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Investigating the causes of late fruit drop in 'Regina' sweet cherry (Prunus avium)

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Abstract

In the Italian region Trentino - South Tyrol, late fruit drop of ‘Regina’ sweet cherry represents a widespread phenomenon able to significantly reduce the yields and, thus, the profitability of the crop. Its intensity varies from year to year and numerous hypotheses regarding its cause have been formulated including the lack of fertilization, nutrient deficiency of the fruitlets, late effects of frost damages, and competition between vegetative- and reproductive growth. Currently, there is a lack of knowledge about this phenomenon and about its diffusion and intensity in the South Tyrolean climatic conditions.

The presented study aimed to correctly describe the (late) fruit drop pattern of sweet cherry cv. Regina grafted on ‘Gisela 5’ and investigate its internal causes. Several factors such as the limb age, the limb height within the canopy, the limb vigor, as well as the climatic variables were considered in this study.

The present work monitored late fruit drop of ‘Regina’ during the three-year period 2020-22 in several orchards located at different elevations in South Tyrol, in sub-alpine environment.

In the first season, a method to describe the fruit drop pattern was defined and validated. The second season was devoted to a province-based screening of the phenomenon in order to identify potential influences of environmental (altitude, temperature), physiological (limb height above ground) and management factors (pruning). The multisite trial involved 6 commercial orchards homogeneous in age and rootstock located at different elevations, from 225 up to 1175m a.s.l.; flowers/fruit number as well as shoots growth were recorded weekly. The third season was dedicated to find confirmation of the hypothesis formulated during the previous year. The multisite comparison was maintained but reduced to only two orchards to allow more frequent samplings.

From these studies, it emerged that late fruit drop is a complex phenomenon showing variable intensity: the percentage of late fruit drop ranged from 7 to 76% of the fruitlets set, depending on the orchard and on the year considered. Two main waves of fruitlets drop have been observed: the first one was composed by unfertilized parthenocarpic fruitlets, probably caused by late or missing fertilization, that immediately after bloom already showed smaller diameters and symptoms of senescence; the second one (the focus of this study) was composed by fully developed fruits that at a certain point decreased their growth rate and got senescent. All the late dropped cherries showed an aborted embryo. This sudden change has been observed to be concomitant both with prolonged periods of low temperatures (or sudden severe decreases in the daily Growing Degree Hours accumulation) and with extraordinary high temperatures close to or above 30°C. Temperature extreme fluctuations recorded later in the season (from about 50

DAFB) were not followed by new waves of fruit drop; therefore, the existence of a “sensitivity phase” could be assumed. Late fruit drop appeared as a self-regulation of crop load performed by the cherry tree in a situation where the climatic conditions were limiting.

Other factors, such as the position of the limb within the canopy (close to the tree top or to its basal branches) and its orientation (sunny vs. shady side) played only a marginal role sometimes hard to interpret. Excessive vigor of the limb can increase late fruit drop intensity but is not its main cause.

Finally, the soil-, leaf-, and fruitlet analysis data presented suggest that fruit drop was not linked to nutrient deficiencies.

1 Chapter 1: Literature review and aim of the study

The aim of this literature review is to gain an understanding of the main biological and physiological processes behind successful fruit development, from flower initiation to maturity, and of the factors influencing fruit set.

1.1 Floral biology and fruit set

In sweet cherry (*Prunus avium*) most of the flower buds are found on the spurs and to a lesser extent on the 1-year-old shoots. The former are very short fruiting formations that end in an apical woody bud and bear many flower buds close together. The latter, on the other hand, bear flowers mainly in their basal and sometimes in their median part, in greater or lesser portions depending on the cultivar and on the vigor of the tree. The flower bud density, defined as number of flower buds per linear meter (or cm) of branch, and the number of flowers per flower bud are highly influenced by the cultivar, the rootstock, and by the climatic conditions (Garcia-Montiel et al., 2010). On average, each flower bud contains 3 hermaphrodite flowers, ranging between 1 to 7 (Herrero et al., 2017), but no leaf primordia (Sønsteby et al., 2019). Like other fruiting trees belonging to the Rosaceae family, flower initiation and primordial development occurs during the previous late summer and may be anticipated or delayed depending on cultivar, light conditions, nutrition, vigor, and climate (Sønsteby et al., 2019).

1.1.1 From flower initiation to fertilization

Studies carried out on the cultivar ‘Regina del mercato’ have shown that morphological changes in the meristematic apex are recognizable as early as mid-May (transition from a cone-like to a dome-like meristematic apex); the first flower primordia begin to be visible in late June, and by mid-July the first stages of differentiation of the calyx and later those of the corolla are noted. By mid-August the ovary and the style are differentiated, and by mid-September the anthers are evident (Bargioni, 1978).

In the spring, when the chilling hours are fulfilled and the air temperature starts to rise, during the two weeks preceding anthesis, ovary and nucellus grow rapidly; at the beginning of this period the megaspore mother cell is formed, and the macrogametophyte is ready approximately 7 to 3 days before anthesis. At about the same time, microsporogenesis occurs. In general, despite the efforts of the breeding programs, sweet cherry cultivars still tend to have a rather high chilling requirement ranging from 400 to 1700 hours below 7°C (Bargioni, 1978; Guo et al., 2020). Two ovules are formed in the ovary, and these ovules slowly grow for a period of 55 to 14 days before flowering. After the pollination, an acceleration in growth occurs but, with some exceptions, 7 to 2 days before flower opening one of the two ovules suddenly stops growing and is aborted (Hedhly et al., 2012). Anther dehiscence occurs at flowering when the stigma shows maximum receptivity. Bargioni et al. (1978) reported that the average number of pollen grains per anther can be variable depending on the cultivar, ranging from the 876 of the 'Ciliegia di Revoli' up to the 2968 of the 'Precoce di Bagno di Ripoli'. The vigor of the rootstock as well as the position on the tree can significantly affect the flowering time (generally with the basal branches reaching full bloom some days earlier than the top). 4 to 7 days usually elapse between opening and petal fall of the same flower, and blooming can be accomplished in 7 to 10 days under ideal climatic conditions; rainy springs or low air temperatures can greatly prolong these periods (Erez et al., 1998).

The pollen has an elliptical or spherical shape (Guo et al., 2020). Although some transport of pollen by wind cannot be ruled out, pollination of sweet cherry is essentially entomophilous. In general, ideal conditions for a successful pollination are that it occurs shortly after flower opening and that temperatures in the days immediately following are not limiting; the work of Sagredo et al. (2017) showed that for 'Regina' the effectiveness for fertilization was strongly reduced as the anthesis progressed. The same observation was reported for several sour cherry varieties (Lech et al., 1983). The stigma is receptive starting from petals opening and is covered with abundant secretion. When pollen grains land on the stigma they hydrate and germinate producing a pollen tube; this happens within 24 hours from pollination (Hedhly et al., 2012). Pollen germination rate (PGR) can be highly variable: examples were reported by several authors who measured an *in Vitro* PGR of 6% for 'Santina', 7% for 'Black Pearl', 34% for 'Brooks' (Guo et al., 2020), 16% for 'Tumberlay', 77% for 'Incrocio Bargioni 111' (Bargioni et al., 1978), >95% for both 'Lapins' and 'Summit' cultivars (Else et al., 2004) and 41% for 'Stella' (Choi et al., 2001). Rate fluctuations must be interpreted as function of several parameters including incubation time, temperature, and percentage of sucrose in the germination medium. Under the same environmental conditions, PGR was found to be positively correlated with pollen grain size (Guo et al., 2020). The pollen grains move along this newly forming cell wall. Excluding the living tip, which is viable and able to extend, the pollen tube is essentially the empty cell wall left behind. Later, the pollen tube passes through the style: in cherry it is of the solid type with an inner transmitting tissue where the pollen tubes grow between cells. Here the cells of the transmitting tissues, which are rich in starch, provide the carbohydrates required to build the new cell wall. This provision of resources is not unlimited and strong competition takes place in the stigma between the elongating pollen tubes. Although hundreds of pollen grains land on the stigma, in the end only 1 to 3 are then able to reach the ovary (Herrero, 2017). Although externally the style appears as a cylinder, internally it is funnel-shaped and leaves less space and fewer carbohydrates to the pollen tubes as they proceed downward. The ovary contains two ovules and fertilization of at least one of the two is the prerogative of fruit set (Lech et al., 1983). The ovary is made up of several concentric wrappings: the female gamete, the egg cell, is contained into the embryo sac, which in turn is contained in the nucellus, which is wrapped by two integuments. The pollen tube will have to pass through all these envelopes to meet the female gamete. First, the pollen tube faces the obturator and passes through the micropyle, a narrow corridor formed by the two integuments, enters the nucellus and reaches the embryo sac where it discharges the two spermatids (fertilization). The first spermatid fuses with the egg cell forming the zygote that will develop into the embryo while the second one will fuse with the polar nucleus forming the endosperm. The time between pollination and fertilization is called the progamic phase (Hedhly et al., 2012). The Effective Pollination Period (EPP) is defined as the number of days during which pollination is able to result in a fruit: this is determined by the longevity of the ovule minus the time lag between pollination and fertilization (Bound et al., 2014). The main parameters that can influence EPP are stigmatic receptivity, pollen tube growth kinetics and ovule longevity; these are in turn influenced by physiological and environmental factors such as cultivar, pollen quality, plant growth regulators, nutrition, and temperature. This period can range between 4 to 8 days, and it is influenced by air temperatures at bloom (Garcia-Montiel et al., 2010; Hedhly et al., 2012; Sagredo et al., 2017); for example, EPP lasts 4 days for 'Bing' and more than 10 days for 'Stella' but with variations from year to year. 'Regina' ovule longevity can vary from 1 to 7 days depending on the season (Bound et al., 2014). In cherry the amount of time after pollination that is needed for the pollen tube to successfully reach the base of the style and meet the ovary can be variable: 24h for 'Katalin' and 48h for 'Kordia' pollinizing 'Regina' (Sagredo et al., 2017) or more generally from 48 to 96 hours (Bargioni, 1978; Hedhly et al., 2012). The speed of pollen tube growth depends on air temperature (Lech et al., 1983; Garcia-Montiel et al., 2010).

In conclusion, several conditions must be met for a successful pollination: availability of viable and compatible pollen, transfer of pollen at the time when the stigmas are receptive, growth of the pollen tube when the embryos are mature, and ovules are viable.

1.1.2 Failures in pollination

In cherry some pollen tubes arrest their growth during this pathway: this can be related to the ageing of the pistil or to the lack of secretions during these steps (Herrero et al., 2017). Problems in successful fertilization can occur in each of the territories traversed by the pollen tube. The encounter between pollen and stigma can be threatened by short stigmatic receptivity, which reduces the EPP. Once the encounter between stigma and pollen has occurred, the pollen tube passes through the style where intense competition between extending tubes occurs. The most severe selection is known as pollen-pistil incompatibility.

Flower quality appears to be strongly influenced by its carbohydrate content. In other crops (apricot and avocado) the starch content of flowers appears to be strongly correlated with their fate (Herrero, 2017). Some authors suggested that, since a single flower bud can contain numerous flowers, even intra-bud competition is conceivable (Gibeaut et al., 2017). Thus, it appears that flower life, beginning during the previous summer with flower differentiation, continuing through winter until spring, can have a strong effect on the fruit set. Although cropping is an annual event, and only 55 to 100 days are needed from full bloom to harvest, the process that elapse from the first biochemical signal leading to flower initiation up to harvest covers a much longer period of time estimated about 15 months (Lang, 2001).

If the orchard is not properly designed, with enough viable and compatible pollinizers with similar bloom timing, problems of pollen stigma encounter may occur. Although in some areas one cultivar may prove to be an excellent pollinizer for a second one, in a different climate this may not be the case. There is a lack of knowledge regarding the cold requirements of different varieties and the control of dormancy. If the breaking of dormancy (both endo- and ecodormancy) is not simultaneous for the two varieties, we may have mismatches in flowering and lack of successful pollination (Herrero, 2017). Sagredo et al. (2017) reported very low fruit set and poor crop load for the combination of the two partially cross-compatible cultivars 'Kordia' and 'Regina' in the southern Chile in both years of the study. He calculated a 10% fruit set for both 'Kordia' and 'Regina' in the first season (low spring temperature) and a 10% for 'Regina' but a 5% for 'Kordia' in the second one (higher spring temperature). Other authors reported a much higher fruit set for 'Kordia' in Italy's alpine region "Trentino – South Tyrol" (about 38% if impollinated by 'Regina') and a variable fruit set for 'Regina' (ranging from 8,5% up to 18,8% if impollinated by 'Kordia') depending on the year and on intensity of the fruitlets drop (Zago et al., 2011). All the authors agreed that 'Regina' flowering coincided only with the second half of 'Kordia' blooming period. Bargioni (1978) emphasized that the concept of best pollinizer should be considered valid only for a given growing area and not in an absolute sense, too many being the causes that can lead to mismatches in flowering time and embryo sack longevity (climatic pattern, rootstock, cultural technique). Zago et al. (2011) also emphasized that the concept of best pollinizer is not necessarily bi-directional: in fact they reported 'Regina' as the best pollen donor cultivar for 'Kordia' and 'Kordia' as the worse pollen donor cultivar for 'Regina'. Another component of the cropping variability can be the distance from the pollinizer: it has been shown that the fruit set and the yields decreased as the distance between the ‘Regina’ tree considered and the pollinizer ‘Sam’ increased (Núñez-Elisea et al., 2008).

In sour cherry, Lech et al. (1983) detected a considerable amount of pollen on the stigma of the flowers in both of the seasons considered. They also reported that not all of them were able to germinate and that a very high percentage of the germinated ones showed stunted growth or swollen ends. About 10-12 tubes were able to penetrate the style to its basis but only 2-3 of them grew into the ovary. Although at the end of blooming almost every flower showed pollen tubes at the base of the style, the fruit set was poor, indicating that a very high amount of variables are able to influence fruit set.

1.1.3 Pollen-pistil incompatibility

Self-incompatibility is a trait that characterizes most sweet cherry cultivars and some sour cherry cultivars. It takes the name S-RNase-based gametophytic Self Incompatibility (SI) and is genetically determined by a single S-locus, with multiple alleles, able to regulate pollen tube growth. The multi-allelic S-locus encodes for a ribonuclease (S-RNase) expressed by the style and for an S-locus F-box (SFB) protein expressed by the pollen. During pollen tube growth, these two elements interact in an allele-specific manner eventually resulting in an SI reaction. Tube growth is inhibited when style and pollen express the same allele; fertilization can only occur in the case where the SFB allele expressed by the haploid pollen is different from the two S-RNase alleles expressed by the diploid style. This SI-system prevents self-pollination as well as cross-pollination between varieties sharing the same S-genotype (Herrero, et al., 2017).

The first self-fertile sweet cherry seedling was obtained in the 1950s by crossing ‘Emperor Francis’ as pistil donor and with the ‘Napoleon’ pollen previously exposed to X-radiations. Self-compatible varieties appear to have several advantages including being universal pollen donors due to the S4' allele and depending less on pollinator activity. However, Choi et al. (2001) observed higher fruit set in open-pollinated self-fertile genotypes than in the self-pollinated ones, emphasizing the importance of additional pollen donors. Surprisingly, they also found no statistically significant difference between the fruit set obtained from fully compatible pollination (4 different allels) and half compatible pollination.

1.1.4 Sterility phenomena due to climatic factors

Climatic factors such as temperature and humidity can accentuate the phenomenon of sterility (Sagredo, et al. 2017). They may intervene before, during or after anthesis either directly or indirectly. In addition to the well-known direct damage from late frosts on flower buds or on the flowers themselves, some studies indicate how at temperatures below 21°C pollen tube growth is slowed, so that the spermatids may reach the embryo sac when the egg cell has already begun to degenerate. The optimum temperature of about 25°C has been reported by Bargioni (1978), but its response is dependent on the genotype and on its adaptation to cool or to milder latitudes. Stigmas are particularly sensitive to high temperatures: their receptivity lasts 5 days at 10°C, 2 days at 20°C, and just 1 day at 30°C (Herrero, 2017). Temperature can also affect ovary performance. A slight increase in temperature during the phase from pollination to fertilization can result in low fruit set due to a high percentage of flowers having both ovules degenerated. It is quite common to find high percentages of degenerated ovules already during anthesis or even just before (Bargioni, 1978); it has been proposed that the short longevity of the ovule and of the embryo sac may explain low fruit set percentages, especially when a delay in pollination occurs (Bound et al., 2014; Herrero, 2017). Hedhly et al. (2012) studied the effect of increased temperature at bloom in field conditions. Thanks to a polyethylene cage, the authors were able raise the average temperature by 1,4°C and by 3°C in the first and in the second season, respectively. This has hastened pollen tube growth, halved the number of tubes in the style, accelerated ovule degeneration, decreased the percentage of fertilized flowers and drastically reduced fruit set and crop load. On the other hand, other authors reported that embryo development may be compromised also if the temperature is maintained for some time below 18°C (Bargioni, 1978). Low temperatures can also prevent or slow the activity of pollinating insects. A generic negative effect of low temperatures and rainy weather at anthesis on fruit set of 'Regina' (Bound et al., 2014) and of other varieties (Neilsen et al., 2014) has been reported. Direct damages can be caused also by water consisting mainly of pollen washed away from anthers and stigmas, and bursting of granules due to their excessive swelling (Bargioni, 1978). High temperature (20-25°C) in the days before fruit set could also play a role: in sweet cherry it resulted in earlier flowering, reduced pistil size as well as low fruit set (Choi et al., 2001). Sønsteby et al. (2019) reported a higher proportion of non-breaking flower buds (dead or dormant) in trees exposed to elevated temperature during the previous summer than the control.

1.2 Fruit development and its vascular flows

1.2.1 From fertilization to harvest

According to Bargioni (1978), after the fusion of the two spermatids with the egg cell and the polar nucleus, the ovary begins to develop. The ovary first grows rapidly for about two weeks after full bloom (depending on the cultivar and on the climatic conditions); then suddenly its development almost stops, and its growth continues imperceptibly for about 14 days. This stationary phase is followed by a second period of rapid growth that continues until the fruit is ripe. The first period of rapid development of the ovary is matched by an equally rapid development of the nucellus and the integuments (Azarenko et al., 2008), which subsequently grow slowly, and with modest increase in size, until the fruit is almost ripe. The endosperm and the embryo develop very slowly for a relatively long period after fertilization but, during the stationary phase of ovary growth, they rapidly resume growth until about 30 days after full bloom (Bargioni, 1978); then, their development continues slowly to complete near fruit maturity.

Parallel to ovary growth is the formation of the fruit according to a process well described by many authors (Flore, et al., 1999; Else et al., 2004; Blanusa et al., 2005; Ayala et al., 2008; Azarenko et al., 2008; Morandi et al., 2019; Mancini et al., 2021). The fruit consists of four distinct parts: the exocarp (or skin), the fleshy mesocarp, the lignified endocarp, and the embryo (Flore et al., 1999). Cumulative growth, expressed as volume or fresh weight increase, has been described as a double-sigmoidal curve consisting of three steps: the initial phase of exponential growth of the fleshy mesocarp, beginning with fertilization and commonly associated with cell-division (Else et al., 2004) or cell-division and expansion (Azarenko et al., 2008) (Stage I); the "lag phase," a period characterized by minimal growth of the mesocarp and simultaneous development of the embryo and of the seed-associated tissues ("pit hardening" or Stage II) (Azarenko et al., 2008); and a second phase of exponential growth, commonly associated with cell-expansion and dry matter accumulation (Ayala et al., 2008), that ends with fruit ripening ("final swell" or Stage III) (Flore et al., 1999). However, some authors have suggested that the traditional approach that identifies fertilization as the starting point of the first phase of exponential growth (Stage I) ignores about half of this phase as it begins in pre-anthesis (about two weeks before anthesis, coincident with bud scale separation) with an increase in ovary's cell number (cell division) starting at the completion of eco-dormancy. At anthesis, 47% of a mature sour cherry fruit cells are already present (Tukey et al. (1939) cited by Gibeaut et al. (2017)).

The fruit growth depends on its size as well as on its potential Relative Growth Rate (RGR) in the time interval considered: thus, the ovary growth in pre-anthesis can have significant consequences on fruit growth in post-anthesis. Indeed, a positive correlation has been found between ovary size at anthesis and final fruit size at commercial harvest (Ayala et al., 2004; Gibeaut et al., 2017). In the work of Azarenko et al. (2008), diameter measurements from shuck split up to commercial harvest were plotted as a function of Growing Degree Hours (GDH, calculated from peak bloom) revealing a double-sigmoidal curve. The second derivative was used to identify concavity changes or end points of the function for each stage of development. Stage II duration was found to be generally correlated with the time of maturity (longer in late-ripening varieties such as 'Regina' or 'Sweetheart' than in early-ripening ones). Stage III duration was found to be less valid in predicting ripening time, although the late-ripening varieties showed the longest stage III duration. Interestingly, in both years considered, end points of stage II and III were found to be similar in 'Bing' despite differences in fruit size. The same authors reported that the fruit development from full bloom up to commercial harvest of 'Regina' accounts for 16.830 GDH. Differently, other authors asserted that time indices of early, middle, and late varieties are similar until the end of the Stage II and that the differences in ovary size and seasonal development are attributable only to the duration of the second exponential growth period SIII (Gibeaut et al., 2017).

Surprinsigly, Gibeaut et al. (2017) reported that unfertilized 'Bing' flowers (obtained thanks to bee-exclusion netting) were able to grow into parthenocarpic fruits, which were retained up to more than 30 DAFB and finally dropped. Although these fruits showed a smaller volume if compared to the fertilized ones already after fruit set, their growth rate were similar until 15/20 DAFB.

1.2.2 Xylem, phloem and transpiration flows

There are four flows to which the fruit is subjected, and the net sum of these determines its daily increment: the xylem and the phloem sap flow, both through the pedicel, and the osmotic uptake and the transpiration losses, via the fruit skin (Morandi et al., 2019; Mancini, et al., 2021). Brüggenwirth et al. (2016) studied the water relations of 'Sam' grafted on rootstock 'Gisela 5' from 19 to 76 DAFB, thus from stage II up to mature stage III. The pit dry mass increased significantly during SII while it remained constant during SIII; in contrast, the pericarp showed very little growth during SII and then accelerated during SIII. Interestingly, Gibeaut et al. (2017) specified that at 30 and 45 DAFB the pit already accounted for 70% and 98% of its final volume, respectively: thus, the so-called "lag phase" (SII) is nothing more than the time required for the last one-third of pit growth ('Chelan', 'Bing' and 'Sweetheart' on 'Mazzard'). RGR of total dry weight accumulation showed a peak at 62 DAFB and then decreased until fruit maturity at 72DAFB (Brüggenwirth et al., 2016). Similarly, Mancini et al. (2021) reported a RGR peak at 51 DAFB for the variety 'Blaze Star' grafted on rootstock 'MaxMa 14' (commercial harvest 56 DAFB). Daily growth patterns, characterized by net volume losses during the day and net increases during the night, have emerged (circadian growth); however, this trend faded until it was no longer evident at late SIII (Brüggenwirth et al., 2016). Fruits detached but left inside the tree canopy decreased in volume by losing water due to transpiration (more pronounced during the day than during the night) and did not show the nocturnal increase. Surprisingly, the circadian rhythm observed on intact fruits was also observable on stem-girdled fruits (without phloem flow) but only in SII and early SIII but not in late SIII. Even if girdled, the fruits in the SII and early SIII were still able to grow but with a lower growth rate than the intact ones. This was not the case of late SIII: here girdled fruits showed net volume losses like those of the detached fruits. Daily phloem flows were low during the SII and the early part of SIII, increased during the middle part of SIII (55 DAFB) and then peaked at 70 DAFB near maturity. Initially, xylem flows were significantly more abundant than phloem flows and remained constant from SII until the middle part of SIII; at about 55 DAFB xylem flows began to decrease tending to zero at 76 DAFB (commercial harvest). Xylem flow and transpiration appeared similar and opposite during SII until 56 DAFB. At mature SIII, no simple relationship between the two flows was evident. From the fruit set to the beginning of SIII (about 44DAFB) xylem and phloem flows contributed with constant proportions to the incoming flow and more precisely 85% for xylem and 15% for phloem. From this point on, the proportions were reversed leading the phloem to become dominant near maturity. Similar trends and values were reported by Morandi et al. (2019). The correlation between dry matter accumulation in the fruit per unit time and phloem flow appeared linear suggesting that the concentration of phloem sap remained almost constant over time. At mature SIII, the phloem flow showed a maximum peak during the day and a minimum peak at night similarly and opposite to the transpiration losses. At all developmental stages considered, transpiration followed the pattern of VPD (Brüggenwirth et al., 2016). However, it is not clear how the intensity of the transpiration losses changes throughout the season: Blanpied (1972) reported that transpiration rate decreased from the pit hardening up to the overripening phase; differently, Brüggenwirth et al. (2016) measured a high transpiration at SII and mature SIII but significantly lower values at early SIII. Morandi et al. (2019) asserted that as fruit size increases, fruit specific transpiration (mg of transpired H_2O per g of fruit per day) decreased from about -100 mg g^{-1} d^{-1} at 30 DAFB to -5mg g^{-1} d^{-1} at 50 DAFB (‘Grace star’ grafted on rootstocks ‘CAB6P’ and ‘Gisela 6’, harvested at 61 DAFB).

1.3 Photoassimilate production and distribution

Carbohydrates partitioning among different organs, reproductive and vegetative, is a function of the remobilization of storage carbohydrates from reserve organs, the (photo)synthesis of new carbohydrates, and the ability of the sinks to call different resources from the partitioning system.

1.3.1 Storage carbohydrates accumulation and partitioning

Storage reserves are organic compounds and nutrients accumulated in excess of current requirements that are essential to sustain the plant during periods of stress, during dormancy, and at the initial stages of spring growth when leaves have not yet reached their full photosynthetic capacity (Measham et al., 2014; Ayala et al., 2015). Nonstructural carbohydrates (NSCs) in sweet cherry are mainly starch, sorbitol, sucrose, fructose, glucose and raffinose and their composition can vary both qualitatively and quantitatively throughout the season (Flore et al., 1999); they accumulate in various organs, including buds, branches, stems, and roots (Measham et al., 2014). In general, just before bud break, the concentration of NSCs decreases in all perennial tissues except the buds where it increases. Thereafter, NSCs slowly increase with increasing leaf area until harvest; from this point on, accumulation is rapid (Flore et al., 1999). Ayala et al. (2015) studied the distribution of carbon storage reserves using 'Regina' grafted on rootstock 'Gisela 6'. During the previous late summer and early autumn, some trees were pulsed with high level of $^{13}CO_2$. At leaf fall as well as during the following spring, different organs were sampled using GC-MS. The first sampling (at leaf abscission) revealed that the highest ^{13}C relative contents (%) were found in 2- and 3-year-old trunk wood, in the roots (both coarse and medium), and in the vegetative buds; significant levels were also found in 1-year-old branch wood, flower buds, and fine roots. The current-season growth (CSG) wood and the bark of various organs showed a much lower level of ^{13}C. At leaf fall, leaf ^{13}C content was 74% lower than immediately after pulsing (likely, due to export and respiration). At bud break, the highest ^{13}C concentrations were found in the flower buds, in the vegetative buds, and in the coarse roots. Significant levels were also measured for the medium and fine roots, and in the 1-year-old wood of the branches. Finally, very low levels were measured in the trunk and bark. At budbreak, most organs showed similar or lower ^{13}C concentrations than those measured at leaf fall; the only exceptions were primarily the flower buds and secondarily the vegetative buds. The greatest reduction in ^{13}C content was measured in the 2- and 3-year-old wood of the trunk. No significant differences were observed between leaf fall and bud break for roots and 1-year-old branch wood. Similar observations were reported by (Cittadini et al., 2008). High levels of ^{13}C were detected at first- (6DBFB) and full bloom (0DAFB), thereafter, the concentration decreased in all organs until it stabilized between 21 DAFB and 35 DAFB. Considering only the aerial part, at the first bloom the highest ^{13}C concentrations were measured in spur flowers, single flowers, and NFS-leaves (the other organs were not yet developed). At full bloom, the ranking was: single flowers, spur flowers, CSG-leaves, NFS-leaves, and FS-leaves. From full bloom to fruit set, ^{13}C levels dropped in all organs albeit with small significant differences. At fruit set (7 DAFB) slightly higher concentrations were shown by the fruits and FS-leaves. At 14 DAFB, fruits had the highest ^{13}C levels followed by FS-leaves. In subsequent sampling the levels were low but always higher than the natural abundance; interestingly, the NFS-leaves were always significantly richer in ^{13}C than the other organs. Once the dry weight of the different organs in each developmental stage was measured, the absolute amount of ^{13}C recovered was calculated: the main organ of accumulation from full bloom to 28 DAFB were the NFS-leaves. Only at 6DBFB, the greatest accumulation was found in CSG-leaves (Ayala et al., 2015).

It is evident that competition between reproductive and vegetative development for storage reserves remobilization is highest at full bloom. The strongest sink activity, expressed as ^{13}C atom % excess per individual organ with respect to its natural ^{13}C abundance level, is exerted by the reproductive organs; however, considering the total mass of organs, it is evident that the vegetative structures exert the greatest sink strength for reserve remobilization (expressed as the amount of ^{13}C partitioned to a specific organ in terms of dry matter).

Measham et al. (2014) studied the effect of different pruning treatments on summer and winter buds NSCs. In the trial, pruning consisted of the elimination of current season extension growth; it was carried out at 4 different times: during stage II, during early stage III (3 weeks before harvest), during late stage III (1 week before harvest) and in postharvest (1 week after harvest). The buds were sampled during the current summer and the following winter. In general, in the summer buds the total soluble solids (TSS) content was double than that of starch; at the same time, sucrose content was low while glucose and fructose content was high. The time of pruning significantly influenced the concentration of soluble solids in the summer buds: the unpruned treatment showed the highest fructose/glucose/sucrose/TSS concentration followed by the postharvest pruned treatment. The lowest NSC content emerged from the shoots pruned at stage II and III: probably the high sink demand by fruit at these stages competed with the needs of the buds. The treatment pruned during stage II showed renewal extension growth. During dormancy, the winter buds showed high sucrose and low glucose and fructose concentrations. The differences observed between the different treatments on summer buds were no longer evident on the winter buds with the only exception of the shoots pruned during stage II, which showed significantly lower sucrose and TSS values probably because of the competition for resources between bud development and shoots regrowth. NSCs in winter buds were much more abundant than those found in summer buds, indicating that accumulation occurs late. The cultivar showed a significant effect on TSS and NSCs content in summer and winter buds. 'Kordia' showed a significant increase in starch content from summer- to winter buds, but, surprisingly, the same evolution was not detected for 'Sweetheart', in which the starch content was constant at both sampling dates (Measham et al., 2014). As reported by Flore et al. (1999) and Measham et al. (2014), during dormancy starch is the most common storage material and sucrose the most common soluble carbohydrate; Flore et al. (1999) also reported that at bud break the most common soluble carbohydrate was represented by sorbitol. Surprisingly, Measham et al. (2014) reported that high crop loads resulted in higher concentration of NCSs in winter buds than low crop loads, probably due to higher sink strength.

1.3.2 Vegetative growth and photoassimilate production

Flore et al. (1999) summarized the principal physiological properties of assimilate supply in sweet cherry. Photosynthetic assimilation rate (*A*) was reported to be about 17,9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ CO_2 and controlled by many environmental factors (cultivar, training system, rootstock, etc.) and by the sink strength of the various organs (crop load, distance of the leaves from the fruit, etc.). Light saturation point was reached at 30% to 50% of full sun. As shade increased, cherry tree leaves increased their surface area, were flatter, thinner, contained more chlorophyll per unit weight, and exhibited lower light compensation points. Shoots were longer and finer, internodes were shorter, leaf area increased as did the number of laterals, but dry weight decreased. As for many *Prunus* species, the relationship between temperature and photosynthesis was described as parabolic, but sweet cherry photosynthesis appeared to be unaffected by temperature increases in the 17°C 30°C range. VPD between leaf and air increased with increasing temperature; VPD >1.5 kPa resulted in closure of the stomata and decreased photosynthesis in sour cherry. The green fruitlet showed positive net photosynthesis that could make up a significant percentage of the photoassimilates used for growth and respiration.

The natural growth habitus of the sweet cherry tree is that of a competitive forest tree in constant search for light. From the terminal buds of the previous year's growth develop the most vigorous shoots: this will allow the canopy, from year to year, to develop more and more in height and breadth, maximizing the capture of sunlight while shading lower and interior branches (or competing trees) (Lang, 2001). There are essentially three leaves populations: 1) Current Season Shoot (CSS)-leaves form and expand as the shoot grows, initially acting as sinks for reserves but, once mature, supporting much of their own growth (Ayala et al., 2008) and becoming sources. Furthermore, after terminal bud set, they contribute to the synthesis and accumulation of reserve substances; 2) Non-Fruiting Spur (NFS)-leaves represent the evolution into the next season of the extension shoots. They show 6-8 leaves per node, bear no fruits, and once expanded act as source for numerous sinks both acropetally and basipetally (Ayala et al., 2008); 3) Fruiting Spur (FS)-leaves have 7-9 leaves as well as numerous fruits at each node and represent the evolution of the previous season NFS (Lang, 2001). FS-leaves and NFS-leaves reach their maximum development rapidly in the season while the current season shoots create new leaf area even up to harvest (Ayala et al., 2008).

The influence of different leaf populations on fruit quality was investigated using ‘Hedelfinger’ grafted on ‘Gisela 5’ and ‘Ulster’ on ‘Gisela 6’ (Ayala, et al., 2004). Six different limb treatments were established on 2-year-old branches by using girdling and defoliation to isolate the various leaf populations acting as source: (C) control; (T1) whole branch girdled at its base before the intersection with trunk; (T2) FS segment girdled at both sides; (T3) FS+NFS segment girdled at both sides; (T4) girdled as (T3) plus removal of all leaves in the FS segment; (T5) girdled as (T3) plus removal of all leaves in the NFS segment. In both cultivar/rootstock combinations, the (T1) variant returned larger and heavier fruits with higher sugar content than all other treatments including the control, indicating that a certain amount of photoassimilates that should have been exported was redirected to the fruit by girdling. A marked effect of the cultivar was evident: for example, the (T3) variant showed worse quality than the control (C) for the 'Ulster'/'Gisela 6' combination and better quality (equivalent to (T1)) for the 'Hedelfinger'/'Gisela 5' combination. This suggests how important is the contribution of photosynthates exported from the current season shoots for certain varieties (or rootstocks). The (T4) variant was detrimental in both combinations (export from NFS-leaves only); interestingly, the (T2) variant (export form FS-leaves only) did not differ from the control for 'Hedelfinger'/'Gisela 5' but negatively affected the fruit quality in the 'Ulster'/'Gisela 6' combination, highlighting the role of FS-leaves photoassimilates or of lateral shoots for certain varieties (Ayala, et al. 2004). These observations indicate that FS-, NFS-, and current season shoots-leaf populations alone are not sufficient for optimal fruit development (with some varietal differences).

Sønsteby et al. (2019) studied the effect of temperature on growth and floral initiation in 'Van' and 'Lapins' grafted on 'Gisela 6'. The experiment was conducted in Ås in southern Norway: by means of a phytotron, the trees were kept at 12°C for 6 weeks until June 13, then distributed at varying temperatures (12, 15, 18, and 21°C) until October 2 under natural daylight conditions. Shoot growth followed a sigmoid pattern and the growth rate increased with increasing temperature; at the end of the trial annual shoots were twice as long at 21°C as at 12°C. For each treatment, the growth rate started to slow down from mid-August and then stopped completely in mid-September. Dissection of the basal buds of the current season shoots on August 15 (after 6 weeks of exposure to different temperatures), showed that only a small proportion were generative. At this stage, the variants kept at the highest temperatures showed a more advanced flower differentiation process. However, 6 weeks later, the variants 18°C and 21°C had not significantly increased the percentage of flower buds in contrast to those at 12°C and 15°C. By October 2, the treatment with the highest percentage of generative buds was the 12°C one for both 'Lapins' and 'Van' (Sønsteby et al., 2019). In a second experiment, the same authors studied the effect of high temperatures (approximately 20,5°C) at different times and for different period lengths during the season. Once again, shoot growth proved to be positively correlated with air temperature. The highest growth rates were recorded for trees kept at high temperatures from June to August, the lowest growth rates for those left outdoors during the same period. The variant left outdoors in June (13,3°C on average) immediately increased the growth rate if exposed to high temperatures in July (21,4°C) and August (20,1°C). However, variants exposed to high temperatures only in August were no longer able to recover with increased growth, and, ended up with the same shoot length of the outdoor trees. Finally, trees exposed to high temperatures in June or June and July immediately ceased to grow at high rates when exposed to the lower outdoor temperature. For almost all the variants, growth ended by mid-August. Interestingly, the authors observed an overlap between the time when shoot growth ceases (the so-called terminal bud set) and the first morphological evidence of floral initiation (Sønsteby et al., 2019). The control mechanism of shoot growth seems to be regulated by air temperature, photoperiodic sensitivity as well as internal factors.

1.3.3 Current season photoassimilate partitioning and sink effect

Reproductive organs (flowers and fruits) and vegetative organs (shoots and roots) represent sinks for carbohydrates, nutrients, water, and phytohormones. The sink effect can be expressed in several ways: 1) by increasing *A* values; 2) by increasing the translocation of photosynthates to sinks; and 3) by increasing dry matter production per unit leaf area (Flore et al., 1999). The sink strength of fruits varies throughout the season and peaks during the SIII (Ayala et al., 2018). External factors such as drought can affect the development of a sink very differently depending on when they occur. In spring, a drought phenomenon would be primarily perceived by extension shoots and only to a lesser extent by the fruit. For example, during SII, when the fruit accumulates little dry matter, any water stress (or precipitation) would have limited effect on the fruit growth. During SIII, fruits are the main sink (to the point that competition with shoots growth may occur); at this time any water stress could adversely affect both fruit growth and shoots extension (Flore et al., 1999; Mancini et al., 2021). During fruit growth, shading reduces fruit sugar content, fruit size, fruit set, and stimulates fruit drop; it also reduces flower induction (Flore et al., 1999). In sweet cherry the reproductive cycle and the vegetative growth occur simultaneously: phenomena of competition may occur between the reproductive sinks and the vegetative sinks as well as between the reproductive sinks themselves (i.e., the growing fruitlets). The parameter Fruit Number to Leaf Area Ratio (FNLAR) can be used as a measure of the crop load. In general terms, the main elements of variability in the FNLAR are pollination efficiency, climatic factors (as rain, relative humidity, and air temperature) and fruit-drop as well as the vigor of the vegetative growth (in turn influenced by rootstock, air temperature, training system and soil humidity). Cittadini et al. (2008) founded that mean fruit weight, titratable acidity, fruit firmness and soluble solids content of ‘Bing’ decreased linearly with increasing FNLAR. So, FNLAR appears to be the main limiting factor for many qualitative parameters, but storage carbohydrates may also play a role. Interestingly, no correlation was found between FNLAR and the mean shoot growth or the trunk cross-sectional area increment. The authors suggested that, although minimal competition might exist between vegetative growth and reproduction, the fruit remained the main sink (Cittadini et al., 2008), but the vigorous 'Mahaleb' rootstock could also have played a role.

The sink demand of an organ and its ability to compete with other sinks in attracting photosynthates depends on its developmental stage during the season. The amount of carbon available for each sink depends on the supply of photosynthates from sources but also on the organ's sink demand. The work of Ayala et al. (2018) studied the partitioning to different competing sinks of pulsed ^{13}C fixed by different leaf populations (FS, NFS and ES) at the following phenological stages: 25 DAFB (SI), 40 DAFB (SII), 44 DAFB (early SIII), 56 DAFB (mid-SIII) and 75 DAFB (late-SIII, terminal bud set); the combination 'Ulster' grafted on 'Gisela 6' was used. At 25 DAFB the ES were shorter, with fewer leaves, and stored less ^{13}C than the spurs (both fruiting and non-fruiting); for every other sampling date the ^{13}C content fixed by the different leaf populations was similar. Fruits directly exposed to $^{13}CO_2$ showed high levels of ^{13}C at 25 DAFB, lower at 40 and 44 DAFB and minimal at 56 and 75 DAFB, thus indicating a progressive loss of photosynthetic capacity. The highest relative ^{13}C distribution to the fruit was found when FS leaves were the pulsed source (from 57,3% to 63,2% of the total ^{13}C recovered by the specific leaf population, with a peak of 79,1% at 56DAFB), followed by NSF (from 31,3% to 45,8%, with a peak of 70,9% at 56DAFB) and finally by ES (from 17,5% to 28,3%, with a peak of 59,2% at 56DAFB). Interestingly, the greatest increase in fruit fresh weight was recorded exactly between 44 and 56 DAFB. The sinks of FS were primarily the fruit followed by the FS leaves and to a lesser extent the FS wood. NFS had the fruit and NFS leaves as the main sink, and to a lesser extent the FS wood and NFS wood. For both FS and NFS, allocation rates to other sinks plummeted at 56 DAFB when the fruit became the primary sink, then rose again at 75 DAFB. In all sampling dates except for 56 DAFB, the sinks of ES were primarily the ES leaves, secondarily the fruit, and to a lesser extent the FS wood, the NFS wood, and the ES wood. At 56 DAFB the export of ES to ES leaves collapsed from about 50% to 28,1%. In general, ES did not seem to represent a great sink for FS and NFS. The lowest ^{13}C export from ES to fruit (17%) was measured at the beginning of SIII (44 DAFB) when shoots were elongating rapidly. Similar results were shown by the same authors in a previous publication; however, in this last work a higher amount of C fixed by NFS was partitioned to ES growth (Ayala et al., 2008). The distribution of ^{13}C between the pericarp (epicarp + mesocarp) and the pit (endocarp + embryo) is highly variable during fruit development: at 25 DAFB (late stage I, beginning of the lag phase) 74,2% of the ^{13}C fixed by FS leaves and its fruits is stored in the endocarp, this percentage remains similar at 40 DAFB and then decreases to 16.6% at 75 DAFB when the pericarp becomes the main sink (Ayala et al., 2018). In summary, Ayala et al. (2018) reported that fruits were the main sink for the ‘Ulster’/’Gisela 6’ combination; similar conclusions were reported in other works (Cittadini et al., 2008; Ayala et al., 2008). On the other side, Kappel (1991) found out that growing shoots represented a greater sink for photosynthates than the fruit for the ‘Lambert’/*Prunus avium* combination. A good explanation to this apparent contradiction can be found in the work of Morandi et al. (2019), who stated that dwarfing rootstocks are able to reduce the sink strength of growing shoots by shifting the balance of water and photosynthate partitioning to the fruits.

1.4 Factors influencing fruit set in sweet cherry

1.4.1 The causes of variable fruit set

Low fruit set has been described as the main factor that can limit sweet and sour cherry profitability by many authors (Bubàn, 1996; Else et al., 2004; Blanusa et al., 2005; Blanusa et al., 2006; Núñez-Elisea et al., 2008; Davarynejad et al., 2009; Bound et al., 2014; Davarynejad et al., 2014; Sagredo et al., 2017; Stopar, 2018; Guo et al., 2020; Askarieh et al., 2021). Fruit set percentage can be calculated as the number of fruits at harvest per total flowers at bloom (Garcia-Montiel et al., 2010). Fruit set percentages can vary widely: from 15% up to 56% for ‘Bing’ on ‘Mahaleb’ (Cittadini et al., 2008), from 4% to 25% for ‘Sunburst’ on ‘Santa Lucia 64’(Hedhly et al., 2012), from 0,5% to 38,7% for ‘Kordia’ on ‘Gisela 5’, and from 3% to 43,7% for ‘Regina’ on ‘Gisela 5’ (Zago et al., 2011). Many hypotheses have been investigated to give an explanation to the variable fruit set. Sagredo et al. (2017) proposed that the lack of flowering synchronization between ‘Kordia’ and ‘Regina’ could negatively affect pollen availability and hence fruit set. Improper combination of pollen donors and pollinated cultivars has been indicated also by Choi et al. (2001), Núñez-Elisea et al. (2008), and Davarynejad et al. (2009). Also pollen quality and bee activity have been pointed out as important factors for successful pollination (Choi et al., 2001; Guo et al., 2020). Choi et al. (2001) and Sagredo et al. (2017) reported that high temperature at bloom could reduce EPP, and Racskó et al. (2007), Hedhly et al. (2012), and Herrero et al. (2017) that the same condition could accelerate ovules degeneration. More generally, some authors reported that high temperature at bloom could negatively affect fruit set (Davarynejad et al., 2009; Hedhly et al., 2012). On the other hand, also low temperature during flowering have been reported to have detrimental effect on the subsequent fruit set (Webster et al., 2006; Bound et al., 2014) due to frost damages (Neilsen et al., 2014) as well as by reducing pollen tube growth rate and hence shortening the EPP (Bargioni, 1978; Choi et al., 2001; Hedhly et al., 2012). Many authors suggested that the fruit set could be negatively influenced by rainy and cloudy weather at bloom (Davarynejad et al., 2009; Hedhly et al., 2012; Stopar, 2018) or more generally by insufficient light conditions (Webster et al., 2006; Racskó et al., 2007; Stopar, 2018). Bradbury (1929), Bargioni (1978), Blanusa et al. (2006), Racskó et al. (2007), Bound et al. (2014), and Herrero (2017) suggested that competition for resources such as carbohydrates could also play a role. ‘Kordia’ and ‘Regina’ have been reported to be particularly prone to poor fruit set on the vigorous ‘Mazzard F12/1’ and ‘Colt’ rootstocks (Bound et al., 2014). Interestingly, Hedhly et al. (2009) reported that flower emasculation, a common practice to carry out controlled pollination, accelerated ovule degeneration and reduced fruit set.

Finally, many authors reported that excessive fruit drop could significantly reduce final fruit set (Bradbury, 1929; Blanusa et al., 2005; Blanusa et al., 2006; Racskó et al., 2007; Davarynejad et al., 2014; Sagredo et al., 2017; Stopar, 2018).

1.4.2 The effect of plant growth regulators on fruit set and drop

Many authors have examined the effect of endogenous content of plant hormones or of the application of plant growth regulators on fruit set and fruit drop in sweet and sour cherry. However, the reported results cannot be easily compared because the works have focused sometimes on the post-bloom initial fruit set, sometimes on the fruit drop (early or late), or even only generally on the total fruit set.

Despite cherries are commonly recognized as non-climacteric fruits, high levels of ethylene were associated with flower abscission and “June drop” of immature fruitlets (Blanpied, 1972). On the other hand, ZR (zeatin riboside), GA (gibberellic acid), IAA (indole-3-acetic acid) levels in fruits were described as positively correlated with fruit retention (Guo et al., 2020); furthermore, the same authors reported that high ABA (abscisic acid) concentration in cherries could lead to fruit dropping. However, Blanusa et al. (2006) suggested that the role of ABA might be secondary in cherry fruit dropping process and might be a consequence rather that a cause of fruit abscission. Different hormone mixtures have been tried to promote the fruit setting of sweet and sour cherry: Webster et al. (1979) applied a mixture containing GA_3, DPU (NN’-diphenylurea, a cytokinin) and one of the auxins NOXA (2-naphthoxyacetic acid), NAA (1-Naphthaleneacetic acid), and 2,4,5-TP (α-(2,4,5-trichlorophenoxy)-propionic acid). In most cases the treated branches showed higher fruit set than the control, but the differences were not always significant. The results were very variable, and in some years and with some cultivars the treated variants showed lower fruit set than the control. The application of 2-NOXA+GA_3 resulted in increased fruit set also for sour cherry (Bubàn, 1996). Stopar (2018) tried a similar approach using GA_3, NAA, BA (6-Benzylaminopurine, a cytokinin), GA_{4+7}, and Prohexadione calcium. Only branches treated with GA_3+NAA showed an increase in fruit set, but the effect was not significantly different from the control for all the varieties considered. Furthermore, the effects varied consistently between the two seasons studied. GA_3, NAA, and their combinations have been studied by Askarieh et al. (2021). In this work, GA_3+NAA increased the fruit set of both the varieties considered and reduced fruit drop. Webster et al. (2006) studied the effects of $GA_{3,}$ NAA and AVG (aminoethoxyvinylglycine, an inhibitor of ethylene biosynthesis) on two different varieties. For the first cultivar only the GA_3+NAA variant significantly increased the fruit set, while none of the treatments had any significant effect on the percentage of abscinding fruits throughout the season for the second one. The effect of multiple applications of AVG was investigated by Bound et al. (2014): 'Kordia' treated with AVG showed a 57% higher fruit set at harvest than the control; in 'Regina' no difference was observed at 3 weeks after bloom but at harvest the treated variants showed a 33% higher fruit set than the control (indicating an effect on the late fruit drop).

In conclusion, despite a more solid effect was achieved with both NAA and AVG, the consistency of the results seems to be dependent on many factors as the application timing, the variety, the vigor of the tree, and the climatic conditions of the season.

1.4.3 The fruit drop pattern in sweet and sour cherry

The fruit drop pattern has been widely described for sour cherry (*Prunus cerasus*) and to a lesser extent for sweet cherry (*Prunus avium*). Bradbury (1929) described the fruit drop pattern of the sour cherry cultivars 'Early Richmond' and 'Montmorency'.

He identified three waves of fruit drop:

1) fruits of the first wave were characterized by a shorter pedicel, the style and the calyx cup did not detach at the end of flowering, had a smaller ovary, and showed a yellowing pedicel and pistil. Usually both ovaries of the aborted fruits appear shriveled. The first wave was completed within the first two and a half weeks after anthesis;

2) fruits of the second wave showed heterogeneous sizes, were narrower at the stem end, less turgid, and first characterized by a dull green color that then turned yellow. The ovules of aborting fruits showed signs of shriveling, while that of growing fruits was swollen and turgid. This wave of dropping reached its peak in the third week after anthesis;

3) Fruits of the third wave showed a considerable size of about 9-10mm while the developing ones had already reached 9-11mm. As those of the second wave, they showed a greenish-yellow color,

> dull appearance, and lack of turgidity. The ovule of healthy fruits was turgid, completely occupied the ovarian cavity and was surrounded by a lignified endocarp. In contrast, in aborting/dropping fruits, the ovary was shriveled, and the lignification of the endocarp was bounded to the distal end of the fruit. The third wave is conventionally called “runoff”, “red drop” or "June drop", but it does not necessarily have to occur in June; timing varies with geographic location and climatic conditions of the season. The third wave of dropping occurred three weeks after the second one (thus 6 weeks after anthesis). In some seasons, an overlapping of the dropping waves was observed.

