Swedish University of
Agricultural Sciences

Temperature requirement for germination of *Solanum nigrum* seeds

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Uppsala 2009

To my beloved brother

Ali

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Abstract

Temperature requirement for germination in *Solanum nigrum* seeds.

Solanum nigrum has become an increasingly difficult weed in field-grown vegetables in the southern part of Sweden during the last decade. To be able to predict the timing of seedling emergence in the field, which is important for the timing of weed control measures, a study was made with the aim to investigate the timing of seed germination in the weedy species *Solanum nigrum* L., as affected by the level of dormancy and temperature conditions. Germination requirements of non-stratified seeds and seeds stratified at 5 and 15 °C were investigated. Germination was tested at i) constant temperatures ranging from 6 to 38 °C, ii) alternating temperatures with 15 °C constant amplitude and increasing mean temperatures from 16 to 33.5 °C, and iii) alternating temperatures with constant mean temperature of 25 °C and increasing amplitude from 0 to 20 °C. It was concluded that seed germination of *S. nigrum* depends on the level of dormancy and, to a large extent, on the current temperature. Seeds with lower level of dormancy germinate at constant temperatures and alternating temperatures result in a high percentage of germination in seeds with different levels of dormancy.

Keywords: Germination, seed dormancy, *Solanum nigrum*, stratification, temperature.

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Introduction

Solanum nigrum L. (black nightshade) is a common weed throughout the world, reported as a weed in 61 countries and 37 crops (Holm *et al.*, 1991). Therefore, effectively controlling this weed is vital for successful crop production. Within the range of available weed control practices, mechanical and chemical controls are the most commonly used methods under field conditions. The success of these control methods depends on reaching the highest number of individuals in the seedling stage. Therefore, to increase their effectiveness we must be able to predict the timing at which certain weed species emerge. The aim of the presented study was to increase the knowledge on temperature requirement for germination in S. nigrum. This knowledge is essential for predicting the time of emergence.

Solanum nigrum

Nightshades belong to the Solanaceae family, which also includes potato, tomato, and eggplant. The genus shows large variations in properties, e.g. fruit type, and many species display morphological variation in response to the environment. Historically there has been confusion in the classification of *Solanum* species and the relations between them still have not been resolved taxonomically (Defelice, 2003). The two main nightshade species occurring as weeds in Sweden are *S. nigrum* L. (black nightshade) and *S. physalifolium* Rusby (green nightshade) (Berlin, 2003). *S. nigrum* is an annual herbaceous plant that can grow 50 cm tall. The stem is with or without hair and is strongly branched with egg-shaped leaves. *S. nigrum* has white flowers, and is primarily self-pollinating**.** The berries are black, or in rare cases green, round and 6-10 mm in diameter). Like all *Solanum* species, the nightshades contain the poison solanin. The levels vary between different species, varieties and organs. The nightshades as weeds reduce the yield by competition with the crop, as well as reducing or destroying the crop quality if the berries are harvested with the crop (Defelice, 2003). The poisonous and badly tasting berries constitute a special problem in garden pea production since they have the same size and colour as peas and cannot be separated automatically (Andersson, personal communication). In North America the nightshades constitute a larger problem in small seeded vegetable crops like tomato and broccoli than in big seeded soybeans. Also, in North America the black nightshade has been reported to be a host for nematodes, bacteria, fungi, mycoplasma, and viruses that can be damaging to a wide range of crops. But, this property has been exploited by using nightshades as a trap crop with the ability to reduce pest infestations (Defelice, 2003).

Dormancy

To survive in the soil a seed must not only remain viable, but germination must be avoided until conditions for seedling establishment and growth are good. A viable seed may fail to germinate because of quiescence, dormancy, or both. Quiescence may be enforced by the environment, like in air-dry seeds or in imbibed seeds below the base temperature for germination of non-dormant seeds (Labouriau, 1970; Garcia-Huidobro *et al*., 1982). Dormancy is a state of the seed that does not permit germination, although conditions for germination may be favorable with respect to temperature, water, and oxygen. Timing of seed germination can be critical for the survival of a natural plant population, and dormancy plays a major role in such timing (Lambers *et al.,* 1998).

