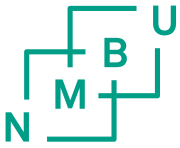
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**Preface and acknowledgements**

This master thesis is written at NMBU at the Institute of Plant Sciences as part of the research project “Mycotoxin contamination in Norwegian food and feed – Modelling, reductive approaches, and risk assessment with regards to the whole food chain” (2010-2014) and “Expansion of the Genetic Diversity in Oat”, financed by the Genetic Resource Centre (2011-2014). I gratefully received a scholarship from Yara Norge in 2012, on the basis of the topic’s possible importance to food safety.

I would like to thank my supervisors Åsmund Bjørnstad and Selamawit Tekle Gobena for all advice and help throughout the thesis process. I am also grateful to all others who helped me get through smaller and greater obstacles on the way. Of these I would like to mention Anja Ruud and Morten Lillemo, the ladies at the imaging center, and all the good ladies and gentlemen at SKP; Marit Holmøy, Tone Melby, Dag Wenner, Gry Skjeseth, and all others, both for advice in greenhouse and laboratory and for a welcoming atmosphere. I would also like to thank all that put their time at my disposal at Vollebekk and the good helper at Cigene; Sylvia Sagen Johnsen. Last but not least, thanks to Marit Evjen and Margit Oami Kim for good times, and Paul Schofer for almost patiently reading through.

Sissela Sund Stråbø,
January 2015,
Volda

# Abstract

Anther extrusion (AE) is a trait that has been studied in wheat for its influence on *Fusarium* head blight. *Fusarium* is a disease causing great problems in Nordic small grain production, particularly in oat. However, AE is little studied in oat. In this thesis the variation of AE was studied in RILs of two oat crosses, Svea × Stormogul and Fiia × Stormogul, and in a collection of 146 genotypes, mainly of Nordic origin. Both crosses showed high heritability for AE over two greenhouse experiments, and was estimated to be controlled by 4 and 6 genes respectively. The association between AE and other traits was assessed, with lemma color indicating linkage clearest. Two experiments with drought before anthesis gave lower AE in all genotypes exposed to drought. Variation of extrusion was not found between branches in the panicle, but between the 1$^{st}$ and 2$^{nd}$ florets. Neither the difference in swelling of lodicules nor the lengths of filaments seemingly cause differences in AE. The association between AE and DON was not convincing, but the percent florets with remaining anthers gave a 'fan shape' when plotted against DON. Emasculated florets infected with *Fusarium graminearum* indicated the same effect, with lowered DON level when anthers were removed.

# Samandrag

Støvknappfelling er ein eigenskap studert i kveite for påverknaden den har på aksfusariose. *Fusarium* er ein sjukdom som skaper store problem i nordisk kornproduksjon, spesielt i havre. Likevel er eigenskapen lite studert i havre. I denne masteroppgåva er variasjonen i støvknappfelling undersøkt i RIL av to havre kryssingar, Svea × Stormogul and Fiia × Stormogul, og i ei samling av 146 genotypar, hovudsakleg av nordisk opphav. Begge kryssingane viste sterk arvegrad for støvknappfelling gjennom begge drivhuseksperimenta, og talet på gen som styrer eigenskapen blei estimert til høvesvis 4 og 6. Assosiasjonen mellom støvknappfelling og andre eigenskapar vart vurdert, lemma farge viste den sterkaste linken. To eksperiment med tørke før blomstring ga redusert støvknappfelling i alle genotypane utsatt for tørke. Det vart ikkje funne variasjon i støvknappfelling mellom greinene i risla, men det var variasjon mellom 1. og 2. småblome. Verken skilnad i aukinga i svellingsskjel eller skilnader i filament lengde ser ut til å stå bak skilnader i støvknappfelling. Assosiasjonen mellom støvknappfelling og DON var ikkje overtydande, men prosenten småblomster med støvknappar sittande igjen ga ei 'vifteform' når plotta mot DON. Emaskulerte småblomster smitta med *Fusarium graminearum* viste til same effekt, med lågare DON nivå når støvknappane var fjerna.

# List of acronyms

AE – Anther extrusion

DON – Deoxynivalenol

DTF – Days to flowering from sowing

DTH – Days to heading from sowing

DUS – Distinctness, uniformity and stability

EST – Expressed sequence tag

FRA – Percent florets with remaining anthers

FHB – Fusarium head blight

GLM – General linear model

IAA –Indole-3-acetic acid

ICA – Indole-3-carboxylic acid

REML – Restricted maximum likelihood

RH – Relative humidity

RIL – Recombinant inbred lines

SKP – Center for climate regulated plant science (Senter for klimaregulert planteforskning)

QTL – Quantitative trait loci/locus

2,4-D – 2,4-Dichlorophenoxyacetic acid

# List of contents

# 1. Introduction

*Fusarium* species cause great problems in temperate grain producing climate zones around the world. The pathogens causing Fusarium Head Blight (FHB) give reduced germination rates, lowered yields, and production of mycotoxins that make the grains unsuitable for food and feed (Gagkaeva, Gavrilova, Yli-Mattila, & Loskutov, 2013). The most prevalent *Fusarium* mycotoxin in Norway is deoxynivalenol (DON). DON is found in almost all samples of unrefined grain, compound feed for animals and cereal food products. Since cereals are a very important part of the Norwegian diet, *Fusarium* and DON impose a potential threat to food safety (Bernhoft et al., 2013).

DON goes by the name vomitoxin since a high intake of DON gives nausea, vomiting and diarrhea in humans. The review of the Norwegian Scientific committee for Food Safety uncovered that young children are exposed to DON levels 2 to 3.5 times the tolerable daily intakes (TDIs).Vomiting and diarrhea is the effect on livestock as well, in addition to feed refusal and reduced fertility. Pigs are particularly vulnerable to the mycotoxins, while ruminants are quite tolerant (Bernhoft et al., 2013; FAO/WHO, 2002). The EU limit for DON content in unprocessed oat for food is set to 1750 µg/kg and to 500 µg/kg in processed oat (Scudamore, Baillie, Patel, & Edwards, 2007), other DON limits and guidance values for DON in food and feed can be seen in appendix 1.

In Northern Europe the highest levels of DON are found in unprocessed oat (Yli-Mattila et al., 2013). In 2009 as much as 76% of oat samples for feed exceeded the limit for DON content in Norway (Norwegian veterinary institute, 2009). The Norwegian scientific committee for food safety reported in 2013 a doubling of *Fusarium* infection in cereal seeds of oat, barley and wheat during the last decade. Both changes in cultivation practices and climate have increased the incidence of *Fusarium*. Reduced tillage together with less crop rotation, the use of semi-dwarf cultivars, soil compaction and more humid weather during the growing season are some of the factors contributing to the *Fusarium* problem (Bernhoft, Torp, Clasen, Løes, & Kristoffersen, 2012; Lu, Lillemo, Skinnes, He, Shi, Ji, Dong, & Bjørnstad, 2012; Terzi, Tumino, Stanca, &Morcia, 2014).

The *Fusarium* problem has other severe consequences for Norwegian oat production than merely the problem of high DON contents. From 2008 to 2013 the decrease in oat production in Norway was at 34.8 percent, 213.6 tons. This decrease is partly caused by a reduction in

growing area, but also a severe decline in yield. The yield was reduced from 4.2 tons per ha in 2008 to 3.1 tons per ha in 2013 and *Fusarium* is a main reason for the decline (Statistics Norway, 2013).

An important tool in lowering infection rates of *Fusarium* and DON content in food and feed is breeding. Improvements through breeding can be reached by using avoidance and resistance traits. Both passive resistance mechanisms (as avoidance through traits as morphology, anther extrusion, and flowering time) and active resistance mechanisms against *Fusarium* have been detected in wheat and barley. Five different types have been defined for *Fusarium* resistance: Type I – resistance to invasion or initial infection, Type II – resistance to fungal spread, Type III – resistance to toxin accumulation, Type IV – resistance to kernel infection, Type V – tolerance (Mesterházy, Bartók, Mirocha, & Komoróczy, 1999) many of which have pleiotropic effects on other traits of commercial importance.

Florets with high anther extrusion (AE) impact *Fusarium* incidence in wheat (Gilsinger, Kong, Shen, & Ohm, 2005; Lu et al., 2013). Studies in wheat and barley have shown that AE is a highly variable trait (Abdel-Gahni *et al.*, 2005). The trait shows different degrees of extrusion, in addition to cleistogamous florets where anthers are completely enclosed ( Nair et al., 2010;Graham & Browne, 2009; Kubo *et al*., 2013; Skinnes, Tarkegne, Dieseth, & Bjornstad, 2008). Some claim that AE is a stable character (Ceccarelli, 1978; Skinnes *et al.*, 2013), while others have concluded that the trait is highly influenced by environmental factors, especially drought (Sage & Isturiz. 1974; Abdel-Gahni, *et al.*, 2005) Very few studies have been done in oat.

This thesis will focus upon flowering type in oat as studies have pointed to that different flowering traits may affect *Fusarium* infections. *Fusarium* infections mainly occur during flowering and the infection pathway is strongly associated with anthers, as these are colonized first, both in oat, and wheat (Tekle, Dill-Macky, Skinnes, Tronsmo, & Bjornstad, 2012; Pugh, Johann, & Dickson, 1933). Studies of the floral trait AE are important to evaluate its potential use in breeding.

This thesis aims to:

• uncover whether genetic variation in AE is present in oat, by screening a broad collection with emphasis on Nordic gene material, and its inheritance through RILs of two crosses, ‘Svea’ × ‘Stormogul’ and ‘Fiia’ × ‘Stormogul’. Further the relation between AE and other traits, and inheritance of AE was investigated.

• look at how stable AE is and determine its sensitivity to environmental factors. Possible drought effects on AE could weaken the usefulness of the trait. The lodicules responsiveness to auxin and the extent of filament elongation could explain the mechanism behind different degrees of AE. To assess the stability of the trait the relationship between visually scored AE and the number of remaining anthers per floret in field and greenhouse, in addition to the variation within the panicle.

• look for a similar relationship between AE and *Fusarium* infection in oat as that found in other small grains. If high AE would give a negative correlation with disease incidence, and the trait shows high variability and heritability the trait could be very useful in resistance breeding.

# 2. Background

## 2.1. Oat production

Oat is a younger crop than barley and wheat, brought into domestication as a weed in the two older crops. The main cultivated species of oat is *Avena sativa.* Oat production has been tightly connected to the horse throughout history, from the Romans discovered its value as feed for their war horses to the decline in oat production as the tractor replaced horses in agriculture and cars on the roads (Bjørnstad, 2010; Marshall & Sorrells, 1992). From 1962 global oat yield has decreased from 50 million tons to 21 million tons in 2012. On world basis oat is taking up less than one percent of the total cereal production, with Russia and Canada as top producers (FAOSTAT, 2014). Despite oat being beneficial to health (Andon & Anderson, 2008), most of the oat is grown for feed.

In the Nordic countries oat is third in importance of the cereals, and the Nordic countries rank third in amount oat produced globally. Oat is well adapted to the north as it sustains the cooler climate and has low demand on soil quality and nutrition (Bjørnstad, 2010; Fredlund et al., 2013). In 2013 oat covered 24% of the total grain area in Norway, corresponding to 68 400 ha, a reduction of 900 ha from 2012.

## 2.2. Oat panicle morphology

Grasses are built-up by many smaller “subunits” called phytomers. The phytomers have an upper half node with leaf primordium and a lower half node with root or shoot primordia, the area between the two half nodes is the internode. The culm has phytomers with long internodes, but further up in the plant, in the inflorescence, internodes get shorter and shorter. The phytomere units are attached to each other so that leaves alternate, with 180° angle from one leaf to the next (Figure 1) (Moore 1995; Murai, 2013).

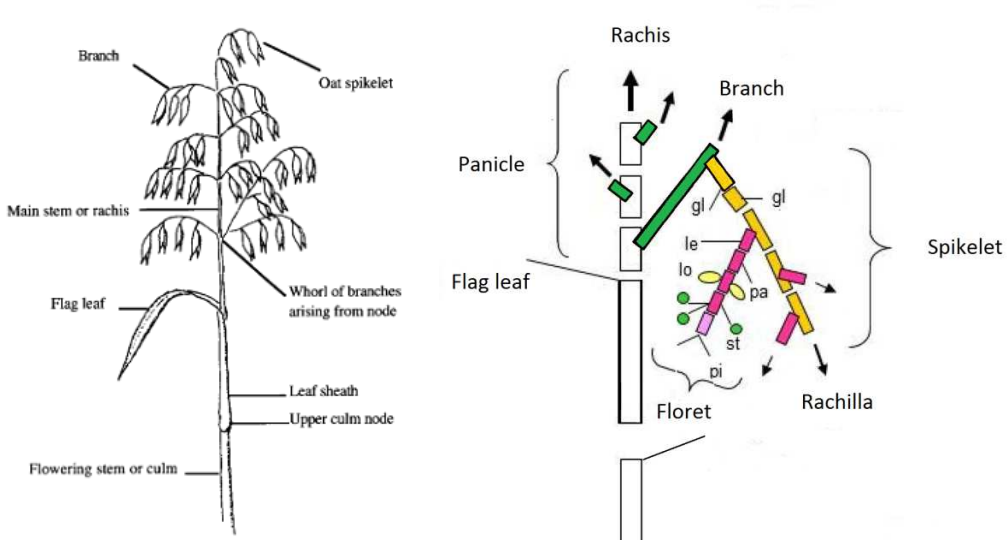



**Figure 1 – Left: Oat panicle from [www.inspection.gc.ca](http://www.inspection.gc.ca) Right: Schematic illustration of the phytomeric structure of the panicle. The spikelet is built up with glumes (gl) at the base of the rachilla. The lemma (le) is the first bract element where the floret axis is attached to the rachilla, followed by the palea (pa), lodicules (lo), stamens (st) and the pistil (pi) the distal end (figure from Murai, 2013, -modified by the author).**

The inflorescence of oat is called a panicle (Figure 1). The panicle has a main stem, rachis, with branches (pedicels) attached to its four to nine nodes. Spikelets are attached to the branches by rachilla, the spikelet axis. The spikelet is composed of, normally two or three, florets enclosed by two glumes. The florets sit on opposite sides of rachilla. Each floret is composed of lemma and palea (forming the hull), two lodicules, three stamens and a pistil, from the proximal end. Floral parts are modified leaves sitting on phytomers with short internodes. The phytomere structure of the floret can be seen in Figure 1.

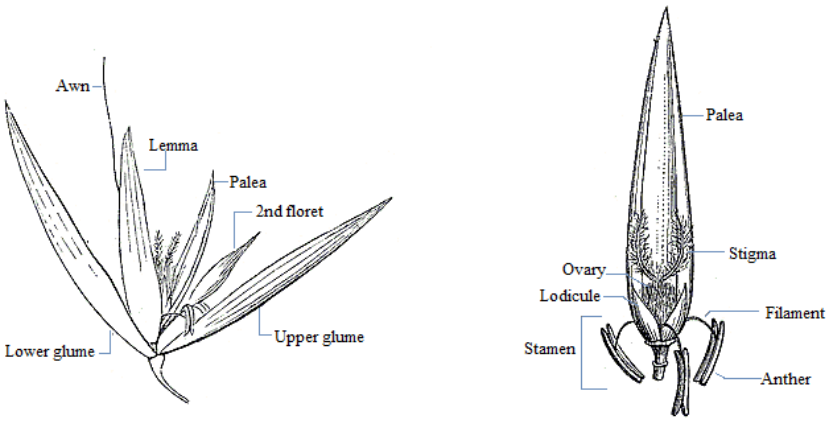



**Figure 2 - Oat floret, taken from [http://delta-intkey.com/angio/www/graminea.htm](http://delta-intkey.com/angio/www/graminea.htm), and modified by the author. The drawing shows all floral organs with glumes protect the whole floret and the hull, consisting of lemma and palea, protecting the reproductive organs of the individual florets.**

The lemma is quite similar to the glumes in structure. In wheat they have scarcely lignified outer epidermis consisting of medium-sized cells with thick cell walls, the inner epidermis has thinner cell walls and is only lignified at the wings. Both the inner and outer epidermis have a sclerotic hypodermis. In the lemma this sclerotic hypodermis is only between the epidermis and parenchyma (chlorenchyma) that is associated with the vascular bundles. The palea is thinner, with smaller cells and less chlorenchyma than the lemma. The palea has hairs on the two keels that fold in towards the ovary ( Percival, 1921; Marshall & Sorrells, 1992; Ribichich, Lopez, & Vegetti, 2000).

The two scale-like lodicules are attached directly above the hull, ready to push open the floret during anthesis. The lodicules and the reproductive organs are joined to the axis close together. The epidermal layer of the lodicules is composed of rough roundish cone cells, while the inner cell layers consist of parenchymatic cells. At the top the lodicules have a non-swelling, membranous wing, while it is the distensible parenchyma cells of the cushion like, fleshy region that swell. A vascular strand enters the base of each lodicule from the rachilla. The vascular strand parts into three as it enters the lodicule (Craig & O'Brien, 1975; White, 1995; Heslop-Harrison & Heslop-Harrison, 1996; Ladizinsky, 2012; Yoshida, 2012) The lodicules lean on the bifid ovary, with the stamens connected to the axis between them. The three stamens consist of a filament with a single vascular strand and a bilobed anther at the tip. Above the ovary the oat pistil has feathery stigmas. (Marshall & Sorrells, 1992; White, 1995C

Ladizinsky (2000) defined three major domestic traits for oat that all involve changes within the inflorescence: 1. a tougher rachilla giving seeds that do not shatter (Marshall and Sorrel, 1991), 2. hairless and bright hulls and lemmas, and 3. simple and few or no awns.

## 2.3. Anthesis

### 2.3.1. The mechanism of anthesis

Lodicules open florets at anthesis by swelling rapidly and pushing the lemma and palea apart while anthers move out as filaments extend. Filament elongation and lodicule expansion happens due to inflow of water as a response to increased osmotic pressure. The cell walls of the distensible cells have cellulose fibrils layers laid out in a lattice pattern, allowing the cells to expand rapidly without bursting. For long the osmoticum was suspected to be sugar

because of the sweet taste of lodicules. Heslop-Harrison and Heslop-Harrison (1996) showed that there was a rapid influx of potassium ions in both filament and lodicules at flowering, leading to higher osmotic pressure.

The hormone involved in this process is auxin. Honda, Turuspekov, Komatsuda, and Watanabe (2005) found high levels of the auxin indole-3-acetic acid (IAA) in barley anthers before flowering. The IAA levels were lowered in the anthers at flowering when lodicules had acquired a high content of the IAA metabolite indole-3-carboxylic acid (ICA). This implies that auxin moves from the anther, through the filament and ends up in the lodicule where it signals the start of the potassium influx. The content of IAA and ICA in the filaments was not described.

The lowering of IAA content at flowering only occurred in chasmogamous florets. Anthers in cleistogamous florets obtained an even higher IAA content in the anthers and no increase in ICA content in the lodicules (Honda *et al*,. 2005). The abnormally developed lodicules of cleistogamous barley do not react to applied auxin, while lodicules of chasmogamous barley respond to applications of active auxins such as IAA and 2,4-D. Application of these auxins induce flower opening, and keeps the flowers open longer than they otherwise would (Wang, Ning, Pourkheirandish, Honda, & Komatsuda, 2013).

Simultaneously as the auxin moves from the anther to the lodicules another mechanism may be responsible for transporting water into the filaments at anthesis. In *Arabidopsis* anther dehiscence is linked to transport of water from anthers into the filaments. Production of jasmonic acid in the upper part of the filament promotes water transport from the locules of the anthers, through the tapetum (anther walls), and into the filaments. This leads to desiccation of the locules and opening of the stomium (Ishiguro, Kawai-Oda, Ueda, Nishida, & Okada, 2001).

The rapid water influx during elongation tears the xylem apart. The cylindrical shape of the extended filament is kept by the helical orientation of the microfibrils that limits any lateral expansion. In the unextended filaments the lateral cell walls are folded and pleated while the end walls are thickened (Heslop-Harrison & Heslop-Harrison, 1996).

### 2.3.2. Factors inducing anthesis

Lodicule swelling is closely synchronized with AE and dehiscence. Synchronization is dependent upon different types of signaling. Mechanical stimuli of the pistil and the anther induce anther dehiscence. Matsui, Omasa, & Horie (2000) suggested that the expansion of lodicules produce a mechanical stimulus of the pistil when expanding. In cleistogamous florets where the lodicules do not function, it is the anthers pressing against the stigma that initiates the dehiscence (Heslop-Harrison & Heslop-Harrison, 1996; Matsui et al., 2000). As oat mainly are self-pollinated the anthers will usually have shed much of their pollen ahead of or during floret opening, and fertilized the receptive stigma before foreign pollen has the chance of entering (Marshall & Sorrells, 1992).

Swelling of the lodicules is induced in mature florets by environmental stimuli, like light or mechanical stimuli (e. g. wind). These environmental cues ensure timing of pollination to ideal weather conditions. In rye temperature and humidity were not found to be important stimuli (Heslop-Harrison & Heslop-Harrison, 1996), but in early studies in wheat a minimum temperature of 12-16 ° C was found as a requirement for flowers to open. During rain flowers do not open at all (De Vries, 1971; Pugh et al., 1933).

### 2.3.3. The speed of anthesis

The reaction to appropriate stimuli is very rapid. Full opening of the florets can be reached within few minutes. Findings in wheat and rye are very similar on timing and extension of anthers. The whole process of anthesis lasts between 8 and 30 minutes, on average 20 minutes, where the flower stays open for 5-15 minutes. Temperature and other environmental factors can speed up or slow down the process. The extension of anthers starts simultaneously with the opening, and full extension is reached within 2-4 minutes of floret opening. In this short time filaments are extended from 2-3 mm to 7-10 mm. Before the floret starts closing again all pollen is shed (De Vries, 1971; Heslop-Harrison & Heslop-Harrison, 1996; Percival, 1921).

The angle at which the floret opens and how long it stays open can affect the degree of AE. A small angle or short duration (especially both together) may hinder anthers from extruding, leaving anthers stuck between lemma and palea. Both duration of flowering and opening angle varies between wheat cultivars, and with weather conditions (Percival, 1921; De Vries, 1971; Gilsinger et al., 2005).

The flowering time of oat panicles is long compared to the other small grain cereals. For the whole panicle to flower, from the top floret to the innermost florets at the basal node, about ten to eleven days may pass. Wheat on the other hand only needs four to five days (Rajala & Peltonen-Sainio, 2011).

## 2.4. The genetics of anthesis

### 2.4.1. The ABCDE model of flower development

The ABCDE model is built upon findings in the two plant model species *Arabidopsis thaliana* and *Antirrhinum majus.* In this model the floret is partitioned into four ring-like whorls. Whorl 1 (from the outside) corresponds to the sepal, whorl 2 the petals, whorl 3 the stamens and whorl 4 the pistils. The model predicts that each whorl is specified by a class of genes, A, B, C, D, E, or combination of these gene classes. Of the gene classes only the A class *APETALA2 (AP2)* gene does not encode MADS-box proteins (Ning et al., 2013; Yoshida, 2012).

This model may in part be transferred to grasses, as many of the genes involved in flower development are highly conserved. Studies in maize and rice have shown that lodicules are modified petals. Lodicule development is, in similarity with eudicot petals, controlled by a combination of A- and B-class genes (Ambrose et al., 2000; Kang, Jeon, Lee, & An, 1998; Kyozuka, Kobayashi, Morita, & Shimamoto, 2000; Luo, Guo, & Li, 2013).

