



# A hopeful study of hop

– agronomic performance of historically cultivated hop varieties grown in Uppsala

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*En studie av svenska kulturarvssorter av humle odlade i Uppsala*

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## Abstract

Historically cultivated grown hop usually have an early maturation, this is important for developing  $\alpha$  and  $\beta$  – acid. In addition, these varieties often come with an interesting history and have the potential to give the local beer an even more local connection. The objective of this study was to investigate the agronomic-quality of historically cultivated Swedish hop varieties in the search for an early maturing high  $\alpha$  - acid-producing hop variety, that could give both good yield and good quality. 11 hop varieties were grown in a field in Krusenberg outside Uppsala, ten Swedish varieties and one variety from the Czech Republic. The trial was investigated by going out to the field trial once a week during the growing season to note development stages. Mean of development time between the varieties was compared. Overall plant health was also noted but not quantified. Hops were harvested when they reached assumed maturation, based on smell, and looks of cones. The cones were dried in 60 °C in 2-3 hours until they reached 10 % moisture. Analysis of  $\alpha$  and  $\beta$  – acid content was performed along with total essential oil, and proportion of myrcene  $\alpha$ -humulene,  $\beta$ -caryophyllene, farnesene, linalool, limonene, and geraniol. The study conclude that Korsta is the variety that seems most interesting as it has the highest yield, alpha acid, and essential oil. As the variety has high myrcene values it might be suitable for brewing fruitier ales or IPA. Hulla Norrgård is another interesting variety with early maturation, big cones, and a high  $\alpha$  – acid. Hulla norrgård is also high myrcene containing variety, indicating that it would be suitable for fruitier ales or IPA. Bonneråd was amongst the lower  $\alpha$  – acid varieties but had a high total essential oil content. This variety could be interesting for lager beers as it has low myrcene and moderate levels of  $\alpha$ -humulene,  $\beta$ -caryophyllene, and farnesene. Tvärud is another variety with moderate levels of  $\alpha$ -humulene, and myrcene an could be suitable for lager style beers.

# Populärvetenskaplig sammanfattning

Intresset för lokalproducerade råvaror har ökat den senaste tiden så även intresset för att brygga öl. Detta går bland annat att se på den stora ökningen i antal registrerade bryggerier, från 42 bryggerier 2010 till 392 bryggerier 2017. Konsumenternas intresse driver på framställandet och produktionen av öl med unika profiler. Detta öppnar upp möjligheter för svensk humleproduktion, vilket dessutom kan vara ett sätt för landsbygdsföretag att diversifiera sig. Intresset för och efterfrågan på svensk humle ökar, men det behövs mer kunskap om de äldre sorterna. I detta projekt undersöktes vilka kloner av historiskt odlad humle som har en hög kvalitet och samtidigt kan ge en bra skörd. Humle är en perenn slingrande växt som är tvåbyggare. Det betyder att han- och honblommorna finns på olika individer. Det är endast honväxten som är intressant i odling, då dess kottar innehåller de eftertraktade kemiska ämnena som bidrar till ölets arom och ger beska. Humle har en lång historia i Sverige och var till och med lagstadgad att odla under närmare 400 år. Lagen efterföljdes inte överallt men tack vare detta så finns det väl dokumenterat vart humlen odlades, vilket har varit till hjälp under den nationella inventeringen av humle. Det finns idag ett klonarkiv av humlesorter med en lång och intressant historia i Sverige. Svensk kulturarvshumle har ofta en tidigare mognadstid vilket är intressant för de svenska odlingsförutsättningarna.

Det här examensarbetet har gått ut på att jämförda 11 sorter av humle, med fokus på mognadstid innehåll av alfasyra (beska) och humleoljor (arom). Av sorterna i försöket räknas 10 som kulturarvssorter och en Saaz-klon. Arbetet genomfördes genom ett fältförsök placerat i Krusenberg cirka 1 mil utanför Uppsala samt en litteraturstudie. fyra sorter valdes ut som extra intressanta då de visade sig ha höga halter av alfasyra samt högt innehåll av de mest intressanta humleoljorna. Humle intressant för ale-stilen var Korsta som gav en hög skörd, hög alfasyra 12 % och ett högt innehåll av myrcen. Myrcen har karaktär av tallbarr, koda och amerikanske humlesorter har ofta ett högt innehåll av denna. Även Hulla Norrgård har en hög alfa syra på 10,5 % samt även den ett högt innehåll av myrcen. Humle intressant för lagerbryggaren tros vara Tvärud och Bonneråd, som båda har lägre myrcenhalt och aningens lägre alfasyra ca 6 %. Dessa sorter har mer utav humleoljorna som karakteriseras av örtighet, blommighet och skog, dessa humleoljor är vanligtvis eftertraktade i de klassiska europeiska humlesorterna.

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# 1. Introduction

## 1.1. Background

The interest in small-scale and locally produced beers has increased rapidly in the latest years (Systembolaget 2020). This can also be seen in the increased amount of registered brewers, which increased from 42 in 2010 to 392 in 2017 (Björnsbacka, 2018). Several of the newly registered brewers are microbrewers with less than nine employees. The increased interest for small-scale and locally produced goes hand in hand with the trending concept of New Nordic Food and Local Food (Bech-Larsen et al., 2016). Historically cultivated hop has the potential to give the local beer an even more local connection.

Hop has a long history in Sweden as the earliest findings date back to around 800-1000 AD (Carlsson, 2012, Karlsson Strese, 2015). Hop was then primarily used in the brewing process for its preservative properties. A law from 1440 claimed that every farmer should grow at least 40 poles of hop, this amount increased in 1474 to 200 poles (Karlsson Strese, 2015). During industrialization, new technology made it possible to brew beer in bigger batches, with more secure techniques. The interest in homebrew decreased and so did the demand for Swedish hop as hop was imported from central Europe (Karlsson Strese et al., 2010, Karlsson Strese, 2015). At the beginning of the 20<sup>th</sup> century, the interest in Swedish hop increased as imported hop were harder to come by (Karlsson Strese, 2016). A Swedish breeding program and commercial hop cultivation was initiated in 1921 for selecting a few clones that seemed promising (Karlsson Strese et al., 2010). The program lasted until 1959. When the import of European hop started again the breeding program was abandoned as there was no longer any commercial production of hop in Sweden (Karlsson Strese et al., 2010). A project to establish a Swedish gene bank for once cultivated hop by localizing and verifying the remnants of cultivated hop (Karlsson Strese et al. 2010) led to a description of about 60 varieties (Karlsson Strese, 2016). The varieties are referred to as Swedish varieties and they grow at the clonal archives at SLU in Alnarp. Historically cultivated hop many times have an interesting cultural history (Karlsson Strese, 2016) and could add an even more local touch to locally produced beer. It is therefore of interest to further study the agronomical properties of the Swedish hop varieties to see if they have the qualities required to grow and produce quality hop in Sweden.

The most important restriction for hop growing does not come from the soil and climatic conditions, but the light conditions or the length of the day. The switch from the vegetative to the generative growth phase takes place with a day length of 16 to 18 hours. These conditions are only met between the 35<sup>th</sup> and 55<sup>th</sup> parallel in the northern and southern hemispheres. Since this zone only covers the tips of South America, Africa, and Australia as well as New Zealand in the southern hemisphere, the main hop growing countries have established in the northern hemisphere (IHGC, 2020, Biendl et al., 2014). In Central Europe, there can be summers when the hops' water requirements are not fully met. This is associated with lower yields and low  $\alpha$  - acid contents. In the northern hemisphere, the most important prerequisite for good yields and ingredients is enough rain in June, July and August with about 100 mm/m<sup>2</sup> each (Biendl et al. 2012). One of the desired agronomical qualities for hop grown in Sweden is early flowering and maturing.  $\alpha$  - and  $\beta$  - acids, that contributes to the bitterness, start developing at the flowering stage and increases towards the ripening of the cones and stabilizes before full ripeness (Howard and Tatchell, 1956). This period is influenced by weather and a temperature from 16 – 20 degrees, depending on varieties, is considered optimal (Kučera and Krofta, 2009). The hop aroma comes from the essential oils that increase even after the peak of  $\alpha$  - and  $\beta$  - acids (Menary and Doe, 1983). Early maturation is also positive as the risk for pests and fungi attacks increases the longer the cones are left on the bines (Jensen, 2016). Another desired agronomical quality is resilience towards pests and a low susceptibility towards diseases. A more resilient crop needs fewer plant protection products which are following the integrated pest management principles. Studies have been made on the chemical and sensory characteristics of hop grown in Sweden (Lantau and Ottosson, 2013), although not solely on Swedish hop varieties.

The objective of this study was to investigate the agronomic-quality of ten historically cultivated Swedish varieties in the search for an early maturing high  $\alpha$  - acid-producing hop variety, that could give both good yield and good quality in Uppsala.

## 1.2. Hypothesis

Out of the ten Swedish hop clones, three of the varieties are cultivated and commercially sold on the “Elitplantstationen” as they are free from virus. With this background, the hypothesis is that these three varieties, Korsta, Hulla Norrgård and Näs will be the best performing varieties in the study. Other varieties that have been described as interesting for brewers are Gamla Källmon, Böksnäs, Tvärud, Svalöf S, Bonneråd as they have high essential oil content.

## 2. Theory

### 2.1. Biology of hop

Hop is an herbaceous perennial. Meaning that the plant has non-woody stems that reach full height in one season before dying back over the winter. Hop is a vigorous plant that can grow up to 30 cm in a day with the right conditions and reach a height of approximately 9 m in one season (Václav Fric and Rybáček, 1991). The climbing shoots of hopes are termed bines. The bine winds around and clings on to the support by using small hooked hairs (Biendl et al., 2014). Hop belongs to the same family as hemp, *Cannabaceae*, and the same orders as nettles, *Urticales* (Biendl et al., 2014, Karlsson Strese, 2015).

Hop is dioecious where only the female plant is used in commercial production as the wanted product is Lupulin from the unpollinated female inflorescence (Karlsson Strese et al., 2010). If a female hop plant is pollinated, seeds are produced in the cones which change the brewing qualities. The high fatty acid content in the seeds can have a negative impact on foam and taste stability of the beer (Forster et al., 2014). To avoid pollination all male plants are removed from the surroundings (Karlsson Strese et al., 2010, Karlsson Strese, 2015). Genetic recombination of hop is therefore uncommon, nevertheless, it should be mentioned that hop is not a strictly dioecious plant but can sometimes have both male and female flowers. The remnants of cultivated plants that have been found in the inventory could therefore either be the same genotype as once was planted or a result of cross-pollination (Karlsson Strese et al., 2010).

In commercial hop farms, it is common to replant the hop yard every 10 to 20 years to keep high productivity (Turner et al., 2011) although the plant can live for much longer (Wample and Farrar, 1983) as the clones in this study is an example of.



## 2.2. Chemical composition in hops

The cones consist of leaves, bracts, which are connected to a central axis also called strig (Figure 1). The strig is a zig-zag formed stalk and at each bend, there is a floret that is covered by bracts. The bracts are egg-shaped and arranged in a way that forms a cone. Between the bracts and the strig are the lupulin glands. The lupulin contains most of the wanted beer components (resins, essential oils, polyphenols) giving the beer its characteristic bitter taste and aroma, except for the polyphenols that are located in bracts and bracteoles (Kunze, 1999, Biendl et al., 2014).



*Figure 1: Cone of the variety Svalöf S, yellow powder between bracts and next to strig is lupulin.*

The total resin content ranges from 15 to 30 %, of the total hop cone weight, (Figure 2). Resins are usually divided into soft and hard resins depending on their solubility in hexane or methanol, respectively (Biendl et al., 2014). The percentage of soft resins of the total weight of the dry cone can range from 10-30 %. The hard resins usually make up 2 – 3 % of the total weight of the dry cone. The content of soft and hard resins may vary with hop variety, growing conditions, harvest time and post-harvest conditions (De Keukeleire, 2000, Almaguer et al., 2014). The total soft resin content consists of  $\alpha$  - acids,  $\beta$  - -acids and an uncharacterized soft resin fraction (Almaguer et al., 2014, Biendl et al., 2014). Alpha - acid is considered to be important resin for beer brewing as it contributes to the bittering of the beer.

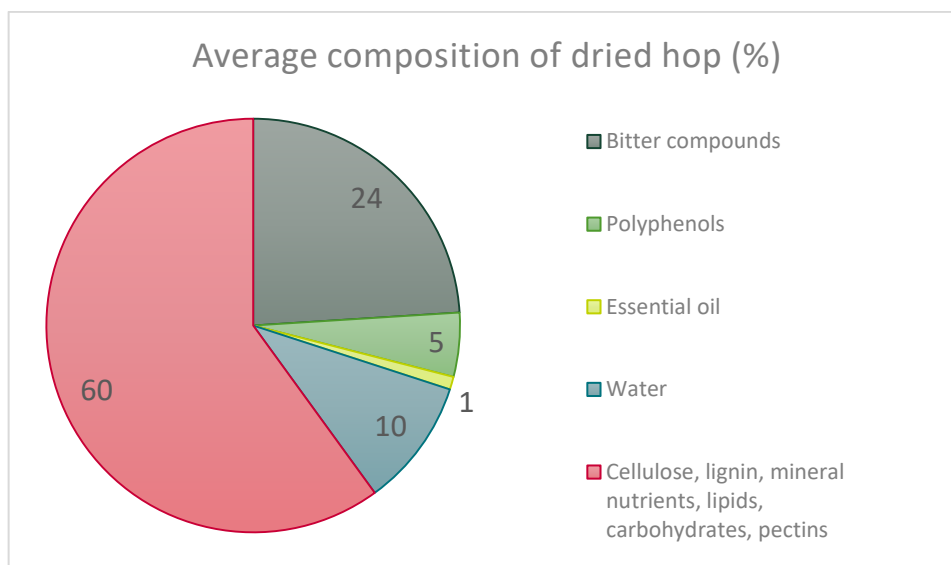


Figure 2: Example of average composition (in percent) of different dried hop cones, the original figure is to be found in Biendl et al. (2014).

### 2.2.1. Alpha - acids

Depending on the wanted type of beer,  $\alpha$  - acids content is of interest. In the latest years, it has been common to brew bitter ales (ex. Indian Pale Ales). Hop with higher  $\alpha$  -acid content is more efficient for producing a bitter beer, i.e. less hop is needed, and thus the focus in the hop industry has been producing high  $\alpha$  -acid hop varieties (Sparhawk, 2016). During development from flowers to cones, levels of  $\alpha$  - and  $\beta$ -acids synthesis increase (De Keukeleire et al., 2003). The accumulation rate has been noted to vary between hop varieties. Alpha -acids content is usually expressed at the percentage of the dry weight of cones. Alpha -acids content in the same varieties has been recorded to vary from year to year (Kučera and Krofta, 2009). The accumulation of  $\alpha$  - acid is as most intensive weeks before harvest (Hecht et al., 2004). The weather during flowering, cone forming, and cone ripening is usually considered to be an important factor. Smith (1974) found an optimum temperature in July to August for the  $\alpha$  - acids synthesis. The temperature varies between varieties (Fuggle, Northern Brewer, Hallertau, and Saaz), was between 16 to 17 °C (Kučera and Krofta, 2009).

