Germination dynamics of soybean cultivars in relation to common spring germinating weeds

Sojabönsorters groningsdynamik i förhållande till vanliga vårgroende ogräs

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Master thesis• (30 hec)
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Agriculture programme – Soil/Plant
Uppsala 2021

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Credits:	30 hec
Level:	Second cycle (A2E)
Course title:	**Master Thesis in Biology, A2E – Agriculture Programme Soil/Plant**
Course code:	**EX0898**
Programme/education:	
Course coordinating dept:	**Department of Aquatic Sciences and Assessment**
Place of publication:	**Uppsala, Sweden.**
Year of publication:	**2021**
Cover picture:	Sofie Pålsson Andersson

Keywords: Soybean, cardinal temperatures, base temperature, cold tolerance, germination, Nemoral climatic zone, weeds

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Publishing and archiving

Abstract

Soybeans are one the most traded crops in the world with main production areas in north and south America. Europe only produces 5 % of its domestic need for soybean. In the Nemoral zone of northern Europe no commercial cultivation of soybeans exists today. However, between 1940 -1960 there existed a breeding programme for soybeans in Sweden, but it never reached a commercial scale. Soybeans are interesting to introduce to Swedish organic farming for replacing imported soymeal in animal diets as well as for direct human consumption. To expand the soybean production further north more knowledge about soybeans maturity timing and temperature requirements during the major phenological stages needs to be acquired. An important trait for cultivation of soybeans in the Nemoral zone is capacity to germinate under the low temperatures which in this zone are common in spring. This thesis aims to study three germination dynamics parameters of soybeans; the base temperature for germination (T_b), growing degree days to 50 % germination (GDD_{50}) and the maximum germination rate (b). Secondly, this study aims to investigate if there is an increased tolerance of germination under low temperatures for modern cultivars compared to historic Swedish cultivars. Thirdly, germination dynamic parameters of the most common weed species were studied with the aim to gather more information about weed-crop competition dynamics and possible implications on non-chemical weed management practices. In total 12 soybean cultivars and 10 weed species were studied. Germination experiments were carried out in climate chambers with 12 constant temperatures between 0 – 40 °C. Log logistic functions were used for estimation of the mentioned germination dynamic parameters. The results showed that there were significant differences regarding T_b, GDD_{50} and b between the studied soybean cultivars. There were also significant differences between modern cultivars and the historic cultivar ‘Fiskeby V’. Germination dynamic parameters of the studied weed species varied greatly. Based on the study results it can be concluded that a selection of soybean cultivars with contrasting germination dynamic parameters compared to the main weed species can help to optimise the timing of non-chemical weed management measures. Further it can be concluded that the studied parameters could be included in the future soybean breeding programs aiming for the adaptation of this crop to northern climates.

Keywords: Soybean, cardinal temperatures, base temperature, cold tolerance, germination, Nemoral climatic zone, weeds

Germination of soybean and weeds in cold temperatures

Popular Science summary

Soybeans feeds both humans and animals around the world. Most of the world production of soybeans is grown in the north and south America. In the colder and northern parts of Europe almost no soybean can be found in the farmers' fields. However, this could be about to change.

Even though many might think of soybean as a tropical plant, it is not entirely true. Soybeans are grown in both Japan, China, Canada, Poland and Germany, which do not at all have a tropical climate. Further north, in Sweden the springs are commonly cold and not suitable for a plant like soybean, that mostly prefers warm and sunny days. Therefore, if soybeans are to be grown in colder climate they need to be though and germinate even if the spring is a bit chilly. If soybeans could be grown in Sweden the reliance on imported soybean would be reduced. This thesis has studied the lowest possible temperature for when twelve different soybean cultivars can germinate and how fast the germination occurs. Additionally, to see if new and modern cultivars are better at germinating in cold environment, they are compared to three old Swedish cultivars. The historic cultivars were bred to tolerate the colder climate in Sweden. The cold springs are not the only obstacle for a Swedish soybean production. Another problem, especially for organic farms, is weeds taking over the field. To manage weeds, organic farmers heavily relay on mechanical management. If the temperature for gemination of weeds is also known, it can help to choose both management method and also which soybean cultivar that stands the best chance in competition to weeds. The obtained information about both soybean and weed need of temperature and germination speed was compared to see if it could fit possible mechanical management methods. To study the seeds temperature needs for germination, the seeds were grown in several different temperatures inside climate chambers. All the results from germinating seeds were later analysed with models to find at which temperature and growing degree days seeds could germinate.

The results showed that some soybean cultivars were more adapted to grow in cold temperatures than others. Also, that modern cultivars germinated better than the old historic cultivars. So, the breeding has been promoting the cultivars to tolerate lower temperatures. If scientist want to develop even better cultivars in the future, they could use the temperature needed for gemination to find cultivars that survive in cold springs. For weeds and soybean seeds together, it could be seen that depending on the weed present in field there are different options for controlling them. Early and fast germinating weeds could be controlled with stale seed bed or blind harrowing. Later emerging weeds can be controlled with harrowing in the crop. Who knows, with an improved breeding for cold tolerant soybeans and good management methods for weeds, it is possible that in the future soybeans can be seen in farmers' fields across the northern Europe.

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Abbreviations

(b)	Maximum germination rate
GDD_{50}	Growing degree-day to 50 % germination
T_b	Base temperature for germination
GDD	Growing degree day

1. Introduction

1.1. Soybean

1.1.1. Soybean cultivation

Soybean is primarily cultivated for oil and protein production (Hicks 1978) for both human and livestock consumption. As well as for the production of biofuels (Avila et al. 2013). The leading producers of soybean globally are Brazil, United States (U.S.) and Argentina. The total world production of soybeans was 399 million tonnes during 2019/2020, of them Brazil accounted for a production of 128,5 million tonnes (USDA 2021). During the market year of 2019/2020 Europe imported 15.7 million tonnes soybeans and 17.6 million tonnes soybean meal (USDA 2021). For Sweden the import of soybeans during 2019 was 27 760 tonnes (Strandberg & Lind 2020).

On approximately 1 million ha of its arable land, the EU 27 (Europe without UK) produced 2.6 million tonnes soybeans in 2020 (European Commission 2020), which is covering approximately 5 % of the annual domestic need. The main producers of domestic soybean within the European Union (EU) are Italy, France and Romania. The EU is far from being self-sufficient in soybean production (European Commission 2018). The EU is committed to reduce this gap, since domestic production of soybeans can provide many advantages. Also, EU-consumers request non-genetically modified crops that are produced under high environmental standard (European Commission 2018; Lamichhane et al. 2019).

Belonging to the family of legumes soybeans naturally contribute to increasing soil fertility by N_2-fixation (Hicks 1978; European Commission 2018; Jähne et al. 2019). This can have positive effects on crops like wheat, maize and rapeseed. A more diverse corporation, with legumes included, can also lower the pressure from pests (European Commission 2018). Current challenges for domestic production of soybeans and other legumes are the absence of agronomic expertise and the lack of varieties adapted to European growing conditions (European Commission 2018).

In Sweden, soybean cultivation is currently not existing on a commercial scale (SLU 2018). A major challenge to be considered is the cool climate, with a short growing season. The southern parts of Sweden could be suitable for soybean production (Fogelberg 2009). These are mostly classified as part of the Continental and Nemoral climatic zones of Europe (Figure 1) (Metzger et al. 2005). The Continental zone is characterized by being dominated by fields for crop production. The temperature varies throughout the year and the growing season for the Swedish Continental parts is approximately 213-227 days. In contrast, the Nemoral zone is more characterized by forest and cultivated grassland. In the Nemoral zone the temperatures are cooler, but not inadequate for crop production. The growing seasons for the nemoral parts of Sweden are on average 190-196 days (Metzger et al. 2012). A soybean of early varieties needs about 80-90 days after seeding for germination. However, if temperatures are below 20 °C the growth will be restricted and slow (Rizov & Rodriguez Cerezo 2015).

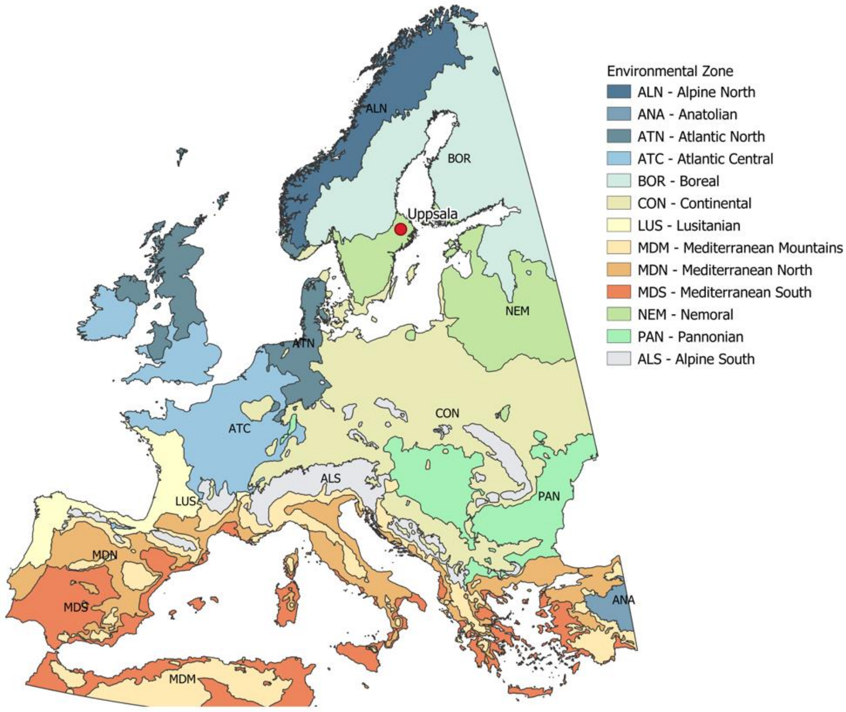

Figure 1. A climatic stratification of Europe according to Metzger et al. (2005). The southern parts Sweden belongs to the Nemoral and Continental environmental zones.

However, during the 1940's a breeding program for soybeans was started at the Fiskeby breeding station, located outside Norrköping (58°N), Sweden. The breeding program resulted in a cultivar sequence of cultivars named Fiskeby. The breeding program included varieties with origin in Hokkadio, Sakhalin and Kurli Islands in Japan, known for their tolerance towards low temperatures. The well-known cultivar 'Fiskeby V' was developed by crossing Japanese and continental varieties (varieties from Bonn and Müncheberg) and was registered as a cultivar in 1968 (Holmberg 1973). Two other cultivars originating from Fiskeby breeding station are ‘Bråvalla’, registered in 1975 (GENBIS 2021a) and ‘Träff’, registered in 1978 (GENBIS 2021b). Cultivation trials performed during 1960-1972 showed that the Japanese and Swedish cultivars were able to develop flower and set pods below the earlier considered biological minimum temperature of 17 °C. In addition, continental cultivars, from Canada and Siberia could tolerate lower germination temperatures but needed higher temperatures during flowering than the Fiskeby cultivars. In germination experiments, ‘Fiskeby V’ showed the ability to germinate at 8 °C day temperature and 6 °C night temperature (Holmberg 1973). Despite this historic period of soybean breeding in Sweden, cultivation of soybeans under Scandinavian climatic conditions is still challenging. More recent experience from Swedish soybean cultivars trials, performed between 2006 and 2009 confirmed the possibility to grow very early soybean cultivars in Sweden. However, these cultivation experiments also showed, that soybeans are very sensitive to frost during early development stages (Fogelberg 2009). Moreover, low temperatures during flowering and pod set can cause complete loss of flowers and pods resulting in severe yield losses (Kurosaki et al. 2004). The usually high precipitation rates in Sweden during harvest period in October-November are further complicating soybean cultivation (Fogelberg 2009). Soybeans are interesting to introduce to Swedish organic farming for replacing imported soymeal in animal diets as well as for direct human consumption. Organic farms in Sweden need to produce 60 percent of the animal feed on the individual farm. Today farmers mainly use peas (*Pisum sativum*), rapeseed (*Brassica napus*) and faba beans (*Vicia faba*) in the fodder to animals. Due to pests, there is a limitation of how often these legumes can be cultivated. An example is root rot (*Aphanomyces euteiches*) on peas. But in contrast to the peas, the oospores do not germinate in the presences of soybean roots (Shang et al. 2000). Therefore, an introduction of new legumes, like soybean, can increase the domestic legume production in Sweden (SLU 2018). Another challenge for the Swedish soybean production is that soybeans germinate slower below temperatures of 20 °C (Rizov & Rodriguez Cerezo 2015) being an disadvantage in the competition of weeds (Pester et al. 1999). At the Swedish University of Agricultural Sciences (SLU) an ongoing project about soybean and lupin named “IMPULSE - Fostering organic cultivation of grain legumes in northern latitudes – A feasibility study for soybean and lupine cultivation in Sweden” is evaluating and studying the possibilities of Swedish organic soybean production. This thesis has been using cultivars and weed samples from this project.