### 2.4.2. The genetic background of flowering types in cereals

MicroRNAs are post transcriptional regulators important in the development of the flower, and they are highly conserved in angiosperms. The microRNAs have roles in floral transition, floral pattering, and the development of floral organs. The microRNA miR172 works in both monocotyledons and dicotyledons as a regulator in transitions between developmental stages, and in floral patterning by specifying floral organ identity (Luo et al., 2013; Zhu & Helliwell, 2011). In lodicules that develop normally miR172 cleaves the mRNA of the *Cly1* (*HvAP2,* homologue of *AP2* in barley) gene. The effect of the cleavage is to reduce the abundance of the protein HvAP2, a protein that suppresses lodicule swelling. Two distinct synonymous substitutions (*cly1.b* and *cly1.c*) in the *AP2*-like *Cly1* locus affect the miR172 targeting site, and both mutations give the cleistogamous phenotype (Nair et al., 2010). The necessity of a correct balance between miR172 and *AP2*-like genes was confirmed by miR172 overexpression in rice. The overexpression increases the number of lodicules and lead to

elongated lodicules in spikelets that were unable to close again after flowering (Wang et al., 2013; Zhu & Helliwell, 2011). Three bread wheat homoeologs has been found for the *Cly1*-gene; *TaAP2-A, TaAP2B-*, and *TaAP2-D.*The homoeologs all have a very similar structure to *Cly1* and sit in a region syntenous with that of *Cly1* in barley, the distal region of the long arm of the group 2 chromosomes (Ning et al., 2013). An oat homologue has not yet been isolated in oat, but the expressed sequence tag (EST) has been identified (G. Lazo, (06.05.2014), personal communication).

The difference between lodicules of cleistogamous and non-cleistogamous genotypes of barley can already be seen in the white anther stage, with less cell activity in the cleistogamous lodicules. At the green anther stage the lodicules of the cleistogamous are half the size of lodicules in non-cleistogamous genotypes, with the greatest difference shown in the depth of the lodicules. Normally developed lodicules is a fully dominant trait, only the homozygote *cly1/cly1* gives cleistogamous flowers (Nair et al., 2010; Wang et al., 2013).

While the *Cly1* gene explains whether the lodicules function or not, genes at a linked locus, *Cly2*, seem to determine when flowering occurs in barley. *Cly2* controls whether flowering occurs before the spike has left the boot, or after. The *Cly2* allele causing premature flowering has a dominant epistatic effect on *Cly1*, because even if flowers have functioning lodicules these will have decreased in size again before the spikes leaves the boot. The premature flowering will leave the florets closed to the environment and give the protection of a closed floret (Wang et al., 2013).

Mutations in the miR172 binding site of HvAP2, that gives defect barley lodicules, also give shorter rachis internode lengths in barley. (Turuspekov, Kawada, Honda, Watanabe, & Komatsuda, 2005) Both synonymous and non-synonymous substitutions have been detected giving dense panicles, where the non-synonymous seem to give even denser panicles than synonymous substitutions. When the single nucleotide changes hinder cleavage of the *HvAP2* mRNA it also was shown to delay spikelet maturity at the awn initiation stage (Houston et al., 2013). The barley cultivar Golden Promise is cleistogamous through both mechanisms and has a dense spike.

For AE to occur flowering has to happen after emergence from the boot and the lodicules have to function. The degree of floret opening impacts the degree of AE (Gilsinger *et al.*, 2004). Heslop-Harrison & Heslop-Harrison (1996) saw elongation of the filament as the main

force of AE, while Sage (1974) saw synchronization of the flower mechanisms as more crucial since opening seemed to fail more often than filament elongation.

No mutation has been found in filament extension in barley (Wang et al., 2013). Although studies of genes that regulate anther development (*GAMYB, ARF6 and ARF8*), have described knockouts or overexpression of genes and/or their regulators that affect filament length (Achard, Herr, Baulcombe, & Harberd, 2004; Fujioka et al., 2008; Luo et al., 2013) In wheat however 'U24', a variety considered to be cleistogamous because of its very small angle of floret opening despite fully swelling lodicules, the filaments do not elongate (Ning et al., 2013).

In wheat the trait was expressed differently within the ear, the basal region was showing higher extrusion as it had a larger amount of open florets (Sage & Isturiz, 1974; Percival, 1921). Sage & Isturiz (1974) also found that the size of the floret did not affect the number of anthers extruded. Anther size is positively correlated with AE in both barley and wheat (Hammer, 1975; Kubo, 2013; Langer, Longin & Würschum, 2014)

### 2.4.3. Inheritance of the anther extrusion trait in small grains

While it is generally agreed upon that AE is an additive trait, there are quite different results on the degree of heritability (h) and number of genes involved. In barley Ceccarelli (1978) suggested that AE, in the sense of open or not opening flowers, was controlled by a single gene where the extruding type was completely dominant. This is likely to describe the inheritance of the *Cly1* gene, as AE was scored as extruding or not. Later Sage & Isturiz (1974) concluded that AE in wheat was dependent on the equal additive effects of at least two genes, but was a trait with low heritability because of strong environmental influence. Atashi-Rang and Lucken (1978) had similar heritability results, but found indications that additive and non-additive variance had an equal effect on AE. The non-additive could unknowingly describe the *cly* genes. In later studies high heritability has been found in wheat (Singh, Arun, & Joshi, 2007; Skinnes Semagn, Tarkegene, Marøy, Bjørnstad, 2010) (H= 84 and $h^2$=0.91 respectively) and intermediate to high heritability (0.3 to 0.7) for the trait in barley (Abdel-Ghani, Parzies, Ceccarelli, Grando, & Geiger, 2005). Skinnes et al., (2010) discovered 3 major and 2 minor QTLs in wheat that explained 53.6% of the phenotypic variation. Kubo (2013) found three QTLs for recessive genes giving closed flowering in 'U24', these were also thought to affect the degree of AE

## 2.5. *Fusarium* head blight

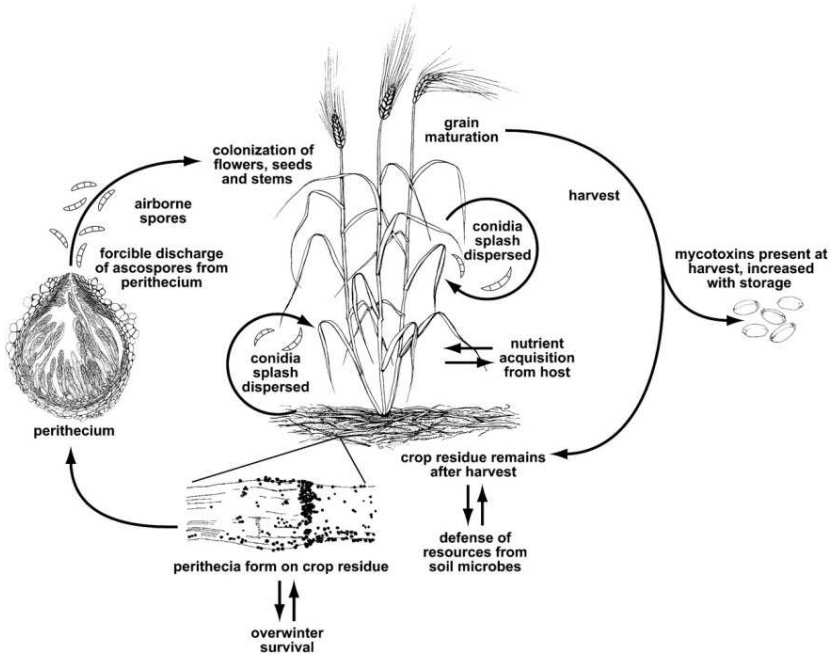



**Figure 3 - The Life cycle of *Fusarium graminearum* on wheat. From Trail (2009)**

### 2.5.1. *Fusarium* life cycle and infection process

Figure 3 shows the life cycle of *F. graminearum* in wheat. *Fusarium* overwinters as binucleate hypha and in the case of *Fusarium graminearum* also as perithecia initials in crop debris. Throughout the season great amounts of inoculum is produced as conidia forming on infected plants and on crop residue during damp conditions. The conidia mainly spread by water splash from sporodochia. *F. graminearum* has an additional primary inoculum as ascospores that spread widely by wind as they burst out of the ripe perithecia (Trail, 2009; Bernhoft et al., 2012). Rain is not only necessary for in-field spread of conidia, but it also gives the free water or high humidity needed for spore germination (Bernhoft et al., 2012), and is essential for the hypha not to desiccate (Skadsen & Hohn, 2004).

The fungus struggles to attack the glumes, lemma and palea from the outer (adaxial) side, where the cell walls are thick and lignified. Open florets give spores direct access to the exposed ovary and extruded anthers can be a pathway into the florets as the fungus colonizes the anther and grow into the floret through the filament. Inside the florets the fungus is protected against desiccation and has tissue that is more easily infected within reach (Pugh et al., 1933; Skadsen & Hohn, 2004; Tekle et al., 2012)

When *Fusarium* has entered the floret it first colonizes degenerating tissues such as anthers ( Ribichich et al., 2000; Tekle et al., 2012). The role of anthers in infection process of *F. graminearum* has been discussed since it was described by Dickson & Wineland in 1921, the same year as Percival (1921) described retained and extruded anthers in wheat. The preference *Fusarium* has for growing on anthers has been explained by the high content of choline and betaine in anthers. Choline and betaine stimulates the growth of *Fusarium* both *in vitro* and *in vivo* and is found in much higher concentrations in anthers than any other part of the wheat plant (Pearce, Strange, & Smith, 1976). Miller, Chabot, Ouellet, Harris, and Fedak (2004) confirmed the predilection *Fusarium* has for anthers, while Engle, Lipps, Graham, & Boehm (2004) on the other hand doubted the anther theory, finding all floral parts equally prone to infection.

In oat, barley, and wheat the optimal time of infection is at anthesis. In wheat infections can start from early flowering to dough stage, but for both wheat and oat the severity of infection is much greater when it occurs at anthesis than at other times (Pugh et al., 1933; Tekle et al., 2012; Yoshida, 2012). Tekle et al. (2012) found that inoculation of oat at flowering resulted in complete decay of the caryopsis or infected kernels with low germination rate and high content of mycotoxins. The effect of inoculation decreased strongly with time, with no difference in *Fusarium* damage between the uninoculated control and the inoculated two weeks after inoculation. In cleistogamous barley the optimal time of infection is delayed (Yoshida, 2012). This delay strengthens the theory that anthers play an important role in the infection process as the optimal time of infection in the cleistogamous barley was matched with the time when anthers were pushed out by the caryopsis. The pushing out of the anthers gives *Fusarium* both anthers to colonize and an opening to enter.

After colonizing anthers, the fungus spreads to other tissues, the insides of the palea and lemma. Since the epidermis on the inner side of these bracts have thinner cell walls, they are much easier for the fungus to penetrate than the outside (Pugh et al., 1933). Ribichich et al. (2000) confirmed these findings in wheat and Tekle *et al* (2012) found the same infection pathway in oat.

Production of deoxynivalenol (DON) starts almost immediately after infection (Jansen, Von Wettstein, Schäfer, Kogel, Felk & Maier, 2005). Deoxynivalenol acts as a strong protein inhibitor in plants (Mesterházy, 2002), and is the only one of several toxins in the thricothecenes group produced by the *Fusarium* species acting as a virulence factor in wheat. (Proctor, Hohn, & McCormick, 1995; Desjardins, Proctor, Bai, McCormick, Shaner,

Buechley, & Hohn, 1996; Bernhoft, 2012). Since it is a virulence factor the magnitude of DON production reflects the aggressiveness of a *Fusarium* isolate (Mesterházy, 2002). The use of knock out mutants shows both the lack of virulence and *Fusarium* symptoms when the ability of producing DON is gone (Proctor et al, 1995; Desjardins et al., 1996; Jansen et al., 2005)

In contrast to wheat where prematurely bleached tissue, often bands of several florets, are easily recognizable in the field, for oat visual symptoms of *Fusarium* infection are often absent during the growing season (Gagkaeva et al., 2012). The symptoms that can develop in oat are brownish, necrotic seeds and pink mycelia can appear on the glumes in periods of high humidity (Bjørnstad & Skinnes, 2008).

The growth of *F. graminearum* and production of DON has the same optimum temperature, 25°C. Although this is higher than the mean temperature in a Norwegian summer DON levels have increased in the recent years. This is likely due to two other factors that strongly affect DON production; humid weather or high water activity and high temperature. Usually many *Fusarium* species occur together, but which species is the dominant varies between regions and weather conditions (Fredlund et al., 2013). *F. graminearum* is taking over as the main deoxynivalenol (DON) producer in Norway after *F. culmorum*. *F. graminearum* has a higher optimum temperature for growth than *F. culmorum*, and is expected to become even more prevalent as the climate changes (Bernhoft et al., 2012; Yli-Mattila et al., 2013)

### 2.5.2. Resistance to *Fusarium* head blight in small grain cereals

FHB resistance in wheat and barley is encoded by several genes, giving a complex quantitative trait (Dahleen, Morgan, Mittal, Bergitzer, Brown, & Hill). In wheat more than a hundred QTLs connected to FHB have been detected (Burstmayr, Ban, & Anderson, 2009).

Of the five already mentioned *Fusarium* resistance parameters DON can break the type II resistance by spreading from florets through the rachis in wheat. In barley DON does not seem to have the same virulence effect and *Fusarium* can spread from the barley floret by growing on the outside of the spikelets instead of through the rachis (Jansen, et al., 2005). In oat the long peduncles prevent such spread and give Type II resistance (Tekle, 2012).

Anther extrusion had highly significant negative correlations on FHB in wheat (Skinnes *et al*. 2010). Kubo et al. (2013) found that closed flowers gave lower FHB incidence, with the highest incidence in genotypes with intermediate AE. In line with these findigs were those of Gilsinger et al., (2004) where wide flower openings gave higher disease incidence. Plant

height has shown strong negative correlation with FHB severity in wheat, and is a known avoidance mechanism for *Fusarium (*Mesterhazy, 1995; Niks et al., 2011; Sage & Istruiz, 1974. QTL for low AE co-localized with *Rht-B1b* dwarfing allele and increased susceptibility after spawn and spray inoculation (Lu et al., 2013). Wang (2013) also found the distance between the ligule and spike to be closely related to AE.

In oat less susceptibility has been observed in naked genotypes (Gagkaeva et al., 2012), but in hulled oats much of the DON can be removed by dehulling (Gavrilova, Gagakeva, Burkin, Kononenko, and Loskutov, 2008; Bernhoft, et al., 2013). Langseth, Høie and Gullord (1995) found indications of a similar relationship between FHB and PH in oat as in wheat in a study of five oat cultivars. Later studies have not given much support to that relationship. Gavrilova,et al., (2008) did not find any correlation between Fusarium incidence and plant height in their study of germplasm from the VIR collection. He, et al. (2013) did not find any clear evidence of a connection between FHB and PH or DON and PH in the RILs of their two crosses, although some years the results showed correlations.

# 3. Materials and methods

## 3.1. Genetic variation of anther extrusion in oat

To investigate genetic variation and the inheritance of AE recombinant inbred lines of two crosses (Svea × Stormogul and Fiia × Stormogul) were used. A collection of oat genotypes was screened for AE to investigate the degree of AE in a wider collection and trends in AE in relation to age and origin of genotypes. In both the crosses and the wider collection additional traits were scored to look for their possible relation to AE: height, days to flowering and heading, lemma color, presence of awn, and panicle traits.

### 3.1.1. Anther extrusion in the Stormogul crosses – greenhouse trials 2012 and 2013

**Plant material.** The Swedish oat cultivar 'Stormogul' (Svalöf,1901) is an old black seeded genotype made by pure line selection in 'Plym black tartar' (Bengt Mattsson, 1997). 'Stormogul' was crossed with the Finnish genotype 'Fiia' (Boreal plant breeding Ltd, 2002) and the Swedish genotype 'Svea' (Svalöf, 1976) respectively in 2010 (by Å. Bjørnstad). The crosses were originally made to explore the early drought resistance found in Stormogul and other of the black oat (Mattsson, 1997) However the low DON levels and the high AE of Stormogul led to AE studies. Recombinant inbred lines (RILs) (148 lines each from Svea×Stormogul (S×S) and Fiia×Stormogul (F×S)) were made by the single seed descent method. Individual plants in the F6:7 generation were used in 2012 and F6:8 generation in 2013.

**Experimental design.** The experiment was conducted twice. The first experiment took place during the summer 2012 and the replication took place in winter 2013.

* The 2012 experiment was planted July in the greenhouse at the Center for Climate Regulated Plant Research (SKP). In each cross the pots were arranged in 11columns × 14 rows on a table in an augmented design with Stormogul, Fiia/Svea and Hurdal as checks (2 of each per cross). Data from the 2012 trial were collected by Selamawit Tekle Gobena.
* In the replication planted on August 2$^{nd}$ 2013 the augmented design was expanded to include five checks, with Norum and Typhon as the newcomers, and 4 of each check per cross. The pots were then arranged in 12 columns and 24 rows. 168 pots were too many per table and each cross therefore filled 1.5 tables each.

**Growth conditions.** For planting 2 liter pots were filled with Gartnerjord (Tjerbo, Norway). The greenhouse settings were an ambient day-length of 16 hours, from 06.00 to 22.00, air temperature was set to 18 °C day and to 15 °C night, and relative humidity was set to 75%.

**Traits scored.** In 2012, the RILs were scored for AE, plant height, and earliness. In 2013 the RILs were scored for AE, plant height, earliness, lemma color, presence of awns, panicle type, panicle density, and panicle erectness.

*Anther extrusion was scored visually on a scale from 0 to 9; where 0 was equal to no AE and 9 was equal to 100 % extrusion throughout the panicle (where max is 3 anthers per floret). In the greenhouse trials the panicles on the main shoots were examined.

*Earliness was scored as both days to heading (DTH) and days to flowering (DTF). DTH scored when the whole panicle had emerged from the leaf sheath, (Zadoks stage 59 (Zadoks, Chang, & Konzak, 1974)). DTF was scored when open flowers were observed in one or more panicles.

*Panicle type (Figure 4) was scored as equilateral and unilateral (Diederichsen, 2009). The intermediate types were scored as equilateral.

*Panicle density was scored as wide or compact. Panicle erectness was scored as erect, semi-erect or drooping.

* In Figure 4, five different levels of erectness are shown. For panicle erectness, the groups semi-erect and horizontal were grouped into semi-erect, and drooping and strongly drooping were grouped into drooping.

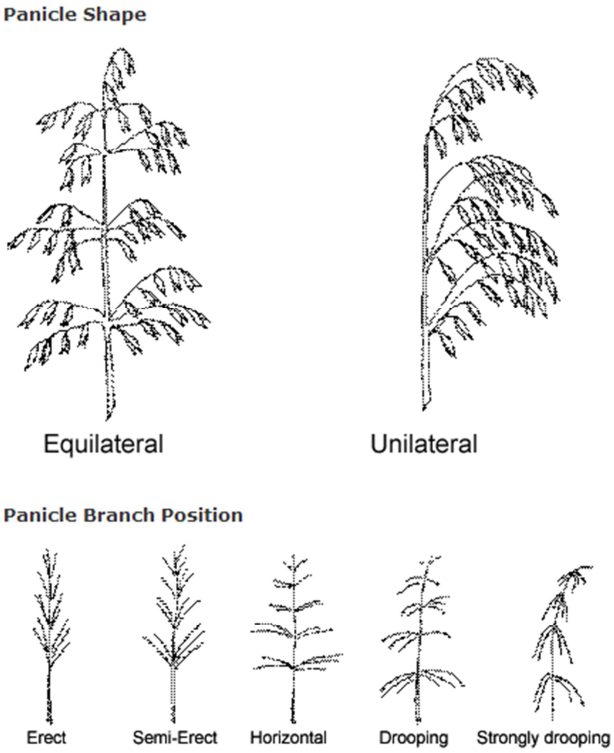



**Figure 4 - Panicle type (in figure called shape) and panicle erectness (CFIA, 2012).**

* Lemma color was scored as white and brown (all shades).

* Presence of awns was scored if more than a third of the panicles had awns.

* Plant height was measured (on the tallest straw) from the soil up to the base of the panicles.

**Statistical analyses**. Two statistical programs were used for the data treatment IBM SPSS Statistics 19 and Genstat. REML analyses of AE, height and DTF were run in Genstat by Åsmund Bjørnstad. Entry and year was set as fixed variables. The statistical analyses were run for this experiment and the replication together to look at year effects of AE. The adjusted entry means from REML analyses were used for further analyses in SPSS by the author. The adjusted entry means for the two years collected were used for analyses of correlations with other traits. Univariate ANOVA by GLM method with Pearson correlation and t-tests, and linear regression were used for means and correlations between AE and the other traits. The analyses used is mentioned in the results of the specific cases.

Heritability is a measure of the genetic proportion of phenotypic variance. In narrow sense heritability, the percentage of additive variance is expressed (Sleper & Phoelman, 2006). The formula for narrow sense heritability and calculation of the minimum number of genes was

taken from (Singh, Ma & Rajaram, 1995). Numbers for the calculation were read out of the REML results.

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

$$\sigma_g^2 = \text{additive genetic variance} = \frac{(\sigma_L^2 - \sigma_E^2)}{r}$$

$$\sigma_p^2 = \text{phenotypic variance} = \sigma_g^2 + \frac{\sigma_E^2}{r}$$

$$\sigma_L^2 = \text{variance of } \mathbf{F_6}(\mathbf{F_g}) \text{ lines}$$
$$= \text{average variance of differences}$$

$$\sigma_E^2 = \text{error of variance} = \text{residual variance (error)}$$

$$\text{r} = \#\text{ of replications}$$

The formula used for calculating the numbers of genes that contribute to the quantitative trait from Singh et al (1995) is based upon Wrights method. For the formula to be correct 5 assumptions are made: 1. No linkage, 2. No epistasis, 3. No dominance, 4. Equal effect and 5. No transgressive segregation. Breaking any of the assumptions will result in underestimation of gene number. (Sleper & Phoelman, 2006).

$$\boldsymbol{n = D^2 / \left[ \frac{8\sigma_g^2}{2 - \left( \frac{1}{2^{(g-2)}} \right)} \right]}$$

***D*** = estimation of the genetic range of the parents = ($\bar{x}$P1-$\bar{x}$P2)

***D*** was multiplied by the heritability to obtain a more stable estimate, by removing the environmental influence.

$g$ = the generation number (here 6)

The field trial was a randomized complete block design with two replications. Anther extrusion, DTH, DTF and height were scored as before. Since wind and rain easily remove extruded anthers it was necessary at times to check florets for retained anthers as a control measure. Five or more panicles were studied more closely for AE, in addition to a general overview of the plot. Heading and flowering was scored when approximately 50% of the panicles had emerged and flowered respectively.

### 3.1.2. Screening for anther extrusion in the oat core collection

**Plant materials.** The 146 genotypes were of diverse origins, but with a focus on Nordic material. Oat from 14 countries were represented in the collection, these were; USA, Russia, Canada, Germany, Mongolia, The Netherlands, UK, Ethiopia, Australia and China in addition to the Nordic countries. The Russian and Mongolian were from the collection of the Vavilov Research Institute of Plant Industry (VIR), the Chinese genotypes were current naked oat genotypes obtained from Dr. Ren. Both old and modern genotypes were present in the collection. Appendix 1 contains the full list of genotypes with origin and year of release. Year of release was not known for all genotypes and the Nordgen database ("SESTO Gene bank documentation system," 2014) and (Bengt Mattsson, 1997) was used to fill in missing information in the dataset.

**Experimental design.** The experiment was laid out in a randomized block design with two replicates. Bessin, Svea, Fiia and Stormogul were checks in addition to two RILs from the F×S population, #134 & #126. The two RILs represented high and low AE genotypes, 0 and 8 respectively (as scored in the 2012 experiment). The checks had, as the other genotypes, two replicates.

**Growth conditions.** The genotypes were sown on December 21$^{st}$ 2012 in the greenhouses at the research farm Vollebekk. The greenhouse settings were as described in 3.1.1. apart from RH that could not be regulated at Vollebekk.

**Traits scored.** The genotypes were scored for: AE, height, earliness, lemma color, panicle type and density as described in 3.1.1. For lemma color an additional category, yellow, was scored. Country of origin and year of release of the genotypes in the core collection were used to indicate changes of AE over time and compare AE in material of different origin.

**Statistical analyses.** One-way ANOVA was used on AE in the screening. For analyzing correlations between AE and the other scored traits GLM univariate ANOVA with Tukey HSD and Pearson correlation was used.

## 3.2. Environmental stability of anther extrusion

### 3.2.1 Sensitivity of anther extrusion to drought

Two drought trials were carried out with the aim of revealing whether drought will affect the degree of AE.

**Plant material.** Three genotypes that had been scored with low, medium, and high AE in the screening (3.1.2.),Typhon, GN9004 and Norum respectively. Typhon (AE=0) and Norum were compared in both years while the breeding line GN9004 was only subjected to drought in 2014.