In the treatment where pollination was prevented, parthenocarpic development of the fruitlets was observed, but they were completely dropped during the first two waves (closely resembling the described aborting fruits). Unfertilized parthenocarpic sweet cherry fruitlets were observed also by Bargioni (1978). He observed this phenomenon mainly in years when the seasonal trend proceeds with relatively low temperatures after flowering. These fruitlets were finally dropped when the retained ones started the second exponential phase of growth (between late stage II and early stage I). In the work of Bradbury (1929), aborting and developing fruit from the first wave were examined: almost all of them showed pollen tubes along the style or in the ovarian cavity; however, most of the tubes showed a random growth pattern. Only 6,5% showed tubes in the nucellus. 91% of the aborting fruits contained two shriveled ovules while 100% of the healthy fruits showed at least one functional ovule. The author stated that the apparently random growth of the pollen tubes coupled with the fact that degeneration of both ovules took place before the pollen tubes could reach the ovarian cavity, suggested that the factors determining the degeneration, rather than the lack of fertilization, were the primary causes of first fruit drop. Of the dropping fruits belonging to the second wave, 96% showed at least the embryo or a pollen tube at the ovarian cavity/micropyle/nucellus. Of the late dropping fruits, only 5,7% did not show any embryos. Embryo development appeared to be very heterogeneous; however, large embryos were found more frequently in the developing fruits than in the aborted ones (similar observations regarding the size of the embryo were reported by Racskó et al. (2007)). Interestingly, Bargioni (1978) reported that if embryo abortion occurs in late ripening sweet cherry varieties it will result in fruit drop. In contrast, in early ripening cultivars, embryo abortion may occur, it will determine a slowing down in fruit growth but will not be followed by fruit drop. The same observation was reported by Lech et al. (1983) and Racskó et al. (2007). Recently, Mancini et al. (2021) monitored the growth of several fruitlets using the middle-early ripening varieties ‘Blaze star’: during the last phase of fruit drop, some fruits stopped growing but were not dropped and reached maturity although with a lower fruit diameter.

Many authors have divided the fruit drop pattern of sweet and sour cherry in different waves. For example, Lech et al. (1983) claimed that in sour cherry three periods of fruit drop can be observed: the first occurred at the end of flowering when unfertilized flowers dropped; the second period coincided with the phase of rapid embryo growth; the third period occurred during pericarp development. Blanusa et al. (2005) reported that the inconsistency of sweet cherry cropping in the UK was due to abscission of unpollinated flowers, flowers that failed to set, and fruitlet that were not able to reach maturity (the latter reported as the main peak). They wrote that the intensity of abscission varied with fruit developmental stage and from year to year. Davarynejad et al. (2014) studied 9 different sour cherry cultivars and identified 4 different abscission waves: the first appeared during the second week after pollination, the second at the third one, the third at the fourth one, and finally the fourth dropping period occurred between the fourth and the fifth week after pollination. 4 abscission peaks were already present in Davarynejad et al. (2009); also here, seasonal changes for fruit set and fruit drop were observed. Guo et al. (2020) identified two different peaks for different sweet cherry varieties: despite all the varieties considered reached the full bloom at the same time, the first peak was on different dates for each cultivar, while the second one occurred simultaneously for all of them. Each peak showed different intensities for each variety.

1.4.4 Possible causes of fruit drop

Bradbury (1929) stated that abortion of fruitlets (=failure to reach maturity) could be traced back to many causes:

1) self- or cross-sterility (no fruit set after cross- or self-pollination);
2) lack of pollination due to unfavorable climatic conditions before, after or at full bloom;
3) lack of fertilization after pollination (e.g., unfavorable environmental conditions for pollen tube growth or degeneration of both ovules);
4) improper nutrition of the tree or of parts of the tree;
5) competition among flowers for nutrients.

The author stated that since 95% of the total blossoms analyzed in the trial had been pollinated, the fruit drop (up to 75%) could not be attributed to a lack of pollination. He concluded that the most likely cause of embryo abortion (and hence of fruit drop) was a lack of proper nutrition; the hypothesis was supported by the surprisingly high fruit set observed after severe frost damages that reduced the number of viable flowers (Bradbury, 1929). Lech et al. (1983) asserted that fruit drop was caused either by the simultaneous atrophy of both ovules (1st wave), by the atrophy of the embryos (2nd wave), or by the atrophy of the seeds (3rd wave) due to physiological reasons. Fruit drop is a complex physiological process in which an abscission zone (AZ) at the base of the peduncle is activated. As already mentioned, the process is regulated by plant hormones such as auxins, abscisic acid, and ethylene but its cause should be attributed to interior factors as competition for photoassimilates (Bargioni, 1978; Blanusa et al., 2005). Interestingly, higher fruit drop rates have been reported for the north side of the tree (Flore et al., 1999; Davarynejad et al., 2009). Blanusa et al. (2006) suggested that source limitation was the main factor inducing late-season fruit abscission. They reported that fruit rapidly abscised from phloem-girdled spurs with low LA:FN ratio, while fruits on spurs with a high ratio were typically retained. Lower level of sorbitol was measured in the fruits characterized by a low LA:FN ratio; interestingly, ABA concentration increased as sorbitol concentration declined in fruitlet prone to drop. Furthermore, exogenous application of ABA induced fruit abscission. IAA are produced in the seeds by the endosperm (Racskó et al., 2007) and exported from the fruit via phloem flow and via the polar auxin transport pathway (PAT) (Blanusa et al., 2005). The flux of IAA through the AZ appears to regulate the ethylene sensitivity of cells in this layer, carbohydrates partitioning, and fruit retention (Blanusa et al., 2005). Very low capacity for PAT was measured in pedicels of fruitlets destined to drop (Else et al., 2004). The fruit drop reduction due to the application of plant growth regulators such as IAA can be explained by the compensatory effect of exogenous auxin which prevents the formation of the AZ by inhibiting the enzymatic activity of polygalacturonase and pectinase (Askarieh et al., 2021) and preserves the photoassimilates supply to the fruit. These last examples show how carbohydrates and plant hormones are strictly interconnected.

Racskó et al. (2007) reported that in warm climates rapid shoot growth can promote fruit drop. Similarly, Hedhly et al. (2012) drastically enhanced flower and fruit drop by increasing air temperature at bloom by 1-3°C in orchard conditions using a plastic cage. Increased temperature resulting from greenhouse cultivation of stone fruits (peaches, nectarines, and sweet cherry) has been described to promote shoot growth and to reduce fruit set. In this work, for sweet cherry, as temperature increased, the percentage of dropping fruit increased linearly (Erez et al., 1998). As already mentioned, a positive correlation between air temperature and shoot growth was found by (Sønsteby et al., 2019). Interestingly, (Lalatta and Sansavini, 1983 cit. Racskó, et al., 2007) reported that high elevations above the sea level were less afflicted by fruit drop. In addition, Racskó et al. (2007) wrote that in sweet cherry fruit drop can occur if a cool and rainy weather during fruit set is followed by a rapid increase in air temperature.

1.5 Aim of the study

In the scientific literature, numerous valid works about floral biology, fruit development, and photoassimilate production and partitioning have been found. Many of them identified low fruit set as the major limiting factor for the profitability of this fruit crop. However, only a few of these investigated the causes of this phenomenon, while most addressed this problem only with hormonal treatments.

Many of the papers reviewed in this chapter seem to confuse the phenomenon of fruit drop with the concept of low fruit set. Defining fruit drop as the percentage of flowers that did not reach maturity (and thus as *fruit drop = 1 - %fruit set*) does not allow us to discern between the damages to styles caused by late spring frost, the flower drop, the drop of unfertilized fruitlets, the late drop of fruitlets set, or even the damages caused by birds or mammals.

The following main knowledge gaps were identified:

- A clear definition and quantification of the late fruit drop phenomenon;
- The evolution pattern of fruit drop over the time (from full bloom to harvest);
- The growth kinetics of the fruits destined to drop;
- The effect of climatic factors on fruit drop;
- The role of nutrients (micro- and macro-) on fruit drop.

Furthermore, minor knowledge gaps regarding the effect of physiological- and management factors on the fruit drop pattern have been found.

Late fruit drop of the cv. Regina represents a widespread phenomenon in the Italian region Trentino - South Tyrol, able to significantly reduce the yields and, thus, the profitability of the crop. Its intensity varies from year to year and numerous hypotheses regarding its causes have been formulated.

It can be defined as the premature detachment of the fruitlet set. This phenomenon is highly variable between the growing areas: it is influenced by many factors, the most important of which are very likely the cultivar and the climatic conditions during the season.

The present work monitored the late fruit drop of ‘Regina’ during the three-year period 2020-22 in several orchards located at different altitudes (from 225 up to 1175m a.s.l.) homogeneous in age, pollinizer, and rootstock. Weekly samples were carried out to identify potential influences of climatic- (altitude, temperature, solar radiation, etc.), physiological - (shoot/branch position in the plant, competition with extension shoots, typology of branch/shoot bearing flower buds) and management factors (long or short pruning) on fruit drop pattern. The objective of this trial was to clarify the dynamics of late fruit drop in the South Tyrolean climatic conditions, to quantify the extent of this phenomenon, and to investigate some of the proposed hypotheses in order to be able to set an appropriate control strategy in the future.

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2 Chapter 2: A first approach to model the (late) drop pattern of sweet cherry cv. Regina in South Tyrol (Italy)

2.1 Abstract

‘Kordia’ and ‘Regina’ represent the standard varietal combination in South Tyrolean cherry growing. Unfortunately, in the last few years, due to high percentages of late fruit drop, very unstable yields have been observed for the cv. Regina. In this work, a sound experimental protocol has been developed to correctly describe the (late) fruit drop pattern of sweet cherry cv. Regina grafted on Gisela 5, that could be later used on a broader scale during the following seasons. The trial was carried out at the experimental orchard of the Laimburg Research Centre in year 2020: number of flowers or fruitlets on each tagged limb was counted weekly from 2DAFB up to the day prior to harvest. The evolution of about 2550 flowers and the growth of 240 individually labelled fruitlets on 48 limbs with different features were monitored during the season. The fruit set evolution appeared very similar for both the 1-year-old shoots and the 2-year-old branches. Several waves of fruit drop were observed, and the final fruit set was reached at the beginning of veraison (between 37 and 44 DAFB). Fruit dropped late in the season showed aborted seeds. The position of the 1-year-old shoots within the canopy (high or low) did not significantly influence the fruit set evolution during the time (*p* = 0,192), nor the number of new shoots per limb (*p* = 0,278) or the average new shoot length per limb (*p* = 0,449). Conversely, “high” 2-year-old branches emitted a higher number of new shoots per limb (*p* = 0,028), with greater average shoot length (*p* = 0,026) and an overall lower absolute value of fruit set although not entirely significant (*p* = 0,062). Pruning the 1-year-old shoots in spring prolonged the late fruit drop by one week (*p* = 0,010) and accelerated the shrinking of the cherries which dropped between 42 and 49 DAFB; however, no significant difference on the final fruit set was detected between pruned and unpruned 1-year-old shoots. Pruned 1-year-old shoots emitted the same number of new shoots as the unpruned ones (between 3 and 4 per limb), but the latter showed a lower sum of shoots length per limb normalized by the limb length (*p* = 0,005). This ratio was interpreted as a measure of the limb vigor and used as predictor in a linear regression analysis having the final fruit set as the dependent variable; the analysis revealed that for each unit increase of the predictor (therefore, by increasing the vigor) the final fruit set decreased by 1,8%. Since the factor considered explained only partially the studied phenomenon (R-square = 0,132), further factors need to be investigated in the future. In general, the growth rate of dropping cherries started to decrease at least 2 weeks before the actual abscission; in particular, the data collected showed how from 14 to 7 days before the abscission, dropping cherries still showed positive but significantly lower AGR values than those of the retained ones; the AGR

values of the dropping cherries became negative only during the last week before the abscission. This information suggest that the physiological causes of late fruit drop should be investigated at least two weeks before the actual drop.

2.2 Introduction

Although the Italian Alpine region Trentino - South Tyrol represents a small part of the national sweet cherry production, it stands out for its late ripening time (from early July to late August) and high quality of production. Since the '90s, local farmers decided to invest in high quality – late ripening varieties such as 'Kordia' and 'Regina' grafted on Gisela 5 and trained as central leader (Spindle) in semi-intense planting systems (1500 - 2000 trees/ha). These two partially cross-compatible cultivars represent a standard combination in many cherry production areas such as the North of Italy (Zago, et al., 2011), Croatia (Milinović, et al., 2016), Austria (Hajagos, et al., 2012), Slovenia (Usenik, et al., 2008), Germany (Schuster, et al., 2014), Tasmania (Bound, et al., 2014), and Southern Chile (Sagredo, et al., 2017). In recent years, due to high percentages of late fruit drop, for the cv. Regina very unstable yields have been observed. A relatively high number of publications focused on the reduction of the physiological drop (or, more generally, on increasing the fruit set) for different varieties, but only a few described the phenomenon in detail and/or investigated its physiological causes. The aim of this study was to develop an experimental protocol enabling the description of the fruit drop pattern of 'Regina' in the South Tyrolean climatic conditions; the effect of several factors such as limb position in the plant (low/high), the age of the limb (1-year-old shoots/2-year-old branches) and pruning of the 1-year-old shoots were investigated.

Research hypothesis n°1: frequent sampling is indispensable to properly describe and quantify the late fruit drop pattern of sweet cherry.

Research hypothesis n°2: physiological and management factors have an influence on the fruit drop pattern and the vegetative growth of sweet cherry.

2.3 Materials and methods

The study was carried out in 2020 on 8 mature (year of planting 2012) sweet cherry trees, cv. Regina (with 'Kordia' as pollenizer) grafted on the dwarfing rootstock 'Gisela 5'. The trial was carried out in the valley floor of the "Adige" river (225m a.s.l.) in the experimental orchard of the Laimburg Research Centre in South Tyrol, Italy. Trees were equal in age, trained as central leader, spaced 1.8 x 3.5m, and located on the same row; only trees with similar vigor (i.e. with a similar trunk cross-sectional area) were chosen. The orchards were managed according to integrated cultural practices in terms of fertilization, irrigation, plant protection, and pruning. An exhaustive summary of the agronomic practices recommended by the local agricultural extension specialist (https://www.beratungsring.org) can be found in the official guidelines published annually (https://www.beratungsring.org/info/organisation/broschueren/kirsche.html). No thinning and no plant growth regulators were applied during the trial. The orchard was covered both by an anti-hail net and a plastic rain-cover. Full bloom occurred on April 6th, veraison at the end of May, and fruit were harvested on June 25th, 80 days after full bloom (DAFB). At full bloom, none of the trees suffered frost damages. The orchard was equipped with a weather station.

Prior to flowering, 6 limbs per tree were tagged: two 1-year-old shoots with basal flower buds left unpruned, two 1-year-old shoots with basal flower buds shortened to 3/4 vegetative buds, and two 2-year-old fruiting branches without associated laterals, one in the lower (0 – 1.5 m) and one in the upper (3.0 – 4.0 m) part of the crown respectively. The choice of these particular limb types was made because in the literature no information about the effect of pruning the 1-old-shoots, of the limb age, and of the limb position within the canopy on the late fruit drop pattern (and intensity) was found. This basic technical knowledge may provide important information on which to base future approaches to pruning and tree training.

FLOWER AND FRUIT COUNTING, FRUIT SET

The number of flowers or fruitlets on each limb was counted weekly from 2DAFB up to the day prior to harvest. Each fruiting spur (FS) was counted separately. Fruit with symptoms of abscission were counted until natural detachment occurred.

Total fruit set was calculated for each limb as follows (Askarieh, et al., 2021):

$$Total\ fruit\ set\ \% = \frac{Fruit\ number\ at\ harvest\ time}{Total\ flowers\ number} * 100 \tag{1}$$

At full bloom, fruiting limb length was registered.

SEASONAL FRUIT GROWTH

At 28 DAFB, 5 drupelets without any evidence of abscission (at least 13 mm of diameter) were tagged on each limb using a progressive number from 1 to 240. Overall, 30 fruits per tree and 40 fruits for each limb type were tagged. Starting from 36 DAFB, these fruits were calibrated every week using a digital Bluetooth caliper (T. R. Turoni, Forlì – Italia) up to harvest. Fruit growth rates (both positive and negative) were expressed as absolute size increment per unit of time (AGR). The following equations, as reported by (Morandi, et al., 2019), were used:

$$AGR = \frac{d_{t1} - d_{t0}}{t_1 - t_0} \tag{2}$$

Where d is the fruit diameter in mm and t the time expressed as DAFB.

Fruit measurements were performed in the morning.

FRUIT SURFACE CONDUCTANCE AND DRY MASS

Surface conductance (g_c) was determined according to (Gibert, et al., 2005). During the growing season, skin conductance was monitored using 10 fruits for each date. Once the pedicel was removed, and diameter and mass measured, they were kept 24 h at room temperature (ranging from 19,5 to 20,4°C) with constant relative humidity (ranging from 88,2 to 92,2%); these variables were monitored by a data logger. After 24 h, fruits were weighed again and skin conductance [m h^{-1}] was calculated using the following equation (Corelli Grappadelli, et al., 2019):

$$g_c = \frac{Tf\ (h)}{Sf * \frac{M_W}{R * Temp[K]} * P * (H_f - H_a) * 100} \tag{3}$$

Where T_f is the transpiration per unit of time [g h^{-1}] expressed as the weight loss, h time in the constant T/RH room, Sf is the fruit surface area (with the fruit conventionally considered spherical) at the time of sampling [cm^2], M_w is the molecular mass of water [18 g mol^{-1}], R is the gas constant [83 cm^3 bar mol^{-1} K^{-1}], $Temp[K]$ is the room temperature in Kelvin, P is the saturation vapor pressure [bar] (P = 0,008048 * exp(0,0547 x (Temp -273.15))), H_f is the relative humidity within the fruit (assumed to be 100%), and H_a is the relative humidity of the atmosphere. Fruits were sampled on 5 dates from 44 up to 79 DAFB (the day before harvest).

On five sampling dates starting from 44 up to 79 DAFB, flesh slices of 10 fruits were weighed (fresh weight, FW) and oven-dried to constant mass at 65°C for about 10 days. Successively, they were weighed again for dry weight (DW). Dry matter % for each fruit was determined.

HARVEST YIELD AND SHOOT LENGTH

At commercial harvest, fruit number, total fruit weight of each limb, and trunk cross-sectional area (TCSA, 20 cm above the grafting point) were measured. Each tree was harvested separately. In the following days, all new shoots on the tagged limbs were counted and measured.

STATISTICAL ANALYSIS

All the flower/fruit counts were elaborated using the General Mixed Model (GMM) with Repeated Measures (RM) procedure in IBM SPSS Statistics 27; with tree as the random factor and limb as the repeated subject at each sampling time. The same approach, but with limb as random factor and the fruit as the subject repeated was used for fruit diameter determinations. The covariance type (COVTYPE) of the RM was chosen using the "Aikaike Information Criterion" (AIC). The GMM was also used to compare the vegetative parameters (with the tree as the random factor). Estimated Marginal Means (EMMs) were compared using pairwise multiple comparison with Sidak adjustment. The more common Tukey test has been discarded since it leads to inflated alpha level when the sphericity assumption is not met (and mixed models don't require the sphericity assumption), and therefore it is unsuitable for performing pairwise comparisons in a repeated measures design (Scott, 1980). Of the most frequently tests used with these models, LSD was discarded since it is vulnerable to Type I errors, and Bonferroni to Type II errors. Linear Regression analysis was used, having the vegetative parameters as predictors and the fruit set at harvest as dependent variable. Effects were considered significant at $p \leq 0.05$.

ABBREVIATIONS

DAFB = Days after full bloom;
GMMRM = General Mixed Models with Repeated Measures;
EMMs = Estimated Marginal Means;
AGR = Absolute Growth Rate;
s.e. = standard error

2.4 Results

2.4.1 Flower and fruit counting, fruit set

p-value "Pruning"	**p-value "Position"**	**p-value "Sampling (time)"**	**p-value "Pruning * Position"**	**p-value "Pruning * Sampling"**	**p-value "Position * Sampling"**	**p-value "Pruning * Position * Sampling"**
,560	,484	**,000**	,860	**,010**	,192	,091

Table 1: p-values for the different factors considered obtained as output of the Mixed Model with Repeated Measures having the fruit set in % as the dependent variable. 1-year-old shoots. Bold values are considered significant at α ≤ 0,05.

Table 1 report the outputs of Type III Tests of Fixed Effects after the mixed model with repeated measures procedure in which the subject was the limb. This analysis studied the effect of different factors on the evolution of fruit set of 1-year-old shoots during the season. Of the 11 flower/fruit counts made, the last one (at harvest, 79 DAFB) was excluded from the analysis since the lower number of cherries counted was not due to physiological fruit drop but caused by bird damage. The factors considered were "Position" of the limb within the canopy ("high" and "low"), "Pruning" (1-year-old shoots shortened or left unpruned) and the day of "Sampling"; the interactions between the factors were also studied. The model returned the factor "Sampling" and the interaction "Pruning * Sampling" as significant.

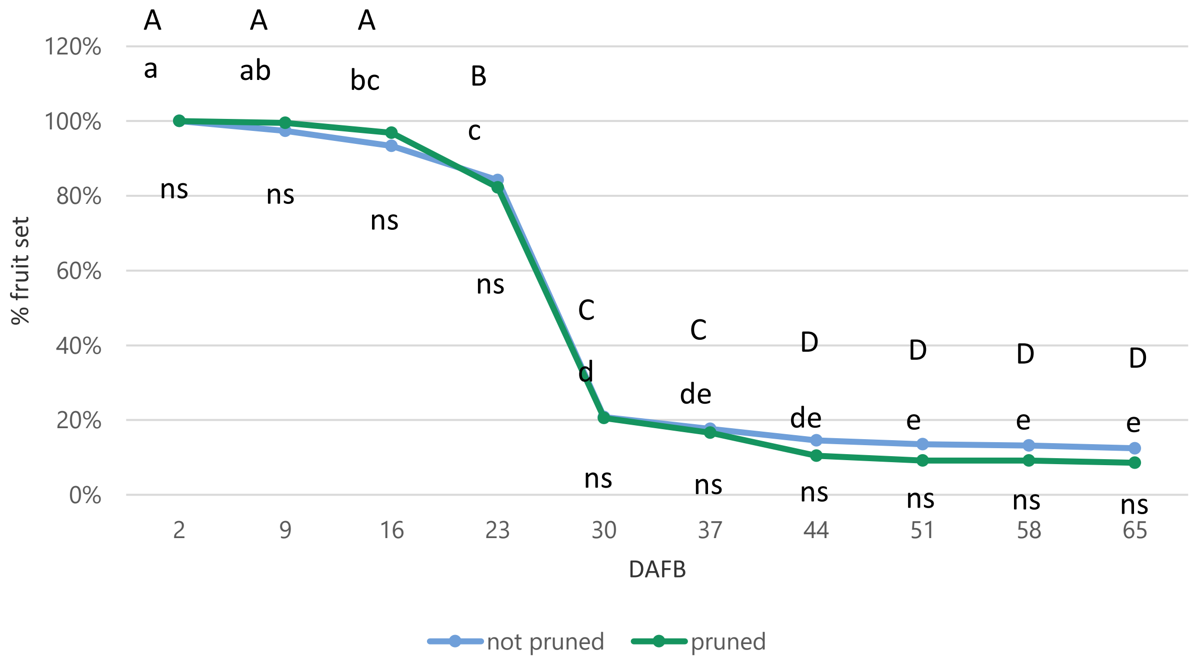

Figure 1: effect of the factor "Pruning" on the evolution of the fruit set on the 1-year-old shoots. ns/: not significant/significant «Pruning * Sampling»; Lowercase letters: "Sampling * Pruning" referred to «not pruned»; Uppercase letters: "Sampling * Pruning" referred to «pruned».*

Figure 1 represents the evolution of fruit set during the time calculated using the formula (1); each point represents the estimated marginal mean of the percentage of total fruit set referred to a specific sampling day expressed in days after full bloom (DAFB). The green and the blue lines represent the evolution of fruit set of pruned and unpruned shoots, respectively. No significant difference was observed between these two treatments for each sampling time separately ("Pruning * Sampling"). The final fruit set calculated at harvest was not different between the pruned and the unpruned shoots. The overall final fruit set was about 10,5 %. In contrast, although the evolution during the season appeared very similar, some significant differences have been measured: in the "unpruned" limbs final fruit set was reached already at 37 DAFB, while in the "pruned" ones a significant decrease in fruit set was observed also between 37 and 44 DAFB. Hence, in the thesis "pruned" the final fruit set was reached one week later than in the "unpruned" one. No effect of position of the 1-year-old shoots within the canopy ("high" vs "low") was detected.

p-value "Position"	**p-value "Sampling"**	**p-value "Position * Sampling"**
,062	**,000**	,231

Table 2: p-values for the different factors considered obtained as output of the Mixed Model with Repeated Measures having the fruit set in % as the dependent variable. 2-year-old branches. Bold values are considered significant at α ≤ 0,05.

Table 2 reports the output of Type III Tests of Fixed Effects after the mixed models with repeated measures procedure in which the subject was the limb, repeated at each sampling time. This analysis studied the effect of the "Position" of the 2-year-old branches ("high" and "low") and of the day of "Sampling" on the total fruit set. The interaction of the two factors was also considered. Again, of the 11 flower/fruit counts made, the last one (at harvest) was excluded from the analysis. The model returned only the factor "Sampling" as significant. It is worth noting that the p-value of the factor "Position" (,062) was only slightly higher than the alpha-value (,05).

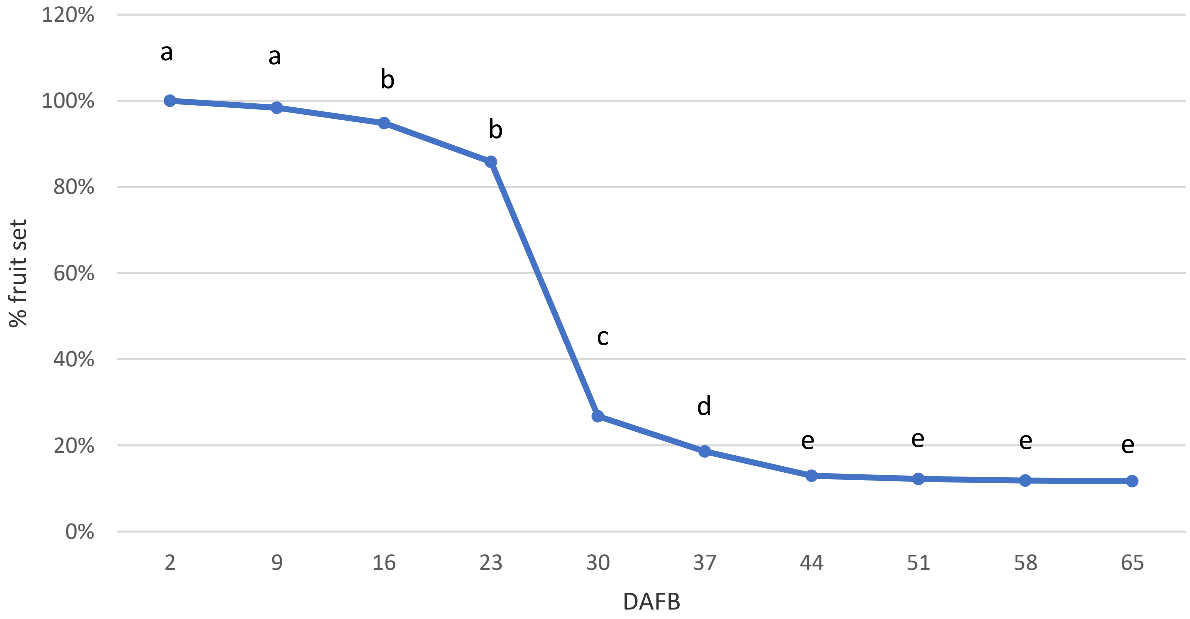

Figure 2: effect of the factor day of "Sampling" on the evolution of the fruit set on the 2-year-old branches

Figure 2 represents the evolution of fruit set of the 2-year-old branches calculated using the formula (1). Each point describes the estimated marginal mean (EMM) of the percentage of fruit set at the sampling day. The final fruit set was reached at 44 DAFB. Despite the fact that 2-year-old branches positioned close to the top showed a much lower final fruit set (8,5%) than those of the lower part of the crown (14,9%), no significant difference was detected by the model (Table 3).

	1-YEAR-OLD SHOOTS										**2-YEAR-OLD BRANCHES**					
	Sampling day		EMMs Pruning * Sampling day				EMMs Position * Sampling day				Sampling day		EMMs Position * Sampling day			
DAFB	EMMs	s.e.	"not pruned"	s.e.	"pruned"	s.e.	"high"	s.e.	"low"	s.e.	EMMs	s.e.	"high"	s.e.	"low"	s.e.
2	1,00	0,00	1,00	0,00	1,00	0,00	1,00	0,00	1,00	0,00	1,00	0,00	1,00	0,00	1,00	0,00
9	0,98	0,01	0,97	0,01	1,00	0,01	0,98	0,01	0,99	0,01	0,98	0,01	0,97	0,01	0,99	0,01
16	0,95	0,01	0,93	0,01	0,97	0,01	0,94	0,01	0,96	0,01	0,95	0,01	0,92	0,01	0,97	0,01
23	0,83	0,02	0,84	0,03	0,82	0,03	0,81	0,03	0,85	0,03	0,86	0,03	0,82	0,04	0,90	0,04
30	0,21	0,02	0,21	0,03	0,21	0,03	0,19	0,03	0,22	0,03	0,27	0,03	0,26	0,04	0,28	0,04
37	0,17	0,02	0,18	0,03	0,17	0,03	0,16	0,03	0,18	0,03	0,19	0,02	0,17	0,03	0,20	0,03
44	0,12	0,02	0,15	0,02	0,10	0,02	0,12	0,02	0,13	0,02	0,13	0,02	0,10	0,02	0,16	0,02
51	0,11	0,02	0,13	0,02	0,09	0,02	0,11	0,02	0,12	0,02	0,12	0,02	0,09	0,02	0,15	0,02
58	0,11	0,02	0,13	0,02	0,09	0,02	0,11	0,02	0,12	0,02	0,12	0,02	0,09	0,02	0,15	0,02
65	0,11	0,02	0,12	0,02	0,09	0,02	0,10	0,02	0,11	0,02	0,12	0,02	0,09	0,02	0,15	0,02
p-value	**,000**		**0,01**				**0,192**				**,000**		**0,231**			

Table 3: Evolution of the fruit set in % for the factors (and their interactions) considered. P- values are considered significant at α ≤ 0,05.

		N° OF NEW SHOOTS PER LIMB	**AVERAGE SHOOTS LENGTH PER LIMB [CM]**	**SUM OF SHOOTS LENGTH PER LIMB [CM]**	**LIMB LENGTH AT FB [CM]**	**SUM OF SHOOTS LENGTH PER LIMB / LIMB LENGTH [-]**
FACTOR “PRUNING”	EMMs "pruned"	3,50	37	125	33	4,01
	EMMs "not pruned"	3,94	27	104	43	2,31
	p-value	**,487**	**,008**	**,283**	**,033**	**,005**
FACTOR “POSITION”	EMMs “high”	4,06	31	125	42	3,01
	EMMs “low”	3,38	33	104	35	3,31
	p-value	**,278**	**,449**	**,305**	**,122**	**,593**
INTERACTION “PRUNING * POSITION”	EMMs “pruned, high”	4,00	33	136	38	3,60
	EMMs “pruned, low”	3,00	41	115	29	4,42
	EMMs “not pruned, high”	4,13	28	114	46	2,43
	EMMs “not pruned, low”	3,75	25	93	41	2,20
	p-value	**0,619**	**0,127**	**0,979**	**0,658**	**0,346**

Table 4: effect of the factors “Pruning”, “Position” and their interaction on the vegetative parameters considered. 1-year-old shoots. P- values are considered significant at α ≤ 0,05.

		N° OF NEW SHOOTS PER LIMB	**AVERAGE SHOOTS LENGTH PER LIMB [CM]**	**SUM OF SHOOTS LENGTH PER LIMB [CM]**	**LIMB LENGTH AT FB [CM]**	**SUM OF SHOOTS LENGTH PER LIMB / LIMB LENGTH [-]**
FACTOR “POSITION”	EMMs “high”	4,63	37	169	36	4,93
	EMMs “low”	2,88	21	64	36	2,04
	p-value	**0,028**	**0,026**	**0,100**	**0,983**	**0,016**

Table 5: effect of the factor “Position” on the vegetative parameters considered. 2-year-old branches. P- values are considered significant at α ≤ 0,05.

2.4.2 Vegetative parameters

Table 4 summarizes the effect of the factors considered on different vegetative parameters (1-year-old shoots). The numbers of new shoots emitted by the tagged limbs during the season was not affected by either "Pruning", "Position" or their interaction. The spring pruned 1-year-old shoots emitted significantly longer shoots than the unpruned ones; in contrast, the average shoot length per limb was not influenced by either the "Position" factor or the interaction "Pruning * Position". Despite the greater sum of shoot length measured on the pruned limbs and on those close to the top, no significant effect of the factors "Pruning" and "Position" was found. As expected, limb length at full bloom was affected by the factor "Pruning", with the pruned limbs significantly shorter than the unpruned ones. The ratio between the sum of new shoot length measured at harvest and the limb length (from which they originated) at full bloom was studied. At the end of the season, new shoots grown on unpruned limbs reached a final length twice that of the limb from which they originated, while new shoots grown on the pruned ones reached a final length equal to four times that of their limbs. While no significant effect of "Position" was found on 1-year-old shoots, this factor seemed to have a greater effect on the 2-year-old branches (Table 5). In this group, branches closer to the top of the tree produced a greater number of new shoots than those on in lower part; furthermore, the new shoots originated from the latter were significantly shorter. The ratio between the sum of new shoot length measured at harvest and the limb length at full bloom is significantly greater for the branches close to the top (with equal limb length at full bloom).

Possible linear regressions between vegetative parameters and fruit set were investigated; the results are reported on Table 6.

	1-year-old shoots			**2-year-old branches**		
Predictor	p-value	R-square	B	p-value	R-square	B
n° of new shoots per limb	,597	-	-	,225	-	-
Average shoots length per limb [cm]	,167	-	-	,378	-	-
Sum of shoots length per limb [cm]	,101	-	-	,141	-	-
Limb length at full bloom [cm]	,923	-	-	,258	-	-
Sum of shoots length per limb / limb length [-]	,041	,132	-,018	,546	-	-

Table 6: p-values of the different vegetative parameters interpreted as predictors in a linear regression analysis having the final fruit set (65 DAFB) as dependent variable. For significant predictors, the R-square as well as the regression slope (B) were printed.

As shown, the only predictor having a significant effect on the final fruit set at harvest was the ratio between the sum of shoot length per limb and limb length; the corresponding R-square value is 0,132, which states that 13,2 % of the total variance of the dependent variable (the fruit set) can be explained by the model. The "B" value (the regression slope) is slightly negative and indicates that for each unit increase of the predictor the final fruit set at harvest decreased by 1,8%. The same effect was not found for the 2-year-old branches.

2.4.3 Seasonal fruit growth

	1-year-old shoots	**2-year-old branches**
Time of detachment	% of cherries dropped out of total fruits labeled	% of cherries dropped out of total fruits labeled
Between 36 and 42 DAFB	13%	22%
Between 42 and 49 DAFB	18%	20%
Between 49 and 60 DAFB	8%	8%
Between 60 and 70 DAFB	2%	0%
Between 70 and 79 DAFB (bird damage)	11%	14%
79 DAFB, Harvest	48%	36%
Total	100%	100%

Table 7: percentage of cherries detached in each time interval considered based on the total number of cherries tagged (160 for the 1-year-old shoots and 80 for the 2-year-old branches).

At 36 DAFB, 160 fruits without any evidence of abscission were calibrated for the first time on the 1-year-old shoots. Of these, 13% dropped between the first and the second calibration, 18% between the second and the third, 8% between the third and the fourth, and only 2% between the fourth and the fifth (2 fruits). Unfortunately, during the last week before commercial harvest an additional 11% of the retained cherries were eaten/damaged by birds; at 79 DAFB only 48% of the tagged cherries reached the commercial harvest (Table 7). Considering those damaged by birds as "not dropped", we can assert that 59% of the tagged fruit did not drop. Similarly, the seasonal growth of 80 fruits with the same characteristics was studied on 2-year-old branches. Of these, 22% dropped between the first and the second calibration, 20% between the second and the third, 8% between the third and the fourth, and none between the fourth and the fifth. Again, 14% of the cherries were severely damaged by birds during the last week before harvest; only 36% reached the commercial harvest at 79 DAFB. Overall, on the 2-year-old branches, 50% of the tagged fruit did not drop. Figure 3 shows the growth pattern of the non-dropping cherries categorized by limb age. For both limb types, cherries followed the second half of the typical double sigmoidal curve. The average diameter increased on each day of sampling with the only exception of the sampling 79, when it did not increase. Cherries on 1-year-old shoots and 2-year-old branches showed very similar fruit size at harvest. For a better understanding of its growth, the fruit absolute growth rate (AGR) was taken into account. The AGR values of all the non-dropping cherries were studied using the Mixed Models with Repeated Measures procedure with the fruit as the subject repeated at each sampling. This analysis reported the day of "Sampling" and the interaction between "Position" and "Sampling" as significant factors for the 1-year-old shoots (Figure 4). The distribution of the AGR intensities during the season assumes a bell-shaped form in which the highest values were measured at the fourth sampling (hence referring to the time ranging from 49 and 60 DAFB). The analysis of the interaction revealed that the position of the 1-year-old shoots with respect to the crown (=the height from the ground) influenced the fruit growth pattern: between 36 and 49 DAFB the fruits of the top shoots showed higher AGRs than those of the low shoots. The opposite significant trend was observed between 49 and 70 DAFB. Overall, the growth trend of the cherries on the higher shoots appeared as shifted to the left, hence a few days in advance if compared with that of the lower shoots. During the last week before commercial harvest no positive AGR values were measured indicating a loss in diameter. No significant interaction was found for this day of sampling.

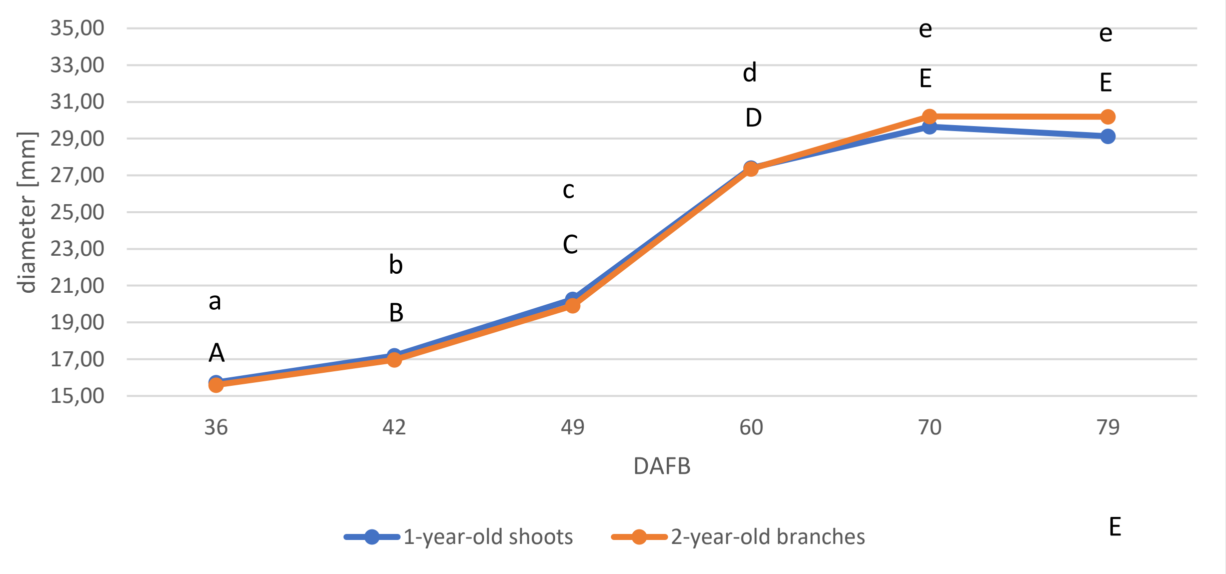

Figure 3: Transversal diameter development of retained fruit from 36 DAFB up to harvest. Lowercase letters indicate the differences among the sampling times of the fruits borne on 2-year-old branches, uppercase letters indicate the differences among the sampling times of those borne on 1-year-old shoots. EMMs compared using Sidak pairwise multiple comparison.

The distribution of the AGR intensities during the season for the 2-year-old branches resembled that of the 1-year-old shoots (Figure 5). Also in this case, the highest absolute values were measured between 49 and 60 DAFB. Here, the top branches showed significantly higher AGR values only between 42 and 49

DAFB, whereas an opposite significant trend was measured between 60 and 70 DAFB. Differently from what has been observed for the 1-year-old shoots, cherries of the lower branches still showed slightly positive AGR values during the week before harvest; at the same time, cherries of the higher branches were shrinking (but no significant difference was obtained for the interaction at this day of sampling, p-value .085)

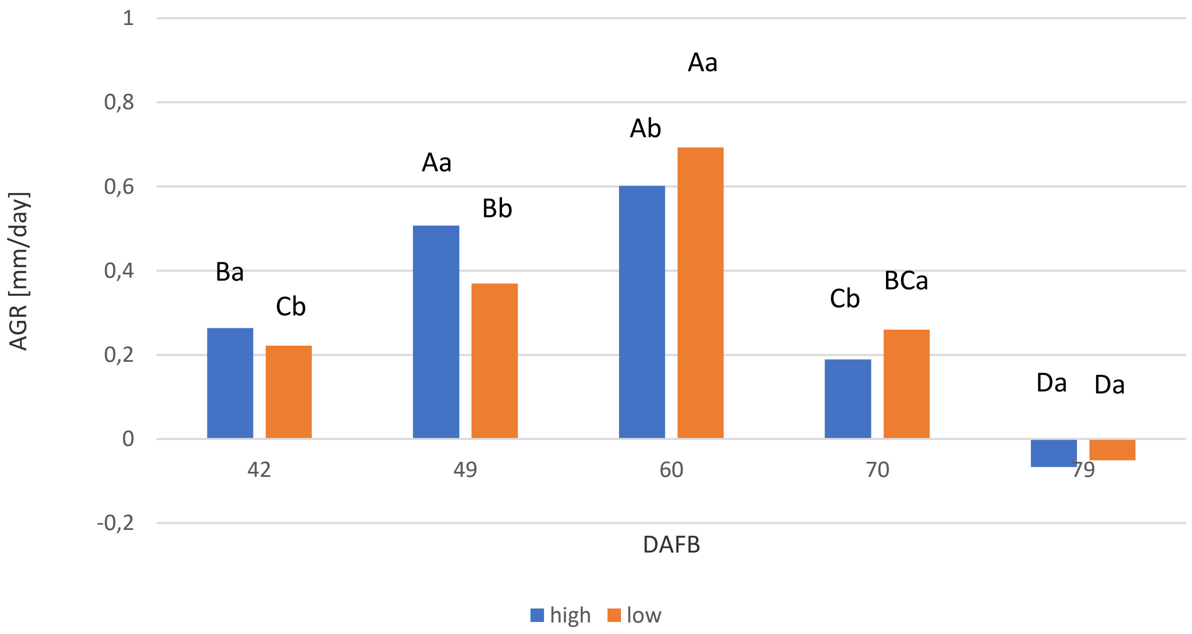

*Figure 4: AGR values of retained fruits borne on 1-year-old shoots at different height within the crown. The lowercase letters indicate the differences between the two positions at each sampling time ("Position * Sampling"); the uppercase letters indicate the differences among the days of a specific position ("Sampling * Position"). EMMs compared using Sidak pairwise multiple comparison.*

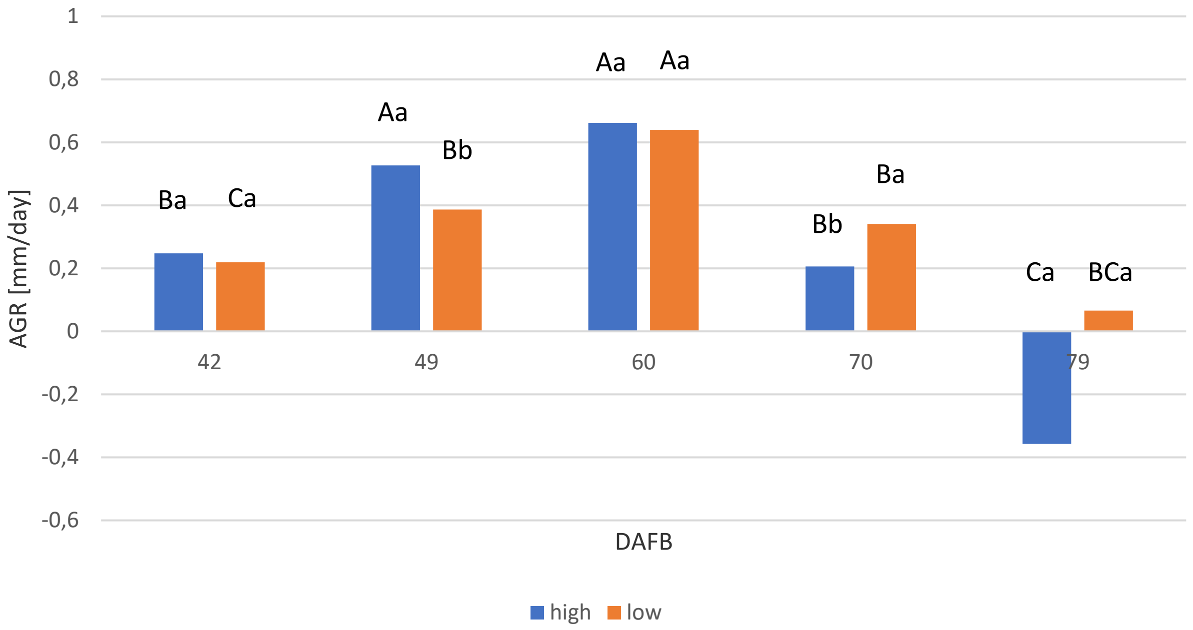

*Figure 5: AGR values of retained fruits borne on 2-year-old branches at different height within the crown. The lowercase letters indicate the differences between the two positions at each sampling time ("Position * Sampling"); the uppercase letters indicate the differences among the sampling times of a specific position ("Sampling * Position"). EMMs compared using Sidak pairwise multiple comparison.*

The growth pattern of the dropping fruits vs. that of the retained ones was studied. During the data analysis, tagged cherries were divided into different groups based on the "Time of detachment" factor (table 7). For each time interval between two consecutive samplings, the mean AGR value was calculated for each group separately. For example, in order to study the AGR values from 36 to 42 DAFB fruits were divided into the following groups: those that would drop between 42 and 49 DAFB, those that would drop between 49 and 60 DAFB, those that would drop between 60 and 70 DAFB, those that would be damaged by birds between 70 and 79, and finally those that would reach maturity. The effect of the different factors on the AGR values are reported into Table 8. Between 36 and 42 DAFB the model detected a significant effect of the group (reported as "Time of detachment") and of the interaction "Pruning * Time of detachment" (Table 8, first row).

Time interval considered	p-value “Pruning”	p-value “Position”	p-value “Time of detachment”	p-value “Pruning * Position”	p-value “Pruning * Time of detachment”	p-value “position * Time of detachment”	p-value “Pruning * Position * Time of detachment”
36 – 42 DAFB	,215	**,003**	**,000**	,797	**,000**	,053	,367
42 – 49 DAFB	,619	,617	**,000**	,615	,962	,540	,274
49 – 60 DAFB	,965	**,005**	**,000**	,561	,967	,311	,190

Table 8: p-values of the different factors (and of their interactions) on the AGR value in the time interval considered for the 1-year-old shoots. The factor “Time of detachment” refers to the different groups of detachment. EMMs compared using Sidak pairwise multiple comparison. Bold values are considered significant for α ≤ 0,05.

Table 9 reports the results of the pairwise comparisons between the different groups (factor: “Time of detachment”, variable AGR). During the time ranging between 36 and 42 DAFB the cherries that will drop between 42 and 49 DAFB are already showing a negative AGR significantly different from that of the other groups. As expected, the AGR value of the fruits damaged by birds and that of the cherries that will reach the commercial harvest are identical. Interestingly, despite cherries destined to drop between 49 and 60 DAFB were already showing lower absolute values than the retained ones, their AGR was not considered significantly different from that of the other groups by the statistical model.

Time of detachment	AGR	s.e.	Sig.
42 – 49 DAFB	-,057	,028	b
49 – 60 DAFB	,185	,047	a
60 – 70 DAFB	,208[a]	,088	a
70 – 79 DAFB (damaged by birds)	,250	,034	a
79 DAFB (Harvest)	,241	,017	a

Table 9: EMMs of AGR values of cherries characterized by different time of detachment from 36 to 42 DAFB. [a] = very small sample size (2 fruits). 1-year-old shoots. EMMs compared using Sidak pairwise multiple comparison.

Not only the factor “Time of detachment” was significant but also its interaction with the factor “Pruning”. The results are reported in Figure 6: here, lowercase letters indicate any significant effect of the factor “Pruning” within each group. Interestingly, inside the group that will drop between 42 and 49 DAFB cherries of the pruned shoots are decreasing their growth rate in a more intense way than those of the unpruned ones. Within every other group the interaction was not significant.

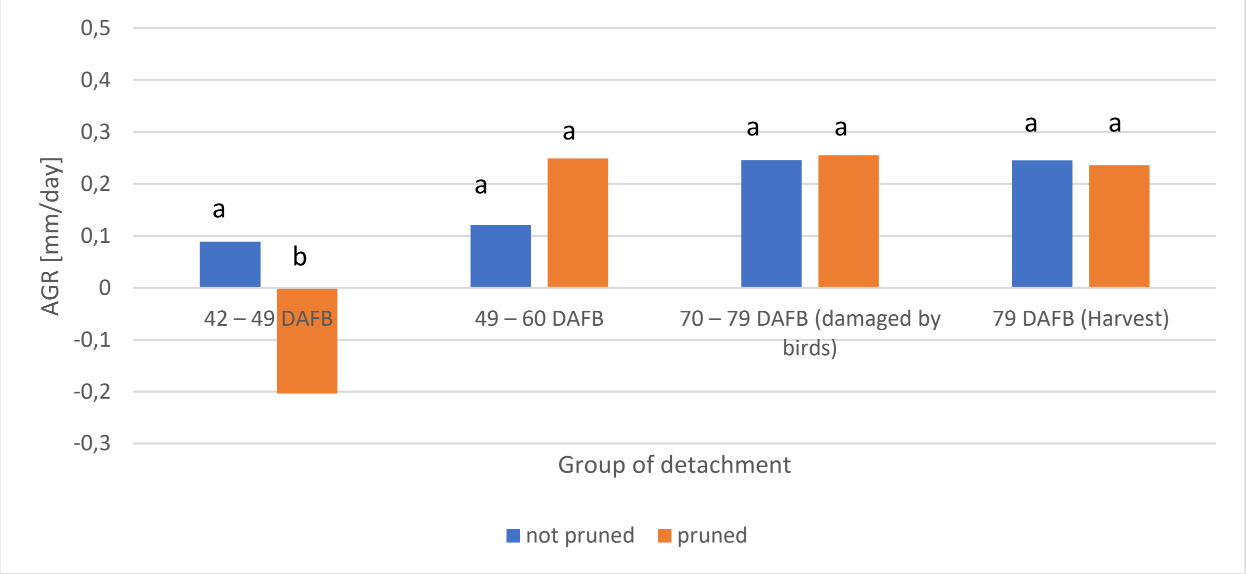

*Figure 6: the diagram represents the AGR values from 36 to 42 DAFB of cherries belonging to groups characterized by different time of detachment. Lowercase letters indicate the differences between the "pruned" and the "not pruned" thesis within a certain group ("Pruning * Time of detachment"); 1-year-old shoots. EMMs compared using Sidak pairwise multiple comparison.*

Between 42 and 49 DAFB the model detected only the significant effect of the group; EMMs of the AGR are reported in Table 10 separately for each group. Again, cherries that will drop before the next sampling (Time of detachment = 49 – 60 DAFB) are already showing a negative AGR, significantly different from that of the other groups. Due to the very poor sample size (2 cherries), the group of cherries dropped between 60 and 70 DAFB was not studied separately.

