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Title: Seed Dormancy in Domesticated and Wild Sunflowers (*Helianthus annuus* L.): Types, Longevity and QTL Discovery

Abstract approved:

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Carol A. Mallory-Smith

Elite inbred sunflower (*Helianthus annuus* L.) lines were found to have short-lived embryo and seed covering dormancies. Seed dormancy of wild sunflowers (*H. annuus*, *H. argophyllus* and *H. exilis*) was found to be controlled primarily by the seed covering (seed coat and pericarp) and embryo dormancy was short-lived (four to eight weeks). Native American Landraces (NALs) had low to moderate embryo and seed covering dormancy, which was more similar to elite lines. The seed covering in the NALs contributed more to seed dormancy than did the seed covering in the elite lines. The seed coat itself was implicated in seed dormancy and the length of dormancy caused by the seed coat varied by accession and variety. Excising ¼ of the seed, rather than removing the entire seed covering, increased germination but also increased the number of seed that decayed. Dormancy of embryos from 19 wild sunflower accessions ranging in south to north latitude from Texas to Saskatchewan, Canada was found to be highly variable; however seed covering dormancy was similar among all the wild accessions in the northern latitudes. Germination of accessions in the most southern latitudes was greater ($P<0.0001$) than germination of accessions from more northern latitudes. The seed covering had to be completely removed in order to maximize germination of wild accessions from all latitudes. Seed germination of whole achenes of elite lines was greatest under alternating conditions of 12 hours of light and 12 hours of darkness.

Recombinant inbred lines (RILs) of an elite by wild cross (HA89 x ANN1238) were evaluated for seed dormancy quantitative trait loci (QTL). QTL were mapped to 14 of the 17 linkage groups, for the time course of 0 to 24 weeks of seed after-ripening. Twenty four QTL related to whole seed dormancy were found. Whole seed dormancy QTL explained between 9-30% of the phenotypic variation observed. Seven QTL were found related to embryo dormancy. QTL related to embryo dormancy explained from 12-23% of the phenotypic variation observed. Delay of germination percentage (DOGp) of each RIL was calculated using the Richards function (1959) to detect QTL related to 25, 50 and 75% germination. Twelve DOGp QTL were detected to help further explain seed dormancy QTL. Four strong regions harboring several QTL each were identified which sets the stage for more in depth future analyses.

# Seed Dormancy in Domesticated and Wild Sunflowers (*Helianthus annuus* L.): Types, Longevity and QTL Discovery

by
Robert L. Brunick

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Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Robert L. Brunick, Author

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# CHAPTER 1

## GENERAL INTRODUCTION

Domesticated sunflowers (*Helianthus annuus L.*) are an important oil seed crop worldwide. Sunflowers are native to North America where wild and weedy forms of the species are abundant. Wild types of *H. annuus* are adapted to the widest number of environments of all the species of sunflower where its habitat ranges from coast to coast and to the southern border of the USA and north of the US-Canadian border. Sunflowers are one of the few food crops originating in North America and were domesticated approximately 4500-3500 years ago by Native Americans (Diamond, 1999). Native American Landraces played an important part in the culture of many Indian tribes in the West. Sunflower achenes provided a rich source of fatty acids and pigments were extracted from achenes and used as dyes. Sunflowers are grown commercially for both oil and confection use. They are also widely used in the ornamental industry where they are used in fresh cut flower arrangements. Sunflowers are also produced commercially for use as wild bird and squirrel feed. Sunflowers are one of the native species to the USA that could be used in land reclamation and restoration, but seed dormancy in wild sunflowers prohibits this on a large scale (Seiler, 1998).

Wild sunflowers are often difficult to control in agronomically important crops and are an important weed in many of the cropping systems in the USA (Arias and Reisburg, 1994). Sunflower seed dormancy plays an important role in both commercial sunflower production and in managing wild sunflowers in crops and along roadsides and ditches that border crop fields (Snow et al. 1998). Sunflower achenes which are commonly referred to as seeds, may have embryo, seed coat and pericarp dormancy and are likely to have combinations of the three. Seed dormancy in wild sunflower species is primarily controlled by the seed coat and pericarp and can last for several years (Heiser, 1969 and Seiler, 1998). Little is known about embryo dormancy among the wild sunflower biotypes.

Wild sunflowers are good candidate donors for agronomic traits such as drought tolerance, salt tolerance, disease resistance, water use efficiency and biomass production and can easily be crossed with cultivated types; however, seed dormancy limits the use of some wild accessions for breeding. In addition, other traits linked to dormancy are not known. Embryo dormancy which can last up to eight weeks limits the cycling of breeding germplasm and interferes with sunflower breeding operations when breeders have nurseries in both northern and southern hemispheres or when attempts are made to rapidly cycle material in greenhouses. Dry storage will typically overcome seed dormancy of domesticated elite sunflower types but not wild sunflowers (Seiler, 1998); therefore, easy to use, consistent alternative methods need to be devised to germinate wild sunflower seeds.

The mechanisms and duration of dormancy in wild sunflowers and the requirements for germination are not well understood. Sunflower seed dormancy can be difficult to break, especially in wild accessions, but techniques such as soaking seeds in acidic, hydrogen peroxide or hot water solution or by heating seeds to extreme temperatures (Akinola et al. 2000); soaking in gibberellic acid solutions (Chandler and Jan, 1985; Seiler, 1998); scarifying and dehulling (Chandler and Jan, 1985; Brunick et al. 2008a; Brunick et al. 2008c); increasing atmospheric oxygen concentrations (Gay et al. 1991); or by simply allowing seeds to soak in moist pots in varying climatic conditions (Heiser et al. 1969) have proven to increase germination. The optimum temperature for germinating sunflower seeds has been reported to be 25$^{o}$C; seeds can germinate at 40$^{o}$C but many seeds fail to germinate at temperatures above 45$^{o}$C (Gay et al. 1991).

The seed coat itself has been found to inhibit germination by limiting oxygen to the embryo (Gay et al. 1991) and dormancy can be controlled by a combination of embryo and seed covering (Kelly, 1992; Nikolaeva, 1977). Essential requirements of after-ripening, light, moisture or hormone levels must be met in order for an embryo to germinate. However, the seed covering can inhibit some of these essential elements from reaching the embryo by blocking out moisture or gasses (Kelly, 1992) and the seed coverings may contain chemicals that directly inhibit germination (Sondheimer, 1966; Wareing and Foda, 1957). Seed dormancy related to both embryo and seed covering has

been shown to vary widely within other species (Foley, 2001). The hormone balance theory, where abscisic acid (ABA) and gibberellic acid (GA), must be in balance in order for germination to occur, has also been implicated in seed dormancy (Karssen and Lacka, 1986).

Seed dormancy is influenced by the environment, seed genetics (Bentsink et al. 2006; Simpson 1990; Strand 1991) and the resulting genetic x environment interaction (King et al. 1989). In species such as wild oat, the heritability of seed dormancy has been determined to be 50% with the environment affecting the remaining 50% of the response (Jana and Naylor, 1980).

The wild *H. annuus* sunflowers have adapted to more climates throughout North America than any of the many other species of sunflower (Seiler and Reisberg, 1997). Wild sunflowers are found from the southernmost part of Texas to north of the USA-Canadian border. Linder (2000) and Baskin (1973) indicated that latitude may have an impact on seed dormancy. Linder (2000) found that accessions of wild *H. annuus* seeds from lower latitudes were more likely to germinate than seeds from higher latitudes. Germination of the biotypes was correlated to the fatty acid profile of the accessions tested and the selection pressures under those specific germinating temperatures. Localized adaptations have led to numerous individual populations that have unique genotypic and phenotypic traits regarding seed germination (Linder, 2000), seed oil concentration (Fick et al. 1976; Seiler 1985, 1994) and fatty acid profile (Knowles et al. 1970; Fernandez-Martines and Knowles, 1976; Dorrell and Whelan, 1978; Thompson et al. 1978).

The environment in which the mother plant grew during seed formation had a great impact on seed dormancy (Fenner, 1991). Year-to-year differences in the degree of embryo dormancy in sunflower have been reported (Le Page-Degivry et al. 1990) and temperature at the time of maturation can impact the level of dormancy in sunflower seeds. Stage of maturity at harvest can also impact seed dormancy. Sunflower seed dormancy is weaker when harvested before physiological maturity and storage time and temperature cannot overcome dormancy in late maturing achenes (Seiler, 1998). Seeds harvested at physiological maturity have been shown to have maximum seed dormancy.

Seed dormancy of cultivated sunflowers can typically be overcome during dry storage (Corbineau et al. 1991). The optimum moisture level for storage of sunflower seed has been reported to be approximately 3% dry weight (Vertucci and Roos, 1990, 1993).

Long term seed covering dormancy has been bred out of domesticated sunflowers, whereas, wild types have prolonged seed covering dormancy. Many of today's cultivars are descendants from both the wild *H. annuus* types and sunflowers grown by the Native Americans centuries ago (Heiser 1945; Heiser 1951; Castetter 1935; Wilson 1917). However, little is known about the seed dormancy of Native American Landraces.

There have been no other reports of multiple time course germination studies in sunflower. A few studies had been done on a few wild accessions and elite cultivars but the germination studies were only conducted at one after-ripening time interval. Two studies reported QTL in sunflower (Gandhi et al. 2005; Al-Chaarani et el. 2005), but these studies were conducted at a single after-ripening time period in different populations.

The objectives of this research were to: 1) evaluate seed treatments in both elite and wild sunflowers and find the most effective methods to germinate seeds. 2) to learn the differences in dormancy related to the embryo, seed coat and pericarp and determine if there were differences between domesticated and wild sunflowers regarding these tissues. 3) evaluate germplasm from various latitudes and determine if there were differences in seed dormancy type and longevity among accessions related to latitude. 4) phenotype seed dormancy in an elite by wild recombinant inbred line (RIL) population and scan the chromosomes for seed dormancy quantitative trait loci (QTL) over five after-ripening time periods in a large time course study.

# CHAPTER 2

## Embryo, Seed Coat, and Pericarp Dormancy in Domesticated and Wild Sunflower

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**ABSTRACT**

Common sunflower (*Helianthus annuus* L.) is a globally important oilseed crop indigenous to North America. Wild and weedy types are serious pests in soybean and other crops grown in the USA and have deep seed dormancy; whereas, domesticated and cultivated types have shallow seed dormancy. The physiological and genetic mechanisms underlying sunflower seed dormancy are poorly understood. The goals of our studies were to identify the role played by the embryo, seed coat, and pericarp in seed dormancy among domesticated and wild sunflower genotypes. We estimated the effects of after-ripening time (0, 4, and 8 wks) and seed treatments (embryo, embryo + seed coat, and embryo + seed coat + pericarp) on the seed germination of two elite inbred lines (HA89 and RHA3737), one wild *H. annuus* (ANN1238), one wild *H. exilis* (Ames27234), four elite x wild (HA89 x ANN1238) recombinant inbred lines (RILs). The RILs were selected because they had different seed dormancy phenotypes detected in a previous experiment. Significant differences were observed among genotypes and seed treatments ($P < 0.0001$). Seed dormancy was short-lived, less than 8 weeks, and was primarily embryo-induced in both elite inbred lines. By contrast, seed dormancy was more protracted and primarily induced by the seed coat and pericarp in both wild populations (embryo dormancy was negligible).

**INTRODUCTION**

Wild sunflowers (*Helianthus annuus* L.) likely possess a great number of highly desirable genes that would be useful for further developing agronomically suitable oil and confection-type cultivars. There is a demand to enhance garden and ornamental sunflower types with novel plant and flower characteristics using compatible wild relatives. Seed dormancy in wild sunflower species makes it difficult to incorporate wild germplasm into sunflower breeding programs by minimizing the capacity of plant breeders to rapidly cycle germplasm. Currently, there is also a desire to use native wild sunflowers in land reclamation and restoration in the United States (Seiler, 1998). However, seed dormancy often inhibits the use of wild sunflower species for use in land reclamation and restoration because germination rates can be very low and unpredictable.

Sunflower achenes, which are commonly referred to as seeds, may have embryo, seed coat, and/or pericarp dormancy and are likely to have combinations of the three. In order to effectively use wild sunflower materials in breeding programs, it is essential to understand the mechanisms that control seed dormancy, and the duration of each type of dormancy. Embryo dormancy is often short lived (4-8 weeks); however, seed coat and pericarp dormancy can persist for longer periods of time (>32 weeks) (unpublished data). The authors have tried unsuccessfully to germinate whole achenes of the wild *H. annuus* ANN1238 which were after-ripened at room temperature for two years. Upon removal of the seed coat and pericarp, the embryos readily germinate. Genotypes that have prolonged seed coat and pericarp dormancy are the most difficult to incorporate into breeding programs. Typically, most cultivated types show little to no embryo or pericarp and seed coat dormancy and usually germinate within a few months from harvest, but wild sunflowers can have prolonged dormancy and low or uneven germination. The type of dormancy in the wild sunflowers is not well understood.

Desirable genes were likely selected against during the domestication process when non-dormant types were selected. Understanding the controls of seed dormancy in both wild and elite germplasm will likely provide useful information to assess which traits may have been selected against. Furthermore, to more completely understand the seed dormancy role prior to the transition of domestication, discovering the linkages/relationships between non-dormant cultivated types and desirable genes in wild types that were selected against during the domestication process is important.

The breaking of sunflower seed dormancy has been shown to be hastened by soaking seeds in acidic, hydrogen peroxide or hot water solutions or by heating seeds at extreme temperatures (Akinola et al. 2000); soaking in gibberellic acid solutions (Seiler, 1998); scarifying and dehulling (Chandler and Jan, 1985); increasing atmospheric oxygen concentrations (Gay et al. 1991); or by simply allowing seeds to soak in moist soil in pots in varying climatic conditions (Heiser et al. 1969). However, germinating the seed is time consuming, variable, and results are unpredictable, especially with wild germplasm.

Gay et al. (1991) evaluated the inhibitory germination effects that the seed coat had on non-dormant seeds of the sunflower cultivar ‘Mirasol’ and reported that the seed

coat delayed or inhibited germination, especially at low oxygen concentrations. In these non-dormant seeds, germination increased as oxygen concentration increased. In addition, the seed coat decreased germination of non-dormant seeds when seeds were exposed to high temperatures.

Our objective was to evaluate the length of embryo, seed coat, and pericarp dormancies in physiologically mature non-after-ripened and after-ripened sunflower seeds of elite inbred lines, wild accessions, and recombinant inbred lines (RILs).

## MATERIALS AND METHODS

Seed was produced at Corvallis, OR in the summer of 2004. In order to minimize seed after-ripening on the mother plant, all genotypes were harvested at physiological maturity, as defined by Schneiter and Miller (1981). After harvest, seed was immediately dried at 39$^{o}$C for 17 h, cleaned and stored in vacuum-sealed (model ASTM F-1249) KAPAK® bags, to keep out moisture, and stored at 4$^{o}$C ± 2$^{o}$C to decelerate after-ripening. Seed stored at 4$^{o}$C is referred to as non-after-ripened seed. Seed was after-ripened for 4 or 8 wk at 20$^{o}$C ± 2$^{o}$C in a germination chamber. The after-ripening temperature of 20$^{o}$C was chosen to simulate after-ripening at room temperature.

The genotypes evaluated were: RHA373, HA89, ANN1238 (wild *H. annuus*), RIL71, RIL114, RIL140, RIL182, and Ames27234 (wild *H. exilis*). The $F_8$ RILs (HA89 x ANN1238) were selected for this experiment based on dormancy testing in a previous experiment. The RILs were developed from a population previously described by Burke et al. (2002). The selected genotypes were expected to segregate for embryo, seed coat, and pericarp + seed coat dormancy.

For germination time 0, the seeds were removed from 4$^{o}$C and treatments begun immediately. Seeds were removed from the after-ripening chamber at 4 or 8 wk for the first and second after-ripened germination times, respectively. Whole seeds were used as the control group.

Seeds were disinfected prior to imbibition by agitating in a 50/50 solution of sodium hypochlorite and water (3% v/v) for 90 s. Seeds were then rinsed with distilled, de-ionized water to remove the disinfectant. Four replicates of 25 seed of each genotype

were placed into Petri plates on blue blotter paper with 5 ml of distilled water. Petri plates were kept in Ziploc® bags inside the germinator to decrease evaporation. Seeds/embryos were germinated at 25°C with 12 h of light and dark periods. Seeds were allowed to imbibe and on the fifth day the pericarp + seed coat (NOPSC) or pericarp only (NOP) were removed. It was necessary to wait five days to apply the treatment in order to completely remove the seed coat and pericarp without injuring the embryo. A dry embryo cannot be removed from the seed coat and pericarp whereas an imbibed achene makes removal of the embryo possible. The altered seeds were compared to seeds left whole. In order to remove the pericarp or the pericarp + seed coat in the *H. annuus* genotypes, achenes were split along the longitudinal axis and embryos were pulled from the pericarp without damaging the embryo. In the treatment where the seed coat was also removed, if necessary, a scalpel was used to cut the seed coat between the cotyledons and remove the embryo without damage. Any embryos that were inadvertently damaged were discarded. In order to separate *H. exilis* (Ames27234) embryos from the pericarp + seed coat, it was necessary to excise ¼ of the blunt end of the seed in order to squeeze the embryo out of the pericarp + seed coat. The embryo could not be removed from just the pericarp for *H. exilis* due to the extremely small size of the achenes. As a result, only two of the treatments (whole seed and NOPSC) were applied to *H. exilis*. After removal of the pericarp + seed coat or the pericarp only, the embryos were placed on new blotter paper to decrease chances of fungal infection. Germination data were collected each day to evaluate the speed of germination of each genotype for all treatments (data not shown) up to day 21. When the radical pierced the pericarp of the whole achene or when the radical was visibly elongated in the altered seeds, a seed was considered germinated.

The experiment was conducted as a completely randomized design with four replicates. Raw data of each genotype were analyzed using Proc Mixed model in statistical analysis system (SAS). Statistical difference was defined as $P<0.05$. The fixed effects (genotype, treatment and after-ripening time), and all interactions (genotype x treatment, genotype x after-ripening time, treatment x after-ripening time and, genotype x treatment x after-ripening time) were found to be significant ($P<0.0001$). Therefore, each genotype and treatment was evaluated independently. All genotypes were fitted to linear

and quadratic models for germination. All genotypes fit the linear model (P-value<0.0001) and genotypes ANN1238 (P-value <0.0001), RHA373 (P-value<0.0001), RIL71 (P-value<0.005), and RIL140 (P-value <0.0005) fit the quadratic model.

## RESULTS

### Dormancy in Elite Lines

HA89 demonstrated embryo, seed coat, and pericarp + seed coat dormancy at 0 weeks after-ripening. When the pericarp only was removed, germination did not differ from the whole seed at any after-ripening time period (P=0.27). However, removal of the pericarp + seed coat increased germination by more than 20% (Fig. 2.1) (P<0.0001) over removal of the pericarp only at both the 4 and 8 wk after-ripening times. These results demonstrate that as embryo dormancy fades, the seed coat accounted for approximately 20% of the total dormancy up to at least 8 wk after-ripening.

RHA373 had low embryo dormancy and high seed coat and pericarp + seed coat dormancy as non-after-ripened seed. Both pericarp and seed coat played a significant role in dormancy in non-after-ripened seed. The germination rates of 16, 21, and 74% for the whole seed, NOP, and NOPSC treatments, respectively, provide evidence that the seed coat and the pericarp contribute differently to seed dormancy (Table 2.1). Pericarp + seed coat accounted for 58% (16%-74%) of the dormancy in non-after-ripened seed while seed coat alone only accounted for 5% (16%-21%). By wk 4 of after-ripening, embryo and pericarp + seed coat dormancy had completely faded; germination in the control treatment was 97%. In RHA373 embryo, seed coat and pericarp + seed coat dormancy are extremely short lived. Because the achenes of RHA373 were extremely soft when imbibed for five days, the achene could not be cut to remove the embryo in the NOPSC treatment, to remove the pericarp + seed coat, without destroying the seed and embryo. As a result, only whole seed were evaluated of RHA373 at wk 4 and 8 after-ripening time periods. At these after-ripening time periods, on 5 d, when the treatments were to be applied, the germination rates of RHA373 in the NOPSC treatments were 60 and 92%, respectively; germination in the NOP treatments were 63 and 93%,

respectively, while germination of whole seed was 76 and 90%, respectively. Because the NOPSC and NOP treatments were applied at 5 d, some of the genotypes had germinated seeds before the treatments were applied. The germination percentages at 5 and 21 d after imbibition are presented in Table 2.1.

The differing roles of embryo and seed coat dormancy expressed in HA89 and RHA373 are likely due to the germplasm sources used to generate these two elite lines. In HA89, embryo and pericarp + seed coat dormancy played nearly equal roles at 0 wk after-ripening; while for non-after-ripened seed of RHA373, the seed coat and pericarp + seed coat were almost solely responsible for seed dormancy. In RHA373, embryo and pericarp + seed coat dormancy faded within 4 wk. In contrast in HA89, embryo and pericarp + seed coat dormancy were still present at 4 wk after-ripening and decreased germination by approximately 40 and 60%, respectively. If only a few plants are needed for making crosses, enough seeds will germinate readily within 4 wk after harvest. These responses, however, are genotype specific and are expected to vary, perhaps widely.

**Dormancy in Wild *H. annuus* and *H. exilis***

In ANN1238 at 0 wk after-ripening, there is complete pericarp + seed coat dormancy (0% germination in the control), nearly complete seed coat dormancy (5% germination in the NOP treatment), and partial embryo dormancy (42% germination in the NOPSC treatment). At 4 and 8 wk after-ripening, embryo dormancy was nearly gone, with 92 and 95% germination, respectively, for the NOPSC treatment. The NOPSC treatment increased germination ($P<0.0001$) over the whole seed and NOP treatments when treatments were compared over after-ripening time periods. In the NOP treatment, there was strong evidence that seed coat dormancy continued to at least 8 wk. In the NOP treatment, germination at 4 and 8 wk after-ripening was 39 and 36%, respectively, while germination in the NOPSC treatment was 92 and 95%. These results, demonstrate that embryo dormancy was short lived, approximately 4 wk. Over the course of 8 wk of after-ripening, no whole seeds germinated which indicated that the pericarp + seed coat completely inhibited germination during this same time period ($P=1.000$). *H. exilis* seeds (Ames 27234) in the NOPSC treatment showed little to no embryo dormancy at 0, 4, and

8 wk after-ripening as evidenced by the percent germinations of 95, 100, and 99, respectively, while whole seeds had germination percentages of 0, 1 and, 0, respectively, during the same time periods. As a result, we concluded that seed dormancy of *H. exilis* was controlled entirely by the pericarp + seed coat ($P<0.0001$).

**Dormancy in RILs**

With 0 wks after-ripening, RIL71 showed nearly complete embryo dormancy. RILs 140 and 182 germinated at 51 and 100% which was different than RILs 71 and 114 which had 5 and 14% germination rates, respectively, in the NOPSC treatment. Without after-ripening, RIL71 and RIL114 germinated most like the elite parent while RIL140 germinated most like the wild parent. However, in the NOP treatment the seed coat reduced germination significantly ($P<0.0001$) of RILs 114 and 140 to 2 and 0%, respectively. The seed coat alone accounts for 48 and 100% of the dormancy in these two RILs, respectively. These two RILs were nearly identical in germination to that of the parents in the NOP treatment, while RIL182 germinated at 25%. RIL182 shows the classic signs of transgressive segregation as it had greater germination than both parents in the NOP and NOPSC treatments as non-after-ripened seed.

At 4 wk after-ripening, seed coat dormancy was still present in RILs 71, 114, and 140. Germination of these RILs in the NOP treatment was 7, 12, and 9%, respectively, while germination of RIL182 in the NOP treatment was 53%. The seed coat dormancy had nearly faded in RIL182 at 4 wk after-ripening while it still predominated in the other three RILs. Embryo dormancy was markedly reduced in RILs 71, 114, and 140 after 4 wk of after-ripening. Germination of these RILs increased to 60, 93, and 97%, respectively, in the NOPSC treatment which demonstrated that the pericarp + seed coat dormancy was longer lived than embryo dormancy in these RILs, which is more like that of the wild parent. Of the RILs, with the exception of RIL182, at 4 wk after-ripening, the NOP treatment caused no increase in germination over that of whole seed. Therefore, this suggests that in the early stages of after-ripening, seed coat alone played a strong role in dormancy. If germination of only a few seeds was required for breeding purposes,

removal of both the pericarp + seed coat would improve germination in most genotypes and could be performed successfully 4 wk after harvest.