**Experimental design.** The first experiment was sown in June 2013, and the second in November 18. 2013. The experiment had two treatments, drought and control. The first trial had three full replications (3× 2 pots per genotype and the second experiment had two replications (2×2 pots per genotype).

**Traits scored.** Anther extrusion was scored on all plants as described in 3.1.1. (between July 30$^{th}$ and August 05$^{th}$ 2013, and between January 12$^{th}$ and January 18$^{th}$). Anthers remaining in the florets were counted after anthesis (as in 3.2.2.).

**Growth conditions.** The experiments were carried out in the greenhouses at SKP with conditions as in 3.1.1. At Zadoks stage 59 half of the pots were subjected to drought from right before flowering while the rest were watered as normally. The drought stressed plants were kept under drought conditions throughout flowering, but were fed with a minimum of water after the first days to avoid wilting.

**Statistical analyses.** The AE of drought and control plants were compared by paired samples t-test.

### 3.2.2. Degree of anther extrusion

The control counts were made as a measure of checking how the visually scored AE scale (0-9) was correlated to its ideal of 0 to 100 % AE, and to evaluate scoring in different environments, greenhouse and field. The control counts were also used to investigate if there

was variation of remaining anthers within the panicle; checked by differences between branches and between the 1st and 2nd floret.

**Plant materials.** Control counts were performed on selected plots in the field trial and on both treatments in the drought trials, where the control treatment of Norum was used to evaluate visual scoring and panicle variation.

**Scoring.** All anthers present between palea and lemma were counted (also the ones trapped halfway at the tip between the lemma and the glume). For the study of variation within the panicle the position of the anthers were noted. The position was noted as branch number from the top node in the panicle and floret number (1st or 2nd floret).

**Statistical analyses.** Linear regression was used to look at the correlation between AE and remaining anthers. Univariate ANOVA was used to compare branches, and independent t-test was used to comare the 1st and 2nd floret.

### 3.2.3 Sensitivity of anther extrusion to auxin

As the *cly1* gene found in cleistogamous barleys gives non-functioning lodicules these experiments were performed to test the hypothesis that differences in degree of AE could be reflected in the lodicule size. The study of Ning (2013) was used as a basis for the experiments. The two preliminary experiments were no great success, but after finding and following Wang's (2013) instructions on the importance of the right anther stage, yellow, for lodicule reaction to auxin the two last experiments gave results.

**Plant material.** Typhon, GN9004, Odal and Norum were present in the preliminary experiments. In the two last experiments only Typhon, GN9004, and Norum were used. In the screening (3.1.2.) the genotypes had been registered with AE 0, 4, and 8,5 respectively. With an AE of 0 Typhon was the genotype that had greatest expectation of a diverging lodicule size. The two cleistogamous barley genotypes Tiril and Golden Promise were used as controls in addition to the chasmogamous barley genotype Tampar in the last two experiments. The Tiril and Golden Promise are both homozygous for *cly1* and do not have any 2,4-D response.

**Growth conditions.** Plants were grown in parallel with other experiments at SKP and had the same greenhouse conditions as described in 3.1.1.

**Procedure.** The laboratory and microscopy work took place at the laboratory at SKP. In the first experiment with auxin sensitivity whole panicles were set in a 100 ppm 2.4- D solution overnight. In the second experiment spikelets where the 1st floret was expected to be close to anthesis were picked. From the third and fourth experiments florets were opened with tweezers and made sure to be in the yellow anther stage, but before anthesis.

From the second to the fourth, and final, experiment spikelets were placed on cotton in tissue culture boxes with 2×2 cm wells and lids. The cotton had been drenched with ~6 ml 100 ppm 2,4-D solution. The 2,4-D solution was made by first dissolving crystallized 2,4-D (Sigma Aldrich) in 96% ethanol and then diluting with milliQ water. In all experiments, the spikelets were kept in the 2,4-D solution for 24 hours before examination.

The examination was common for all four experiments. When examined the base of the spikelet was first cut off and the lemma torn up. A scalpel was used to separate the lodicule from the base and palea. If pulling out the palea the ovary would often loosen from the lodicules. Lodicules were studied using Leica M205C stereo microscope and analyzed using Leica Application Suite (LAS V3.7). Lodicule area was measured from the front using the area tool and the diameter was measured both from front (width) and side (depth) using the two-point-distance-line tool. When measuring the diameter the widest point of the lodicule was chosen.

**Statistical analyses.** Univariate ANOVA with Tukey HSD was used to look at differences between genotypes in lodicule reaction to auxin.

### 3.2.4. Filament length

Another hypothesis that could explain differences in AE is differences in filaments elongation. Experiments were conducted where filament length was measured before and after anthesis in genotypes of different AE.

**Plant material.** Florets were picked from the Stormogul crosses and controls in the drought trial (3.2.1.) in the greenhouse at SKP in 2013. Among them were Norum and Typhon.

**Procedure.** After several tests the most successful floret pickings proved to be of florets that were just at the start of anthesis. To examine the filaments the florets were dissected. If the palea was pulled out of the lemma the ovule often broke off either with the filaments attached or with the filaments remaining at the base (in the lemma) together with the lodicules. A

better approach was therefore first using scissors to carefully cut of the base where the lemma and palea was attached. Cutting close to the point of attachment of the lemma and tearing the lemma using tweezers and needle could give an easy detachment of the hulls. Letting the filaments be attached to the lodicules, sometimes also to the ovule, for the measurements was usually best as the filaments were not easy to detach at the right point and tended to curl up. To standardize the results only the 1$^{st}$ floret in a spikelet was examined. A Leica M205C stereo microscope was used to examine the florets. Pictures were analyzed with Leica Application Suite (LAS) V3.7 using the segment tool (Figure 5).

A problem in measuring filament length is determining if the filaments are fully extended or under extension. It did look like the filaments during extension were firm and wavy (not extending equally on both sides of the filament). Based on that observation florets were classified by stage; 1: before filament extension; 2: under extension; and 3: end of extension. Because of the short time the filaments stay extended and fresh, and because they all naturally flower at the same time of day, it is not possible to check many florets. A maximum of ten was managed at a time. Keeping the florets in a lidded petri dish on moist cotton did not lengthen the shelf life.

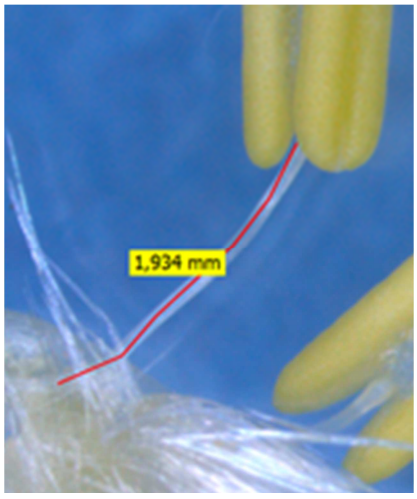



**Figure 5 - Filament length was measured from the basis of the anther to the point of attachment under the ovule.**

**Statistical analyses.** A univariate ANOVA was used to compare groups and filament length before and after elongation.

## 3.3. Effect of anther extrusion on deoxynivalenol content

### 3.3.1. *Fusarium* greenhouse trial

The aim of the *Fusarium* greenhouse trial was to look for connections between AE and *Fusarium* infection in oat through measuring DON content in a controlled environment. If the same pattern is as in wheat the higher DON levels would be found when the AE is low.

**Plant material.** Ten RILs from each cross representing the variation in AE were selected; Stormogul, Hurdal, Svea and Fiia were used as controls.

**Experimental design.** The experiment had three replications with pots placed randomly within three blocks.

**Growth conditions.** The experiment was planted at SKP August 3. 2012 with greenhouse set as in 3.1.1..

**Procedure.** Plants were spray inoculated with macroconidial suspension of *F. graminearum* isolates V101118, V101177 and V101018, containing 100.000 spores/mL at flowering. The isolates were obtained from the Norwegian Veterinary Institute. The plants were inoculated three times from different sides with approximately 2.3 mL inoculum. The inoculum was grown on mung bean agar (recipe in appendix 3A) to produce macroconidia (prepared by Yalew Tarkegne).Following inoculation panicles were covered with plastic bags to raise the RH for successful infection. Bags were removed after three days.

**Traits scored and toxin analyses.** The plants were scored for DTF, AE and the % of florets with remaining anthers in the panicle (only noted if anthers any were present or not ~25 florets per panicle). Samples from the greenhouse inoculation trial were sent to the University of Minnesota, Department of Plant Pathology, for toxin analysis. The samples were analyzed by GC-MS to detect DON. The procedure is shown in appendix 4.

**Statistical analyzes.** Linear regression and Pearson correlation.

### 3.3.2. *Fusarium* field trial

The *Fusarium* field trial was another trial with the intention of looking at the connection between AE and *Fusarium* through DON, but in a natural environment.

**Plant material**. A selection of RILs from the Stormogul crosses that represented the AE variation in the Stormogul crosses was used. The selection consisted of 79 lines from the SXS and 43 lines from the FXS crosses. Seeds had been multiplied in New Zealand over the winter.

**Growth conditions.** The selection was sown in field at Østre Voll, the Vollebekk research farm, 20.05.2013.The previous crop was a one-year old meadow. The field was plowed in the autumn 2012 and harrowed twice in the spring 2013 before sowing, and fertilized with 103 kg N/ha, 14.1 kg P/ha, and 47 kg K/ha (Yaramila$^{TM}$, 22-3-10 NPK). 2.2 l/ha Areane S (88 g/ha fluoroxypyr + 44 g/ha clopyralid + 440 ml/ha MCPA) was used against weeds. The insecticide used was Perfectione, 0.8 l/ha (400 g/l dimethoate) and the fungicide Zenit (450 g/ha fenopropidine + 125 g/ha propikonazol) was used against powdery mildew at 1 l/ha. Mist irrigation (9 min/h) was on from 19.00- 22.00 every evening following inoculation. During the dry period in early July (Figure 6 Temperature and precipitation coinciding with anthesis the irrigation time was extended to 19.00-23.00 until ten days after the last flowering date.

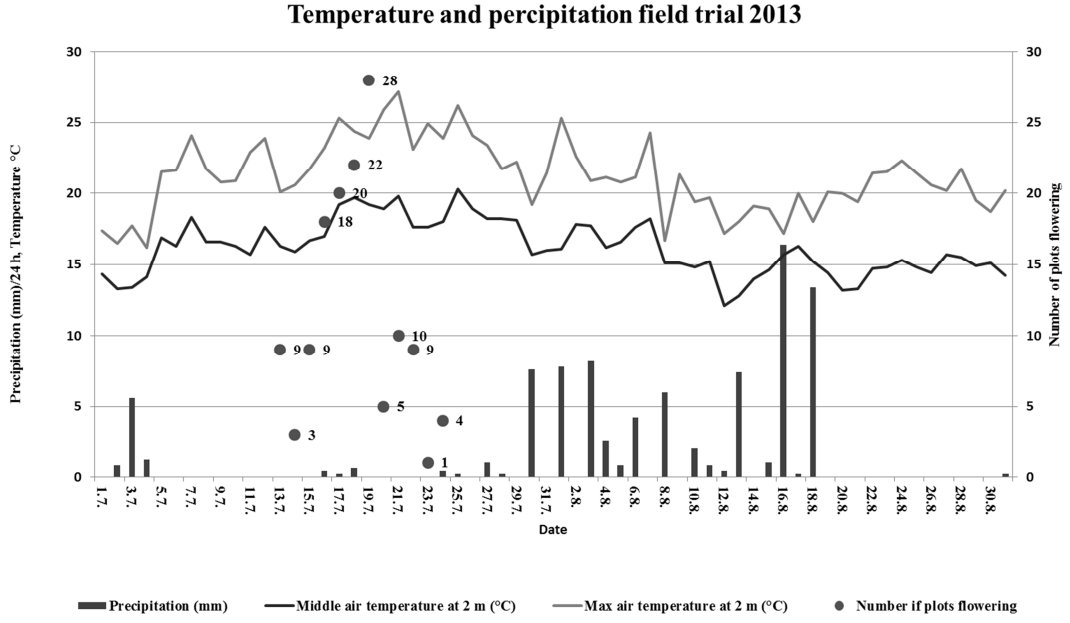



**Figure 6 Temperature and precipitation around flowering time, July and August 2013. The number of plots flowering is shown by dots.**

**Experimental design.** The field trial was a randomized complete block design with two replications.

**Inoculation method.** The field was spawn inoculated by *F. graminearum* colonized oat kernels. The inoculum was made by Yalew Tarkegne following the protocol by Dr. Bernd Rodemann (Julius Kühn Institute, Braunschweig, Germany). Four *F. graminearum* isolates, obtained from the Norwegian veterinary institute, were used; V101023, V101118, V101018, and V101177. The isolates were first cultivated 7 days on potato dextrose agar (PDA, see appendix 3.B) at ambient temperature and light. To make liquid *F. graminearum* culture five pieces of PDA from each isolate were transferred to 100 ml ionized water containing 1 g oat flour. This mixture was kept on a horizontal shaker set at 90 rpm for seven days in ambient light and temperature.

Belinda oat was sterilized before it was mixed with the liquid culture. 2 kg oat was soaked overnight and sterilized by autoclaving for 60 min at 121 °C in heat stable polyethylene bags. After the oat had cooled down to room temperature, they were inoculated with 100 ml of the liquid culture. The bags were kept 3 weeks at room temperature and ambient light to promote mycelial growth. This was followed with another 3 weeks with kernels spread on trolleys and misted with water to promote growth of perithecia. The ready inoculum was spread in the field at a density of $10g/m^2$ when the plants were at Zadoks stage 31-32 (Zadoks et al., 1974).

**Traits scored.** Anther extrusion, DTH, DTF and height were scored as in 3.1.1. Five or more panicles were studied more closely for AE in addition to a general overview of the plot. In the field wind and rain remove extruded anthers which at times made it necessary to check florets for remaining anthers as a control measure. DON content was estimated on a selection of plots where the AE scored was close to the AE scored in the greenhouse. The toxin data was analyzed with ELISA in order to get results in time. The kit used was AgraQuant® Deoxynivalenol Assay 0.25/5.0 from Romer Labs® (providing DON levels). Sylvia Sagen Johnsen led the ELISA procedure in cooperation with the author. The procedure from Romer labs were followed, with some modifications in sample preparation after advice from Heidi Udnes Aamot. Method described in appendix 5.

**Statistical analyses.** Linear regression and Pearson correlation.

### 3.3.3. Emasculation experiment

The intention of the emasculation experiment was to further confirm the effect of anthers and AE on DON content. If the absence of anthers affect the *Fusarium* infection and DON content a change in DON content was expected. The largest change would be expected between the emasculated and the control of the genotype with lowest AE where all three anthers usually remain in the florets (here Typhon).

**Plant material.** Norum, GN9004, and Typhon were used as they represent an AE spectrum from 0-8.

**Experimental design.** The experiments had four plants per genotype where half of the panicles in the pots were used as control. For the toxin analyses it proved necessary to join the samples of each treatment of each genotype to obtain large enough sample sizes.

**Growth conditions.** Planting took place in the greenhouse at SKP November 18$^{th}$ 2013. The greenhouse settings were as in 3.1.1. A plastic tent was raised in the room to give plants higher RH in the days after inoculation.

**Procedure.** When more than 50% of the panicles in a pot had flowered, half of the spikelets in the panicles were emasculated. The other half of the panicles were left as controls. The emasculation was done by opening the florets with tweezers and removing the anthers. The florets were closed and marked following emasculation (Figure 7). The emasculation was done with care to avoid damaging the floret, although some damage (of the husk) was inevitable. To get as many seeds as possible from each plant emasculation was done over a couple of days to allow more spikelets to flower. Florets that had not yet flowered when the emasculation was done were removed.

The emasculated plants were spray inoculated with macroconidial suspension of *F. graminearum* with a spore concentration of 100.000 spores per ml. The panicles were sprayed from three sides (approximately 1.7 ml) and, in the second round of emasculation trial, moved into a plastic tent erected in the greenhouse to increase the RH around the inoculated panicles. The RH in the tent was approximately 100%. The first round of plants had a morning drop in RH during the first 24 h. This might have led to a poorer infection than wanted. The plants stayed in the tent for two days before being moved back in the growth room. Panicles were harvested at yellow maturity.

In a preliminary emasculation trial the moisture of was too high with the RH. These plants had plastic bags over the panicle for three days, followed by RH of 80% in the growth room and too close contact with the nozzles (giving wet panicles). This led to excessive growth of white-pinkish mycelia (Figure 7). To avoid too high infection in the second round of experiment, the time after inoculation with high humidity was decreased to two days and the plastic bag was replaced with a plastic tent.

**Figure 7 – From the preliminary emasculation trial. Emasculated spikelets are marked with black marker and controls have no mark. The over-humid environment led to the extensive growth of Fusarium mycelium, seen as pink pillows on spikelets. The extreme infection lead to something that could resemble head blight in oat, spikelets of a dead panicle are seen in the photo.**

**Toxin analyses.** The toxin data was analyzed by ELISA as in 3.3.2. An unfortunate decision was made when analyzing the emasculation trial. The samples were diluted 1:10, as was fitting the field trial samples since the DON levels there were high. The emasculation samples had much lower values, but at the reading they were thought to be within the curve. Later calculations showed that the values of the diluted samples were under the limit for accurate values of 0.2 ppm. There was not enough left of the dry samples for a rerun, and because of deterioration of DON levels in the extracts these were not kept. The results will anyway be analyzed and discussed, but with knowledge of their uncertainty.

**Statistical analyses.** A paired T-test was used to analyze for differences in DON levels between controls and emasculated samples.

# 4. Results

## 4.1. Genetic variation of the anther extrusion trait and its relation to other traits in oat

**Figure 8 – Left shows a genotype scored with AE 0 and right is agenotype scored with AE 6**

Figure 8 illustrates some of the variation found while scoring. AE 0 shows gaping spikelets, but no observable anthers.

### 4.1.1. Anther extrusion in the Stormogul crosses

**Table 1 – Means and range of AE by year shown for parents and RILs from the predicted numbers from REML analyses for both crosses.**

| **Fiia × Stormogul** | | | | | | | **Svea × Stormogul** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Means** | | | | | | | **Means** | | | | | |
| **Year** | **Stormogul** | **Fiia** | **Parental** | **RILs** | | | **Year** | **Stormogul** | **Svea** | **Parental** | **RILs** | | |
| 2012 | 6.5 | 3 | 4.75 | 3.87 | | | 2012 | 6.5 | 3 | 4.75 | 4.11 | | |
| 2013 | 6 | 3.5 | 4.75 | 3.91 | | | 2013 | 5 | 2 | 3.5 | 3.76 | | |
| Mean | 6.25 | 3.25 | 4.75 | 3.91 | | | Mean | 5.75 | 2.75 | 4.25 | 3.93 | | |
| Range | 0-8.5 | | | | | | Range | 0-9 | | | | | |
| | **Wald** | **n.d.f** | **F** | **d.d.f** | **P** | **SE** | | **Wald** | **n.d.f** | **F** | **d.d.f** | **P** | **SE** |
| Genotype (g) | 3179.84 | 152 | 20.92 | 18 | <0.001 | 0.63 | g | 1880.96 | 152 | 12.37 | 18 | <0.001 | 0.74 |
| Year (y) | 0.24 | 1 | 0.20 | 18 | 0.658 | - | y | 16.72 | 1 | 16.72 | 18 | <0.001 | - |
| g×y | 719.59 | 147 | 4.90 | 18 | <0.001 | 0.89 | g×y | 437.93 | 149 | 2.94 | 18 | 0.005 | 1.04 |

Dominance of high AE was observed in the F1 genotypes by Å. Bjørnstad (2010). The REML analyses showed significant differences for AE between genotypes in both crosses (P <0.001). In S×S the year effect was significant (P <0.001), but the Wald Statistic was much smaller for year effect than for genotype effect. In F×S there was no significant difference in variance between years (P 0.658). In both years both populations had significantly lower means than the parents (Table 1).

Figure 9 of the RIL frequencies shows normal distribution of AE, and tendencies of transgression. A significance level of 0.1 was needed to show any significant transgression using Fisher's Least Significant Difference (LSD). The significant transgression was towards higher AE in both populations apart from S×S in 2013 where there was significant transgression towards lower AE. The presence of genotypes more extreme than the parents in both directions points to both parents adding genetic variance both for higher and lower AE. Transgression might have been more distinguishable in a larger population.

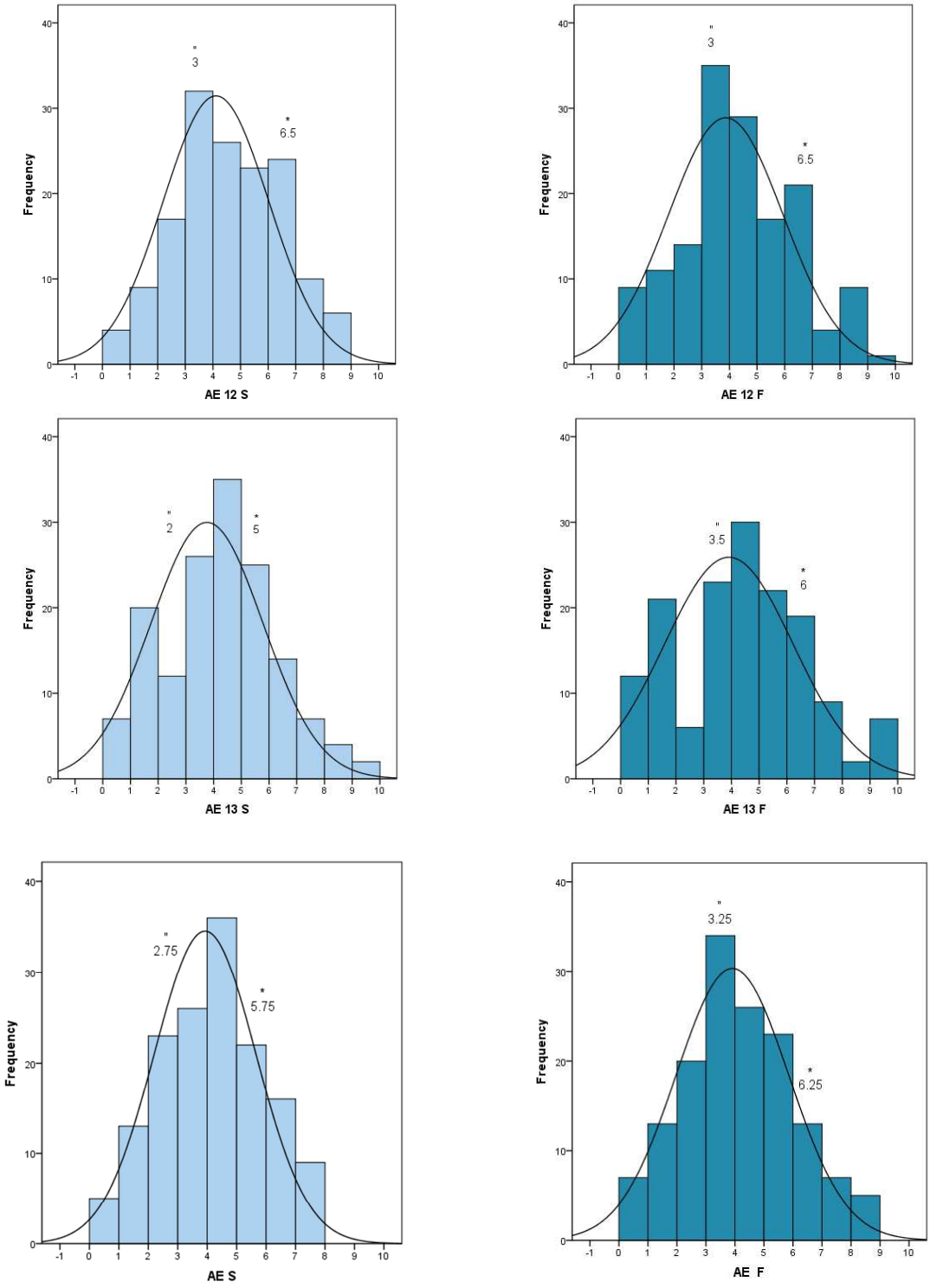



**Figure 9 - Frequency distributions of AE in the Stormogul crosses, showing normal curves. S × S is found to the left and F × S to the right, years are shown from the top and down: 2012, 2013 and the mean of the two years respectively. The parent values are shown above the histogram with Stormogul as the right one.**

**Table 2 – The calculated narrow sense heritability and estimated gene number of the AE trait in the two Stormogul crosses (estimated entries over both years). The error of variance and the variance of the $F_6$ lines is also shown.**

| *Anther extrusion* | $\sigma_L^2$ | $\sigma_E^2$ | $h^2$ | Estimated gene # |
|---|---|---|---|---|
| F×S | 0.7954 | 0.1343 | 0.91 | 6 |
| S×S | 1.097 | 0.1852 | 0.83 | 4 |

Both populations showed high heritability for AE. Because of the larger variance of the S×S population both the narrow sense heritability and the estimated gene number was lower than in F×S.

