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Hawthorn (*Crataegus monogyna)* grown in Lower Austria: exploring the phenolic content and antioxidant capacity of extracts obtained from different organs

Master thesis
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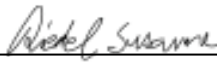
04.03.2021	
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Abstract:

Besides the high value as a landscape element in agriculture, Hawthorn (*Crataegus spp.)* finds application as an herbal drug in modern medicine and phytotherapy. Due to the high content of secondary metabolites, *Crataegus* is used for the treatment of chronic heart disease and high blood pressure. Phenolic compounds as proanthocyanidins and flavonoids are notably present. For medicinal products, all organs of *Crataegus* are used. 80% of the raw material, which is processed for pharmaceutical purposes, comes from wild collections. Future challenges such as meeting the prospective demand for raw material and providing consistent quality to a regular price need more research and development of cultivations strategy. With that aim, we surveyed the potential of *Crataegus monogyna* in Lower Austria. The intention was to explore the potential of different organs obtained in spring (leaves and flowers) and autumn (fruits).

The diverse organs were examined for their total phenolic content (Folin-Ciocalteau) and antioxidant capacity (FRAP). Moreover, a pruning treatment was tested concerning the effects on production-related factors such as the quantity of metabolites. Contiguous to the content analysis, fruit parameters were recorded to investigate the impact of pruning. In the first year, fruits present no significant difference in total phenolic content (TPC) and antioxidative capacity (TAC) between pruned plants and non-pruned plants. Yet, the unpruned group's measurements showed that increasing color properties, such as saturation, go along with decreasing TAC values. All organs exhibit varying TPC and TAC values. Upper leaves (<2m) and flowers showed the highest concentration of total phenolics with 52,51 mg/g DW and for flowers 52,52 mg/g DW. The fruits had an average value of 20,83 mg/g DW hence a significantly lower phenolic content than the leaf and flower parts ($p<0.05$). The TAC of base leaves was significantly lower than the TAC of flowers and upper leaves ($p<0.05$). Even though base leaf and unpruned fruit samples did not possess a relationship between TPC and TAC, a significant positive correlation between the TPC and the TAC of all organs together was ascertained ($p < 0.001$).

This investigation lays the foundation for further research projects on the cultivation of hawthorn in Lower Austria.

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Abbreviations

e.g.	for example
et al.	and others
kg	kilogram
g	gram
mg	milligram
µg	microgram
l	liter
ml	milliliter
µl	microliter
mm^2	square millimeter
m	meter
cm	centimeter
mm	millimeter
kgf	kilogram pro force
°C	degree centigrade
l	length
b	width
d	diameter
n.a.	not analyzed
n.i.	not investigated
DW	dry weight
OPC	oligomeric proanthocyanidins
TPC	total phenolic content
TAC	total antioxidative capacity
TFC	total flavonoid content
sp.	The abbreviation sp. or spec. is used as an addition after the genus name for an unspecified species within the biological taxonomy
spp.	several species
HPLC	High-Pressure-Liquid-Chromatograph

Table of Content

1. Introduction

In Europe, hawthorn (*Crataegus spp.)* is cultivated as a shrub or a small tree and traditionally used as a hedge for landscapes. In agriculture, hawthorn finds application as a windbreaker to decrease transpiration and enhance the microclimates of crop fields at the same time (Phipps et al., 2003). Furthermore, hawthorn serves as a valuable habitat for wildlife hence increases the biodiversity of the agrarian landscape (Sparks and Martin, 1999; Phipps et al., 2003). *Crataegus spp.* is native to Europe, northwest Africa, western Asia (Dickinson and Phipps, 1986; Phipps et al., 2003) and presents various species and subspecies (Christensen, 1992a). Besides the high value as a landscape element, hawthorn features a high content of health-promoting secondary metabolites. These beneficial compounds are present in all plant parts such as leaf and flower (*Crataegi folium cum flore)* and fruit (*Crataegi fructus*). In particular, the species *Crataegus monogyna* is primarily used as an herbal drug in phytotherapeutic treatments for cardiovascular diseases and heart insufficiency. Different types of tissue, like leaves, flowers, and fruits, contain distinct amounts of specific metabolites. The group of phenolics are considered to be responsible for the beneficial effects in treatments with *Crataegus* products and are notably present in hawthorn (Kaul, 1998; Melzer and Saller, 2005; Froehlicher et al., 2009; Edwards et al., 2012; Nabavi et al., 2015). Due to different amounts of specific compounds (Edwards et al., 2012), a lack of clinicopharmacologic research (Kaul, 1998), and for reasons of economy, the mainly further processed parts are leaves and flowers in order to produce extracts for pharmaceutical purposes (Kirakosyan et al., 2003; Sonnenschein and Plescher, 2005; Peschel et al., 2008).

Besides distinctive extraction procedures, also species or cultivar, cultivation style, and origin of the raw material influence the phytochemical profile hence the quality of herbal products (Tanko et al., 2005). Although a steady demand for hawthorn raw material is expected until 2020, the cultivation of *Crataegus* leaf and flower drugs has not been established yet (Fachagentur Nachwachsende Rohstoffe e.V., 2013b). Currently, almost 80% of the hawthorn raw material, which is industrially processed for the pharmaceutics and food industry, comes from wild collections (Kaul, 1998; Sonnenschein and Plescher, 2005; Fachagentur Nachwachsende Rohstoffe, 2017).

Though, there is no scientific data available for the procedure, the quantity, and the expenses for the harvest of raw material (leaves, flowers, and fruits) from wild collections (Sonnenschein and Plescher, 2005). Besides the detrimental impact of human interference on flora and fauna (Lange, 2006), plant material gained from wild collections is problematic due to inconsistent contents and accumulations of toxic elements (Lange, 2006; Schippmann et al., 2006; Fachagentur Nachwachsende Rohstoffe, 2017). Especially the leaves with flowers of hawthorn, which are collected in their natural habitat (Sonnenschein and Plescher, 2005; Orhan et al., 2007), are prone to accumulate heavy metals from industrial pollutions, as lead, zinc (Bewley and Campbell, 1980) and in particular cadmium (Bewley and Campbell, 1980; Sonnenschein and Plescher, 2005). Another challenging aspect is to meet the constant demand for raw material since seasonal and natural fluctuation influences the disposable amount (Schippmann et al., 2006; Fachagentur Nachwachsende Rohstoffe, 2017).

Future challenges as meeting the demand for raw material at a high quality and adequate costs need more information and agronomic cultivation strategies. With the purpose of filling missing knowledge and establishing the cultivation of *Crataegus ssp*. in Northern Europe, several scientists examined various *Crataegus* species and cultivars to develop potential cultivation strategies (Sonnenschein and Plescher, 2005; Peschel et al., 2008). The results showed that species or cultivar, the growing location, exposure to sunlight (Peschel et al., 2008), and harvest time (Sonnenschein and Plescher, 2005; Peschel et al., 2008) affect the phytochemical profile of all hawthorn raw material organs (Sonnenschein and Plescher, 2005; Peschel et al., 2008).

Furthermore, different management styles, as the intensity of pruning, significantly influence the yield of fruits (Croxton and Sparks, 2002). However, no research investigated the impact of a pruning treatment on the phytochemical profile of hawthorn's raw material. Contrary to other European countries, such as the United Kingdom, hawthorn is less present in Austrian hedges (Schramayr and Baumgartner, 2016) although hawthorn is very well adapted to Austria's environmental conditions and establishes quickly (Schramayr and Baumgartner, 2016). According to estimations, 40 to 50 million individuals of *Crataegus* occur in Lower Austria (Schramayr, 2016c).

Based on these assessments, this investigation aims to provide an impression about the potential of hawthorn for farmers as a supplementary source of income in lower Austria. Therefore, this thesis explores the potential of different organs obtained in spring (leaves and flowers) and autumn (fruits). Leaves, flowers, and fruits of wild-grown *Crataegus monogyna* in Lower Austria are examined for their polyphenolic content and antioxidant capacity. Moreover, a pruning treatment is tested concerning the effects on production-related factors as quality of metabolites and the feasibility of harvest. Contiguous to the content analyzes, physical fruit parameters as size, weight, and color of the fruits are measured to investigate the pruning treatment's impact. In consideration of previous research and based on the selected methods, the study was designed to answer the following research questions:

- **Does pruning affect the fruit parameters and quality (with focus on total phenolic content and total antioxidative capacity) of the fruits in the first year?**

- **Is there a difference in the content of total phenolics and total antioxidative capacity between leaves, flowers, and fruits?**

- **Have light exposed leaves (sample height<2m) a higher content of phenolic compounds and a higher antioxidative capacity than shaded leaves (sample height >1m)?**

- **Is there a correlation between the content of total phenolics and the total antioxidative capacity?**

- **Is there a distinction in the quality and quantity of secondary metabolites of *Crataegus* plant material selected in Lower Austria and Crataegus plant material from other countries of origin?**

As before mentioned, hawthorn is one of the most applied herbals drugs, various parts of the world (Liu et al., 2011; Edwards et al., 2012; Lund et al., 2017), and yet

scientific knowledge about many production-related aspects of *Crataegus monogyna* is still lacking. The data of this investigation lay the foundation for further research projects about the cultivation of hawthorn in Lower Austria hence contributes to developing a cultivation strategy of hawthorn in general.

2. Literature Overview on *Crataegus monogyna*

2.1. General overview

In the following chapters, all essential information and the current state of the research about the genus *Crataegus* and especially the species *Crataegus monogyna* will be addressed and united in order to review the potential of different organs for farmers in Lower Austria as a supplementary source of income during the whole year. Firstly the classification, phylogenetic history, and a morphological description of the species *Crataegus monogyna* will be introduced. Different phytochemical properties and how they can be influenced will be exemplified. In particular, the complexity of the reproductive biology of *Crataegus monogyna* will be ascertained. Followed by the cultivation of *Crataegus monogyna,* breeding of diverse cultivars, and the connected problems of the cultivation will be discussed. In the end, the practical value and application of *Crataegus monogyna* as a hedge plant or further processed herbal drug will be evaluated.

2.2. Taxonomy and Distribution of *Crataegus monogyna*

Clade	*Mangoliphyta*
Clade	*Rosidis*
Order	*Rosales*
Family	*Rosaceae*
Subfamily	*Spiraeoideae*
Tribe	*Pyreae*
Subtribe	*Pyrinae,(formerly Maloideae)*
Genus	*Crataegus*
Species	*Crataegus monogyna*

Table 1 Classification of *Crataegus monogyna* modified by (Kaul, 1998; Potter et al., 2007)

The species *Crataegus monogyna* is also known under the common name hawthorn, one seed hawthorn, or single-seeded hawthorn. *Crataegus monogyna* is classified to the genus *Crataegus* and is native to Europe, northwest Africa, and western Asia (Phipps et al., 2003). In particular, Iran and Turkey are deemed to be the genetic hotspot regions of *Crataegus* species with the richest hawthorn gene pool (Ercisli, 2004; Yanar M. et al., 2011; Gundogdu et al., 2014; Khadivi-Khub et al., 2015; Gharaghani et al., 2016; Khadivi et al., 2019).

Members of the genus *Crataegus* usually present a limited range of morphological variation within the genus (Edwards et al., 2012). All species crop as woody polycormic shrub or trees with similar leaves types and inflorescences. These typical pentamerous, unspecialized flowers develop into polypyrenous drupes (Campbell et al., 2007; Potter et al., 2007).

Depending on the climate zone, species of *Crataegus* develop mesophytic or xerophytic traits (Phipps et al., 2003). In Europe, two hawthorn species are most widely spread, the sibling species *Crataegus leavigata* and *Crataegus monogyna* (Kaul, 1998; Phipps et al., 2003).

Crataegus monogyna is affiliated to the family *Rosacea*, the tribe *Pyreae* and the genus *Crataegus* (Dickinson and Phipps, 1986; Kaul, 1998; Pilaske, 1999; Phipps et al., 2003; Evans and Dickinson, 2005; Schramayr, 2016a; Verein Naturvermittlung, 2016). The family *Rosaceae* is subdivided among four families based on the fruits' morphology and the base chromosome number (Evans and Campbell, 2002). These four subfamilies, *Amygdaloideae, Maloideae, Rosoideae,* and *Spiraeoideae,* are further divided into tribes and subtribes (Potter et al., 2007). The subfamily *Spiraeoideae* is heterogeneous and has a base chromosome number x=17 (Evans and Campbell, 2002; Potter et al., 2007). According to the phylogenetic analysis of Potter et al. 2007, which are based on combined sequence data from nuclear and chloroplast loci, the genus *Crataegus* is classified to the supertribe *Pyrodeae*, the tribe *Pyreae* and the subtribe *Pyrinae* (Potter et al., 2007). Contrary to the limited range of morphological variation within the genus *Crataegus,* the single species of this genus represents great variations within populations due to polymorphism, hybridization, apomictic propagation systems, and further reasons, as external effects (Khadivi-Khub et al. 2015).

However, to understand the morphology and reproductive biology of *Crataegus monogyna* with a view on potential cultivation methods, it is essential to know the characteristic of the subtribe *Pyrinae*, formerly the *Maloideae*. Campell et al. 2007 approached to design a possible resolution of relationships phylogeny of subtribe *Pyrinae* with PCR and DNA sequencing methods. The scientists acknowledge with these collected data previous suggestions about relationships of the pome-bearing genera and could create a molecular-based clade of *Crataegus-Mespilus*. The potential rapid, ancient radiation and intergeneric hybridization determined a significant part of the group's taxonomic history (Campbell et al., 2007). However, hybridizations between many members of *Pyrinae* still occur. Due to reproductive biology, the relatively short time of speciation of the members of the subtribe, it is challenging to reconstruct the phylogeny of *Pyrinae* hence the phylogeny of *Crataegus* (Dönmey, 2004; Campbell et al., 2007; Schramayr, 2016a).

This complex relationship of the genera *Crataegus* and the related genera *Mespilus* within the subtribe *Pyrinae* (Lo et al., 2007) is reflected in a large number of more than 250 reported varieties worldwide (Evans and Campbell, 2002; Phipps et al., 2003). According to Christensen estimation in 1992, the number of species vacillates up to possible 1200 species (Christensen, 1992a).

As before mentioned, *Crataegus monogyna,* as a member of the subfamily *Spiraeoideae,* has the base chromosome number x=17. Contrary to other polyploidy *Crataegus* species and *Crataegus* hybrids, *Crataegus monogyna* is diploid hence presents a basic chromosome number of 2n (2x) = 34. However, polyploidy is a common phenomenon among the *Crataegus* species (Evans and Campbell, 2002; Dickinson et al., 2007; Khadivi-Khub et al., 2015). Although *Crateagus leavigata* possesses the same diploid chromosome number as *Crataegus monogyna (Bartha, 2014),* the closed relatives of *Crataegus monogyna* are considered to be *Crataegus meyeri, Crataegus pseudoheterophylla,* and *Crataegus songarica* (Phipps et al., 2003).

The strong inclination of *Crataegus ssp.* for hybridization, apomixis, and polyploidy results in a challenging identification of individual *Crataegus* species (Phipps et al., 2003; Dönmey, 2004; Ercisli, 2004; Gundogdu et al., 2014). In general, the identification is traditionally performed on the basis of morphological characteristics (Byatt, 1975; Phipps et al., 2003; Dönmey, 2004; Dönmez, 2008; Khadivi-Khub et al., 2015; Schramayr, 2016a; Güney et al., 2018). Yet newly established methods

investigate chemotaxonomic and genetic properties of hawthorn hence are used to identify species and distinguish them from each other (Lund et al., 2017; Güney et al., 2018). According to a chemotaxonomic investigation by Prinz et al. 2007, *Crataegus monogyna* contains, in comparison to *Crataegus pentagyna*, the flavonoid 4'''-acetylvitexin-2''-O-rhamnoside, which might be useful to define and differ *Crataegus monogyna* species from others (Prinz et al., 2007). Further, new examinations demonstrated that single species, genetic variations, and relationships between *Crataegus* genotypes could be reliable determined by using molecular markers as simple sequence repeat (SSR) markers (Güney et al., 2018).

In the following, the typical morphological traits, along with the typical phytochemical profiles of *Crataegus monogyna,* will be introduced and discussed.

2.3. Morphology of *Crataegus monogyna*

According to several studies about the morphological varieties of *Crataegus monogyna*, high variations of phenotypes within the species have been reported (Dickinson and Phipps, 1984; Phipps et al., 2003; Dönmey, 2004; Ercisli, 2004; Yanar M. et al., 2011; Khadivi-Khub et al., 2015; Schramayr, 2016a). This diversification of morphological traits within one species can be caused by a combination of several factors as the reproductive biology of the species, which results in hybridization, introgression, and apomixis, and as environmental stress factors, as climate conditions (Phipps et al., 2003; Campbell et al., 2007; Peschel et al., 2008; Khadivi-Khub et al., 2015). All these factors conduct that besides within the species also within a population, a great variation is shown (Khadivi-Khub et al., 2015). In the following, distinctive morphological features of *Crataegus monogyna* will be introduced, along with a view on potential variations of these traits.

Growth Habit

Members of the genus *Crataegus* usually grow as multi-branched, ranging shrubs or trees. Hawthorns can develop different growth habits as single-trunked, multitrunked, irregularly trunked, or branched out to the ground (Kaul, 1998; Yanar M. et al., 2011; Khadivi-Khub et al., 2015). In general, average hawthorn plants feature a height of 2 up to 5 m (Yanar M. et al., 2011). However, *Crataegus monogyna* trees can reach a height of more than 10m (Phipps et al., 2003).

Figure 1 Wild-grown Crataegus monogyna subjects with a multitrunked growth habit in Lower Austria May 2019

Branching system

Hawthorn, as a woody member of *Rosaceae*, develops a characteristic ramification with a division in long shoots and spur shoots. The vegetative long shoots, which grow at the tips of the twigs, contribute to an enlargement of the plant. The spur shoots are generated lateral to the above and grow between 1-2 mm within a vegetations period. Only the short shoots produce generative organs as flowers and the subsequent fruit-set (Phipps et al., 2003). Due to the slow growth of the spur shoots, fruits only occur on 2-year-old wood (Croxton and Sparks, 2002).

Thorn

Crataegus monogyna develops indeterminate stout formed thorns with a length of 5-24mm (Phipps et al., 2003; Albarouki and Peterson, 2007). Contrary to prickles, thorns are morphologically modified shoots, which ceased growth. By stopping the growth in girth, no bast layer is built. Hence the epidermis of the shoot dries out and starts to harden (Schramayr, 2016b). In general, cultivars of *Crataegus monogyna*

present thorns (Phipps et al., 2003; Schramayr, 2016b). However, in Anatolia, Turkey, thornless variations of *Crataegus monogyna* ssp. *monogyna* Jacq have been reported. To investigate morphological characteristics (fruit weight, dimensions, fruit skin color, flesh/seed ratio, plant habits, and thorn situation) of several hawthorn *(Crataegus* spp.) genotypes, the scientists sampled two genotypes of the species *Crataegus monogyna ssp*. *monogyna* Jacq from Malatya, Turkey. Both genotypes showed a wide variation among genotypes for most of the morphological properties. The genotypes in the lower altitude of 1476 m grew as trees with medium acanthaceous branches. At the same time, the other group in the upper altitude of 1570m grew as thornless *Crataegus monogyna* tress (Yanar M. et al., 2011). A possible explanation for the diversity in the number of developed thorns is that within the species *Crataegus monogyna,* a wide phenotypical variation exists, which might be caused by specific environmental factors (Phipps et al., 2003; Albarouki and Peterson, 2007; Yanar M. et al., 2011; Khadivi et al., 2019). Since other members of the subfamily *Spiraeoideae,* as the firethorn, *Pyracantha,* and blackthorn, *Prunus spinosa*, (Potter et al., 2007) develop more thorns in the case of feeding damage (Schramayr, 2016b). However, the intensity of developed thorns depends primarily on the cultivar and not on environmental factors as stress and climatic conditions (Phipps et al., 2003; Albarouki and Peterson, 2007; Yanar M. et al., 2011).

Foliage

Crataegus monogyna is deciduous hence the distinctively small, nearly glabrous, and deeply dissected green leaves are present only in the warmer season (Ellenberg and Leuschner, 2010). Mature leaves are often 5-7-lobed, and the lateral leaf-sinus extend to at least two-thirds to the midrib with strongly formed veins to leaf-sinus. The leaf base can variate from wedge-shaped to concave. The leaf-lobe shape of *Crataegus monogyna* is acute and generally longer than broad (see. Image 2.). The leaf margin is entirely or just at the apex toothed. (Byatt, 1975; Gosler, 1990; Phipps et al., 2003; Schramayr, 2016b). The upper leaf is characterized by dark green to brownish green color, whereas the leaf's underside is brighter with a greyish green color. Also, the underside presents a narrow reticulate venation with slight bulging principal veins (Kaul, 1998).

As in figure 2 illustrated, the leaf shape can variate strongly on one *Crataegus monogyna* tree or polycromic shrub (Byatt, 1975; Dickinson and Phipps, 1984; Gosler, 1990; Christensen, 1992b; Schramayr, 2016b). Since extension shoots show a much higher variation of leaf shapes than the short shoots, usually just leaves of short shoots are used for adequate identification of *Crataegus monogyna* (Phipps et al., 2003; Schramayr, 2016b). However, due to the high variation of leaf shape and size, called leaf heteroplasty, it is important to differentiate the canopy into diverse leaf types. According to the taxonomy concept of Dickinson und Phipps 1984, the various leaf types of the genus *Crataegus* can be dived into the leaves and stipules of elongated shoots, short shoots, and sterile shoots (Dickinson and Phipps, 1984; Dönmey, 2004). Further, leaves and stipules can be differentiated into sun and shade leaves (Schramayr, 2016a).On elongated shoots, leaves either evolve from the main branches' axis or develop from the apex of previous years' branches. In general, present extension shoots are the largest leaves on the plant and bear no flowers (Dickinson and Phipps, 1984; Phipps et al., 2003; Dönmey, 2004). Since these leaves serve mainly for photosynthesis, they differ in their morphology from the rest and show the highest variation in shape and size (Schramayr, 2016b, 2016a).

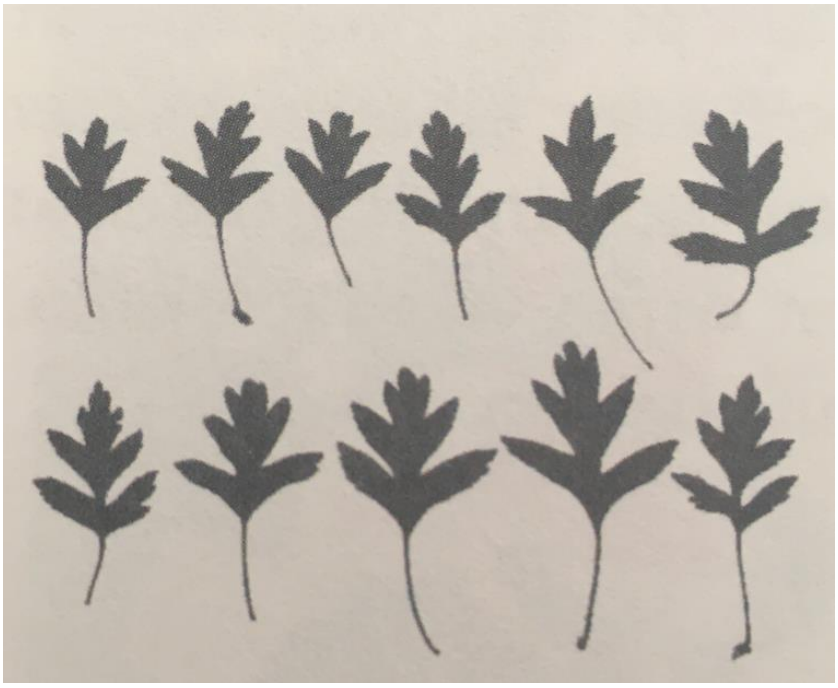

Figure 2 Variation of leave shape of Crataegus monogyna within one plan Reproduced out (Der Weißdorn - ein unterschätzter Alleskönner) with permission (Freya Verlag ,Schramayr, 22.03.2021)

Contrary to the main leaves, stipules of the elongated shoots feature deeper incisions (Dönmey, 2004) and no fully developed lamina (Schramayr, 2016a). The short shoots' leaves clustered on the top, below the inflorescence (Phipps et al., 2003), and bear flowers after the second year (Croxton and Sparks, 2002). Similar to the long shoot leaves, the leaves of the flowering shoot's present also variation

in shape and size. The leaves of sterile shoots are similar to generative shoots in shape and size, but these develop a rosette of leaves at the end of the short shoots without flowering (Dickinson and Phipps, 1984; Dönmey, 2004).

Stomata

In general, *Crataegus* species obtain on the leaf underside numerous large stomata of the anomocytic type. The leaf's upper side consists of unregular polygonal cells and only a few stomata of the anamocytic type (Kaul, 1998). However, Albarouki et al. 2007, who examined the morphology of various *Crataegus* species in Syria, reported that contrary to the other *Crataegus* species, *Crataegus monogyna* grown in Syrian did not possess stomata on the upper leaf side (Albarouki and Peterson, 2007). According to observations in Italy, leaves of *Crataegus monogyna* grown under high light environments represent a significantly higher number and density of stomata than leaves developed in shade conditions (Granata et al., 2020).

Flower

In Austria and Germany, Hawthorn usually flowers from April to May (Pilaske, 1999; Schramayr, 2016b). The start of the flowering period is induced by warm weather conditions (Guitián and Fuentes, 1992). Thus, the actual time of flowering always depends on environmental conditions (Dönmey, 2004) as well as the number of flower production, which is also affected by the habitat (Gosler, 1990). During this flower-bearing period, which usually lasts 1 to 2 weeks, *Crataegus monogyna* develops white flowers grouped in small trusses, also called inflorescences (Phipps et al., 2003). These inflorescences constitute 5 to 15 small single glabrous flowers with 10-25mm diameter (Albarouki and Peterson, 2007). The actinomorphic flowers consist of a brownish-green colored hypanthium, which contains at the upper end five triangle-shaped sepals. The five white petals are roundish to broad shaped and short (Evans and Dickinson, 2005). The carpels of hawthorn are constructed by the hypanthium that lies beneath the petals and sepals. In addition, *Crataegus monogyna* plenty stamens, which produce the pollen, also originate from the hypanthium (Kaul, 1998; Phipps et al., 2003). The hypanthium of *Crataegus monogyna* is fused with more than half of the ovary (Potter et al., 2007) and develops just one pistil and loculus (Kaul, 1998; Phipps et al., 2003). On the inner surface of the hypanthium, nectary is produced (Evans and Dickinson, 2005). According to a light and scanning electron microscopy examination in Turkey 2008, the pollen of *Crataegus monogyna* did not feature characteristic morphological traits

of taxonomic value to distinguish this species from other *Crataegus* species (Dönmez, 2008). To be attractive insects for pollination, the hermaphroditic flowers of *Crataegus monogyna*, as in Image 3 seen, produce, and release secondary metabolites.

Figure 3 Flowers of *Crataegus monogyna* subject in Lower Austria, April 2019

Contrary to other related Rosaceae plants, *Crataegus monogyna* does not set on producing odorant essential oils to attract pollinators. Hawthorn chooses another strategy to attract pollinators and produce the volatile amine, the triethylamine. Since the gas triethylamine usually emerges from microbiological destruction processes of proteins, the odor signature of triethylamine is noticed by humans as decomposed edibles or as fish-like smell (Schramayr and Paszkiewicz, 2016). This amine gas is especially attractive to specific insect groups as *Hymenoptera* and *Diptera* (Gosler, 1990; Schramayr and Paszkiewicz, 2016).

Fruit

After successful pollination, the flowers begin immediately to develop into polypyrenous drupes (Phipps et al., 2003; Potter et al., 2007). The berry-like fruits start green colored, swell, and evolve with continuing ripening stages the typical red color (Phipps et al., 2003). The mature fruits are spherical to broadly ellipsoid shaped (see. Fig.4). Hawthorn fruits posse an open apex since the hypanthium has never fully closed over the flower. This opening is generally enclosed by a residual calyx or calyx remnants. The dried and curled up remains of the stamen and styles occult the top of the fruit. The

Figure 4 Sample fruits from *Crataegus monogyna* grown in Lower Austria October 2019

fruit flesh emanates from the flower's hypanthial tissue and is comparable with the fleshy part of an apple fruit. In the center of the fruit are very hard, seedlike organs the nutlets, which originated from the carpels in the flowers. Nutlets or also called pyrenes are bony carpels and contain the actual seeds. However, many authors use the term “seed,” but suppose the nutlets or pyrenes (Phipps et al., 2003). *Crataegus monogyna* is generally one-seeded hence contains one pyrene. Yet, due to cross-pollination and the resulting hybridization, some fruits might develop two or more nutlets/seeds on a *Crataegus monogyna* individual. (Yanar M. et al., 2011; Gundogdu et al., 2014; Khadivi-Khub et al., 2015; Vašková and Kolarčik, 2019).

Since a widely fleshy, edible layer, outside the true ovary, servers technically to build a pome, the drupes of hawthorn are rather classified as fruits than berries (Kaul, 1998; Phipps et al., 2003). In general, the small fruits show a diameter between 6 - 16mm (Kaul, 1998; Pilaske, 1999; Phipps et al., 2003). Yet, research has demonstrated that size, weight, and red coloring of the *Crataegus monogyna* fruits variate (Phipps et al., 2003; Albarouki and Peterson, 2007; Yanar M. et al., 2011; Gundogdu et al., 2014; Khadivi-Khub et al., 2015; Schramayr, 2016b) depending on genetic factors, climatic factors and soil structure (Sonnenschein and Plescher, 2005; Gundogdu et al., 2014; Khadivi-Khub et al., 2015). However, also pruning treatments affect the size and the weight of the hawthorn fruits (Sparks and Martin, 1999; Croxton and Sparks, 2002). The fruits' maturity dates are primarily determined by the type of cultivar (Sonnenschein and Plescher, 2005). The visual fruit color of *Crataegus monogyna* is red (Phipps et al., 2003; Ercisli, 2004; Sonnenschein and Plescher, 2005; Khadivi-Khub et al., 2015; Mraihi et al., 2015). The pigment content of the fruits' reddish color can usually be adequately determined with CIE (L*, a*, b*) color space values (Egea et al., 2010). The measured color is localized in a three-dimensional color space by these CIE (L*, a*, b*) coordinates (McGuire, 1992).
According to investigations from Turkey, Iran, Spain collected fruits also show variation in the reddish color characteristics, from dark blueish red to bright red (Dönmey, 2004; Egea et al., 2010; Khadivi-Khub et al., 2015). In conformity to various observations in Europe, fruit ripening occurs later as latitude and altitude decrease. According to the observers, this shift of fruit ripening might be evidence for *Crataegus's* adaption o benefit from the maximum abundance of fruit-eating birds in the different regions (Guitián and Fuentes, 1992). However, even after the

maturity date, the berrylike fruits remain attached to the shrubs or trees for an extended period throughout autumn and winter, hence providing an important nutritional source for wildlife (Osborne, 1984; Pilaske, 1999; Sparks and Martin, 1999; Phipps et al., 2003).

2.4. Phytochemical profile of *Crataegus monogyna*

Crataegus monogyna shows a wide variety of secondary metabolites (Kaul, 1998; Edwards et al., 2012; Nabavi et al., 2015). Contrary to primary metabolites, secondary metabolites are not associated with essential cellular functions and actively enhance the plant's fitness. Yet they might be fundamental for reproduction, as the attraction of insects and animals to warrant fertilization and/or seed dispersal and viability in their natural environment. For instance, as protection against abiotic stress such as radiation or biotic stress such as pathogens and herbivores (Böttger et al., 2018b). Hence secondary metabolites are often species-specific, and the portion alters under certain environmental conditions (Croteau et al., 2000). The by-products or intermediates of primary metabolism build mostly the basic structure for secondary metabolites. Depending on the structure and biosynthetic pathways, secondary metabolites are distinct and classified into specific groups. The main groups based on their pathways and chemical structure are terpenoids, alkaloids, polyketides, quinones, cyanogenic, glycosides and phenylpropanoids (Croteau et al., 2000; Kabera et al., 2014; Böttger et al., 2018b).

