Effects of temperature sum on vitamin C concentration and yield of sea buckthorn (*Hippophae rhamnoides*) fruit: optimal time of fruit harvest

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To investigate the effects of temperature sum on vitamin C concentration (V_c), yield and maturity of sea buckthorn fruit (*Hippophae rhamnoides* L.) and to predict the optimal harvest time, berries were collected from eight genotypes at an interval of about one week from August 16 to December 2. Maturity was visually observed, berry weight measured and V_c determined. Berries matured at 1165-1316 degree-days (d.d.). V_c reached maximum at about 1229 d.d., while fruit size and yield reached maximum at 1380 d.d.. Mathematical models of polynomial equations were highly significant for predicting the effects of temperature sum on V_c, maturity and fruit yield. Optimal harvest time for maximizing V_c, yield or economic income could be determined according to differential equations. Great variations in V_c, fruit maturity and fruit size suggested good opportunities for selection and breeding. Low rank correlations in vitamin C concentration during fruit maturity, however, call for special attention in selection and breeding.

Key words: fruit size, maturity, mathematical models, breeding

Introduction

Sea buckthorn, *Hippophae rhamnoides*, is widely distributed on the Eurasian continent. Its agricultural, nutrient, medical and ornamental value makes the plant a very promising subject for domestication. The nutrient and medical values have commanded great attention during the last decade. The birth of the special Chinese journal "Hippophae" in 1988 has provided researchers with information on the importance of the plant in China.

The berries of sea buckthorn are among the richest sources of vitamin C in edible fruits. There are a number of reports on the vitamin C concentrations (V_c) of *H. rhamnoides* berries. Results have revealed a large variation of V_c among subspecies, populations and genotypes of *H. rhamnoides* (TIAN 1985, LI et al. 1988, PLEKHANOVA 1988, WANG et al. 1990, ZHAO et al. 1991, WAHLBERG and JEPPSSON 1990, 1992). YAO et al. (1992) have investigated the variation of V_c between and within natural sea buckthorn populations in Finland. All these reports indicate good opportunities for selection and breeding. Nonetheless, ROUSI and AULIN (1977), YANG et al. (1988), LIU et al. (1990), WAHLBERG and JEPPSSON (1990) found a significant decline of V_c during fruit maturity. Their findings imply that harvest time is very critical for securing a high V_c in sea buckthorn berries. These studies showed the relations between V_c and the calendar dates of fruit collection. However, many studies have shown that dates of biological developmental stages vary greatly from year to year due to yearly climatic fluctuations.

Temperature sum or heat sum T_s degree-days (d.d.), particularly the effective temperature sum (>5°C), has been widely used for observing the phenology of horticultural, agricultural and silvicultural plants. Results have constantly indicated that T_s is a better predicator than calendar date for budbreak, flowering, fruit setting, harvest time and yield (DANIEL and BAJTAY 1984, KRISTENSEN et al. 1987, RYSAVA and PORUBA 1987, HARI and HÄKKINEN 1991).

The purpose of the present study was to investigate the effects of temperature sum T_s on fruit maturity, vitamin C concentration V_c and fruit yield in sea buckthorn. The study also addressed relationships among these variables. A model is proposed to predict the optimal time of harvesting sea buckthorn berries. In addition, ranking and selection of genotypes for breeding are discussed.

Material and methods

From August 16 to December 2, 1990, berry samples were collected separately from eight individual bushes of a plantation growing in Helsinki harbour. Collections were made at approximately weekly intervals, except for the last three collections, which were taken at intervals of two and three weeks. The plantation was of Danish origin and 18 years old, consisting of about 400 bushes. Bushes were selected at a regular interval in the plantation, and the collected sample was represented by eight genotypes.

When sea buckthorn berries mature, their colour usually turns yellow, orange or red depending on the genotype. These colours are normally taken to indicate fruit maturity. In the present study, the process of fruit maturity of sea buckthorn berries was observed on the basis of colour changes. The percentage of matured berries (M_p) on each bush, i.e. the proportion of yellow, orange or red berries on each bush, was recorded according to visual observation during collection at the site. This percentage M_p was used as a quantitative measure of the degree of fruit maturity on a bush or group of bushes. When M_p was 100%, the bush had reached full maturity.