**Table 3 - Means of height and AE in the two crosses over the two years**

| | Stormogul × Fiia | | Stormogul × Svea | |
|---|---|---|---|---|
| Year | Mean | | Mean | |
| | Height | AE | Height | AE |
| 2012 | 102.37 | 3.87 | 102.69 | 4.11 |
| 2013 | 146.93 | 3.91 | 158.50 | 3.76 |
| Both | 124.65 | 3.91 | 130.60 | 3.93 |

**Table 4- Pearson correlation of height between years and between height and AE (1-tailed significance, $\alpha$=0.5). Significant numbers labelled with*.**

| Stormogul × Fiia | | | Stormogul × Svea | | |
|---|---|---|---|---|---|
| Height | 2013 | AE | Height | 2013 | AE |
| 2012 | .479* | .090 | 2012 | .376* | .045 |
| 2013 | | .096 | 2013 | | -.152* |
| Both | | .108 | Both | | |

**Plant height.** For height (Table 3 and Table 4) the REML analyses showed significant differences between the years (P <0. 001), as illustrated in the correlation of height between the years. The only moderate correlation of height between the two years was at least partly due to poorly adjusted lamps in 2013 in addition to late heading. This yearly difference was particularly strong in S×S which also had more late heading genotypes in 2013 than F×S. The effect of this is seen in the GLM analyses of S×S where height had a significant effect on AE in 2013 (t -2.248, sig .026), while in 2012 it did not (t 1.364, sig .175). However, this correlation to AE (F 2.683, sig .072, $R^2$ .035) was not significant over years. As Table 4 shows, AE and height are generally not correlated in the two crosses.

**Table 5- Pearson correlation between DTF in the two years, and between DTF and AE (1-tailed significance, α=0.5). Significant numbers are labelled with*.**

| Stormogul × Fiia | | | Stormogul × Svea | | |
|---|---|---|---|---|---|
| **DTF** | **2013** | **AE** | **DTF** | **2013** | **AE** |
| **2012** | .773* | -.129 | **2012** | .770* | -.042 |
| **2013** | | -.218* | **2013** | | -.159* |
| **Mean** | | -.199* | **Mean** | | -.131 |

**Earliness, days to flowering.** In general, the deviation between DTF and DTH was not great (±0.9 days). Some genotypes would start flowering after emerging from the leaf sheath, but before the panicle was finished heading. DTH was not analyzed further. For DTF there was a difference between the two years, with the flowering period starting after the same amount of days in the two years but stretching out an additional 8 (F×S) to 20 (S×S) days extra in 2013 (more details can be found in appendix 6). The year difference might be partially due to poorer light conditions in 2013 as the lamps were not adjusted, heading was late (and leading to shorter days, less additional light from the outside, at the time of flowering). But the great difference between populations, and specifically between the Stormogul checks (0-1.1 versus 3.8-4.1), in the two populations indicates unequal environments in the respective rooms the plants were in (most likely temperature). Other controls tha Stormogul were not (significantly) affected. This delay might have caused the year differences seen in the populations (Table 5). DTF and AE showed no correlation in 2012, and a very weak, but significant, negative correlation in 2013 (Table 5).

**Panicle traits.** Table 6 shows the correlation between different panicle traits and AE. The panicle types did not have significant effects in either of the populations, the differences are not large and in addition it is opposite which type has the highest AE. Panicle density did not have a significant effect on AE in S×S, but on F×S. The mean difference was, however, larger in S×S than in F×S (of the panicle traits only matched by mean AE difference of lemma color). This was due to the large differences in sample size where there were very few of the compact panicles (that seem to have an associated with lower AE). Panicle erectness on the other hand had a significant effect on AE in both populations, with highest AE on erect panicles and lower as the panicle was less erect.

**Table 6 - Effect of panicle traits on AE. Independent T –test and univariate ANOVA*, 2-tailed, sig. α =.05.**

| | | Fiia × Stormogul | | | | | Svea × Stormogul | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Panicle trait** | | **N** | **Mean** | **SE** | **t/F*** | **Sig.** | **N** | **Mean** | **SE** | **t/F*** | **Sig.** |
| **Type** | Unilateral | 92 | 3.78 | .21 | -1.04 | .299 | 76 | 4.06 | .21 | 1.00 | .318 |
| | Equilateral | 56 | 4.1 | .23 | | | 74 | 3.78 | .19 | | |
| **Density** | Compact | 4 | 2.88 | .66 | -1.57 | .280 | 18 | 3.06 | .4 | -2,34 | .021 |
| | Wide | 144 | 3.96 | .16 | | | 131 | 4.06 | .15 | | |
| **Erectness** | Erect | 35 | 4.37 | .36 | 4.662* | .010 | 29 | 4.38 | .3 | 4.58* | .012 |
| | Semi-erect | 73 | 3.83 | .24 | | | 65 | 4.2 | .22 | | |
| | Drooping | 40 | 3.65 | .25 | | | 55 | 3.4 | .22 | | |
| **Awned** | Awned | 109 | 3.98 | .13 | -.717 | .474 | 108 | 3.9 | .16 | .47 | .640 |
| | Not | 39 | 3.82 | .18 | | | 40 | 4.05 | .29 | | |
| **Color** | White | 74 | 3.17 | .21 | 5.2 | .000 | 78 | 3.59 | .18 | 2.54 | .012 |
| | Brown | 73 | 4.7 | .20 | | | 72 | 4.3 | .21 | | |

Further, Table 6 shows no effect of awnedness on AE. Lemma color had significant correlations to AE in both populations with large mean differences (Table 7), especially in F×S. Brown lemmas are associated with higher AE than white lemmas.

**Table 7 - Independent t-test result of color in RIL; Mean difference in AE, significant differences are marked with *.**

| **Fiia × Stormogul** | | **Svea × Stormogul** | |
|---|---|---|---|
| **Seed color** | **Brown** | **Seed color** | **Brown** |
| **White** | 1.53 * | **White** | .71 * |

### 4.1.5. Screening of the oat core collection

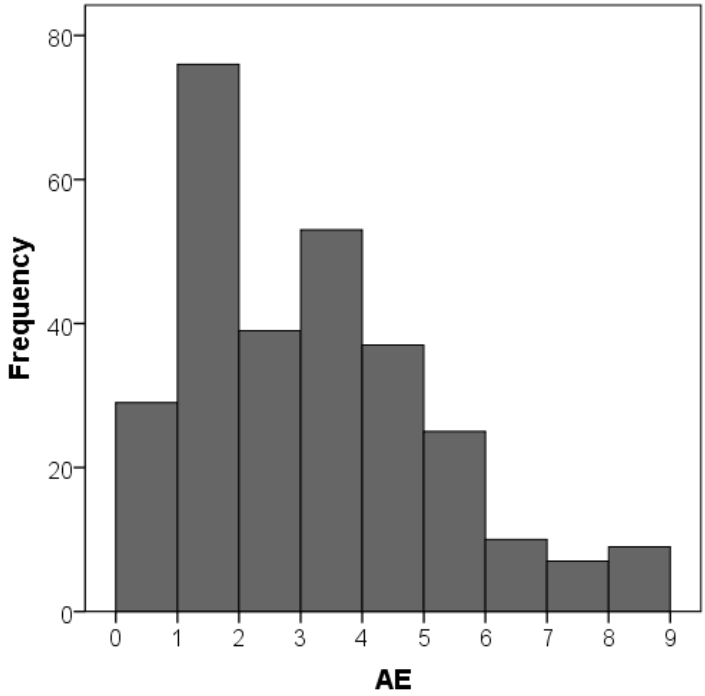



**Figure 10 - The distribution of AE in the core collection. The mean AE was 2.75, with .836 as SE of the controls.**

**Table 8 - One way ANOVA of AE between genotypes in the core collection with 1-tailed significance, α 0.05.**

| AE | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| **Between Groups** | 1124.486 | 145 | 7.755 | 8.188 | .000 |
| **Within Groups** | 134.500 | 142 | .947 | | |
| **Total** | 1258.986 | 287 | | | |

There was genetic variation of AE in the core collection (Figure 10, Table 8). The main part of the collection had AE scores between 0 and 6. The standard error of the controls showed a relative stable scoring and stability of genotypes.

**Table 9 – Pearson correlation between AE and three traits with * 1-tailed significance, $\alpha$ 0.05.**

| Trait | Correlation | Pearson correlation |
|---|---|---|
| **Days to flowering** | None | -.073 |
| **Plant height** | None | .141* |
| **Year of release** | Moderate | -.363* |

**Correlation of earliness, days to flowering, and year of release.** Linear regression was used to look for correlation between AE and DTF, plant height and year of release by genotype. Table 9 shows that only year of release had a moderate negative correlation to AE ($R^2$ .132, t – 5.231, sig .000). A paired T-test showed no significant difference between replications in any of the three traits: AE, days to flowering and height (respectively: t 0.061, sig .091; t 1.703, sig .952; t -1.843 sig .067).

**Panicle traits.** In the core collection panicle type (Table 10) had a significant correlation to AE, unlike in the Stormogul crosses, with equilateral panicles having lower AE. Similarly with F×S panicle density was not significant, but in the case of the core collection the AE means was almost identical. Color was significantly different in the core collection, as in both crosses, with the brown/black colored lemmas correlated to higher AE. There was no difference between white and yellow lemmas in AE (Table 11)

**Table 10 - Effect of panicle traits on AE. Independent T –test and univariate ANOVA*, 2-tailed, sig. α = .05.**

**Core collection**

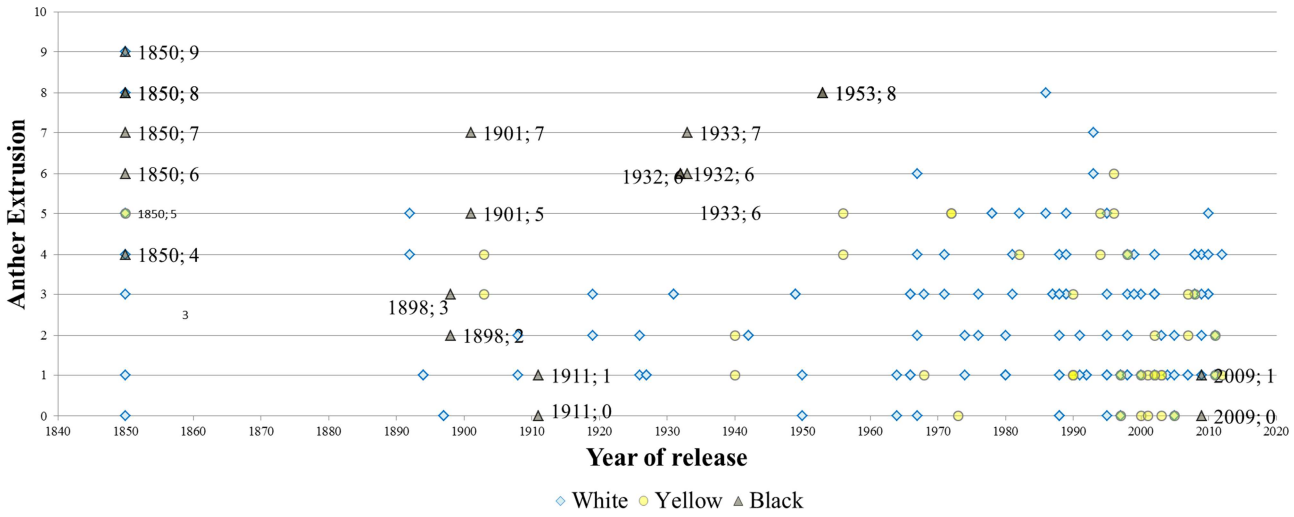
| Panicle trait | | N | Mean | SE | t/F* | Sig. |
|---|---|---|---|---|---|---|
| **Type** | Unilateral | 151 | 3.1 | .17 | 2.72 | .007 |
| | Equilateral | 137 | 2.41 | .18 | | |
| **Density** | Compact | 18 | 2.72 | .50 | .73 | .942 |
| | Wide | 270 | 2.76 | .13 | | |
| **Color** | White | 193 | 2.55 | .14 | 9.37* | .000 |
| | Yellow | 55 | 2.53 | .27 | | |
| | Brown | 40 | 4.05 | .4 | | |

**Table 11 - Tukey HSD of color in the core collection; Mean difference in AE, significant differences are marked with *.**

**Core collection**

| Seed color | Yellow | Brown |
|---|---|---|
| **White** | 0.03 | 1.50 * |
| **Yellow** | | 1.52 * |

**Figure 11 - The spread of seed color in the core collection over anther extrusion and year of release.**




**Year of release.** Figure 11 shows how the predominance of different seed colors change with year of release of genotypes in the core collection. In table 11, the mean AE difference between the three seed colors are shown. The regression analyses (Table 9) gave a weak trend towards lower AE over years, but the trend could also be read as towards less variation (Figure 11). Especially from 1995 and onwards less variation can be found in AE, with variation gathering at the lower end of the scale. Most of the genotypes from before 1990 were of norwegian or swedish origin, so to compare AE between countries only genotypes released in the time span 1990-2012 were analysed (Figure 12). The Norwegian and Swedish genotypes did not show a clear trend within this timespan with values between 0.5-4, 1-4.5 respectively. The German genotypes showed the least variation of all, with AE values between 0-2.5. The Finnish genotypes of this collection move towards lower AE (linear regression: ($R^2$ .765, t – 6.746, sig .000). Panicle type and panicle density have opposite results in the two populations in regards to significant differences between groups. When a univariate ANOVA was run on RILs with all panicle traits as fixed variables (-awn because of inference) only color had a significant effect on AE (F 9.381, sig .002).

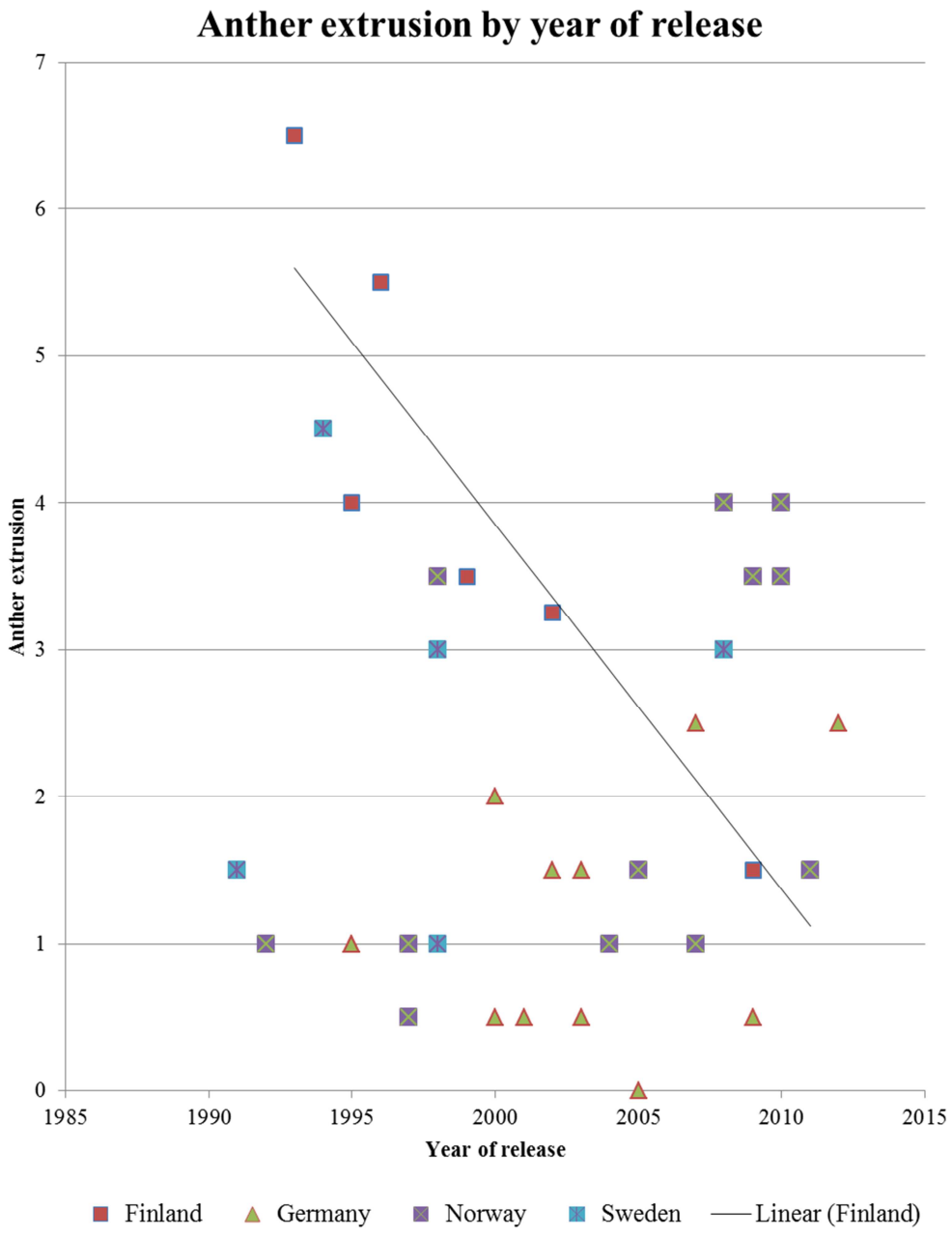



**Figure 12 - Anther extrusion by years - 1990-2015. (The regression line for the Finnish genotypes has a fit of $R^2$ .765)**

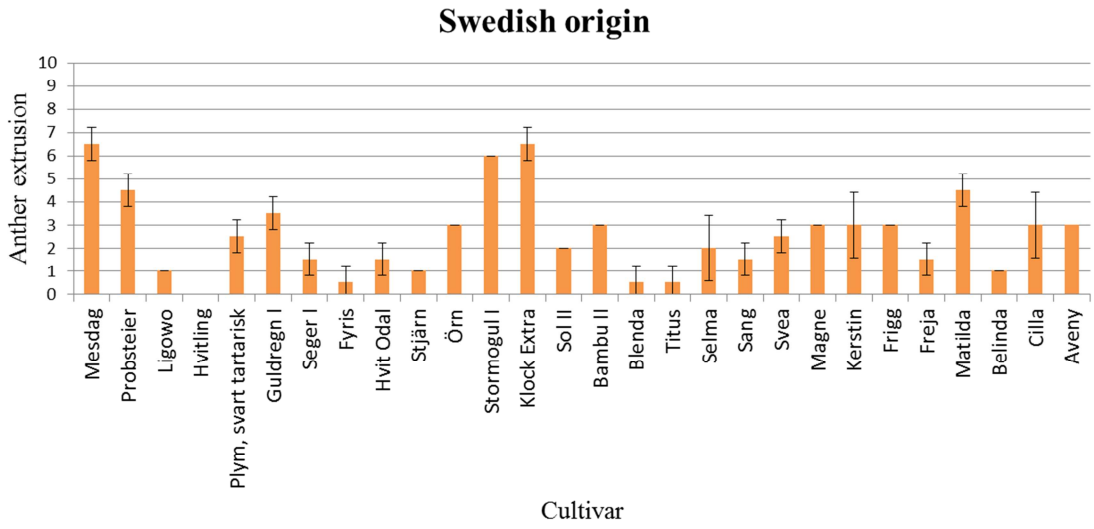



**Figure 13 - The average AE of Swedish genotypes arranged by year of release**

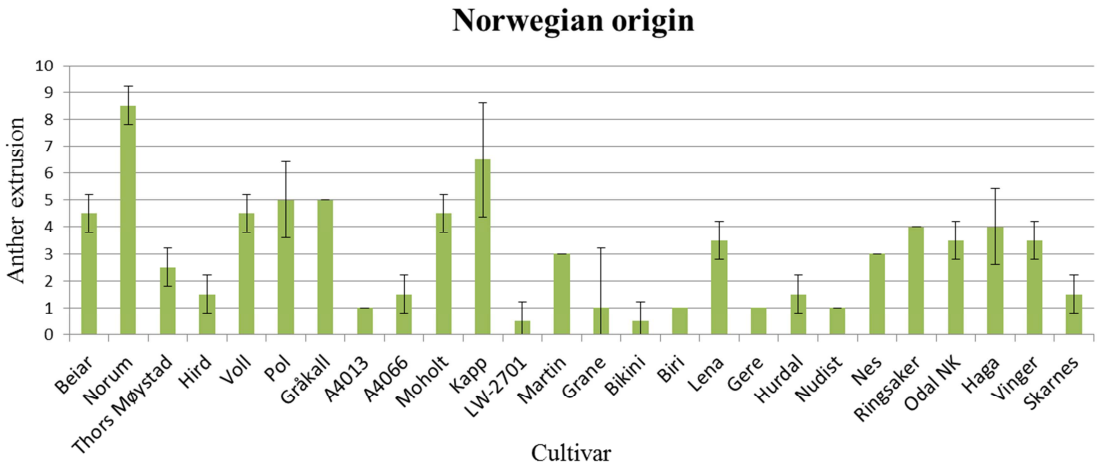



**Figure 14 - Average AE of Norwegian genotypes arranged by year of release**

**Country of origin.** Figure 13 to Figure 16 display the AE of genotypes from Sweden, Norway, Germany and Finland respectively. The German genotypes clearly stood out having low AE scores. Univariate ANOVA showed a significance effect of country of origin on AE (f 9.2, sig .000). A Tukey HSD was also run on the data for multiple comparisons, only three of the five countries analyzed for the years 1990-2012 differed significantly; Germany, Norway, and Finland (Table 12).