In *Crataegus monogyna,* there are generally compounds of phenylpropanoid and terpenoid groups attributed as the most crucial secondary metabolites (Kaul, 1998; Edwards et al., 2012). Especially about the phenolic compounds occurring in hawthorn, which are primarily synthesized by phenylpropanoid, phenylpropanoid-acetate, and related biochemical pathways (Croteau et al., 2000), much research has been done (Kaul, 1998; Froehlicher et al., 2009; Liu et al., 2011; Nabavi et al., 2015). However, also other compounds as fatty, organic, and phenolic acids up to sugar and sugar alcohols have been detected and extracted from different organs of hawthorn (Edwards et al., 2012; Nabavi et al., 2015). According to several studies, different types of tissue, like leaves, flowers, and fruits, contain distinct compositions of beneficial compounds (Kaul, 1998; Froehlicher et al., 2009).

The essential compounds of *C. monogyna* and in which plant organ they are primarily present will be introduced in the following. Since different authors used different classification systems for the detected phenolic compounds in *Crataegus sp.* (Froehlicher et al., 2009; Rasmussen, 2011; Pavlovic et al., 2019), a short description and the used classification will be defined based on published basic scientific literature (Kaul, 1998; Croteau et al., 2000; Böttger et al., 2018a; Vuolo et al., 2019).

2.4.1. Phenolic Compounds

As before mentioned, phenolic compounds or phenols are characterized by their chemical structure and the phenylpropanoid or related pathway they are derived from. Phenols consist of a phenyl ring and a specific "acidic hydroxyl or phenolic group.

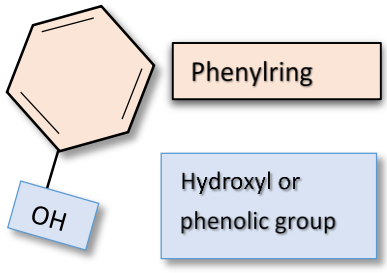

Figure 5 Chemical structure of phenol modified by (Croteau et al. 2000).

Phenolic compounds can be based on their synthesized pathway (Croteau et al., 2000) and structure classified as hydroxycinnamic acids, lignans, and flavonoids (Vuolo et al., 2019). In the genera of *Crataegus,* many substances of these classes occur in samples of *C. monogyna*.

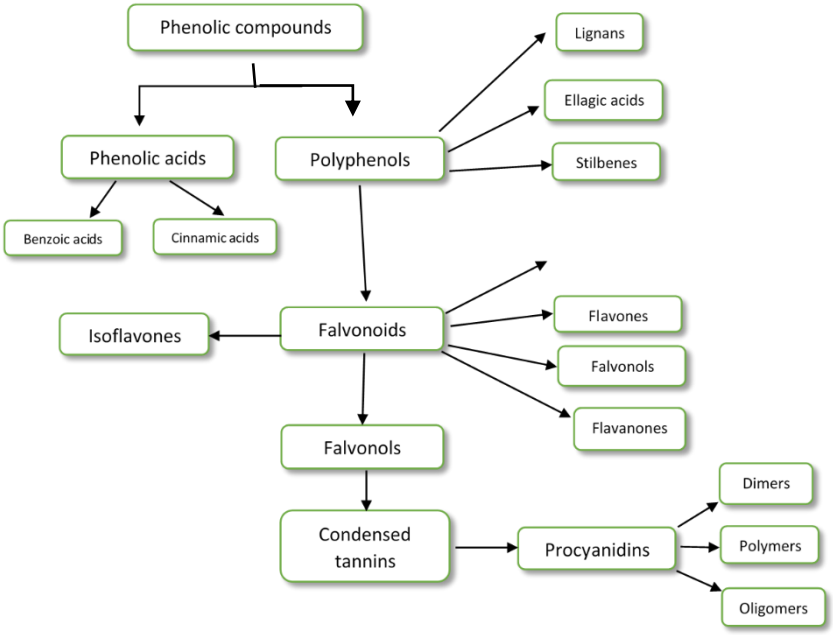

Figure 6 Schematic classification of phenolic compounds modified by (Vuolo et al., 2019).

Table 2 Concentrations of main compounds as reference values for specific *Crataegus monogyna* organs

	Concentration (mg/g)							
	Total phenols	References	Flavonids	References	Proanthocyanidin	References	Anthocyanidins	References
Fruit	16.426–57.07	(Bernatonienė et al., 2008; Froehlicher et al., 2009)	4.46–147.3	(Prinz et al., 2007; Froehlicher et al., 2009)	1.058–22.2	(Froehlicher et al., 2009; Mraihi et al., 2015)	0.150–0.580	(Froehlicher et al., 2009; Mraihi et al., 2015)
Flower	9.7–98.89	(Bahorun et al., 1994; Froehlicher et al., 2009)	10.4–1026.6	(Prinz et al., 2007; Froehlicher et al., 2009)	9.295–17.946	(Leskovac et al., 2007; Froehlicher et al., 2009)	–	(Froehlicher et al., 2009)
Leaf	n.i.		24.95–28.60	(Orhan et al., 2007)	n.i.		n.i.	

In particular hydroxycinnamic acids, flavonoids, and condensed tannins have been detected (Edwards et al., 2012). To determine the phenolic content, in general, the unselective Folin-Ciocalteus method is performed. This procedure provides information about the total phenolic content, TPC, thus the amount of all phenolic compounds from simple phenolic acids to complex flavonoids and condensed tannins, of a specific sample (Kaul, 1998; Waterhouse, 2005).

Hydroxycinnamic acids

Hydroxycinnamic acids are derived from nonphenolic molecules of cinnamic acid (Vuolo et al., 2019) and are generated in the phenylpropanoid pathway as hydroxy derivatives (Croteau et al., 2000). Some of these hydroxycinnamic acids, for example, sinapic acid (Froehlicher et al., 2009), chlorogenic acids (Rodrigues et al., 2012), and ferulic acid, have been detected in *Crataegus monogyna* organs (Edwards et al., 2012).

Flavonoids

Although Flavonoids are present in most plant tissue types, they are not essential for plant survival, yet they contribute to the numerous physiological mechanisms of the plant. Flavonoids occur as monomers, dimers, and higher oligomers and are mostly stored in vacuoles (Croteau et al., 2000). This class of secondary products is conducive to a plant's color characteristic (Panche et al., 2016). In addition, flavonoids are involved in enhancing the adaption to abiotic and biotic stress. Especially in the case of frost hardiness, drought resistance higher levels of flavonoids have been detected in various plant organs (Kirakosyan et al., 2004; Nakabayashi et al., 2014) and might play a functional role in plant drought adaption (Nakabayashi et al., 2014) and freezing tolerance (Panche et al., 2016). Several studies examined the total flavonoid content of *Crataegus* leave (Peschel et al.,

2008; Coimbra et al., 2020) and fruits (García-Mateos et al., 2013; Simirgiotis, 2013). Generally, flavonoids are, according to their chemical structure, further subdivided into groups. These subgroups are commonly bound to sugars, called glycosides, for storage in the plant. On the one hand, the bonded forms of flavonoids are more stable than the free version. On the other hand, the bonded flavonoids represent in investigations a relatively poor bioavailability. Bonded flavonoids are conjugated with *O*- or *C*-glycosides. These sugars can be galactose, glucose, rhamnose, and apiose. Generally, the sugar residues are linked to 3-, 7-, or 4-hydroxyl groups for *O*-glycosides. At the same time, the sugar residues of the *C*-glycosides are linked directly to *C*-6 or *C*-8 (Vuolo et al., 2019).

In *Crataegus monogyna,* there are represent several classes, such as flavonols, flavanones, flavans, anthocyanidins, isoflavones, neoflavones (Nabavi et al., 2015). However, according to a literature review about hawthorn chemistry, the authors conclude that the flavonoid content represents just as much variability within species as among species (Edwards et al., 2012).

Flavonols

Crataegus monogyna present various flavonol-glycosides in the different organs (Kaul, 1998; Orhan et al., 2007; Froehlicher et al., 2009; Edwards et al., 2012; Nabavi et al., 2015). Generally, flavonols contain a ketone group (Panche et al., 2016). According to several investigations, flavonols feature antioxidant properties and other biological activities (Panche et al., 2016; Brodowska, 2017). Especially quercetin (Edwards et al., 2012), hyperosid (= quercetin-3-*O*-galactoside) (Prinz et al., 2007), rutin (= quercetin-3-*O*-rutinoside) (Konyalioglu et al., 2017), isoquercitrin (=quercetin-3-*O*-glucoside) (Prinz et al., 2007) and Spiraeoside (=quercitin-4-*O*-glycoside) and have been detected in *Crataegus monogyna (Edwards et al., 2012)*.

Flavones

Flavones exhibit a similar chemical structure as flavonol compounds (Brodowska, 2017). The subclasses apigenin and luteonin are significantly represented in *Crataegus monogyna* (Nabavi et al., 2015). According to several studies, vitexin-2”-*O*-rhamnoside (Orhan et al., 2007; Froehlicher et al., 2009), orientin (= orientin-2”-*O*-rhamnoside), isoorientin, (=acetylvitexin-2”-*O*-rhamnoside), -2”-*O*-rhamnoside (Edwards et al., 2012), -8-methoxykaempferol-3-*O*-glucoside, vitexin-4”-*O*-

glucoside*(Prinz et al., 2007; Edwards et al., 2012)* Luteolin-7”-*O*-rutinoside, Kaempherol-3”-*O*-galactoside, Kaempherol-3”-*O*-glucoside and Apigenin-7”-*O*-glucoside (Mraihi et al., 2015) have been ascertained in *Crataegus monogyna.*
All these flavonols and flavones compounds are generally based on glycosylation and/or acetylation reactions of naringenin (Edwards et al., 2012; Vuolo et al., 2019), which belongs to the class of flavanones (Panche et al., 2016).

Flavanols/Flavan-3-ols

The contained flavanols are derivates of flavans and present as intermediate products in many different tissue types (Panche et al., 2016). The single-molecule is catechin. Catechin can occur as monomer, dimers, trimers, or oligomers and is mostly the basic product for tannins (see Image X). They have high antioxidative potential and are colorless (Brodowska, 2017; Santos et al., 2017). Particularly, the monomer (-)-epicatechin is present in *Crataegus monogyna* (Froehlicher et al., 2009). Due to its free radical scavenging capacity, Catechin prevents protein to oxidate. Besides, does catechin feature the ability to reduce the covalent modification of proteins, which are induced by reactive oxygen species or by-products of oxidative stress (Brodowska, 2017).

Procyanidin

Dimers, trimers, and oligomeric structures as Catechins or Epicaechins condensate to tannins (Panche et al., 2016; Brodowska, 2017) and constitute single or double bounded procyanidins (Nabavi et al., 2015). Condensed tannins are generally more complex and uniform than hydrolyzable tannins (Vuolo et al., 2019). The production of oligomer procyanidin (OPC) provides a defense mechanism against herbivores, protection against UV radiation, and adverse climate conditions (Kaul, 1998). The dimeric proanthocyanidin B2 is the most abundant condensed tannin in *Crataegus monogyna* (Edwards et al., 2012; Nabavi et al., 2015).

Anthocyanidins

In the phenylpropanoid pathway, anthocyanin is synthesized. These water-soluble vacuolar pigments occur in all tissues (Croteau et al., 2000). Anthocyanidin glycosides (anthocyanidins) are the main constituents of flowers and berries, giving them their distinctive color. Depending on the pH and the UV radiation, anthocyanin develops a wide variety of colors from red, blue, purple to black (Panche et al.,

2016). Five anthocyanidins, cyanidin-3-*O*-glucoside, and cyanidin-3-*O*-rutinoside, pelargonidin-3-*O*-glucoside, cyanidin-3-*O*-pentoside, peonidin-3-*O*-glucoside have been detected in fruits of *C. monogyna* (Rodrigues et al., 2012). The most abundant anthocyanidin glycoside in fruits is cyanidin-3-*O*-galactoside (Froehlicher et al., 2009). Yet cyanidin-3-*O*-glucoside is considered to be responsible for the typical reddish coloring of *C. monogyna* fruits. Anthocyanidins accumulate in the peel of *Crataegus* fruits (Mraihi et al., 2015).

2.4.2. Other non-phenolic compounds

In various parts of *C. monogyna* have been also non-phenolic compounds as tocopherols, ascorbic acid, fatty acids (Barros et al., 2011), carotenoids (Egea et al., 2010), minerals (Özcan et al., 2005) verified. But also organic acids, sugar (Gundogdu et al., 2014), triterpenic acids, amines (European medicines Agency, 2016a), and residues of sugar (Edwards et al., 2012) have been detected. All these mentioned compounds also contribute to the antioxidative character of *Crataegus monogyna* (García-Mateos et al., 2013).

2.4.3. Antioxidative capacity

The antioxidant potential of phenolic compounds and plant extracts is commonly *in vitro* determined due to a single electron transfer reaction or a hydrogen atom transfer reaction. There are two types of reaction to investigate the antioxidant activity of a sample, the redox types and the thermodynamics competition types. In this examination, a redox type, the ferric reducing ability of plasma (FRAP) assay, is introduced. In general, the redox type reacts that present variations of coloring correlated with antioxidant concentration species in the sample, which is expressed in Trolox. The FRAP method is based on reducing ferric to ferrous ions at a low pH (3.6) caused by antioxidants and constitutes a colored complex (Vuolo et al., 2019). Various studies proved that phenolic compounds possess a high antioxidant capacity, hence act as potent antioxidants *in vitro* (Rasmussen, 2011; Rodrigues et al., 2012; Martín Ortega and Segura Campos, 2019). Since phenolic compounds inhibit biomolecules, such as proteins, polyunsaturated lipids, nucleic acids, and sugars from being submitted to oxidative damage through free radical-mediated

reactions, in many studies about plants, a positive correlation between the total antioxidative capacity and the TPC or total flavonoid content, TFC, has been demonstrated (Cantín et al., 2009; Contreras-Calderón et al., 2011). Further, due to their protective function of biomolecules, many beneficial effects include anti-inflammatory, cardioprotective, neuroprotective, antitumor, antidiabetic, and antiaging properties are allocated to them (Kaul, 1998; Lopes et al., 2017; Santos et al., 2017; Martín Ortega and Segura Campos, 2019).

2.4.4. The occurrence of secondary metabolites in all organs

According to review articles, the fruits of the hawthorn are chemically similar to the other organs. However, the composition and the amount of the substances are distinctive between the organs (European Agency for the Evaluation of Medicinal Products, 1999; Edwards et al., 2012; Nabavi et al., 2015). Generally, the levels of flavonoids differ, as flowers present a TFC of 2,5%. In comparison, leaves contain 1.8% TFC and fruits 0.5% TFC (European Agency for the Evaluation of Medicinal Products, 1999). Also, the constitutes alter with changing environmental conditions (Kirakosyan et al., 2004; Konyalioglu et al., 2017), population (Peschel et al., 2008), or maturity stages of the plant (Sonnenschein and Plescher, 2005; Pavlovic et al., 2019). The antioxidative properties also distinguish between the different plant organs (Konyalioglu et al., 2017).

In *Crataegus monogyna,* leaves and flowers contain higher total procyanidin levels, especially B2 dimers, as the fruits (Edwards et al., 2012). Flowers present tocopherols and ascorbic acid content next to other organs. Further, they showed the best n-6/n-3 fatty acids ratio (Barros et al., 2011). Generally, flavonol glycosides are mainly concentrated in the flowers (Nabavi et al., 2015). Yet fruits present in some studies higher levels of hyperoside (quercetin-3-O-galactoside) than the flowers but at the same time smaller levels of vitexins than the leaves (Froehlicher et al., 2009; Edwards et al., 2012). Especially minerals, such as Ca, Fe, K, Mg, Na, Ni, P, and Zn are present in fruits (Özcan et al., 2005). Phenolic compounds, like anthocyanins, flavonols, and flavones accumulate mostly in the peel of *Crataegus monogyna* fruits with the exception of procyanidins, which are most abundant in the seed (Mraihi et al., 2015). Contrary to other *Crataegus* fruits, the containing

substances of fruits of *Crataegus monogyna* have not been intensively investigated (Edwards et al., 2012). However, one study about the content of organic acids and sugars in fruits of various *Crataegu*s species, showed that *Crataegus monogyna* had in comparison to other *Crataegus* species a relatively low ascorbic acid content and yet possesses in contrast to other species the highest glucose and fructose content. Another interesting factor was ascertained, that the two different cultivars of *Crataegus monogyna* showed different composition of metabolites (Gundogdu et al., 2014).

To sum up, the phytochemical profile of *Crataegus monogyna* distinguishes between the different organs and depends on several factors: environmental conditions, genetic properties, and maturity stages of the plant.

2.5. Reproductive Biology of *Crataegus monogyna*

As mentioned before, members of the subtribe *Pyrinae* are prone to apomixis, polyploidy, and hybridization (Dickinson et al., 2007). Hence, *Crataegus monogyna* presents complex reproductive biology and is capable of sexual or asexual reproduction (Phipps et al., 2003). In the following important terms of reproductive mechanisms for the genus, *Crataegus* will be defined.

Sexual reproductive mechanisms

Autogamy

Autogamy is a sexual reproduction mode that describes self-pollination, also called selfing, within a single flower. During autogamic reproduction, gametes are derived from a single individual union. The process is also called inbreeding. Self-pollination between flowers emanated from one individual is known as geitonogamy. However, there are no differences between the genetic product of autogamy, and geitonogamy replication hence the progeny is identical (Simpson, 2019).

Hybridization

Hybridization is the sexual reproduction of different species, the so-called interspecific hybridization, or of genetically different individuals as different populations, specific selections, breeding lines, or cultivars (Murray, 2017; Simpson, 2019). The descendants of this crossbreeding are hybrids. Hybridization aims to

transfer genes from one individual to another hence to extend the genetic variability of the parents' generation. The effect of hybridization is used as a standard tool in plant breeding. By exploiting the heterosis effect, which often occurs when genetically different plants are crossed, the Hybrids' deliberated traits show an increased efficiency contrary to the parental generation (Murray, 2017).

Introgressive hybridization

Introgressive hybridization or vicinism describes genetic material transfer by the recurrent crossing of one species and another species or close relatives like subspecies. The results of vicinism depend on the strength of the internal barriers of a species. Introgression produces in genetic populations with weak internal barriers, subspecies, or varieties, which grade imperceptibly and smoothly into the parental species. The population with strong internal barriers carries parts of the barrier system into the introgressed species and therefore, forms semi-isolated subspecies or races within it. Contrary to species with strong internal barriers, the vicinism tends to transfer these barriers into the populations of the recurrent parent. (Anderson, 1953).

Asexual reproductive mechanisms

Apomixis

Apomixis is a reproduction mechanism, which has been reported in over 300 species. The family Rosacea of the angiosperm families also presents this propagation method. In general enables apomixis plants the autonomous seed production without the processes of meiosis and fertilization (Mazzolari et al., 2017). In gametophytic apomixis, the embryo originates from a diploid egg in the embryo sac, (Kandemir and SAYGILI, 2015). In general, there are three major types of the asexual, clonal production of seeds, the apospory, the diplospory, which belong to the gametophytic apomixis, and adventitious embryonic (Hanna and Bashaw, 1987). Since for the genera *Crataegus* apomictic mechanisms apospory has been reported (Phipps et al., 2003), only the apospory type will be introduced. Generally, the divers' apomictic mechanisms are classified based upon the cell's incurrence and development from which the embryo evolves (Hanna and Bashaw, 1987). In the case of the apospory apomixis, the development of the endosperm and embryo

occurs in the unreduced embryo sacs of the ovule. Customary one or a few somatic cells of the nuclei accumulate to a megagametophyte in the ovule. The megagametophyte is comparable to a megaspore (Kandemir and SAYGILI, 2015). In general, the main advantages of apomixis is the clonal production of seeds hence the development of hybrids or genotypes that breed true regardless of heterozygosity (Hanna and Bashaw, 1987)

Polyploidy

Polyploidy is a phenome when a cell contains more than two sets of chromosomes. Polyploidy occurs during meiosis at the chromosome duplication. In particular, in the family of Rosaceae, polyploidy is frequently allied with the occurrence of reproductive mechanisms as gametophytic apomixis and hybridization (Dickinson et al., 2007). Polyploidy occurs either within a species, the so-called autopolyploidy, or between different species, allopolyploidy (Simpson, 2019). Among the *Crataegus* species, the occurrence of polyploidy is a widespread phenomenon. As before mentioned, *C. monogyna*, as a member of the subfamily *Spiraeoideae,* has the base chromosome number x=17. However, *C. monogyna* is diploid hence present a basic chromosome number of 2n (2x) = 34, contrary to other close related polyploid *Crataegus* species and *Crataegus* hybrids. (Evans and Campbell, 2002; Dickinson et al., 2007; Khadivi-Khub et al., 2015).

Reproductive methods of *Crataegus monogyna*

Since hawthorn, as a member of the subtribe *Pyrinae*, is prone to apomixis, hybridization, and vicinism, various reproductive modes of *Crataegus monogyna* have been reported. For example, apomictic replication of *Crataegus monogyna* in Turkey (Dönmey, 2004) and North America (Dickinson and Phipps, 1986), partially self-compatibility of populations in Spain (Guitián and Fuentes, 1992) and autogamy propagation in Britain (Gyan and Woodell, 1987).

Crataegus monogyna flowers only once a year for a short duration of one or two weeks. At this time, all flowers open for pollination (Phipps et al., 2003). By producing and releasing the before mentioned volatile amine, triethylamine (Schramayr and Paszkiewicz, 2016), *Crataegus monogyna* attracts specific pollinators (Gosler, 1990; Chacoff et al., 2008; Schramayr and Paszkiewicz, 2016). During an examination in 2005 about the impact of quantity and quality of pollen on

the propagation of a *Crataegus monogyna* population grown in northern Spain, scientists performed 94 countings of flowering branches. Overall, 310 insects attended *Crataegus monogyna* flowers within 10 minutes of counting (Chacoff et al., 2008). Since fertilization occurs when an insect transfers the pollen from the stamen to the stigma (Phipps et al., 2003), a visit was only counted when the visitor contacted a reproductive part of the flower, whether the anthers and, or the stigma. According to the observation, the pollinators visited the flowers, especially on sunny to slightly cloudy days with low wind velocity. The flowers were with nearly 88% of all visits primarily toured by *Diptera* species. Of all visits, only 10% were conducted by Honeybees, and 1% occasionally by beetles and vespids (Chacoff et al., 2008). Further, monitorings from other regions confirm these occurrences and abundances of pollinators as *Hymenoptera* and *Diptera.* (Gosler, 1990; Guitián and Fuentes, 1992; Schramayr and Paszkiewicz, 2016).

Overall, animal-mediated pollination is presumed to be the most important and employed strategy for hawthorn to achieve fertilization (Phipps et al., 2003).

Since insects mainly fertilize the hermaphrodite flowers of hawthorn in the wild, particularly cross-pollination of different species occurs. The consequent hybridization and vicinism of *Crataegus monogyna* are essential in producing the spatial distribution of phenotypic variation, which can be observed in populations (Gosler, 1990).

Especially open pollination, along with a high degree of heterozygosity of *Crataegus monogyna*, results in an acceleration of genetic variability and a highly variable phenotype (Gundogdu et al., 2014; Khadivi-Khub et al., 2015). The incidence of *Crataegus* hybrids is common in all distributed areas (Byatt, 1975, 1975; Gosler, 1990; Christensen, 1992a, 1992b; Dönmey, 2004; Peschel et al., 2008; Schramayr, 2016a).

In Europe, *Crataegus laevigata* and *Crataegus monogyna* are most abundant hence many populations share the same geographic area, various hybrids of that sympatric species crop up in populations and pattern hybrid swarms (Byatt, 1975; Dönmey, 2004). Since both species are diploid with 2n=2x=34, it is presumed that interspecific hybridization is partly responsible for the extensive morphological variability (Gosler, 1990). In Britain, the resulted fruit-set of recurrent crossing between *Crataegus monogyna* and *Crataegus laevigata* within a population and the pollen of both parental and intermediate types were examined. According to the

observations, the fruit-set developed only 2 % from self- pollination, whereas 30-59 % of fruit set derived from inter- or intra-specific cross-pollination. Additionally, no pollen sterility in hybrids was ascertained (Bradshaw, 1971). In conformity with several observations of vicinism of *Crataegus sp.* and the definition of introgressive hybridization of Anderson, *Crataegus monogyna* posseses a weak internal barrier (Anderson, 1953). Hence it is presumed that there are external barriers of ecological origin to hybridization of sympatric *Crataegus* species or populations (Bradshaw, 1971; Byatt, 1975; Christensen, 1992b). However, these external barriers depend on the environmental conditions hence differ between the regions. In central and north Europe is the maturity date of anthesis between *Crataegus monogyna* and *Crataegus laevigata* flowers different.

Further, there are barriers based on adaption since *Crataegus laevigata* more shade tolerant than *Crataegus monogyna* hence produces more flowers under shading conditions. Contrary to Europe are in Greece, more external barriers to hybridization have been described as altitudinal range and substrate preferences (Christensen, 1992b). Further takes the break of northern hawthorn species' dormancy, who are native to 7 or lower (see Fig.6 & 7.), two winters, whereas southern species germinate after one (Phipps et al., 2003). These external barriers derive from hawthorn's habitat and affect little the individual's survival but significantly influence the flower production and the subsequent fruit-set. Consequently, the morphological direction of a population and the subsequent generations might be determined by the impact of the habitat on the individuals' specific fertility (Gosler, 1990). According to a British study, a sympatric hawthorn population's structure strongly correlates with the soil type. Hence hybrids adapt better and establish more in the habitat (Byatt, 1975).

Even though fertilization via pollination is presumed to be the most crucial strategy to warrant a successful reproduction (Phipps et al., 2003), the case of pollen limitation occurs. This limitation whether airs out of the quantity of the available pollen or the quality of the pollen. In the case of the quantitative disposability, the pollen might be restricted because of individuals' isolation, rarely present pollinators, or the competition of plants for the pollinators' services. Contrary to the quantity is the quality of pollen, especially for self-incompatible species, a significantly limiting factor because the pollen of closely related individuals deposit on the stigma of a self-incompatible individual generally does not induce successful fertilization

(Chacoff et al., 2008). Fertilization is presumed as successful when transferred pollen, regardless of his origin as the same individual, close relative, or another species, germinates on the stigma and grows into the egg cell (Phipps et al., 2003). In general, the genus *Crataegus* is considered self-incompatible to ensure cross-pollination, extending the progeny's genetic variation and avoiding inbreeding depression. However, some plants of the genera *Crataegus* are capable of changing their propagation strategy in case of environmental stress, as pollen limitation (Guitián and Fuentes, 1992; Chacoff et al., 2008). *Crataegus monogyna* is one of those and features self-compatible traits hence is capable of propagating autogamous (Gyan and Woodell, 1987; Phipps et al., 2003). Self-pollination scenarios of *Crataegus monogyna* have been reported in different regions of Europe (Gyan and Woodell, 1987; Guitián and Fuentes, 1992; Chacoff et al., 2008). Nevertheless, the results of self-pollination are, in most cases, less successful than the results of cross-pollination (Bradshaw, 1971; Guitián and Fuentes, 1992). This observation was confirmed in an investigation about the effects of pollen's quality and quantity on the reproduction of a population of Crataegus monogyna wildly grown in northern Spain. By performing seven treatments on Crataegus monogyna, the scientists provoked different reproductive mechanisms, as apomixis, spontaneous autogamy, pure self-pollination, or cross-pollination.

Self-pollinated flowers present a significantly higher abortion rate of developing fruits than cross-pollinated flowers. Therefore, the authors assumed the phenom of post-zygotic selective embryo abortion. The mother plant chooses between embryos or embryos vie with each other for resources from the mother. However, there was no difference between the number of developed pollen tubes for both treatments. Consequently, the sciences presumed that pollen quality might be a limiting factor for fruit production in this species than quantity (Chacoff et al., 2008).

Besides the several introduced sexual replication forms of *Crataegus monogyna,* also asexual propagation, as apomixis has been reported in Turkey and North America (Dickinson and Phipps, 1986; Dönmey, 2004). Individuals of *Crataegus monogyna* perform under specific environmental conditions, apospory apomixis hence are capable of producing genetic ident seeds without meiosis and fertilization (Dickinson and Phipps, 1986; Phipps et al., 2003). However, in contrast to the investigation of Dickinson and Phipps, 1986 in North America, Spanish scientists detected during the examinations that fruit set in emasculated flowers is very rare

present, which evidences that apomixis without pollen induction is not usual (Chacoff et al., 2008).

To sum up, the reproductive biology of *Crataegus monogyna* is changeable due to weak internal barriers prone to introgression, polyploidy, and apomixis. Therefore the lines between species and sub-species are not always clear to separate and affect the cultivation of those (Christensen, 1992a; Albarouki and Peterson, 2007).

2.6. Cultivation of *Crataegus monogyna*

Although some hawthorn species are cultivated on a small scale for fruit production as for example, in North America (Payne and Krewer, 1990) and China (Liu et al., 2016), cultivation for the production of leaves and flowers drugs has not been established, yet. Investigations on the first-time cultivation and cultivation are complicated by various factors as that hawthorn bears its first flowers at the earliest three years after starting the first-time cultivation hence only produces real yields after about 5 years (Phipps et al., 2003; Sonnenschein and Plescher, 2005; Peschel et al., 2008; Fachagentur Nachwachsende Rohstoffe e.V., 2013a). Anyway, the prerequisite for the successful cultivation of a plant species is the prior precise analysis of its location requirements such as soil conditions, climate, light conditions, and soil moisture. Furthermore, knowledge of the reproductive mechanism, i.e., flower biology, germination conditions, and the plant species' vegetative reproducibility, is presupposed. Likewise, the possible biomass increase per vegetation period and area unit, as well as the sensitivity to frost, will determine the success of cultivation. There are significant differences in the working procedure between annual and perennial plant species, perennials, and woody plants (Sonnenschein and Plescher, 2005).

In the following, knowledge about several essential aspects and requirements for the cultivation of hawthorn, in particular for *Crataegus monogyna*, will be compiled.

2.6.1. Habitat requirements

The indicator values reflect a plant population's occurrence in relation to certain environmental factors under field conditions, i.e., with the distinctive interspecific competition. Therefore, the indicator values say nothing about the “requirements“,

thus the behavior in cultivation. Yet, these indicator values of wild species can give an impression about certain growing requirements for successful cultivation. The authors Ellenberg and Leuschner described in their overview of Central Europe's vegetation ecology indicator values of *Crataegus monogyna* populations. Contrary to other species, *Crataegus monogyna* prefers open habitats as a half-light plant, hence usually in full light, but also in the shade to about 30 % of relative light intensity (Ellenberg and Leuschner, 2010). Individuals of *Crataegus monogyna* are phytosociological parts of various plant societies, including the *Fagetalia* and as a character species of the *Prunetalia* society (Schuck, 2014). Hence naturally grown *Crataegus monogyna* establishes numerous shrubbery and pre-forest societies on not extreme soils. Simultaneously, *Crataegus monogyna* is located just in climatic warmer forest societies as a relict of the former shrubby-assented pre-forset environment (Schramayr and Baumgartner, 2016).

Climatic requirements

Crataegus monogyna populations grow in altitudes from deep to montane in moderate climate zones. The main abundance is submontane temperate areas of large parts in Central Europe. According to the climate, classification are *Crataegus monogyna* populations as oceanic to suboceanic, extending to East and West Europe (Ellenberg and Leuschner, 2010). As the wild-grown hawthorn population, cultivated hawthorn is also determined by climatic requirements. According to the in 2003 Phipps et al. modified a map of the hardiness zones after Heinze and Schreibe 1984, the best cultivation locations for *Crataegus monogyna* are in the hardiness zones 5-7. Austria is located in in the hardiness zones 5 and 6. This means that depending on the cultivar *C. monogyna* can withstand an average extreme minimum temperature of -29°C to -12°C (Phipps et al., 2003).