After each collection, berry weight (weight/100 berries) was measured immediately in three replications, except for bush 8 which bore too few berries to allow this measurement. The samples were then kept at -20°C until determination of V_c.

V_c was determined by high-performance liquid chromatography (HPLC) on the collection day or the following day according to the method developed by VUORELA et al. (1986). To avoid any loss of vitamin C by breaking of berries, approximately 10 g of unbroken berries were exactly weighed from each bush. After weighing, each sample was immediately immersed in 20 ml of extractant solution, then homogenized and centrifuged. In order to make the sample concentration fall within the calibration range, 1 ml of sample solution was diluted with 2 ml of extractant. A 10 μl aliquot of this diluted sample solution was used in the HPLC system for analysis. An external standard was used after every four samples had been chromatographed.

The calibration curve ($r^2 = 0.998$) was based on six replications for each amount of injection. The computation of V_c was based on the exact weight of each berry sample (including seed weight). Following the convention, V_c was expressed as mg% (milligrams of vitamin C per 100 grams of berries).

The above method detects only L-ascorbic acid. In addition, what is usually called vitamin C also includes the oxidized form of L-ascorbic acid (dehydroascorbic acid). However, its amount seems to be generally small in mature fruits (MAPSON 1970) and is ignored in most analyses (YAO et al. 1992).

The effective temperature sum (over 5°C), simply called temperature sum T_s, was calculated based on the daily mean temperature observed at the Kaisaniemi Weather Station (Ilmatieteen laitos 1990), one kilometer from the plantation. T_s reached 1419.4 d.d. on November 2 and then ceased to increase any further. SAS programs RSQUARE and GLM (SAS Institute 1985) were used for calculation and data analysis including modelling.

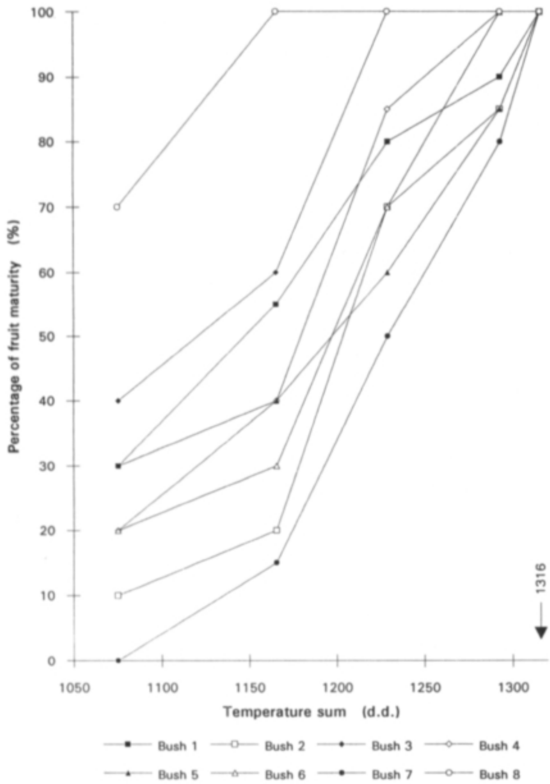

Fig. 1. Effects of temperature sum on fruit maturity.

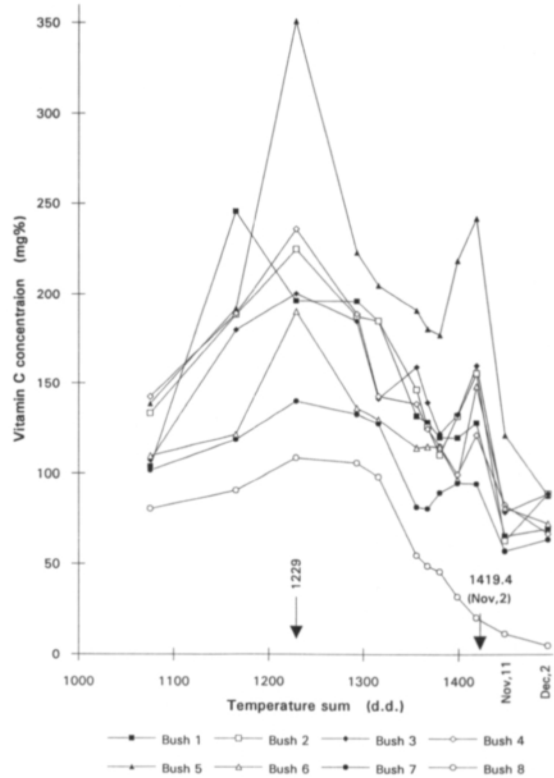

Fig. 2. Effects of temperature sum on vitamin C concentration. (Points for Nov. 11 and Dec. 2 on the X axis given in time scale, see text).