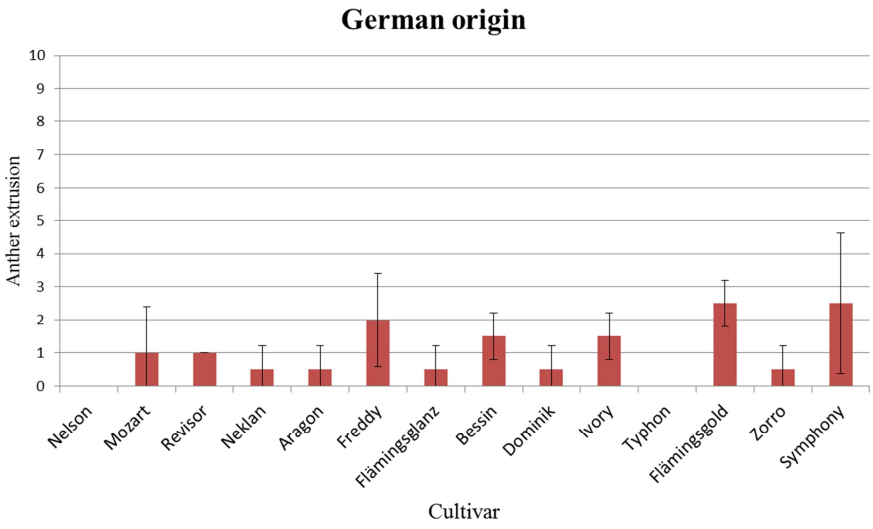



**Figure 15 - Average AE of German genotypes arranged by year of release**

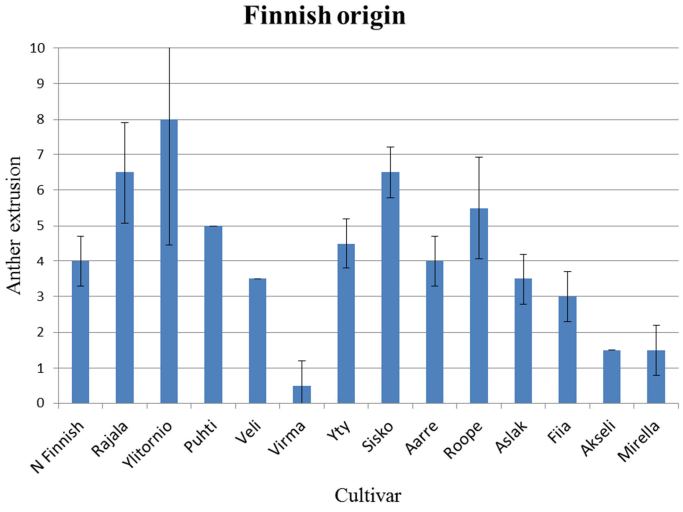



**Figure 16 - Average AE in Finnish genotypes arranged by year of release**

**Table 12 – Significances between countries in AE, those significantly different are marked. (Tukey HSD with mean square(error)=1.881, α=0.05)**

| Mean AE | Country | Finland | Sweden | Norway | USA |
|---|---|---|---|---|---|
| 3.64 | **Finland** | | | | |
| 2.56 | **Sweden** | .342 | | | |
| 2.33 | **Norway** | .036 | .994 | | |
| 1.50 | **USA** | .053 | .704 | .704 | |
| 1.11 | **Germany** | .000 | .055 | .016 | .983 |

## 4.2. Environmental sensitivity and stability of anther extrusion

### 4.2.1. Effect of drought on anther extrusion

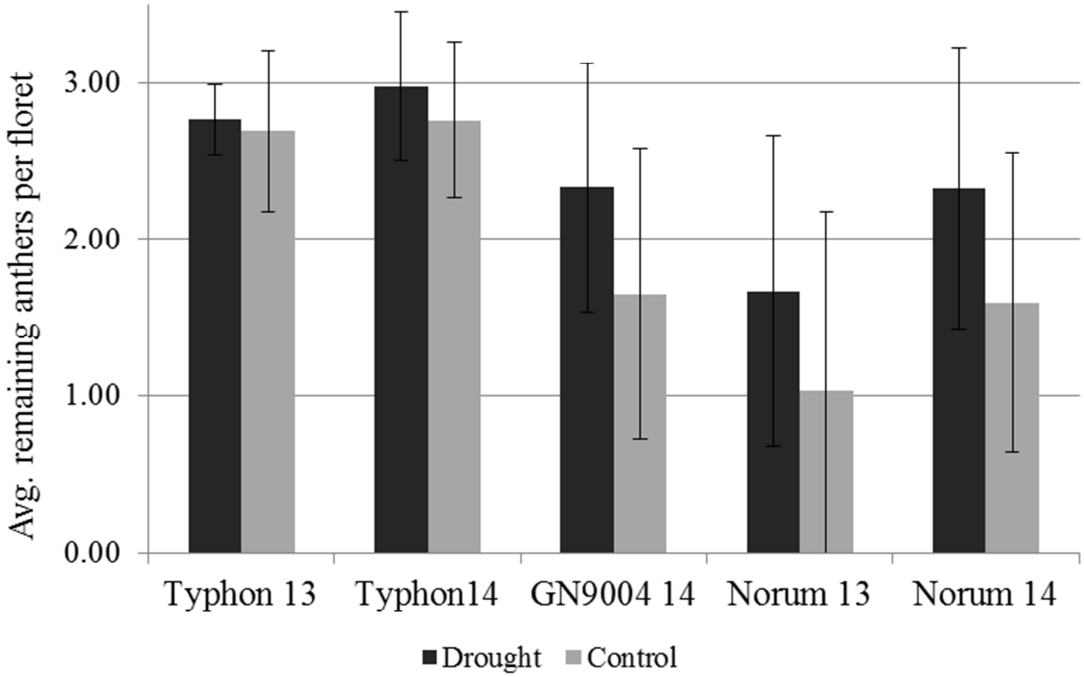



**Figure 17 - Drought effect on the number of remaining anthers for the different genotypes. The genotypes are sorted from high to low AE. A difference can also be seen between years.**

Figure 17 shows the drought effect on the three different genotypes in two subsequent years. All had a higher average of remaining anthers under drought conditions than under control conditions, with a greater response in higher AE genotypes. A paired samples t-test showed significant differences in all genotypes between drought and control. The drought response is clear in the experiments although the difference in the number of remaining anthers is great between the two experiments for the same genotype. In 2014, the number of remaining anthers was higher for both Typhon and Norum, and the difference between control and drought slightly lower (Table 13).

**Table 13 – Paired samples t-test, showing the mean differences between the two treatments, drought and control, by genotype in 2013 and 2014.**

| Genotype | Year | Mean difference | SE Mean | t | Df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|
| **Typhon** | 2013 | .218 | .043 | 5.068 | 164 | .000 |
| **Typhon** | 2014 | .060 | .057 | 1.054 | 150 | .294 |
| **Norum** | 2013 | .641 | .088 | 7.322 | 269 | .000 |
| **Norum** | 2014 | .522 | .090 | 5.770 | 231 | .000 |
| **GN9004** | 2014 | .612 | .109 | 5.640 | 133 | .000 |

In order to examine the effect of drought better the remaining anthers were looked at. The florets were grouped by the number of remaining anthers (3, 2, 1, or 0) and shown as the percentage of total number of florets (Figure 18). The figure shows that Norum clearly has the highest percentage of florets where all anthers are shed. The fraction of florets where all anthers remain after flowering increased strongly under drought conditions in Norum and GN9004. With limited chance of less extrusion Typhon still showed a marked response to drought (20% more florets with remaining anthers in September 2013 and 5% change in 2014). The change in % florets with all anthers remaining was larger between drought and control in the 2013 (September) experiment, while the % florets with no remaining anthers was larger in the 2014 (February) experiment. There were large differences between the drought trial in autumn 2013 and winter 2014, where the Norum control has 24% florets with no remaining anthers in 2013 against 40 % in 2014.

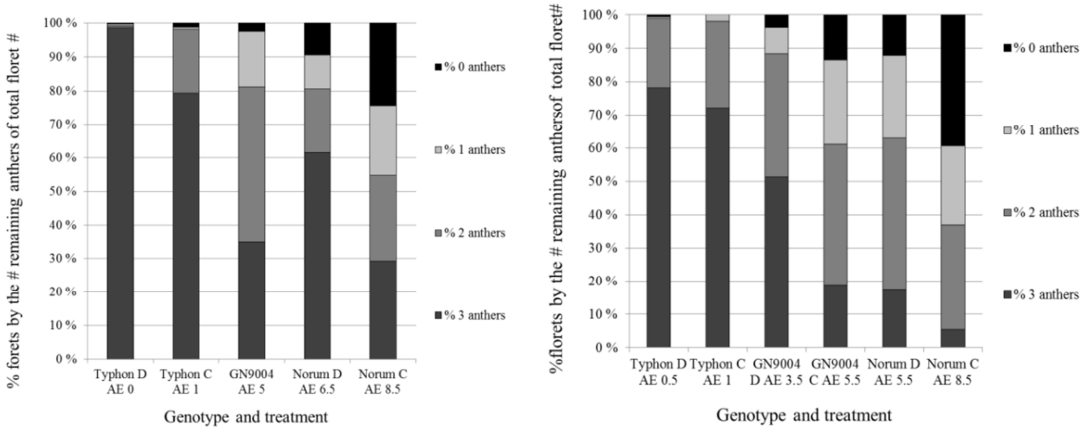



**Figure 18 – The percent of florets with 3, 2,1, and 0 remaining anthers of total florets counted in the drought trials of 2013 (left) and 2014 (right). The genotypes are sorted from low to high AE. D=drought, C=control.**

### 4.2.2. Degree of anther extrusion

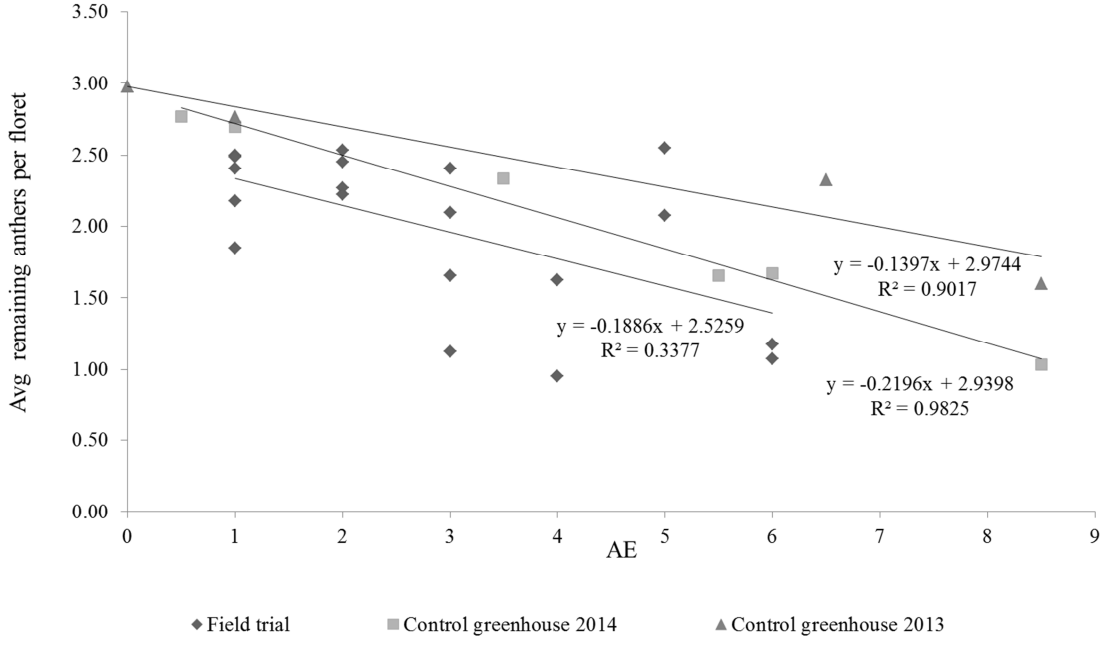



**Figure 19 – Correlation between the number of anthers remaining in florets after anthesis and the scored AE value of the controls in the two drought trials and the *Fusarium* field trial.**

Figure 19 shows the regression lines of control counts from three trials: the field trial and the controls of the two drought trials. The average remaining anthers per floret found in the control count was plotted against the AE scores. The three regression lines have slightly different slopes, and also different fits. The slope differences represent how the AE scale was scored in the different trials, and the differences in fit clearly show that the field environment gives much less precise estimation of AE than the greenhouse. The figure also shows that in none of the cases (drought controls) the average number of remaining anthers was low enough for the highest scores given. The AE 0 scores were more correct. As no average was below a mean of 1 a AE higher than ~6 should not have been scored.

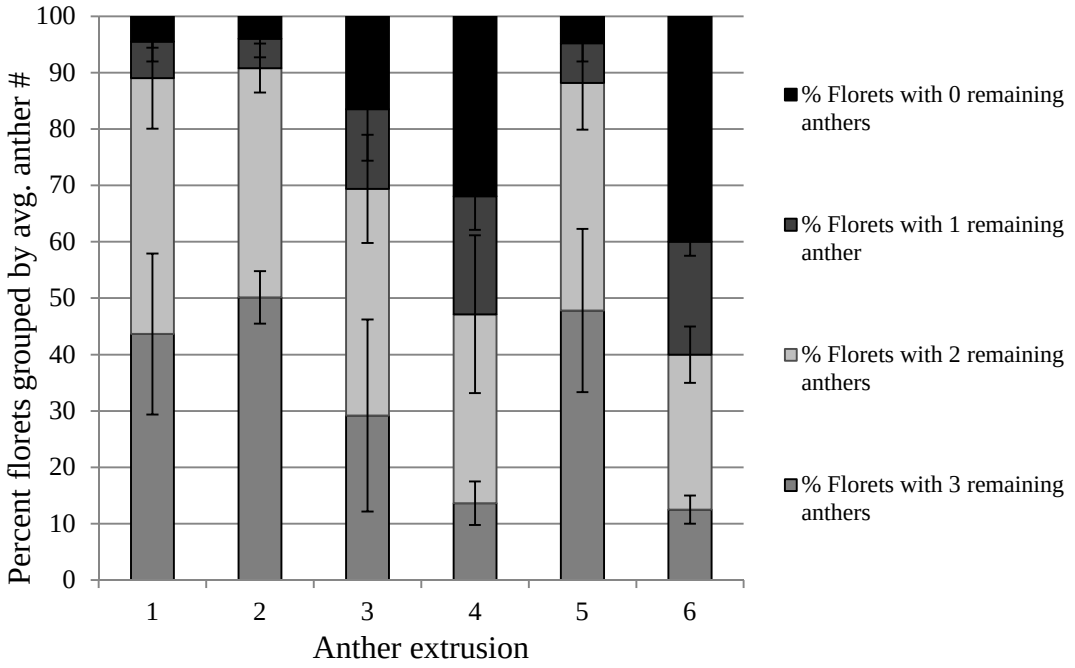



**Figure 20 - Percent florets containing 0, 1, 2 or 3 anthers after anthesis (standard deviations between panicles). From the *Fusarium* field trial in 2013.**

The controls from the field trial (Figure 20) show that the AE scored does not follow the expected pattern of remaining anthers. The genotypes scored with AE 5 break the pattern as they showed a distribution of remaining anthers close to the distribution found in AE 1 and AE 2. The distributions of the genotypes scored with AE 1,2, and 5, were so close that they could be expected to show the same AE. Compared to the distributions from the drought controls results (Figure 18) the AE 5s in Figure 19 were overestimated while AE 4 and AE 6 were underestimated. In the field trial as much as 40% of the florets were without anthers of those scored with AE six (Figure 20), almost equivalent to the Norum control in the greenhouse that was up to 39% with a score with AE 8.5.

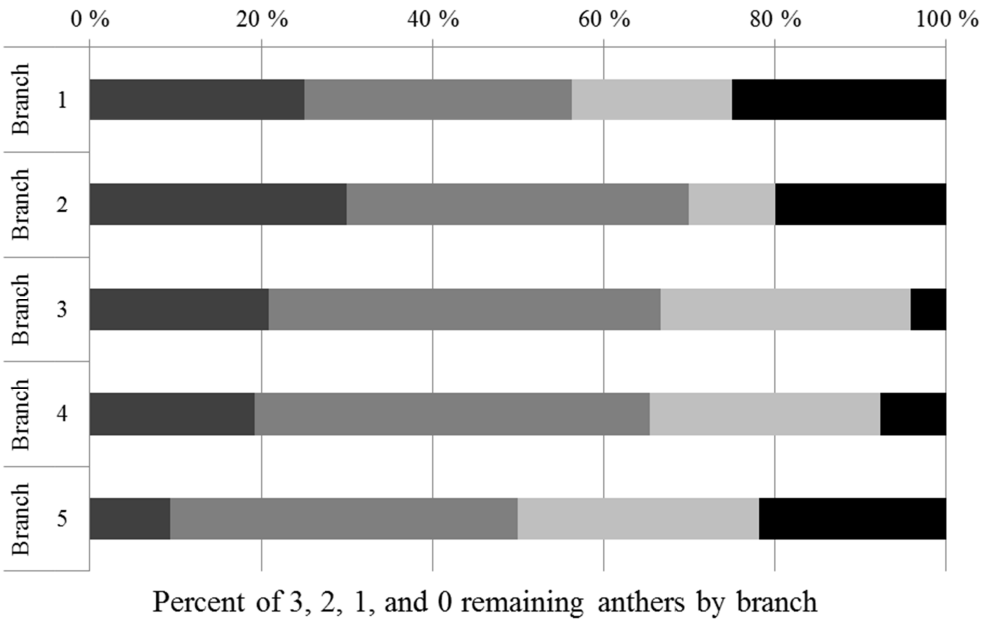



**Figure 21-Remaining anthers by branch. The percentages of florets with 3, 2, 1 or 0 remaining anthers of total floret number sorted by branch, in Norum. Branches are numbered from the top of the panicle.**

Figure 21 shows the distribution of remaining anthers by branch number. Since Norum had shed most anthers it was expected to have most variation. Univariate ANOVA of the Norum controls from the drought trial in September 2013 showed no significant differences between branches (F 1.026, Sig .403), but there was a significant difference between the 1st and 2nd floret (Figure 22), analyzed using independent t-test (t 42,953, sig .000).

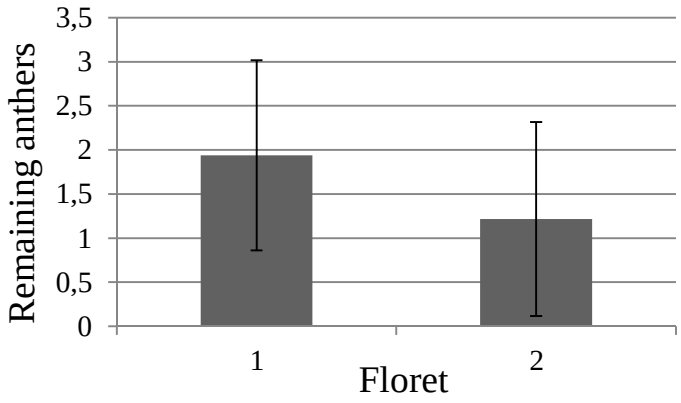



**Figure 22 - Floret numbers and remaining anthers. Florets are counted from the base of the spikelet.**

### 4.2.3. Sensitivity of anther extrusion to auxin

The first attempt with auxin response in whole panicles had no visible response. As the flowering period of oat is so stretched out in time a whole panicle could not be expected to react. In Wang's (2013) article it was stated that many drew wrong conclusions about lodicules not responding to auxin, because they did not take the very short period where lodicules were auxin responsive into account.

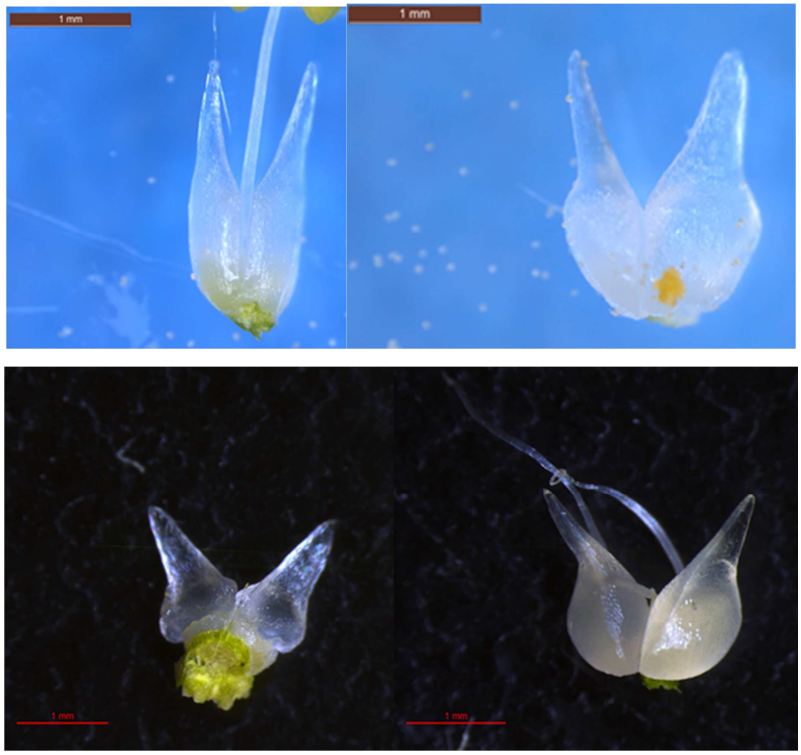



**Figure 23 – Top: Lodicues from Norum pre anthesis and post auxin treatment. Bottom: Typhon pre anthesis and post auxin treatment.**

Lodicules from all three oat genotypes reacted to the auxin treatment. Lodicules from Norum and Typhon before and after swelling can be seen in Figure 23. Lodicule depth, width and area with standard error are listed for every genotype in Table 14, both in untreated and treated state. A univariate ANOVA showed that both genotype and stage (before and after swelling) had significant effect on the depth (respectively F 32.914, sig .000, F 200.929 sig .000).

**Table 14 – Lodicule depth (mm), width (mm) and area ($mm^2$) with means and standard errors, before and after swelling by genotype. The three barley controls are in green.**

| Measurement | Genotype | Untreated | | | Treated | | |
|---|---|---|---|---|---|---|---|
| | | **N** | *Mean* | *S.E* | **N** | *Mean* | *S.E* |
| Depth | Golden Promise | 41 | .072 | .018 | - | As before | |
| | Tampar | 32 | .054 | .020 | 14 | .624 | .031 |
| | Tiril | 14 | .077 | .035 | - | As before | |
| | Norum | 11 | .379 | 0.35 | 5 | 1.08 | .052 |
| | Typhon | 6 | .219 | 0.47 | 11 | .772 | .035 |
| | GN9004 | 3 | .474 | .067 | 1 | .953 | .115 |
| Width | Norum | 4 | .372 | .196 | 12 | .882 | .113 |
| | Typhon | 2 | .067 | .277 | 12 | .931 | .113 |
| Area | Norum | 8 | 1.378 | .062 | 5 | 1.857 | .079 |
| | Typhon | 5 | 1.385 | .079 | 12 | 1.905 | .051 |
| | GN9004 | 1 | 1.160 | .176 | 2 | 1.908 | .124 |

A Tukey HSD (Table 15) showed the grouping of genotypes by depth. The three oat genotypes were not significantly different from each other, but significantly different from the two barley groups. The two significantly different barley groups are the cleistogamous and the chasmogamous (Tampar), showing that the effect of genotype in the ANOVA rather should be described as a genus effect. By analyzing the oat on its own genotype effects were found on depth (F 4.932 sig .014). But the genotype differences in depth change showed no connection with AE in oat, and were not comparable to the barley groups differences. The size of lodicules both before and after swelling on the other hand does coincide with AE. Width and area did not show any effect of genotype.

**Table 15- Homogenous subsets of lodicule depth using a harmonic sample size of 12.273, a mean square (error) of .013 and α=.05.**

**Tukey HSD, homogenous subsets of lodicule depth**

| | | |
|---|---|---|
| 41 Golden Promise<br>14 Tiril<br>Sig. 1.000 | | |
| | 46 Tampar<br>Sig. 1.000 | |
| | | 17 Norum<br>4 GN9004<br>16 Typhon<br>Sig .998 |
| Barley | | Oat |

### 4.2.4. Filament studies


**Figure 24- Norum before (left) anthesis, at the start (middle) of anthesis and at towards the end (right) of anthesis (stage 1, 2, and 3 respectively).**

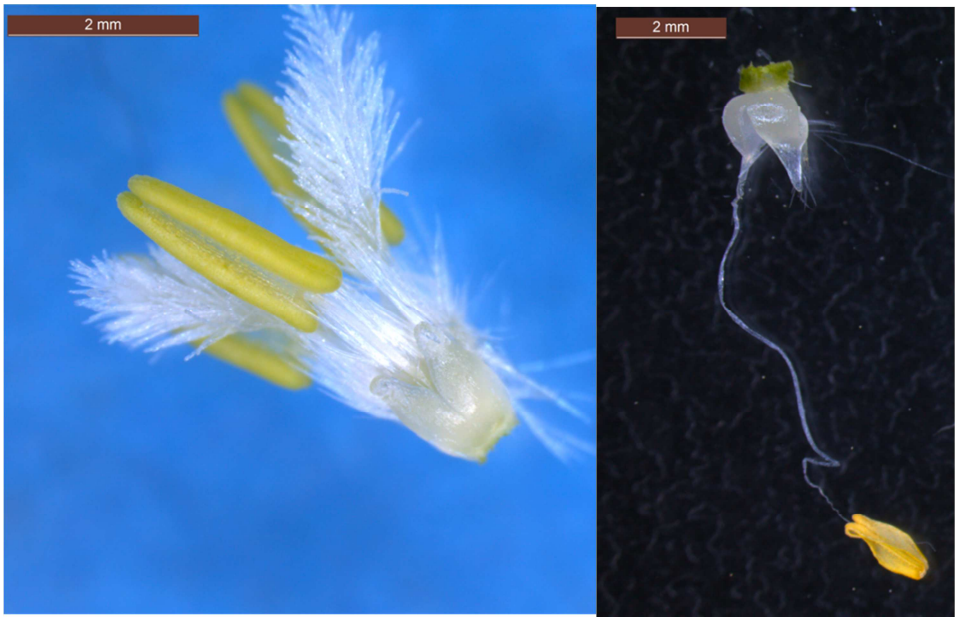



**Figure 25 - Typhon before (left) filament extension and at end (right) extension, (stage 1 and 3 respectively).**

Figure 24 and Figure 25 show filament extension in Norum and Typhon. Florets prior to filament extension can be seen to the left in both figures; both pictures are taken with filaments sitting together with ovary and lodicules. The pictures to the right show filaments that are fully (or close to fully) extended. The middle photo in figure 22 show filaments under extension; they still have the fat, rigid look of the unextended filaments, but an undulating shape.

