

Article

Improving of Cherry Fruit Quality and Bearing Regularity by Chemical Thinning with Fertilizer

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Abstract: The study's objective was to evaluate the influence of thinning on the quality and regularity of yield of 'Regina' cherries grown on a dwarf Gisela 5 rootstock. The experiments were conducted in the years 2009-2012 in Western Poland. Trees were thinned using a chemical agent, ammonium thiosulphate (ATS) at doses of 20g ATS × L⁻¹, 30g ATS × L⁻¹ and 40g ATS × L⁻¹, and by hand, and the results of chemical and hand thinning were compared with those obtained for unthinned trees. The course of weather conditions in winter and during flowering had a significant impact on yield. Chemical thinning is known to be an effective method to regulate fruiting of pome trees, but it can also be successful if applied to cherry trees, as reflected by the alternate bearing index, which was the lowest after applying ATS at a dose of 40g. ATS treatment improved fruit quality in full crop years. Thinning resulted in, among other things, larger and darker fruit and higher content of total soluble solids and titratable acidity. Treatment with 40g ATS × L⁻¹ significantly stimulated the vegetative growth of trees.

Keywords: TSS; skin colour; thinning of flowers; fruit quality; firmness; ammonium thiosulphate; tree vigour

1. Introduction

Sweet cherries are among fresh fruits that have been experiencing a global growth in production and trade [1]. According to 2017 data, global cherry production was 2,443,407 t⁻¹ (FAOSTAT). The world's largest producers were Turkey, the United States, Iran and Uzbekistan, which account for about 54% of the global cherry production. Italy and Spain, are the largest cherry producers in Europe, while Chile is South America's top producer. In Poland, cherry production is about 20 thousand t⁻¹ per year, and is increasing due to the intensification of fruit growing.

The use of dwarf rootstocks in the cultivation of cherries contributes to the intensification of production [2,3]. It increases productivity per area unit, but often leads to a deterioration in fruit quality [4,5]. Competition and market requirements force attention to quality and favour large, firm and high-sugar fruit [6,7]. The key to obtaining fruit with the proper and desired quality characteristics is the regulation of fruiting [8–10]. Trees that produce too much fruit due to the limited supply of carbohydrates and nutrients produce small fruit with a low firmness [11].

The use of dwarf rootstocks for cultivars with a tendency to yield small fruit significantly worsens the fruit size. This is especially evident in self-fertile cultivars, such as 'Lapins', 'Sweethart', which tend to create a large number of flower buds. With a large fruit set and weak tree growth, there is a problem with the right size of the fruit [12–15]. This dependence was found when assessing the Tabel® Edabriz dwarf rootstocks for cherries and the 'Summit' trees grafted on them. Removing 30 to 50% of fruit spurs resulted in the best fruit quality (>28 mm) [16]. The optimum quality of cherry fruit was achieved with 10 fruit per cm² of limb cross-sectional area [7]. The ratio of leaves to

fruit is also important. An increase in the leaves-to-fruit ratio is associated with an increase in the mass and content of the extract and an increase in the ratio of sugars to acids. With a larger area of the leaves per fruit, the colour of the fruit was darker and the fruit itself matured earlier (Usenik, Orazem, and Stampar 2010). It is important to develop cultivation methods that will allow to obtain an acceptable yield while maintaining high quality of fruit [4,7,11,18].

Fruit thinning is a practice commonly applied in fruit growing [19]. Depending on the fruit species, chemical, manual or mechanical thinning is used. Mechanical thinning is an alternative to manual thinning, which is a laborious process [20,21] and which is increasingly difficult to apply from year to year due to the shrinking availability of workforce [22–24]. Mechanical thinning is typically applied to apples, pears and peaches [25–29]. However, the first devices were designed for the treatment of trees trained as narrow spindle, axis or fruit-bearing wall rather than for trees maintained in a broad spindle or vase shape [30]. Even if grown on draft rootstocks, cherry trees generally develop pretty large crowns and the thinning of cherries with the same devices as those used for apples or pears is very difficult and often impossible. This drawback can be solved by hand-held mechanical devices which can be applied to trees trained according to various systems [21], but fruit thinning with hand-held devices requires much more effort than chemical thinning and its efficiency is disputable, so it seems that the best solution is to work out an effective chemical thinning method.

Chemical thinning of fruit to improve its quality and prevent alternate bearing is commonly used in apple and pear cultivation [4,31]. It also reduces manual effort. Hand thinning of sweet and sour cherries is unprofitable due to labour intensity [18,32]. Compared to the pome species, more flowers must be left on the trees of stone species in order to obtain a profitable commercial yield of good quality. For peaches, this should be about 25% [33], while sweet and sour cherries require 75% flowers to remain on trees for a profitable commercial yield [34].

The options of chemical thinning of stone fruit are limited [28]. Tests have been conducted on thinning methods such as burning formulations, growth regulators and photosynthesis inhibitors [35,36]. Chemical thinning may be carried out during flowering or shortly thereafter to reduce the load on trees during the growing period [37]. Removing flowers and fruitlets in the initial growth period preserves more assimilates thus reducing competition between the vegetative and generative organs of the tree. This contributes to stronger vegetative growth but also stimulates the differentiation of flower buds and improves fruit quality and yield size [6].

Fruiting can be regulated using gibberellic acid (GA₃) to reduce flower bud induction, balance the amount of fruit and improve fruit quality during the post-application season [35,38]. Gibberellin used during the initiation of flower buds in the induction period allows to reduce the total number of flowers in the next growing season. The date of use is important, as bud development can be influenced only for a limited period each year. Thus, the period of induction and differentiation of flowers must be known for each species or for each cultivar [38,39]. In studies on the thinning of cherries also molasses, potassium soap, Tergitol, 2%-4% vegetable oil emulsion or copper were tested [40–42].

The disadvantage of thinning flowers is the risk of yield loss if frosts and unfavourable conditions for pollination and fertilization occur after thinning. Chemicals, such as ammonium thiosulphate (ATS), urea, hydrogen cyanamide and other sulphur-based compounds [18,41,43,44] used in such conditions cause damage to the stigma, style, anther and pollen, which prevents pollination of damaged flowers

ATS is used to thin apple and peach flowers and is considered to be ecologically sustainable. However, it can have phytotoxic effects on leaves and consequently reduce photosynthesis, which may affect the fruit size [43]. However, there are also reports that there are no visible signs of leaf damage caused by the use of 20 g L⁻¹ ATS [45]. Two-percent ATS was used twice to thin the cherries of the 'Bing' cultivar grown on the 'Gisela 5' rootstock - for the first time when 10% of flowers were developed and again when 90% of flowers were fully open. The result was a reduction in yield and improved fruit quality. In other studies, ATS effectively reduced the yield of cherries, but did not improve the fruit mass or size or the extract content [43].

In the context of the aforementioned findings, the aim of the work was to examine the impact of ammonium thiosulphate on productivity parameters and the regularity of fruiting of 'Regina' cherry trees grown on a dwarf 'Gisela 5' rootstock. The study also assessed how thinning using ATS affects tree growth and fruit quality.

2. Materials and Methods

The study was conducted from 1/03/2009 to 8/05/2013. The experimental field part was carried out in an orchard in western Poland, in an area situated at 52°5'3-36" north latitude and 15°50'5-58" east longitude. The experimental material consisted of cherry trees of the 'Regina' cultivar grown on a 'Gisela 5' rootstock, planted as one-year old trees in spring 2001 in north-south rows with 3.5 × 2.0 m spacing. The pollinator cultivar was 'Kordia' growing in blocks evenly distributed in the orchard, each block consisting of 4-6 trees. The experiment was set up in a randomized block design with 4 repetitions per each combination. There were four trees in each repetition, i.e. a total of 16 trees per combination. Each repetition was separated from the next one by two divider trees.

The following thinning treatments were applied in each year of the study:

1. Control (K)
2. Flower thinning with ammonium thiosulphate solution at a dose of 20g ATS × L⁻¹
3. Flower thinning with ammonium thiosulphate solution at a dose of 30g ATS × L⁻¹
4. Flower thinning with ammonium thiosulphate solution at a dose of 40g ATS × L⁻¹
5. Fruitlet hand thinning

Crystalline 98% ammonium thiosulphate (Rosier S.A., Moustier, Belgium) was used for chemical thinning treatments (Rosier S.A., Moustier, Belgium). Doses were 20g ATS × L⁻¹, 30g ATS × L⁻¹ and 40g ATS × L⁻¹. Canopy spraying was performed with a Stihl SR420 sprayer at a working liquid dose of 0.35 l/tree (500 l of liquid × ha⁻¹). Chemical thinning of flowers was performed in the 80±6% open flower buds phase [45]. The date was set annually by monitoring the percentage of flowers developed on marked indicator branches of two trees in each treatment. At the time of chemical thinning, the mean percentage of developed flowers was 78.4% in 2009, 83.3% in 2010, 85.6% in 2010 and 76.6% in 2012.

Manual thinning of fruitlets was performed when their diameter was between 8mm and 12mm. Fruitlets were removed from all branches of a tree where their number exceeded 100 fruitlets × m⁻¹ of the shoot length or 4 fruitlets per spur. The percentage of removed fruitlets depended on the number of set fruit and was determined each time by comparing the number of fruitlets on the marked indicator branches on each tree before and after thinning (**Error! Reference source not found.**).

Table 1. Dates of crop load regulation treatments of 'Regina' sweet cherry trees in 2009-2012.