At 8 wk after-ripening, embryo and pericarp + seed coat dormancy were greatly reduced in RIL71; germination of whole seed was 95%. Seed coat dormancy decreased between 4 and 8 wk after-ripening in RILs 114 (germination in the NOP treatment was 72%) and 140 (germination in the NOP treatment was 75%). However, pericarp + seed coat dormancy was still present at a high level in RIL114 (germination of control was 7%) and at moderate levels in RILs 140 (germination of control was 59%) and 182 (germination of control was 52%). This result demonstrated that the seed coat alone accounted for part but not all of the pericarp + seed coat dormancy in these RILs. Recombinant inbred lines 140 and 182 germinated in the control treatment much more similar to the elite parent, HA89. In contrast, at 8 wk after-ripening, 93% of the dormancy in RIL114 was related to pericarp + seed coat dormancy which was more like the dormancy of the wild parent.

## DISCUSSION

The pericarp is comprised of several distinct layers (Hanausek, 1902; Roth, 1977). The role that each of these layers plays in germination is not known. It is theoretically possible that each layer contributes differently to the level of pericarp dormancy. If this is the case, these different layers would help explain the gradations, which we observed, in pericarp + seed coat dormancy, that is, the dormancy that is caused by the pericarp alone. In this study, the entire pericarp explains only part of the dormancy differences seen between the NOPSC treatment and whole seed. According to Bewley and Black (1982) seed dormancy can either be coat-imposed or controlled within the embryo itself. In sunflower, coat-imposed dormancy would include both the seed coat and the pericarp. In addition, Debeaujon et al. (2000) stated that the seed coat was partially responsible for dormancy in *Arabidopsis* because it excluded water and or oxygen from the embryo or by mechanically inhibiting the radical from emerging. Corbineau and Come (1992) found that sunflower seed envelopes inhibited germination of physiologically mature seeds at

high temperatures of 35$^{o}$C and greater. Several types of dormancy ranging from physical to chemical have been implicated in seed dormancy by Ballard (1973) and Torrey (1976).

Roth (1977) demonstrated that the seed coat and pericarp were closely held together, but do not coalesce completely. In ANN1238, the seed coat and pericarp are nearly fused. Separating the embryo plus the seed coat from the pericarp and to leave the entire seed coat intact to the embryo (NOP treatment) was difficult. Based on visual observation, some of the ANN1238 embryos in the NOP treatment lacked the entire seed coat; however, all of the embryos in this treatment had some part of the seed coat completely surrounding the embryo. As a result, germination in this treatment may be higher than if all of the seed coat had remained intact. The seed coat consists of three distinct layers (Gassner, 1973); therefore, it is theoretically possible that each layer plays a role in dormancy, or at least contributes to the level of dormancy. Because we have demonstrated that the seed coat played a major part in dormancy, the level of dormancy may vary depending on the number of layers of seed coat that remained intact for the NOP treatment. If one or more parts of a layer were removed, it is likely that this contributed to the germination of some of the ANN1238 seeds in the NOP treatment.

Abscisic acid (ABA) has been identified as a major inhibitor in seed germination although not related to dormancy caused by the seed covering (Sondheimer, 1968), but has been implicated in dormancy in the sunflower embryo itself (Le Page-Degivery et al. 1990). The pericarp and seed coat clearly inhibit germination, but whether this inhibition was chemical, physical, or a combination of the two, is not yet known.

Further research needs to be conducted in order to more fully understand the dormancy controlled by the pericarp and seed coat in sunflower. Additional wild *H. annuus* genotypes need to be included, by geographical collection site. If the accessions were produced in a single environment, it may be possible to determine if the dormancy was regulated more by environment or genetics. In addition, it may be incorrect to refer to seed coat and pericarp inhibition of germination as dormancy when the embryo, the actual growth structure, is non-dormant.

Additional research is needed to determine if: 1) there are dormancy differences between wild *H. annuus* accessions; 2) there are evolutionary advantages to seeds having

pericarp + seed coat dormancy but short-lived embryo dormancy; 3) biological factors are the most important factors in reducing seed coat and pericarp dormancies, and 4) the length of time an embryo can be non-dormant and remain viable while the seed coat and pericarp retard germination.

## CONCLUSIONS

Embryo dormancy was short lived (0 to 8 wks) in all sunflower genotypes; whereas, seed coat and pericarp + seed coat dormancies were longer lived, especially in wild material. The seed coat and pericarp + seed coat were almost solely responsible for the seed dormancy in wild material. Elite material may have both short-lived embryo and short-lived seed coat and pericarp + seed coat dormancies, but varies by variety. Seeds that have after-ripened for four or more weeks can be readily germinated by simply removing the seed coat and pericarp. Although this is not practical on a large scale, it is practical on a small scale in order to germinate seeds to produce enough plants to make crosses or evaluate germplasm.

Because germination of wild sunflowers is mostly influenced by the seed coat and pericarp, it is not likely that wild accessions will be able to be used for land restoration and reclamation unless seed coat and pericarp dormancies are bred out of the wild accessions that are most desired for such purposes.

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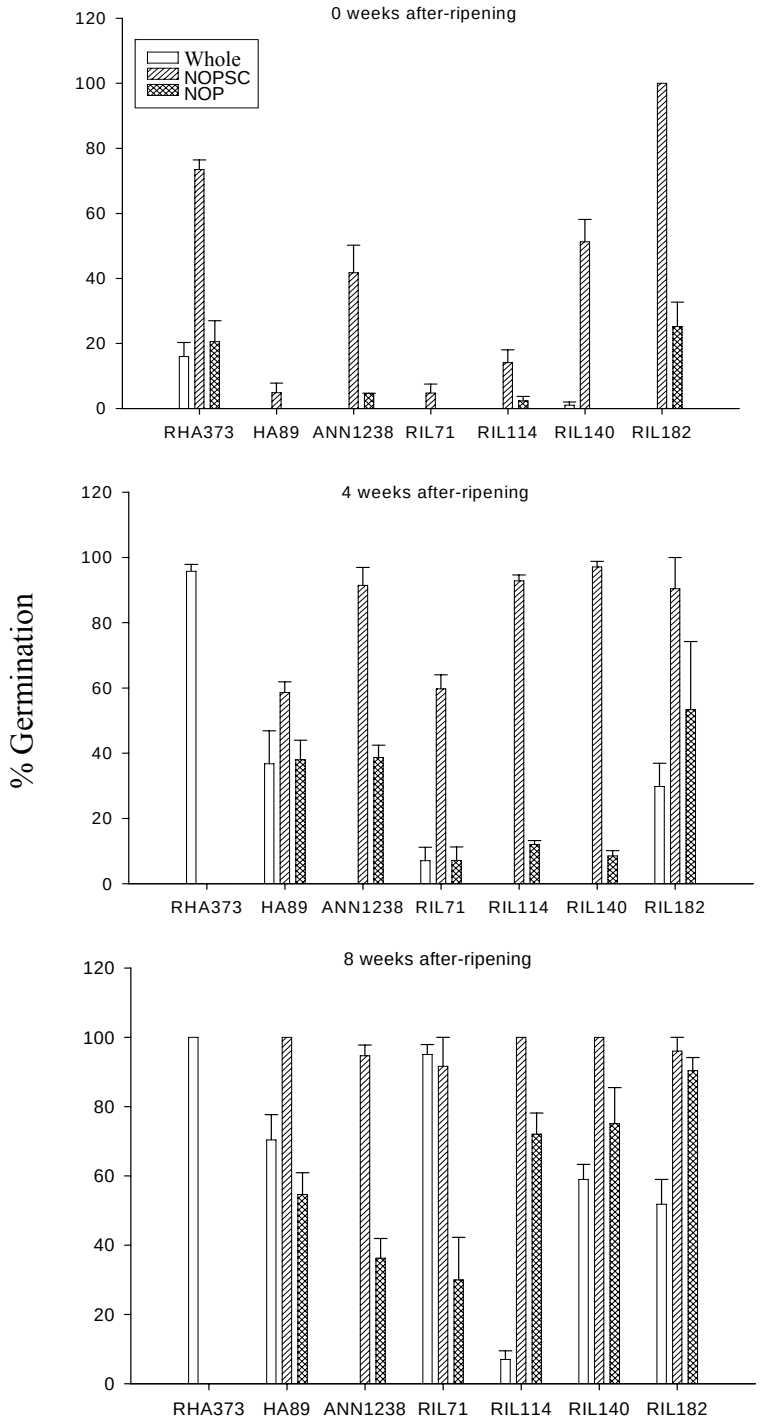


Fig. 2.1. Percent germination of sunflower genotypes on day 21. Treatments are whole seed, pericarp removed (NOP), or pericarp and seed coat removed (NOPSC) at 0, 4, or 8 weeks after-ripening. Error bars denote standard error.

Table 2.1. Percent germination on days 5 and 21 for sunflower genotypes, the treatments are whole seed, pericarp removed (NOP), or pericarp and seed coat removed (NOPSC) at 0, 4, or 8 weeks after-ripening.

After-ripening Time 0

| | Whole | | NOP | | NOPSC | | |
|---|---|---|---|---|---|---|---|
| Genotype | 5D | 21D | 5D | 21D | 5D | 21D | LSD0.05[a] |
| RHA373 | 0 | 16 | 0 | 21 | 0 | 74 | 11 |
| HA89 | 0 | 0 | 0 | 0 | 0 | 5 | 6 |
| ANN1238 | 0 | 0 | 0 | 5 | 0 | 42 | 17 |
| RIL71 | 0 | 0 | 0 | 0 | 0 | 5 | 6 |
| RIL114 | 0 | 0 | 0 | 2 | 0 | 14 | 7 |
| RIL140 | 0 | 1 | 0 | 0 | 0 | 51 | 15 |
| RIL182 | 0 | 0 | 0 | 25 | 0 | 100 | 15 |
| Ames27234 | 0 | 0 | NA | NA | 0 | 95 | 7 |

After-ripening Time 4

| | Whole | | NOP | | NOPSC | | |
|---|---|---|---|---|---|---|---|
| Genotype | 5D | 21D | 5D | 21D | 5D | 21D | LSD0.05[a] |
| RHA373 | 76 | 97 | 63 | NA | 60 | NA | NA |
| HA89 | 0 | 37 | 1 | 38 | 0 | 59 | 28 |
| ANN1238 | 0 | 0 | 0 | 39 | 0 | 92 | 14 |
| RIL71 | 1 | 7 | 0 | 7 | 0 | 60 | 16 |
| RIL114 | 0 | 0 | 0 | 12 | 0 | 93 | 3 |
| RIL140 | 0 | 0 | 0 | 9 | 0 | 97 | 5 |
| RIL182 | 25 | 30 | 21 | 53 | 18 | 90 | 49 |
| Ames27234 | 0 | 1 | NA | NA | 0 | 100 | 3 |

After-ripening Time 8

| | Whole | | NOP | | NOPSC | | |
|---|---|---|---|---|---|---|---|
| Genotype | 5D | 21D | 5D | 21D | 5D | 21D | LSD0.05[a] |
| RHA373 | 90 | 96 | 93 | NA | 92 | NA | NA |
| HA89 | 65 | 71 | 51 | 55 | 54 | 100 | 21 |
| ANN1238 | 0 | 0 | 0 | 36 | 0 | 95 | 10 |
| RIL71 | 87 | 95 | 78 | 30 | 86 | 92 | 33 |
| RIL114 | 7 | 7 | 10 | 72 | 12 | 100 | 14 |
| RIL140 | 54 | 59 | 45 | 75 | 49 | 100 | 20 |
| RIL182 | 49 | 52 | 34 | 90 | 28 | 96 | 13 |
| Ames27234 | 0 | 0 | NA | NA | 0 | 99 | 3 |

NA = not applicable, treatment was not applied

[a]LSD0.05 compares treatment means for day (D) 21 by genotype within a row

# CHAPTER 3

## Impact of Various Seed Treatments on the Germination of Domesticated and Wild Sunflower

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**ABSTRACT**

The physiological and genetic mechanisms underlying sunflower (*Helianthus annuus* L.) seed dormancy are poorly understood. The goals of these studies were to identify the length and type of seed dormancy in domesticated elite lines and several wild accessions and to evaluate various seed treatments to maximize germination. The effects of after-ripening time (0, 4, or 8 wk), light and dark conditions, and seed treatments including naked seeds, removing the pericarp (embryo + seed coat) or no modification (whole achenes) (embryo + seed coat + pericarp) on germination were studied. Germination of seeds of three elite inbred lines, two wild *H. annuus*, one *H. argophyllus* species and four wild x elite $F_1$ hybrids was measured. Differences were observed among genotypes and seed treatments ($P < 0.0001$). In all three elite inbred lines, seed dormancy was short-lived, less than 8 wk, and was primarily embryo-induced. In contrast, seed dormancy in the wild populations was stronger and primarily induced by the seed coat and pericarp. In the wild x elite hybrids, there was low to moderate embryo dormancy at 4 and 8 wk after-ripening and strong seed coat and pericarp dormancy in 75% of the hybrid populations. In the elite x wild hybrid (HA89 x ANN1238), embryo dormancy persisted up to 8 wk. In contrast, in the hybrid of RHA373 x ANN1811 embryo dormancy was low, seed coat and pericarp dormancy were primarily responsible for seed dormancy at all after-ripening times. Consistent increases in germination were not obtained with cold or heat treatments. Germination tended to be better if seeds were placed in alternating light and dark conditions versus constant dark.

**INTRODUCTION**

Wild sunflowers (*Helianthus annuus* L.) are native to North America while domesticated sunflowers are an important oil seed crop world wide. Sunflowers are widely grown as ornamentals in home gardens and seed is produced commercially and sold in mixes of wild bird and squirrel feed. Many wild sunflower species possess desired attributes such as disease resistance, drought tolerance and biomass production which could be of value for sunflower breeding programs. Wild sunflower species also have novel ornamental traits which are of particular interest to breeders of garden and ornamental sunflowers. However, seed dormancy is one barrier that limits the direct use

of wild material as ornamentals and as donors for desirable agronomic traits. Seiler (1998) noted that there is a desire to use native wild sunflowers in land reclamation and restoration in the United States. It is often difficult to use wild sunflower species for such purposes because germination is frequently low and unpredictable (Heiser et al., 1969).

Sunflower achenes have three primary tissues, embryo, seed coat and pericarp, which may contribute to seed dormancy. Typically in most sunflower genotypes, all three tissues influence the level of dormancy in the seed. In order to effectively use wild sunflower species in future breeding programs, it would be helpful to understand how each of these three structures acts, either independently or collectively, to maintain seed dormancy. Embryo dormancy is often short lived (4-8 wk); however, seed coat and pericarp dormancy can persist for long periods of time (>32 weeks) (unpublished data). Cultivated sunflowers typically show little to no dormancy and usually germinate within a few months of harvest. In contrast, wild sunflowers typically have prolonged dormancy and low or erratic germination. Based on the authors' experience, there is variability among accessions of the same species as well as among species. The type and duration of dormancy in the wild sunflowers and the requirements for germination are not well understood.

The breaking of sunflower seed dormancy has been shown to be hastened by soaking seeds in acidic, hydrogen peroxide and/or hot water solutions and by heating seeds at extreme temperatures (Akinola et al. 2000); soaking in gibberellic acid solutions (Seiler, 1998); scarifying and dehulling (Chandler and Jan, 1985); increasing atmospheric oxygen concentrations (Gay et al. 1991); and by simply placing seeds in moist pots in varying climatic conditions (Heiser et al. 1969). However, germinating the seed is often time consuming, with variable, unpredictable results, especially with wild germplasm.

Seed dormancy can either be caused by the embryo or seed covering or a combination of the two (Kelly 1992). In the case of the latter, the seed covering can prevent water or gasses from reaching the embryo or the covering may contain chemicals which inhibit germination. In order for an embryo to germinate, essential requirements regarding light, temperature, or hormones levels must be met. When all types of dormancy are present and the seed covering is removed, germination still will not result

(Kelly 1992). In species where the seed covering also controls dormancy, successfully germinating the seeds becomes more complicated and difficult because removing the seed covering to expose the embryo can result in damage to or death of the embryo. Gay et al. (1991) evaluated the inhibitory germination effects of the seed coat on non-dormant seeds of the sunflower cultivar 'Mirasol', and reported that the seed coat delayed or inhibited germination especially at low oxygen concentrations. In these non-dormant seeds, germination increased as oxygen concentration increased and the seed coat reduced germination when the seeds were exposed to high temperatures.

We evaluated the length of dormancy in physiologically mature non-after-ripened and after-ripened seeds of three elite inbred lines, two wild *H. annuus* accessions, one *H. exilis* accession, and one *H. argophyllus* accession. We also evaluated the impacts of various treatments on seed germination.

## MATERIALS AND METHODS

Seed was produced at Corvallis, OR in the summer of 2004 for all of the studies except the seed used in the achene excision experiment two which was produced in 2005. In order to minimize seed after-ripening on the mother plant, all achenes were harvested at physiological maturity, as defined by Schneiter and Miller (1981). After harvest, seed was immediately dried at 39$^{o}$C for 17 h, cleaned and vacuum sealed (model ASTM F-1249) in KAPAK® bags to keep moisture out, and stored at 4$^{o}$C ± 2$^{o}$C to decelerate after-ripening. Seed stored at 4$^{o}$C was used in all studies as the control (time 0) and referred to hereafter as non-after-ripened seed. Seed was after-ripened at 20$^{o}$C ± 2$^{o}$C to simulate after-ripening at room temperature.

For all studies the following methods were used unless otherwise noted. Seed was disinfected prior to imbibition by agitating in a 50/50 solution of sodium hypochlorite and water (3% v/v) for 90 s. Seed was then rinsed with distilled, de-ionized water to remove the disinfectant. Four replicates of 25 seed per replicate of each genotype were placed into Petri plates on blue blotter paper with 5 ml of distilled water. Petri plates were kept in Ziploc® bags inside the germinator to decrease evaporation. Seeds were germinated at 25$^{o}$C with 12 h of light and 12 h of dark. Germination data were collected

up to day 21. Germination was defined as either when the radical pierced the pericarp of the whole achene or for the altered seed when the radical was visibly elongated.

The experiment was conducted as a completely randomized design with four replicates. Raw data of each genotype were analyzed using Proc Mixed model in statistical analysis system (SAS). For the impact of light on germination experiment, the fixed effects were genotype, treatment and after-ripening time. For all other experiments, the fixed effects were genotype, treatment and after-ripening time and light was treated as a random effect.

**Impact of Varying Temperatures on Germination**

For the temperature experiment, seed was after-ripened for 2, 4, or 8 wk. The genotypes evaluated were two elite lines (RHA373, HA89); two wild *H. annuus* (ANN1238, ANN1811 (PI494567)); one wild *H. argophyllus* (ARG1805); and one wild *H. exilis* (Ames27246). The genotypes were expected to differ in seed dormancy type and longevity. Seeds were placed in envelopes and exposed to constant temperatures of -20$^{o}$, 20$^{o}$, or 50$^{o}$C for the 24 h prior to being placed in Petri dishes and put in a germinator.

**Impact of Light on Germination**

Whole seeds were evaluated for germination in both light and dark conditions. The genotypes evaluated were: three oilseed elite lines (RHA373, RHA801, HA89); two wild *H. annuus* (ANN1238, ANN1811); one wild *H. argophyllus* (ARG1805); and four $F_1$ crosses, (HA89xANN1238, NMS373xANN1811) and the reciprocal crosses. The genotypes were selected in order to detect if there were differences in germination response in the light versus dark. In addition, the reciprocal crosses were selected to evaluate possible differences in seed dormancy due to maternal tissues (i.e. the seed coat and pericarp). Because seed was limited, it was not possible to make all pair-wise comparisons. For the alternating light/dark treatment, seed was exposed to 12 h of light and 12 h of dark. For the dark treatment, Petri plates were wrapped in aluminum foil to eliminate light for the duration of the experiment.

**Impact of Achene Excision on Germination**

The seed treatments were either cutting off the blunt end of the seed or removal of the seed coat and pericarp (NOPSC) leaving naked embryos. For both the cut seed and the NOPSC seed, ¼ of the blunt end of the seed was removed on day five. It was necessary to wait five days to apply the treatment in order to completely remove the seed coat and pericarp without injuring the embryo. A dry embryo cannot be removed from the seed coat and pericarp whereas an imbibed achene makes removal of the embryo possible. Seeds were placed on clean blotter paper after the treatments were applied. The after-ripening times were 0, 4, or 8 wk. For achene excision experiment two, the number of decayed seeds among treatments was counted.

The genotypes evaluated in experiment one were: two oilseed elite lines (RHA373, HA89); two wild *H. annuus* (ANN1238, ANN1811); one wild *H. argophyllus* (ARG1805); and four $F_1$ crosses (HA89xANN1238, NMS373xANN1811) and the reciprocal crosses. The reciprocal crosses were selected to evaluate possible differences in seed dormancy due to maternal tissues (i.e. the seed coat and pericarp). Because seed was limited, all pairwise comparisons were not possible.

The genotypes evaluated in experiment two were: two oilseed elite lines (RHA373, HA89); two wild *H. annuus* (ANN1238, ANN1811); one wild *H. argophyllus* (ARG1805); and four $F_1$ crosses (HA89xANN1238, NMS373xANN1811) and the reciprocal crosses.

**RESULTS AND DISCUSSION**

**Impact of Temperature on Germination**

The fixed effects of genotype and after-ripening time were significant for the overall model, ($P < 0.0001$). Temperature as a fixed effect for the overall model was not significant ($P = 0.9188$) and was therefore treated as a random effect. However, the three-way interaction of genotype x temperature x after-ripening time was significant, $P = 0.0055$.

For RHA373 and ANN1811, temperature affected germination at specific after-ripening times so the after-ripening time by temperature interactions were significant,

P = 0.0375 and 0.0354, respectively. Therefore, individual after-ripening times were evaluated for the genotype's response to temperature.

All genotypes were tested for fit to linear, quadratic, and cubic models for germination to after-ripening time. ANN1811 fit the linear model ($P < 0.0001$) and Ames27246 fit the quadratic model ($P = 0.0137$). Genotypes HA89, RHA373 and ARG1805 fit the cubic model with associated P values of $< 0.0001$, 0.0186, and $< 0.0001$, respectively.

HA89 seeds were dormant until 8 wk after-ripening. At 8 wk after-ripening, seeds germinated at 90, 80, and 91% for the control, heat, and cold treatments, respectively. The temperature treatments were ineffective in reducing dormancy of HA89 (Fig. 3.1).

Germination of RHA373 seeds were most influenced by after-ripening time ($P < 0.0001$). In general, as after-ripening time increased, germination of RHA373 increased. However, there were two after-ripening time periods where temperature treatments were found to be important. At 0 wk after-ripening, the heat treatment increased germination compared to the control by 45% ($P = 0.0133$) and at 2 wk after-ripening the heat treatment decreased germination by 37% compared to the cold treatment ($P = 0.0345$).

Germination of ANN1811 increased as after-ripening time increased ($P < 0.0001$). For ANN1811, in the control treatment, germination increased progressively over the after-ripening times from 18 to 67%. The heat treatment increased germination of the non-after-ripened seed compared to the control treatment ($P = 0.0122$). Wild *H. annuus* genotypes can range widely in seed dormancy as seen here by the differences in total germination between ANN1811 and ANN1238 (Fig. 3.1). These results demonstrate that ANN1811 had moderate seed dormancy and that ANN1238 was highly recalcitrant with very deep seed dormancy. No seed of ANN1238 germinated during the entire experiment despite the fact that a tetrazolium chloride test showed 96% viable seed.

For *H. exilis* and *H. argophyllus*, after-ripening time affected the pattern of germination. In the control treatment for *H. argophyllus*, the dormancy cycled over the course of the after-ripening time periods. Germination of *H. argophyllus* was 18 and 24% in non-after-ripened seeds and seeds after-ripened for 4 wk, respectively. While for

the after-ripening periods of 2 and 8 wk, germination was 12 and 4%, respectively. Germination in *H. exilis* increased slightly, but not significantly, at each after-ripening time period in the control treatment. For *H. exilis*, at 8 wk after-ripening, the heat treatment reduced germination compared to the cold treatment by 13% ($P = 0.0020$) and by 10%, compared to the control ($P = 0.0179$).

