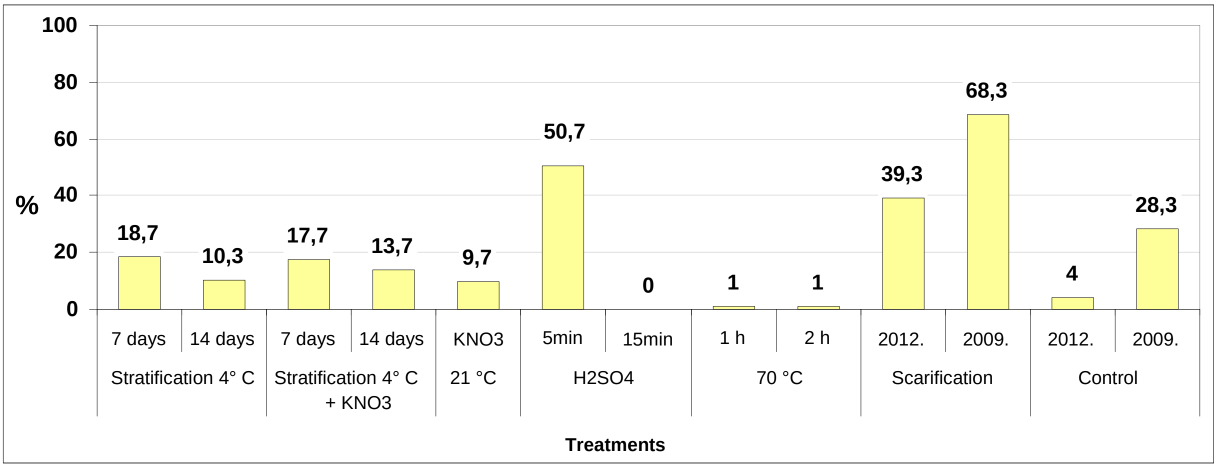

Figure 14. Total germination *Abutilon theophrasti* dived by treatments *(Obajgor, 2013)*.

The best germination was obtained with the chemical method, dipping the seeds in 77 % H_2SO_4 acid for 5 minutes obtaining 50,7% of seed germinated and with the mechanical scarification of seeds of the year with 39,3 % of seed germinated. These results are not statistically different each other for P = 0,05 (anova test). In addition to the seeds harvested and tested in the same year (2012), was also tested seeds of three years old (seeds of 2009). The results has shown that the seed coat becomes softer and loses the seed dormancy propriety through years, in fact, untreated seeds of three years old had shown significantly higher germination (28,3%) than of seeds of 2012 (4%). Mechanical (manual) scarification of three years old seeds has shown a germination of 68.3%, which was significantly higher compared to untreated seed (28.3%) of the same age. Regarding *C. album* and *A. retroflexus*, the freshly collected mature seeds are dormant with primary dormancy, and they need to spent time at cool and moist conditions, as in the winter, in order to germinate. For these species, different treatments were tested:

1) 7 and 14 days of vernalization at 4 °C;
2) treatment with 0,02% KNO_3 solution;
3) 7 and 14 days at 4 degrees combined with the use of KNO_3;
4) treatment with H_2SO_4;
5) treatment in water at 40 and 70 degrees for one hour.

The best solution was the use of KNO_3 joined to the chilling period of 7 days for *C. album*, while it was sufficient to use the vernalization for 7 days for *A. retroflexus*. In germinator at 24 degrees the 3 replicates of *A. retroflexus* did not germinate. The seeds of these replicates have been disinfected with sodium hypochlorite, as for the others species. Therefore it was hypothesized that the problem would be the use of sodium hypochlorite. Subsequently, in the tests at 16 and 20 degrees, the seeds were disinfected with hydrogen peroxide, but again the seeds have not germinated. At this point the second hypothesis was that the use of a normal refrigerator for the vernalization at 4°C should be the cause of the failure germination, because the possible changes of temperatures could have determined interruptions of the vernalization period. Subsequently, for 28 degrees, more attention was paid in the vernalization process but in any case poor results were obtained. Finally it was concluded that, as shown for *A. theoprasti* by Obajgor Tihana, the cause of the poor germination may be the freshness of the seeds and that the tests will be conducted in a few months time to obtain better germination results.