Treatment	Dates of thinning treatments			
	2009	2010	2011	2012
Control	-	-	-	-
Flower thinning with 20 g ATS	22.04	27.04	22.04	26.04
Flower thinning with 30 g ATS	22.04	27.04	22.04	26.04
Flower thinning with 40 g ATS	22.04	27.04	22.04	26.04
Fruitlet hand thinning	30.05	1.06	28.05	30.05

Agricultural practices

The trees were trained as spindle crowns. The height of the trees was limited to 3.30 m from the ground. The trees were pruned after fruit harvests, with the focus on stimulating vegetative growth by either removing older weakly growing shoots or shortening them to over ten centimeters long

stumps. The trees grew in herbicide strips, while the inter-rows were covered with grass which was regularly cut. The trees were protected against diseases and pests in accordance with recommendations for commercial orchards.

Course of meteorological conditions

Climatic conditions were monitored using U12-011 sensors (Onset, Bourne, MA, USA) located in the tree crown at a height of 1.5 m from the ground level. Temperature and relative humidity were measured at a frequency of 24 readings per day. In addition, data from the weather station located in the orchard were analysed (**Error! Reference source not found.**).

Table 2. Air temperatures in experimental plot at bloom time in 2009-2012.

	2009	2010	2011	2012
Mean air temperature during bloom period	13.7	11.4	13.5	17.3
Mean air temperature during 7 days after bloom	12.8	10.6	14.3	15.4
Minimum air temperature during bloom period	-0.2	2.5	-2.4	2.0
Minimum air temperature during 7 days after bloom	2.5	5.0	-2.4	3.7
Maximum air temperature during bloom period	29.1	27.1	27.5	33.6
Maximum air temperature during 7 days after bloom	27.5	21.0	30.7	32.8

Tree vegetative growth parameters

The following effects of experimental treatments were evaluated:

- percentage of the fruit set in relation to the number of flowers,
- percentage of natural fruitlets drop,
- percentage of developed fruit which has reached full maturity in relation to the number of flowers

Measurements were conducted on one indicator branch within each tree. In the green bud phase, the number of bouquet spurs, the number of flower and leaf buds on bouquet spurs, as well as the number of flower and leaf buds on one-year shoots were counted. In the white bud phase, the total number of flowers on the indicator branches and the mean number of flowers per flower bud were assessed.

Assessment of frost damage of flower buds and flowers

Spring frosts occurred in May 2011, while in February 2012 there were frosts causing damage to buds. Figure 1 and Figure 2 show the temperature changes.

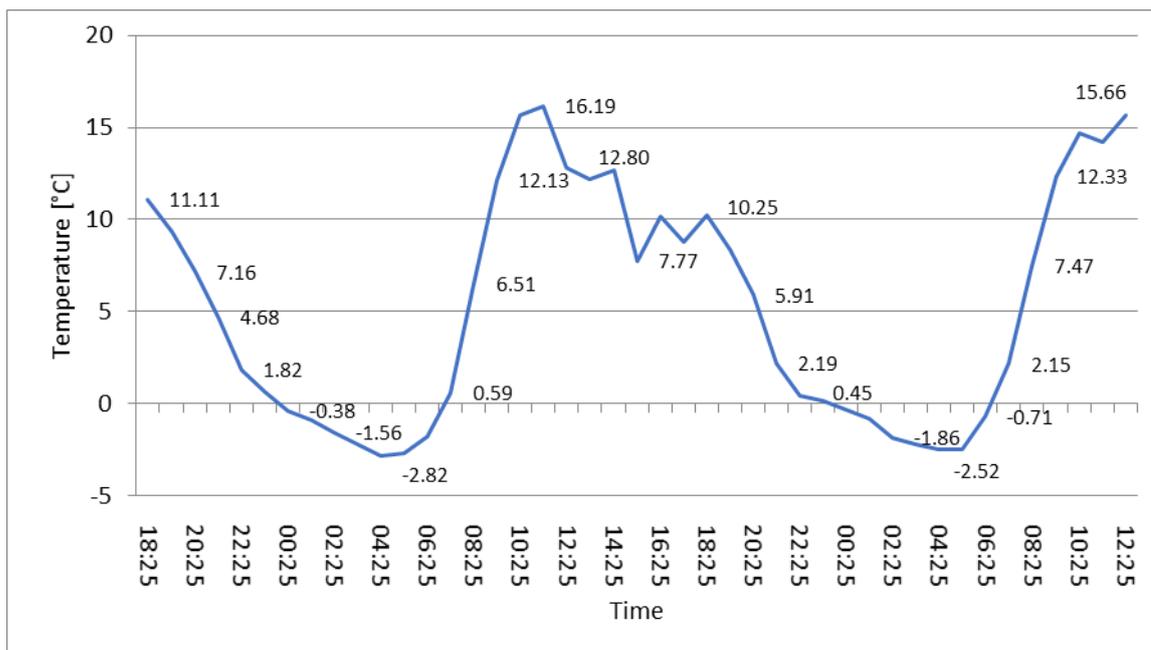


Figure 1. Air temperature fluctuations measured at 1.5 m height from 3 to 5 May 2011

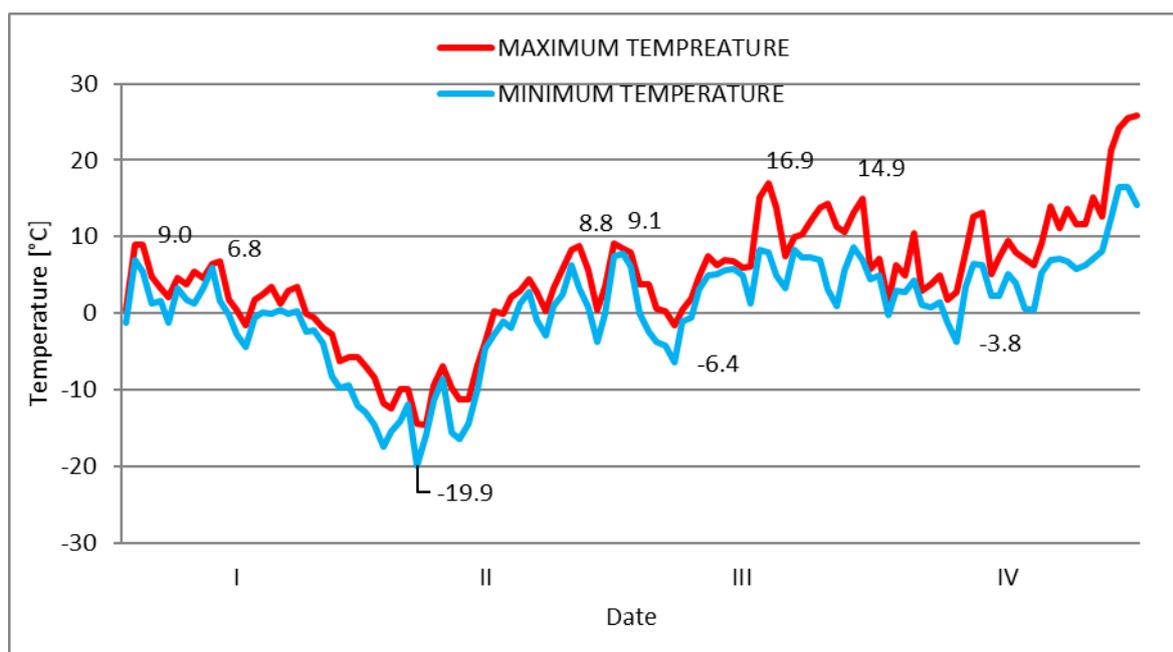


Figure 2. Minimum and maximum temperatures from January to April 2012

Yielding and tree productivity parameters

The assessment of tree productivity between 2009 and 2012 was based on yield per tree (kg/tree), yield per area unit (t/ha), yield relative to the trunk cross-sectional area (kg/cm²) (yield efficiency), yield relative to the total length of one-year shoots (kg/m) and the yield regularity index (alternate bearing index - ABI). The alternate bearing index was calculated from the formula proposed by [46–48]:

$$I = \frac{\sum_{t=2}^n (y_t - y_{t-1}) / (y_t + y_{t-1})}{n-1}$$

where

n- number of years

y_t- yielding over the years

The alternate bearing index of 0 thus expresses the regular yield, while its value close to 1 indicates the alternating yield. All fruit was harvested at the same time. The harvest date was based

on the fruit skin colour: fruit was collected when most of it reached colour fraction No 4-5 according to the CTIFL colour scale (Centre Technique Interprofessionnel des Fruit et Legumes, France)[49,50]. Harvest was carried out on 11 July 2009, 21 July 2010, 28 June 28 2011 and 15 July 5 2012. After harvest, the fruit with stalks was weighed from each tree to an accuracy of 0.01 kg.

Assessment of fruit quality and tree vegetative growth

The division of yield into quality fractions was based on measurements of the cross-sectional diameter (in a plane perpendicular to longitudinal axis) and the firmness of the fruit. Measurements were made with a Firmtech 2 (Bioworks, Victor, NY, USA) Fruit Firmness Tester, with an accuracy of 0.01g for firmness and 0.01mm for diameter measurement. The study was conducted in four repetitions, each time on 100 randomly selected fruit with stalks meeting the quality requirements for commercial fruit (no damage, diameter over 23.0mm). The measurement was made at room temperature on the fruit of each repetition. The result was expressed in $\text{g} \times \text{mm}^{-1}$, where firmness was determined as the force needed to bend the skin by 1mm.