During seed development on the mother plant, there is an increase in concentration of abscisic acid (ABA). This phytohormone is involved in the prevention of precocious germination, synthesis of reserve proteins, the development of desiccation tolerance, and the induction of primary dormancy (Lambers *et al.,* 1998).

According to Baskin & Baskin (1998), there are six types of seed dormancy:

A: Physiological dormancy (PD) is caused by physiological inhibiting mechanisms of the embryo that prevent radicle emergence.

B: Morphological dormancy (MD); the embryo is immature or not fully developed at fruit maturity.

C: Morphophysiological dormancy (MPD) occurs in seeds with rudimentary or linear embryos. It is a combination of morphological and physiological dormancy, i.e., the underdeveloped embryo has physiological dormancy.

D: Physical dormancy; the primary reason for the lack of germination is the impermeability of seed coats to water.

E: Physical plus physiological dormancy; seeds have impermeable coats and dormant embryos.

F: Chemical dormancy; chemically dormant seeds do not germinate due to the presence of inhibitors in the pericarp. Removal of the pericarp or leaching of the fruits breaks chemical dormancy.

Seeds of winter annuals germinate in autumn and fulfil their life cycle in spring or early summer. If seeds are conditionally dormant they germinate only at temperatures that do not occur in the habitat in late spring or summer (Baskin & Baskin, 1983). Dormant or conditionally dormant seeds of obligate or facultative winter annuals become non dormant at high but not at low temperatures (Baskin & Baskin, 1986). They must be exposed to high summer temperatures for several months to germinate at autumn temperatures (Baskin & Baskin, 1984; Roberts & Nilson, 1982).

Seeds of summer annuals germinate in spring or summer, and the resulting plants complete their life cycle before or at the time of frost in autumn. At maturity in autumn, seeds are dormant or conditionally dormant, depending on the species. Conditionally dormant seeds will germinate at high (35/20, 30/15 °C) but not at low (15/6, 20/10 °C) temperatures. During winter, dormant seeds of summer annuals become conditionally dormant and then eventually non-dormant. When seeds become conditionally dormant, they will germinate only at high temperatures (Baskin & Baskin, 1983). When seeds become non-dormant, they can germinate over a range of temperatures including those in the habitat in spring. Seeds that are conditionally dormant in autumn also come out of dormancy during winter and the minimum temperature for germination declines (Baskin & Baskin, 1998).

Dormancy in S. nigrum

S. nigrum is regarded as a spring and summer germinating species and has a peak of germination in June in southern Sweden (Andersson, personal communication). Induction of dormancy or conditional dormancy in seeds of spring and summer germinating summer annuals is controlled by temperature. Maximum soil temperature was the factor inducing dormancy in summer annuals that stopped germinating in late July or in late August in Japan (Watanabe & Hirokawa, 1975). In addition, seeds entered dormancy earlier at 0-5 cm than at 10 to 15 cm soil depths.

Germination

Species from a wide range of plant families, life cycle types, and plant communities exhibit differences in germination characteristics of seeds collected at different locations. Depending on the species, germination responses vary with latitude, elevation, soil moisture, soil nutrients, temperature, kind and density of plant cover, and degree of habitat disturbance of the sites where the seeds matured (Baskin & Baskin, 1998). Requirements for germination differ among species, but generally, species with small seeds tend to require light for germination more than large seeded species (Milberg *et al*., 2000). The timing of seed germination is triggered by environmental cues. The most common cues are light quality and quantity, temperature, moisture and gases (O_2 and CO_2). These generally vary on large scales but they also vary locally.

The effect of temperature

Long (1989) recognized three separate physiological processes in seeds that are affected by temperature:

– Temperature together with moisture content determine the rate of deterioration in all seeds,
– Temperature affects the rate of dormancy loss in dry seeds and the patterns of dormancy change in moist seeds,
– In non-dormant seeds temperature determines the rate of germination.