1.1.2. Genetic and phenotypic characteristics of soybeans

Soybean (*Glycine max L.*) plants reaches a height of 90 - 120 cm with high variability between cultivars (Hicks 1978). Soybeans leaves are trifoliate (Ritchie et al. 1985) and the plant is building 19-24 nodes where the leaves are attached (Hicks 1978). Plants can commonly build up to 6 branches. The number of branches depends on the space for each individual plant (Ritchie et al. 1985).

The flowering period is initiated by temperature and photoperiod and is influenced by genotype. Under optimal growing conditions the flowering is initiated 6 - 8 weeks after the plant emergence. In all leaf axils flowers are produced. Soybean flowers are self-pollinated. Flowering can last 3-4 weeks (Hicks 1978), with later development of flowers at the branches than the ones on the main stem (Ritchie et al. 1985). Soybeans are facultative short day plants, and therefore growth (Cao et al. 2017), plant height, flowering (Whigham & Minor 1978; Cao et al. 2017), maturity and seed weight will respond to daylength (Whigham & Minor 1978). Therefore, daylength regulators at which latitudes different cultivars are suitable to grow (Cao et al. 2017). If soybean cultivars are grown outside their day length optimum, plants will remain longer in the vegetative stage, not entering the reproductive growth stage (Hicks 1978). However, there is great variability in day length sensitivity between cultivars (Hicks 1978; Whigham & Minor 1978), especially early maturing often have a reduced or absent day length sensitivity (Cao et al. 2017).

After flowering, plants generally produce up to 5 pods in each node. Pods will develop after 10 – 14 days following. Every pod consists of up to 5 seeds. Even if the pollination time varies amongst the flowers the pods will all mature within a period of approximately one week. The soybean seed is characterized by an oval shape and the hilium, the visible seed scar (Figure 2) formed after detachment of the ovary. The seedcoat encloses the embryo with 8 – 10 cell layers (Hicks 1978).

Figure 2. The characteristic oval shape and seed scar of the soybean seed. The cultivars from left to right are 'Bråvalla', 'Fiskeby V' and 'Träff'.

1.1.3. Classification systems for soybean

Maturity date is an important trait to determine the adaptation to different cultivation areas (Liu et al. 2017), technically allowing a classification of cultivars into maturity groups (Song et al. 2019). There is up-to-date no worldwide standard on how to classify soybean into different maturity groups for different regions (Song et al. 2019). Since soybeans are facultative short-day plants (Cao et al. 2017), cultivars respond differently to given latitudes (Song et al. 2019). Maturity traits include the time to first flower, the vegetative period. Also, the time from flowering until maturity, the reproductive growth. As well as the full growth period. Maturity traits decides at which temperature and light settings soybeans are adapted to grow in (Jia et al. 2014).

The U.S. maturity group classification system for soybean cultivars is based on development response to photoperiod (Setiyono et al. 2007) and latitude (Whigham & Minor 1978; Liu et al. 2017). It consists of thirteen maturity groups ranging from 000 (very early) to X (late) maturing cultivars (Song et al. 2019). New cultivars are given a maturity group by being compared with cultivars of known maturity group in different environments (Liu et al. 2017; Song et al. 2019). Because new cultivars are bred that develop faster than cultivars belonging to maturity group 000, it is suggested to add a 0000 (extremely early) maturity group to the system (Jia et al. 2014; Liu et al. 2017; Song et al. 2019). The maturity group classification gives an indication for the area suitable for a specific cultivar. Nonetheless, cultivars belonging to the same maturity group can still diverge regarding time to maturity and development rate. As an example, the time to reach maturity can differ with 3 weeks between cultivars within the same group. Another limitation with the U.S. maturity system is, that it can be misleading if used outside North America. Especially if the environment differs from what the cultivars have been grown and classified in (Whigham & Minor 1978).

In Europe the majority of available cultivars belong to the earliest maturity groups, ranging from maturity group 000 – II (Kurasch et al. 2017; Schoving et al. 2020), depending on latitude. The very early cultivars (000) are mostly grown in northern latitudes, such as the Netherlands and Germany (Kurasch et al. 2017). These northern and cold areas have not traditionally been regarded as appropriate for soybean cultivation. Therefore, there is an absence of sufficient information about suitable cultivar maturation time for these regions (Jia et al. 2014). However, time to reach maturity is not the only key trait for cultivation in northern latitudes. For cultivation in the short growing season of northern latitudes (Jähne et al. 2019) another important trait is the ability of germinating under cool temperatures (Bramlage et al. 1978). Especially since the germination and seedlings are threatened by suboptimal and subzero temperatures at the beginning of the growing season (Schoving et al. 2020).

1.2. The effect of temperature on soybean germination

1.2.1. Germination

Germination is a complex biochemical and physiological process. The process of germination starts with the uptake of water through the seedcoat. Soybean seeds have a high demand of water for germination, the moisture content need to be around 50 % of the seeds dry weight (Hicks 1978; Ritchie et al. 1985). In comparison, maize (*Zea mays*) only absorbs around 30 % and sugarbeet (*Beta vulgaris*) 31 % water of their respective dry weight. The nutrient reserves within the seed are spent to produce the shoot and the roots. Under optimal conditions, the radicle emerges 1 - 2 days after planting. Thereafter, the radicle grows downwards and branch roots develop when the radicle reaches a length of 2-3 cm. The branch roots attach the seed to the soil and keep it in place. The hypocotyl arch grows upwards and breaks through the soil surface (Hicks 1978). The germination of soybeans are epigeal, meaning that they pull the cotyledons up above the soil surface (Rathore et al. 1981). The food reserves within the cotyledons provide enough nutrients until the first node and leaves appear (Ritchie et al. 1985). As soon as the first leaves are developed photosynthesis starts to provide energy for the seedling (Hicks 1978).

1.2.2. Cold tolerance and responses during germination

Germination, growth, reproduction and yield of soybeans are affected by sub-optimal temperatures (Dhingra 2015). The capacity to germinate in cold soil can be beneficial from an agronomical point of view (see 1.2.4 Current recommendations for agricultural practice, page 19). Robinson et al. (2017) describe germination at low temperatures as a suitable measure of cold tolerance. The cultivar Fiskeby V is mentioned as a cultivar carrying the genetic potential for germination at low temperatures (Robison et al. 2017). Further there seems to be a difference in cold sensitiveness depending on seed coat coloration (Tully et al. 1981; Kurosaki et al. 2004). Pigmented seeds seem to have slower imbibition of water and therefore the germination is less sensitive to cold environment (Tully et al. 1981). Soybeans seeds can experience severe damage already within a few minutes of imbibition of cold water. Imbibition of cold water is detrimental since it inhibits the reconstruction of the seed’s phospholipid membrane (Bramlage et al. 1978). When seeds can’t reorganize the phosphor lipids during the imbibition it will cause a rapid water inflow (Tully et al. 1981) and leakage of cell ions from the seeds. The leakage will empty the seeds nutrient reserves and additionally seed suffers from internal deformation (Bramlage et al. 1978). Additionally, pathogenic microorganisms get an opportunity to attack and grow on the seed (Bramlage et al. 1978). Sometimes the cold damage is visible to the naked eye when seeds break apart (Tully et al. 1981).

1.2.3. Cardinal temperatures

As previously pointed out, plants can only tolerate and grow within a species specific temperature range (Dhingra 2015) and temperature impacts metabolic reactions, diffusion rate of gases and solubility of nutrients in the plant (Avila et al. 2013). The temperature range where growth and development is possible for a certain plant species or crop cultivar is defined by three so called cardinal temperatures: T_b, the base temperature, is the lowest temperature allowing growth and development. Different growth and development stages have different base temperature values. T_{max}, is the maximum temperature the plant or seed can tolerate. T_{opt}, is the temperature most optimal for growth and development (Schoving et al. 2020).

Cardinal temperatures have been determined by trials for seeds germination (Lamichhane et al. 2019; Schoving et al. 2020), plant growth (Souza et al. 2013; Schoving et al. 2020) and pollen germination (Salem et al. 2007) (Table 1). The lower threshold for soybean, T_b, is reported to range from 2 - 4 °C for germination processes (Lamichhane et al. 2019; Schoving et al. 2020), 10-11 °C for vegetative growth and development (Souza et al. 2013) or 13.2 °C for pollen germination (Salem et al. 2007). T_{opt} is ranging between 30-31 °C for all physiological stages (Salem et al. 2007; Souza et al. 2013; Lamichhane et al. 2019; Schoving et al. 2020). According to Souza et al. (2013) and Schoving et al. (2020), T_{max} is at 40 °C. For pollen germination the T_{max} is reported to be 47.2 °C (Salem et al. 2007). Depending on development stage the response to temperatures rising above 40 °C will differ. If the plant is in a vegetative state it will rush the start of flowering. During the reproductive stage, high temperatures will cause a decline in both number of seeds and seed weight (Avila et al. 2013).

Table 1. The cardinal temperatures T_b, T_{opt} and T_{max} for soybean seed, plants and pollen germination according to literature.

T_b	T_{opt}	T_{max}	Method	Reference
10 – 11 °C	31 °C	40 °C	Plants	Souza et al. (2013)
2 °C	30 °C	40 °C	Seeds & Plants	Schoving et al. (2020)
4 °C	30 °C	-	Seeds	Lamichhane et al. (2019)
13.2 °C	30.2 °C	47.2 °C	Pollen germination	Salem et al. (2007)

1.2.4. Current recommendations for agricultural practice

Depending on planting date during the growing season, the soybean plants will partly experience shorter or longer days. Further into the season the days will become longer (Whigham & Minor 1978), therefore, earlier sowing can contribute to induce early flowering and hence a prolonged generative phase (Schoving et al. 2020). It could also be possible to cultivate varieties with later maturation, this is beneficial since later maturity groups are often characterised by higher yields (Lamichhane et al. 2019). However, the planting must occur considering the temperatures mentioned in chapter 1.2.2. In the northern latitudes, the growing season is short and bearing a high risk of cold night temperatures early and late in the growing season (Jähne et al. 2019). Further, if the seed is planted too deep in the soil there is a risk of soil temperatures being suboptimal which can slow down the development of the seedling (Ritchie et al. 1985). In the southern part of Europe, heat waves and drought are problematic. Early sowing might here reduce the irrigation requirements (Schoving et al. 2020). In France, farmers are recommended to sow earlier to avoid the seed bed drying, this since soybean seeds do not tolerate water stress (Lamichhane et al. 2019). For the northern parts of Europe, the climate change will lead to increased temperatures (IPCC 2014), which might push the cultivation areas further north (Schoving et al. 2020). According to Lamichhane et al. (2019) sowing should occur as soon as the temperature are adequate, and the field is possible to access.

1.3. Weeds

In the following a brief overview of the species-specific biology and ecology of weeds considered in this study will be given. The selected weed species are a collection of the most common weeds occurring in soybean trials in Sweden. Seeds of the used ecotypes have been harvested within cultivar testing trial for soybeans in Sweden. The weeds are included in the thesis for studying the relationship between soybean and weeds germination dynamics.

Mechanical weed management for soybeans, such as stale seedbed, blind harrowing and hoeing within the crop can be used to control weeds in organic farming. However, in U.S. soybean trials by Place et al. (2009), stale seedbed with rotary hoeing had little or no effect on the yield of organic soybean (Place et al. 2009). According to Pester et al. (1999) an early removal of weeds is needed to obtain the best possible harvest. Early and rapid emergence of soybean is beneficial in aspect to a cultivar weed competitiveness. Further, it is also favorable if the leaves as soon as possible shadows and closes the rows (Pester et al. 1999).