The size results are given by specifying the percentage of fruit in the following size categories (diameter):

- below 22.0 mm;
- from 22.0 mm to 24.0 mm;
- 24.0 mm to 26.0 mm;
- 26.0 mm to 28.0 mm;
- 28.0 mm to 30.0 mm;
- over 30.0 mm.

The above-mentioned division into quality fractions assumes that fruit with a diameter of more than 28.0 mm is 'premium class' class fruit. The mean weight was assessed on the basis of mass measurements of 200 cherries with stalks from each repetition. Accuracy was up to 0.01 g.

Extract (TSS) and total acidity (TA) were measured in the juice of 40 randomly selected cherries. TSS content was evaluated using a refractometer PR-101a (Atago Co. Ltd., Fukaya-shi, Japan). TA was marked by adding 45 ml of distilled water to 5 ml of juice, titrating 0.1 N with sodium hydroxide to pH 8.2. TA was expressed as a percentage of malic acid [51].

Fruit skin colour was measured using a CR20 colorimeter (Konica-Minolta, Tokyo, Japan) on a sample of 40 cherries in each repetition. The results are presented in the form of a CIE Lab coordinates of the $L^*a^*b^*$ color space and h° . The value of HUE angle (h°) is calculated from the formula:

$$H^\circ_{ab} = \tan^{-1} (b^*/a^*)$$

The growth strength of trees was assessed based on the length of one-year shoots. The measurement included all shoots exceeding 5.0 cm in length on two trees in each repetition between 2009 and 2012. The measurement accuracy was 1.0 cm. The growth strength was also determined by counting the mean number of one-year shoots per tree and calculating the mean shoot length and the total length of all one-year shoots. In addition, the circumference of the trunks at a height of 20 cm from the ground surface was measured. All measurements were taken after the vegetation period.

Climatic condition

The total annual rainfall figures varied considerably during the study period. In 2009 and 2011, the total annual rainfall was 573.5 mm and 574.0 mm, respectively, whereas it exceeded 700 mm in the remaining years. In 2009, which was the driest year, the total rainfall was 265.4 mm during the growing season (IV-IX). Precipitation was distributed very unevenly within individual years. In 2011, the total rainfall was 0.8 mm in November and 198.7 mm in July. 2010 was the coldest year, with an mean annual temperature of 7.7°C. The lowest temperatures were recorded in January 2010 and February 2012, with the lowest temperature recorded below -20°C. In 2012, winter

damage occurred in February after a warmer spell in January, when the temperature reached 9.0°C (

Table 1).

Table 1. Recorded temperatures and total rainfall in the year 2009 -2012

Month	Total rainfall (mm)				Temperature (°C)							
	2009	2010	2011	2012	2009		2010		2011		2012	
					\bar{X}	min.	\bar{X}	min.	\bar{X}	min.	\bar{X}	min.
I	30.9	38.2	32.9	66.2	-2.7	-18.6	-6.5	-20.2	0.3	-9.8	0.5	-13.1
II	44.6	17.1	13.6	35.7	-0.7	-7.2	-1.1	-8.9	-2.4	-16.1	-3.7	-20.0
III	66.5	43.6	36.1	12.0	4.0	-5.1	3.9	-9.4	4.7	-6.1	6.6	-6.4
IV	15.1	26.9	16.0	36.1	12.7	0.8	9.3	0.6	12.2	2.8	9.3	-3.8
V	86.7	101.0	35.3	50.6	13.9	4.0	11.2	3.9	14.7	0.8	15.4	3.2
VI	73.1	10.4	44.1	114.0	15.2	5.8	17.6	7.9	18.4	9.9	15.9	6.0
VII	51.5	96.0	198.7	174.0	19.0	10.2	22.0	12.2	17.5	8.6	19.0	8.5
VIII	10.2	106.1	53.2	54.0	19.8	8.2	18.0	10.3	18.4	8.0	19.1	10.2
IX	28.8	117.4	44.5	41.5	15.8	5.8	12.4	4.9	15.8	7.4	14.6	4.6
X	68.1	10.0	35.0	21.5	7.1	0.1	6.9	-0.9	9.7	-1.3	8.5	-3.5
XI	42.6	122.6	0.8	62.2	6.6	-2.7	4.7	-9.4	4.0	-2.9	4.8	-3.2
XII	55.4	59.9	64.0	34.3	-1.2	-16.0	-5.5	-15.7	3.3	-2.7	-0.8	-12.8
Mean	573.5	749.1	574.0	702.1	9.12	-1.2	7.74	-2.1	9.72	-0.1	9.09	-2.5

All data were statistically analysed. One or two-factor analysis of variance was carried out in accordance with the experiment design, using Statistica v. 13.3 (TIBCO Software Inc., USA). The differences between mean values were checked for statistical significance using Duncan's test, at a significance level $\alpha=0.05$. Correlation factor scans and determination analysis were done using Microsoft Excel tools Office 365.

3. Results and discussion

Effect of thinning treatments on yield and productivity

Tree yield depended on the year of research and averaged between 3.1 t/ha in 2011 and 18.1 t/ha in 2009 (**Figure 3**). Yield variability was caused by low temperatures during flowering and fertilization. The 75.6% decline in yields in 2010 was due to poor pollination and flower fertilization, which was confirmed by fruitlet drop by as much as 82.0% (**Error! Reference source not found.**). Honey bee, which pollinates most flowers in orchards, is not active at temperatures below 12°C [52]. The optimum temperature for flower fertilization is about 20°C [53,54]. In the following year (2011), low yield was caused by frosts of -2.82°C immediately after flowering (Figure 1). The mean share of frozen fruitlets was 95.0%. Cherry buds and flowers are often damaged by spring frosts, which limit the areas of cherry cultivation [55,56]. Lethal temperatures for stages from green bud to full flowering range from -5°C to -2°C depending on the cultivar [57]. Temperature drops below 0°C result in flower mortality of almost 80%. Flower mortality depends on cultivar and late cultivars are considered to be less susceptible to damage [58]. However, in our study on 'Regina', which is a late cultivar, the damage was significant.

ATS applications were found to have an impact on tree yields in 2009 and 2012 (**Error! Reference source not found.**). Chemical thinning of flowers resulted in a yield decrease by 29.4% at 20g ATS, by 43.0% at 30g ATS and by 48.9% at 40g ATS. No effect of hand thinning was observed. In 2012, yield differences ranged from 15.7% to 41.5%, but a significant difference was found only after the application of ATS at a dose of 40g (**Error! Reference source not found.**). The literature mentions

a similar effect after applying 2% ATS to 'Bing'/'Gisela 5' cherries [41]. In other studies, ATS was effective with 20.4 or 30.6 g/l, while the dose of 10.2 g/l had no effect on the fruit set [59]. ATS effectiveness on crop was also confirmed by studies conducted in Switzerland on four self-sterile varieties of cherries [43].

Crop load measured by yield efficiency (kg/cm^2) was different in each year, and the effects of chemical thinning were significant only in 2009 and 2012 (**Error! Reference source not found.**).

In 2009 and 2012 chemical and manual thinning significantly influenced also the yield relative to the total length of one-year shoots. In 2009 it was the highest on control trees ($1.17 \text{ kg}\times\text{m}^{-1}$), significantly lower after manual thinning ($0.96 \text{ kg}\times\text{m}^{-1}$), even lower after chemical thinning of flowers (**Error! Reference source not found.**). In 2012, the pattern was similar, although the differences were not so clear.

The mean yield did not vary much, but was lower after 30gATS and 40gATS (**Error! Reference source not found.**). Also the thinning of flower buds of 'Bing' cherries during the dormancy period was found to have a similar effect on the yield and yield efficiency [7,11]. However, in other studies conducted on cherries, the removal of only 20% of flowers and 20% of buds significantly increased the yield efficiency [32].

Thinning of flowers and fruitlets had a significant impact on the regularity of fruiting. The alternate bearing index (ABI) was the highest on unthinned trees ($\text{ABI}=0.69$). The smallest yield fluctuations ($\text{ABI}=0.35$) were observed on trees whose flowers were thinned with 40gATS (**Figure 4**). Despite the opinion that cherries grown on dwarf rootstocks show less tendency towards biannual bearing compared to other fruit species [4], this study clearly showed alternate bearing in the unthinned cherry trees. However, it should be stressed that alternate bearing may be induced to a larger extent by climate conditions and agrotechnical treatments [60].