Results

Effects of temperature sum on fruit maturity

Observations showed that the sea buckthorn berries changed from green to yellowish green, then to greenish yellow and finally to yellow, orange or red during their maturity process. There was a large variation in fruit maturity among genotypes (Fig. 1). Bush 8, the earliest one, was 64 d.d. (one week) earlier than bush 3, 128 d.d. (two weeks) earlier than bushes 4 and 6, and 151 d.d. (three weeks) earlier than the rest. Recent years' phenological observations have confirmed that bush 8 is one of a few genotypes characterized by early flowering, budbreak, growth cessation, maturity and leaf secession. The results indicated that there are good opportunities for selection and breeding for early maturity.

Effect of temperature sum on vitamin C concentration

The effects of temperature sum T_s on vitamin C concentration V_c are shown in Fig. 2. There were two peaks in V_c for all bushes, except that there was only one poorly defined peak for bush 8. The first peak was distinct and high for all bushes except that it was flat for bushes 7 and 8. The second peak was also obvious though it was much less pronounced than the first one. The first peak of V_c appeared at 1229 d.d. (August 31) for all but for bush 1 at 1165 d.d. (August 24).

Bushes 7 and 8 always ranked lowest, while bush 5 almost invariably ranked highest for V_c. The remaining five bushes varied considerably with collection time and frequently changed their ranks. This indicates that except for the genotypes with extreme values of V_c, selection of genotypes for

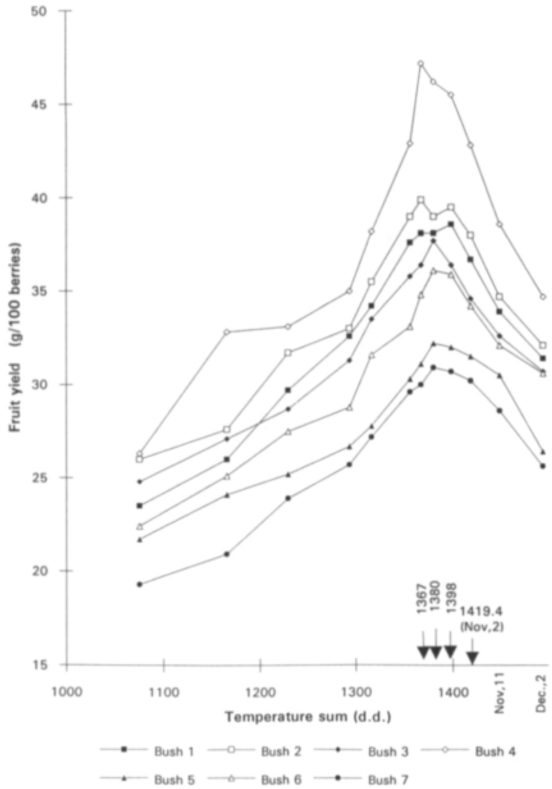

Fig. 3. Effects of temperature on fruit yield (fruit size). (Points for Nov. 11 and Dec. 2 on the X axis given in time scale, see text).

high V_c based on one measurement at one time should be used with caution. In fact it would be difficult to separate genetic variance from experimental error variation in such genotypes.

Effects of temperature sum on fruit yield

Several years' observation has shown that premature drop and developmental failure of sea buckthorn fruit occur only in the early stages of fruit development. A normally developing berry in July almost always proceeds towards maturity. Therefore the number of berries on a bush after the end of July remains basically unchanged (a constant) and thereafter the fruit yield (Y_f) of a given bush or plantation is determined only by the average size or weight of fruit. In this case the fruit weight or size is an index of fruit yield, and the effects of T_s on fruit weight or fruit size are equivalent to the effects of T_s on fruit yield of a given bush or plantation. Therefore in the present study, the effects of T_s on fruit weight, fruit size and fruit yield are used interchangeably whenever convenient. However, when different bushes or genotypes are compared, fruit weight or size is no longer a proportionate index of fruit yield.