Solis

Crataegus monogyna grows on almost all soil types, but preferably calcareous, base-rich, dry to fresh clay soils in warm layers (Schuck, 2014). Therefore, *Crataegus monogyna* populations are considered weak acid to moderate base indicators of soils (Phipps et al., 2003; Ellenberg and Leuschner, 2010). Population extends, preferably on chalky soils and never on strongly acidic soils such as peat (Gosler, 1990). The single-seeded hawthorn is particularly competitive on deep and

well aerated, stony soils because it develops a remarkably compact, vigorous, deep root system. Hence short drought periods are tolerated, whereas high groundwater levels and soil compaction are avoided (Schuck). The optimum growth conditions provide light-dry to medium-humid soils. *Crataegus monogyna* mostly occupies nitrogen-poor and moderately nitrogen-rich locations (Byatt, 1975; Phipps et al., 2003; Ellenberg and Leuschner, 2010).

2.6.2. Sowing, planting, and subsequent care of *Crataegus monogyna*

Generally, it is recommended to sow 250 seeds/m^2. The germinated plants reach approximately a height of 40 cm in the first year (Schuck, 2014).
Depending on the purpose of planting, as plantation or hedge, the distances within the rows can variate (Sonnenschein and Plescher, 2005). The transplanting is most successful with a leafless plant between late fall and early spring without temperatures below zero. The root system should not be exposed to frost (Phipps et al., 2003). In general, the juvenile plants of *Crataegus* are planted in rows with a distance of 0,5m-1m. Smaller distances are usually used to establish dense-growing hedges or windbreakers quickly (Schramayr, 2016d). On a windy location, it is recommended to stake the small plant until the root system has extended. After planting out in the first two summers, it is advisable to water the young plants during dry periods (Phipps et al., 2003). Three to four years after planting, the grown juvenile plants start at the earliest to develop flowers (Sonnenschein and Plescher, 2005; Peschel et al., 2008). Approximately after five years, the plant bears fully fruitful flowers hence produces real yields (Sonnenschein and Plescher, 2005). *Crataegus monogyna* is a dominant shrub species hence prefers open habitats (Gosler, 1990) and does establish well in sunny areas but not under shade conditions (Christensen, 1992b; Phipps et al., 2003). Contrary to other closely related species, *Crataegus monogyna* copes better with dry periods, yet hot, dry summers without groundwater sources might be problematic for the species (Phipps et al., 2003).

2.6.3. Pruning treatments

Currently, there is no scientific data available for a generally valid instruction for pruning *Crataegus monogyna* concerning leaf flower drug or fruit production for pharmaceutical, food- or cosmetical purpose. Yet, in 1996 scientists in Thüringen, Germany, established a pilot plant to cultivate hawthorn to test production-related factors as formative pruning treatments of comparable pomology cultures on hawthorn (Sonnenschein and Plescher, 2005). Nevertheless, the deliberated purpose of pruning determines the time, intensity and implementation, whether manual or mechanical, of distinguishing pruning treatments (Sparks and Martin, 1999; Croxton and Sparks, 2002; Sonnenschein and Plescher, 2005). Date and intensity of the pruning influence, the yield of fruits (Croxton and Sparks, 2002), the biomass production, and the leaf-flower ratio of *Crataegus* (Peschel et al., 2008). In general, thinning out pruning treatment in too dense, aging fruit trees in the old age phase is particularly important because it stimulates the tree to grow again, which correlates with a higher yield, better fruit quality, and longer life (Spornberger et al., 2013).

Formative pruning

As mentioned before, the scientist Sonnenschein and Plescher investigated various formative prunes, pictured in Image x, and other agronomic valuable treatments for C*rataegus ssp* in a pilot plantation. The formative pruning of the plants once per year was considered to be the most important nursing management. Especially removing suckers to establish a single trunk is recommended for commercial cultivation of hawthorn (Sonnenschein and Plescher, 2005).

Manual pruning treatment

Hawthorn is a commonly pruned manual (Sonnenschein and Plescher, 2005). For hawthorn with ornamental purpose as a hedge, the best time for pruning is generally during dormancy in late winter to early spring before the bud-burst inserts (Phipps et al., 2003). Adult plants can be pruned strongly back once to twice per year (Schramayr, 2016a). It is essential to prevent the establishment of any suckers on grafted individuals. Otherwise, they are capable of taking over the scion (Phipps et al., 2003).

However, other pruning treatments for the cultivation of hawthorn with pharmaceuticals, food- or cosmetic purpose might be more suitable to promote quantity or quality of fruit, leaf, and flower drugs. In particular, with a view on the flower and the consequent fruit production, the pruning treatment has a significant impact on the fruits (Sparks and Martin, 1999; Croxton and Sparks, 2002). Regularly trimmed hedges result in rarely producing flowers and fruits (Phipps et al., 2003). Due to the fact that flowering and subsequent fruit-set occur on 2-year-old wood (Croxton and Sparks, 2002). However, trimming branches stimulates vegetative growth and delivers strong twigs (Peschel et al., 2008).

Mechanical pruning treatment

Since the mechanical harvesting of woody plants, which is necessary for economic cultivation, causes problems, special machines are required. These machines are engineered for the respective species of trees and shrubs and are only profitable if they are cultivated to an appropriate commercial extent. In 2004 the first time a contour cutting device was employed in *Crataegus* plantation. Hence the pruning by hand was reduced to a minimum (Sonnenschein and Plescher, 2005).

2.6.4. Harvest

Currently, there is no scientific data available for the procedure, the quantity, and the expenses for the harvest of raw material (leaves, flowers, and berries) from wild collections. As maintained by a study of 2019, the harvest date determines the phytochemical composition of the various hawthorn organs significantly (Pavlovic et al., 2019).

Leaf and flower drug harvest

Since the highest total flavonoid content in leaves and flowers is archived shortly before *Crataegus spp*. is full in bloom, the harvest date to yield leaf and flower material is mostly around full bloom (Sonnenschein and Plescher, 2005). In the field, it is recommended to gain the highest total flavonoid content of the leaf flower drug raw material to harvest before 50% of the flowers are open. Alternatively, pure leaves could be picked in late summer (Peschel et al., 2008).

Fruit harvest

The harvest of fruits should be picked by hand and conducted at the time of full ripeness. Different cultivars show different maturity dates of the fruits (Sonnenschein and Plescher, 2005). The harvest date influences the phytochemical composition of the *Crataegus* fruits significantly *(Pavlovic et al., 2019).* According to estimations, the manual harvest of hawthorn fruits imposes high costs for the cultivation of hawthorn (Sonnenschein and Plescher, 2005).

2.6.5. Pathology of *Crataegus ssp.*

In comparison to other woody plants, *Crataegus monogyna* is subjected to several diseases (Schuck, 2014). In the following, pathogens with the most impact on agronomic aspects will be introduced. In Chapter 2.7. the importance of *Crataegus monogyna* as a host plant for various crucial pathogens of diverse fruit and horticulture cultivars in Europe respectively, Austria, will be discussed.

2.6.5.1. Bacteria

Erwinia amylovora is probably one of the most important diseases for *Crataegus monogyna*. An infection with *Erwinia amylovora* causes fire blight on flowers, leaves, and shoots of numerous woody plants from the family of *Rosaceae* (Schuck, 2014). It can be recognized by the black discoloration of the leaves and shoots, the hook-shaped downward curvature of young shoots and the bark necrosis, from which later sometimes a milky, initially white and even later a yellow-brown bacterial slime leaks (Schuck, 2014; Pfiffinger G. and Peham J., 2016; AGES, 2020b). *Crataegus monogyna* is considered highly susceptible and was often presumed as the starting point for the disease's epidemic spread in 1970’s (Zeller, 1979; Paulin et al., 1993). *Crataegus* is besides *Malus* and *Pyrus* also classified as a main host. This disease is hazardous for trees and shrubs of the family *Rosaceae* hence is subject to registration. According to the council directive, 2009/29/EC of 8 May 2000 on protective measures against the introduction into and spread within the community of harmful organisms to plants or plant products (OJ No 169, 10.07.2000, p. 1) is the fire blight agent *Erwinia amylovora* is a quarantine pest hence the introduction and spread into areas of the EU is prohibited. In the event of the occurrence of the

fire blight disease, competent authorities are allowed to limit locally and temporally cultivations of suspicious cultivars. In addition, in the distance located, highly susceptible species can be arranged from the endangered areas (AGES, 2020b). A general ban on affected cultivars exists, however, not (Schuck, 2014). Since hawthorn-free areas present the same intense infestation density as areas with hawthorn (Schramayr, 2016c).

Within short distances, the bacterium is transmitted to other host plants by humans, such as cutting tools, shoes, clothing, car tires, hands, and various pollinators. The bacteria can spread very quickly within a crop under suitable conditions, as warm and humid weather with temperatures above 18 °C and more than 70 percent humidity, and accelerated by rain, wind, and hail. Over longer distances, fire blight is spread by contaminated plant material or contaminated objects. Migratory birds can also carry the pathogen away (AGES, 2020b).

2.6.5.2. Mycosis

Gymnosporangium clavariaeforme is a rust fungus species, which develops their spores on *Crataegus* in Europe and causes the hawthorn rust (see Fig.7) on leaves of Crataegus (Schuck, 2014). This rust fungus species undergoes a constant host change between genera of Cupressaceae and genera of *Rosacea*, as *Crataegus, Malus, Pyrus,* and *Sorbus.* During the winter, the causing agent hibernates mainly on several species of *Juniperus* and *Cupressus*. In spring, the fungus releases its spores, spread by wind or insects, to infect species of *Crataegus* (Dervis et al., 2010). According to a study, spring rain promotes spore spread and hence the resulting infection of the rust disease on hawthorn (Huitao, 2007). After a successful establishment on the secondary host, orange-colored spots appear on infested leaves. In these mycelium spots, the spores of the fungus develop. During summer, proliferations develop branches, twig and leaves (Pfiffinger G. and Peham J., 2016). The infected branches can stall (Schuck, 2014).

Figure 7 Rust fungus on Crataegus monogyna subject 26.05.2019

Podosphaera clandestina is the causative agent of hawthorn powdery mildew and a very harmful pathogen, in particular on the soft shoots of clipped hedges and young nursery stock. The infested young shoots are covered with the typical white mycelium of *Podosphaera clandestina* and lead to shooting curvature (Schuck, 2014). Germination of the spores occurs with temperatures ranging from 5 to 25°C along, and relative humidifies as low as 50%. The propagation increases with increasing relative humidity and an optimum temperature of 18±20°C. Leaves gradually show higher resistance to infections along with the increasing age (Xu and Robinson, 2000).

Myriellina cydoniae, the diasporic fungus, was detected for the first time on *Crataegus monogyna* in Germany in 2001. Usually, the fungus parasitizes on the species *Cydonia oblonga and* causes leaf scorch, but occasionally it also infests *C. monogyna***.** The fungus creates a pattern of damage on the leaves, which are recognizable as dark brown spots up to 6 mm in size and often sharply bordered by the leaf veins. Humidity-promoting conditions such as in densely growing hedges with a low air-circulation inside, support the appearance of the fungus. Hence, this disease can reach epidemic proportions in Crataegus hedges and can lead to branches' death in case of repeated infestation (Kehr and Butin, 2003).

Neonectria ditissima**,** the appel cranker disease results from an infestation with the fungal pathogen *Neonectria ditissima.* The fungus presents a broad host range that includes apple *(Malus)* and pear *(Pyrus)* as well as numerous broad-leaved trees such as *Alnus, Betula, Crataegus, Fagus, Fraxinus, Ilex, Juglans, Populus, Quercus, Ribes, Salix, Tilia,* and *Ulmus (Weber, 2014).* An infection presents typical symptoms as the orange to brown coloring and flakiness and fragility of the bark (Pfiffinger G. and Peham J., 2016).

2.6.5.3. Pest

Besides fungal and bacterial pathogens, also a large number of animal pests, especially mites, lice, and butterfly larvae, attack the hawthorn (Pilaske, 1999; Schuck, 2014; Pfiffinger G. and Peham J., 2016). Only a selection of the most common pests shall be addressed here.

***Dasysneura crataegi*,** the hawthorn leaf gall mite, which causes roundly, convex shaped galls, is a commonly occurring pest on Crataegus monogyna (Pfiffinger G. and Peham J., 2016).

Dysaphis crataegi, the hawthorn leaf louse causes, especially during flowering, A conspicuous damage symptom at hawthorn (Sonnenschein and Plescher, 2005). Aphid species of the genus *Dysaphis* prefer hawthorn as winter host. The leaves are clearly red in color and usually also blistered, sometimes even folded. Mostly on the underside of the leaves, the 1.5-2.5 mm small and dark-colored aphids establish. During summer, the winged stages of aphids alter the host and hence generally leave *Crataegus* plants. The preferred summer hosts are primarily species of *Apiaceae,* especially carrot, parsley, celery. In autumn, the winged aphid stages migrate back to the hawthorn with the aim to lay cold-resistant winter eggs in cracks of the trunk (Zeĭnalov and Kanygina, 1988; Massimino Cocuzza and Barbagallo, 2017).

Cacopsylla melanoneura, the hawthorn leaf sucker is a psyllid and sucks on the leaf and flower clusters, causing only slowly unfolding the clusters in spring. The infected parts color brownish and can also die off in case of a massive infestation. In addition, the adult leaf suckers and especially the larvae produce sugary, sticky excrements, so-called honeydew on the leaves and shoots, which serve as a breeding ground for sooty mildew fungi. This results in blackish spots as secondary damage (Pfiffinger G. and Peham J., 2016; AGES, 2020a).

***Prociphilus pini*,** the hawthorn-pine root aphid, also can induce reddish coloring of the leaves. The also called hawthorn blood louse sucks in twigs and causes cancerous growths (Schuck, 2014).

In addition, the hawthorn serves numerous butterfly caterpillars as a food plant, for instance, the *Trichiura crataegi L*. (hawthorn moth), *Cilix glaucata* (silver moth)and *Orgyia recens* (blackthorn moth) (Schuck, 2014).

2.6.5.4. Virus

Apple chlorotic leaf spot virus (ACLSV), is one of the most important harmful viruses for fruit trees, especially *Malus* and *Pyrus*, are affected. However, a transfer of ACLSV via seed material or aphids was not detected (Sweet, 1980).

Since the ACLSV commonly develops typical symptoms in pome fruit trees, such as *Malus, Pyrus*, and *Crataegus*, only a long time after infection, it is complicated to detect the virus in the early stages of infection. The virus accumulates with the development of trees or shrubs and reduces plants' growth, decreases the yield and quality, and hence causes profound losses in fruit production (Guo et al., 2018).

2.6.6. Propagation of *Crataegus monogyna*

Hawthorn's propagation is carried out by seed, by cuttings (Morgenson, 1998; Phipps et al., 2003), and by grafting (Peschel et al., 2008).

Seed

Since *Crataegus monogyna* belongs to the heterogenous subfamily *Spiraeoideae* and the subtribe *Pyrinae,* which is characterized by various reproductive modes (Evans and Campbell, 2002; Dickinson et al., 2007; Potter et al., 2007), divers propagation forms of *Crataegus monogyna* have been reported (Chacoff et al., 2008). This reproductive biology can result in problems for the propagation of a specific cultivar via seeds, like infertility and lack of true breeding. In general, the pyrenes are planted in shallow pan or flat with a thin mesh to avoid interferences. The planted pyrenes are stored at ambient temperature for germination (Phipps et al., 2003). Especially the double winter seed dormancy of northern species might be a critical factor for propagation via seeds (Phipps et al., 2003). The authors Young and Young reported that the dormancy might be broken by a treatment of the dry pyrene with concentrated sulfuric acid for up to 4 hours (Young and Young, 1992). However, the success of this treatment was not confirmed in a study of 1998 in North Dakoda, North America. The effect of stratification and acid treatment on the germination of three different Hawthorn species was investigated. The results showed that the ratio of warm and cold periods has a more substantial impact on the cultivars as the acid treatment, which was not beneficial at all for dormancy breaking of two cultivars. Overall the author concluded that an acid treatment might be an useful to reduce the warm periods for specific cultivars (Morgenson, 1998). Nevertheless, this treatment might be attractive aid in breeding for *Crataegus* species in northern temperate zones. After the germination, when the young

seedlings developed their first three leaves, the plants are potted into larger pots. The subsequent care is similar to other woody plants (Phipps et al., 2003).

Cuttings

To induce rooting of cuttings of hawthorn is generally very difficult (Morgenson, 1998) and necessitates specific knowledge about the cultivar (Gosler et al., 1994; Phipps et al., 2003). According to reports of successful propagation via cuttings of mayhaw's a hawthorn species, it is advisable to use extremely young, still growing, greenwood cuttings. These young cuttings should be treated with rooting hormones, and the sensitive growing tips should be removed. Further, these cuttings need to be kept under mizzle and also be protected from direct solar radiation until they developed an adequate root system (Phipps et al., 2003).

Grafting

Since the production of cuttings is complicated, grafting is a simple and effective method to produce genetically identical individuals hence clones. Grafting of *Crataegus* cultivars is the most common way of reproduction and hence the preferred commercial technique for propagation. In general, it is almost any hawthorn species compatible (Phipps et al., 2003). However, mostly one-year-old seedling rootstocks of *Crataegus monogyna* are usually used in nurseries for ornamental or other *Crataegus* grafts. Young, strong twigs of *Crataegus* cultivars with the desired strain commonly are used as scions and are grafted on the seedling rootstock (Peschel et al., 2008). The most appropriate time to grow enough understock from seed and graft dormant scions is in spring about bud-bursts or in fairly late summer. In open land, it is recommended to conduct both tasks during overcast and mild weather. Contrary to the outside is the leaf outdone in a lath house, in which the solar radiation is reduced, onto actively growing stocks or grafting onto a dormant stock, also called bench-grafting, in the winter at 2-5°C (Phipps et al., 2003).

New propagation approaches

In 2009 scientists investigated the potential of micropropagation of *Crataegus monogyna* with multinodal segments in an *in vitro* culture system. With a survival rate of 80% percent of the *in vitro* rooted plantlets after the transplantation to the soil, they established an *in vitro* protocol (Iapichino and Airò, 2009).

2.6.7. Breeding of *Crataegus monogyna*

Besides the natural hybridization of wild cultivars of *Crataegus monogyna* over the last decades, new cultivars were bred for specific purposes (Phipps et al., 2003; Schramayr, 2016a). The primary breeding purposes are biomass production, edible quality of the fruit, growth habit, thorniness, drought, and disease resistance (Phipps et al., 2003).

Generally, the breeding of *Crataegus monogyna* cultivars is conducted with controlled pollination. To avoid self-pollination, the young flowers are emasculated. Afterward, hand carefully pollinated with xenogamous pollen of the desired parent and bagged to prevent the contact of external pollen (Phipps et al., 2003). However, they are also self-pollination, a useful tool to get inbred lines. The flowers are bagged and hand-pollinated with geitonogamous pollen (Chacoff et al., 2008).

The breeding objectives for *Crataegus monogyna* are mostly determined by the wanted end product, such as leaf flower drugs for pharmaceutical purposes, edible fruits for human consumption, or as an ornamental plant (Phipps et al., 2003). For fruit production purposes, several breeding objectives are essential to ensure an attractive edible quality of the fruit. One of the preferable traits is a higher fruit weight along with higher flesh to seed ratio (Ercisli, 2004). Only in the genotype *Crataegus pinnatifida* the nutlets are very strong reduced hence possess a desirable flesh seed ratio. Also, a uniform size, shape, and color are desirable for fruit production (Phipps et al., 2003). The various phytochemical compositions of different cultivars are also important traits for breeding programs (Liu et al., 2011; Gundogdu et al., 2014).

Besides fruit quality and phytochemical properties, agronomic traits are also crucial factors for breeding programs. The density of flowers, fruits, and leaves of strongly and healthy growing trees is an essential agronomic criterion. Especially the high biomass production and the foliage quality are essential for the leaf flower drug production (Peschel et al., 2008) as well as for ornamental purposes like windbreakers (Phipps et al., 2003; Ercisli, 2004). Another crucial agronomic aspect in breeding programs is the resistance to drought and disease. In particular, the resistance to fire blight and other pathogens, as rust fungal, is the most crucial breeding traits (Zeller, 1979; H. Scherm and A. T. Savelle, 2003; Phipps et al., 2003). Contrary to the leaf flower drug production, other agronomic characteristics

concerning the harvest are relevant for the fruit production, such as growth habit and thorniness (Sonnenschein and Plescher, 2005). Since spring frost can be a limiting factor in northern climate zones, breeding approaches to develop late-blooming cultivars hence minimize the potential of spring freeze to compromise flowers.

Therefore, late-blooming types might be potential donor parents in breeding programs to devise new late-blooming cultivars for colder climatic conditions (Khadivi-Khub et al., 2015). However, researches about genetics and breeding of *Crataegus* species are still very limited. Since in 2018, the successful development of SSR markers of hawthorn was published, no markers for breeding systems of *Crataegus* existed before. These polymorphic SSR markers are considered to be a solid tool for the detection of relationships of *Crataegus* and their genotypes genetic variations. The gained knowledge of the basic molecular analysis of the SSR marker of various hawthorn genotypes will support to maintain *Crataegus* germplasm collections and therefore can be applied in new breeding programs by assisting with the choice of parents for future cultivar breeding programs (Güney et al., 2018)

To sum up, the wanted Crataegus end-product and correspondently applied sector, such as pharmaceuticals, ornamental, food- or cosmetical purposes, determine the breeding program's objectives.

2.6.8. Introduction of some cultivars of *Crataegus monogyna*

In garden and landscape gardening, numerous forms are differentiated, and cultivars are categorized by morphological characteristics in four major groups (Jablonski, 2020).

Flower color: Pink ‘Rosea’; Red ‘Punicea’;

Flowering twice: Mild winter December and May ‘Biflora.’

Fruit color: Yellow ‘Aurea’ (Phipps et al., 2003) ‘Goldstein’ (Jablonski, 2020)

Growth and twig characteristics: ‘Stricta’ branches erect; Stems twisted ‘Flexuosa’ (Phipps et al., 2003); ‘Compacta' grafted on the high trunk (Jablonski, 2020)

A study about the resistance to fire blight cultivar ‘Compacta' also showed a lower susceptibility than other cultivars (van Teylingen, 2002). However, it is to mention that due to control measures against fire blight, several cultivars got lost during the last decades (Jablonski, 2020), and also, the natural gene pool of hawthorn is threatened by genetic erosion. According to several observations of wild populations in Iran have been during the last decades, native and wild hawthorn plants disappearing rapidly (Khadivi-Khub et al., 2015).

2.7. *Crataegus monogyna* as a host plant

Since *Crataegus's* exterminations were one of the most applied control strategies against fire blight in 1970, hawthorn still has a partial bad reputation along with pomology farmers (Jablonski, 2020). Even though in hawthorn, free areas show the same intense infestation density as areas with established hawthorn species (Schramayr, 2016c). However, in comparison to other woody plants is *Crataegus monogyna* subjected to several other diseases (Kehr and Butin, 2003) and serves as a host for some agricultural relevant pathogens and pests (Sweet, 1980; Tedeschi and Alma, 2004; Weber, 2014; Massimino Cocuzza and Barbagallo, 2017).

Besides *Malus* and *Pyrus*, hawthorn was regarded to be one of the most important secondary hosts for the bacterium *Erwinia amylovora* (Zeller, 1979; Paulin et al., 1993). Together with other cultivars, *Crataegus monogyna* is indexed as very susceptible to fire blight. Especially in Germany, France, and North America present *Crataegus monogyna* a high susceptibility, whereas, in Denmark, *Crataegus monogyna* has been reported as moderately resisting. Consequently, it is presumed that *Crataegus* monogyna present within the species variability of predisposition (Zeller, 1979; Paulin et al., 1993), which can be useful knowledge for breeding programs. However, a study about the level of susceptibility of three hawthorn species, *Crataegus monogyna, Crataegus laevigata*, and *Crataegus persimilis* to *Erwinia amylovora* in 2009 showed that theses hawthorn species, mostly planted as ornamental plants in gardens, are convenient hosts for the bacterium and therefore it should be considered to crop them in close distance to pome orchards (San et al., 2009). According to an investigation in the Netherlands, *Crataegus's* flowering

period is a determining factor for a potential infection. Flowering hawthorn individuals are to be seven times at a higher risk of getting infested than non-flowering hawthorns (Schouten and van Teylingen, 1990). However, other observations could not establish a significant relationship between occurring infestations of flowering *Crataegus* and fire blight incidence in neighboring orchards (van Teylingen, 2002).

Nevertheless, the Austrian Agency for Health and Food Safety recommends abdicating the planting of fire blight host plants as ornamental and wild plants in home gardens and public green areas in order to reduce the pathogen potential and thus the possibilities of spreading the bacteria on crops. (AGES, 2020b). However, there are cultivars of *Crataegus monogyna*, as *Crataegus monogyna 'Compacta,'* which show an intermediate resistance or respectively a moderate susceptibility against fire blight (van Teylingen, 2002). Since no complete resistance Rosacea cultivars exist is beside the application of diverse pesticides, monitoring, choosing more resistant cultivars, and the adequate application of hygiene measurements the most recommended strategy against fire blight (AGES, 2020b)

Contrary to fireblight, other pathogens occur more and less intense depending on the region. Especially in southern parts of Europe, the incidence of diverse pathogens might be indirectly connected to the abundance of *Crataegus ssp.*

Dysaphis crataegi, also called hawthorn-carrot aphid, migrates during the life cycles between hawthorns (Crataegus laevigata, Crataegus monogyna) as the spring host and several herbaceous plant species (mainly *Apiaceae*) as summer hosts. Hence *Crataegus* might influence the abundance of *Dysaphis crataegi* on several horticultural cultivars. In the last decades, the occurrence of *Dysaphis crataegi* on some horticultural cultivars has been reported in different parts of southern Europe. An infestation with *Dysaphis crataegi* can be critical for fruit development and actively damaging the fruit or vegetable(Massimino Cocuzza and Barbagallo, 2017). Another risk is the passively caused damage with the transmission of viruses via the sucking of aphids. In conformity with several studies, *Dysaphis crataegi* is proved to be a vector for some virus species, as the watermelon mosaic virus (Karl and Schmelzer, 1971) or the celery mosaic virus (Karl and Wolf, 1974).

The growing region has a major impact on the significance of hawthorn as a distributor or host of various pathogenes. In conformity with the recent studies, the

general contribution of *Crataegus species* in the spread of apple proliferation phytoplasma via the psyllid *Cacopsylla melanoneura* can be refuted. In northern Italy the psyllid has been reported as a vector for transmitting the apple proliferation phytoplasma, *Candidatus Phytoplasma mali* (Tedeschi and Alma, 2004). In general, *cacopsylla melanoneura* migrate between their “reproduction hosts” and “overwintering hosts.” The reproduction hosts, also the main host, are species of *Crataegus.* With few previous studies about *Candidatus Phytoplasma mali,* scientists investigated *Crataegus monogyna* as a reservoir for *Candidatus Phytoplasma mali,* and the German population's transfer potential *Cacopsylla melanoneura* hence evaluated the risk of *Cacopsylla melanoneura* for apple-growing areas in Germany and neighboring countries. The examination showed that no natural infection of hawthorn with *Candidatus Phytoplasma mali* was detected in German populations.

Additionally, it was not possible to infect *Crataegus monogyna* plants artificially. Hence it is presumed to *Crataegus monogyna* does not constitute a suitable habitat for the phytoplasma. During the observation, the psyllid reveals preferences to reproduce on hawthorn. In the event of lacking *Crataegus* as the main host plant for oviposition or after accidental establishing on apple, females are capable of adapting host-specific molecular cues and altering their preference to *Malus* species as a new suitable host plant for reproduction (Mayer et al., 2009).

In reference to the gained knowledge about various pathogens' lifecycles and distribution strategies, the danger of hawthorn as a host plant is with the right management measures reducible to a minimum.

2.8. Practical Value of *Crataegus monogyna*

Due to the high content of secondary metabolites of various *Crataegus* species worldwide, leaf flowers and berries are used for a long time as herbal drugs. Moreover, the vegetative structure of *Crataegus* species is responsible for the high practical value as a hedge plant in husbandry and plays a significant role in the conversation (Phipps et al., 2003). In the following, the main applications and the practical value of *Crataegus monogyna* will be addressed.

2.8.1 Agricultural value

Pomology

Crataegus monogyna is commonly used as a rootstock for grafting (Ercisli, 2004; Peschel et al., 2008; Khadivi-Khub et al., 2015). It is graft compatible with *Mespilus* L. (medlar), pear (*Pyrus* L.), and quince (*Cydonia* Mill.) and makes a hardier rootstock than quince. However, the hawthorn's thorny suckering habit might be problematic for large-scale cultivation (Phipps et al., 2003). *Crataegus* species are suitable for quinces and pear as frost-resistant rootstocks (Ercisli, 2004).

Husbandry

Due to the shrubby vegetative growth, hawthorn is traditionally used as a hedging plant. Phipps et al. described in his analysis the agricultural value of *Crataegus* hedges. Agricultural hedges were primarily generated by planting and natural invasions along fence lines. *Crataegus* hedges were primarily planted as windbreakers and to enclosure livestock (Phipps et al., 2003). Hedges, in general, present major beneficial agro-ecological values as the control of soil erosion. Hedgerows affect the water flow on a field hence influence the erosion of soil. Besides the water fluxes also the windbreaking effect controls the soil erosion on the field. Additionally, the windbreaker effect, which extends downwind to a considerable distance of 8-10 times of hedgerow height, modifies the microclimate significantly on the field (Burel and Baudry, 1995). Another beneficial impact is the reduction of evaporation on the field. Overall, hedgerows stable the crop yield (Phipps et al., 2003).

Further benefits are the value of hedges as habitat for wildlife, especially for beneficial organisms (Osborne, 1984; Phipps et al., 2003). However, the aspects of *Crataegus* in hedges will be further discussed in Chapter X. In some cases, hedges are valuable sources of firewood for farmers. For example, in France, woody plants and shrubs were commonly used to heat the house. According to estimations, it is possible to harvest three to eight tons of dry weight per 100 m of hedgerow every 9 years (Burel and Baudry, 1995). *Crataegus sp.* is predominantly due to the specific density of approximately 0,85g/cm^{3,} one of the hardest woods in the central European region (Peham et al., 2016a). Therefore it is mainly used for building tools, arrows, and walkingsticks (Schuck, 2014).

Currently, Crataegus species are common in hedges in the European area, mostly present in England, Ireland, and France. In Austria, Crataegus species contribute only 3% of species composition in hedges (Schramayr and Baumgartner, 2016), which might be caused by former pest control measurements against fire blight in the 1970s (Jablonski, 2020).

However, hawthorn possesses a passive value as a hedge for husbandry; it can also be used actively in agriculture, for example, as valuable food sources.
In former agricultural structures, hawthorn fruits were used as an alternative or additional food source in pig husbandry (Schuck, 2014). Since modern animal husbandry science less present leaf fodder shrub and trees, Crataegus' foliage is to be considered a valuable food source for cattle, goats, and sheep. Moreover, hawthorn extracts, in the form of crushed fruits, are also added as health-promoting supplements in several ped foods. Since hawthorn fruits have the perfect shape for European birds, there are also commonly added to bird foods (Grahofer and Scharmayr, 2016).

2.8.2. Conservation with the assistance of *Crataegus monogyna*

Whether in a naturally occurring population or planted in hedgerows, *Crataegus monogyna* presents many ecological benefits (Hille-Rohde, 1989; Phipps et al., 2003; Blanusa et al., 2019).
The hawthorn provides habitat and food for more than 300 species of leaf-eating insects (*phytophages*) and the caterpillars of more than 100 butterfly species (Hille-Rohde, 1989). Besides, *Crataegus* leaves constitute a suitable food source for many mammals, especially for fawns (Grahofer and Scharmayr, 2016).
Like all hawthorns, *C. monogyna* facilitates more than one group of pollinators, including primary hoverflies, bumblebees, honey-bees, and several beetle species as the longicorn (Hille-Rohde, 1989; Pfiffinger G. and Peham J., 2016). According to a study in England about the impact of native and non-native trees on the occurrence of insectivorous birds (*Paridae*) and the abundance of insects (*Hemiptera)*, the native *Crataegus monogyna* showed during the observations the greatest insect abundance (Helden et al., 2012).