1.3.1. Annuals

Chenopodium album (Lambs quarters, Svinmålla) is a competitive weed in cultivations of summer annual and row crops, e.g., it is mentioned to be a serious weed in maize, soybean, wheat (*Triticum aestivum*) and potato (*Solanum* *tuberosum*) (Bajwa et al. 2019). *C. album* can grow in soils with both low and high pH. Moreover, germination can occur in various temperatures, stretching from 5 – 30 °C (Bajwa et al. 2019). *C. album* is a summer annual that germinates during late spring in Sweden, with germination being at its highest in May (Håkansson 1983). Cold winters break seed dormancy and lead to an intense germination period during spring (Grundy et al. 2003) and germination generally stops during summer (Håkansson 1983). Additionally, germination will be initiated when the seeds experience fluctuating temperatures and light. One plant can produce up to 70,000 seeds and the seeds can survive in the soil seedbank for up to 39 years (Bajwa et al. 2019). Experiments have shown that *C. album* can be controlled with a combination of harrowing before and after crop establishment (Lundkvist 2009).

Another annual weed with predominantly spring germination is *Silene noctiflora* (Night-flowering catchfly, Nattglim), although some germination occurs during autumn (McNeill 1980). *S. noctiflora* can be found as a weed in both cereals and legumes (Qaderi & Reid 2008). Germination of seeds seems to occur in a temperature range between 6 and 31 °C (McNeill 1980) and germination is improved if seeds are exposed to short light impulses. Soil cultivation expose seeds to light and leads to an impulse of germinating seeds (Milberg 1997). One plant produce more than 2500 seeds and they are viable for at least 5 years (Qaderi & Reid 2008).

Some weeds can act as both winter and summer annuals, such as *Matricaria inodora L. (*Scentless Mayweed, Baldersbrå) (Bochenek et al. 2010). In dense populations it can also occur as a biennial (Buckley et al. 2001). *M. inodora* is common in annual crops throughout northern and eastern Europe, the dispersal to southern parts of Europe is limited due to sensitiveness towards drought (Ellis & Kay 1975). M. *inodora L.* germinates mostly during spring (Bochenek et al. 2010), with a peak in May (Håkansson 1983). The seeds have no obligatory dormancy (Buckley et al. 2001), yet they require light for germination. One plant can produce on average 34,000 seeds, but there has been recorded extreme seed production of one million seeds on one plant (Kay 1994).

Capsella bursa-pastoris (Shepherd´s purse, Lomme) is a facultative winter annual, germinating in both autumn and early spring (Baskin & Baskin 1989a). *C. bursa-pastoris* is one of the most common weeds in the world (Neuffer & Hurka 1999). It originates from Europe and can be found in Africa, Asia and North America. The weed habitat stretches far north, even beyond the arctic circle (Baskin et al. 2004). In Sweden *C. bursa-pastoris* seeds will germinate throughout the cropping season, starting in April and continue until the end of September. Germination peaks seems to occur in May and August (Håkansson 1983). To break dormancy of the seeds, a period of temperatures below 15 °C is needed. Seeds germinate slowly at a temperature of 4 °C with an optimum temperature for gemination at around 9 – 10 °C (Popay & Roberts 1970). It has been shown that almost no seeds of *C. bursa-pastoris* germinates under complete darkness (Popay & Roberts 1970). Seeds can stay within the seedbank for decades (Yang 2018). *C. bursa-pastoris* is an alternative host for the soybean cyst nematode (*Heterodera Glycines).* In U.S. both *C. bursa-pastoris* and *Thlaspi arvense* have been identified as alternative host for the nematodes reproduction (Venkatesh et al. 2000).

Thlaspi arvense (Field pennycress, Peningört) is a facultative winter annual plant (Håkansson 1983) widespread in temperate regions (Holm et al. 1997). In Sweden, the germination of *T. arvense* occurs from April until November. However, the numbers of germinating seeds is reduced during summer (Håkansson 1983). During winter the seeds will enter a secondary conditional dormancy. The seeds will continue to germinate as long as the temperatures during spring are low, but the germination will stop when the temperatures rise. *T. arvense* seeds that germinate during spring will act as a short-term summer annual. The same response can be seen in *C. bursa-pastoris* (Baskin & Baskin 1988). Fresh seeds of *T. arvense* need at least 5 days of temperatures around 5 °C to be able to germinate. More seeds germinate if they are exposed to light and soil disturbance increase spring germination (Baskin & Baskin 1989b).

Similar to the other winter annuals *Papaver rhoeas* (Common Poppy, Valmo) germinates both in autumn and spring (Karlsson & Milberg 2007). *P. rhoeas* prefer growing in calcareous soils (Cirujeda et al. 2006) and seeds will persist in the seedbank for about 5 years (Karlsson & Milberg 2007). The seed dormancy for *P.rhoeas* is very strong and most of the seeds germinate during the autumn from September to December (Milberg & Andersson 1997). P. *rhoeas* dormancy is released by cold temperatures.

1.3.2. Perennials

The perennial weed *Cirsium arvense,* (Field thistle, Tistel) is a problematic weed. *C. arvense* mainly spreads in fields by vegetative root and stem parts. Even if vegetative reproduction plays a major role in the reproduction of *C. arvense,* propagation via seeds is important. The optimum germination for seeds has been reported to occur at high temperatures between 26 and 35 °C from Polish and British seeds (Bochenek et al. 2009). Light stimuli will increase the number of seeds that germinate (Wilson 1979).

One of the most important perennial weeds in Sweden, is *Sonchus arvensis* (Field milk Thistle, Åkermolke) (Fogelfors & Lundkvist 2009). *S. arvensis* is mainly reproducing by buds and stem parts, but seed dispersal is also significant (Taab et al. 2018). Each plant can produce up to 10 000 achenes (seeds) that are mainly spread by wind. The seeds can germinate immediately or short after dispersal. Seeds do not require light, but it will enhance the germination. Optimum temperatures for germination are at 25 – 30 °C (Lemna & Messersmith 1990). *S.*

arvensis causes yield losses in soybeans of 50-80 % loss if the weed are left uncontrolled in the field (Zollinger & Kells 1993).

Rumex crispus (Curled dock, Skräppa) is a perennial weed that is troublesome in both pasture and annual crops (Baskin & Baskin 1985; Zaller 2004; Pye & Andersson 2009), especially in organic farming (Baskin & Baskin 1985). *R. Crispus* can grow and adapt to very different environments but grows preferably in soils with high nitrogen content (Zaller 2004). It also favor environments where competition from crops is low (Baskin & Baskin 1985). Rumex can spread by rot parts as well as by seed. It is a competitive weed since it has a quick establishment and produces up to 40,000 per plant (Zaller 2004; Pye & Andersson 2009). The seeds can remain viable for up to 80 years. Whether the seeds have any dormancy seems to vary, Baskin & Baskin (1985) reported no dormancy in seeds while reported dormancy during early summer (Van Assche et al. 2002). Baskin & Baskin (1985) concluded that seeds will not germinate if buried, but they will not be in a dormant state. Germination of *R. Crispus* is possible throughout the year, with peaks during early spring and autumn (Zaller 2004). Tillage stimulates germination of seeds and can be used for management of *R. crispus* population, e.g. through stale seedbed preparation before crop seeding (Pye & Andersson 2009). Even though tillage might be an advantage for seedling emergence, it can have different effect on shoots from root parts. Tillage can limit the reproduction from root parts but can also favor it depending on method and timing (Zaller 2004).

Plantago major (Broad-leaved plantain, Groddblad) is a plant native to Europe. Plantago is commonly found in pastures and row crops. It spreads with seeds and vegetatively by ramets. The seeds of *P. major* can survive in soil for up to 21 years, but there has been records of seeds surviving for up to 39 years (Hawthorn 1974). The seeds will mostly germinate during spring, from April to June, but germination is possible throughout the summer (Roberts & Boddrell 1984). The seeds need light stimuli for germination (Hawthorn 1974).

2. Aims and hypotheses

To expand soybean production to the nemoral climate zone (Metzger et al. 2005) an informed and trait- based breeding approach is needed which requires more detailed knowledge about temperature requirements of soybeans in different development stages. The temperature requirements for germination and early development are of particular interest due to the relatively cold spring temperatures in the Nemoral climatic zone. Therefore, this master thesis aims to highlight differences in germination dynamics for early maturing soybean cultivars. In addition, three historic cultivars from Sweden are tested in comparison to the modern varieties. Moreover, the germination dynamics of the most common weed species will be included in the study. The weed species are included to deepen the understanding about the relation between germination of soybeans and weeds allowing an informed selection of weed management tools in organic farming. This study aims to answer the following questions:

1. Are there significant differences in terms of germination dynamic parameters between soybean cultivars belonging to similar maturity groups in terms of germination dynamic parameters?
2. Do new soybean cultivars have improved cold tolerance for germination compared to historic Swedish cultivars?
3. What are the base temperatures for germination (T_b) for the most common weed species found in ongoing soybean field trials in Sweden and how do they compare to the germination dynamic parameters of the tested soybean cultivars?

For answering these research questions, three specific hypotheses have been tested in this study:

1. **Cultivar differences in germination dynamics:** Although belonging to similar maturity groups, the tested modern cultivars are differing significantly in terms of base temperature for germination (T_b), required growing degree-days for reaching 50 % germination (GDD50) as well as in their overall germination rate (b).

2. **Breeding progress:** Modern cultivars have a significantly lower base temperature for germination as well as growing degree-day requirement for reaching 50 % germination and overall germination rate compared to the tested historic cultivars.
3. **Germination dynamics of weeds:** the most common weed species occurring in Swedish soybean production systems are differing significantly in terms of base temperature for germination (T_b), required growing degree days for reaching 50 % germination (GDD50) as well as in their overall germination rate (b).

3. Material and Methods

3.1. Germination experiments

3.1.1. Selection of seed samples

In this experiment the base temperatures for germination (T_b) of twelve soybean cultivars and ten weed species have been tested (Table 2 and 3) (Figure 3 and 4). All soybean cultivars belong to the early or very early maturity groups. Weed seeds were harvested from organic soybean cultivar trials in Lönnstorp (55° latitude) and Lövsta (59° latitude), Sweden.

Table 2. Soybean cultivars included in experiment in alphabetic order and maturity group classification according to the U.S. Maturity group classification system. The cultivars 'Fiskeby V', 'Bråvalla' and 'Träff' are Swedish historic cultivars that do not have assigned maturity groups.

Cultivar	Maturity group classifications
Abaca	000
Annushka	000
Bilyavka	0000
Bråvalla	Historic
Fiskeby V	Historic
Gallec	000
Merlin	000
Obelix	000
Sculptor	000
Taifun 8	000
Tofina	000
Träff	Historic

Table 3. Weed species included in experiment. The species Latin, English and Swedish name and also from which cultivar trial location and year the weeds were harvested in.

Species	English name	Swedish name	Harvest place
Capsella bursa-pastoris	Shepherd´s purse	Lomme	Lövsta 2020
Chenopodium album	Lambs quarters/Goosefoot	Svinmålla	Lönnstorp 2020
Cirsium arvense	Creeping/Field thistle	Åker tistel	Lövsta 2020
Matricaria inodora	Scentless mayweed	Baldersbrå	Lönnstorp 2020
Papaver rhoeas	Common poppy	Valmo	Lönnstorp 2020
Plantago major	Broad-leaved plantain	Groddblad	Lövsta 2019
Rumex crispus	Curled Dock	Skräppa	Lövsta 2020
Silene noctiflora L.	Night-flowering Catchfly	Nattglim	Lövsta 2020
Sonchus arvensis	Field milk Thistle/ Gutweed	Åkermolke	Lövsta 2020
Thlaspi arvense	Field pennycress	Penningört	Lönnstorp 2020

Figure 3. The dry soybean seed and names of the twelve soybean cultivars included in the germination experiments of the thesis.

Figure 4. The seeds and Latin name of the weed species included in the germination experiments of the thesis.

3.1.2. Experimental design

For the experiments, climate chambers, at a constant temperature, were used for the experiments. The temperatures tested were 0 °C, 2 °C, 4 °C, 6 °C, 8 °C 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 40 °C. Over the whole extend of the experiments and in all climate chambers, potential temperature fluctuations were recorded with Tinytags Plus 2 temperature loggers.

For each crop cultivar and weed species, 60 seeds were placed in Petri dishes, 9 cm in diameter, on two layers of filter paper (Ahlstrom Munksjö Munktel). The only exception was the soybean cultivar 'Bråvalla', where due to a lack of seeds only 50 seeds were placed in each Petri dish. For each temperature regime, three repetitions, respectively Petri dishes, per cultivar and weed species were prepared. The seeds were counted and placed in the dish by hand. Only intact seeds were selected, broken and empty seeds were discarded.