3.5 Temperature studied and seed germinator setting

Base temperatures were calculated with the method of the intercept, explained below in the "base temperature" chapter. This method of study concerns of incubating seeds at a range of constant temperatures and monitoring germinations. Seven different temperatures for each of the studied species were tested: 4, 8, 12, 16, 20, 24, 28° C.

To perform the tests were used a germination chamber equipped with UV lamps in the ceiling and on the shelves. The petri dishes were placed in the second shelf of a bookcase and a thermometer was used to check if the constant temperature was maintained in the position of the plates *(figure 15)*. Taking 20 °C settings as an example the germination chamber has been calibrated for:

- 12 hours of light, beginning at 6 AM (6:00) with a temperature of 20 °C with 50% humidity;
- 12 hours dark, beginning at 6 PM (18:00) with a temperature of 20 °C with 50% humidity.

For the other temperatures were used the same settings of light-time and humidity.

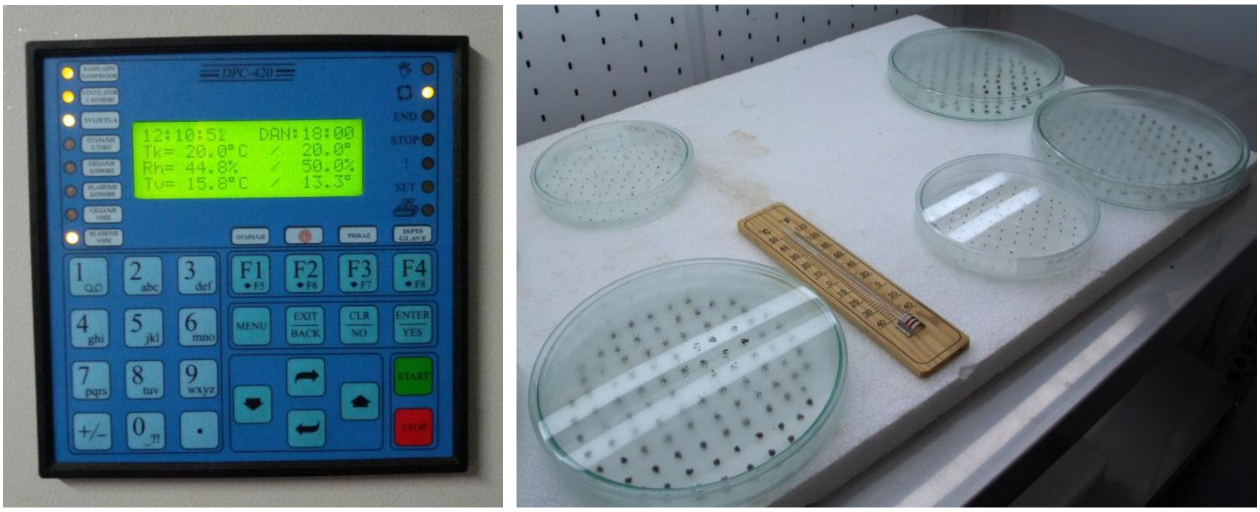

Figure 15. Germinator's settings for 20°C and Petri dishes inside the germinator *(photos: Dario Magosso)*.

3.6 Base temperature

Base temperatures were calculated with the method proposed by Roché et al. *(1997)*. Three replicates of 100 seeds of each of the four species were incubated at a range of constant temperatures in 14 and 19 cm-diam Petri Dishes with 5 ml or 10 ml of water. Germination was recorded at about 24-h intervals until no further germination occurred for 4 days. The seeds were defined as germinated at the time of visible radicle emergence. The germination time course was analyzed using a logistic function in the Bioassay97 program *(Onofri, 2001)* and the time necessary for 50% germination was estimated. A linear regression, estimated using the bootstrap method *(Efron, 1979)*, provided the best fit of germination rate (reciprocal of time to 50% germination) against incubation temperature *(figure 16)*. The base temperature was estimated as the intercept of the regression line with the temperature axis *(Masin et al., 2005; Masin et al., 2010)*.