The heat and cold treatments were ineffective in breaking seed dormancy in *H. annuus* (ANN1238), *H. argophyllus* and *H. exilis*. There were distinct patterns in germination among the genotypes tested; primarily due to after-ripening time. However, seed dormancy differed among genotypes and genotypes responded differently to cold and heat treatments. The heat and cold treatments were ineffective in eliminating or decreasing seed dormancy in most cases. Therefore, these temperatures are not recommended for use as a method to break sunflower seed dormancy. Germination of a particular genotype may be increased by heat at a specific after-ripening time period; however, this was not generally the case and a heat treatment at the wrong time may decrease germination.

**Impact of Light on Germination**

Genotypes ANN1238 and ARG1805 were evaluated in all treatments and at all after-ripening times. As a result, these two genotypes were evaluated in a separate analysis. In the overall analysis, the three way interaction of genotype x after-ripening time x treatment was significant indicating that the effect of after-ripening time and treatment on germination was different between these two genotypes. For genotypes ANN1238 and ARG1805 there was no difference in germination between alternating light/dark (light) and dark treatments ($P = 0.1589$) (Table 3.1; Fig. 3.2). Both genotypes germinated successfully in the NOPSC treatment compared to the whole seeds ($P < 0.0001$) at 0, 4, and 8 wk after-ripening. The cut treatment provided a germination advantage compared to whole seeds for ARG1805 at all after-ripening times; while, the cut treatment only provided a germination advantage at 8 wk after-ripening for ANN1238 compared to whole seeds. The NOPSC treatment provided a germination advantage compared to the cut treatment in ANN1238 at all three after-ripening times, but at only 0, and 4 wk after-ripening times for ARG1805.

Genotypes were analyzed separately in order to determine the impact of after-ripening time and treatment on each genotype. In the overall model for the remaining genotypes, there was a germination advantage for whole seeds germinated in the light treatment compared to those germinated in the dark treatment, but this was only for after-ripening times 4 and 8; P-values were 0.0002 and < 0.0001, respectively. Germination was negligible for many genotypes at 0 wk after-ripening in both the light and dark treatments.

In the overall analysis, the NOPSC light treatment provided an advantage over the whole seed light treatment at all after-ripening times ($P < 0.0001$). When comparing the whole seed light and dark treatments, there were differences between treatments for individual genotypes at specific after-ripening times and the germination advantage was always for the whole seeds germinated in the light. In all treatments, although the difference was small, most genotypes germinated better when placed under light conditions.

Wild genotypes had lower rates of germination when the seeds were left whole ($P < 0.0001$). The seed coat and pericarp were primarily responsible for seed dormancy in wild types. However, whole seed of ANN1811 germinated whereas whole seed of ANN1238 did not. Wild types ANN1238 and ARG1805 had low embryo dormancy and high seed coat and pericarp dormancy; while ANN1811 had low embryo and low to moderate seed coat and pericarp dormancies. Results were similar for wild x elite hybrids. Wild x elite hybrids, which have wild seed coat and pericarp, had lower germination rates when the seed coat and pericarp were left intact. Whole seed, which shows dormancy effects of embryo + seed coat + pericarp, of the ANN1811 x RHA373 hybrid germinated at 8, 19, and 87% at 0, 4, and 8 wk after-ripening, respectively. This compared to the RHA373 x ANN1811 hybrid germination rates of 2, 29, and 16% for the same after-ripening time periods respectively. This result is interesting because the elite line RHA373 germinated at 1, 46, and 84%; while ANN1811 germinated at 19, 81 and 73% at 0, 4, and 8 wk after-ripening, respectively. This indicates that there may be an interaction between the seed coat and pericarp dormancy alleles when RHA373 and ANN1811 are hybridized. This interaction may be responsible for the greater rates of

germination for the three out of the four hybrid genotypes in the NOPSC light treatment. For example, at 0 wk after-ripening, RHA373 germinated at 1% while ANN1811, RHA373 x ANN1811 and ANN1811 x RHA373 germinated at 62, 85 and 97%, respectively.

**Impact of Achene Excision on Germination**

Earlier experiments by Gay et al. (1991) demonstrated that the seed coat retarded germination of non-dormant viable sunflower embryos by excluding oxygen. Ballard (1973) showed that the pericarp excluded water which prevented germination. We had hoped that excising the end of the achene would increase germination equal to the NOPSC treatment, and thus save the vast amount of time required to remove the entire seed coat and pericarp; however, results were mixed and were genotype specific. Excising is not recommended for wild types.

The NOPSC treatment provided a germination advantage in the following genotypes during the following after-ripening times: ANN1238 at 0, 4 and 8 wk; ANN1811 at 0 and 4 wk; ANN1238 x HA89 at 4 and 8 wk; HA89 x ANN1238 at 8 wk; ANN1811 x RHA373 at 0 and 4 wk; RHA373 x ANN1811 at 0, 4 and 8 wk; ARG1805 at 0, 4 and 8 wk and, RHA373 at 4 and 8 wk. Seeds of HA89 did not germinate until after-ripened for 8 wk. Embryo, seed coat and pericarp dormancies of HA89 started to fade at 8 wk compared to that of RHA373 which started to fade at 4 wk; germination of HA89 and RHA373 were 29 and 46%, respectively, for the whole light treatment for these two genotypes. The wild types, ANN1238 and ARG1805, germinated successfully at all three after-ripening times when the seed coat and pericarp were removed.

For ARG1805, the cut treatment increased germination compared to the whole, light treatment at all three after-ripening times; however, the NOPSC treatment provided the greatest germination advantage for this genotype at all three after-ripening times. For ANN1238, the cut light treatment was different from the whole achene with light treatment only for 8 wk after-ripening. The NOPSC treatment always provided the greatest germination advantage for ANN1238.

The number of seeds that decayed in each of the treatments in experiment two was counted. RHA373, HA89, ANN1238 and ARG1805 were compared in one analysis

these four genotypes were evaluated in all treatments under all after-ripening times) while the other genotypes were compared in a separate analysis. For ANN1238 and ARG1805, the number of decayed seeds was determined not to be genotype specific ($P = 0.6037$) but rather was dependent upon the interacttion of genotype x after-ripening time x treatment ($P < 0.0001$). The cut treatment increased the number of decayed seeds ($P < 0.0001$). No seeds of ANN1238 decayed for the whole, light treatment. In contrast, the percentages of decayed seeds of ANN1238 in the cut treatment were 7, 96, and 68% and were 55, 16, and 23% in the NOPSC treatment, respectively (Table 3.2). Seeds that have embryo dormancy and do not germinate when the seed coat and pericarp are removed may be at a higher risk for decay than seeds that germinate readily when the seed coat and pericarp are removed. When seed of ARG1805 were excised, seed decayed similarly to those of ANN1238. Whole seed of ANN138 and ARG1805 at 0, 4, and 8 wk after-ripening did not decay. Seeds of ARG1805 in the cut treatment decayed at 16, 70, and 70% while seeds in the NOPSC treatment decayed at 31, 42, and 50% at 0, 4, and 8 wk after-ripening, respectively. Even though seeds in the NOPSC and cut treatments typically had higher rates of decayed seeds than whole seeds, the NOPSC treatment also provided the greatest germination advantage.

Elite lines that are in early stages of after-ripening may be germinated more successfully when the seed coat and pericarp are removed. This result, however, was genotype specific and can be expected to vary as seen with RHA373 and HA89. Whole seeds of elite lines in this experiment had high germination rates when after-ripened for 8 wk. Wild types that have strong seed coat and pericarp dormancies germinated successfully when the seed coat and pericarp were removed even in the early stages of after-ripening. The tissues need to be removed completely rather than just cutting off the blunt end of the seed, as in the cut treatment, in order to maximize the number of seedlings recovered. Removing the seed coat and pericarp increased germination and decreased the number of seeds that decayed.

In experiment two, genotypes were tested by group (wild *H. annuus* or elite). It was of interest to know the differences between the numbers of seeds that decayed in the treatments because often times altered seeds decay when they do not germinate. We do

not know if they decay because they do not germinate or if they do not germinate because they decay. Analysis showed that there were no differences in the number of decayed seeds between the wild and elite groups ($P < 0.0001$). There were differences for the number of decayed seeds between treatments: whole versus NOPSC ($P = 0.0003$), whole versus cut ($P = <0.0001$) and NOPSC versus cut ($P < 0.0001$). The cut treatment always had higher rates of decay than the other two treatments and the NOPSC treatment had a higher rate of decay than the whole treatment. However, the differences between the estimates for the means of the comparisons were negligible (3.5% or less). Although there was a statistical difference of the number of decayed seeds by treatment, there does not seem to be a large biological difference.

Although some wild *H. annuus* seeds will germinate with the seed coat and pericarp intact, the germination rates can be expected to be very low. Despite the labor involved to remove the seed coat and pericarp, in general, we recommend that the entire seed coat and pericarp be removed to successfully germinate wild *H. annuus* and *H. argophyllus* seeds which will also decrease the number of seeds that decay. Cutting the seeds allowed free flow of oxygen and water to the embryo, but elimination of those elements did not seem to act as the major cause of dormancy in these wild types. Therefore, chemicals in the seed coat and pericarp may have been inhibiting germination. The cut treatment provided a germination advantage compared to the whole treatment for certain genotypes (ANN1238 and ARG1805) which have strong seed coat and pericarp dormancies. Cutting the seed partially relieved dormancy and provided an improvement in germination at 8 wk after-ripening in the wild types. The primary role of the seed coat and pericarp in some genotypes may be to exclude water and gasses which are essential for germination. However, because the germination rates in the cut treatment in the many wild accessions was not equal to the NOPSC treatment the seed coat and pericarp may contain chemical inhibitors when left intact to the embryo partially or fully inhibit germination.

## CONCLUSIONS

In sunflower seeds, several factors combine to inhibit germination. These consist of an after-ripening requirement of the embryo, seed coat and pericarp. The latter may

mechanically or chemically inhibit germination through exclusion of environmental components such as gasses and liquids, or by chemicals contained in the seed coat and pericarp themselves. When the seeds are after-ripened for as little as 4 to 8 wk, the embryo is ready for germination in both elite lines and wild types. Nevertheless, in most wild types, the seed coat and pericarp must be removed before the embryo will germinate. There seem to be various levels of seed coat and pericarp dormancies among the wild types as well as between species. In addition, embryo dormancy varies between genotypes and species. Whether these different levels of dormancy in the seed coat and pericarp are physical, chemical, or both are not known. In general, germination was greater in light than in dark. It is recommended based on these studies to germinate sunflower seeds under alternating 12 h of light and dark. Among the seed treatments tested, the NOPSC treatment provided a large germination advantage in genotypes that had strong seed coat and pericarp dormancies. Cutting off the blunt end of the seed typically was not effective for increasing germination. After-ripening seed of wild accessions will have little impact on increasing germination. Temperature treatments were ineffective at increasing germination for most genotypes. Therefore, we recommend that the seed coat and pericarp be removed from wild accessions with low germination (1-5%) to maximize germination.

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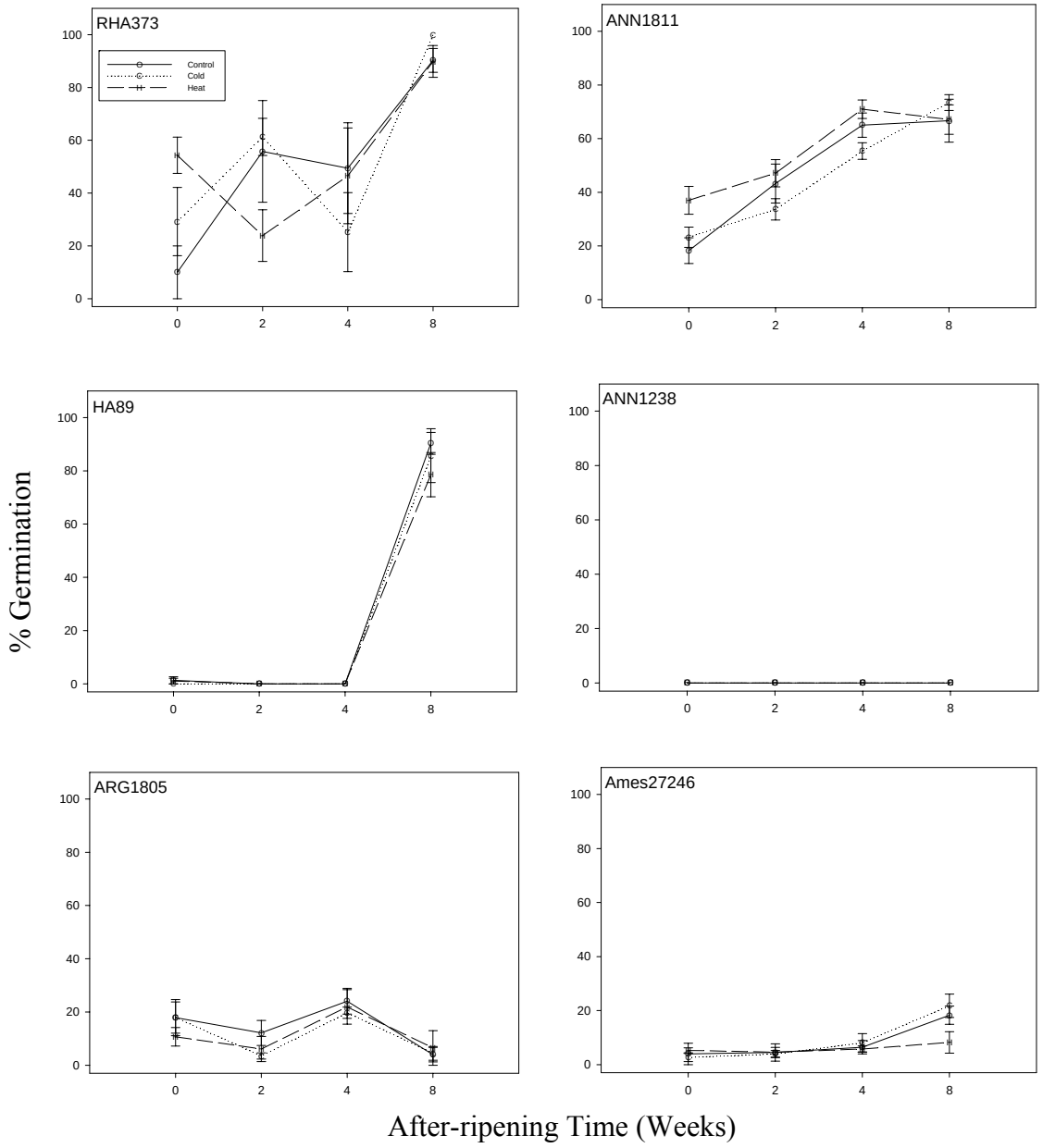


Fig. 3.1. Percent germination of sunflower accessions on day 21 at 0, 2, 4, or 8 weeks after-ripening. Seeds were exposed to temperature treatments of -20, 20 or 50$^{o}$C for the 24 hour period prior to imbibition. Error bars denote standard error.

Table 3.1. Comparisons between germination treatments of sunflower accessions. The treatments are: whole dark (WD) versus whole light (WL), WL versus NOPSC (no pericarp or seed coat), WL versus cut and NOPSC versus cut seed for achene excision experiment one.

| | | Treatments | | Treatments | | Treatments | | Treatments | |
|---|---|---|---|---|---|---|---|---|---|
| Genotype | AR Time | WD vs WL (P-value) | Advantage | WL vs NOPSC (P-value) | Advantage | WL vs Cut (P-value) | Advantage | NOPSC vs Cut (P-value) | Advantage |
| ANN1238 | 0 | 1.0000 | ns | <0.0001 | NOPSC | 1.0000 | ns | <0.0001 | NOPSC |
| | 4 | 1.0000 | ns | <0.0001 | NOPSC | 0.7931 | ns | <0.0001 | NOPSC |
| | 8 | 1.0000 | ns | <0.0001 | NOPSC | <0.0001 | Cut | <0.0001 | NOPSC |
| ANN1811 | 0 | 0.4195 | ns | <0.0001 | NOPSC | | | | |
| | 4 | 0.0015 | WL | <0.0001 | NOPSC | | | | |
| | 8 | <0.0001 | WL | 0.0897 | ns | | | | |
| 1238x89 | 0 | 1.0000 | ns | 0.2770 | ns | | | | |
| | 4 | 1.0000 | ns | <0.0001 | NOPSC | | | | |
| | 8 | 0.9780 | ns | <0.0001 | NOPSC | | | | |
| 89x1238 | 0 | 1.0000 | ns | 0.5481 | ns | | | | |
| | 4 | 1.0000 | ns | 1.0000 | ns | | | | |
| | 8 | 0.7097 | ns | <0.0001 | NOPSC | | | | |
| 1811x373 | 0 | 0.5507 | ns | <0.0001 | NOPSC | | | | |
| | 4 | 0.0819 | ns | <0.0001 | NOPSC | | | | |
| | 8 | <0.0001 | WL | 0.3742 | ns | | | | |
| 373x1811 | 0 | 0.0073 | WL | <0.0001 | NOPSC | | | | |
| | 4 | 0.7334 | ns | <0.0001 | NOPSC | | | | |
| | 8 | 0.9759 | ns | <0.0001 | NOPSC | | | | |
| ARG1805 | 0 | 0.8611 | ns | <0.0001 | NOPSC | 0.0003 | Cut | <0.0001 | NOPSC |
| | 4 | 0.0171 | WL | <0.0001 | NOPSC | <0.0001 | Cut | <0.0001 | NOPSC |
| | 8 | 0.0423 | WL | <0.0001 | NOPSC | <0.0001 | Cut | 0.1715 | ns |
| RHA373 | 0 | 0.9237 | ns | 0.3370 | ns | 0.8655 | ns | 0.4279 | ns |
| | 4 | 0.4448 | ns | <0.0001 | NOPSC | 0.1411 | ns | <0.0001 | NOPSC |
| | 8 | 0.8840 | ns | <0.0001 | NOPSC | | | | |
| HA89 | 0 | not estimable | | not estimable | | | | | |
| | 4 | not estimable | | not estimable | | | | | |
| | 8 | 0.6596 | ns | 0.8278 | ns | | | | |

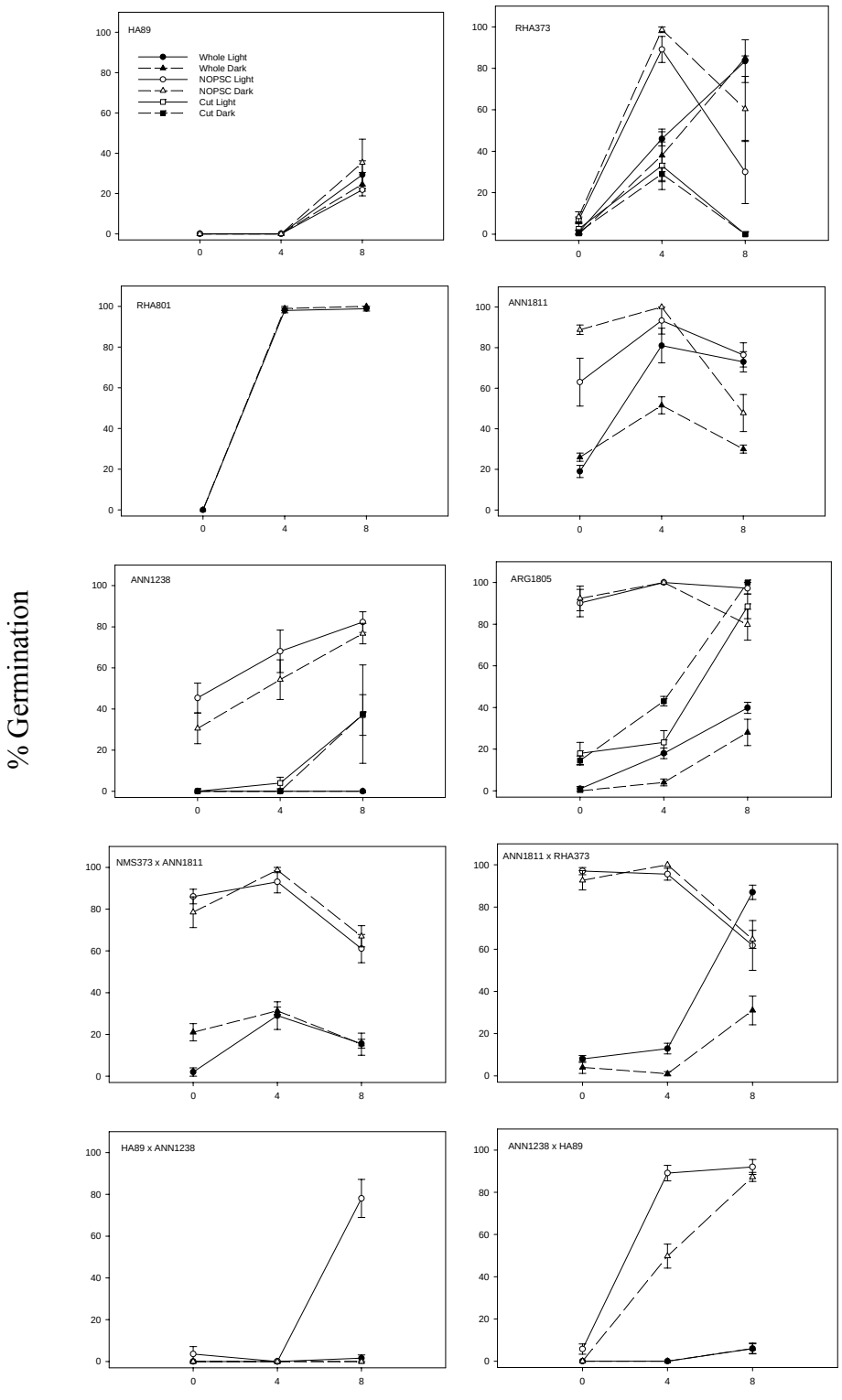


Fig. 3.2. Percent germination of sunflower accessions on day 21 at 0, 4, or 8 weeks after-ripening. Treatments are whole seed or embryos (NOPSC) germinated in alternating 12 hours of light and 12 hours of darkness (light) or germinated in constant darkness.

Table 3.2. Percent of decayed sunflower seeds at 0, 4, or 8 weeks weeks after-ripening for achene excision experiment two. The treatments are whole seed, cut seed (excised) or no pericarp or seed coat (NOPSC).

| | | After-ripening time (weeks) | | |
|---|---|---|---|---|
| Genotype | Treatment | 0 | 4 | 8 |
| ANN1238 | Whole | 0 | 0 | 0 |
| | Cut | 7 | 96* | 68* |
| | NOPSC[a] | 55* | 16* | 23* |
| ARG1805 | Whole | 0 | 0 | 2 |
| | Cut | 16* | 70* | 70* |
| | NOPSC | 31* | 43* | 50* |
| RHA373 | Whole | 0 | 0 | 20 |
| | Cut | 20* | 42* | 25 |
| | NOPSC | 27* | 19* | NA |
| HA89 | Whole | 1 | 0 | 5 |
| | Cut | 0 | 38* | NA |
| | NOPSC | 24* | 10* | 72* |
| 1238x89 | Whole | 0 | 0 | 0 |
| | NOPSC | 30* | 6 | 1 |
| 1811x373 | Whole | 0 | 0 | 0 |
| | NOPSC | 33* | 18* | 8 |
| 89x1238 | Whole | 1 | 0 | 25 |
| | NOPSC | 34* | 27* | 58* |
| 373x1811 | Whole | 2 | 0 | 10 |
| | NOPSC | 29* | 5 | 11 |
| ANN1811 | Whole | 0 | 0 | 0 |
| | NOPSC | 34* | 5 | 11* |

[a]NOPSC = no seed coat or pericarp

* Denotes significance at the 0.05 levels of probability within a genotype and between treatments for either 0, 4, or 8 weeks. after-ripening

# CHAPTER 4

## Latitude Influence on Seed Dormancy of Wild Sunflower (*Helianthus annuus* L.)

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**ABSTRACT**

Latitude impacts on dormancy of elite sunflower (*Helianthus annuus* L.) lines and wild sunflower accessions was evaluated Genotypes were grown in a common nursery at 44.57$^{o}$ N latitude at Corvallis, OR in 2005. Collections of wild *H. annuus* accessions ranged in latitude of origin from the southern region of Texas (27.17$^{o}$ N) to the southern region of Saskatchewan Canada (50.37$^{o}$ N). Differences in germination of whole seed were observed among wild *H. annuus* accessions by latitude ($P<0.0001$). Whole seed dormancy of accessions from the most extreme southern latitudes was much lower than whole seed dormancy in accessions from more northern latitudes. Differences were attributed to the embryo and the seed covering ($P<0.0001$) over 4, 8, and 16 weeks after-ripening (AR). Accessions from extreme lower latitudes had lower embryo dormancy than accessions from more northern latitudes. When genotypes were grouped by one of three types (elite, wild, or Native American Landrace), differences in dormancy were observed among groups ($P<0.0001$) woth Native American Landraces germinating most like elite lines.