Soybean seeds were soaked in 30ml of distilled water per Petri dish. For breaking seed dormancy, weed seeds were soaked in a 2 mM gibberellic acid, 0.2 % KNO_3 solution (30ml per Petri dish) (Popay & Roberts 1970; Bochenek et al. 2009). The soybeans and weeds were each sorted separately by repetition and placed in plastic bags to avoid evaporation. Petri dishes containing soybean seeds were additionally wrapped in black plastic bags for eliminating light. Petri dishes containing weed seeds were wrapped in transparent plastic bags allowing a light stimulus of eight hours per day (Hawthorn 1974; Wilson 1979; Baskin & Baskin 1988). At the time petri dishes were placed in the climate chambers, the starting date and time was recorded.

3.1.3. Data collection

The number of germinated seeds per petri dish was counted every 24 h. For every counting occurrence, date, time and number of germinated, damaged or mouldy seeds were noted. Seeds that had germinated where counted and then removed from the Petri dish. Soybean seeds were considered as germinated when the radicle had reached a length of 2 mm (Andrade et al. 2018). Weed seeds were considered as germinated as soon as the radicle was visible (Baskin & Baskin 1985; Baskin et al. 2004). The weeds were in the climate chambers until all seeds had germinated or the seeds were destroyed by mould. For colder temperatures where no mould was developed the seeds were kept in the climate chambers for up to 30 days.

Figure 5. Germinated soybean seeds of the cultivar 'Obelix' with the radicle emerged to a length of more than 2 mm.

Figure 6. Germinated seeds of S. arvensis in 4 °C with the radicle emerged from the seedcoat.

3.2. Data analysis of seed germination

For analysing the soybean cultivars and weed species responses to germination in different temperatures, three germination parameters were studied by creating germination curves. Three germination dynamics parameters were determined, the base temperature for germination (T_b), the required growing degree-days for reaching 50 % germination (GDD_{50}), as well as the maximum germination rate around GDD_{50} (b) (Figure 7).

For this purpose, log-logistic models were fitted to the data and analysed with the statistical software R (version 1.4.1103). The temperatures included in the modelling and data analysis approach are shown in Table 4 for soybean and Table 5 for weeds. The temperatures for the analysis were selected from the germination experiment based on being within the suboptimal temperature range for germination. Temperatures at which no germination occurred during the germination experiments were excluded. The modelling and data analysis was done following the protocol of Mesgaran et al. (2019), in the following the major steps are summarised.

Table 4. Selected temperatures for soybean cultivars included in the fitting of log logistic Growing Degree-day model for the germination analyses.

Cultivar	Temperatures included in model (°C)
Abaca	6, 8, 10, 15, 20, 25
Annushka	6, 8, 10, 15, 20, 25
Bilyavka	6, 8, 10, 15, 20, 25
Bråvalla	6, 8, 10, 15, 20, 25
Fiskeby V	6, 8, 10, 15, 20, 25
Gallec	8, 10, 15, 20, 25
Merlin	8, 10, 15, 20, 25
Obelix	6, 8, 10, 15, 20, 25
Sculptor	6, 8, 10, 15, 20, 25
Träff	8, 10, 15, 20, 25
Taifun 8	6, 8, 10, 15, 20, 25
Tofina	6, 8, 10, 15, 20, 25

Table 5. Selected temperatures for weed species included in the fitting of log logistic Growing Degree-Day model for the germination analyses.

Cultivar	Temperatures included in model (°C)
Capsella bursa-pastoris	2, 4, 6, 8, 10, 15
Chenopodium album	4, 6, 8, 10, 15
Cirsium arvense	8, 10, 15
Matricaria inodora	4, 6, 8, 10, 15, 25
Papaver rhoeas	4, 6, 8, 10, 15
Plantago major	10, 15, 25, 30
Rumex crispus	*8, 10, 15, 25*
Silene noctiflora L.	*8, 10, 15*
Sonchus arvensis	2, 4, 6, 8, 10, 15
Thlaspi arvense	4, 6, 8, 10, 15

As a first step, a three-parameter log-logistic function was fitted to the observed germination data and for each temperature. With G_{max} as the upper limit of the function, t_{50} the time until 50 % germination and b the slope around 50 % germination. The time to germination (t_g) was calculated for each temperature by using the inverse form of the logistic function above as well as the growth rate (GR) was calculated as the reciprocal of t_g.

$$f_{(x)} = \frac{G_{max}}{1 + \exp(b(\log(x) - Log(t_{50})))}$$

For calculating a first estimate of the base temperature for germination (T_b), GR was plotted against temperature and a linear model was fitted to the data following

$$GR = b * (T - T_b)$$

With T as the measured temperature, and b the slope for the function.
In the following step, growing degree-days was calculated according to

$$GDD = Chamber\ temperature - T_b \times t$$

with chamber temperature, the actual temperature (°C) setting of the respective climate chamber and t, the time (days).

Maximum germination rate (G_{max}) (%) varied between soybean cultivars as well as between weed species. Therefore, the measured germination data (g) was normalised for allowing a direct comparison of the model parameter across cultivars and weed species. The germination data was normalised according to

$$g_{norm} = \frac{g}{G_{max}}$$

g_{norm} is ranging between 0 and 1 with 1 indication the average G_{max} of the respective cultivar or weed species.

Being now able to estimate GDD for every germination observation, a non-linear log logistic function was fitted to the data according to

$$g_{norm}(t,T) = \frac{1}{1 + \left(\frac{(T - T_b)t_g}{GDD_{50}}\right)^b}$$

With g_{norm} the cumulative normalised germination, tg as time to germination and T as temperature. From the function T_b, GDD_{50} and b were estimated.

T_b, GDD_{50} and b were analysed for significant differences between soybean cultivars and weed species. The analysis was done with a two-sample z-test. Since T_b, GDD_{50} and b are mean values for each soybean cultivars or weed species the statistical analysis was done with a z-test. Because of the comparison of means between groups (soybean cultivars or weed species) a post-hoc test was not possible. Instead, the z-test allowed to compare the parameter means between each soybean cultivar pair or weed species pair separately. Parameter estimates (m) and their standard error (s) for Tb, GDD50 or b were compared according to

$$z = \frac{m_1 - m_2}{\sqrt{s_1^2 + s_2^2}}$$

The results were significant if $|z| \geq 1.96$ at level 0.05.

3.2.1. Biological interpretation of the parameter estimates

The cardinal base temperature for germination (T_b) is defined as the minimum temperature at which germination of a seed can take place.

Growing Degree Day to 50 % germination (GDD_{50}) denotes growing degree-days required until 50 % germination is reached. This point also marks the turning point also marks the turning point of the log-logistic function or rather the retransition from the linear into the asymptotic part of the model (Figure 7).

b at 50 % germination is the slope of the linear part of the GDD_{50} indicating the maximum germination rate of a certain soybean cultivar or weed species.

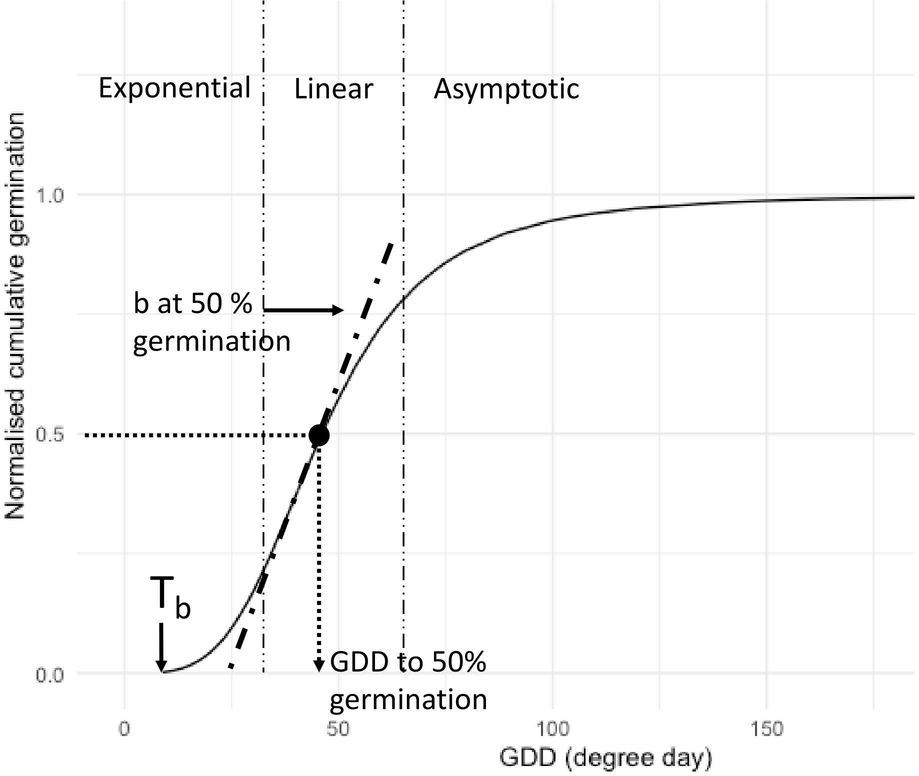

Figure 7. Description of a Growing Degree-Day models estimated parameters placement and phases of the germination curve. The germination parameters studied are cardinal base temperature (Tb), Growing Degree-Day to 50 % germination (GDD50) and b at 50 % germination. The germination model has three phases: exponential, linear and asymptotic.

4. Results

4.1. Soybean

4.1.1. Estimated base temperature for germination (T_b)

The estimated base temperature for germination (T_b) varied between the tested cultivars (Table 6 and Figure 8). The lowest T_b estimated was 4.2 °C for the cultivar ‘Obelix’ and highest was for ‘Gallec’ at 7.1 °C. For the Swedish historical varieties ‘Fiskeby V’, ‘Träff’ and ‘Bråvalla’ calculated base temperatures were 6.7 °C, 4.6 °C and 4.3 °C. The cultivar ‘Bilyavka’, the only one belonging to maturity group 0000, had an estimated T_b of 5.8 °C. The mean T_b for all soybean cultivars was 5.2 °C. During the germination tests no germination was observed at temperature lower than 4 °C. Significant results were obtained between several cultivars, details can be seen in Appendix 1. To see the significant differences of cultivars compared with ‘Fiskeby V’ see Table 6.

*Table 6. Results of estimated base temperature for germination (Tb), Growing Degree-Day at 50 % germination (GDD50) and b at 50 % germination (GDD50) for all soybean cultivars with standard error. Results marked with * are significant different to the cultivar ‘Fiskeby V’.*

Cultivar	Maturity group	T_b (°C)	T_b Standard error	GDD_{50}	GDD_{50} Standard error	b at GDD_{50}	b Standard error
Abaca	000	5.115 *	0.2020	46.077	2.4106	3.694	0.3936
Annushka	000	5.653	0.5658	52.812	6.4252	4.021	0.9746
Bilyavka	0000	5.837	0.5248	60.014*	6.0840	3.537	0.6871
Bråvalla	Historic	4.305*	0.2967	45.653	4.7064	2.048	0.2928
Fiskeby V	Historic	6.680	0.2965	38.629	3.3318	3.037	0.4552
Gallec	000	7.102	0.3495	36.899	3.8018	2.815	0.4891
Merlin	000	4.749	0.3470	46.940	3.5226	4.775*	0.7212
Obelix	000	4.206*	0.1334	41.956	1.9806	4.910*	0.6157
Sculptor	000	4.632*	0.1586	38.975	2.3714	7.342*	1.9154
Taifun 8	000	4.828*	0.1813	42.262	2.7740	2.398	0.2687
Tofina	000	4.509	0.1620	36.556	2.4264	3.555	0.4959
Träff	Historic	4.563*	0.1490	37.634	1.3656	5.941*	0.3917