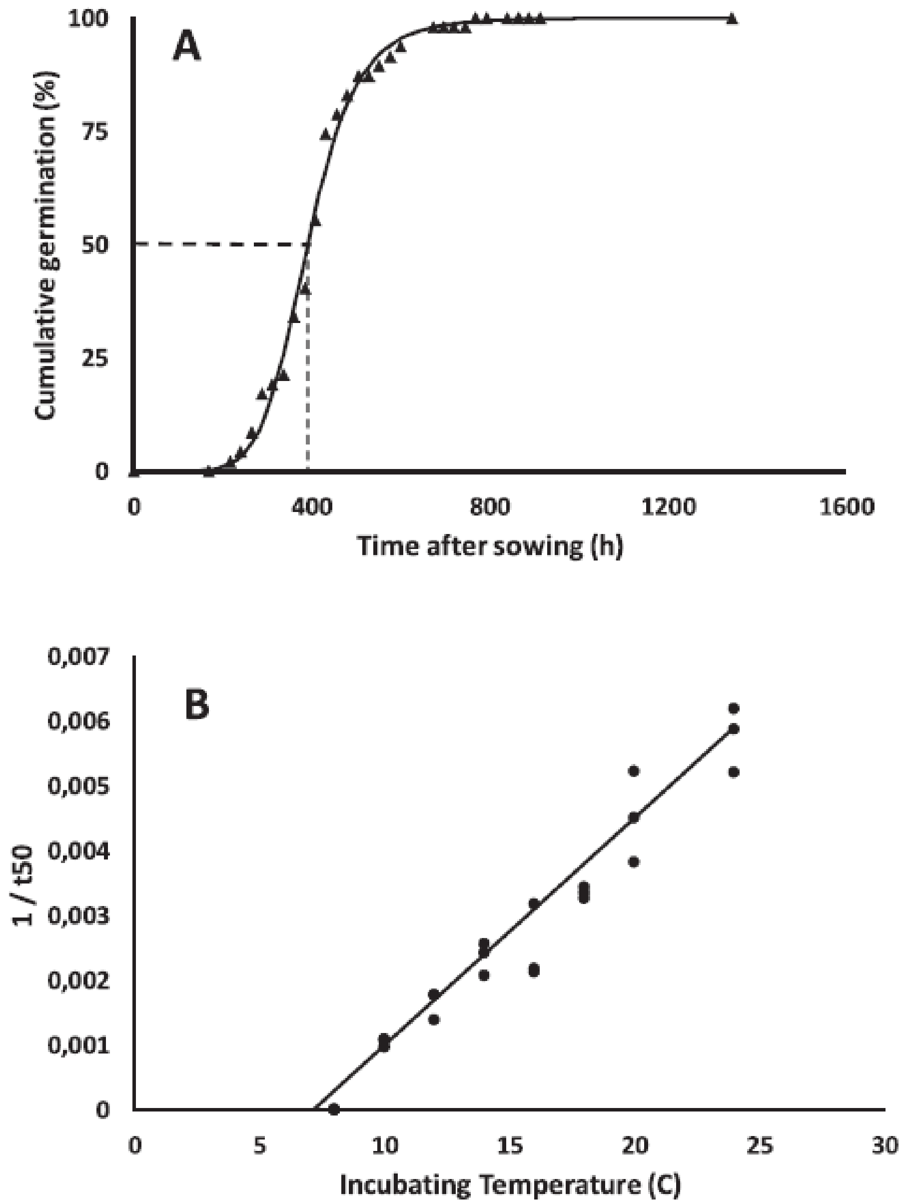

Figure 16. Example of the methodology for estimation of the base temperature (T_b) for germination using the method of reciprocal time to median germination. The first graph (A) shows the procedure to estimate germination of half the germinated seeds (t_{50}) of a single replicate at a given incubating temperature through a logistic function. The points are the observed germination data and the solid line represents the predicted value determined from the logistic function. The second graph (B) displays the procedure of estimation of T_b for germination for a given experimental lot: the points are the calculated germination rate ($1/t_{50}$) of the three replicates at different incubating temperatures and the solid line represents the linear regression line. The Tb for germination was estimated as the intercept of the regression line with the incubating temperature axis.