**INTRODUCTION**

Wild sunflowers (*Helianthus annuus* L.) are native to the United States and are commonly found throughout the landscape, most typically observed along roadside ditches, fence lines, and pastures. The wild sunflowers have adapted to more climates throughout North America than any of the many other species of sunflower (Seiler and Reisberg, 1997). Wild sunflowers are found from the southernmost part of Texas and north to the US-Canadian border. Localized adaptations have led to numerous individual populations that have unique genotypic and phenotypic traits regarding seed germination (Linder, 2000), seed oil concentration (Fick et al. 1976; Seiler 1985, 1994) and fatty acid profile (Knowles et al. 1970; Fernandez-Martines and Knowles, 1976; Dorrell and Whelan, 1978, Thompson et al. 1978).

Sunflower seeds have germination energy stored as triacylgylcerols also known as fatty acids. Linder (2000) stated that the latitude in which a species is adapted directly influences the ratio of unsaturated-saturated fatty acids stored in seed. Based on this evidence wild *H. annuus* accessions adapted to a specific latitude might be expected to

have unique fatty acid profiles when compared to accessions from different latitudes. Accessions of *H. annuus* at lower latitudes had higher proportions of saturated fatty acids and were thus selected to germinate at higher temperatures, while accessions at higher latitudes had lower proportions of saturated fatty acids were thus selected to germinate at lower temperatures (Linder, 2000). Seed produced at lower latitudes germinate at higher temperatures, on average, than seed produced at higher latitudes and thus the ratio of unsaturated-saturated fatty acids may be based on a typical germination temperature for the area (Linder, 2000).

Wild *H. annuus* sunflower accessions are difficult to germinate, which can influence the accessions used for breeding material. The two primary forms of seed dormancy are embryo or seed covering (seed coat + pericarp) or a combination of the two (Nikolaeva 1977). Seed dormancy related to both embryo and seed covering has been shown to vary widely within other species (Foley, 2001). Low and inconsistent germination among the wild sunflower accessions has undoubtedly limited their use in certain breeding programs. It has also limited their use as a wild flower in home gardens and in land reclamation and restoration (Seiler, 1998). Year-to-year differences in the level of embryo dormancy in sunflower seeds and temperature at the time of maturation can impact the level of seed dormancy (Le Page-Degivry et al. 1990). Genetics certainly plays a large role in seed dormancy; however, the environment can affect up to as much as 50% of the phenotypic variation seen in the germination of wild oat seed (Jana and Naylor, 1980).

Because seed dormancy in sunflower is controlled by both the embryo and the seed covering, there are likely differences in the inheritance of dormancy among the wild *H. annuus* sunflowers that have adapted to various regions across North America. In this study the variability in seed dormancy among 19 wild *H. annuus* sunflower accessions that were collected in latitudes as far south as 27.17$^{o}$ N (the southern tip of Texas) and as far north as 50.37$^{o}$ N (Canadian border in Saskatchewan), seven elite lines, and two Native American Landraces were evaluated for dormancy as whole seeds and embryos.

**MATERIALS AND METHODS**

Seed was produced at Corvallis, OR in the summer 2005. The genotypes evaluated were: 19 wild *H. annuus* plant introductions (PI) accessions (PI435434, PI494567, PI468475, PI435619, PI613720, PI586856, PI586849, PI586872, ANN1238, PI586869, PI586879, PI597890, PI613711, PI613722, PI613723, PI613750, PI592326, PI592325, PI592316); five oilseed elite lines (RHA373, RHA801, HA89, HA384 and Ames1847); two confectionary elite lines (Ames7574 and PI599976), and two Native American Landraces (PI432509, PI600718). Planting stock seed of all wild accessions, except ANN1238, was provided by the USDA seed repository at Ames, IA in 2005. Seed of ANN1238 and the elite inbred lines were produced in Corvallis, OR in 2004.

In order to minimize seed after-ripening on the mother plant each genotype was harvested at physiological maturity, as defined by Schneiter and Miller (1981). Seed was harvested and then immediately dried at 39$^{o}$C for 17 h. Seed was cleaned and vacuum sealed in KAPAK® bags (model ASTM F-1249) to lock out moisture and stored at 4$^{o}$C ± 2$^{o}$C to decelerate AR. Seed for this experiment was grown in a common nursery west of the original sites of adaptation of the specific wild *H. annuus* accessions tested at 44.57$^{o}$ N latitude.

Seed used for germination treatment at time 0 was non-after-ripened and stored at 4$^{o}$C since harvest. The after-ripening times (ART) were 4, 8, or 16 wk. Seed was held at 20$^{o}$C ± 2$^{o}$C in a germination chamber and removed at each ART for the germination tests. The AR temperature of 20$^{o}$C was chosen to closely simulate AR at room temperature.

Seed was disinfected prior to imbibition by agitating in a 50/50 solution of sodium hypochlorite and water (3% v/v) for 90 s. Seed was rinsed with distilled, de-ionized water to remove the bleach solution. For each genotype, four replicates of 25 seeds each were placed into Petri plates on blue blotter paper with 5 ml of distilled water. Petri plates were kept in Ziploc® bags inside the germinator to minimize evaporation and maintain a more constant moisture level. Germination data were collected up to 21 d for each ART. A seed was considered germinated when the radical pierced the pericarp of the whole achene or when the radical was visibly elongated in the altered seeds.

Germination of whole seed was compared to seed without pericarp and seed coat (NOPSC) and cut seeds at the same ART. For the cut treatment and the NOPSC treatment, approximately ¼ of the blunt end of the achene was cut off. For the NOPSC treatment, the seed was squeezed out of the seed coat and pericarp to isolate the embryo. The NOPSC and the cut treatments were applied on day five. It was necessary to wait five days to apply the treatment in order to completely remove the seed coat and pericarp without injuring the embryo. A dry embryo cannot be removed from the seed coat and pericarp whereas an imbibed achene makes removal of the embryo possible.

Fatty acid concentrations in sunflower kernel oils were measured by gas chromatography of fatty acid methyl esters. Samples were prepared by grinding four replicates of 10 achenes in 10 ml of HPLC grade hexane using a Polytron (Brinkmann Instruments, Westbury, New York). The mixture was allowed to settle for 20 to 30 min before transferring 0.5 ml of the supernatant to a 16 x 100 mm glass tube. Capped samples were heated for 15 min at 50$^{o}$C in a heat block. The hexane was evaporated under a gentle stream of nitrogen gas before adding 0.1 ml of ethyl ether and 0.1 ml 0.1M of KOH in methanol and heating the samples for 5 min at 50$^{o}$C. The transesterification reaction was neutralized by adding 0.1 ml of 0.15M HCl to each tube, followed by 2.0 ml of hexane. Samples were mixed by swirling and allowed to settle. Then 0.5 ml of the upper phase (hexane) was transferred to a gas chromatography vial, with a disposable pipette, and capped. Using a split ratio of 1:80, 1.0 µl samples were injected onto an Agilent Technologies (Palo Alto, California) DB-23 µm column mounted in an HP6890 gas chromatograph (Hewlett-Packard, Wilmington, Delaware). Oven temperatures were ramped up from an initial temperature of 50$^{o}$C to 185$^{o}$C in 30$^{o}$C/min increments and held at 185$^{o}$C for 4.5 min. Total run time was 10 min. Fatty acid concentrations were calculated using ChemStation Software (Agilent Technologies). Palmitic, stearic, oleic, and linoleic acid peaks were identified using standards purchased from NU-CHEK PREP (Elysian, MN).

The experiment was conducted as a completely randomized design with four replicates. Raw data of each genotype were analyzed using Proc Mixed model in statistical analysis system (SAS). Statistical difference was defined as $P<0.05$. For the

wild *H. annuus* types, the fixed effects of treatment and latitude, were significant (P<0.0001) but ART was not significant (P=0.6791). For wild types, the interaction of latitude x treatment was significant (P=0.0127) and the interactions of treatment x ART (P=0.8456), latitude x ART (P=0.7190) and latitude x treatment x ART (P=0.7948) were not significant. Since ART was not significant, it was dropped from the model to explore the relationship between treatment and latitude to find a model to explain germination of wild *H. annuus* types within the range of latitudes tested here. The mixed model in SAS was used to find a relationship between latitude and germination. Percent germination was used as the dependent variable while taking latitude and the interaction of latitude x treatment (to find the difference between whole seed and NOPSC treatment at a given latitude) to the $2^{nd}$, $3^{rd}$, and $4^{th}$ powers. The AIC model with latitude cubed was the best fit for the model and can be used to predict germination of *H. annuus* accessions by treatment. Fatty acids were analyzed using the Proc Mixed model in SAS with genotype as the class level and the fatty acid (i.e. C16.0) as the dependent variable.

**RESULTS AND DISCUSSION**

Germination of whole seed of wild *H. annuus* accessions was influenced by latitude of the site of origin (P<0.0001). Germination of wild *H. annuus* whole seed was highly variable among accessions (0-45%), and naked embryos of accessions from similar latitudes, did not germinate similarly (Fig. 4.1). The two genotypes that originated in Southern Texas had much higher rates of germination (P<0.0001) at all ARTs, as both whole seed and as embryos, compared to the genotypes which originated in more northern latitudes, even the accession originating in northern Texas (Table 4.1). After-ripening had no affect on the percentage of germination among the wild types tested (P=0.6791). In contrast, elite lines typically had an ART by germination interaction. There was no interaction effect of treatment by ART. This may indicate one of two things, either the embryos were not after-ripened for a long enough period of time or that the treatment effect did not increase as ART increased. If the latter is true, one could expect that the level of germination of these accessions in the wild would be the same from year-to-year. Hence, embryo dormancy is stronger in these accessions.

There was a latitude by treatment effect (P=0.0127). The latitude by treatment effect indicates that the embryo + seed coat + pericarp dormancy of each accession may be due to the adaptation to the local area. All accessions had a significant increase in germination, although not equal, when the seed coat and pericarp were removed (P<0.0001). Based on this result, it was clear that there were differences in the degree of embryo dormancy in the wild *H. annuus* accessions tested. When the seed coat + pericarp were removed, germination of genotypes from different latitudes had an increase in germination. For accessions that originated north of $29^{o}$ latitude, the seed coat and pericarp were primarily responsible for seed dormancy.

ART did not affect germination of the wild types; therefore, to investigate the relationship between treatment and latitude, ART was dropped from the model. Germination was modeled using linear, quadratic and cubic models. The cubic model best explained germination (Fig. 4.2). The response in germination for a genotype originating at a specific latitude varied by latitude. Models to estimate the germination of a wild *H. annuus* accession by each treatment for a specific latitude were found to be: NOPSC = 2091.32-150.74*latitude + 3.6488*latitude$^2$ – 0.0290*latitude$^3$ and germination for whole achenes = 805.76 – 57.03*latitude + 1.3397*latitude$^2$ - .01033*latitude$^3$. These models provide a method to estimate germination of wild *H. annuus* genotypes, between the latitudes tested, and to make comparisons between treatments for specific latitudes.

The genotypes were separated into three groups (elite, wild, and Native American Landrace (NAL)) and the germination response to treatment was analyzed. The elite inbred lines have shallow seed dormancy (Fig. 4.3). Elite lines have both weak seed coat and pericarp dormancies and low to moderate embryo dormancies. Dormancy fades rapidly and removing both the seed coat and the pericarp provided only a small germination advantage at 8 and 16 wk AR in the elite lines. For the NALs, removing the seed coat + pericarp provided a large germination advantage at 0, 4, and 16 wk AR and a slight germination advantage at 8 wk AR. The two NALs germinated much more similar to the elite inbred lines than the wild types. This was not surprising because the NALs were domesticated as a food crop centuries ago and today's cultivars are likely

descendents of those early domestications (Heiser 1945; Heiser 1951; Castetter 1935; Wilson 1917). The NALs had weak embryo dormancy and moderate seed coat + pericarp dormancy. There would be sufficient germination of the NALs, even shortly after harvest, for breeding purposes without removing the seed covering. The wild *H. annuus* types have strong seed coat and pericarp dormancy. Except for Accessions 494567, 435434 and 586869, the only means to get seeds to consistently germinate more than 1 or 2% was to remove the seed coat and pericarp. Removing the seed coat and pericarp on the wild *H. annuus* accessions increased germination 40% at all ARTs.

The fatty acid profiles of the wild *H. annuus* accessions (Table 4.2) were evaluated in order to determine if the fatty acid types (saturated fatty acid versus unsaturated fatty acids) were different based on latitude of origin. The three accessions from Texas were pooled and the averages of each fatty acid were determined. The single average of these three accessions was calculated in order to separate the most extreme southern accessions from the northern accessions and look at saturated versus unsaturated fatty acids. The accessions from Texas had different fatty acid profiles than the other accessions ($P<0.001$). The three accessions originating at the lower latitudes had higher levels of saturated fatty acids than accessions originating at higher latitudes (Table 4.2; Fig. 4.4). Linder (2000) found similar results regarding both *H. annuus* and *H. maximiliani* and noted that the differences in fatty acid ratios of saturated:unsaturated may allow biotypes to become adapted to local conditions. Linder (2000) also noted that within *H. annuus* types germination results showed that biotypes germinating at lower temperatures were selected for lower proportions of saturated fatty acids, and biotypes with higher proportions of saturated fatty acids were selected for germinating at warmer temperatures. However, Linder (2000) left 75% of the seed coat and pericarp intact when germinating those seed. Results from this study and a previous study by the authors (chapter two) showed that the seed coat and pericarp inhibited germination even when the seed was cut and the seed coat + pericarp were left mostly intact. Results of excising seeds of wild sunflower accessions were highly variable and genotype specific. Based on these facts, whole seed dormancy (embryo + seed coat + pericarp) most likely plays a larger role in germination than fatty acid type and germination temperature.

In *Arabidopsis*, the seed coat restricted germination through the presence of phenolic compounds (Debeaujon et al. 2000). This may very well be the case for *H. annuus* as well. When seeds were excised, in the cut treatment, the seed coat was still intact with the seed, but the embryo was exposed to increased levels of water and gasses compared to whole seeds. Therefore, it seems likely that chemicals in the seed coat inhibited germination rather than the exclusion of water and gasses as some have hypothesized. If chemicals in the seed coat are indeed the inhibitor, removing part of the seed coat only partially relieved seed dormancy.

## CONCLUSIONS

Differences in whole seed dormancy of wild *H annuus* accessions were found based on latitude. Accessions from the most southern latitudes germinated as whole seed when accessions from more northern latitudes did not. Accessions of wild *H. annuus* have very different levels of embryo dormancy and seed covering dormancy. These differences were partly found to be dependent upon latitude; accessions from the extreme part of Southern Texas had much less whole seed dormancy and embryo dormancy than the accessions from the more northern latitudes. After-ripening seed of the wild *H. annuus* accessions did not result in an increase in germination, but removing the seed covering did increase germination. The fatty acid profiles of the accessions from Texas were found to be different than the other accessions but we do not believe that these differences were responsible for the differences in embryo dormancy, at least not at the germination temperature tested here. The NALs germinated more similarly to elite lines than wild types; however, germination of the NALs was controlled more by the seed covering than was germination of the elite lines.

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Fig. 4.1. Germination percentages of wild *Helianthus annuus* accessions after-ripened for 0, 4, 8, or 16 weeks. The treatments are: control (whole seed), or no pericarp and no seed coat (NOPSC). Accessions are arranged from most southern to most northern latitude from left to right.

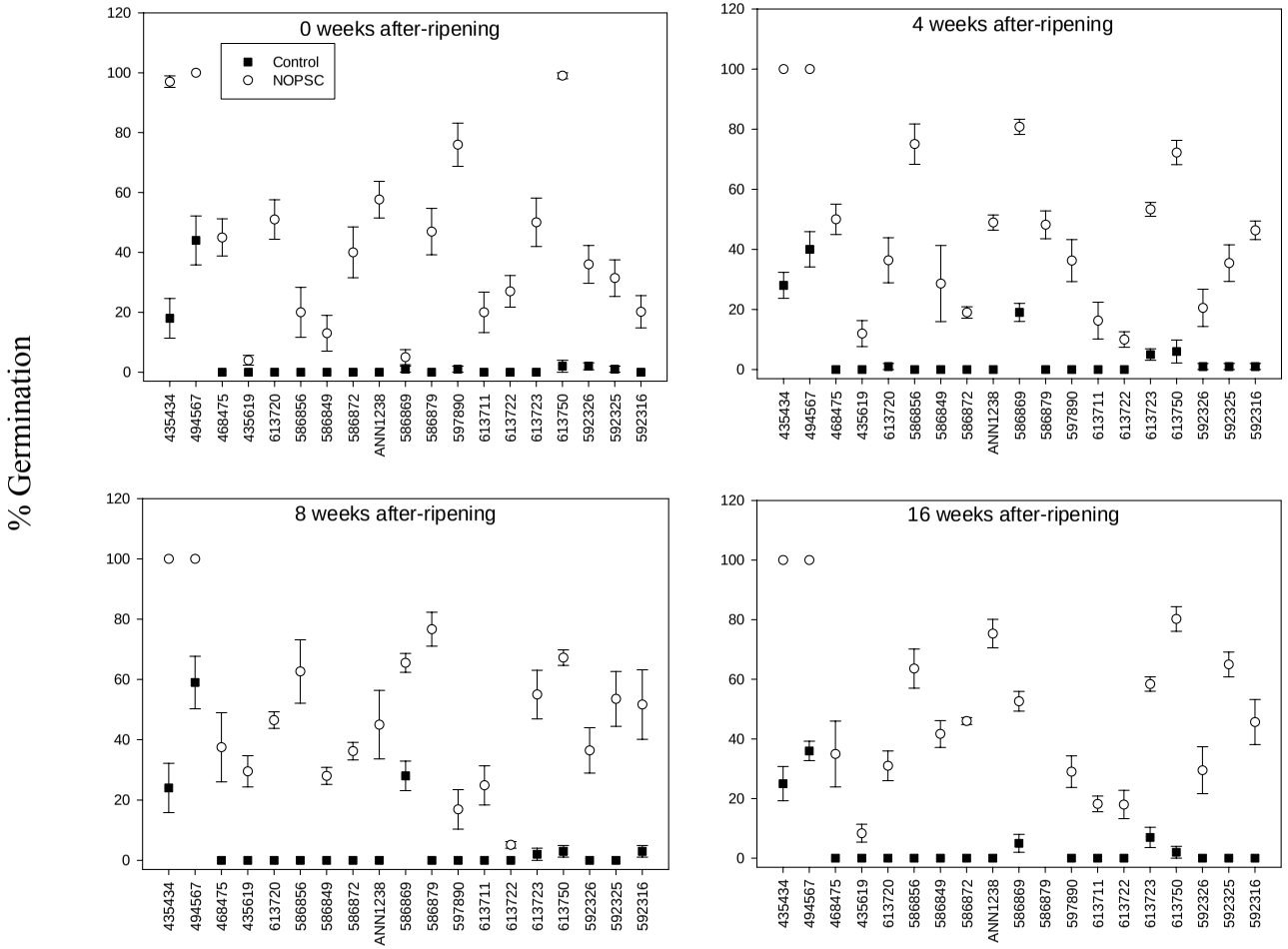

Table 4.1. Percent germination of sunflower accessions at 5 and 21 days after imbibition. The treatments are whole seed, pericarp and seed coat removed (NOPSC) and cut seed.

| | | | Whole Seed | | | | | | | | NOPSC | | | | | | | | Cut | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | ART[a] 0 | | ART 4 | | ART 8 | | ART 16 | | ART 0 | | ART 4 | | ART 8 | | ART 16 | | ART 8 | |
| Genotype | Site[b] | Grp[c] | 5D | 21D | 5D | 21D | 5D | 21D | 5D | 21D | 5D | 21D | 5D | 21D | 5D | 21D | 5D | 21D | 5D | 21D |
| HA89 | | E | 56 | 79 | 88 | 94 | 63 | 95 | NA | NA | 54 | 100* | 95 | 100 | 62 | 100 | NA | NA | 60 | 100 |
| Ames1847 | | E | 99 | 99 | 100 | 100 | 54 | 99 | 61 | 100 | 98 | 100 | 100 | 100 | 64 | 100 | 74 | 100 | 64 | 100 |
| Ames7574 | | E | 100 | 100 | 78 | 88 | 94 | 99 | 89 | 100 | 100 | 100 | 70 | 100 | 89 | 100 | 77 | 100 | 93 | 100 |
| RHA373 | | E | 82 | 89 | 91 | 100 | 39 | 84 | 51 | 95 | 84 | 75 | 87 | 100 | 23 | 100 | 62 | 100 | 29 | 100 |
| RHA801 | | E | 100 | 100 | 91 | 94 | 84 | 100 | 95 | 100 | 98 | 100 | 84 | 43 | 86 | 100 | 100 | 100 | 86 | 100 |
| HA384 | | E | 90 | 99 | 82 | 97 | 77 | 100 | 54 | 100 | 70 | 100 | 48 | 100 | 84 | 100 | 60 | 100 | 72 | 100 |
| PI599976 | ND | E | 67 | 96 | 95 | 100 | 40 | 95 | NA | NA | 48 | 69 | 75 | 100 | 45 | 100 | NA | NA | NA | NA |
| PI600718 | ND | NAL | 60 | 75 | 88 | 97 | 47 | 98 | NA | NA | 71 | 100* | 79 | 100 | 45 | 100 | NA | NA | NA | NA |
| PI432509 | AZ | NAL | 2 | 10 | 64 | 73 | 71 | 85 | 65 | 72 | 8 | 97* | 42 | 100* | 75 | 96 | 63 | 100* | 70 | 100* |
| PI592316 | SAS | W | 0 | 0 | 0 | 1 | 1 | 3 | 0 | 0 | 0 | 20* | 0 | 47* | 0 | 52* | 1 | 46* | 2 | 16* |
| PI592325 | SAS | W | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 31* | 0 | 35* | 0 | 53* | 0 | 65* | 0 | 12* |
| PI592326 | MAN | W | 2 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 36* | 0 | 20* | 0 | 36* | 0 | 29* | 0 | 8 |
| PI613750 | ND | W | 1 | 2 | 1 | 6 | 0 | 3 | 1 | 2 | 1 | 99* | 1 | 72* | 3 | 67* | 1 | 80* | 3 | 44* |
| PI613723 | ND | W | 0 | 2 | 3 | 5 | 1 | 2 | 7 | 7 | 0 | 50* | 3 | 53* | 3 | 55* | 3 | 58* | 3 | 17* |
| PI613722 | SD | W | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 27* | 0 | 10* | 0 | 5 | 0 | 18* | 0 | 5 |
| PI613711 | SD | W | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20* | 0 | 16* | 0 | 25* | 0 | 18* | 0 | 2 |
| PI597890 | SD | W | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 76* | 0 | 39* | 0 | 17* | 0 | 28* | 0 | 11* |
| PI586879 | NE | W | 0 | 0 | 0 | 0 | 0 | 0 | NA | NA | 0 | 46* | 0 | 51* | 0 | 77* | NA | NA | 0 | 14* |
| PI586869 | NE | W | 0 | 1 | 2 | 19 | 2 | 28 | 0 | 5 | 0 | 5 | 1 | 80* | 1 | 66* | 0 | 53* | 1 | 61* |
| PI586872 | NE | W | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 40* | 0 | 19* | 0 | 36* | 0 | 46* | 0 | 21* |
| ANN1238 | NE | W | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 58* | 0 | 49* | 0 | 45* | 0 | 76* | 0 | 17* |
| PI586849 | KS | W | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13* | 0 | 28* | 0 | 28* | 0 | 42* | 0 | 7 |
| PI586856 | KS | W | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20* | 0 | 77* | 0 | 62* | 0 | 64* | 0 | 27* |
| PI613720 | KS | W | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 51* | 0 | 36* | 0 | 47* | 0 | 31* | 0 | 14* |
| PI435619 | OK | W | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 12* | 0 | 30* | 0 | 8* | 0 | 4 |
| PI468475 | TX | W | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 45* | 0 | 50* | 0 | 38* | 0 | 35* | 0 | 3 |
| PI435434 | TX | W | 2 | 0 | 0 | 28 | 2 | 24 | 3 | 25 | 2 | 96* | 0 | 100* | 3 | 100* | 4 | 100* | 1 | 99* |
| PI494567 | TX | W | 4 | 44 | 0 | 40 | 0 | 59 | 3 | 36 | 1 | 100* | 1 | 100* | 1 | 100* | 2 | 100* | 0 | 98* |

[a]ART = After-ripening Time; 0, 4, 8 or, 16 weeks

[b]Site denotes the state or Canadian province of original collection site for the accession

[c]Group (Grp) denotes: elite (E), wild (W) or Native American Landrace (NAL)

* Denotes a significant difference at the 0.05 level of probability for the treatment compared to whole seed at the given ART

NA = not applicable; there was not enough seed for the treatment

Fig. 4.2. Model for germination of wild *Helianthus annuus* accessions by origin of latitude; whole seed explains dormancy related to embryo + seed coat + pericarp and the NOPSC treatment explains embryo dormancy.

