



Effects of different overwintering conditions on spring phenology of the seasonal leaves of different woodland strawberry (*Fragaria vesca*) genotypes

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Abstract

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Abstract: Woodland strawberry (*Fragaria vesca*) is a perennial herb in the Rosaceae family with dimorphic leaves, summer and winter leaves, adapted to seasonal climate. Woodland strawberry produces a new set of leaves in spring that are photosynthetically active throughout the summer season (summer leaves), and the leaves senesce in autumn when they are replaced by a new set of leaves (winter leaves). The winter leaves retain photosynthetic capacity under the snow cover throughout the winter season, which prolong the photosynthetic period of the species. With the world-wide climate warming, the thickness of winter snow is decreasing, which can affect overwintering and spring phenology of plants. This thesis focuses on springtime ecophysiology and phenology of the senescing winter leaves and the formation of new summer leaves of woodland strawberry genotypes of different European origin. The 15 different genotypes of woodland strawberry are from Iceland, Italy and Norway, and they originate from different environments that are geographically separated from each other, so the populations are genetically distinct. In this study, these genotypes were kept at two different overwintering sites, coastal site at the Åland islands with mild temperatures, and continental site in Lammi with a persistent snow cover. According to the results all 15 genotypes showed earlier development of the summer leaves and earlier senescence of winter leaves in the group with Åland as overwintering site than in the group with Lammi. Another important finding is that the first summer leaves produced in spring begun to senesce shortly after they are fully developed and were replaced by later formed summer leaves. Specifically, the dates of summer leaf formation, flowering and stolon production were advanced, and the dates of winter leaf senescence were also advanced. The value of different leaf types to chlorophyll fluorescence was also lower at the Åland site. Therefore, it can be concluded that overwintering conditions have an effect on the subsequent phenological development in spring. In the context of global climate change, the spring development of woodland strawberry will be earlier, and the senescence of winter leaves will also be earlier.

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1 Introduction

1.1 Life cycle of woodland strawberry (*Fragaria vesca*)

Woodland strawberry (*Fragaria vesca*) is a perennial herb of the Rosaceae family that lives widely in Asia, Europe, and North America (Darrow 1966). Woodland strawberries grow in open terrain, such as meadows and forest edges in the high latitudes of the Northern Hemisphere, and the flowering period is usually between May and July. As a perennial herb growing at high latitudes, a serious challenge in its life cycle is inevitably the seasonal climate, in particular, freezing temperatures below 0°C, extreme dryness, and lack of light in winter. Due to these harsh winter conditions, woodland strawberries take advantage of the summer months when light and temperature conditions are good for reproduction. The leaves are specialized and divided into two types: winter leaves and summer leaves (Åström *et al.* 2015). These two types of leaves alternate each year: summer leaves are produced in spring and die off in autumn, and winter leaves are produced in autumn and die off in spring. This growth mechanism allows woodland strawberries to have green leaves to sustain photosynthetic activity throughout the year (Chabot 2007).

Woodland strawberries, like most strawberries, have a seasonal flowering habit. There are two types of flowering habits: seasonal flowering and recurrent flowering. The woodland strawberry, which is distributed in high latitudes, is a seasonal flowering plant that lives in an environment with short daylength in winter and long daylength in summer (Heide *et al.* 2013). The flowering period is determined by the differentiation pattern of the plant's meristematic tissues, with the inflorescences produced by apical buds flowering earlier in the plant's life cycle and the inflorescences produced by axillary buds flowering later in comparison (Costes *et al.* 2014).

The axillary meristems can be differentiated into lateral branches or stolons, and the ratio of stolons to lateral branches of the inflorescence has been inferred to be dominated by gibberellins (Hytönen and Kurokura 2020). In general, woodland strawberries do not start to differentiate reproductive stems (inflorescences) or stolons until the plants are fully mature. However, the production of reproductive stems is closely related to the growth of the plant itself and the external environment,

so the growth and development of reproductive stems is also a criterion for judging the growth status of woodland strawberries (Costes *et al.* 2014). Stolons, also known as runners, are actually long shoots produced by strawberries with long internodes that sprout new rosettes at the nodes (Andrés *et al.* 2021). Due to the presence of flowers and stolons, woodland strawberry has both sexual and asexual means of reproduction, which provides a great advantage in terms of gene retention and dispersal in extreme environments (Körner 2003).

1.2 Life under snow

Living in high latitudes means facing seasons of high variability. Across a wide range of high latitudes, different organisms have their own different strategies for surviving the winter (Marchand 2014). Annual plants often cope with winter conditions by producing seeds before the onset of winter, allowing the seeds to lay dormant over the winter and germinate and grow again in the following spring. On the other hand, perennial plants suspend their physiological activity during the winter and resuming it in the following spring. Woodland strawberries produce winter leaves adapted to winter conditions to maintain physiological activity under winter snow and to resume physiological activity levels more quickly in the following spring. This thesis focuses on the effect of different winter conditions on the spring phenology of woodland strawberries.

A very integral aspect of the winter ecology is snow, especially in the high latitudes. This is because at high latitudes, the snow can cover the entire ground in winter and protects plants from cold as an efficient insulator (Körner 2003). If the ground loses the protection of snow in late winter, the moisture in the soil and vegetation will freeze more easily and then sublimate and dissipate into the air. Soils will become dry, and vegetation will be subject to drought stress in addition to freezing. A certain thickness of snow can maintain the soil surface temperature close to 0°C at an outside air temperature of -33°C. The density of the snowpack also influences its insulating effect, as a denser snowpack (higher density) reduces the ability of the snowpack to insulate heat (Körner 2003).

Interestingly, according to Körner and Larcher (1988), cold climates are not intuitive for plants. That is, cold does not act on all plants in a single form. Plants with their origins in cold regions such as high mountains and high latitudes make certain adjustments to their structural and functional characteristics to adapt to their environment. The winter leaves of the woodland strawberry, for example, differ in anatomy from the summer leaves. In winter leaves, the leaf cells are more closely packed in the mesophyll and the proportion of air spaces between cells is lower (Åström *et al.* 2015). Lundell *et al.* (2008) showed that *Vaccinium vitis-idaea* L. maintained photosynthetic capacity throughout the winter. As an evergreen perennial dwarf shrub that also lives at high latitudes in the Northern Hemisphere, *V. vitis-idaea* also maintains photosynthetic capacity of leaves under snow. However, the results of Lundell *et al.* (2008) also indicate that photosynthetic inhibition occurs immediately after the end of winter and the melting of the snow. This may be a stressful phenomenon for the leaves in response to after snow melting: the exposure to high light levels.

1.3 Common spring phenology in Northern Hemisphere

Phenology refers to the cyclical phenomena of living organisms, including the germination, flowering, and fruiting of plants, as well as the hibernation of animals and the migration of migratory birds. This thesis focuses on the specific phenomena of spring phenology, senescence of winter leaves, summer leaf production, flowering and stolon formation. The leaf formation is the most typical, and arguably the most intuitive representation of the "plant phenology" that can be observed. It often occurs in spring and is considered a sign of the arrival of spring. The sprouting of leaf buds is an extremely critical physiological activity for the plant itself, and in trees, it has an irreplaceable key role in the development of the canopy and thus the degree of photosynthesis (Fu *et al.* 2012). In the Northern Hemisphere, the leaf phenology of trees has extensively been studied. A large amount of data has been used to develop relevant spring phenology models that can be applied to predict future phenology and to analyze the relationship between leaf phenology and environmental factors (Sarvas 1972; Sarvas 1974; Linkosalo 2000; Fu *et al.* 2012). In the past studies, leaf phenology

was considered to have a high correlation with temperature. The previous hypothesis was that the higher the spring temperature, the earlier the leaf production and the earlier the leaf development would be completed and unfolded (Cannell 1985). However, after continuous data collection and repeated analysis, researchers have found that spring leaf production and expansion are becoming less and less temperature dependent. That is, even in the context of global climate change, spring temperatures are getting warmer and warmer, and the time of spring leaf production and unfolding is not as much earlier as expected (Hikosaka *et al.* 2010; Fu *et al.* 2012). However, for perennials, both trees and herbs, winter climate change may have an impact on spring phenology as well. This aspect has not been studied as much as the effect of spring climate change on spring phenology, which is why this thesis is based on the spring phenology after overwintering at different winter climate conditions.

Plant flowering is also one of the important phenological phenomena, especially evident in cash crops. There are two aspects of plant flowering phenology: the date of flower unfolding and the duration of flowering. Major phenological studies have focused on the date of flower unfolding, while studies focusing on the duration of flowering have received little attention (Bock *et al.* 2014). This is also related to the traditional way of investigation, but now that continuous data collection has become common, it is possible to explore the relationship between flowering duration and environmental factors (Bock *et al.* 2014). The results of an extensive data analysis (a 27-year weekly floral observation dataset of 232 plant species in Guernsey, English Channel) (Bock *et al.* 2014), concluded that, over time, the date of flowering onset was significantly earlier, and the duration of flowering was significantly shorter due to the climate change. Moreover, the effect of temperature change on flowering duration was more pronounced than on the date of flowering onset.