Time of detachment	**AGR**	**s.e.**	**Sig.**
49 – 60 DAFB	-,038	,098	b
60 – 70 DAFB	,544[a]	,184	a
70 – 79 DAFB (damaged by birds)	.489	,070	a
79 DAFB (Harvest)	,429	,036	a

Table 10: EMMs of AGR values of cherries characterized by different time of detachment from 42 to 49 DAFB. [a] = very small sample size (2 fruits). 1-year-old shoots. EMMs compared using Sidak pairwise multiple comparison.

The same analysis was performed for the fruit of the 2-year-old branches; Table 11 summarizes the p-values of the different factors (and of their interaction) on the AGR value. In both time intervals considered (from 49 DAFB no fruit drop was observed), the fruits that were going to drop during the following weeks showed significantly different growth rates than those of the retained ones (factor "Time of detachment").

Between 36 and 42 DAFB (Table 12) cherries that were going to drop between 42 and 49 DAFB showed negative AGR values. Differently, cherries that were going to drop later (between 49 and 60 DAFB) were still showing positive AGR values but much lower than those of the retained ones. Similarly, between 42 and 49 DAFB (Table 13), the cherries that were going to drop between 49 and 60 DAFB showed negative AGR values significantly lower than those of the retained cherries. As expected, in both time intervals considered, no significant difference was found between the cherries picked at commercial harvest and those damaged by birds.

TIME INTERVAL CONSIDERED	P-VALUE "POSITION"	P-VALUE "TIME OF DETACHMENT"	P-VALUE "POSITION * TIME OF DETACHMENT"
36 – 42 DAFB	,744	**,000**	,612
42 – 49 DAFB	**,044**	**,000**	,481

Table 11: p-values of the different factors (and of their interactions) on the AGR value in the time interval considered for the 2-year-old branches. The factor "Time of detachment" refers to the different groups of detachment. EMMs compared using Sidak pairwise multiple comparison. Bold values are considered significant for α ≤ 0,05.

Time of detachment	AGR	s.e.	Sig.
42 – 49 DAFB	-,038	,041	b
49 – 60 DAFB	,047	,077	ab
70 – 79 DAFB (damaged by birds)	,229	,055	a
79 DAFB (Harvest)	,228	,028	a

Table 12: EMMs of AGR values of cherries characterized by different time of detachment from 36 to 42 DAFB. 2-year-old branches. EMMs compared using Sidak pairwise multiple comparison.

Time of detachment	AGR	s.e.	Sig.
49 – 60 DAFB	-,165	,119	b
70 – 79 DAFB (damaged by birds)	,496	,085	a
79 DAFB (Harvest)	,422	,043	a

Table 13: EMMs of AGR values of cherries characterized by different time of detachment from 42 to 49 DAFB. 2-year-old branches. EMMs compared using Sidak pairwise multiple comparison.

2.4.4 Fruit surface conductance and dry mass

In order to describe the evolution of the internal quality during the season, the dry matter percentage was measured 5 times from 43 DAFB until harvest (Figure 7). The dry matter accumulation followed a linear tendency as shown by the dotted line with the only exception of the sampling at 74 DAFB.

Similarly, the skin conductance (g_c) was measured from 44 DAFB up to harvest. Figure 8 reports the evolution of this parameter during the time interval considered. Skin conductance showed a negative curvilinear trend, with high values early in the season (about 3,30 m/h at the first sampling, a few days before veraison). Although from 51 until 72 DAFB the values remained more or less constant, a significant decrease was observed at the commercial harvest. Anyway, g_c never became negative during the time considered. A cubic equation was chosen to describe the evolution of g_c during the season (with x = DAFB) because this model gained the lowest AIC (Akaike's information criterion).

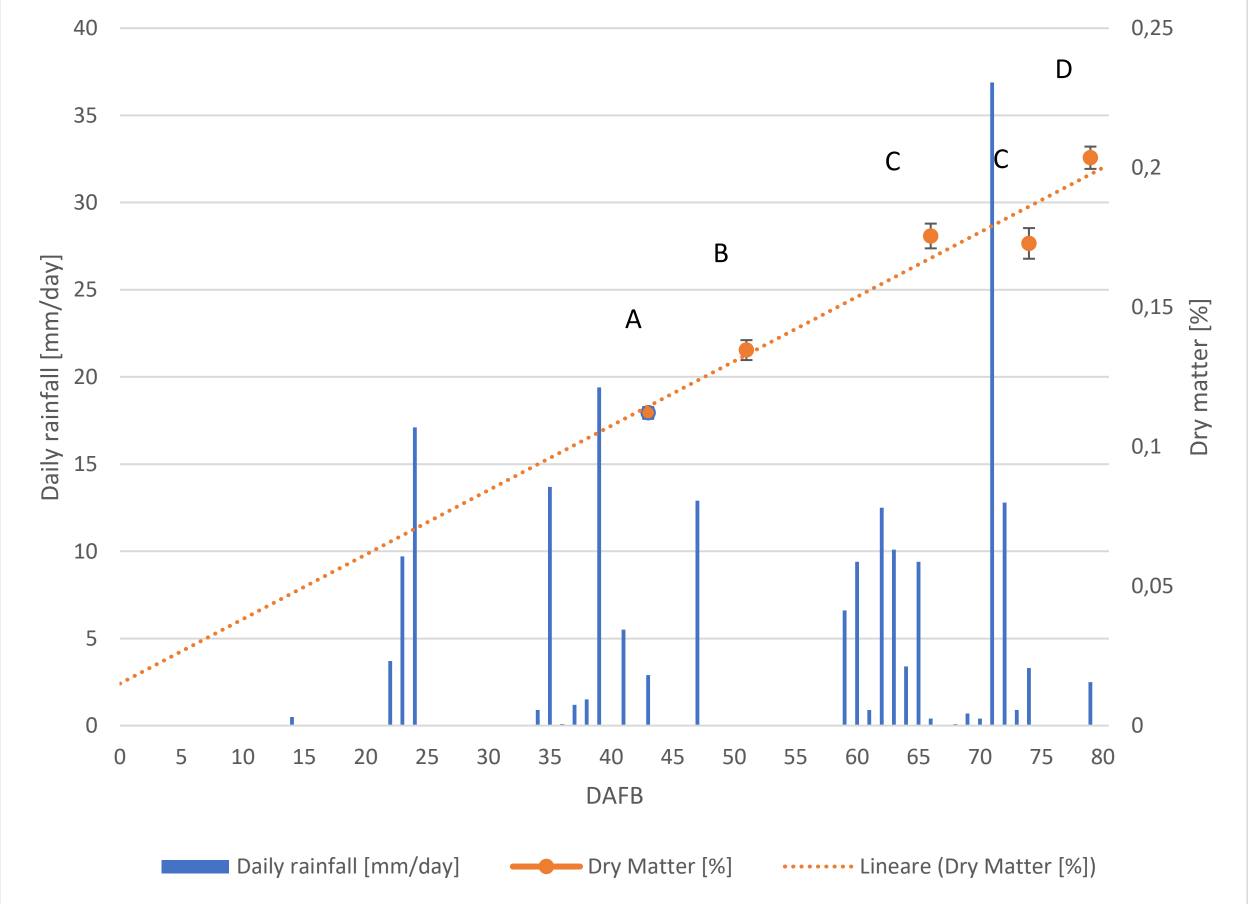

Figure 7: EMMs of dry matter [%] accumulation from 43 until 79 DAFB and rainfall distribution from 0 until 79 DAFB. Letters indicate the differences among the days of the dry matter %. EMMs compared using Sidak pairwise multiple comparison.

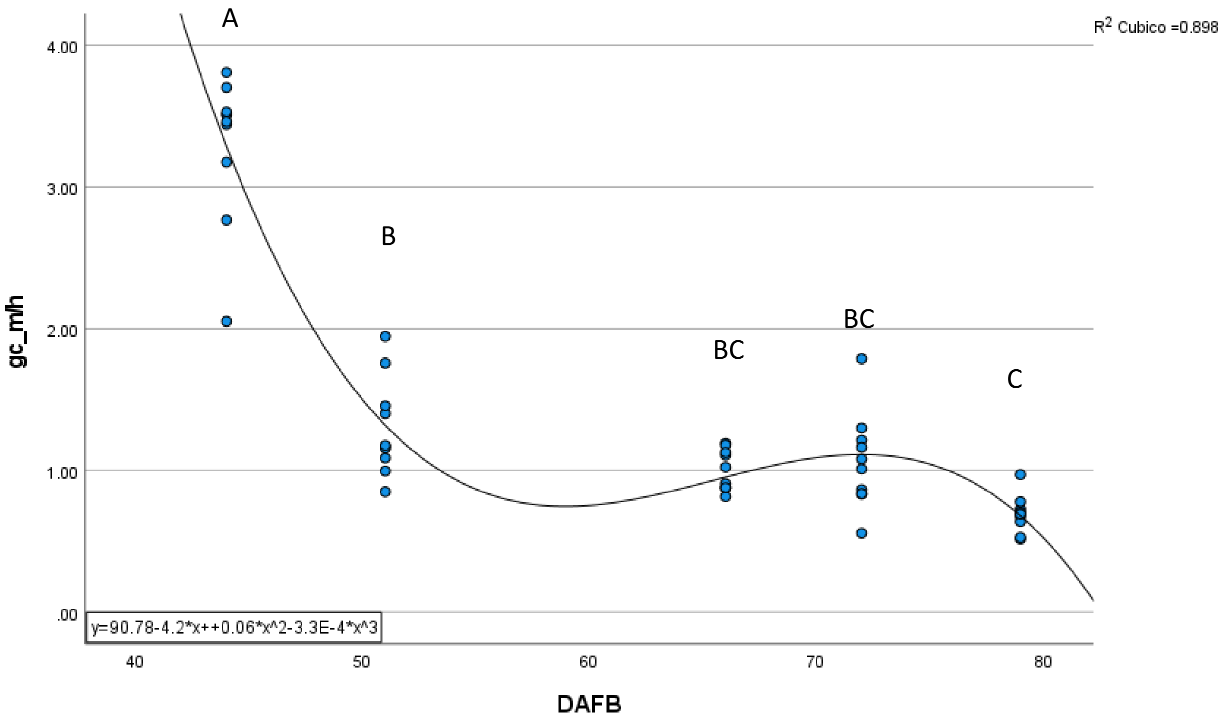

Figure 8: EMMs of surface conductance [m/h] from 44 until 79 DAFB. Letters indicate the differences among the days of surface conductance. EMMs compared using Sidak pairwise multiple comparison. The corresponding cubic equation is printed in the box.

2.4.5 Harvest

At commercial harvest, each tree was picked separately, the trunk diameter was measured, and the yield efficiency (the ratio between the yield in kg and the trunk cross sectional area in cm^2) calculated as reported in Table 14.

Tree n°	**Yield [kg/tree]**	**Yield efficiency [kg/cm^2]**
1	3,09	0,07
2	4,53	0,13
3	4,69	0,09
4	3,19	0,06
5	1,67	0,03
6	2,30	0,04
7	3,51	0,08
8	5,71	0,12
Average	**3,59**	**0,08**

Table 14: Yield per tree [kg] and yield efficiency [kg/cm^2] for each tree considered in the study.

Figure 9: Different phases of fruit drop of ‘Regina’. “A”: fruitlets with smaller sizes show symptoms of fruit drop at 23 DAFB; “B”: fully developed cherries show first symptoms of late fruit drop at 31 DAFB (dull appearance, pink over color); “C”: late-dropping cherries show the typical orange / pink coloration of late-dropping fruits at 37 DAFB. On the same photo, a retained green cherry can be seen on the background.

2.5 Discussion

2.5.1 Flower and fruit counting, fruit set

In the first season, a method to describe fruit drop was defined and validated. Different types of fruiting limbs (1-year-old shoots with basal flower buds and 2-year-old branches with fruiting spurs), at different positions on the tree (basal vs. top limbs) were monitored on a weekly basis in an orchard at the Laimburg Research Centre. In addition, the role of pruning 1-year-old shoots was studied. In both 1-year-old shoots and 2-year-old branches the pattern of fruit set, expressed as the ratio between the number of flowers and fruits (Askarieh, et al., 2021), resembled a sigmoidal curve. A curve with a similar shape was obtained by Hedhly et al. (2009), who studied fruit set of 'Vignola' and 'Sunburst' pollinated by 'Napoleon' and 'Burlat', respectively. Interestingly, the same fruit drop pattern was described also for *Prunus persica* cv. Sudanell (Blanco, 1987). In our trial, starting from full bloom, the number of flowers/fruitlets remained constant for about two weeks after which it started to decrease; at 16 DAFB, the end of petal fall, the fruit size appeared very heterogeneous. At the same time the unpollinated/unfertilized/damaged flowers started to dry out and detach. One week later, at about 23 DAFB the young fruitlets characterized by a smaller size turned yellowish with pink spots on the stylar end; bigger cherries showed instead a bright green color (Figure 9, "A"). All these yellowish fruitlets eventually dropped by 30 DAFB. At the same time, first symptoms of late fruit drop became visible: a certain percentage of fully developed cherries turned dull and yellow (Figure 9, "B"). At 37 DAFB the late-dropping cherries developed a pink / orange color and gradually dropped during the following weeks (Figure 9, "C"). Every late dropped cherry showed an aborted seed and incomplete lignification of the endocarp. For all the treatments, the most intense phase of fruit drop was observed between 23 and 30 DAFB and involved the smallest cherries, probably resulting from late, incomplete, or missing pollination. These considerations are consistent with those reported by Bradbury (1929), who described the fruit drop pattern of *Prunus cerasus* (cv. Early Richmond and Montmorency) as characterized by three waves of fruit drop (a proper description is reported in Chapter 1). In our trial, the first wave was not observed (or confused with flower drop), but the description of the second and the third ones perfectly matches our observations. Unfertilized parthenocarpic fruitlets abscised during the first two waves. Interestingly, he also reported that 93,3% of the fruit belonging to the third wave (= late drop) showed an embryo, concluding that the factors determining the abortion, rather than the lack of fertilization, were the primary cause of fruit drop. With respect to the 2-year-old branches, the statistical model used returned only a significant effect of the day of sampling (DAFB) on the variable considered (the fruit set). Although the lower branches showed a much higher absolute value of the final fruit set than those on top (Table 3, 65 DAFB), the factor "Position" was not significant. This happened probably because of the high variability of the data or because of the relatively small sample size (n = 8). With respect to the 1-year-old shoots, the time of sampling was again found to be significant on the evolution of fruit set; interestingly, also the interaction between this factor and the "Pruning" turned out to be significant. On the unpruned 1-year-old shoots the last phase of fruit drop was observed between 30 and 37 DAFB, and from this point on the percentage of fruit set did not differ from the final one. On the other hand, on the pruned shoots, the same phase of late fruit drop lasted one week longer, and the final fruit set was reached at 44 DAFB (Figure 1). Despite the lower absolute value of the final fruit set measured on the pruned 1-year-old shoots, no significant difference with the unpruned ones was found by the model. No significant effect was found also for the factor "Position". Regardless of the factors considered, final fruit set ranged between 9% and 15%, which is consistent with the final fruit set of Regina pollinated by Kordia measured by Zago et al. (2011) in low crop load years in the same region (Trentino – South Tyrol). Similar final crop loads for 'Regina' pollinated by 'Kordia' (about 15%) were reported for the south of Chile by Sagredo et al. (2017). Interestingly, Zago et al. (2011) reported a high variability in 'Regina' fruit set, ranging from 8,5% up to 18,8% in relation to the year. Bound et al. (2014) reported an intermediate fruit set of 60% at 21 DAFB for 'Regina' pollinated by 'Kordia' which is slightly lower than our measurements for the same period (about 80%). Surprisingly, the same article reported a final fruit set of 23%, much higher than ours, emphasizing how it was the last (=late) phase of fruit drop that had a decisive effect on final fruit set. No information about how limb position within the crown and/or pruning influence fruit set was found in literature.

2.5.2 Vegetative parameters

For 1-year-old shoots, the height within the canopy (factor "Position") seemed to have no effect on their vegetative parameters. Spring pruned 1-year-old limbs were shorter at full bloom, but grew longer new shoots at the end of the season, and showed a higher ratio between the sum of the (new) shoot length per limb at terminal bud set and limb length at FB. Since it normalizes the overall seasonal growth to the length of the limb from which the new shoots originated, this ratio was interpreted as a measure of the vigor of the limb. The factor "Pruning" had no effect on the number of new shoots emitted per limb as well as on the overall length (sum) of the new shoots per limb. Surprisingly, the factor "Position" significantly influenced the vegetative growth of the 2-year-old branches. Higher branches emitted a higher number of new shoots, showed a higher shoot length, and a higher ratio between the sum of the (new) shoot length per limb and the limb length at FB. Possible linear regressions between the vegetative parameters and the fruit set values have been considered. None of the parameters seemed to be correlated to final fruit set, except for the ratio between the sum of the (new) shoot length per limb and the limb length at FB on the 1-year-old shoots. The “B” value (the regression slope) is slightly negative and indicates that for each unit increase of the predictor the final fruit set at harvest decreased by 1,8%, which is consistent with the final fruit set differences shown in Table 3. This predictor explained 13,2% of the whole variance of the dependent variable; the low R-square does not compromise the model, but it simply suggests that other explanatory variables certainly play a role. In sweet cherry, competition between fruit set and the vegetative growth has been hypothesized and studied by many authors (Blanusa et al., 2006; Cittadini et al., 2008; Ayala et al., 2015; Morandi et al., 2019).

Pruning the 1-year-old shoots did affect their leaf demography: by pruning the 1-year-old shoots we removed 23% of their initial length; the new shoots emitted were significantly longer in the pruned thesis but, interestingly, their number was the same for both treatments (ranging between 3,5 and 3,94 new shoots per limb). An extension shoot formed in the previous year, will evolve into a limb bearing fruits on its basal part, and showing either non-fruiting spurs or current season shoots in its middle and terminal part (Lang, 2001; Ayala et al., 2004). Since the number of current season shoots was not influenced by pruning, the treatment changed the ratio between the number of current season shoots and the number of the non-fruiting spurs, and hence the ratio between their leaf populations. Non-fruiting spurs reach their maximum development rapidly in the season, while the current season shoots create new leaf area even up to harvest (Ayala, et al., 2008). A few years ago, Ayala et al. (2018) reported that at 25 DAFB (more or less at the same time as most of our late-dropping cherries stopped growing) 46% of the total carbon fixed by non-fruiting spurs was exported to the fruit, while only 27% of the carbon fixed by the extension shoots was exported to the fruit (‘Ulster’ on ‘Gisela 6’). If this observation was also true for the combination ‘Regina’ on ‘Gisela 5’, we could suppose that promoting the vegetative growth by pruning the 1-year-old shoots in the spring not only could increase the competition for stored reserves between vegetative and reproductive growth, but could also affect the efficiency of the newly-synthetized carbohydrates exported from the sources to the fruits by changing the composition of the leaf populations. The same authors studied the dependence of the sweet cherry cv. Regina grafted on ‘Gisela 6’ on storage reserves. In this work, the highest dependence was measured at first and full bloom, thereafter, the concentration decreased in all organs until it stabilized between 21 DAFB and 35 DAFB. Interestingly, in our trial, dropping fruits of the same cultivar, ceased growth in the same period, i.e. when the plant went from depending on reserves to becoming photosynthetically self-sufficient (Ayala, et al., 2015).

In our trial, limbs were not girdled in order to keep the studied organs as representative as possible; however, despite long-distance carbon import/export could not be excluded, in sweet cherry the primary export of carbohydrates seems to be directed towards the fruit closest to the source (Ayala, et al., 2008).

2.5.3 Seasonal fruit growth

At 28 DAFB, 5 fruits without any evidence of abscission were tagged on each tree (240 in total). One week later, at 36 DAFB, the first calibration was performed. For both limb types, retained cherries followed the second half of the typical double sigmoidal curve described by many authors (Flore et al., 1999; Else et al., 2004; Azarenko et al., 2008). The average diameter increased at each time of sampling with the only exception of the sampling 79 DAFB, in which it did not increase (and, in some cases, decreased). Unfortunately, at 36 DAFB, a high percentage of the tagged fruits was already showing the first symptoms of dropping; therefore, measurements only described the last phases of growth of the dropping cherries, thus impeding the identification of the onset of late fruit drop. For both limb ages considered (1-year-old shoots, 2-year-old branches), tagged cherries dropped gradually and with similar timing. The heaviest drops were measured between 36 and 42 DAFB and between 42 and 49 DAFB; a residual percentage dropped between 49 and 60 DAFB, after which, no relevant physiological fruit drop was observed. Cherries that dropped between 36 and 42 DAFB were calibrated only once, therefore no AGR was calculated for this group. The AGR of retained cherries resembled a bell-shaped function, with the most intense growth between 49 and 60 DAFB, similar to the findings of Mancini et al. (2021) who studied ‘Blaze star’ grafted on ‘MaxMa 14’. For both limb ages, cherries closer to the top reached maturity (i.e. stopped their growth) some days earlier than those on the lower limbs. Negative AGR values measured at the last sampling showed that overripe cherries started decreasing their volume. The growth rate of dropping cherries started to decrease at least 2 weeks before the actual abscission; in particular, the data collected showed how from 14 to 7 days before the abscission, dropping cherries still showed positive AGRs but they were significantly lower than the retained ones; the AGR values of the dropping cherries became negative only during the last week before the abscission. Negative AGR values on dropping cherries were measured also by Mancini et al. (2021). Interestingly, the statistical model used returned the interaction “Pruning * Time of detachment” as significant in the time between 36 and 42 DAFB for the 1-year-old shoots. From 36 to 42 DAFB, the growth of the cherries that would drop between 42 and 49 DAFB (Figure 6) seemed to be significantly influenced by the pruning: cherries of the unpruned shoots were still showing positive AGRs (albeit significantly lower than those of the retained ones) while cherries of the pruned shoots were already showing very negative values. This result is difficult to interpret, however it seems that pruning somehow accelerated the dropping process.

2.5.4 Fruit surface conductance and dry mass

The dry matter accumulation measured from 43 DAFB up to harvest followed a linear trend. A similar linear trend was reported by Ayala et al. (2018) from 45 to 75 DAFB for the combination 'Ulster' on 'Gisela 6'. Interestingly, a temporary stationary phase was observed at 74 DAFB, probably due to the intense rainfalls of the previous days. The evolution of skin conductance followed a negative curvilinear trend, which was found to be very well described by a cubic function. Fruit's ability to lose water in form of water vapor during transpiration represents an important factor in regulating fruit pressure potential, and hence in attracting xylem and phloem flows; fruit surface conductance was found to be strongly related to specific transpiration and to xylem flow (Rossi, et al., 2022). Brüggenwirt et al. (2016) reported that during the early stages of fruit growth, xylem flow and transpiration appeared similar in size and opposite, but, at late SIII, xylem contribution to the cherry vascular flows drastically decreased, and no relationship between xylem flow and transpiration was evident. The drop in fruit surface conductance shown late in the season in our trial can be attributed to the accumulation of waxes on the fruit surface, as well as to the progressive drop in stomatal and xylem functionality. The skin conductance values reported for sweet cherry by Rossi et al. (2022) for the cv. Black Star grafted on 'Gisela 6' early and late in the season are fully compatible with our results, with the only difference that our pre-harvest values were slightly higher, probably due to the different cultivar considered. The evolution of the skin conductance of sweet cherry fruits as reported in Figure 8 is consistent with the observations made on other stone fruits such as Prunus persica (Gibert, et al., 2005) and Prunus salicina (Corelli Grappadelli, et al., 2019).

2.6 Conclusions

In this work, it was investigated which approach could correctly describe the (late) fruit drop pattern of sweet cherry cv. Regina grafted on 'Gisela 5'. The aim of this preliminary work was to develop a method that could be functional to a similar monitoring on a broader scale during the following seasons. For both the limb ages considered, the evolution of the % fruit set resembled a sigmoidal curve conditioned by at least two intense fruit drop waves. The first one involved only smaller fruitlets, very likely the unfertilized parthenocarpic ones and the late-fertilized ones; a very plausible situation since full bloom of 'Kordia' (an early blooming pollinizer) shows only a partial overlapping with that of 'Regina' (late blooming). The second wave, which represented the focus of this work, involved fully developed cherries, which, once dropped, showed an aborted seed. For this group, the cause of drop is to be found in the physiology of the plant rather than in a lack of fertilization. In the season considered, late fruit drop started at 30 DAFB and was concluded with the beginning of veraison. The position of the limb within the canopy did not influence the fruit drop pattern both for the 1-year-old shoots and the 2-year-old limbs; however, it is worth noting that, due to the high variability observed, the sample size was probably too small (1 orchard, 8 trees) to properly detect the effect of the factor. On the contrary, pruning the 1-year-old shoots in spring prolonged the late fruit drop by one week and accelerated the shrinking of the cherries which dropped between 42 and 49 DAFB. The pruning of 1-year-old shoots increased the average length of the new shoots emitted during the season and changed the ratio between non-fruiting spur leaves and current-season shoot leaves. The ratio between the sum of shoots length per limb at terminal bud set and the limb length at full bloom showed to be a significant predictor of the final fruit set in the linear regression analysis for the 1-year-old shoots. 2-year-old branches belonging to the basal part of the canopy seem to behave very differently during the season than those close to the top. The first ones re-formed the fruiting spurs and did not invest much into the vegetative growth, while the second ones emitted a great number of new shoots which completely upset the original composition of the leaf population. The tagging of single fruitlets was performed quite late in the season, therefore only the last part of the growth pattern of late dropping cherries was measured. In general, the growth rate of dropping cherries started to decrease at least 2 weeks before the actual abscission; in particular, the data collected showed how from 14 to 7 days before the abscission, dropping cherries still showed positive but significantly lower AGR values than those of the retained ones; the AGR values of the dropping cherries became negative only during the last week before the abscission. This information suggest that the physiological causes of late fruit drop should be investigated at least two weeks before the actual drop. The data collected for % dry matter, skin conductance, and yield efficiency indicate that the orchard chosen for the trial was representative of the South Tyrolean cherry growing. Since the factors considered (“Pruning” and “Position”) did not show a great significant effect on the evolution of fruit set, future trials should also include other factors such as the climatic variables.

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3 Chapter 3: A province-wide screening of ‘Regina’ late fruit drop: influence of environmental, physiological and management factors

3.1 Abstract

‘Kordia’ and ‘Regina’ represent the standard varietal mix in South Tyrol cherry growing. Within the same season, although the similar agronomic conditions, the intensity of late fruit drop of ‘Regina’ in different sites can be very variable, in some cases limiting the profitability of the crop. In the present work, the evolution of fruit set and vegetative growth of 6 orchards located at different elevations (from 225 to 1175 m a.s.l.) was monitored weekly to study the effect of different climatic conditions. In total, 6300 flowers and of 1920 fruitlets were studied. In this work, as soon as much of the first wave of fruit drop ceased, the number of fruits per limb prior to late fruit drop (n_0) was collected. Therefore, relative fruit set was calculated as the ratio between the fruit number at harvest and the n_0; finally, intensity of late fruit drop was expressed as *1 – Relative fruit set*. Again, the pattern of total fruit set resembled a sigmoidal curve with some sharp decreases due to frost damages. In the season considered, late fruit drop intensity was very heterogeneous, varying from 12,40% of the fruitlets set in “Laimburg” to the 76,10% of “Fragsburg”. The factor “Position” within the canopy (“high” vs “low”) showed no significant effect on the relative fruit set, the number of new shoots, the number of non-fruiting spurs, and their linear density in the limbs considered. Conversely, spring pruned 1-year-old shoots showed, in absolute terms, less extension shoots than the unpruned ones but, interestingly, the extension shoots linear density remained constant in 5 orchards of 6. Shortening the 1-year-old shoots strongly influenced the number of non-fruiting spurs both in absolute and relative terms: the unpruned limbs showed both a higher number of non-fruiting spurs per limb and a higher non-fruiting spurs density per linear meter in almost all the orchards, with strong consequences on the leaf populations of the following season. Pruned shoots showed significantly lower relative fruit set only in “Fragsburg” and “Martell”; interestingly, these two orchards also showed the most vigorous shoot growth. A linear regression analysis between the vigor of the limb (expressed as the sum of new shoots’ length normalized by the limb length) and its relative fruit set was performed considering the sites all together: the analysis revealed that for each unit increase of the predictor (therefore, by increasing the vigor) the final fruit set decreased by 4,7%. Again, the factor considered explained only partially the total variance of the studied phenomenon (R-square = 0,072). The fact that the drupelets have been individually labelled already at shuck split, made it possible, a posteriori, to divide them into different groups based on their time of detachment and to use these as fixed factors in the data analysis; furthermore, knowing the average fruit diameter of every group at the first sampling,

allowed us to ignore those that were already smaller than the retained ones. In doing so, it was possible to discern between the different waves of fruit drop, and, more specifically, between the detachment of unpollinated/unfertilized fruitlets and the physiological late fruit drop. Prolonged periods of low temperatures and/or sudden severe reduction in the daily Growing Degree Hours accumulation were followed by a decrease in the average growth rate of both extension shoots and fruitlets. Fruits that slowed down their growth were not able to recover and detached in the following weeks. The most intense late fruit drop was observed in those orchards where temperature drops occurred between 20 and 30 DAFB. In the soil analysis no significant deficiencies were detected; similarly, the leaf analysis performed at the time of late fruit drop did not show any particular deficiency in the two orchards characterized by the most intense fruit drop.

3.2 Introduction

Following from the previous chapter, the objective of the present study was to perform a Province-wide screening of 'Regina' late fruit drop in order to identify potential influences of environmental, physiological and management factors. Although the rootstock- and varietal choice, as well as the agronomic management, are relatively homogeneous in this area due to a centralized advisory service, within the same season the intensity of late fruit drop of 'Regina' in the different orchards can be very variable, in some cases limiting the profitability of the crop. South Tyrol is a typical mountain region characterized by a sub-alpine climate. Despite the modest size of this province, its climatic conditions can be very heterogeneous as they are strongly influenced by numerous factors such as, for example, elevation and exposure. In the present work, this characteristic of the area has been exploited to study the effect of different climatic conditions on both vegetative- and fruit growth, and above all on the evolution of fruit set. Orchards from 225m to 1175m a.s.l. have been used in this study. In the scientific literature, many hypotheses have been investigated to give an explanation to the variable fruit set (for further information see Chapter 1); however, these works focused on floral biology, pollinators, and plant growth regulators as key elements of a "general" fruit set, but none of these studied in detail the intensity of late fruit drop and its dependence on environmental factors.

Research hypothesis n°1: climatic conditions have an influence on the fruit drop pattern.

Research hypothesis n°2: excessive vigor can increase late fruit drop intensity but is not its main cause.

Research hypothesis n°3: nutrient deficiencies are not the main cause of late fruit drop.

3.3 Materials and methods

The study was carried out in 2021 in 7 mature sweet cherry commercial orchards located at different elevations (from 225 up to 1175m a.s.l.) in South Tyrol, Italy. Each orchard had 'Kordia' as the main cultivar and 'Regina' as pollinizer (about 2/3 and 1/3); each orchard was covered both by an anti-hail net and a plastic anti-rain film. With the only exception of the orchard "Laimburg", the whole orchards were surrounded by anti-insect nets. The plant distance was about 1,5-1,8 m between the trees and 3,5 m between the rows, plant density was about 1714-1428 trees/ha.

In each orchard 8 trees with similar trunk diameter and vigor were identified. All the trees studied were in full production (about 10-year-old), trained as central leader and grafted on the dwarfing rootstock 'Gisela 5'. The orchards were managed according to integrated cultural practices in terms of fertilization, irrigation, plant defense, and pruning. An exhaustive summary of the agronomic practices recommended by the local agricultural extension specialist (https://www.beratungsring.org) can be found in the official guidelines published annually (https://www.beratungsring.org/info/organisation/broschueren/kirsche.html). No thinning and no plant growth regulators were applied during the trial. Full bloom occurred from early April up to mid-May and fruit were harvested from late June up to mid-August, depending on the elevation and the precocity of the location. Each orchard was provided with a weather station, the elevation was recorded using GeoBrowser Maps (https://maps.civis.bz.it). At pre- and full bloom frost damages on 500 flowers per cv. were measured. Unfortunately, due to the severe frost damages (> 80%) the orchard "Schluderns" at 900m a.s.l. was discarded. The phenological stages were characterized using the BBCH scale (Fadon, et al., 2015).

Prior to flowering, 4 limbs per tree were tagged: two 1-year-old shoots with basal flower buds left unpruned, and two 1-year-old shoots with basal flower buds shortened leaving three to four vegetative buds, one in the lower (0 – 1,5 m) and one in the upper (3,0 – 4,0 m) part of the canopy respectively. The rationale for the chosen treatments is the same of Chapter 2; however, in this trial treatments were repeated on a broader level in order to quantify their effects in different orchards. Since in the first trial the fruit set evolution appeared very similar for both the 1-year-old shoots and the 2-year-old branches, the latter were not considered in this trial.

FLOWER AND FRUIT COUNTING, FRUIT SET

The number of flowers or fruitlets on each tagged limb was counted weekly from pre- or full bloom up to the end of late fruit drop, for a total of 10/13 samplings per each orchard. Fruits with symptoms of abscission were counted until natural detachment occurred.

At full bloom, fruiting limbs length was measured.

SEASONAL FRUIT GROWTH

As soon as it was possible to distinguish persisting fruitlets from unfertilized ones (about 23-30 DAFB, depending on the orchard), 10 fruits (if present) without any evidence of abscission were tagged on each limb using a progressive number from 1 to 320. Overall, 40 fruits per tree and 80 fruits for each limb type were tagged. Starting from the same day, these fruits were calibrated on a weekly basis using a digital Bluetooth caliper (T. R. Turoni, Forlì – Italia) up to the end of late fruit drop. Fruit growth rate (both positive and negative) was expressed as absolute size increment in the unit of time (AGR). The following equation, as reported by Morandi et al. (2019), was used:

$$AGR = \frac{d_{t1} - d_{t0}}{t_1 - t_0}$$

Where *d* is the fruit diameter in mm and *t* the time expressed as DAFB.

As soon as much of the first waves of fruit drop ceased, approximately at stage 77 as described by Fadon et al. (2015), the number of fruits per limb prior to late fruit drop (n_0) was collected.

The following parameters were considered:

Total fruit set was calculated for each limb as follows (Askarieh, et al., 2021):

$$Total\ fruit\ set\ \% = \frac{Fruit\ number\ at\ harvest\ time}{Total\ flowers\ number} * 100 \tag{1}$$

Relative fruit set (referred to n_0) was calculated for each limb as follows:

$$Relative\ fruit\ set\ \% = \frac{Fruit\ number\ at\ harvest\ time}{Fruit\ number\ at\ n0} * 100 \tag{2}$$

Intensity of late fruit drop was calculated for each limb as follows:

$$Intensity\ of\ late\ fruit\ drop\ \% = (1 - Relative\ fruit\ set\ \%) \tag{3}$$

Additionally, two orchards managed by the same farmer but located at different elevations ("Lengstein D." and "Lengstein J.") were provided with fruit gauges able to perform continuous measurement of fruit diameter interfaced to a wireless data-logger system (Wi-Net s.r.l., Cesena, Italy) (Morandi, et al., 2007). In each orchard, 8 fruits were monitored from about 30 DAFB up to harvest.

SEASONAL SHOOT GROWTH

As soon as vegetative buds started to grow (a few days after full bloom), the new shoots on the tagged limbs were counted and measured on a weekly basis; about 9/12 samplings were performed for each orchard until the end of late fruit drop.

GROWING DEGREE HOURS

Daily cumulative Growing Degree Hours (GDH) were calculated from full bloom using an asymmetric curvilinear model as reported by Azarenko et al. (2008). Between 4°C (base temperature) and 25°C (optimum), the following formula was used:

$$GDH = \left[\frac{25°C-4°C}{2}\right] * (1+\cos\left(\pi + \frac{\pi(hourly\ temperature-4°C)}{25°C-4°C}\right)) \tag{4}$$

For temperature above optimum, the critical temperature of 36°C was considered:

$$GDH = (25°C-4°C) * (1+\cos\,(\frac{\pi}{2}+\frac{\pi}{2} * \frac{hourly\ temperature-25°C}{36°C-25°C})) \tag{4}$$

FRUITLET AND LEAF ANALYSES

When the first signs of late fruit drop became evident, retained fruitlets and dropping fruitlets were separately sampled. Nitrogen (N) concentration was determined by means of open digestion with H_2SO_4 and catalyst, detection with colorimetry; for P, K, Ca, Mg: open digestion with H2SO4 and catalyst, detection with ICP-OES. The microelements B, Fe, Mn, Cu, Zn, Na, S, Si, by means of EPA 3052 1996 and EPA 6010D 2018 (Microwave digestion and quantification with ICP-OES).

At the same time, leaves of the marked trees were collected in order to determine their nutrient status: the nitrogen (N) concentration was determined by means of DIN EN ISO 16634-1:2009 (Elementar Analysis according to Dumas) while P, K, Ca, Mg, B, Fe, Mn, Cu, Zn, Na, S, Si concentration with EPA 3052 1996 and EPA 6010D 2018 (Microwave digestion and quantification with ICP-OES).

HARVEST YIELD AND FINAL SAMPLING

At commercial harvest, total fruit weight per tree and trunk cross-sectional area (TCSA, 20 cm above the grafting point) were measured. Each tree was harvested separately. At the same time, the last fruit counting, the last calibration, and the last measurement of shoot length were performed.

In addition to this, at harvest a soil sample (0-20 cm depth) for each orchard was collected and investigated for type, texture, organic substance concentration, pH, and P/K/Mg/B/Mn/Cu/Zn concentration; finally, the leaf tissue analysis was repeated.

STATISTICAL ANALYSIS

All the flowers/fruits counts were elaborated using the General Mixed Model (GMM) with Repeated Measures (RM) procedure in IBM SPSS Statistics 27; within the model the tree was selected as the random factor and the limb as the subject repeated at each sampling time. The same approach but having the limb as the random factor and the single fruit as the subject repeated was used for the diameter measurements. The covariance type (COVTYPE) of the RM was chosen using the “Aikaike Information Criterion” (AIC). The GMM was also used to study the vegetative growth (with the tree as the random factor). Estimated Marginal Means (EMMs) were compared using pairwise multiple comparison with Sidak adjustment. The more common Tukey test has been discarded since it leads to inflated alpha level when the sphericity assumption is not met (and mixed models don’t require the sphericity assumption), and therefore it is unsuitable for performing pairwise comparisons in a repeated measures design (Scott, 1980). Of the most frequently tests used with these models, LSD was discarded since it is vulnerable to Type I errors, and Bonferroni to Type II errors. Linear Regression analysis was used having the relative fruit set at harvest as dependent variable. Effect was considered significant at $p \leq 0.05$.

ABBREVIATIONS

DAFB = Days after full bloom;
GMMRM = General Mixed Models with Repeated Measures;
EMMs = Estimated Marginal Means;
AGR = Absolute Growth Rate;
GDH = Growing Degree Hours;
ASL = Average Shoot Length;
s.e. = standard error.

3.4 Results

Location	**Elevation**	**Full bloom**	**Harvest**	**% Frost damaged flowers**
Laimburg	225 m a.s.l.	06.04.2021	30.06.2021	1 %
Fragsburg	705 m a.s.l.	18.04.2021	19.07.2021	1 %
Lengstein D.	785 m a.s.l.	20.04.2021	22.07.2021	7 %
Lengstein J.	920 m a.s.l.	03.05.2021	28.07.2021	70 %
Vöran	1175 m a.s.l.	12.05.2021	06.08.2021	47 %
Martell	1135 m a.s.l.	19.05.2021	14.08.2021	11 %

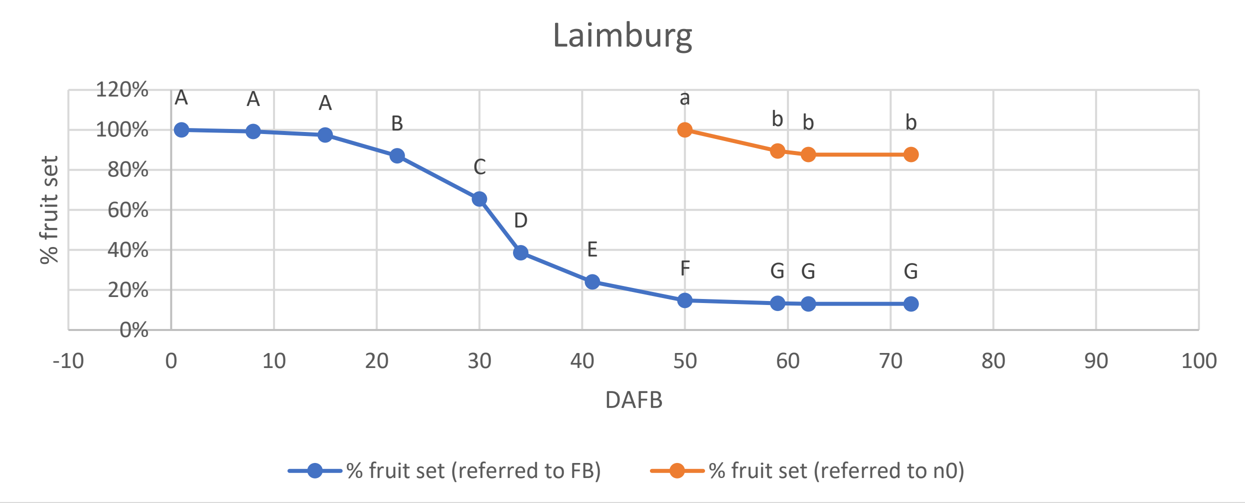
Table 1: name of the locality, elevation, date of full bloom and harvest, and percentage of total frost damages of the orchards involved in the study.

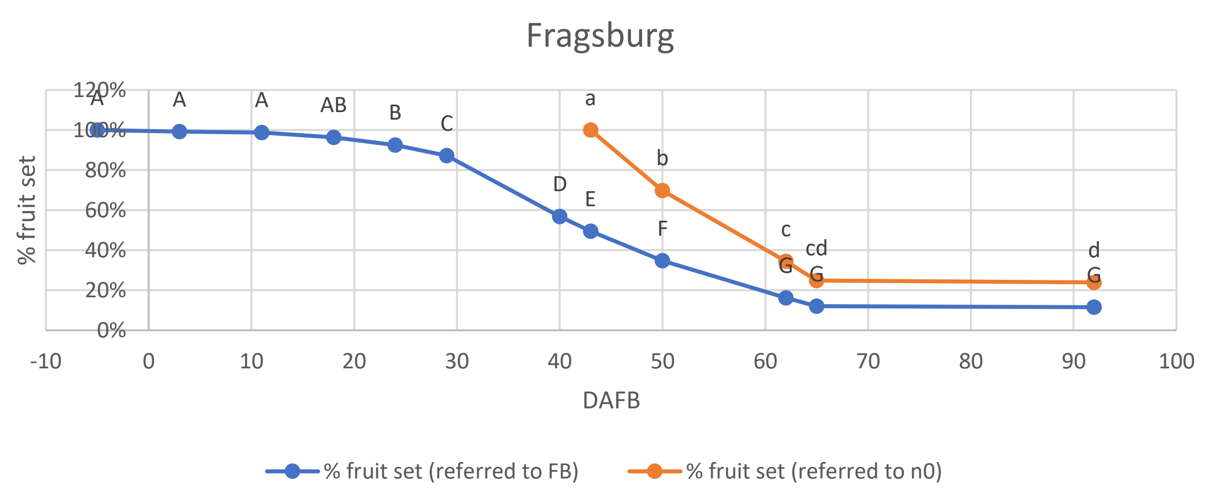
3.4.1 Flowers and fruits counting, fruit set

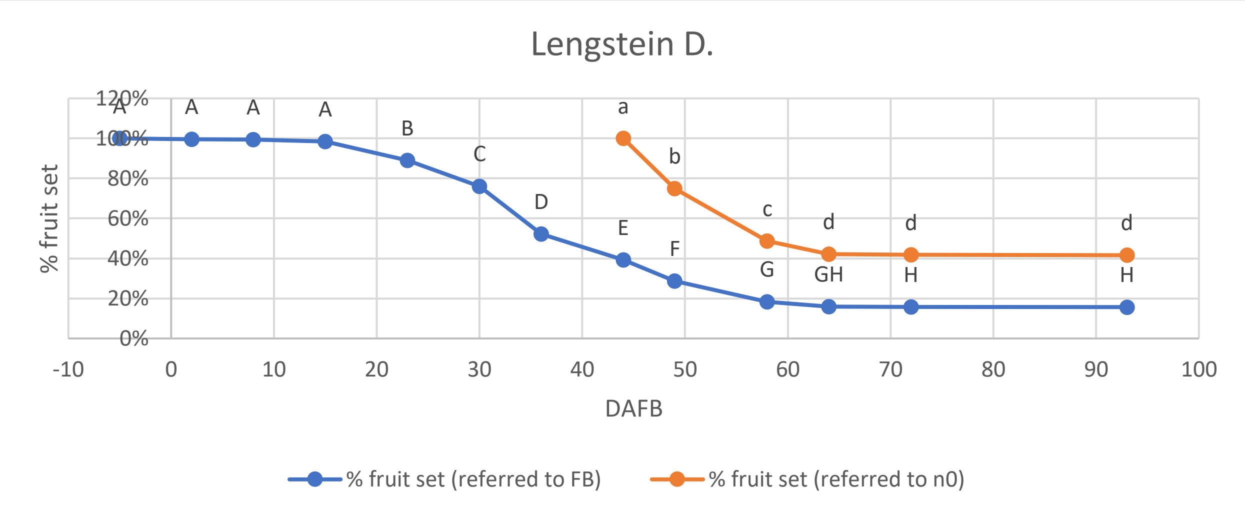
Orchard	**p-value "Pruning"**	**p-value "Position"**	**p-value "Sampling (time)"**	**p-value "Pruning * Position"**	**p-value "Pruning * Sampling"**	**p-value "Position * Sampling"**	**p-value "Pruning * Position * Sampling"**
Laimburg	,630	,143	**,000**	,879	,320	**,011**	,121
Fragsburg	,405	,228	**,000**	,898	,109	**,039**	,063
Lengstein D.	,435	,399	**,000**	**,025**	,962	**,002**	,209
Lengstein J.	,857	,124	**,000**	,735	,995	**,030**	,373
Vöran	,287	**,005**	**,000**	,725	,927	,171	,957
Martell	,607	**,006**	**,000**	,969	,729	**,005**	,950

Table 2: p-values for the different factors considered obtained as output of the Mixed Model with Repeated Measures having the % of total fruit set as the dependent variable. Bold p-values are considered significant at α ≤ 0,05.

Table 2 reports the output of the Type III Tests of Fixed Effect after the Mixed Model with repeated measures procedure in which the subject was the limb. This analysis studied the effect of different factors on the evolution of the total fruit set of the 1-year-old shoots during the season from full bloom to harvest (calculated using the formula (1)). Each orchard was analyzed separately. Of the 12 flower/fruit counts made in the "Laimburg" orchard, the last one (at harvest, 85 DAFB) was excluded since it was severely influenced by birds' damage. The factors considered were the "Position" of the limb within the canopy ("high" and "low"), the "Pruning" (1-year-old shoots shortened to 3/4 vegetative buds or left unpruned) and the day of "Sampling"; the interactions between the factors were also studied. For every orchard the model returned the factor time of "Sampling" as significant as well as the interaction "Position * Sampling" with the only exception of the orchard "Vöran", where an overall effect of the factor "Position" was recorded. Figure 1 represents the evolution of fruit set both referred to the number of flowers at FB (blue line, calculated using eq. 1) and to the number of fruits at n0 before the beginning of late fruit drop (orange line, calculated using eq. 2).


Laimburg
% fruit set
120%
100%
80%
60%
40%
20%
0%
-10
0
10
20
30
40
50
60
70
80
90
100
DAFB
A
A
A
B
C
D
E
F
G
G
G
a
b
b
b
% fruit set (referred to FB)
% fruit set (referred to n0)


Fragsburg
% fruit set
120%
100%
80%
60%
40%
20%
0%
-10
0
10
20
30
40
50
60
70
80
90
100
DAFB
A
A
A
AB
B
C
D
E
F
G
G
G
a
b
c
cd
d
% fruit set (referred to FB)
% fruit set (referred to n0)


Lengstein D.
% fruit set
120%
100%
80%
60%
40%
20%
0%
-10
0
10
20
30
40
50
60
70
80
90
100
DAFB
A
A
A
A
B
C
D
E
F
G
GH
H
H
a
b
c
d
d
d
% fruit set (referred to FB)
% fruit set (referred to n0)

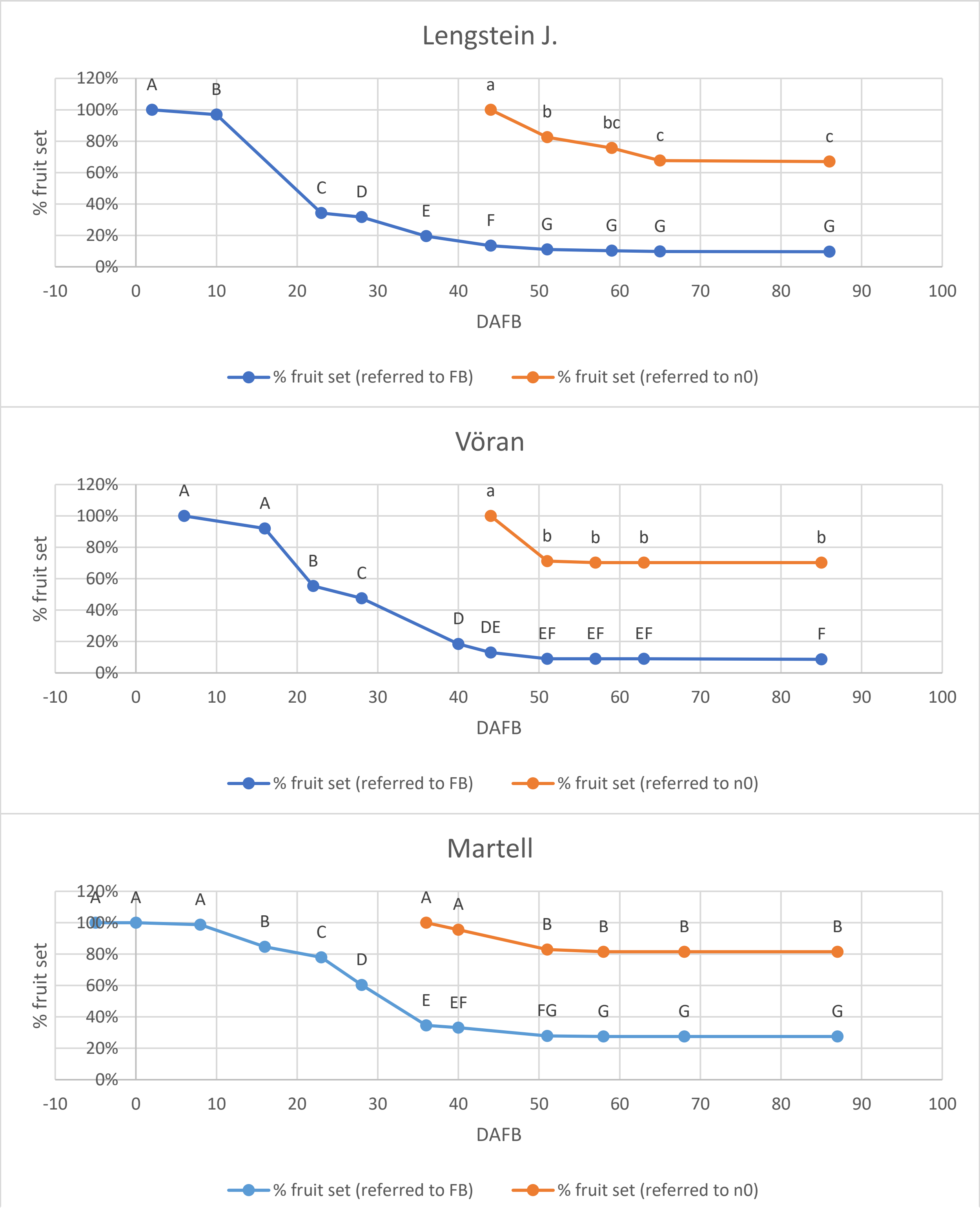


Figure 1: evolution of the total- (referred to FB) and the relative (referred to n0) fruit set over the time expressed as Days After Full Bloom. Percentages are referred to the day of sampling. Means followed by the same letter are not significantly different at α ≤ 0,05. For every orchard, each point represents the average of the % fruit set of 32 limbs tagged on 8 trees.