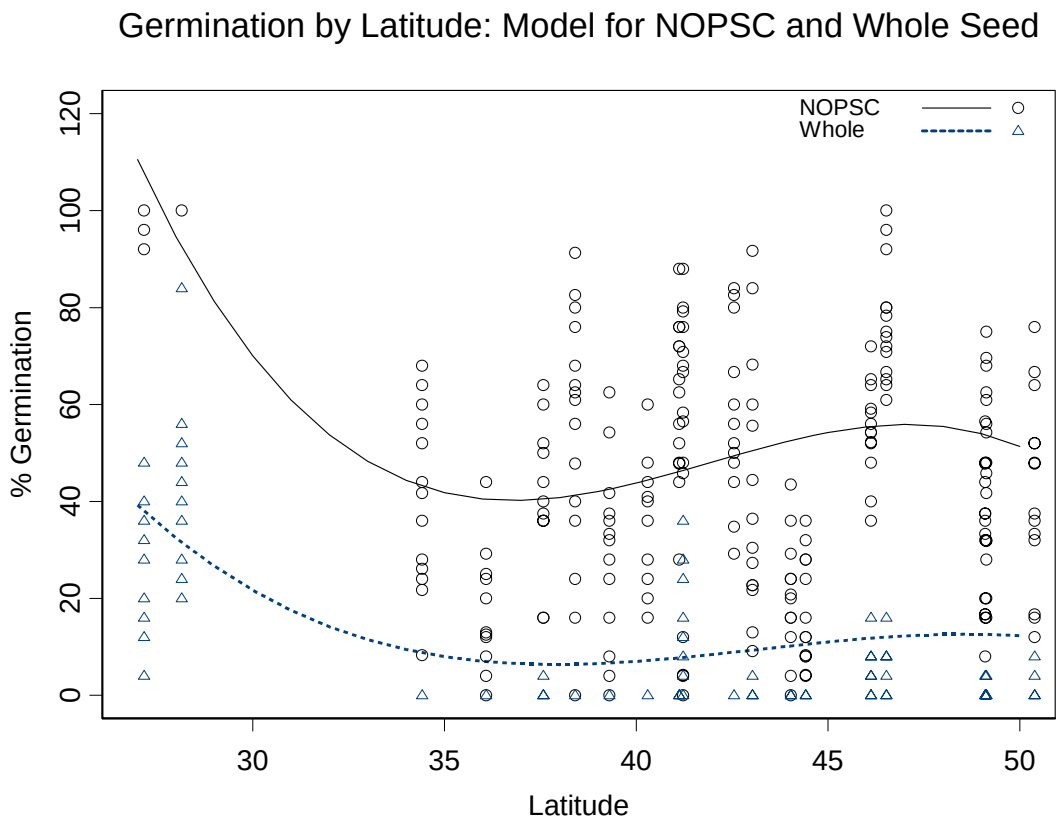

Fig. 4.3. Percent germination of elite, wild and Native American Landrace sunflower seeds by group and by treatment. The control explains whole seed dormancy and the no pericarp and no seed coat (NOPSC) treatment explains embryo dormancy. Seeds were after-ripened for 0, 4, 8, or 16 weeks. Error bars denote standard error.

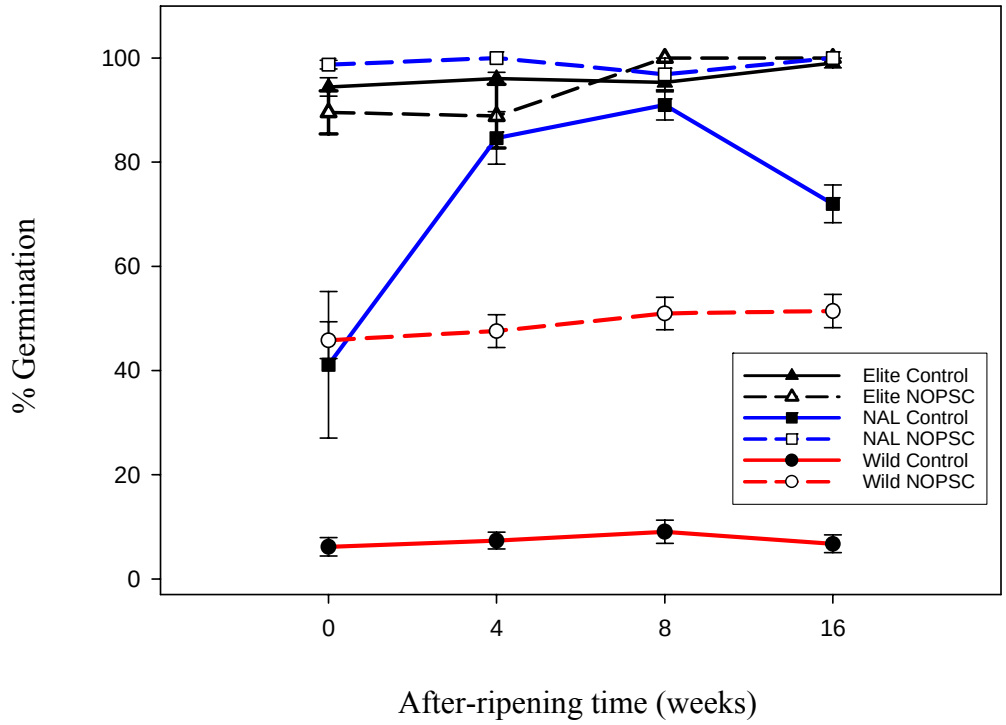

Table 4.2. Means of fatty acid profiles for the 19 wild *Helianthus annuus* accessions in order by latitude from south to north.

| | | | Fatty acid | | | |
|---|---|---|---|---|---|---|
| Accession (PI)[a] | Site of Origin[b] | Latitude | C16:0 | C18:0 | C18:1 | C18:2 |
| 435434 | TX | 27.17 | 6.35 | 4.94 | 14.22 | 74.49 |
| 494567 | TX | 28.15 | 5.99 | 4.50 | 15.10 | 74.27 |
| 468475 | TX | 34.42 | 6.03 | 3.04 | 13.96 | 76.83 |
| 435619 | OK | 36.09 | 5.73 | 3.85 | 14.06 | 76.19 |
| 613720 | KS | 37.58 | 5.70 | 3.17 | 12.39 | 78.60 |
| 586856 | KS | 38.41 | 6.10 | 3.08 | 11.61 | 79.08 |
| 586849 | KS | 39.30 | 5.41 | 3.51 | 12.46 | 78.41 |
| 586872 | NE | 40.30 | 5.08 | 2.99 | 13.27 | 78.51 |
| ANN1238 | NE | 41.12 | 4.74 | 3.42 | 19.58 | 72.20 |
| 586869 | NE | 41.22 | 5.39 | 3.55 | 12.29 | 78.65 |
| 586879 | NE | 42.55 | 5.46 | 3.50 | 11.93 | 78.96 |
| 597890 | SD | 43.03 | 5.15 | 3.14 | 12.88 | 78.71 |
| 613711 | SD | 44.03 | 5.54 | 3.08 | 16.42 | 74.81 |
| 613722 | SD | 44.42 | 5.05 | 2.86 | 15.27 | 76.61 |
| 613723 | ND | 46.12 | 4.98 | 2.78 | 15.47 | 76.56 |
| 613750 | ND | 46.52 | 4.47 | 2.35 | 14.95 | 78.10 |
| 592326 | CAN | 49.10 | 4.24 | 2.46 | 15.66 | 77.40 |
| 592325 | CAN | 49.13 | 4.43 | 2.84 | 15.51 | 77.03 |
| 592316 | CAN | 50.39 | 4.60 | 2.46 | 14.53 | 78.14 |
| Average of TX PIs | TX | 29.91* | 6.12* | 4.16* | 14.43 | 75.2* |

[a]PI indicates plant introduction from the National Plant Germplasm Collection, Ames, IA

[b]Site of original collection for the accession

*Indicates signifigant difference at the P<0.05 level of probability within a column. This is the the average of the accessions from Texas; therefore, it excludes the Texas accessions and shows that all other accessions are different when compared to accessions from Texas.

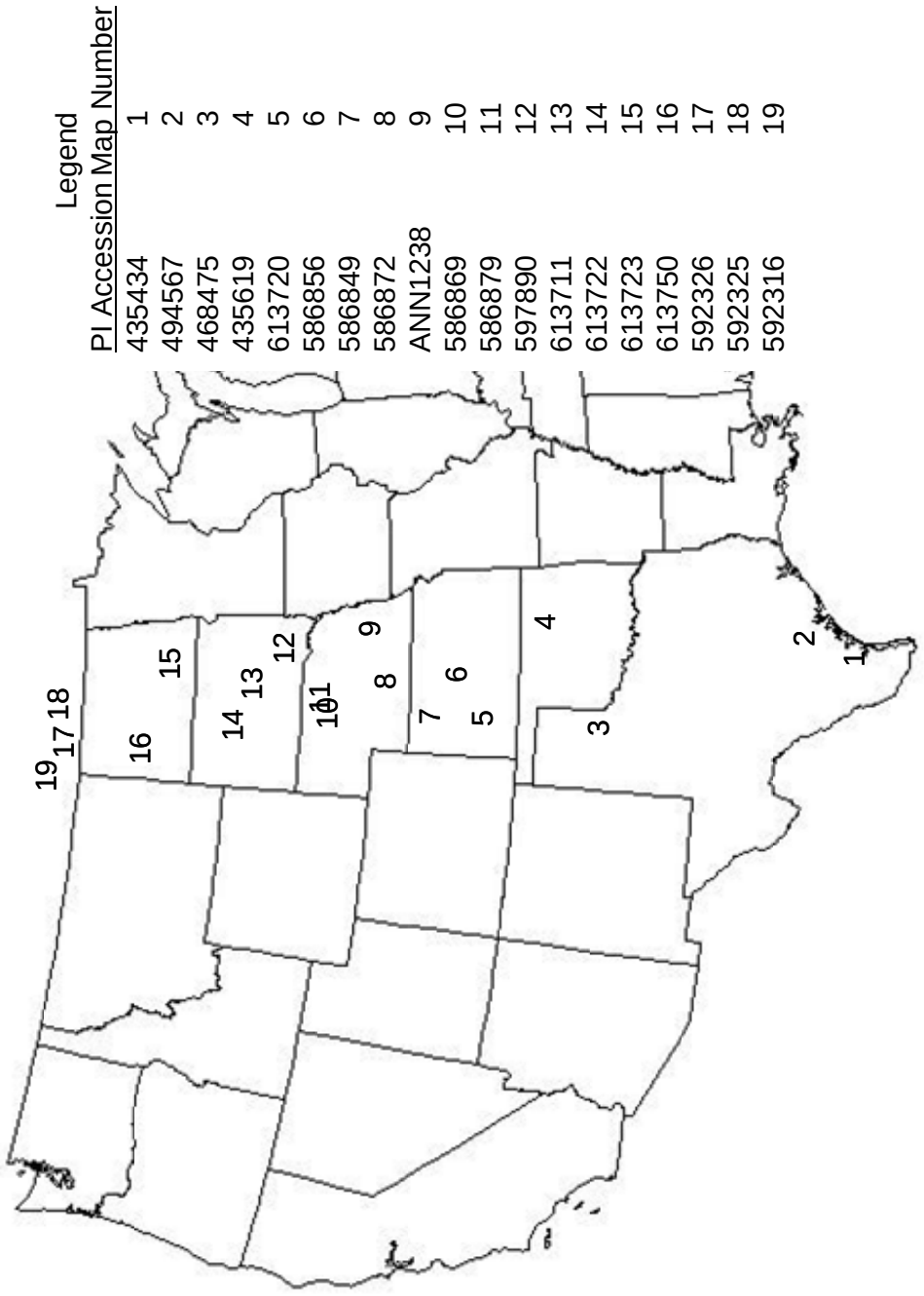


Fig. 4.4. Map with approximate locations of original collection sites of the 19 wild *Helianthus annuus* accessions used in this germination study.

# Chapter 5

## Novel Sunflower Seed Germplasm Development

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**ABSTRACT**

Two populations of novel sunflower (*Helianthus annuus* L.) germplasm were developed. Seed of wild *H. annuus* accession ANN1238 was screened under a cage in the field, to isolate the plants from natural pollinators, for self-compatibility. Differences in self-compatibility were found ($P<0.0001$). The plant that had the highest number of seed autonomous set was used as the progenitor for the development of a wild inbred line to the $S_5$ generation. The second population was developed by backcrossing the seed coat and pericarp dormancy alleles from wild *H. argophyllus* into the elite line NMS801. The segregating generations were screened and backcrossed to NMS801 to the $BC_3$ generation.

**INTRODUCTION**

Novel sunflower germplasm development is needed in order to produce populations to better understand the genetics of sunflower. Wild sunflowers possess many traits of interest to sunflower researchers and breeders but many of these traits, such as seed dormancy, are not well understood.

Wild sunflowers are typically self-incompatible (Heiser, 1954). Miller and Fick (1997) noted that the three factors controlling self-fertility are: genetics, the environment, and floral morphology. Pinthus (1959) found that temperatures influence self-fertility. Roath and Miller (1980) and Vranceanu et al. (1978) also found that there is a large genetic x environmental interaction related to self-fertility. Self-infertility in sunflower is sporophytic in nature (Habura 1957; Fernandez-Martinez and Knowles 1978). Habura (1957) reported that self-infertility was controlled by at least two multiallelic S (self-incompatible) loci and Fernandez-Martinez and Knowles (1978) reported that at least five different S alleles at a single locus were responsible.

Sunflower seed dormancy in wild types has proven to cause difficulties for their use in breeding programs (Heiser et al. 1969; Akinola et al. 2000; Seiler, 1998; and Chandler and Jan, 1985). Both the embryo and the seed covering contribute to seed dormancy (Ballard 1973; Gay et al. 1991; Kelly 1992). The length and types of seed dormancy are variable among the species.

Our goals were to develop wild *H. annuus* (ANN1238) fully self-compatible germplasm and to introgress seed coat and pericarp dormancy genes from wild *H. argophyllus,* accession 1803 (ARG1803) into the elite line, nuclear male sterile (NMS) NMS801. These unique germplasms will allow researchers to more fully understand the inheritance of traits from wild sunflowers, to more accurately determine the loci of seed dormancy genes, and to understand the mechanisms of seed dormancy.

## MATERIALS AND METHODS

### Development of Self-compatible Wild *H. annuus* Germplasm

During the summer of 2004 in Corvallis, Oregon, ANN1238 was grown in isolation under a cage. Ten plants were randomly selected under the cage and bags were placed on four heads of each of the ten plants. Heads were manually pollinated (M) over the course of flowering or left to set seed autonomously (A). The numbers of seed per head were counted and raw data were analyzed in SAS using Proc GLM .

When the seed had after-ripened for 30 d, germination studies were initiated. Fifteen seed were disinfected with a 10% sodium hypochlorite solution for 5 min and then rinsed with distilled water. Seed were placed in a Petri plate on blotter paper with 5 ml of distilled water and allowed to imbibe for 5 d. On day five, the seed coat and pericarp were removed, and the embryos were placed on clean blotter paper and allowed to germinate. It was necessary to wait five days to apply the treatment in order to completely remove the seed coat and pericarp without injuring the embryo. A dry embryo cannot be removed from the seed coat and pericarp whereas an imbibed achene makes removal of the embryo possible. Seedlings were transplanted into two liter pots, transferred to the greenhouse and grown with 16 hr supplemental light. Over the course of flowering, the heads were manually pollinated several times by rubbing heads with a paper towel to increase the number of seed set per head. Seeds were harvested at maturity and allowed to after-ripen at room temperature for 30 d before initiating the next round of germination.

**Development of Seed Coat and Pericarp Dormant Elite Inbred Line NMS801 by Introgressing Dormancy Alleles from *H. argophyllus***

A non-dormant, male sterile NMS801 plant was crossed with a dormant ARG1803 plant. One progeny resulting from this cross was backcrossed, in the greenhouse, to NMS801 to produce the $BC_1$ generation. Progeny from the $BC_1$ generation were grown under a cage in isolation and intermated (cross pollinated with half siblings) using bees in the summer of 2004. The $BC_1S_1$ seed was harvested by family (Families 1-29). The $BC_1S_1$ seed was screened for seed coat + pericarp dormancy after the seed had after-ripened for 30 d at room temperature. It was necessary to after-ripen the seed to insure that the embryo dormancy had lapsed and that seed coat + pericarp dormancy were controlling germination of seed. The seed was disinfected with a 10% sodium hypochlorite solution for 5 min and then rinsed with distilled water. The seed was placed in Petri plates on blue blotter paper in 5 ml of water and water was added as needed to keep the seed moist for 30 d. The Petri plates were kept at room temperature in alternating 12 hr of light and 12 hr of darkness under a grow light. Seed that had not germinated after 30 d was considered to have seed coat and pericarp dormancy alleles contributed from ARG1803. On day 30, the seed coat and pericarp were removed from the un-germinated seed and the naked embryos germinated within one to two days. The plants resulting from these selections were backcrossed to a sterile NMS801 plant in the greenhouse producing $BC_2$ seed. During summer 2005, $BC_2$ seed was planted in the field. Seventy-eight individual plants were bagged and manually pollinated to produce $BC_2S_1$ seed. The $BC_2S_1$ seed was harvested at physiological maturity and after-ripened for 30 d at room temperature and then disinfected and germinated as previously described. For the seed that remained un-germinated on day 30, the pericarp and seed coat were removed and the naked embryos germinated within one to two days (data not shown). The plants resulting from these selections were backcrossed to sterile individual NMS801 plants producing the $BC_3$ generation.

## RESULTS AND DISCUSSION

### Development of Self-compatible Wild *Helianthus annuus* Germplasm

For the plants grown under the cage in 2004, the number of seed set per head per plant was different ($P<0.0001$). The plant identified under the cage as 2004/10A was allowed to self and had the greatest number of seeds set per head per plant. This plant was selected as the progenitor for the development of the self-compatible line. The four heads harvested from 2004/10A produced 14, 14, 20, and 21 seeds per head. The development of $S_5$ lines of the inbred wild *H. annuus* ANN1238 is shown in Table 5.1. Throughout the selection and advancement of this line, the plants were selected for number of seed set and for the most vigorous plants. Because of the continuous selfing, the plants produced in the later generations were severely stunted and displayed the typical signs of inbreeding depression.

This germplasm is held by Dr. Steven J. Knapp at the University of Georgia, Athens, Georgia. Dr. Knapp may be contacted at SJKnapp@uga.edu regarding information about this population.

### Development of Seed Coat and Pericarp Dormant Elite Inbred Line NMS801 by Introgressing Dormancy Alleles from *H. argophyllus*

The NMS801 line has weak seed coat and pericarp dormancy; whereas, ARG1803 has strong seed coat and pericarp dormancy. The population developed from this project will be used to identify the locations of the dormancy introgressions from ARG1803 into NMS801. The pedigrees of these backcross populations are shown in Table 5.2. This population will help provide an understanding of the dormancy genes in the wild *H. argophyllus* accession and perhaps for wild material in general. *Helianthus argophyllus* was used to create this backcross population in order to evaluate germplasm from a novel wild source and to screen backcross progeny for silver leaf traits harbored by *argophyllus* species. Dormant lines advanced in this program did not contain the silver leaf traits that are typical of *H. argophyllus*.

This germplasm is held by Dr. Steven J. Knapp at the University of Georgia, Athens, Georgia. Dr. Knapp may be contacted at SJKnapp@uga.edu regarding information about this population.

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Table 5.1. Developmental chart of wild *Helianthus annuus* accession ANN1238, self-compatible inbred line, through continuous selfing.

| Generation | Selfing | Timeline |
|---|---|---|
| $S_0$ plant | 2004/10 | Summer 2004 |
| $S_1$ seed | ↓ | Autonomous seed set under a cage; harvested October 2004 |
| $S_1$ plant | 2004/10-D | Plant D was manually-selfed in greenhouse December/January 2004-05 |
| $S_2$ seed | ↓ | |
| $S_2$ plant | 2004/10-D-2 | Plant 2 was manually-selfed in greenhouse June/July 2005 |
| $S_3$ seed | ↓ | |
| $S_3$ plant | 2004/10-D-2-O | Plant O was manually-selfed in greenhouse Oct/Nov 2005 |
| $S_4$ seed | ↓ | |
| $S_4$ plants | 2004/10-D-2-O-1 | Plants 1, 2, 3, 4, 7, 15 and 16 were maually-selfed in greenhouse March/April 2006 |
| $S_5$ seed | 2004/10-D-2-L-2 | Plant 1 was recommended for advancement based on seed set of 350 per plant. |
| | 2004/10-D-2-L-3 | |
| | 2004/10-D-2-O-4 | |
| | 2004/10-D-2-O-7 | |
| | 2004/10-D-2-O-15 | |
| | 2004/10-D-2-O-16 | |

Table 5.2. Developmental chart of backcross derived NMS801 germplasm with introgressions of seed coat and pericarp dormancy alleles from *Helianthus argophyllyus.*

| Backcross | Generation | Description |
|---|---|---|
| | NMS801[a] x ARG1803 | |
| | ↓ | |
| NMS801 x | $F_1$ | $F_1$ = (NMS801 x ARG1803)-1 |
| | ↓ | |
| | $BC_1$ | Intermate under cage with bees - summer 2004 |
| | ↓ | $BC_1$ Families = (NMS801 x ARG1803)-1...,29 |
| NMS801 x | $BC_1S_1$ | Harvested individual half-sibbed/selfed families from cage |
| | ↓ | Individual $BC_1S_1$ plants that had pericarp+seedcoat dormancy were backcrossed to NMS801 |
| | | These individual plants are numbered: 1- 2004/6A, 1- 2004/6B, 1- 2004/8, 1-2004/17, 1- 2004/22A |
| | $BC_2$ | 78 Individual plants were bagged and selfed in the field - summer 2005 |
| | ↓ | |
| NMS801 x | $BC_2S_1$ | Individual $BC_2S_1$ plants that had pericarp+seedcoat dormancy were backcrossed to NMS801 |
| | ↓ | These individual plants are numbered: 1-2004/17-11A, 1-2004/17-11C, 1-2004/22A-4A, 1-2004/22A-4C, 1-2004/22A-22E, 1-2004/22A-13, 1-2004/22A-22B |
| | $BC_3$ | |

[a]NMS801 - nuclear male sterile, eltie line, *H. annuus* ; ARG1803 - *H. argophyllus*

# CHAPTER 6

## Genetic Mapping of Seed Dormancy Loci in a Domesticated x Wild Sunflower Recombinant Inbred Line Population

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**ABSTRACT**

Sunflower seed dormancy QTL were mapped in an elite by wild recombinant inbred line (RIL) $F_8$ population (HA89 x ANN138). Seed dormancy of the wild parent was found to be almost entirely controlled by the seed covering with almost no embryo dormancy, while the elite parent had moderate embryo dormancy and little to no seed covering dormancy. Germination of the RILs was strongly controlled by the seed covering in the early after-ripening time periods. Forty-three QTL were discovered over the time course of 0 to 24 weeks of after-ripening. QTL responsible for seed dormancy related to the embryo, whole seed, and delay of germination percentage (DOGp) for 25, 50, and 75% were identified. Both large and small effect QTL controlling whole seed dormancy were discovered and explained between 8.9-30.4% of the phenotypic variation, while 11.6-23.1% of the phenotypic variation observed in embryo dormancy was explained. Multiple linkage groups harboring several important QTL regions each were identified.