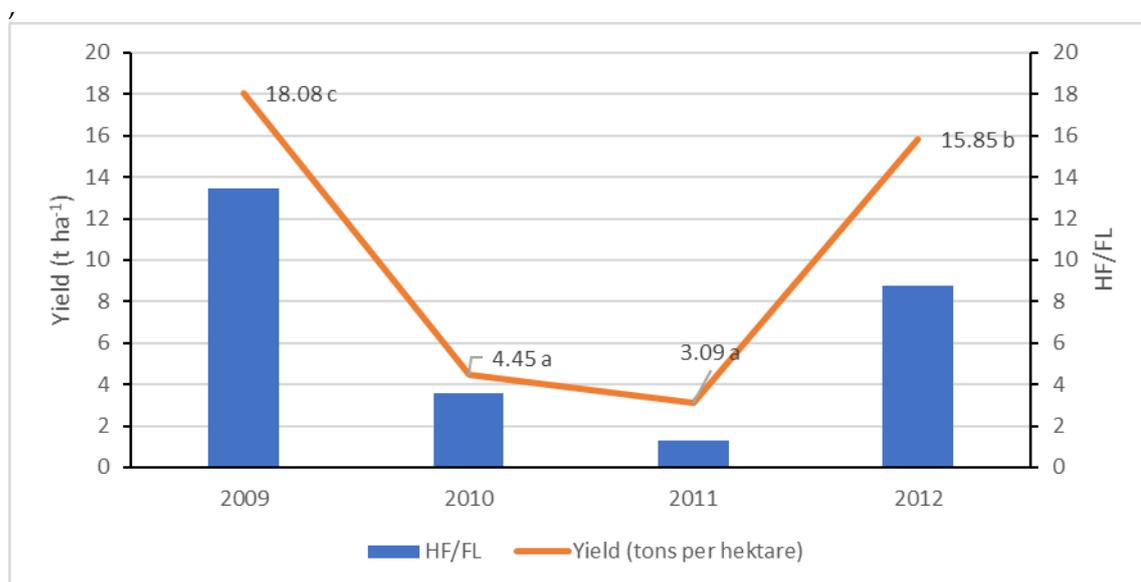


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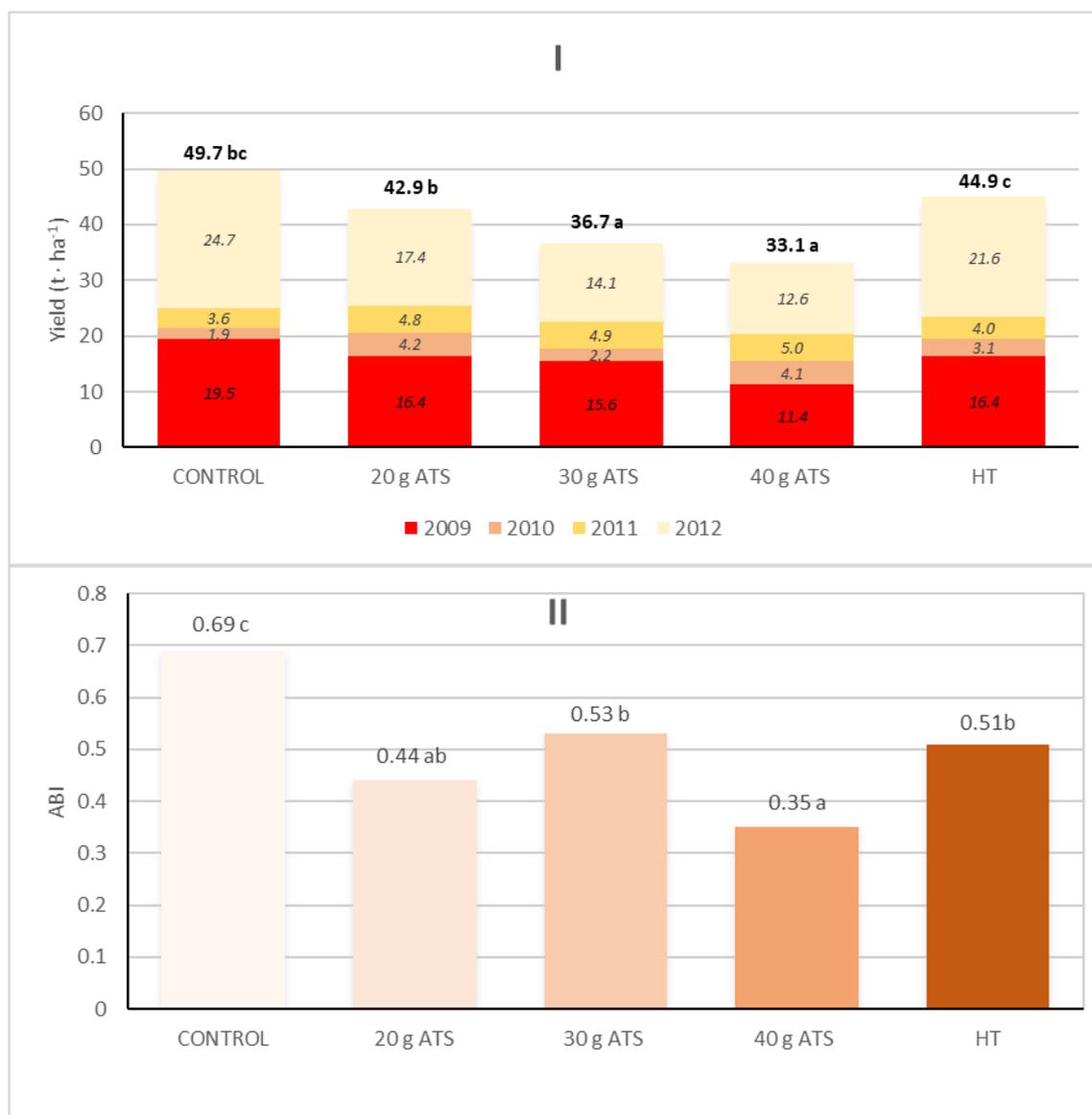
Table 4. Crop load regulation effect on fruit set and fruit drop of Regina sweet cherry trees in 2009-2012.

Treatment	The share of dropped fruit in set fruit (%)		Percentage of opened flowers at the time of chemical thinning (%)		The ratio of developed fruit to the number of flowers (%)	
2009						
Control	48.3	e ¹	83.5	efg	19.7	g
20g ATS	4.5	ab	80.8	cdef	13.3	f
30g ATS	2.9	ab	76.7	abc	8.5	de
40g ATS	3.0	ab	77.8	abcd	7.7	cde
Hand thinning	13.9	c	79.4	bcde	17.9	g
2010						
Control	87.8	g	97.4	i	3.4	abc
20g ATS	85.8	g	85.8	gh	2.8	ab
30g ATS	82.5	g	81.1	cdefg	3.3	abc
40g ATS	84.3	g	82.9	efg	3.6	abc
Hand thinning	69.4	f	94.6	i	4.6	abcd
2011						
Control	2.5	ab	88.6	h	0.8	a
20g ATS	2.1	ab	85.2	fgh	1.1	a
30g ATS	0.8	a	88.8	h	1.2	a
40g ATS	3.8	ab	82.9	efg	1.7	ab
Hand thinning	1.7	ab	82.3	defg	1.7	ab
2012						
Control	28.5	d	77.1	abc	13.7	f
20g ATS	4.2	ab	76.6	abc	6.1	bcd
30g ATS	3.5	ab	77.3	abc	5.0	abcd
40g ATS	9.7	bc	75.9	ab	7.8	cde
Hand thinning	15.4	c	73.2	a	11.2	ef
Mean 2009 - 2012						
Control	41.7	b	86.6	c	9.4	b
20g ATS	24.1	a	81.6	b	5.8	a
30g ATS	22.4	a	80.7	ab	4.5	a
40g ATS	25.2	a	78.9	a	5.2	a
Hand thinning	25.1	a	82.4	b	8.9	b

Table 5. Crop load regulation effect on productivity parameters of Regina sweet cherry trees in 2009-2012.

Treatment	Yield per tree (kg×tree ⁻¹)		Yield per area (t×ha ⁻¹)		Yield efficiency (kg of fruit per cm ² TCSA ²) (kg×cm ⁻²)		Yield to total 1-year-old shoot length (TSL) (kg×m ⁻¹)	
2009								
Control	17.3	g ¹	24.7	g	0.23	g	1.17	i
20g ATS	12.2	de	17.4	de	0.17	e	0.65	g
30g ATS	9.9	bcd	14.1	bcd	0.13	d	0.41	f
40g ATS	8.8	bc	12.6	bc	0.10	cd	0.31	e
Hand thinning	15.1	fg	21.6	fg	0.20	f	0.96	h
2010								
Control	2.5	a	3.6	a	0.03	a	0.06	a
20g ATS	3.4	a	4.8	a	0.04	a	0.08	a
30g ATS	3.4	a	4.9	a	0.03	a	0.09	ab
40g ATS	3.5	a	5.0	a	0.03	a	0.10	ab
Hand thinning	2.8	a	3.9	a	0.03	a	0.08	a
2011								
Control	1.3	a	1.9	a	0.01	a	0.03	a
20g ATS	3.0	a	4.2	a	0.03	a	0.05	a
30g ATS	1.5	a	2.2	a	0.01	a	0.03	a
40g ATS	2.8	a	4.1	a	0.02	a	0.05	a
Hand thinning	2.1	a	3.1	a	0.02	a	0.04	a
2012								
Control	13.7	ef	19.5	ef	0.11	cd	0.25	de
20g ATS	11.5	cde	16.4	cde	0.10	cd	0.22	cde
30g ATS	10.9	cde	15.6	cde	0.07	b	0.19	bcd
40g ATS	8.0	b	11.4	b	0.09	bc	0.14	abc
Hand thinning	11.5	cde	16.4	cde	0.11	cd	0.24	cde
Mean values 2009 - 2012								
Control	8.7	c	12.4	c	0.10	b	0.38	c
20g ATS	7.5	bc	10.7	bc	0.09	b	0.25	b
30g ATS	6.4	ab	9.2	ab	0.06	a	0.18	a
40g ATS	5.8	a	8.3	a	0.06	a	0.15	a
Hand thinning	7.9	c	11.2	c	0.09	b	0.33	c

¹ two-way analyses of variance; data marked with the same letter are not significantly different at $\alpha=0.05$ (Duncan's test).