Each point represents the estimated marginal mean of the percentage of total or relative fruit set referred to a specific sampling day expressed in days after full bloom (DAFB). The evolution of the total fruit set appears very similar in every orchard resembling a sigmoidal curve. The initial stationary phase coincided with the flowering time and lasted more than 2 weeks in most of the orchards; this was followed by a gradual decrease of the total number during the initial fruit set. Final fruit set was reached at different times between 51 and 65 DAFB depending on the orchard considered. The total fruit set varied between 9% and 28%, while the relative fruit set varied between 24% and 88%. The evolution of fruit set referred to n0 allows to visualize the reduction of the production caused by late fruit drop. The estimated marginal means of the fruit set evolution as function of the Growing Degree Hours is reported in Figure 2 for each orchard separately. From 0 to 5000 GDH most of the orchards followed a very similar evolution. A drastic drop in the % of fruit set was observed in the orchards heavily damaged by the spring frost (Table 1): here a high number of flowers were killed by the low temperatures and were not able to develop into fruitlets. Overall, the fruit set evolution over the GDH appears very similar in each orchard. Furthermore, in each site 'Regina' Growing Degree Hours requirement to reach harvest was about 24000.

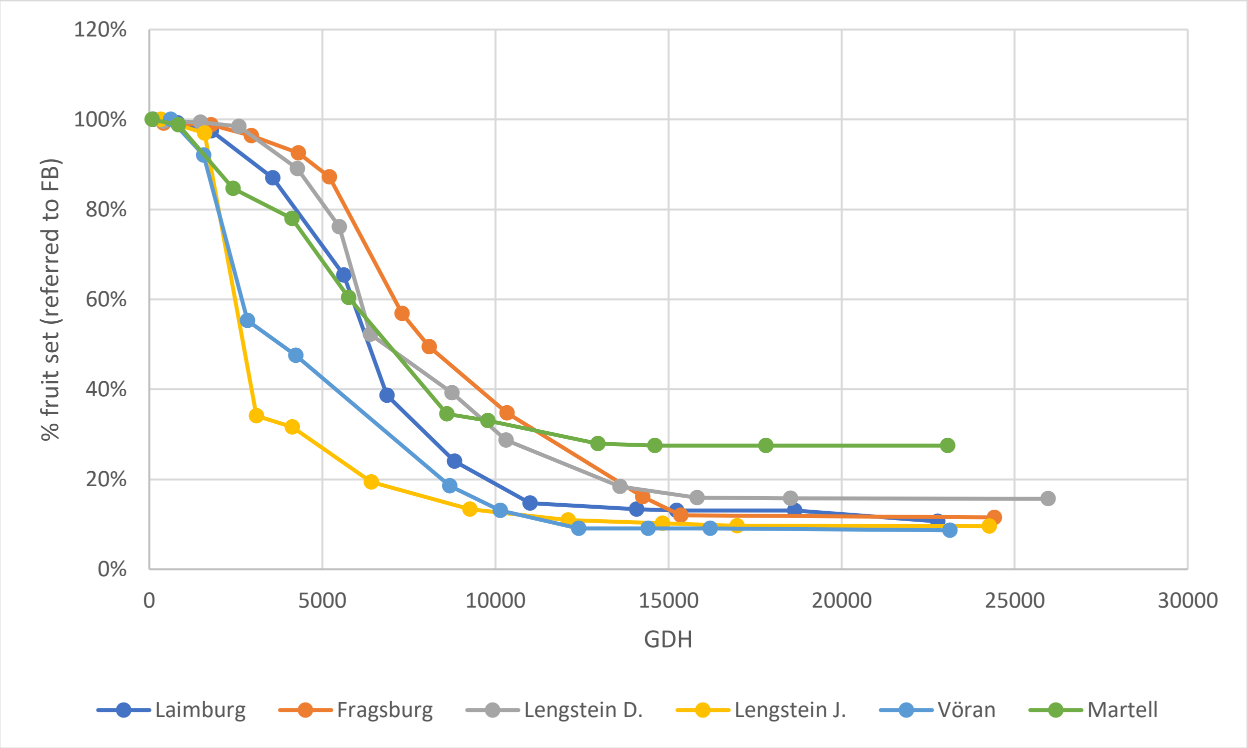


Figure 2: Evolution of total fruit set (eq. 1, referred to FB) over the daily Growing Degree Hours (eq. 4) accumulation from full bloom to harvest. In each orchard, the total fruit set was calculated from the % fruit set of 32 limbs tagged on 8 plants. The first counting was performed at full bloom, the last one at commercial harvest.

The percentage of relative fruit set referred to n0 was used to calculate the intensity of late fruit drop (eq. 3). Figure 3 represents the intensity of late fruit drop from n0 to harvest. The orchards are reported according to the increasing elevation (for further details see Table 1).

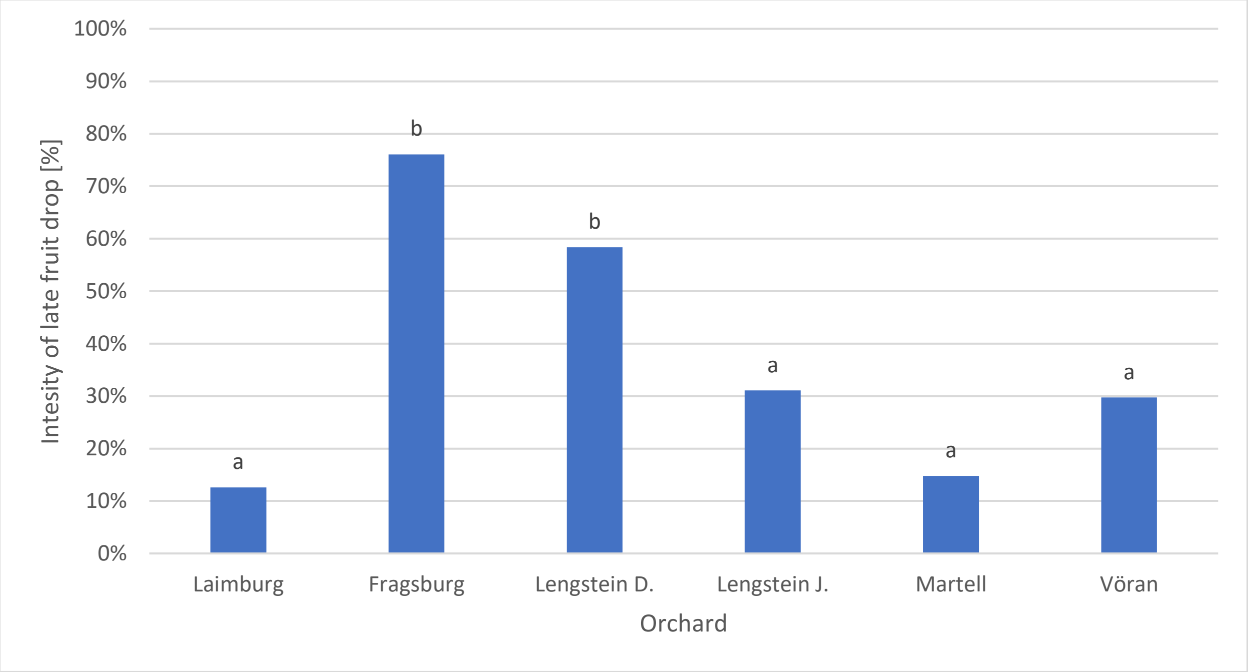


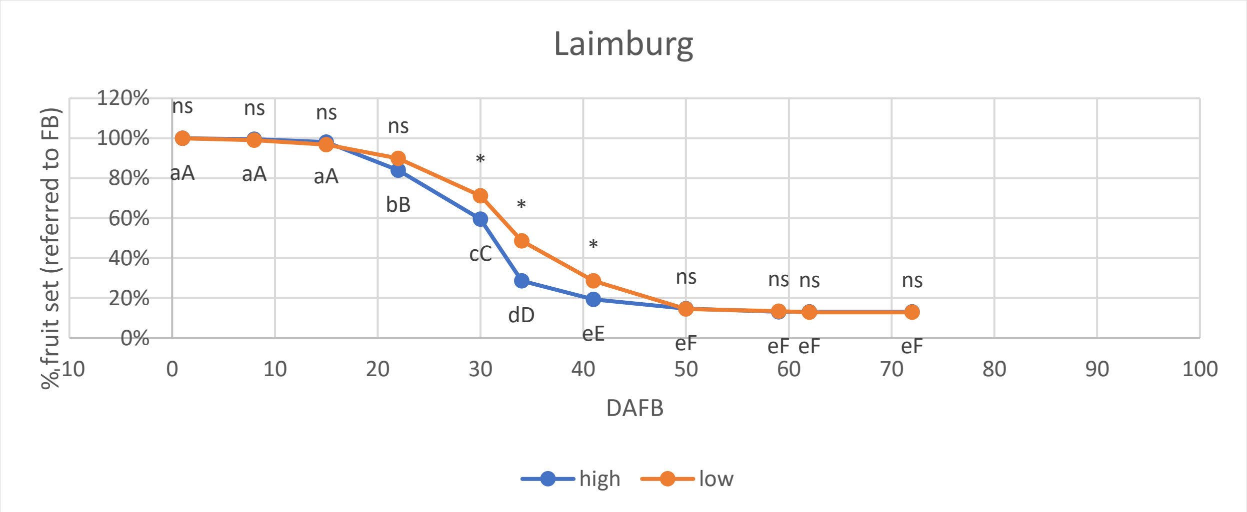
Figure 3: Intensity of late fruit drop referred to harvest expressed as % of the fruitlets set calculated using eq. (3). Means followed by the same lowercase letter are not significantly different at α ≤ 0,05.

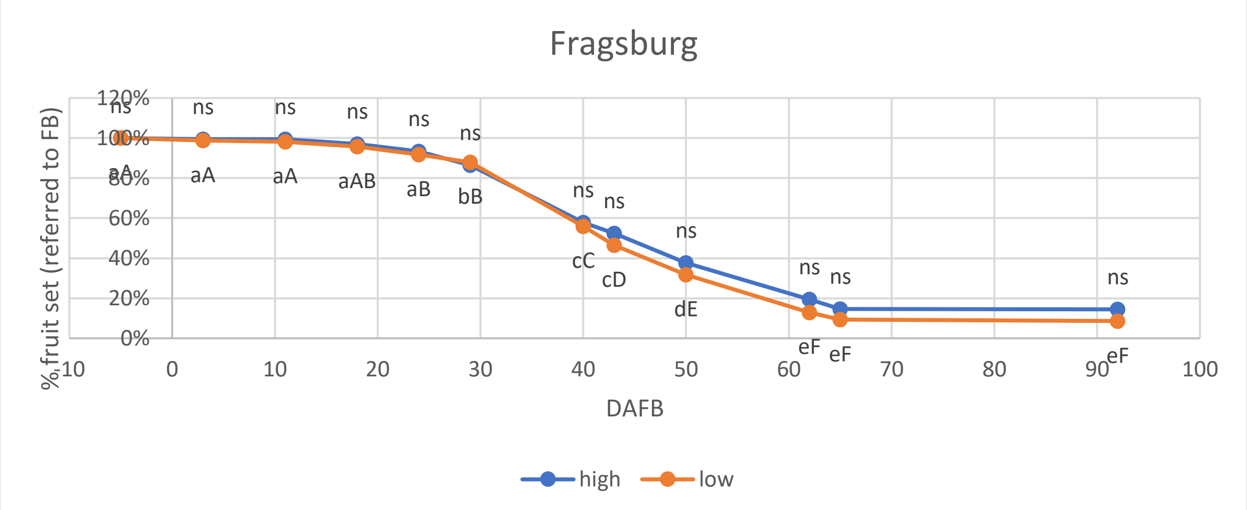
Figure 4 represents the interaction “Position * Sampling”: although it was significant in every orchard, a clear and universal trend was not identified. At the “Laimburg” site, the total fruit set of the upper limbs (“high”) was significantly higher than that of the lower limbs (“low”) at 30, 34, and 41 DAFB; furthermore, the lower limbs reached the final fruit set 9 days later than the upper limbs (50 and 41 DAFB, respectively). At the “Fragsburg”, “Lengstein D.”, and “Lengstein J.” sites, the evolution of the total fruit set at the two heights appears very similar, while for the “Vöran” and “Martell” sites the upper limbs show considerable higher fruit set than the lower ones.

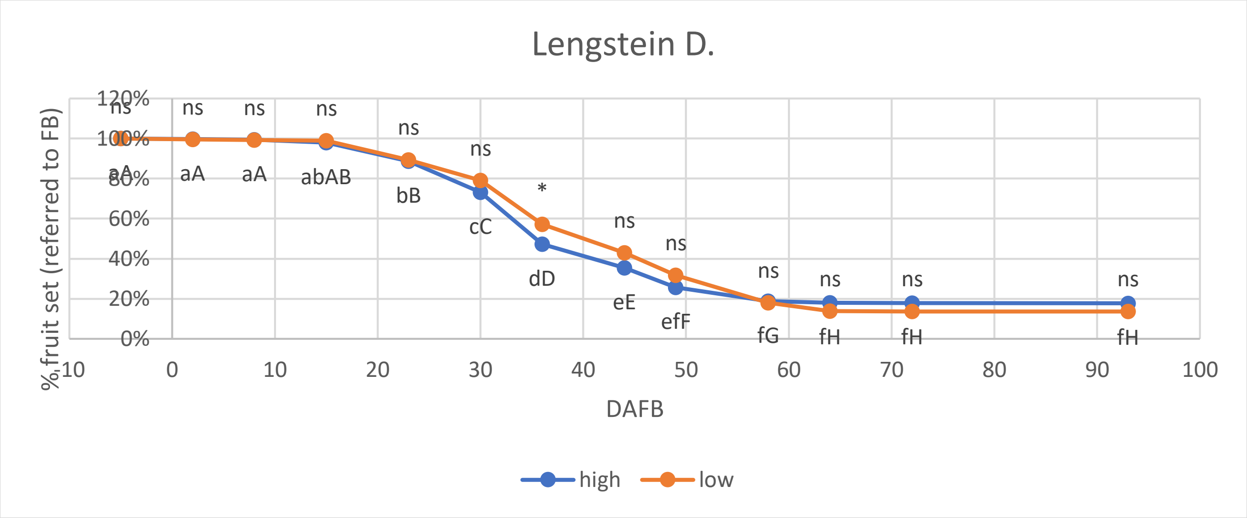
Orchard	**p-value “Pruning”**	**p-value “Position”**	**p-value “Sampling (time)”**	**p-value “Pruning * Position”**	**p-value “Pruning * Sampling”**	**p-value “Position * Sampling”**	**p-value “Pruning * Position * Sampling”**
Laimburg	,416	,980	**,012**	,388	,704	,237	,678
Fragsburg	,122	,240	**,000**	,835	**,021**	,602	,370
Lengstein D.	,825	,165	**,000**	,062	,455	,060	,100
Lengstein J.	,769	,635	**,003**	,625	,250	,785	,898
Vöran	,891	,390	**,001**	,948	,919	,806	,920
Martell	**,023**	,265	**,000**	,249	**,012**	,495	,556

Table 3: p-values for the different factors considered obtained as output of the Mixed Model with Repeated Measures having the % of relative fruit set (eq. 2) as the dependent variable. Bold p-values are considered significant at α ≤ 0,05.

Unlike Table 2, Table 3 summarizes the effect of the different factors considered on the evolution of relative fruit set (referred to n0, calculated using the formula (2)) during the last part of the season. As expected, since the % of fruit set changes over time, the factor “Sampling” was always significant. The factor “Pruning” or its interaction with the time of “Sampling” was found statistically significant for both “Fragsburg” and “Martell”; in both cases, pruned limbs showed an increase in late fruit drop rate and a significantly lower final fruit set than the unpruned ones (Figure 5).


Laimburg
120%
100%
80%
60%
40%
20%
0%
% fruit set (referred to FB)
-10
0
10
20
30
40
50
60
70
80
90
100
DAFB
ns
ns
ns
ns
*
*
*
ns
ns
ns
ns
aA
aA
aA
bB
cC
dD
eE
eF
eF
eF
eF
high
low


Fragsburg
120%
100%
80%
60%
40%
20%
0%
% fruit set (referred to FB)
-10
0
10
20
30
40
50
60
70
80
90
100
DAFB
ns
ns
ns
ns
ns
ns
ns
ns
ns
ns
ns
ns
aA
aA
aA
aAB
aB
bB
cC
cD
dE
eF
eF
eF
high
low


Lengstein D.
120%
100%
80%
60%
40%
20%
0%
% fruit set (referred to FB)
-10
0
10
20
30
40
50
60
70
80
90
100
DAFB
ns
ns
ns
ns
ns
ns
*
ns
ns
ns
ns
ns
ns
aA
aA
aA
abAB
bB
cC
dD
eE
efF
fG
fH
fH
fH
high
low

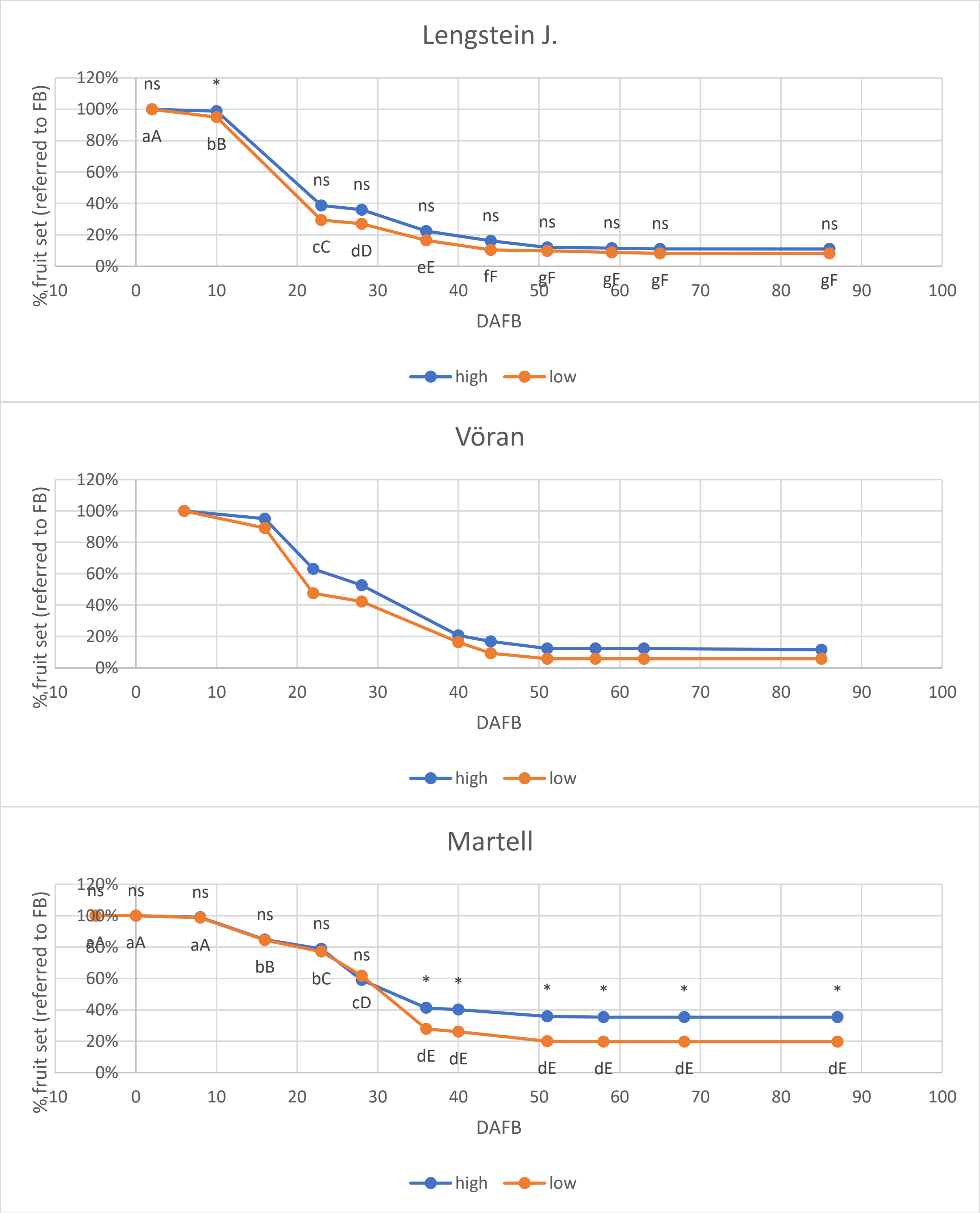


*Figure 4: effect of the interaction "Position * Sampling" on the evolution of the total fruit set (eq. 1, referred to FB) over the time expressed as Days After Full Bloom. ns/*: not significant/significant "Position * Sampling" at α ≤ 0,05. Lowercase letters "Sampling * Position" referred to "high"; uppercase letters "Sampling * Position" referred to "low". In "Vöran" no letters have been reported since is the factor "Position" and not its interaction with the factor "Sampling" that is significant.*

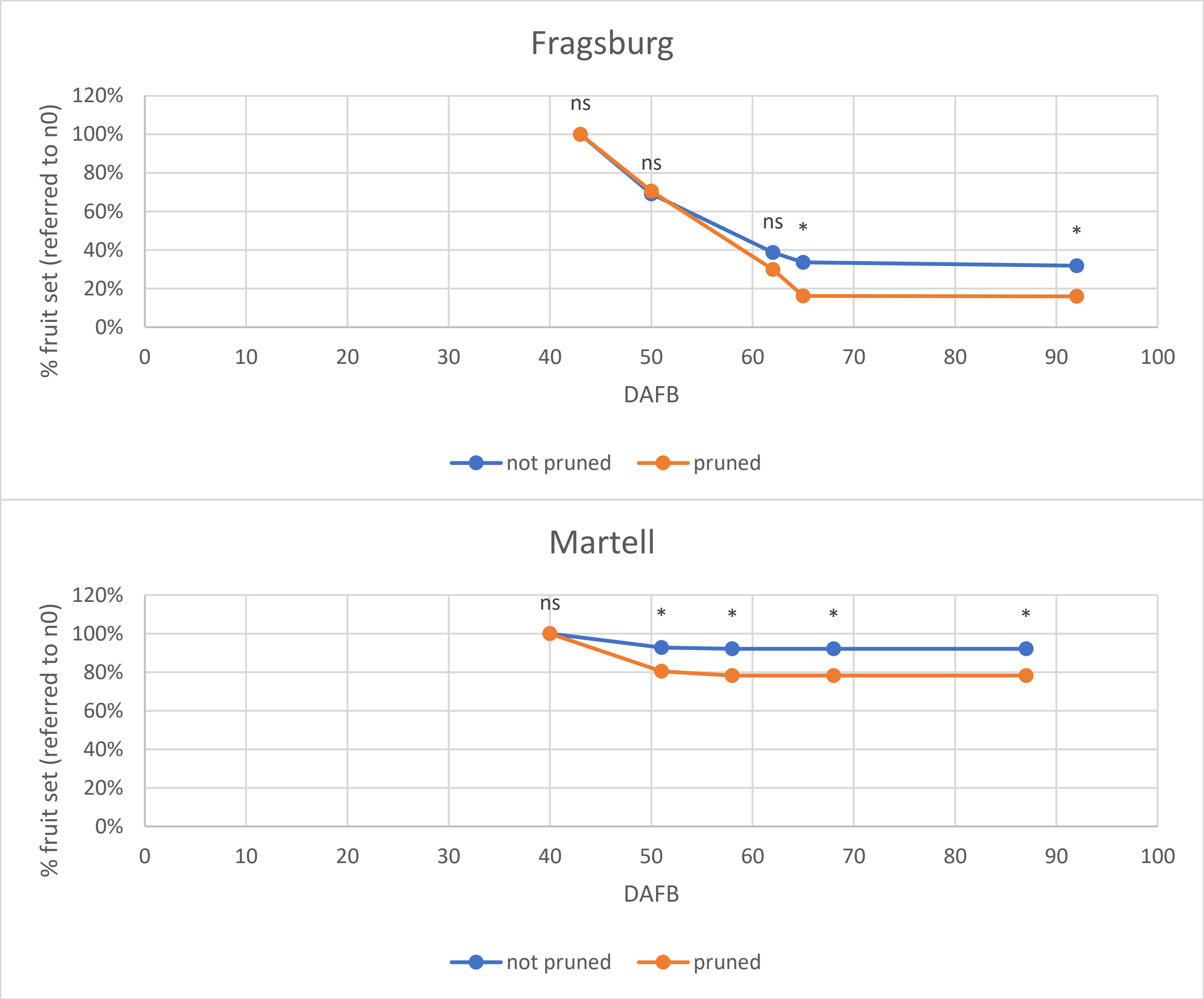


*Figure 5: effect of the interaction "Pruning * Sampling" on the evolution of the relative fruit set (eq. 2, referred to n0) over the time expressed as Days After Full Bloom. ns/*: not significant/significant "Pruning * Sampling" at α ≤ 0,05.*

3.4.2 Vegetative parameters

Orchard	p-value “Pruning”	p-value “Position”	p-value “Sampling (time)”	p-value “Pruning * Position”	p-value “Pruning * Sampling”	p-value “Position * Sampling”	p-value “Pruning * Position * Sampling”
Laimburg	**,000**	,491	**,000**	,963	**,000**	,081	,992
Fragsburg	**,000**	,533	**,000**	,166	**,026**	,268	,304
Lengstein D.	**,014**	,411	**,000**	,730	**,000**	,924	1,000
Lengstein J.	,797	,824	**,000**	,286	**,000**	,999	,113
Vöran	**,031**	,536	**,000**	,573	**,000**	,169	,784
Martell	**,000**	,289	**,000**	,768	**,000**	**,000**	,809

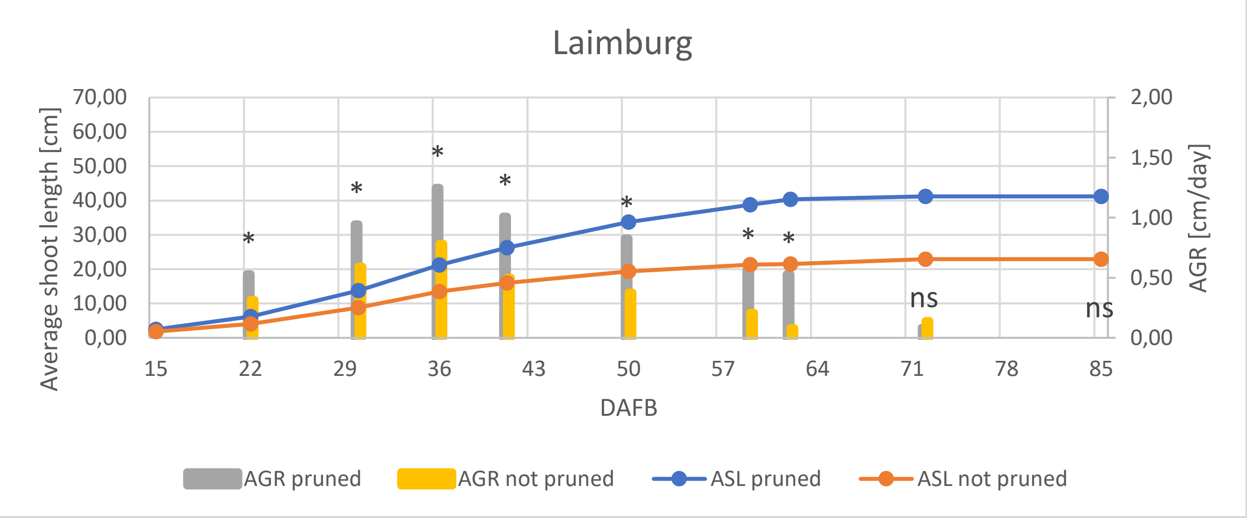
Table 4: p-values for the different factors considered obtained as output of the Mixed Model with Repeated Measures having the average shoot length (ASL), calculated as the average length of all the extension shoots per limb (spurs excluded), as the dependent variable. Bold p-values are considered significant at α ≤ 0,05.

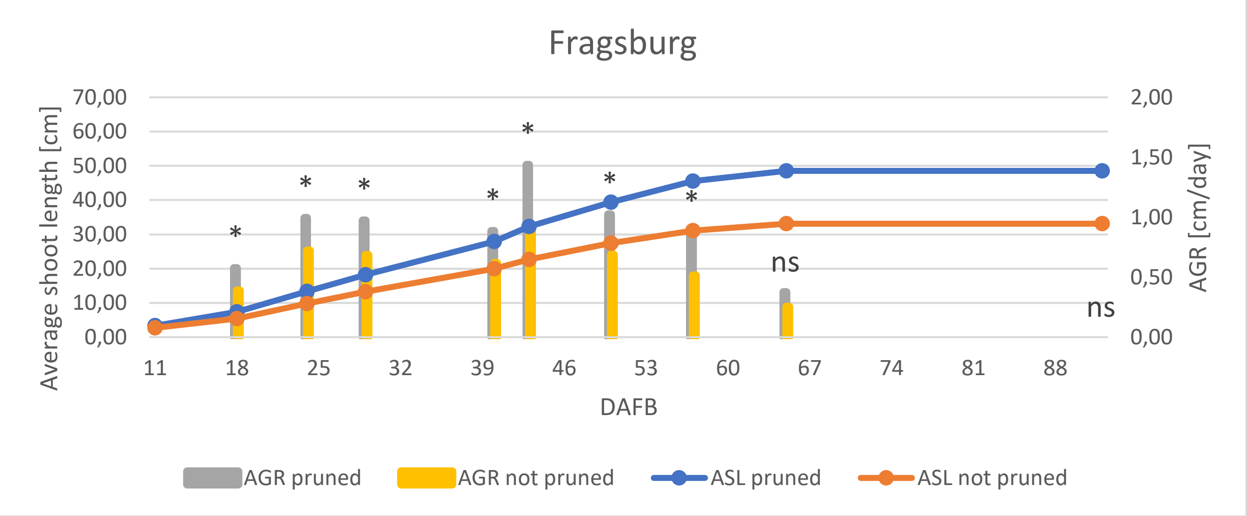
Table 4 reports the effect of the different factors considered on vegetative growth during the season; the dependent variable is the average shoot length (ASL), calculated as the average length of all the extension shoots per limb (spurs excluded). The mixed model returned the factor “Sampling”, which should be interpreted as the time, and the interaction “Pruning * Sampling” as significant; furthermore, for all but one orchard (“Lengstein J.”) the factor “Pruning” showed a significant overall effect on the shoot growth. Figure 6 shows the Average shoot length (ASL) expressed in cm and the Average growth rate (AGR) [cm/day] of pruned and unpruned limbs over the time for the 6 orchards individually. Pruning the 1-year-old limb in the spring, has increased the average AGR values of their extension shoots, causing longer ones at terminal bud set. Significantly higher AGR values for the “pruned” treatment than for the “not pruned” one have been observed for the first time at 22 DAFB in “Laimburg”, 18 DAFB in “Fragsburg”, 36 DAFB in “Lengstein D.”, 44 DAFB in “Lengstein J.”, 41 DAFB in “Vöran”, and 23 DAFB in “Martell”. Overall, the vegetative response to the pruning was severely affected by the site. Taking the AGR parameter into account, we can observe how it reached its maximum peak at different times in each site: in “Laimburg” at 36 DAFB, in “Fragsburg” at 43 DAFB, in “Lengstein D.” at 44 DAFB, in “Lengstein J.” at 51 DAFB, in “Vöran” at 40 DAFB”, and in “Martell” at 28 DAFB. Despite the distribution of the AGR values resembles a bell-shaped curve for most of the orchards, in “Fragsburg” the growth followed a clear bimodal trend with a minor peak at 24 DAFB; similarly but to a lesser extent, the same trend was observed also in “Lengstein D.”. The estimated marginal means of the average shoot length are reported in Figure 7 for each orchard separately; to make the comparison between different locations meaningful, the vegetative growth was expressed as a function of the Growing Degree Hours calculated following Azarenko et al. (2008). Based on the collected data, the orchards “Fragsburg” and “Martell” showed the greatest ASL, followed by “Laimburg”, “Vöran” and “Lengstein D.”, and finally “Lengstein J.”. In every orchard the ASL increase followed a very similar evolution; terminal bud set was reached in every location at about 18’000 GDH.

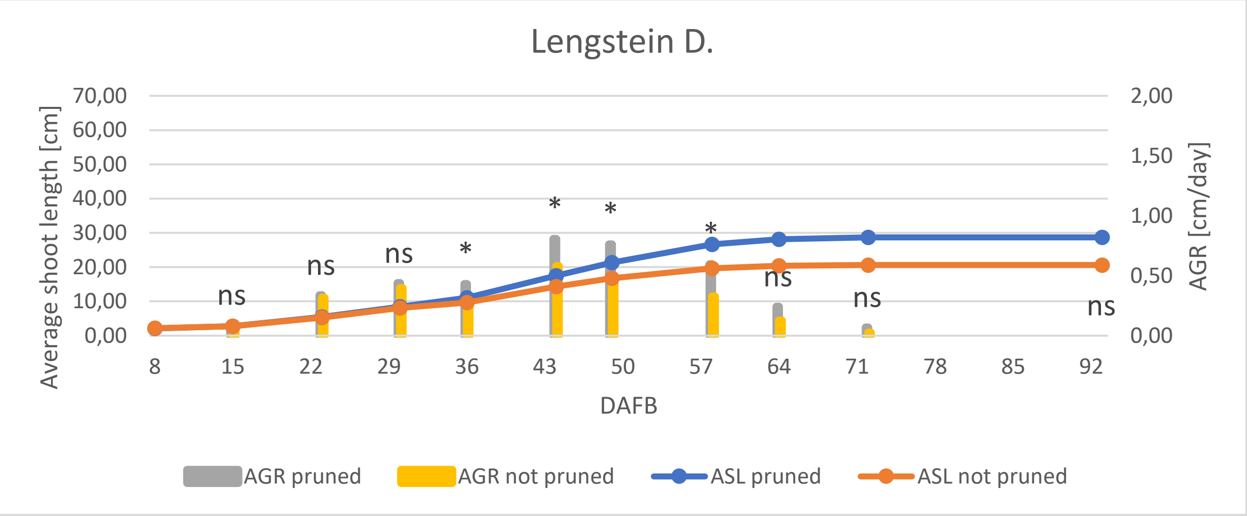
	n° of new shoots [-]		**n° of non-fruiting spurs [-]**		**new shoots density per limb [n°/m]**		**non-fruiting spurs density per limb [n°/m]**	
Orchard	pruned	not pruned	pruned	not pruned	pruned	not pruned	pruned	not pruned
Laimburg	2,88	6,31	0,38	4,50	11,07	11,45	1,59	9,09
p-value	**0,00**		**0,00**		0,79		**0,00**	
Fragsburg	2,75	4,94	0,25	2,94	9,72	8,98	0,88	4,77
p-value	**0,01**		**0,00**		0,63		**0,00**	
Lengstein D.	2,31	2,44	1,06	4,38	7,30	5,28	3,61	9,88
p-value	0,82		**0,00**		0,09		**0,01**	
Lengstein J.	0,56	1,69	3,19	6,06	1,81	3,19	9,89	13,57
p-value	0,08		**0,01**		0,23		0,17	
Vöran	1,88	2,81	1,19	4,38	5,63	6,24	4,12	9,08
p-value	**0,05**		**0,00**		0,56		**0,02**	
Martell	1,81	1,13	1,13	6,75	5,58	2,15	3,53	12,79
p-value	**0,03**		**0,00**		**0,00**		**0,00**	

Table 5: effect of the factor “Pruning” on the number of new extension shoots per limb [-] and their linear density [-/m], and on the number of non-fruiting spurs [-] and their linear density [-/m]. The estimated marginal means are reported individually for each site. Bold p-values are considered significant at α ≤ 0,05.

Table 5 summarizes the effect of the factor “Pruning” on the development during summer of the vegetative buds of the 1-year-old shoots pruned or not in the previous spring. In the data analysis, every vegetative growth shorter than 4 cm at terminal bud set was considered a non-fruiting spur. As shown, since a considerable portion of the wood has been removed, pruned 1-year-old limbs showed less extension shoots than the unpruned ones (in absolute terms) in 4 orchards of 6; interestingly, the number of extension shoots per linear meter remained constant in 5 orchards of 6 with the only exception of “Martell”. On the other hand, shortening the 1-year-old limbs strongly influenced the number of non-fruiting spurs both in absolute and relative terms: the unpruned limbs showed both a higher number of non-fruiting spurs per limb and a higher non-fruiting spurs density per linear meter in almost all the orchards. The same analysis was conducted also for the factor “Position” but, since no significant effect was observed, data were not reported.


Laimburg
Average shoot length [cm]
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
AGR [cm/day]
2,00
1,50
1,00
0,50
0,00
15
22
29
36
43
50
57
64
71
78
85
DAFB
*
*
*
*
*
*
*
ns
ns
AGR pruned
AGR not pruned
ASL pruned
ASL not pruned


Fragsburg
Average shoot length [cm]
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
AGR [cm/day]
2,00
1,50
1,00
0,50
0,00
11
18
25
32
39
46
53
60
67
74
81
88
DAFB
*
*
*
*
*
*
*
ns
ns
AGR pruned
AGR not pruned
ASL pruned
ASL not pruned


Lengstein D.
Average shoot length [cm]
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
AGR [cm/day]
2,00
1,50
1,00
0,50
0,00
8
15
22
29
36
43
50
57
64
71
78
85
92
DAFB
ns
ns
ns
*
*
*
*
ns
ns
ns
AGR pruned
AGR not pruned
ASL pruned
ASL not pruned

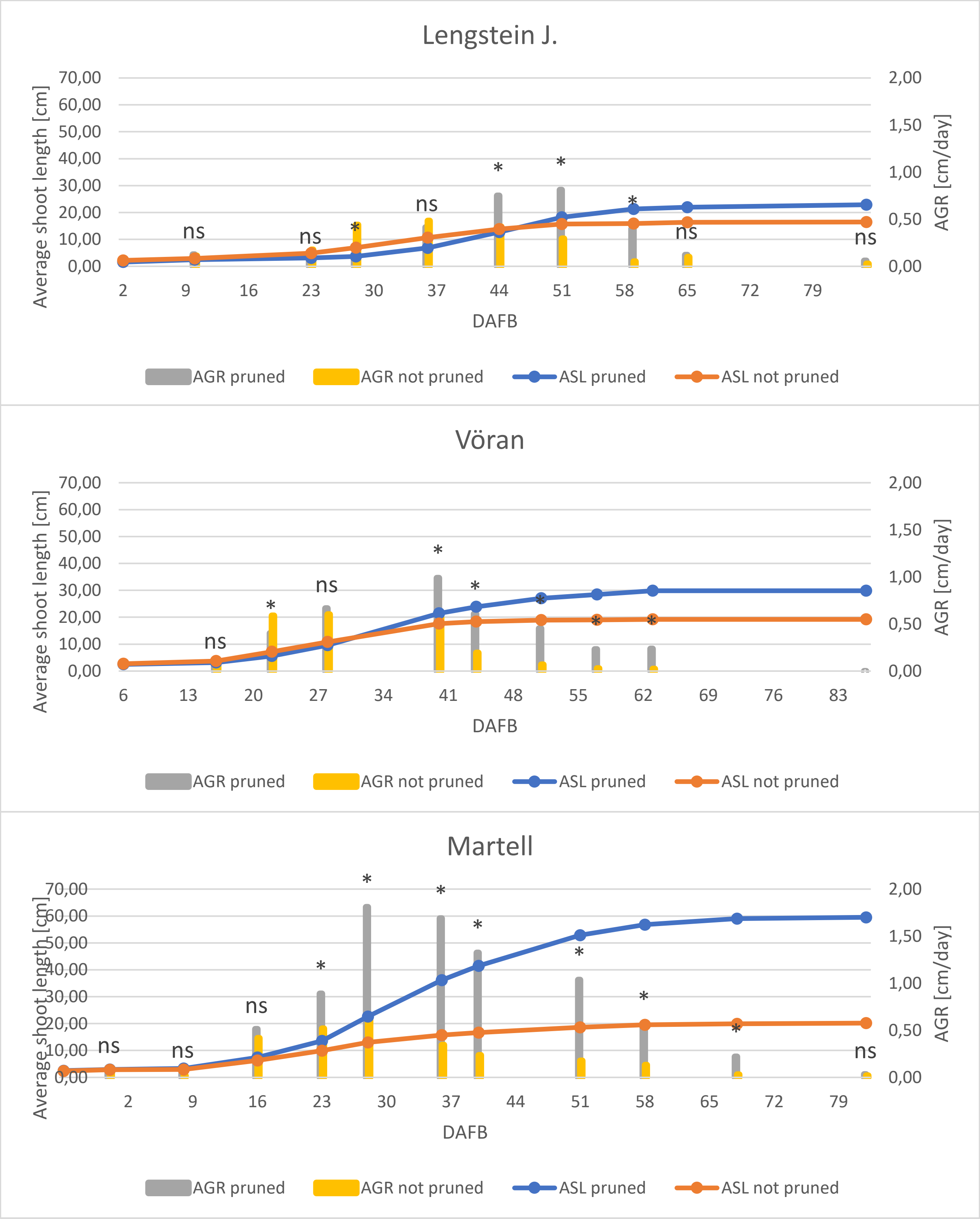


Figure 6: evolution of the average shoot length (ASL) over the time expressed as Days After Full Bloom. ASL are referred to the day of sampling. The statistical analysis refers to the AGR values of the ASL. ns/: not significant/significant "Pruning * Sampling" at α ≤ 0,05. For every orchard, each point represents the average length of all the shoots borne on 32 limbs tagged on 8 trees.*

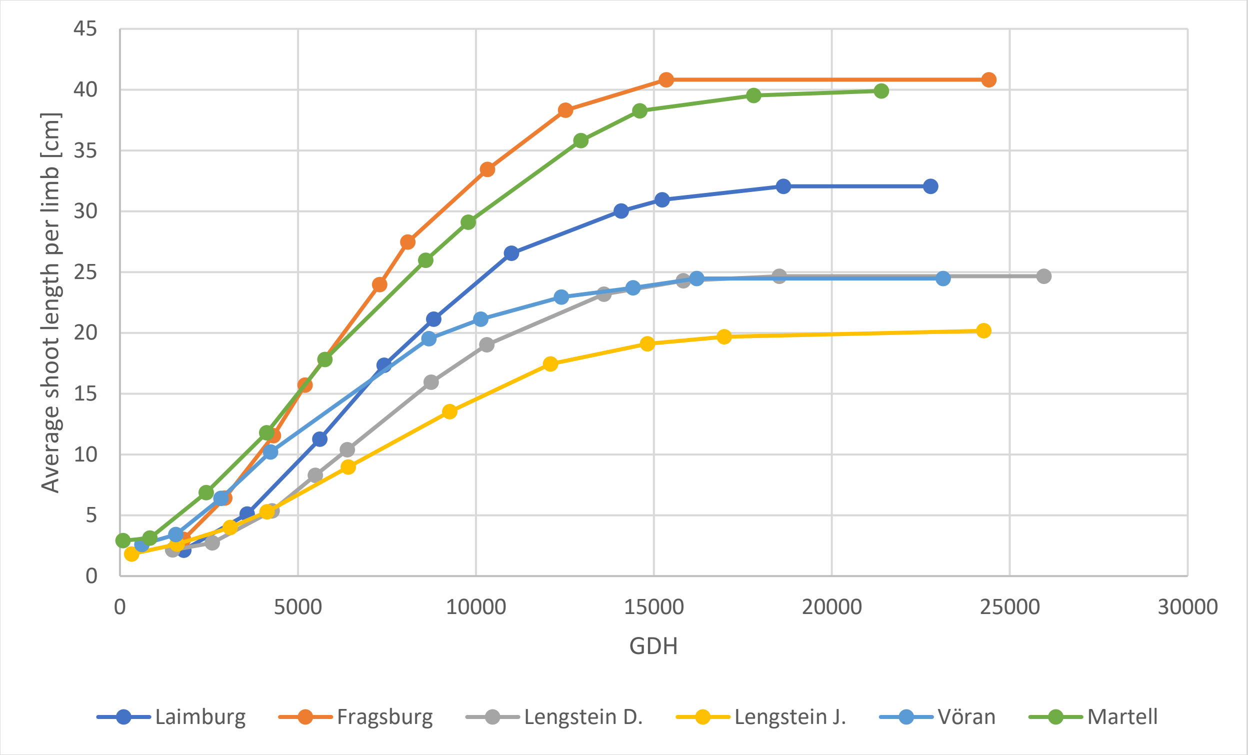


Figure 7: Evolution of the average shoot length per limb over the daily Growing Degree Hours (eq. 4) accumulation from full bloom to harvest.

The ratio between the sum of the length of new shoots at terminal bud set and the limb length (from which they originated) at full bloom was used as a measure of the vigor of the limb. In other words, the sum of the new shoots' length was normalized by the limb length: if this had not been done, a long limb with many short shoots would have turned out to be as vigorous as a shorter limb with fewer longer shoots. Table 6 reports the significance of this ratio as predictor of the relative fruit set (referred to n0) in a linear regression analysis. Studying each location separately, the predictor was significant only in the "Fragsburg" orchard but, interestingly, by putting all the data together the predictor turned out to be highly significant. The corresponding R-square value is 0,072, which states that 7,2 % of the total variance of the dependent variable (the relative fruit set) can be explained by the model. The "B" value (the regression slope) is negative and indicates that for each unit increase of the predictor the final fruit set at harvest decreased by 4,7%. For the "Fragsburg" orchard this ratio explained 17,5% of the total variance. The linear regressions for all the orchards considered are represented in the Figure 8.

Orchard	**p-value**	**R-square**	**B**
Laimburg	,286	-	-
Fragsburg	,017	,175	-,047
Lengstein D.	,069	-	-
Lengstein J.	,184	-	-
Vöran	,129	-	-
Martell	,082	-	-
All together	,000	,072	-,047

Table 6: p-values of the ratio between the sum of the length of new shoots at terminal bud set and the limb length (from which they originated) at full bloom [m/m] interpreted as predictor in a linear regression analysis having the final relative fruit set as dependent variable. For predictors significant at α ≤ 0,05, R-square as well as the regression slope (B) were printed.

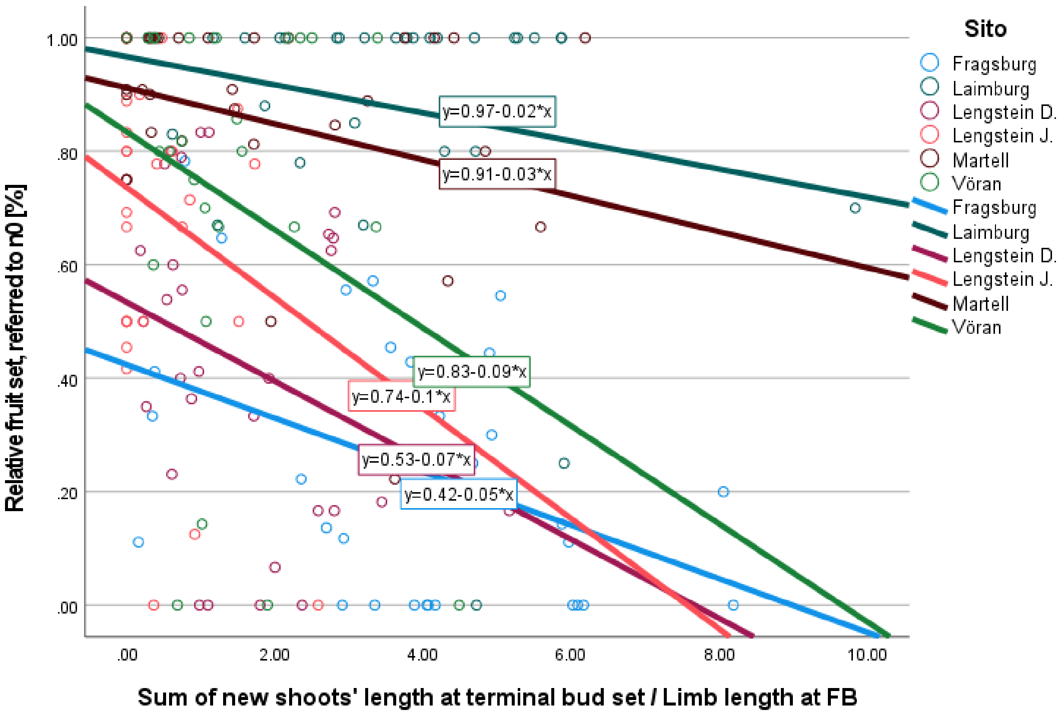


Figure 8: linear regression of the ratio between the sum of the length of new shoots at terminal bud set and the limb length (from which they originated) at full bloom [m/m] interpreted as predictor of the final relative fruit set.