Alpha -acids consist mainly of humulone, adhumulone, and cohumulone (Biendl et al., 2014). Alpha -acids, as well as  $\beta$ -acids, have very low solubility in water and nearly no bitter taste when untreated (De Keukeleire, 2000). To evolve the characteristic bitterness, the  $\alpha$  - acids need to be heated to chemically converted to the more water-soluble iso- $\alpha$  - acids. This conversion happens when hop is added to boiling wort. The wort consists of water and unfermented maltose from barley or other cereals (Biendl et al., 2014, Ryan et al., 2019). After the isomerization, the

molecules humulone, adhumulone, and cohumulone are transformed into iso-humulone, iso-cohumulone, and iso-adhumulone that contribute with the characteristic bitterness (Forster et al., 2014). The amount of humulone and cohumulone (20-50%) varies with hop varieties and the amount of adhumulone is invariable at around 15 % of the mixture. The different  $\alpha$  - acids generate different smells and flavour, as iso-cohumulone generates a harsher bitterness than the other two iso- $\alpha$ -acids (De Keukeleire, 2000).

Lantau and Ottosson (2013) compared  $\alpha$  - and  $\beta$  - acids of hop varieties grown in Sweden and internationally. They concluded that Swedish grown varieties often have slightly lower amounts of  $\alpha$  - acids compared to the same varieties grown internationally. They noticed no differences in  $\beta$  - acid content between Swedish and internationally grown hop.

### 2.2.2. Beta - acids

Beta-acids also have poor solubility in water when untreated and they do not undergo isomerization the same way  $\alpha$  - acids do during boiling (Biendl et al., 2014). The oxidation of  $\beta$ -acids contributes to a bitter profile, this happens at a slower rate than the thermal isomerization of  $\alpha$  - acids (Ryan et al., 2019). Studies by Haseleu et al. (2009) showed that there are products from boiling  $\beta$ -acids that have bittering potential in addition to the isomerized  $\alpha$  - acids (Haseleu et al., 2009). The hop acids inhibit the growth of gram-positive bacteria and contribute to a sterile beer after wort boiling (De Keukeleire, 2000).

### 2.2.3. Essential oils

The essential oils contribute to scent and aroma. Examples of typical aromas from different hop varieties are spicy, herbal, citrusy, fruity, piney and floral (Goiris et al., 2002, Praet et al., 2015, Barry et al., 2018, Ryan et al., 2019). The essential oil content in hop ranges between 0.2 % to 3 % d.w. and the amount also varies between variety and growing conditions. These compounds are very volatile, and they are easily evaporated in the brewing process (Biendl et al., 2014). Therefore, the hop aroma perceived in the beer is rarely the same as the aroma of the green undried cones. The essential oil fraction is rather complex and not fully understood yet and it has been suggested to consist of over 1000 compounds whereas around 440 have been chemically identified (Roberts et al., 2004, Almaguer et al., 2014).

Four essential oils that often are referred to by brewers are myrcene, farnesene,  $\beta$  - caryophyllene and  $\alpha$  - humulene (Systembolaget, 2020, Högström, 2020). Myrcene is often the largest component in hop essential oil, independent of variety. It is also

mentioned to be responsible for the smell of green hop (Velde and Verzele, 1986, Almaguer et al., 2014, Forster et al., 2014) but it is also related to a resinous, piney and spicy aroma. Myrcene is interesting when brewing fruity ales and IPA (Högström, 2020). Farnesene,  $\alpha$  - humulene and  $\beta$  - caryophyllene are interesting for the lager brewers (Högström, 2020); they are also of interest if making more traditional European lager (Systembolaget, 2020). Beta- Caryophyllene could also be of interest for brewing traditional ale (Högström, 2020). Linalool is associated with floral and citrusy aroma (Schönberger and Kostelecky, 2011) and geraniol with floral more towards rose like aroma.

*Table 1: Table over 7 compounds in essential oil and their aroma description from hop flavour database (ASBC).*

Compound name	Aroma description
Myrcene	Herbs, metallic, resinous, spicy, balsamic, geraniumlike, green, peppery, terpene, balsam, plastic, sweet carrot, slight piney, celery, lemon, woody
Limonene	Citrusy, orange, green, fruity
Geraniol	Floral, citrus, rose-like, flowery, lime, lemon, hyacinth, geranium
Linalool	Flowery, fruity, floral, citrus, rosewood-like, aniseed, terpenic, rose-like, hoppy, coriander seed
$\alpha$ - Humulene	Balsamic, flowery, grassy, herbal, spicy, woody, clove oil.
$\beta$ - Caryophyllene	Woody (cedar), spicy, flower, turpentine, clove, lime, green, terpene.
Farnesene	Woody, green apple, sweet, lavender.

Studies have been made on the possibilities to distinguish hop varieties by looking at the content of hop acids and essential oils (Likens and Nickerson, 1967, Kovačević and Kač, 2002) concluding that it is possible to distinguish varieties. Although, there is a need for reference samples of each specific variety tested. To distinguish a variety is more difficult with a minor difference in hop acids and essential oil.

## 2.2.4. Polyphenols

Polyphenols contribute with an aroma to the beer and can be found in bracts and bracteoles, except for the polyphenol xanthohumol that is produced in the lupulin glands (Biendl et al., 2014). The polyphenol content ranges from 3 to 8 % depending on variety and can consist of several compounds, some specific to hop (Forster et al., 2014). Polyphenols act as strong antioxidants as they are easily oxidized. This contributes to protecting the beer against oxidation (Ibid). Xanthohumol is a polyphenol that has been investigated in several studies for its potential cancer-preventive properties (Stevens and Page, 2004). There is a possibility that xanthohumol and related prenylflavonoids from hops could have potential for application in cancer prevention programs and in prevention or treatment of (post-)menopausal “hot flashes” and osteoporosis i.e. when bones become weak and brittle.

## 2.3. Fertilization and irrigation of hop in Sweden

The need for fertilization is dependent on several factors, as weather conditions and the soil's capacity to supply nutrients. Other factors as plant density, expected yield and the total biomass of the plant is also important as this affects the overall removal of nutrients from the soil (Gingrich et al., 2000, Kvarmo et al., 2020b). As hop produces a large amount of biomass, the need for nutrition is relatively large in the spring. Nutrients should be added 30-45 days after emergence, usually from mid-May to mid-June, for the plant to be able to use it during its vegetative growth stage, which lasts until the beginning of July. Nutrients are then translocated from leaves to cones (Gingrich et al., 2000, Darby, 2013).

### 2.3.1. Macronutrients

#### *Nitrogen (N)*

Both the plant biomass and the cones contain N that is thereby removed from the soil at harvest. The goal with fertilization is to add the amount of N that the plant could potentially remove from the soil. Usually, the whole plant weight is unknown but can be roughly estimated, as it is three times as large as the cone yield per plant. The whole plant biomass contains approximately 3 % of N (Darby, 2013). The addition of N should be between 80 to 150 kg N/ha depending on expected yield and factors earlier mentioned (Neve, 1991, Gingrich et al., 1994, Jensen, 2016). According to Kořen (2008), the typical plant density on a hop yard is between 2000 – 3000 plants/ha. An example of typical plant distances is 3.2 m x 1.5 m in Hallertau, Germany, and 4 m x 0.9 m in Yakima valley, Washington State, USA (Dodds, 2017). It is recommended to divide the N application into two or three

applications to reduce the risk of N leaching. Ideally, N should be available before the rapid biomass growth from late May to early July (Gingrich et al., 1994 and 2000, Lizotte and Serrine, 2020). Nitrogen should not be applied after the plant has flowered as this can lead to unwanted vegetative growth instead of cone development (Lizotte and Serrine, 2020). Over-application of N not only increases the risk of runoff but it can also increase problems such as powdery mildew and two-spotted spider mite infestations (O'Neal et al., 2015, Takle and Cochran, 2017).

#### *Phosphorous (P)*

The need for P is also dependent on the yield and soil supply of P. A yield of 2017 kg hops/ha removes approximately 22 kg P/ha (Gingrich et al 1994). Approximately 25 to 30 % of the total plant P is found in the cones. P is important for plant growth as it takes parts in key functions as energy transfer and photosynthesis (Båth 2004).

#### *Potassium (K)*

The need for K is relatively large, approximately 80 to 140 kg K/ha, whereas 25 % of this is found in the hop cones (Gingrich et al., 1994, Jensen, 2016). Depending on soil type and its K content the need for fertilization varies. Potassium is not incorporated in the biomass, but it plays an important role in plant metabolism. Potassium affects the thickness of the cell wall and a thicker cell wall makes the plant hardier against fungal infections (Heimer, 2004). Symptoms appear as brown spots and chlorosis and can first be seen in older and semi-old leaves as K is mobile in plants (Båth 2004).

#### *Magnesium (Mg)*

Magnesium is easily transported in the soil and low clay content implicates an increased risk of Mg-deficiency. Clay-soils have larger storage of Mg and the risk of Mg-leakage is higher on sandy soils (Båth, 2003). Magnesium is a central part of the chlorophyll molecule and is therefore important for photosynthesis. Deficiencies are first seen in the older leaves as Mg is mobile in plants (Båth 2004).

#### *Sulphur (S)*

Sulphur deficiency can reduce growth and inhibit protein synthesis and thereby cause an accumulation of nitrate in the plant. The symptoms are as for N deficiency yellow leaves although for S the deficiency occurs first in the older leaves and not in the young (Båth 2003). Sulphur-deficiency can also cause delayed and reduced flowering (Heimer 2004).

### *Calcium (Ca)*

Calcium is important for plant structural and physical stability. Symptoms of Ca-deficiency can be necrosis, soft mushy spots on the leaf. Calcium is not easily moved in plants and deficiency is first seen on younger leaves, growing zones, and fruits (Báth 2003).

### 2.3.2. Micronutrients

Amongst micronutrients, the most reported deficiencies in hop are Zinc (Zn) and Boron (Neve 1991). Zinc is essential for photosynthesis; it is important for protein synthesis and the balance of growth hormones (Magnusson 2003). Hop is sensitive to Zn deficiency as it is important for optimal growth, internode elongation, and cone setting (Lizotte and Sirenne 2020).

Boron (B) is important for carbohydrate transport in plants (Lizotte and Sirenne 2020). Boron deficiency causes damages in the plant growth zone (Kvarnmo et al 2020) and a symptom of this is delayed development of new shoots (Neve 1991). Boron is released from organic material and deficiencies are therefore more common in soils with low soil organic compounds (Kvarnmo et al 2020). Manganese (Mn) is another micronutrient that is essential for photosynthesis in plants. Manganese is also a building block in an enzyme that protects the plant tissue against the harmful effects of free radicals. Manganese is considered one of the most important micronutrients for resilience against fungal pathogens (Magnusson, 2003). Both B and Mn are toxic when accumulated in higher concentration than the plant need. Soil pH is important as it affects the plant availability of nutrients. A pH lower than 5.7 increases the amount of Mn in the plant tissue (Gingrich 1994).

Hop is sensitive towards higher levels of sodium (Na) and chlorine (Cl). Excess of Na can lead to toxicity visible as necrosis in leaf tips (Jensen, 2016, Lizotte and Serrine, 2020).

### 2.3.3. Water availability and climate

Hop has been noted to be fairly drought tolerant with root systems that can grow deep as two meters (Naawuka 2017). According to Jensen (2016), hop rarely need to be watered in Nordic conditions when growing on soil with good water holding capacity. Hop have been reported to use 610 to 715 mm water per season (Evans, 2003, Nakawuka, 2013). Most of the water is required between training and flowering for optimal growth (Darby et al., 2016). The crop uses between 60 mm (Kučera and Krofta, 2009) and 100 mm per month in June, July and August (Jensen,

2016). Možny et al. (2009) studied the impact of climate change on the hop variety Saaz and documented an increase in yield in correlation to higher precipitation. Svoboda et al. (2008) showed a 21 % increase in hop cone yield with irrigation as their test site was in a region with yearly precipitation lower than 500 mm. Water stress during the cone development stage has been reported to affect the yield and quality of cones (Nakawuka et al., 2017). Studies by Srečec et al. (2008) show that a higher temperature, meaning evapotranspiration higher than 4.5 mm/day during cone formation causes a decrease in  $\alpha$  - acid content in the variety Aurora. The study could also show a positive correlation between total rainfall and  $\alpha$  -acid content.

## 2.4. Pathogens & pests

Several pathogens can affect hop. For further reading “Field guide for integrated pest management in hop” can be recommended (O’Neal et al., 2015). A common problem in hop production is fungal diseases such as hop downy mildew, *Pseudoperonospora humuli*, (humlebladmögel) and powdery mildew, *Sphaerotheca humuli* (Humlemjöldagg). Other common pathogens are hop aphids, *Phorodon humuli* (humlebladlus) and spider mites *Tetranychus urticae* (spinnkvalster). Other pests that are potentially harmful against hop is butterfly larvae (Neve 1991, O’Neal et al., 2015). In Sweden, the larvae of *Hypena rostralis* (Humlenäbbfly), have been noticed to feed on hop (Artdatabanken, 2020). As hop is a clonally reproduced perennial, often cultivated from rhizome cuttings, there is usually little genetic diversity in the hop yards (Neve 1991). A low genetic diversity could mean a lower resilience towards diseases (Finckh and Wolfe, 1997). There are studies of planting different cultivars of a crop that have a reducing effect on pest and disease pressure. Although this is depending on the pathogen sensitivity of the varieties in the mixture (Mundt, 2002).

### 2.4.1. Downy mildew (Humlebladmögel)

*Pseudoperonospora humuli* is an obligate parasite that causes downy mildew. The disease can slow down the development of shoots and prevent cone formation and can lead to major productivity loss in hop (Salmon and Ware, 1929, Runge and Thines, 2012). When the infection reaches the cones, their further development stops and get a brown coloration. The cones can sometimes have a striped appearance (Figure 6) as bracteoles tend to get browner than the bracts (O’Neal et al., 2015). Hop cultivars vary in their resilience towards *P. humuli* but no variety is completely immune (Václav Fric and Rybáček, 1991, O’Neal et al., 2015). The first appearance of downy mildew can be seen in spring as chlorotic, stunted shoots (see Figure 4 and Figure 5; Johnson and Skotland, 1985, Václav Fric and Rybáček,



1991). These stunted shoots are the primary infection of the disease and are often called basal spikes. Later in the season, infected shoots can grow as laterals on the bine (Ware, 1926). The emerging of basal spikes can be predicted using degree models (Gent et al., 2010, O’Neal et al., 2015). Through emerging sporangia on the infected tissue, the infection spread to healthy parts of the plant or plants nearby see Figure 3 (Hoerner, 1932, Neve, 1991). The sporangia contain zoospores that with the help of its two flagella can move in moisture on a plant leaf and enter the leaf through the stomata (Neve 1991). The sporulation of sporangia occurs when the night temperature reaches above 6 °C, and with relative humidity above 90%. Between the temperatures 15 – 20 °C, there is an increased risk of sporulation. Another risk factor for disease spreading is daytime rain, as the zoospores enter through an open stomata (O’Neal et al., 2015).