The effects of temperature sum T_s on fruit yield Y_f are presented in Fig. 3. The variation of Y_f of sea buckthorn with maturity appears as a curve with a single peak. With the increase of T_s, Y_f increased steadily until 1367 d.d. (September 30) when bushes 4 and 2 reached their maximum values. At 1380 d.d., bushes 3, 5, 6, 7 and virtually the entire plantation reached their maximum yield, while bush 1 reached its maximum yield at 1398.

The ranking of bushes based on the fruit weight was rather consistent in contrast to the rankings based on V_c. This suggests that genotypes with large berries will generally retain this characteristic regardless of collection time and that selection for genotypes with large berry size based on a single measurement in time is reliable.

Relationships among vitamin C concentration, fruit yield, vitamin C yield and fruit maturity

From the results (Figs. 1, 2 and 3) we know that the maximum V_c appeared at 1229 d.d., 87 d.d. earlier than full maturity (1316 d.d) and 151 d.d. earlier than maximum fruit yield Y_f (1380 d.d.). To enable a meaningful examination of the general relations among V_c, Y_f and M_p, averaged values of V_c, Y_f and M_p over the eight bushes were expressed as a percentage of their corresponding maximum values (Fig. 4).

From 1075 d.d., the first collection, to 1229 d.d, V_c, Y_f and M_p all increased with T_s. At 1229 d.d., V_c reached its maximum, while Y_f and M_p reached 77% of their maxima. Thereafter V_c started to decrease, while the fruit yield Y_f and fruit maturity M_p continued to increase until M_p reached 100% at 1316 d.d.. Beyond this V_c continued to decrease

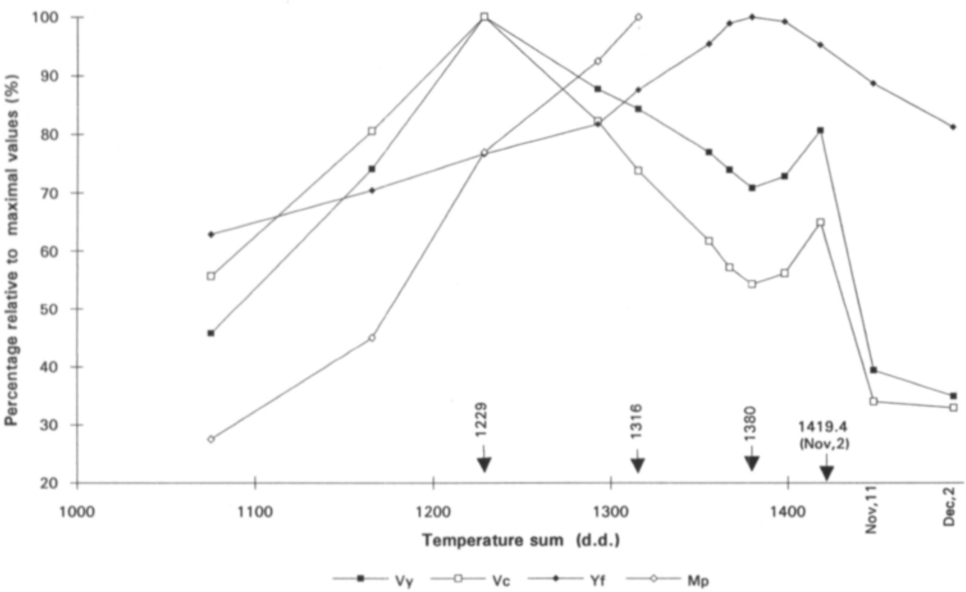

Fig. 4. Relationships among vitamin C concentration (V_c), fruit yield (Y_f), fruit maturity (M_p) and vitamin C yield (V_y). (Points for Nov. 11 and Dec. 2 on the X axis given in time scale, see text).

while Y_f still kept increasing until Y_f reached its maximum at 1380 d.d.. At this temperature sum V_c reached its minimum value, 54.3% of the maximum. Again, V_c began to increase and Y_f to decrease until V_c approached its second peak, whereafter both decreased sharply.