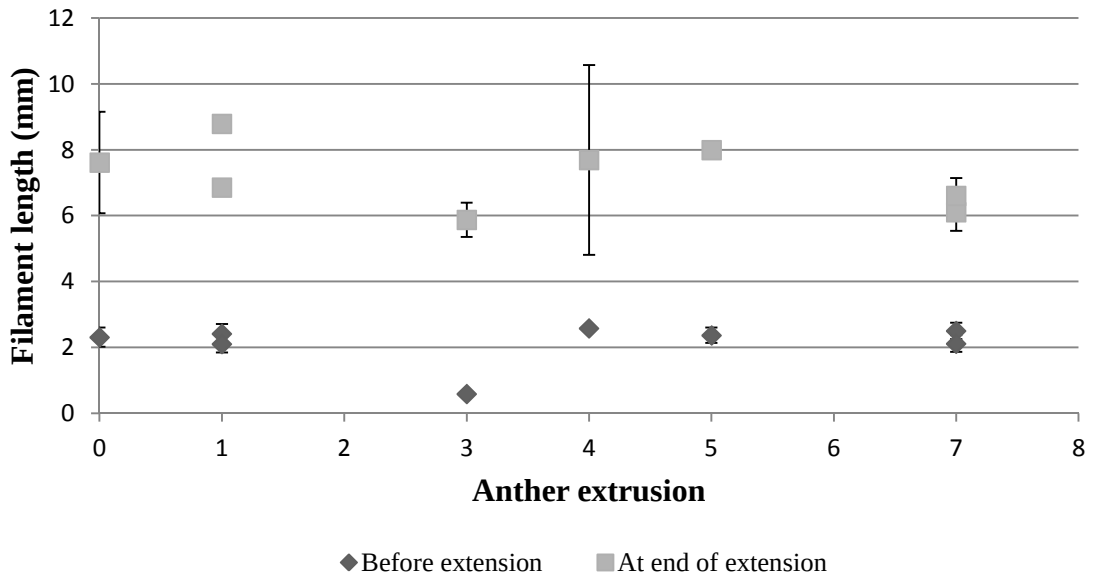



**Figure 26 – Filament length (mm) before and after filament extension plotted against AE. The numbers are average lengths of selected FXS lines (2013), including Norum (at AE 7) and Typhon (at AE 0).**

Figure 26 shows the filament length of F×S lines before and after filament extension. The figure shows that there was no sign of effect of genotype or AE on filament length. A univariate ANOVA (IBM SPSS Statistics 19) confirmed this by not showing any significant differences between the genotypes (F 1.192, Sig .311). A clear difference in filament length was seen before and after extension in all lines (F 241.392, sig .000).

**Table 16 – Table showing average filament lengths in stage 1 and 3, and maximum length measured for the selected FXS lines and Typhon and Norum. * Both accession number and AE are from 2013. * Values based on only one measurement.**

| AE | RIL | Average length by stage | | Max length |
|---|---|---|---|---|
| | | 1 | 3 | |
| 0 | Typhon | 2,31 | 7,61 | 9,85 |
| 1 | 146 | 2,10 | 6,85* | 6,85 |
| 1 | 2 | 2,41 | 8,79* | 8,79 |
| 3 | 85 | 0,58 | 5,87 | 6,59 |
| 4 | 86 | 2,58* | 7,69 | 12,61 |
| 5 | 134 | 2,37 | 7,99* | 7,99 |
| 7 | 32 | 2,50 | 6,11 | 6,68 |
| 7 | Norum | 2,12 | 6,60 | 7,14 |

## 4.3. Anther extrusion and deoxynivalenol in oat

### 4.3.1. *Fusarium* greenhouse trial

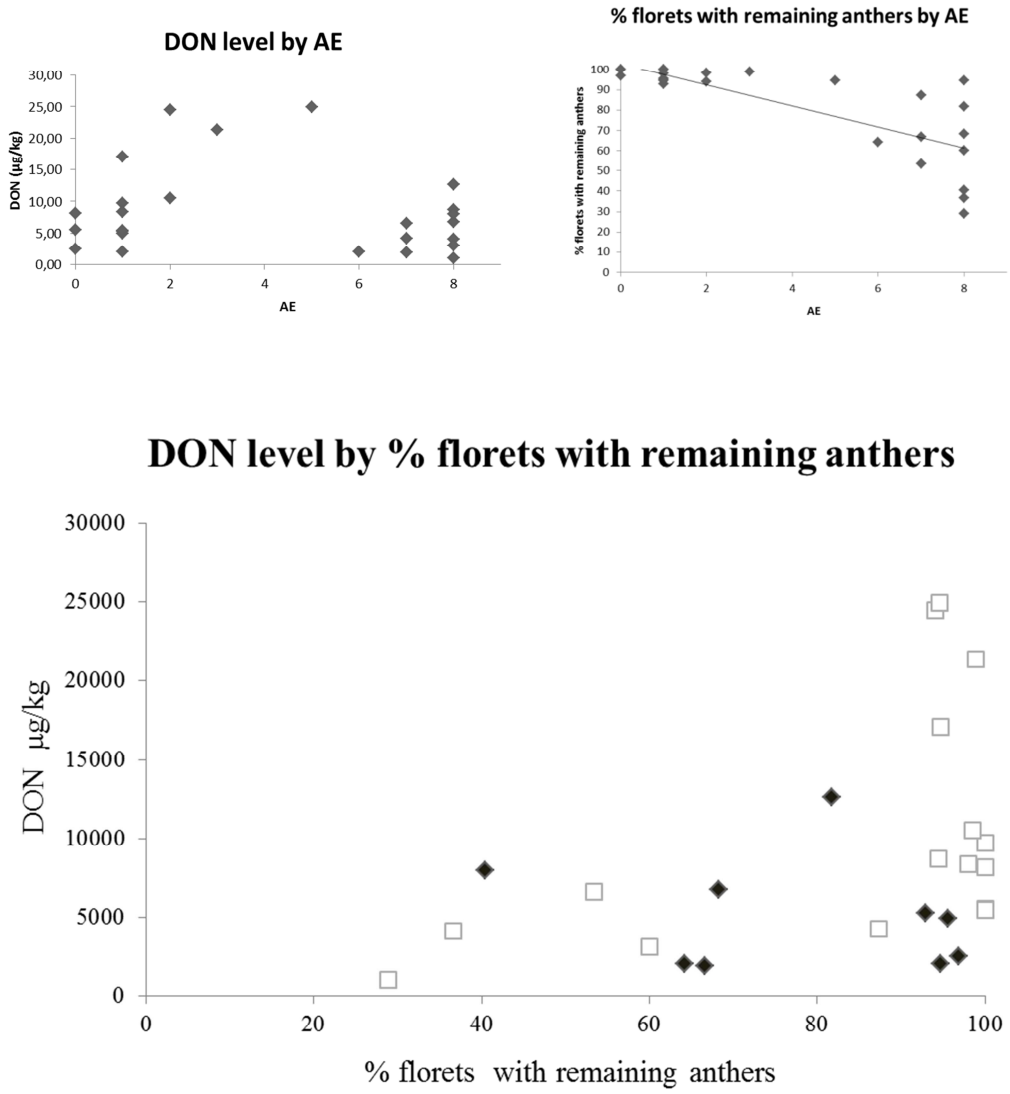



**Figure 27 - Top left: The correlation between deoxynivalenol and AE, $R^2$=.016. Top right: Correlation between AE and % florets with remaining anthers of total floret number in the panicle, $R^2$=.540. Bottom: Deoxynivalenol (µg/kg) levels from greenhouse *Fusarium* trial in 2012 correlated with the % of florets with remaining anthers, $R^2$=.332.**

In the main figure in Figure 27 a fan shape is seen where the highest DON values are found when <80 % of florets had anthers remaining (1-3 anthers). None of the genotypes with black lemma color were among the ones with highest DON content. The relationship between AE and DON (top left) did not show a clear trend, with the low-intermediate AE types having the highest DON levels. This can be explained, at least partially, by the fan shape relationship between % florets with remaining anthers and AE (top right) where the

preciseness of scoring seem to go down as AE goes up. The highest DON values were found from AE 2 to AE 5. A linear correlation is not ideal to describe a fit as other underlying resistance mechanisms are expected to give a fan shape. Other resistance mechanisms could also be the reason for the low DON levels of AE 0 and AE 1, as they have close to 100% florets with remaining anthers a group that has the whole range of DON levels.

A linear regression (although as said not ideal for studying resistance mechanisms) was run on DON and the other registered traits in the *Fusarium* greenhouse trial. Apart from the moderate positive significant correlation between DON and percent florets with remaining anthers, DON had a strong negative correlation to DTF and a moderate negative correlation to PH (Table 18). The majority of data points are below 17500 µg DON/kg oat which might let outliers affect the DTF and PH equations too strongly at high DON content (Figure 28Figure 24). Most of the plants flowered around 60 days after sowing. Days to flowering had a strong correlation to plant height, but this did not cause any problems with colinearity when run together in a linear regression as they both had VIF of 1.5. Percent florets with remaining anthers also have moderate and weak significant correlations to DTF and PH. This could describe the genotype that sheds most anthers as late flowering and tall, which matches the description of Stormogul.

**Table 17 - Pearson's correlation between deoxynivalenol (µg/kg) content, AE, days to flowering, plant height, and % florets with remaining anthers in the *Fusarium* greenhouse trial. Significant values* - 1-tailed significance.**

**Greenhouse trial**

| Mean and st. deviation | | Pearson correlation | | | |
|---|---|---|---|---|---|
| | | DON | AE | DTF | PH |
| 8776.81 ± 8084.37 | DON | 1.000 | -0.125 | -0.637* | -0.342 |
| 3.91 ± 3.26 | AE | -0.125 | 1.000 | 0.174 | 0.124 |
| 59.43 ± 3.83 | DTF | -0.637* | 0.174 | 1.000 | 0.584* |
| 99.13 ± 13.90 | PH | -0.342* | 0.124 | 0.584* | 1.000 |
| 0.83 ± 0.22 | FRA | .332* | -.735* | -.322* | -.245* |

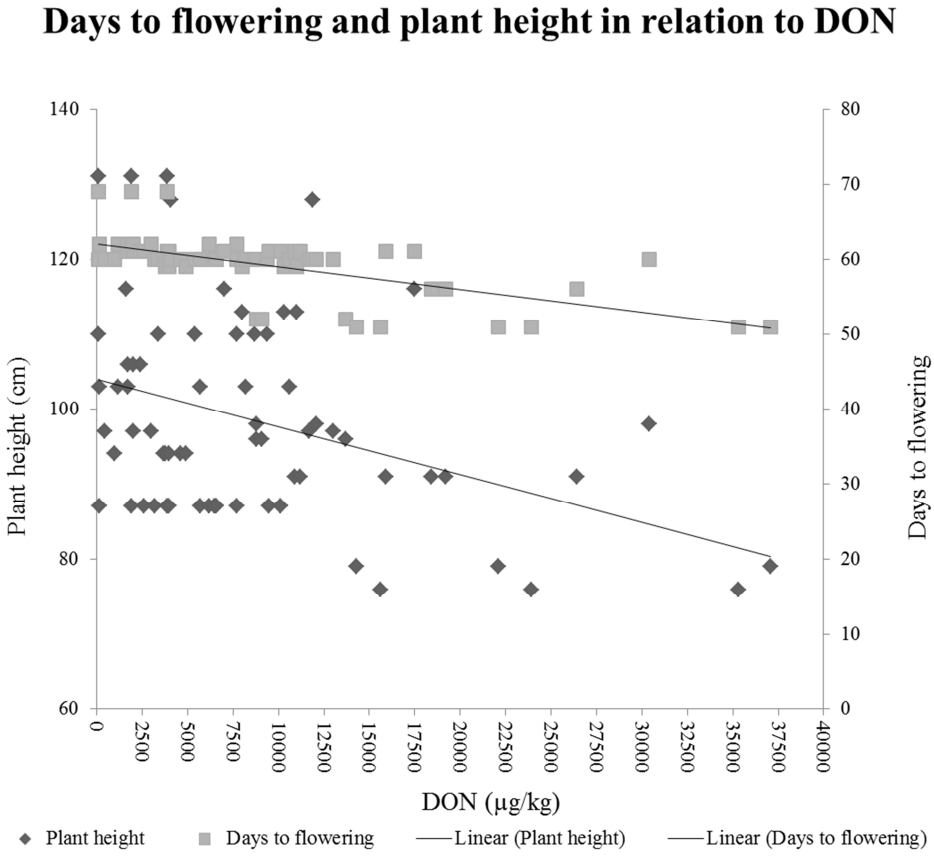



**Figure 28 – Correlation between DON and days to flowering(y=88.629-1.344(x/1000), $R^2$=.406), and DON and plant height (y=28.513-.199(x/1000), $R^2$=117), in the greenhouse *Fusarium* trial. The plant height for the genotypes was gathered from the Stormogul cross screenings in greenhouse 2012.**

### 4.3.2. *Fusarium* field trial

The seed multiplication in New Zealand had been problematic because of phenological issues. Many plants did not head, or took very long to head resulting in poor seed quality. Many of the seeds had a more "old fashioned" shape with longer and thinner kernels than the more modern ones. These seeds plugged the sowing machine resulting in mixed up plots with different phenotypes. Another issue in the field was the dry conditions seemingly holding back anthesis (Figure 6) which may have affected the results. Although there were no sign of drought stress neither on the plants nor on the soil surface, the plants clearly reacted to the extra hour of watering. The subsequent day, July 23$^{rd}$, a large part of the plots flowered throughout the main part of the panicles, enough for the flower opening to be heard in the field. Many of the plots had the first flowers open, and therefore DTF already registered, before the extended watering period. The reaction of the plants to the extra water was still clear. DTF shows a peak after a few mm of rainfall on July 16$^{th}$ – 18$^{th}$.

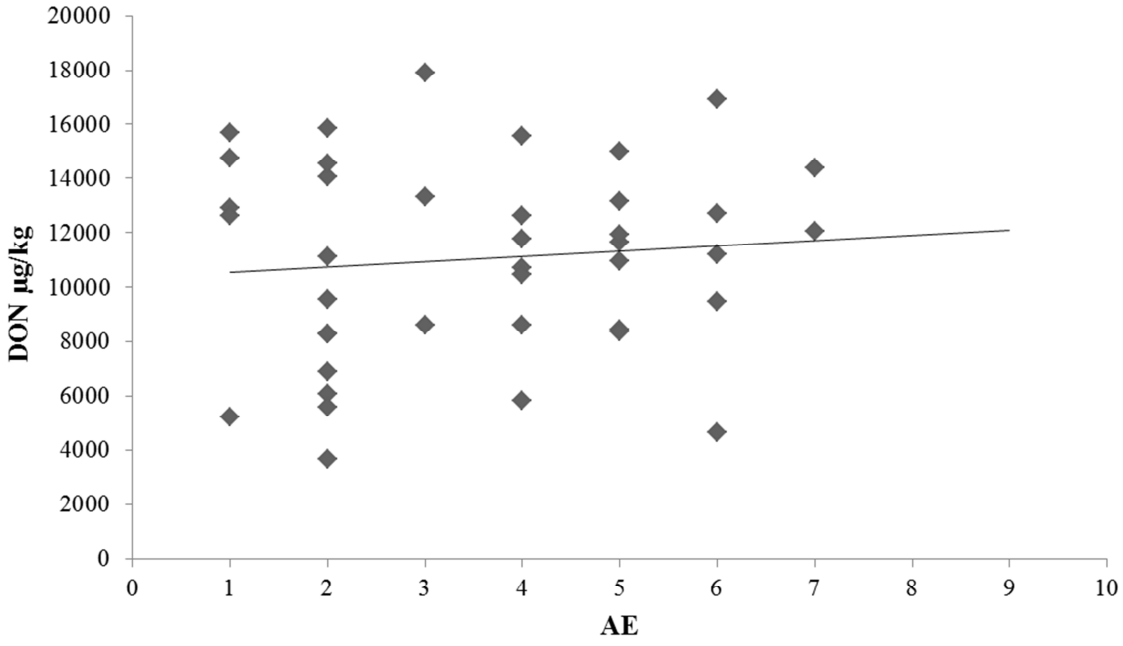



**Figure 29 – Correlation between DON content and AE in the field trial, $R^2$=0.02.**

Figure 29 shows a very poor linear relationship between the registered AE and DON level in the field trial ($R^2$=0.02). A poor linear relationship does not necessarily mean any connection as other resistance mechanisms also participates, but here there is a general lack

of any pattern or tendency. DON level and AE was not correlated in the field trial, and no correlation was found between DON and the other traits either (Table 18). The poor estimation in field is likely to have given poor results (Figure 19).

The ELISA procedure gave variable results when compared to available GC-MS results and controls. There was no particular pattern in the deviation. ELISA gave both higher and lower values than GC-MS. The deviations of the repetitions in ELISA were much smaller than the deviation between the methods, which shows that the ELISA result in some ways were stable enough. Although most numbers were in the area 2-4ppm above or below the GC-MS results the two controls of 10 ppm and 9.7 ppm had ELISA values of12.87±1.17 ppm and 16.73 ± 0.32 ppm respectively. The uncertainty given by the method will be taken into consideration in the discussion of both field data and data from the emasculation trial (4.3.3) where ELISA was used. The same problems with ELISA were experienced by another master student using the method.

**Table 18 - Pearson's correlation between DON (µg/kg) content, AE, DTF, plant height, and % florets with remaining anthers in the *Fusarium* field trial. Significant values* - 1-tailed significance.**

**Field trial**

| | | | Pearson correlation | | | |
|---|---|---|---|---|---|---|
| Mean | S.E. | | DON | AE | DTF | PH |
| 11108.27 | 389.49 | DON | 1.000 | 0.100 | -0.122 | -0.165 |
| 3.67 | .198 | AE | 0.100 | 1.000 | 0.007 | 0.053 |
| 58.43 | .201 | DTF | -0.122 | 0.007 | 1.000 | -0.044 |
| 102.17 | 1.453 | PH | -0.165 | 0.053 | -0.044 | 1.000 |

### 4.3.3. Emasculation experiment

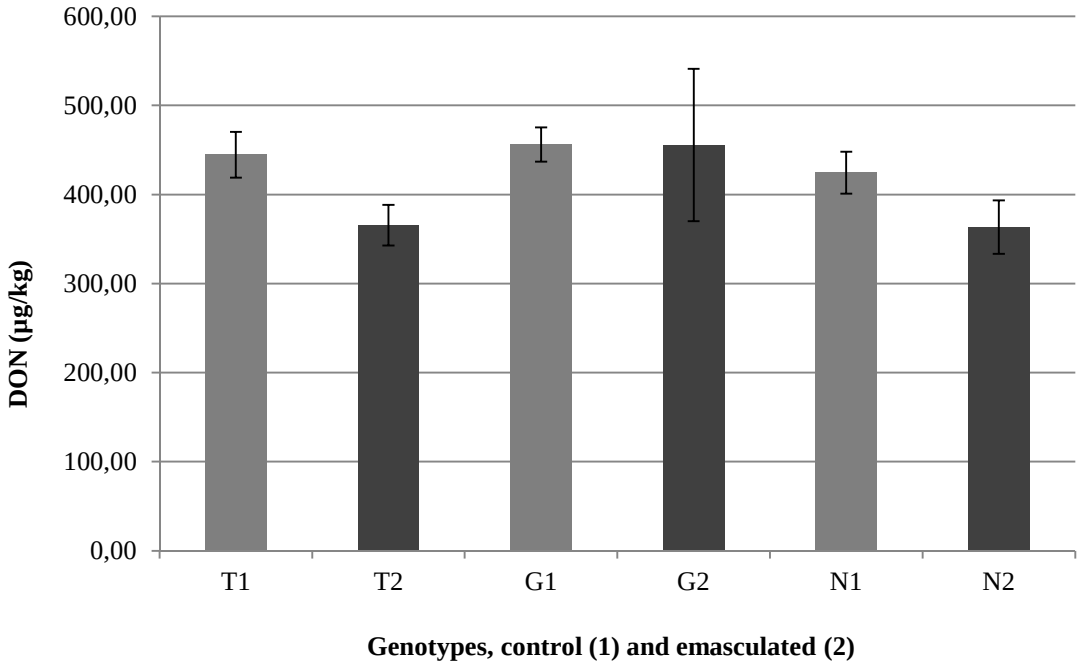



**Figure 30- DON (µg/kg) in controls (1) and emasculated plants (2). Genotypes are placed from lowest to highest AE, from the left: Typhon, GN9004 and Norum. The standard deviation shown is between the two replications of a sample in the ELISA wells.**

Despite the problems with dilution and reading values the results of the emasculation were much as expected. A paired T-test between emasculated and control by genotype only showed a significant difference in DON content between emasculated and control in Typhon, which was hypothesized to have the greatest difference. A paired T-test showed no significant difference in toxin level between emasculated and control (t=2.062, sig. 0.094). However if GN9004 is taken out because of the high deviation in G2, there is a significant difference (t=12,142, sig.=0.001). One of the replicas of G2 showed a higher DON level than any of the other in the emasculation trial, and a very high standard deviation for G2 (90 µg/kg versus 24 µg/kg). This could be due to a mistake made in that single well.

During the emasculation large differences were observed in the stiffness of hulls and glumes between genotypes. Norum had soft, thin and lighter green color while Typhon had stiff darker glumes. GN9004 was somewhere in between, closer to Typhon. In regards to color Typhon and GN9004 were closer to the modern genotypes.

# 5. Discussions

For AE to be an interesting trait as part of breeding for lower *Fusarium* incidence in oat variation of the trait, environmental stability and a high heritability of the trait is necessary. Here the different findings of the thesis will be discussed separately before a general discussion and conclusion.

## 5.1. The variation of anther extrusion trait, its heritability and its relation to other traits

### 5.1.1. Genetic variation of anther extrusion in oat

Genotypic effects of AE were strong in both populations, though with a higher year effect in S×S than F×S (Table 1). The low number of replications might have increased the year effect. Anther extrusion showed continuous quantitative frequency distribution in both populations in accordance with findings in wheat and barley (Skinnes et al., 2010); Abdel-Ghani et al., 2005) Although the transgression was not significant, the data insinuates that both parents add variance in both directions since lines with lower and higher AE values than the parents occur in the populations. Sage and Isturiz (1974), Skinnes et al.,(2010) and Abdel-Ghani et al., (2005) all found transgression in their respective wheat and barley populations). A larger population size might have proven transgression in the RILs. The transgression tendency shows breeding potential for genotypes with higher AE.

The quantitative frequency distribution indicates that the trait is controlled by several genes which each contribute with small or moderate effects. For additive inheritance the mean of the progeny is expected to be identical to the parental mean. The RILs of both populations had significantly lower mean AE than their respective parental means (Figure 9) while an identical mean was expected. This difference in mean could be caused by dominance (Sleper &Phoelman, 2006). Dominance cannot be observed in the F6 generation as a high level of homozygosity is reached by then. However the dominance observed in the 1$^{st}$ filial was of a higher AE and therefore makes dominance an unlikely cause of the lower RIL mean. Two other factors that can affect the progeny mean is the presence of an epistatic (Snape & Simpsons, 1981) effect and selection during development of the RILs. Accidental selection cannot be excluded, but is unlikely as it was taken care to avoid any selection (Bjørnstad, personal communication, April, 2014). An epistatic effect can be

observed through different genotypic frequencies over generations (Snape & Simpson, 1981), as only the F6 generation is observed in this case this theory cannot be tested here. Epistasy is still a plausible cause as anthers are extruded through a series of steps.