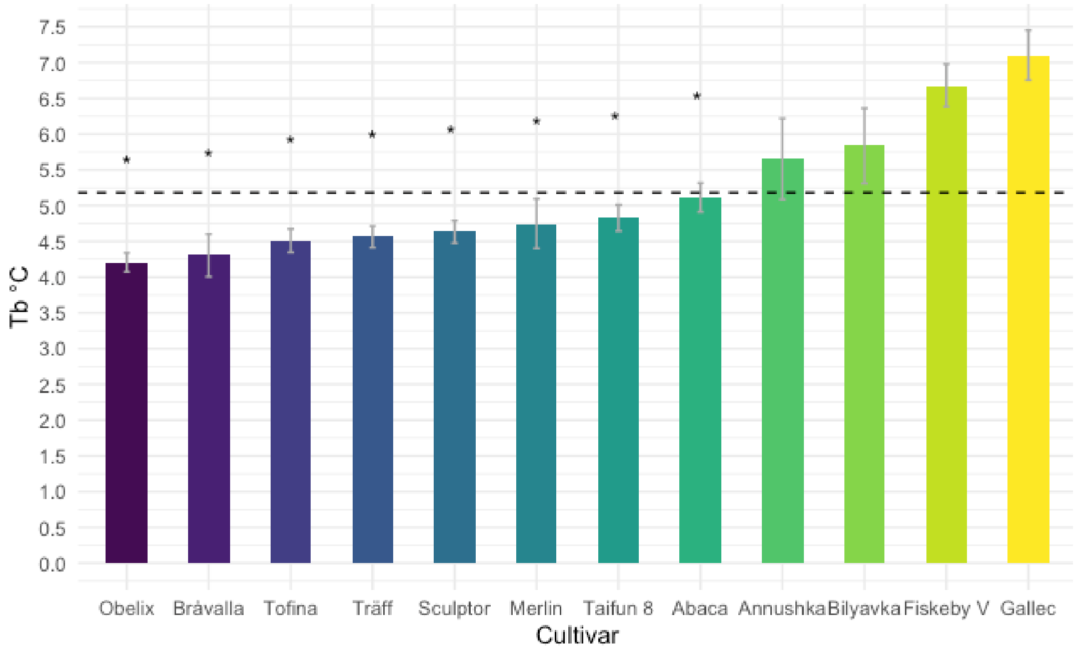

*Figure 8. Estimated T_b for Soybean. Cultivars that are significant different to the cultivar 'Fiskeby V' are marked with *. Black horizontal line represents the mean T_b across all cultivars at (5.2 °C). Grey vertical lines are standard error for each cultivar.*

4.1.2. Estimated Growing Degree-Days until 50 % germination (GDD_{50})

The results for estimated Growing Degree-Days to reach 50 % (GDD_{50}) can be seen in Table 6 and Figure 9. The cultivar with the lowest growing degree-day (GDD) requirement was 'Tofina' with an estimation of 36.5 GDD. Whereas the cultivar 'Bilyavka' need 60.0 GDD to reach 50 % germination. The two Swedish cultivars 'Träff' and 'Fiskeby V' had similar value for GDD_{50} with 37.6 respectively 38.6 GDD, while 'Bråvalla' needed 45 GDD to reach 50% germination. The mean GDD_{50} for all soybeans was 43.7 GDD. Only the cultivar 'Bilyavka' was significant different to 'Fiskeby V' for GDD_{50}. There were significant differences between other cultivars, 'Tofina' that had the lowest result for GDD_{50} germination was significant different to 'Merlin', 'Abaca', 'Annushka' and 'Bilyavka'. For significant result of the other cultivars, details can be seen in Appendix 2.

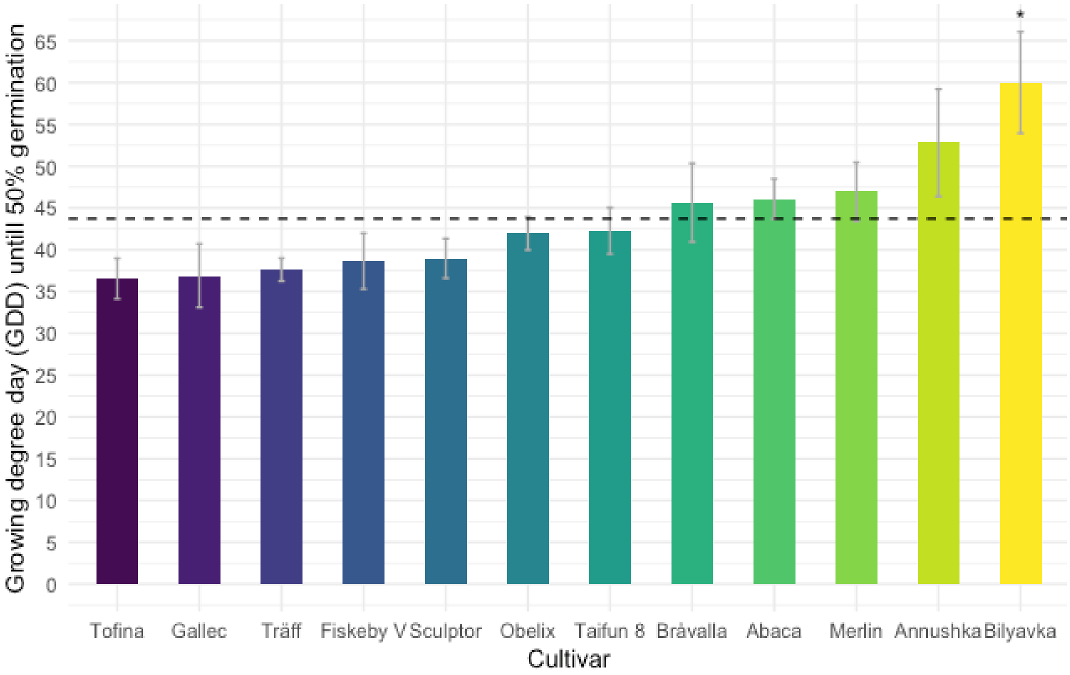

*Figure 9. Estimated Growing Degree-Day to 50 % (GDD$_{50}$) germination for Soybean. Cultivars that are significant different to 'Fiskeby V' are marked with *. Black horizontal line represents the mean GDD$_{50}$ germination across all cultivars (43.7 Growing Degree-Days (GDD)). Grey vertical lines are standard error for each cultivar.*

4.1.3. Estimated germination rate at 50 % germination (b)

The results of estimated germination rate at 50 % germination (b) can be seen in Table 6 and Figure 10. The slope of the temperature curves at 50 % germination (b) differs between the lowest value of 2.05 for 'Bråvalla' and the highest 7.34 for 'Sculptor'. The mean germination rate for soybeans was 4.0. For the other Swedish cultivars 'Fiskeby V' have a germination rate (b) of 3.04 and 'Träff' have the second highest germination rate (b) of 5.94. 'Merlin', 'Obelix', 'Träff' and 'Sculptor' were all significant different to 'Fiskeby V'. For all significant differences between other cultivars see Appendix 3.

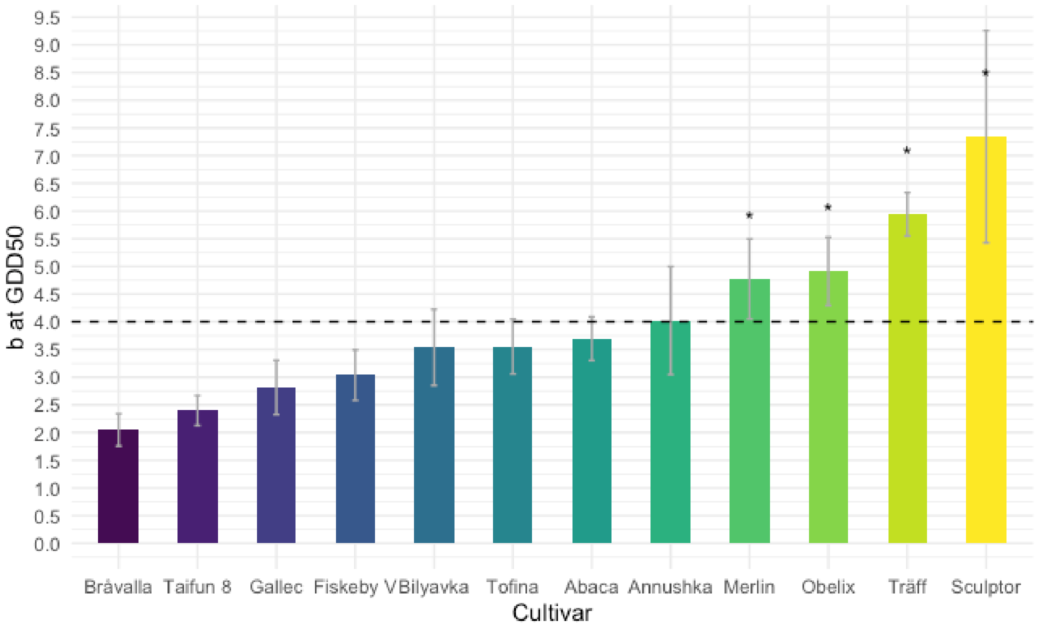

*Figure 10. Estimated b at 50 % germination (GDD_{50}) for soybean cultivars. Cultivars that are significant different to 'Fiskeby V' are marked with *. Black horizontal line represents the mean b at 50 % germination across all cultivars (4.0). Grey vertical lines are standard error for each cultivar.*

4.2. Weeds

4.2.1. Estimated base temperature for gemination (T_b)

The estimated base temperature for germination (T_b) for weeds ranged between *T. arvense* with a T_b of 1.9 °C to *P. major* with a T_b of 6.3 °C, see Table 7 and Figure 6. The results are presented in Table 7 and Figure 11. *P. rhoeas*, *S. arvensis* and *T. arvense* all have a T_b lower than the mean of 3.6 °C. Out of 10 weed species 7 had a T_b lower than 4 °C. *T. arvense* and *P. major* were significant different to all other weeds. The rest of the weeds are significant different to a minimum of 6 other species. For exact details on significant differences between weed species see Appendix 4.

Table 7. Results of estimated base temperature for germination (T_b), Growing Degree-Day at 50 % germination (GDD_{50}) and b at 50 % germination (GDD_{50}) for all weed species with standard error. Significant differences between weed species can be found in Appendix 4, 5 and 6.

Species	T_b (°C)	T_b Standard error	GDD_{50}	GDD_{50} Standard error	b at GDD_{50}	b Standard error
Capsella bursa-pastoris	3.0527	0.1112	48.5175	1.4529	4.5041	0.2892
Chenepodium album	3.285	0.1311	106.8922	2.622	4.2142	0.1991
Cirsium arvense	4.9682	0.2775	91.8807	5.1951	3.8301	0.4029
Matricaria cinodora	4.98784	0.09642	25.50552	1.5412	1.535549	0.09654
Papaver rhoeas	2.3751	0.1646	75.8021	2.8796	4.622	0.2847
Plantago major	6.2886	0.3163	41.9458	2.7018	6.4056	1.169
Rumex rispus.	3.103	0.1122	67.4811	1.7524	10.9392	1.2216
Silene noctiflora L.	3.37025	0.09739	66.01541	1.68015	6.35767	0.38865
Sonchus arvensis	2.6399	0.0387	37.4441	0.6074	6.6847	0.4047
Thlaspi arvense	1.9871	0.0423	51.1085	0.6226	8.3213	0.389

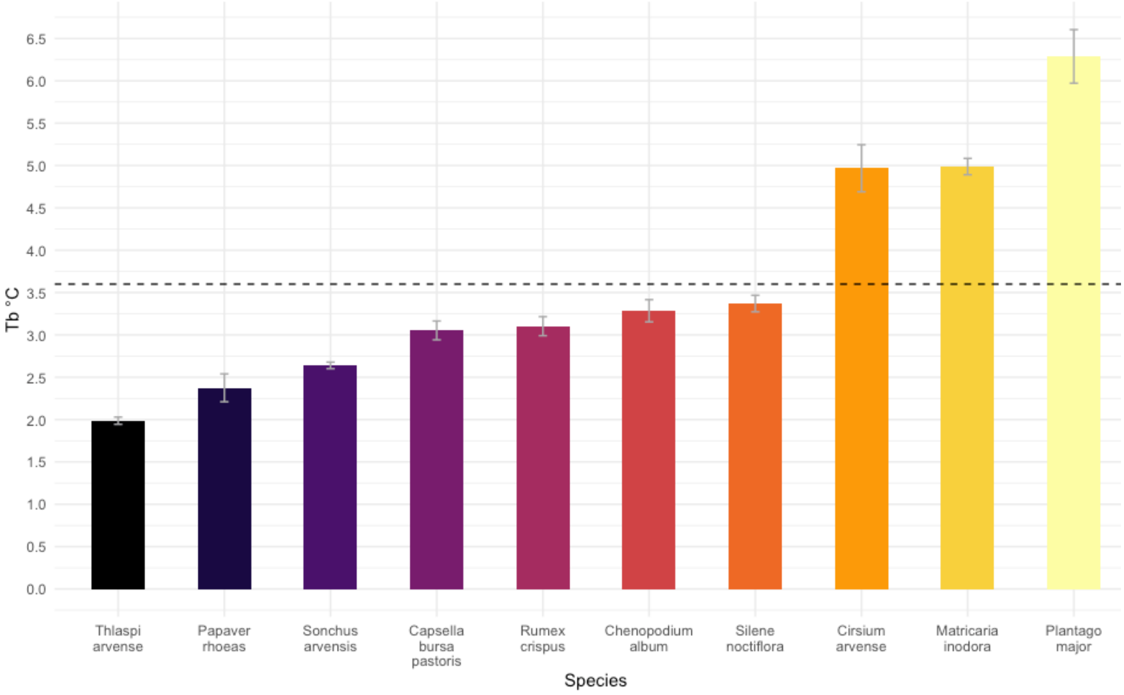

Figure 11. Estimated T_b for weed species. Black horizontal line represents the mean T_b across species (3.6 °C). Grey vertical lines are standard error for each species. Significant differences between weed species can be found in Appendix 4.