In addition to foliage and flowering events, the woodland strawberry, as a clonal plant, produces asexual reproduction stolons in spring (Kymäläinen 2016). Therefore, the production of stolons, or runners, is a necessary element to record the spring phenology of woodland strawberry (Blanke and Cooke 2004). Since the stolon is an organ to clone the parent, its production also means that the woodland strawberry can spread its genes in a certain range. This movement through asexual reproduction

gives the woodland strawberry a mobility, allowing it to transfer its genetic material in harsh conditions for the plants to spread (Körner 2003; Zhang and Zhang 2007). Clonal growth is necessarily related to the allocation of resources to the plant proper and will also be closely related to environmental conditions. The correlation between environmental conditions and asexual reproduction of cloned plants can be explored when individuals are in the certain age. Life span theory predicts that there is encroachment between different types of reproductive methods (Williams 1966). When plants invest too much in one trait (reproductive mode), they will inevitably invest less in other traits. Thus, the production of stolons itself also implies the adaptation of the parent plant to the existing survival environment and the ability to put resources into asexual reproduction.

1.4 Climate change: winter warming in northern Europe

Climate change, as a long-discussed topic, does not present itself in a single form. The increased frequency of extreme weather phenomena, summer heat, storms, hurricanes, tsunamis, and winter warming are all part of climate change. Climate change in winter is reflected in the increase in minimum and average temperatures, the movement of the snow line towards the poles, and the change in winter precipitation (Jylhä *et al.* 2008). Changes in winter temperatures can lead to unstable snowpack conditions, reduced snow thickness, changes in density, and repeated thawing of the snowpack (Körner 2003). As well, the intensity of the most severe extreme precipitation events increases with global warming, according to the conclusions of scholarly studies (Myhre *et al.* 2019). This is manifested in winter by greater frequency of more erratic snowstorms, which create instability for plants adapted to winter conditions. The adaptation of plants living in the Northern Hemisphere is gradually occur in accordance with climate change. Depending on the changes in climatic conditions, the phenology of different plants will show a certain plasticity (Nicotra *et al.* 2010).

1.5 Project description and hypotheses

This master's thesis focuses on the effects of different overwintering conditions on spring phenology of the seasonal leaves of different woodland strawberry (*Fragaria vesca*) genotypes. For the purpose of the study, senescence of winter leaves, chlorophyll fluorescence of winter and summer leaves, summer leaf formation, flowering and stolon production were measured and observed. This master's thesis is part of the PhD project of Sonja Still and a larger collaboration project between two research groups at different faculties at the University of Helsinki: Plant Ecophysiology and Climate Change (PECC) at the Faculty of Biological and Environmental Sciences, and Strawberry Research Group at the Faculty of Agriculture and Forestry.

By observing the phenological events in spring after overwintering in different locations for different genotypes of woodland strawberry, it is possible to reveal the relationship between different winter environments and spring phenology. Based on the dimorphism of woodland strawberry leaves (Åström *et al.* 2015), woodland strawberry summer leaves will form and mature in the spring. Therefore, all genotypes will produce summer leaves in the spring and during summer. Depending on the overwintering site, the timing of summer leaf production will vary. At the same time, winter leaves will remain functional for a period of time in spring before senescence. The hypothesis of this thesis is that the senescence process of winter leaves in woodland strawberry will be different depending on the overwintering conditions.

According to the possible effects of different overwintering conditions on the different genotypes of woodland strawberry plants, another hypothesis of this thesis is that different overwintering conditions will affect the spring phenology of woodland strawberries of different genotypes.

2 Materials and methods

2.1 Woodland strawberry genotypes

Woodland strawberry is a perennial herb of the Rosaceae family, and it is a promising plant for model organisms. Hilmarsson *et al.* (2017) had analysed 298 woodland strawberry plants by used microsatellite markers to analyse their population structure

and distribution of genetic diversity. As a collaboration project, the plants were collected by the research group of Timo Hytönen. The material for the study presented in this thesis consisted of fifteen European woodland strawberry genotypes that have originally been collected from wild in three European countries: Italy (labelled IT2, IT8, IT12), Iceland (labelled ICE2, ICE4, ICE16), and Norway (labelled NOR17, NOR16, NOR13, NOR11, NOR29, NOR27, NOR5, NOR6, NOR9) (Hilmarsson *et al.* 2017). These plant materials were not only used in this thesis, but also used in a doctoral thesis of Sonja Still.

Table 1 provides detailed information on the collection of these 15 genotypes, using the World Geodetic System-1984 Coordinate System, and the information in Table 1 was used to create Figure 1 based on Google Maps. The distribution of the 15 genotypes in terms of geographic location is shown in the map (Fig. 1). Altitude data are not included in Table 1 because they were not fully recorded at the time of collection; only the three Italian genotypes and NOR5 had altitude data. (IT2: 590m; IT8: 810m; IT12: 934m; NOR5: <50m.)

The three genotypes from Italy were from Trentino in northern Italy and the three genotypes from Iceland were from southern Iceland. The nine genotypes from Norway were divided into three groups that were collected from three different locations in northern Norway: Kåfjord, Tromsø and Alta. The different source locations mean that the 15 genotypes may have different overwintering performance and spring phenology, which can provide different comparative data for spring phenology observations.

Table. 1 Original location of 15 genotypes of woodland strawberry used in this thesis (coordinate system: World Geodetic System-1984 Coordinate System).

Genotype	Collection place	Latitude	Longitude
IT2	Italy: Tenno, Ville del Monte, Tennosee	45.94	10.81
IT8	Italy: Garniga Terme, Trentino	46.01	11.08
IT12	Italy: da Lagolo, Trentino	46.05	11.01
ICE2	Iceland: Hraunteigur	64.00	-19.96
ICE4	Iceland: Búrfellsskógur, Vesturhlíð	64.07	-19.86
ICE16	Iceland: Djúpa	64.17	-17.75
NOR17	Norway: Kåfjord	69.43	20.97
NOR16	Norway: Kåfjord	69.45	20.95

NOR13	Norway: Indre Nordnes	69.54	20.38
NOR11	Norway: Kvaløya, Tromsø	69.52	18.38
NOR29	Norway: Kvaløya, Tromsø	69.53	18.38
NOR27	Norway: Kvaløya, Tromsø	69.69	18.73
NOR5	Norway: Alta, Rafsbotn, Rishaugen	70.02	23.56
NOR6	Norway: Alta, Rafsbotn, Elvehaugen	70.03	23.49
NOR9	Norway: Alta, Leirbotn, Indre Skillefjordnes	70.16	23.28

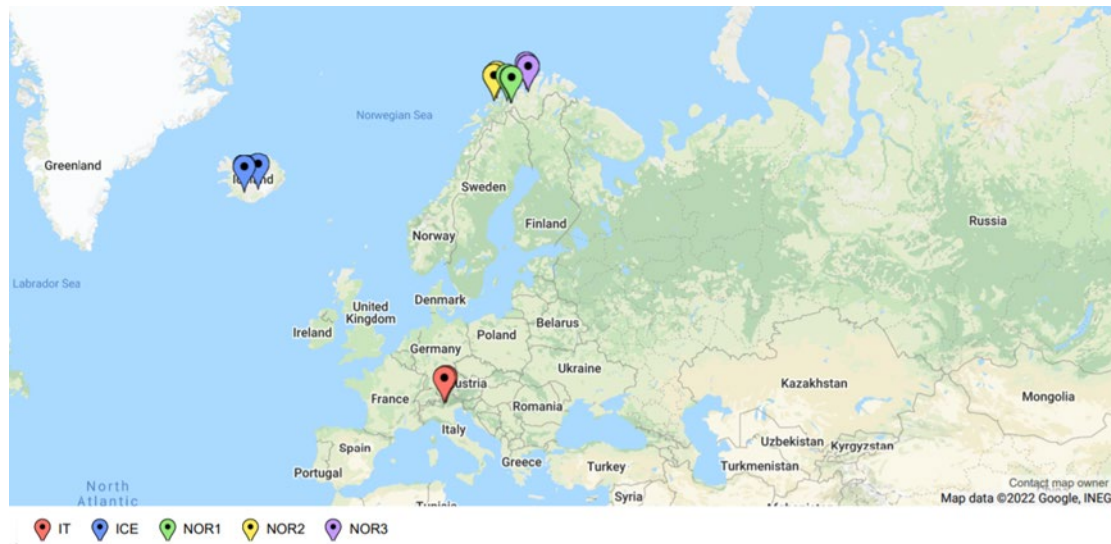


Figure. 1 Map of the original location of the 15 genotypes of woodland strawberry used in this thesis (Google Map 2021). IT: Italy genotypes (IT2, IT8, IT12), ICE: Iceland genotypes (ICE2, ICE4, ICE16), NOR1: Kåfjord, Norway genotypes (NOR17, NOR16, NOR13), NOR2: Tromsø, Norway genotypes (NOR11, NOR29, NOR27), NOR3: Alta, Norway genotypes (NOR5, NOR6, NOR9).