As mentioned before, the fruits of hawthorn are perfectly shaped for European birds (Grahofer and Scharmayr, 2016) and are an attractive food source for several mammals and birds. As a reward, the spatial distribution of hawthorn seeds is warranted (Osborne, 1984; Pilaske, 1999; Phipps et al., 2003). In central Europe, fruits constitute an important energy source in hard winters for about 32 bird species. In particular are to be mentioned crows, pheasant, titmouse, chaffinch and brambling, waxwing, common redpoll, and several thrush types (Hille-Rohde, 1989). Further, Crataegus' branch system with the dense structure and thorns offers protection for several bird species (Osborne, 1984; Pfiffinger G. and Peham J., 2016; O'Sullivan et al., 2017). Especially in Lower Austria, the bird species red-backed shrike nest in *Crataegus* species (Pfiffinger G. and Peham J., 2016). Besides providing habitat for various species, Crataegus planted in hedges is also a valuable corridor for movement and species survival in agricultural landscapes (Burel and Baudry, 1995). To sum up, *Crataegus* in hedgerows represent an essential component in Agri-environmental landscapes and provide a wide range of ecosystem services such as habitats, food sources, and wildlife corridors for animals, acting as wind-breaks and preventing soil erosion (Brown et al., 2016).
However, the value for wildlife conservation is based on the conducted management treatments (Sparks and Martin, 1999; Croxton and Sparks, 2002; O'Sullivan et al., 2017; Blanusa et al., 2019). Especially, intense and frequently pruning affects the precious branch habitat for birds (O'Sullivan et al., 2017) and the flower production along with subsequent fruit set (Sparks and Martin, 1999; Croxton and Sparks, 2002; Blanusa et al., 2019).

2.8.3. Ornamental Value

Before the control measurements against fire blight in the 1970s, *Crataegus* species were traditionally planted for ornamental purposes in gardens (Schramayr, 2016c; Jablonski, 2020). Especially in hedgerows, *Crataegus monogyna* was preferred. Due to its dense growing branch system, attractive seasonal foliage colour, and a large number of flowers(Phipps et al., 2003), it was used as a visual cover, as noise protection, and to determine property lines (Blanusa et al., 2019).

2.9 Pharmaceutical properties, toxicology and quality standards for *Crataegus monogyna* as a herbal drug

Due to the in Chapter 2.4. introduced, secondary metabolites hawthorns are traditionally used as herbal drug in China, Europe, North (Phipps et al., 2003), and Central America (García-Mateos et al., 2013).

In Europe, especially extracts of *Crataegus monogyna* and *Crataegus laevigata* have been and still are commonly applied for hypertension and heart failure (Pilaske, 1999; Phipps et al., 2003; Rasmussen, 2011). Currently, 48 phytopharmaceuticals and 50 homeopathic products exist and hence show that hawthorn is one of the most important medicinal plants processed into pharmaceuticals in Germany (Fachagentur Nachwachsende Rohstoffe e.V., 2013a). On the European market there are also several commercial preparations available as tablets or liquids, such as Crategutt® and Oxacant® (Phipps et al., 2003). Official herbal monographs on *Crataegus ssp.* were published by the European Pharmacopeia, by the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency's (EMA) and by the german Commission E, a brains trust of the federal institute for drugs and medical devices (Kaul, 1998; WHO, 2004; European medicines Agency, 2016a). Drug monographs contain accumulated scientific knowledge to define quality standards for medicinal plants (Knöss et al., 2014).

The german Commission E replaced the old monograph of *Crataegus* and published four recent monographs of Crataegus spp. in 1994. Each monograph discusses the pharmaceutical properties and toxicology of certain used plant parts. The three monographs are *Crataegi flos*, the hawthorn flower, *Crataegi folium,* the leaves of hawthorn, *Crataegi folium cum flore*, the flower, and leaves of hawthorn, and *Crataegi fructus,* the fruits of hawthorn (Kommission E, 1994b, 1994a; Kaul, 1998). In addition, *Crataegus monogyna* as a medicinal plant is recorded by the World Health Organization (WHO) in the monographs on selected medicinal plants Vol.2 and in European Scientific Cooperative on Phytotherapy (ESCOP) (WHO, 2004; European medicines Agency, 2016a). The ESCOP and the WHO are no official libraries. Still, they are a big contribution to understanding the evaluation of herbal drugs and discussion at the international level (Knöss et al., 2014).

In 2014 the European pharmacopeia published just a monograph on *Crataegus spp. folium cum flore* (European medicines Agency, 2016b). Due to a lack of clinical trials and studies about pharmaceutical properties of *Crataegi frucutus*, the effectiveness in specific indications has not been proven, and the European pharmacopeia cannot promote a therapeutic application of this plant organ (Kaul, 1998). Based on this monograph, the HMPC published the latest European herbal monograph on *Crataegus spp*. *folium cum flore* in 2016. Flowering-bearing branches of diverse cultivars of *Crataegus* were investigated regarding contents, dosage, indication and pharmaceutical form (European medicines Agency, 2014, 2016b).

"Whole or cut, dried flower-bearing branches of Crataegus monogyna Jacq. (Lindm.), Crataegus laevigata (Poir.) DC. (syn. Crataegus oxyacanthoides Thuill.; Crataegus oxyacantha auct.) or their hybrids or, more rarely, other European Crataegus species including Crataegus pentagyna Waldst. et Kit. ex Willd., Crataegus nigra Waldst. et Kit. and Crataegus azarolus L.

It contains minimum 1.5% of total flavonoids, expressed as hyperoside (dried drug). (European medicines Agency, 2016b)"

The minimum content of procyanidin is not determined in the European pharmacopeia. However, in the literature, mostly a minimum content of 1%-4% is referred (Rahfeld, 2011).

Further also the used plant organs parts are along with the preparation types and the application area defined. Herbal teas consist of *Crataegi folium cum flore* and *Cartaegi fructus* (Melzer and Saller, 2005) and used as a cardiovascular tonic, tonic for the senile heart, and for the regulation of blood pressure and cardiovascular, nervous disorders. Fresh juice of *Cartaegi fructus* is recommended for patients with a mild form of coronary failure, senile heart, circulatory disorder, hypertension, and atherosclerosis. Generally, as cardiotonic, circulatory stimulant or care product for heart and circulation. A fluid extract can be produced from all organs to support the heart and circulation. Especially in old age preventive are fluid extracts recommended to treat empirically expected natural fluctuations in organs' function, treat exhaustion, and increase irritability. Powder of leaves and flowers are applied against nervous cardiac function disorders to control blood pressure, irritability, palpitations, and exhaustion. Dry extract of *Crataegus* is considered to strengthen the heart and keep it performance-capable. The liquid extract is utilized for cardiac

nervous disorders and beginning organic cardiac insufficiency, activation of circulation to regulate blood pressure. Tinctures are recommended as the invigoration of the myocardium and, therefore, to support a patient's aging heart (European medicines Agency, 2016a). Clinical studies prove its effectiveness when the heart's performance is declining. This is because hawthorn enhance the heart's ability to contract and reduces blood vessels' resistance. As a result, cardiac output and blood circulation increase, and the heart tolerates oxygen deficiency better (Kaul, 1998; Melzer and Saller, 2005). The monograph "Hawthorn leaves with flowers" of Commission E recommends a daily dose of 160 to 900 mg of an aqueous-alcoholic hawthorn extract with a defined content of flavonoids of 4 to 20 mg and of oligomeric procyanidins of 30 to 160 mg (European medicines Agency, 2016a). At the same time, clinical studies recommend 600 to 900 mg of extract per day. The treatment should be continued for at least 6 weeks (Rasmussen, 2011). The above-mentioned effect is also recognized for a combination of *Crataegus* leaves, flowers, and fruits (Kaul, 1998; Melzer and Saller, 2005). Based on previous research, Rodrigues et al., 2012 conducted an in vtiro research about the effect of extracts obtained from Crataegus monogyna flower buds and fruit parts on the growth inhibitory activity on the human tumor cell lines. The authors revealed an antiproliferative activity of *Crataegus monogyna* extracts and accentuated that the tested samples did not show toxicity for non-tumor cells (Rodrigues et al., 2012).

In this thesis, all three parts, flowers, leaves, and berries of *Crataegus monogyna,* will be examined with the view of their as TPC and TAC to explore commercial relevant phytochemical properties.

2.9.1. Trade and Origin of drug raw material

In 2011 raw material of hawthorn was one of the most demanded medicinal plants in Germany. According to the market analysis of the agency for renewable raw material, “FNR,” the demand for Crataegus' raw material was 960t. The market size for *Crataegus spp*. was 1.9 million euros and constituted 3% of the total demand for raw material in 2011. The market price fluctuates between 2.00-2.86 € per kg of hawthorn raw drug material. According to estimations, a steady demand for Hawthorn is expected until 2020 (Fachagentur Nachwachsende Rohstoffe, 2017).

As before mentioned, some Crataegus species are cultivated on a small scale for fruit production, for example, in North America (Payne and Krewer, 1990) and China (Dai et al., 2007; Liu et al., 2016), cultivation for the production of leaf and flower drug has not yet been established (Fachagentur Nachwachsende Rohstoffe e.V., 2013a). As before mentioned, there is currently no scientific data available for the procedure, the quantity, and the expenses for the harvest of raw material, such as leaves, flowers, and fruits from wild collections (Sonnenschein and Plescher, 2005). The most raw material of Crataegus used for the production of phytopharmaceuticals are almost exclusively obtained by wild collection in various Eastern European countries such as Albania, Poland, Romania, Bulgaria, and Hungary as well as in Russia (Kaul, 1998) and China (Fachagentur Nachwachsende Rohstoffe e.V., 2013a).

2.9.2. Further processing methods

The raw material of Hawthorn can be further processed in many ways (Kaul, 1998; Phipps et al., 2003), whereby the processing method should be chosen suitable for the wanted endproduct (Tanko et al., 2005). Depending on the wanted end product, Crataegus's fresh raw material will be directly or firstly dried and then further processed (Kaul, 1998). Fresh material, as leaves and flowers, is traditionally directly extracted as raw drug dry extract (Kaul, 1998), whereas fruits commonly squeezed to juice, used for direct consumption (Phipps et al., 2003), or fermented to gain hawthorn wine (Liu et al., 2016; Liu et al., 2018). Especially in China and Mexico, fresh and dried fruits are established as edible supplements in nutrition (Zhang et al., 2001; García-Mateos et al., 2013).

Besides utilizing fresh fruits for extracts (García-Mateos et al., 2013), various methods are applied to dry fruits of hawthorn (Aral and Beşe, 2016). Depending on the wanted end product, such as medicinal purposes, fruits are lyophilized (Zhang et al., 2001; Egea et al., 2010; Rodrigues et al., 2012; Mraihi et al., 2015) or dried at room temperature always protected from UV-radiation (Tadić et al., 2008; Liu et al., 2011). The drying method affects various physiological factors, such as color (Aral and Beşe, 2016) and the phytochemical profile of the fruits (Liu et al., 2016; Coklar et al., 2018). According to a study about TPC's alternation in hawthorn fruits under different drying procedures, freeze-drying represents the smallest TPC and

TAC loss, though it is the most expensive method (Coklar et al., 2018). Hence it is mostly applied in the pharmaceutical and biotechnological industries (García-Pérez et al., 2015). Even though the microwave treatment had a negative influence on the color of the fruit, the authors recommend with view on the health-promoting compounds for the less cost intense microwave drying instead of oven drying large scale processing (Coklar et al., 2018). Contrary to dried fruits for direct consumption, fermented hawthorn products, such as wine and hawthorn drinks, present a beneficial change in their phytochemical profile with drying pretreatments, such as heat and microwaves (Liu et al., 2016; Liu et al., 2018). However, convective dryers are commonly applied in the further processing industry due to the convenience and comparatively low cost (García-Pérez et al., 2015).

Contrary to the fruits, no scientific data about the optimum drying process of leave and flower material is available. However, recommendations for private persons exist for drying leaves and flowers in order to produce tea. Leaves and flowers should be dried at room temperature or in the oven at 60°C (Pilaske, 1999). In various studies, different drying methods were applied and described, such as freeze-drying (Kirakosyan et al., 2003; Hellenbrand et al., 2015; Lund et al., 2017), hot air drying at 60°C for 1-2 days (Pilaske, 1999; Demiray, 2007), or drying in humidity chambers at 30 °C and 30% humidity to constant weight (Shortle et al., 2014).

The most dried raw material is extracted and further processed to extracts, tinctures, fluids, or dry extracts in tablets or capsules (Kaul, 1998). Various studies exhibit that the applied method significantly influences the composition and amount of the extracted hawthorn compounds (Ngoc et al., 2019). To extract polyphenols are too basic extraction types applied, the classical solvent-based solid-liquid extraction method and the supercritical fluid extraction (SFE), which does not require any solvent (Shortle et al., 2014). The solid-liquid extraction is rested on the transfer of polyphenolic compounds from plant material into a solvent such as methanol, ethanol, or an aqueous alcohol mixture at a specific temperature level for a specific time period via various techniques (Kim and Lee, 2005). Since crushing the plant material maximize the extraction of TPC/TFC/OPC of hawthorn material, most preparations protocols prescribe crushed material as solid constitute (Ngoc et al., 2019). Several extraction modes such as maceration (Kirakosyan et al., 2004; Orhan et al., 2007; Bernatonienė et al., 2008; Froehlicher et al., 2009), infusion

(Badalica-Petrescu et al., 2014), soxhlet (Calişkan et al., 2012) microwaves (Martino et al., 2008) or ultrasonic (Coimbra et al., 2020) have been performed to isolate polyphenols from different plant organs of *Crataegus monogyna*. According to Martiono et al. 2008 the microwave and ultrasonic extraction protocols gain the highest output of the desirable phenolic compounds of *Crataegus monogyna.* The authors recommend for commercial production of *Crataegus* extracts, particularly the optimized microwave protocol combined with isocratic HPLC, to gain a high extraction efficiency and high reproducibility within a short screening period (Martino et al., 2008).

For a standardized application and production of extracts, the European Pharmacopoeia governed the minimum TFC in herbal preparation of hawthorn leaf and flower extracts, which is in the following defined:

"Hawthorn leaf and flower liquid extract, quantified definition:
- Quantified liquid extract produced from hawthorn leaf with flower. It contains 0.8-3% of flavonoids, expressed as hyperoside. The extract is produced from the herbal drug and ethanol (30-70% V/V) by an appropriate procedure.
- Dry extract produced from hawthorn leaf and flower. It contains for aqueous extracts minimum 2.5% of total flavonoids, expressed as hyperoside (dried extract) and for hydroalcoholic extracts minimum 6% of total flavonoids, expressed as hyperoside (dried extract). The extract is produced from the herbal drug by a suitable procedure using either water or a hydroalcoholic solvent at least equivalent in strength to ethanol (45% V/V) (European medicines Agency, 2016a).*"*

Even though the class of flavans, with the procyanidins and the oligoprocyianidins, is considered to be the most health-promoting substances (Kaul, 1998), no minimum content is determined in the monograph. Only the minimum flavonoid content, expressed as hyperoside, is determined as a value determining component for specific herbal preparations (Kaul, 1998; European medicines Agency, 2016a). However, products sold on the market contain standardized content of OPC's, as for example, well-studied *Crataegus* special extract WS 1442. This standardized aqueous-alcoholic (45% ethanol) *Crataegus ssp*. extract contains 18.75% oligomeric procyanidins (OPC) (Zorniak et al., 2017).

One reason for the insufficient definition for a minimum of specific compounds in the European Pharmacopoeia might be the lack of standardized protocols for quality analysis of *Crataegus* extracts (Veit and Wittig, 2005; Hellenbrand et al., 2015). Besides, hawthorn extracts many other botanical preparations often present

variable quality in the content of health-promoting compounds (Tanko et al., 2005). Especially liquid extract types need standardized protocols since the continuing polymerization of OPC leads to an increase of polymeric procyanidins PPC and a decrease of the OPC at the same time (Krawczyk and Petri, 1991). A comparison of content values, in particular for the procyanidins in hawthorn drugs and preparations, is only possible if the information is available on the analytics used. Therefore the dosage and batch conformity of preparations can only be assessed if the method used for quality analysis is known (Veit and Wittig, 2005). However, in 2015 scientists developed a protocol for the isolation identification and quantification of oligomeric and polymeric procyanidins from Crataegus spp. leave and flowers with an ICH-Q2 validated UHPLC method with fluorimetric detection. This analytical method can be used for large scale quantification of procyanidin clusters of *Crataegus* batches within 10 minutes (Hellenbrand et al., 2015).

Since the European Pharmacopoeia does not distinguish between the *Crataegus* species (Lund et al., 2017) and has not governed a minimum on the content of single relevant substances, it is important to provide further research and to develop protocols for uniform isolation and quantification of the health-promoting compounds with the aim to constitute adequate quality standards for specific treatments of with these herbal substances.

2.10. Potential application of *Crataegus monogyna*

Due to the vegetative and phytochemical profile, the species *Crataegus monogyna* presents a broad scope for further potential application with its versatile properties. For example, the hawthorn wood was used as part of a sensor jacket, which was developed for cosmonauts' outer space. Furthermore, the white flowers of Crataegus possess a qualitative high tinctorial ability to color material yellow (Peham et al., 2016b). Another example is the potential of raw material extracts of *Crataegus monogyna* for UV-protection. In 2013 scientists investigated the photoprotective UVA and UVB potential of three inflorescence extracts and their photostability in cosmetic emulsions. The results represent that the formulations, which comprise polyphenols, as phenolic acids and flavans, and feature a broad spectrum of UV protection and a high photostability and remarkable antioxidant nature, conform to official requirements for sunscreen products. Contrary to

classical sunscreens, which commonly consist of a combination of UVA and UVB chemical filters and other physical components, these broad sunscreen protective substances are based on renewable resources and biodegradable (Jarzycka et al., 2013). Especially with view on ecological aspects, new biodegradable substances extracted from herbal plant material might be a very attractive alternative for the cosmetic or industry.

3. Materials and Methods

This experimental study is composed of three main parts:

- Testing and evaluating the effect of pruning
- Laboratory examination with focus on TPC and TAC of different organs of *Crataegus monogyna*

3.1. Plant material

For a convincing trail, the examined plants had to fullfill specific requirements such as similar age, size, cultivar of plants, and grown under the same environmental conditions without human intervention. The research material of *Crataegus monogyna* hails from Lower Austria and comprehends three types of organs, leaves, flowers, and berries. In autumn 1999, juvenile plants of *Crataegus* were bed out in shifted rows along the incline and were similarly exposed to the sun. The individuals are no clones. Hence, they have not the same genotype. The perennials were not cultivated to trees, thus grown naturally as shrubs. All plants grew without the application of fertilizer or pest management. Furthermore, the crop has never been pruned.

3.2. Experimental framework conditions

3.2.1. Experimental plot

The trail field is located in Willendorf/Steinfeld, the area of Schneebergland (N47°47, 27.338 E16°4,399). This area comes under a moderate climate zone and is 585m above sea level (ZAMG, 2020). The experimental plantation is an open East-South-oriented incline with 30m^2 and 14 individuals. In accordance with eBod, the digital

soil map service in Austria, the soil type of the experimental plant is as humid chernozem categorized. The soil characteristics at this plant position are wet-dry conditions mixed with a neutral to a slightly basic pH-value of 6,6-7-2. This profound, humus-rich, chalky ground displays a low permeability of water; hence tempo of the drainage is low. In general, the value of the soil is as valuable agricultural land classified (Ebod, 2018).

3.2.2. Climatic conditions

The meteorological data for the experimental plot is received from the station Puchberg in the region Schneebergland, 13 kilometer away. Based on the recorded ZAMG data of the last 5 years, this area showed an average annual temperature of 9.2°C, annual precipitation of 1248mm, and a total sunshine duration of 1626h. During the vegetation period of 2019, an average annual temperature of 9.7°C was measured. In this valley location, the average annual temperature maximum was at 15,8°C and the minimum at 4.9 °C. The recorded amount of the annual precipitation was at 1000 mm. The time from May until September with over 90mm per month is usually the high-precipitation period of the year. However, in June, July, and August, the rainfall was under 90 mm/m^2 lower than the years before. In general, the annual percepcipitation with 1000 mm was lower in 2019 as typical for this region. The months with most perceptions of the year were registered in May with 164mm and January with 119mm. A total sunshine duration of 1709h was recorded for this region over the entire vegetations period, whereas June was with 247h the month with the most sunshine hours. (ZAMG, 2020).

3.3. Experimental Design

3.3.1. Pruning System

Aforementioned, the pilot plant was not managed during the entire growing period. The experimental plantation was neither irrigated, fertilized, nor pruned. Additionally, no pest management was conducted at the research site. To investigate the effects of pruning, the 14 test plants were divided into two groups, a pruned group and a control group without pruning. Each group contained seven individuals of *Crataegus monogyna*. The tested pruning treatment was chosen with

regards to previous studies and practical knowledge (Sparks and Martin, 1999; Croxton and Sparks, 2002; Spornberger et al., 2013). Moreover, the pruning treatment was modified to a mix of a soft cut for thinning out and a harvest cut for raw material, such as leaves and flowers, at once. Therefore, at time of full blossom mostly steep and dense competitor growing branches were cut out to gain raw material, increase light exposure and yet not to influence at once the yield of the fruits significantly within the vegetation period. Furthermore, all deadwood was cut out. Approximatly one third of every individual's biomass was cut out. The intention was to receive an impression if it is possible to use this pruning treatment as a farmer in order to gain raw material for sideline use and enhance the fruit yield, concerning crop management and quality, in one step.

3.3.2. Data collection

During the examined vegetation period from March 2019 to November 2019, the crop was continuously controlled for pest infestation and plant diseases. Infested parts of plants were documented, and samples for identification were taken. Further, the perimeter of the shrub trunks of both groups was measured. The collection of the plant material was carried out at two different times. The expanded BBCH- scale of Meier et al. for the description of the phenological growth stages of pome fruit aid to orientation for the date of sample taking (Meier et al., 1994).

Leaf and Flower

Right before full flowering (30th April 2019), at a BBCH of 65, which means at least 50% of flowers were open and the first petals started failling (Meier et al., 1994), the plants of the first group were pruned with the aim of harvesting raw material at the same time. Leaf and flower samples were harvested from the cuttings for destructive analyses. The leaves were sampled from the higher portions of the canopy (>2,5m) and the lower (<1m) of each plant. Of every pruned individual, the yield of the whole cut material was single scaled in kilograms. Following by weighting only leaves and only flowers in kg of every single plant in order to investigate the relation between vegetative parts and generative parts. Directly after the harvest, the selected samples were stored at -80°C in the laboratory of the Institue of Viticulture and

Pomology at the University Research Center Tulln (UFT) for defining the contained biochemical compounds.

Fruits

The fruit harvest was conducted at a time of full ripeness with a BBCH of 89 (Meier et al., 1994) for destructive and non-destructive analyses (7th October 2019). For ascertaining physical properties, 100 fruit of each plant were randomly picked by hand, and the weight, size, and color was directly recorded in the laboratory of the Division of Viticulture and Pomology of the University of Natural Resources and Life Sciences in Vienna. For determination of the secondary metabolites, the fruits were stored at -80°C directly after the harvest in the laboratory of the Institue of Viticulture and Pomology at the University Research Center Tulln (UFT).

3.4. Analytical procedures

The non- destructive examinations were conducted in the laboratory of the Division of Viticulture and Pomology of the University of Natural Resources and Life Sciences in Vienna. The destructive analyses for defining the total phenolic content were carried out in the laboratory of the Institute of Viticulture and Pomology at the University Research Center Tulln (UFT). The ascertainment of total antioxidative capacity was performed in the laboratories of the Department of Post-Harvest Technology of Horticultural Products at Mendel University in Brno.

3.4.1. Determination of physical fruit parameters

In the laboratory of the Institute of Viticulture and Pomology of the University of Natural Resources and Life Sciences in Vienna, various physical parameters of overall 1400 berries of 14 different *Crataegus monogyna* individuals were measured.

Weight of fruits

The weight in grams of each fruit was measured by an electronic balance, Denvert Instrument SI-603, to an accuracy of 0.05g.

Volume and fruit form ratio of fruits

The size was determined in height (h), length (l), and width (w) in mm with a digital vernier caliper. The height was the measured distance from the pedicel cavity to the fruit pistil. The values of width and length were ascertained as the horizontal distance in the right angle and the horizontal distance on the opposite side. Based on these values, the volume (V) of the fruits in cm^3 (Shahbazi and Rahmati, 2013; López-Ortega et al., 2016) and fruit form ratio (FFR) (Spornberger et al., 2014; Schüller et al., 2015) was calculated with the formulas:

$$V=\left(\frac{4\times\pi\times r^3}{3}\right)\times\frac{1}{1000} \qquad r=\frac{(h+l+w)}{6} \qquad FFR=\frac{h^2}{(l*w)}$$

The calculated fruit form ratio serves as a reference to describe the shape of a fruit.

FFR value: <1: flat spherical-shaped fruits

=1: spherical-shaped fruits

>1: obround-shaped fruits

Pedicel removal force

Further, the pedicel removal force, the force which is needed to detach the fruit from the pedicel, was recorded in kilogram-force (kfg) with a penetrometer AFG500N. This value serves as reference suitability if the fruits are suitable for a shakedown harvest technique.

Color of the fruits

The colorimetry of the fruit skin was performed with the aid of a Chromameter CR-400/410 and represented by the CIELab scale. These CIELab coordinates are expressed as lightness (L*), red (+a*)-green(-a*) and a yellow(+b*) -blue(-b*) ratio of chroma (Cab*). Resultant saturation (C*) and Hue value (H°) were defined (McGuire, 1992). Before the colorimetric analyses for each plant were performed, the calibration to a standard white reflective plate was conducted.

$$C^* = \sqrt{(a^{+2} + b^{*2})}$$

$$h° = \arctan\left(\frac{a^*}{b^*}\right)$$

3.4.2. Determination of biochemical compounds

3.4.2.1. Preparation for analyzing biochemical compounds

Freeze- drying of the samples

For the biochemical analyses, the samples of the different organs of each plant were crushed to powder by using a Retsch mixer mill while keeping the tissues frozen using liquid nitrogen. The ground frozen samples were freeze-dried for three days at -105 ° C in a Christ beta 2-4 LD plus LT lyophilizator. During processing, all frozen samples were cooled continuously with liquid nitrogen and protected from solar radiation.

Extraction of the samples

To extract the polyphenolics of *Crataegus* samples, the method of ultrasound-assisted aqueous methanol extraction of polyphenols was performed according to a protocol of Kim and Lee (Kim and Lee, 2005). The extraction was performed using 0.01g of freeze-dried sample powder into a 50% methanol-water (MeHO) solution. Plant powder solutions were sonicated for 30 minutes and centrifuged for 10 minutes at 13000 rpm at 15 ° C. During processing all freeze-dried samples were protected continuously from solar radiation.

3.4.2.2. Determination of total phenolic content (TPC) by the Folin–Ciocalteu colorimetry

Folin–Ciocalteu colorimetry is a commonly applied procedure to determine the total phenolic content (TPC) of a sample. This method is based on the chemical reduction of a reagent. The Folin–Ciocalteu colorimetry method, a calibration standard, and a buffer solution were created according to a modified version of the protocol of Waterhouse (2005).

For the calibration standards, a gallic acid calibration standard was prepared. 0.25 g gallic acid was dissolved in 5 ml ethanol and then with 50ml distilled water diluted too create a solution of 2500 mg/l. The standard solution was diluted with water to 0, 50, 100, 250, 500, 750, 1000 mg/l for the calibration standard curve. For the buffer solution, a sodium carbonate solution was produced. In 400 ml double distilled water, 100 g sodium carbonate (Na_2CO_3) was dissolved and heated. The

solution rested for 24 hours at room temperature. The sodium carbonate solution was filtered through a Whatman no. 1 filter paper and with replenished with 250 ml double distilled water.

Due to the small sample volumes, the total phenol contents were analyzed according to Waterhouse (2005) described microscale protocol for the Folin–Ciocalteu colorimetric method. The reaction was conducted in a Eppendorf Tub, where 20 µl of the MeHO-sample solution and one blank, which contained distilled water, were mixed with 1.58 ml distilled H_2O. To the mixed solutions 100 µl Folin-Ciocalteau reagent were added and mixed again. After 5 minutes of incubation 300 µl of the sodium carbonate buffer solution was added and mixed again. After 2 hours incubation time at room temperature, the absorbance of the sample solutions was measured by a spectrophotometer at 765nm. The procedure was repeated two times. The results were expressed as milligrams of gallic acid equivalent (GAE) per 100 gramm of dried sample weight (Waterhouse, 2005).

3.4.2.3. Determination of total antioxidant capacity (TAC) by ferric reducing antioxidant power (FRAP) method

Freeze-dried leaves and flower samples of *Crataegus monogyna* were extracted by 80% ethanol for five days with permanent shaking at laboratory temperature. The extraction ratio was around 1:10 (dry plant material: extracting agent). After extraction, supernatants were separated from sediments by centrifugation for 5 min at 3.500 rpm. Diluted supernatants were measured on total antioxidant capacity (TAC) by ferric reducing antioxidant power (FRAP) method with Trolox as standard. TAC values were calculated as mg of Trolox per gram dry weight sample (mg/g DW) (Soural et al., 2019).

3.5. Statistical Analysis

The collected data were evaluated with the IBM statistical program SPSS Statistics 26.

The variables were tested to a normal distribution with the Kolmogorov–Smirnov–Test *($p<0,001$).*

The average measured values were calculated according to the treatment or organ and expressed as mean value ± standard deviation. To test differences between the not normally distributed variables for significance, nonparametric tests, as the Mann-Whitney-U-Test and the Kruskal-Wallis-ANOVA, were performed. The statistical significance was set at the 5% level. Further, the Spearman's Rho correlation coefficients were also determined to investigate the relationship between total phenolics and antioxidant capacity as well as fruit parameters of fruits *(p*< 0.001).

4. Results

In order to answer the research questions of this study, we performed several measurements. For ascertaining the pruning effect on the subjects in the first year, the vegetative growth, the yields of leaf and flower drugs were documented. Further, the fruits' physical and phytochemical properties were measured to investigate the impact of pruning. Demonstrating the differences in the content of total phenolics and total antioxidative capacity between leaves, flowers, and fruits, all organs were examined for the phytochemical characteristics. The examination of light exposed leaves and shaded leaves clarifies whether external factors, like exposure, might cause a higher content of phenolic compounds and a higher antioxidative capacity within the organ type. All measured TPC and TAC values were set in a relationship to show which organs contain correlations between the content of total phenolics and the total antioxidative capacity. The data should indicate a distinction in the quality and quantity of secondary metabolites of *Crataegus* plant material selected in Lower Austria and *Crataegus* plant material from other countries of origin. Thus, to answer whether Lower Austria represents a good cultivation site for *Crataegus* as a niche crop or not. In the following, all results of this study are set in statistical ratio and represented.

4.1. The average yield of leaf and flower drug

The weight of cut brunches of all pruned trees was 79 kg, 25.47 kg was leaf (18.24 kg), and flower (7.23 kg) raw material, which results in a 2.5: 1 ratio (see Table 3.).

Table 3 Weight of pruned branches and yield of leaf- and flower material 30.04.2019

Weight of pruned branches and yield of leaf- and flower material

Pruned Individual	Leave mass [kg]	Flower mass [kg]	Total mass [kg]
P1	1,2	0,9	5,3
P2	2,9	0,8	13,4
P3	4,9	2,3	22,2
P4	0,84	0,23	3,5
P5	6,2	2,3	16,9
P6	0,7	0,2	5,5
P7	1,5	0,5	12,2
Total yield [kg]	18,24	7,23	79
Total yield LF [kg]			25,47

Table 4 Growth of new shoots in 14.08.2019

	Total length growth [cm]	Total number of new shoots
1P	24,3	3
2N	n.s.	n.s.
3P	90,3	6
4N	17,80	7
5P	153,8	11
6N	n.s.	n.s.
7P	308,4	28
8N	124,7	7
9P	245,05	14
10N	98,4	3
11P	20,3	4
12N	n.s.	n.s.
13P	n.s.	n.s.
14N	n.s.	n.s.
Total length growth [cm]	1083,05	
Total number of new shoots	83	

Table 5 Growth of new shoots for both groups in 14.08.2019

	PrunedGroup	ControlGroup
Sum of length [cm]	817,85	240,90
Average length [cm]	116,83	34,41
Number of new shoots	66	17
Ratio of new shoots	79,51%	20,48%

Figure 8 Picture of Crataegus plant 7 and 8

4.2. Vegetative growth of the pruned and not pruned group

The wild-grown individuals (numbers 7, 9, 10, and 15) in the center of the plantation showed remarkable less vegetative growth in the lower part (< approx.. 1.80m) as in the upper part of plants (see Fig. 9.).