4.2.2. Estimated Growing Degree-Days until 50 % germination

The results for estimated Growing Degree-Days until 50 % germination (GDD_{50}) can be seen in Table 7 and Figure 12. GDD_{50} varied between the different weed species. *M. inodora* only required 25.5 Growing Degree Days (GDD) to reach 50 % germination while in *C. album* 106,9 GDD were necessary. Other species with low requirement of GDD are *S. arvensis*, 37.4 GDD, and *P. major*, 41.9 growing degree-days. Mean of GDD_{50} for weeds is 61.2 GDD. *C.album*, *C.arvense*, *M.* inodora and *P.roheas* are significant different to all weeds. The rest of the weeds are significantly different to all weeds except one. For exact details on significant differences between weed species see Appendix 5.

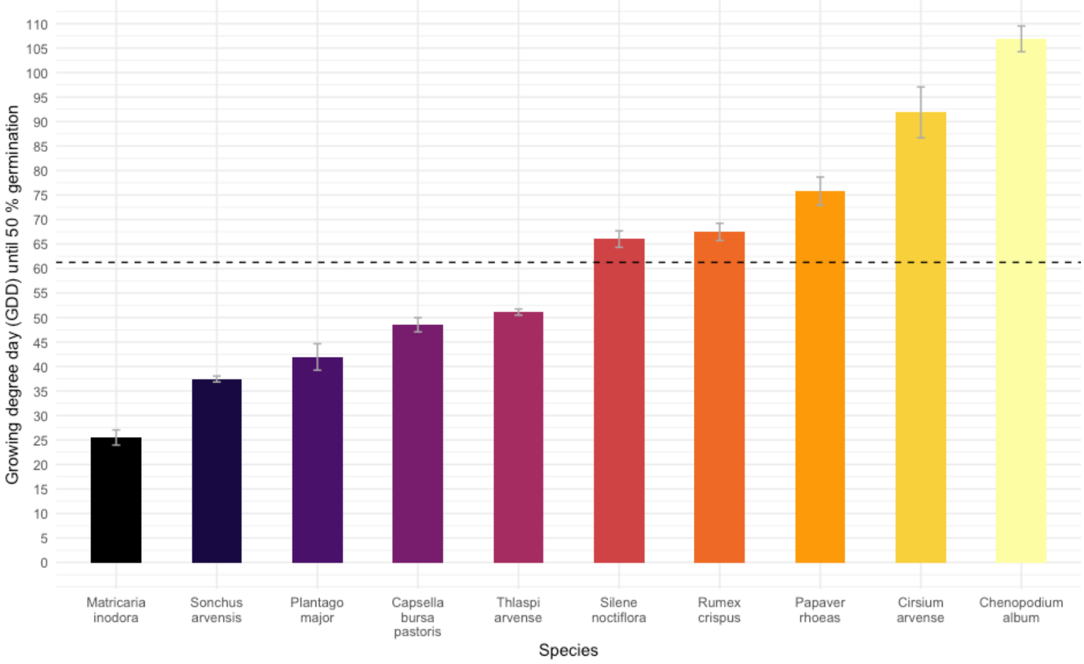

Figure 12. Estimated Growing Degree-Day until 50 % (GDD50) germination for weed species. Black horizontal line represents the mean GDD50 across species (61.2 Growing Degree-Days (GDD)). Grey vertical lines are standard error for each species. Significant differences between weed species can be found in Appendix 5.

4.2.3. Estimated germination rate at 50 % germination (b)

The highest germination rate at 50 % germination (b) was reached by *R. Crispus* with a b value of 10.94, see Table 7 and Figure 13. *T. arvense* have the second highest germination rate (b) at 8.32. *S. arvensis*, with a b of 6.68, follows on third place. The lowest germination rate (b) was found for *M. inodora* at 1.53. Also, *C. arvense* at 3.83 and *C. album* at 4.21 have lower germination rate than the mean of 5.7. *R. crispus* and *M. inodora* are significant different to all other species. *P. major* is only significantly different to 4 other weed spices. For exact details on significant differences between weed species see Appendix 6.

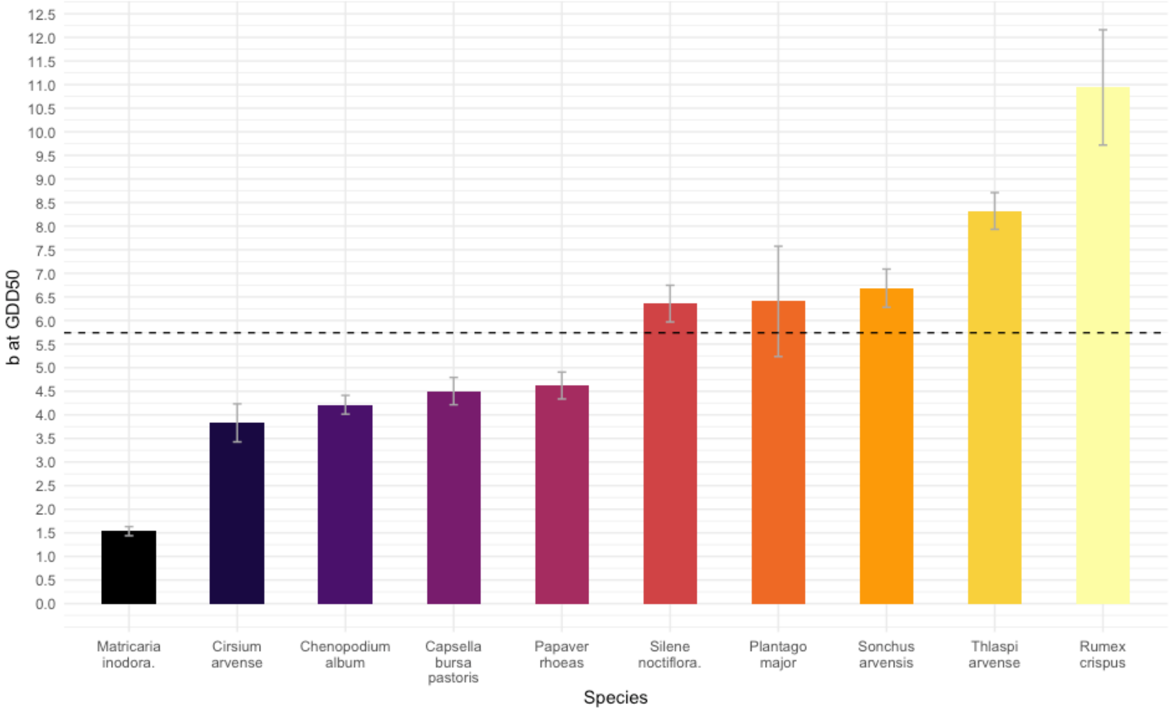

Figure 13. Estimated germination rate (b) at 50 % germination (GDD_{50}) for weed species. Black horizontal line represents the mean b at 50 % germination across species (5.7). Grey vertical lines are standard error for each species. Significant differences between weed species can be found in Appendix 6.

4.3. Examples of germination curves

In Figure 14 – 15 examples of germination curves for some soybean cultivars and weed species are presented. In Figure 14 the weed species *M. inodora* and *T. arvense* are compared. *M. inodora* has, in comparison to *T. arvense*, a lower germination rate (b), which results in a much longer germination period and flatter curve. In Figure 15 the soybean cultivars ‘Obelix’ and *T. arvense* are compared to each other. ‘Obelix’ has the lowest T_b for soybeans while *T. arvense* has the lowest T_b for all weeds. The curves have about the same shape, but *T. arvense* has a faster germination rate (b) and therefore a slightly steeper curve.

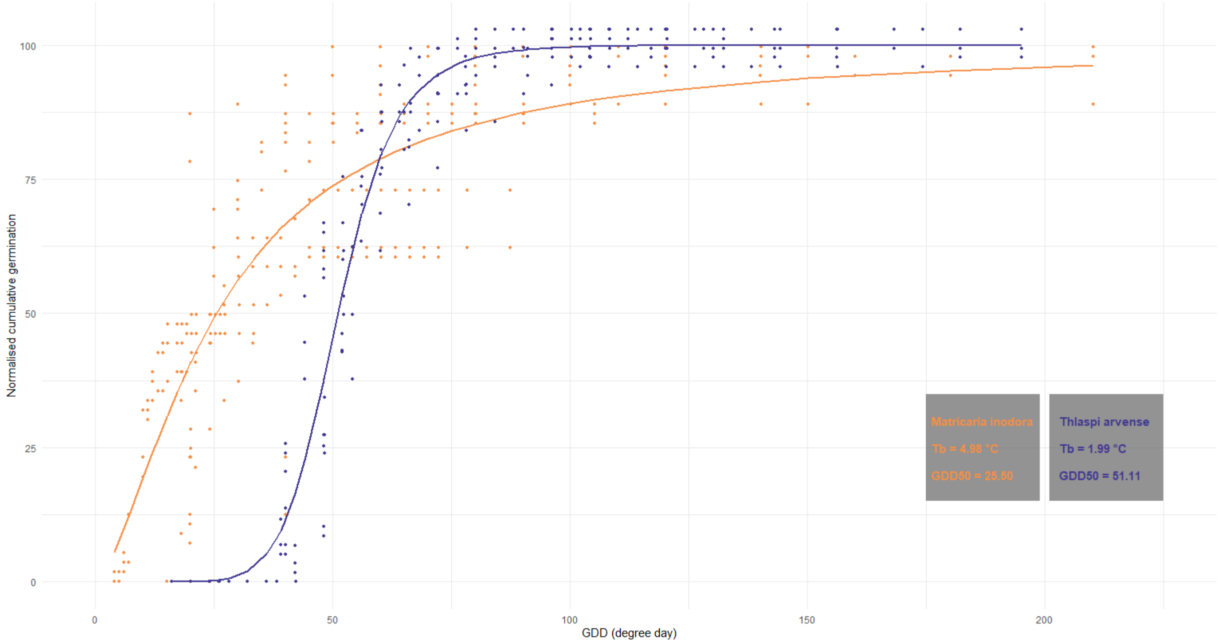

Figure 14. The germination curves for the weeds M. inodora *and* T. arvense. T.arvense *has a lower Tb but higher GDD50 and faster germination rate (b) than* M. inodora. *Since* T. arvense *has a faster germination rate (b), the curve is steeper.*