2.2 Experimental setup

2.2.1 Plant material

The original plants were kept in a greenhouse at Viikki, Helsinki, Finland (N 60.2272, E 25.0178; WGS84). Fifteen different genotypes were studied in this experiment, with 20 ramets of each genotype. They were planted in pots measuring 12 x 12 cm and 13 cm high. The soil type used was Kekkiläs peat-based soil. These plants were used in the doctoral thesis of Sonja Still, and they were cultivated by her from 2020. The data of this thesis on these plant materials were collected from April to July 2021.

For the experiments the plants were divided into two groups and then transported for overwintering to an open meadow of Nåtö biological Station on Åland Islands (60.0465,

19.9743; WGS84) on 29 October 2020, and to an open meadow of Lammi biological Station in Hämeenlinna (61.0465, E 25.0178; WGS84) on 31 October 2020.

2.2.2 Overwintering sites and marking of different leaves

Lammi is in the interior of southern Finland and has a typical continental winter climate: cold winter climate with average temperatures around -10°C and a stable snowpack (around 40 cm in winter). Åland Islands, located in the archipelago between Finland and Sweden, has a typical coastal winter: milder winter climate with temperatures around 0°C and a thin snowpack that melted early (around 15 cm) (Finnish Meteorological Institute, data from October 2020 to April 2021, 2022).

Before transporting the plants to the overwintering sites, three winter leaves were marked using cotton threads of different colors (purple, yellow, white). The leaves marked with white cotton thread were latest winter leaves for all genotypes. These winter leaves were the main target for observation after overwintering.

However, due to the temperature rise in early April, the snow on the leaves had already melted on Åland Islands when the plants were retrieved from the overwintering location. Those plants that overwintered on Åland Islands were brought back to Viikki on 4 April 2021. Sonja Still recorded the number of summer leaf formation of these plants at the same date. When the Lammi samples were brought back to Viikki on 5 April 2021, there was still a small amount of snow remaining on the leaves, and therefore the chlorophyll measurements could not be done immediately. It was therefore decided to let the residual snow melt naturally in the field of Viikki overnight, and the measurements and recording officially started the next afternoon (6 April).

The research question of this thesis is to investigate what effects the different genotypes had on the spring phenology, after overwintering in different conditions. All individuals were observed on the condition of winter leaves and on summer leaf formation. Summer leaves have different morphology than winter leaves, with larger leaf lamina and longer petioles, and are produced in the spring and during the summer.

In order to observe summer leaves, on 4 May, blue rubber bands were used to mark the leaves in the main rosette of each plant. Rubber bands are placed around the petioles of the youngest fully grown leaves that emerge from the rosette. Based on the information provided by these tags, the start date for collecting summer leaf data (11 May) was determined. These marks will not cause damage to the leaves or plants, because the rubber band could easily be placed around the petiole.

Unexpectedly, the first summer leaves marked in the spring (with blue rubber bands) started to senesce shortly after getting fully developed, and only three measurement timepoints were taken. Meanwhile, a new set of leaves began to emerge. The newly produced leaves were comparatively larger and had longer petioles than the previous leaf (Fig. 2), hence the new leaves had the typical features of a summer leaf. To obtain complete data, I chose to mark the newly produced leaves with red rubber bands and named them later formed summer leaf.



Figure. 2 Early and later formed summer leaves of woodland strawberry. These summer leaves emerged in spring were marked with different color rubber bands: the first summer leaf with blue and the later formed leaf with red. (Photograph: Zhenzhen Liu, 31.5.2021)

2.2.3 Timeline of observations and measurements

All plants were transferred back to Viikki from the overwintering sites on 5 April and chlorophyll fluorescence were measured using FluorPen FP 100-MAX-D/USB on labelled winter leaves starting on 6 April. Observations and recordings of summer leaf production began on 3 April and were provided by Sonja Still between 3 April and 13 April and continued until all plants had produced new leaf (27 April). The new summer leaf of woodland strawberry was formed at the middle of the rosette. The main inflorescence grew at the middle of the rosette, at the beginning of spring, it could be observed as flower buds. Woodland strawberry produces multiple buds at the same time: terminal bud and axillary buds. In order to keep the rosette with only one apical shoot, the axillary buds were excluded when they were found. Also the number of stolons were counted. Stolons were not easy to notice at the beginning of their formation. The elongation speed of stolons is quite high, so only those stolon length over 5 cm would be counted and recorded. After the counting and recording of stolons longer than 5 cm was completed, the stolons were removed to avoid confusion in the count.

During the summer leaf growth and unfolding, two sets of leaves produced at different dates were marked with rubber bands of different colors. Chlorophyll fluorescence were measured of the blue rubber band marked summer leaf starting on 11 May and of the red rubber band marked summer leaf starting on 11 June. The remaining spring events: flowering onset and stolon production were recorded starting on 12 May and 17 May, respectively. All measurements and records were ended on 28 June.

2.3 Fv/Fm of summer and winter leaves

FluorPen is a portable battery-powered fluorometer that can quickly and accurately measure chlorophyll fluorescence parameters. It is equipped with LED emitter, optically filtered and precisely focused to deliver light intensities of up to 3,000 $\mu\text{mol}\cdot\text{m}^2\cdot\text{s}^{-1}$ to measured plant tissue. Through different measurement pulse intensities, FluorPen can trigger the chlorophyll in the tested leaves to quickly be in different specific states (such as the minimum chlorophyll fluorescence and the

maximum chlorophyll fluorescence) (Öquist and Chow 1992; FluroPen Manual and User Guide 2018).

The experiment included the use of FluorPen FP 100-MAX-D/USB to measure F_v/F_m . F_v/F_m is a stable, rapid dark adaptation assay for measuring maximum quantum yield of PSII in leaves. F_v/F_m is a normalized ratio for comparing and measuring dark adaptation states under known plant stress. Furthermore, it correlates with the carbon assimilation of plant photosynthesis and is generally measured between 0.79 and 0.83 in healthy plants. A lower value indicates that the plant is under some kind of stress affecting PSII (Photosystem II) (Baker and Rosenqvist 2004).

To get F_v/F_m , the following parameters will be involved: F_m , F_0 , F_v , and the result F_v/F_m . F_m , maximum fluorescence yield, which is the fluorescence yield when the PSII reaction centres are completely closed. It can reflect the electron transfer through PSII. F_0 , minimum fluorescence in dark-adapted state, also called basic fluorescence, is the fluorescence output when the reaction centres of PSII are fully open. F_v , variable fluorescence from dark-adapted leaf, demonstrate the ability of PSII to perform primary photochemistry. F_v/F_m , it is the maximum photochemical quantum yield of PSII, reflecting the optimal/maximal PSII efficiency of PSII, measured after 20 min of leaf dark adaptation. Under non-stress conditions, the change of this parameter is very small, and it is not affected by species and growth conditions. Under stress conditions, this parameter decreases significantly (Baker and Rosenqvist 2004).

When measuring F_v/F_m , the measured leaves need to be dark adapted first. Using the detached clips to place the sample for around 20 minutes in the dark for preparing. Those detached clips have open and closed position. Before measurement closed clips were used on the winter leaves, during the measurement, clips were open for FluorPen (Fig. 3).

FluorPen measurements were used to monitor the photosynthetic efficiency of the leaves. Measurements with the FluorPen were started on the winter leaves of the white cotton thread group from 6 April, once the snow on the leaves had completely melted. After the summer leaves were produced, the first formed summer marked with blue rubber bands were measured from 11 May. From 31 May, new summer

leaves marked with red rubber bands were measured. All measurements were stopped after 28 June.



Figure. 3 Leaf clips for dark adaptation on the woodland strawberry winter leaves. There are 2 types of leaves on the plants: the dried leaves are the summer leaves from last summer; the green leaves are the winter leaves that overwintered under snow. (Photograph: Zhenzhen Liu, 13.4.2021)

2.4 Set up and application of senescence scale

In spring, the winter leaves of woodland strawberries senesce and the summer leaves form and replace the winter leaves to maintain the physiological activities of the plant. Therefore, the observation of winter leaf senescence is also part of the spring phenological observation.

Senescence is a continuous process, so it is difficult to use a specific value to show the status of plants. In order to be able to quantify the senescence process more visually, the creation of a senescence scale is indispensable. The senescence scale was established to serve as a rubric to assess the senescence extent of woodland strawberry winter leaves.

The leaves of woodland strawberry consist of a petiole and three leaflets (NatureGate 2022). Depending to the senescence degree of the three leaflets, the levels of the senescence scale can be classified into five (0,1,2,3,4). The explanations for the scale are in the figure legend (Fig. 4).