¹ one-way analyses of variance; data marked with the same letter are not significantly different at $\alpha=0.05$ (Duncan's test)

Figure 4. (II) Crop load regulation effect on cumulative yield of 'Regina' sweet cherry trees in 2009-2012 (I) and alternate bearing index – ABI (II)

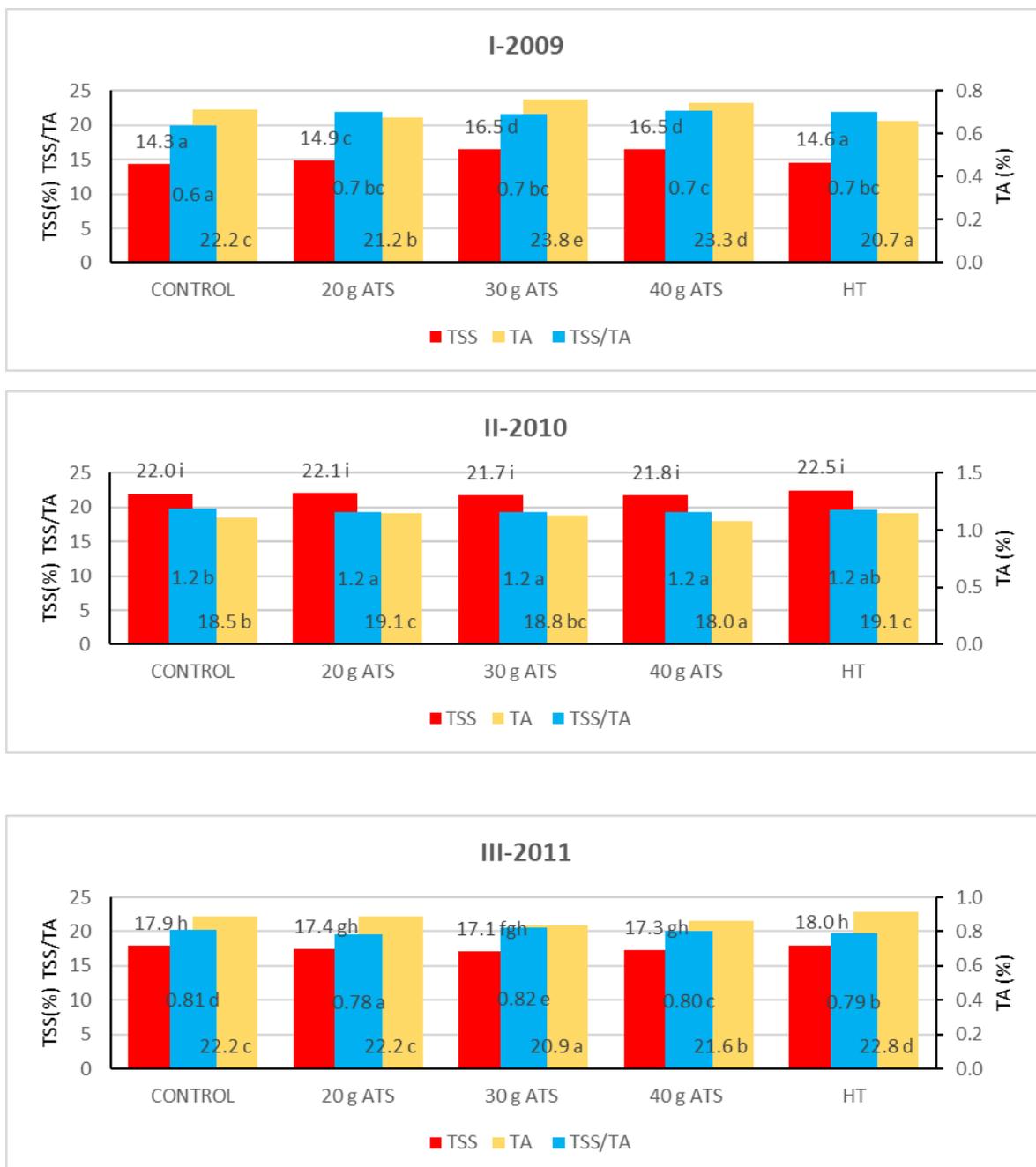
Total soluble solids and acidity

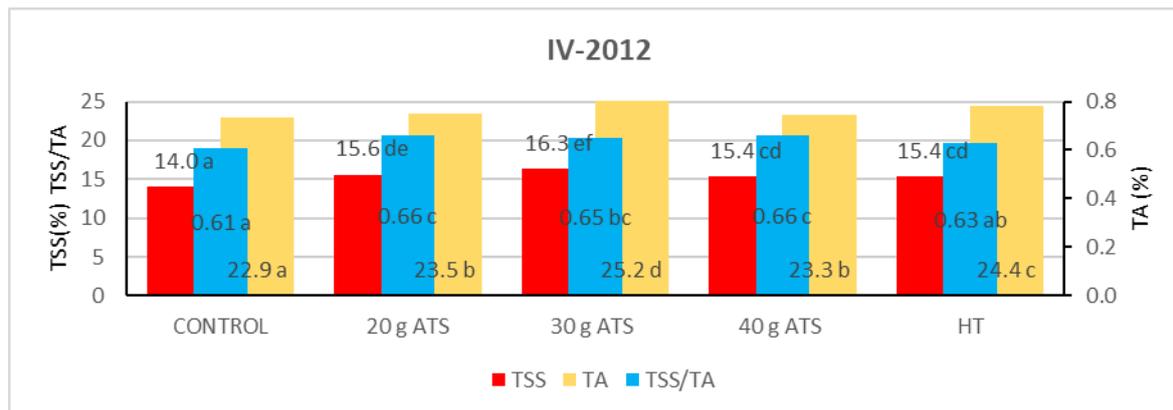
TSS and TA in the fruit depended on the test season. Different course of weather conditions and the sum of active temperatures determine not only the time of maturation but also the chemical composition of the fruit [61]. According to earlier studies on cherries, TSS in the fruit could be more influenced by the cultivar and the course of weather conditions than by thinning operations [59] and the year [43]. Thinning had an effect on TSS in the full crop years (2009 and 2012), whereas it affected acidity only in 2012. In 2009, the highest extract content was identified in fruit from trees treated with the two highest doses of ATS. In 2012, any thinning procedure increased TSS in the fruit. The mean TSS values for all four years of research showed a positive influence of all thinning methods on this quality trait and the increase ranged from 2.9% to 5.3% (**Figure 5**). This effect was already observed in previous studies [11,17] and was explained by a lower fruit/leaf area ratio and reduced

competition for assimilates between fruit. Similar conclusions were proposed by (Bound and Others 2013), who stated that fruit yield was negatively correlated with quality parameters.

As with TSS, thinning was found to affect TA in 2009 and 2012. In 2009, fruit from control trees had the lowest acidity level. In 2012, overall acidity increased in fruit from trees thinned with ATS at doses of 20g and 40g. The multi-year mean value also shows that the thinning of fruit increases its overall acidity, which is in accord with some findings on the effects of flower bud thinning [62].

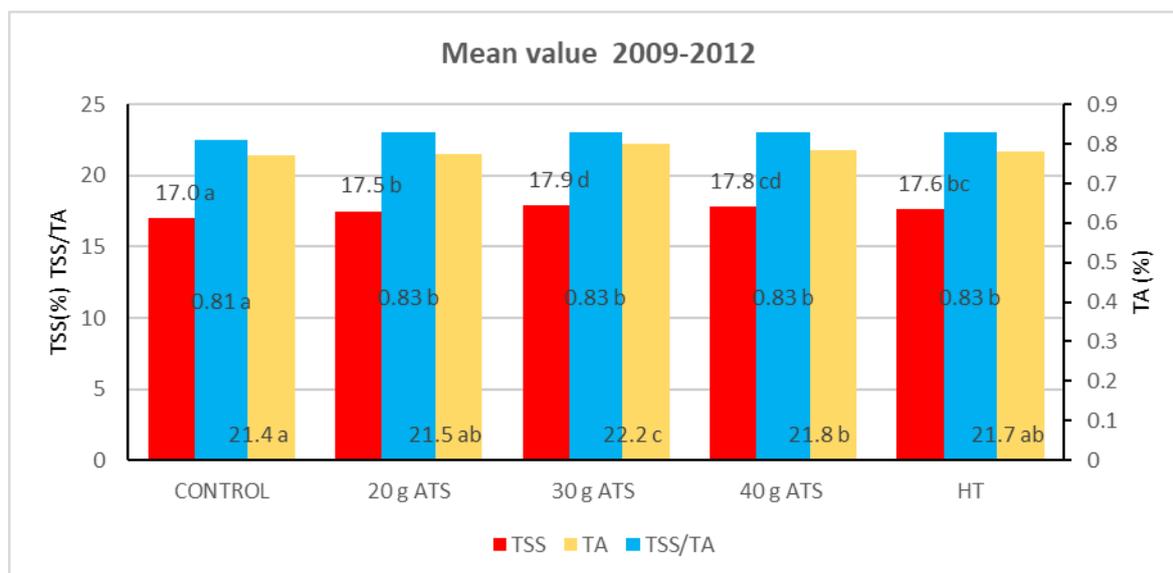
The TSS/TA ratio changed after all treatments only in 2012, while in 2011 after thinning with 30g and 40g ATS and after fruitlet hand thinning. The four-year mean value did not show the effect of thinning on this trait (**Figure 5**). The influence of chemical thinning on the TSS/TA ratio had previously been demonstrated for Japanese plum [8]. In cherry cultivation, the higher number of fruit/leave area ratio resulted in a higher TSS/TA ratio [17]. In this study, TSS and TA were positively correlated regardless of the test season and treatment (correlation coefficient = 0.97, see **Figure 7**).