In August 2019, four months after the pruning, the trees 1, 3, 4, 5, 7, 8, 9, 10, and 11 developed overall 83 new shoots in the lower part of the plant (< approx. 1.80m) with a complete length of 1083.05 cm. The sum length of 66 shoots was 817.85cm for the pruned group and 240.9cm for the 17 shoots of the control group. Especially the pruned individuals 7 and 9 exhibit several new shoots (see Fig. 10 and Fig. 11). Plant number 7 developed twenty-eight new shoots with a complete length of 3084 cm. On plant number 9, fourteen new shoots were counted. However, not all of the seven subjects of the pruned group feature new shoots below hence the average length of 116.83cm per group is the more representative value. The unpruned plants had an average length growth of 34.41cm and showed less new shoots: seven for plant number 8 and three for plant 10 in the lower part. To sum up, the pruned group provided 79,51% of new shoots and 75.51% of the total length growth, whereas the unpruned group contributes 20.48% of the shoot number and 22.24% to the growth development (see Table 4 and Table 5.).

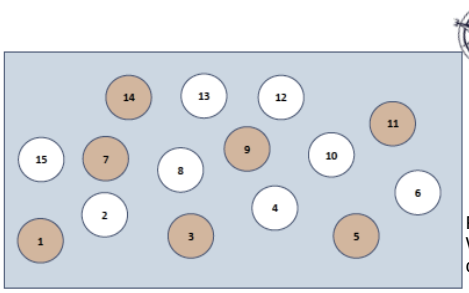

Figure 9 Arrangement of the investigated plants. White-colored individuals unpruned group and orange-colored pruned group

Figure 10 New developed shoots of pruned plant number 7 (August 2019)

Figure 11 New developed shoots of pruned plant number 9 (August 2019)

4.3. Comparison of fruit parameters and phytochemical properties of the fruits of the pruned and not pruned group

The statistical analysis showed that besides the length and width of fruits, the differences concerning the other fruit parameters and phytochemical properties between pruned and not pruned groups are not significant. The average means of the fruit parameter trends to result in a slight difference in the tested features among both groups (Tab. 6, 7, 8).

Table 6 The physical features and measured colour properites of the investigated Crataegus monogyna fruits. Data are expressed as mean value ± standard deviation ($p<0.05;n=12$).

Feature	GROUP		CONTROL GROUP		Significance (p-value)
	Pruned		Not Pruned		
Geometrical features	$\bar{x} \pm \sigma$		$\bar{x} \pm \sigma$		
Height (mm)	10.5	±0.08	10.2	±0.07	0.589
Length (mm)	9,82	±1,0	8,73	±0,05	0.041*
Width (mm)	9,67	±1,05	8,47	±0,06	0.041*
Fruit Form Index (FFI)	1,36	±0,49	1,48	±0,25	0.31
Volume (cm3)	0,54	±0,14	0,41	±0,062	0.18
Weight (g)	0,66	±0,15	0,47	±0,08	0.065
Tensil force (kgf)	0,31	±0,07	0,24	±0,06	0.093
Colour Parameters					
Lightness (L*)	37,88	±13,66	29,66	±0,98	0.093
Red-green ratio (a*)	36,38	±5,17	31,646	±2,16	0.065
Yellow-blue ratio (b*)	20,16	±9,86	13,37	±2,064	0.310
Saturation (C*)	43,49	±10,74	34,77	±2,52	0.065
Hue (H°)	24,43	±5,86	22,19	±6,89	0.39

Table 7 The phytochemical features of the investigated Crataegus monogyna fruits. Total phenolic content (mg eq. gallic acid/100 g DW) and total antioxidative capacity (mg/ 100 g DW) are expressed as mean value ± standard deviation ($p<0.05;n=12$).

Phytochemical features	GROUP	CONTROL GROUP	Significance (p-value)
	Pruned	Not Pruned	
	$\bar{x} \pm \sigma$	$\bar{x} \pm \sigma$	
Total phenolic content (mg GAE/100g DW)	2880.2 ±1902.7	1416.0 ±811.8	0.73
Total antioxidative capacity (g/100g DW)	1827.6 ±1407.4	1672.5 ±450.6	0.38

Yet, since all values are not normally distributed and the means of both groups show a high standard deviation, the mean ranks are used for a valid interpretation of the data (Table 8).

Table 8 Ranks of all values across pruning treatment

Ranks of all values across pruning treatment

	Treatment	N	Mean Rank		Treatment	N	Mean Rank
height	No	6	5,83	Lightness	No	6	4,67
	Yes	6	7,17		Yes	6	8,33
	Total	12			Total	12	
length	No	6	4,33	a-value	No	6	4,5
	Yes	6	8,67		Yes	6	8,5
	Total	12			Total	12	
width	No	6	4,33	b-value	No	6	5,33
	Yes	6	8,67		Yes	6	7,67
	Total	12			Total	12	
weight	No	6	4,5	C	No	6	4,5
	Yes	6	8,5		Yes	6	8,5
	Total	12			Total	12	
FFR	No	6	7,76	Hue	No	6	5,5
	Yes	6	5,33		Yes	6	7,5
	Total	12			Total	12	
Volume	No	6	5	TAC	No	7	8,57
	Yes	6	8		Yes	7	6,43
	Total	12			Total	14	
Tensilforce	No	6	4,67	TPC	No	7	5,43
	Yes	6	8,33		Yes	7	9,57
	Total	12			Total	14	

4.3.1. Physical properties

Weight

The results represent that the fruits of the pruned plants weigh on average 0.66g and of the not pruned 0.46g. Since the weight of the fruits correlates with volume (*r=0.92; p=0.00; n=12*); hence, the fruits also showed a slight trend of an averagely higher volume of the pruned berries. In comparison to the unpruned plants, the

pruned ones also showed a positive correlation between the weight of the fruits, length, and width *(r=0.94; p<0.05; n=6).*

Fruit Form Index

According to the calculated Fruit Form Index (FFI) the average means of the pruned and the not pruned plants is <1 hence both groups contain obround-shaped fruits. Contrary to the unpruned group, fruits of the pruned group have significantly higher values for length and width *(p=0.41; p<0.05; n=12).* In both groups width and length correlates *(r=0.94; p<0.05; n=6).*

Tensile force

There were no significant differences between both groups concerning the tensile force.

Color properties

The pruned group has, on average, according to the L*, a*, b* and C* values, a higher amount of brighter and more reddish fruits in comparison to the control group (Tab. 6 and 8). In the pruned group, the L*-values showed a strong positive relationship with the b*-value (*r=1.0; p=0.05; n=6)*. Regardless of the treatment, the L*-values correlated with a*-value (*r=0.85; p=0.05; n=12)*. and highly with b-*value (*r=0.88; p=0.05; n=12)*. In the unpruned group, a strong negative correlation between the measured lightness values (*r=-0.83; p=0.04; n=6*) and the antioxidative capacity (*r=-0.83; p=0.04; n=6*) was ascertained at statistical significance of 5%. Further the a-values (*r=-0.94; p=0.05; n=6)* and the calculated saturation (C* Chroma) values showed also a strong negative correlation to the TAC. The measurements showed that increasing saturation, lightness, and a- values go along with decreasing TAC values. Furthermore, a strong positive relationship between the Saturation C* and the lightness *(r=0.82, p= 0.42 n=6)* and the weight *(r=0.83, p= 0.42 n=6)* and volume *(r=0.83, p= 0.42 n=6)* of the fruits was established for the control group. In general, for smaller fruits, lower saturation, and lightness values were measured (see appendix).

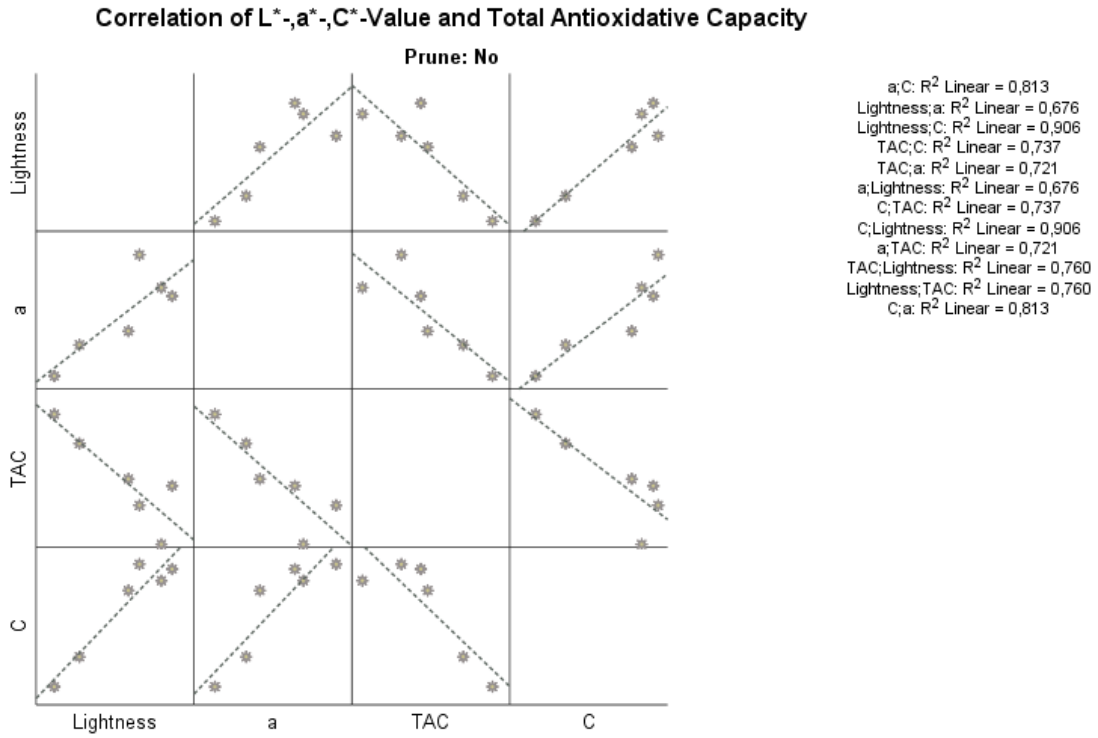

Figure 12 Graphic depiction of significant correlation of L*-, a*-, C*-values, and TAC of the unpruned group (*p<0.05,* n=6)

4.3.2. Phytochemical properties

In this study, the fruits present no significant difference in total phenolic content (TPC) and the antioxidative capacity (TAC) between pruned plants and non-pruned plants. The pruned plants contained a TPC of 2880 mg/100g DW, averagely 1464 mg per 100g dry weight more phenolics than the control group. The maximum of phenolics of the pruned group is at 6036 mg/100g DW, whereas the control group shows a maximum of 2686 mg/100g DW. The minimum of 591 mg/100g DW was in the control group and for the pruned at 631 mg/100g DW. Contrary to the TPC, the TAC was with 1739 mg/100g DW of the pruned and 1617 mg/100g DW of the not pruned, almost similar. The pruned group showed a maximum TAC of 46302 mg/100g DW and a minimum of 893 mg/100gDW. The maximum TAC of the control group was determined at 2308 mg/ 100g DW and the minimum TAC at 1026 mg/ 100g DW. Due to the high standard deviation of the means of the both groups (see Table 7), the mean ranks show better the differences between the TPC and TAC values within and between the treatment groups (see Table 8).

The pruned group showed a mean rank value of 8.57. Hence a higher mean rank in TPC than the pruned group, which had a TPC mean rank of 6.43. At the same time, the pruned group had for the TAC a rank of 9.57 and the control group a rank of 5.43 for the TAC off all measured values.

In the pruned group, a significant positive relationship between the phenolic content and the antioxidant activities of the fruits was established with a strong positive correlation value of (r =0.92*; n=7, p* < 0.001). Whereas for the unpruned group, no positive relationship between TPC and TAC was found. Regardless of the treatment, no positive relationship between the TPC and TAC was ascertained for the fruits in this study (see appendix).

4.4. TPC and TAC of all plant organ types

The three different types of organs showed significant differences in the total phenolic content TPC *(p= 0.01; p<0.05; n=56).*
Upper leaves with 5252 mg/100g DW and flowers with 5252 mg/100g DW contain the highest average content of total phenolics. Followed by based leaves with an average TPC of 4325 mg/100gDW. The fruits had an average TPC value of 2083 mg/100g DW, hence a significantly lower phenolic content in comparison to upper (*p=0.019*) and based (*p=0.017)* leaf and flower (*p=0.002)* parts (*p<0.05; n=56*).

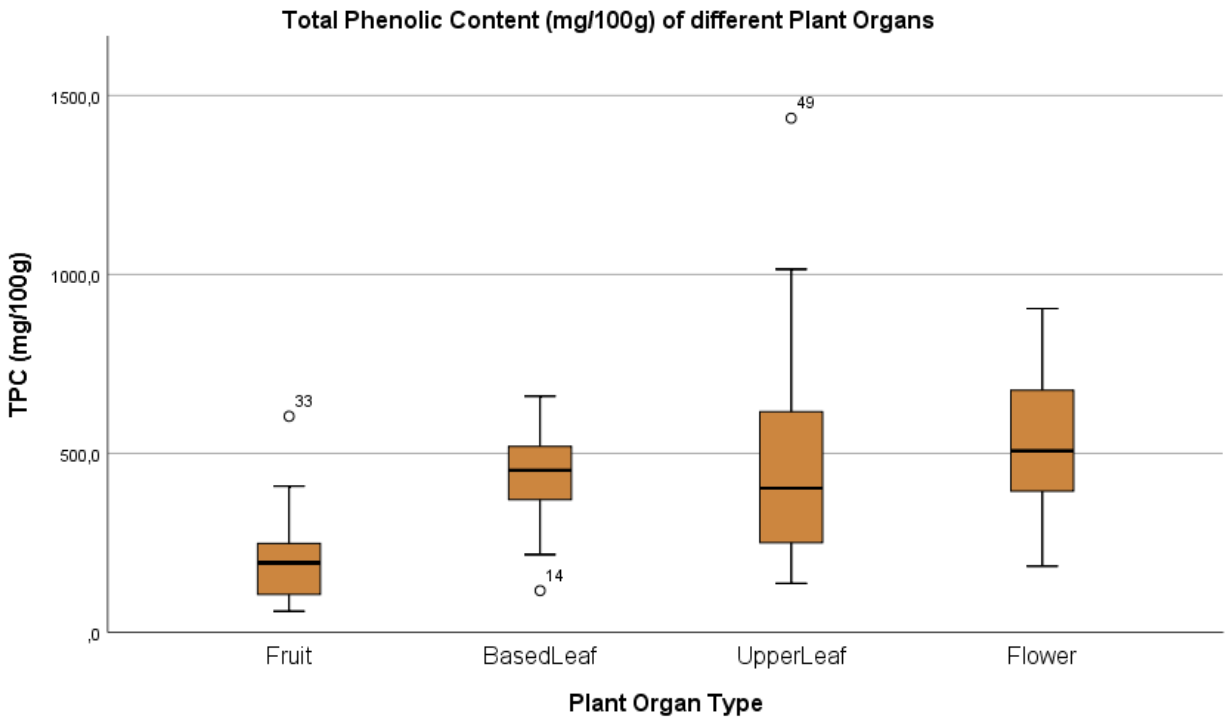

Figure 13.: TPC (mg/100g DW) of different Crataegus plant organ types in comparison *(p<0.05; n=56).*

TAC was measured for fruits, basal leaves, upper leaves, and flowers of *Crataegus monogyna*. In general, a significant difference in the total antioxidative capacity was detected between fruits, leaves, and flowers *(p= 0.003 p<0.05; n=56).* Contrary to the TPC content, the fruits showed an average of 1679 mg/100g DW, no significant difference in the antioxidative capacity between fruit, leaf, and flower samples.
However, there is a significant difference (*p<0.05; n=56*) in the content of TAC between the content of based leaves to the content of flowers *(p=0.004)* and upper leaves *(p=0.003)*. For the base leaves, the average value for all 14 measurements was 1016 mg/100g DW.
The TAC values of the flower samples were mostly higher, and the average was 2346 mg/100g DW.

A similar average as for the flower samples was detected for the upper leaves with values 2005mg/100g DW. In general, flower and upper leaf samples showed a comparable average of TAC; hence the relationship of the TAC and TPC was tested according to the Spearman-Rho-test. Resulting in a significant positive relationship

between the phenolic content TPC and the antioxidant activities, TAC of all plant organs was established *(r= 0.27; p= 0.4; n=56; p<0.05).* Especially for leaf *(r =0.54; p=0.04; n=14; p < 0.05)* and flower (*r =0.67; p=0.008; n=14; p < 0.001*) samples a significantly strong positive correlation was established. However, for the fruit and base leaf samples, no positive relationship between the TPC and TAC was ascertained in this study (see appendix).

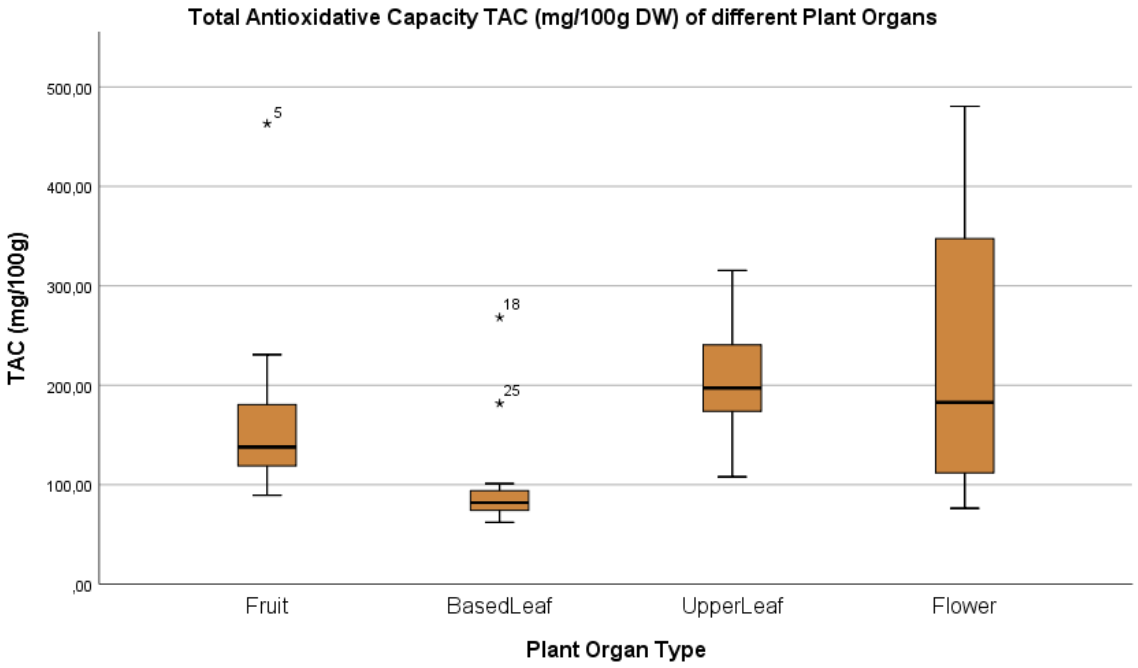

Figure 14.: TAC (mg/g DW) of Crataegus fruit, leaf, and flower organs in comparison (*p<0.05; n=56*).

Taken together the results, fruits' physical and phytochemical properties presented no significant difference between pruned plants and non-pruned plants in the first year. All organs exhibit varying TPC and TAC values. Leave samples from different proportions of the plant displayed a disparity in the TAC and TPC concentrations within the organ part. As with the fruits, pruned group individuals present a positive relationship between TPC and TAC, whereas unpruned plants lack this correlation. The unpruned group measurements showed that increasing color properties, such as saturation, lightness, and a- values, go along with decreasing TAC values. Even though basal leaves and unpruned samples did not possess a relationship between TPC and TAC, a significant positive correlation between the TPC and the TAC of all organs together was ascertained. The mean TPC and TAC values from subjects' organs serve to distinguish the concentration of secondary metabolites of *Crataegus*

plant material selected in Lower Austria and *Crataegus* plant material from other countries of origin. Based on this study's data and evidence, the answer whether Lower Austria represents a good cultivation site for *Crataegus* as a niche crop or not will be additionally answered in the following.

5. Discussion

5.1. The impact of pruning on fruit parameters, total phenolic content, and total antioxidative capacity of the fruits in the first year

In this one-year-long experiment, no significant differences between the physical and phytochemical fruit properties of the pruned and the non-pruned plants were ascertained. The results arise out of several factors. Firstly, measuring the impact of pruning on fruits accurately takes more than one vegetation period, since flowering and subsequent fruit-set develops on 2-year-old wood (Croxton and Sparks, 2002). Further, the investigated number of plants was small since finding suitable subjects to examine the fruit parameters of *Crataegus monogyna* grown in Lower Austria needed to comply with specific requirements. Due to the propensity to hybridization, apomixis, and polyploidy of *Crataegus* species (Byatt, 1975; Dönmey, 2004; Güney et al., 2018), the first necessary step was to identify individuals of *Crataegus monogyna* adequately. Some investigations about hawthorn do not distinguish between the species of the genera *Crataegus* (Özcan et al., 2005; Tadić et al., 2008; Zarrinkalam et al., 2018), which may obscure the results. In particular genetical factors such as species (Yanar M. et al., 2011; Mraihi et al., 2015) or cultivars define vegetative growth, morphological and phytochemical traits of leaf flower material (Peschel et al., 2008), as well as the maturity date, secondary metabolite content and other physical properties of fruits (Sonnenschein and Plescher, 2005; Gundogdu et al., 2014). Additionally, *Crataegus monogyna* represents a great variation of morphological and characteristics within the species subjected to environmental factors (Khadivi-Khub et al., 2015). Hence it was essential to locate several *Crataegus monogyna* plants with similar age, size, cultivar, and grown under the same environmental conditions without human intervention in order to design a convincing trail. Besides the small number of subjects, the duration of the observation is a critical aspect and might obscure the gained results of this study.

Most experiments in terms of pruning effects last for over two years and include 10 or more repetitions to establish the impact of pruning (Strik and Poole, 1991; Croxton and Sparks, 2002; Drake and Clark, 2003; Radünz et al., 2014). Due to the short observation, no significant effect of the pruning treatment on the fruit parameters was ascertained during the vegetation period of 2019. However,

previous studies proved that a pruning treatment affects the yield of hawthorn fruits (Sparks and Martin, 1999; Croxton and Sparks, 2002), biomass production, and the leaf flower ratio (Phipps et al., 2003; Peschel et al., 2008). In 1997 scientists investigated the impact of various hedgerow management treatments concerning the yield of *Crataegus monogyna* fruits in the UK with the intention of examining the influence of intense hedgerow management on biodiversity. Each pruning treatment was repeated three to five times within 6 years before the impact on the fruit yield was measured. A significant disparity was proven in hawthorn fruits' yield between the pruning treatments and the unmanaged control group. A soft thinning out treatment increases the yield and the weight of *Crataegus* fruits. Besides, the result also demonstrated that the yield of fruits varies from year to year (Sparks and Martin, 1999). In conformity with other observations, the intensity and less the pruning date determine the fruit set (Strik and Poole, 1991; Sparks and Martin, 1999). However, prunings' frequencies significantly impact the yield of flowers (Phipps et al., 2003) and fruits. An annual cutting reduces the yield significantly, whereas pruning in a three-year cycle improves yield because flowering and subsequent fruit-set emerge on 2-year-old wood (Croxton and Sparks, 2002).

Contrary to fruit production, annual trimming stimulates vegetative growth and delivers strong twigs hence affect biomass production (Phipps et al., 2003; Peschel et al., 2008). Since the pruned group developed almost 80% of new shoots, the results of this study confirm these observations. In particular, the pruning style affects the leaf-flower ratio of *Crataegus* (Peschel et al., 2008). As before mentioned (Chapter. 3.3.1.) the applied pruning treatment was softly thinning out with the intention of harvesting leaf and flower material, increasing the sun exposure, stimulating the vegetative growth, and yet not affect the yield of the fruits significantly at once. Due to the consideration that a high intense prune to time around blossom influences the yield of fruits within the growing season (Strik and Poole, 1991; Spornberger et al., 2013). In respect to previous case studies, it is presumed that the actual effects of tested pruning on the generative and the vegetative properties in this study are adequately measurable in the following vegetation periods.

Though it is to mention that in this study, the pruned group showed significant differences in length and width and a positive relationship between fruit length, width, and weight compared to the unpruned individuals. Contrary to the group

without pruning, the results of the pruned group support the observed correlation of fruit length, width, and weight of investigated *Crataegus monogyna* fruits in Iran. Khadivi-Khub et al., 2015 established other relationships between morphological fruit traits, such as a highly positive correlation between flesh weight and fruit weight (Khadivi-Khub et al., 2015). Since the flesh weight was not invested in this observation, the missing positive relationship between fruit length, width, and weight in the unpruned group might be explained by a low flesh weight of unpruned fruits.

Furthermore, for the control group, a positive correlation between the calculated Chroma (C*)- value and weight and volume was detected. Hence, the saturation of unpruned fruits increased along with weight and volume, which means that smaller fruits possessed a darker dull reddish color, whereas bigger fruits featured a brighter reddish color. In this study, the sample fruits showed beside the positive correlation of lightness and red-green ratio (a-value), also a very strong positive relationship between the lightness and the yellow-blue ratio (b-value). Hence the brighter the fruit, the higher the yellow and red content, and the darker the fruit, the lower the green and blue content (see. Chapter 4.3.1.). Similar observations are supported by a study about the phytochemical properties of various hawthorn species wild-grown in turkey. Based on the ascertained correlations, smaller fruits also possessed a darker, less reddish, and more blueish color spectrum than longer brighter fruits, which contained a higher reddish and yellow content (Calişkan et al., 2012). In contrast to the unpruned fruits, the pruned samples lacked these significant correlations between physical and color traits.

Since a positive relationship between color-giving pigments, size and antioxidative capacity exists (Moyer et al., 2002; Boo et al., 2012; Cömert et al., 2020), this investigation also examined possible relationships between the morphological and phytochemical traits. The results displayed overall no significant differences between the measured color properties of both groups. Further, no significant alteration was detected in total phenolic content (TPC) and antioxidative capacity (TAC) of fruits between the pruning treatment and the control group in the first year.

However, the results indicate a negative correlation between the color traits and the TAC of unpruned fruits. The lightness, the green-reddish ratio, and the fruits'

calculated saturation correlated negatively with the TAC values. Hence smaller fruits with a darker, less reddish color spectrum featured a higher antioxidative capacity. Also, Calişkan et al., 2012 came during their examination to this finding (Calişkan et al., 2012). According to examinations, smaller fruits generally tend within species to present higher TAC (Moyer et al., 2002). This relation might be explained by the fact that most bioactive compounds, especially of *Crataegus monogyna,* are present in the peel (Mraihi et al., 2015), and smaller fruits have a relatively larger peel area in relation to the volume when compared to larger fruits. Consequently, a larger peel area might lead to a higher TAC per fruit. (Calişkan et al., 2012).

Besides the size also the color properties of fruits are linked to antioxidative activity and the total amount of phenolics (Moyer et al., 2002). The samples of this investigation showed a decline of TAC along with an increase of a*-value. Adjacent to the relationship between TAC and saturation, Calişkan et al., 2012, additionally established a negative correlation between saturation, b*-value, and TPC. Consequently, the brighter and the higher the yellow content of the fruit is, the lower is the measured TPC (Calişkan et al., 2012). In contrast to the results of Calişkan et al., 2012 no significant correlation between the TPC and color properties could be discovered in this study.

An explanation for the alternation of the TAC along with the changing color properties might be that darker fruits with a less reddish color spectrum posed based on visual color perception different phytochemical profiles. According to a color classification based on the antioxidative power of fruits and vegetables, the colors magenta, blue and red possess the highest antioxidative capacity averagely. At the same time, plants with yellow or green visual color perception showed a lower antioxidative capacity (Cömert et al., 2020). The polyphenolics as the class of coloring matter are mainly responsible for magenta, blue and red color of plant tissue hence are connected to a high antioxidative capacity. In most cases increases the TAC with and increasing TPC (Moyer et al., 2002; Boo et al., 2012).

However, the unpruned group did not feature this relationship. Calişkan et al., 2012 and one other study about wild-grown *Crataegus monogyna* fruits (Egea et al., 2010) validate the missing relation between TAC and TPC in the unpruned group.

Even though no significant differences have been detected between the TPC and TAC of both groups, the pruned group featured a strong positive between TAC and

TPC, whereas the unpruned group lacked it. The composition of phenolic profil and the featured structural properties of single phenolic compounds might be responsible in the unpruned group for this missing correlation at a similar TPC level. The relationship of the chemical structure of phenols and their antioxidative capacity might be an explanation (Robards et al., 1999). In many studies, antioxidant activity is mostly detected in association with flavonoid content (Montoroa et al., 2005; Fiol et al., 2012). Since the antioxidant activity of flavonoids is closely related to the position and degree of hydroxylation of the molecule, flavonoids that are usually present as glycosides show a different antioxidative activity than their respective aglycones (Robards et al., 1999). Therefore, the unpruned group might present structural variant compounds with different antioxidative character, which leads to the fact that their TAC value tends to be not related to their TPC values.

Yet, contrary to the control group, the TPC and TAC values of pruned fruits are linked to each other. This leads to the assumption that besides environmental and genetic factors also increased light exposure, which was created by the pruning treatment, might affect the TPC and TAC of the subjects. Various investigations showed that the light intensity has a strong impact on the phytochemical profile, particularly on the phenolic content of fruits (Peschel et al., 2008; Zoratti et al., 2014; Arena et al., 2017). Especially experiments about the effects of pruning treatments demonstrated, besides the yield, positive changes in color, and biochemical composition of fruits (Strik and Poole, 1991; Sparks and Martin, 1999; Drake and Clark, 2003; Radünz et al., 2014). As *Crataegus monogyna,* several species present the highest amount of phenolics in the fruits' peel (Moyer et al., 2002; Zoratti et al., 2014; Mraihi et al., 2015), so it is presumed that plants accumulate polyphenolics in the epidermis for protection against UV radiation (Croteau et al., 2000; Kolb et al., 2001; Zoratti et al., 2014; Böttger et al., 2018b). Therefore, the positive effects of diverse pruning treatments are mostly linked to increased light exposure (Drake and Clark, 2003; Radünz et al., 2014). All the observations correspond with the latest studies, which provided evidence that polyphenolic biosynthesis is induced by solar, particularly UV-B, radiation (Kusano et al., 2011; Falcone Ferreyra et al., 2012). To conclude, an elevated light exposure facilitates an increase in the level of total phenolics, in particular anthocyanins, in many different kinds of fruits (Radünz et al., 2014; Alonso et al., 2016; Arena et al., 2017).

Since the pruning treatment induced no differences in phenolic concentrations, this study's results have not confirmed these observations. Yet, based on previous case studies (Strik and Poole, 1991; Radünz et al., 2014), it is presumed that in this research, the effects of the tested prune and the associated increasing light exposure of the fruits on phenolics contents are adequately measurable in the following vegetation periods. For instance, in a three-year-long study about the effects of various intense pruning systems on yield and the anthocyanin content of cranberries, no significant differences were measured in the first year. Whereas in the second year already, the berries without pruning treatment contained significantly lower anthocyanin concentration. In contrast to the unmanaged group, berries of pruned groups with light and moderate-intensity showed an enhancement of the anthocyanin content (Strik and Poole, 1991).
Yet, it should be taken into account that not just the light intensity affects the TPC of fruits, other abiotic and biotic factors also impact the TPC and TAC of fruits (Falcone Ferreyra et al., 2012). Pursuant to a two-year-long examination about TPC and TAC changes under different conditions in cultivated *B. microphylla* fruits, the authors concluded that besides the light intensity also fertilization, temperature, humidity play a significant role in the amount of the TPC and TAC (Arena et al., 2017).