3.4.3 Seasonal fruit growth

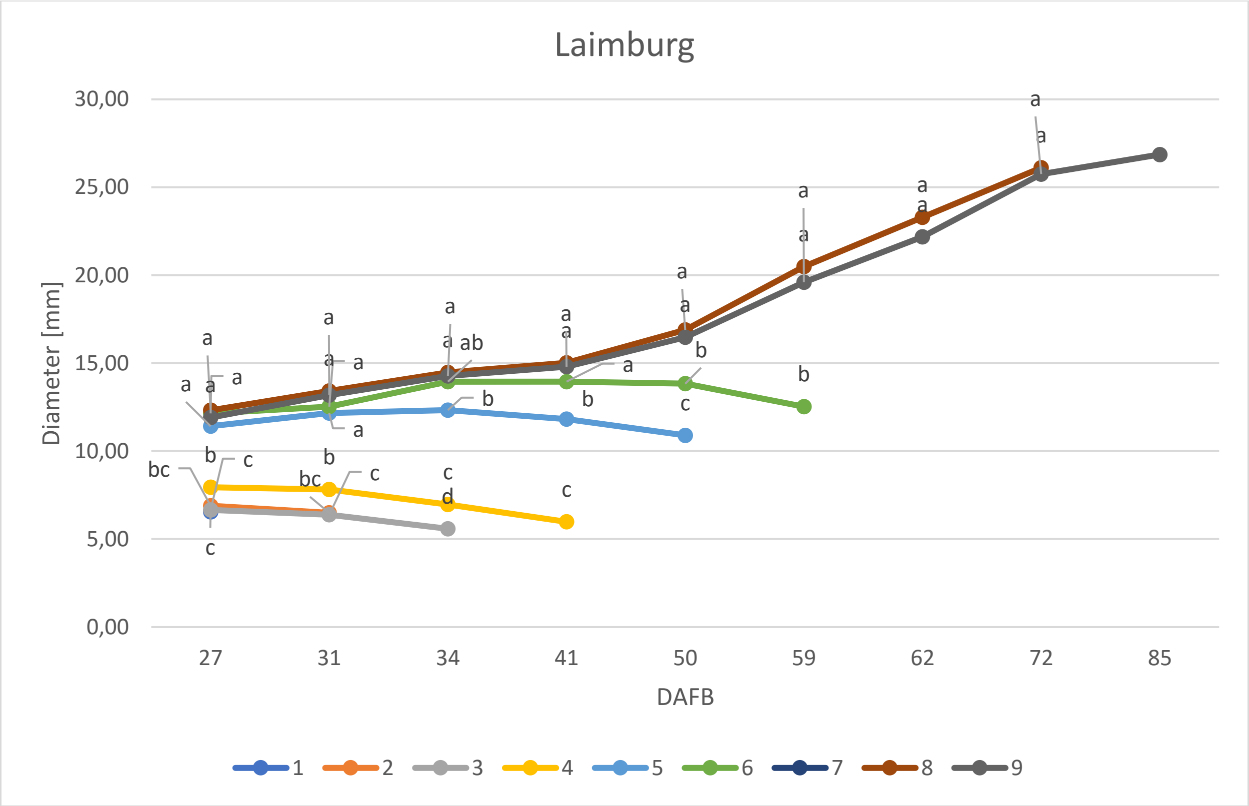
At petal fall, i.e. as soon as it was possible, 320 drupelets were individually labelled with a progressive unique number in each orchard (10 each limb). From this point onwards they were calibrated once a week until the end of late fruit drop (which always coincided with the beginning of the veraison). During the data analysis, the labelled fruits of each location were grouped according to the time of detachment; Table 6 summarizes the time of detachment of each group and its relevance in terms of % of the tagged fruitlets. The group “Harvested” includes the cherries that reached commercial harvest without dropping. For obvious reason, in the “Laimburg” orchard the amount of non-dropping cherries consists of the sum of group 9 (Harvested) and group 8 (those damaged by birds during the last week before harvest).

LAIMBURG			FRAGSBURG		
Time of detachment	**Group n°**	**% of the tagged fruitlets**	**Time of detachment**	**Group n°**	**% of the tagged fruitlets**
27 – 31 DAFB	1	0 %	30 – 35 DAFB	1	0 %
31 – 34 DAFB	2	5 %	35 – 46 DAFB	2	4 %
34 – 41 DAFB	3	13 %	46 – 49 DAFB	3	13 %
41 – 50 DAFB	4	23 %	49 – 56 DAFB	4	12 %
50 – 59 DAFB	5	10 %	56 – 63 DAFB	5	26 %
59 – 62 DAFB	6	3 %	63 – 70 DAFB	6	24 %
62 – 72 DAFB	7	0 %	70 – 98 DAFB	7	1 %
72 – 85 DAFB	8*	10 %	HARVESTED	8	21 %
HARVESTED	9	36 %			
LENGSTEIN D.			**LENGSTEIN J.**		
Time of detachment	**Group n°**	**% of the tagged fruitlets**	**Time of detachment**	**Group n°**	**% of the tagged fruitlets**
23 – 30 DAFB	1	0 %	28 – 36 DAFB	1	4 %
30 – 36 DAFB	2	2 %	36 – 44 DAFB	2	28 %
36 – 44 DAFB	3	12 %	44 – 51 DAFB	3	11 %
44 – 49 DAFB	4	22 %	51 – 59 DAFB	4	9 %
49 – 58 DAFB	5	25 %	59 – 65 DAFB	5	3 %
58 – 64 DAFB	6	7 %	65 – 85 DAFB	6	1 %
64 – 72 DAFB	7	1 %	HARVESTED	7	43 %
72 – 93 DAFB	8	0 %			
HARVESTED	9	31 %			
VÖRAN			**MARTELL**		
Time of detachment	**Group n°**	**% of the tagged fruitlets**	**Time of detachment**	**Group n°**	**% of the tagged fruitlets**
28 – 40 DAFB	1	13 %	26 – 28 DAFB	1	2 %
40 – 44 DAFB	2	34 %	28 – 36 DAFB	2	9 %
44 – 51 DAFB	3	11 %	36 – 40 DAFB	3	15 %
51 – 57 DAFB	4	11 %	40 – 51 DAFB	4	6 %
57 – 63 DAFB	5	0 %	51 – 58 DAFB	5	4 %
63 – 85 DAFB	6	2 %	58 – 68 DAFB	6	0 %
HARVESTED	7	29 %	68 – 82 DAFB	7	1 %
			HARVESTED	8	62 %

Table 7: percentage of cherries detached in each time interval considered based on the total number of cherries tagged (about 320 in each orchard). In the "Laimburg" orchard, Group n° 8 represents the amount of cherries damaged by birds.*

During the data analysis, once having virtually tagged every cherry with its "Group of detachment", the latter was used as fixed factor in the Mixed Model analysis with Repeated Measures. Every orchard was analyzed separately. In this way, the diameter evolution of each wave of fruit drop could be plotted individually over the time as shown in Figure 9. Overall, at the first day of calibration, about 23 – 30 DAFB, a substantial percentage of fruits already showed a significantly smaller diameter: at this time these fruits had already stopped growing and were slowly getting senescent. This first wave of fruit drop in the “Laimburg” location involved Groups n° 1, 2, 3, and 4, in the “Fragsburg” location Groups n° 2 and 3, in the “Lengstein D.” location Groups n° 2 and 3, in the “Lengstein J.” location Groups n° 1 and 2, in the “Vöran” location Groups n° 1 and 2 and, finally, in the “Martell” location Groups n° 1, 2, and 3. This first phase of fruit drop should not be confused with the late fruit drop that is the focus of this study: in this work, only the drupelets that at the first calibration showed a diameter not significantly different from that of the retained ones (= Group “Harvested”) and which subsequently detached were considered part of the “late fruit drop” of ‘Regina’. Therefore, the proper late fruit drop consisted of Groups n° 5 and 6 in “Laimburg” (cumulatively representing 13% of the tagged fruits); of Groups n° 4, 5, and 6 in “Fragsburg” (62% of the tagged fruits); of Groups n° 4, 5, 6 in “Lengstein D.” (54 % of the tagged fruits); of Groups 3, 4, and 5 in “Lengstein J.” (23 % of the tagged fruits); of Groups 3 and 4 in “Vöran“ (22% of the tagged fruits); of Groups 4 and 5 in “Martell” (10% of the tagged fruits).

Figure 10 represents the AGR values of fruit growth calculated for each “Group of detachment” and each orchard separately. This way of representing growth data allows us to estimate with good accuracy the moment when a given “Group of detachment” starts to grow differently from the group that will reach harvest. For example, in the “Laimburg” orchard Group n° 5 is already growing significantly slower than Group n° 9 between the first and the second sampling (thus between 27 and 31 DAFB); similarly, at the same time Group n° 6 is characterized, in absolute terms, by a much lower AGR value compared to that of Group n° 9. For Group n° 6 the statistical analysis returned an intermediate situation, in which its AGR value was not different both from that of both Group n° 9 and of Group n° 5, but this result was very likely influenced by its small sample size. In the “Fragsburg” orchard, Groups n° 4 and n° 5 show lower AGR values than Group n° 8 already between the first two samplings (thus between 24 and 29 DAFB). In the same time interval, Group n° 6 shows a slightly lower AGR value than that of Group n° 8 (in absolute terms) but without any statistical significance; a clear distinction can be observed between 29 and 40 DAFB.


Laimburg
Diameter [mm]
30,00
25,00
20,00
15,00
10,00
5,00
0,00
27
31
34
41
50
59
62
72
85
DAFB
a
a
a
a
a
a
a
a
a
a
a
a
a
a ab
a
b
b
b
a
b
c
a
a
a
a
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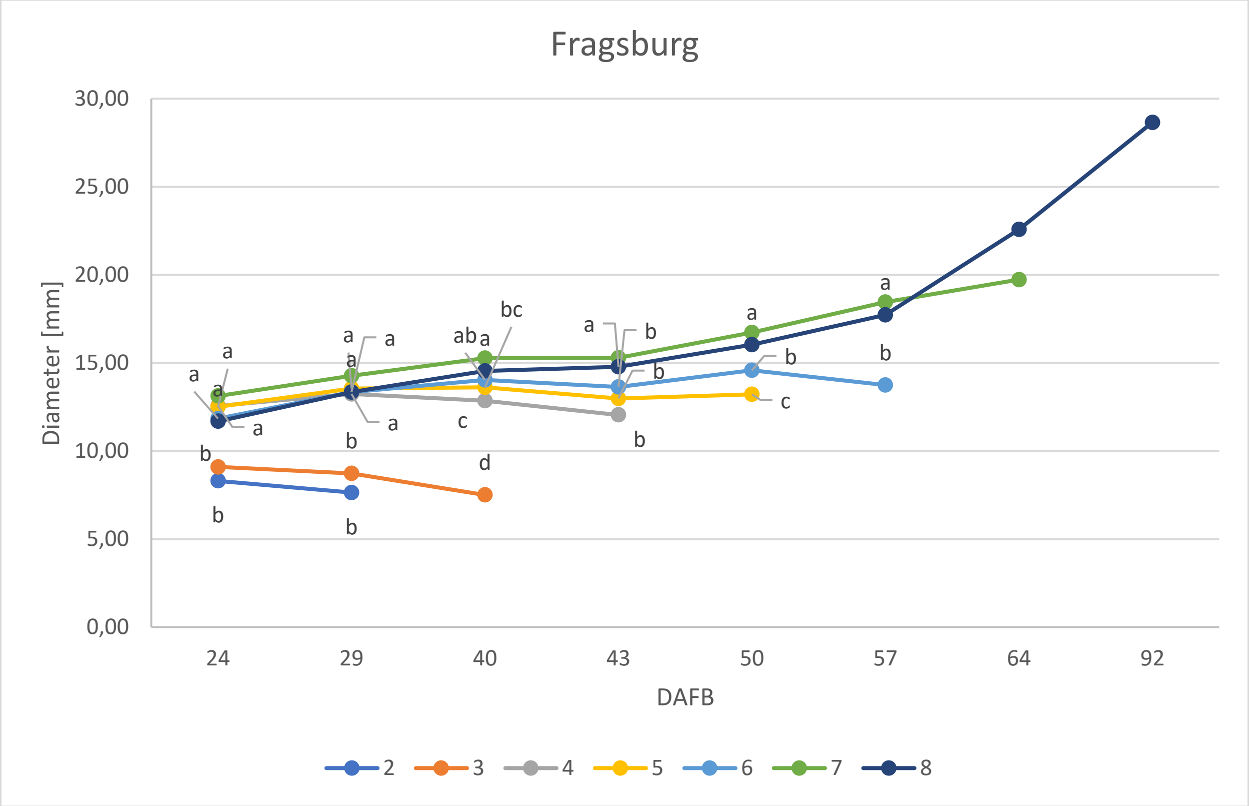
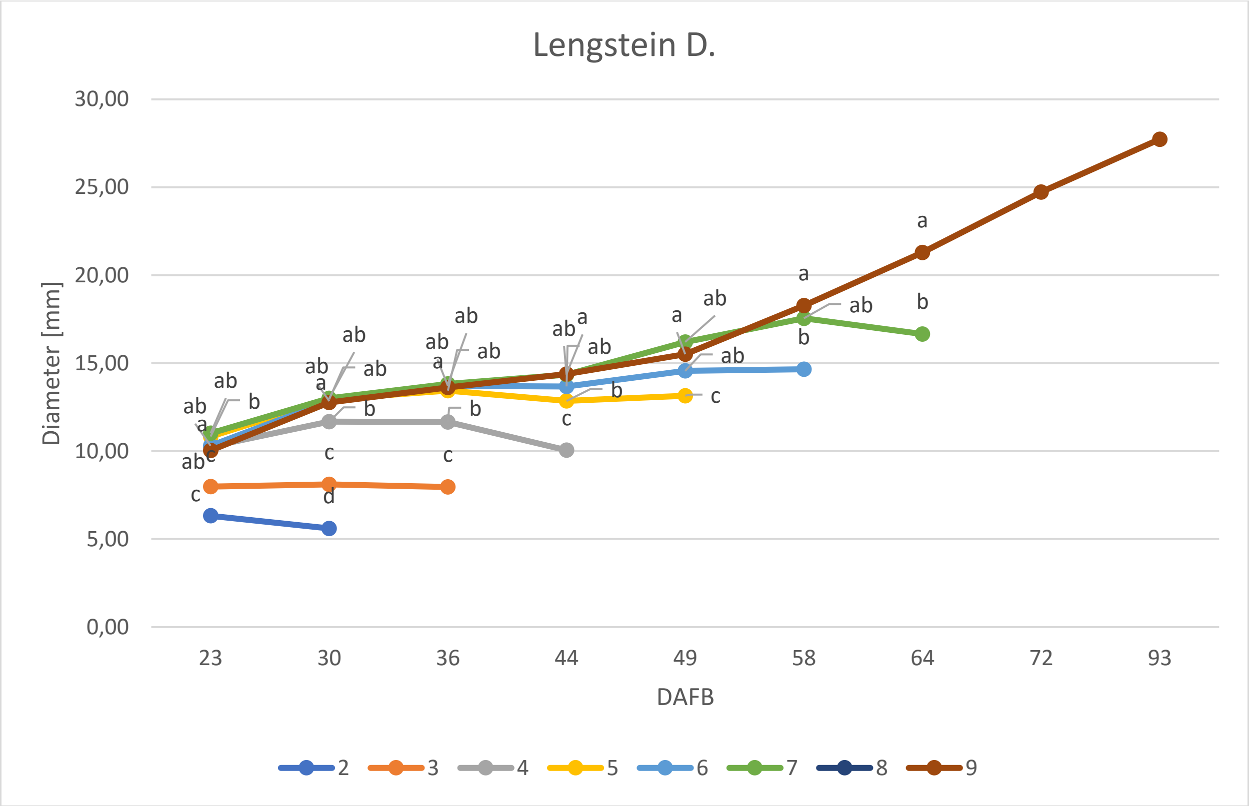
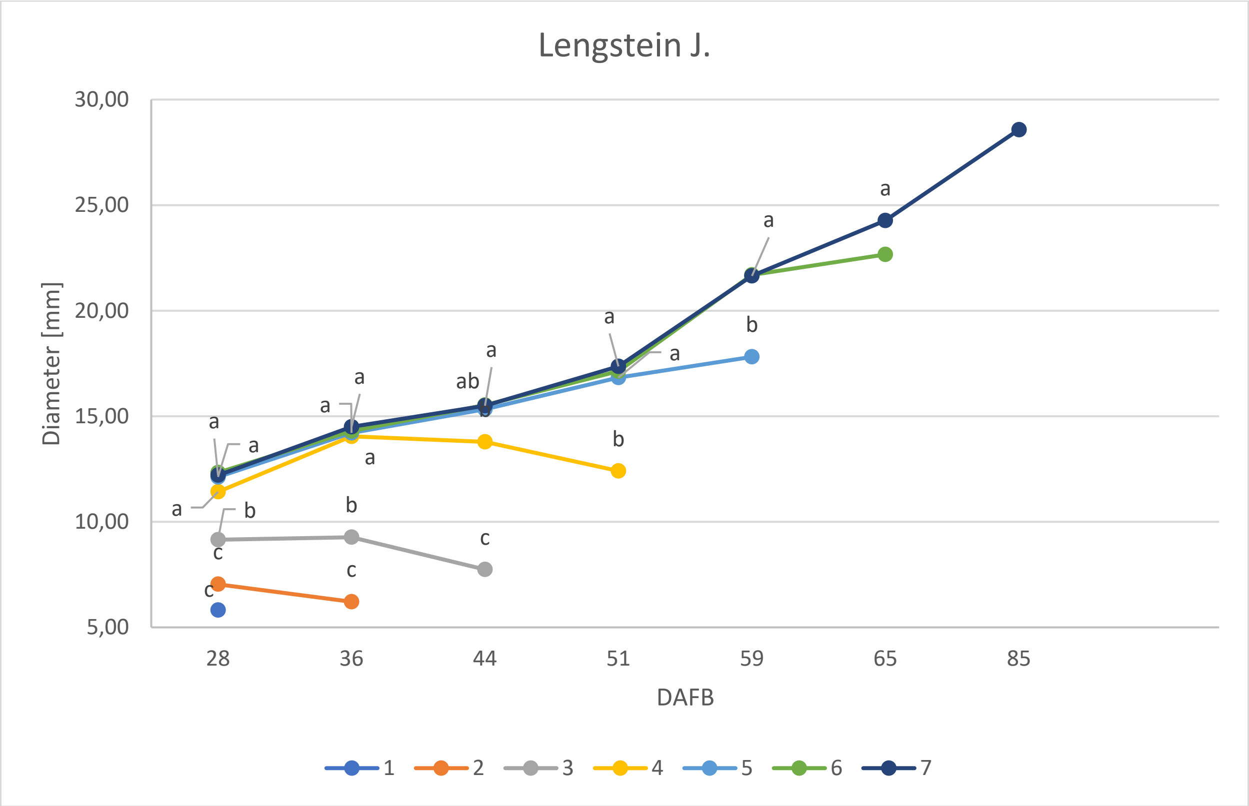
3
4
5
6
7
8
9


Fragsburg
Diameter [mm]
30,00
25,00
20,00
15,00
10,00
5,00
0,00
24
29
40
43
50
57
64
92
DAFB
a
a
bc
a
a
a
ab
a
a
b
a
a
a
b
b
b
a
a
c
c
b
b
b
b
d
b
b
2
3
4
5
6
7
8


Lengstein D.
Diameter [mm]
30,00
25,00
20,00
15,00
10,00
5,00
0,00
23
30
36
44
49
58
64
72
93
DAFB
2
3
4
5
6
7
8
9


Lengstein J.
Diameter [mm]
30,00
25,00
20,00
15,00
10,00
5,00
28
36
44
51
59
65
85
DAFB
1
2
3
4
5
6
7

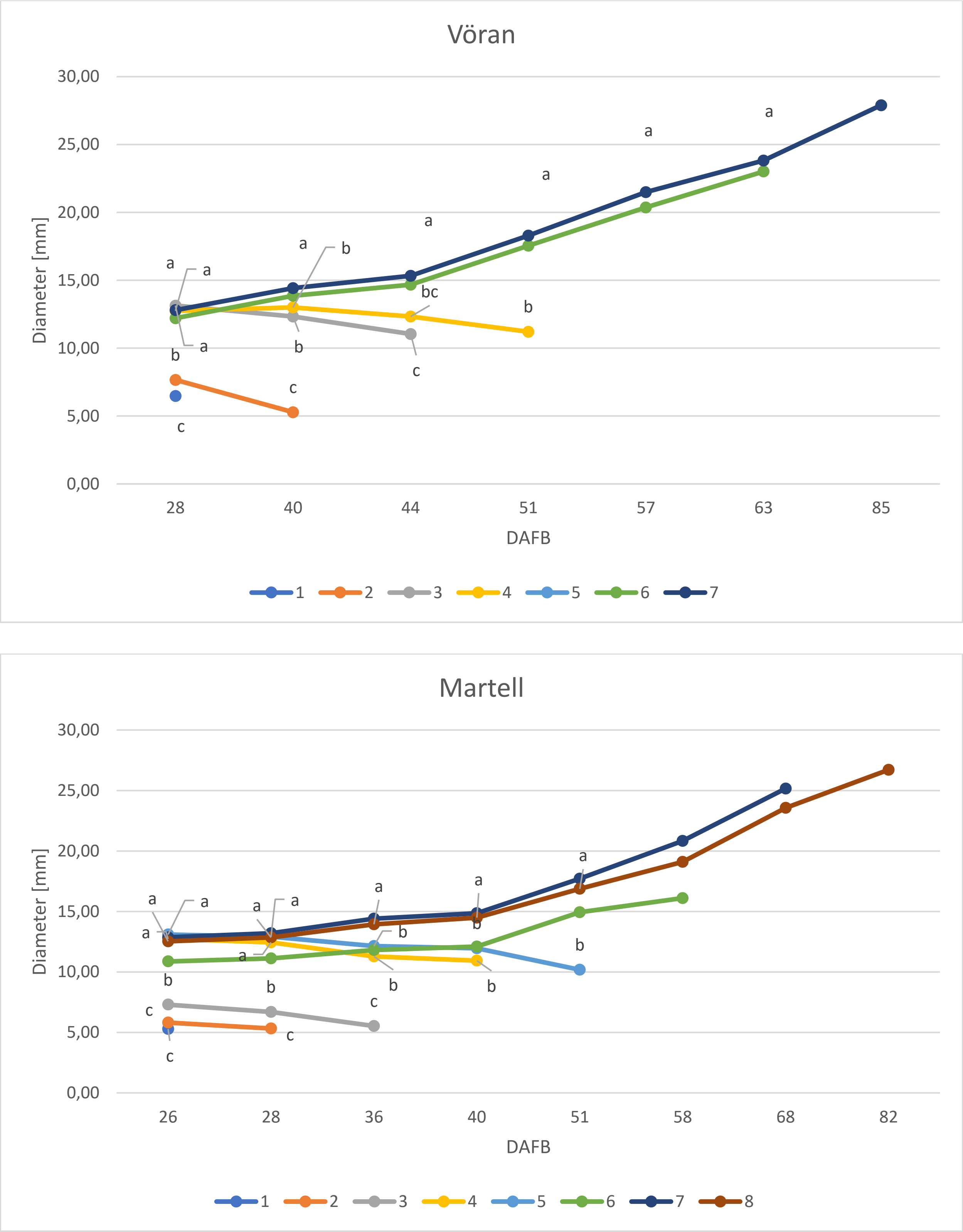


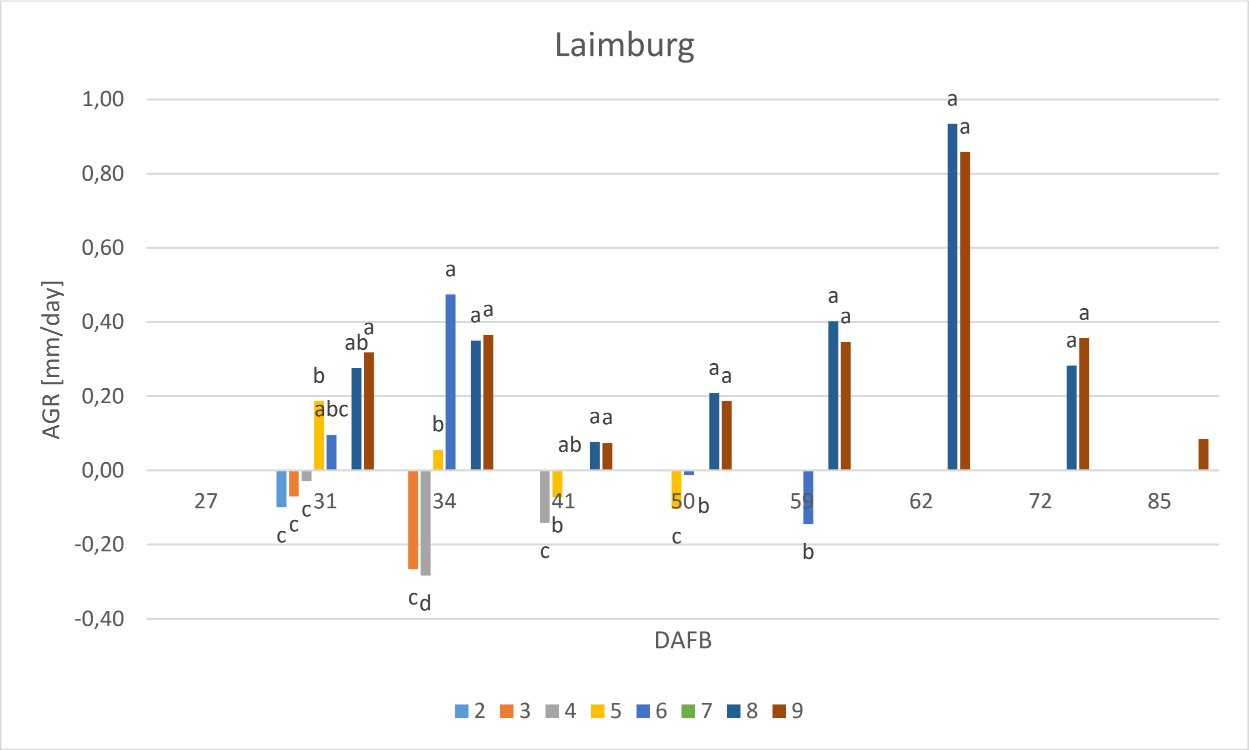
Figure 9: *Measurements of ‘Regina’ sweet cherry fruit growth in different orchards; diameter increase over the time (expressed as DAFB) is reported individually for each Group of detachment (characterized by different colors). The codes of the colored bars refer to the Groups of detachment shown in Table 7. Estimated marginal means of the fruit diameter in the interaction “Group of*

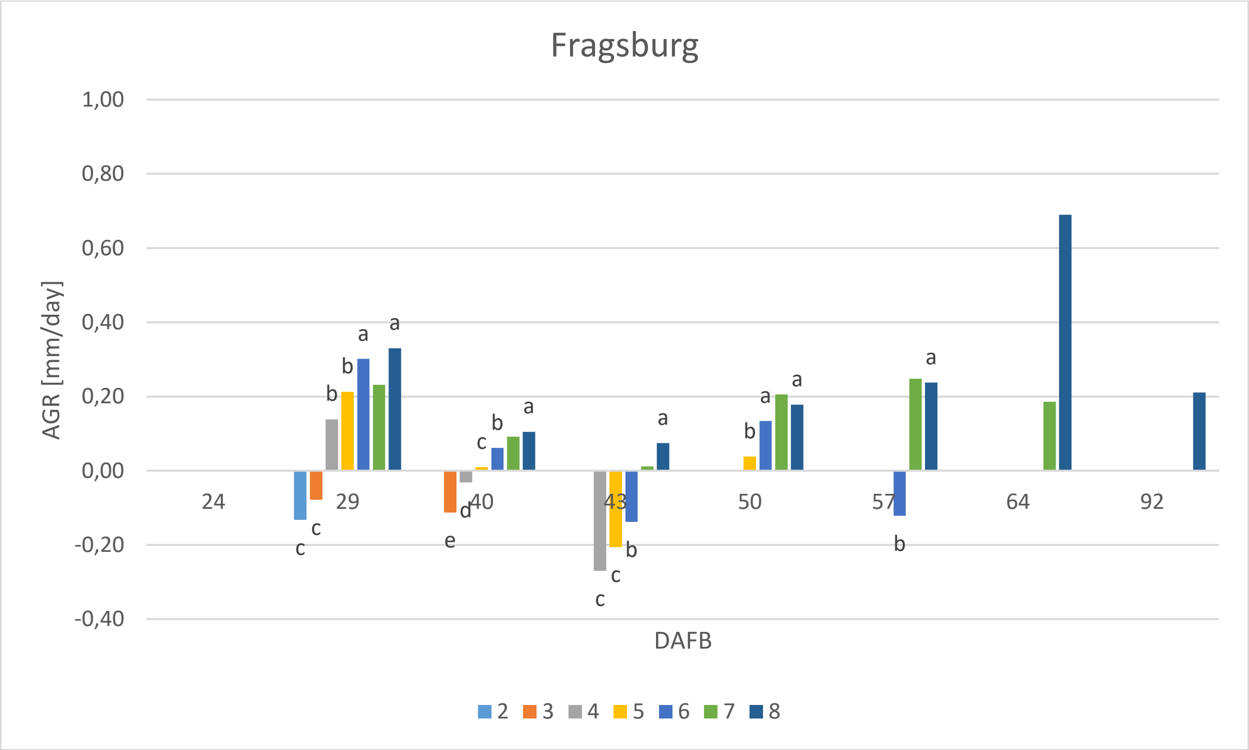
*detachment * Sampling" followed by the same lowercase letter are not significantly different at α ≤ 0,05 within the day of sampling. Groups with a sample size lower than 2% have been excluded from the statistical analysis. 320 fruitlets have been tagged and measured in each orchard until natural detachment or harvest.*

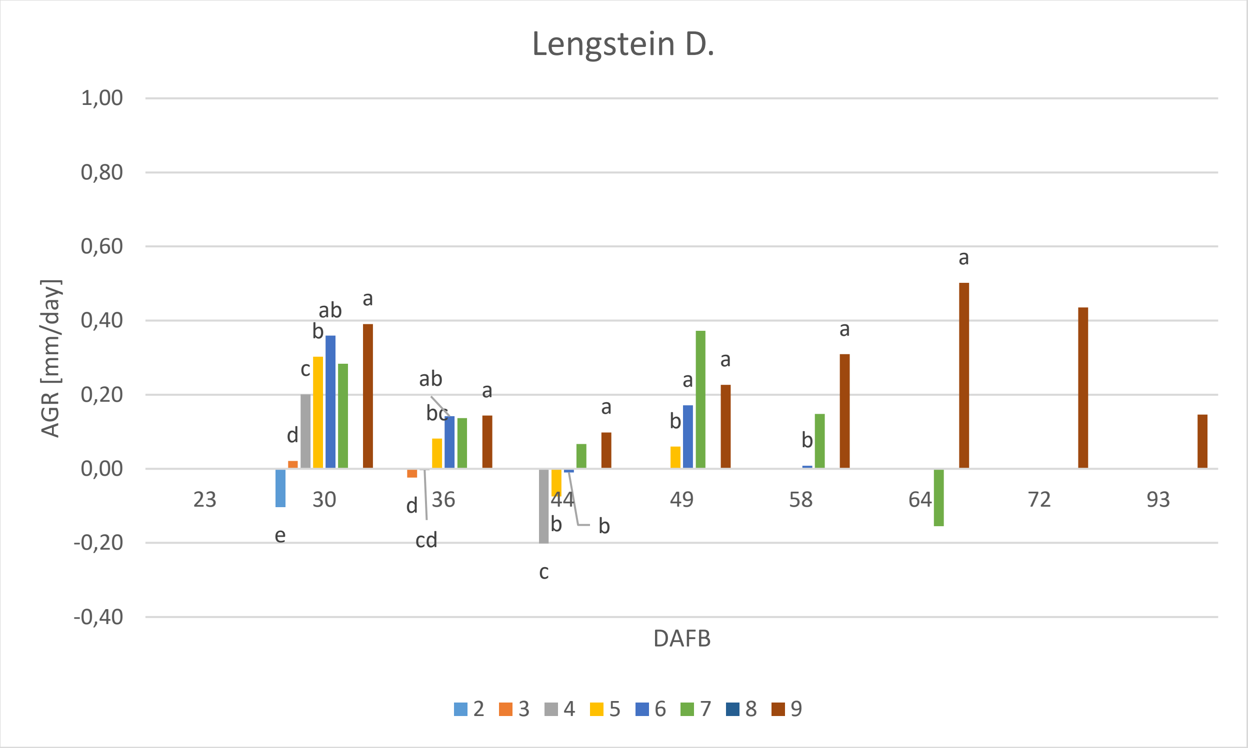
In the "Lengstein D." orchard Groups n ° 4 and 5 show significantly lower AGR values already between the first and the second sampling (thus between 23 and 30 DAFB). In both the time intervals between 23 and 30 DAFB and 30 and 36 DAFB Group n° 6 appears to be in an intermediate situation: at both sampling days its AGR values are at the same time not significantly different from those of Group n° 5 and of Group n° 9. Group n° 6 AGR values finally dropped between 36 and 44 DAFB. In the "Lengstein J." orchard it is debatable if Group n° 3 should be included or not into the actual "late" fruit drop. Despite the high average size of its fruitlets (9,15 mm) at the first sampling, its diameter is significantly different from that of Group n° 7. Group n° 3 already shows a significantly lower AGR between 28 and 36 DAFB. During the same period, Group n° 4 shows an average AGR comparable with that of Group n° 7, but the first statistical difference emerges shortly after, between 36 and 44 DAFB. Group n° 5, a residual part representing 3 % of the total tagged fruits, reduce its growth rate between 51 and 59 DAFB. In the "Vöran" orchard, both Groups n° 3 and 4 show a significantly lower growth rate than that of Group n° 7 already between the first and the second sampling (thus between 28 and 40 DAFB). A similar situation can be found in the "Martell" orchard where both Group n° 4 and n° 5 show a significantly lower AGR already at the second sampling (thus between 26 and 28 DAFB). In general, it is worth noting that, if a certain Group of detachment was showing a lower AGR than the group of the retained cherries already between the first and the second sampling, this does not necessarily mean that those fruits stopped growing exactly at that time interval; in fact, it cannot be excluded that such an event occurred shortly before the first sampling.

In both Figure 9 and 10 "Groups of detachment" characterized by a too small sample size (<2% of the tagged fruits) were represented on the graph but excluded from the statistical analysis.

A more detailed representation of fruit growth path can be obtained using fruit gauges able to perform continuous measurement of fruit diameter: Figure 11 represents an example of diameter increase/decrease of both retained (F4 and F5) and dropping (F2) cherries in the "Lengstein J." orchard. In the "Lengstein D." orchard the fruit gauges were installed too late and, consequently, were not able to portray the exact moment when the fruit stopped growing. Since the sensors were installed at 29 DAFB, it is difficult to tell from Figure 11 whether the cherry F2 stopped growing at 32 DAFB or if it was already shriveling at 29 DAFB.


Laimburg
1,00
0,80
0,60
0,40
0,20
0,00
-0,20
-0,40
AGR [mm/day]
27
31
34
41
50
59
62
72
85
DAFB
a
a
a
aa
ab
a
b
abc
b
aa
ab
a
a
a
a
a
c
c
c
cd
b
c
b
c
b
■ 2 ■ 3 ■ 4 ■ 5 ■ 6 ■ 7 ■ 8 ■ 9


Fragsburg
1,00
0,80
0,60
0,40
0,20
0,00
-0,20
-0,40
AGR [mm/day]
24
29
40
43
50
57
64
92
DAFB
a
a
b
b
c
b
a
a
b
a
a
a
c
c
e
d
c
c
b
b
■ 2 ■ 3 ■ 4 ■ 5 ■ 6 ■ 7 ■ 8


Lengstein D.
AGR [mm/day]
1,00
0,80
0,60
0,40
0,20
0,00
-0,20
-0,40
23
30
36
44
49
58
64
72
93
DAFB
a
ab
b
c
d
e
ab
bc
a
d
cd
a
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c
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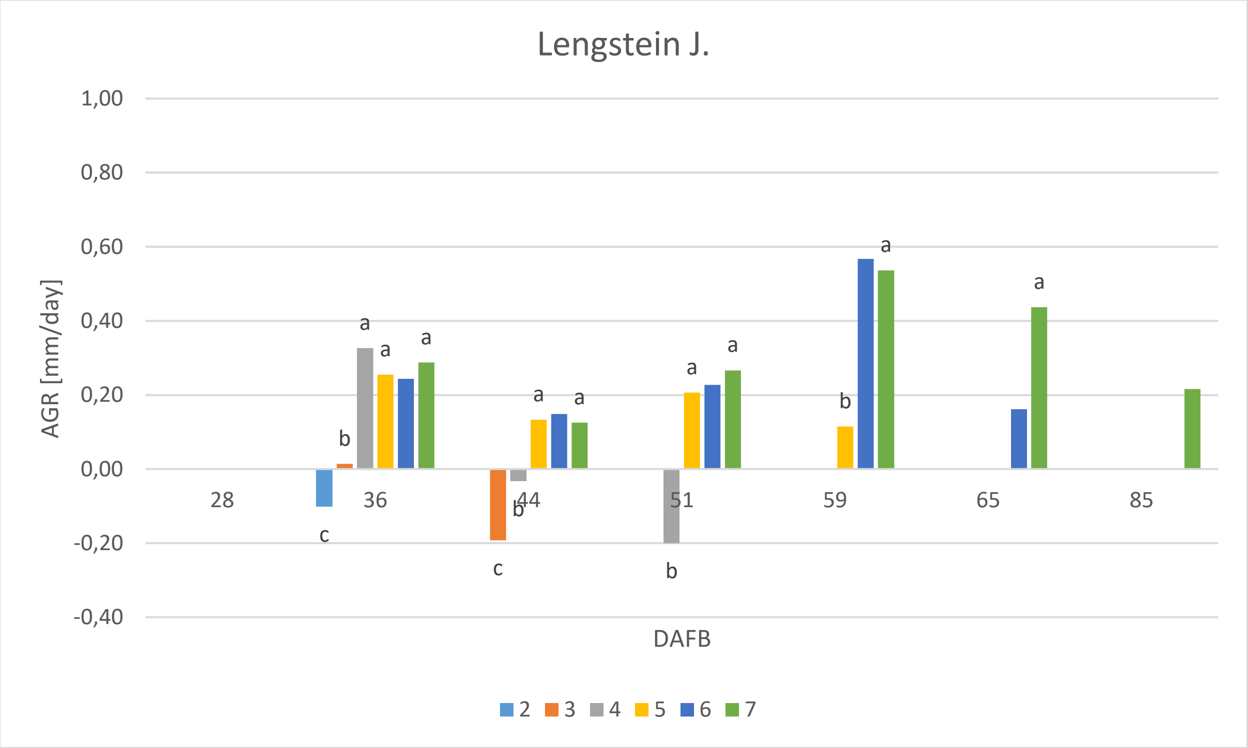
3
4
5
6
7
8
9


Lengstein J.
AGR [mm/day]
1,00
0,80
0,60
0,40
0,20
0,00
-0,20
-0,40
28
36
44
51
59
65
85
DAFB
a
a
a
b
c
a
a
b
c
a
a
b
a
b
a
a
2
3
4
5
6
7

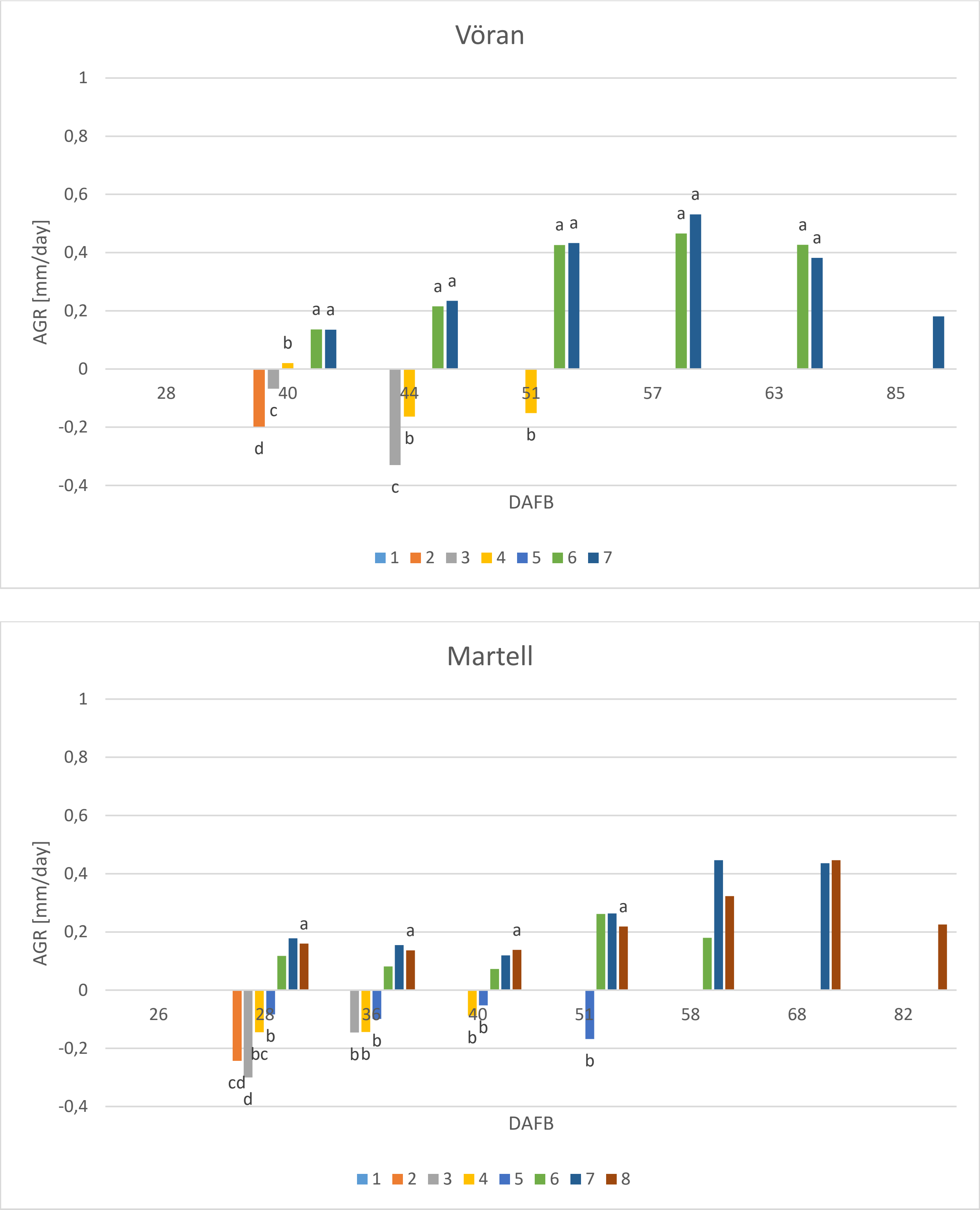


*Figure 10: Measurements of 'Regina' sweet cherry fruit absolute growth rate (AGR) in each orchard; AGR evolution over the time (expressed as DAFB) is reported individually for each group of detachment (characterized by different colors). The codes of the colored bars refer to the Groups of detachment shown in Table 7. Estimated marginal means of AGR in the interaction "Group of detachment * Sampling" followed by the same lowercase letter are not significantly different at α ≤ 0,05 within the day of*

sampling. Groups with a sample size lower than 2% have been excluded from the statistical analysis. 320 fruitlets have been tagged and measured in each orchard until natural detachment/harvest.

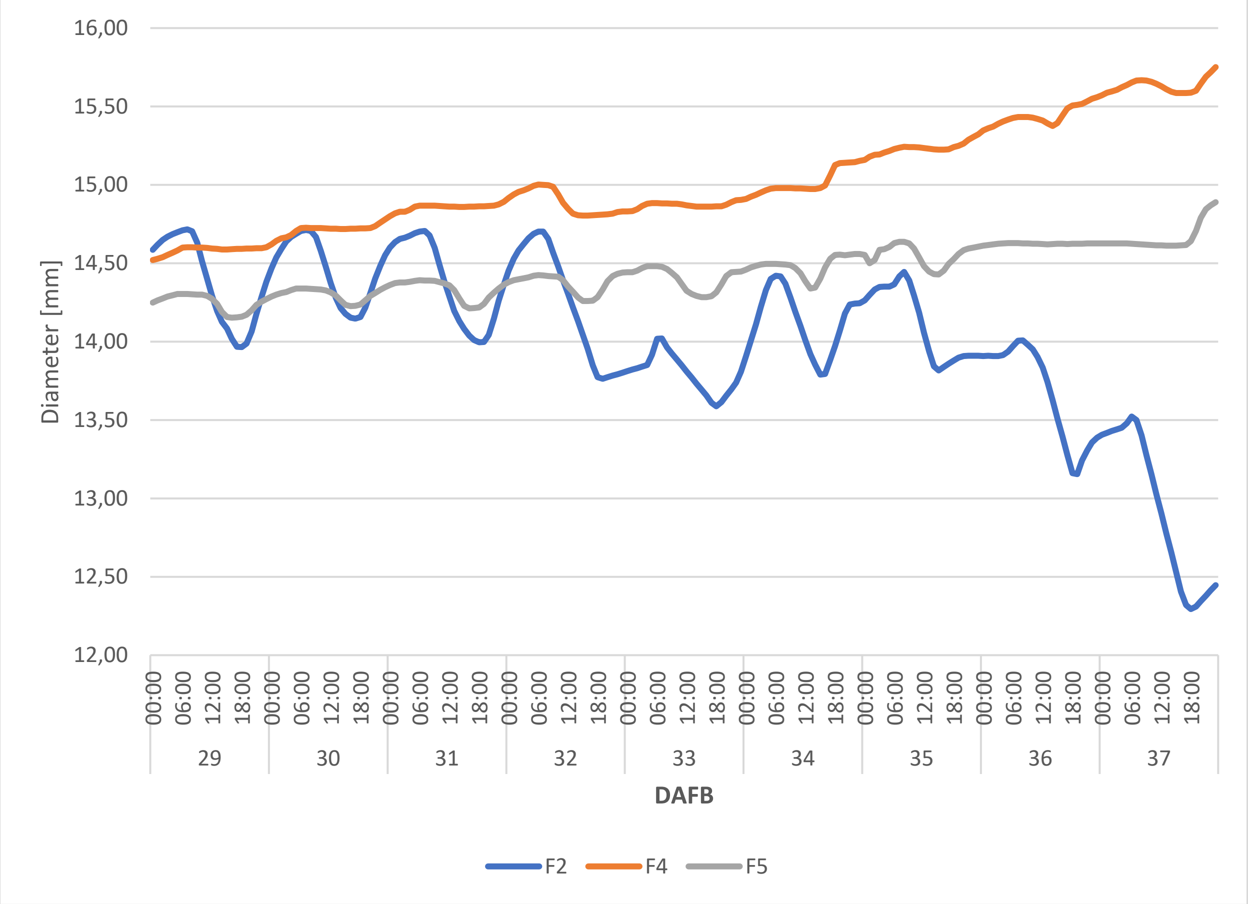


Figure 11: Patterns of 'Regina' fruit diameter growth over 9 days measured using custom-built gauges interfaced with a data logger (Morandi, et al., 2007). Both retained and dropping fruits are shown.

3.4.4 Soil, leaf, and fruitlet analyses

Throughout the season, a number of soil-, leaves-, and fruitlets analysis were carried out to exclude any deficiency-driven fruit drop. Table 8 reports the results of the soil analysis performed at harvest. The orchards considered show medium to high organic matter concentration, acidic to neutral pH, and an average good macro- and micronutrient concentration. The soil texture analysis revealed that the principal component of every soil was the sand, followed by silt, and to a lesser extent by clay. The leaf tissue analysis was performed in every orchard both at the beginning of late fruit drop (Table 9a) and at harvest (Table 9b). At late fruit drop time, the nitrogen-, phosphorus-, potassium-, and magnesium concentration are perfectly within the recommended ones at each location. Conversely, in relation to the calcium concentration, the orchards "Laimburg", "Fragsburg", "Lenstein D.", and "Vöran" show a slight deficiency. In relation to the micronutrients concentration the situation is more variable, with all the orchards but “Martell” showing Boron deficiencies, and the “Laimburg” showing Manganese deficiency.

Orchard	Soil organic matter [%]	pH [-]	Phosphorus [mg/100 g]	Potassium [mg/100 g]	Magnesium [mg/100 g]	Boron [mg/kg]	Manganese [mg/kg]	Copper [mg/kg]	Zinc [mg/kg]	Sand (2000 - 63 µm) [%]	Silt (63 - 2 µm) [%]	Clay (< 2 µm) [%]
Laimburg	2,5	7	27	34	16	0,59	17	16	11	41,5	49,5	9
Fragsburg	3,8	6	17	28	14	0,57	37	25	8	46	35	19
Lengstein D.	5,1	6,6	20	35	29	1,19	78	21	13	65	23	12
Lengstein J.	3,2	5,6	16	21	20	0,28	44	10	2	56	24	20
Vöran	5,1	5,4	22	46	21	0,97	76	24	21	64	22	13
Martell	6,3	6,5	91	36	17	1,49	30	29	27	50	38	12

Table 8: Percentage of soil organic matter, pH, texture and macro- and micronutrients concentration of the different orchards. Sampling was performed at harvest following the "W" pattern.

Orchard	N [%]	P [%]	K [%]	Ca [%]	Mg [%]	B [mg/kg]	Fe [mg/kg]	Mn [mg/kg]	Cu [mg/kg]	Zn [mg/kg]
Optimal range	2,60 - 2,80	0,18 - 0,30	1,60 - 2,00	1,20 - 2,00	0,30 - 0,50	30 - 60		30 - 100	5 - 12	15 -50
Laimburg	2,9	0,2	2,1	1,0	0,3	21,1	49,2	22,9	15,8	21,1
Fragsburg	3,3	0,3	1,7	1,1	0,3	20,8	59,0	88,5	81,6	22,6
Lengstein D.	3,5	0,3	1,9	1,1	0,4	17,4	62,7	127,2	8,7	34,9
Lengstein J.	3,1	0,3	1,8	1,3	0,4	29,4	86,5	74,4	103,8	17,3
Vöran	3,6	0,3	1,9	0,9	0,3	27,8	85,3	111,4	8,7	15,7
Martell	3,9	0,4	3,1	1,4	0,4	34,9	108,1	219,7	8,7	64,5

Table 9a: Leaves macro- and micronutrients concentration of 'Regina'. The sampling was performed as soon as the first symptoms of late fruit drop appeared. The "optimal range" is defined after Aichner et al. (2004)

Orchard	N [%]	P [%]	K [%]	Ca [%]	Mg [%]	B [mg/kg]	Fe [mg/kg]	Mn [mg/kg]	Cu [mg/kg]	Zn [mg/kg]
Optimal range	2,60 - 2,80	0,18 - 0,30	1,60 - 2,00	1,20 - 2,00	0,30 - 0,50	30 - 60		30 - 100	5 - 12	15 -50
Laimburg	2,77	0,28	2,92	1,46	0,37	60,70	67,60	19,10	45,10	15,60
Fragsburg	2,98	0,22	1,83	1,55	0,38	43,10	62,10	55,20	25,90	15,50
Lengstein D.	2,86	0,21	1,61	1,77	0,47	30,90	68,70	55,00	61,90	13,70
Lengstein J.	3,16	0,27	1,68	1,40	0,42	36,20	86,20	65,50	31,00	12,10
Vöran	3,44	0,27	1,83	1,34	0,39	48,40	91,60	157,30	6,90	15,60
Martell	3,38	0,30	2,49	1,90	0,40	41,50	70,80	107,10	10,40	25,90

Table 9b: Leaves macro- and micronutrients concentration of 'Regina'. The sampling was performed in each orchard at commercial harvest. The "optimal range" is defined after Aichner et al. (2004)

At the commercial harvest the nutrient status has remained unchanged: nitrogen-, phosphorus-, potassium-, calcium-, and magnesium values were still inside the optimal range. No Boron deficiency was found, but the Manganese deficiency was detected again in “Laimburg”.