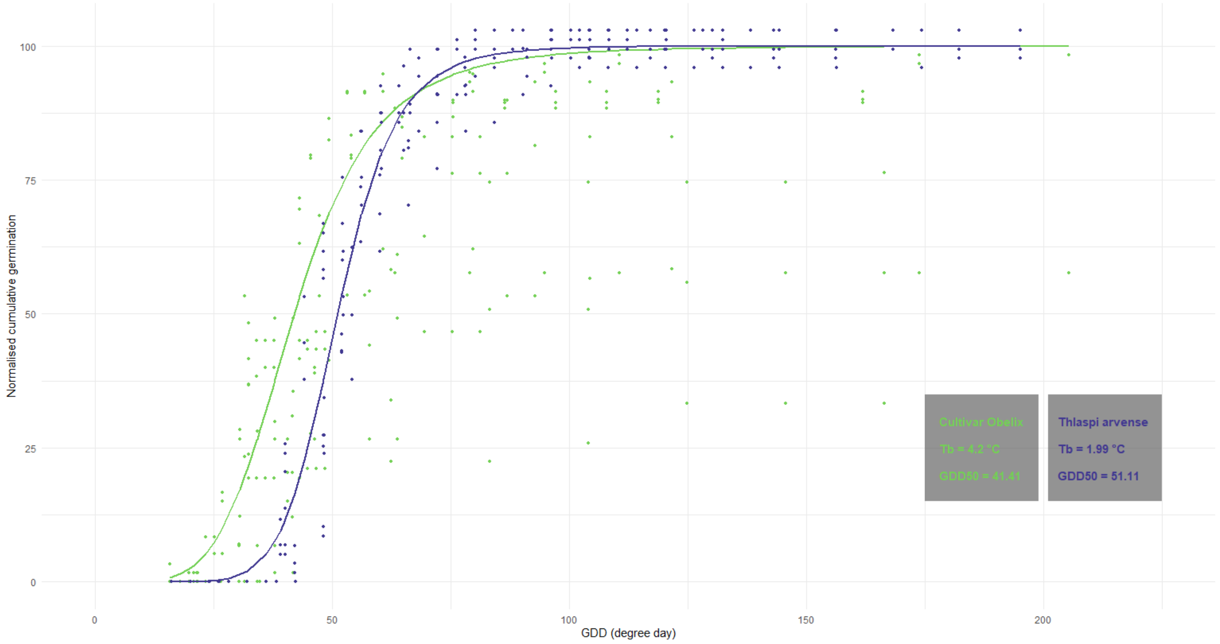

Figure 15. The germination curve for the soybean cultivar 'Obelix' and weed species T. arvense. T. arvense *has a lower* T_b *but a higher* GDD_{50} *and faster germination rate (b) than the soybean cultivar 'Obelix'. Since* T. arvense *has a faster germination rate (b), the curve is steeper.*

5. Discussion

To expand soybean production to the Nemoral zone, detailed knowledge about temperature requirements of soybeans in different development stages is required. Detailed knowledge about temperature requirements can improve the targeted breeding and selection of cultivars for these zones. The temperature requirements for germination are of specific interest due to cold spring temperature in the Nemoral zone (Fogelberg 2009; Jähne et al. 2019). The studied germination parameters were base temperature (T_b), Growing Degree-Day to 50 % germination (GDD_{50}) and maximum germination rate (b). T_b should be as low as possible for all early development stages to ensure that the seeds tolerate the Nemoral climate. To reduce the risk of pathogen attacks on seeds (Bramlage et al. 1978) a low number of GDD_{50} and a high germination rate (b) seems also preferable. The results showed that some of the included cultivars significantly differed from each other in terms of T_b, GDD_{50} and germination rate (b). Cultivars that showed suitable characteristics for germination in cold temperatures are for example 'Obelix', 'Tofina' and 'Träff'.

Following current U.S. classification system very early cultivars would be considered suitable for cultivation in the Nemoral zone. However, the results suggest that selecting cultivars based on maturity group is only partly a useful strategy. The maturity group classification does not ensure best possible cold tolerance at the germination stage, since that is not included in the classification system. 'Bilyavka' (0000) is considered as an extremely early maturing cultivar but the results show that 6 cultivars from maturity group 000 have significantly lower T_b results. Since the maturity group system is based on responses to photoperiod and latitude (Whigham & Minor 1978; Liu et al. 2017) additional information on T_b, GDD_{50} and germination rate (b) could promote the selection of both early maturing and more cold tolerant cultivars suitable early germination in the Nemoral zone.

To evaluate if modern cultivars have better cold tolerance during germination stage compared to historic cultivars they were compared with the oldest cultivar included, 'Fiskeby V'. 'Fiskeby V' was registered in 1968 (Holmberg 1973) and has since been included in breeding programs to develop more cold tolerant cultivars. Eight of the cultivars showed significantly lower T_b differences to the historic cultivar 'Fiskeby V'. In addition, four cultivars also had significantly higher germination rate (b), but none had significantly lower GDD_{50}. Although, the focus of soybean breeding has mainly been to improve early maturing traits, it seems like newer varieties have, intentionally or unintentionally, been bred towards a lower T_b and higher germination rate (b) as well. Thus, the second hypothesis is accurate for T_b and germination rate (b). 'Fiskeby V' had the second highest T_b value of 6.7 °C, which is comparable to the breeder's germination trial where 'Fiskeby V' germinated at 8 °C day temperature and 6 °C night temperature (Holmberg 1973). Among the Swedish cultivars there are significant improvements of T_b for the later developed cultivars 'Bråvalla' and 'Träff' compared to 'Fiskeby V'. No modern cultivar showed significant improvements compared to 'Träff' regarding germination responses of T_b, GDD_{50} and germination rate (b) in cold temperatures. 'Fiskeby V' has been considered as a main part of breeding for early maturing cultivars, inheriting god genetic potential for cold temperature germination (Robison et al. 2019). However, the obtained results suggest that 'Träff' might be a more suitable choice for early germination traits for cold environments in breeding. The exact crossings details and pedigree of the Swedish cultivars have unfortunately been lost.

The T_b for the studied selection of common Swedish weeds ranges from 2 °C to 6.3 °C and almost all weeds are significantly different from each other. Further, the GDD_{50} and germination rate (b) also show significant differences among species. This is supporting the third hypothesis, that there are significant differences amongst weed species. T_b for the weeds *C. bursa-pastoris* (3 °C), *C. album* (3.3 °C), *S. noctiflora* (3.4 °C) and *T. arvense* (2 °C) are all lower than the germination temperatures mentioned in literature. Differences between results and literature can depend on the temperature interval used in the experiments (Popay & Roberts 1970), the origin of the seeds (ecotype) (McNeill 1980; Baskin & Baskin 1989b) and the method used in the germination trials (Popay & Roberts 1970; Baskin & Baskin 1988). Popay and Roberts (1970) states that the germination of *C. bursa-pastoris* is possible at temperatures as low as 4 °C, and that the germination rate will be slow. Both Bajwa et al. (2019) and McNeill (1980) reported minimal germination of 5 °C for *C. album* and 6 °C for *S. noctiflora*, respectively. *S. noctiflora* seeds belong to the German ecotype (McNeill 1980), but other details of the trial to acquire these temperatures are unknown. Baskin & Baskin (1989b) set the baseline germination temperature for *T. arvense*, originating from Kentucky, U.S., at 5 °C. Baskin & Baskin (1989b) tested non-dormant seeds at temperatures of 1 °C and 5 °C and concluded that seeds in these temperatures mostly re-entered dormancy and did not germinate. In contrast to the experiment of Baskin & Baskin (1989b), the seeds in this experiment could not re-enter dormancy since gibberellic acid had been applied. Even though dormancy might hinder germination at suboptimal temperatures (Baskin & Baskin 1989b; Karlsson & Milberg 2007) the T_b results reflect the lowest tolerated temperature for seeds that have already started the germination process. In addition, by normalizing the data the results presented in this thesis are only true for non-dormant and viable seeds.

Seven of the tested weed species have a T_b below the soybean minimum of 4.2 °C. For GDD_{50} the weeds range from 25 to 106 GDD, compared to 36.5 to 60 GDD for soybean. For the parameter b, the range of results was quite similar for soybean and weed species.

To ensure a good soybean yield in organic farming, weeds need to be managed by different mechanical methods in organic farming. The differences in germination dynamics of the selected weeds and soybean cultivars could potentially be used for an informed weed management approach in soybean cultivation. Weed control in organic farming is predominantly based on mechanical measures. The timing of these measures is key for their success and therefore understanding the dynamics of early crop and weed development could help to improve timing and efficiency of mechanical weed control.

Because of the weeds ability to germinate faster at lower temperatures than soybean, early seeding bears the risk that the crop is quickly overgrown by the much faster establishing weeds (Pester et al. 1999). Lamiche et al. (2019) recommend that soybeans should be sown as soon as temperatures are adequate. To this recommendation it can be added that the sowing should be done when temperatures are beneficial for both weed management and crop establishment. On the other hand, the earliness and low temperature requirement of some weeds pose the opportunity to control weeds before soybean sowing or emergence. Early weeds like *S. arvense*, *P. Rhoeas*, *T. arvense* and *C. bursa-pastoris* germinate at low temperatures and require few growing degree days for germination. Those weeds could be controlled for example with a stale seedbed and/or blind harrowing. If blind harrowing is a suitable management tool, it could be beneficial if the chosen soybean cultivar has a slower germination rate, allowing faster weeds to germinate and be eliminated before crop emergence. For controlling later emerging weeds with higher temperature requirements and slower germination rate, a selection of a cultivar with low T_b and GDD_{50} as well as high germination rate could be beneficial. If the soybean establishes faster than the weeds it can better gain a growth advantage (Pester et al. 1999) and better compete for resources. A faster establishing soybean also enables weed management by hoeing in the crop. For hoeing in the growing crop, the soybean must be big enough to tolerate the treatment. If the weeds at the same time are small, they can be removed by effectively.

Not only do early and fast emerging weeds pose difficulties for soybean production. Slow and late emerging weeds like *C. arvense, C. album, P. major* and *M. inodora* can become troublesome as well since their germination period exceeds the available time window for efficient weed control. *C. album* can tolerate low temperatures, but have a high requirement of growing degree days which enables it to germinate and grow alongside the soybean. *C. album* has been recognized as serious weed in soybean cultivation for other parts of the world (Bajwa et al. 2019) and *C. album* originating from the Nemoral zone also seems to have the potential to become problematic. For *C. album*, Swedish weed trials in peas have shown that a combination of mechanical treatment before and after crop establishment is an effective management method (Lundkvist 2009). Due to the generally slow emergence, this could also be a management choice for soybean cultivation. *C. album* will be troublesome independently from the chosen cultivar. A cultivar with high competitiveness throughout the growing season would be preferable. Also, *P. major* and *M. inodora* results suggest a long and late germination period compared to other weeds. The literature support this: *P. major* can germinate throughout the summer period (Hawthorn 1974; Håkansson 1983) while *M. inodora* reaches optimum germination late in spring, around may (Håkansson 1983). The results of the relationship between T_b, GDD_{50} and germination rate (b) of soybeans and weeds indicate that both managing method and soybean cultivar should be selected depending on the main weed flora within the field. A fast-germinating cultivar is not always beneficial; a slower cultivar can be superior for eliminating weeds before soybean emergence.

It has to be considered that the experiments behind the estimated values were performed in a controlled environment, different to conditions normally experienced by seeds under field conditions. In this experiment fluctuating temperatures were not tested. Under field conditions temperatures will fluctuate, both above and below the estimated T_b. Additionally, water stress was not included as a factor. Sufficient water availability to the seeds is crucial for germination (Hicks 1978; Ritchie et al. 1985). Thirdly, dormancy of weed seeds was broken chemically in this experiment. Under field conditions the right environmental conditions must be fulfilled to break seed dormancy (Baskin & Baskin 1985; Milberg 1997; Lundkvist 2009). Due to these limitations, it could be argued, just like Lamichhane et al. (2019) points out, that the germination rate (b) will in fact be slower under field conditions. At last, for perennial weeds it is also important to consider that weed management can not only focus on seeds. As Zaller (2004) points out, tillage can be suitable for seed management but in contrast it may promote growth from root parts instead.

Improvements and recommendation for further investigation of soybean and weeds T_b, GDD_{50} and germination rate (b) are to include more temperatures at degrees below 10 °C. For better fitting of model, it would be beneficial to have steps of 1 °C for temperature regimes. It would give more exact information about the actual T_b value. Moreover, as discovered in this experiment, the quality of soybean seeds is important. In this experiment the germination percentages were low for some cultivars. Possible explanations for this could be physical damage by post-harvest handling or that the seeds were too old (harvested in 2019 and used in

November 2020). The lowered germination quality could also be caused by the drought during the growing year 2019. In addition, for further investigation it could be relevant to explore if the seed coloration of Bråvalla could have had an impact on the cold resistance, it could be possible that Bråvalla has slower uptake of cold water which make it less sensitive to cold damage (Tully et al. 1981). It would also be interesting to test the T_b for the vegetative reproduction parts as stem and roots for perennial weeds. This since vegetive reproduction of perennial plays a significant role in the dispersal of perennial weeds (Roberts & Boddrell 1984; Zaller 2004; Bochenek et al. 2009; Taab et al. 2018). Combined knowledge of T_b for vegetative parts and seed could help improving strategies for field management.