Even though no significant impact of the pruning treatment was ascertained on morphological traits, TPC, and TAC, the different occurrence of the various relationships between physical and phytochemical properties in both groups is noteworthy. Contrary to the fruits, the impact on phytochemical characters of leaf and flower material will be quantifiable in the next vegetation periods. Yet, the phytochemical properties of all organs were examined and compared to each other.

5.2. Variations of TPC and TAC of all plant organs

The results of this study highlight significant differences in the concentrations of phenols and antioxidative capacity between the distinctive organ types. In particular, leaves and flowers showed higher concentrations of total phenols than fruits. Hence the results support a broad range of investigations about *Crataegus monogyna* (Kaul, 1998; Peschel et al., 2008; Froehlicher et al., 2009; Edwards et al., 2012).

In general, different organs of hawthorn contain different types and quantities of specific secondary metabolites, which change along with the development or maturity stage (Orhan et al., 2007; Peschel et al., 2008; Barros et al., 2011; Edwards et al., 2012; Rodrigues et al., 2012). During different harvesting periods, these alternations in the TPC and TAC were monitored in different hawthorn organs over one vegetation period by Pavlovic et al. (2019). The results demonstrated a seasonal variation in the phenolic profiles' quantity and composition and the antioxidant activity of different hawthorn organs (Pavlovic et al., 2019). The phytochemical properties, as the total procyanidin content, of the *Crataegus monogyna* fruits, alter during ripeness (Hiermann et al., 1986; Kaul, 1998). In 2012 the bioactive compounds of *Crataegus monogyna* were analyzed and quantified with HPLC–DAD–ESI/MS and verified other studies' results. Unripe fruits present the highest TPC, TFC, and phenolic acids. In particular, flavanols, as the monomeric (-)-epicatechin, dimeric and trimeric procyanidin, and chlorogenic acids as 3-*O*-caffeoylquinic acid were abundantly present. Compared to ripe and overripe fruit, the unripe fruits did not feature several anthocyanins. The overripe fruits present the significantly highest anthocyanin concentration of all maturity stages. Further, the overripe stages generally contain more TFC, TPC, and phenolic acids than the mature stages. Hence the harvest time influences TPC, TFC, and the content of procyanidins remarkably (Rodrigues et al., 2012).

Another study from Germany, which included the fruit diameter of the various stages in relation to the drug concentrations per fruit, confirms the influence of harvest date on drug concentrations (Hiermann et al., 1986). The first harvest date was conducted in June at an unripe fruit stage and revealed the highest procyanidin content per fruit. The posterior harvest dates in June and especially in September present the significant lower procyanidin content. In October, ripened fruits displayed a slight increase in procyanidin content again (Hiermann et al., 1986).

With regard to these research results, the low TPC of the fruits might be explained by the harvest time in this study. Since the harvest of the fruits was conducted at full ripeness with the aim to gain fresh fruits for consumption and not to attain the highest TPC of fruits. However, it should be considered if the fruits are consumed fresh, dried, or used a powder for pharmaceutical purposes and then decide about the harvest timing. Based on the relation of beneficial compounds per fruit to fruit diameter and volume, a harvest date of the fruits at the time of full ripeness is still

considered the best method to obtain the highest yield of fruit weight and phenolic compounds (Kaul, 1998).

Besides the date of harvest, the extraction method might also a factor affecting the results in this study. Shortle et al. (2014) showed in a study that specific organs of *Crataegus monogyna* present under different extraction methods various yields of TPC and TAC. Extracts of leaves and flowers yielded higher TPC and TAC values when the ultrasound bath was used, whereas fruit extracts showed higher TPC and TAC values with the traditional maceration extraction method. Shortle et al. indicated that the different yields of TPC and TAC might be explanied by the different tissue types of the fruits. Therefore fruit samples might need more time in the sonicated mode to get an effective solvent penetration into the fruit tissue (Shortle et al., 2014). However, in this study, all organs were similarly extracted by performing the ultrasonicated extraction technique according to Kim and Lee's protocol, which might affect the yield of TPC and TAC analyzes for the fruits (Kim and Lee, 2005).

In contempt of the significantly lower TPC, the fruits, regardless of the treatment, present no difference in TAC compared to flowers, upper and basal leaves. Furthermore, all fruit samples together demonstrate no correlation between the TPC and TAC. This result may have arisen from the fact that the treatment and control group were summarized for establishing correlations for fruits in general. Yet, it is to mention that the TAC of a sample is not only based on the polyphenolic content (Mallet et al., 1994; Grassmann et al., 2007; Locato et al., 2013; Cömert et al., 2020). Other non-phenolic compounds, as ascorbic acid and carotenoids, also notably contribute to the fruits' high antioxidative activity (García-Mateos et al., 2013; Gundogdu et al., 2014; Cömert et al., 2020). In this study, other beneficial compounds, such as ascorbic acid (García-Mateos et al., 2013; Gundogdu et al., 2014; Mraihi et al., 2015), and nutrients, like potassium, magnesium, and calcium (Özcan et al., 2005) that are remarkable present in fruits of hawthorn are not analyzed hence are not taken into consideration. Anyway, other investigations about bioactivity of wild-grown *Crataegus monogyna* fruits report similar observations (Egea et al., 2010; Calişkan et al., 2012).

In opposition to the fruits, flower samples exhibit a strong relationship between TPC and TAC values. Moreover, our results support that the flower parts of *Crataegus ssp.* mostly contain similar or even higher amounts of bioactive compounds as the other plant organs and, therefore, confirm several examinations (Kaul, 1998; Edwards et al., 2012).
However, upper leaves also feature a similar high TPC value and also a high TAC value. In equation with flowers, leaves are obtainable at a much earlier stage of plant development, crop up in much greater quantity, hence can be harvested for a much more extended time period (Kirakosyan et al., 2003).

Though, leaf samples harvested from a lower portion did not represent the same amount of TPC and TAC of leaves sample from a higher portion. Notably, the base leaves' TAC value was significantly lower. Hence next to the fruits TAC, the antioxidative power of leaf samples harvested from a lower portion did not correlate with the TPC, too.
This distinction of these values within the organ part could be produced as a result of various factors. One possible explanation could be the different exposure to the sun. In our study, the leaves of the higher portions of the canopy (>2,5m) from the East-South oriented shrubs were potentially more exposed to the sunlight than the base leaves (<1m).
Light conditions substantially impact morphological (Granata et al., 2020) and phytochemical development of the leaves of *Crataegus monogyna*. During an investigation about the variability of total flavonoids in hawthorn to evaluate production-related factors of industrial leaf and flower drug starting material, the scientists Peschel et al. (2008) tested the sun exposure's influence on leaves and leaf flower drug material in a simple test. The analyzed samples were taken from hawthorn trees equally at the north and south sides, whereby the own shading the sun exposure varies considerably, at six different harvest dates. The result showed that regardless of the harvest date, all samples grown on the tree's southern side contained a higher TFC than the northern side's leaf samples. Additionally, it was ascertained that pure leave samples harvested from branches in the most shadowed tree center present the lowest TFC (Peschel et al., 2008). As mentioned, the latest studies provided evidence that polyphenolic biosynthesis is induced by UV radiation (Kusano et al., 2011; Falcone Ferreyra et al., 2012) and therefore affect

the total phenolic content of specific plant tissues (Croteau et al., 2000; Peschel et al., 2008). With regards to these rationales, the differences of TPC and TAC within the plant organ and the missing relation between the TPC and TAC of the unpruned individuals and lower leaf samples might be explainable.
However, it is to mention that besides UV radiation, other stress factors influence the TPC and TAC levels of plants significant (Croteau et al., 2000; Kolb et al., 2001; Kirakosyan et al., 2003).

In particular, one study about the impact of abiotic and biotic stress on secondary metabolites of *Crataegus monogyna* clarifies the effect of stress on the production of polyphenolic compounds. In this experiment, the plants stressed by drought and cold featured a significantly higher amount of polyphenols than the biotic-stressed and non-stressed control group. The treatments flooding, dunking the roots into water, or herbivory, continuous removing of leaves, did not affect or even decrease polyphenolic level in contradistinction to the applied drought and cold stress (Kirakosyan et al., 2004). An additional study also revealed that both treatments trigger the production of specific phenolic compounds hence leading to different phenolic compositions. The drought stress induces a higher level of flavanols, like catechines, whereas the cold stress induces the production of flavonols, as quercitin. For both stress inductions, the antioxidant capacity increased together with the TPC (Kirakosyan et al., 2003; Kirakosyan et al., 2004).
Even though some sample groups examined individually could not establish a TPC and TAC relationship, for all samples summarized together the results support the positive relationship between the total phenolic content and the antioxidant capacity in this experiment.

5.3. Comparison of the quality and quantity of secondary metabolites of *Crataegus* plant material selected in Lower Austria with from other countries of origin

Since raw material of various species and hybrids of hawthorn is approved by the European pharmacopeia (European medicines Agency, 2016a), this study additionally aimed to compare the quality and quantity of secondary metabolites of plant material from other countries of origin with the selected plant material from

Lower Austria. However, due to various factors, a simple comparison of TPC and TAC values would obscure the equation.

Since especially genetical factors are responsible for the amount and composition of phenolic compounds (Peschel et al., 2008), the content of phenols and the antioxidative capacity differ between species and cultivar (Sonnenschein and Plescher, 2005; Prinz et al., 2007; Gundogdu et al., 2014; Tahirović et al., 2015; Lund et al., 2017). Due to the significantly different phytochemical profile of the species, researchers established chemotaxonomic classification to distinguish one species from each other (Prinz et al., 2007; Lund et al., 2017). Hence it is essential to compare the same species or cultivar and, in the best case, clones with the same genetic material to identify the influence of the growing area. Peschel et al. 2008 investigated in their study about the evaluation of production-related factors of industrial hawthorn leaf and flower drug starting material regarding the variability of total flavonoids, the influence of the location side on hawthorn clones. They concluded that the dissimilarities of the TFC within one clone at two different location sites are often higher than the differences between two clones at one location site (Peschel et al., 2008). Besides the genetic factors, an equal development stadium of the comparing sample is crucial for a significant comparison. Numerous studies proved an alternation of the amount and the composition of specific compounds in all organs of *Crataegus monogyna* during the different development stages (Hiermann et al., 1986; Kaul, 1998; Sonnenschein and Plescher, 2005; Orhan et al., 2007; Peschel et al., 2008; Rodrigues et al., 2012; Pavlovic et al., 2019).

As our results confirm, distinctive parts of *Crataegus monogyna* contain different amounts and variations of phenolic compounds, it is always essential to compare the TPC, TFC, and TAC obtained from a similar organ. Comparing Crataegus species with values obtained from extracted leaves and flowers with values from extracted flowers might result in an obscure equation leading to the next crucial point, the influence of analytical methods on the phytochemical profile of *Crataegus*. An examination revealed that specific organs of *Crataegus monogyna* present under distinctive extract modes of different TPC and TAC yields (Shortle et al., 2014).

Crataegus monogyna material extracted in an ethanolic solution contain threefold more phenolics than the aqueous extract (Bernatonienė et al., 2008).

Even the materials' state, as fresh or dry, impacts bioactive compounds' yield and composition, hence affecting the results (Froehlicher et al., 2009; Ngoc et al., 2019).

Since distinctive drying techniques alter physical properties (Aral and Beşe, 2016) and phytochemical profiles (Chan et al., 2009; Liu et al., 2016; Zielinska and Michalska, 2016; Coklar et al., 2018), also the preparation style might impact the values used for the comparison. Nevertheless, it is to mention that the applied procedure depends on the intended purpose of the extracted substances. In analytics, aqueous methanol is commonly preferred as a solvent for extracting polyphenols from freeze-dried plant material due to its efficiency, high boiling point, and for the reason of economy (Kim and Lee, 2005). However, for commercial production of plant material extracts, hot air drying is usually performed since freeze-drying is a more complex procedure and needs more expensive equipment hence is higher in costs (Tanko et al., 2005). In addition, ethanol and hot water are used instead as solvents since they leave no harmful residues. Therefore no extra removal of the remainders is necessary and saves costs (Shi et al., 2005).

Concerning all these delicate factors, just some values from literature were suitable for comparison since they were gained with similar methods (Froehlicher et al., 2009; Shortle et al., 2014; Coimbra et al., 2020).

In this study, in particular fruits of the pruned group, averagely feature a higher TPC in comparison to reported values of wild-grown *Crataegus monogyna* fruits from North France (Froehlicher et al., 2009) and Ireland (Shortle et al., 2014).
Nevertheless, the TPC and TAC values of all examinations, regardless of the analytical method, generally do not deviate significantly from the values of this study (Bernatonienė et al., 2008; Froehlicher et al., 2009; Egea et al., 2010; Shortle et al., 2014; Tahirović et al., 2015). However, a slight trend is seen between the TPC of individuals grown in a dryer Mediterranean area (Özcan et al., 2005; Orhan et al., 2007; Coimbra et al., 2020) and grown in more humid climate zones (Shortle et al., 2014). However, no study currently puts the TPC and TAC in relation to the total yield of leaves, flowers, and fruit material. Therefore, it is unclear whether hawthorn under specific stresses that might increase TPC and TAC makes a trade-off between biomass production and acclimation to stress in terms of secondary metabolite production.

5.4. Evaluation of *Crataegus monogyna* grown in Lower Austria

To sum up, according to my observation, *Crataegus monogyna* grows in Lower Austria very well and features comparable TPC and TAC values to plant material from other regions. However, for an adequate evaluation of the actual influence of the cultivation area Schneeberg Landregion in Lower Austria, more research needs to be done. In particular, the possible yield of leaf, flowers, and fruits for farmers and the contained bioactive compounds in relation to the average yield need to be investigated.

Though, it can be concluded that this region has the potential as a cultivation site for *Crataegus ssp.* and can be an attractive supplementary source of income for farmers during the whole year. After all, medicinal plants are among the renewable raw materials, and the cultivation of new species extends the possibility of alternative land use in agriculture, at least in some niches (Sonnenschein and Plescher, 2005). Besides the monetary aspects, the farmer can profit from various beneficial agro-ecological values as the control of soil erosion, the windbreaker effect, and the increased biodiversity (Burel and Baudry, 1995; Phipps et al., 2003). Depending on the individual production aim, the expenditure of time, and management potential, the farmer has the possibility to benefit from leaves and flowers in spring or fruits in autumn. Each production target has its own advantages and disadvantages. Based on the Sonnenschein and Plescher examination, single trunk education is considered to be the most efficient educational form (Sonnenschein and Plescher, 2005).

For hawthorn fruit production, pruning treatments in a two-year-cycle or even three-year-cycle might be promising management strategies to increase flower or fruit yield, light in the trees, and stimulate biomass production since flowering only occurs on 2-year-old wood (Croxton and Sparks, 2002) and the yields of fruits vary from year-to-year (Sparks and Martin, 1999).

Besides pruning treatments, other management effects can also influence the yield of *Crataegus monogyna* fruits significantly, especially in agricultural landsides as in Lower Austria. According to an examination about the effects of pesticide spray drift on hawthorn hedgerows. The impact of different doses of the sulfonylurea herbicide metsulfuron-methyl at different spraying times gravely influenced the reproduction

system and inhibited the development of fruits on *Crataegus monogyna.* Sulfonylurea herbicides are primarily applied in cereal cultivation in Europe.
Besides the lost yield for the farmer, this highly significant reduction of the fruits also constitutes negative consequences for biodiversity (Kjaer et al., 2006) since various wildlife species rely on the fruits as a food source (Hille-Rohde, 1989). Contrary to fruit production, no significant effects on hawthorn leaves and flowers were measured regardless of the doses and spraying times (Kjaer et al., 2006).
In particular, the area of Schneebergland presents many cropping farms, and an application of sulfonylurea herbicides should not be taken into account close to a hawthorn fruit production site.

Yet, the area of Schneebergland has as a location site one major advantage. Due to the altitude, fewer orchards are present, hence cultivating *Crataegus ssp.* does not create a conflict with fruit production. However, the few fruit farmers might see hawthorn cultivation as critical even though other observations could not establish a significant relationship between occurring infestations of flowering *Crataegus* and fire blight incidence in neighboring orchards (van Teylingen, 2002).
A possibility to avoid a conflict with pomiculture is to adapt the management and change the production target from yielding hawthorn fruits to leaf and flower drugs. According to an investigation in the Netherlands, *Crataegus's* flowering period is a determining factor for potential infection with fire blight. Flowering hawthorn individuals are to be seven times at a higher risk of getting infected than non-flowering hawthorns (Schouten and van Teylingen, 1990). Therefore, a harvest cut of leaf and flower drugs right before flowering might protect hawthorn against an infection. Further, a harvest before flowering has the positive side effect of presenting the highest flavonoid content (Sonnenschein and Plescher, 2005). So in case of an occurrence of fireblight in the area, the farmer can shift the production from fruit to leaf raw material production hence might prevent hawthorn from being a host.
Regarding the accumulated knowledge of previous examinations and this observation, Lower Austria possesses great potential as a cultivation site for *Crataegus* raw material and fruits.

5. Summary

This one-year-long investigation gives us an impression of the biochemical potential from different organs of *Crataegus monogyna* grown in Lower Austria.

Due to the short duration of one vegetation period, this examination did not evince significant distinction in the pruning treatment effects concerning production-related factors as fruit parameters, quality of metabolites, and yield in the first year. However, individuals of the pruned group present a positive relationship between TPC and TAC, whereas unpruned plants lack this correlation. Nevertheless, it is presumed that the impact of tested pruning is adequately measurable in the upcoming vegetation periods.

Although separately examined, not all samples possess these relationships. However, a positive correlation between TPC and TAC was established for all plant organs. Contrary to the strong positive relationship between TPC and TAC for flower and upper leaf samples, the base leaf sample did like the unpruned fruits, not feature these correlations. Leaves harvested from lower portions present a significantly lower TAC than the flower samples. Thus samples from different proportions of the plant displayed a disparity in the TAC and TPC concentrations within the organ part.In contrast to leaf and flower samples, fruits possessed a significantly lower TPC. Overall, there was a distinction between TPC and TAC within the plant organ leaf. With few on TPC and TAC's established relations, this difference might be related to sun exposure.

Generally, there is a variation in the amount of TPC and TAC between the three investigated plant organs. Flowers contained the highest amount of TPC and TAC, followed by upper leaves. The comparison of plant organs with different origins based on literature values is problematic due to various factors. Tough comparing similar studies showed that *Crataegus monogyna* grown in Lower Austria possesses similar phytochemical properties as raw material from other location sides.

However, to evaluate the impact of the cultivation side, the clones' examination would provide less affected results. Due to the subjects' identical genetic material, the variation within the species or cultivar is reduced to a minimum, and the impact of the extern factor might be adequately measurable. Additional, it is essential that in future studies about the pruning effect on *Crataegus ssp.* a higher number of repetitions is generated and to work over two years. Regarding the former

investigation and this one-year observation, it can be concluded that *Crataegus spp.* is an attractive choice as a windbreak-hedge for their fields in Lower Austria. The shrub decreases transpiration and increases biodiversity, simultaneous the farmer profiting with relatively little effort from obtained raw material and fruits as a supplementary income source during the whole year. An additional attractive aspect is that most management activities are performed in less work intense months for farmers. The most promising management measurements are a single trunk education and a thinning-out pruning treatment in a two to three-year cycle to increase flower or fruit yield, light in the trees, and stimulate biomass production.

Yet, establishing the production of *Crataegus monogyna* raw material as a niche crop in Lower Austria, more research concerning production-related factors needs to be done. Especially the manual pruning and the harvest of leaf flower drugs and fruits contribute to high production factors. The tested pruning treatment is also a manual harvest cut and might be attractive for a small number of individuals, but a larger number should be harvested by machine. Developing automatic devices for pruning and harvest is indispensable in making an inculturation of *Crataegus monogyna* affordable for farmers and the market.

To sum up, this one-year-long observation showed that the average TPC and TAC values from all hawthorn organs grown in Lower Austria are comparable to those from literature hence meet the requirements of pharmaceutical raw material. Therefore Crataegus spp. can be an attractive choice for farmers as windbreak-hedge for fields. The shrub decreases transpiration and increases biodiversity, simultaneous the farmer profiting with relatively little effort from obtained raw material and fruits as a supplementary source of income during the whole year. Hence the cultivation of Crataegus species as medicinal plants extends the possibility of alternative land use in agriculture and might be promising for establishing a new niche for farmers in Lower Austria. This investigation lays the foundation for further research projects on the cultivation of hawthorn in Lower Austria.

6. Appendix

6.1. Fruit values across pruning treatment

Descriptive Statistics[a]

Prune		N	Minimum	Maximum	Mean	Std. Deviation
No	height	6	9,44	11,32	10,4823	,80333
	length	6	8,72	11,27	9,8247	1,02630
	width	6	8,58	11,13	9,6736	1,04754
	weight	6	,54	,87	,6607	,14747
	FFR	6	,98	2,25	1,3615	,48735
	mm2	6	396,61	732,08	541,4828	141,99278
	Tensilforce	6	,198	,373	,30877	,071977
	Lightness	6	28,68	64,42	37,8849	13,65839
	a	6	30,81	45,62	36,3849	5,17399
	b	6	10,77	36,27	20,1613	9,86447
	C	6	32,67	59,65	43,4968	10,74129
	Hue	6	19,08	31,49	24,4263	4,15078
	Valid N (listwise)	6				
Yes	height	6	9,35	11,03	10,1788	,70131
	length	6	8,26	9,35	8,7340	,47299
	width	6	7,90	9,24	8,4742	,58909
	weight	6	,37	,58	,4668	,08245
	FFR	6	1,13	1,75	1,4786	,24930
	mm2	6	341,45	492,59	410,9886	62,96804
	Tensilforce	6	,158	,334	,23685	,057554
	Lightness	6	28,22	30,71	29,6572	,98174
	a	6	28,64	34,62	31,6466	2,16087
	b	6	11,01	16,03	13,3731	2,06437
	C	6	30,89	36,93	34,7687	2,51818
	Hue	6	19,53	25,82	22,1942	2,32924
	Valid N (listwise)	6				

Descriptive Statistics[a]

PRUNE		N	Minimum	Maximum	Mean	Std. Deviation
No	TPC	6	591,00	2686,00	1416,0000	811,86206
	TAC	6	1026,03	2307,55	1672,5233	450,63848
	Valid N (listwise)	6				
Yes	TPC	6	631,00	6036,00	2880,1667	1902,69655
	TAC	6	893,75	4631,53	1827,5816	1407,40271
	Valid N (listwise)	6				

Tests of Normality

Prune		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
No	height	,270	6	,196	,841	6	,134
	length	,204	6	,200*	,911	6	,442
	width	,179	6	,200*	,910	6	,439
	weight	,278	6	,164	,833	6	,115
	FFR	,334	6	,035	,793	6	,051
	mm2	,251	6	,200*	,868	6	,219
	Tensilforce	,275	6	,176	,860	6	,188
	Lightness	,339	6	,030	,719	6	,010
	a	,228	6	,200*	,914	6	,461
	b	,273	6	,181	,874	6	,243
	C	,235	6	,200*	,886	6	,296
	Hue	,249	6	,200*	,943	6	,684
	TAC	,340	6	,029	,691	6	,005
	TPC	,250	6	,200*	,929	6	,573
Yes	height	,215	6	,200*	,912	6	,449
	length	,202	6	,200*	,875	6	,249
	width	,207	6	,200*	,863	6	,200
	weight	,243	6	,200*	,921	6	,515
	FFR	,190	6	,200*	,936	6	,629
	mm2	,245	6	,200*	,886	6	,297
	Tensilforce	,242	6	,200*	,938	6	,645
	Lightness	,217	6	,200*	,921	6	,513
	a	,167	6	,200*	,982	6	,960
	b	,173	6	,200*	,923	6	,527
	C	,300	6	,097	,832	6	,112
	Hue	,227	6	,200*	,942	6	,677
	TAC	,169	6	,200*	,987	6	,980
	TPC	,171	6	,200*	,928	6	,564

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Kruskal-Wallis Test- Fruit paramteres across pruning treatment

Ranks

	Treatment	N	Mean Rank
height	,00	6	5,83
	1,00	6	7,17
	Total	12	
length	,00	6	4,33
	1,00	6	8,67
	Total	12	
width	,00	6	4,33
	1,00	6	8,67
	Total	12	
weight	,00	6	4,50
	1,00	6	8,50
	Total	12	
FFR	,00	6	7,67
	1,00	6	5,33
	Total	12	
mm2	,00	6	5,00
	1,00	6	8,00
	Total	12	
Tensilforce	,00	6	4,67
	1,00	6	8,33
	Total	12	
Lightness	,00	6	4,67
	1,00	6	8,33
	Total	12	
a	,00	6	4,50
	1,00	6	8,50
	Total	12	
b	,00	6	5,33
	1,00	6	7,67
	Total	12	
C	,00	6	4,50
	1,00	6	8,50
	Total	12	
Hue	,00	6	5,50
	1,00	6	7,50
	Total	12	
TAC	,00	7	8,57
	1,00	7	6,43
	Total	14	
TPC	,00	7	5,43
	1,00	7	9,57
	Total	14	

Test Statistics[a,b]

	height	length	width	weight	FFR	mm2	Tensilforce	Lightness	a	b	C	Hue	TAC	TPC
Kruskal-Wallis H	,410	4,333	4,333	3,692	1,256	2,077	3,103	3,103	3,692	1,256	3,692	,923	,641	2,564
df	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Asymp. Sig.	,522	,037	,037	,055	,262	,150	,078	,078	,055	,262	,055	,337	,423	,109

a. Kruskal Wallis Test

b. Grouping Variable: Treatment

Mann-Whitney U Test - Fruit parameter across pruning treatment

Test Statistics[a]

	height	length	width	weight	FFR	mm2	Tensilforce	Lightness	a	b	C	Hue	TAC	TPC
Mann-Whitney U	14,000	5,000	5,000	6,000	11,000	9,000	7,000	7,000	6,000	11,000	6,000	12,000	13,000	8,000
Wilcoxon W	35,000	26,000	26,000	27,000	32,000	30,000	28,000	28,000	27,000	32,000	27,000	33,000	34,000	29,000
Z	-,641	-2,082	-2,082	-1,922	-1,121	-1,441	-1,761	-1,761	-1,922	-1,121	-1,922	-,961	-,801	-1,601
Asymp. Sig. (2-tailed)	,522	,037	,037	,055	,262	,150	,078	,078	,055	,262	,055	,337	,423	,109
Exact Sig. [2*(1-tailed Sig.)]	,589[b]	,041[b]	,041[b]	,065[b]	,310[b]	,180[b]	,093[b]	,093[b]	,065[b]	,310[b]	,065[b]	,394[b]	,485[b]	,132[b]

a. Grouping Variable: Treatment

b. Not corrected for ties.

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of height is the same across categories of Prune.	Independent-Samples Mann-Whitney U Test	,589[a]	Retain the null hypothesis.
2	The distribution of length is the same across categories of Prune.	Independent-Samples Mann-Whitney U Test	,041[a]	Reject the null hypothesis.
3	The distribution of width is the same across categories of Prune.	Independent-Samples Mann-Whitney U Test	,041[a]	Reject the null hypothesis.
4	The distribution of weight is the same across categories of Prune.	Independent-Samples Mann-Whitney U Test	,065[a]	Retain the null hypothesis.
5	The distribution of FFR is the same across categories of Prune.	Independent-Samples Mann-Whitney U Test	,310[a]	Retain the null hypothesis.
6	The distribution of mm2 is the same across categories of Prune.	Independent-Samples Mann-Whitney U Test	,180[a]	Retain the null hypothesis.
7	The distribution of Tensilforce is the same across categories of Prune.	Independent-Samples Mann-Whitney U Test	,093[a]	Retain the null hypothesis.
8	The distribution of Lightness is the same across categories of Prune.	Independent-Samples Mann-Whitney U Test	,093[a]	Retain the null hypothesis.
9	The distribution of a is the same across categories of Prune.	Independent-Samples Mann-Whitney U Test	,065[a]	Retain the null hypothesis.
10	The distribution of b is the same across categories of Prune.	Independent-Samples Mann-Whitney U Test	,310[a]	Retain the null hypothesis.
11	The distribution of C is the same across categories of Prune.	Independent-Samples Mann-Whitney U Test	,065[a]	Retain the null hypothesis.
12	The distribution of Hue is the same across categories of Prune.	Independent-Samples Mann-Whitney U Test	,394[a]	Retain the null hypothesis.
13	The distribution of TAC is the same across categories of Prune.	Independent-Samples Mann-Whitney U Test	,485[a]	Retain the null hypothesis.
14	The distribution of TPC is the same across categories of Prune.	Independent-Samples Mann-Whitney U Test	,132[a]	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is ,050.

a. Exact significance is displayed for this test.