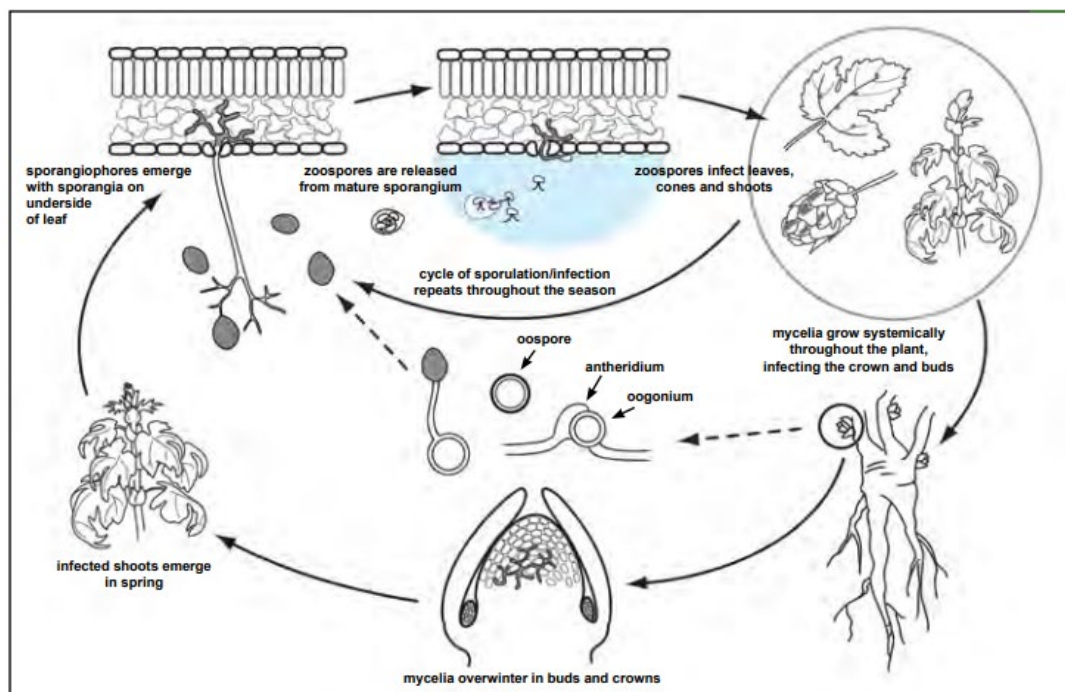


Figure 3: The life cycle of *Pseudoperonospora humuli* on hop. (Prepared by V. Brewster in O’Neal et al., 2015)



Figure 4: Downy mildew basal spike on the variety Tvärud (24th June 2020)



*Figure 5: Downy mildew basal spike on the variety Hulla Norrgård, (19 of May 2020)*

Infected leaves get dark angular spots next to the leaf veins (Figure 7) but also darker greyish bigger spots on the leaf surface (Runge and Thines, 2012), which is characteristic for downy mildew. The darker spots consist of sporangiophores (Neve1991). Another typical sign of downy mildew are little yellow spots on the leaf (Eyck 2015)



*Figure 6: Hop downy mildew on cones, "File: INFECTED CONES.jpg" by Michelle Marks is licensed under CC BY-SA 4.0*



*Figure 7: Dark angular spots on hop leaf by Downy mildew, (photo by D. H. Gent in O'Neal et al., 2015)*

The secondary infection grows into and overwinters in the perennial hop rhizome (Neve 1991; Ware 1926). Shoots from infected rhizomes can have weaker growth and could also have a reduced yield (Neve 1991).

#### 2.4.2. Powdery mildew (Humlemjöldagg)

Powdery mildew caused by *Podospaera macularis* is a fungal disease that can be recognized by its characteristic white mould look (Figure 9). It can cause reductions in both yield and quality. The infection of flowers and young cones causes abortion or deformation of cones. The infected cones can develop an unwanted mushroom-like aroma. Powdery mildew causes accelerated senescence in cones, and the affected cones become reddish-brown (Neve, 1991, O’Neal et al., 2015, Probst et al., 2016). An experiment by Gent et al. (2010) showed a reduction of  $\alpha$  - acid with 0.33 % in the cultivar galena by every 1 % increase of powdery mildew in the cones. Young cones are more susceptible to infection and become less susceptible as they mature. A late attack does not affect  $\alpha$  - acid levels directly but indirectly by the decrease in yield due to dry and brittle cones caused by the accelerated senescence (O’Neal et al., 2015).

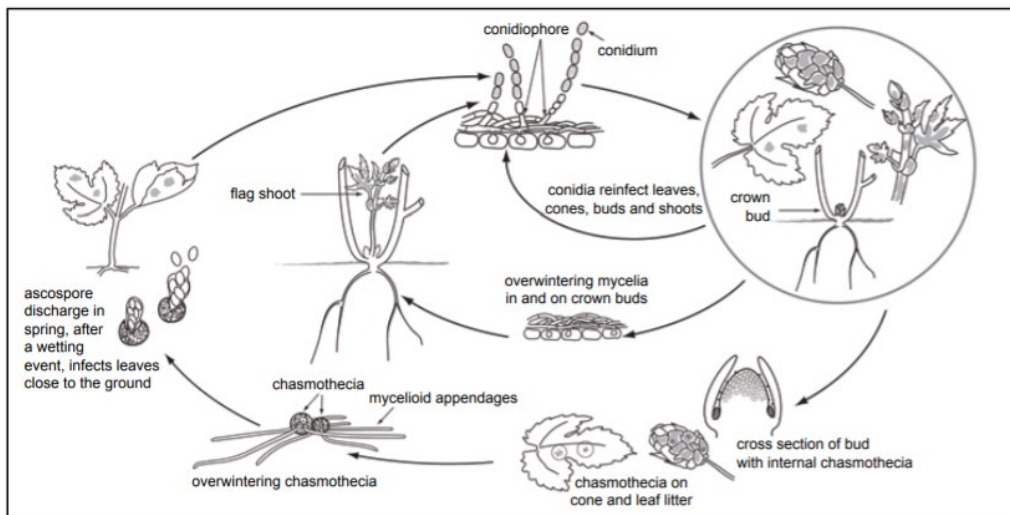


Figure 8: Life cycle of Powdery mildew on hop. The sexual stage shown by arrows on the bottom and left side of the figure (Prepared by V. Brewster in O’Neal et al., 2015)



Figure 9: Powdery mildew *Podosphaera macularis* on *Humulus lupulus*. Photo: David Gent, USDA Agricultural research service, CC Attribute 3.0 licence.



Figure 10: Hop shoot infected with powdery mildew, typically called flag shoot. Image from (Gent et al., 2008).

The disease overwinters in a vegetative state as a hypha in dormant infected buds on the rhizome. It can also overwinter in the soil and on plant remnants on old bines. The shoots coming from the infected bud are typically stunted and whiter like they have been dusted in talk, compared to the healthy shoots. These shoots are referred to as flag shoots. The flag shoot release asexual spores (conidia) that are spread by the wind and that can infect other healthy parts of the plant or other plants. Powdery mildew does not need a water film to infect the plant and can therefore spread in dry weather (Eyck, 2015 and O’Neal et al., 2015,). A symptom of powdery mildew can appear as small chlorotic spots on the top of the leaf, indicating that the plant is suffering from malnutrition. The plant is not being able to produce enough chlorophyll to make the leaf green and thereby not being able to use the sunlight into chemical energy in an optimal way. Spore producing white powdery spots can be seen on the undersides of the leaf (Eyck, 2015).

### 2.4.3. Prevention and control of fungi

The pathogen pressure is affected by the surroundings of the hop yard, places next to a river or other low-lying places with a risk of cool air pooling increases the pathogen pressure. It is also of importance to choose healthy starting material when starting a new hop yard or when filling in a gap in an old. Spacing between plants is important as this increases the possibilities for air circulation and sunlight exposure (Eyck, 2015). To reduce the inoculum and the development of downy and powdery mildew, old bines and the first growth in the spring are removed. This can be done by crowning, pruning, and scratching (Gent et al., 2009, Gent et al., 2012). Crowning means cutting off the top 2 – 5 cm of the crown and the plant before bud break. The pruning can be done mechanically or chemically by cutting off the old bines as well as the new shoots. This can be done by machine or by manually with a knife depending on the number of plants. Scratching means the removal of the top 2-5 cm of the crown within the soil surface by using a machine with two spinning disks that scratch the soil (O’Neal et al., 2015). This practice should be done as late as possible to reduce the severity of downy mildew (O’Neal et al., 2015). Nevertheless, it is important to keep in mind that the treatment also shortens the time for the plants to reach their full height before the end of June. Bines need three to four weeks to regrow after pruning before they are ready for training (Sirrinc, 2016). A study by Darby et al. (2019) concluded that due to their short growing season, an early crowning (second week of April) was the best option. This is due to the decrease in yield by shortening the growing season by a late crowning (second week of May).

Cutting off the earliest growth also provides a more coherent growth of the plants, as the first irregular shoots are removed (Darby 2019). These practices also remove something referred to as Bull shoots (O’Neal et al., 2015) that are fast emerging and strong growing shoots. Bull shoots have a hollow core and are therefore more sensitive to breakage than other shoots and the recommendations are to avoid training them. Around midsummer it is a common practice to hill-up soil around the crown, this promotes root and rhizome development around the top of the crown. This practice also buries diseased shoots next to the crown and prepares the plant with a protective layer for next year’s pruning (O’Neal et al., 2015). It is important to remove any diseased shoots that emerge from the soil as well as keeping the area around the bine open and sunny to allow air circulation and for the area to dry up. This means removing weeds and subsequent hop shoots as this helps to contain moisture and shadow around the bine. Further, when the bines have reached a height of 1.2 meters leaves should be stripped from the bine, recommendations are to do this continuously and not all at once. This should be continued as the bine grows until the bine is without leaves the first meter (Eyck, 2015).

### *Fungicides*

Copper-based fungicides are used internationally in hop farming both in organic and conventional growing systems. Studies have been made on the effects of Cu on earthworms that showed an avoidance of earthworms at higher Cu levels than 553 mg Cu/kg soil (Van Zwieten et al., 2004). Microbial communities have also been recorded to change the structure and local spatiality of the communities at higher Cu concentrations (Van Zwieten et al., 2004, Ge and Zhang, 2011, Mackie et al., 2013). In Sweden, copper-based fungicides are not allowed to be used in organic or conventional farming (Ascard et al., 2017). Although it is permitted to fertilize the foliage with Cu if needed (Kvarmo et al., 2020a).

Up to recently, there have been no approved pesticides in Sweden to use in hop farming as the industry has been too small. Now there is permission (also in organic farming) to use a fungicide called VitiSan (K bicarbonate).. From 2019, it is permitted in Sweden to use the fungicide Aliette on conventionally grown hop (LRF trädgård, 2020).

#### **2.4.4. Hop aphid (*Bladlus*)**

The hop aphid *Phorodon humuli*, is a common problem in hop farms in the northern hemisphere. As the aphid feed from the plant phloem, it weakens the plant by robbing the plants of nutrients that can lead to a lower yield. The honeydew produced and secreted by the aphids could increase the risk of secondary infections, such as sooty mould. Aphids can also serve as a vector for viruses. An aphid infestation late in the season is problematic as aphids feed on cones and lower the quality (Neve 1991). The aphids overwinter by laying eggs on species of *Prunus* that hatches into wingless females in spring. This first generation of aphids reproduces asexually by giving birth to living young females. This can occur for another generation until winged females are produced when the temperature threshold is reached, usually this occurs in the middle of May. Aphids enter a winged state and migrate to hop. There the asexual reproduction continues until the end of summer when winged males and females are being produced. These fly back to *Prunus* to mate and lay eggs. The eggs will hatch into asexual females in the spring (Eyck, 2015).



Figure 11: Hop aphids on the variety Hulla Norrgård and a possible secondary soot infection      Figure 12: Leaf of the variety Bonneråd with aphids

Generally, aphids are harder to fight when they get inside the cones as this makes it harder for predatory insects and insecticides to reach them. Therefore, insecticides are usually used right before flower formation (Eyck, 2015). Making the hop yards more attractive for beneficial insects by conservation biology control is a discussed method (Grasswitz and James, 2009).

#### 2.4.5. Two spotted spider mite (Spinnkvalster)

Two spotted spider mites, *Tetranychus urticae*, are very small insects. Nevertheless, they can reduce both yield and quality. The mites thrive under hot and dry conditions. They are called spider mites as they spin a web over the surface of the leaf as protection while feeding on the plant (Eyck, 2015). The two spotted spider mite is considered a major agricultural pest in several crops (Fonseca et al., 2020). The mites, as the aphids, are fed on the plant's phloem on leaf and cones. Symptoms of this are weak and bronzing plants as well as red and brittle cones. Female mites overwinter in the hop yard, emerging in spring, and start feeding on the hop shoots. The egg-laying starts in about two days and eggs are hatched only a few days after. Fertilized eggs hatch females and unfertilized male mites. The reproduction rate is high and a female can lay up to 16 eggs/day and 240 during their lifetime (Eyck, 2015). Dry conditions and high N fertilization rate have been connected to two spotted spider mites (Iskra et al., 2019). If using insecticides, it is important to use a selective one, as there is a high risk of quick mite population growth when there are no beneficial insects to prey on the mites (Eyck, 2015). Studies comparing the abundance and phenology of pesticide-treated and pesticide-free hop have shown that natural enemies can be as effective as using miticides in regulating mite population (James et al., 2001).

#### 2.4.6. *Hypena rostralis* (Humlenäbbfly)

*Hypena rostralis* is a moth where the caterpillar has been recorded to eat on the leaf and cones of hop. In a study by Campbell (2019), *H. rostralis* was noticed not to feed on cones if the leaf was available. In their study they did not see severe damage by *H. rostralis* compared to other common lepidopterans (moth and butterfly) (Campbell, 2019).

#### 2.4.7. Pest controlling methods

Increased plant diversity can reduce pest pressure and increase populations of beneficial insects, known as conservation biological control. Plant diversifying creates more opportunities for beneficial insects to get shelter and alternative food sources as other prey, nectar and pollen (Hossain et al., 2002, Zehnder et al., 2007, Grasswitz and James, 2009). Nevertheless, Grasswitz and James (2009) looked at the influence of hop yard ground flora had on hop aphid, two-spotted spider mite and hop looper, as well as this affected their natural enemies. They did see a significant increase of the natural enemies in the ground flora compared to the control plots. Although they did not see the same result on the hop plant itself. This could have many reasons some mentioned are the choice of method and that the natural enemies might prefer the ground cover instead of the hop. They conclude that ground flora shouldn't be recommended with the only intention of increasing natural enemies on the hop plant. Over-application of N can cause plants to produce succulent. This has been linked to increasing problems with aphids and spider mites (Gent et al., 2015).

Against the larvae of *Hypena rostralis* it is important to remove the larvae from the plant. This can be done in several ways either using a biocide with *Bacillus thuringiensis* early in the season or by shaking the plants to get the larvae to fall to the ground. As the larvae easily can climb the bine again it is important to remove or kill them (Pettersson, 2011).

### 2.5. Harvest

The time of harvest can be decided by different parameters such as the peak of  $\alpha$  - acid or higher essential oil content. Studies made by Murphey and Probasco (1996) show that  $\alpha$  - acid content in hop peak when cones reached 22-24 % dry matter. The dry matter can be calculated by weighing field fresh cones (green weight) and drying them and re-weighing them. The dry weight percentage is calculated by dividing the dry weight by the green weight (Madde and Darby, 2012). It is important to keep in mind that the essential oil content increases after the development of  $\alpha$  - acids is completed (Stevens, 1961, Almaguer et al., 2014).



Although at a later harvest more time for possible infections of the cones is given (Calderwood and post 2015). The cones preferably are harvest in dry weather, as the time for drying increases if they are wet. Sensory assessments of the cones is also a good way of knowing the harvest optimum. Immature hop have a smell of green grass and overripe hop smell more of garlic and onions. The sound of a cone crushing can also be a sign as a mature cone have a sound reminding of a baby rattle (Calderwood and Post 2015).

From the start of flowering and during the development of cones accumulation of  $\alpha$  - and  $\beta$  - acids increase significantly (De Keukeleire et al., 2003). Matsui et al. (2016) studied the impact of different pruning and harvest dates on chemical components and yield. The difference in time of pruning gave no significant effect on the time of flowering, chemical components nor yield. Although in this study harvest time influenced the amount of essential oils in the cone. Five different harvesting times were tested, from the middle of August to the middle of September. According to the study, the essential oils and linalool increased during the time of the harvest period. Brewing trials were also done and according to their results, the hoppy aroma is stronger in beer brewed with later harvested hop (Matsui et al., 2016). Lafontaine et al. (2019) made a study on the impact of harvest date on the aroma profile on the hop variety Cascade. The authors concluded that the maturity of the hop when harvested had a significant impact on the aroma of cascade hop (Lafontaine et al., 2019).