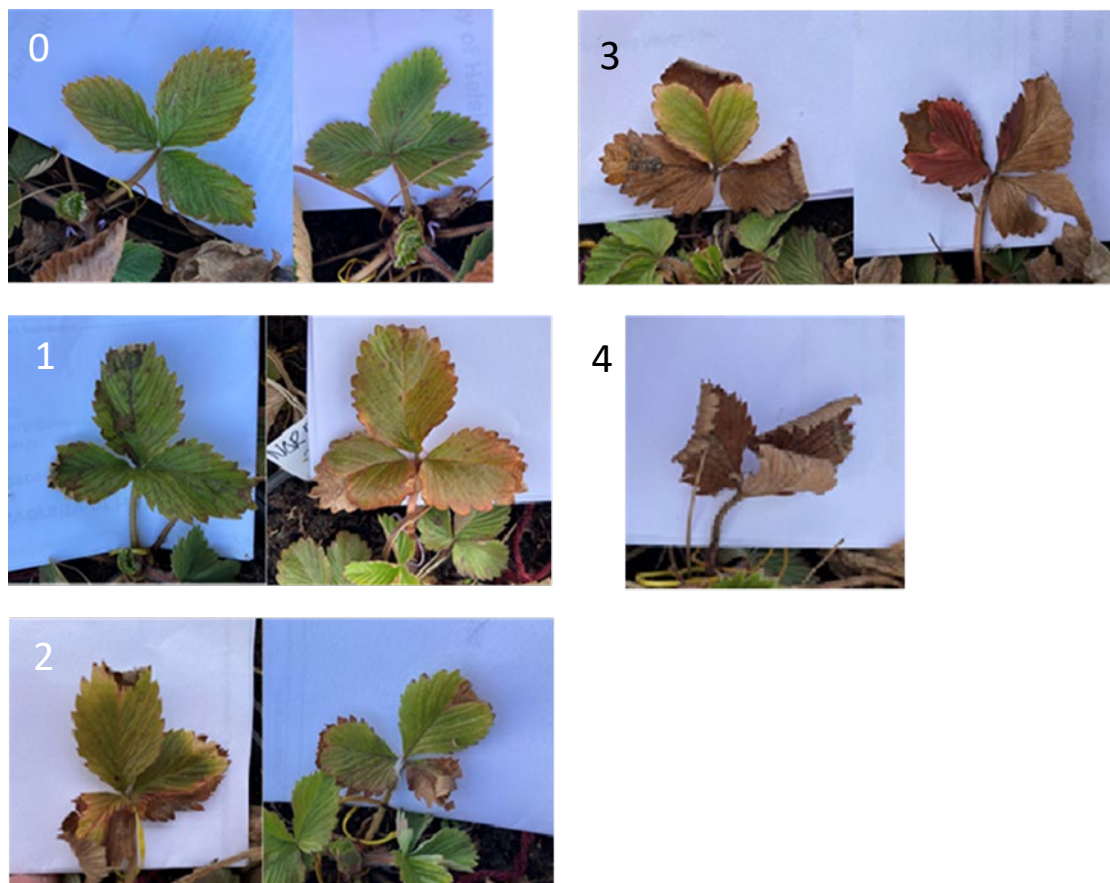


Figure. 4 Senescence scale of woodland strawberry winter leaves. **0.** Healthy leaf: Fully healthy leaves, complete structure, green and uniform color, occasionally small spots. **1.** Slightly dried leaf: Larger yellow or brown spots appear on the leaf surface, or about 1/3 of the area (less than or equal to) begin to become chlorotic, but the whole leaf is still intact. **2.** Mid-dried leaf: Over 1 leaflet turns into brown or dried, or over 1/3 but less than 2/3 of the leaf area begin to become chlorotic, parts of leaf begin to dry and curl, petiole begin to dry. **3.** Severely dried leaf: Over 2 leaflets or 2/3 of the leaf area begin to become chlorotic, dry and curl, but the leaves are still photosynthesizing at this time, petiole starts to bend. **4.** Died leaf: The whole leaf and petiole are dry and curl, becomes very brittle and easily broken, complete loss of functionality.

The observation and recording of the leaf senescence process started on 6 April and was carried out once a week. The concept of senescence scale was set up before the start of observation and recording, but the specific data and pictures were collected between 6 April and 28 June.

2.5 Observations of flower development

The observation of flowers began on 27 April. At the beginning, only the formation of flower buds was recorded. This was preparation for accurately recording the time of the flowering onset of each plant. The flower buds usually formed in the center of the rosette, surrounded by newly produced summer leaves. And near the time of flowering, the inflorescence usually elongated significantly, exposing the bracts to the leaves. When flowering was observed on plants that had produced buds, it was recorded as the date of initial flowering. The first time a flower was observed to open completely was on 12 May. In order to accurately record the date of flowering onset, the frequency of observation was set to record once every 1-2 days.

The observation and recording of flower development can more intuitively compare the different genotypes and different overwintering locations in the spring phenology of woodland strawberry. The date of flowering onset is the spring phenology event in general and represents the state of development of woodland strawberry in spring. Woodland strawberry has many lateral buds that develop simultaneously with the apical meristem, and the lateral buds can either develop into stolons or branch crowns. It is possible to remove the stolons and branch crowns during development without

harming the plant. Removing the axillary buds removes distracting factors from the recording, allowing for more accurate and faster observations.

2.6 Observations of stolon development

The observation period started on 5 April 2021, but the production of stolons began on 17 May 2021. During the first two weeks the work focused on counting and removing all stolons that were longer than 5 cm of each woodland strawberry plant. Shorter ones were allowed to stay on the plant until they had grown longer. This process continued every week throughout the 7-week study period, from 17 May to 28 June 2021.

The stolon is an important organ for vegetative reproduction in woodland strawberries. Strawberries use stolons to grow horizontally in order to find a more suitable growing environment and to spread their individuals. Therefore, it shows that stolons are, in favourable conditions, produced and developed after the plant has reached a certain level of growth and development. This means that while observing stolon production I also need to remove the stolons that have been recorded. This avoids both interference of spreading individuals between pots and double counting of it.

2.7 Data analysis

The data was statistically analyzed using IBM SPSS Statistics 28 (IBM, USA) statistical software. The statistical analysis was performed mainly on four different spring phenology (date of summer leaf production, date of flowering onset, date of first stolon production, date of winter leaf senescence), and the F_v/F_m of three different sets of leaves (winter leaves, first summer leaves, later formed summer leaves).

Data on the overall number of stolons produced, and the rate of senescence of winter leaves were not statistically analyzed. This is because senescence rates are continuous data judged quantitatively according to the senescence scale I established and are not

applicable for statistical analysis. The date of the first stolon production was regarded sufficient as an illustrative factor for spring phenology.

Repeated measures ANOVA analysis with Tukey's post hoc test was performed for Fv/Fm of the three different sets of leaves, with genotype and overwintering site as independent variables. One-way ANOVA with Tukey's post hoc test was performed for the date of flowering onset and the date of first summer leaf production, again with genotype and overwintering site as independent variables. One-way ANOVA using the Tukey post hoc test required equal sample sizes across different genotypes. However, practically the date of first stolon production and the date of winter leaf senescence did not have same sample sizes for different genotypes. Therefore, one-way ANOVA analysis with Dunnett-T3 post hoc test was chosen.

3 Results

3.1 Winter leaves of woodland strawberry

3.1.1 Chlorophyll fluorescence of woodland strawberry winter leaf

The winter leaf chlorophyll fluorescence was measured during the spring in order to see how the photosynthesis capacity is maintained during the spring. The effect of two different overwintering environments on winter leaf can also be derived from chlorophyll fluorescence measurements. The period of chlorophyll fluorescence measurements on woodland strawberry winter leaves was from 6 April to 28 June (2021). The temperature in Viikki is also recorded as a parameter and is shown in the Fig. 5 the temperature in Viikki decreases significantly between 23 April and 10 May, but generally maintains a fluctuating upward trend.

General trend of chlorophyll fluorescence of all genotypes and treatments is winter leaves recovering in the spring, then the values remain high until May. After that, due to senescence of the winter leaves, Fv/Fm ratios decrease again (Fig. 5, A). Individuals that overwintered at Lammi had higher Fv/Fm ratios in winter leaves at the first week and the values were decreasing slower than those overwintered at Åland (Fig. 5, A). The Italian genotypes, IT2, IT8, IT12 showed differences in chlorophyll fluorescence between overwintering sites only in early spring and no differences between

overwintering sites at the end of the observation (Fig. 5, B). The NOR 6, NOR 9 genotypes from Alta, Norway were found to consistently have lower values (Fig. 5, F). However, NOR 5 from the same location had approximately similar values to the other genotypes.