¹ two-way analyses of variance; data marked with the same letter are not significantly different at $\alpha=0.05$ (Duncan's test)

Figure 5. Crop load regulation effect on total soluble solids (TSS), titratable acidity (TA) and TSS/TA ratio of 'Regina' sweet cherry fruit in 2009 (I), 2010 (II), 2011 (III) and 2012 (IV).



¹ one-way analyses of variance; data marked with the same letter are not significantly different at $\alpha=0.05$ (Duncan's test)

Figure 6. Crop load regulation effect on mean total soluble solids (TSS), titratable acidity (TA) and TSS/TA ratio of 'Regina' sweet cherry fruit in the period 2009-2012.

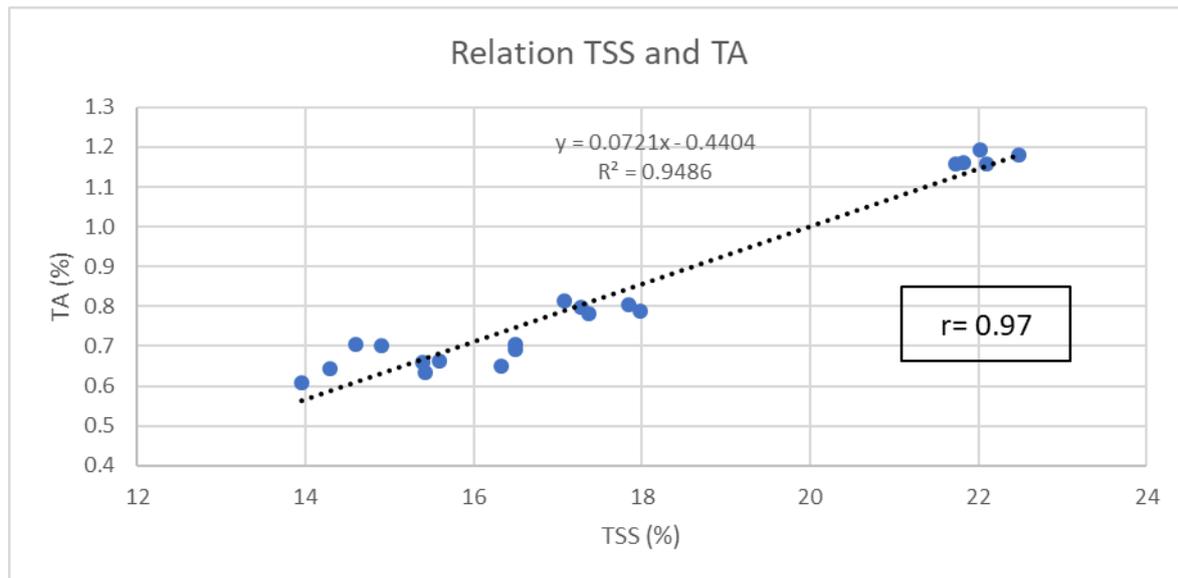


Figure 7. Relation between total soluble solids (TSS) and titratable acidity (TA) of 'Regina' sweet cherry fruit in 2009-2012 regardless of treatment

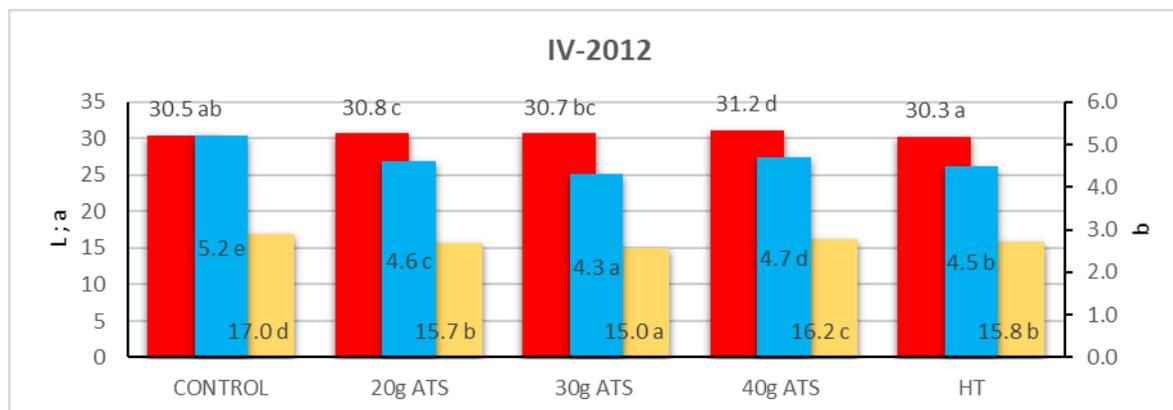
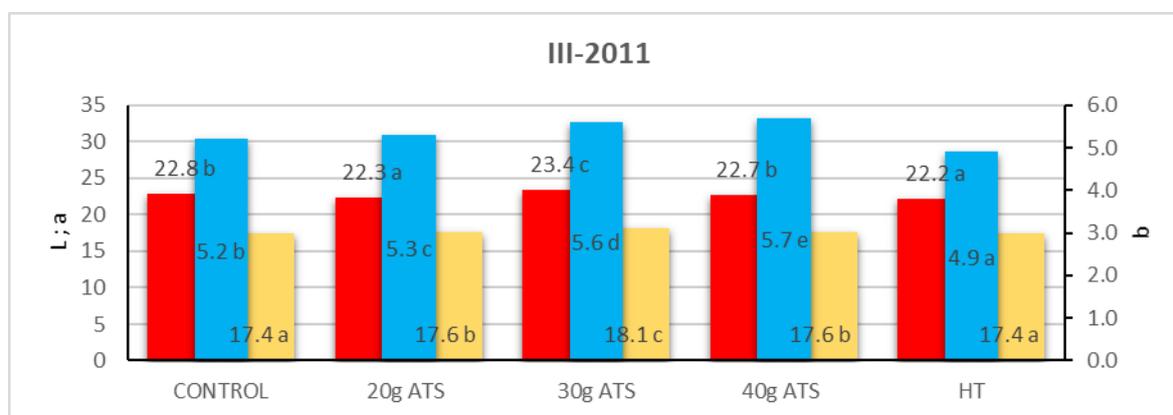
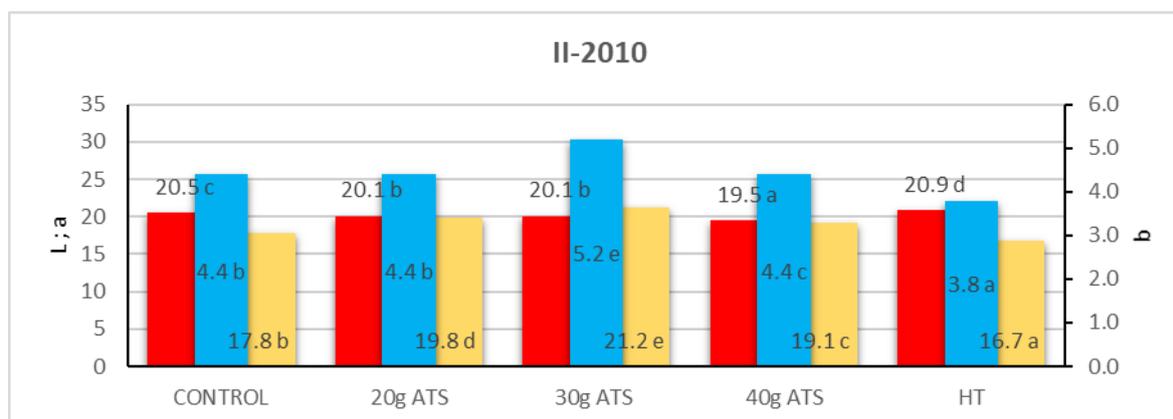
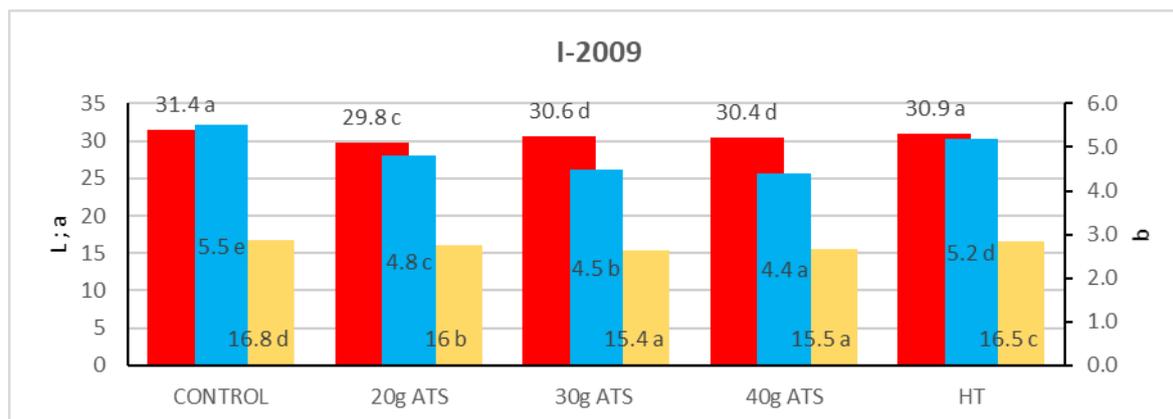
Fruit colouring

Fruit ripening involves biochemical changes that affect the taste, firmness, colour and other quality parameters of the fruit (Bureau et al. 2009), which very often depend on the course of climatic conditions during the growing season [63]. One aspect of fruit maturation is the change in the fruit skin colour from green to red, which is due to the accumulation of anthocyanins and the degradation of chlorophyll [64]. The change in the fruit skin colour can be used as one of the basic indicators of the optimum harvest date [65]. In the present study, measurements of the fruit skin colour coordinates showed that thinning treatments had an effect on the colour of the fruit at harvest. Fruit with a significantly darker fruit skin colour (L^*) observed in 2009 and 2010 came from trees sprayed with ATS at a dose of 40g. This agrees with the results of thinning experiments conducted on flower buds of the 'Lapins' cherry, which revealed that fruit from thinned trees was darker regardless of treatment intensity [66]. A similar effect was achieved by intensive cherry pruning [63].