The possible epistatic effect and the transgression, though not found significant, both defy the prerequisites of the gene number calculated. The gene number might therefore be underestimated. The high inheritance and gene numbers calculated (Table 2) for the two populations is in correspondence with Skinnes et al (2010)'s findings in wheat. The year effect on AE in the S×S population is reflected in a slightly lower heritability and by the lower gene number calculated in S×S than in F×S. Heading and flowering was particularly delayed in S×S in 2013. The delay gave shorter ambient day-lengths (although with constant artificial light) during flowering for the last RILs, which might point to light as an environmental factor affecting AE that would not be relevant in field. Overall the significant genetic variance and high heritability of the AE trait points to it as a trait that easily can be taken in use for breeding purposes.

**Plant height.** There are no signs of correlation between plant height and AE in either of the crosses (Table 5). This is not similar to what Lu et al., (2013) found in wheat, where there was a significant moderate correlation between AE and height ($r$=0.43). In wheat it was questioned whether it would be possible to develop short phenotypes with high AE, none of these findings point to any problems in combining the two characters in oat.

**Days to flowering.** As temperature affects DTF (Marshall & Sorrels, 1992), the differences in DTF between populations could be due to temperature. The temperature was set equal in the two rooms, but was not double checked. Stormogul was the parent showing delay in 2013 and is likely to have brought genes into the cross causing the delay in the RILs (Appendix 6). Stormogul's behavior both in New Zealand and the distributions in the greenhouse trial indicates that it might be a winter oat, where both weak vernalization demand and shorter photoperiod can lead to later flowering (Holland, Portyanko, Hoffmann, & Lee, 2002). Days to flowering only had a weak significant correlation to AE in 2013, when average DTF was higher than normal. The results from the experiments did not give any indication on association between AE and DTF.

**Panicle traits.** Of the panicle traits panicle type and presence of awns did not seem to have any linkage to AE. Panicle density did not give a clear answer, much because of the few

genotypes with compact panicles. The compact panicles have a lower mean AE than the wide panicles which could be said to match what is found in wheat where the more compact spikes have less extrusion (Percival, 1921), where the dense spikes leaves less space for opening. In oat where the peduncles are long, even though they might be shorter in the compact panicles, the same density is not reached. Panicle erectness and lemma color on the other hand both seem to be linked to AE. For panicle erectness the most erect type showed the highest AE, but the mean AEs did not differ greatly. For lemma color there is a clear mean difference between the black and the white (from 0.7 in S×S to 1.5 in F×S), where brown color is linked to higher AE. There are a few exceptions pointing to this linkage not being too tight, giving the possibility of separating the two traits. Figure 27 shows black genotypes quite evenly along the scale of percent flowers with remaining anthers.

### 5.1.2. Screening of core collection

The core collection showed variation for AE, but with fewer reaching the top of the scale. Although genotypes with high AE scores are found in the screening, the distribution indicates that there are fewer sources for high AE than low AE.

**Plant Height**. There was no significant correlation between PH and AE in the core collection either, confirming that an association between AE and PH is unlikely in oat.

**Days to flowering.** In the core collection there was no correlation between AE and DTF. Together the crosses and the collection indicate that there is no such association in oat. Genotypes that stick out by having flowering very late all belong to the group of genotypes that are the basis of Nordic gene material (Figure 32-Appenix 6).

**Panicle traits.** Historical factors like breeding are likely to stand behind the representation of different genes in the core collection. The results from the collection can support findings in the crosses. Opposite of the results in the crosses panicle type shows a correlation to AE in the core collection, showing a very weak association that is not found in the crosses. Panicle density was not correlated to AE, supporting the results of the crosses. In the crosses, there was also no AE mean difference between the compact and wide panicles. The compact phenotype was a minority in the collection, 6% of the panicles. For panicle compactness to affect AE it must be through a very short rachilla

giving compactness within the spikelets. The same effect of compactness as seen in wheat (Percival, 1921) cannot be expected in oat because of the more open structure of oat panicles compared to wheat spikes.

The uneven distribution of seed color and AE over time in the collection could affect the correlation between AE and lemma color (Figure 11). But in the collection a clear association was found between lemma color and AE supporting the findings in the crosses. Also in the core collection there are examples of black genotypes with a low AE score, for instance the German 'Zorro' which had scores of 0 and 1 in the screening. Scoring color in just black and white is a simplification as the genetics between lemma color are quite complex with at least 11 genes. The fact that all shades of brown and gray were termed black or brown here might have had an impact on the result. "Weak links" could come from the fact that the different dark colors have different genes behind them (e.g. gray shades versus brown shades) (Marshall & Sorrels, 1992). That said the genetically different yellow and white did not differ in mean AE at all. The increase of yellow genotypes over time could be due to two other traits that are linked to lemma color (Figure 11), seed shattering and the number of seeds per spikelets. Brown lemma color is linked to seed shattering and has fewer filled seeds than yellow genotypes (Ladizinsky, 1995; Ladizinsky, 2000).

**Trends seen by year of release and country of origin.** The core collection can give an indication on AE trends from landraces until modern cultivars (and genotypes) (Figure 11)Figure 11 - The spread of seed color in the core collection over anther extrusion and year of release., but with reservation as it is uncertain how well the collection represents different time periods and countries. The collection shows a weak trend towards lower AE over time, particularly during the last 25 years (Figure 12). The weak trend towards lower AE over the years could be a result of indirect breeding. Open flowering and high AE promotes outcrossing (Waines & Hedge, 2003; Abdel-Gahni et al., 2005; Langer et al. 2014) so the demands of uniformity and stability (DUS) for a modern cultivar by the international union for the protection of new varieties of plants [UPOV] (2010) might have been a factor in a trend away from the genotypes with the highest AE. Breeding for high AE contradicts resistance breeding against diseases that infect through the open flower such as smut and ergot (Pedersen, 1960; Wood & Coley-Smith, 1980) and is an unfavorable trait in that respect.

The trend towards lower AE could also be described as a trend towards less variability. Studying the same core collection as in this thesis He & Bjørnstad (2012) found little diversity in the modern Nordic oat gene pool. The Nordic genotypes were distinct from the German group, the least diverse group of all. This pattern is reflected in AE, with little variability in the modern genotypes and very little diversity among the German genotypes that all were below AE 3 (Figure 13 toFigure 16 and Table 12). For modern genotypes Finland showed most variation, with a steep (very possibly random) trend line towards low AE (Figure 12). As the modern Nordic material only reaches intermediately high AE older plant material is a better source for breeding high AE genotypes. While Germany is a source if low AE genotypes are wanted.

## 5.2. Environmental sensitivity

### 5.2.1. Effect of drought on anther extrusion

Drought clearly gave lower AE. This is similar to what Abdel-Gahni et al. (2005) describes in barley, where he also finds that the AE in barley is affected by drought. He also assumes that observed effects of DTH seen on AE might be due to early drought. As water is a key operator in the processes of opening the florets and extruding the anthers, and as also De Vries (1971) suggests prolonged drought might hamper the water uptake to the lodicules.

If drought hinders swelling of the lodicules the effect would either be no flower opening at all, reduced flower opening or delayed flower opening. The observations in the field could imply the latter (4.3.2). The drought trials in the greenhouse, however, showed reduced AE, which indicates that there has been a reduction in flower opening. This might be because the lodicules do not swell as fully as they otherwise would or that the florets open for a shorter time period. Although duration of flowering and angle of opening are correlated (Arya & Sethi, 2005), lowered osmotic pressure only giving partially swollen lodicules is a more likely theory than fully swollen lodicules that lose their pressure quickly. It is likely that there are differences in how the plants react by the severity of the stress.

In the field drought would result in fewer anthers extruded, simultaneously time the drought conditions would be unfavorable for *Fusarium*. This would probably give less infection at flowering time, but might then resemble the effect observed in cleistogamous

barley by Yoshida (2007) where the infection time is moved to when anthers are pushed out by the caryopsis. Drought in early summer followed by high precipitation in July tends to give high DON levels in oat (Langseth *et al.,* 1995). The effect observed here of drought on the number of retained anthers might be a factor causing those high DON levels.

There was just as substantial a difference seen between years within genotypes as the effect of drought on genotypes, with more remaining anthers in 2014. The year effect is most likely due to environmental signals. The only obvious environmental difference between the trials is in ambient photoperiodic influence. The 2013 trial flowered in the last end of September (12.3h sun) and the 2014 trial flowered in the last half of January (5 h sun), giving the January trial more than five hours less of surrounding daylight (Bull, Helleland, Johannessen, Leifsen & Vannebo, 2013). Although the artificial lights were on for 16 h a day the light quality will have been different. The oat plant is known to be photoperiod sensitive, for that reason crossing oat plants are not done early in the year (J.A. Dieseth, personal communications, July 2014). It could be looked into whether light quality has an effect on AE, but for field conditions the light quality would not be an issue, although genotypes might perform 'better' than what a greenhouse screening in a dark period would indicate.

### 5.2.2. Degree of anther extrusion

The control of AE revealed a gap between expected AE and the actual number of remaining anthers (chapter 4.2.2.). The genotypes scored with high AE gave the impression of having all anthers out, or close to all anthers out, something the control count proved to be far from the truth. This means that the high AE genotypes are far from the ideal 9 on the scale where all anthers are extruded, and for an ideal effect of AE used as an avoidance mechanism against *Fusarium* the breeding material should have higher AE then observed here.

The control showed that the different experiments have been scored differently (slopes), which is not ideal for comparison. The gap between the ideal score and the score from the trials also give less certainty for interpretation of the results. Scoring during flowering could have caused some of the errors in the early trials. When the floret stands wide open it gives an impression of having all anthers extruded, while some or all of these anthers

might be trapped again later. The best time for scoring AE was therefore found to be right after flowering (after 14.00).

The control count also revealed great differences between scoring in the greenhouse and in the field. While the two greenhouse controls showed good fits ($R^2$=0.90 and $R^2$=0.98) the field control did not ($R^2$=0.34). Langer et al. (2014) found more stable results when scoring AE in wheat by counting anthers compared to scoring visually in field. The scale used was similar to the one used in these experiments, 1-9. Their correlation between the counted AE and other traits was stronger than their visually scored AE and other traits, and the counted AE gave higher heritability. Their conclusion was still that visual scoring gave good enough results for screening with many genotypes, where counting anthers would be too time consuming. The same cannot be concluded for oat with its long flowering time, where the best would be to only score AE visually in the greenhouse. Scoring AE by counting would be preferable in smaller screenings and in field trials. Counting anthers removes the subjective element and gives more stable and repeatable results.

When it comes to the distribution of anthers in the panicle no differences were found between branches. Differences were found between the 1$^{st}$ and 2$^{nd}$ floret, where the 2$^{nd}$ floret rather surprisingly had fewer remaining anthers than the 1$^{st}$. Following the principle in wheat where anther retention is connected to dense spikes and to smaller flower openings (Percival, 1921) the 2$^{nd}$ floret was expected to have higher retention than the 1$^{st}$.

### 5.2.3. Measurement of lodicule size and effect of auxin

All genotypes tested responded to auxin. A difference in measurement could give the 0 genotype away at once Wang et al., (2010) measured AE as either extruding or not, where one visible anther was enough for a genotype to be classified as extruding. On Typhon one anther would extrude in a panicle at occasions (more in one of the drought experiments). This one extruded anther would have been enough to disqualify Typhon as a *cly1* candidate by their measurements.

The great differences in the measurements were between oat and barley and between cleistogamous and chasmogamous barley. The results found when only size gain in oat was tested did not fit with AE. Looking at the sizes pre and post flowering of oat lodicules though could imply a connection between lodicule size and AE, that a difference in final

size could give smaller or larger openings independent of how much the lodicules swell. The measurements were not accurate enough to give a clear answer on the differences in final size, so to determine whether or not the lodicule size is significantly different and correlated with AE a more thorough study is needed.

#### 5.2.4. Measurements of filament lengths

No genotype effect was found of filament length. The measurements were not easy to perform, but the results were still clear enough to determine that filament length is not the reason for differences in AE in these genotypes. This corresponds to an experiment in wheat where no correlation was found between AE and filament length (Arya & Sethi, 2005).

If neither difference in lodicule swelling nor in filament length explains the differences found in AE some other mechanism may be causing it. Several studies have found strong correlations between the duration of flower opening and AE, the angle of opening between glumes and AE, and between duration of opening and angle of opening (Arya & Sethi, 2005; Gilsinger et al., 2005; Sing et al., 2007). If the size of the lodicules does not decide how far the glumes are pushed apart the observed differences between glumes might affect how easily they are pushed apart. These observed differences could be degrees of glume toughness. In wheat the gene tenacious glume (Tg) controls glume toughness and is one of the threshability genes in wheat (Sood, Kuraparthy, Bai & Gill, 2009). The idea of that tougher glumes hindering the lodicules in opening could explain differences in AE, however Atashi-Rang & Lucken (1978) did not find any correlation between AE and glume tenacity in wheat in their two experiments.

### 5.3. Effect of anther extrusion on deoxynivalenol content

#### 5.3.1. Correlation between deoxynivalenol, anther extrusion and other traits in the greenhouse

There was no significant correlation between DON and AE in the greenhouse *Fusarium* trial, but a negative tendency of AE on DON (r = -0.125, Table 17). The pattern found (Figure 27, top left) is similar with Kubo et. al (2013)'s finding that the genotypes with intermediate AE were the most susceptible. In Kubo's findings the zero types were completely cleistogamous, which the auxin sensitivity test showed that the representatives

for the zeros, Typhon, was not. (And the parents for the crosses are most likely not carriers of any *clyI* allele). However different degrees of floret opening were observed and can have given a cleistogamy-like effect in genotypes with small floret openings. The higher DON levels of intermediate AE genotypes could be due to a higher risk of anthers trapped between lemma and palea. The correlation between length of opening time and width of flower opening (Arya & Sethi, 2005) could increase the chance of anthers being trapped in the intermediate types, giving an ideal entrance for *Fusarium.* The lower DON levels in the higher AE genotypes might therefore be caused by long enough floret openings and lower chance of entrapment of anthers.

The results from the control count have an impact on how the DON results can be read. The high AE genotypes cannot be expected to have the same results in Fusarium trials as the ideal panicle that would throw out all anthers. The ones given a high score in the experiment might have had a larger percent of seeds where all anthers are out, but they may also have a good portion of seeds that are ideal for infection where anthers are stuck between lemma and palea.

The AE results do not fit very well with the results from percent florets with remaining anthers (Figure 27, top right). The accuracy decreases as AE increases the. DON and the percentage of florets with remaining anthers form a ‘fan shape’ where the wide end is with 100 % florets with remaining anthers low AE (Figure 27, bottom). The exact AE cannot be told as only presence of anthers were registered in this case, judging by the distributions where all anthers were counted the AE is likely to be close to 0, maybe stretching from 0 to 2. It would have been interesting to see if the % florets were only 1 or 2 anthers are shed would have made much difference from a same percentage of florets where 3 anthers are shed, if the greatest effect of AE is due to the percentage of florets where all anthers are shed.

All the high DON levels are found in the wide end of the fan together with the whole spectrum of DON levels. The spread in DON level in the wide end could be due to fewer anthers in some of the florets as 100% florets represents not only AE 0, but also intermediate types (Figure 27, top right). Parts of the spread in the wide end is likely due to other active resistance mechanisms (Mesterhazy, 1995).The ‘fan shape’ is equivalent to the relationship between AE and DON Skinnes et al., (2010) found, suggesting that AE affects

DON in a similar way in oat as in wheat. Running the experiment on a larger population though could have given clearer results. There is no clear pattern between 30-70 % florets with remaining anthers (Figure 27, bottom), where an experiment on a larger population might have shown if there is a further effect of fewer remaining anthers. Table 18

One of the resistance mechanisms causing spread at 100% can be black lemma. None of the black seeded RILs reach the highest DON levels. In barley black seeded genotypes are known to be more resistant than yellow. The resistance is ascribed to the higher lignin and pehonlic content and pigments in black seeds. Black barley has been shown to have 17-59% less DON in experiments under natural conditions, while hulless and black genotypes had 46% less DON than other genotypes. (Choo, Vigier, Ho, Ceccarelli, Grando, & Franckowiak, 2003; Choo, Vigier, Savard, Blackwell, & Martin, 2014). Although the genes for the "black" colors in oat are different the biochemical background that gives an effect on *Fusarium* might be similar.

Plant height had negative significant correlation to DON level in the RILs in accordance with the findings in wheat (Lu *et al*.,2012) and Langseths findings in oat. He, et al, (2013) did not find a clear indication on a correlation between plant height and DON in only one of three trials. In the field the higher DON level in short plants could be due to the taller plants escaping inoculum, but in this case when the experiment took place in the greenhouse using spray inoculation a genetic factor like linkage or pleiotropy is more likely to explain the correlation. He *et al*. (2013) found strong correlations between DTH and FHB severity in oat in accordance with the findings in this experiment. Stormogul which has been quite late in several of the greenhouse experiments and is likely to have brought genes into the populations that give much later genotypes than is typical for modern cultivars.

For oat where the long peduncles hinder spread within the panicle spikelets could possibly be seen as more individual units than they can in wheat and barley spikes. Near infrared hyperspectral imaging (HSI) could be a good measurement of *Fusarium* incidence. The method can classify asymptomatic, mildly damaged and severely damaged kernels (Tekle, Måge, Segtnan, & Bjørnstad 2014). Comparing both AE and the percent of florets with remaining anther (and number of remaining anthers) to the number of damaged kernel

could give a precise measurement of incidence for oat genotypes that represents the variation in AE throughout the panicle better.

### 5.3.2. Correlation between anther extrusion and deoxynivalenol in the field

There was no correlation and no pattern between AE and DON in the field trial. The reason is more likely due to errors than too the actual relationship between AE and DON. Two things complicate the reading of these results; 1) the poor relationship between remaining anthers and visually scored AE (Figure 19), and 2) the uncertainty of how good the ELISA results are. When both scoring of the trait and the disease is uncertain, the results are quite dubious. Other factors complicated comparison of field trial with the greenhouse trial. The drought experiment showed that drought lowers AE. Many of the genotypes, especially the early ones, experienced drought. The mix up of plots was also a problem for comparison as the real identity of the genotype was not always certain. The greatest conclusion that can be drawn from the field trial is still that scoring of AE visually is not ideally done in the field.

### 5.3.3. Effect of emasculation on deoxynivalenol content

Despite the issues with dilution the results came out much as expected. Poor quality of the inoculum could be behind the low DON values. The experiment points to emasculation, and in other words high AE, giving lower DON levels. This supports the findings in the greenhouse *Fusarium* experiment where the absence of anthers gave low DON levels. Strange & Smith (1971) found a similar effect of emasculation in wheat with less infection in the emasculated than the un-emasculated.

# Conclusions

This study shows that AE in oat is a trait with high heritability governed by several genes. Although variation in AE was present in both crosses and the collection the control count showed that the top of the scale is not reached by any genotypes. The role of lodicules in creating differences in AE did not give a clear answer, and could be studied further, as could the potential role of glume toughness. Some of the experiments showed correlations between DON and the presence of anthers implying that it could be a valuable trait in breeding for *Fusarium* resistance. However, the sensitivity of AE to drought reduces the value of the trait as an avoidance mechanism. And while the breeding of higher AE genotypes could lower *Fusarium* incidence it could simultaneously increase the incidence of seed borne diseases. The possible resistance mechanism of lemma color is an interesting trait to study further in oat.

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# Appendices

## 1. EU limits for DON in food and guidance values for DON in feed

**Table 19 – EU limits for DON in food and feed.**

| EU limits for DON in food | DON µg kg-1 | EU guidance values for DON in feed | DON µg kg-1 |
|---|---|---|---|
| Unprocessed oat | 1750 | Complementary and complete feeding-stuffs for pigs | 900 |
| Cereal flour | 750 | Cereal and cereal products | 8000 |
| Bread pastries, biscuits cereal snacks, and breakfast cereals | 500 | Maize by-products | 12000 |
| Processed cereal-based foods for infants, young children and baby food | 200 | | |
| (Scudamore, Baillie, Patel, & Edwards, 2007). | | (Streit et al., 2012) | |

## 2. Genotypes in the oat screening

| Entry Number | Accession name [a] | Country of origin [b] | Year of release | Entry Number | Accession name [a] | Country of origin [b] | Year of release |
|---|---|---|---|---|---|---|---|
| 96 | 53-6 | US | <1953? | 95 | L986-1 | US | |
| 37 | A4013 | Norway | | 54 | Lena | Norway | 1998 |
| 36 | A4066 | Norway | | 125 | Leningrad-Russia Omiho var mutica | Russia | |
| 12 | Aarre | Finland | 1995 | 68 | Ligowo | Sweden | 1894 |
| 129 | Akseli | Finland | 2009 | 51 | LW-2701 | Norway | 1988 |
| 112 | Amur-Russia local var flava | Russia | Landrace | 64 | Magne | Sweden | 1987 |
| 18 | Aragon | Germany | 2000 | 39 | Martin | Norway | 1988 |
| 16 | Aslak | Finland | 1999 | 83 | Matilda | Sweden | 1994 |
| 121 | Australia Numbat var inermis | Australia | 1998 | 61 | Mesdag | Sweden | Landrace |
| 102 | Avena abyssinica | Ethiopia | | 131 | Mirella | Finland | 2011 |
| 87 | Aveny | Sweden | 2008 | 76 | MK 5-1050 'Sol II' | Sweden | 1942 |
| 66 | Bambu II | Sweden | 1949 | 38 | Moholt | Norway | 1982 |
| 32 | Beiar | Norway | Landrace | 107 | Mongolia local var inermis | Mongolia | |
| 84 | Belinda | Sweden | 1998 | 108 | Mongolia local var inermis | Mongolia | |
| 24 | Bessin | Germany | 2002 | 23 | Mozart | Germany | 1995 |
| 41 | Bikini | Norway | 1997 | 29 | Mustang | Holland | 1971 |
| 55 | Biri | Norway | 1997 | 6 | N Finnish | Finland | Landrace |
| 59 | Blenda | Sweden | 1950 | 17 | Neklan | Germany | 1997 |
| 103 | China Hull-less var. mongolica | China | | 20 | Nelson | Germany | 2002 |
| 114 | China Uimai var maculata | China | | 46 | Nes | Norway | 2008 |
| 104 | China, Hull-less var chinensis | China | | 49 | NK03112 | Norway | |
| 106 | China, Hull-less var mongolica | China | | 57 | Norum | Norway | |
| 105 | China, Naked var mongolica | China | | 43 | Nudist | Norway | 2007 |
| 85 | Cilla | Sweden | 1998 | 45 | Odal NK | Norway | 2009 |
| 91 | Clintford | US | 1966 | 126 | Omsk-Russia Sibirskii kormovoi var | Russia | |
| 30 | Condor | Holland | <1967 | 86 | Plym, svart tartarisk | Sweden | Landrace |
| 2 | Dansk | Danmark | | 35 | Pol | Norway | 1967 |
| 115 | Denmark Trelle Dwarf v obtusata | Denmark | | 110 | Primorye-Russia local v obtusata | Russia | |
| 21 | Dominik | Germany | 2003 | 67 | Probsteier | Sweden | <1892 |
| 14 | Fiia | Finland | 2002 | 7 | Puhti | Finland | 1978 |
| 19 | Flämingsglanz | Germany | 2001 | 88 | Pure line potato oat | UK | <1850 |
| 25 | Flämingsgold | Germany | 2007 | 5 | Rajala | Finland | Landrace |
| 28 | Freddy | Germany | 2000 | 77 | Ramiro | NL | |
| 82 | Freja | Sweden | 1991 | 1 | Regal | Danmark | 1966 |
| 65 | Frigg | Sweden | 1989 | 27 | Revisor | Germany | 1995 |
| 73 | Fyris | Sweden | 1911 | 146 | RIL FxS 126, AE=8 | UMB | |
| 47 | Gere | Norway | 2004 | 145 | RIL FxS134, AE=0 | UMB | |
| 138 | GN07045 | Norway | | 44 | Ringsaker | Norway | 2008 |
| 48 | GN07133 | Norway | | 101 | Robust | Canada | |
| 50 | GN07134 | Norway | | 13 | Roope | Finland | 1996 |
| 139 | GN08009 | Norway | | 109 | Sakha-Inacutia local v montana | M2008 | |
| 140 | GN08250 | Norway | | 113 | Sakhalin-Russia Bisuandorodu v aure | Russia | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 133 | GN09004 | Norway | | 79 | Sang | Sweden | 1974 |
| 137 | GN09005 | Norway | | 72 | Seger I | Sweden | 1908 |
| 134 | GN09078 | Norway | | 78 | Selma | Sweden | 1968 |
| 136 | GN09146 | Norway | | 11 | Sisko | Finland | 1993 |
| 40 | Grane | Norway | 1992 | 53 | Skarnes | Norway | 2011 |
| 34 | Gråkall | Norway | 1972 | 118 | South Korea Meguiru A. byzantina | South Korea | |
| 71 | Guldregn I | Sweden | 1903 | 62 | Stjärn | Sweden | 1927 |
| 52 | Haga | Norway | 2010 | 70 | Stormogul I | Sweden | 1901 |
| 99 | High oil #3 | US | | 80 | Svea | Sweden | 1976 |
| 100 | High oil #7 | US | | 127 | Sverdlovsk-Russia Pamyati Bal v mut | Russia | |
| 33 | Hird | Norway | <1940 | 128 | Sverdlovsk-Russia Stayer var mutica | Russia | |
| 42 | Hurdal | Norway | 2005 | 135 | SW071119 | Norway | |
| 74 | Hvit Odal | Sweden | 1926 | 132 | Symphony | Germany | 2012 |
| 69 | Hvitling | Sweden | 1897 | 58 | Thors Møystad | Norway | |
| 15 | Ivory | Germany | 2003 | 60 | Titus | Sweden | 1964 |
| 93 | J706-1 | US | | 22 | Typhon | Germany | 2005 |
| 92 | J740 | US | | 120 | USA Lemont A. Byzantina | US | |
| 97 | J-75 | US | | 8 | Veli | Finland | 1981 |
| 94 | J-762-1 | US | | 130 | Vinger | Norway | 2010 |
| 116 | Japan Kuromi N1 var brunnea | Japan | | 9 | Virma | Finland | 1988 |
| 111 | Japan local var brunnea | Japan | | 56 | Voll | Norway | 1956 |
| 31 | Kapp | Norway | 1986 | 98 | X2-1 | US | |
| 3 | Kareela | Finland | | 117 | Yekaterinburg-Russia Univ 1 v mutic | Russia | |
| 123 | Kemerovol-Russia Levsha var inermis | Russia | | 4 | Ylitornio | Finland | Landrace |
| 81 | Kerstin | Sweden | 1988 | 10 | Yty | Finland | 1989 |
| 119 | Kirov-Russia Argamac v mutica | Russia | | 89 | Z595-7 | US | 1990 |
| 122 | Kirov-Russia Vyatskii Gol v inermis | Russia | | 90 | Z615-4 | US | 1990 |
| 75 | Klock Extra | Sweden | 1933 | 26 | Zorro | Germany | 2009 |
| 124 | Kursk-Russia L'govskiig var mutica | Russia | | 63 | Örn | Sweden | 1931 |