It is interesting to note that between 1229 and 1418 d.d., Y_f and V_c were always negatively correlated (Fig. 4). One may wonder whether the amount of vitamin C in the berries was constant (maybe dynamically) during this period, and the variation in V_c caused only by growth in fruit size. To answer this question, a new measure, vitamin C yield V_y was calculated. V_y was defined as V_c multiplied by Y_f, $V_y = V_c Y_f$, i.e. total amount of vitamin C in 100 berries (or a berry, or a bush). If the variation in V_c was caused only by an increase or decrease in fruit size, V_y should be constant during fruit maturity. However, this was not the case. Fig. 4 tells us that the amount of vitamin C in a berry V_y was not constant during the fruit maturity and post-maturity processes. Therefore the variation of V_c was caused not only by fruit size Y_f but also, and more importantly, by the variation of total amount of vitamin C in a berry, i.e. the amount of vitamin C synthesized and decomposed in a berry during the fruit maturity and post-maturity processes.

Modelling the effects of temperature sum on vitamin C concentration, fruit yield, vitamin C yield and maturity

The results above showed that M_p, V_c, Y_f and V_y were all functions of temperature sum T_s. Therefore mathematical models could be built to simulate and predict the effects of T_s on M_p, V_c, Y_f and V_y, and furthermore to determine the optimal harvest time. Since the temperature sum showed no further increase after November 2 and the fruit of sea buckthorn is rarely harvested later than the end of October, only the data before the end of October were used for modelling and the resulting models were applicable to the same period.

Mathematical models could be built for each bush or genotype if necessary. However, the following mathematical models in Table 1 for simulating and predicting the effects of T_s on M_p, V_c, Y_f

Table 1. Models for predicting fruit maturity, vitamin C concentration, fruit yield and vitamin C yield ($P < 0.015$ for all parameters in the equations).[1]

Model	R^2	F	P
1 $M_p = -313 + 314T_s$	0.978	130.6	0.0014
2 $V_c = 372051 - 1225143T_s + 1505770T_s^2 - 818346T_s^3 + 165946T_s^4$	0.987	97.0	0.0001
3 $Y_f = -27081 + 88365T_s - 107706T_s^2 + 58159T_s^3 - 11734T_s^4$	0.989	116.5	0.0001
4 $V_y = 92721 - 304691T_s + 373578T_s^2 - 202492T_s^3 + 40950T_s^4$	0.973	45.3	0.0004
5 $V_c = 180607 - 594730T_s + 730956T_s^2 - 397255T_s^3 + 80557T_s^4$	0.987	97.0	0.0001
6 $Y_f = -72799 + 237540T_s - 289533T_s^2 + 156340T_s^3 - 31542T_s^4$	0.989	116.5	0.0001
7 $V_y = 157816 - 518579T_s + 635794T_s^2 - 344605T_s^3 + 69687T_s^4$	0.973	45.3	0.0004

[1] M_p = percentage of matured berries, T_s = effective temperature sum, V_c = vitamin C concentration, Y_f = fruit yield, V_y = vitamin C yield. (see text for further explanation).

and V_y are based on the average values of the eight bushes.

The equations in Table 1 are divided into two groups. The upper group predicts the values of the variables V_c, Y_f, V_y and M_p in the units defined in the text, and the lower group predicts the percentage relative to their maximum values. In both cases T_s in the equations is the temperature sum divided by 1000. If one's purpose is to know actual values, equations in the upper group are used. If the purpose is to know the value of a variable relative to its maximum or to determine the optimal harvest time, the lower group of equations are more suitable. The R^2, F and P values indicated that all models listed in Table 1 have a good predictive power. If the power of polynomial equations was reduced to 2, R^2 was still as high as 0.942 for Y_f but dropped to 0.656 and 0.730 for V_c and V_y, respectively.

Predicting and determining optimal time of harvest

With the above equations, the optimal time of harvest, which maximized an objective, can be mathematically determined. This was done by taking differentials of above equations and solving for T_s from the differential equations, i.e. by solving the equation $dF(T_s)/dT_s = 0$. The solutions for T_s must be multiplied by 1000 to give the temperature sum.