6. Conclusions

In conclusion this experiment has shown that the base temperature for germination (T_b), Growing Degree-Day to 50 % germination (GDD_{50}) and germination rate (b) differ between soybean cultivars belonging in the very early maturity groups. Therefore, it is suggested that T_b, GDD_{50} and germination rate (b) should be included as parameters together with maturity group when breeding soybean cultivars for the Nemoral zone. Secondly, modern cultivars have developed improved cold tolerance compared with the historic reference cultivar Fiskeby V. Thirdly, the T_b, GDD_{50} and germination rate (b) for common Swedish weeds differ significantly. And lastly, the opportunity to mechanically manage weeds in organic farming depends on the relationship of T_b, GDD_{50} and germination rate (b) between soybean and weeds. Weeds with a long or delayed germination period compared to soybeans will possibly be difficult to manage.

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Acknowledgements

I want to thank my supervisors Alexander Menegat and Steffen Dahlke for all help and support during the work with my thesis. It has been a pleasure to learn everything about cardinal temperatures together with you. Thank you! I also want to thank Eva, Nisse and Alexander for helping me with weed counting.

Appendix 1

Results of Z test for soybean cultivars T_b. Significant differences marked with *. Results are significant if $|z| \geq 1.96$.

Cultivar	z (T_b) Obelix	z(T_b) Bråvalla	z(T_b) Tofina	z(T_b) Träff	z(T_b) Sculptor	z(T_b) Merlin	z(T_b) Taifun 8	z(T_b) Abaca	z(T_b) Annushka	z(T_b) Bilyavka	z(T_b) Fiskeby V	z(T_b) Gallec
Obelix		0.30	1.45	1.79	2.06*	1.46	2.76*	3.75*	2.49*	3.01*	7.61*	7.74*
Bravalla	0.30		0.60	0.78	0.97	0.97	1.50	2.26*	2.11*	2.54*	5.66*	6.10*
Tofina	1.45	0.60		0.25	0.54	0.63	1.31	2.34*	1.94	2.42*	6.42*	6.73*
Traff	1.79	0.78	0.25		0.32	0.49	1.13	2.20*	1.86	2.33*	6.38*	6.68*
Sculptor	2.06*	0.97	0.54	0.32		0.31	0.81	1.88	1.74	2.20*	6.09*	6.44*
Merlin	1.46	0.97	0.63	0.49	0.31		0.20	0.91	1.36	1.73	4.23*	4.78*
Taifun 8	2.76*	1.50	1.31	1.13	0.81	0.20		1.06	1.39	1.82	5.33*	5.78*
Abaca	3.75*	2.26*	2.34*	2.20*	1.88	0.91	1.06		0.90	1.28	4.36*	4.92*
Annushka	2.49*	2.11*	1.94	1.86	1.74	1.36	1.39	0.90		0.24	1.61	2.18*
Bilyavka	3.01*	2.54*	2.42*	2.33*	2.20*	1.73	1.82	1.28	0.24		1.40	2.01*
Fiskeby V	7.61*	5.66*	6.42*	6.38*	6.09*	4.23	5.33*	4.36*	1.61	1.40		0.92
Gallec	7.74*	6.10*	6.73*	6.68*	6.44*	4.78	5.78*	4.92*	2.18*	2.01*	0.92	

Appendix 2

Results of Z test for soybean cultivars GDD$_{50}$. Significant differences marked with *. Results are significant if $|z| \geq 1.96$

Cultivar	z(GDD$_{50}$) Obelix	z(GDD$_{50}$) Bråvalla	z(GDD$_{50}$) Tofina	z(GDD$_{50}$) Träff	z(GDD$_{50}$) Sculptor	z(GDD$_{50}$) Merlin	z(GDD$_{50}$) Taifun 8	z(GDD$_{50}$) Abaca	z(GDD$_{50}$) Annushka	z(GDD$_{50}$) Bilyavka	z(GDD$_{50}$) Fiskeby V	z(GDD$_{50}$) Gallec
Obelix		0.72	1.72	1.80	0.96	1.23	0.09	1.32	1.61	2.82*	0.86	1.18
Bravalla	0.72		1.72	1.64	1.27	0.22	0.62	0.08	0.90	1.87	1.22	1.45
Tofina	1.72	1.72		0.39	0.71	2.43*	1.55	2.78*	2.37*	3.58*	0.50	0.08
Traff	1.80	1.64	0.39		0.49	2.46*	1.50	3.05*	2.31*	3.59*	0.28	0.18
Sculptor	0.96	1.27	0.71	0.49		1.88	0.90	2.10*	2.02*	3.22*	0.08	0.46
Merlin	1.23	0.22	2.43*	2.46*	1.88		1.04	0.20	0.80	1.86	1.71	1.94
Taifun 8	0.09	0.62	1.55	1.50	0.90	1.04		1.04	1.51	2.65*	0.84	1.14
Abaca	1.32	0.08	2.78*	3.05*	2.10*	0.20	1.04		0.98	2.13*	1.81	2.04*
Annushka	1.61	0.90	2.37*	2.31*	2.02*	0.80	1.51	0.98		0.81	1.96	2.13*
Bilyavka	2.82*	1.87	3.58*	3.59*	3.22*	1.86	2.65*	2.13*	0.81		3.08*	3.22*
Fiskeby V	0.86	1.22	0.50	0.28	0.08	1.71	0.84	1.81	1.96	3.08*		0.34
Gallec	1.18	1.45	0.08	0.18	0.46	1.94	1.14	2.04*	2.13*	3.22*	0.34	

Appendix 3

Results of Z test for soybean cultivars germination rate (b) at 50 % germinaton. Significant differences marked with *. Results are significant if $|z| \geq 1.96$

Cultivar	z (b) Obelix	z(b) Bråvalla	z(b) Tofina	z(b) Träff	z(b) Sculptor	z(b) Merlin	z(b) Taifun 8	z(b) Abaca	z(b) Annushka	z(b) Bilyavka	z(b) Fiskeby V	z(b) Gallec
Obelix		4.20*	1.71	1.41	1.21	0.14	3.74*	1.66	0.77	1.49	2.45*	2.66*
Bravalla	4.20*		2.62*	7.96	2.73	3.50	0.88	3.35*	1.94	1.99*	1.83	1.34
Tofina	1.71	2.62*		3.78	1.91	1.39	2.05*	0.22	0.43	0.02	0.77	1.06
Traff	1.41	7.96*	3.78*		0.72	1.42	7.46*	4.05*	1.83	3.04*	4.84*	4.99*
Sculptor	1.21	2.73*	1.91	0.72		1.25	2.56*	1.87	1.55	1.87	2.19*	2.29*
Merlin	0.14	3.50*	1.39	1.42	1.25		3.09*	1.32	0.62	1.24	2.04*	2.25*
Taifun 8	3.74*	0.88	2.05*	7.46*	2.56	3.09*		2.72*	1.61	1.54	1.21	0.75
Abaca	1.66	3.35*	0.22	4.05*	1.87	1.32	2.72*		0.31	0.20	1.09	1.40
Annushka	0.77	1.94	0.43	1.83	1.55	0.62	1.61	0.31		0.41	0.91	1.11
Bilyavka	1.49	1.99*	0.02	3.04*	1.87	1.24	1.54	0.20	0.41		0.61	0.86
Fiskeby V	2.45*	1.83	0.77	4.84*	2.19*	2.04*	1.21	1.09	0.91	0.61		0.33
Gallec	2.66*	1.34	1.06	4.99*	2.29*	2.25*	0.75	1.40	1.11	0.86	0.33	

Appendix 4

Results of Z test for weed species T_b. Significant differences marked with *. Results are significant if $|z| \geq 1.96$

Species	z (T_b) *C.bursa pastoris*	z(T_b) *C. album*	z(T_b) *C. arvense*	z(T_b) *M. inodora*	z(T_b) *P. rhoeas*	z(T_b) *P. major*	z(T_b) *R. crispus*	z(T_b) *S. noctiflora*	z(T_b) *S.oleraceus*	z(T_b) *T. arvense*
C. bursa pastoris		1.35	6.41*	13.15*	3.41*	9.65*	0.32	2.15*	3.51*	8.96*
C. album	1.35		5.48*	10.46*	4.32*	8.77*	1.05	0.52	4.72*	9.42*
C. arvense	6.41*	5.48*		0.07	8.04*	3.14*	6.23*	5.43*	8.31*	10.62*
M. inodora	13.15*	10.46*	0.07		13.70*	3.93*	12.74*	11.80*	22.60*	28.50*
P. rhoeas	3.41*	4.32*	8.04*	13.70*		10.98*	3.65*	5.20*	1.57	2.28*
P. major	9.65*	8.77*	3.14*	3.93*	10.98*		9.49*	8.82*	11.45*	13.48*
R.crispus	0.32	1.05	6.23*	12.74*	3.65*	9.49*		1.80	3.90*	9.31*
S. noctiflora	2.15*	0.52	5.43*	11.80*	5.20*	8.82*	1.80		6.97*	13.03*
S. oleraceus	3.51*	4.72*	8.31*	22.60*	1.57	11.45*	3.90*	6.97*		11.39*
T. arvense	8.96*	9.42*	10.62*	28.50*	2.28*	13.48*	9.31*	13.03*	11.39*	

Appendix 5

Results of Z test for soybean cultivars GDD_{50}. Significant differences marked with *. Results are significant if $|z| \geq 1.96$

Species	z(GDD_{50}) *C.bursa pastoris*	z(GDD_{50}) *C. album*	z(GDD_{50}) *C. arvense*	z(GDD_{50}) *M. inodora*	z(GDD_{50}) *P. rhoeas*	z(GDD_{50}) *P. major*	z(GDD_{50}) *R. crispus*	z(GDD_{50}) *S. noctiflora*	z(GDD_{50}) *S.oleraceus*	z(GDD_{50}) *T. arvense*
C. bursa pastoris		19.47*	8.04*	10.86*	8.46*	2.14*	8.33*	7.88*	7.03*	1.64
C. album	19.47*		2.58*	26.76*	7.98*	17.25*	12.50*	13.13*	25.80*	20.70*
C. arvense	8.04*	2.58*		12.25*	2.71*	8.53*	4.45*	4.74*	10.41*	7.79*
M. inodora	10.86*	26.76*	12.25*		15.40*	5.29*	17.99*	17.77*	7.21*	15.40*
P. rhoeas	8.46*	7.98*	2.71*	15.40*		8.57*	2.47*	2.94*	13.03*	8.38*
P. major	2.14*	17.25*	8.53*	5.29*	8.57*		7.93*	7.57*	1.63	3.30*
R.crispus	8.33*	12.50*	4.45*	17.99*	2.47*	7.93*		0.60	16.20*	8.80*
S. noctiflora	7.88*	13.13*	4.74*	17.77*	2.94*	7.57*	0.60		15.99*	8.32*
S. oleraceus	7.03*	25.80*	10.41*	7.21*	13.03*	1.63	16.20*	15.99*		15.71*
T. arvense	1.64	20.70*	7.79*	15.40*	8.38*	3.30*	8.80*	8.32*	15.71*	

Appendix 6

Results of Z test for weed species germination rate at 50 % germination (b). Significant differences marked with *. Results are significant if |z|≥1.96

Species	z (b) *C.bursa pastoris*	z (b) *C. album*	z (b) *C. arvense*	z (b) *M. inodora*	z (b) *P. rhoeas*	z (b) *P. major*	z (b) *R. crispus*	z (b) *S. noctiflora*	z (b) *S.oleraceus*	z (b) *T. arvense*
C. bursa pastoris		0.83	1.36	9.74*	0.29	1.58	5.13*	3.83*	4.38*	7.87*
C. album	0.83		0.85	12.11*	1.17	1.85	5.43*	4.91*	5.48*	9.40*
C. arvense	1.36	0.85		5.54*	1.61	2.08*	5.53*	4.52*	5.00*	8.02*
M. inodora	9.74*	12.11*	5.54*		10.27*	4.15*	7.67*	12.04*	12.38*	16.93*
P. rhoeas	0.29	1.17	1.61	10.27*		1.48*	5.04*	3.60*	4.17*	7.67*
P. major	1.58	1.85	2.08*	4.15*	1.48		2.68*	0.04	0.23	1.55
R.crispus	5.13*	5.43*	5.53*	7.67*	5.04*	2.68*		3.57*	3.31*	2.04*
S. noctiflora	3.83*	4.91*	4.52*	12.04*	3.60*	0.04	3.57*		0.58	3.57*
S. oleraceus	4.38*	5.48*	5.00*	12.38*	4.17*	0.23	3.31*	0.58		2.92*
T. arvense	7.87*	9.40*	8.02*	16.93*	7.67*	1.55	2.04*	3.57*	2.92*	