## 3. Agar recipes

### A. Mung Bean Agar (MBA)

40 g mung beans

1 liter distilled water

15 Difco agar

### B. Difco Potato Dextrose Agar (PDA)

Per Liter:

4.0 g Potato starch from infusion

20.0 g Dextrose

15.0 g Agar

Final PH 5.6 ± 0.2

## 4. GC-MS procedure

### A. Purification Column Preparation

1. Wear dust mask
2. Weigh 200g of C18 and 600g of Aluminum Oxide and pour in to a 2000ml flask
3. Cap the flask and mix thoroughly
4. Use a plunger to push one filter paper to the bottom of a 5cc syringe(with out needle)
5. Weigh 1g of C18 : Aluminum oxide mixture and pour the mixture into the syringe
6. Place another filter paper on the top of C18:aluminium Oxide mixture with a plunger and leave the plunger in place

### B. Sample Grinding

1. Wear safety goggles, dust mask, lab coat, gloves and earmuff and work in the hood
2. Make sure that the grinder and the cup is clean
3. Pour a sample from its bag into the cup
4. Secure the cup to the grinder by turning it counter clockwise and then tightening the knob under the cup
5. Set the timer to 2 min
6. Turn the grinder on and then press the button on top of the timer. The grinder will automatically shut off after 2 min
7. Wait until the blade quits spinning, then turn off the grinder and loose the knob under the cup
8. Turn the cup clockwise to take it out of the grinder
9. Pour the sample back into the bag
10. Clean the grinder and cup, Begin by first brushing the grinder and the cup with a brush. Then use compressed air from air hose to spray the grinder and cup with denatured ethanol and wipe then with paper towels

### C. Sample Extraction

*i. Extraction solvent (84:16 of acetonitrile: water mixture) preparation*

1. Wear a pair of lab gloves and work in the hood
2. Use a 2000ml graduated cylinder, and add 320ml distilled water to it
3. Add 1680ml of acetonitrile to the graduated cylinder
4. Use a funnel and pour the Acetonitrile: water mixture into a clean empty 1L Acetonitrile bottle, and mix thoroughly.

*ii. Extraction*

1. Wear dust mask

2. Weigh 4g of ground sample and place it into a labeled 50mL centrifuge tube.
3. Make sure the label on the centrifuge is the same as that on the sample bag
4. Wear a pair of lab glove
5. Add 16ml of acetonitrile: water (84:16) using a solvent distributer in the hood
6. Cap the tube tightly and place the tubes in a tube rack
7. Place the rack horizontally in a shaker and shake for 2hrs
8. Filter 4ml of extract through purification column prepared earlier
9. Transfer 1ml of the filtrate into a labeled 1 dram vial. Make sure the label on the vial is the same as that on the centrifuge tube.
10. Dry samples under nitrogen in the hood and make sure samples are completely dry. A small amount of water in the sample vial will react with the derivatizing reagent.
11. Cap the sample vials and store them in a freezer if TMS derivatization is not followed immediately.
12. Place a piece of filter paper in a funnel, and dispose leftover solution through the funnel into a 4L waste bottle in the hood
13. Dispose the filter paper in a trash can, and cap the waste bottle
14. Dispose centrifuge tubes and plastic syringe columns into a trash can
15. Dispose glass test tubes into a glass container while wearing safety goggles.

*iii. TMS Derivatization*

1. Wear a pair of lab gloves
2. Make sure sample vials are at room temperature before opening the cap
3. Add 100µl 0f TMS reagent (TMSI:TMCS= 100:1) into a sample vial in the hood, and cap the vial
4. Rotate the vial to ensure the reagent coating the side of the vial
5. Place sample vials rack in a shaker and shake for 10 min
6. Add 1ml of Mirex isooctane solution (4mg/ml) in the hood and shake the rack a few times
7. Add 1ml of HPLC water and cap the sample vials
8. Vortex the vials so that the milky isooctane layer becomes transparent and set the vial on bench for at least 20min
9. Transfer the upper layer (isooctane layer) into a labeled 1.5ml GC vial in the hood and cap it. Make sure the label on the GC vial is the same as that on the 1 dram sample vial.
10. Dispose plastic pipette tips into a trash can
11. Dispose leftover solution the funnel into a 4l waste bottle in the hood and cap the waste bottle.
12. Dispose glass vials into a glass container while wearing safety goggles

## 5. ELISA procedure

### Sample preparation

A) Field trial

1. 70 grams frozen sample were ground in a Stein Laboratory mill (model M-2, Stein Laboratory Inc. Atchinson, Kansas) at a time until approximately 75% of the sample could pass through a 20-mesh screen. (Close to 2 min grinding.) The hulls represented most of the larger pieces.

B) Emasculation trial

Only samples from the second trial were analyzed. The extreme infection in the preliminary trial would probable to overrule the predicted differences between emasculated and control. Samples from the emasculation trial were too small for the mill and had to be crushed by hand in a mortar.

1. The mortar and pestle were chilled with liquid nitrogen.
2. Sample was poured in and liquid nitrogen was poured over.
3. The sample was ground. Fineness was judged visually to get approximately the same coarseness as the field samples. Under extraction they proved to have less of the finest material that was found in the mill ground samples.

### Extraction

1. 5 grams of samples were transferred to a tube and 25 ml of distilled water was added.
2. The samples were shaken vigorously by hand for three minutes.
3. Samples were centrifuged at 3000 rpm for 1 min.
4. 1 ml supernatant was transferred to another tube.
5. The supernatant was diluted with distilled water to a concentration of 1:10.

### Assay

1. The kit was taken out and brought to room temperature before running the assay.
2. 6 Dilution strips were placed in a microwell strip holder, and 6 antibody coated microwell strips in another microwell strip holder.
3. 12 mL conjugate was placed in a container and was distributed to the microwells, 200 µL into each dilution well with a multichannel electronic pipette.

4. 100 µl of each standard (0, 0.25, 1.0, 2.0, 5.0,) and sample were added to the dilution wells after planned design. Each sample had one replicate, and a control oat sample with known DON had three replicates. The three control samples were placed spread as to check for differences over the “plate”.
5. A multichannel pipette was used to mix samples and conjugate in the wells by pipetting up and down three times.
6. 100 µl of the contents from each well was transferred directly to the corresponding Antibody Covered microwell.
7. The mix was incubated for 15 minutes in room temperature.
8. After incubation the content was poured out of the wells and the wells were washed five times with wash solution. Paper towels were laid down on a flat surface to remove as much residual water as possible. The undersides of the wells were dried with a towel.
9. 100 µl of the Substrate into each well with a multi-channel pipette in a closed hood.
10. Incubated for 5 min.
11. 100 µl of Stop solution was added to each well, and color changed from blue to yellow.
12. The plate was red in Perkin Elmer Victor 3 1420 Multilabel Plate Counter with a 450 nm filter (@454).
13. A dose response curve was made from each plate reading based on the standards, and calculated DON levels were adjusted by the dilution factor.

## 6. Days to flowering

The REML analasys for DTF showed a significant difference between years (F pr <0.001) and between the genotypes in both populations (F pr <0.001). The DTF mean of the parents was not significantly different from the DTF mean of the genotypes.

**Table 20 - Mean days to flowering of parents and RILs 2012-2013.**

| Stormogul × Fiia | | | | | Stormogul × Svea | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | | | | | Mean | | | |
| Year | Stormogul | Fiia | Parental $\bar{x}$ | RILs | Year | Stormogul | Svea | Parental $\bar{x}$ | RILs |
| **2012** | 70 | 53 | 61 | 60 | **2012** | 69 | 56 | 63 | 61 |
| **2013** | 78 | 53 | 68 | 63 | **2013** | 89 | 57 | 70 | 68 |
| **Mean** | 74 | 53 | 65 | 62 | **Mean** | 79 | 56 | 69 | 65 |
| **SE** | | | | 1.940 | **SE** | | | | 2.396 |

Fisher's LSD $_{\alpha=0.05}$ showed significant transgression in both directions in both years for F×S, while in S×S transgression was not signficant in 2012 and only towards late flowering in 2013 (at significance level 0.1 transgression was significant in both directions in 2013 and towards later flowering in 2012 in the S×S population). Days to flowering follows an approximately normal distribution in both populations and years, Figure 31 – Frequencies of days to flowering in the two Stormogul crosses, showing normal curves. S × S is found to the left and F × S to the right, years are shown from the top and down: 2012, 2013 and the predicted means (REML) of the two years. The value of Stormogul is marked with * and the other parent with ".. In both populations the distribution is wider, stretching towards towards later floweing in 2013, whearas only S×S show traces of a similar stretch in 2012. The difference between years in DTF, as seen in the frequency distributions in Figure 31, is reflected in the correlation, Table 5.

The start of flowering is identical in the two years and populations (50 DTF), and although many genotypes are later in the second year, there are also more genotypes in both populations flowering early. The change in span between the years is also quite similar in the two populations, 28 days in S×S and 31 days in F×S. While Fiia has the same mean

DTF in the two years and Svea flowered one day later on average, the average DTF of Stormogul was 8 (F×S) and 20 (S×S) days later in 2013 than 2012. The average additional days to flowering of the RILs in 2013 were 2.77 days in the F×S population and 6.15 days in the S×S population.

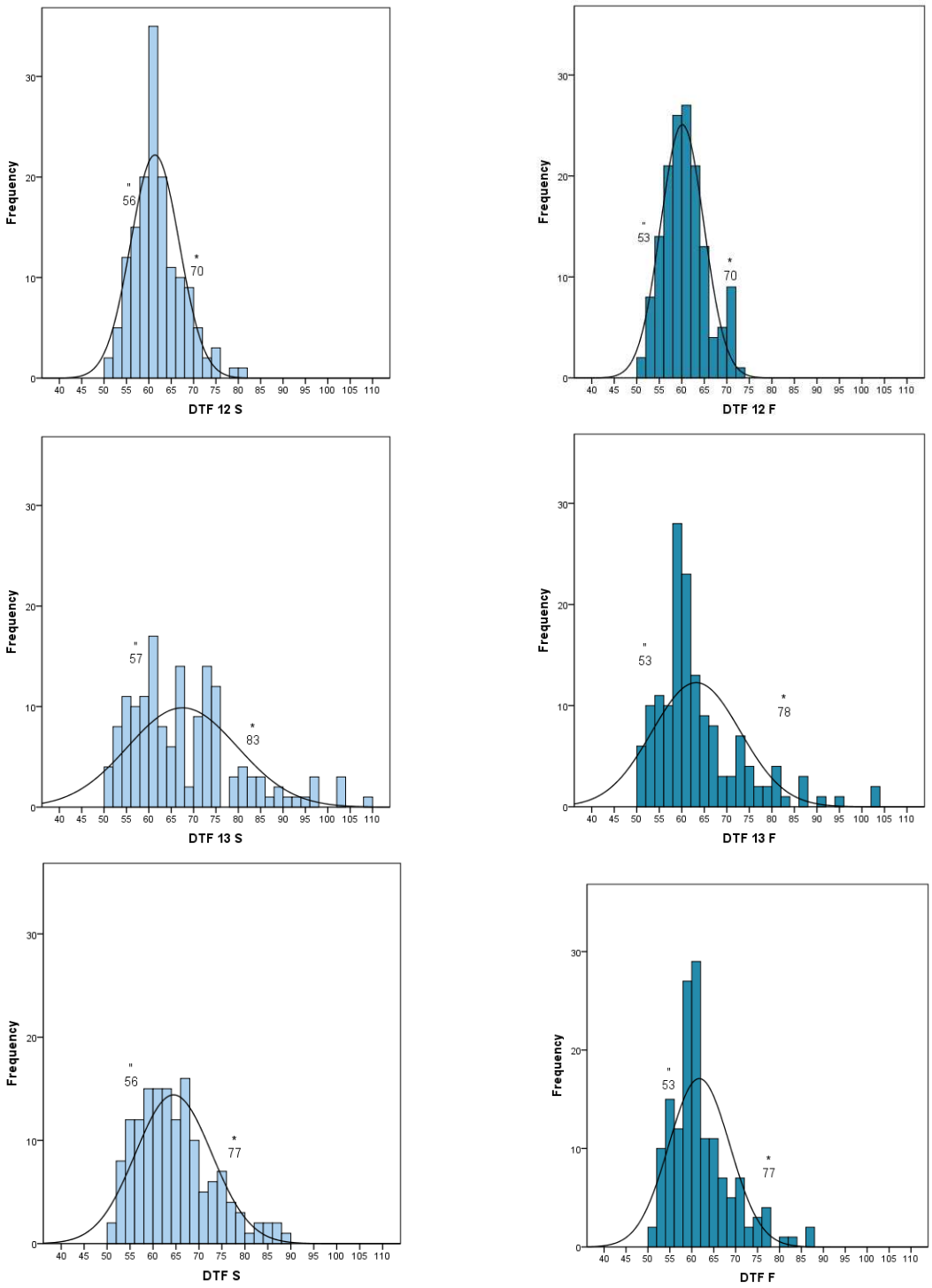



**Figure 31 – Frequencies of days to flowering in the two Stormogul crosses, showing normal curves. S × S is found to the left and F × S to the right, years are shown from the top and down: 2012, 2013 and the predicted means (REML) of the two years. The value of Stormogul is marked with * and the other parent with ".**

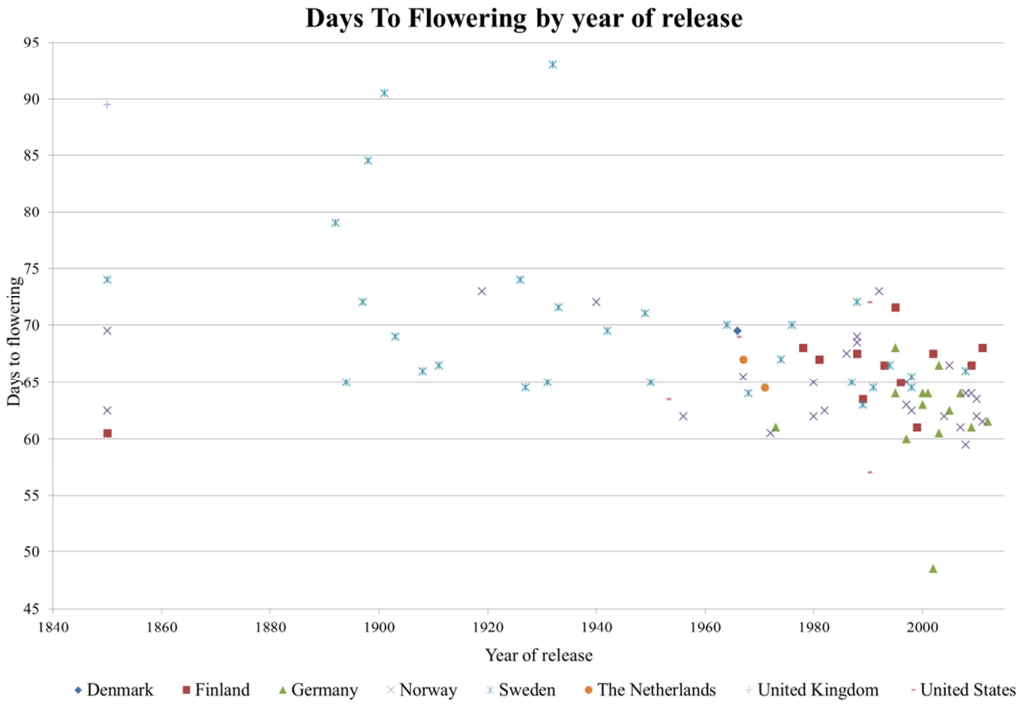



**Figure 32 - Days to flowering by year of release in the core collection, the landraces are all set to 1850 together with the oldest dated genotypes.**

Days to flowering (Figure 32) showed a moderate negative correlation with year of release (Pearson correlation -.340 sig. 000) ($R^2$ .132, t -4.852, sig .000), but no significant difference in origin. The number of days to flowering is less variable between countries than AE was, and most of the plants flowered ~60-75 days after sowing. Only a very early German cultivar, Bessin, was registered outside that timespan in more recent years, the other cultivars were older and had late flowering. One of these was a British cultivar, Pure Line Potato, and the rest were labelled as Swedish; Plym Black Tartare, Probsteier, Stormogul I, and Stormogul II. The two first of the Swedish belong to material that is part of the base material of the Nordic gene pool. Stormogul I is a selection from Plym Black Tartare, and Stormogul II is from a cross between Probsteier progeny (Klock) and Stormogul I. In other words this group of outliers is closely related. That said, Probsteier is maybe the most important progenitor the Nordic oat family (appendix 6).

## 7. A digression on unfertilized florets of cleistogamous Tiril and Golden promise

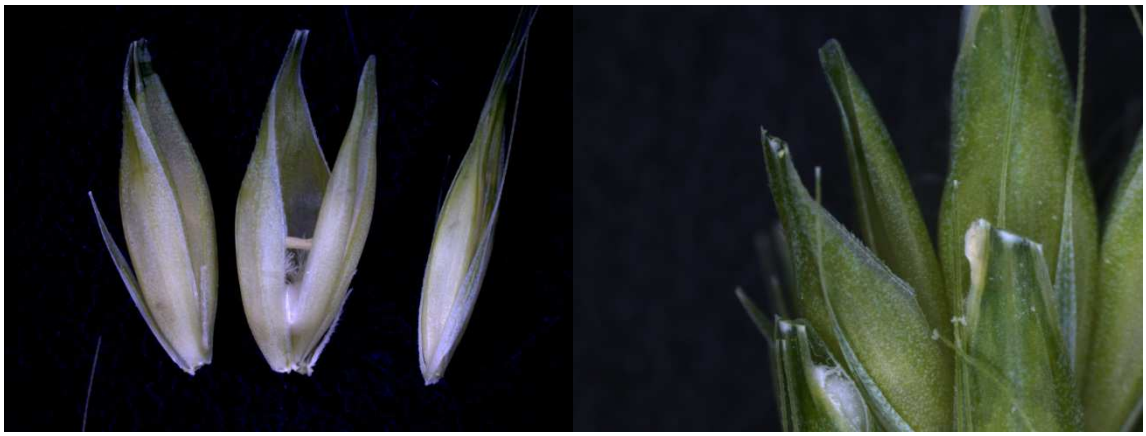


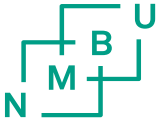
**Figure 33 - The Barley Tiril with sterile florets. Left: Sterile floret in the middle, florets after anthesis on the side, the left one older than the right. Right: Sterile floret on spike.**

An observation was made in the cleistogamous barleys Tiril and Golden Promise that could lead to a misunderstanding regarding the dysfunctional lodicules. Measurements of lodicules did not show any swelling, but ovaries on the other hand had swelled in the unfertilized florets. Ovaries in the unfertilized had a significant larger area than fertilized ovaries where the caryopsis developed beneath (t -5.581, sig .000).

Landrace
< 1900
1901-1905
1906-1910
1911-1915
1916-1920
1921-1925
1926-1930
1931-1935
1936-1940
1941-1945
1946-1950
1951-1955
1956-1960
1961-1965
1966-1970
1971-1975
1976-1980
1981-1985
1986-1990
1991-1995
1996-2000
2001-2005
2006 <

DK
FI
FR
DE
NL
NO
SE
UK
US
NN

8.5
Norum 206
4.5
Probsteier (Dansk Havre)
Hedemarkshavre
Dalahavre
Trønderhavre
1
Ligowo
2.5
Brie
0
Hvitling
Abundance
Milton
Anderbecker Beseler
6
Black Tartar Plym
Guldregn I
Gul Næsgaard
Stormogul I
Klock I
1.5
Seger I
Klock II
Lochow's Gelbhafer
2.5
HJA TA 091-3
Perle
Nova (Abed)
Thor (Thors Møystad?)
Grenader
HJA TA 02272
vasa
Kytö
Esa
1
1.5
Abeds Sølv
Nidar
Engelbrekt I
Hvit Odal
Klock III
French black oat
Stjärn
3
6.5
6
Kron
Bambu
Örn
Klock Extra
1.5
Engelbrekt II
Stormogul II
Hird
Nidar II
2
0.5
Sol II
12894
3
Minor Abed
Palu
Saxo
Blenda
Bambu II
Sisu
Blixt
4.5
Eho
Voll
AB4757
0.5
Hannes
Titus
JO 50-2395
5
Ryhti
Pol
Unisignum
SV01771
WZ62060
Selma
Karin
Condor
Clintland
H662
H8461
5
1.5
3.5
0
2.5
Gråkall
Sang
Dula
Mustang
Nelson
5
3.5
Puhti
Svea
Tador
4.5
Rhiannon
Moholt
SV56697
SV0177
Veli
4.5
3
3
Yty
Martin
Frigg
Marengo
NZ-1
4
1
6.5
1.5
Aarre
Grane
Sisko
JO 1033
Freja/Frode
Silvano
5.5
3.5
1
0.5
3.5
1
Roope
Lena
Biri
Bikini
Aslak
A90017
SWB95011
Belinda
NK92575
SW951865
3.25
1.5
Fiia
4
1.5
Hurdal
3.5
3
3.5
3
Ringsaker
Akseli
Vinger
Nes
Odal
Aveny



Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no