**INTRODUCTION**

Seed dormancy has been described as an intact, viable seed failing to germinate when placed in favorable germination conditions (Hilhorst, 1995; Bewley, 1997). A seed will typically germinate when additional environmental cues are no longer necessary to initiate germination (Thompson, 2000). Seed germination is affected by several environmental factors, e.g., light (Bewley and Black, 1994; Pons, 2000 and Baskin and Baskin, 2004) and temperature (Leupold et al. 1988; Koornneef and Karssen, 1994) in addition to genetics (Jana and Naylor, 1980).

The roles of abscisic acid (ABA) and gibberallic acid (GA) in seed germination are not completely clear, but both strongly affect seed germination. ABA has been shown to inhibit germination of whole seed (Hilhorst and Karssen, 1992) and isolated embryos (Le Page-Digivry et al. 1990). Decreased levels of ABA increased seed germination in wheat (*Triticum aestivum* L.) (King, 1976) and soybean (*Glycine max* L.) (Ackerson, 1984). Conversely, ABA level and seed germination do not seem to be negatively correlated in some plants (Bianco et al. 1994; Kermode et al. 1989). Two

ABA loci are tightly linked with two delay of germination (DOG) loci in *Arabidopsis* (*Arabidopsis thaliana*), suggesting ABA may play a role in seed dormancy in this species (Bentsink et al. 2006). Although the role of GA in germination is not completely understood, germination typically increases as ABA levels decrease and GA levels increase (Bewley and Black, 1994).

Seed germination is a genetically complex trait, often controlled by many loci which are strongly affected by environment and genotype x environment (G x E) interactions in pre- and post-harvest stages. Conversely, several major QTL controlling seed dormancy have been reported in wheat (Torada et al. 2005) and barley (*Hordeum vulgare* L.) (Han et al. 1999; Li et al. 2004; Prada et al. 2004). When QTL with large effects are discovered often times additional QTL with smaller effects are detected in the same population(s) (Hori et al. 2007). When loci underlying a complex trait are not known, quantitative trait locus (QTL) analyses are often initially used to identify and demarcate genomic regions harboring QTL, as the first step towards identifying genes and interactions.

Seed dormancy is an important trait to consider in plant breeding. The level of seed dormancy in a particular variety can determine its success. An adequate level of seed dormancy is important to balance when selecting for other traits but may prove difficult due to gene interactions. For example, genes tightly linked to dormancy genes would be inherited together possibly complicating the selection process. The effects of seed dormancy loci are usually temporal and strongly affected by pre- and post-harvest environmental effects and post-harvest storage conditions. Seed dormancy QTL in cultivated rice lost their effect when the seed was dried (Lin et al. 1998). Seed dormancy QTL have been classified as early, constant, or late expression in weedy rice (Gu et al. 2004). Similar temporal effect groupings have been described in *Arabiidopsis* (Alonso-Blanco et al. 2003).

Gu et al. (2005) used phenotypic and marker assisted selection (MAS) to introgress weedy rice (*Oryza sativa*) seed dormancy QTL into non-dormant breeding material. Seed dormancy in the introgressed lines was explained by the effects of three QTL, a trigenic epistatic effect, and G x E after-ripening time interactions. One of the

three QTL had a large-effect, accounting for 67 and 50% of the phenotypic variance in germination at 7 and 14 days of after-ripening, while, another accounted for 7 and 12% at the same after-ripening times. QTL in wild oat (A*vena fatua L.*) have also been determined to control approximately 50% of the phenotypic variation of germination (Jana and Naylor, 1980). Fennimore et al. (1999) found that a three-locus model explained dormancy in recombinant inbred lines (RILs) of wild oat (two dominant alleles and one recessive allele). The three loci were associated with random amplified polymorphic DNA (RAPD) markers in the $F_2$ population. Two of the markers explained 12.6 and 6.8% of the phenotypic variation in germination while the third marker was determined to be linked in repulsion with one of the other markers.

Several small and large-effect seed dormancy QTL have been identified in malting barley (Ullrich et al. 1993; Han et al. 1996), and account for as much as 50% and as little as 5% of the phenotypic variation in seed dormancy. Seed dormancy is often highly heritable. Heritability for seed dormancy ranged from 56-84% in white wheat (Anderson et al. 1993). Prada et al. (2004) reported a heritability of 86% in malting barley and identified a single QTL accounting for 52% of the phenotypic variability.

Pre-harvest sprouting (PHS), a trait analogous to seed dormancy, is a problem in wheat in some cultivars and growing areas. PHS is usually correlated with seed dormancy and decreases grain quality, thereby decreasing crop value and storability. Several, mostly small-effect seed dormancy and PHS QTL (Osa et al. 2003; Groos et al. 2002) have been identified in wheat and account for 4.2 to 11.4% of the phenotypic variation.

Seed dormancy in sunflower (*Helianthus annuus* L.) is controlled by both the embryo and seed covering. The seed covering is physically complex and incorporates both the seed coat and pericarp. The seed coat (Gassner, 1973) and pericarp (Hanausek, 1902 and Roth, 1977) have been described in detail and both have been shown to inhibit germination (Chandler and Jan, 1985; Corbineau and Come, 1992; Debeaujon et al. 2000). Several methods have been evaluated to germinate sunflower seeds such as heating seeds at extreme temperatures (Akinola et al. 2000); soaking in gibberellic acid solutions (Seiler, 1998); increasing atmospheric oxygen concentrations (Gay et al. 1991);

and allowing seeds to soak in moist pots in varying climatic conditions (Heiser et al. 1969); however, none have been as regularly successful as dehulling (Chandler and Jan, 1985; chapter one; chapter 3). Optimum sunflower seed germination has been demonstrated to occur at 25$^{o}$C (Gay et al. 1991). After a three day drying period, isolated embryos of sunflower showed a decrease in ABA content but not an increase in germination; however, additional dry storage up to six weeks promoted germination without a decrease in ABA content (Bianco et al. 1994). Gibberellic acid (GA) has been used to successfully increase germination of wild sunflowers (Seiler, 1997).

Wild sunflowers are especially difficult to germinate even when after-ripened for long periods (unpublished data). Seed dormancy of wild sunflowers is primarily controlled by the seed covering (Seiler, 1997; chapter one, chapter three) while dormancy due to the seed covering in elite lines is short lived (chapter one; chapter two; chapter three). Excising a small portion of the seed covering and embryo to allow permeation of water and gasses only provided a slight advantage in germination compared to removing the entire seed coat and pericarp (chapter one; chapter three). However, when the seeds were excised typically 50% or greater of the seeds decayed before they could germinate (chapter two).

Gandhi et. al. (2005) mapped three seed dormancy QTL to linkage groups 3, 11 and 15 in a wild x domesticated sunflower (*H. annuus*) backcross population, but only sampled one post-harvest storage stage (four weeks of after-ripening). The three QTL accounted for 9.7, 16.5 and 12.1% of the phenotypic variation in seed germination. Al-Chaarani et al. (2005) identified several seed dormancy QTL in a sunflower RIL population developed from a cross between elite (domesticated) lines. QTL on linkage groups 1 and 8 accounted for 13 and 18% of the phenotypic variation, respectively. Four other QTL were identified, one each on linkage groups 3 and 9 and two on linkage group 6 and accounted for 16, 11, 9 and 8% of the phenotypic variation. Collectively, the QTL accounted for half of the phenotypic variation.

Seed dormancy has been greatly reduced by domestication and breeding in sunflower, and is significantly more pronounced and persistent in wild than domesticated sunflower germplasm (Heiser et al. 1969; Seiler, 1997; chapter one; chapter three), although limited

germplasm has been screened for temporal and spatial seed dormancy variability in sunflower, genetic and physiological mechanisms underlying dormancy are still not known. In chapter one we reported that there are differences between elite lines, Native American Landraces (NALs) and wild *H. annuus* accessions regarding embryo and seed covering dormancy. Seed dormancy of the NALs was more similar to the elite lines. Germination suppressed by the seed covering in the NALs was more pronounced than in the elite lines but not nearly as much as in wild *H. annuus* accessions. Embryo dormancy of the NALs was twice as strong (half the germination) as the elite lines before after-ripening, then was nearly gone at four and eight weeks and increased again at 16 weeks. Dormancy of wild *H. annuus* accessions is primarily controlled by the seed covering; however, the range in germination of naked embryos from different accessions is highly variable (chapter three).

Genetic analyses of seed dormancy in wild sunflower are complicated by self-incompatiblity (Gandhi et al. 2005), which has previously necessitated the development and analysis of heterogeneous, non-inbred, sibbed seed populations. Normally, very large seed samples are needed for dormancy phenotyping, particularly when temporal factors, e.g., post-harvest after-ripening time (ART), affect germination and time-course analyses are needed to deconvolute seed dormancy QTL effects (Alonso-Blanco et al. 2003; Foley, 2002; Schaar et al. 1997; Wan et al. 2005; Hori et al. 2007; Torada et al. 2005). We developed a novel sunflower recombinant inbred line (RIL) population from a cross between a self-compatible wild population (ANN1238) and elite oilseed inbred line (HA89), which facilitated the production of the large quantities of seed needed for a time-course analyses of seed dormancy and the discovery of seed dormancy QTL in a population segregating for domesticated and wild alleles. Here, we describe forward genetic analyses of seed dormancy QTL identified by phenotyping seeds of HA89 x ANN1238 RILs after-ripened from four to 24 weeks.

## MATERIALS AND METHODS

Seed germination phenotyping and genetic analysis were performed on 97 ($F_8$ seed and $F_7$ plants) RILs developed by modified single-seed-descent from a cross

between CMS-HA89 (elite, non-dormant B-line) and ANN1238 (a wild, dormant accession). The RILs were expected to segregate for embryo and seed covering dormancy and were developed from a population previously described by Burke et al. (2002).

Seed was produced at Corvallis, OR in the summer of 2004. In order to minimize seed after-ripening on the mother plant, all genotypes were harvested at physiological maturity, as defined by Schneiter and Miller (1981). Harvested seed was immediately dried at 39°C for 17 h. Seed was cleaned and stored in vacuum sealed KAPAK® bags, (model ASTM F-1249), at 4°C ± 2°C to decelerate after-ripening and lock out moisture. Seed stored at 4°C was used at 0 wks after-ripening and is referred to as non-after-ripened seed.

Seed was after-ripened (AR) for 4, 8, 16, or 24 wk at 20°C ± 2°C in a germination chamber. The after-ripening temperature of 20°C was chosen to simulate AR at room temperature. For the initial germination, time 0, seed was removed from 4°C and treatments begun immediately.

Seed was disinfected prior to imbibition by agitating in a 50/50 solution of sodium hypochlorite and water (3% v/v) for 90 s. The seed was then rinsed with distilled, de-ionized water to remove the bleach solution. At each ART, four replicates of 25 seed each were placed in Petri plates on blue blotter paper with five mls of distilled water. All Petri plates were kept in Ziploc® bags inside the germinator to decrease evaporation.

Seed was germinated at 25°C ± 2°C in a germination chamber with 12 h of light and dark periods. Germination data were collected up to 21 days. When the radical pierced the pericarp of the seed a seed was considered germinated. For the evaluation of embryos, at ART4, on day 22, ¼ of the blunt end of the seed was excised in order to remove the embryo from the seed covering (seed coat + pericarp) (by doing this we eliminated the seed covering dormancy and were able to evaluate the embryo alone). Twenty-five naked embryos from each RIL were placed on clean blotter paper with distilled water and evaluated for germination. Germination data of naked embryos were collected for three days. When the radical was visibly elongated on a naked embryo it was considered germinated.

**QTL Analysis**

Composite interval mapping method (CIM; Jansen and Stam, 1994; Zeng, 1994) was performed to detect putative QTL associated with the variations in seed dormancy as a mean of four replicates from a single environment and to estimate their effects. QTL analyses were carried out using PLABQTL Version 1.2 software (Utz and Melchinger, 1996), which combines interval mapping by the regression approach (Haley and Knott, 1992) and selected markers as cofactors. Initially, using the *first* statement and simple interval mapping method (SIM), an analysis was conducted to select the cofactors. Finally, CIM analysis was done by pre-selected cofactors and *cov* statement. Genome-wide threshold values ($\alpha = 0.05$) for declaring the presence of QTL were estimated from 1000 permutations (Churchill and Doerge, 1994; Doerge and Churchill, 1996). The QTL positions were determined at the maximum point of the LOD-curve plot in the region under consideration. The proportion of phenotypic variance explained by each individual QTL was calculated (Utz et al. 2000). QTL additive effects were estimated according to Falconer and Mackay (1996).

**Dependent Variables and Developmental Modeling of Seed Dormancy**

QTL analyses were performed on three dependent variables: (i) germination percentage of whole achenes (seeds) after-ripened for *x* days; (ii) germination percentage of naked embryos (seeds minus the pericarp and seed coat) after-ripened for 28 days; and (iii) delay of germination (DOG) of a specified percentage (*p*) of seeds after-ripened for *x* days ($DOG_p$), e.g., $DOG_{50}$ is the number of days of after-ripening needed for 50% of the seeds of an accession to germinate. $DOG_p$ variables were estimated for HA89, ANN1238, CMS-HA89 x ANN1238, ANN1238 x HA89, and 160 CMS-HA89 x ANN1238 RILs for $p = 25$, 50, and 75% using a generalized sigmoid-shaped logistic function (Richards 1959). Richards (1959) function [1] was hypothesized to accurately model changes in seed dormancy (seed germination) in sunflower as a function of after-ripening time:

$$G = a + \frac{(h - a)}{(1 + te^{-b(x-m)})^{\frac{1}{t}}} \quad [1]$$

where *G* is the observed germination percentage, *a* is the lower asymptote, *h* is the upper asymptote, *b* is the gradient (non-linear rate of change), *m* is the time of maximum gradient, *t* is the shape parameter, and *x* is after-ripening time (days). When $a = 0$, the lower asymptote was equal to 0% germination and when $h = 1$, the upper asymptote was equal to 100% germination (the natural lower and upper limits for seed germination). The shape parameter *t* controls the symmetry (lopsidedness) of the sigmoidal curve. The curve is symmetric when $t = 1$. Setting $G = p$ and rearranging, the delay of germination in sunflower was predicted from solutions of:

$$DOGp = m - \ln \left[ \frac{\left(\frac{p - a}{h - a}\right)^{-t} - 1}{t} \right] / b \quad [2]$$

$DOG_p$ was estimated from the original dependent variables (germination percentages for four replications/RIL/after-ripening time) by setting $a = 0$, $h = 1$, and $t = 1$, finding non-linear regression solutions for *m* and *b*, and predicting delay of germination ($DOG_p$) percentages for $p = 25$, 50, or 75%. Non-linear regression analyses were performed using PROC NLIN, the Statistical Analysis System (SAS, Cary, NC) non-linear regression program (Appendix A).

## RESULTS

### Genetic Background of the Domesticated x Wild (HA89 x ANN1238) RIL Mapping Population

Recombinant inbred lines (RILs) were developed by modified single seed descent from a hybrid between CMS-HA89 and ANN1238; multiple $F_3$ to $F_6$ individuals were bulked within each lineage (tracing to a single $F_2$) and a single $F_7$ individual was harvested to produce $F_8$ (RIL) seed for phenotyping. CMS-HA89, a cytoplasmic-genic male-sterile (A) inbred line, is near-isogenic to HA89, an elite self-compatible sterility maintainer (B) inbred line. ANN1238 is a weakly self-compatible wild population (Burke et al. 2002). Self-compatibility was absent or sufficiently weak in each lineage to enable self-pollination. Bulking seeds within lineages through the $F_6$ generation ensured the survival of most lineages, many of which were severely depressed by inbreeding. Selfed HA89 and sibbed ANN1238 seeds were phenotyped for seed dormancy.

CMS-HA89 was previously found to have early or short-term embryo dormancy and minimal pericarp dormancy, whereas ANN1238 was previously found to have minimal embryo dormancy and prolonged pericarp dormancy (chapter one). These phenotypes were confirmed in the present study (Table 6.1). Because seed dormancy is more pronounced and prolonged in wild germplasm (Seiler, 1998; Heiser, 1969; Snow et al. 1998), the discovery of a self-compatible wild population displaying seed dormancy was essential for developing inbred progenies and producing seed on the scale necessary for replicated time-course analyses of seed dormancy. RIL seed production was difficult in many lineages because of inbreeding depression. CMS-HA89 x ANN1238 progeny segregated for numerous morphological and developmental traits and, with succeeding generations of inbreeding, displayed successively greater inbreeding depression, primarily manifested through decreased plant height, biomass, seed yield, and fecundity. Genetic loads of HA89 and ANN1238 were predicted to be greatly different. HA89 has excellent fecundity and productivity and, as such, became a commercially important female inbred line and was an founder of numerous commercially important female inbred lines. The wild parent was outbred and presumed to have a much greater genetic load than CMS-HA89, a presumption born out by phenotypes of the RILs, most of which

had lower fecundity and biomass than HA89. Seeds for the dormancy study were produced by manually selfing bagged inflorescences of multiple plants of the parents and RILs. We produced 25 to 2000 seeds/plant among the RILs, 2000 seeds/plant of ANN1238, and 600 seeds/plant of HA89.

**Genetic Mapping in HA89 x ANN1238**

Previously mapped SSR markers were screened for polymorphisms between the parents of the RIL population to identify a genome-wide framework of SSR markers spanning each of the 17 previously identified linkage groups (x = 17) (Burke et al. 2002; Tang et al. 2002; Yu et al. 2003; Gandhi et al. 2005); 110 SSR markers were genotyped in the RIL mapping population, supplied loci dispersed throughout the genome, and assembled into 17 linkage groups spanning 1,019.8 cM (Figure 6.1). SSR loci supplied nearly complete genome coverage in the HA89 x ANN1238 RIL mapping population and the 17 linkage groups were concordant with previously identified linkage groups (Burke et al. 2002; Tang et al. 2002; Yu et al. 2003; Gandhi et al. 2005). Recombination was lower in the HA89 x ANN1238 RIL mapping population than elite x elite reference mapping populations (e.g., 1,566.7 cM in RHA280 x RHA801), both in the present study (Figure 6.1) and in an earlier study with HA89 x ANN1238 $F_2$ progeny (Burke et al. 2002). Recombination was similarly suppressed in a wild x primitive land race (ANN1238 x Hopi) $F_2$ mapping population (Wills and Burke 2007). The short length (reduced recombination), while decreasing mapping resolution, did not affect genome coverage.

**Seed Dormancy Developmental Profiles for Freshly Harvested and After-Ripened Seeds of Elite Inbred Lines, Wild Populations, and Elite x Wild Hybrids**

Seed dormancy among elite inbred lines can be highly variable. For example, whole seeds of RHA373 and HA89 will not germinate readily as non-after-ripened seed. The seed coat (primarily), and pericarp was found to control dormancy in RHA373 but when the seed coat and pericarp were removed the embryo germinated even when non-after-ripened. In contrast, removing the seed covering of HA89 did not increase germination at

the same after-ripening time (chapter one). Similar results were found in the present study (Figure 6.2). Whole achenes of the RILs would not germinate after four weeks of after-ripening; however, when the seed coat and pericarp were removed most RILs germinated. Germination of wild types of *H. annuus, H. argophyllus* and *H. exilis* was nearly or entirely controlled by the seed covering. Germination of wild types of whole achenes seldom exceeds 1-2% (chapter one; chapter three). Embryo dormancy was variable among wild *H. annuus* accessions and all accessions significantly increased in germination when the seed covering was removed (chapter three). In the present study, germination of hybrids was also tested. Germination of whole achenes of HA89 x ANN1238 was low throughout the entire study; while, germination of embryos was low initially, it increased in the later after-ripening times. Surprisingly, whole achenes of ANN1238 x HA89 germinated but the level of germination remained low (0-6%) throughout the study. Naked embryos of ANN1238 x HA89 germinated more similarly to naked embryos of ANN1238. Snow et al. (1998) found similar results; wild-crop hybrids had greater germination than the wild types.

DOG variables were used to eliminate the problems associated with zero germination rates of whole achenes, which confound QTL analyses, and were found to be strongly correlated (Figure 6.3).

**DISCUSSION**

The QTL analyses described here builds a foundation for more in-depth analyses of the genetics of seed dormancy in sunflower and identifies QTL underlying differences in seed dormancy between domesticated and wild sunflower genotypes. One of the peculiar outcomes of the present study was the apparent absence of embryo dormancy in the wild parent (ANN1238), in contrast to the presence of short-term embryo dormancy in the domesticated parent (HA89). These two components of dormancy had been previously investigated (chapter one). Short-term embryo and long lived pericarp dormancies have been widely observed and complicates rapid cycling of generations in breeding programs (Heiser, 1969; Chandler and Jan, 1985; Seiler, 1998; chapter one; chapter three). HA89 has been widely used in commercial breeding programs and has undoubtedly been one of

the most important sources of embryo dormancy alleles found in modern elite inbred lines. Still, embryo dormancy is a comparatively small component of seed dormancy in sunflower (chapter one; chapter three). Our analyses focused on pericarp dormancy, which seems to be the primary and perhaps only component of long-term seed dormancy (chapter one; chapter three). The embryo dormancy found in both elite and wild genotypes seems to be short-lived (chapter one). The embryo and pericarp components of seed dormancy can only be teased apart by comparing germination of whole achenes (seeds) and seeds lacking the pericarp, seed coat, or both (embryos). Whole achene analyses are straightforward and can be done on a large scale, whereas embryo only analyses require manually separating the pericarp, seed coat, or both from the embryo, which is difficult or virtually impossible in most elite oilseed and wild genotypes. We mapped embryo dormancy QTL at the earliest time point only because embryo dormancy had disappeared by the second time point in the wild parent (chapter one; Table 6.1; Figure 6.1).

Because of the challenges of producing inbred progenies and large seed samples from inbred elite x wild hybrid progeny, and attendant complications posed by inbreeding depression, other strategies are needed for forward genetic analyses of seed dormancy in sunflower and, more specifically, for increasing genetic resolution. Three strategies have promise: the development and analysis of wild introgression lines (WILs); the development and analysis of intermated elite x wild progenies; and association mapping using elite (non-dormant) and wild (dormant) germplasm (Brouwer and St. Clair, 2004; Frary et al. 2004; Fridman et al. 2004; Gur and Zamir, 2004; Goodstal et al. 2005; Liu and Burke, 2006; Schauer et al. 2006; Liu and Burke, 2006; Kolkman et al. 2007).

Wild introgression lines (WILs) could be developed by targeting individual segments dispersed throughout the genome or multiple segments harboring putative seed dormancy QTL identified by low-resolution QTL mapping (Gandhi et al. 2005; Wills and Burke, 2006; Figure 6.4). The former (whole genome) WIL strategy focuses on a single segment per WIL, which has the advantage of masking deleterious pleiotropic and epistatic effects produced by the wild genetic background (Frary et al. 2004; Gur and Zamir, 2004). Conversely, the effects of individual segments (QTL) may not accurately recapitulate the

seed dormancy syndrome, which is apparently controlled by several QTL with comparatively small effects in sunflower (Table 6.2; Figure 6.4). Nevertheless, single segment WILs should be powerful for validating individual seed dormancy QTL.

Because the sequential assembly of multiple segment WILs, using QTL identified through the analysis of individual segment WILs as a guide, is laborious and time-consuming, a more rapid and efficient strategy would be to assemble WILs carrying multiple wild segments predicted to harbor seed dormancy QTL. Selection of false-positive QTL (wild segments) should not detract from the goal of validating and cross-validating QTL identified by low-resolution analyses (Figure 6.4), but does increase the number of progeny needed to identify the recombinants and construct multiple segment WILs (Frisch and Melchinger, 2005). Most domestication traits in sunflower have a complex architecture (Burke et al. 2002; Gandhi et al. 2005; Wills and Burke, 2007), in contrast to many other crop species where a comparatively small number of loci with large effects have been identified for important domestication traits (Liu et al. 2007; Lee et al. 2005).

QTL mapping resolution can be greatly increased by using inbred progenies produced from intermated biparental hybrid populations (Falque 2005; Lee et al. 2002; Falke et al. 2006; Fu et al. 2006; Szalma et al. 2007). We are presently developing an intermated $S_1$ population from an elite x wild hybrid using a self-compatible inbred line (ANN1238-$S_6$) developed from the ANN1238 population. Selfing outbred individuals in an advanced intermated generation (e.g., $\geq I_4$ where $I_1 = F_1$) should yield sufficient seed per $S_1$ line for seed dormancy analyses, skirt the inbreeding depression problem, enable the development and testing of a much larger number of progenies ($S_1$ lines), and increase the power and resolution of QTL analyses three-fold compared to an $F_2$ or RIL analysis, e.g., the analysis of 400 $I_4$-$S_1$ lines is predicted to be 12-fold more powerful than the analysis of 100 RILs, as was done in the present study (Lynch and Walsh, 2000).