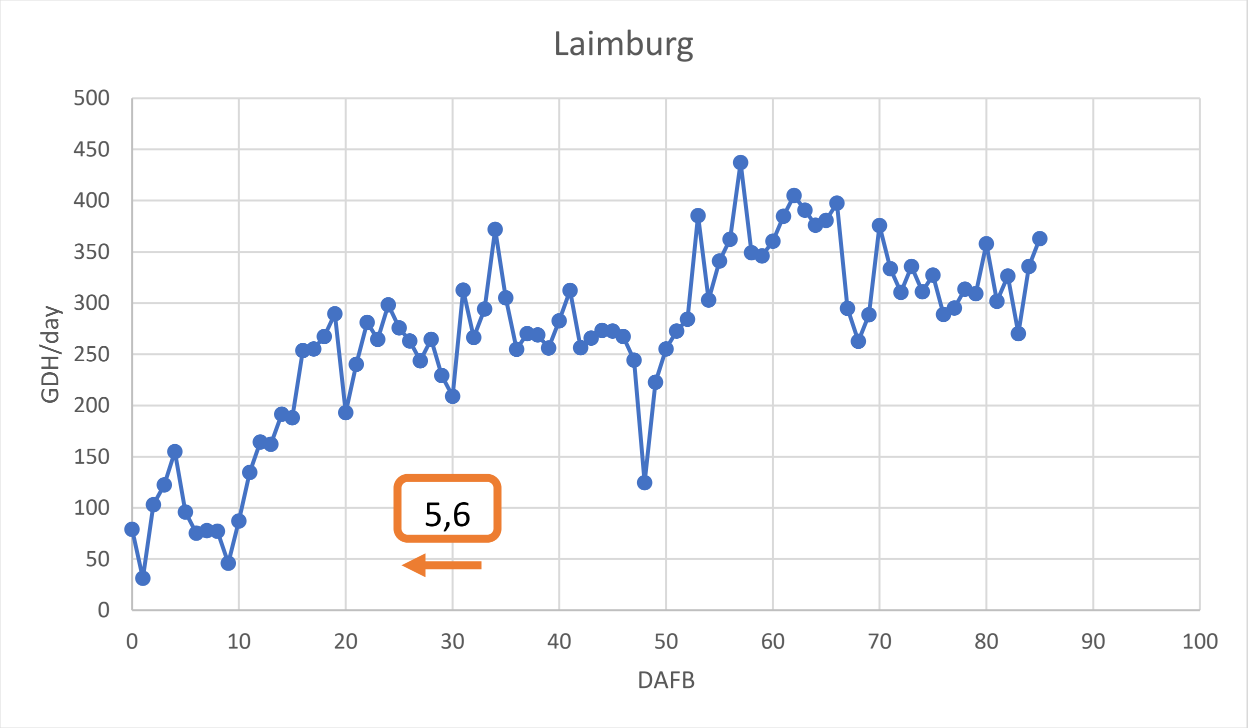
The fruitlets sampling was performed on different dates depending on the phenology of each site. The following data represent an average of the 6 locations. The fruitlets analysis was carried out in the form of 3 replications per orchard, and a basic ANOVA was performed to compare dropping cherries with the retained ones; in this analysis the orchards have been set as random factor (Table 10). Before reading the results, we should consider that, at the time of the analysis, the average fruit weight of the retained fruits was 33% higher than that of the dropping ones. This can be easily explained considering that if the symptoms of late fruit drop become evident (e.g., from an overall dull appearance to a yellowish color), it means that the fruit is at a very advanced stage of the senescence/dehydration process, and, therefore, its weight will naturally be lower than retained fruits. The loss of water due to transpiration modifies the average weight of the fruit as well as the concentration of the macro- and micronutrients. Based on these considerations, it is not surprising that dropping cherries show a significantly higher concentration of phosphorus, potassium, calcium, magnesium, iron, manganese, copper, and zinc; although the difference was not significant, also the concentration of the microelements sulfur and sodium was found to be higher in the dropping cherries. However, it is worth noting that the nitrogen concentration between the two categories was not statistically different but even higher, in absolute terms, in the retained fruits; finally, an identical concentration was detected for boron and silicon.

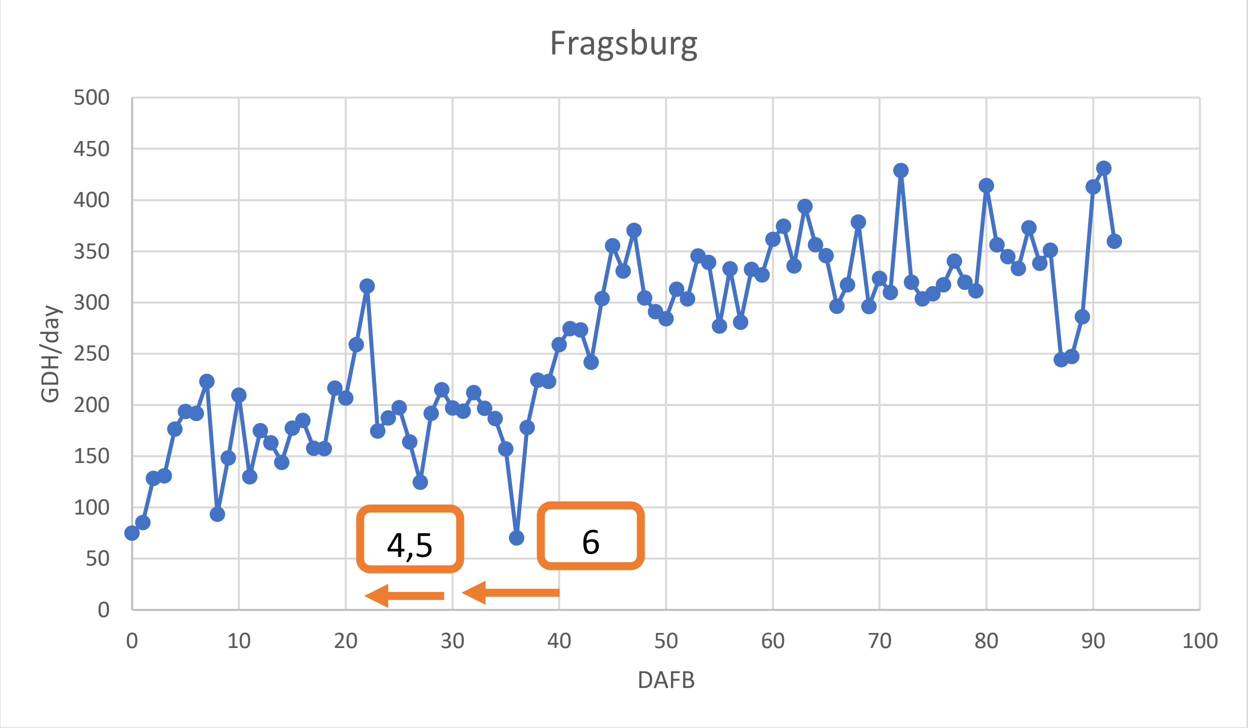
Element	**Retained fruitlets**	**Dropping fruitlets**	**Sig.**
Nitrogen [mg/100 g]	299,42	277,12	0,087
Phosphorus [mg/100 g]	22,67	29,97	**0,000**
Potassium [mg/100 g]	279,05	300,50	**0,001**
Calcium [mg/100 g]	31,81	43,50	**0,000**
Magnesium [mg/100 g]	18,68	23,80	**0,000**
Boron [mg/kg]	20,43	20,04	0,859
Iron [mg/kg]	3,96	4,53	**0,000**
Manganese [mg/kg]	2,38	3,53	**0,000**
Copper [mg/kg]	1,17	1,46	**0,002**
Zinc [mg/kg]	2,31	3,30	**0,000**
Sodium [mg/kg]	11,77	12,43	0,673
Sulfur [mg/kg]	135,89	140,43	0,327
Silicon [mg/kg]	95,86	96,05	0,987
Average fruit weight at the time of the analysis [g]	1,99	1,32	**0,000**

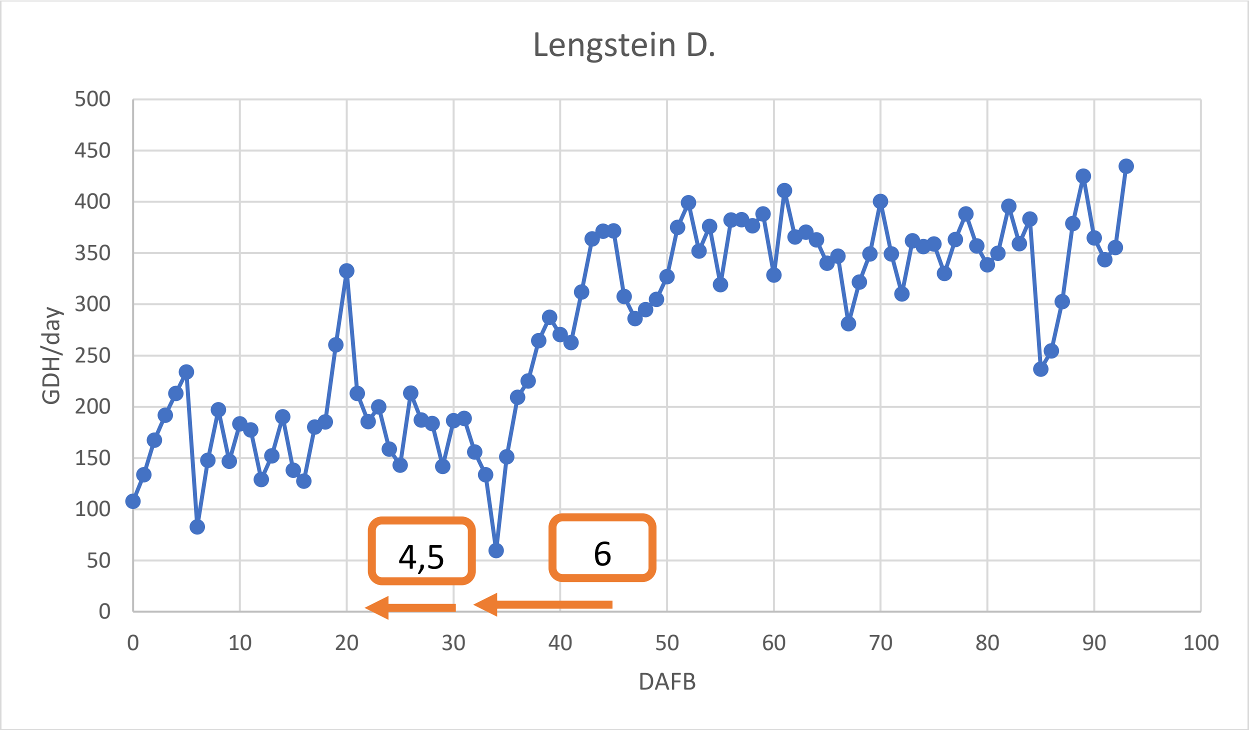
Table 10: concentration of macro- and micro nutrients in retained and dropping cherries cv. Regina. Bold p-values are considered significant at α ≤ 0,05. The sampling was performed as soon as the first symptoms of late fruit drop appeared. Dropping cherries were manually picked from the tree.

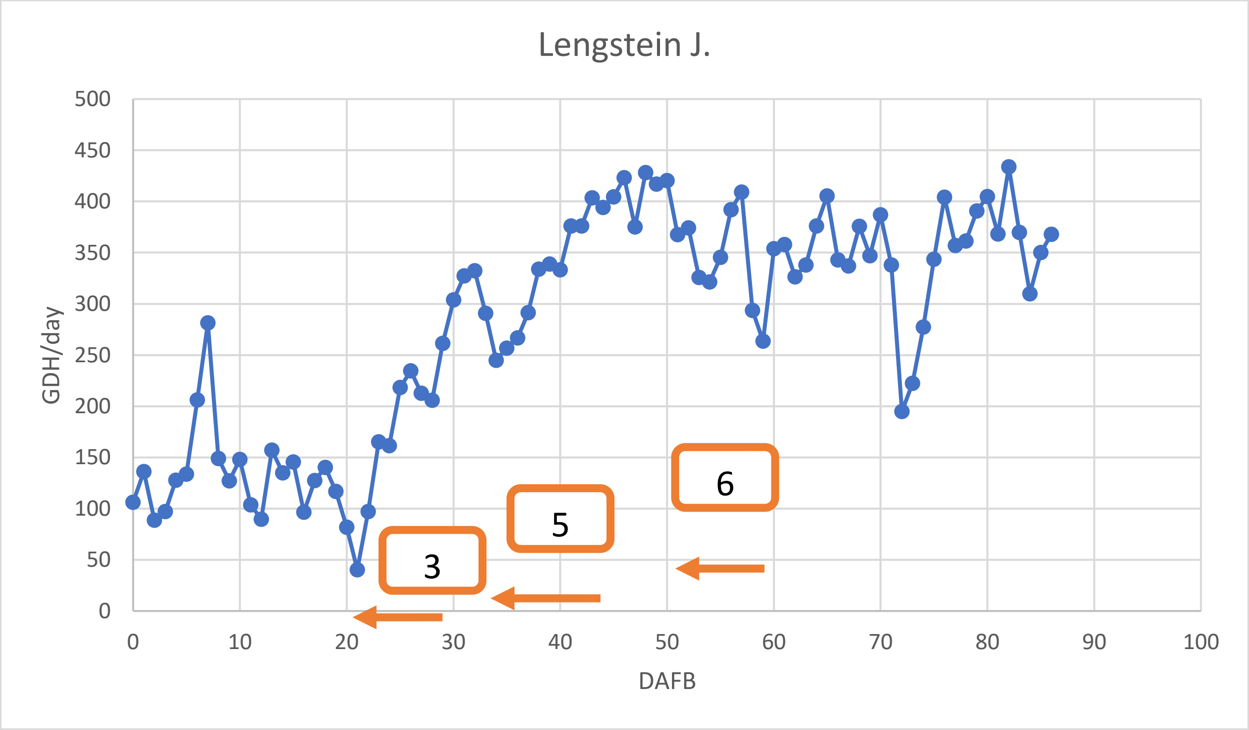
3.4.5 Meteorological data

Each orchard was provided with a weather station. During the data analysis the hourly average temperature was converted to Growing Degree Hours using the formula (4); each point of Figure 12 represents the sum of the GDH for each day (expressed as DAFB). In every diagram, the arrows indicate the time interval in which the Group of detachment specified within the orange box started to show a significantly different AGR than that of the retained cherries. Since the orchards are located at different elevations and the GDH are expressed as a function of the DAFB, the 6 different curves refer to different days of the year. In Figure 13 the GDH are reported as the average of 5 days for each orchard separately (e.g. from day 0 to day 4 after full bloom, from day 5 to day 9 after full bloom, and so on). Here we can observe that the different locations were subject to very different climatic conditions, especially during the first half of their reproductive cycle. During the first 9 DAFB the average GDH accumulation per day of "Laimburg", "Vöran" and "Martell" was much lower than that of "Fragsburg", "Lengstein D.", and "Lengstein J." but, interestingly, during the following 30 days this trend was completely reversed. The orchards “Laimburg”, “Vöran” and “Martell” started to accumulate GDH constantly, whereas, at the same time, the remaining three sites were unable to considerably increase their GDH accumulation rate. At each location, the climatic situation stabilizes towards 50 DAFB.






Lengstein D.
GDH/day
DAFB
0
50
100
150
200
250
300
350
400
450
500
0
10
20
30
40
50
60
70
80
90
100
4,5
6


Lengstein J.
GDH/day
DAFB
0
50
100
150
200
250
300
350
400
450
500
0
10
20
30
40
50
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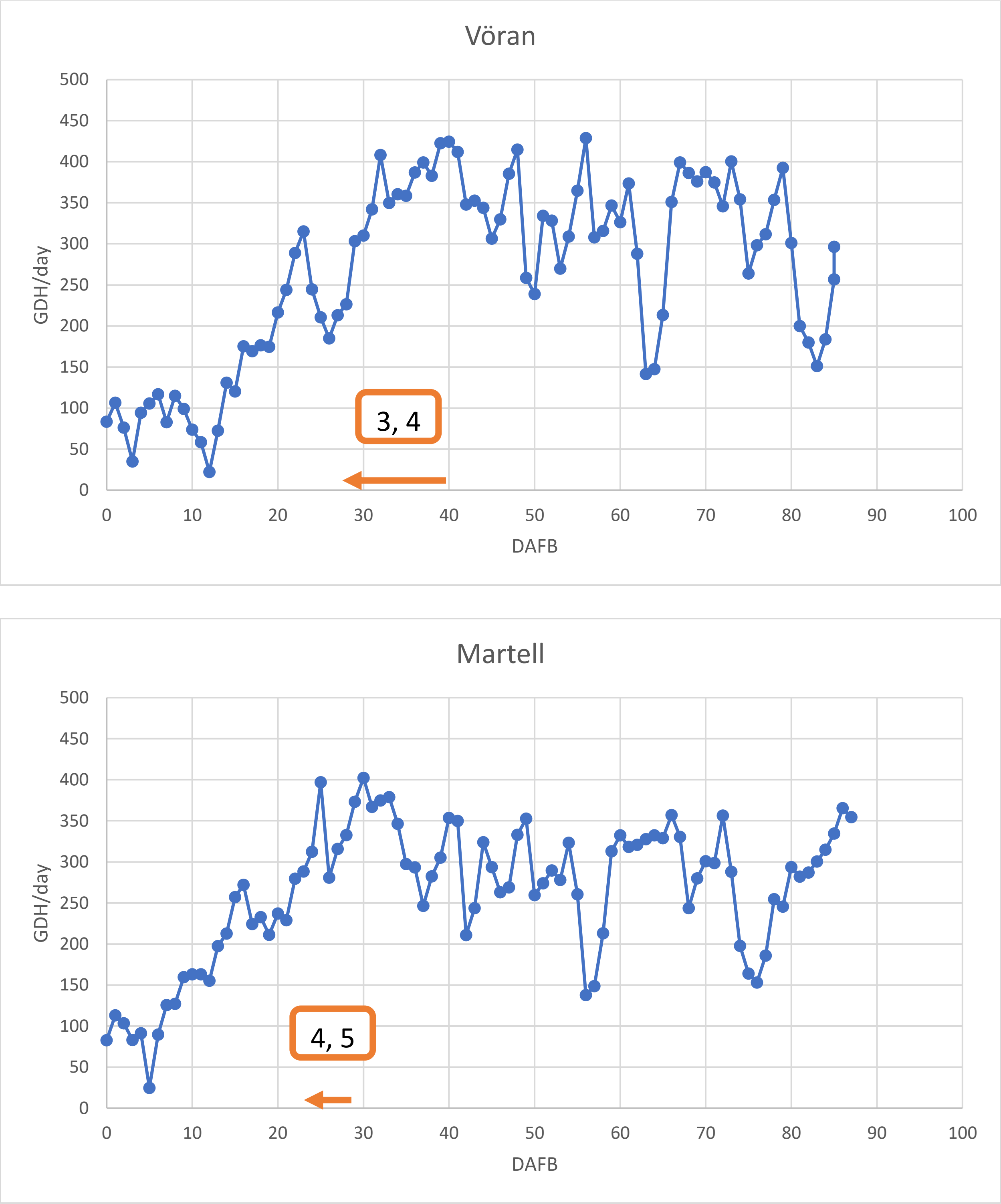

Figure 12: Growing Degree Hours evolution over the time expressed in DAFB, for each orchard separately. Each point represents the daily sum of the GDH calculated from the hourly average temperature of each location. The orange arrows indicate the period in which a certain Group of detachment (specified inside the orange box, for further information see Table 7) showed for the first time a significantly lower Absolute Growth Rate (AGR) than the retained cherries.

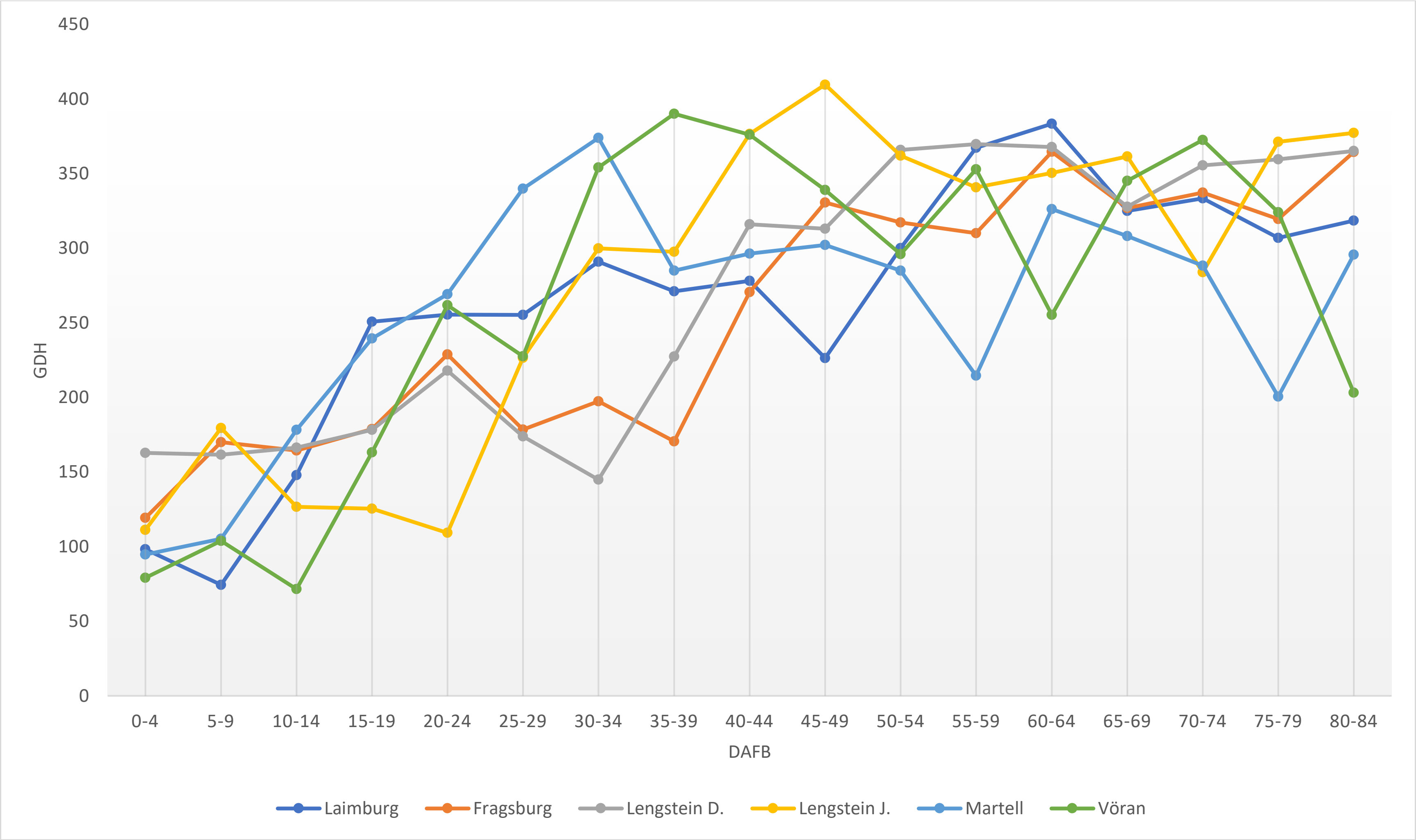

Figure 13: Growing Degree Hours evolution over the time of the different orchards; each point represents the 5 days average of daily GDH accumulation for each orchard separately.

3.4.6 Harvest

Figure 14 shows the average yield per tree at commercial harvest and its yield efficiency expressed as g/cm^2. As shown, high frost damages (e.g., “Lengstein J.”) or intense late fruit drop (e.g., “Lengstein D.”) do not necessarily compromise yield. In the same way, the absence of frost damages and a very weak late fruit drop do not necessarily ensure a gratifying harvest (e.g., “Laimburg”). This is because the final yield per hectare is a function of numerous factors, such as: the number of flowers left after the summer/spring pruning, the frost damages, the pollination efficiency, the fruit drop waves, the thinning, and finally the fruit size.

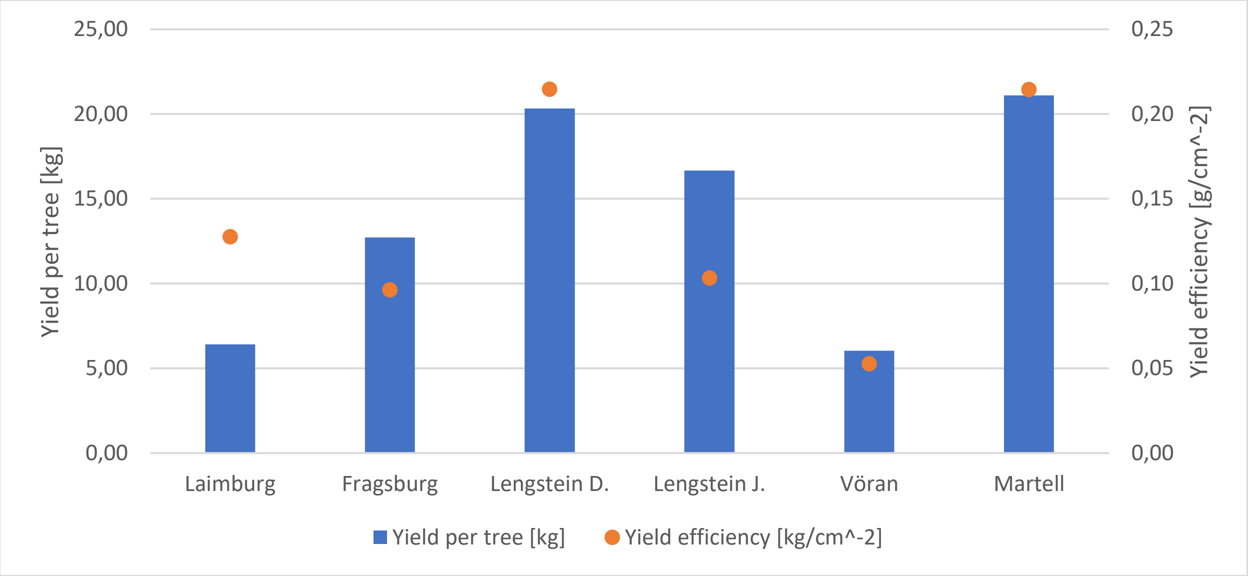

Figure 14: Average yield per tree at harvest and yield efficiency calculated as grams of cherries per cm^2 of trunk cross-sectional area of the corresponding tree.

3.5 Discussion

3.5.1 Flowers and fruits counting, fruit set

Similar to the previous season, the evolution of total fruit set, expressed as the percent ratio between the number of flowers and fruits (Askarieh, et al., 2021), resembled a sigmoidal curve in all the orchards considered (Figure 1). As already mentioned in the second chapter, a curve with a similar shape was obtained by Hedhly et al. (2009) who studied the fruit set evolution of ‘Vignola’ and ‘Sunburst’ pollinated by ‘Napoleon’ and ‘Burlat’, respectively. The gradual decrease in the number of flowers/fruitlets after the initial stationary phase (about 0 – 15 DAFB) was not observed in the “Lengstein J.” and in the “Vöran” orchards, where the severe frost damages prior to FB drastically reduced the number of flowers able to be pollinated / fertilized (Table 1 for more detailed information); interestingly, as shown in Figure 2, once the frost damaged- and the unfertilized flowers detached, the evolution of the fruit set of these two orchards becomes similar to that of all the others. Since the rate of fruit set varies over time, it makes sense that the factor day of “Sampling” was found to be significant in every orchard. The final total fruit set was reached at different times in each orchard: at about 59 DAFB (14080 GDH) in the “Laimburg” location, at 62 DAFB (14254 GDH) in “Fragsburg”, at 64 DAFB (15827 GDH) in “Lengstein D.”, and at 51 DAFB in “Lengstein J.” (12099 GDH), “Vöran” (12405 GDH), and “Martell” (12955 GDH). Even considering that the sampling was done once a week, and that, therefore, a certain margin of error is inherent, it seems clear that, in the season considered, the orchards located at higher elevations reached their final total fruit set earlier than those at the lower ones (both as DAFB and as GDH). Although either the factor “Position” or its interaction with the factor “Sampling” have been found to be significant in all the orchards, a clear and universal trend was not identified: In the “Laimburg” orchard, the upper branches reduced their fruit set faster (from 30 to 41 DAFB) and reached their final total fruit set earlier than the lower ones, but, in absolute terms, no significant effect on the final fruit set has been observed; in “Fragsburg”, although a slightly higher final fruit set was shown by the upper branches, again no significant difference was detected. Similar to the first two orchards, in “Lengstein D.”, the upper branches significantly reduced their fruit set earlier in the season, reached the final total fruit set about one week before the lower ones, and showed (only in absolute terms, without any significant difference) a slightly higher final fruit set. In the “Lengstein J.” location, no significant differences were detected. Completely different is the case of “Vöran” where an overall higher fruit set of the upper branches was detected already at shuck split. In “Martell” a higher fruit set on the upper branches was also observed but only from 28 DAFB to harvest. This phenomenon should be read at least from two different perspectives: the first one is the better light condition of the top of the canopy compared to the lower branches (probably as in the case of “Martell”) whereas the second one is the heterogeneous distribution of frost damages between the lower and the upper branches (very likely the case of “Vöran”), with the latter usually showing a lower percentage of damaged flowers. Eventually, the sweet cherry tree marked acrotony could also have played a role (Lauri, et al., 2008). In the scientific literature, no exhaustive information about the effect of the position within the canopy is given for sweet cherry; however, Luchsinger et al. (2002) measured a higher fruit size and overall quality in the ‘Angelus’ peaches picked from the top of the central leader than in those of the lower branches, asserting that the light availability and distribution within the canopy could have played a role. Furthermore, a higher fruit set was found in the upper parts of the tree canopy exposed to higher irradiance for persimmon (*Diospyros kaki* L.) (George, et al., 1996). The height of sweet cherry fruiting branches from the ground, their position in the canopy, and their orientation were reported to have little influence on the fruit set of different cultivars (Roversi, et al., 1996); in this work, a generally better fruit set occurred on the higher and external branches, and on branches with a south-east orientation, which is consistent with our observations. Finally, a marginal effect of better light exposure on fruitlets net photosynthesis in the early stages of development (Flore, et al., 1999) and, therefore, on fruit set cannot be excluded.

The idea of calculating the relative fruit set on the basis of the number of fruitlets set, arises from the need of “cleaning up” the total fruit set from the influence of secondary factors in order to be able to compare the intensity of late fruit drop in different locations; for example, the total fruit set is also function of the frost damages and the pollination efficiency, both site-specific factors. The n0-stage was attributed to each orchard individually as soon as most of the unfertilized fruit had detached but no late drop (of bigger fruits) had yet occurred. This approach is based on the considerations reported by Bradbury (1929), that the first wave of fruit drop is composed by smaller unfertilized parthenocarpic fruitlets, and that the second wave (our focus) is composed by fully developed drupelets showing an aborted embryo. A certain percentage of late fruit drop was observed at all the locations, but with consistent differences in its intensity. The evolution of the relative fruit set seems expolinear, characterized by an initial rapid decrease in fruit number (more or less marked, depending on the location) followed by a stationary phase. At the “Laimburg” location, late fruit drop lasted from 50 to 59 DAFB, in “Fragsburg” from 43 to 65 DAFB, in “Lengstein D.” from 44 to 64 DAFB, in “Lengstein J.” from 44 to 65 DAFB, in “Vöran” from 40 to 51 DAFB, and in “Martell” from 36 to 51 DAFB. Since the less intense late fruit drop was recorded in “Laimburg” (225 m a.sl.) and “Martell” (1135 m a.s.l.) and the most intense at “Lengstein D.” (785 m a.s.l.) and “Fragsburg” (705 m a.s.l.), no linear correlation between the increase/decrease in elevation and the percentage of late fruit drop can be assumed (Figure 3). Interestingly, repeating the Mixed Model procedure using the relative fruit set instead of the total one as dependent variable (Table 3), revealed no effect of either the “Position” or of its interaction with the “Sampling”. Based on this information, we can assume that if the position of the branch within the canopy could influence the total fruit set, this is not true for the relative fruit set; in other words, the influence of the position is exerted early in the season, but no effect was measured on the late fruit drop. The factor “Pruning” showed a significant effect on the relative fruit set of both “Martell” and “Fragsburg” (here in form of the interaction “Pruning*Sampling”); in both cases, pruned limbs showed an increase in late fruit drop rate and significantly lower final fruit set (Figure 5). It is worth noting that these two orchards are also the most vigorous ones as shown in Figure 7. In Sweet cherry the reproductive cycle and the vegetative growth occur simultaneously: therefore, phenomena of competition may occur between the reproductive sinks and the vegetative sinks. In both “Fragsburg” and “Martell”, at the time when Groups of detachment n° 4 and n° 5 started to grow slower than the retained cherries, the AGR of shoot growth was very high if compared to the other orchards. Ayala et al. (2015) reported that competition between reproductive and vegetative development for storage reserves remobilization was highest at full bloom; however, competition cannot be excluded also at later stages since storage reserves were found in both reproductive and vegetative organs until 35 DAFB (‘Regina’ on ‘Gisela 6’). A more detailed explanation about the effect of pruning the 1-year-old shoots will be provided in the next paragraphs.

3.5.2 Vegetative parameters

As already discussed before, since the average shoot length increases with the time, it is consistent that the factor day of “Sampling” is significant at each location. Despite the well-known acrotony of sweet cherry, in this trial no effect of the factor “Position” on the vegetative parameters was found. On the contrary, the factor “Pruning” severely influenced the vegetative growth: since most of the vegetative buds have been removed by pruning, pruned limbs showed a significantly lower absolute number of new shoots per limb in 4 orchards out of 6. Interestingly, the linear density of new shoots per limb length remained unchanged in 5 orchards out of 6, suggesting an internal self-regulating mechanism able to keep the number of the extension shoots constant regardless of the limb’s management (Table 4). Extension shoots borne on pruned limbs showed a greater average shoot length than those borne on the unpruned ones in all the orchards except for “Lengstein J.”. Specifically, the latter suffered from several factors during the season that reduced its vegetative growth (as shown in Figure 6, Figure 7, and Table 4) such as the very cold temperatures in the spring and an overall problem of root asphyxia due to high water table. Both pruned and not pruned limbs showed a similar growth rate distribution over time (grey and yellow AGR bars, Figure 6) in most of the orchards, but with the former having significantly higher AGR values in most of the season. In “Laimburg”, the maximum peak of shoot growth was reached early in the season, between 29 and 36 DAFB, for both the pruned and the unpruned limbs. Differently, in “Fragsburg” a bimodal distribution, with a first peak between 18 and 24 DAFB and a second one between 40 and 43 DAFB, was observed. In “Lengstein D.” a weak bimodal distribution can be recognized especially for the unpruned limbs, where a first peak was reached between 23 and 30 DAFB and the maximum peak between 36 and 44 DAFB. In “Lengstein J.”, the AGR distribution over time of the pruned and the unpruned limbs appeared very different, with the former showing a maximum peak between 28 and 36 DAFB, and the latter between 44 and 51 DAFB, but, again, the environmental conditions of this orchard could have played a role. In “Vöran” the extension shoots borne on the unpruned limbs, instead of showing a maximum peak, grew at a constant rate from 22 to 40 DAFB; on the contrary, the pruned ones showed a clear maximum peak between 28 and 40 DAFB. In “Martell” both treatments reached their maximum peak between 23 and 28 DAFB. In general, the shoot growth seemed to be strongly temperature driven (as shown in Figure 7): the sigmoidal functions found in each location reached their stationary phases (terminal bud set) only after a certain number of GDH have been accumulated. A similar connection with the GDH accumulation could be seen in the total fruit set evolution represented in Figure 2. Limb management turned out to have a significant influence on the formation of non-fruiting spurs: pruned limbs bore fewer non-fruiting spurs (in absolute terms) with a dramatically lower linear density per meter of limb. Pruning the 1-year-old shoots did affect their leaf population: non-fruiting spurs reach their maximum development rapidly in the season, while the current season shoots create new leaf area even up to harvest (Ayala, et al., 2008). As already reported and discussed in the second chapter, at 25 DAFB half of the carbon recovered by non-fruiting spurs is exported to the fruits, while only 27% of the carbon fixed by the extension shoots is exported to the fruits and 50% to the extension shoot leaves to sustain the vegetative growth (Ayala, et al., 2018). However, since no girdling was performed on the studied limbs, carbohydrates contribution from other parts of the canopy cannot be excluded. The analysis of the linear regression between the vigor of the limb (expressed as the sum of new shoots’ length normalized by the limb length) and its relative fruit set returned that for each unit increase of the predictor the relative fruit set at harvest decreased by 4,7%. Interestingly, in the “Fragsburg” orchard, the most vigorous one, the predictor explained 17,5% of the total variability. In conclusion, the “Pruning” treatment proved to be capable of modifying the extension shoots growth kinetics in the same season but also, at the same time, the leaf populations that will support fruiting in the following one. Differently, the “Position” of the limbs within the canopy did not show any significant effect on the extension shoots growth kinetics. Furthermore, the extension shoots growth plotted over the GDH accumulation returned a similar curve for each site, indicating how the growth kinetics are strong dependent on temperature. Finally, linear regression analysis showed that, overall, only 7,2% of the total variability of the relative fruit set can be explained by an excess of vigor. Therefore, it can be concluded that, despite higher vigor was significantly correlated with greater intensity of late fruit drop, it was not its main cause. In sweet cherry, competition between fruit set and the vegetative growth has been hypothesized and/or studied by many authors (Blanusa et al., 2006; Cittadini et al., 2008; Ayala et al., 2015; Morandi et al., 2019) but further studies are needed to investigate the balance between the vegetative- and the reproductive sink strength.

3.5.3 Seasonal fruit growth

The fact that the drupelets have been individually labelled already at shuck split, made it possible, a posteriori, to divide them into different groups based on their time of detachment; furthermore, knowing the average fruit diameter of every group at the first sampling, allowed us to ignore those that were already smaller than the retained ones. In doing so, it was possible to discern between the different waves of fruit drop, and, more specifically, between the detachment of unpollinated/unfertilized fruitlets and the physiological late fruit drop. A good example is the "Vöran" orchard where out of 320 fruits tagged only 29% reached maturity, but, thanks to the division into groups of detachment, we were able to estimate that only 24% of the tagged ones actually belonged to the late drop wave and that all the others (Group n° 1 and n° 2) were already shrinking at shuck split. Generally, the high number of unpollinated/unfertilized drupelets can be explained either as a lack of overlapping between the full bloom of 'Regina' and that of 'Kordia' (Zago et al., 2011; Sagredo et al., 2017) (a very common situation at the lower elevation in South Tyrol), or as an effect of late frost. The latter could be either direct ('Regina' flowers killed by frost) or indirect: since 'Kordia' is less frost tolerant than 'Regina' (Vercammen, et al., 2017), the reduction of viable flowers of the former can lead to less pollen availability for the latter. In most of the orchards, at a certain point of the season, some groups of cherries started to grow slower than the retained ones. Initially, their AGR values are still slightly positive, but, at a later time, probably as soon as the incoming phloem flow ceases, they turn negative when the fruits start to shrink. Mancini et al. (2021) hypothesized that whenever the fruit growth stops, the xylem flow can maintain for few days the sink of water, but without a night enlargement (due to the lack of the phloem contribution) the fruit is destined to drop. These observations are consistent with the fact that the actual detachment of cherries showing symptoms of late drop could be hastened by the rising temperature due to the higher transpirational losses via the fruit skin and probably also due to the increased activity of enzymes involved in senescence and abscission process (see paragraph 1.4.4, Chapter 1). It is worth noting that several weeks may pass from the time the AGR of a group of detachment turns significantly lower than that of the retained cherries to the time of the actual drop. Since in most of the orchards the first significantly lower AGR were recorded in the first 30 days after full bloom (at about 1/3 of the reproductive cycle of this variety), the adjective "late" should be reconsidered. It is interesting to note that, in both the "Fragsburg" and the "Lengstein D." orchards (i.e. those with the most intense late fruit drop) Group n° 5 and n° 6 initially showed negative AGR values between 40 and 43 DAFB, and between 36 and 44 DAFB, respectively, but surprisingly, soon afterwards, they tried to recover; however, despite the positive AGR values recorded between 43 and 50 DAFB and between 44 and 49 DAFB, respectively, they finally dropped. Retained cherries followed the typical double sigmoidal curve.

3.5.4 Soil, leaf, and fruitlet analyses

In the soil analysis no significant deficiency was detected: on average, soils showed a medium to high organic matter concentration, they were light with good drainage, and had an average good macro- and micronutrient concentration. No soil features were considered limiting for sweet cherry growing according to Aichner et al. (2004). The leaf analysis performed at the time of late fruit drop did not show any particular deficiency in the two orchards characterized by the most intense fruit drop. Overall, although some slight deficiencies were found, again, none of them were found to be limiting according to Aichner et al. (2004). Usenik et al. (2007) investigated the possible correlation between the boron content of dormant buds (increased by foliar applications in the fall) of 'Hedelfinger' and 'Summit' on the following season's fruit set. The results showed that the tree response to foliar application of micronutrients was inconsistent, and that the low fruit set measured could have been influenced by several factors, as the tree age or the climatic conditions. In the work of Jimenez et al. (2007), shoot mineral concentration appeared to be positively correlated with yield efficiency but, surprisingly, shoot potassium concentration showed instead a negative correlation. However, in our work the potassium leaf concentration of the two orchards showing the most intense fruit drop ("Fragsburg" and "Lengstein D.") was not higher than in the others. The same work highlighted the effect of different rootstock on the leaf mineral nutrition but, in our trial only the combination 'Regina' on 'Gisela 5' was used. As already mentioned in the results, since the dropping fruits were already shrinking (i.e., losing water), it is not surprising that their micro- and macronutrients concentration was significantly higher. Since no publication on the nutrient status of dropping cherries has been found, our results are hardly comparable. However, Abruzzese et al. (1995) studied the nutrient status of retained and dropped apples during June drop. Similar to our results, they measured a significantly lower fruit fresh weight for the abscised apples than for the retained ones. Again, calcium concentration was higher in the abscised apples, but, differently from our results, the potassium concentration was lower. Interestingly, the protein concentration was much higher in the retained apples, which is consistent with the higher nitrogen concentration detected in the retained cherries. A possible explanation is that the shrinking of the young fruitlets prior to fruit drop causes a concentration of most of the mineral nutrients but probably nitrogen compounds follow a different fate. A separate consideration deserves the contribution of boron to fruit set: although many authors claim that this microelement plays a key role in the reproductive process, at the same time contradictory reports concerning the effect of boron nutrition on fruit set of stone fruits have been published (Michailidis, et al., 2023). For example, Hanson et al. (1985) reported that fall foliar sprays of Boron showed opposite effects on the fruit set of *Prunus domestica* depending on the spring temperature (Hanson, et al., 1985). A few years later, the same author studied the effect of fall sprays of Boron on the fruit set of sour cherry: boron applications had inconsistent effects on fruit set, yield and yield efficiency in six of seven sites, while in only one increased the fruit set by 110% (Hanson, 1991). The effect of fall sprays of Boron on the fruit set of ‘Butte’ and ‘Mono’ almonds was also studied: the treatment significantly increased the fruit set of ‘Butte’ and only to a lesser extent that of ‘Mono’; this difference was explained by reporting that, of the two cultivars, ‘Butte’ showed lower tissue Boron concentration before the exogenous application in the fall than ‘Mono’, and therefore responded more significantly in both fruit set and yield (Nyomora, et al., 1997). Similarly, Boron application on sweet cherry significantly increased the fruit set of ‘Hedelfinger’ (but just in one block of two) but not of ‘Summit’ (Usenik, et al., 2007). Despite in our trial the highest Boron concentration in both soil and leaves (during the drop) was found in the orchard showing the lower intensity of fruit drop (“Martell”), a clear trend is difficult to delineate. In fact, the soil of the “Lengstein D.” orchard is the second richest in Boron, but this site is also the one that, together with “Fragsburg”, showed the highest fruit drop intensity. Analogously, despite the leaf Boron concentration of the “Laimburg” orchard during the fruit drop was very similar to that of “Fragsburg” and “Lengstein D.”, its relative fruit set at harvest was much higher than the latter two. Overall, it is very hard to accurately delineate the contribution of this microelement to fruit set: in the data presented in Table 10, the fruit Boron concentration was similar in both retained and dropping fruitlets despite the latter were already senescent; despite the overall statistical analysis suggests that the abscission-destined fruits had lower Boron level than the retained ones, the analysis of the single orchard is not as unambiguous: in fact, although at each site the retained fruitlets showed a higher fresh weight than the dropping ones, the Boron concentration of the latter was sometimes higher (“Laimburg”, “Lengstein D.”), equal (“Lengstein J.”), or even lower ("Fragsburg”, “Martell”) (data not shown). In the future, a better understanding of the physiological, anatomical, metabolic, and transcriptomic impact of Boron supply on the sweet cherry fruit set will be provided by new approaches to the topic (Michailidis, et al., 2023).

In conclusion, the data presented suggest that fruit drop was not linked to any nutrient deficiency.

3.5.5 Meteorological data

Although the cherry orchards were part of the same province, the weather conditions from full bloom to harvest were very different for each one. The fact that they were located at different altitudes (about

1000 meters difference from the lowest to the highest) resulted in great differences in their phenology: therefore, a particular climatic condition may have been experienced by two locations at very different stages of their reproductive cycle. For example, prolonged periods of temperatures below zero in pre-bloom, able to cause significant frost damages, have been recorded both in "Lengstein J." and in "Vöran", but the same cold wave did not affect the "Laimburg", "Fragsburg", and "Lengstein D." orchards thanks to their lower elevation. Interestingly, although the "Martell" orchard is only 40 meters lower than the "Vöran" one, their difference in phenology (both for full bloom and harvest) was about one week; apparently, the fact that "Martell" had a later phenology made it less susceptible to this frost event.

Many of the processes studied in this work seem to depend on the temperature trends. For example, it is very likely that the bimodal AGR distribution of the vegetative growth measured in both "Fragsburg" and "Lengstein D." was caused by a temperature drop. In the case of "Fragsburg" we can observe a first peak of AGR between 18 and 24 DAFB followed by a slight decrease in average shoot growth rate until 40 DAFB (Figure 6). Likewise, the weather station installed in "Fragsburg" recorded a marked increase in air temperature from 18 to 22 DAFB, followed by a sudden drop (316 GDH have been measured at 22 DAFB and only 125 at 27 DAFB) and long period of low temperatures. At 36 DAFB only 70 GDH have been measured, but after this minimum peak, the temperature started to increase again and reached the average values of the season at 40 DAFB. In the case of "Lengstein D.", which is located at a similar elevation and was therefore subject to the same climatic phenomena of "Fragsburg", a first peak of shoot growth rate was measured between 23 and 30 DAFB, followed by a significant reduction in the growth rate between 30 and 36 DAFB. The maximum peak of AGR was measured between 36 and 44 DAFB. Similarly, the weather station installed at the edge of the orchard measured a period of relatively stable temperatures until 31 DAFB followed by a severe drop: at 31 DAFB 189 GDH have been measured, while only 60 GDH have been measured at 34 DAFB. From this point on, GDH accumulation started to increase again, reaching a maximum peak at 45 DAFB exactly as the average shoot growth rate. The fact that this bimodal growth appeared less marked in the "Lengstein D." orchard than in the "Fragsburg" one, is probably due to the much higher vigor of the latter as shown in Figure 7. A severe drop in the daily GDH accumulation has been observed also in the other orchards, but much later in the season: at 48 DAFB in the "Laimburg" location, at 72 DAFB in "Lengstein J.", at 63 DAFB in "Vöran", and at 56 DAFB in "Martell" but none of these was followed by a reduction in the AGR of the extension shoots; therefore, the existence of a "sensitivity phase" can be assumed. The cessation of sweet cherry shoot growth after a marked drop in air temperature was also reported by other authors (Sonsteby, et al., 2019).

In “Fragsburg” and “Lengstein D.” the most intense late fruit drop was recorded. In these two sites, both Group of detachment n° 4 and 5 showed a lower growth rate already between 24 and 29 DAFB (“Fragsburg”) and 23 and 30 DAFB (“Lengstein D.”). In this period, 38% and 47% of the tagged fruits, for each location respectively, became senescent. This phenomenon could be traced back to the severe temperature drop recorded simultaneously: from 316 GDH at 22 DAFB to 125 GDH at 27 DAFB in “Fragsburg”, and from the 333 GDH at 20 DAFB to 142 GDH at 29 DAFB in “Lengstein D.”. Again, in both sites, fruitlets of Group n° 6 started to grow significantly slower than the retained ones over a period of time overlapping the minimum peak of daily GDH accumulation. Surprisingly, in both “Fragsburg” and “Lengstein D.”, Group n° 5 and n° 6, although they already showed a negative AGR as described above, tried to recover, and showed increasing AGR values (in some cases even comparable to those of the retained fruits) with increasing temperature; however, something irreversible had happened and they all finally dropped. A similar minimum temperature peak has been recorded also in “Vöran” (22 GDH at 12 DAFB), but since it occurred earlier in the season it probably influenced more the initial fruit set (in fact a high percentage of small/unfertilized drupelets has been recorded in this orchard) than the later physiological drop of the fruitlets set. A similar situation was recorded in the “Lengstein J.” orchard, where the lowest peak in temperature was recorded at 21 DAFB (40 GDH): here, again, a high percentage of unfertilized fruitlets have been observed but not an intense late fruit drop phenomenon. As reported by Guerrero-Prieto et al. (1985), low temperatures at the blooming time can reduce the total fruit set by slowing down the pollen tube growth and therefore increasing the risk of ovule degeneration. In “Martell” and “Laimburg” small and rapid variations in the daily GDH accumulation could be found at the same time as the different Groups of detachment started to grow slower, but their low intensity was proportional to the low severity of late fruit drop.

These data lead to hypothesize temperature as the driving factor of many biological processes within the sweet cherry tree. However, it is still unclear whether the initial “switching off” of the fruitlet set was caused by either a prolonged period of temperatures below a certain physiological threshold or by a single negative peak.