## 2.6. Drying

Fresh hop cones have a water content of 80 % and can be used directly for beer brewing. Although the cones are sensitive towards microorganisms and oxidation that quickly can lower the quality. The brewing needs to be well-timed with the harvest, to keep the time between harvest and brewing as short as possible. To prolong their quality the cones are usually dried shortly after harvest (Forster et al., 2014). There are several suggestions for temperature and time for drying. According to Kunze et al. (1999), the drying should be at a maximum of 60 °C to a water content of 8-12 %. According to Forster et al. (2014), drying should occur at 65 °C until a water content of 9-11 %. A study by Rybka et al. (2018) showed that drying hop reduces the intensity of the aroma depending on the drying temperature. The cones dry from outside to the inside of the cone and the strig often has higher moisture than the bracteoles. The goal is to get a uniform moisture content in the cones, and this can be done by conditioning them for a couple of hours (Biendl et al., 2014).

## 3. Material and Methods

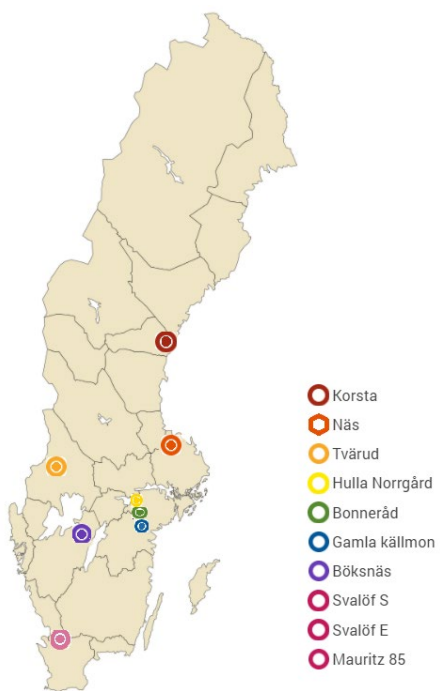
### 3.1. Literature

A literature survey over previous studies concerning growing hop,  $\alpha$  - acid was performed in PRIMO and google scholar using the search words “Hop”, “hop”+ “germplasm”, “hop”+ “ $\alpha$  - acid”, “humulus lupulus” + ”cultivation”.

### 3.2. Description of clones

Eleven hop clones were used in this study. Out of the 11 clones, 7 of the hop varieties have been found feral in Sweden (Figure 13) and six varieties have been described in the book by Karlson Strese (2016). Korsta is the clone from the furthest north and was found outside of Sundsvall. Böksnäs is the furthest south clone, outside of Tiveden. Näs is found approximately 80 km outside of Uppsala and is included in the “grönt kulturarv” brand. Hulla Norrgård, Bonneråd and Gamla Källmon all come from around Katrineholm. Hulla Norrgård is also included in the “grönt kulturarv” brand. Svalöf E, Svalöf S and Mauritz 85 are a result of the Swedish breeding program. Mauritz 85 is mentioned as the most promising clone from the breeding program (Strese, 2016). Svalöf S is the only clone with pink pistils in this study (Figure 14). Three of the varieties are cultivated and sold on the “Elitplantstationen” as they are free from viruses. Other varieties that have been described as interesting for brewers is Gamla källmon, Böksnäs, Tvärud, Svalöf S, Böksnäs as they have high essential oil content.

Tvärud comes from Järperud close to the Norwegian border but is not described in the book. Zlatan is a Saaz clone growing in Uppsala that a student from the Czech Republic brought to Sweden 30 years ago. The  $\alpha$ -acids content according to the study made by Strese (2016) can be seen in Table 2. A hypothesis of time of flowering and harvest were made from earlier studies of the varieties Karlson Strese (2016) grown in Alnarp, Skåne. Earlier studies of essential oil content show that Hulla Norrgård has a high percentage of Myrcene of the total oil (Table 3).



*Figure 13: Map over 10 of the 11 hop varieties in the report.*



*Figure 14: Flowers of the variety Svalöf S with pink pistils.*

Table 2: Table 2 Varieties used in this study and their  $\alpha$ -acids content according to the study made by Karlsson Strese (2016).

	$\alpha$ -acid% (K. Strese 2016)
Gamla Källmon	7.2
Hulla Norrgård	8.1
Bonneråd	4.5
Mauritz 85	4.1
Svalöf S	5.3
Svalöf E	4.4
Korsta	7.9
Böksnäs	3.7
Näs	6.3

Table 3: Essential oil content in % of total oil content (Strese 2016)

	Myrcene (%)	$\beta$ -Caryophyllene (%)	$\alpha$ -Humulene (%)	Farnesene (%)
Hulla Norrgård	43,2	5,5	15,5	20
Korsta	28,7	11,9	32,9	0,2
Mauritz 85	22,2	9,6	23,4	10,2
Näs	20,6	5,6	13,4	29,2
Svalöf E	22,2	13,8	43,4	3
Svalöf S	31,1	10,6	22,9	9,4

Table 4: Flowering (pink) and Harvest (Yellow) according to Karlsson Strese (2016)

Varieties	June			July			August			September		
Bonneråd												
Böksnäs												
G. Källmon												
Hulla NG												
Korsta												
Mauritz 85												
Näs												
Svalöf E												
Svalöf S												

### 3.3. Experimental plot

The study was conducted between April 2020 and September 2020 outside of Krusenberg, Uppsala, Sweden (59.742N, 17.684E). The plot was designed in six rows; every row contained doublets of all the 11 varieties from start. Spacing between rows was 3.5 m and 2 m between plants. Bamboo sticks (diameter of 3-4 cm) were put 65 – 70 cm into the soil and supported the climbing plants with a length of 3.7 m. The hop experimental plot had a plant density of 1330 plants per hectare. In total 132 plants were planted in 2017 whereas 12 plants of each variety. Not all plants survived until 2020 (Table 4). The varieties Näs, Svalöf E were represented with 5 and 7, Gamla källmon, Svalöf S and Zlatan with 10 plants in the trial, respectively.

Table 5: Number of plants per variety in 2020

Variety	Plants
Bonneråd	9
Böksnäs	12
Gamla Källmon	10
Hulla Norrgård	12
Korsta	12
Mauritz 85	10
Näs	5
Svalöf E	7
Svalöf S	10
Tvärud	11
Zlatan	10

#### 3.3.1. Soil

Soil texture and pH were measured in September. The soil in the field trial had an average of clay 4.1 % (<0.0002), silt 7.7 % (0.0002-0.06) 74.7 % fine sand (0.06-0.2 mm, 12.3 % medium sand (0.2-0.6 mm), sand 1.2 % (0.6–2 mm). The top layer (0-20 cm) had an organic matter content of 2.4 %, the second layer (20-40 cm) had 1.2 % and the third layer had very low organic matter content at 0.3 % (Table 5). The pH ranged from 5.8 in the top layer to 6.6 in the third layer.

Table 6: Soil pH and organic matter content

	pH	Organic matter
0-20	5.8	2.4
20-40	5.9	1.2
40-60	6.6	0.3

### 3.3.2. Weather

The weather during the growing season of 2020 could be considered as normal, with slightly warmer and slightly dryer autumn. Data was collected from the weather station in Ultuna 11.5 km from the field trial (Figure 15).

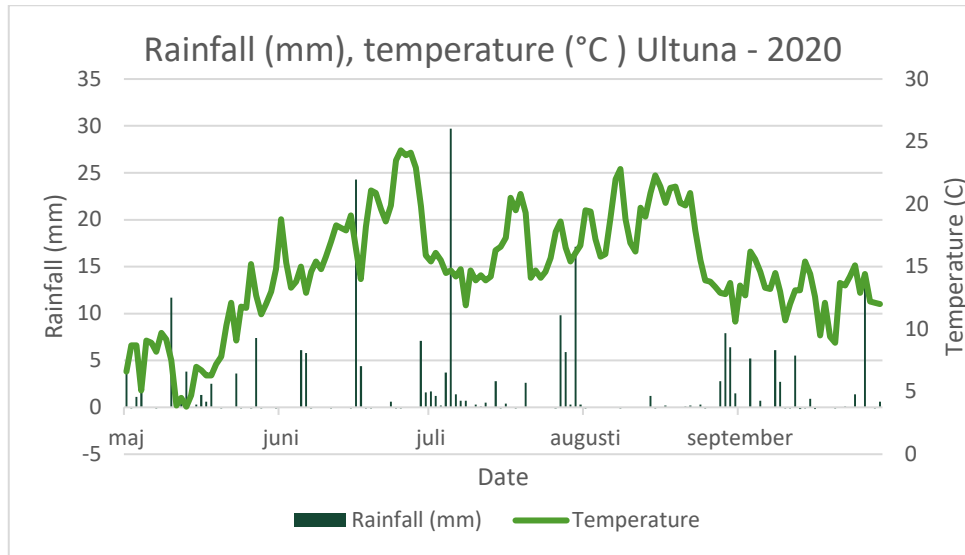


Figure 15: Rainfall (mm) and temperature (°C) during growing season 2020 in Ultuna (Fältforsk)

April 2020 was 2°C warmer and dryer than compared to the reference period 1961-1990 (Table 6). May was a few degrees colder and with 6 mm more in rainfall than the reference years of 1961-1990. June had the highest average temperature with 18.5°C since 1917 and had 10 mm more rainfall compared to the reference period. July had 16.4°C in monthly average and 14 mm more rainfall than the reference period. Compared to the reference period 1961-1990, August was 3°C warmer and had 46 mm less rainfall. September was 3°C warmer and had 14 mm less rainfall than the reference years of 1961-1990 (SMHI 2020).

Table 7: Monthly average temperature and rainfall for Uppsala 2020 and 1961 – 1990 (reference period). (SMHI 2020)

(SMHI, 2020)Month	Average Temperature °C 2020	Average temperature °C 1961 -1990	Average rainfall mm 2020	Average Rainfall mm 1961-1990
April	6.8	4.1	16	29
May	9.3	10.4	39	33
June	18.5	15.0	55	45
July	16.4	16.4	89	75
August	18.3	15.2	19	65
September	13.3	10.9	36	59

### 3.3.3. Maintenance

The hop plants were first pruned at the beginning of May and ten bines were kept. On the 15<sup>th</sup> of May, bamboo poles were placed in the soil, by drilling a hole next to the plant. The training was done at the beginning of June and 4-6 stems were kept having a backup bine if one would break from wind or be hurt by the training. The field was looked after once a week and shoots from the ground were pulled off. When basal spikes were discovered they were noticed and removed. Leaf stripping the bottom of the bine, 20 – 30 cm was done in the first week of August. Below is a schedule of maintenance during the field trial, inspired by the timing of hop production management schedule made by Michigan state university (2020).

Table 8: Culture stages season 2020

Month	April				May				June				July				August				September			
Week	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Plant stage	Spring regrowth				Vegetative growth				Reproductive growth				Preparation for dormancy											
Growth stages	Sprouting	Leaf development		Side shoots				Flowering				Maturing of cones												
		Elongation of bines				Cone development																		
Plant-support																								
Training																								
Weeding																								
Leaf stripping																								
Fertilization																								
Pest scouting																								
Harvest																								

### 3.3.4. Fertilization

The field was fertilized once with 1 kg Biofer 10-3-1 which is a pelletized organic fertilizer made from blood and bone meal from slaughter rests (Gyllebogöding 2020). The amount per hectare was calculated by multiplying the plant density of the field trial, 1330 plants/ha, with the amount of added nutrients g/plant, see Table 8. The trial did not get any irrigation.

Table 9: Macronutrients in Biofer 10-3-1 used as fertilizer on hop during field trial 2020

Nutrient	g nutrients/kg Biofer	kg nutrients/ha
Nitrogen (N)	100	133.0
Calcium (Ca)	44	58.5
Phosphor (P)	26	34.6
Potassium (K)	10	13.3
Iron (Fe)	8.4	11.2
Natrium (Na)	7	9.3
Sulphur (S)	5	6.6
Magnesium (Mg)	2	2.7

Table 10: Micronutrients in Biofer 10-3-1 used as fertilizer on hop during field trial 2020

Nutrient	mg nutrients/kg Biofer	g nutrients/ha
Zink (Zn)	110	146.3
Mangan (Mn)	23	30.6
Boron (B)	22	29.3
Copper (Cu)	10	13.3
Chrome (Cr)	10	13.3
Nickel (Ni)	1.4	1.9
Iodide (I)	0.51	0.7
Cadmium (Cd)	0.03	0.04
Mercury (Hg)	0.02	0.03

The estimated plant need of NPK and the added amount of NPK calculated on 2 m<sup>2</sup>, can be seen in Table 10. The need of K is calculated to 10 g/ha and the added amount is 1.3 indicates that there is probably a K deficiency of approximately 8.7 g K /m<sup>2</sup> or 87 kg K/ha.‘

Table 11 NPK in kg/ 2 m<sup>2</sup> and kg/ha

Nutrients	kg/ha	Need		Added	
		need/2m <sup>2</sup>	kg/ha	kg/2m <sup>2</sup>	kg/2m <sup>2</sup>
Nitrogen, N	130	13	133.0	13.3	13.3
Phosphor, P	20	2	34.6	3.5	3.5
Potassium, K	100	10	13.30	1.3	1.3

### 3.3.5. Weed management

Under-vegetation (i.e. weeds) appeared in the field during the season. Hand-weeding around poles were done weekly in May, June and July. The field was mechanically treated for weeds twice, once the first week of May and the second time in the last week of august (31/8).



### 3.4. Method

The trial was investigated by going out to the field trial once a week during the growing season to look for stunted plants, damaged or cupped leaves, discoloration, chlorosis, bronzing, failure to thrive in general. Also to keep an eye open for a huge group of insects as it usually is not worth worrying for individuals. Development stages were also noted during the season.

#### 3.4.1. Field measurement

Field measurements were inspired by a study made by Rossini et al. (2016) where phenological surveys were performed every week during the growing season using the BBCH centesimal scale (Rossbauer et al., 1995). In this study, growth stages (GS) reaching the top of the pole 3 m (GS 38), the start of flowering (GS 61), the start of cone setting (GS 71) and cone ripe for harvest (GS 89) was recorded for each plant. Temperature data in form of growing degree-days (GDD) was used to compare when the different growing stages accrued in the different varieties. The formula used for GDD was:

$$GDD = \sum_{days} \left( \frac{T_{max} + T_{min}}{2} \right) - T_{base}$$

GDD describes the accumulated heat until a certain phenological stage occurs. This is calculated by adding the maximum daily temperature with the daily minimum temperature divided by two to get a daily average. Then the temperature where the plant phenology does not progress,  $T_{base}$ , is subtracted. In the study by Rossini et al. (2016) the temperature of 5 degrees is used as  $T_{base}$ .

As in the study by Johnson (1991) and Rossini et al. (2016) degree-days were summed after the latest period of five consecutive days with a degree-day value equal to or less than zero. In Ultuna 2020 at the height of 1.5 m this occurred on the 1<sup>st</sup> of May. Temperature data used for calculation of degree-days came from LantMet at SLU/fältforsk from the weather station at SLU Ultuna 12.1 km from the hop field in Krusenberg. Additionally, GDD was also calculated for 2019 time of harvest, which corresponds to growing stage 89, cone maturity. The degree-days count started the 16<sup>th</sup> of April and hop were harvested from 17<sup>th</sup> August until 23<sup>rd</sup> of September.

#### 3.4.2. Harvest and drying

Hop were harvested when they reached the esteemed maturation. Maturation was decided by the look and smell, and the fact that they left a trace of resin when the cones were crushed and rubbed against the skin. The varieties matured in different timespans, from the 17<sup>th</sup> of August until the 23<sup>rd</sup> of September.