4. RESULTS AND DISCUSSION

In this session the results of *A. retroflexus* are not discussed because of the insufficient germination for the statistical analysis of the data.

4.1 *Abutilon theophrasti*

Final germination percentage of *A. theophrasti* was above 50% at each temperature *(table 13)*, the highest germination was observed at 16 and 24 °C with an average of 79 and 77%. Even the lowest percentage of germination (47 % at 28 °C) was sufficient for the statistical analysis of the germination trend.

Table 13. Percentage of final germination observed for each species at the different constant temperatures.

	Final germination (%)						
	4 °C	8 °C	12 °C	16 °C	20 °C	24 °C	28 °C
R1	16	77	58	92	61	82	47
R2	6	75	55	76	65	67	55
R3	21	74	58	69	62	83	50
mean	14	75	57	79	63	77	51
st.dev.	7,64	1,53	1,73	11,79	2,08	8,96	4,04

As an example, in *figure 17* it is reported the cumulated germination at 24 °C. It is possible to observe that the germination starts on the third day, it means that there is a lag phase before the beginning of germination during which the seeds accumulate the thermal time (degree days) needed for germination, then the curve shows the typical S-shaped behavior, with the initial phase approximately exponential. The inflexion point is reached after about 4 days (3,97 ± 0,072, mean ± standard error). The curve reaches its maximum the eighth day, with 83 seeds (83,34 ± 1,20) germinated on the 100 sowed in the petri dish. The curve fits satisfactory the data of germination (R^2 = 0,99).

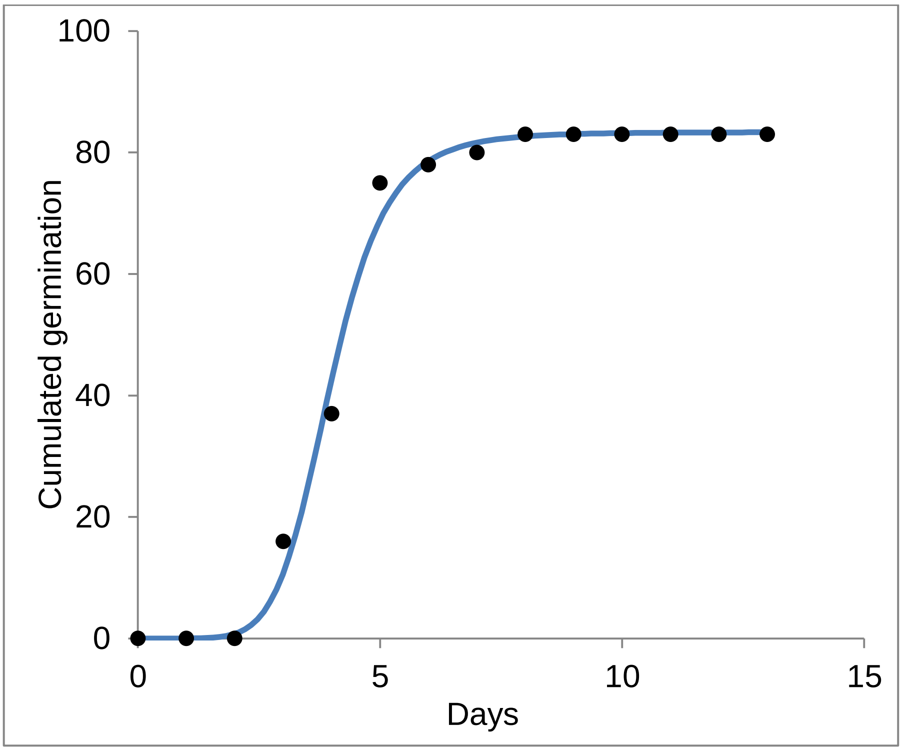

Figure 17. Cumulated germination of *A. theophrasti* incubated at 24 °C. The black circles indicates the observed germinations and the blue line the simulation.