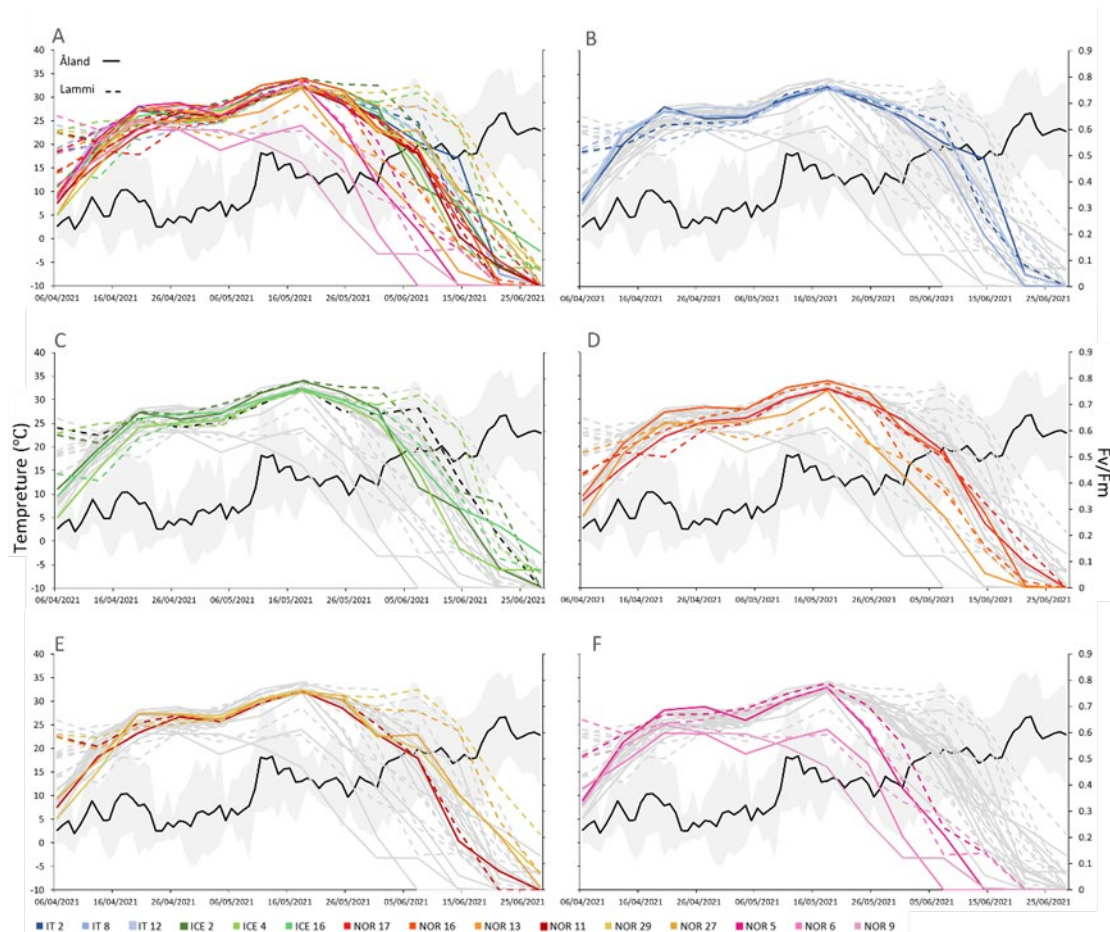


Figure. 5 Fv/Fm measurements of woodland strawberry winter leaves and temperatures from 6 April to 28 June (2021). **A.** Winter leaf Fv/Fm curves and temperature data for 15 genotypes of woodland strawberries overwintered at Åland Island and Lammi (30 data sets in total). The left vertical axis is the temperature (°C) during this period, and the right vertical axis is Fv/Fm. **B.** Italy winter leaf genotypes (IT2, IT8, IT12). **C.** Iceland winter leaf genotypes (ICE2, ICE4, ICE16). **D.** Norway Kåfjord winter leaf genotypes (NOR17, NOR16, NOR13). **E.** Norway Tromsø winter leaf genotypes (NOR11, NOR29, NOR27). **F.** Norway Alta winter leaf genotypes (NOR5, NOR6, NOR9).

The repeated measures ANOVA for Fv/Fm ratio in winter leaves during 6 April to 28 June shows $p < 0.001$, $F = 926.784$, $df = 12$. It indicates that the observation of different

Fv/Fm ratio between the genotypes is statistically significant. The interaction of overwintering sites and genotypes for Fv/Fm shows $p < 0.001$, $F = 10.939$, $df = 29$. This also showed that the results were statistically significant after repeated measurements for different overwintering sites as well as different genotypes over 6 April to 28 June.

In post hoc Tukey tests, NOR 13, NOR 29, NOR 5, NOR 6, and NOR 9 genotypes from Norway all differed significantly from the other genotypes (Table. 2). Among them, the winter leaf Fv/Fm ratios of NOR 13 and NOR 9 that had overwintered at Lammi were only significantly different from those of genotypes from different regions that also had overwintered at Lammi (Table. 2). Fv/Fm ratio of winter leaves in different genotypes and overwintering site differed significantly. In contrast, NOR 9, which had overwintered in Åland, was significantly different from almost all treatments except NOR 13, NOR 6, and NOR 5, which also had overwintered in Åland.

Table. 2 Fv/Fm ratio statistical significance of woodland strawberry winter leaf between 15 genotypes and 2 overwintering sites in post hoc Tukey tests.

Genotype	Overwintering site	Sig.	Genotype	Overwintering site	Sig.
NOR 13 Åland	IT 12 Lammi	0.046	NOR 6 Åland	NOR 17 Åland	0.024
	ICE 2 Lammi	0.003		NOR 17 Lammi	0.014
	ICE 4 Lammi	0.003		NOR 16 Åland	0.002
	NOR 29 Lammi	0.000		NOR 11 Åland	0.041
	NOR 27 Lammi	0.002		NOR 11 Lammi	0.001
NOR 13 Lammi	ICE 2 Lammi	0.046		NOR 29 Åland	0.001
	ICE 4 Lammi	0.045		NOR 29 Lammi	0.000
	NOR 29 Lammi	0.001		NOR 27 Åland	0.000
	NOR 27 Lammi	0.043		NOR 27 Lammi	0.000
NOR 29 Lammi	ICE 4 Åland	0.014		NOR 5 Lammi	0.042
	NOR 17 Åland	0.050	NOR 9 Åland	IT 2 Åland	0.000
	NOR 16 Lammi	0.012		IT 2 Lammi	0.000
	NOR 13 Åland	0.000		IT 8 Åland	0.005
	NOR 13 Lammi	0.001		IT 8 Lammi	0.000
	NOR 11 Åland	0.030		IT 12 Åland	0.000
	NOR 5 Åland	0.000		IT 12 Lammi	0.000
	NOR 5 Lammi	0.028		ICE 2 Åland	0.000
	NOR 6 Åland	0.000		ICE 2 Lammi	0.000
	NOR 6 Lammi	0.003		ICE 4 Åland	0.021
NOR 9 Åland	0.000	ICE 4 Lammi		0.000	
NOR 9 Lammi	0.000	ICE 16 Åland	0.000		

NOR 5 Åland	ICE 2 Lammi	0.010		ICE 16 Lammi	0.000
	ICE 4 Lammi	0.010		NOR 17 Åland	0.006
	NOR 29 Lammi	0.000		NOR 17 Lammi	0.003
	NOR 27 Lammi	0.009		NOR 16 Åland	0.000
NOR 6 Åland	IT 2 Åland	0.001		NOR 16 Lammi	0.026
	IT 2 Lammi	0.000		NOR 11 Åland	0.010
	IT 8 Åland	0.020		NOR 11 Lammi	0.000
	IT 8 Lammi	0.001		NOR 29 Åland	0.000
	IT 12 Åland	0.000		NOR 29 Lammi	0.000
	IT 12 Lammi	0.000		NOR 27 Åland	0.000
	ICE 2 Åland	0.002		NOR 27 Lammi	0.000
	ICE 2 Lammi	0.000		NOR 5 Lammi	0.011
	ICE 4 Lammi	0.000	NOR 9 Lammi	IT 12 Lammi	0.024
	ICE 16 Åland	0.000		ICE 2 Lammi	0.001
	ICE 16 Lammi	0.001		ICE 4 Lammi	0.001
				NOR 29 Lammi	0.000
				NOR 27 Lammi	0.001

3.1.2 Senescence rate of woodland strawberry winter leaf

The winter leaf senescence rate of woodland strawberry was obtained by comparing the observed winter leaf condition with the senescence scale (Fig. 4). A total of five phases in the senescence scale are represented in different percentages in the Fig. 6. There were visible differences in the winter leaf senescence rate of the different genotypes and overwintering sites (Åland and Lammi).

Overall, the winter leaves on the plants that overwintered in Lammi maintained their leaves for a longer period compared to Åland (Fig. 6). Woodland strawberries that had overwintered in Lammi had later onset of leaf senescence in winter and maintained 0- and 1-phase senescence for a longer period. This finding was more evident in the IT 8, ICE 4, NOR 17, NOR 16, NOR 11, NOR 29, NOR 27, NOR 6, NOR 9 genotypes. Compared to the other genotypes, winter leaves of NOR 6 and NOR 9 genotypes entered 4-phase senescence earlier (Fig. 6).