Cones were dried in a thermostatic oven (at 60°C for 2 – 3 hours) with forced ventilation until a water content of 7-11 % was reached. The water content in the cones was measured with humimeter FLH hop moisture meter (Schaller Messtechnik GmbH, St. Ruprecht an der Raab, Austria). Samples were dried according to methods available in the literature (Kunze, 1999). Time was depending on the wetness of the cones when they were harvested. Cones were left in the dryer during the night and packed with a vacuum sealer in airtight bags in the morning after the harvest. The bags were then stored at 3 °C until analysis was performed.



Figure 16: Drying of cones in cotton bags.

### 3.4.3. Analyses of $\alpha$ - and $\beta$ - acids and essential oil

Three hop cone samples from each variety were sent to SLU in Alnarp for analysis of  $\alpha$  - and  $\beta$  - acids and essential oils.

Samples were coarsely milled with an Retsch Cutting Mill SM200. The milled sample was divided into two 50 ml falcon tubes. The samples were then milled until a particle size of 0,5 mm was reached. One falcon tube was freeze-dried until reached a stable weight.

#### *$\alpha$ - and $\beta$ - acids*

The analyse was made on freeze dried samples. The method was in accordance with HPLC Analysis of Alpha- and Beta-Acids in Hops (Baker et al., 2008), with some smaller changes. Instead of the International Calibration Extract (ICE)-2 reference, ICE-3 reference was used. Reference varieties were Mosaic, Cascade och Amarillo (BullDog brews). Quantification was made with a DAD-detector 326 nm, control of molecule with MSD 6120. LC-DAD-MS Agilent 1260 system with MSD 6120,

column YMC triart C18ExRS plus 150\*3 3  $\mu\text{m}$ , injection volume 5  $\mu\text{l}$ , flowrate 0.6 ml/min, eluent 85/15  $\text{H}_2\text{O}$ /Methanol 0.1%  $\text{HCOOH}$ .

### *Essential oil*

Samples were made on dried fine milled samples. Total essential oil content was measured using spectrophotometer. Using Folin-Ciocalteu's method (1915) and further developed by Dewanto et al. (2002).

For analysing the percentage of hop essential oils, extractions were made of finely milled ( $\leq 0.5$  mm) non freeze-dried samples. Shortly, 1 g of hop to 10 ml of ethanol (99.98%) placed in glass tube with an airtight lid. Sample was placed in an ultrasonic bath (30min) then placed in 2° C during 16 h. Thereafter samples are centrifuged and the supernatants transferred to a GC-vial and is sealed with a tight-fitting membrane. Sample was kept in freezer until time of analyse.

For determination of the relative percentage of each major essential oil compound by GC-FID-MS method, the method *Determination of volatile compounds in different hop varieties* (Aberl and Coelhan, 2012) was used as a basis. Small changes were made. 2  $\mu\text{L}$  was injected directly in the colon. Portions of the hop oil are treated and injected into the GC being volatilized and separated. The response of each compound will be expressed in terms of % of the total oil. Identification of the essential oil component was made with mass spectrometry detector (Kishimoto et al., 2005), and compared to the National institute of standards and technology (NIST) database for mass spectrometry.

## 3.5. Statistics

Collected data consist of estimated growing stages during the season, weight in grams of the cones after harvest. Content of  $\alpha$  - and  $\beta$  - acids as well as the analyse of essential oils. The chemical analyse was made on three out of eleven plants of each variety. All data have been processed in MS Excel. The statistical analysis has been performed in the statistical program JMP® Pro 15 (SAS Institute Inc., Cary, NC, 1989–2020). Statistics of the growing stages were performed between the six blocks on each variety to compare the difference between the means. The weight was also compared between the six blocks, the highest yielding plant was chosen in each block, as the highest potential yield is of interest in this trial. Means were compared in Oneway Anova ( $p < 0.05$ ). Tukey's honestly significant difference test was performed on the means to examine if the means between the varieties differed. Means followed by different letters in rows are significantly different ( $\alpha=0.05$ ).

## 4. Results

### 4.1. Development stages

#### 4.1.1. Elongation of bines – reaching the top of the pole (GS 38)

All the plants in the trial emerged at week 18. Bonneråd and Gamla källmon were the earliest varieties to reach the top of the pole (3 meters) with a mean of 317 GDD at week 25. The latest to grow to the top was Zlatan with a mean of 511 GDD at the end of week 27. Between these varieties, there was a difference of 194 GDD and approximately 2 weeks. The only significant difference (p-value < 0.05) was between Zlatan and the rest of the varieties,

*Table 12: Growing degree-days (GDD) for reaching the top of the pole, flowering, start of cone setting, and maturity of the 11 varieties in the study. Means followed by different letters in rows are significantly different by the Tukeys HSD test ( $\alpha=0.05$ )*

Variety	Top of the pole (GS 38)		Start of flowering (GS 61)		Start of cone setting (GS 71)		Cone ripe for harvest (GS 89)	
	Thermal time (GDD)	week number	Thermal time (GDD)	week number	Thermal time (GDD)	week number	Thermal time (GDD)	week number
Bonneråd	317 b	25 b	679 bc	30 bc	848 ef	32 c	1194 d	37 c
Böknäs	355 b	26 b	739 b	31 ab	925 cd	33 b	1275 c	37 c
Gamla källmon	317 b	26 b	508 d	27 d	697 g	30 d	1048 f	34 f
Hulla Norrgård	327 b	26 b	585 cd	29 bcd	850 de	32 c	1135 e	36 d
Korsta	362 b	26 b	598 cd	29 bcd	850 de	32 c	1147 e	36 e
Mauritz	352 b	26 b	735 b	30 ab	955 c	33 b	1252 c	37 c
Näs	350 b	26 b	593 cd	29 bcd	771 fg	32 c	1086 fg	36 e
Svalöf E	363 b	26 b	881 a	32 a	1111 a	35 a	1368 a	39 a
Svalöf S	355 b	26 b	744 b	31 ab	970 bc	33 c	1324 b	38 b
Tvärud	355 b	26 b	545 d	28 cd	781 ef	32 a	1082 f	34 f
Zlatan	511 a	26 b	951 a	32 a	1033 b	35 a	1368 a	39 a

#### 4.1.2. Start of flowering – (GS 61)

Gamla källmon was the earliest variety to start flowering at 508 GDD in 27 weeks and Zlatan was the latest variety with 951 GDD in 32 weeks. Between these varieties, there was a difference of 443 GDD and 5 weeks. Tvärud, Hulla Norrgård, and Näs were amongst the earlier varieties to start flowering and had a heat accumulation of 545 GDD, 585 GDD and 593 GDD, respectively. This corresponds to week 28, 29 and 29. Zlatan and Svalöf E were significantly later than the rest of the varieties.

#### 4.1.3. Start of cone setting – (GS 71)

The earliest with cone setting was Gamla källmon with 697 GDD in 30 weeks and the latest variety was Svalöf E with 1111 GDD in 35 weeks. The difference between these varieties was 414 GDD and 5 weeks. Näs, Tvärud, and Bonneråd were amongst the earliest varieties with 771 GDD, 781 GDD and 848 GDD accumulates respectively. This corresponds to week 32. Late genotypes for cone setting were Mauritz 85, Svalöf S, and Zlatan with a heat accumulation of 955 GDD, 970 GDD, and 1033 GDD respectively. This corresponds to week 33 for Mauritz 85 and 35 for Zlatan and Svalöf E.

#### 4.1.4. Cone ripe for harvest (GS 88)

The earliest varieties with cones ripe for harvest were Gamla Källmon, Tvärud and Näs with an accumulation of 1048 GDD, 1082 GDD, and 1086 GDD each. This corresponds to week, 34, 34 and 36. Middle rated in maturation time were Hulla Norrgård, Korsta, and Bonneråd with an accumulation of 1135 GDD, 1147 GDD, and 1194 GDD, respectively. This corresponds to week 36, 36 and 37.

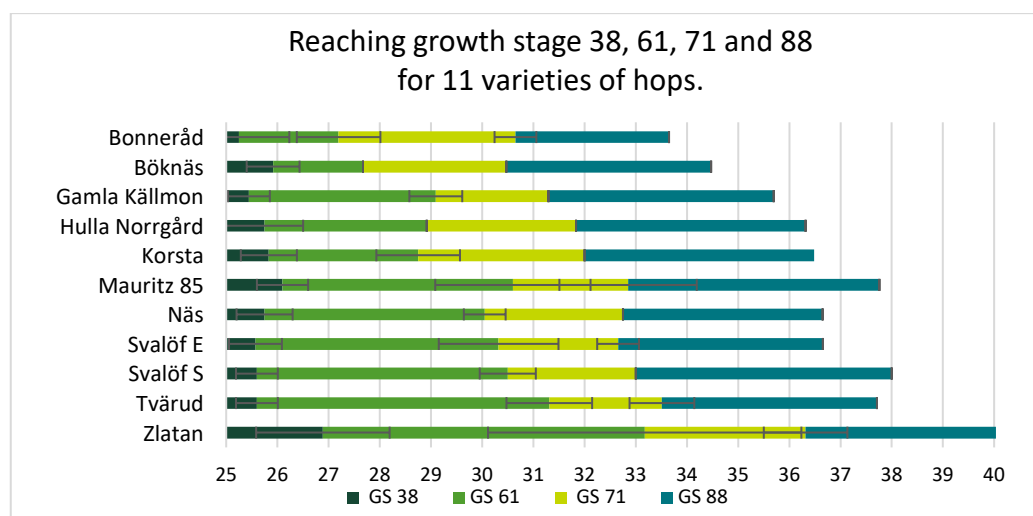


Figure 17: Graph over the thermal time for the varieties in the study to reach the four different growth stages

There were differences in the time of flowering and harvest, whereas Bonneråd and Mauritz 85 started flowering approximately one week later than expected. Hulla Norrgård and Korsta started at the end of June instead of from the middle of July. Gamla Källmon started flowering at the end of June as expected. Hulla Norrgård, Korsta, Näs, Svalöf E, Böknäs and Svalöf S flowered approximately two weeks later than expected. The harvest was later compared to the expected harvest according to the hypothesis made from Karlsson Strese (2016) for all varieties (Table 12).

Table 13: Date of flowering (●) and harvest (■) of the hop variety included in this study compared to the hypothesis made from Karlsson Strese (2016) flowering (pink) and harvest (yellow).

	June		July		August				September				
Bonneråd				●	●						■		
Böknäs					●	●					■		
Gamla Källmon	●	●	●					■					
Hulla Norrgård			●	●	●						■		
Korsta			●	●	●					■			
Mauritz 85				●	●						■		
Näs			●	●	●					■			
Svalöf E						●	●						■
Svalöf S					●	●						■	
Tvärud		●	●	●				■					
Zlatan						●	●	●					■

## 4.2. Yield

The yield was measured on cones with a water content of 9-11 % (dry weight). Korsta and Hulla Norrgård gave the highest yields in 2020 with 161 and 152 g/plant respectively. Bonneråd had an average yield of 137 g/plant. Mauritz 85, Tvärud, and Näs had a yield of 119, 111 and 109 g/plant. At around 100g/plant and below came Svalöf S, Gamla Källmon, and Svalöf E with 101, 93 and 88 g/plant. The lowest yield in 2020 came from the varieties Böknäs and Zlatan with 71 and 42 g/plant (Table 13). The only significant difference ( $p < 0.05$ ) in yield in all years was between Korsta and Zlatan. The yield was calculated to kg/ha per variety for 2019 and 2020, with a plant density of 1330 hop plants per hectare.

Table 14: Average cone yield (g/plant) of the cultivars under during 2019 – 2020. Means followed by different letters in rows are significantly different from each other; Tukeys HSD test ( $\alpha=0.05$ )

	2019	2020
Bonneråd	93 AB	136 AB
Böksnäs	54 AB	71 AB
Gamla Källmon	72 AB	93 AB
Hulla NG	133 AB	152 AB
Korsta	171 A	161 A
Mauritz 85	42 B	119 AB
Näs	100 AB	109 AB
Svalöf E	150 AB	88 AB
Svalöf S	130 AB	101 AB
Tvärud	162 A	111 AB
Zlatan	54 AB	42 B

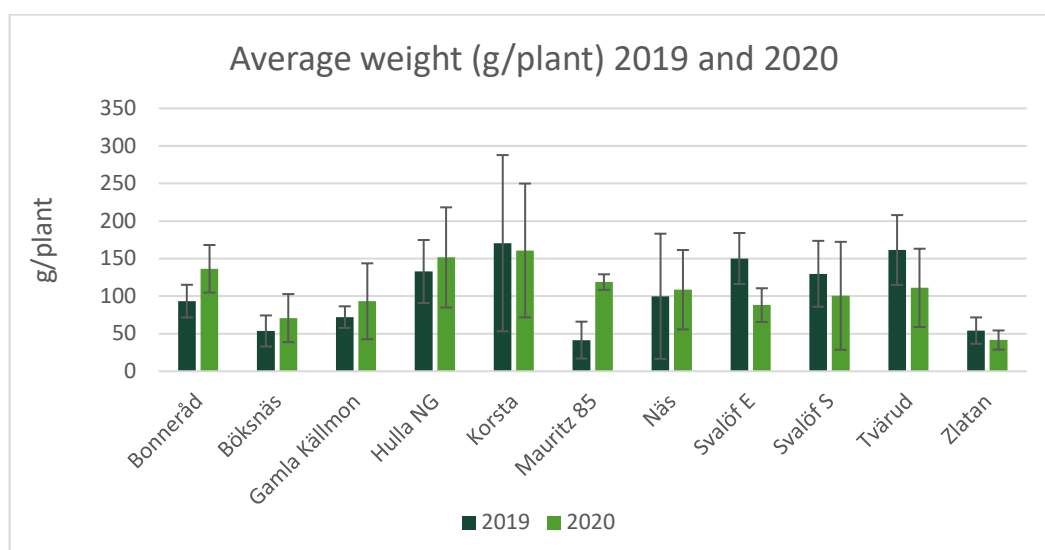


Figure 18: Graph over the average weight of the 11 varieties in 2019 and 2020

### 4.3. $\alpha$ - and $\beta$ - acid content

Alpha - acid content varied between the highest content in Korsta at 12.5 % to 4.3 % in Mauritz 85 (Table 14). Hulla Norrgård had an average content of 10.5 %  $\alpha$  - acid. There was no significant difference between the two highest  $\alpha$  - acid-containing varieties although Korsta was significantly different from the rest of the varieties (except for Hulla Norrgård) and Hulla Norrgård did not differ in comparison between the means from Korsta, Svalöf S and Gamla Källmon. Svalöf S and Gamla Källmon had an  $\alpha$  - acid content of 7.4 % and 7.1 % they were not significantly different to the lower  $\alpha$  - acid-containing varieties. Tvärud, Näs and

Bonneråd had a mean of 6.2 %, 5.8 % and 5.6 %  $\alpha$  - acid. Böksnäs, Zlatan and Svalöf E had an average of 4.9 %, 4.7 % and 4.5 %.

Table 15:  $\alpha$  - and  $\beta$  - acid content in the 11 varieties tested in the trial 2020. Means followed by different letters in rows are significantly different from each other; Tukeys HSD test ( $\alpha=0.05$ )

Variety	Mean $\alpha$ – acid (%)	Mean $\beta$ – acid (%)
Bonneråd	5.6 C	5.8 BCD
Böksnäs	4.9 C	4.3 D
Gamla Källmon	7.1 BC	6.0 BCD
Hulla Norrgård	10.5 AB	7.8 B
Korsta	12.5 A	10.1 A
Mauritz 85	4.3 C	5.2 CD
Näs	5.8 C	7.0 BC
Svalöf E	4.5 C	6.1 BCD
Svalöf S	7.4 BC	5.2 CD
Tvärud	6.2 C	7.3 BC
Zlatan	4.7 C	4.3 D

Beta -acid content was significantly highest ( $p < 0.05$ ) in Korsta at 10.1 %.  $\beta$  -acid levels were lowest in Böksnäs and Zlatan at 4.3% the value did not significantly differed from Mauritz 85 and Svalöf S at 5.2%, Bonneråd at 5.8%, Gamla Källmon at 6.0% or Svalöf E at 6.1%(Table 14). Näs had a  $\beta$  -acid content of 7.0 %, Tvärud 7.3 % and Hulla Norrgård 7.8 %.