Analyzing the linear regression across all temperatures *(figure 18)*, it is possible to observe that the germination rate increases as the incubation temperature increases following a linear trend. The accuracy of the linear estimation is high with an R^2 of 0,90 (R^2 = 1 means perfect estimation).

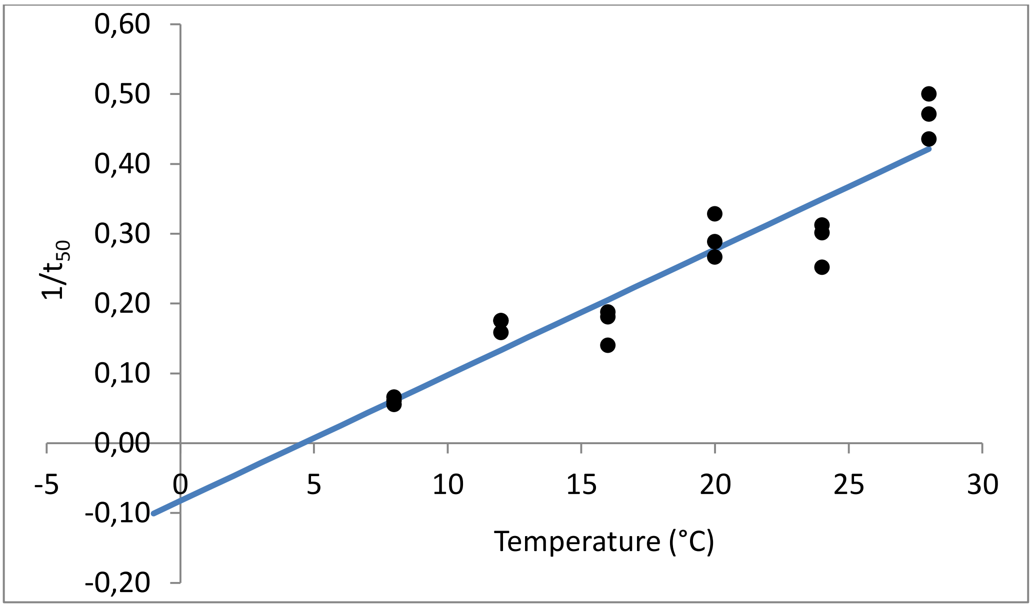

Figure 18. The solid line represents the linear regression line (y = 0,018x - 0,0826, R^2 = 0,90) and the points are the calculated germination rate ($1/t_{50}$).

4.2 *Chenopodium album*

In germination tests, *C. album* reached the highest germination percentage among the studied species, some replicates at 16 and 20 °C showed 100% of germination *(table 14)*. High percentage of germination was observed for this species also at 24 °C (mean germination percentage of 98), while germination decreased significantly with incubating temperature below 12 °C and above 28 °C.

Table 14. Percentage of final germination observed for each species at the different constant temperatures.

	Final germination (%)						
	4 °C	8 °C	12 °C	16 °C	20 °C	24 °C	28 °C
R1	10	46	37	100	100	97	60
R2	13	37	51	94	90	98	55
R3	8	26	35	97	99	99	66
mean	10	36	41	97	96	98	60
st.dev.	2,52	10,02	8,72	3,00	5,51	1,00	5,51

The linear regression across all temperatures *(figure 19)* fits the data satisfactory (R^2 = 0,98).