## CONCLUSIONS

The parents showed extreme phenotypes for seed germination which necessitated the discovery of seed dormancy QTL. Forty-three QTL were identified over five ARTs for both seed dormancy and embryo dormancy which explained between 8.9-30.4 and 11.6-23.1%, respectively, of the phenotypic variation observed and 12 DOG QTL explained between 10.9-33.7% of the phenotypic variation observed. Multiple QTL were discovered across several ARTs on each of linkage groups 4, 11, 12, 15, 16 and 17 which demonstrates the presence of important clusters of seed dormancy loci in these regions. The seed dormancy QTL discovered on linkage groups 11 and 15 are important because another study by Gandhi et al. (2005) found QTL in these same regions in a $BC_1$ elite x wild population. The majority of favorable alleles for seed dormancy were contributed by the wild parent which is consistent with other QTL studies in sunflower when elite x wild populations are studied.

This is the first study that we are aware of that separates embryo dormancy from seed covering dormancy in sunflower and to find QTL associated with each. More seed covering dormancy QTL were discovered (24) than were QTL associated with embryo dormancy (seven). This should not be surprising since the seed covering controls seed germination long after the embryo is no longer dormant. The number and magnitude of the QTL discovered demonstrate the sheer complexity of seed dormancy in sunflower. The discovery of these seed dormancy QTL provides a great deal of information about seed dormancy in sunflower, but further research is required to further refine the locations of the loci and to understand the cascading effects of seed dormancy genetics.

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Table 6.1. Germination percentages of sunflower seeds of HA89, ANN1238 and hybrids after-ripened for 0 to 24 weeks. Treatments are whole seed or embryos; for evaluation of embryos, seed coat and pericarp were removed (NOPSC).

| Whole Seed | | | | Embryo (NOPSC) | | | |
|---|---|---|---|---|---|---|---|
| Genotype | ART[a] | % Germination | SE | Genotype | ART | % Germination | SE |
| HA89 | 0 | 0 | 0 | HA89 | 0 | 0 | 0 |
| | 4 | 0 | 0 | | 4 | 0 | 0 |
| | 8 | 41 | 2 | | 8 | 70 | 7 |
| | 16 | 56 | 4 | | 16 | 100 | 0 |
| | 24 | 61 | 20 | | 24 | 100 | 0 |
| ANN1238 | 0 | 0 | 0 | ANN1238 | 0 | 45 | 7 |
| | 4 | 0 | 0 | | 4 | 68 | 10 |
| | 8 | 0 | 0 | | 8 | 63 | 5 |
| | 16 | 0 | 0 | | 16 | 90 | 6 |
| | 24 | 0 | 0 | | 24 | 99 | 1 |
| 89x1238 | 0 | 0 | 0 | 89x1238 | 0 | 4 | 4 |
| | 4 | 0 | 0 | | 4 | 0 | 0 |
| | 8 | 6 | 2 | | 8 | 78 | 9 |
| | 16 | 10 | 9 | | 16 | 100 | 0 |
| | 24 | 11 | 6 | | 24 | 50 | 7 |
| 1238x89 | 0 | 0 | 0 | 1238x89 | 0 | 6 | 2 |
| | 4 | 0 | 0 | | 4 | 89 | 4 |
| | 8 | 6 | 2 | | 8 | 92 | 4 |
| | 16 | 1 | 1 | | 16 | 93 | 7 |
| | 24 | 4 | 3 | | 24 | 85 | 3 |

[a]ART is after-ripening time (weeks)

Fig. 6.1. Genetic linkage map of HA89 x ANN1238 recombinant inbred line sunflower mapping population.

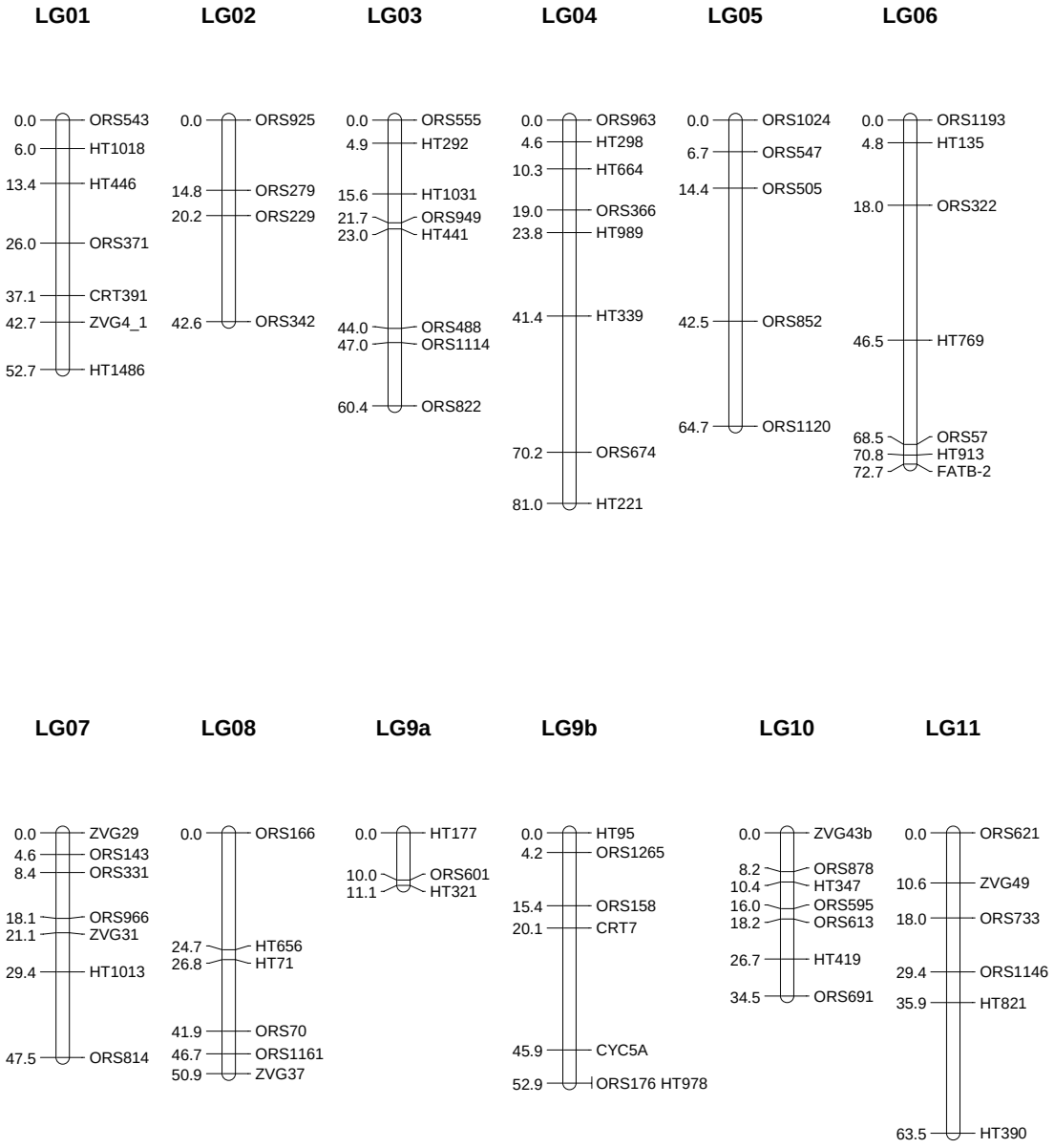


Fig. 6.1 (continued). Genetic linkage map of HA89 x ANN1238 recombinant inbred line sunflower mapping population.

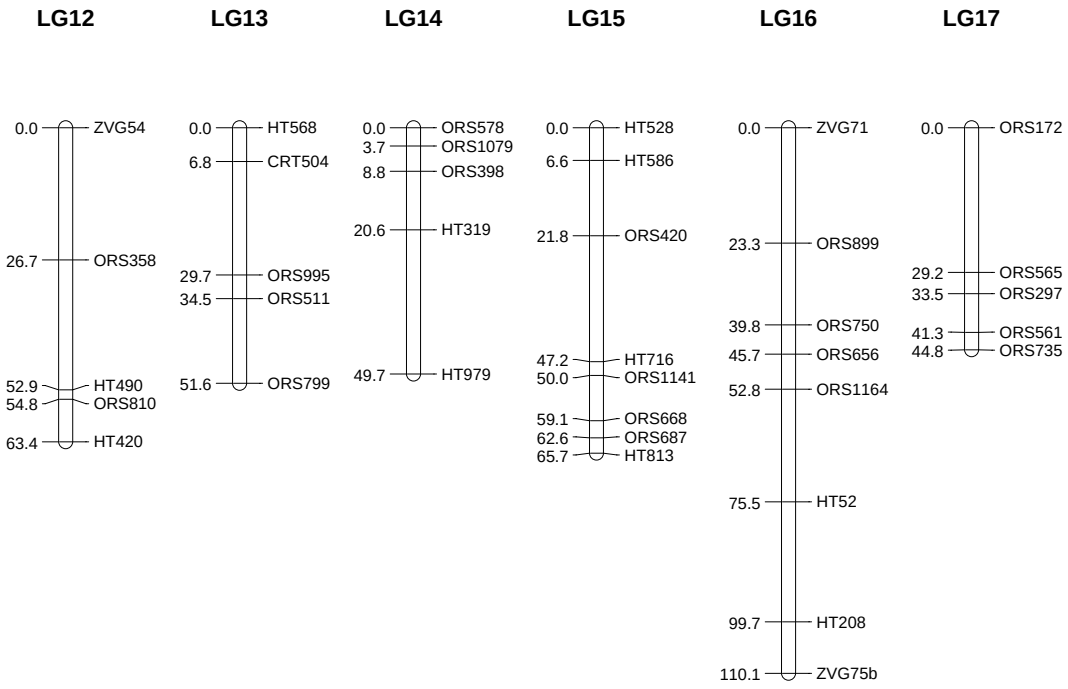
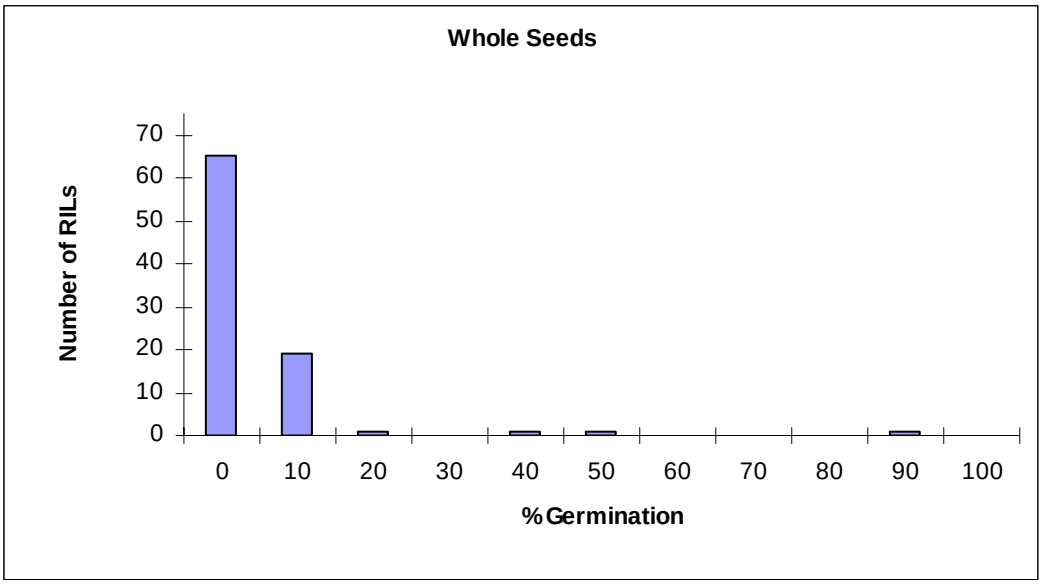


Fig. 6.2. Percent germination of sunflower seeds at four weeks after-ripening of the 97 RILs in the HA89 x ANN1328 mapping population. Treatments are whole achenes (seeds) or naked embryos.



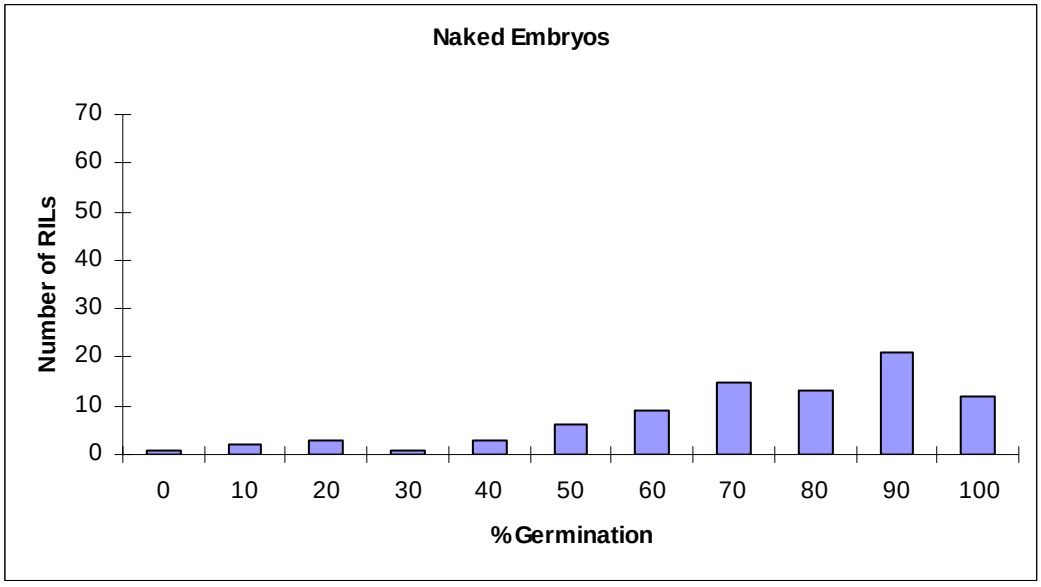
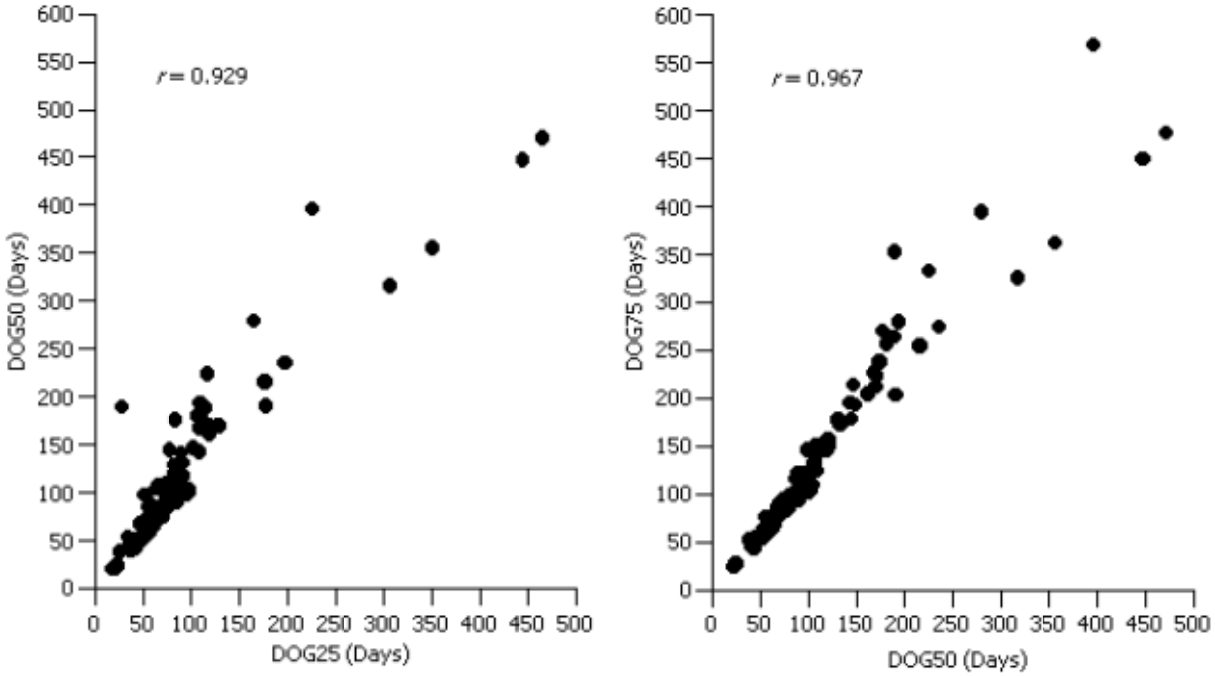



Fig. 6.3. Scatter plot graphs showing correlation of delay of germination (DOG) percentage variables for HA89 x ANN1238 RIL sunflower mapping population at 25, 50 and 75% germination.



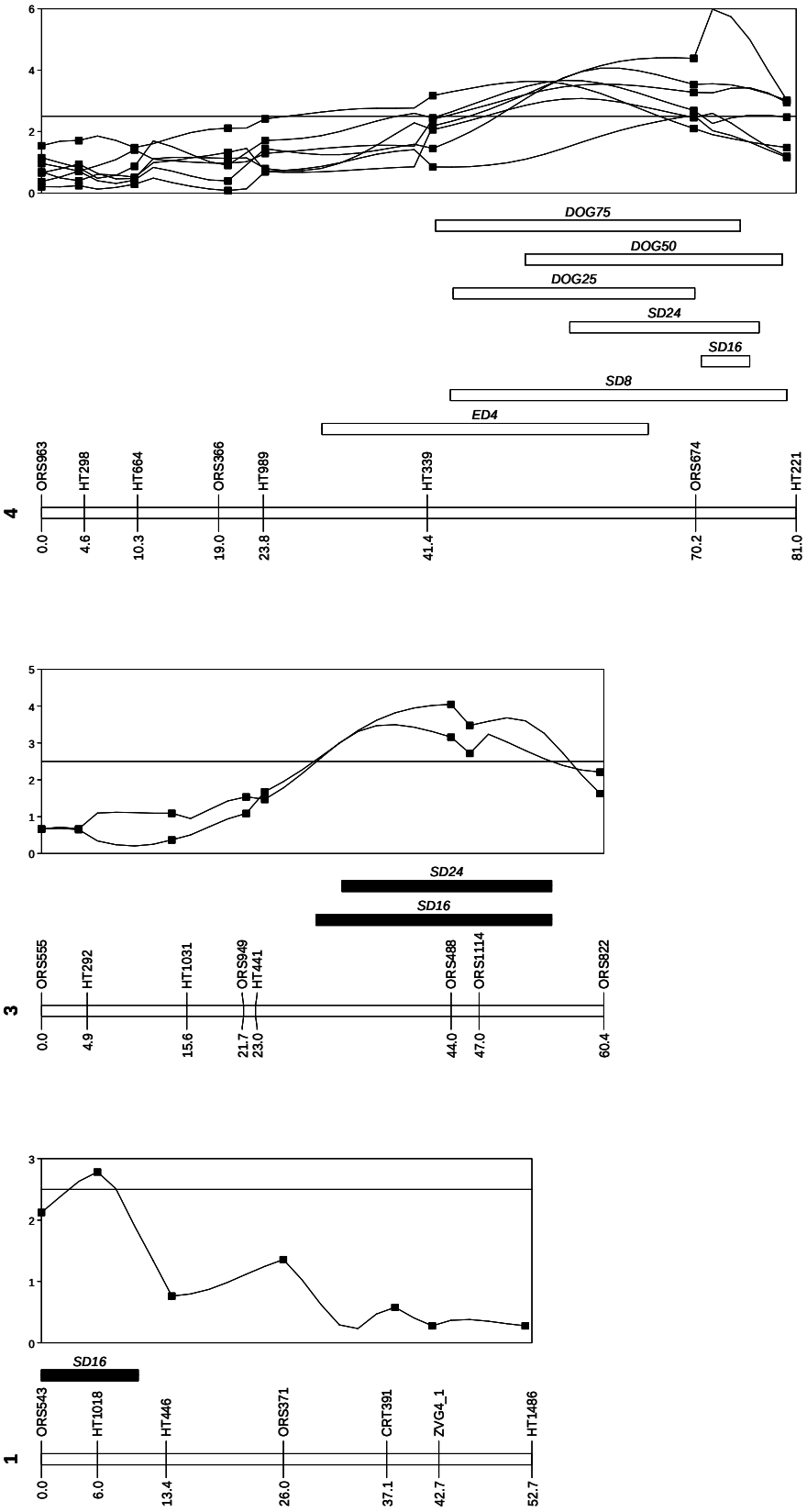



Fig. 6.4 Quantitative trait loci for seed dormancy (SD), embryo dormancy (ED) and delay of germination (DOG) (vertical bars are 1.0 LOD support intervals) identified by composite interval mapping in the HA89 x ANN1238 RIL sunflower mapping population.

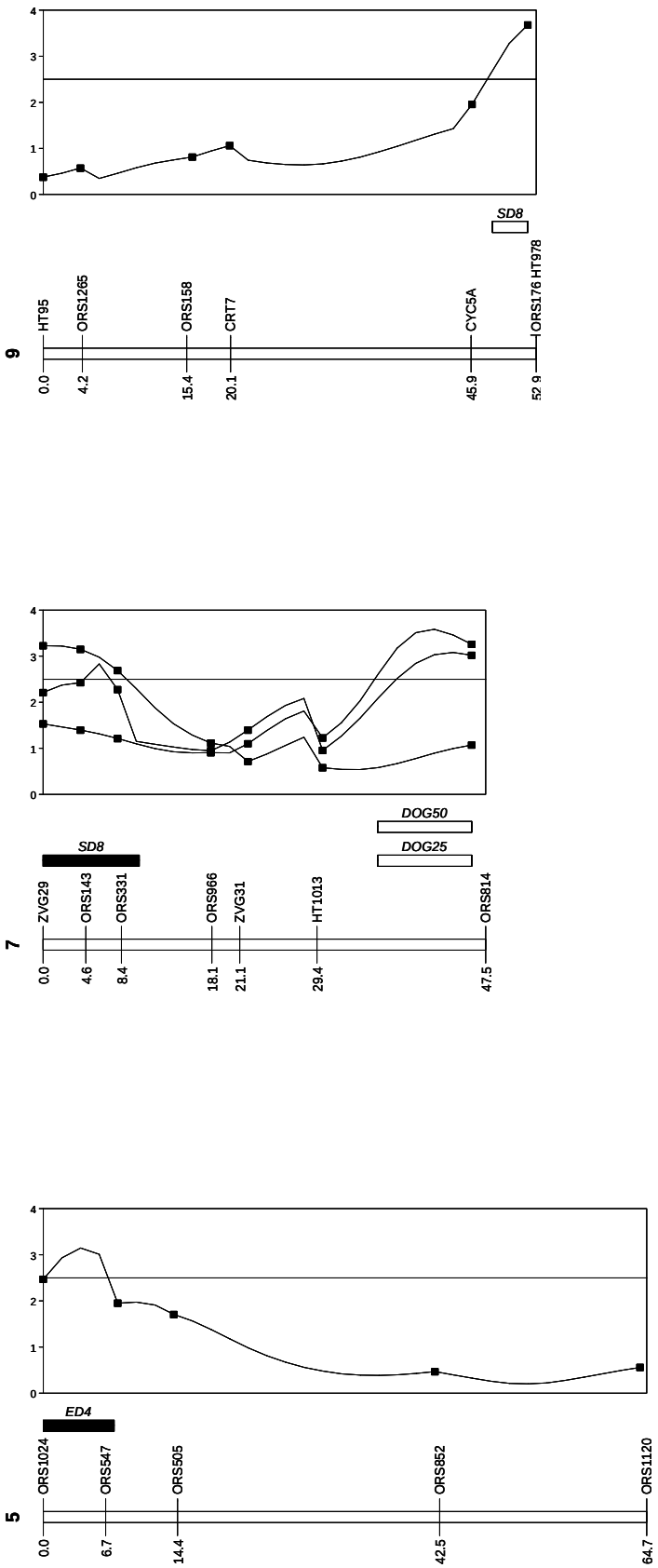



Fig. 6.4 (continued) Quantitative trait loci for seed dormancy (SD), embryo dormancy (ED) and delay of germination (DOG) (vertical bars are 1.0 LOD support intervals) identified by composite interval mapping in the HA89 x ANN1238 RIL sunflower mapping population.