Korsta, Hulla Norrgård and Svalöf S had the highest mean  $\alpha$  - acid in 2020. In 2019, Gamla Källmon had the highest mean, not being significantly different from Korsta, Hulla Norrgård, Svalöf S, Zlatan and Svalöf (Table 15).



Table 16:  $\alpha$  - acid content (%) in the 11 varieties tested in the trial for 2019 and 2020. Means followed by different letters in rows are significantly different from each other; Tukeys HSD test ( $\alpha=0.05$ )

Vairety	Mean $\alpha$ -acid (%) 2019	Mean $\alpha$ -acid (%) 2020
Bonneråd	3.3 EF	5.6 C
Böksnäs	4.5 DE	4.9 C
Gamla Källmon	6.6 A	7.1 BC
Hulla	6.0 ABC	10.5 AB
Norrgård		
Korsta	6.2 AB	12.5 A
Mauritz 85	2.2 F	4.3 C
Näs	4.5 DE	5.8 C
Svalöf E	5.8 ABCD	4.5 C
Svalöf S	5.0 BCD	7.4 ABC
Tvärud	4.8 CD	6.2 BC
Zlatan	5.9 ABCD	4.7 C

Alpha - acid content during 2019 and 2020 can be seen in Figure 21. Zlatan had the highest standard deviation. Zlatan E and Mauritz 85 were the only two varieties that did not have an increase in average  $\alpha$  - acid content from 2019 to 2020.

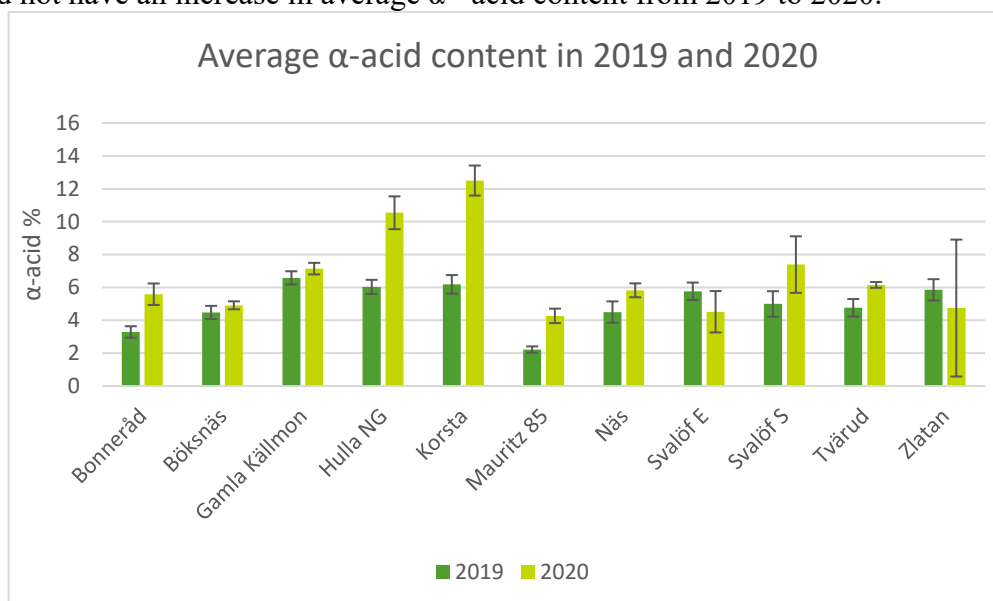


Figure 19: Average  $\alpha$  - acid content during 2019 and 2020

Korsta had the highest mean  $\beta$  - acid in 2019 and 2020 (Table 16) and was significantly higher than the other varieties.

Table 17:  $\beta$  - acid content (%) in the 11 varieties tested in the trial over 2019 and 2020. Means followed by different letters in rows are significantly different from each other; Tukeys HSD test ( $\alpha=0.05$ )

	$\beta$ -acid (%) 2019		$\beta$ -acid (%) 2020
Bonneråd	5.0	C	5.8 BC
Böksnäs	5.4	BC	4.3 C
Gamla Källmon	5.1	C	6.0 BC
Hulla Norrgård	6.7	B	7.8 B
Korsta	9.1	A	10.1 A
Mauritz 85	4.7	C	5.2 BC
Näs	5.1	BC	7.0 B
Svalöf E	5.9	BC	6.1 BC
Svalöf S	4.7	C	5.2 BC
Tvärud	6.7	B	7.3 B
Zlatan	5.7	BC	4.3 C

Zlatan had the highest standard deviation in average  $\beta$  - acid content (Figure 22). Böksnäs was the only clone that had a decrease of  $\beta$  - acid from 2019 to 2020.

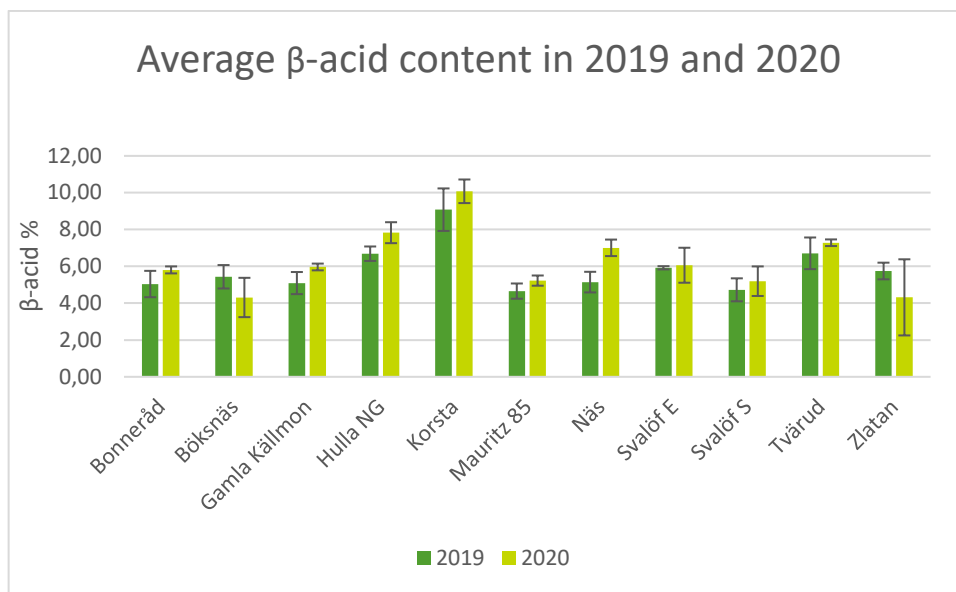


Figure 20: Average  $\beta$  - acid content in varieties 2019 and 2020

#### 4.4. Essential oil

In 2020, the essential oil content in the varieties differed at most with 458.3 mg /100 g hop cones (dry weight) between Korsta, that had 714.0 mg and Zlatan 255.7 mg. Bonneråd, Hulla Norrgård and Böksnäs had 651.3, 549.8 and 455.3 mg/100 g d.w. Tvärud, Gamla Källmon and Svalöf S had 433.3, 371.3 and 356.7 mg/100 g d.w. Svalöf E, Mauritz 85 and Näs had 278.7, 271.0 and 258.7 mg/ 100 g d.w. The

only significant difference was between Korsta and the varieties Svalöf E, Mauritz 85, Näs and Zlatan, see Table 17 and Figure 23.

Table 18: Average total essential oil content in mg oil per 100 g hop cones (dry weight) for the 11 varieties in the study. Means followed by different letters in rows are significantly different from each other; Tukeys HSD test ( $\alpha=0.05$ )

	Total essential oil content (mg oil per 100 g d. w. hop) 2019	Total essential oil content (mg oil per 100 g d. w. hop) 2020
Bonneråd	441.0 AB	651.3 A
Böksnäs	541.0 A	455.3 AB
Gamla Källmon	427.3 AB	371.3 AB
Hulla Norrgård	313.8 AB	549.8 AB
Korsta	419.3 AB	714.0 A
Mauritz 85	223.0 B	271.0 AB
Näs	332.3 AB	258.7 B
Svalöf E	521.5 A	278.7 AB
Svalöf S	366.3 AB	356.7 AB
Tvärud	326.8 AB	433.3 AB
Zlatan	225.3 B	255.7 B

Korsta, Bonneråd, Hulla Norrgård and Tvärud increased their total essential oil content from 2019 to 2020. Svalöf E, Böksnäs and Näs decreased in total oil content. Zlatan, Mauritz 85, Svalöf S had small changes between the years (Figure 23).

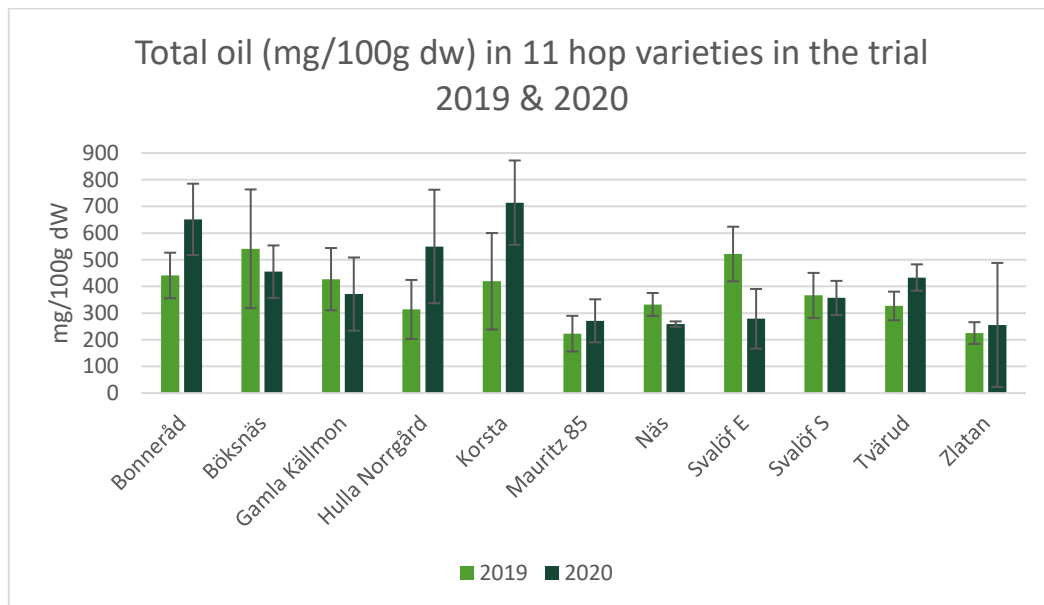


Figure 21: Graph over average essential oil content mg/100 g hop cone (dry weight) of the 11 varieties in the field study 2019 and 2020.

The essential oil content in this study was measured in percentage of the total essential oil content (Table 18). Some oil contents were below detection limit and are either written as <0.02% or <0.04%.

Table 19: Percentage of 7 essential oils in the 11 varieties on the study 2020. Means followed by different letters in rows are significantly different from each other; Tukeys HSD test ( $\alpha=0.05$ )

	$\alpha$ -Humulene	$\beta$ -Caryophyllene	Geraniol	Farnesene	Limonene	Linalool	Myrcene
Bonneråd	47.1 ab	12.6abcd	<0.04	30.0abcd	<0.02	0.72abc	9.6 d
Böksnäs	54.6 a	15.1ab	<0.04	10.5cdef	0.030 b	0.61abc	19.2 bcd
Gamla Källmon	23.0 c	9.4cde	<0.04	38.9ab	0.030 b	0.91ab	27.8 abc
Hulla Norrgård	27.6 c	8.3de	<0.04	32.9abc	0.045 ab	0.91a	30.1 ab
Korsta	51.4 a	14.1abc	<0.04	0.3f	0.167 a	0.96a	33.2 ab
Mauritz 85	31.2 bc	10.5bcde	<0.04	35.8ab	<0.02	0.49cde	21.9 bcd
Näs	21.0 c	6.9e	<0.04	53.1a	<0.02	0.15de	18.9 bcd
Svalöf E	53.2 a	14.0abc	0.12	7.1def	0.050 ab	0.10e	25.4 abc
Svalöf S	27.2 bc	10.5bcde	<0.04	30.5abcd	0.040 b	0.19de	31.6 ab
Tvärud	63.1 a	17.0a	<0.04	5.1ef	0.040 ab	0.51bcd	14.2 cd
Zlatan	28.5bc	8.1de	0.05	25.0bcde	0.055ab	0.81abc	37.6a

The results of the seven different essential oils are given in percentage of total essential oil (Figure 24).

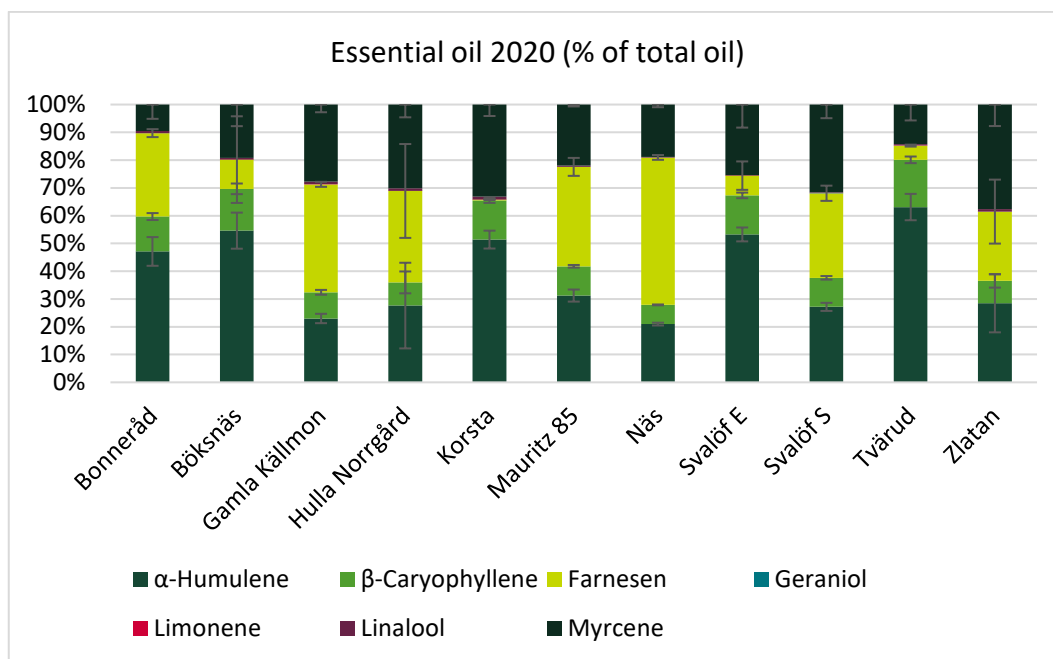


Figure 22: Percentage of essential oil content in 11 varieties in the study.

Essential oil content calculated to mg/100 g d.w. hop cones (Table 18).