Alternating temperatures are required for the seed germination of many weeds (Booth *et al.*, 2003). According to Roberts & Lockett (1978), the effect of temperature fluctuations depends on the:

- Amplitude (difference between maximum and minimum temperatures),
- Mean temperature,
- Thermoperiod.

Emergence

Emergence usually refers to the appearance of a shoot above the soil or a root from the seed. The timing of seedling emergence is important because it determines whether an individual will be able to compete with its neighbors (Forcella *et al*., 2000). The timing of seedling emergence is determined by the interaction of seed size; dormancy, germination, and the rate of stem and root elongation with abiotic factors (Meyer & Allen, 1998; Forcella *et al*., 2000; Roman *et al*., 2000). In an ordinary seedbed, weeds may emerge at very different times in relation to the crop. In a good seedbed, the majority of the plants of cereals and many other crops emerge within 1-2 days following the first emergence, but the period is often longer (Håkansson, 2003). In crops such as cereals whose stand close rapidly and exert a strong competition against weeds from early stages, weed plants emerging late largely exhibit a very weak growth and sometimes a high mortality. However, many weeds can grow and reproduce when light improves in the maturing crops. Crops with stands closing late, such as sugar beet, potato and vegetable, exert a weak competition in a long period of early growth. Late emerging weed plants can often grow vigorously and create problems in these crops. Even at low plant densities, early-emerged weeds must be actively controlled (Håkansson, 2003).

Emergence of S. nigrum

According to (Håkansson, 2003) certain species prone to late germination such as *S. nigrum*, are troublesome in weakly competitive crops in many areas. Plant emergence is reduced with increased seed depth placement.

Zhou *et al.* (2005) studied the relationship of depth and emergence in *S. nigrum*. They concluded that the maximum emergence of seedlings occurred at planting depths of 2 cm or less. Emergence of seedlings decreased with increased seeding depth larger than 2 cm. No emergence occurred when seeding depth reached 8 cm.

Roberts & Lockett (1978) showed that the emergence in *S. nigrum* started in late April or early May, and ceased at the end of August. Within this period, the timing and extent of emergence depended partly on rainfall and partly on the time of soil disturbance. However, the great number of seedlings usually appeared in May and June.

Objective of the study

The aim of this study was to determine whether there is a difference in temperature requirements for seed germination in *S. nigrum* seeds with different levels of dormancy. Seeds were subjected to three pre-treatments and subjected to germination tests with i) constant temperatures, ii) constant temperature amplitude with increasing mean temperature, and iii) constant mean temperature with increasing temperature amplitude.

Material and methods

Seeds

The seeds were collected in April 2006 from plants that had been grown in a greenhouse at ca. 17/8°C day/night. Seeds were after harvest stored for nine months at 6 °C. To test the initial level of dormancy before starting the experiment a preliminary germination test was carried out at 25/15 °C, 16/8 hours light/darkness, and 18/8 °C, 16/8 hours light/darkness. The test was terminated after two weeks.

Stratification

To enable an experiment with seeds with different levels of dormancy, three seed portions were used: i) ”Fresh seeds” (SF) taken directly from the fridge at the start of the experiment, ii) seeds stratified at 5 °C (S5), and iii) seeds stratified at 15 °C (S15). Before stratification, 10 samples of 50 seeds were weighed and the mean weight was calculated. Fifty seeds (0.042 g) were evenly spread in each of 87 Petri dishes (ø 55 mm) on two filter papers moistened with de-ionized water. The dishes were immediately sealed with parafilm and aluminium foil, and thereafter placed in stratification cabinets.

Table 1. Constant, maximum, minimum and mean temperature and temperature amplitude in three experiments investigating temperature requirements for seed germination in *Solanum nigrum*

	Temperature (˚C)								
Experiment 1									
Temp	6	10	14	18	22	26	30	34	38
Experiment 2									
Day (16 hr)	21	23.5	26	28.5	31	33.5	36	38.5	
Night (8 hr)	6	8.5	11	13.5	16	18.5	21	23.5	
Mean temp	16	18.5	21	23.5	26	28.5	31	33.5	
Amplitude	15	15	15	15	15	15	15	15	
Experiment 3									
Day (12 hr)	25	27.5	30	32.5	35				
Night (12 hr)	25	22.5	20	17.5	15				
Mean temp	25	25	25	25	25				
Amplitude	0	5	10	15	20				

Experiment 1: Constant temperatures

Seeds with three levels of dormancy (SF, S5, and S15) were tested for germination at nine constant temperatures, 16 hr in light and 8 hr in darkness (Table 1). The experiment started on 23 March 2007 and was terminated after two weeks.