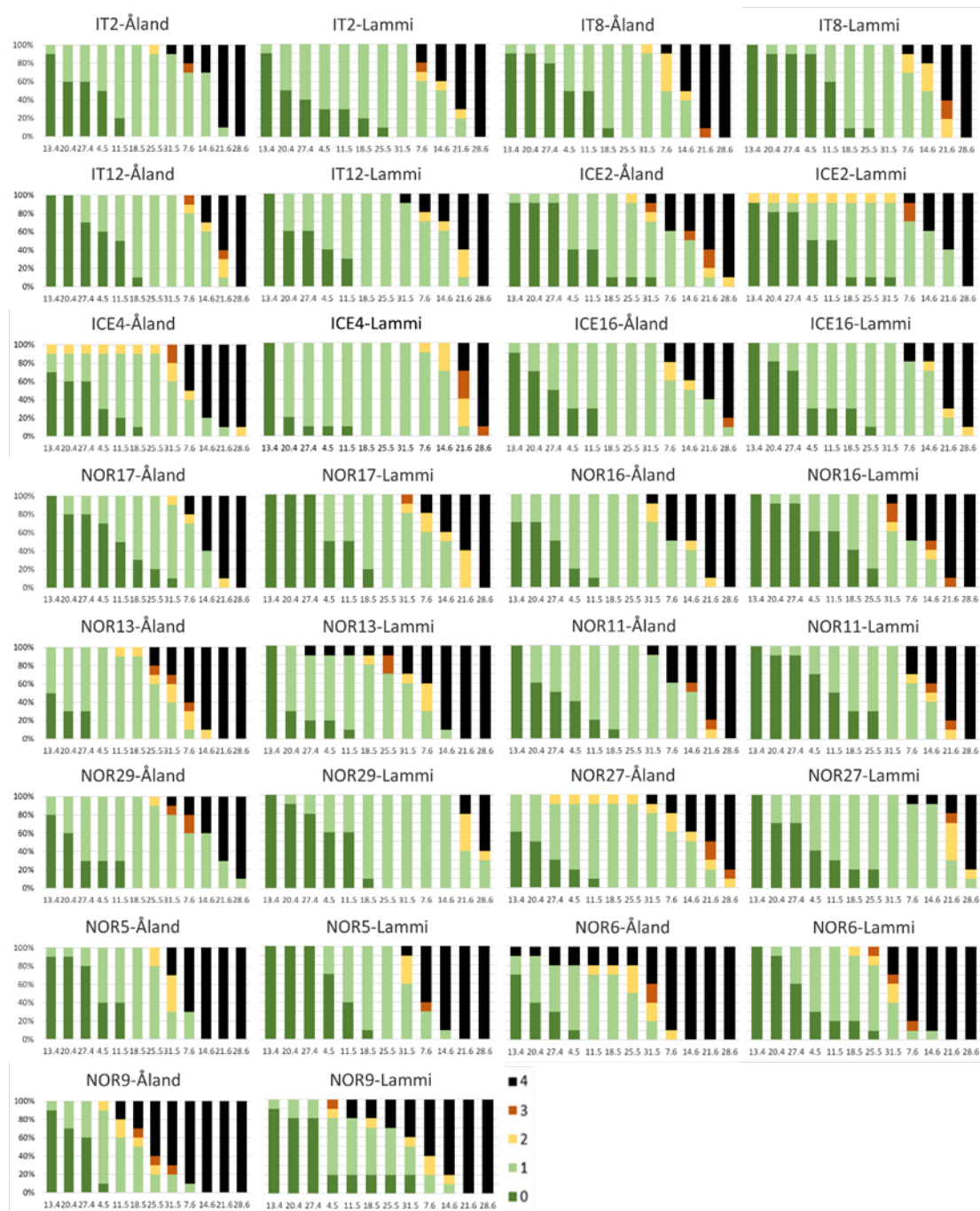


Figure. 6 Senescence rates of 15 genotypes woodland strawberry winter leaves that overwintered in Åland Island and Lammi (2021). The senescence scale has a total of 5 levels (0-healthy leaf, 1-slightly dried leaf, 2-mid-dried leaf, 3-severely dried leaf, 4-died leaf), which are shown in different colors in the pictures. The woodland strawberries were observed from 13 April to 28 June (2021). The results are presented using the percentage of different senescence stages within the same sets.

3.1.3 Senescence date of woodland strawberry winter leaf

The winter leaves maintained functionality for some time in Viikki in the spring, and entered the senescence in May. The senescence of the leaves begun in the end of May

and was completed by the end of the data recording phase (Fig. 7). Among all the different genotypes of winter leaf senescence dates, only ICE 4 showed significant differences in the comparison of different overwintering sites. Other significant differences were found mainly between genotypes such as NOR 9 and IT 2, IT 8, IT 12, ICE 16, NOR 29, NOR 27.

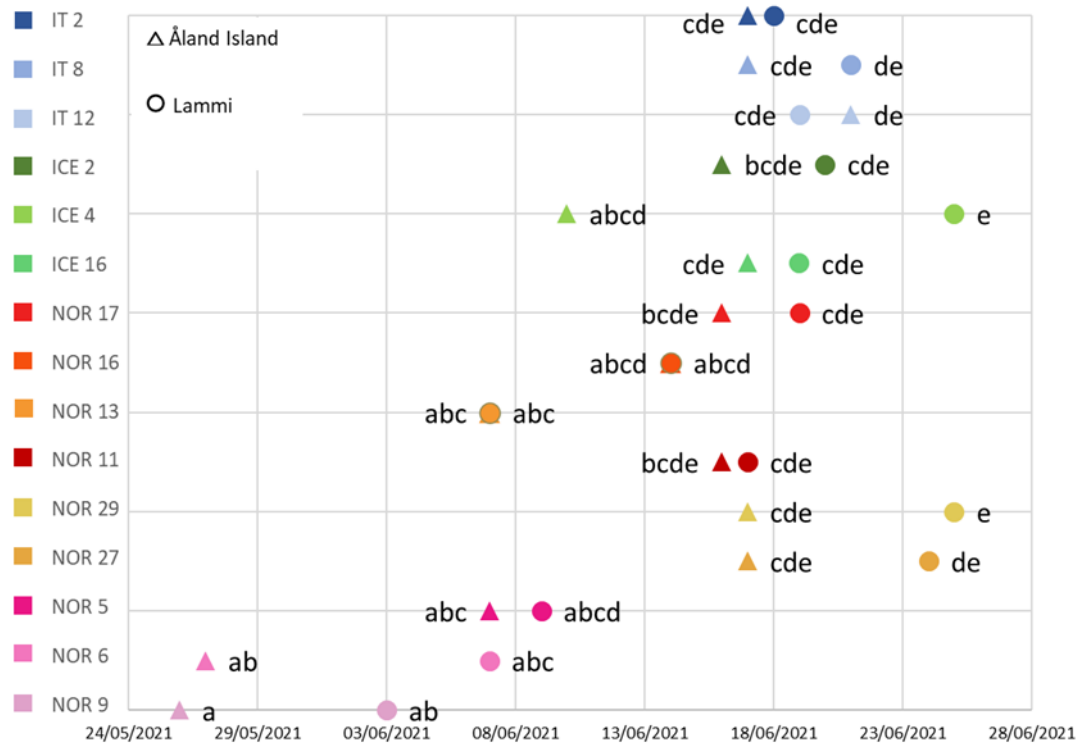


Figure. 7 Woodland strawberry winter leaves senescence date in the spring 2021. The recording period begun on 24 May and ended on 28 June (2021). Significance level $\alpha = 0.05$; different letters indicate different levels of significance ($F=6.052$, $df=29$, $p<0.001$).

3.2 First summer leaf of woodland strawberry produced in spring

3.2.1 First summer leaf formation date of woodland strawberry

First summer leaf formation in woodland strawberries occurred earlier than what was expected in the experimental design. First summer leaf formation in the center of the plant rosette had been noticed when the woodland strawberries were transported back to Viikki from Åland in the beginning of April. The date of first summer leaf formation is mainly concentrated in the middle and early April (Fig. 8). Almost all first summer leaf formation date of woodland strawberries that had overwintered in

Lammi was concentrated between 17 April and 20 April. Nearly half of the 15 genotypes of woodland strawberry showed significant differences in the date of first summer leaf formation depending on the overwintering site (IT 2, NOR 17, NOR 16, NOR 13, NOR 11, NOR 27, NOR 6).