In the scientific literature, many articles studied the effect of either high or low temperatures on the total fruit set of several sweet cherry cultivars; most of these works focused on stigma receptivity and ovule viability (Zhang, et al., 2018), ovule degeneration (Hedhly, et al., 2009), pollen germination and pollen tube growth (Milatovic, et al., 2017), and bee flight (Roversi, et al., 1996), but none of these studied in detail the effect on the physiological drop. However, our results are consistent to those published by

Vosnjak et al. (2022): in this very interesting work, sweet cherry trees cv. Grace star have been exposed to low temperatures without frost for two consecutive nights under field conditions at 36 DAFB; the average temperature of 2,4°C and 4,9°C were measured during the first and the second night of exposure, respectively. Very similar temperatures have been observed at the end of May in both "Fragsburg" and "Lengstein D." which at that time were at 36 and 34 DAFB, respectively. In this article, low temperatures significantly altered leaf physiological and biochemical parameters: exposure reduced leaf net photosynthesis, stomatal conductance, and total chlorophyll. Interestingly, a long-term negative effect has been measured: recovery took longer to the trees that were exposed twice. In this work the stress caused by low temperature was indicated as the possible cause of fruit drop. Fruit drop is a complex physiological process in which an abscission zone (AZ) at the base of the peduncle is activated. As already mentioned in the first chapter, the process is regulated by plant hormones such as auxins, abscisic acid, and ethylene. Blanusa et al. (2006) suggested that source limitation was the main factor inducing late-season fruit abscission: they reported that ABA concentration increased as sorbitol concentration declined in sweet cherry fruitlet prone to drop, showing how carbohydrates and plant hormones are strictly interconnected, and how nutritional deficiencies can potentially start the abscission process. From another point of view, it cannot be excluded that the lack of nutrition primarily leads to the abortion of the embryo and that the resulting lack of IAA flux originally produced by the seed may increase the ethylene sensitivity of the cells of the abscission zone (Askarieh et al., 2021).

The role of tree carbon balance and competition amongst organs in early apple fruit growth and drop has been widely investigated by Lakso (Lakso, et al., 1990); findings have been used to develop and validate a dynamic simulation model able to integrate variation induced by weather and carbohydrate supply : demand balances (Lakso, et al., 2015). Based on this model, fruit abscission can be enhanced if carbon supply is less than the total demand; in apple, carbon supply develops linearly with leaf area growth, but a critical period of carbon deficit can occur about 1-3 weeks after bloom when the maximum number of sinks are growing (large fruit number after initial fruit set, characterised by exponential growth) (Lakso, 2011). During this time, fruit abscission is sensitive to particular climatic conditions such as low light or high temperatures (especially during the night) able to reduce the net photosynthesis or increase the respiration rate, respectively (Lakso, et al. 2001). Sweet cherry carbohydrate balance has not yet been fully modelled, however, presented data, with due distinctions, seem to be consistent with the carbon deficiency-driven fruit drop hypothesis formulated for *Malus domestica*.

3.6 Conclusions

Late fruit drop in sweet cherry is a complex phenomenon probably determined by multiple factors. However, the idea of calculating the late drop intensity as a function of the number of the fruitlets set (n0) has allowed a better understanding of its evolution. Many physiological processes described in this chapter seemed to be temperature-driven: prolonged periods of low temperatures and/or sudden severe decreases in the daily Growing Degree Hours accumulation were followed by a reduction in the average growth rate of both extension shoots and fruitlets. Several weeks may pass from the time the AGR of a group of detachment turns significantly lower than that of the retained cherries, to the time of the actual drop; therefore the "late" fruit drop is nothing more than a late detachment of a fruit that has been "switched off" by the tree much earlier in the season. A recent publication (Vosnjak, et al., 2022) reported that exposure of sweet cherry trees to low temperatures without frost at 36 DAFB significantly reduced leaf net photosynthesis, stomatal conductance, and total chlorophyll content, which is consistent with our data.

Based on this experience, we can interpret late fruit drop as a self-regulation of crop load performed by the cherry tree in a situation of lack of resources, probably carbohydrates, determined by limiting climatic conditions. If this were true, it would explain why no intense fruit drop was observed at "Lengstein J." and "Vöran" despite the very low temperatures recorded in post bloom: in these two orchards the crop load had been already severely reduced by late frost, and this probably made the plant more resilient to further physiological stresses. Severe drops in the daily GDH accumulation, but much later in the season, have been observed also in the other orchards; since none of these was followed by a reduction of growth, the existence of a "sensitivity phase" could be assumed. Interestingly, the period in which the studied orchard responded to low temperatures by diminishing their crop load correspond to the time the plant transitions from being dependent on reserves to becoming photosynthetically self-sufficient (Ayala, et al., 2015).

Since girdling was not performed, carbohydrate contribution from other parts of the canopy cannot be excluded. However, the relative fruit set of the 1-year-old shoots seemed to be negatively influenced by a strong emission of extension shoots (either as an effect of pruning or because of high intrinsic vigor). This could be explained as an effect of competition for resources or because of the different contribution to fruit development of extension shoots and non-fruiting spurs (Ayala, et al., 2018).

Finally, the soil-, leaf-, and fruitlets analysis presented suggest that fruit drop is not linked to nutrient deficiencies.

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4 Chapter 4: Late fruit drop of ‘Regina’ sweet cherry (Prunus avium): a self-regulation of crop load under critical climatic conditions

4.1 Abstract

In the present work, the evolution of fruit set in two orchards was monitored twice a week from pre- or full bloom up to the end of late fruit drop, for a total of 17/19 samplings per each orchard. In total, 1113 flowers and 640 fruitlets were studied. As in the previous season, the late fruit drop intensity was based on the number of the fruitlets set prior to late drop (n_0). The evolution of the total fruit set followed a sigmoidal trend while that of the relative fruit set an expolinear one. Limb exposure (sunny vs. shady side of the canopy) significantly affected the total fruit set evolution over the time, probably by hastening or retarding the actual detachment of already dropping cherries; however, no significant effect was found on the total fruit set at harvest. The factor “exposure” did not affect the average shoot length at harvest but in “Fragsburg” the extension shoots belonging to the southern part of the canopy showed a more prolonged vegetative growth. The “Martell” orchard showed a lower linear density of new shoots per limb (*p-value = <,001*), a greater linear density of non-fruiting spurs (*p-value = <,001*), and an overall lower vigor than “Fragsburg”. Probably due to an overall lack of growth, no significant linear regression between the limb vigor and the relative fruit set was found in any location. In the season considered, late fruit drop intensity varied from 26% of the fruitlets set in “Martell” to the 41% of “Fragsburg”. As in the previous season, the Groups of detachment were used as fixed factors in the data analysis. Late fruit drop affected fully developed fruitlets which at some point decreased their growth rate and got senescent. As already observed in the previous season, both the vegetative and the reproductive growth seems to be temperature driven. For example, in “Martell” the growth rate of the extension shoots suddenly dropped between 16 and 22 DAFB: interestingly, at the same time, at least two minimum peaks of GDH accumulation can be found (145 GDH at 15 DAFB and 48 GDH at 19 DAFB). Interestingly, between 14 and 16 DAFB fruitlets belonging to “Group of detachment” n° 7 showed a lower growth rate than the retained ones despite a similar diameter, a sign that, until shortly before, their AGR values were the same. Furthermore, at the same time, cherries of the “Group of detachment” n° 8 started to reduce their growth rate until they reached a negative value at 29 DAFB. The last minimum peak of GDH accumulation was measured exactly between 27 and 33 DAFB (172 GDH at 28 DAFB). In “Fragsburg” a sharp decline of the growth rate of both retained fruitlets (from 0,81 mm/day to 0,25 mm/day) and extension shoots (from 1,24 cm/day to 0,70 cm/day) was observed between 26 and 31 DAFB. This appeared to be concomitant with the time when the maximum temperature measured outside the orchard approached 30°C (29,1°C

at 26 DAFB, on 12 May). Furthermore, between 31 and 38 DAFB most “Groups of detachment” showed significantly lower AGR values than retained cherries and started to be senescent; at 35 DAFB the maximum temperature reached outside the orchard was 32,15°C. Based on these considerations, sweet cherry late fruit drop appears as a self-regulation of crop load performed by the cherry tree in a situation where the climatic conditions are limiting.

4.2 Introduction

In year 2021, the orchards “Fragsburg” and “Martell” showed opposite trends: “Fragsburg” (705 m a.s.l.) was characterized by early phenology and intense late fruit drop while “Martell” (1135 m a.s.l.) by late phenology and poor late fruit drop; in both orchards, promoting vegetative growth by pruning the 1-year-old limbs in the spring significantly increased late fruit drop intensity. Aim of the 2022 trial was to repeat the same samplings performed in 2021 but with higher frequency (every 3/4 days) in order to confirm or refute the previously formulated hypothesis. In addition to this, 2022 trial took into account also the exposure of the limb to light (sunny vs. shady side of the canopy). Finally, to investigate the possible interdependence between fruit set and the vegetative growth of the extension shoots, 50% of the latter have been pinched immediately after flowering.

Research hypothesis: late fruit drop of ‘Regina’ sweet cherry is linked to limiting climatic conditions.

4.3 Materials and methods

The study was carried out in 2022 in two sweet cherry orchards located at different elevations in South Tyrol, Italy; the same trees were also used in the 2021 trial. Each orchard had ‘Kordia’ as the main cultivar and ‘Regina’ as pollinizer; each orchard was covered both by an anti-hail net and a plastic anti-rain film and surrounded by anti-insect nets. The plant distance was about 1,5-1,8 m between the trees and 3,5 m between the rows, plant density was about 1714-1428 trees/ha.

In each orchard 8 trees with similar trunk diameter and vigor were identified. All the trees studied were in full production (about 10-year-old), trained as central leader and grafted on the dwarfing rootstock ‘Gisela 5’. The orchards were managed according to integrated cultural practices in terms of fertilization, irrigation, plant defense, and pruning. An exhaustive summary of the agronomic practices recommended by the local agricultural extension specialist (https://www.beratungsring.org) can be found in the official guidelines published annually (https://www.beratungsring.org/info/organisation/broschueren/kirsche.html). No thinning and no plant growth regulators were applied during the trial. Full bloom occurred on April 16 and May 10, while harvest was on July 11 and August 8 (the tree tops were harvested on August 1) for “Fragsburg” and “Martell”, respectively.

Each orchard was provided with a weather station, the elevation was recorded using GeoBrowser Maps (https://maps.civis.bz.it). At pre- and full bloom frost damages on 500 flowers per cv. were measured. The phenological stages were characterized using the BBCH scale (Fadon, et al., 2015).

Prior to flowering, 4 limbs per tree were tagged: two 1-year-old shoots with basal flower buds on the sunny side of the crown (south/south-east), and two 1-year-old shoots with basal flower buds on the shady side (north/north-west), all located in the middle part of the crown (average height 2m). “Fragsburg” rows had an east-west orientation, while the “Martell” orchard was realized with northeast – southwest oriented rows.

FLOWER AND FRUIT COUNTING, FRUIT SET

The number of flowers or fruitlets on each tagged limb was counted twice a week from pre- or full bloom up to the end of late fruit drop, for a total of 17/19 samplings per each orchard. Fruits with symptoms of abscission were counted until natural detachment occurred.

At full bloom, fruiting limbs length was measured.

SEASONAL FRUIT GROWTH

About two weeks after full bloom, as soon as it was possible to distinguish adhering fruitlets from unfertilized ones, 10 fruits (if present) without any evidence of abscission were tagged on each limb using a progressive number from 1 to 320. Overall, 40 fruits per tree and 80 fruits for each limb type were tagged. Starting from the same day, these fruits were calibrated twice a week using a digital Bluetooth caliper (T. R. Turoni, Forlì – Italia) up to the end of late fruit drop. Fruit growth rate (both positive and negative) was expressed as absolute size increment in the unit of time (AGR). The following equation, as reported by Morandi et al. (2019), was used:

$$AGR = \frac{d_{t1} - d_{t0}}{t_1 - t_0}$$

Where *d* is the fruit diameter in mm and *t* the time expressed as DAFB.

As soon as much of the first waves of fruit drop ceased, approximately at stage 77 as described by (Fadon, et al., 2015), the number of fruit set prior to late fruit drop (n_0) was collected.

The following parameters were considered:

Total fruit set was calculated for each limb as follows (Askarieh, et al., 2021):

$$Total\ fruit\ set\ \% = \frac{Fruit\ number\ at\ harvest\ time}{Total\ flowers\ number} * 100 \tag{1}$$

Relative fruit set (referred to n_0) was calculated for each limb as follows:

$$Relative\ fruit\ set\ \% = \frac{Fruit\ number\ at\ harvest\ time}{Fruit\ number\ at\ n0} * 100 \tag{2}$$

Intensity of late fruit drop was calculated for each limb as follows:

$$Intensity\ of\ late\ fruit\ drop\ \% = (1 - Relative\ fruit\ set\ \%) \tag{3}$$

Fruit measurements were performed in the morning.

SEASONAL SHOOT GROWTH AND PINCHING

As soon as vegetative buds started to grow (about full bloom), the newly formed extension shoots on the tagged limbs were counted and measured twice a week; about 14 samplings were performed for each orchard until the end of late fruit drop. To investigate the possible interdependence between the growth of fruitlets and extension shoots, at about 22/23 DAFB depending on the location, on half of the tagged limbs (hence, one on each side of the canopy), all the shoots longer than 4 cm were pinched (i.e., the apical meristem was removed).

GROWING DEGREE HOURS

Daily cumulative Growing Degree Hours (GDH) were calculated from full bloom using an asymmetric curvilinear model as reported by Azarenko et al. (2008). Between 4°C (base temperature) and 25°C (optimum), the following formula was used:

$$GDH = \left[\frac{25°C - 4°C}{2}\right] * (1 + \cos\left(\pi + \frac{\pi(hourly\ temperature - 4°C)}{25°C - 4°C}\right)) \tag{4}$$

For temperature above optimum, the critical temperature of 36°C was considered:

$$GDH = (25°C - 4°C) * (1 + \cos(\frac{\pi}{2} + \frac{\pi}{2} * \frac{hourly\ temperature - 25°C}{36°C - 25°C})) \tag{4}$$

HARVEST YIELD AND FINAL SAMPLING

At commercial harvest, total fruit weight of each limb and trunk cross-sectional area (TCSA, 20 cm above the grafting point) were measured. Each tree was harvested separately. At the same time, the last fruit counting, the last calibration, and the last measurement of shoot length were performed. The yields per tree reported for “Martell” consist of the sum of the two harvest (01.08 and 08.08).

STATISTICAL ANALYSIS

All the flowers/fruits counts were elaborated using the General Mixed Model (GMM) with Repeated Measures (RM) procedure in IBM SPSS Statistics 27; within the model the tree was selected as the random factor and the limb as the subject repeated at each sampling time. The same approach but having the limb as the random factor and the single fruit as the subject repeated was used for the calibrations. The covariance type (COVTYPE) of the RM was chosen using the “Aikaike Information Criterion” (AIC). The GMM was also used to study the vegetative growth (with the tree as the random factor). Estimated Marginal Means (EMMs) were compared using pairwise multiple comparison with Sidak adjustment. The more common Tukey test has been discarded since it leads to inflated alpha level when the sphericity assumption is not met (and mixed models don’t require the sphericity assumption), and therefore it is unsuitable for performing pairwise comparisons in a repeated measures design (Scott, 1980). Of the most frequently tests used with these models, LSD was discarded since it is vulnerable to Type I errors, and Bonferroni to Type II errors. Linear Regression analysis was used having the relative fruit set at harvest as dependent variable. Effect was considered significant at $p \leq 0.05$.

ABBREVIATIONS

DAFB = Days after full bloom;
GMMRM = General Mixed Models with Repeated Measures;
EMMs = Estimated Marginal Means;
AGR = Absolute Growth Rate;
GDH = Growing Degree Hours;
ASL = Average Shoot Length;
s.e. = standard error.

4.4 Results

Location	**Elevation**	**Full bloom**	**Harvest**	**% Frost damaged flowers**
Fragsburg	705 m a.s.l.	16.04.2022	11.07.2022	0%
Martell	1135 m a.s.l.	10.05.2022	08.08.2022*	20%

Table 1: name of the locality, elevation, date of full bloom and harvest, and percentage of frost damaged flowers of the orchards involved in the study. (*) = tree tops were harvested on August 1.

4.4.1 Flowers and fruits counting, fruit set

Orchard	**p-value "Exposure"**	**p-value "Pinching"**	**p-value "Sampling"**	**p-value "Exposure * Pinching"**	**p-value "Exposure * Sampling"**	**p-value "Pinching * Sampling"**	**p-value "Exposure * Pinching * Sampling"**
Fragsburg	**<.001**	.959	**<.001**	.750	**<.001**	.182	.998
Martell	**.021**	.472	**<.001**	.132	**<.001**	.988	.092

Table 2: p-values for the different factors considered obtained as output of the Mixed Model with Repeated Measures having the % of total fruit set as the dependent variable. Bold p-values are considered significant at α ≤ 0,05.

Table 2 reports the output of Type III Tests of Fixed Effect after the Mixed Model with Repeated Measures procedure in which the subject was the limb. This analysis studied the effect of different factors on the evolution of total fruit set of the 1-year-old shoots during the season from full bloom to harvest (calculated using the formula (1)). Each orchard was analyzed separately. The factors considered were the "Exposure" of the limb to the sun (sunny – vs. shady side of the canopy), the "Pinching" (removal of the apical meristem of the extension shoots on 50% of the tagged limbs) and the day of "Sampling" (i.e., the evolution over the time); the interactions between these factors were also studied. For both orchards the model returned the factor time of "Sampling" and "Exposure", as well as their interaction, as significant. Figure 1 represents the evolution of fruit set both referred to the number of flowers at FB (blue line, calculated using eq. 1) and to the number of fruits at n0 before the beginning of late fruit drop (orange line, calculated using eq. 2). Each point represents the estimated marginal mean (EMM) of total and relative fruit set referred to a specific sampling day expressed in days after full bloom (DAFB). The total fruit set initially showed a stationary phase of different duration depending on the location (23 days for "Fragsburg" and 7 days for "Martell") followed by a drastic reduction. As soon as most of the unfertilized fruitlets detached, the “n0” stage was determined (i.e., the number of drupelets set before the late fruit drop phase). The late fruit drop intensity was calculated based on this number. Final total fruit set was reached at different times between 44 and 48 DAFB depending on the orchard considered. The total fruit set at harvest varied between 21% and 36%, while the relative fruit set varied between 59% and 74% (for “Fragsburg” and “Martell” respectively).

Figure 2 represents the effect of the interaction “Exposure * Sampling” on total fruit set. Interestingly, in both orchards the orientation of the limb showed no significant effect neither during the early- (during the bloom) nor at the late stages of fruit ripening but only in the central phase during the initial fruit set. No significant difference between the total fruit set at the different exposures was found at harvest.

Furthermore, no significant effect of “Pinching” on the total fruit set was detected at both locations.

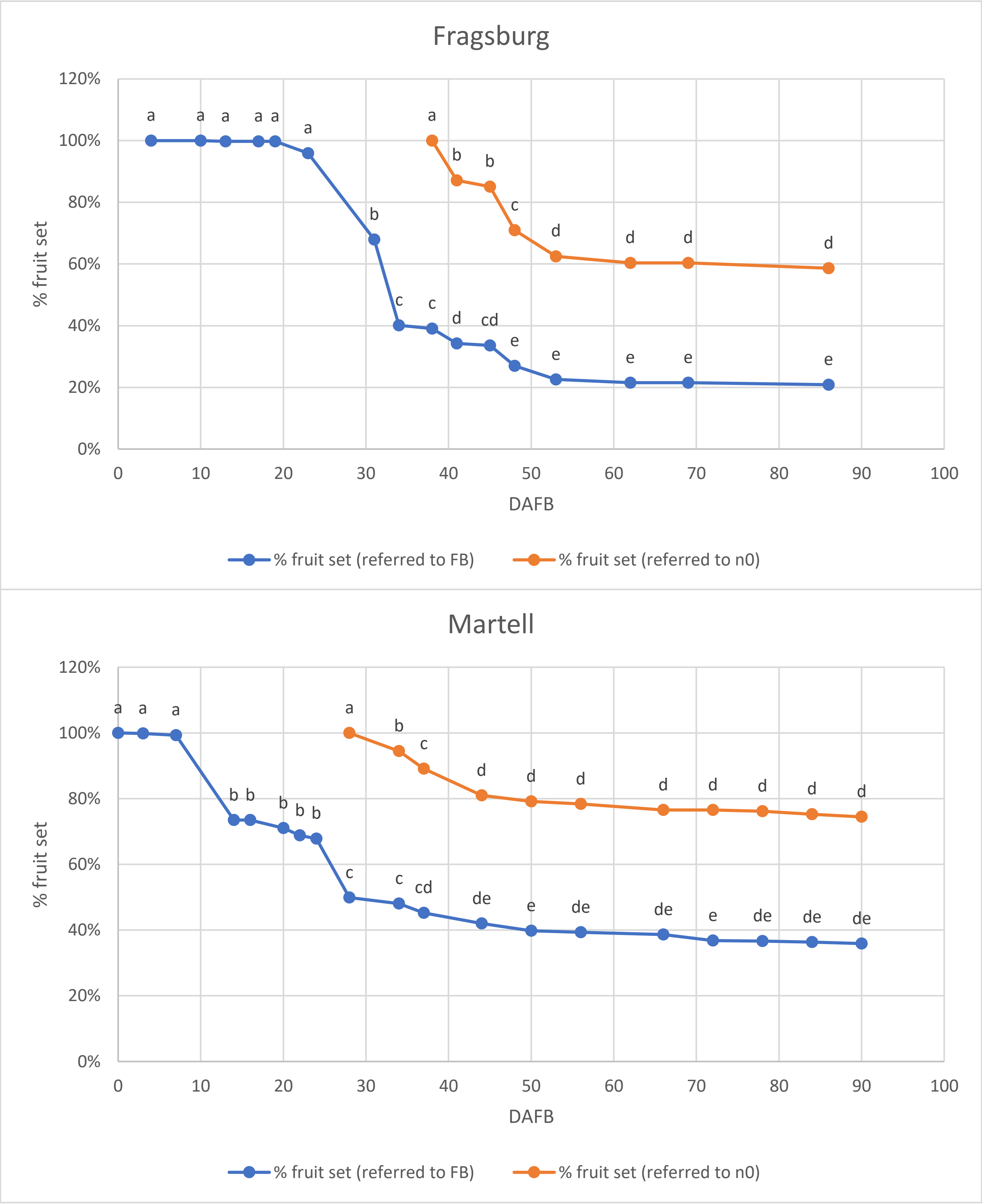

Figure 1: evolution of the total- (referred to FB) and the relative (referred to n0) fruit set over the time expressed as Days After Full Bloom. Percentages are referred to the day of sampling. Means followed by the same letter are not significantly different at α ≤ 0,05. For every orchard, each point represents the average of the % fruit set of 32 limbs tagged on 8 trees.

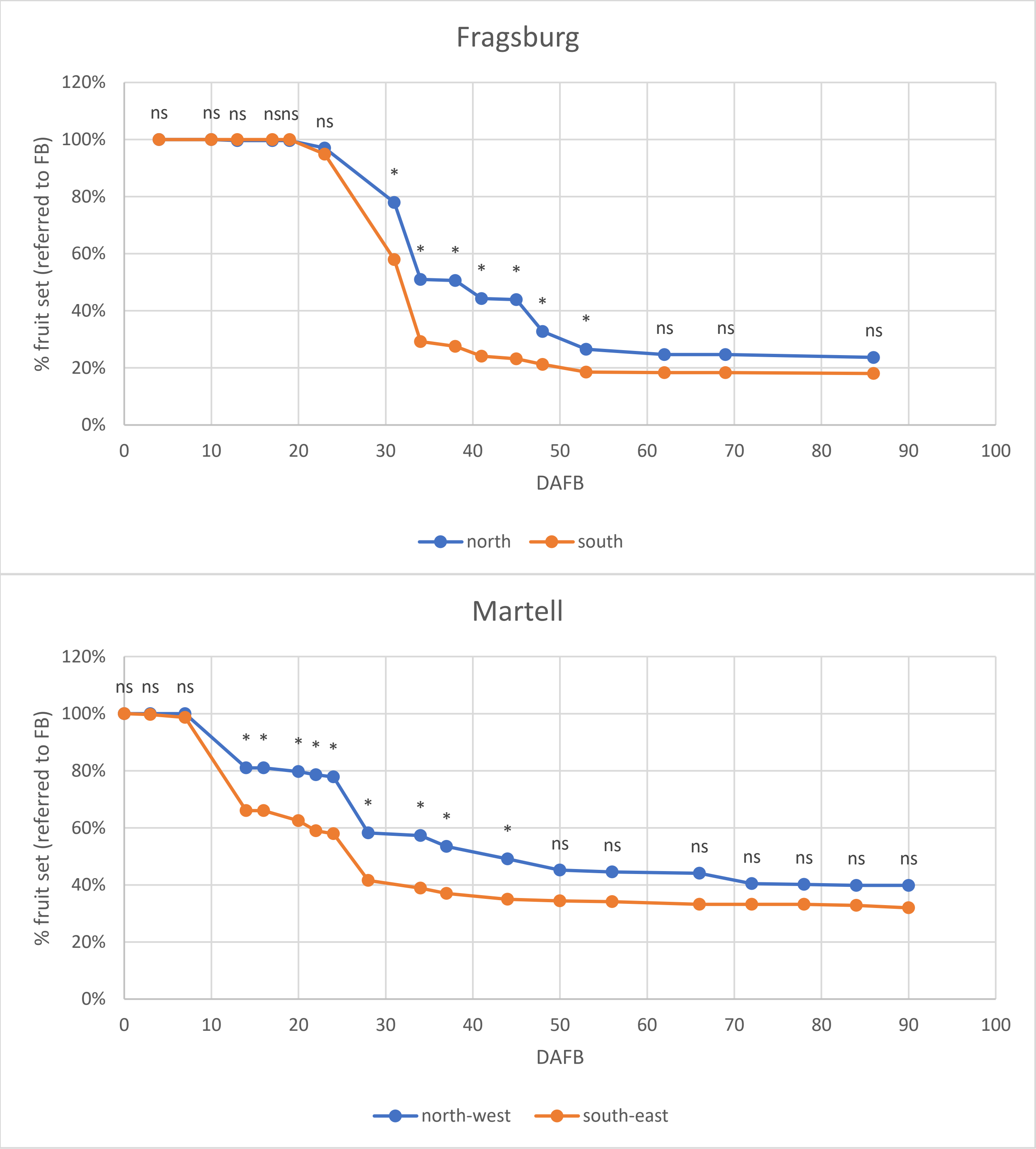

*Figure 2: effect of the interaction "Exposure * Sampling" on the evolution of the total fruit set (eq. 1, referred to FB) over the time expressed as Days After Full Bloom. ns/*: not significant/significant "Exposure * Sampling" at α ≤ 0,05.*

Orchard	**p-value "Exposure"**	**p-value "Pinching"**	**p-value "Sampling"**	**p-value "Exposure * Pinching"**	**p-value "Exposure * Sampling"**	**p-value "Pinching * Sampling"**	**p-value "Exposure * Pinching * Sampling"**
Fragsburg	**.028**	.286	**<.001**	.917	**<.001**	.359	.305
Martell	.548	.226	**<.001**	.877	.534	.452	.999

Table 3: p-values for the different factors considered obtained as output of the Mixed Model with Repeated Measures having the % of relative fruit set (eq. 2) as the dependent variable. Bold p-values are considered significant at α ≤ 0,05.

Table 3 summarizes the effect of the different factors considered on the evolution of relative fruit set (referred to n0, calculated using the formula (2)) during the last part of the season. As expected, since the % of relative fruit set changes over time, the factor "Sampling" was found significant in both orchards. The factor "Exposure" and its interaction with the factor "Sampling" was significant only in the "Fragsburg" orchard; the effect of the interaction "Exposure * Sampling" on the relative fruit set is represented in Figure 3: here, after an initial overlapping of the two curves, from 48 DAFB until harvest the limbs with a southern exposure showed a significantly higher relative fruit set (i.e., a significantly lower late fruit drop). No effect of "Pinching" or its interactions with other factors was found to be significant.

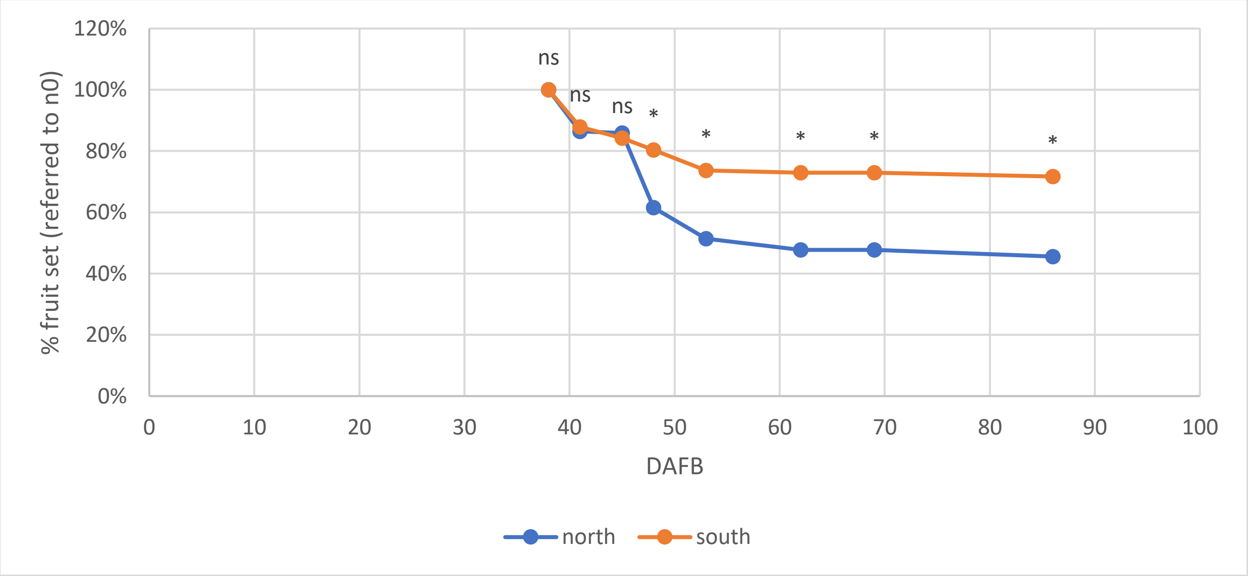

*Figure 3: effect of the interaction "Exposure * Sampling" on the evolution of the relative fruit set (eq. 2, referred to n0) over the time expressed as Days After Full Bloom in "Fragsburg". ns/*: not significant/significant "Exposure * Sampling" α ≤ 0,05.*

4.4.2 Vegetative parameters

Orchard	p-value “Exposure”	p-value “Pinching”	p-value “Sampling”	p-value “Exposure * Pinching”	p-value “Exposure * Sampling”	p-value “Pinching * Sampling”	p-value “Exposure * Pinching * Sampling”
Fragsburg	.946	**<.001**	**<.001**	.938	**.004**	**<.001**	.904
Martell	.983	.452	**<.001**	.215	.879	**<.001**	.072

Table 4: p-values for the different factors considered obtained as output of the Mixed Model with Repeated Measures having the average shoot length (ASL), calculated as the average length of all the extension shoots per limb (spurs excluded), as the dependent variable. Bold p-values are considered significant at α ≤ 0,05.

Table 4 summarizes the effect of the factors considered on the average shoot length calculated as the average length of all the extension shoots of the limb; any new growth shorter than 4 cm was considered a spur and therefore excluded. The factor “Sampling” was found to be highly significant in both orchards. The “Pinching” consisted in removing the apical meristem of the extension shoots as soon as they became clearly distinguishable from the spurs; this was done at 23 DAFB in the “Fragsburg” orchard and at 22 DAFB in the “Martell” one. Despite the pinching was performed at a similar time in both locations, shoot growth kinetics at the time of the manipulation was very different: in “Fragsburg” “Pinching” affected shoots that were showing an intense exponential growth, while in “Martell” they were already reducing their growth rate approaching a plateau-like phase. Furthermore, in the season considered the two orchards showed very different vigor, with “Fragsburg” having at terminal bud set an average shoot length per limb of 26,46 cm and “Martell” only 11,12 cm. These elements should be taken into account in the comprehension of Figure 4: in “Fragsburg” the “Pinching” stopped the growth of the shoots only temporarily, and these were able to recover (i.e., showing AGR values comparable to the not pinched ones) late in the season at about 45, 53 and 62 DAFB; on the contrary, in “Martell” pinched shoots completely stopped growing but, since the intact ones were already showing very low growth rates, unlike “Fragsburg”, no significant difference in ASL was found at terminal bud set between the two treatments.

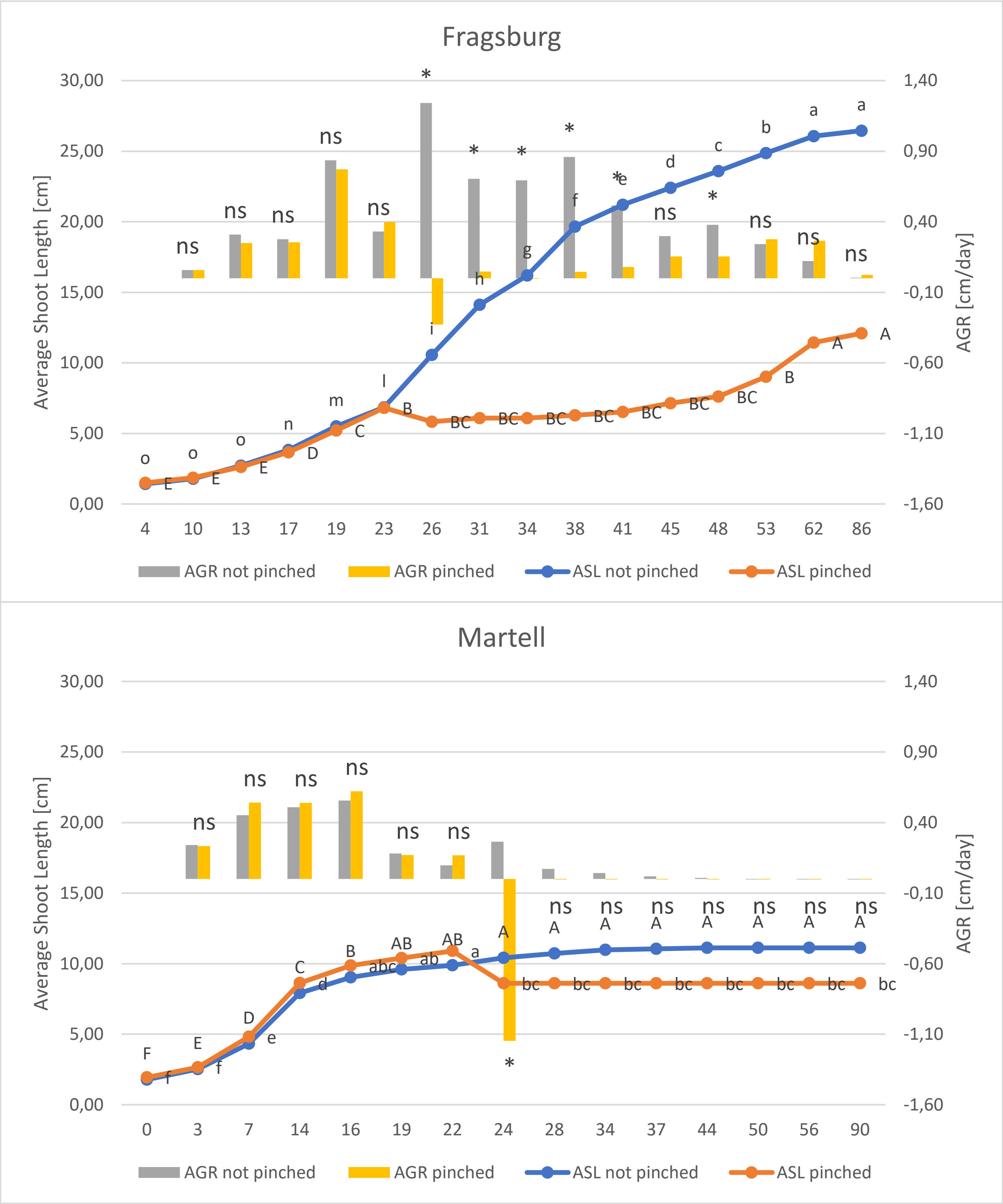

Figure 4: evolution of the average shoot length (ASL) over the time expressed as Days After Full Bloom. ASL are referred to the day of sampling. ns/: not significant/significant "Pinching * Sampling" at α ≤ 0,05 (dependent variable: AGR). Lowercase letters: "Sampling * Pinching" referred to "pinched", uppercase letters "Sampling * Pinching" referred to "not pinched" (dependent variable ASL); means followed by the same letter are not significantly different at α ≤ 0,05. For every orchard, each point represents the average length of all the shoots borne on 32 limbs tagged on 8 trees.*

The Mixed Models analysis summarized in Table 4 returned the interaction “Exposure * Sampling” as significant for the “Fragsburg” location: the EMMs are reported in Figure 5. The average shoot length at terminal bud set was not statistically different, but the extension shoots belonging to the southern part of the canopy showed a more prolonged vegetative growth. This tendency was not found in “Martell”.

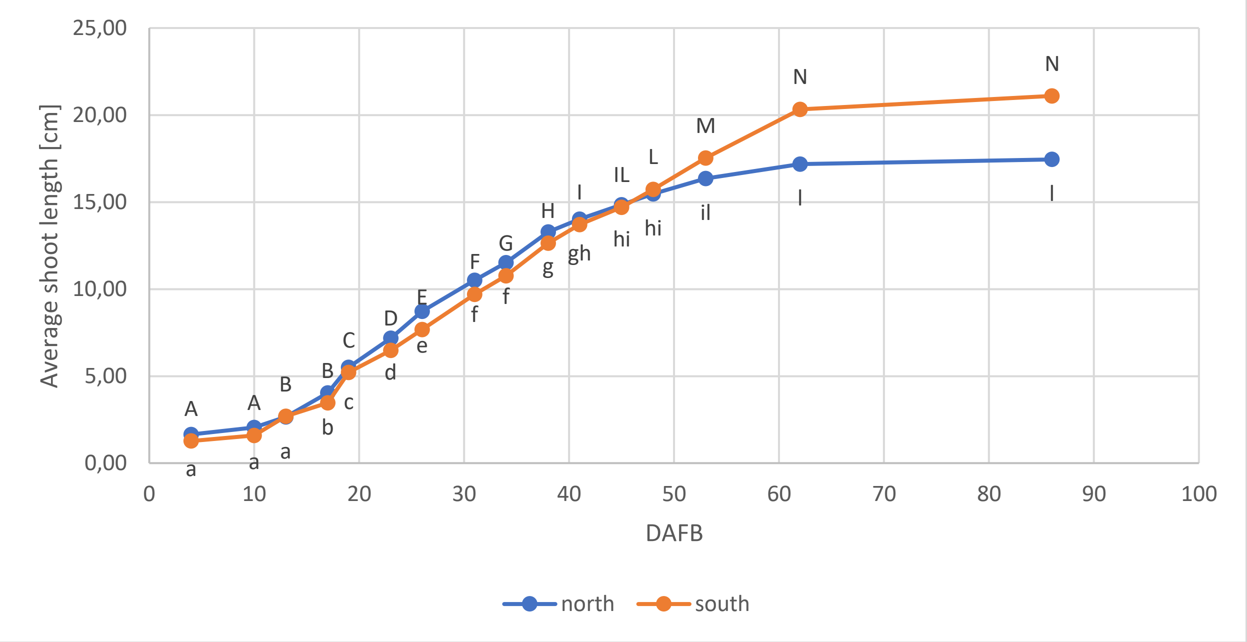

*Figure 5: effect of the interaction “Exposure * Sampling” on the average shoot length (ASL) over the time expressed as Days After Full Bloom in “Fragsburg”. “Exposure * Sampling” was not significant on any sampling day. Lowercase letters: “Sampling * Exposure” referred to “north”, uppercase letters “Sampling * Exposure” referred to “south”; means followed by the same letter are not significantly different at α ≤ 0,05.*

	EMMs Limb length [cm]	**n° of new shoots [-]**	**n° of non-fruiting spurs [-]**	**new shoots density per limb [n°/m]**	**non-fruiting spurs density [n°/m]**
Fragsburg	42	2,88	4,50	6,83	11,00
Martell	29	0,94	4,78	3,74	15,37
p-value	**<,001**	**<,001**	0,71	**<,001**	**<,001**

Table 5: effect of the factor “Orchard” on the length of the limbs (at FB), on the number of new extension shoots per limb [-] and their linear density [-/m] (at terminal bud set), and on the number of non-fruiting spurs [-] and their linear density [-/m] (at terminal bud set). The estimated marginal means are reported individually for each site. Bold p-values are considered significant at α ≤ 0,05.

Similar to the previous season, the ratio between the sum of the length of new shoots at terminal bud set and the limb length (from which they originated) at full bloom was studied as a potential predictor of relative fruit set at harvest (referred to n0). In 2022, no significant effect of the predictor was observed in any location. The results are summarized in Table 6.

Orchard	p-value	Shoots length/Limb length [-]
Fragsburg	.244	1.57
Martell	.271	.07

Table 6: p-values of the ratio between the sum of the length of new shoots at terminal bud set and the limb length (from which they originated) at full bloom [m/m] interpreted as predictor in a linear regression analysis having the final fruit set as dependent variable.

4.4.3 Seasonal fruit growth

At petal fall, i.e., as soon as it was possible, 320 drupelets were individually labelled with a progressive unique number in each orchard (10 each limb). From this point onwards they were calibrated twice a week until the end of late fruit drop (which always coincided with the beginning of veraison). As in the previous season (Chapter 3), during the data analysis labelled fruits of each location were grouped according to their time of detachment (from here on called "Group of detachment"); table 7 summarizes the time of detachment of each group and its relevance in terms of % of the tagged fruitlets. The group "Harvested" includes the cherries that reached commercial harvest without dropping. Obviously, the "Time of detachment" coincided with the first day of sampling after the actual detachment.

FRAGSBURG			**MARTELL**		
Time of detachment	**Group n°**	**% of the tagged fruitlets**	**Time of detachment**	**Group n°**	**% of the tagged fruitlets**
20 – 23 DAFB	1	1,30 %	14 – 16 DAFB	1	0,00 %
23 – 26 DAFB	2	1,95 %	16 – 20 DAFB	2	0,00 %
26 – 31 DAFB	3	8,77 %	20 – 22 DAFB	3	0,72 %
31 – 38 DAFB	4	31,17 %	22 – 24 DAFB	4	1,08 %
38 – 41 DAFB	5	1,62 %	24 – 29 DAFB	5	18,35 %
41 – 45 DAFB	6	7,47 %	29 – 34 DAFB	6	2,16 %
45 – 48 DAFB	7	7,79 %	34 – 37 DAFB	7	5,04 %
48 – 53 DAFB	8	4,55 %	37 – 44 DAFB	8	4,32 %
53 – 62 DAFB	9	2,60 %	44 – 50 DAFB	9	0,72 %
62 – 69 DAFB	10	0,00 %	50 – 56 DAFB	10	0,72 %
69 – 73 DAFB	11	0,65 %	56 – 66 DAFB	11	1,08 %
73 – 86 DAFB	12	0,32 %	66 – 72 DAFB	12	0,00 %
			72 – 78 DAFB	13	0,00 %
			78 – 84 DAFB	14	0,72 %
			84 – 90 DAFB	15	0,72 %
HARVESTED	13	31,82 %	HARVESTED	16	64,37 %

Table 7: percentage of cherries detached in each time interval considered based on the total number of cherries tagged (about 320 in each orchard).

During the data analysis, once having virtually tagged every cherry with its “Group of detachment”, the latter was used as fixed factor in the Mixed Model analysis with Repeated Measures. Every orchard was analyzed separately. In this way, the diameter evolution of each wave of fruit drop could be plotted individually over the time as shown in Figures 6 and 7. Overall, at the first day of calibration (on 20 and 14 DAFB for “Fragsburg” and “Martell”, respectively) a substantial percentage of fruits already showed a diameter significantly smaller than the cherries that would reach commercial harvest: at this time (about shuck split) these fruits had already stopped growing and were slowly getting senescent. These first waves of fruit drop in the “Fragsburg” location involved Groups n° 3 and 4, while in the “Martell” location only Group n° 5. This first phase of fruit drop should not be confused with the late fruit drop that is the focus of this study. In this work, only the drupelets that at the first calibration showed a diameter not significantly different from that of the retained ones (i.e. Group “Harvested”) and which subsequently detached were considered part of the “late fruit drop” of ‘Regina’. Therefore, the proper late fruit drop consisted of Groups n° 6, 7, 8 and 9 in “Fragsburg” and of Groups n° 7 and 8 in “Martell”. Groups with a sample size lower than 2% have been excluded from the statistical analysis as well as from the graphical representation. It is worth noting that the more frequent sampling than in the previous season (every 3/4 days instead of once a week) by increasing the number of potential “Groups of detachment” at the same time decreased their consistency and thus the chance of being included in the statistical analysis. However, this affected only minor groups and not the main waves.

Orchard	**p-value “Exposure”**	**p-value “Pinching”**	**p-value “Sampling”**	**p-value “Exposure * Pinching”**	**p-value “Exposure * Sampling”**	**p-value “Pinching * Sampling”**	**p-value “Exposure * Pinching * Sampling”**
Fragsburg	**<.001**	.631	**<.001**	.289	**<.001**	.880	.706
Martell	**<.001**	.325	**<.001**	.836	**<.001**	.922	.312

Table 8: p-values for the different factors considered obtained as output of the Mixed Model with Repeated Measures having the fruit diameter [mm] as the dependent variable. Bold p-values are considered significant at α ≤ 0,05.

Tables 8 summarizes the effect of the factors considered on the fruit growth: the factors time of “Sampling”, “Exposure”, and their interaction were found to be highly significant for both “Fragsburg” and “Martell”; no effect of the “Pinching” was detected. Interestingly, in both orchards, fruits of the southern side of the canopy showed significantly larger diameters than those of the northern side; this effect was detectable throughout the season but not at harvest, when both categories showed the same fruit size (Figure 8).