The germination test was conducted using a temperature gradient incubator (Ekstam & Bengtsson, 1993; see photo page 21), enabling a linear gradient of constant temperatures as well as amplitudes. The incubator consists of an aluminium block with ten milled tracks. Stratified and fresh seeds were placed on two layers of filter paper in the tracks along the gradient. The experimental design was complete randomisation with three replicates. The germinated seeds were counted every second day and removed. The criteria for germination were protrusion of the radicle more than 1 mm. Ungerminated seeds at termination of the germination test were characterised as dormant or dead. Seeds with firm and white endosperm were considered as viable dormant, while those with soft and dark endosperm were considered dead. Pressing the seeds with a pair of tweezers assessed firmness.

Experiment 2: Mean temperature gradient with constant amplitude

Experiment 2 started 12 April 2007. The test encompassed eight mean temperatures with the same, 15 °C, amplitude (Table 1). The time for shift from minimum to maximum temperature and vice versa was 1 hr. Pre-treatment of seeds and handling of seeds during the germination test was as presented for experiment 1.

Experiment 3: Constant mean temperature with amplitude gradient

Experiment 3 started 3 May 2007 and was terminated two weeks later. The germination test encompassed five diurnal temperature amplitudes with the same mean temperature of 25 °C (Table 1) and three replicates. Seeds were tested for 12 hr in light, high temperature and 12 hr in darkness, low temperature. Pre-treatment of seeds and handling of seeds during the germination test were as presented for experiment 1.

Statistics

A statistical analysis was conducted to test the effect of the fixed independent factors pre-treatment, temperature and the interaction between those on germination of *S. nigrum* seeds. A logic analysis was performed, assuming binomially distributed data and a logic link function in the R software v. 2.7 (Andersson, L., 2008).

Results

The statistical analyse revealed highly significant differences between pre-treatments (SF, S5, and S15), temperatures, and pre-treatment x temperature in all three experiments (Tables 2, 3, 4). Germination was largely regulated by temperature but the effect varied depending on pre-treatments.

Experiment 1: Constant temperatures

There was no germination in non-stratified seeds at constant temperatures. Seeds stratified at 5 °C germinated in a range of temperatures between 18 and 34 °C with 42% germination at optimum temperature of 26 °C (Figure 1). Seeds stratified at 15 °C germinated at the same range of temperature but with higher percentage of germination, close to 50%, at 30 °C (Figure 1).