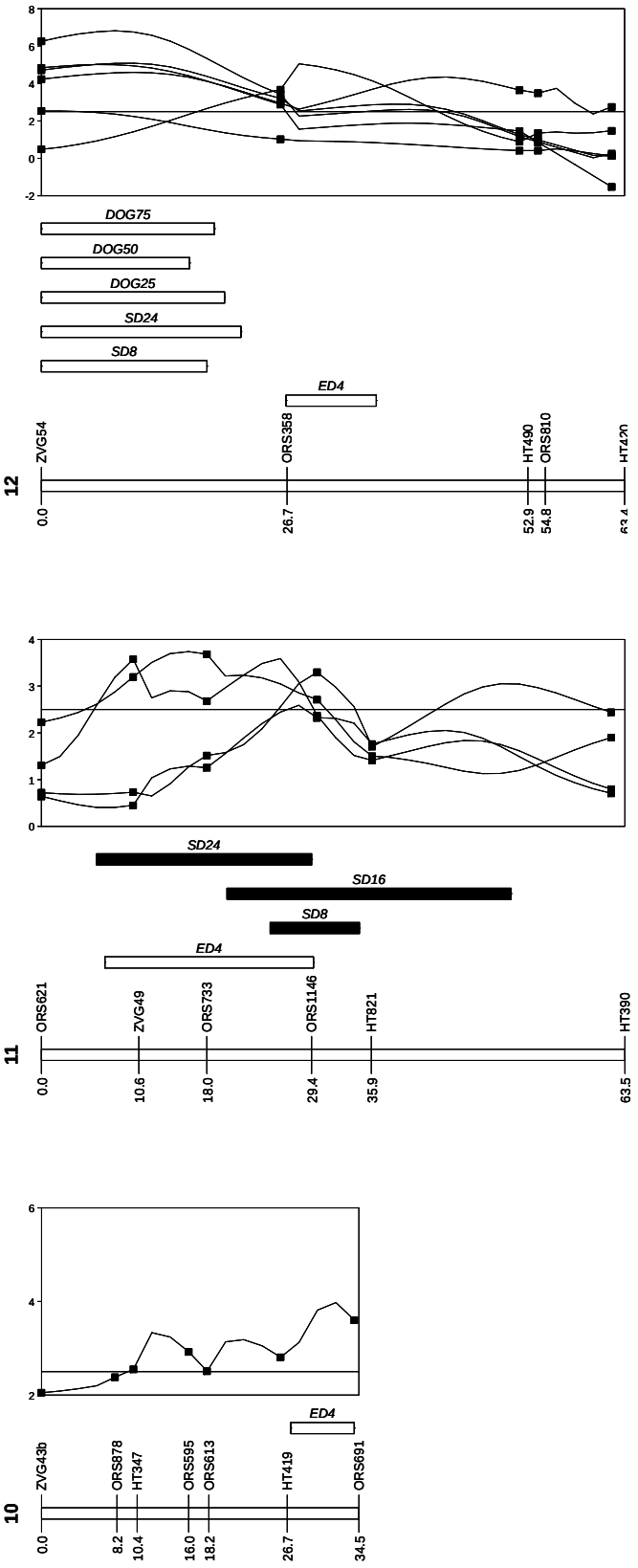



Fig. 6.4 (continued) Quantitative trait loci for seed dormancy (SD), embryo dormancy (ED) and delay of germination (DOG) (vertical bars are 1.0 LOD support intervals) identified by composite interval mapping in the HA89 x ANN1238 RIL sunflower mapping population.

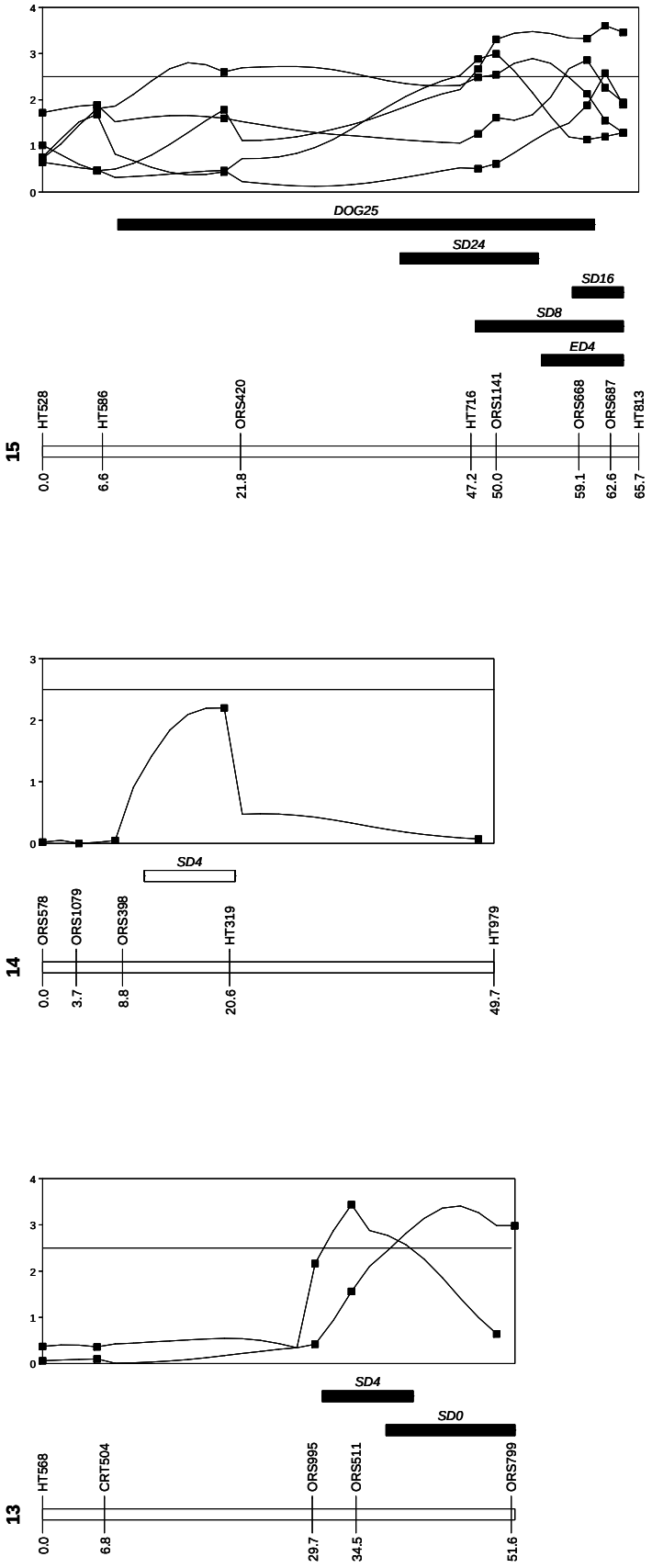



Fig. 6.4 (continued) Quantitative trait loci for seed dormancy (SD), embryo dormancy (ED) and delay of germination (DOG) (vertical bars are 1.0 LOD support intervals) identified by composite interval mapping in the HA89 x ANN1238 RIL sunflower mapping population.

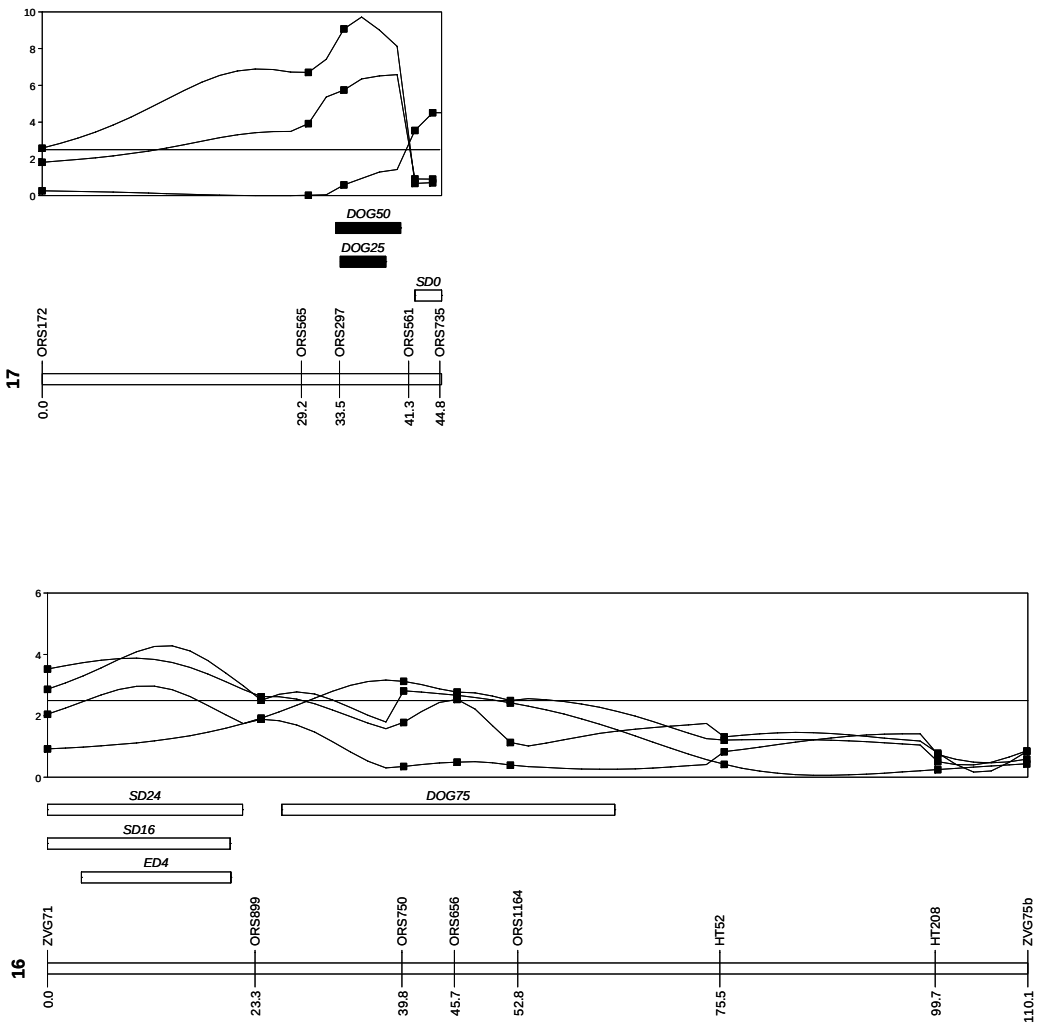



Fig. 6.4. (continued) Quantitative trait loci for seed dormancy (SD), embryo dormancy (ED), and delay of germination (DOG) (vertical bars are 1.0 LOD support intervals) identified by composite interval mapping in the HA89 x ANN1238 RIL sunflower mapping population.

Table 6.2. Seed dormancy (SD), embryo dormancy (ED) and delay of germination (DOG) percentage (p) QTL for HA89 x ANN1238 RIL sunflower mapping population.

| Trait[a] | Linkage Group | Position (cM)[b] | Closest DNA Marker Locus[c] | DNA Marker Interval | LOD | $R^2$ | Additive Effect[d] | Additive Effect Units | Favorable Allele Source[e] |
|---|---|---|---|---|---|---|---|---|---|
| **$SD_{16}$** | 1 | 6 | ORS543 | ORS543, HT1018 | 2.8 | 14.8 | 3.1 | % | Elite |
| **$SD_{16}$** | 3 | 38 | ORS448 | HT441, ORS488 | 3.5 | 15.3 | 14.3 | % | Elite |
| **$SD_{24}$** | 3 | 44 | ORS488 | HT441, ORS488 | 4.1 | 18.0 | 13.6 | % | Elite |
| **$DOG_{25}$** | 4 | 56 | ORS674 | HT339, ORS674 | 3.7 | 13.9 | 3.7 | Days | Wild |
| **$DOG_{50}$** | 4 | 62 | ORS674 | HT339, ORS674 | 4.1 | 15.0 | 4.1 | Days | Wild |
| **$DOG_{75}$** | 4 | 58 | ORS674 | HT339, ORS674 | 3.1 | 11.6 | 4.1 | Days | Wild |
| **$ED_4$** | 4 | 52 | ORS674 | HT339, ORS674 | 3.6 | 15.0 | -10.6 | % | Wild |
| **$SD_{16}$** | 4 | 72 | ORS674 | ORS674, HT221 | 6.0 | 30.4 | -15.4 | % | Wild |
| **$SD_{24}$** | 4 | 70 | ORS674 | HT339, ORS674 | 2.5 | 12.2 | -10.1 | % | Wild |
| **$SD_8$** | 4 | 60 | ORS674 | HT339, ORS674 | 3.5 | 14.5 | -20.8 | % | Wild |
| **$ED_4$** | 5 | 4 | ORS1024 | ORS1024, ORS547 | 3.2 | 13.4 | 8.7 | % | Elite |
| **$DOG_{25}$** | 7 | 44 | ORS814 | HT10131, ORS814 | 3.1 | 15.7 | 4.2 | Days | Wild |
| **$DOG_{50}$** | 7 | 42 | ORS814 | HT10131, ORS814 | 3.6 | 17.6 | 5.4 | Days | Wild |
| **$SD_8$** | 7 | 0 | ZVG29 | ZVG29, ORS143 | 3.2 | 20.4 | 14.7 | % | Elite |
| **$SD_8$** | 9 | 52 | HT978 | CYC5A, HT978 | 3.7 | 16.2 | -39.5 | % | Wild |
| **$ED_4$** | 10 | 32 | ORS691 | ORS878, ORS691 | 4.0 | 20.5 | -15.5 | % | Wild |
| **$ED_4$** | 11 | 16 | ORS1146 | ORS621, ORS733 | 3.7 | 17.4 | -12.6 | % | Wild |
| **$SD_{16}$** | 11 | 28 | ORS1146 | ORS733, ORS1146 | 2.6 | 11.7 | 19.4 | % | Elite |
| **$SD_{24}$** | 11 | 26 | ORS1146 | ZVG49, ORS1146 | 3.6 | 16.3 | 11.0 | % | Elite |
| **$SD_8$** | 11 | 30 | ORS1146 | ORS1146, HT821 | 3.3 | 13.1 | 20.0 | % | Elite |
| **$SD_8$** | 11 | 50 | HT390 | HT821, HT390 | 3.1 | 13.1 | 17.2 | % | Elite |
| **$DOG_{25}$** | 12 | 10 | ZVG54 | ZVG54, ORS358 | 5.1 | 23.2 | 4.0 | Days | Wild |
| **$DOG_{50}$** | 12 | 8 | ZVG54 | ZVG54, ORS358 | 6.8 | 29.4 | 6.7 | Days | Wild |
| **$DOG_{75}$** | 12 | 6 | ZVG54 | ZVG54, ORS358 | 5.0 | 21.8 | 4.8 | Days | Wild |
| **$ED_4$** | 12 | 28 | ORS358 | ORS358, HT490 | 5.1 | 20.4 | -14.2 | % | Wild |
| **$SD_{24}$** | 12 | 10 | ZVG54 | ZVG54, ORS358 | 4.6 | 24.6 | -8.2 | % | Wild |
| **$SD_{24}$** | 12 | 44 | ORS810 | ORS810, HT420 | 4.3 | 19.9 | -7.7 | % | Wild |
| **$SD_8$** | 12 | 0 | ZVG54 | ZVG54, ORS358 | 2.5 | 12.4 | -17.4 | % | Wild |

Table 6.2 (continued). Seed dormancy (SD), embryo dormancy (ED) and delay of germination (DOG) percentage (p) QTL for HA89 x ANN1238 RIL sunflower mapping population.

| Trait[a] | Linkage Group | Position (cM)[b] | Closest DNA Marker Locus[c] | DNA Marker Interval | LOD | $R^2$ | Additive Effect[d] | Additive Effect Units | Favorable Allele Source[e] |
|---|---|---|---|---|---|---|---|---|---|
| **$SD_0$** | 13 | 46 | ORS799 | ORS511, ORS799 | 3.4 | 14.8 | 3.3 | % | Elite |
| **$SD_4$** | 13 | 34 | ORS511 | ORS995, ORS511 | 3.4 | 13.7 | 17.1 | % | Elite |
| **$SD_4$** | 14 | 20 | HT319 | ORS398, HT913 | 2.2 | 8.9 | -8.6 | % | Wild |
| **$DOG_{25}$** | 15 | 54 | ORS1141 | ORS1141, ORS687 | 2.9 | 10.9 | -1.3 | Days | Elite |
| **$ED_4$** | 15 | 60 | ORS668 | ORS668, ORS687 | 2.9 | 11.6 | 15.6 | % | Elite |
| **$SD_{16}$** | 15 | 62 | ORS687 | ORS668, ORS687 | 2.6 | 11.6 | 17.2 | % | Elite |
| **$SD_{24}$** | 15 | 50 | ORS1141 | HT716, ORS668 | 3.0 | 13.8 | 9.6 | % | Elite |
| **$SD_8$** | 15 | 62 | ORS1141 | ORS1141, ORS687 | 3.6 | 14.4 | 25.1 | % | Elite |
| **$DOG_{75}$** | 16 | 38 | ORS899 | ORS899, ORS750 | 3.2 | 12.6 | 3.7 | Days | Wild |
| **$ED_4$** | 16 | 14 | ZVG71 | ZVG71, ORS899 | 4.3 | 23.1 | -11.5 | % | Wild |
| **$SD_{16}$** | 16 | 12 | ORS899 | ZVG71, ORS899 | 3.0 | 18.2 | -15.8 | % | Wild |
| **$SD_{24}$** | 16 | 10 | ORS899 | ZVG71, ORS899 | 3.9 | 23.4 | -21.1 | % | Wild |
| **$DOG_{25}$** | 17 | 36 | ORS297 | ORS297, ORS561 | 9.7 | 33.7 | -7.8 | Days | Elite |
| **$DOG_{50}$** | 17 | 40 | ORS297 | ORS297, ORS561 | 6.6 | 24.1 | -5.2 | Days | Elite |
| **$SD_0$** | 17 | 44 | ORS735 | ORS561, ORS735 | 4.5 | 18.8 | -5.7 | % | Wild |

[a]Numerical suffixes attached to embryo dormancy (ED) and seed dormancy (SD) variable identify the number of weeks of after-ripeing. Numerical suffixes attached to the delay of germination (DOG) variable identify the germination percentage (p) threshold selected for estimating DOG.

[b]Genetic distance (cM) from the upper end of the linkage group (Tang et al. 2002).

[c]DNA marker closest to the QTL LOD peak.

[d]The additive effect = (HA89 - ANN1238)/2, where HA89 is the homozygote mean for the elite line and ANN1238 is the homozygote mean for the wild population.

[e]Favorable alleles decreased seed dormancy; hence additive effects for embryo dormancy (ED) or seed dormancy (SD) were positive if the elite (HA89) allele decreased dormancy (increased germination), whereas additive effects for delay of germiation (DOG) were negative if the elite allele decreased dormancy (reduced DOG)

# CHAPTER 7

## GENERAL CONCLUSION

The results of these studies indicate that sunflower seed dormancy in wild types is not as difficult to overcome as originally anticipated. The diversity of germplasm tested in these studies has provided new insight to the causes behind sunflower seed dormancy and the differences between domesticated (elite) and wild sunflower accessions.

The type of seed dormancy (embryo and seed covering) of elite sunflowers varies by both type and longevity among cultivars. The Native American Landraces (NAL) germinated more like the elite lines when compared to the elite and wild groups. For the elite and NAL groups, dormancy was primarily controlled by the embryo, which faded quickly in dry storage, and had only low to moderate seed covering dormancy. In the elite group the seed coat contributed to dormancy at different levels depending on the variety. Embryos of both elite lines and NALs germinated readily after four to eight weeks of harvest. Removing the seed covering on non-after-ripened seed of elite lines increased germination of some lines but not others. There were differences in the degree of embryo dormancy among elite lines and it was determined to be genotype specific. In general, removing the seed covering on NALs provided a large germination advantage on non-after-ripened seed and a small advantage when the seed had after-ripened for four to eight weeks.

Seed after-ripening did not effect germination of the wild accessions, excluding ANN1811. ANN1811 always showed positive increases in germination when after-ripened. Seed dormancy of *H. annuus* and, *H. argophyllus* was primarily controlled by the seed covering, while seed dormancy of *H. exilis* was controlled almost entirely by the seed covering. The seed coat itself controlled a large amount of seed dormancy in *H. annuus* ANN1238. Although ANN1238 was the only wild accession tested for seed coat dormancy it is likely that dormancy of wild accessions in general is largely controlled by the seed coat. When seed of wild *H. annuus* accessions had not been after-ripened and the seed covering was removed, embryos germinated, at a rate of 10-100%. This range in germination shows the diversity of embryo dormancy among the wild *H. annuus* accessions. Many accessions of wild *H. annuus* had moderate embryo dormancy even

when after-ripened for up to 16 weeks. A moderate percentage of naked embryos of all wild *H. annuus* accessions could be germinated at any after-ripening time throughout all of the studies. For the wild *H. annuus* accessions, latitude effected germination. Whole seed of the most southern accessions would germinate when whole seed from more northern accessions would not germinate. However, seed covering effected germination more so than latitude because most accessions would not germinate unless the seed covering was removed. The seed covering completely suppressed germination up to 16 weeks after-ripening (excluding the most southern accessions), almost equally, in the *H. annuus* accessions when they were grown at a common latitude.

Forty-three seed dormancy QTL were discovered in the recombinant inbred line mapping population HA89 x ANN1238. The vast number of QTL discovered for whole seed dormancy (24), embryo dormancy (7) and delay of germination (DOG) (12) allow the further understanding of the complexity of seed dormancy in sunflowers. Multiple QTL were associated with linkage groups 4, 11, 12, 15, 16 and 17 over the time course of five after-ripening times which indicate locations of important clusters of seed dormancy loci. The seed dormancy QTL discovered on linkage groups 11 and 15 are important because another study by Gandhi et al. (2005) found QTL in this same region in a $BC_1$ elite x wild population. The majority of favorable alleles for seed dormancy were contributed by the wild parent which is consistent with other QTL studies in sunflower when elite x wild populations are studied. The discovery of these seed dormancy QTL is a good starting point, but further research is needed to refine the locations of the loci and to understand the cascading effects of seed dormancy genetics.

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Appendix A. SAS PROC NLIN program developed for predicting the number of days of afterripnening needed for 50% germination of seeds of recombinant inbred lines.

```
data a;
input ril repl artime nseed ngerm;
i=1;
cards;
data b;
set a;
opgerm = ngerm/nseed;
proc sort; by ril artime;
data c;
do ril=0 to 229;
do artime=0 to 50 by .5;
xpgerm=.;
output; end; end;
proc sort; by ril artime;
data d; merge b c; by ril artime;
pgerm=sum(opgerm, xpgerm);
proc sort; by ril; /*
proc gplot data=c; by ril;title1 'Plot of Data';
symbol1 i=hilojoin v=dot c=cyan w=3;
plot pgerm*artime=1; */
proc nlmixed data=d; by ril;title1 'N Lin Analysis';
parms m=10 b=.4; s2e=1; t=1; h=1; a=0;
mu = a+((h-a)*(1+t*exp(-b*(artime-m)))**(-1/t));
model pgerm ~ normal(mu,s2e);
estimate 'DOG 50' m-log((((.5-a)/(h-a))**(-t)-1)/t)/b;
predict h*(1+t*exp(-b*(artime-m)))**(-1/t) out=d;
ods output additionalestimates=e;
proc print data=e;
proc gplot data=d; by ril;title1 'N Lin Analysis';
symbol1 i=join v=none c=red w=3;
symbol2 i=none v=dot c=blue w=3 ;
plot pgerm*artime=2 pred*artime=1 / overlay vaxis=0 to 1 by .1
haxis=0 to 50 by 10;
PROC EXPORT DATA= WORK.D
OUTFILE= "C:\DOG50.xls"
DBMS=EXCEL REPLACE;
SHEET="predicted values";
PROC EXPORT DATA= WORK.e
OUTFILE= "C:\DOG50.xls"
DBMS=EXCEL REPLACE;
SHEET="DOG 50 values";
run;
quit;
```