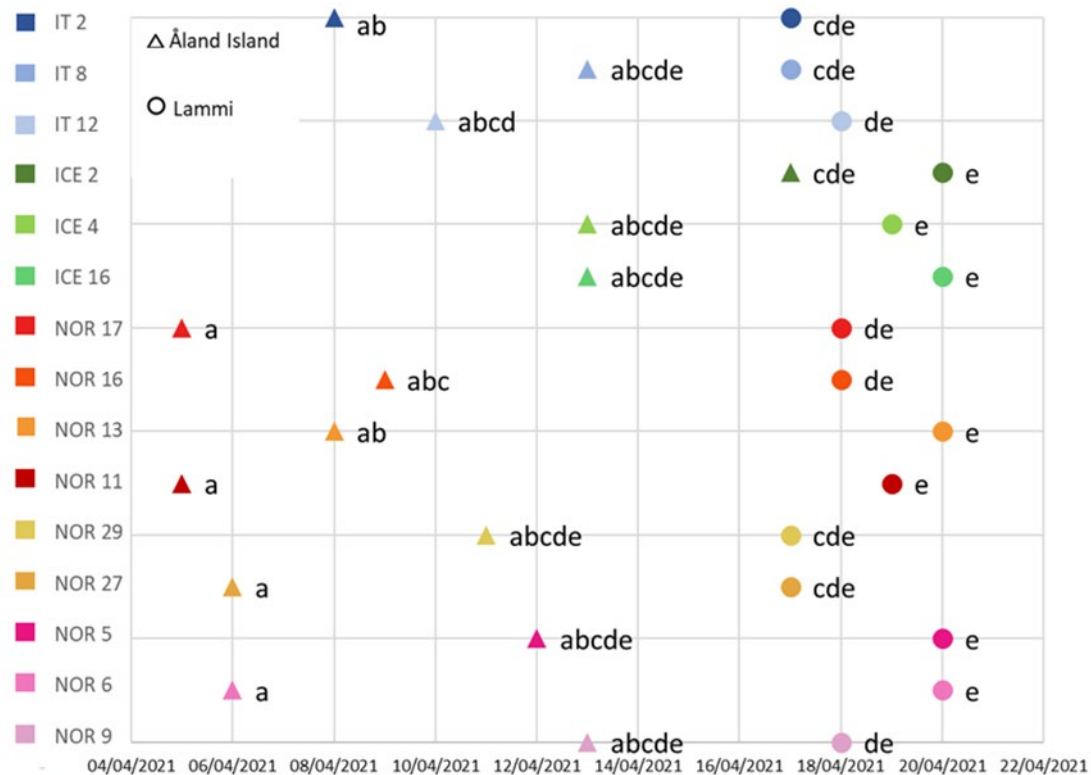


Figure. 8 Woodland strawberry first summer leaf formation date in the spring 2021. This refers to the date when the formation of summer leaves was first observed in the centre of the rosette of a woodland strawberry plant. The recording period begun on 4 April and ended on 22 April (2021). Significance level $\alpha = 0.05$; different letters indicate different levels of significance ($F=10.068$, $df=29$, $p<0.001$).

3.2.2 Chlorophyll fluorescence of woodland strawberry first summer leaf

Chlorophyll fluorescence measurements (11 May) were started when the first summer leaves of woodland strawberries grew to maturity. The purpose of first summer leaf chlorophyll fluorescence measurements is to infer from the functionality of first summer leaves how different overwintering environments affect woodland strawberry plants.

The overall trend of the Fv/Fm ratio of the first summer leaves shows that the effect of different overwintering conditions had no impact on the photosynthetic efficiency. During the first week of the start of the measurements, all values remained at almost the same level. After 18 May, the Fv/Fm ratio started to decrease, and by 28 June, basically the chlorophyll fluorescence of all the different genotypes had dropped to zero (Fig. 9).

Between 11 May and 28 June, the temperature in Viikki decreased slightly, and then gradually increased. After the beginning of warming on 26 May, the Fv/Fm of the first summer leaves of NOR 5, NOR 6, and NOR 9 all started to decrease and were lower than the other genotypes of different origins (Fig. 9, F). Regarding the comparison between different overwintering sites among the same genotypes, the Fv/Fm values of the first summer leaves of the 15 genotypes did not differ significantly.

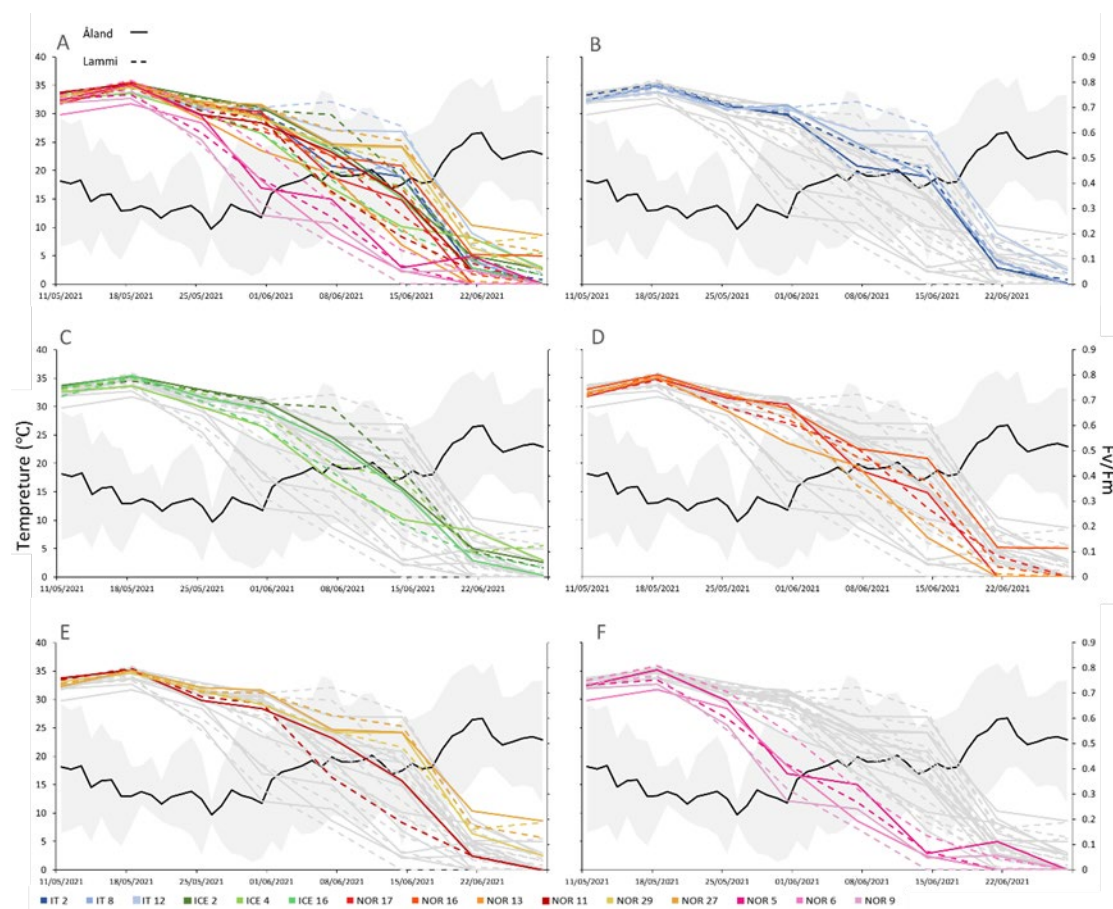


Figure. 9 Fv/Fm measurements of woodland strawberry first summer leaves and temperatures from 11 May to 28 June (2021). **A.** First summer leaf Fv/Fm curves and

temperature data for 15 genotypes of woodland strawberries overwintered at Åland Island and Lammi (30 data sets in total). The left vertical axis is the temperature (°C) during this period, and the right vertical axis is Fv/Fm. **B.** Italy first summer leaf genotypes (IT2, IT8, IT12). **C.** Iceland first summer leaf genotypes (ICE2, ICE4, ICE16). **D.** Norway Kåfjord first summer leaf genotypes (NOR17, NOR16, NOR13). **E.** Norway Tromso first summer leaf genotypes (NOR11, NOR29, NOR27). **F.** Norway Alta first summer leaf genotypes (NOR5, NOR6, NOR9).

The statistical Repeated measures ANOVA analysis for Fv/Fm ratio in first summer leaves during 11 May to 28 June shows $p < 0.001$, $F = 1169.328$, $df = 7$. It indicates that the observation of different Fv/Fm ratio between genotypes is statistically significant. The interaction of overwintering sites and genotypes for Fv/Fm shows $p < 0.001$, $F = 7.629$, $df = 29$. This also showed that the results were statistically significant after repeated measurements for different overwintering sites as well as different genotypes over 11 May to 28 June.

According to Tukey post hoc test, NOR 27 that had overwintered in Åland and IT 12 that had overwintered in Lammi had higher Fv/Fm ratio with significant differences compared to the lower NOR 5, NOR 9, and NOR 13, NOR 6 which had overwintered in Åland (Table. 3). The lower overall Fv/Fm ratio of NOR 9 was significantly lower than most of the groups. NOR 6, which had overwintered in Åland, had more fluctuating levels and was therefore significantly different from some of the groups with more moderate decreases in Fv/Fm ratio.

Table. 3 Fv/Fm ratio statistical significance of woodland strawberry first summer leaf between 15 genotypes and 2 overwintering sites in post hoc Tukey tests.