Thinning of buds reduces the value of the a^* coordinate [66], but in this experiment such an effect was observed only in 2010 in fruit from trees hand thinned or sprayed with ATS at a dose of 30g (**Figure 8**).

The value of the HUE angle (h° parameter) was not influenced by thinning in any of the analysed years. Only the multi-annual analysis showed a trend towards higher h° values in fruit from unthinned trees. The correlation coefficient between h° and TSS was negative (-0.89) regardless of year and treatment (**Figure 10**).

Colour measurements allow to easily assess the relative levels and changes in the content of anthocyanins in cherries [67]. Cherry fruit colour was also found to be related to the content of bioactive compounds in fruit [68]. Prices on the cherry market are associated with fruit colour attributes. The correlation between fruit colour and prices on Poznan-Franowo (Poland) wholesale market in 2007 and 2011 was significant [1]. This clearly demonstrates the importance of colour in evaluating fruit quality and in commerce.



¹ two-way analyses of variance separate for each colour coordinate; data marked with the same letter are not significantly different at $\alpha=0.05$ (Duncan's test)

Figure 8. Crop load regulation effect on the colour of 'Regina' sweet cherry fruit in 2009 (I), 2010 (II), 2011 (III) and 2012 (IV).

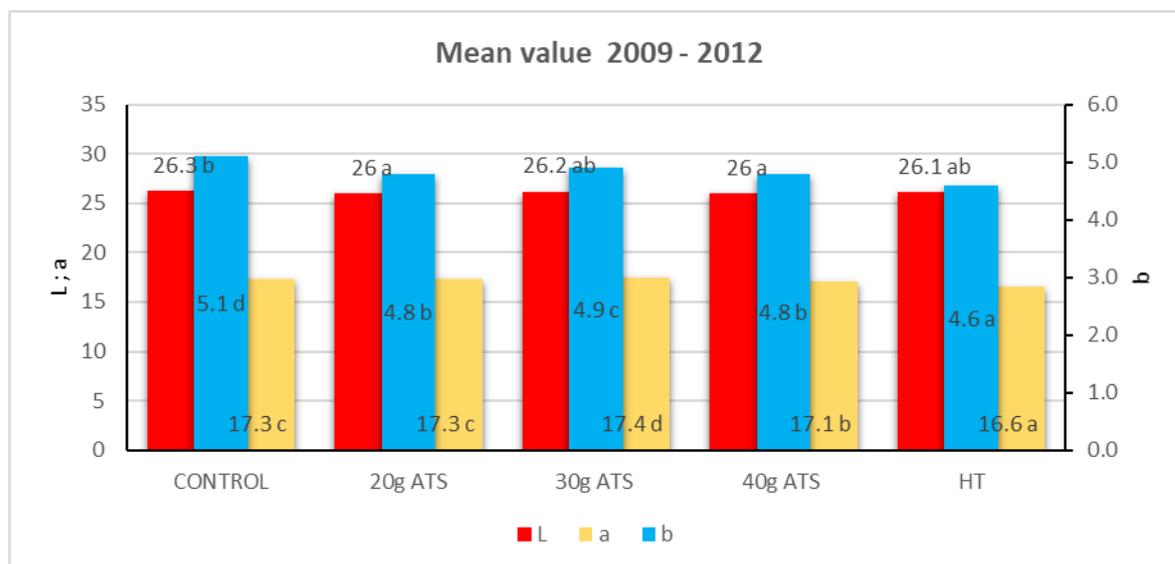


Figure 9. Figure Crop load regulation effect on the colour of 'Regina' sweet cherry fruit – mean values for the period 2009-2012.

Table 2. Crop load regulation effect on h°colour parameter of 'Regina' sweet cherry fruit – mean values for the period 2009-2012.

Treatment	2009		2010		2011		2012		Mean 2009-2012	
Control	18.0	c ²	13.8	ab	16.8	c	17.1	c	16.4	a
20g ATS	16.8	c	12.6	a	16.8	c	16.4	c	15.7	a
30g ATS	16.2	bc	13.7	ab	17.0	c	16.0	bc	15.7	a
40g ATS	15.9	bc	13.0	a	17.8	c	16.1	bc	15.7	a
Hand thinning	17.5	c	12.8	a	15.7	bc	15.9	bc	15.5	a
Annual mean value	16.9	b	13.2	a	16.8	b	16.3	b		

² two-way analyses of variance; ³ one-way analyses of variance; data marked with the same letter are not significantly different at $\alpha=0.05$ (Duncan's test)

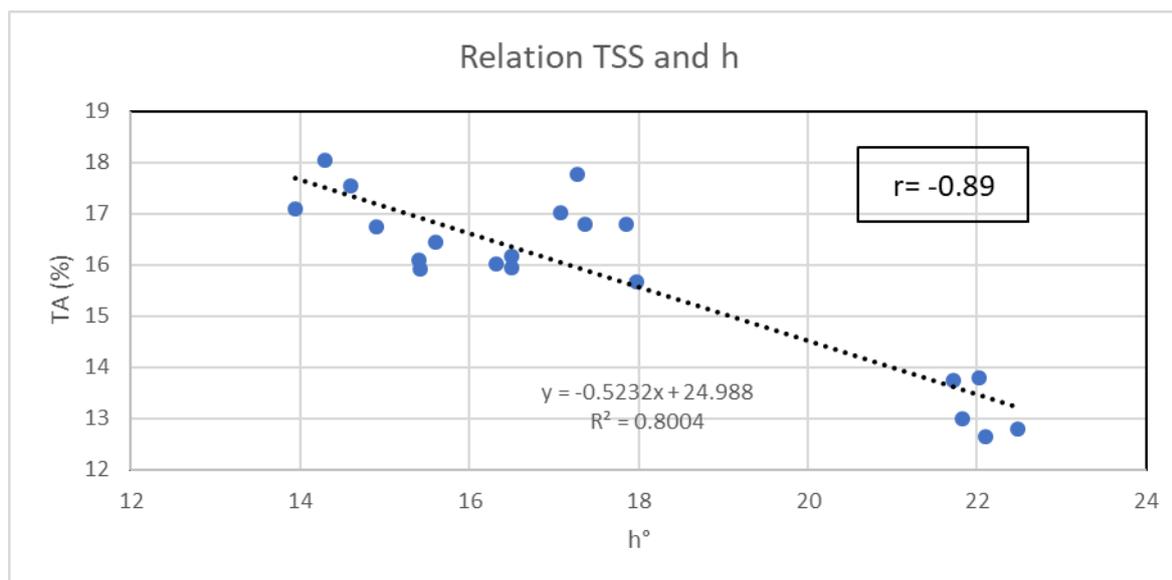


Figure 10. Relation between total soluble solids (TSS) of 'Regina' sweet cherry fruit and fruit skin colour parameter h° in the period 2009-2012 regardless of treatment.

Fruit firmness

Fruit firmness at harvest varied across the years of study, with the highest firmness value observed in 2010 (347.0 g x cm⁻¹) and the lowest in 2012 (248.9 g x cm⁻¹). Chemical thinning at 30g and 40 g ATS increased fruit firmness in each year (**Table 3**). This is consistent with studies where mechanical removal of flower buds resulted in higher firmness of the fruit [62]. Other studies also show that reducing the yield increases the size, the extract content and the firmness of sweet cherries [11]. The removal of spurs during tree dormancy contributed to the crop size reduction and increased fruit firmness [69]. A comparison of yields after different levels of mechanical thinning of cherry buds and flowers (20%, 50% and 80% of the buds removed) revealed that a higher yield was related to a lower fruit quality and a maturation delay [32].

Table 3. Effect of thinning on 'Regina' fruit firmness - mean values for the years 2009-2012.

Treatment	2009		2010		2011		2012		Mean 2009-2012	
Control	325.4	fg	346.7	j	288.2	c	247.2	ab	301.5	ab
20g ATS	325.1	fg	340.0	ij	290.9	c	239.7	a	298.9	a
30g ATS	333.5	gh	350.1	j	296.9	cd	261.7	c	316.8	c
40g ATS	311.8	e	359.3	k	294.6	cd	253.3	bc	304.8	b
Hand thinning	323.1	f	337.3	h	302.9	d	242.5	a	301.5	ab
Annual mean value	324.7	c	347.0	d	294.7	b	248.9	a		

two-way analyses of variance; ³ one-way analyses of variance; data marked with the same letter are not significantly different at $\alpha=0.05$ (Duncan's test)

Fruit size

Fruit size is the basic qualitative criterion of cherries determining their price [1]. The thinning procedures had an impact on this trait, i.e. improved the quality of yield, but only in years when there was no frost damage. In 2009, thinning increased the share of fruit with a diameter of more than 28 mm (**Table 4**). Fruit below 26 mm accounted for 24% of total fruit on the control trees, while any thinning treatment reduced the share of small and medium fruit to less than 10%. Whiting and

Ophardt [4] pointed out that manual thinning of flowers could be used as a reliable method to increase the share of premium fruit (diameters above 26.5 mm). Also, chemical thinning eliminated the smallest fruit below 21.5 mm while increasing the share of fruit above 26.5 mm by more than 400% [41]. In our study, over 90% of fruit collected from thinned trees in both 2009 and 2012 was large and very large (over 26 mm in diameter) (**Table 5**).