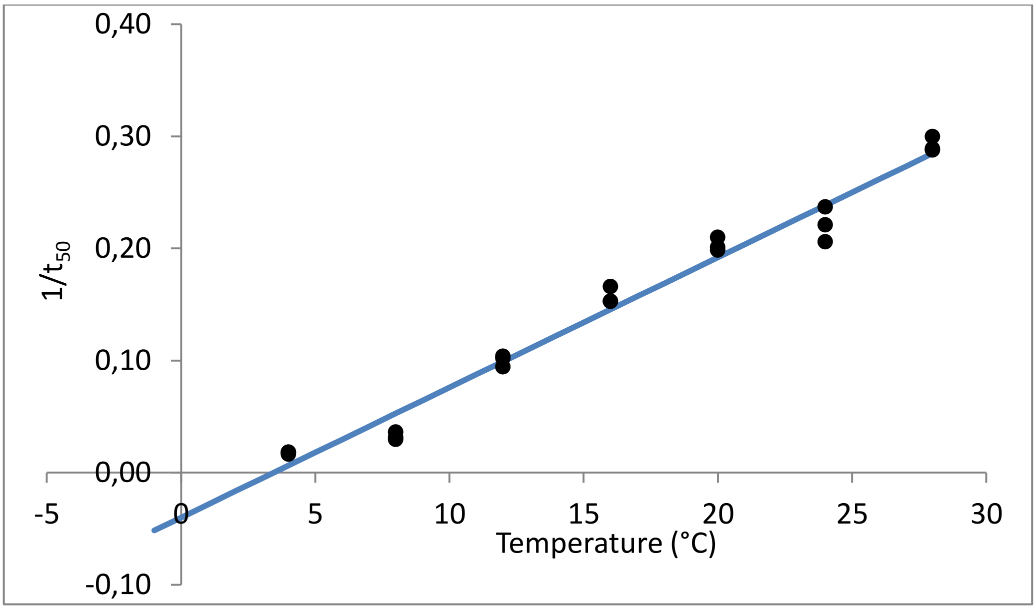

Figure 19. The solid line represents the linear regression line (y = 0,0116x - 0,04, R^2 = 0,98) and the points are the calculated germination rate ($1/t_{50}$).

4.3 *Echinochloa crus-galli*

E. crus-galli germinated with high percentages at 16, 20 and 24 °C, a reduction was observed at temperature of 28 °C and 12 °C, lastly no germination was observed at 8 °C *(table 15)*.

Table 15. Percentage of final germination observed for each species at the different constant temperatures.

	Final germination (%)					
	8 °C	12 °C	16 °C	20 °C	24 °C	28 °C
R1	0	5	92	91	84	75
R2	0	3	93	91	90	67
R3	0	14	90	97	94	61
mean	0	7	92	93	89	68
st.dev.	0,00	5,86	1,53	3,46	5,03	7,02

The linear regression across all temperatures *(figure 20)* fits the data satisfactory (R^2 = 0,94).