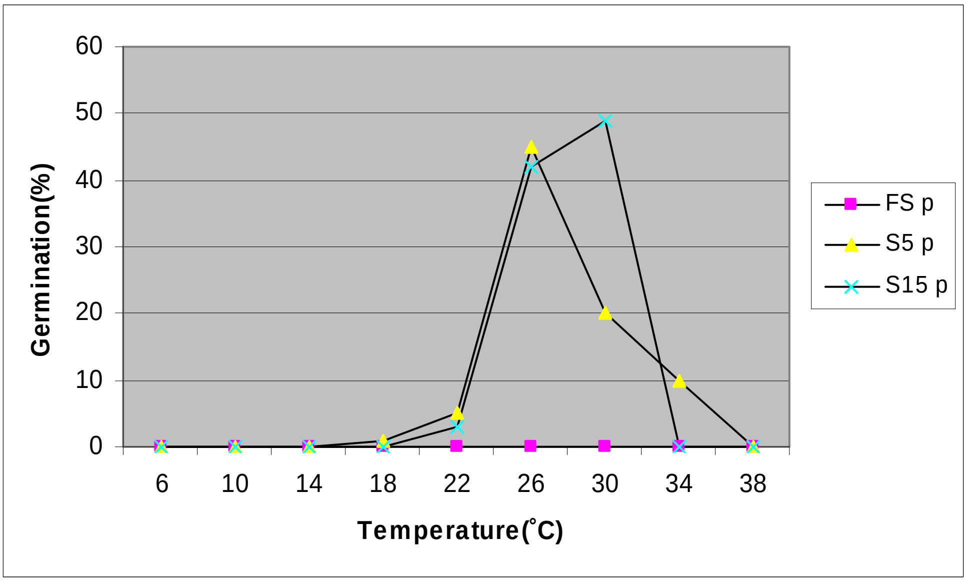

Figure 1. Effect of constant temperatures on germination in *Solanum nigrum*. Seeds were non-stratified (FS), stratified at 5 °C (S5) or stratified at 15 °C (S15).

Table 2. Maximum-likelihood deviance of germination in *S. nigrum* at constant temperature

Factor	df	Deviance	*P*≥
Pre-treatment	2	230.33	0.0001
Temperature	8	694.90	0.0001
Pre-treat x Temperature	16	46.32	0.0001

Experiment 2: Mean temperature gradient with constant amplitude

Germination test at increasing mean temperatures with constant amplitude (15 °C) showed that the level of seed dormancy influenced the response to temperature. The germination of non-stratified seeds was significantly lower than stratified seeds. Seed germination decreased at higher temperatures (Figure 2).

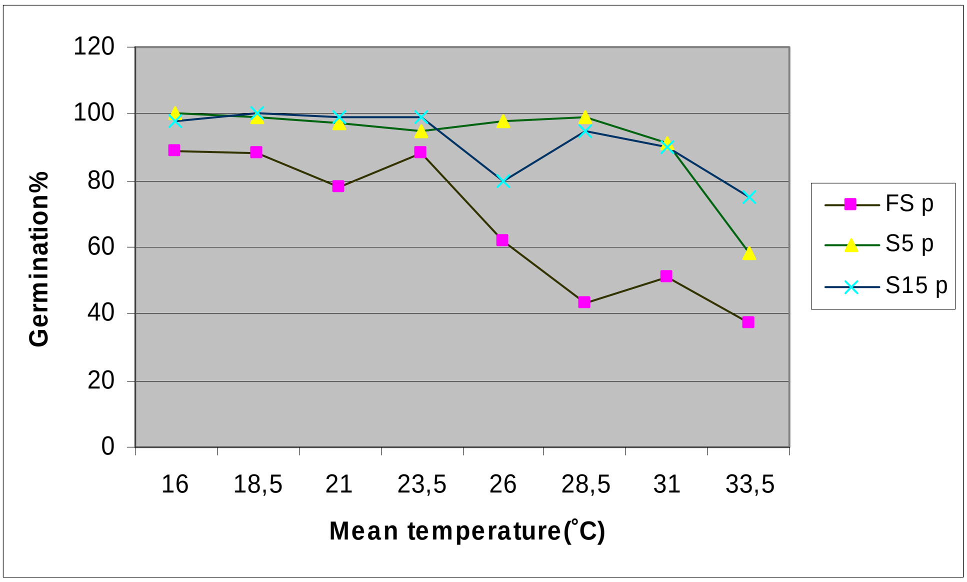

Figure 2. Effect of increasing mean temperatures (amplitude 15 °C) on germination in *Solanum nigrum*. Seeds were non-stratified (FS), stratified at 5 °C (S5) or stratified at 15 °C (S15).

Table 3. Maximum-likelihood deviance of germination in *S. nigrum* at increasing mean temperature

Factor	df	Deviance	*P*≥
Pre-treatment	2	328.75	0.0001
Temperature	7	444.73	0.0001
Pre-treat x Temperature	14	116.25	0.0001

Experiment 3: Constant mean temperature with amplitude gradient

The germination test at increasing temperature amplitude (constant mean temperature 15 °C) revealed a germination peak at 10 °C amplitude. Seeds stratified at 5 °C germinated more than those stratified at 15 °C at all amplitudes except 10 °C. Non-stratified seeds germinated less than stratified seeds (Figure 3).