Table 20: Essential oil content (mg/100 g d.w. hop cones) in the 11 varieties on the study 2020. Means followed by different letters in rows are significantly different from each other; Tukeys HSD test ( $\alpha=0.05$ )

	Myrcene	Limonene	Linalool	$\beta$ -Caryophyllene	Farnesene	$\alpha$ -Humulene	Geraniol							
Bonneråd	66.8	bc	0.1	b	4.1	ab	70.5	abcd	168.4	abcd	264.2	ab	0.2	b
Böksnäs	82.6	bc	0.1	b	3.4	ab	84.4	ab	59.0	cdef	306.2	a	0.2	b
Gamla Källmon	100.6	bc	0.2	b	5.1	a	52.8	cde	218.0	ab	128.9	c	0.2	b
Hulla Norrgård	162.8	ab	0.3	ab	5.1	a	46.8	de	184.6	abc	155.0	c	0.2	b
Korsta	236.2	a	0.4	a	5.4	a	79.0	abc	1.9	f	288.1	a	0.2	b
Mauritz 85	59.2	bc	0.1	b	2.7	bc	59.1	bcde	200.6	ab	175.1	bc	0.2	b
Näs	48.8	c	0.1	b	0.8	c	38.8	e	297.6	a	117.6	c	0.2	b
Svalöf E	64.7	bc	0.2	b	0.6	c	78.8	abc	39.9	def	298.6	a	0.2	b
Svalöf S	111.6	bc	0.2	ab	1.1	c	58.6	bcde	170.8	abcd	152.4	bc	0.5	a
Tvärud	59.9	bc	0.1	b	2.8	bc	95.3	a	28.7	ef	353.9	a	0.2	b
Zlatan	92.8	bc	0.2	ab	4.5	ab	45.2	de	139.9	bcde	159.6	bc	0.3	ab

### *Myrcene*

Korsta had the highest mean of myrcene (236.2 mg/100g d.w.); the average was not significantly higher ( $p > 0.05$ ) than Hulla Norrgård (162.8 mg/100g d.w.). Näs had the lowest mean of myrcene (48.8 mg/100g d.w.) but was only significantly lower than the highest myrcene containing varieties Korsta and Hulla Norrgård.

### *Farnesene*

Näs had the highest average farnesene (297.6 mg/100g d.w.); the average was not significantly different ( $p > 0.05$ ) from Gamla källmon (218.0 mg/100g d.w.), Mauritz 85 (200.6 mg/100g d.w.), Hulla Norrgård (184.6 mg/100g d.w.), Svalöf S (170.8 mg/100g d.w.) and Bonneråd (168.4 mg/100g d.w.). Significantly lowest farnesene containing variety ( $p > 0.05$ ) was Korsta (1.9 mg/100g d.w.). But the average farnesene level in Korsta was not significantly lower than in Tvärud (28.7 mg/100g d.w.), Svalöf E (39.9 mg/100g d.w.) or Böksnäs (59.0 mg/100g d.w.).

### *$\alpha$ -Humulene*

Tvärud had the highest average  $\alpha$ -humulene content (353.9 mg/100g d.w.); the average was not significantly different ( $p > 0.05$ ) from Böksnäs (306.2 mg/100g d.w.), Svalöf E (298.6 mg/100g d.w.), Korsta (288.1 mg/100g d.w.) and Bonneråd (264.2 mg/100g d.w.). Significantly lowest  $\alpha$ -humulene containing variety ( $p > 0.05$ ) was Näs (117.6 mg/100g d.w.) but the average did not significantly differ from Gamla Källmon (128.9 mg/100g d.w.), Svalöf S (152.4 mg/100g d.w.), Hulla Norrgård (155.0 mg/100g d.w.), Zlatan (159.6 mg/100g d.w.) and Mauritz 85 (175.1 mg/100g d.w.).

### *$\beta$ -Caryophyllene*

Tvärud had the highest average  $\beta$ -caryophyllene (95.3 mg/100g d.w.); the average was not significantly different ( $p > 0.05$ ) from Böksnäs (84.4 mg/100g d.w.), Korsta (79.0 mg/100g d.w.), Svalöf E (78.8 mg/100g d.w.) and Bonneråd (70.5 mg/100g d.w.). Significantly lowest  $\beta$ -caryophyllene containing varieties ( $p > 0.05$ ) were Näs (38.8 mg/100g d.w.), Zlatan (45.2 mg/100g d.w.), Hulla Norrgård (46.8 mg/100g d.w.), Gamla Källmon (52.8 mg/100g d.w.), Svalöf S (58.6 mg/100g d.w.) and Mauritz 85 (59.1 mg/100g d.w.).

### *Linalool*

Korsta had the highest average linalool levels (5.4 mg/100g d.w.); the average was not significantly different ( $p > 0.05$ ) from Hulla Norrgård (5.1 mg/100g d.w.), Gamla Källmon (5.1 mg/100g d.w.), Zlatan (4.5 mg/100g d.w.), Bonneråd (4.1 mg/100g d.w.) and Böksnäs (3.4 mg/100g d.w.). Significantly lowest linalool containing varieties ( $p > 0.05$ ) were Svalöf E (0.6 mg/100g d.w.), Näs (0.8 mg/100g d.w.), Svalöf S (1.1 mg/100g d.w.), Mauritz 85 (2.7 mg/100g d.w.) and Tvärud (2.8 mg/100g d.w.).

### *Limonene*

Korsta had the highest average limonene levels (0.38 mg/100g d.w.); the average was not significantly different ( $p > 0.05$ ) from Hulla Norrgård (0.25 mg/100g d.w.), Zlatan (0.23 mg/100g d.w.), Svalöf S (0.23 mg/100g d.w.). Significantly lower average limonene content ( $p > 0.05$ ) had Gamla Källmon (0.20 mg/100g d.w.), Svalöf E (0.17 mg/100g d.w.), Böksnäs (0.13 mg/100g d.w.), Tvärud (0.13 mg/100g d.w.), Bonneråd (0.10 mg/100g d.w.), Mauritz (0.10 mg/100g d.w.) and Näs (0.10 mg/100g d.w.).

### *Geraniol*

The results of Geraniol were below 0.2 mg/100 g d.w.hops, for all varieties except for Svalöf S (0.5 mg/100g d.w.) and (0.3 mg/100g d.w.).

Figure 25–31 show the different amounts of essential oils in the varieties of the trial.

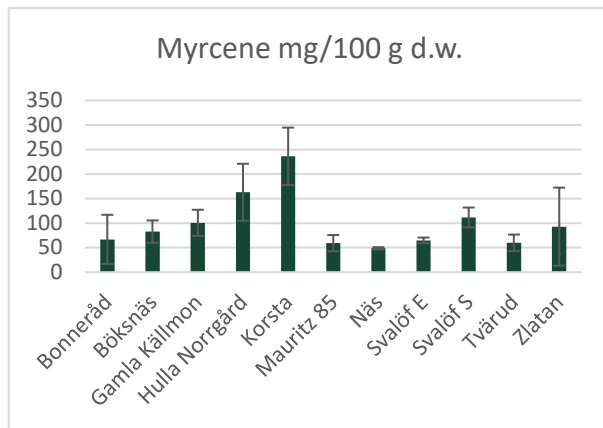


Figure 25: Myrcene content (mg/100 g d.w. hop cones) in varieties of the trial.

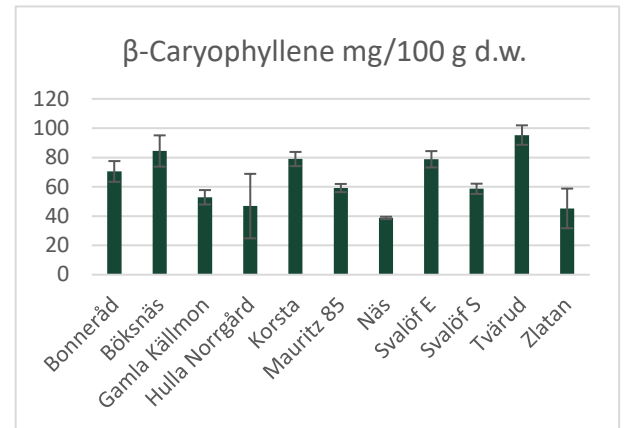


Figure 24:  $\beta$ -Caryophyllene content (mg/100 g d.w. hop cones) in varieties of the trial.

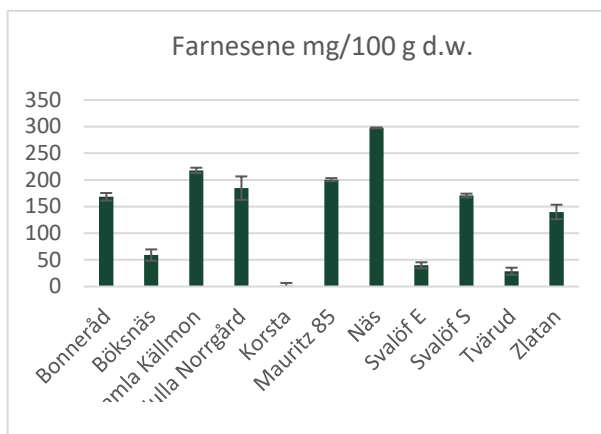


Figure 23: Farnesene content (mg/100 g d.w. hop cones) in varieties of the trial.

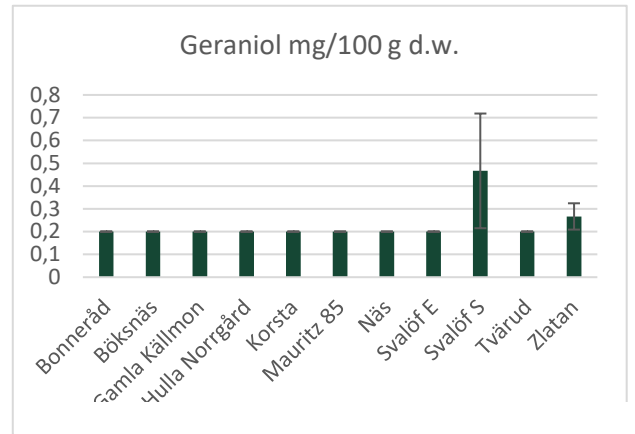


Figure 26: Geraniol content (mg/100 g d.w. hop cones) in varieties of the trial.

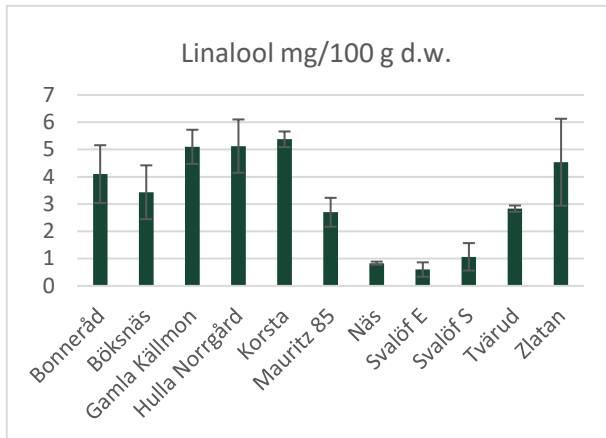


Figure 27: Linalool content (mg/100 g d.w. hop cones) in varieties of the trail.

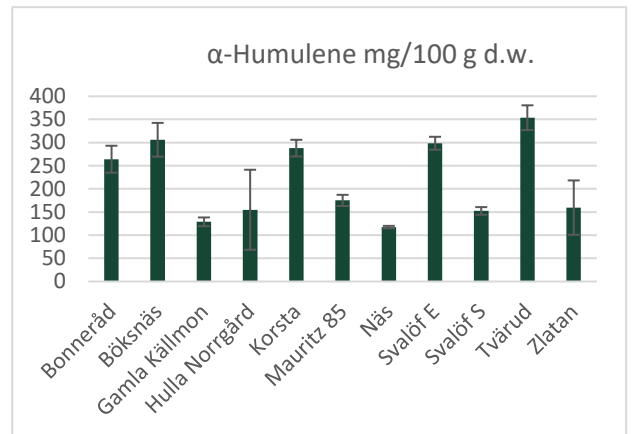


Figure 28: alpha-Humulene content (mg/100g d.w. hop cones) in varieties of the trail.

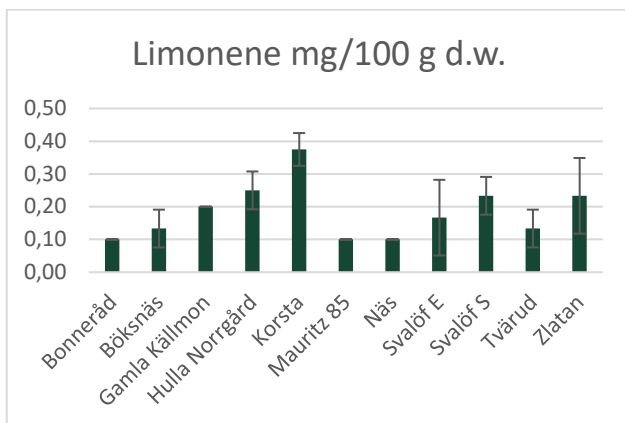


Figure 29: Limonene content (mg/100 g d.w. hop cones) in varieties of the trial.

## Plant Health

### 4.4.1. Pests

#### *Downy mildew*

All varieties were affected by downy mildew. This could be determined by the typical “basal spikes” from early in the season. Spikes from Downy Mildew were spotted in all the varieties except from Svalöf S (Figure 24). The brown colouration on cones were also spotted.





*Figure 30: Downy mildew spike on the hop variety Korsta (1/9 2020) and typical angular spots on the underside of a leaf.*

#### *Phorodon humuli - Hop Aphid*

Plants that reached over-maturity (e.g. Korsta) had noticeable more aphids. Plants with broken bamboo sticks, and where the pole was horizontal, also had more aphids



*Figure 31: Brown cones from the hop variety Korsta with hop aphids on the underside of the leaves.*



*Figure 32: Bonneråd, growing on a bamboo- pole that broke in the middle of the season.*



*Figure 33: Spots on a leaf possible as a symptom of two spotted spider mites.*

#### 4.4.2. Visual deficiency symptoms

Symptoms that indicate nutrient deficiency could be seen in most of the plants. Figure 28 and Figure 29 show a lighter green/yellow shade of the leaves in the lower parts of the bine. Yellow leaves could be a symptom of N deficiency (Gent et al., 2015). There is also a strong possibility that the plants suffered from K deficiency because typical symptoms could be seen. Symptoms developed first on older leaves, appearing as a bronzing between veins and as the season passed these areas turned ashy gray (Figure 35), this fits in to symptoms described by O'Neal et al. (2015).



*Figure 34: Potential N and K deficiency on Svalöf S; shown as yellow leaves in the bottom of the bine and marginal scorch.*



*Figure 35: Potential K deficiency on the variety Zlatan 31 August; shown as bronzing between veins.*

K deficiency have also been reported to have symptoms yellowing towards leaf tips and leaf cupping (Barslund, 2020); see Figure 30.



*Figure 36: Possible K deficiency; shown as brown dots and yellowing in the leaf marginals.*

The fertilizer pellets were not fully dissolved at the beginning of September (Figure 31). This means that probably not all nutrients have been available for the hops during the growing season.



*Figure 37: Biofer pellets next to bamboo stick (10th of September 2020)*

#### 4.4.3. Other observations and cone size

Korsta had noticeable brown cones when picked; this was not quantified (Figure 43 and Figure 44). Korsta got male flowers (Figure 43).

Hulla Norrgård and Gamla Källmon had cones that were perceived as larger and healthier.

Tvärud cones were decent sized although they got more striped brown as time passed (Figure 38).



*Figure 38: Tvärud with striped cones, possible downy mildew.*

Näs had difficulties surviving the first year, as only five plants of 12 survived until year three in the field trial. Cones were perceived as larger and healthier, although the plant differed a lot in yield.

Bonneråd cones were big, although some cones had leaves in between the bracts (Figure 45).