Optimal harvest time for maximizing vitamin C concentration

If the objective is to maximize V_c, vitamin C concentration, from $dV_c/dT_s = 0$ we get $T_s = 1233$ d.d., where T_s means the value of T_s which maximizes V_c. This was 83 d.d. (or about 2 weeks) earlier than the time of full maturity (1316 d.d.), or at the time corresponding to 77% of full maturity. From Figs. 2 and 4 and equations 2 and 5 we know that V_c increased and dropped sharply on either side of its maximum. This sensitivity of V_c to T_s suggests a narrow period of optimal time of harvest for obtaining higher V_c. In other words, to obtain higher V_c, harvest must be carried out within a very short period of time.

Optimal harvest time for maximizing fruit yield

If the objective is to obtain maximum fruit yield Y_f, $dY_f/dT_s = 0$ gives the optimal harvest time $T_s = 1389$ d.d. which was 156 (39 days) and 73 d.d. (26 days) later than those for V_c (1233) and full maturity (1316), respectively. Figs. 3 and 4, and equations 3 and 6 all show that Y_f was insensitive to T_s around its maximum value. This indicates a rather long optimal time of harvest for fruit yield Y_f. Actually, harvest could be conducted for a period of 37 (62) d.d. and still ensured Y_f over 99% (95%) of its maximum. Furthermore because of the slow increase of T_s in later September onwards, 37 (62) d.d. of T_s was equal to about 2 (5) weeks of harvest time.

Optimal harvest time for maximizing vitamin C yield

If the objective is to obtain maximum vitamin C yield V_y, i.e. the purpose of harvest is to extract and to produce natural vitamin C, then $dV_y/dT_s = 0$ gives the solution of $T_s = 1250$ d.d. which was 17 d.d. (2 days) later and 66 d.d. (11 days) earlier than the time for Vc and fruit maturity. The harvest time could also be slightly longer for V_y because V_y was not as sensitive as V_c to T_s after its maximum.

Optimal harvest time for maximizing income I

In the above discussion of optimization, the first consideration was to maximize nutrient concentration or quality and the subsequent considerations were to maximize yield. From the standpoint of commercial production the objective is to maximize the income or profit, i.e. to maximize the expression $I = PY_f$ where I = income, P = price and Y_f = fruit yield as before. If the price is a function of V_c, i.e. $P = P(V_c)$, the equation becomes $I = PY_f = P(V_c)Y_f$. We already know that V_c and Y_f are functions of T_s, so income I is also a function of T_s. To maximize I is to find out the solution for $dI/dT_s = 0$. This could be done mathematically provided that the equation $P = P(V_c)$ was defined, i.e if the relation between the price and V_c were known.

If the price P is a linear function of V_c, i.e. $P = \alpha + \beta V_c$, then $I = PY_f = (\alpha + \beta V_c)Y_f$. If the price is constant regardless of V_c, then $\beta = 0$, $P = \alpha > 0$ and $I = \alpha Y_f$, and the T_s maximizing fruit yield Y_f will also maximize the income I. If the price is proportional to V_c, then $\alpha = 0$, $\beta > 0$, $P = \beta V_c$ and $I = \beta V_c Y_f = \beta V_y$, and the T_s maximizing vitamin C yield V_y will also maximize the income I. If neither α nor β is equal to zero, the T_s maximizing the income must lie between the two T_s values which maximize V_c and Y_f. If α is relatively smaller, the solution of T_s is closer to the T_s maximizing V_c, but if α is relatively larger it is closer to the T_s maximizing Y_f.

Discussion

Many studies have revealed large variations of V_c in sea buckthorn berries among subspecies, populations and genotypes, suggesting good opportunities for breeding and selection for high V_c. Variation of V_c during the fruit maturity process has, however, posed another important question: what is the optimal harvest time?

Results of the present study showed that V_c of sea buckthorn berries reached a maximum at 1229 d.d., 87 d.d. earlier than full maturity (1316 d.d.) and 151 d.d. earlier than the maximum yield (1380 d.d.). At that time the fruit had attained 77% of full maturity and its yield was 77% of the maximum. When the berries reached full maturity at 1316 d.d., the fruit yield had reached 88% of its potential, while V_c had dropped to 74% of its maximum. When yield reached its maximum value at 1380 d.d., V_c dropped to its minimum, about half of its maximum value.