Table 4. Crop load regulation effect on the percentage of size categories of 'Regina' sweet cherry fruit (based on fruit diameter) in 2009

Fruit diameter (mm)	Fruit percentage according to size categories (%)									
	Control		20g ATS		30g ATS		40g ATS		Hand thinning	
< 22 mm	0	a	0	a	0	a	0	a	0	a
22 mm - 24 mm	4	d	0	a	0	a	0	a	0	a
24 mm - 26 mm	20	g	9	e	2	c	0	a	1	b
26 mm - 28 mm	49	j	40	i	26	h	25	h	57	k
28 mm - 30 mm	27	h	48	j	58	k	60	k	39	i
> 30 mm	0	a	3	d	14	f	15	f	3	d

two-way analyses of variance; data marked with the same letter are not significantly different at $\alpha=0.05$ (Duncan's test)

Table 5. Crop load regulation effect on the percentage of size categories of 'Regina' sweet cherry fruit (based on fruit diameter) in 2012

Fruit diameter (mm)	Fruit percentage in size categories (%)									
	Control		20g ATS		30g ATS		40g ATS		Hand thinning	
< 22 mm	0	a	0	a	0	a	0	a	0	a
22 mm - 24 mm	1	b	1	b	0	a	0	a	0	a
24 mm - 26 mm	32	i	5	d	8	e	5	d	6	d
26 mm - 28 mm	47	k	38	j	48	k	37	j	29	h
28 mm - 30 mm	20	g	54	m	35	j	49	kl	52	lm
> 30 mm	0	a	2	c	9	e	9	e	13	f

two-way analyses of variance; data marked with the same letter are not significantly different at $\alpha=0.05$ (Duncan's test)

Vegetative tree growth parameters

Fruiting and vegetative growth are negatively correlated [70]. Competition between fruit and leaves for nutrients significantly weakens growth in years of abundant yield [71–74]. In the present study, the strength and nature of vegetative growth were assessed based on the measurements of the number and length of one-year shoots and the trunk cross-sectional area (Table 6).

The mean number of annual shoots per tree in 2009 ranged from 42.5pc to 68pc. Trees whose fruit was not thinned at all and which had the highest yield had the smallest number of shoots (Table 6). Numerous shoots ending with a flower bud are desirable on the tree, as they allow to maintain high productivity. In the following year, which due to a poor setting was a year of poor yield, excessive growth was observed on unthinned trees, which was manifested in the largest number of shoots (over 100) (Table 6). In 2012, when the yield was high and the impact of thinning could be seen in all parameters measured, the number of shoots on unthinned trees was lower than on trees treated with ATS. Other important parameters characterizing tree vigour are the mean length of the shoots and their total length [75,76]. When it comes to the mean length of shoots, the 2009 results revealed the shortest shoots on trees thinned by hand, and the longest on trees thinned with ATS at

doses of 30g and 40g. The total length of one-year shoots of 14.8 m and 15.7 m, respectively, in the control and manually thinned trees was significantly lower than on trees thinned with 40g ATS (28.5 m). In 2010 and 2011, the differences in the length of shoots were smaller than in the full crop years, in which flowers were not damaged by frosts. The vigour of the tree measured by trunk cross-sectional area was also varied depending of the year of experiment. In the full crop years the trunk grew most strongly on trees thinned with ATS at a dose of 40g. (Table 6). Stronger vegetative growth of trees with a lower yield as a result of thinning is confirmed by other studies where non-fruiting cherry trees were characterized by a stronger increase in the number of shoots and a larger leaf area [77]. However, another experiment involving spur thinning showed that thinning influenced the leaf area, but had no effect on the length of shoots [78]. Undoubtedly, thinning treatments visibly affect tree growth; however, depending on the thinning method, timing, weather conditions prevailing in a given year and thinning agent dosage the effect can be observed on different tree vigour parameters.

Table 6. Crop load regulation effect on vigor parameters of 'Regina' sweet cherry trees in 2009-2012

Treatment	Shoot no. per tree		Mean shoot length		Total shoot length per tree		TCSA	
			(cm)		(m)		(cm ²)	
2009								
Control	42.5	a	34.8	b	14.8	a	74.8	b
20g ATS	48.2	b	39.2	c	18.9	b	73.8	a
30g ATS	56.9	c	42.4	d	24.1	c	78.6	d
40g ATS	68.0	d	41.9	d	28.5	d	84.4	e
Hand thinning	46.8	b	33.5	a	15.7	a	76.5	c
2010								
Control	101.5	e	38.5	a	39.1	c	93.9	a
20g ATS	95.8	d	44.4	d	42.5	d	95.9	b
30g ATS	87.5	c	41.3	b	36.1	a	98.0	c
40g ATS	82.0	a	43.9	c	36.0	a	103.7	d
Hand thinning	83.6	b	44.0	cd	36.8	b	93.8	a
2011								
Control	131.7	b	39.2	a	51.6	a	101.8	b
20g ATS	146.2	e	40.8	b	59.6	e	106.1	c
30g ATS	128.4	a	42.6	e	54.7	b	105.8	c
40g ATS	140.8	d	41.7	d	58.7	d	114.3	d
Hand thinning	139.0	c	41.2	c	57.3	c	99.7	a
2012								
Control	170.0	b	32.3	e	54.9	c	110.0	b
20g ATS	176.3	c	29.5	b	52.0	b	112.1	c
30g ATS	186.8	d	31.2	d	58.2	e	114.8	d
40g ATS	186.8	d	30.4	c	56.8	d	122.6	e
Hand thinning	163.5	a	29.0	a	47.4	a	108.0	a
Mean values 2009 - 2012								
Control	111.4	a	36.2	a	40.1	ab	95.1	a
20g ATS	116.6	a	38.5	bc	43.3	ab	97.0	a
30g ATS	114.9	a	39.4	c	43.3	ab	99.3	a
40g ATS	119.4	a	39.5	c	45.0	b	106.2	b
Hand thinning	108.2	a	36.9	ab	39.3	a	94.5	a

two-way analyses of variance; data marked with the same letter are not significantly different at $\alpha=0.05$ (Duncan's test)

5. Conclusions

Every thinning method has advantages and disadvantages. However, if a method produces the assumed results, it is worth implementing on a larger scale. Mechanical thinning, which is becoming more and more popular, is difficult to apply to cherries due to the size of trees and the risk of increased spread of diseases. Cherry trees are sensitive to bacterial canker [79,80] and mechanical thinning may significantly increase the risk of canker infection as a result of mechanical damage to leaves and shoots. The same negative impact of mechanical thinning has been observed in orchards that experience fireblight infections and therefore mechanical thinning is not recommended in plantations that have been infected with *E. amylovora* within the last 3 years [81].

The above-described drawback can be avoided by using such chemical substances which are cheap, most preferably environment-, user- and consumer-friendly [37] and do not require additional equipment. Many preparations have been withdrawn from the market for environmental reasons in recent years [28,37,82,83]. ATS is one of the substances still in use because it is safe for the environment and fruit [84]. This environmental friendly compound is a cheap and easily available foliar fertilizer [85] and shows very good results in the thinning of pome species [86–88]. It burns the sensitive parts of flowers and destroys the stigma thus preventing pollen germination and flower pollination [37,85]. Its efficiency depends on weather conditions [89]. In apples, the efficiency of ATS varies depending on dosage and cultivar [90].

Climatic conditions have a significant impact on the growth and yield of cherry trees. Low temperatures during flowering reduce yield, but yield can also be negatively affected by frosts in winter and relatively low (although still positive) temperatures during fruit fertilization and initial growth. Chemical thinning of flowers significantly reduces the yield, which is particularly well illustrated by the significantly lower yield parameters. The use of ATS for thinning flowers is an effective method to regulate fruiting, as shown by the alternate bearing index, whose value was the lowest after using ATS at a dose of 40g. The thinning treatments used in the experiment affected fruit quality but only in years of full crop. Total soluble solids and titratable acidity increased as a result of thinning. At the same time, fruit colour and TSS were found to be negatively correlated. The use of ATS allowed achieving darker fruit. Chemical thinning improved fruit quality in the years of full crop and resulted in a higher share of large and very large fruit in the total yield. The vegetative growth of trees was the strongest after applying the highest dose of ATS (40g) in the years of full crop.

Author Contributions: The following statements should be used “Conceptualization, R.K.; methodology, R.K.; software, R.K. and K.R.; validation, R.K. and K.R.; and G.P.L.; formal analysis K.R. and G.P.L.; investigation, R.K.; resources, R.K. and K.R.; data curation, R.K. and K.R. writing—original draft preparation, R.K. and K.R.; writing—review and editing, K.R. and G.P.L.; visualization, R.K. and K.R.; supervision, G.P.L.; project administration, R.K.; funding acquisition, K.R. and G.P.L. All authors have read and agreed to the published version of the manuscript.”.

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