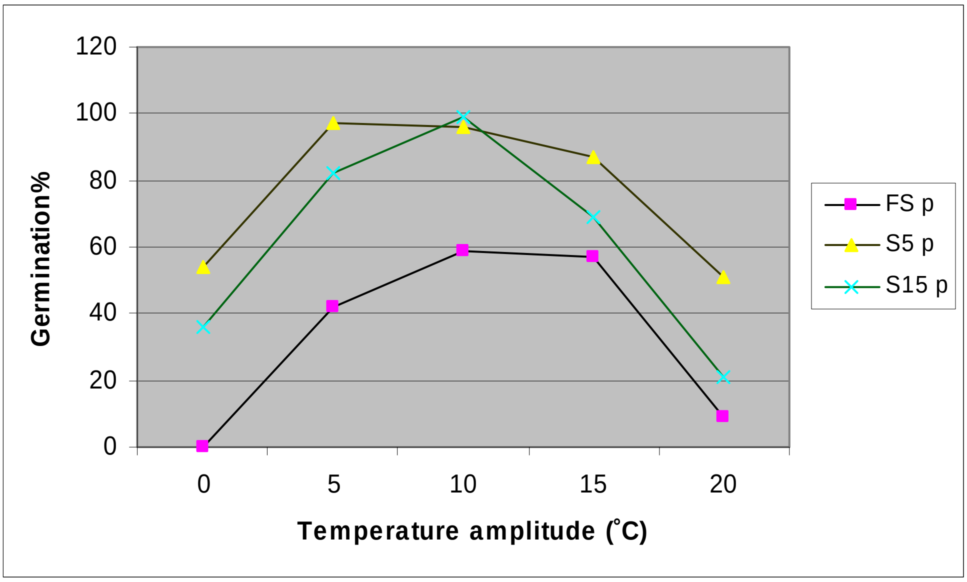

Figure 3. Effect of increasing temperature amplitude on germination in *Solanum nigrum*. Seeds were non-stratified (FS), stratified at 5 °C (S5) or stratified at 15 °C (S15).

Table 4. Maximum-likelihood deviance of germination in *S. nigrum* at increasing amplitude

Factor	df	Deviance	*P*≥
Pre-treatment	2	301.11	0.0001
Amplitude	4	650.90	0.0001
Pre-treat x Amplitude	8	85.81	0.0001

Discussion

Temperature is the single most important factor regulating germination of non-dormant seeds in irrigated, annual agroeco-systems at the beginning of the growth season where light, nutrients, and moisture are typically not growth limiting (Garcia-Huidobro *et al*., 1982). Temperature has a dual role; on one hand it regulates the seasonal changes in dormancy, and on the other hand seed germination. In the present project, I studied the effect of varying, constant, and alternating temperatures on germination. In addition, how different levels of dormancy influences the temperature requirement was also studied.

My results showed that, with 16/8 hours photoperiod, *Solanum nigrum* seeds in Experiment 1 required temperatures between 18 and 34 °C for germination in seeds stratified at 5 and 15 °C. The optimum temperature for germination was between 26 and 30 °C. Finch-Savage & Leubner-Metzger (2006) studied the responses of five *Solanum* species to constant and diurnally alternating tempera-ture, full light, light filtered through green leaves, and darkness. They concluded that germination of *S. nigrum* seeds was generally poor (<20%) at the different constant temperatures between 5-45 °C with optimum 28-33 °C. According to Zhou *et al*. (2005) germination of *S. ptychanthum* under light occurred only when temperatures were 20 °C or higher for 12 to 24 h d^{-1}. Optimum germination occurred within the constant temperature range of 28 to 33°C. This relatively high temperature requirement for germination of *S. nigrum* is probably one reason why this species emerge later in growing season than many other weed species.

The germination test at increasing alternating temperatures showed that a high percentage of seeds germinated at the different levels of dormancy. A large proportion of the fresh seeds germinated at lower alternating temperatures but with tendency of reduced germination at higher temperatures.