Spearman's rho Test- Fruit parameter across Treatment

Correlations

	PRUNE			TAC	TPC
Spearman's rho	No	TAC	Correlation Coefficient	1,000	-,107
			Sig. (2-tailed)	.	,819
			N	7	7
		TPC	Correlation Coefficient	-,107	1,000
			Sig. (2-tailed)	,819	.
			N	7	7
	Yes	TAC	Correlation Coefficient	1,000	,929**
			Sig. (2-tailed)	.	,003
			N	7	7
		TPC	Correlation Coefficient	,929**	1,000
			Sig. (2-tailed)	,003	.
			N	7	7

**. Correlation is significant at the 0.01 level (2-tailed).

Correlations

	Prune			Lightness	a	b	C	Hue	height	length	width	weight	FFR	mm2	TAC	TPC
Spearman's rho	Yes	Lightness	Correlation Coefficient	1,000	,771	1,000**	,943**	,943**	-,486	-,314	-,314	-,543	-,200	-,429	,257	,029
			Sig. (2-tailed)	.	,072	.	,005	,005	,329	,544	,544	,266	,704	,397	,623	,957
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		a	Correlation Coefficient	,771	1,000	,771	,829*	,886*	-,029	-,086	-,086	-,371	-,086	-,029	,486	,143
			Sig. (2-tailed)	,072	.	,072	,042	,019	,957	,872	,872	,468	,872	,957	,329	,787
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		b	Correlation Coefficient	1,000**	,771	1,000	,943**	,943**	-,486	-,314	-,314	-,543	-,200	-,429	,257	,029
			Sig. (2-tailed)	.	,072	.	,005	,005	,329	,544	,544	,266	,704	,397	,623	,957
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		C	Correlation Coefficient	,943**	,829*	,943**	1,000	,886*	-,429	-,257	-,257	-,486	,029	-,371	,486	,257
			Sig. (2-tailed)	,005	,042	,005	.	,019	,397	,623	,623	,329	,957	,468	,329	,623
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		Hue	Correlation Coefficient	,943**	,886*	,943**	,886*	1,000	-,257	-,086	-,086	-,371	-,371	-,143	,371	,086
			Sig. (2-tailed)	,005	,019	,005	,019	.	,623	,872	,872	,468	,468	,787	,468	,872
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		height	Correlation Coefficient	-,486	-,029	-,486	-,429	-,257	1,000	,600	,600	,657	-,257	,771	,200	-,029
			Sig. (2-tailed)	,329	,957	,329	,397	,623	.	,208	,208	,156	,623	,072	,704	,957
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		length	Correlation Coefficient	-,314	-,086	-,314	-,257	-,086	,600	1,000	1,000**	,943**	-,543	,943**	,600	,600
			Sig. (2-tailed)	,544	,872	,544	,623	,872	,208	.	.	,005	,266	,005	,208	,208
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		width	Correlation Coefficient	-,314	-,086	-,314	-,257	-,086	,600	1,000**	1,000	,943**	-,543	,943**	,600	,600
			Sig. (2-tailed)	,544	,872	,544	,623	,872	,208	.	.	,005	,266	,005	,208	,208
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		weight	Correlation Coefficient	-,543	-,371	-,543	-,486	-,371	,657	,943**	,943**	1,000	-,429	,886*	,429	,486
			Sig. (2-tailed)	,266	,468	,266	,329	,468	,156	,005	,005	.	,397	,019	,397	,329
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		FFR	Correlation Coefficient	-,200	-,086	-,200	,029	-,371	-,257	-,543	-,543	-,429	1,000	-,486	-,029	,086
			Sig. (2-tailed)	,704	,872	,704	,957	,468	,623	,266	,266	,397	.	,329	,957	,872
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		mm2	Correlation Coefficient	-,429	-,029	-,429	-,371	-,143	,771	,943**	,943**	,886*	-,486	1,000	,486	,429
			Sig. (2-tailed)	,397	,957	,397	,468	,787	,072	,005	,005	,019	,329	.	,329	,397
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		TAC	Correlation Coefficient	,257	,486	,257	,486	,371	,200	,600	,600	,429	-,029	,486	1,000	,929**
			Sig. (2-tailed)	,623	,329	,623	,329	,468	,704	,208	,208	,397	,957	,329	.	,003
			N	6	6	6	6	6	6	6	6	6	6	6	7	7
		TPC	Correlation Coefficient	,029	,143	,029	,257	,086	-,029	,600	,600	,486	,086	,429	,929**	1,000
			Sig. (2-tailed)	,957	,787	,957	,623	,872	,957	,208	,208	,329	,872	,397	,003	.
			N	6	6	6	6	6	6	6	6	6	6	6	7	7
	No	Lightness	Correlation Coefficient	1,000	,771	,657	,829*	,257	,314	,943**	,886*	,657	-,257	,657	-,829*	-,314
			Sig. (2-tailed)	.	,072	,156	,042	,623	,544	,005	,019	,156	,623	,156	,042	,544
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		a	Correlation Coefficient	,771	1,000	,429	,943**	-,086	,543	,829*	,771	,771	,086	,771	-,943**	-,086
			Sig. (2-tailed)	,072	.	,397	,005	,872	,266	,042	,072	,072	,872	,072	,005	,872
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		b	Correlation Coefficient	,657	,429	1,000	,486	,714	-,314	,600	,543	-,029	-,543	,143	-,486	-,771
			Sig. (2-tailed)	,156	,397	.	,329	,111	,544	,208	,266	,957	,266	,787	,329	,072
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		C	Correlation Coefficient	,829*	,943**	,486	1,000	-,143	,371	,943**	,886*	,829*	-,200	,829*	-,829*	-,257
			Sig. (2-tailed)	,042	,005	,329	.	,787	,468	,005	,019	,042	,704	,042	,042	,623
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		Hue	Correlation Coefficient	,257	-,086	,714	-,143	1,000	-,257	,029	,086	-,543	-,143	-,257	-,143	-,371
			Sig. (2-tailed)	,623	,872	,111	,787	.	,623	,957	,872	,266	,787	,623	,787	,468
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		height	Correlation Coefficient	,314	,543	-,314	,371	-,257	1,000	,257	,371	,600	,771	,657	-,600	,771
			Sig. (2-tailed)	,544	,266	,544	,468	,623	.	,623	,468	,208	,072	,156	,208	,072
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		length	Correlation Coefficient	,943**	,829*	,600	,943**	,029	,257	1,000	,943**	,771	-,371	,771	-,771	-,371
			Sig. (2-tailed)	,005	,042	,208	,005	,957	,623	.	,005	,072	,468	,072	,072	,468
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		width	Correlation Coefficient	,886*	,771	,543	,886*	,086	,371	,943**	1,000	,714	-,257	,886*	-,714	-,200
			Sig. (2-tailed)	,019	,072	,266	,019	,872	,468	,005	.	,111	,623	,019	,111	,704
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		weight	Correlation Coefficient	,657	,771	-,029	,829*	-,543	,600	,771	,714	1,000	,029	,829*	-,657	,143
			Sig. (2-tailed)	,156	,072	,957	,042	,266	,208	,072	,111	.	,957	,042	,156	,787
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		FFR	Correlation Coefficient	-,257	,086	-,543	-,200	-,143	,771	-,371	-,257	,029	1,000	,086	-,200	,886*
			Sig. (2-tailed)	,623	,872	,266	,704	,787	,072	,468	,623	,957	.	,872	,704	,019
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		mm2	Correlation Coefficient	,657	,771	,143	,829*	-,257	,657	,771	,886*	,829*	,086	1,000	-,657	,200
			Sig. (2-tailed)	,156	,072	,787	,042	,623	,156	,072	,019	,042	,872	.	,156	,704
			N	6	6	6	6	6	6	6	6	6	6	6	6	6
		TAC	Correlation Coefficient	-,829*	-,943**	-,486	-,829*	-,143	-,600	-,771	-,714	-,657	-,200	-,657	1,000	-,107
			Sig. (2-tailed)	,042	,005	,329	,042	,787	,208	,072	,111	,156	,704	,156	.	,819
			N	6	6	6	6	6	6	6	6	6	6	6	7	7
		TPC	Correlation Coefficient	-,314	-,086	-,771	-,257	-,371	,771	-,371	-,200	,143	,886*	,200	-,107	1,000
			Sig. (2-tailed)	,544	,872	,072	,623	,468	,072	,468	,704	,787	,019	,704	,819	.
			N	6	6	6	6	6	6	6	6	6	6	6	7	7

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Spearman's rho Test- Fruit parameter regardless pruning treatment

Correlations

			TAC	TPC
Spearman's rho	TAC	Correlation Coefficient	1,000	,257
		Sig. (2-tailed)	.	,375
		N	14	14
	TPC	Correlation Coefficient	,257	1,000
		Sig. (2-tailed)	,375	.
		N	14	14

6.2.TPC/TAC values across plant organs

Descriptive Statistics

ORGAN		N	Minimum	Maximum	Mean	Std. Deviation
BaseLeaf	Phenols (mg/100g)	14	1166,0	6596,0	4325,286	1458,3893
	TACmg100g	14	622,00	2682,00	1015,5000	558,87163
	Valid N (listwise)	14				
Flower	Phenols (mg/100g)	14	1846,0	9046,0	5152,429	2211,2357
	TACmg100g	14	762,00	4806,00	2346,0714	1469,73883
	Valid N (listwise)	14				
Fruit	Phenols (mg/100g)	14	591,0	6036,0	2083,143	1487,3902
	TACmg100g	14	893,75	4631,53	1678,8468	937,27111
	Valid N (listwise)	14				
UpperLeaf	Phenols (mg/100g)	14	1366,0	14366,0	5251,714	3792,8003
	TACmg100g	14	1079,00	3155,00	2004,6429	528,73006
	Valid N (listwise)	14				

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
ORGAN		Statistic	df	Sig.	Statistic	df	Sig.
BaseLeaf	Phenols (mg/100g)	,121	14	,200*	,965	14	,808
	TACmg100g	,361	14	,000	,616	14	,000
Flower	Phenols (mg/100g)	,106	14	,200*	,961	14	,734
	TACmg100g	,195	14	,155	,871	14	,043
Fruit	Phenols (mg/100g)	,200	14	,135	,846	14	,019
	TACmg100g	,232	14	,039	,689	14	,000
UpperLeaf	Phenols (mg/100g)	,209	14	,097	,855	14	,026
	TACmg100g	,159	14	,200*	,971	14	,887

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Kruskal-Wallis Test- TPC/TAC values across plant organ

Ranks

	TYP	N	Mean Rank
TAC (mg/100g)	0	14	28,93
	1	14	13,07
	2	14	37,93
	3	14	34,07
	Total	56	
Phenols (mg/100g)	0	14	13,75
	1	14	32,11
	2	14	31,93
	3	14	36,21
	Total	56	

Test Statistics[a,b]

	TAC (mg/100g)	Phenols (mg/100g)
Kruskal-Wallis H	18,851	15,887
df	3	3
Asymp. Sig.	,000	,001

a. Kruskal Wallis Test

b. Grouping Variable: TYP

⇨ Reject the null hypothesis

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Phenols (mg/100g) is the same across categories of ORGAN.	Independent-Samples Kruskal-Wallis Test	,001	Reject the null hypothesis.
2	The distribution of TAC (mg/100g) is the same across categories of ORGAN.	Independent-Samples Kruskal-Wallis Test	,000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is ,050.

TPC
Independent-Samples Kruskal-Wallis Test
Phenols (mg/100g) across ORGAN

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Phenols (mg/100g) is the same across categories of TYP.	Independent-Samples Kruskal-Wallis Test	,001	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is ,050.

Independent-Samples Kruskal-Wallis Test Summary

Total N	56
Test Statistic	15,887[a]
Degree Of Freedom	3
Asymptotic Sig.(2-sided test)	,001

a. The test statistic is adjusted for ties.

Pairwise Comparisons of ORGAN

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
Fruit-UpperLeaf	-18,179	6,164	-2,949	,003	,019
Fruit-BaseLeaf	18,357	6,164	2,978	,003	,017
Fruit-Flower	22,464	6,164	3,644	,000	,002
UpperLeaf-BaseLeaf	,179	6,164	,029	,977	1,000
UpperLeaf-Flower	4,286	6,164	,695	,487	1,000
BaseLeaf-Flower	-4,107	6,164	-,666	,505	1,000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is ,05.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

TAC
Independent-Samples Kruskal-Wallis Test
Phenols (mg/100g) across ORGAN

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of TACmg100g is the same across categories of TYP.	Independent-Samples Kruskal-Wallis Test	,000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is ,050.

Independent-Samples Kruskal-Wallis Test Summary

Total N	56
Test Statistic	18,851[a]
Degree Of Freedom	3
Asymptotic Sig.(2-sided test)	,000

a. The test statistic is adjusted for ties.

Pairwise Comparisons of ORGAN

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
BaseLeaf-Fruit	-15,857	6,164	-2,572	,010	,061
BaseLeaf-Flower	-21,000	6,164	-3,407	,001	,004
BaseLeaf-UpperLeaf	-24,857	6,164	-4,032	,000	,000
Fruit-Flower	5,143	6,164	,834	,404	1,000
Fruit-UpperLeaf	-9,000	6,164	-1,460	,144	,866
Flower-UpperLeaf	-3,857	6,164	-,626	,531	1,000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is ,05.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Spearman's rho Test- TPC/TAC values across plant organ

Correlations

ORGAN				TAC (mg/100g)	Phenols (mg/100g)
BaseLeaf	Spearman's rho	TAC (mg/100g)	Correlation Coefficient	1,000	,407
			Sig. (2-tailed)	.	,149
			N	14	14
		Phenols (mg/100g)	Correlation Coefficient	,407	1,000
			Sig. (2-tailed)	,149	.
			N	14	14
Flower	Spearman's rho	TAC (mg/100g)	Correlation Coefficient	1,000	,675**
			Sig. (2-tailed)	.	,008
			N	14	14
		Phenols (mg/100g)	Correlation Coefficient	,675**	1,000
			Sig. (2-tailed)	,008	.
			N	14	14
Fruit	Spearman's rho	TAC (mg/100g)	Correlation Coefficient	1,000	-,297
			Sig. (2-tailed)	.	,303
			N	14	14
		Phenols (mg/100g)	Correlation Coefficient	-,297	1,000
			Sig. (2-tailed)	,303	.
			N	14	14
UpperLeaf	Spearman's rho	TAC (mg/100g)	Correlation Coefficient	1,000	,543*
			Sig. (2-tailed)	.	,045
			N	14	14
		Phenols (mg/100g)	Correlation Coefficient	,543*	1,000
			Sig. (2-tailed)	,045	.
			N	14	14

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Spearman's rho Test- TPC/TAC values regardless organ

Correlations

			TAC (mg/100g)	Phenols (mg/100g)
Spearman's rho	TAC (mg/100g)	Correlation Coefficient	1,000	,266*
		Sig. (2-tailed)	.	,048
		N	56	56
	Phenols (mg/100g)	Correlation Coefficient	,266*	1,000
		Sig. (2-tailed)	,048	.
		N	56	56

*. Correlation is significant at the 0.05 level (2-tailed).

References

AGES (2020a). Apfelblattsauger. https://www.ages.at/themen/schaderreger/apfelblattsauger/, accessed 25th May 2020.

AGES (2020b). Feuerbrand. https://www.ages.at/download/0/0/066e2c0ef7ecfc702ae31a0a82e172551984a641/fileadmin/AGES2015/Themen/Landwirtschaft_Dateien/Feuerbrand/Feuerbrand_Folder_2020_1a_Din-lang_BF.pdf, accessed 25th May 2020.

Albarouki, E., and Peterson, A. (2007). Molecular and morphological characterization of Crataegus L. species (Rosaceae) in southern Syria. Botanical Journal of the Linnean Society *153*, 255-263. https://doi.org/10.1111/j.1095-8339.2007.00607.x.

Alonso, R., Berli, F.J., Fontana, A., Piccoli, P., and Bottini, R. (2016). Malbec grape (Vitis vinifera L.) responses to the environment: Berry phenolics as influenced by solar UV-B, water deficit and sprayed abscisic acid. Plant physiology and biochemistry : PPB *109*, 84-90. https://doi.org/10.1016/j.plaphy.2016.09.007.

Anderson, E. (1953). Introgressive hybridization. Biological Reviews *28*, 280-307. https://doi.org/10.1111/j.1469-185X.1953.tb01379.x.

Aral, S., and Beşe, A.V. (2016). Convective drying of hawthorn fruit (Crataegus spp.): Effect of experimental parameters on drying kinetics, color, shrinkage, and rehydration capacity. Food Chemistry *210*, 577-584. https://doi.org/10.1016/j.foodchem.2016.04.128.

Arena, M.E., Postemsky, P.D., and Curvetto, N.R. (2017). Changes in the phenolic compounds and antioxidant capacity of Berberis microphylla G. Forst. berries in relation to light intensity and fertilization. Scientia Horticulturae *218*, 63-71. https://doi.org/10.1016/j.scienta.2017.02.004.

Badalica-Petrescu, M., Dragan, S., Ranga, F., Fetea, F., and Socaciu, C. (2014). Comparative HPLC-DAD-ESI(+)MS Fingerprint and Quantification of Phenolic and Flavonoid Composition of Aqueous Leaf Extracts of Cornus mas and Crataegus monogyna, in Relation to Their Cardiotonic Potential. Not Bot Horti Agrobo *42*. https://doi.org/10.15835/nbha4219270.

Barros, L., Carvalho, A.M., and Ferreira, I.C.F.R. (2011). Comparing the composition and bioactivity of Crataegus Monogyna flowers and fruits used in folk medicine. Phytochemical analysis : PCA *22*, 181-188. https://doi.org/10.1002/pca.1267.

Bartha, D. (2014). Crataegus laevigata. In Enzyklopädie der Holzgewächse: Handbuch und Atlas der Dendrologie. Handbuch und Atlas der Dendrologie / herausgegeben von Andreas Roloff … [et al.], B. Stimm, A. Roloff, U.M. Lang and H. Weisgerber, eds. (Weinheim: Wiley), pp. 1–12.

Bernatonienė, J., Masteikova, R., Majienė, D., Savickas, A., Kėvelaitis, E., Bernatonienė, R., Dvoráčková, K., Civinskienė, G., Lekas, R., and Vitkevičius, K., et al. (2008). Free radical-scavenging activities of Crataegus monogyna extracts. Medicina *44*, 706. https://doi.org/10.3390/medicina44090091.

Bewley, R.J.F., and Campbell, R. (1980). Influence of zinc, lead, and cadmium pollutants on the microflora of hawthorn leaves. Microbial ecology *6*, 227-240.

Blanusa, T., Garratt, M., Cathcart-James, M., Hunt, L., and Cameron, R.W.F. (2019). Urban hedges: A review of plant species and cultivars for ecosystem service delivery in north-west Europe. Urban Forestry & Urban Greening *44*, 126391. https://doi.org/10.1016/j.ufug.2019.126391.

Boo, H.-O., Hwang, S.-J., Bae, C.-S., Park, S.-H., Heo, B.-G., and Gorinstein, S. (2012). Extraction and characterization of some natural plant pigments. 11.12.2020.

Böttger, A., Vothknecht, U., Bolle, C., and Wolf, A. (2018a). Lessons on Caffeine, Cannabis & Co (Cham: Springer International Publishing).

Böttger, A., Vothknecht, U., Bolle, C., and Wolf, A. (2018b). Plant Secondary Metabolites and Their General Function in Plants. In Lessons on Caffeine, Cannabis & Co, A. Böttger, U. Vothknecht, C. Bolle and A. Wolf, eds. (Cham: Springer International Publishing), pp. 3–17.

Bradshaw, A.D. (1971). The significance of hawthorns. In History S. C. f. L. (ed.) Hedges and local history (London), pp. 20–29.

Brodowska, K.M. (2017). Natural Flavonoids: Classification, Potential Role, And Application Of Flavonoid Analogues. https://doi.org/10.5281/ZENODO.545778.

Brown, J.A., Beatty, G.E., Finlay, C.M.V., Montgomery, W.I., Tosh, D.G., and Provan, J. (2016). Genetic analyses reveal high levels of seed and pollen flow in hawthorn (Crataegus monogyna Jacq.), a key component of hedgerows. Tree Genetics & Genomes *12*. https://doi.org/10.1007/s11295-016-1020-0.

Burel, F., and Baudry, J. (1995). Social, aesthetic and ecological aspects of hedgerows in rural landscapes as a framework for greenways. Landscape and Urban Planning *33*, 327-340. https://doi.org/10.1016/0169-2046(94)02026-C.

Byatt (1975). Hybridization between Crataegus monogyna Jacq. and C. laevigata (Poiret) DC. in south-eastern England *1975*.

Calişkan, O., Gündüz, K., Serçe, S., Toplu, C., Kamiloğlu, O., Sengül, M., and Ercişli, S. (2012). Phytochemical characterization of several hawthorn (Crataegus spp.) species sampled from the Eastern Mediterranean region of Turkey. Pharmacognosy magazine *8*, 16-21. https://doi.org/10.4103/0973-1296.93305.

Campbell, C.S., Evans, R.C., Morgan, D.R., Dickinson, T.A., and Arsenault, M.P. (2007). Phylogeny of subtribe Pyrinae (formerly the Maloideae, Rosaceae): Limited resolution of a complex evolutionary history. Plant Syst. Evol. *266*, 119-145. https://doi.org/10.1007/s00606-007-0545-y.

Cantín, C.M., Moreno, M.A., and Gogorcena, Y. (2009). Evaluation of the antioxidant capacity, phenolic compounds, and vitamin C content of different peach and nectarine Prunus persica (L.) Batsch breeding progenies. Journal of agricultural and food chemistry *57*, 4586-4592. https://doi.org/10.1021/jf900385a.

Chacoff, N.P., García, D., and Obeso, J.R. (2008). Effects of pollen quality and quantity on pollen limitation in Crataegus monogyna (Rosaceae) in NW Spain. Flora - Morphology, Distribution, Functional Ecology of Plants *203*, 499-507. https://doi.org/10.1016/j.flora.2007.08.005.

Chan, E.W.C., Lim, Y.Y., Wong, S.K., Lim, K.K., Tan, S.P., Lianto, F.S., and Yong, M.Y. (2009). Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species. Food Chemistry, 113(1), 166-172. Food Chemistry *113*, 166-172. https://doi.org/10.1016/J.FOODCHEM.2008.07.090.

Christensen, K.I. (1992a). Revision of Crataegus Sect. Crataegus and Nothosect. Crataeguineae (Rosaceae-Maloideae) in the Old World. Systematic Botany Monographs *35*, 1. https://doi.org/10.2307/25027810.

Christensen, K.I. (1992b). The Structure of Some Crataegus (Rosaceae) Populations in Greece. Willdenowia *1992*, 65-79.

Coimbra, A.T., Luís, Â.F.S., Batista, M.T., Ferreira, S.M.P., and Duarte, A.P.C. (2020). Phytochemical Characterization, Bioactivities Evaluation and Synergistic Effect of Arbutus unedo and Crataegus monogyna Extracts with Amphotericin B. Current microbiology *77*, 2143-2154. https://doi.org/10.1007/s00284-020-02125-w.

Coklar, H., Akubult, M., Kilinc, S., YILDIRIM, A., and ALHASSAN, I. (2018). Effect of Freeze, Oven and Microwave Pretreated Oven Drying on Color, Browning Index, Phenolic Compounds and Antioxidant Activity of Hawthorn (Crataegus orientalis) Fruit. Not Bot Horti Agrobo *46*, 449-456. https://doi.org/10.15835/nbha46211027.

Cömert, E.D., Mogol, B.A., and Gökmen, V. (2020). Relationship between color and antioxidant capacity of fruits and vegetables. Current research in food science *2*, 1-10. https://doi.org/10.1016/j.crfs.2019.11.001.

Contreras-Calderón, J., Calderón-Jaimes, L., Guerra-Hernández, E., and García-Villanova, B. (2011). Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. Food Research International *44*, 2047-2053. https://doi.org/10.1016/j.foodres.2010.11.003.

Croteau, R., Kutchan, T.M., and Lewis, N.G. (2000). Natural products (secondary metabolites). Biochemistry and Molecular Biology of Plants (Chicester: John Wiley and Sons).

Croxton, P.J., and Sparks, T.H. (2002). A farm-scale evaluation of the influence of hedgerow cutting frequency on hawthorn (Crataegus monogyna) berry yields. Agriculture, Ecosystems & Environment *93*, 437-439. https://doi.org/10.1016/S0167-8809(02)00106-8.

Dai, H., Zhang, Z., and Guo, X. (2007). Adventitious bud regeneration from leaf and cotyledon explants of Chinese hawthorn (Crataegus pinnatifida Bge. var. major N.E.Br.). In Vitro Cell.Dev.Biol.-Plant *43*, 2-8. https://doi.org/10.1007/s11627-006-9008-3.

Demiray, H. (2007). Calcium oxalate crystals of some Crataegus (Rosaceae) species growing in Aegean region. Biologia *62*, 150. https://doi.org/10.2478/s11756-007-0004-9.

Dervis, S., Dixon, L., Doğanlar, M., and Rossman, A. (2010). Gall production on hawthorns caused by Gymnosporangium spp. in Hatay province, Turkey. Phytoparasitica *38*, 391-400. https://doi.org/10.1007/s12600-010-0102-z.

Dickinson, T.A., Lo, E., and Talent, N. (2007). Polyploidy, reproductive biology, and Rosaceae: understanding evolution and making classifications. Plant Syst. Evol. *266*, 59-78. https://doi.org/10.1007/s00606-007-0541-2.

Dickinson, T.A., and Phipps, J.B. (1984). Studies in Crataegus (Rosaceae: Maloideae). IX. Short-shoot leaf heteroblasty in Crataegus crus-galli sensu lato. Can. J. Bot. *62*, 1775-1780. https://doi.org/10.1139/b84-241.

Dickinson, T.A., and Phipps, J.B. (1986). Studies in Crataegus (Rosaceae: Maloideae) XIV. The Breeding System of Crataegus crus-galli Sensu Lato in Ontario. American Journal of Botany, 1169-130.

Dönmey, A.A. (2004). The Genus Crataegus L. (Rosaceae) with Special Reference to Hybridisation and Biodiversity in Turkey. Turkish Journal of Botany, 29-37.

Dönmez, E.O. (2008). Pollen morphology in Turkish Crataegus (Rosaceae). Bot. Helv. *118*, 59-70. https://doi.org/10.1007/s00035-008-0823-5.

Drake, C.A., and Clark, J.R. (2003). Effects of Pruning and Cropping on Field-grown Primocane-fruiting Blackberries. HortSci *38*, 260-262. https://doi.org/10.21273/HORTSCI.38.2.260.

Ebod (2018). Digitale Bodenkarte. eBod- Digitale Bodenkarte Österreich. https://bodenkarte.at/#/center/16.07267,47.79183/zoom/19.3. 02.02.2020.

Edwards, J.E., Brown, P.N., Talent, N., Dickinson, T.A., and Shipley, P.R. (2012). A review of the chemistry of the genus Crataegus. Phytochemistry *79*, 5-26. https://doi.org/10.1016/j.phytochem.2012.04.006.

Egea, I., Sánchez-Bel, P., Romojaro, F., and Pretel, M.T. (2010). Six edible wild fruits as potential antioxidant additives or nutritional supplements. Plant foods for human nutrition (Dordrecht, Netherlands) *65*, 121-129. https://doi.org/10.1007/s11130-010-0159-3.

Ellenberg, H., and Leuschner, C. (2010). Vegetation Mitteleuropas mit den Alpen. In ökologischer, dynamischer und historischer Sicht ; 203 Tabellen (Stuttgart: Verlag Eugen Ulmer).

Ercisli, S. (2004). A short review of the fruit germplasm resources of Turkey. Genetic Resources and Crop Evolution *51*, 419-435.

European Agency for the Evaluation of Medicinal Products (1999). Crataegus. Summary Report of Commitee for veterinary medicinal products.

European medicines Agency (2014). European Union herbal monograph on Crataegus spp., folium cum flore. https://www.ema.europa.eu/en/documents/herbal-monograph/final-european-union-herbal-monograph-crataegus-spp-folium-cum-flore_en.pdf, accessed 23th January 2020.

European medicines Agency (2016a). Assessment report on Crataegus spp., folium cum flore. https://www.ema.europa.eu/en/documents/herbal-report/draft-assessment-report-crataegus-spp-folium-cum-flore_en.pdf, accessed 23th January 2020.

European medicines Agency (2016b). Hawthorn leaf and flower, Crataegus spp., folium cum flore. Science Medicines Health *2016*. https://www.ema.europa.eu/en/medicines/herbal/crataegi-folium-cum-flore, accessed 23th January 2020.

Evans, R.C., and Campbell, C.S. (2002). The origin of the apple subfamily (Maloideae; Rosaceae) is clarified by DNA sequence data from duplicated GBSSI genes. American Journal of Botany *89*, 1478-1484. https://doi.org/10.3732/ajb.89.9.1478.

Evans, R.C., and Dickinson, T.A. (2005). Floral Ontogeny and Morphology in Gillenia ("Spiraeoideae") and Subfamily Maloideae C. Weber (Rosaceae). International Journal of Plant Sciences *166*, 427-447. https://doi.org/10.1086/428631.

Fachagentur Nachwachsende Rohstoffe (2017). Vorstellung der Besonderheiten des Arzneipflanzenanbaus und Förderaktivitäten des Bundesminsteriums für Ernährung und Landwirtschaft (Fachagentur Nachwachsende Rohstoffe e.V. (FNR)). https://mediathek.fnr.de/arzneipflanzen-anbau-und-nutzen.html, accessed 27th March 2020.

Fachagentur Nachwachsende Rohstoffe e.V. (2013a). Arzneipflanzen Anbau und Nutzen. https://mediathek.fnr.de/arzneipflanzen-anbau-und-nutzen.html, accessed 27th March 2020.

Fachagentur Nachwachsende Rohstoffe e.V. (2013b). Marktanalyse Nachwachsende Rohstoffe. https://mediathek.fnr.de/arzneipflanzen-anbau-und-nutzen.html, accessed 27th March 2020.

Falcone Ferreyra, M.L., Rius, S.P., and Casati, P. (2012). Flavonoids: biosynthesis, biological functions, and biotechnological applications. Frontiers in plant science *3*, 222. https://doi.org/10.3389/fpls.2012.00222.

Fiol, M., Adermann, S., Neugart, S., Rohn, S., Mügge, C., Schreiner, M., Krumbein, A., and Kroh, L.W. (2012). Highly glycosylated and acylated flavonols isolated from kale (Brassica oleracea var. sabellica) — Structure–antioxidant activity relationship. Food Research International *47*, 80-89. https://doi.org/10.1016/j.foodres.2012.01.014.

Froehlicher, T., Hennebelle, T., Martin-Nizard, F., Cleenewerck, P., Hilbert, J.-L., Trotin, F., and Grec, S. (2009). Phenolic profiles and antioxidative effects of hawthorn cell suspensions, fresh fruits, and medicinal dried parts. Food Chemistry *115*, 897-903. https://doi.org/10.1016/j.foodchem.2009.01.004.

García-Mateos, R., Ibarra-Estrada, E., and Nieto-Angel, R. (2013). Antioxidant compounds in hawthorn fruits (Crataegus spp.) of Mexico. Revista Mexicana de Biodiversidad *84*, 1298-1304. https://doi.org/10.7550/rmb.35675.

García-Pérez, J.V., Carcel, J.A., Mulet, A., Riera, E., and Gallego-Juarez, J.A. (2015). Ultrasonic drying for food preservation. In Power ultrasonics. Applications of high-intensity ultrasound / edited by Juan A. Gallego-Juárez, Karl F. Graff, J.A. Gallego-Juárez and K.F. Graff, eds. (Elsevier), pp. 875–910.

Gharaghani, A., Solhjoo, S., and Oraguzie, N. (2016). A review of genetic resources of pome fruits in Iran. Genet Resour Crop Evol *63*, 151-172. https://doi.org/10.1007/s10722-015-0334-3.

Gosler, A. (1990). Introgressive hybridization between Crataegus monogyna Jacq. and C. laevigata (Poiret) DC. In the Upper Thames Valley, England. Watsonia *18*.

Gosler, A.G., KELLY, C.K., and BLAKEY, J.K. (1994). Phenotypic plasticity in leaf morphology of Crataegus monogyna (Rosaceae): an experimental study with

taxonomic implications. Botanical Journal of the Linnean Society *115*, 211-219. https://doi.org/10.1111/j.1095-8339.1994.tb01779.x.

Grahofer, E., and Scharmayr, G. (2016). Der Weißdorn als Futterquelle. In Weißdorn. Ein unterschätzter Alleskönner, Verein Naturvermittlung, ed. (Linz: Freya).

Granata, M.U., Bracco, F., Nola, P., and Catoni, R. (2020). Photosynthetic characteristic and leaf traits variations along a natural light gradient in Acer campestre and Crataegus monogyna. Flora *268*, 151626. https://doi.org/10.1016/j.flora.2020.151626.

Grassmann, J., Schnitzler, W.H., and Habegger, R. (2007). Evaluation of different coloured carrot cultivars on antioxidative capacity based on their carotenoid and phenolic contents. International journal of food sciences and nutrition *58*, 603-611. https://doi.org/10.1080/09637480701359149.

Guitián, J., and Fuentes, M. (1992). Reproductive biology of Crataegus monogyna in northwestern Spain. Acta Oecologica, 3-11.

Gundogdu, M., Ozrenk, K., Ercisli, S., Kan, T., Kodad, O., and Hegedus, A. (2014). Organic acids, sugars, vitamin C content and some pomological characteristics of eleven hawthorn species (Crataegus spp.) from Turkey. Biological research *47*, 21. https://doi.org/10.1186/0717-6287-47-21.

Güney, M., Kafkas, S., Keles, H., Aras, S., and Ercişli, S. (2018). Characterization of hawthorn (Crataegus spp.) genotypes by SSR markers. Physiology and molecular biology of plants : an international journal of functional plant biology *24*, 1221-1230. https://doi.org/10.1007/s12298-018-0604-6.

Guo, W., Guo, N., Li, W., and Dai, H. (2018). Transcriptome analysis reveals the hawthorn response to the infection of apple chlorotic leaf spot virus. Scientia Horticulturae *239*, 171-180. https://doi.org/10.1016/j.scienta.2018.05.017.

Gyan, K.Y., and Woodell, S.R.J. (1987). Flowering Phenology, Flower Colour and Mode of Reproduction of Prunus spinosa L. (Blackthorn); Crataegus monogyna Jacq. (Hawthorn); Rosa canina L. (Dog Rose); and Rubus fruticosus L. (Bramble) in Oxfordshire, England. Functional Ecology *1*, 261. https://doi.org/10.2307/2389429.

H. Scherm and A. T. Savelle (2003). Epidemic Development of Hawthorn Leaf Blight (Monilinia johnsonii) on Mayhaw (Crataegus aestivalis and C. opaca) in Georgia. Plant Disease, 539-543.