*Figure 39: Cone from the variety Bonneråd with leaves growing in the cone.*

Böksnäs and Mauritz 85 cones were small and had “open” bracts. Svalöf S cones were small but many; cones were also slightly open (Figure 40)



*Figure 40: Cones from the variety Svalöf S; Small and "open" (18th August 2020)*

Cones on some plants of Svalöf E, were extremely small and few. This might be a symptom of a disease (Figure 40 and Figure 42).



*Figure 41: Small cones of Svalöf E 2020, close up*



*Figure 42: Small cones on Svalöf E 2020*

Zlatan had small to middle-sized cones.

In the variety Korsta, all plants developed male flowers (Figure 43), at beginning of August.



*Figure 43: Korsta block 3 plants 1, male flowers on 10th August 2020.*

Cones of the variety Korsta became brown quickly at beginning of September and in combination with this, more aphid was observed (Figure 44 and Figure 45).



*Figure 44: Korsta 1 september*



*Figure 45: Korsta 10 September*

### *Bamboo sticks*

In the season 2020, the bamboo poles were placed deeper than 2019 (at 0.65 to 0.7 m) to avoid breakage and for increased stability. Three of the bamboo poles broke, one early in the season, and two later. Some of the bamboo-poles were twined with jute-string. At removal of bines from the bamboo-sticks at the end of the season it was a noticeable difference in how hard the bine was twined around the stick depending on if there was a jute-string or not.



*Figure 46: Drilling holes for the bamboo poles.*



## 5. Discussion

The hypothesis was that Korsta, Hulla Norrgård and Näs would be the best performing varieties. Näs had difficulties surviving the first years and gave a low yield and was therefore not chosen as a good variety. The varieties selected were Korsta, Hulla Norrgård and Bonneråd. Korsta is the variety that seems most interesting as it has the highest yield,  $\alpha$ - acid and essential oil. As the variety has high myrcene values, it might be suitable for brewing fruitier ales or IPA. Korsta have male flowers in the beginning of August and it is a risk of pollination if other varieties are flowering at the same time. Hulla Norrgård is another interesting variety with early maturation, big cones and a high content of  $\alpha$  – acid. The results indicate that Hulla Norrgård is also a high myrcene containing variety, indicating that it would be suitable for fruitier ales or IPA. Bonneråd was a lower  $\alpha$  acid variety but with a high total essential oil content. This variety could be interesting for lager beers as it has low myrcene and moderate levels of  $\alpha$ -humulene,  $\beta$ -caryophyllene and farnesene.

Varieties that performed slightly worse were Svalöf E and Zlatan because both were late maturing and had low yield.

### 5.1.1. Plant development

The results in this study show significant differences in plant development for reaching growing stages 38, 61, 71 and 89. The results were compared to previous studies done on the varieties by Strese (2016). There were some small differences in the time of flowering and harvest whereas flowering and harvest occurred later in the trial. The delay could be a result of variables as differences in weather and due to different location of the plants, as the varieties in the study made by Karlson Strese (2016) were grown in Alnarp, Skåne.

When comparing maturation time and looking at the map from where the historical finding place for the varieties something interesting was discovered. The earliest maturing varieties in 2020 were Tvärud, Gamla Källmon, Näs, Hulla Norrgård and Korsta. Korsta is the variety from furthest north and both Näs and Tvärud belong to the “northerners” of the plant collection. The latest flowering varieties were also

the varieties that came from the furthest south. Svalöf S and Svalöf E from Skåne and Zlatan from the Czech Republic.

Can the plant origin possibly affect the time of flowering? As the different time of maturation of birches at Dag Hammarskjölds väg in Uppsala (Eriksson, 2014). Local adaptation is not only affected by latitude, but it is also affected by height over the ocean, proximity to water, wind turbulence nearby mountains etc (Turesson, 1922, Eriksson, 2014).

### 5.1.2. $\alpha$ - acid

When comparing the results of  $\alpha$ - acid levels from 2020 with the measurements done by Karlson Strese (2016), most varieties have a higher alpha acid content in 2020. This indicates that the alpha acid levels were not noticeably negatively affected by the potential nutrient deficiency. In both, this study and the one by Karlsson Strese (2016), the cones were harvested at estimated maturation. Cones might have been picked at different stages at the alpha acid peak curve.

The temperature during the period from the beginning of flowering to cone setting is considered to be important for  $\alpha$ - acid synthesis (Smith, 1974, Kučera and Krofta, 2009). Kučera and Krofta (2009) mentions temperatures between 16 to 17 °C as optimal temperature for the varieties Fuggle, Northern Brewer, Hallertau and Saaz. A later maturation could have a negative effect on the  $\alpha$ - acid synthesis as the average temperature in September was 13 °C.

Four of the varieties are sold commercially and their  $\alpha$ - acid values are used for comparison. Typical values of Korsta, Hulla Norrgård and Mauritz 85 are 7-10%, 6-10% and 2-5% (Humlegården 2020). This corresponds to the  $\alpha$  - acid levels measured in this study. The typical amount of  $\alpha$ -acid in commercially sold pelleted Saaz cones is 2-5 % (Humlegården 2020). The Saaz clone in the trial, Zlatan, had  $\alpha$ - acid levels of 4,7 %, i.e. the plant performed in accordance to the normal span.

### 5.1.3. Yield

The season of 2020 had an average temperature of 18,5 °C in June, 16,5 °C in July and 18.3 °C in August with 163 mm in total rainfall during these three months. As the hop field did not get any irrigation, this might have affected the cone quality, as hop are reported to need at least 100 mm per month in June, July and August (Jensen, 2016). The water need is dependent on the transpiration rate and the water availability in the soil, and this was not determined in this trial. Water stress during the cone development stage has been reported to affect the yield and quality of

cones (Nakawuka et al., 2017). During the dry year of 2018, the trial did not get any irrigation and stayed green throughout the season (Berglund, 2020), this indicates that the water availability in the soil is sufficient.

#### 5.1.4. Essential oils

Essential oils in this study are given s in mg oil / 100 g hop (d.w). This unit has not been found in other studies and thus it is not possible to compare with results of other studies unless mL/100 g or mL /g hop are used as in Karlsson Strese (2016). The results are minimal fluctuating but a similar pattern is visible (Figure 47).

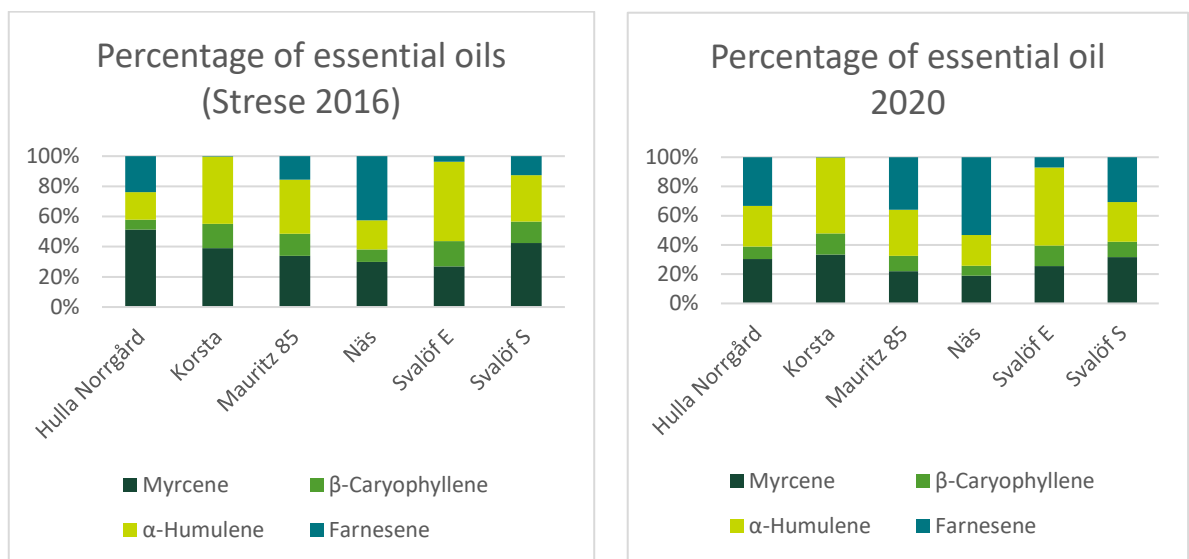


Figure 47: Comparing percentage of essential oil (of total oil content) for myrcene, beta-Caryophyllene, alpha-Humulene and farnesene with values from Strese (2016).

#### 5.1.5. Plant Health

As the trial might have suffered from nutrient deficiency it was impossible to determine the difference between disease and deficiency for an untrained eye. Nevertheless, all varieties were affected by downy mildew, this could be determined by the typical “basal spikes” from early in the season.

To determine nutrient deficiencies in a field visually is a cheap and relatively fast way. Nevertheless, this method is also subjective and has a large margin of error as some nutrient deficiencies may look similar, and sometimes symptoms can mask the existence of other deficiencies. The soil pH was 5.8-5.9 in the top 40 cm and this can affect the availability of nutrients like P, Mg and Mo. It is worth noticing that the hop roots usually grow deeper than 40 cm and pH measured in the layer

40-60 cm was 6.6, which is considered to be a suitable pH for growing hop (Häpi, 2019).

The needed amount of K was not provided as only 10% of the plant need was covered by the added Biofer fertilizer. As mentioned earlier, this did not seem to affect the average  $\alpha$  - and  $\beta$  - acid content compared to values from the commercially sold hop. Nevertheless, signs of deficiency such as marginal scorch, yellow marginals, brown dots and bronzing between veins (Figure 29, Figure 30 and Figure 31) could be seen. Bronzing between veins could be a sign of K deficiency, yellowing between veins could be a sign of Mg deficiency (Gent et al., 2015). Hops are in the *Cannabaceae* family and an assumption is made that hop deficiency symptoms are similar to deficiency symptoms for *Cannabis sativa* (*Cannabaceae*). Symptoms of Mn deficiency could also be observed as interveinal necrotic regions (Cockson et al., 2019).

Liebig's law states that plant growth is limited by the scarcest nutrient. This means that the K deficiency was probably the most limiting factor. Even though the plants seemed to suffer from N deficiency as leaves in the bottom of the bine turned yellow (Figure 29). Not all the Biofer fertilizer was fully dissolved in September. Ideally, N should be available before the rapid biomass growth from late May to early July (Gingrich et al., 1994). Hops have a relatively large N need at the beginning of the season. Biofer pellets need to be dissolved and percolate into the soil before the N can reach the roots. N deficiency negatively affects photosynthetic activity, this in turn also affect the assimilation of other nutrients. N deficiency also negatively affect yield size (Barslund, 2018). Yield did decrease in four of the 11 varieties from 2019 to 2020, although the standard deviation of average yield did differ dramatically between the varieties.

Four out of seven Svalöf E plants had very small cones. This could be a sign of powdery mildew (Madden, 2011). The plants could have been infected when planting or gotten the infection during the three years since planting. Spots on the leaves and discoloration on cones could also come from two spotted mites. The number of insects was not quantified in this report, although there were no major attacks. It was noticed that plants with discoloured cones and plants with broken bamboo sticks had a higher number of hop aphids.

An organic option to enhance biodiversity in the hop field is to sow floor vegetation. Although increased floor vegetation could, as earlier stated, mean a better environment for downy mildew (Eyck 2015).

### 5.1.6. To think about in further studies

This study might best be interpreted as a pilot. The method developed over the time of the field study. A structure for measuring plant health more than by looking at symptoms would have made the results more comparable. As it was difficult to spot the typical signs of the different pathogens as the nutrient deficiency also had symptoms that could make it harder for an untrained eye to see the symptoms/signs of pathogens. It is important to have a good fertilizer plan to be able to see a variety of differences in pathogen pressure. In further studies, it is of great importance to use a fertilizer that is more easily available to the plants, as the Biofer pellets didn't dissolve. Especially for the first fertilizer in spring, the N needs to be plant available. It is also important to choose a fertilizer that contains more K. It would have been interesting to do a soil-plant analysis to see the plant-available nutrients in the soil compared to the nutrient levels in the plant.

To determine when to harvest there are other methods than doing the decision solely based on the smell and looks of the cones. As this is rather subjective and requires training to fully master. Another way to determine the time of harvest is to measure dry matter weight as studies showed that  $\alpha$  - acid peaks at 22-24% dry matter (Murphy and Probasco 1996). The same method to measure maturity would make different studies more comparable. Nevertheless, this method only focuses on the  $\alpha$  - acid, and studies show that the essential oil content peaks later.

It would have been interesting to look at morphological characteristics as in a study by Mongelli et al. (2015) where shape, width, length, fresh weight of cones and shape, width, and length of bracts were measured. In the study, the authors also looked at other qualitative descriptors as leaf shape, cone size, cone shape, cone intensity of green colour, degree of opening bracts, bract ratio width/length, length apex of bract, and bract length. This was done by collecting 40 leaves, cones, and bracts of each variety (Mongelli et al., 2015). Comparing more parameters of the different varieties would give more quantifiable aspects in comparing agronomic performance. Larger cones are faster to harvest. To measure the differences between cone sizes in the varieties a decided number of cones from each variety can be weighed. It is then of great importance that the cones have the same moisture. An alternative to this is to measure the cone length and width.

It is interesting to look further into pests or beneficial insects on hop. Grasswitz (2009) looked at 24 leaves from every variety, not more than 2 leaves per plant, to look for beneficial insects or pests. Before harvest, 24 cones from each variety were randomly selected to look for pests/beneficial insects and the colour of the cones. Aspects as discoloration and shattering of hop were not quantified in this study, although this could have an impact on the lupulin levels in the cones.

Drying is another parameter that needs development in further studies. I harvested during a long period and against the end of the season, the cones had higher moisture content. For further studies preferable the same weight of hop should be put in the cotton bags that go into the dryer. The cones should preferably have approximately the same moisture level so that the time of drying can be the same for all varieties. For a small-scale grower, this is the tricky part as the hop needs to be dried quickly after harvest. This both requires space and equipment. As essential oils easily evaporate at high temperatures more research needs to be done to be able to preserve the aroma. The method of drying probably affects the  $\alpha$  - acid and the essential oil most. Drying hop reduces the intensity of the aroma depending on the drying temperature. There are several suggestions of temperature and time for drying. I dried the hop in at 60 °C for 2 – 3 hours, and there is a possibility that a lower temperature and longer time would be better.

## 6. Conclusion

Agronomic quality was investigated on 10 Swedish historical cultivated varieties and one Saaz clone. All the varieties performed well. Korsta is the variety that seems most interesting as it has the highest yield, alpha acid, and essential oil. As the variety has high myrcene values, it might be suitable for brewing fruitier ales or IPA. Hulla Norrgård is another interesting variety with early maturation, big cones, and a high  $\alpha$  – acid. Hulla Norrgård is also high myrcene containing variety, indicating that it would be suitable for fruitier ales or IPA. Bonneråd was amongst the lower  $\alpha$ -acid varieties but had a high total essential oil content. This variety had low myrcene and moderate levels of  $\alpha$ -humulene,  $\beta$ -caryophyllene, and farnesene. Bonneråd could for example be interesting for brewing a classic pilsner as it has a flowery aroma that could make the beer very interesting. Tvärud was also an early maturing variety with mid high  $\alpha$ -acid content high  $\alpha$ -humulene and low myrcene content. Tvärud could be interesting for brewing for example an India pale lager.

In further studies, it would be interesting to quantify plant health to see what difference there is between the varieties. For a small-scale hop farmer, there are a few things to consider. Looking after your hop is needed throughout all summer, downy mildew spikes need to be removed, fallen bines need to be re-twined. The harvest takes time and drying requires a lot of space and time. If growing several hop varieties different maturing times could be a good idea to be able to have time for both harvest and drying (and brewing).

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