Finch-Savage & Leubner-Metzger (2006), when studying germination of five *Solanum* species, concluded that alternating temperatures stimulated germination. Germination percentage was significantly higher in seeds tested at 20/12 and 30/12 °C compared with those tested at either 20 or 35/12 °C. Seeds of *S. incanum*, *S.*

luteum and *S. nigrum* were capable of germinating in the dark at diurnally alternating temperatures of 28/13 °C. The results indicate that seeds from the four species would germinate on or in the soil if the vegetation covers was removed, resulting in temperature fluctuations.

Riemens *et al.* (2004), through the experiment based on the levels of dormancy and germination of weed seeds, indicated that temperature not only determined the germination percentage but also the germination rate. Low temperatures resulted in a low germination rate whereas high temperatures showed increased germination rate. It can be concluded that temperature has an important role in germination of weed seeds, since it both determines the range of temperatures over which germination is possible (dormancy) and is one of the limiting environmental factors during the germination process itself, influencing both germination rate and percentage.

Seeds of various weed species can remain viable for many years in the soil. In order to understand the dynamic of the weed seed bank of a specific field, it is necessary to know the original location of the weed seed and the dispersal mechanisms of those dispersing plant species. According to Baskin & Baskin (1989), as seed with physiological dormancy after-ripen (time from maturation to germination), they pass through a series of states known as conditional dormancy before finally becoming non-dormant. In the transition from dormancy to non-dormancy, seeds first gain the ability to germinate over a narrow range of environmental conditions. As after ripening continues, seeds become non-dormant and germinate over a wider range of environmental conditions (e.g. darkness). If germination of a non-dormant seed is prevented, subsequent changes in environmental conditions (e.g., low or high temperatures) cause them to enter secondary dormancy. As a result the range of conditions over which they can germinate decreases until they cannot germinate under any set of environmental conditions. Many seeds in soil seed banks exhibit annual dormancy. Through the information on changes in dormancy state, it might be possible to predict when exhumed seed, as from tillage, will actually germinate.

While germination is restricted by the environmental factor (temperature), dormancy is related to the width of the temperature range in which germination can proceed and not to the question whether or not the current field temperature is in that range. In summer annuals induction of dormancy is characterized by an increase of the minimum temperature at which germination can occur (Bouwmeester, 1990). The level of dormancy in seeds is determined by several factors such as maternal environment during maturation, age of the mother plant during maturation and position of the seeds on the plant (Fenner, 2000). Variation in dormancy between years is sometimes described as an adaptation, which provides a better chance for survival of populations in an environment that is both harsh and unpredictable (Milberg & Andersson, 1994).

In this experiment, dormancy was reduced by stratification at 5 °C and 15 °C. Seeds stratified at 5 °C and 15 °C germinated in the same range of constant temperatures and with only small differences in germination percentage. In addition, the significant differences between dry stored and stratified seeds clearly show how temperature requirement for germination depends on the level of dormancy.

Conclusion

Seed germination of *S. nigrum* depends on the level of dormancy and to a large extent on the current temperature. Seeds with a lower level of dormancy germinate at constant temperatures. Alternating temperatures result in a high percentage of germination in seeds with different levels of dormancy.

The optimum constant temperature for germination of *S. nigrum* was within the range of 26 to 30 °C, with a minimum and maximum temperature of about 18 and 38 °C, respectively.

The high temperature requirement for germination largely explains the late emergence in field, and the limitation of *S. nigrum* as a weed problem to field grown vegetables in Sweden. These crops are sown late, with large row distances and are irrigated.

The result of this study might have large implications for agriculture and control of *S. nigrum*. It provides part of the information, which is needed for predicting time of emergence and, thus, contribute to a more efficient weed control.

Acknowledgements

I want to express my thanks to my supervisor Dr. Lars Andersson for his continuous support and interest during the work and to Mr. Alireza Taab who assisted me with the experiments.

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Personal communication

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Photo of incubator:

The gradient incubator consists of 10 milled tracks, with minimum temperature (or amplitude) at one end, maximum temperature (amplitude) at the other and with increasing temperatures (amplitudes) in between.