YANG et al. (1988) found a peak and a valley of V_c during fruit maturity, similar to that observed in the present study. LIU et al. (1990) showed only a single peak of V_c, corresponding to the first peak of this study, because of their short study period. ROUSI and AULIN (1977), WAHLBERG and JEPPSSON (1990) showed only a decline of V_c, corresponding to the right part of the first peak in this study, because of their later beginning and short study period. Actually the peak and later rising of V_c could be found by carefully examining the scattering of WAHLBERG-JEPPSSON's Fig. 3. Therefore the pattern of variation shown in this study appears common in sea buckthorn.

The variation of fruit size during fruit maturity was similar to that found in other studies though they used date rather than temperature sum. YANG et al. (1988) indicated that the secondary rise of V_c was probably due to a decrease of fruit weight. The vitamin C yield V_y calculated in the present study showed that the increase and decrease of V_c during fruit maturity followed the total amount of vitamin C synthesized and decomposed. In rose hips, V_c also shows peak variation during fruit development (UGGLA 1988). However, a decline of V_c is generally found in black currant and other fruits during ripeness (HÅRDH 1964, SISTRUNK et al. 1983), simply because the peak appears at the immature stage before the analysis starts. The second rising of V_c is probably a defending reaction of the postmatured fruit, and the following sharp decrease might indicate the deterioration of fruit, similar to the respiration peak during the storage in apples, pears and other fruits.

In the present study, the author developed mathematical models based on temperature sum T_s providing good predictions of fruit yield, vitamin C concentration and vitamin C yield. The optimal harvest time could be determined by the models. The best harvest time, however, depends on one's objectives. Vitamin C concentration is only one of many nutrient and other quality factors. For commercial production the price of fruit is a function of quality index (integrating all nutrient and other quality factors). If V_c is the only important factor, optimal harvest time can be determined for maximizing V_c, V_y, Y_f or the economic income. Furthermore, the models provide a flexible way of determining optimal harvest time under one or more constraints of quality, weather or labour restrictions.

The present study using temperature sum as predictor overcame some of the problems caused by using calendar date, and the models fitted the actual data very well. However, as the temperature sum reaches a ceiling value in the late autumn, further observations on a "time scale" must be given in calendar dates, as indicated in Figs. 2-4. At this stage of fruit development changes may occur that are independent of the T_s-dependent "biological clock".

The study showed large differences in fruit maturity among genotypes. The earliest genotype was 151 d.d. earlier than the latest ones. The results have also been confirmed by the observations made in recent years by the author. This suggests good opportunities for selection and breeding for early maturity, particularly useful under Finnish conditions. The relatively constant rank of genotypes based on fruit size should make it easy to breed for large berries, an important yield component, particularly in developing an efficient harvesting technique. The low rank correlation among genotypes based on V_c during fruit maturity calls for special attention in selection and breeding. In addition, the large variation of V_c during different collection times makes comparison of different analyses difficult.

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SELOSTUS

Lämpösumman vaikutus tyrnin marjasatoon ja marjojen C-vitamiinipitoisuuteen

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Lämpösumman vaikutusta tyrnin (*Hippophaea rhamnoides*) C-vitamiinipitoisuuteen, marjasatoon ja kypsyysasteeseen tutkittiin kahdeksalla eri genotyypillä. Optimikorjuuajankohdan määrittämiseksi koejäsenistä otettiin näytteitä viikon välein elokuun puolivälistä joulukuun alkuun. Kerätyistä näytteistä määritettiin visuaalisesti kypsyysaste, punnittiin sato ja määritettiin C-vitamiinipitoisuus. Marjat kypsyivät lämpösumman ollessa 1165-1316 astetta. Maksimi C-vitamiinipitoisuus havaittiin noin 1229 asteen lämpösummalla, kun taas marjojen koko ja samalla marjasato olivat suurimmillaan lämpösumman ollessa 1380 astetta.

Havaintojen pohjalta laadittiin matemaattinen malli, jonka avulla voidaan ennustaa lämpösumman vaikutus tarkasteltuihin ominaisuuksiin. Vastaavasti sadonkorjuun optimaalinen aika C-vitamiinipitoisuuden, sadon ja taloudellisen tuloksen kannalta voitiin määrittää mallin pohjalta laaditun yhtälön avulla. Genotyyppien välinen suuri muuntelu tyrnin C-vitamiinipitoisuudessa, marjojen kypsyysasteessa ja marjojen koossa osoittavat tyrnin jalostukselle olevan hyvät mahdollisuudet.