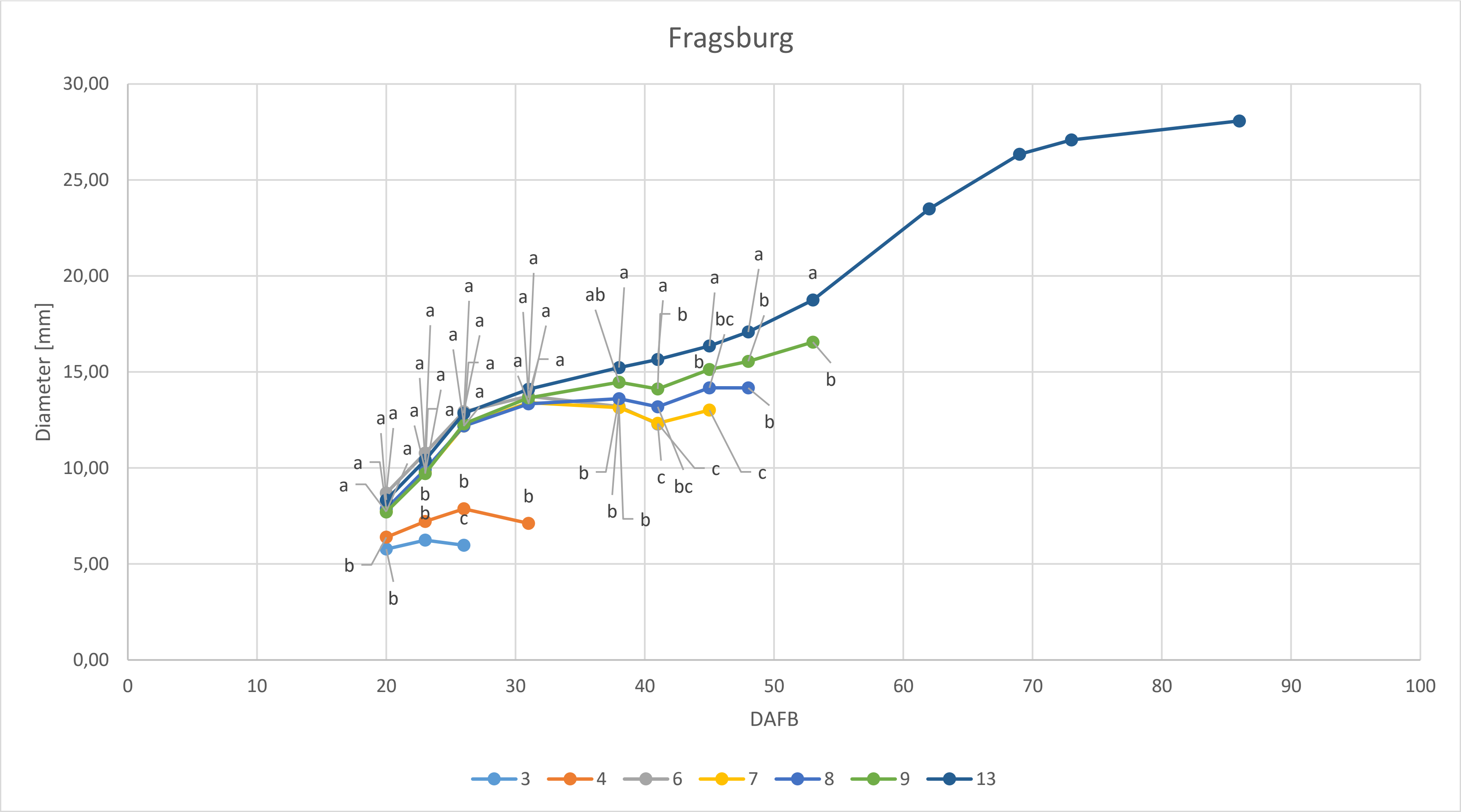

*Figure 6: Measurements of 'Regina' sweet cherry fruit growth in "Fragsburg"; diameter increase over the time (expressed as DAFB) is reported individually for each Group of detachment. The numbers of the legend refer to the Groups of detachment shown in Table 7. Fruit diameter of different "Groups of detachment" in the interaction "Group of detachment * Sampling" followed by the same lowercase letter are not significantly different at α ≤ 0,05 within the day of sampling. Groups with a sample size lower than 2% have been excluded from both the diagram and from the statistical analysis. 320 fruitlets have been tagged and measured in each orchard until natural detachment or harvest.*

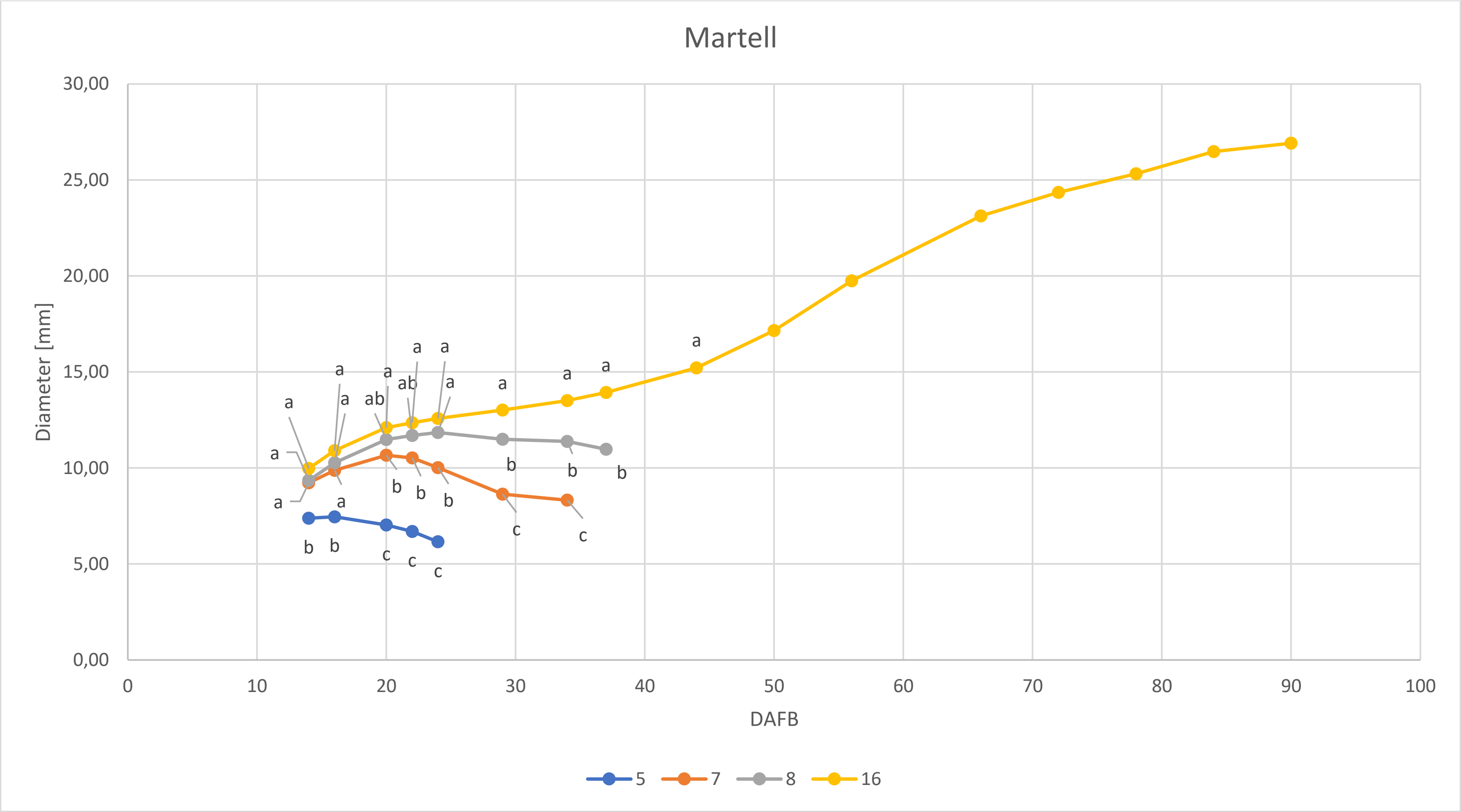

*Figure 7: Measurements of 'Regina' sweet cherry fruit growth in "Martell"; diameter increase over the time (expressed as DAFB) is reported individually for each Group of detachment. The numbers of the legend refer to the Groups of detachment shown in Table 7. Fruit diameter of different "Groups of detachment" in the interaction "Group of detachment * Sampling" followed by the same lowercase letter are not significantly different at α ≤ 0,05 within the day of sampling. Groups with a sample size lower than 2% have been excluded from both the diagram and from the statistical analysis. 320 fruitlets have been tagged and measured in each orchard until natural detachment or harvest.*

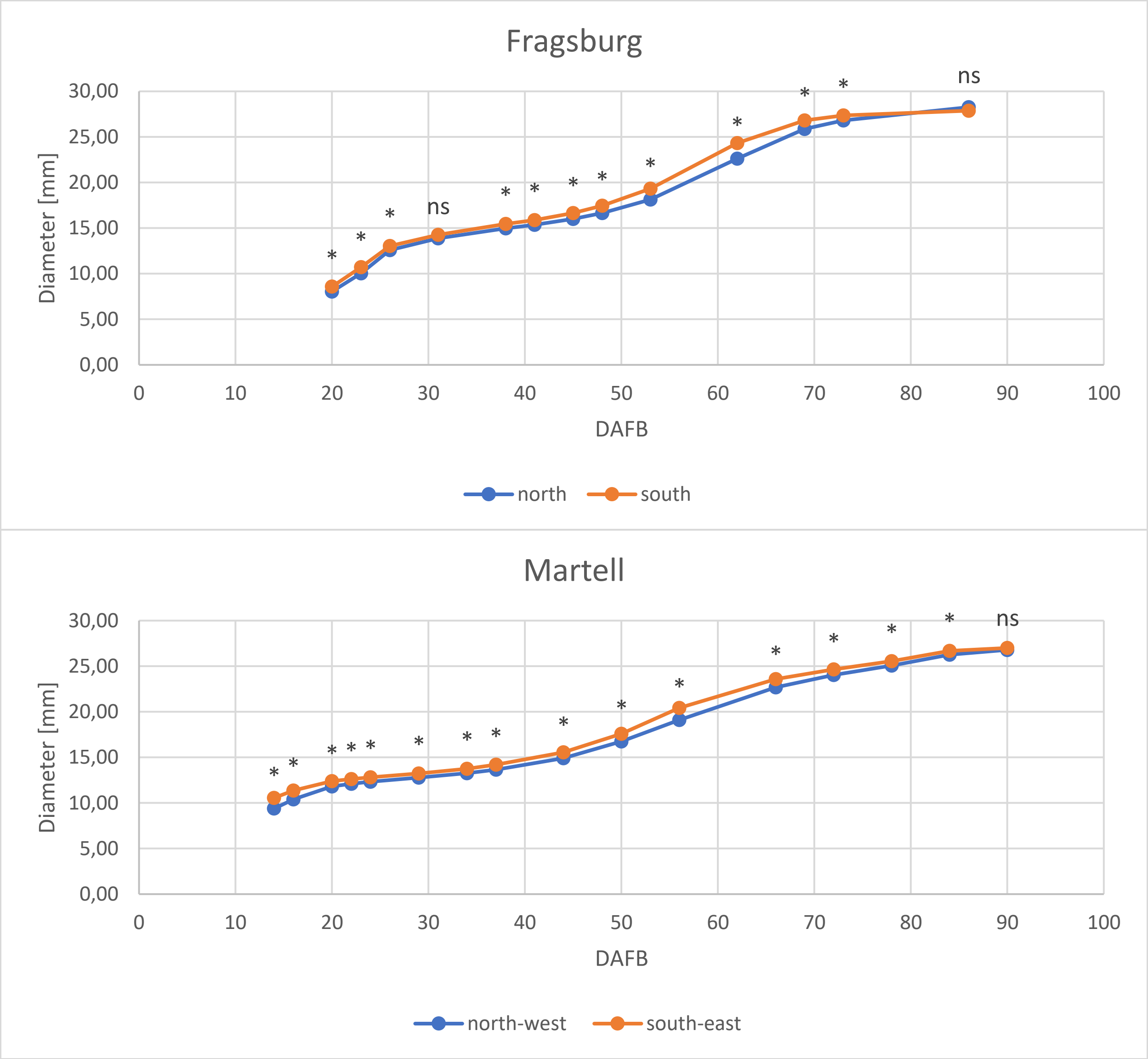

Figure 8: fruit growth over time expressed as Days After Full Bloom. ns/: not significant/significant "Exposure * Sampling" at α ≤ 0,05. For every orchard 320 drupelets have been tagged but the diagram only refers to the retained ones.*

Figure 9 represents the AGR values of the tagged fruits calculated for each "Group of detachment" and each orchard separately. As in the previous season, this way of representing growth data allowed us to estimate with good accuracy the moment when a given "Group of detachment" started to grow differently from the group that would reach harvest. For example, in the "Fragsburg" orchard Groups n° 6, 7, 8, and 9 exhibited an average growth rate similar to Group n° 13 at 23, 26 and 31 DAFB, but between 31 and 38 DAFB Groups n° 6, 7 and 8 started to show significantly lower AGR values. During the same period, Group n° 9 AGR was already characterized by lower absolute values, but the first significant difference was displayed at 41 DAFB. In “Martell”, Group n° 7 growth rate was significantly lower than that of Group n°16 (“Harvested”) already between the first two samplings (14 and 16 DAFB); on the contrary, Group n° 8 showed identical AGR values to Group n°16 up to 20 DAFB, but from that point onwards it progressively reduced its absolute value until it became significantly different (and negative) at 29 DAFB.

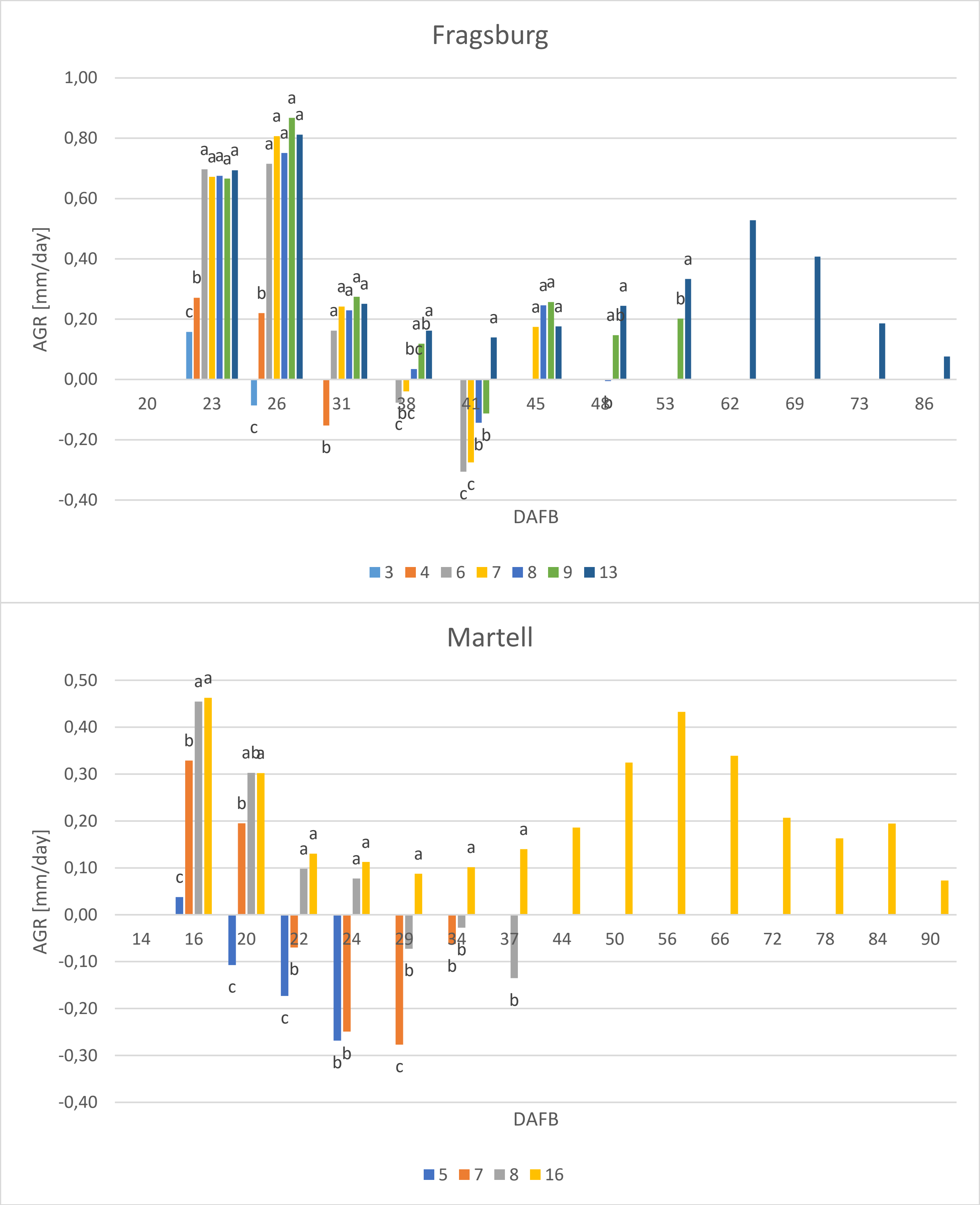

*Figure 9: Measurements of 'Regina' sweet cherry fruit absolute growth rate (AGR) in "Fragsburg" and "Martell"; AGR evolution over the time (expressed as DAFB) is reported individually for each group of detachment. The numbers of the legend refer to the Groups of detachment shown in Table 7. Estimated marginal means of AGR in the interaction "Group of detachment * Sampling" followed by the same lowercase letter are not significantly different at α ≤ 0,05 within the day of sampling. Groups with a sample size lower than 2% have been excluded from both the diagram and from the statistical analysis. 320 fruitlets have been tagged and measured in each orchard until natural detachment/harvest.*

4.4.4 Meteorological data of 2021 and 2022

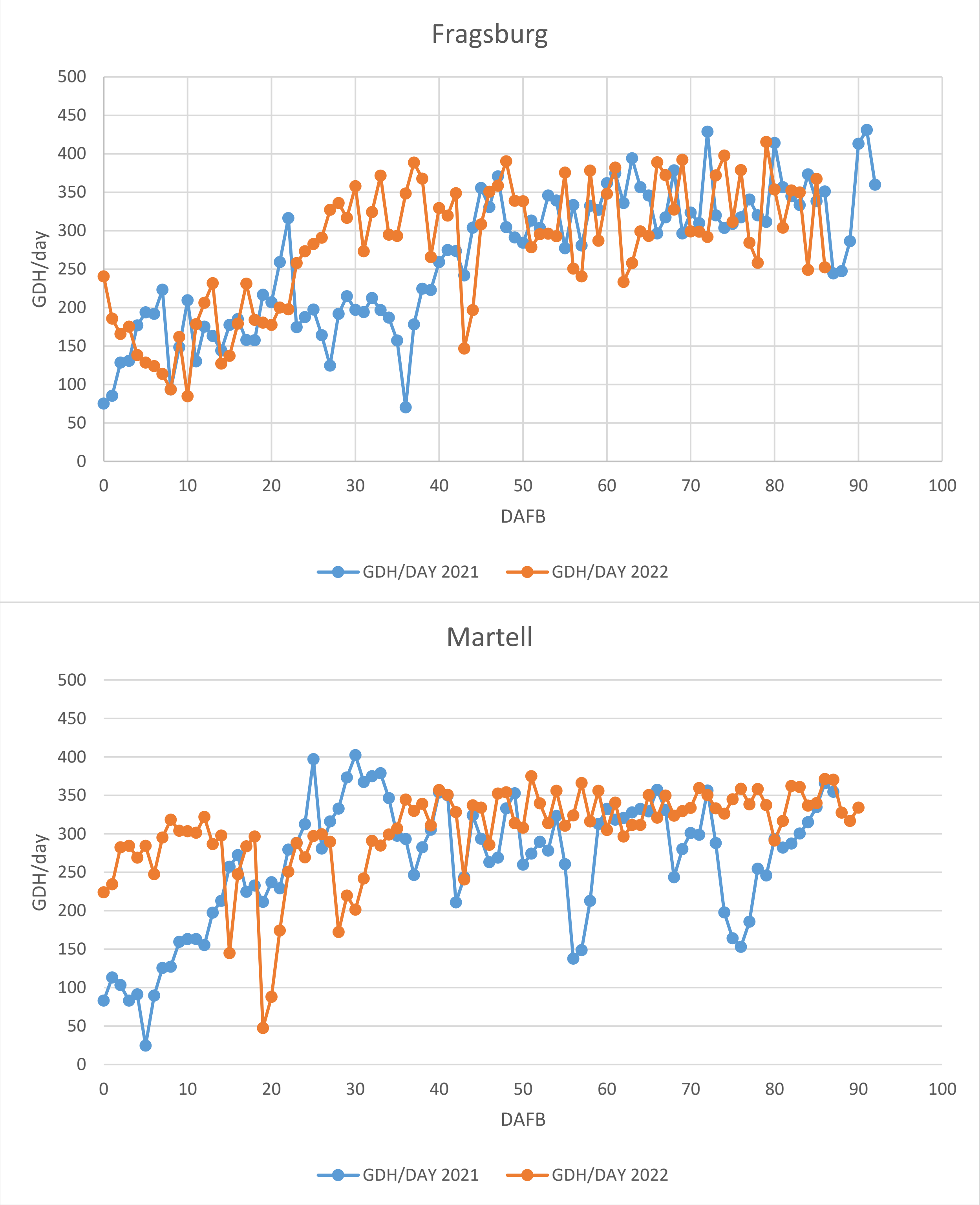

Figure 10: Growing Degree Hours evolution over the time expressed in DAFB, for each orchard separately. Each point represents the daily sum of the GDH calculated from the hourly average temperature of each location.

In Figure 10 the evolution of the daily Growing Degree Hours (GDH) over the time in season 2021 and 2022 is presented. In “Fragsburg”, both years considered were characterized by a cold spring with temperatures often close to zero (but never below). In 2022 the temperature started rising early, at about 22 DAFB while in 2021 it stayed under 200 GDH for two more weeks, even showing two very low minimum peaks. From about 45 DAFB the GDH evolution of both years is similar. In “Martell” the first two weeks after full bloom of 2021 have been characterized by low but gradually rising temperatures (with a very low minimum peak at 5 DAFB), while the same time frame of 2022, on the contrary, by high and constant temperatures. However, if in 2021 between 15 and 35 DAFB the temperature constantly continued to rise, in 2022 it dropped multiple times and became constant only starting from 35 DAFB.

4.4.5 Harvest

Table 9 summarizes the main quantitative and qualitative parameters collected at harvest. Despite the (calculated) number of flowers per tree at full bloom was not significantly different in the two sites, “Martell” managed to produce twice the yield of “Fragsburg”, a higher yield efficiency, and a comparable fruit size. Furthermore, it should be noted that at the time of flowering a 20% of frost damages have been detected in “Martell”.

Orchard	**Average fruit weight [g]**	**Yield per tree [kg]**	**n° of fruits per tree at harvest [-]**	**n° of flowers per tree at FB [-]**	**Yield efficiency [g/cm²]**
Fragsburg	11,13	8,5	773	3838	0,07
Martell	11,50	16,8	1477	4410	0,17
p-value	0,401	**<,001**	**<,001**	,454	**<,001**

Table 9: effect of the factor “Orchard” on the average fruit weight [g], on the yield per tree [kg], on the number of flowers per tree at FB and on the number of fruits per tree at harvest (the latter have been calculated on the basis of % of total fruit set, the average fruit weight and the yield per tree. The estimated marginal means are reported individually for each site. Bold p-values are considered significant at α ≤ 0,05.

4.5 Discussion

4.5.1 Flowers and fruits counting, fruit set

Similar to season 2020 and 2021, the evolution of the total fruit set from full bloom to harvest in year 2022 resembled a sigmoidal curve in both the orchards considered. The initial stationary phase is associated with the full bloom and can last from few days to several weeks depending on the average temperature and the intensity of the frost damages; very likely the 20% frost damages observed prior to flowering in “Martell”, together with the high daily GDH accumulation from 0 to 14 DAFB (about 250-300 GDH per day), determined the rapid decrease in the total fruit set curve observed in between 7 and 14 DAFB; this was not observed in “Fragsburg”, where the low temperatures at bloom retarded the petal fall until 23 DAFB. The evolution of the total fruit set seemed to be conditioned by the exposure of the limb to the sun: despite no significant effect was found at harvest, the evolution of the fruit set of the limbs facing south (or south-east) followed a different path than those facing north (or north-west). From Figure 2 it appears that the detachment of the fruits belonging to the northern side of the canopy is delayed but, interestingly, not prevented or reduced; this initial difference became not significant at 62 DAFB in “Fragsburg” and at 50 DAFB in “Martell”. Since several weeks can pass from the time when the fruit slows down its growth to its actual detachment, we can suppose that the higher temperature of the southern side of the canopy could have only accelerated the natural senescence of the already dropping fruitlets by increasing their transpiration losses. Interestingly, a different consistency of the “Groups of detachment” between the two exposures has been found in “Fragsburg” but not in “Martell” as reported in the additional Figure 11: this makes sense if we consider that “Fragsburg” rows have an east-west orientation, therefore with half of the canopy in full light and the other half characterized by little direct light, while the “Martell” orchard was realized with northeast – southwest oriented rows, thus having a more even distribution of light. Since the relative fruit set was referred to 38 and 28 DAFB in “Fragsburg” and “Martell” respectively, it can be supposed that the retarded detachment of the dropping fruits facing north had significantly influenced the development of this curve. Despite a higher fruit drop rate in condition of poor light (or even on the north side of the canopy) has been reported for sweet cherries (Flore et al., 1999; Davarynejad et al., 2009), it cannot be excluded that the more intense late fruit drop of the fruitlets facing north described in Figure 3 was caused by a “mathematical” rather than a physiological reason. In fact, since on the northern side the senescence was slowed down, it is very likely, that when the “n0” was established a high number of unfertilized (shrinking but not yet detached) fruitlets was still present on the north side.

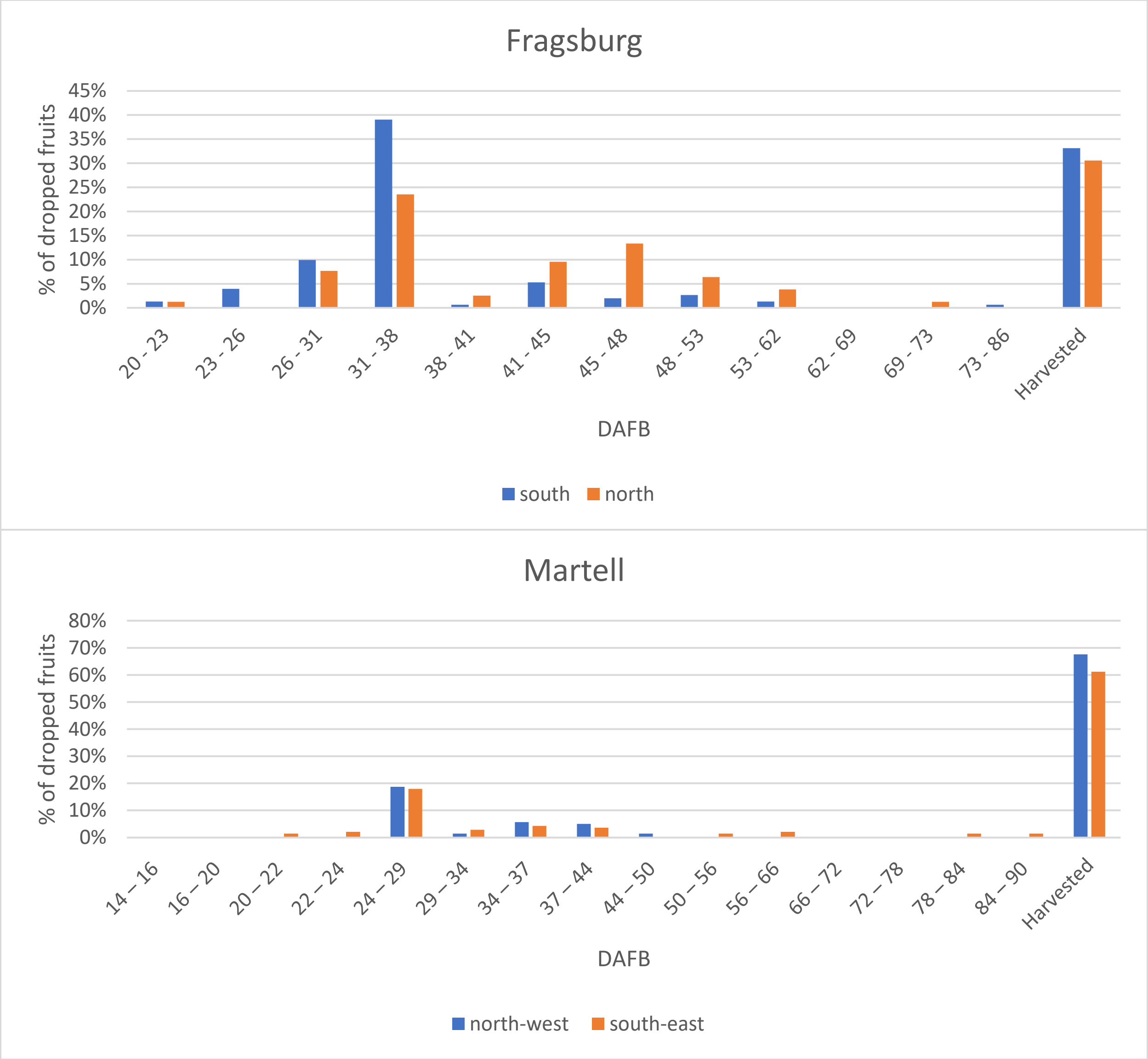

Figure 11: percentage of cherries detached in each time interval considered based on the total number of cherries tagged (about 320 in each orchard). Values for the sunny and the shady side of the canopy are reported separately. Aggregate values can be consulted in Table 7.

In the season considered the late fruit drop accounted for 41% and 26% of the fruits set in “Fragsburg” and “Martell” respectively, which is considered a limited but still significant damage.

In both orchards, no effect of the “Pinching”, neither on the total- nor on the relative fruit set, was observed. This approach was attempted because in both seasons 2020 and 2021 a negative effect of excessive vigor of the limb extension shoots on the relative fruit set was observed (especially in “Fragsburg”). Even assuming that a significant competition for resources between the extension shoots and the growing drupelets could exist, the particular climatic condition of season 2022, characterized by high temperatures and low rainfall, have severely reduced the capacity of the sweet cherry tree to promote vigorous (and competitive) shoots. It is sufficient to observe the ratio between the sum of the length of new shoots at terminal bud set and the limb length (from which they originated) at full bloom (used as a measure of the limb vigor): in year 2021 it was 3,91 and 1,89 for "Fragsburg" and "Martell" respectively, while in year 2022 these values become 1,57 and 0,70. Since the limbs were not girdled, carbohydrates contribution from other parts of the canopy cannot be excluded; however, very likely for competition between the fruit development and the vegetative growth to occur, the latter must be very intense.

4.5.2 Vegetative parameters

As already observed in the previous season, also in year 2022 the growth over the time of the extension shoots followed a sigmoidal development. The start of the vegetative growth coincided with the full bloom. The initial growth was more intense in "Martell" than in "Fragsburg" probably due to the higher spring temperatures. Unexpectedly, in "Martell" the shoot growth stopped already at 19 DAFB, while in "Fragsburg" it lasted until 62 DAFB. In the previous chapter the soil analysis of the two locations can be found. The "Pinching" was able to completely modify the growing behavior of the extension shoots in "Fragsburg": immediately after the removal of the apical meristem, the growth ceased for weeks but, in the last month before harvest, it resumed promoting the emission of lateral shoots. The natural sigmoidal growth of "unpinched" shoots consisted of a first convex part followed by a concave one; on the contrary, the growing behavior of "pinched" shoots consists of two convex parts, which totally makes sense since their growth is composed by two separate emissions of new extension shoots. Probably due to the late application or due to the overall lack of vigor, "Pinching" had no substantial effect in "Martell"; of course, immediately after the removal of the apical meristem a significantly different (and negative) AGR was measured (24 DAFB) in the "pinched" treatment, but no difference in growth rate was detected in any of the subsequent measurements. In "Fragsburg", "pinched" extension shoots showed a significantly lower average length than the "unpinched" ones at terminal bud set. In "Martell", since the vegetative growth ceased approximately on the same day of the "Pinching", no significant difference between the two treatments was found at the end of the season (Figure 4). A minor effect of the "Exposure" factor was found in "Fragsburg": sun-facing extension shoots did not show a greater average length at harvest but only a more prolonged vegetative growth than the north-facing ones. Again, the critical climatic conditions of this season could have played a significant role in the vigor restraint. In general, the two sites differed

by the average extension shoot length (26,46 cm in "Fragsburg" and 11,12 cm in "Martell"), the linear density of extension shoots per limb (6,83 and 3,74 ES/m, respectively), and the linear density of non-fruiting spurs (11,00 and 15,37 nFS/m, respectively) (Table 5). It cannot be ruled out that the overall lower vigor measured in the "Martell" orchard in 2022 may in some way be attributable to the very high production observed in 2021. Despite the negative impact of high crop load on the vegetative growth within the same season is well known (Smith, et al., 2013), no information on the long-term effect was found in literature; however, it can be supposed that the extraordinary yield of 2021 (21,10 kg/tree), by suppressing the vegetative growth in the same season, enhanced the flower bud induction for the following one supporting the sink strength of fruits over that of the extension shoots, resulting in a kind of vicious circle, but without detrimental results.
As already discussed in the previous chapter, different leaf populations contribute differently to each sink. Non-fruiting spurs leaves reach their maximum development rapidly in the season, while the current season shoots create new leaf area even up to harvest (Ayala, et al., 2008); during the early stages, the young fruitlets represent the primary sink of the non-fruiting spur leaves, while only one fourth of the carbon fixed by the extension shoot leaves is exported to the fruits (Ayala, et al., 2018). Interestingly, in the 2022 season characterized by limiting climatic conditions and hence lower vigor, no linear correlation was found between the vigor and the fruit set (Table 6).

4.5.3 Seasonal fruit growth

As already discussed in detail in the previous chapter, the fact that drupelets have been individually labelled already at shuck split made it possible to group them into different waves of drop (or "Groups of detachment"). The same approach of year 2021 has been maintained: only the fruitlets that at the first sampling did not show a significantly different diameter than those of the group "Harvested" (see Table 7) and that subsequently detached were considered part of the late fruit drop. Furthermore, Groups of detachment representing less than 2% of the tagged fruits have been excluded from the graphic representation to make the trends more viewable, as well as from the statistical analysis due their very small sample size. Based on this definition, in "Fragsburg" Groups n° 3 and 4 have been excluded from the "late drop" since their average diameters at 20 DAFB were already significantly smaller than that of Group 13. In the same way, in "Martell" Group n° 5 was not included in the late drop. Drupelets, that immediately after shuck split already showed smaller sizes, were interpreted as a result of a failure in pollination resulting in the development of parthenocarpic fruits (Bradbury, 1929). In "Fragsburg", Groups n° 6, 7, 8 and 9 were considered part of the late fruit drop: the statistical analysis of their growth rates revealed that they were comparable to that of retained cherries until 31 DAFB; from 31 to 38 DAFB, Groups n° 6, 7 and 8 suddenly showed significantly lower AGR values and became senescent; the same was observed for Group n°9 between 38 and 41 DAFB. Retained cherries (Group "Harvested") followed the typical double sigmoidal curve: their growth rate was intense from 20 to 26 DAFB when it suddenly slowed down probably also because of the simultaneous pit hardening; at 38 DAFB the minimum of the growth rate curve was measured, from this point on, the growth slowly resumed reaching at 62 DAFB the maximum of this second phase of growth. In "Martell" only Groups n° 7 and 8 were considered part of the late fruit drop: however, the fact that Group n° 7 showed a significantly lower growth rate already between the first and the second sampling (i.e., between 14 and 16 DAFB) prevented us from identifying the specific moment in which this Group started to grow slower. On the contrary, cherries belonging to Group n° 8 showed similar growth rates to retained cherries from 14 to 20 DAFB; lower (but without any significance) absolute values of AGR have been measured already between 20 to 24 DAFB but the first significant difference appeared between 24 and 29 DAFB. For the "Martell" orchard also retained cherries followed the double sigmoidal curve: the high growth rates measured immediately after bloom (between 14 and 16 DAFB) decreased steadily until they reached a minimum at 29 DAFB; from this point on, they started to increase again and showed a maximum peak at 56 DAFB. It has been suggested that negligible fruit size increases at Stage II of fruit growth could be caused by competition for resources between the lignification of the endocarp (the "pit hardening") and the pericarp development (Zhang, et al., 2013).

In both locations, fruit growth behavior appeared to be influenced by the exposure of the corresponding limbs: south-facing cherries were characterized by greater average diameters in both orchards in almost all the samplings except the last one; since at harvest no significant difference was found, it is likely that the southern exposure had only accelerated the growth process without improving the final fruit size. No effect of the factor "Pinching" was found on the fruit growth; possible causes have been already discussed in paragraph 5.1.

4.5.4 Meteorological data and overview

As already observed in the previous season, both the vegetative and the reproductive growth seems to be temperature driven. For example, in "Martell" the growth rate of the "unpinched" extension shoots suddenly dropped between 16 and 22 DAFB: interestingly, at the same time, at least two minimum peaks of GDH accumulation can be found (145 GDH at 15 DAFB and 48 GDH at 19 DAFB). Interestingly, between 14 and 16 DAFB fruitlets belonging to "Group of detachment" n° 7 showed a lower growth rate than the retained ones (despite a similar diameter, a sign that, until shortly before, their AGR values were similar!);

furthermore, at the same time, cherries of the “Group of detachment” n° 8 started to reduce their growth rate until they reached a negative value at 29 DAFB. The last minimum peak of GDH accumulation was measured exactly between 27 and 33 DAFB (172 GDH at 28 DAFB). It is worth noting that the drop in temperature was responsible only for the first reduction in the growth rate of the extension shoots: very likely, the pit hardening phase, the climatic conditions, as well as the high crop load (16,81 kg per tree at harvest) reduced the sink strength of the vegetative growth redirecting the photoassimilates distribution towards the fruit. A very interesting aspect is the relatively high late fruit drop intensity measured in “Fragsburg” despite the apparently good climatic conditions. Considering only the retained fruitlets (Group “Harvested”, Figure 9), it is surprising how quickly their absolute growth rates dropped between 26 and 31 DAFB; simultaneously, the growth rate of the “unpinched” extension shoots went from 1,24 cm/day to 0,70 cm/day. In “Martell” the same reduction in fruit growth, coinciding with the beginning of the Stage II, was measured, but it appeared as a constant and gradual decrease from 16 to 29 DAFB. The additional Figure 12 represents the evolution over the time of the maximum daily temperatures measured in the “Fragsburg” location.

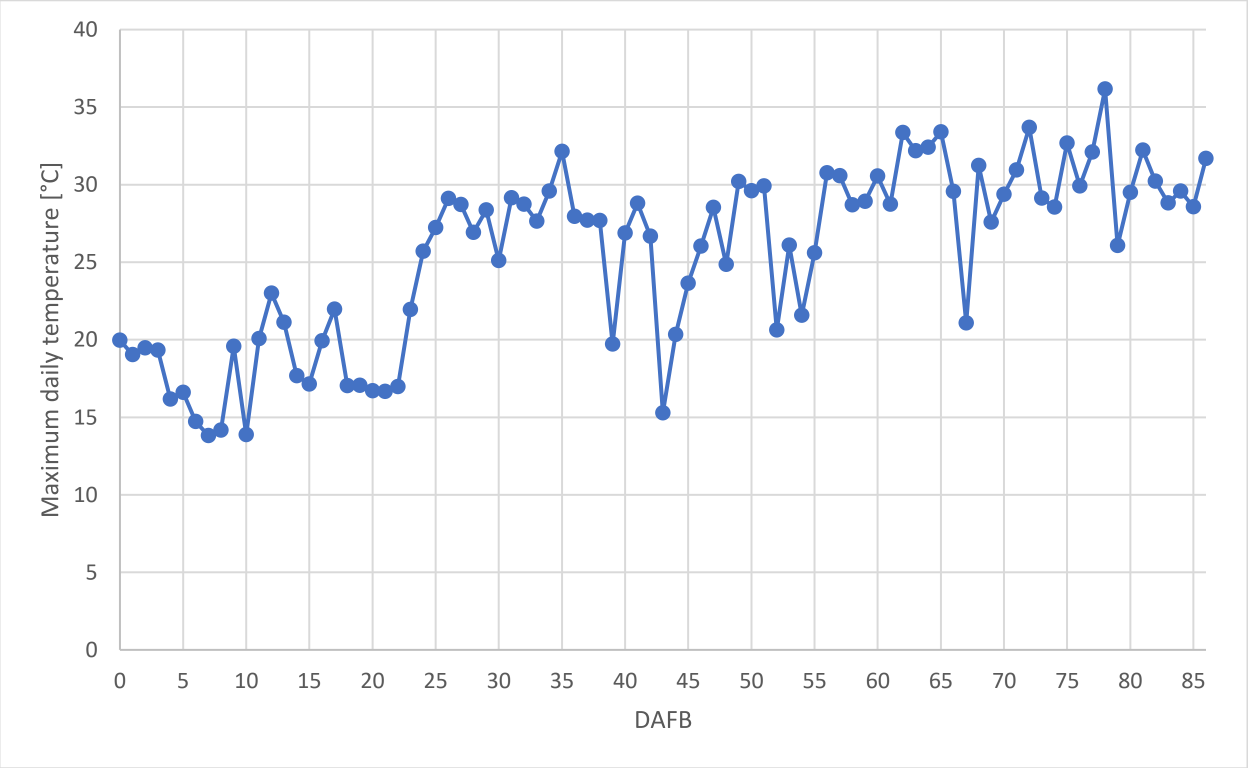

Figure 12: evolution of maximum daily temperatures in “Fragsburg” from FB to harvest in year 2022.

From 20 to 26 DAFB the temperature increase was followed by high values of growth rate. This linear trend stopped as soon as the maximum temperatures approached the 30°C (29,1°C at 26 DAFB, on 12

May). Between 31 and 38 DAFB most “Groups of detachment” showed significantly lower AGR values and started to be senescent; at 35 DAFB the maximum temperature reached was 32,15°C. It is worth noting that the weather station was installed at the edge of the orchard and not inside; therefore, we can suppose that the maximum temperature under the plastic rain cover was even higher. Blanke et al. (2008) studied the effect of plastic rain covers on different aspects in cherry growing; immediately after flowering, despite the outside temperature was about 18°C (April 2005, 1 PM) the air temperature under the cover was already above 30°C. It should be noted that the “Fragsburg” orchard was located at 705 m a.s.l. in the Alpine region “Trentino – South Tyrol” and that this spring temperature differs significantly from the historical averages. It cannot be excluded that the simultaneous phase of pit hardening had increased the competition for resources enhancing the fruit drop. Many authors reported negative effects on fruit set of high temperatures at full bloom (Beppu et al., 1997; Lech et al., 2008; Hedhly et al., 2012; Sarisu, 2017); in particular, Hedly et al. (2012) reported that warm temperatures reduced the number of growing pollen tubes along the style as well as accelerated ovule degeneration. Furthermore, Beppu et al. (2001) reported that nucelli and embryo sac degenerated more rapidly at 25°C than at 15°C. However, not many works focusing on the effect of high temperatures during fruit set were found. Interestingly, Erez et al. (1998) reported that in sweet cherry greenhouse growing, as the temperature increased the percentage of dropping fruits increased linearly. Furthermore, Blanke et al. (2008) reported that in year 2005 a large proportion of the potential crop of ‘Samba’, ‘Burlat’, and ‘Earlise’ grafted on ‘Gisela 5’ was lost due to high fruit drop caused by overheating under the rain cover during fruit set. Beppu et al. (2003) studied the effect of two different temperature regimes (25/15°C and 35/25°C) on 1-year old sweet cherry potted trees (‘Satohnishiki’ grafted on ‘Aobazakura’); after a certain time, both treatments have been temporarily transferred to growth chambers controlled at 15-35°C. The net photosynthetic rate of the trees previously grown at high temperature reached a maximum at 30°C and decreased at 35°C; differently, that of the trees previously grown at low temperature remarkably decreased their net photosynthetic rate already above 25°C, revealing temperature acclimation in photosynthesis. It is worth noting, that the rootstock involved in our trial, ‘Gisela 5’, is known to be very sensitive to water- and heat stress (Stott, et al., 2019).

The different climatic conditions observed in these locations during the two years of monitoring had a direct impact on the evolution of the fruit set. The additional Figure 13 summarizes the “destiny” of the flowers present on the trees at full bloom in both “Fragsburg” and “Martell” in year 2021 and 2022. Blue bars represent the percentage of flowers that did not contribute to the initial fruit set: here, factors as frost damages, pollen availability, pollinators efficiency, as well as the lack of fertilization due to climatic factors (see paragraphs 1.1.2, 1.1.3, and 1.1.4 in Chapter 1) are counted. For example, it is very likely that the lower percentage of initial fruit set detected in year 2021 in “Martell” was largely influenced by the severe temperatures measured in the spring; similarly, in the same orchard, after the mild and constant climatic conditions of the first days after FB in spring 2022, a much higher initial fruit set was observed. The orange bars represent the percentage of late fruit drop referred to the number of flowers. Here, the fruit drop intensity seemed to depend strongly on the temperature trend from the end of bloom to the pit hardening: in both “Fragsburg” and “Martell” the highest percentages of late fruit drop have been detected in years characterized by cold waves exactly in this period (2021 for “Fragsburg” and 2022 for “Martell”).

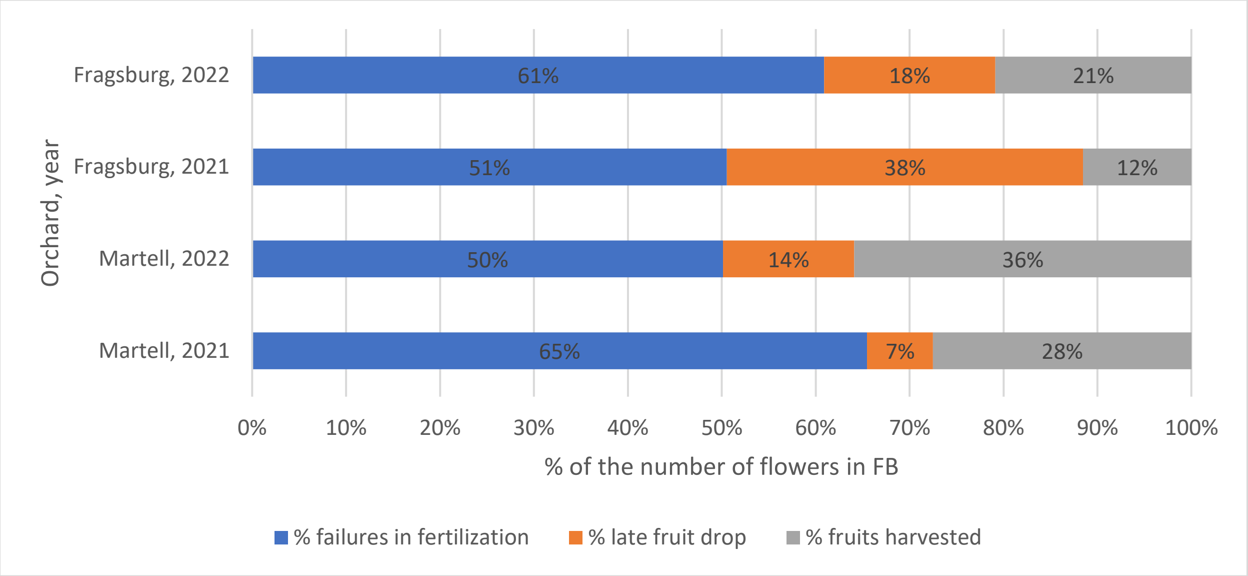

Figure 13: “Destiny” of the flowers present on the tree at full bloom: blue bars represent the percentage of flowers that did not contribute to the initial fruit set, orange bars the amount of flowers initially set but dropped at a later time, grey bars the percentage of flowers that evolved into harvested fruits.

4.6 Conclusions

The evolution of the total fruit set followed a sigmoidal trend while that of the relative fruit set an expolinear one. Despite no significant effect of limb exposure (sunny vs. shady side of the canopy) was found on the total fruit set at harvest, it is possible that northern exposures retarded the actual detachment of already dropping cherries, hence modifying the shape of the fruit drop curve. Probably due to an overall lack of vigor caused by the climatic conditions (high temperatures combined with low precipitations), no effect of “Pinching” the limb’s extension shoots was found on either the fruit set or the fruit growth. On the other hand, “Pinching” drastically changed the growing behavior of the extension shoots.

Again, many physiological processes described in this chapter seemed to be temperature driven. However, if in season 2021 prolonged periods of low temperatures and/or sudden severe decreases in the daily Growing Degree Hours accumulation were followed by a reduction in the average growth rate of both extension shoots and fruitlets, in 2022, surprisingly, the same effect was also caused by extraordinary high temperatures close to or above 30°C immediately after flowering. Considering these two seasons, we can interpret late fruit drop as a self-regulation of crop load performed by the cherry tree in a situation where the climatic conditions are limiting. In the seasons considered, the “sensitivity phase” coincided with a period of natural susceptibility of the tree, such as the initial fruit set, the endocarp lignification, and the time the plant goes from being dependent on reserves to becoming photosynthetically self-sufficient.

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5 Chapter 5: General Discussion and Conclusion

In this thesis, the late fruit drop of ‘Regina’ sweet cherry in the South Tyrolean climatic conditions was studied. ‘Kordia’ and ‘Regina’ represent the standard varietal combination of this Region but, in the last few years, due to high percentages of late fruit drop, very unstable yields have been observed for the cv. Regina.

In Chapter 1, an overview of the main works published on this topic is presented. The late fruit drop can be defined as the premature detachment of the fruitlet set; many of the papers reviewed seem to confuse high late fruit drop rates with the concept of low fruit set. In doing so, it is impossible to distinguish between the different components that contribute to the total fruit set such as, for example, frost damaged flowers, pollination efficiency, flower drop, drop of unfertilized fruitlets, late drop of fruitlets set, or even the damages caused by birds or mammals.

In Chapter 2, a sound experimental protocol has been developed to correctly describe the (late) fruit drop pattern of sweet cherry cv. Regina grafted on ‘Gisela 5’. The trial was carried out at the experimental orchard of the Laimburg Research Centre in year 2020; flowers and fruitlets on each tagged limb were counted weekly from 2 DAFB up to the day prior to harvest. The effect of limb position within the canopy (close to the tree top or to the basal branches), and of pruning the 1-year-old shoots in the spring (in order to promote the vegetative growth), on the fruit set was investigated.

In Chapter 3, the evolution of fruit set in 6 orchards located at different elevations (from 225 to 1175 m a.s.l.) was monitored to study the effect of different climatic conditions (year 2021). Here, as soon as much of the first waves of fruit drop ceased, approximately at stage 77 as described by Fadon et al. (2015), the number of fruits per limb prior to late fruit drop (n_0) was collected. The following definitions were used:

$$Total\ fruit\ set\ \% = \frac{Fruit\ number\ at\ harvest\ time}{Total\ flowers\ number} * 100$$

$$Relative\ fruit\ set\ \% = \frac{Fruit\ number\ at\ harvest\ time}{Fruit\ number\ at\ n0} * 100$$

$$Intensity\ of\ late\ fruit\ drop\ \% = (1 - Relative\ fruit\ set\ \%)$$

In this work, only the drupelets that at petal fall showed a diameter not significantly different from that of cherries that would reach harvest, and which subsequently detached, were considered part of the “late fruit drop” of ‘Regina’.

In Chapter 4, the evolution of fruit set in two locations was monitored (year 2022). In the previous season, these two orchards showed opposite trends: the first one was characterized by early phenology and intense late fruit drop while the second one by late phenology and poor late fruit drop. Aim of the 2022 trial was to repeat the same samplings performed in 2021 but with higher frequency (every 3/4 days) in order to confirm or refute the previously formulated hypothesis. In addition to this, 2022 trial took into account also the exposure of the limb to light (sunny vs. shady side of the canopy). Finally, to investigate the direct competition between fruit set and the vegetative growth of the extension shoots, 50% of the latter have been pinched at about 20 DAFB.

From these studies, it emerged that late fruit drop is a complex and widespread phenomenon, ranging from 7 up to 76% of the fruitlets set, able to significantly affect the profitability of the crop. In all the seasons considered, immediately after petal fall, a substantial percentage of drupelets already stopped growing, showed smaller diameters, and were rapidly getting senescent. This first wave of drop was very likely composed by unfertilized parthenocarpic fruitlets (probably caused by late or missing fertilization due to unfavorable climatic conditions at bloom (Bradbury, 1929)) and it should not be confused with the late fruit drop wave which was composed by fully developed drupelets showing an aborted embryo. Referring the relative fruit set to the number of fruitlets set (n0) allowed us to estimate with good accuracy the intensity of late fruit drop without any interference of the previous wave. The fact that drupelets had been individually labelled already at shuck split (and from this point onwards measured frequently), made it possible to group them into different waves of drop (or “Groups of detachment”). At petal fall, cherries belonging to the late dropping Groups showed the same diameter as those that would reach commercial harvest. Although in the first samplings their growth rate was equal, at a certain point their AGR values drastically decreased, and these fruits got senescent. This sudden change has been observed to be concomitant both with prolonged periods of low temperatures (or sudden severe decreases in the daily Growing Degree Hours accumulation) and with extraordinary high temperatures close to or above 30°C immediately after flowering. In literature, drastic reductions of sweet cherry net photosynthesis have been reported at both high (Beppu, et al., 2003) and low (Vosnjak, et al., 2022) critical temperatures. Late fruit drop appeared as a self-regulation of crop load performed by the cherry tree in a situation where the climatic conditions were limiting. Since the occurrence of these critical conditions during the season (i.e., from FB to harvest) not always corresponded to a new wave of fruit drop, the existence of a “sensitivity phase” can be assumed. Interestingly, in the seasons considered, this coincided with periods of natural susceptibility of the tree, such as the initial fruit set, the endocarp lignification, and the time the plant goes from being dependent on reserves to becoming photosynthetically self-sufficient. Further studies should also consider the role of storage carbohydrates accumulated in the previous season.

Sweet cherry (Prunus avium) is indigenous to the area between the Black and Caspian seas of Asia Minor. It is a species adapted to cool temperate climates, where the danger of late frost is restricted (Lim, 2012); therefore, a regulation of the crop load as a response to critical temperatures seems plausible. Interestingly, although 'Regina' was released as a commercial variety in 1977 and it has been cultivated for more than 20 years in South Tyrol, the first local reports of intense late drop are no older than 10 years. Due to climate change, extreme environmental events such as late frost or heat waves in spring and summer have become more frequent and intense, with direct impact on agriculture.

Excessive vigor was found to be positively correlated with high fruit drop but was not its main cause. Pruning the 1-year-old shoots in the spring to promote the emission of laterals enhanced late fruit drop only in the orchards already characterized by excessive growth.

Other factors, such as the position of the limb within the canopy (close to the tree top or to its basal branches) and its orientation (sunny vs. shady side) played only a marginal role sometimes hard to interpret.

Finally, the soil-, leaf-, and fruitlet analysis data presented suggest that fruit drop was not linked to nutrient deficiencies.

Overall, many of the aspects studied in this work seemed driven by temperature: temperature affected the duration of flowering, the percentage of frost damages, the fruit set evolution over the time as well as both the reproductive and the vegetative growth. The interesting aspect of studying different orchards relatively close together but located at different altitudes was that the climatic events were the same but occurred in different moments during the reproductive cycle, making it possible to study their effects individually. No linear correlation was found between the increase in elevation and the fruit set; however, orchards characterized by a later phenology, and thus reaching full bloom in late-, and not in early, spring, are less subject to unstable weather conditions.

Since in most of the orchards the first significantly lower AGR of the late dropping Groups was recorded in the first 30 days after full bloom (at about 1/3 of the reproductive cycle of this variety), the adjective "late" should be reconsidered; further studies about this topic should focus on the time the fruit slows down its growth rate rather than on its time of detachment.

In conclusion, late fruit drop in sweet cherry is a complex phenomenon probably caused by multiple factors. This work was not intended to be exhaustive in an absolute sense, but rather to start investigating the most common hypothesis and provide new insights about the topic for future studies.

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