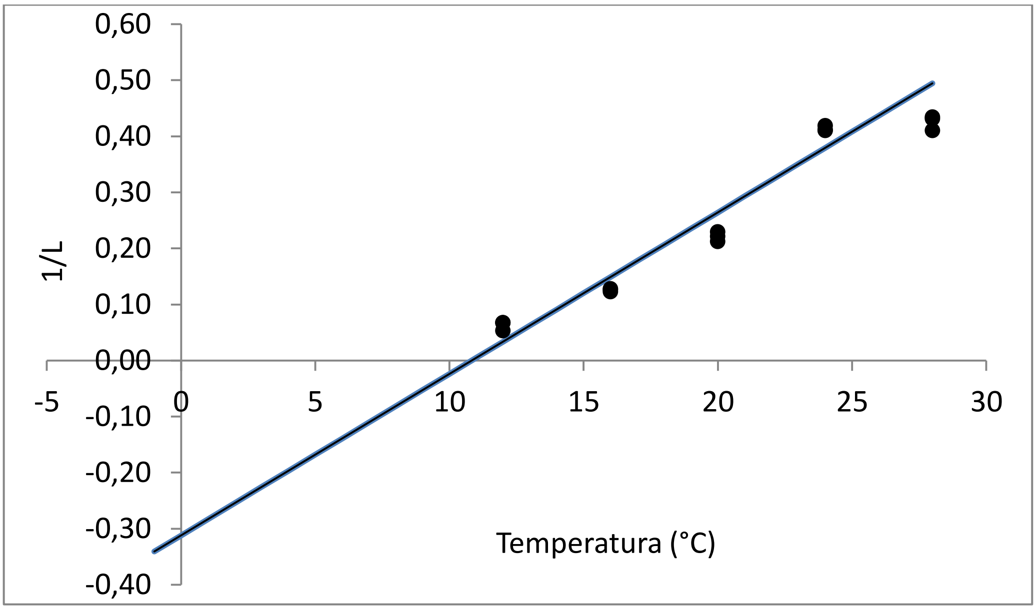

Figure 20. The solid line represents the linear regression line (y = 0,0288x - 0,03119, R^2 = 0,94) and the points are the calculated germination rate ($1/t_{50}$).

4.4 Base Temperature

The base temperature (T_b) estimated with the linear regression analysis (see the chapters above) are reported in *table 16*. The lowest value of T_b among the studied species was observed in *C. album* with 3,4 °C. *A. theophrasti* showed a base temperature of 4,5 °C, slightly higher than *C. album*, whereas *E. crus-galli* has shown a T_b of 10,8 °C, higher than the other studied species.

Table 16. Base temperatures (T_b) estimated with the bootstrap method, 95% confidence interval (95% CI), and coefficient of determination (r^2)

	T_b	95% CI	r^2
ABUTH	4,5	1,23	0,90
CHEAL	3,4	0,36	0,98
ECHCG	10,8	0,27	0,94

Loddo et al. *(2013)* studied three different ecotypes of *A. theophrasti* from Italy, Portugal, and Spain, the base temperatures obtained ranged from 3,1 to 5,0 °C. Also Sartorato and Pignata *(2008)* obtained a value of 6,5 °C, which in any case resulted not statistically different. On the contrary, different values were reported from Dorado et al. *(2009)* in central Spain, who found a base temperature of 6,8 °C in nonchilled seeds and 7,2 °C in chilled seeds buried in soil. The T_b of the Croatian ecotype of *C. album* is similar to that found by Vleeshouwers and Kropff *(2000)*, 2,0 °C, and by Roman et al. *(2000)*, 4,2 °C. Wiese and Binning *(1987)* and Guillemin et al. *(2012)* obtained an higher base temperature, near to 6°C in Wisconsin and Dijon, France. *E. crus-galli* has reached the highest T_b for the species studied. The estimated base temperature is confirmed by Wiese and Binning *(1987)*, Forcella *(1998)*, Sartorato and Pignata *(2008)* that found a value of 10 °C. Anyway for this species more than for the other, the literature reports a great variability in base temperatures among ecotypes of different geographic origins. Higher base temperatures was found from Steinmaus et al. *(2000)*, with 13,8 °C, but also lower temperatures as 5 °C was found by Sadeghloo et al. *(2013)* and 6,2 ± 0,57 by Guillemin et al. *(2012)*. It seems that the tendency for this species is to increase the base temperature from the coolest to the warmest region of origin, with 6,2 °C for a population harvested in north-eastern France *(Guillemin et al., 2012)*, 10 °C for populations from Italy *(Sartorato and Pignata, 2008)* and 13 °C for seeds from California *(Steinmaus et al., 2000)*. Conversely, base temperatures for populations harvested in Wisconsin *(10 °C; Wiese and Binning, 1987)* and in Minnesota *(10 °C; Forcella, 1998)* were higher than those measured in France, even though the yearly mean temperatures are lower in the two American states (6,6 °C) than in north-eastern France (10,6 °C).

Table 17. Base temperatures from literature.