Hanna, W.W., and Bashaw, E.C. (1987). Apomixis: Its Identification and Use in Plant Breeding. Crop Science *27*, 1136-1139. https://doi.org/10.2135/cropsci1987.0011183X002700060010x.

Helden, A.J., Stamp, G.C., and Leather, S.R. (2012). Urban biodiversity: comparison of insect assemblages on native and non-native trees. Urban Ecosyst *15*, 611-624. https://doi.org/10.1007/s11252-012-0231-x.

Hellenbrand, N., Sendker, J., Lechtenberg, M., Petereit, F., and Hensel, A. (2015). Isolation and quantification of oligomeric and polymeric procyanidins in leaves and flowers of Hawthorn (Crataegus spp.). Fitoterapia *104*, 14-22. https://doi.org/10.1016/j.fitote.2015.04.010.

Hiermann, A., Kartnig, T.H., and Azzam, S. (1986). Ein beitrag zur quantitativen bestimmung der procyanidine in crataegus. Scientia Pharmaceutica *54*, 331-337.

Hille-Rohde, S. (1989). Der Weißdorn- Crataegus sp. Ökoporträt *1989*, 1-6.

Huitao, B. (2007). Study on Hawthorn Rust Pathogen Identification, Epidemic Laws and Disease Occurrence Conditions in Western Henan. Journal of Anhui Agricultural Sciences *35*, 5206.

Iapichino, G., and Airò, M. (2009). Multiplication of Crataegus monogyna by In Vitro Culture of Nodal Segments. Acta Hortic., 135-140. https://doi.org/10.17660/ActaHortic.2009.812.13.

Jablonski, E. (2020). Cultivars of European Crataegus - Past and Present. Annales de la Societe Geologique de Belgique, 83-95.

Jarzycka, A., Lewińska, A., Gancarz, R., and Wilk, K.A. (2013). Assessment of extracts of Helichrysum arenarium, Crataegus monogyna, Sambucus nigra in photoprotective UVA and UVB; photostability in cosmetic emulsions. Journal of photochemistry and photobiology. B, Biology *128*, 50-57. https://doi.org/10.1016/j.jphotobiol.2013.07.029.

Kabera, J.N., Semana, E., Mussa, A.R., and He, X. (2014). Plant Secondary Metabolites： Biosynthesis, Classification, Function and Pharmacological Properties. Journal of Pharmacy and Pharmacology *2*, 392.

Kandemir, N., and SAYGILI, İ. (2015). Apomixis: new horizons in plant breeding. Turk J Agric For *39*, 549-556. https://doi.org/10.3906/tar-1409-74.

Karl, E., and Schmelzer, K. (1971). Investigations on the transmissibility of watermelon mosaic viruses by aphid species. Archiv fur Pflanzenschutz *7*, 3-11.

Karl, E., and Wolf, P. (1974). Untersuchungen zur Übertragbarkeit des Selleriemosaik-Virus (celery mosaic virus) durch Blattlausarten. Archives Of Phytopathology And Plant Protection *10*, 75-79. https://doi.org/10.1080/03235407409431103.

Kaul, R. (1998). Der Weissdorn. Botanik, Inhaltsstoffe, Qualitätskontrolle, Pharmakologie, Toxikologie und Klinik (Stuttgart: Wissenschaftliche Verlagsgesellschaft).

Kehr, R., and Butin, H. (2003). Eine neue Blattkrankheit an Weißdorn (Crataegus) – Symptome und Differentialdiagnose. In Jahrbuch der Baumpflege 2003, v. D. Dujesiefken und P. Kockerbeck, ed. (Braunschweig: Thalacker Verlag), pp. 226–229.

Khadivi, A., Heidari, P., Rezaei, M., Safari-Khuzani, A., and Sahebi, M. (2019). Morphological variabilities of Crataegus monogyna and C. pentagyna in northeastern areas of Iran. Industrial Crops and Products *139*, 111531. https://doi.org/10.1016/j.indcrop.2019.111531.

Khadivi-Khub, A., Karimi, S., and Kameli, M. (2015). Morphological diversity of naturally grown Crataegus monogyna (Rosaceae, Maloideae) in Central Iran. Braz. J. Bot *38*, 921-936. https://doi.org/10.1007/s40415-015-0187-1.

Kim, D.-O., and Lee, C.Y. (2005). Extraction and Isolation of Polyphenolics. Pigments, colorants, flavors, texture and bioactive food components/ed. by Ronald E. Wrolstad (Hoboken, N.J.: Wiley-Interscience).

Kirakosyan, A., Kaufman, P., Warber, S., Zick, S., Aaronson, K., Bolling, S., and Chul Chang, S. (2004). Applied environmental stresses to enhance the levels of polyphenolics in leaves of hawthorn plants. Physiol Plant *121*, 182-186. https://doi.org/10.1111/j.1399-3054.2004.00332.x.

Kirakosyan, A., Seymour, E., Kaufman, P.B., Warber, S., Bolling, S., and Chang, S.C. (2003). Antioxidant capacity of polyphenolic extracts from leaves of Crataegus laevigata and Crataegus monogyna (Hawthorn) subjected to drought and cold stress. Journal of agricultural and food chemistry *51*, 3973-3976. https://doi.org/10.1021/jf030096r.

Kjaer, C., Strandberg, M., and Erlandsen, M. (2006). Metsulfuron spray drift reduces fruit yield of hawthorn (Crataegus monogyna L.). The Science of the total environment *356*, 228-234. https://doi.org/10.1016/j.scitotenv.2005.03.019.

Knöss, Reh K, Bodemann S, Kirchner C, Stolte F, and Wiesner J (2014). Rechtliche Rahmenbedingungen. Pharmakon Schwerpunktheft: Komplementäre Therapierichtungen. Pharmakon *2014*, 143-150.

Kolb, C.A., Käser, M.A., Kopecký, J., Zotz, G., Riederer, M., and Pfündel, E.E. (2001). Effects of natural intensities of visible and ultraviolet radiation on epidermal ultraviolet screening and photosynthesis in grape leaves. Plant physiology *127*, 863-875.

Kommission E (1994a). Crataegi flos. Bundesanzeiger: 19.7.1994 *1994*.

Kommission E (1994b). Crategi fructus. Bundesanzeiger: 19.7.1994 *1994*.

Konyalioglu, S., Cebe, G.E., and Aktar, S. (2017). Antioxidant activity of Crataegus Monogyna L flowers. Free Radical Biology and Medicine *108*, S56. https://doi.org/10.1016/j.freeradbiomed.2017.04.197.

Krawczyk, U., and Petri, G. (1991). HPLC Analysis of Procyanidins in Crataegus Extract. Arch. Pharm. Pharm. Med. Chem. *324*, 97-99. https://doi.org/10.1002/ardp.19913240207.

Kusano, M., Tohge, T., Fukushima, A., Kobayashi, M., Hayashi, N., Otsuki, H., Kondou, Y., Goto, H., Kawashima, M., and Matsuda, F., et al. (2011). Metabolomics reveals comprehensive reprogramming involving two independent metabolic responses of Arabidopsis to UV-B light. The Plant journal : for cell and molecular biology *67*, 354-369. https://doi.org/10.1111/j.1365-313X.2011.04599.x.

Lange, D. (2006). International Trade in Medicinal and Aromatic Plants. Actors, volumes and commodities. In Medicinal and aromatic plants. Agricultural, commercial, ecological, legal, pharmacological and social aspects / edited by Robert J. Bogers, Lyle E. Craker and Dagmar Lange, R.J. Bogers, L.E. Craker and D. Lange, eds. (Dordrecht: Springer).

Liu, P., Kallio, H., Lü, D., Zhou, C., and Yang, B. (2011). Quantitative analysis of phenolic compounds in Chinese hawthorn (Crataegus spp.) fruits by high performance liquid chromatography-electrospray ionisation mass spectrometry. Food Chemistry *127*, 1370-1377. https://doi.org/10.1016/j.foodchem.2011.01.103.

Liu, S., Chang, X., Liu, X., and Shen, Z. (2016). Effects of pretreatments on anthocyanin composition, phenolics contents and antioxidant capacities during fermentation of hawthorn (Crataegus pinnatifida) drink. Food Chemistry *212*, 87-95. https://doi.org/10.1016/j.foodchem.2016.05.146.

Liu, S., Zhang, X., You, L., Guo, Z., and Chang, X. (2018). Changes in anthocyanin profile, color, and antioxidant capacity of hawthorn wine (Crataegus pinnatifida) during storage by pretreatments. LWT *95*, 179-186. https://doi.org/10.1016/j.lwt.2018.04.093.

Lo, E.Y.Y., Stefanović, S., and Dickinson, T.A. (2007). Molecular Reappraisal of Relationships Between Crataegus and Mespilus (Rosaceae, Pyreae)—Two Genera or One? Systematic Botany *32*, 596-616. https://doi.org/10.1600/036364407782250562.

Locato, V., Cimini, S., and Gara, L. de (2013). Strategies to increase vitamin C in plants: from plant defense perspective to food biofortification. Frontiers in plant science *4*, 152. https://doi.org/10.3389/fpls.2013.00152.

Lopes, D.B., Queirós, L.D. de, Ávila, A.R.A. de, Monteiro, N.E.S., and Macedo, G.A. (2017). The Importance of Microbial and Enzymatic Bioconversions of Isoflavones in Bioactive Compounds. In Food Bioconversion (Elsevier), pp. 55–93.

López-Ortega, G., García-Montiel, F., Bayo-Canha, A., Frutos-Ruiz, C., and Frutos-Tomás, D. (2016). Rootstock effects on the growth, yield and fruit quality of sweet cherry cv. 'Newstar' in the growing conditions of the Region of Murcia. Scientia Horticulturae *198*, 326-335. https://doi.org/10.1016/j.scienta.2015.11.041.

Lund, J.A., Brown, P.N., and Shipley, P.R. (2017). Differentiation of Crataegus spp. guided by nuclear magnetic resonance spectrometry with chemometric analyses. Phytochemistry *141*, 11-19. https://doi.org/10.1016/j.phytochem.2017.05.003.

Mallet, J.F., Cerrati, C., Ucciani, E., Gamisans, J., and Gruber, M. (1994). Antioxidant activity of plant leaves in relation to their alpha-tocopherol content. Food Chemistry *49*, 61-65. https://doi.org/10.1016/0308-8146(94)90233-X.

Martín Ortega, A.M., and Segura Campos, M.R. (2019). Medicinal Plants and Their Bioactive Metabolites in Cancer Prevention and Treatment. In Bioactive Compounds (Elsevier), pp. 85–109.

Martino, E., Collina, S., Rossi, D., Bazzoni, D., Gaggeri, R., Bracco, F., and Azzolina, O. (2008). Influence of the extraction mode on the yield of hyperoside, vitexin and vitexin-2''-O-rhamnoside from Crataegus monogyna Jacq. (hawthorn). Phytochemical analysis : PCA *19*, 534-540. https://doi.org/10.1002/pca.1081.

Massimino Cocuzza, G.E., and Barbagallo, S. (2017). Dysaphis crataegi (Kaltenbach) (Hemiptera, Aphididae) on fruits of Cucurbita. EPPO Bull *47*, 115-117. https://doi.org/10.1111/epp.12359.

Mayer, C.J., Jarausch, B., Jarausch, W., Jelkmann, W., Vilcinskas, A., and Gross, J. (2009). Cacopsylla melanoneura has no relevance as vector of apple proliferation in Germany. Phytopathology *99*, 729-738. https://doi.org/10.1094/PHYTO-99-6-0729#.

Mazzolari, A.C., Marrero, H.J., and Vázquez, D.P. (2017). Potential contribution to the invasion process of different reproductive strategies of two invasive roses. Biol Invasions *19*, 615-623. https://doi.org/10.1007/s10530-016-1315-y.

McGuire, R.G. (1992). Reporting of Objective Color Measurements *1992*.

Meier, U., Graf, H., Hack, H., Heß, M., Kennel, W., R. Klose, D. Mappes, D. Seipp, R. Stauß, and J. Streif, et al. (1994). Phänologische Entwicklungsstadien des

Kernobstes (Malus domestica Borkh. und Pyrus communis L.), des Steinobstes (Prunus-Arten), der Johannisbeere (Ribes-Arten) und der Erdbeere (Fragaria x ananassa Duch.). 1 *46*, 141.

Melzer, J., and Saller, R. (2005). Weißdorn (Crataegus) bei Herzinsuffizienz. Eine Übersicht. Schweiz. Zschr. GanzheitsMedizin, 362-366.

Montoroa, P., Bracab, A., Pizzaa, C., and Tommasia, N. de (2005). Structure-antioxidant activity relationships of flavonoids isolated from different plant species. Food Chemistry *92*, 349-355. https://doi.org/10.1016/j.foodchem.2004.07.028.

Morgenson, G. (1998). Germination of 3 Crataegus Species. Tree Planters' Notes *49*, 72-74.

Moyer, R.A., Hummer, K.E., Finn, C.E., Frei, B., and Wrolstad, R.E. (2002). Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: vaccinium, rubus, and ribes. Journal of agricultural and food chemistry *50*, 519-525. https://doi.org/10.1021/jf011062r.

Mraihi, F., Hidalgo, M., Pascual-Teresa, S. de, Trabelsi-Ayadi, M., and Chérif, J.-K. (2015). Wild grown red and yellow hawthorn fruits from Tunisia as source of antioxidants. Arabian Journal of Chemistry *8*, 570-578. https://doi.org/10.1016/j.arabjc.2014.11.045.

Murray, B.G. (2017). Hybridization and Plant Breeding. In Encyclopedia of Applied Plant Sciences, 2nd edition, pp. 168–173.

Nabavi, S.F., Habtemariam, S., Ahmed, T., Sureda, A., Daglia, M., Sobarzo-Sánchez, E., and Nabavi, S.M. (2015). Polyphenolic Composition of Crataegus monogyna Jacq.: From Chemistry to Medical Applications. Nutrients *7*, 7708-7728. https://doi.org/10.3390/nu7095361.

Nakabayashi, R., Yonekura-Sakakibara, K., Urano, K., Suzuki, M., Yamada, Y., Nishizawa, T., Matsuda, F., Kojima, M., Sakakibara, H., and Shinozaki, K., et al. (2014). Enhancement of oxidative and drought tolerance in Arabidopsis by overaccumulation of antioxidant flavonoids. The Plant journal : for cell and molecular biology *77*, 367-379. https://doi.org/10.1111/tpj.12388.

Ngoc, P.C., Leclercq, L., Rossi, J.-C., Desvignes, I., Hertzog, J., Fabiano-Tixier, A.-S., Chemat, F., Schmitt-Kopplin, P., and Cottet, H. (2019). Optimizing Water-Based Extraction of Bioactive Principles of Hawthorn: From Experimental Laboratory Research to Homemade Preparations. Molecules (Basel, Switzerland) *24*. https://doi.org/10.3390/molecules24234420.

Orhan, I., Özçelik, B., Kartal, M., Özdeveci, B., and Duman, H. (2007). HPLC Quantification of Vitexine-2″-O-rhamnoside and Hyperoside in Three Crataegus Species and Their Antimicrobial and Antiviral Activities. Chroma *66*, 153-157. https://doi.org/10.1365/s10337-007-0283-x.

Osborne, P. (1984). Bird Numbers and Habitat Characteristics in Farmland Hedgerows. Journal of Applied Ecology *1984*, 63-82.

O'Sullivan, O.S., Holt, A.R., Warren, P.H., and Evans, K.L. (2017). Optimising UK urban road verge contributions to biodiversity and ecosystem services with cost-effective management. Journal of environmental management *191*, 162-171. https://doi.org/10.1016/j.jenvman.2016.12.062.

Özcan, M., Hacıseferoğulları, H., Marakoğlu, T., and Arslan, D. (2005). Hawthorn (Crataegus spp.) fruit: some physical and chemical properties. Journal of Food Engineering *69*, 409-413. https://doi.org/10.1016/j.jfoodeng.2004.08.032.

Panche, A.N., Diwan, A.D., and Chandra, S.R. (2016). Flavonoids: an overview. Journal of nutritional science *5*, e47. https://doi.org/10.1017/jns.2016.41.

Paulin, J.P., Lachaud, G., Cadic, A., and Renoux, A. (1993). Susceptibility of Crataegus species to fire blight. Acta Hortic., 421-426. https://doi.org/10.17660/ActaHortic.1993.338.70.

Pavlovic, J., Mitić, S., Mitić, M., Kocić, G., Pavlović, A., and Tošić, S. (2019). Variation in the Phenolic Compounds Profile and Antioxidant Activity in Different Parts of Hawthorn (Crataegus pentagyna Willd.) During Harvest Periods. Pol. J. Food Nutr. Sci. *69*, 367-378. https://doi.org/10.31883/pjfns/112019.

Payne, J.A., and Krewer, G.W. (1990). Mayhaw. A new Fruit Crop for the south. In Advances in new crops, J. Janick and J.E. Simon, eds. (Portland, Oregon: Timber Press), pp. 317–321.

Peham, J., Garhofer, E., Kral, H., Paszkiewicz, P., and Rosmann, R. (2016a). Der Weißdorn für Experimentierfreudige. In Weißdorn. Ein unterschätzter Alleskönner, Verein Naturvermittlung, ed. (Linz: Freya).

Peham, J., Grahofer, E., Kral, H., Paszkiewicz, P., and Rosmann, R. (2016b). Der Weißdorn für Experimentierfreudige. In Weißdorn. Ein unterschätzter Alleskönner, Verein Naturvermittlung, ed. (Linz: Freya).

Peschel, W., Bohr, C., and Plescher, A. (2008). Variability of total flavonoids in Crataegus--factor evaluation for the monitored production of industrial starting material. Fitoterapia *79*, 6-20. https://doi.org/10.1016/j.fitote.2007.06.010.

Pfiffinger G., and Peham J. (2016). Der Weißdorn als Lebensraum. In Weißdorn. Ein unterschätzter Alleskönner, Verein Naturvermittlung, ed. (Linz: Freya).

Phipps, J.B., Lance, R.W., and O'Kennon, R.J. (2003). Hawthorns and medlars (Portland: Or.: Timber Pr. Royal Horticultural Society plant collector guide).

Pilaske, R. (1999). Natürlich gesund mit Weissdorn. Sanfte Hilfe bei Herzbeschwerden und Bluthochdruck (Augsburg: Midena).

Potter, D., Eriksson, T., Evans, R.C., Oh, S., Smedmark, J.E.E., Morgan, D.R., Kerr, M., Robertson, K.R., Arsenault, M., and Dickinson, T.A., et al. (2007). Phylogeny and classification of Rosaceae. Plant Syst. Evol. *266*, 5-43. https://doi.org/10.1007/s00606-007-0539-9.

Prinz, S., Ringl, A., Huefner, A., Pemp, E., and Kopp, B. (2007). 4'''-Acetylvitexin-2''-O-rhamnoside, isoorientin, orientin, and 8-methoxykaempferol-3-O-glucoside as markers for the differentiation of Crataegus monogyna and Crataegus pentagyna from Crataegus laevigata (Rosaceae). Chemistry & biodiversity *4*, 2920-2931. https://doi.org/10.1002/cbdv.200790241.

Radünz, A.L., Acunha, T.d.S., Giovanaz, M.A., Herter, F.G., and Chaves, F.C. (2014). Intensidade de poda na produção e na qualidade dos frutos de mirtileiro. Intensity of pruning in the production and quality of blueberry fruits. Food Chemistry *36*, 186-191. https://doi.org/10.1590/0100-2945-318/13.

Rahfeld, B. (2011). Mikroskopischer Farbatlas pflanzlicher Drogen (Heidelberg: Spektrum Akademischer Verlag).

Rasmussen, P. (2011). Hawthorn. Crataegus monogyna (Common Hawthorn) or Crataegus laevigata (Midland Hawthorn; Crataegus oxyacantha). Journal of Primary Health Care, 63-64.

Robards, K., Prenzler, P.D., Tucker, G., Swatsitang, P., and Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. Food Chemistry *66*, 401-436. https://doi.org/10.1016/S0308-8146(99)00093-X.

Rodrigues, S., Calhelha, R.C., Barreira, J.C.M., Dueñas, M., Carvalho, A.M., Abreu, R.M.V., Santos-Buelga, C., and Ferreira, I.C.F.R. (2012). Crataegus monogyna buds and fruits phenolic extracts: Growth inhibitory activity on human tumor cell lines and chemical characterization by HPLC–DAD–ESI/MS. Food Research International *49*, 516-523. https://doi.org/10.1016/j.foodres.2012.07.046.

San, S.P., Cullum, J., and Thomidis, T. (2009). An assessment of the relative resistance of three hawthorn species to three strains of Erwinia amylovora using three different inoculation methods. Phytoparasitica *37*, 371-373. https://doi.org/10.1007/s12600-009-0043-6.

Santos, E.L., Maia, B.H.L.N.S., Ferriani, A.P., and Teixeira, S.D. (2017). Flavonoids: Classification, Biosynthesis and Chemical Ecology. In Flavonoids - From Biosynthesis to Human Health, G.C. Justino, ed. (InTech).

Schippmann, U.W.E., Leaman, D., and Cunningham, AB (2006). A comparison of cultivation and wild collection of medicinal and aromatic plants under sustainability aspects. In Medicinal and aromatic plants. Agricultural, commercial, ecological, legal, pharmacological and social aspects / edited by Robert J. Bogers, Lyle E. Craker and Dagmar Lange, R.J. Bogers, L.E. Craker and D. Lange, eds. (Dordrecht: Springer), pp. 75–95.

Schouten, H.J., and van Teylingen, M. (1990). Onderzoek naar de invloed van bloei van wilde meidoorn op bacterievuur in pereboomgaarden. Onderzoek naar de invloed van bloei van wilde meidoorn op bacterievuur in pereboomgaarden.

Schramayr, G. (2016a). Der Weißdorn, seine Systematik und seine Verwandtschaft. In Weißdorn. Ein unterschätzter Alleskönner, Verein Naturvermittlung, ed. (Linz: Freya), pp. 25–43.

Schramayr, G. (2016b). Der Weißedorn und seine morphologischen Besonderheiten. In Weißdorn. Ein unterschätzter Alleskönner, Verein Naturvermittlung, ed. (Linz: Freya).

Schramayr, G. (2016c). Weißdorn- Eine Problempflanze? In Weißdorn. Ein unterschätzter Alleskönner, Verein Naturvermittlung, ed. (Linz: Freya).

Schramayr, G. (2016d). Weißdorn im Garten. In Weißdorn. Ein unterschätzter Alleskönner, Verein Naturvermittlung, ed. (Linz: Freya).

Schramayr, G., and Baumgartner, M. (2016). Der Weißdorn und seine Ansprüche. In Weißdorn. Ein unterschätzter Alleskönner, Verein Naturvermittlung, ed. (Linz: Freya).

Schramayr, G., and Paszkiewicz, P. (2016). Weißdorn und sein eigenartiger Geruch. In Weißdorn. Ein unterschätzter Alleskönner, Verein Naturvermittlung, ed. (Linz: Freya), pp. 97–103.

Schuck, H.J. (2014). Crataegus monogyna. In Enzyklopädie der Holzgewächse: Handbuch und Atlas der Dendrologie. Handbuch und Atlas der Dendrologie / herausgegeben von Andreas Roloff … [et al.], B. Stimm, A. Roloff, U.M. Lang and H. Weisgerber, eds. (Weinheim: Wiley).

Schüller, E., Halbwirth, H., Mikulic-Petkovsek, M., Slatnar, A., Veberic, R., Forneck, A., Stich, K., and Spornberger, A. (2015). High concentrations of anthocyanins in genuine cherry-juice of old local Austrian Prunus avium varieties. Food Chemistry *173*, 935-942. https://doi.org/10.1016/j.foodchem.2014.10.113.

Shahbazi, F., and Rahmati, S. (2013). Mass Modeling of Sweet Cherry (Prunus avium L.) Fruit with Some Physical Characteristics. FNS *04*, 1-5. https://doi.org/10.4236/fns.2013.41001.

Shi, J., Nawaz, H., Pohorly, J., Mittal, G., Kakuda, Y., and Jiang, Y. (2005). Extraction of Polyphenolics from Plant Material for Functional Foods—Engineering and Technology. Food Reviews International *21*, 139-166. https://doi.org/10.1081/FRI-200040606.

Shortle, E., O'Grady, M.N., Gilroy, D., Furey, A., Quinn, N., and Kerry, J.P. (2014). Influence of extraction technique on the anti-oxidative potential of hawthorn (Crataegus monogyna) extracts in bovine muscle homogenates. Meat science *98*, 828-834. https://doi.org/10.1016/j.meatsci.2014.07.001.

Simirgiotis, M.J. (2013). Antioxidant capacity and HPLC-DAD-MS profiling of Chilean peumo (Cryptocarya alba) fruits and comparison with German peumo (Crataegus monogyna) from southern Chile. Molecules (Basel, Switzerland) *18*, 2061-2080. https://doi.org/10.3390/molecules18022061.

Simpson, M.G. (2019). Plant Reproductive Biology. In Plant Systematics (Elsevier), pp. 595–606.

Sonnenschein, M., and Plescher, A. (2005). Industrielle Gewinnung der Arzneidroge. Inkulturnahme und kontrollierter Anbau von Weissdorn (Crataegus spp.). Pharmazie in unserer Zeit *34*, 42-47. https://doi.org/10.1002/pauz.200400103.

Soural, I., Šnurkovič, P., and Bieniasz, M. (2019). l-Ascorbic acid content and antioxidant capacity in less-known fruit juices. Czech J. Food Sci. *37*, 359-365. https://doi.org/10.17221/305/2018-CJFS.

Sparks, T., and Martin, T. (1999). Yields of hawthorn Crataegus monogyna berries under different hedgerow management. Agriculture, Ecosystems and Environment, 107-110.

Spornberger, A., Böck, K., Filipp, M., Kaltenberger, F., and Letzbor-Kalusch, S. (2013). Der professionelle Obstbaumschnitt (Graz: Stocker).

Spornberger, A., Buvac, D., Hajagos, A., Leder, L., Böck, K., Keppel, H., and Vegvari, G. (2014). Impact of a mechanical flower thinning on growth, yield, diseases and fruit quality of sweet cherries (Prunus avium L.) under organic growing conditions. Biological Agriculture & Horticulture *30*, 24-31. https://doi.org/10.1080/01448765.2013.844079.

Strik, B.C., and Poole, A. (1991). Timing and Severity of Pruning Effects on Cranberry Yield Components and Fruit Anthocyanin. HortSci *26*, 1462-1464. https://doi.org/10.21273/HORTSCI.26.12.1462.

Sweet, J.B. (1980). Hedgerow hawthorn (Crataegus spp.) and blackthorn (Prunus spinosa) as hosts of fruit tree viruses in Britain. Ann Applied Biology *94*, 83-90. https://doi.org/10.1111/j.1744-7348.1980.tb03899.x.

Tadić, V.M., Dobrić, S., Marković, G.M., Dordević, S.M., Arsić, I.A., Menković, N.R., and Stević, T. (2008). Anti-inflammatory, gastroprotective, free-radical-scavenging, and antimicrobial activities of hawthorn berries ethanol extract. Journal of agricultural and food chemistry *56*, 7700-7709. https://doi.org/10.1021/jf801668c.

Tahirović, A., Neđad Bašić, Irma Hubijar, Sanela Šito, and Azra Čabaravdić (2015). Comparison of polyphenol content and antioxidant acitivty of extracts from fruits of two crataegus species. Works of the Faculty of Forestry, 38-51.

Tanko, H., Carrier, D.J., Duan, L., and Clausen, E. (2005). Pre- and post-harvest processing of medicinal plants. Plant Genet. Resour. *3*, 304-313. https://doi.org/10.1079/PGR200569.

Tedeschi, R., and Alma, A. (2004). Transmission of Apple Proliferation Phytoplasma by *Cacopsylla melanoneura* (Homoptera: Psyllidae). ec *97*, 8-13. https://doi.org/10.1603/0022-0493-97.1.8.

van Teylingen, M. (2002). Ornamental hosts of Erwinia amylovora and the effect of the fire blight control policy in the Netherland. Acta Hortic., 81-87. https://doi.org/10.17660/ActaHortic.2002.590.9.

Vašková, D., and Kolarčik, V. (2019). Breeding Systems in Diploid and Polyploid Hawthorns (Crataegus): Evidence from Experimental Pollinations of C. monogyna, C. subsphaerica, and Natural Hybrids. Forests *10*, 1059. https://doi.org/10.3390/f10121059.

Veit, M., and Wittig, J. (2005). Vergleichbarkeit und Reproduzierbarkeit. Qualitätskontrolle von Crataegus-Extrakten und -Zubereitungen. Pharmazie in unserer Zeit *34*, 34-40. https://doi.org/10.1002/pauz.200400102.

Verein Naturvermittlung (2016). Weißdorn. Ein unterschätzter Alleskönner (Linz: Freya).

Vuolo, M.M., Lima, V.S., and Maróstica Junior, M.R. (2019). Phenolic Compounds. In Bioactive Compounds (Elsevier), pp. 33–50.

Waterhouse, A.L. (2005). Determination of Total Phenolics. Pigments, colorants, flavors, texture and bioactive food components/ed. by Ronald E. Wrolstad (Hoboken, NJ: Wiley-Interscience).

Weber, R.W.S. (2014). Biology and control of the apple canker fungus Neonectria ditissima (syn. N. galligena) from a Northwestern European perspective. Erwerbs-Obstbau *56*, 95-107. https://doi.org/10.1007/s10341-014-0210-x.

WHO (2004). WHO Monographs on Selected Medicinal Plants. Folium cum Flore Crataegi *2004*, 66-82.

Xu, X.-M., and Robinson, J.D. (2000). Effects of temperature on the incubation and latent periods of hawthorn powdery mildew (Podosphaera clandestina). Plant Pathology *49*, 791-797. https://doi.org/10.1046/j.1365-3059.2000.00520.x.

Yanar M., Ercisli S., Yilmaz KU, Sahiner H., Taskin T., Zengin Y., Akgul I., and Celik F (2011). Morphological and chemical diversity among hawthorn (Crataegus spp.) genotypes from Turkey. Scientific Research and Essays, 35-38.

Young, J.A., and Young, C.G. (1992). Seeds of woody plants in North America (Portland, Or.: Dioscorides).

ZAMG (2020). Gesamtjahresauswertung 2019- Messstation Puchberg. Klima Jahrbuch. https://www.zamg.ac.at/cms/de/klima/klimauebersichten/jahrbuch.

Zarrinkalam, E., Ranjbar, K., Salehi, I., Kheiripour, N., and Komaki, A. (2018). Resistance training and hawthorn extract ameliorate cognitive deficits in streptozotocin-induced diabetic rats. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie *97*, 503-510. https://doi.org/10.1016/j.biopha.2017.10.138.

Zeĭnalov, Y.M., and Kanygina, N.E. (1988). Pests and diseases of middle east species of hawthorn in conditions of Apsheron. Byulleten' Glavnogo Botanicheskogo Sada, 71-75.

Zeller, W. (1979). Resistance and Resistance Breeding in Ornamentals. EPPO Bulletin *9*, 35-44. https://doi.org/10.1111/j.1365-2338.1979.tb02224.x.

Zhang, Z., Chang, Q., Zhu, M., Ho, W.K.K., and Chen, Z.-Y. (2001). Characterization of antioxidants present in hawthorn. Journal of Nutritional Biochemistry, 144-152.

Zielinska, M., and Michalska, A. (2016). Microwave-assisted drying of blueberry (Vaccinium corymbosum L.) fruits: Drying kinetics, polyphenols, anthocyanins, antioxidant capacity, colour and texture. Food Chemistry *212*, 671-680. https://doi.org/10.1016/j.foodchem.2016.06.003.

Zoratti, L., Karppinen, K., Luengo Escobar, A., Häggman, H., and Jaakola, L. (2014). Light-controlled flavonoid biosynthesis in fruits. Frontiers in plant science *5*, 534. https://doi.org/10.3389/fpls.2014.00534.

Zorniak, M., Szydlo, B., and Krzeminski, T.F. (2017). Crataegus special extract WS 1442: up-to-date review of experimental and clinical experiences. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society *68*, 521-526.