	T_b	source
ABUTH	From 3.1 to 5,0	Loddo et al., 2013
	5	Sadeghloo et al., 2013
	6,5	Sartorato and Pignata, 2008
	6,8	Dorado el al., 2009
CHEAL	2,0	Vleeshouwers and Kropff, 2000
	4,2	Roman et al., 2000
	5,9 ± 0,48	Guillemin et al., 2012
	6	Wiese and Binning, 1987
ECHCG	5,014	Sadeghloo et al., 2013
	6,2 ± 0,57	Guillemin et al. 2012
	10	Wiese and Binning, 1987; Forcella, 1998; Sartorato and Pignata, 2008
	13,85	Steinmaus et al., 2000

The aim of this study was to estimate the base temperature of Croatian ecotypes in order to compare them with Italian ecotypes for the same species. This comparison is important to verify the difference or similitude in germination-emergence behavior of ecotypes of species simulated by the model AlertInf and therefore to evaluate the transferability of the model. Comparing the results of this study with the results of Masin et al. *(2010) (table 18)*, obtained studying Italian ecotypes of the same species collected in the Veneto region, it is possible to observe that the base temperatures of *A. theophrasti* and *C. album* are confirmed. The temperature threshold levels were not significantly different between ecotypes, based on 95% bootstrap confidence intervals. Therefore for these two species, it is possible to conclude that the same T_b can be used both for Croatian and Italian ecotypes. An interesting results, considering that the experiments to estimate the temperature thresholds are very time and resource consuming and the necessity of calculating these threshold parameters in different climatic areas is an important limitation in the creation and adoption of weed emergence predictive models, as AlertInf. Unfortunately, this is not the case of *E. crus-galli*, the base temperature of this species collected in Croatia was similar, but statistically different (if the method of the 95% bootstrap confidence intervals is used), to that collected in Italy. The Italian ecotype of which did not germinate if the temperature was less than 11,7 °C, whereas the Croatian ecotype showed a lower base temperature of 10,8 °C. This difference may be explained by the tendency of this species to develop local ecotypes adapted to the environmental conditions of specific geographical locations greater than the other two studied species *(Tasrif et al., 2004)*. This tendency determine differences on various characteristics that may include morphological and growth traits, such as germination parameters. It is confirmed by the wide range of base temperatures estimated among ecotypes of different geographical areas, as previously shown in table 15. In the case of Italy and Croatia, the climate is very similar, but slightly wormer in Italy, therefore the climate seems to influence the thermal response of this species following the same behavior reported in literature (see discussion above): a tendency for this species to increment the base temperature if the climate of the region of origin is warmer. This finding is important to characterize the species and distinguish between those which do not need the re-estimation of the threshold parameters and those for which it is fundamental to recalculate the thresholds when the model is used in a location other than that of creation.

Table 18. Base temperatures and 95% confidence interval of ecotypes collected in Italy (Padova) *(Masin et al., 2010)*.

	T_b	95% CI
ABUTH	3,9	0,59
CHEAL	2,6	0,77
ECHCG	11,7	0,28

5. CONCLUSIONS

Base temperature for germination is a key parameter for developing weed germination and emergence models *(Grundy et al., 2000)*. This threshold, via thermal and hydrothermal time, determines the speed and dynamics at which seeds germinate *(Gardarin et al., 2011)*. The use of thermal or hydrothermal time in predictive model is important to consider the variability between years in germination-emergence timing. The European Union recently published 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 and alternative approaches or techniques (European Parliament 2009). In the case of integrated weed management (IWM), weed emergence models could be innovative tools to aid in achieving these goals *(Grundy, 2003)*. AlertInf is an already used and appreciated predictive model in North-estern Italy. Considering that Croatia has become recently member of the European Union to study the transferability of AlertInf from Italy to Croatia can be seen as part of the ambitious project of spreading the use the Decision Support Systems (DSS) in the European area.

As shown in this thesis, for some weed species the same thermal thresholds can be used also in different geographical areas, without conducting specific experiments that are time and sources consuming. To be able to characterize the species according to their tendency to develop local ecotypes, different in terms of parameters of germination, is fundamental to reduce and simplify the experiments needed for transfer a model from the location of creation to other, with a different climate. The contribution given by this thesis is just an small part of the work needed for transfer AlertInf from Italy to Croatia, further experiments must be conducted for estimate the base parameters of other weed species and the emergence dynamics must be evaluated using independent field data.

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