Genotype	Overwintering site	Sig.	Genotype	Overwintering site	Sig.
NOR 13 Åland	IT 12 Lammi	0.047	NOR 9 Åland	ICE 2 Åland	0.001
	NOR 27 Åland	0.036		ICE 2 Lammi	0.000
NOR 5 Åland	IT 12 Åland	0.028		ICE 4 Lammi	0.003
	IT 12 Lammi	0.008		ICE 16 Åland	0.009
	NOR 27 Åland	0.006		NOR 16 Åland	0.000
NOR 5 Lammi	NOR 27 Lammi	0.017		NOR 16 Lammi	0.017
	IT 12 Åland	0.002		NOR 11 Åland	0.027
	IT 12 Lammi	0.000		NOR 29 Åland	0.000
	ICE 2 Lammi	0.014		NOR 29 Lammi	0.000
	NOR 16 Åland	0.020		NOR 27 Åland	0.000
	NOR 29 Åland	0.018	NOR 27 Lammi	0.000	

	NOR 29 Lammi	0.005	NOR 9 Lammi	IT 2 Åland	0.003
	NOR 27 Åland	0.000		IT 2 Lammi	0.000
	NOR 27 Lammi	0.001		IT 8 Åland	0.000
NOR 6 Åland	IT 2 Åland	0.030		IT 8 Lammi	0.002
	IT 2 Lammi	0.006		IT 12 Åland	0.000
	IT 8 Åland	0.007		IT 12 Lammi	0.000
	IT 8 Lammi	0.021		ICE 2 Åland	0.000
	IT 12 Åland	0.000		ICE 2 Lammi	0.000
	IT 12 Lammi	0.000		ICE 4 Åland	0.049
	ICE 2 Åland	0.002		ICE 4 Lammi	0.001
	ICE 2 Lammi	0.000		ICE 16 Åland	0.003
	ICE 4 Lammi	0.011		NOR 17 Åland	0.027
	ICE 16 Åland	0.031		NOR 17 Lammi	0.031
	NOR 16 Åland	0.001		NOR 16 Åland	0.000
	NOR 29 Åland	0.001		NOR 16 Lammi	0.006
	NOR 29 Lammi	0.000		NOR 11 Åland	0.010
	NOR 27 Åland	0.000		NOR 29 Åland	0.000
	NOR 27 Lammi	0.000		NOR 29 Lammi	0.000
NOR 9 Åland	IT 2 Åland	0.008		NOR 27 Åland	0.000
	IT 2 Lammi	0.001		NOR 27 Lammi	0.000
	IT 8 Åland	0.002			
	IT 8 Lammi	0.005			
	IT 12 Åland	0.000			
	IT 12 Lammi	0.000			

3.3 Chlorophyll fluorescence of woodland strawberry later formed summer leaf

Woodland strawberry summer foliage will theoretically maintain its functionality until autumn when it starts to senesce (Åström *et al.* 2015). In fact, however, the first summer leaves involved in the trial showed unexpected senescence in May. When senescence begins, the later formed summer leaves formed in the centre of the rosette. They were marked for chlorophyll fluorescence measurements to be started after growth maturation. The measurement of later formed summer leaf Fv/Fm ratio is motivated by the consideration of complementary first summer leaf Fv/Fm ratio values. The values of the two sets of summer leaves were compared to explore whether different overwintering conditions also have an effect on the functionality of summer leaves. These later formed summer leaves were measured at the end of May. By this time the outdoor temperature in Viikki had reached basically around 20°C, with a slowly increasing trend.

The general trend of the Fv/Fm ratio of later formed summer leaf remained at a similar level at the beginning. A difference appears from 14 June onwards. The Fv/Fm ratio of later formed summer leaves of different genotypes that had overwintered in different locations started to show differences (Fig. 10). The Fv/Fm ratio of three sets of later formed summer leaves from Italy, Iceland, and the Tromso region of Norway remained stable throughout the period from the end of May to the end of June (Fig. 10, B, C, E). In contrast, NOR 17, NOR 16, NOR 13, NOR 5, NOR 6, and NOR 9 from the Kåfjord and Alta region in Norway had a more pronounced downward trend after 14 June (Fig. 10, D, F). In the comparison between overwintering sites, the Fv/Fm ratios of the later formed summer leaves did not differ significantly and only showed some insignificant differences after 14 June for NOR 5, NOR 6, NOR 9. Leaves that had overwintered in Åland would have lower Fv/Fm ratio after 14 June than plants that had overwintered in Lammi (Fig. 10, F).

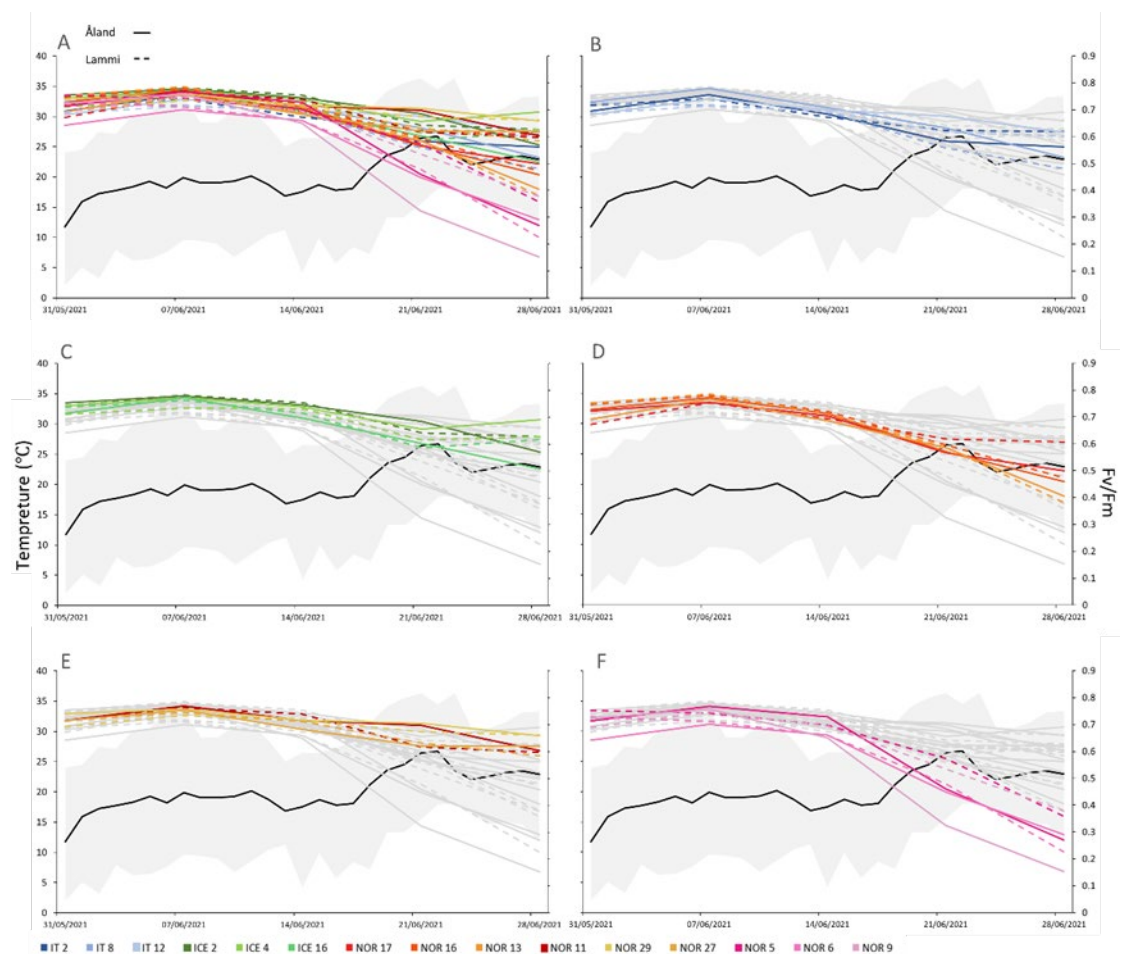


Figure. 10 Fv/Fm measurements of woodland strawberry later formed summer leaves and temperatures from 31 May to 28 June (2021). **A.** Later formed summer leaf Fv/Fm curves and temperature data for 15 genotypes of woodland strawberries overwintered at Åland Island and Lammi (30 data sets in total). The left vertical axis is the temperature (°C) during this period, and the right vertical axis is Fv/Fm. **B.** Italy later formed summer leaf genotypes (IT2, IT8, IT12). **C.** Iceland later formed summer leaf genotypes (ICE2, ICE4, ICE16). **D.** Norway Kåfjord later formed summer leaf genotypes (NOR17, NOR16, NOR13). **E.** Norway Tromso later formed summer leaf genotypes (NOR11, NOR29, NOR27). **F.** Norway Alta later formed summer leaf genotypes (NOR5, NOR6, NOR9).

The statistical Repeated measures ANOVA analysis for Fv/Fm ratio in later formed summer leaves during 31 May to 28 June shows $p < 0.001$, $F = 114.916$, $df = 4$. It indicates that the observation of different Fv/Fm ratio between genotypes is statistically significant. The interaction of overwintering sites and genotypes for Fv/Fm shows $p < 0.001$, $F = 4.914$, $df = 29$. This also showed that the results were statistically significant after repeated measurements for different overwintering sites as well as different genotypes over 31 May to 28 June.

In post hoc Tukey tests, NOR 9 that had overwintered in Åland had significantly lower Fv/Fm ratio than almost all groups of later formed summer leaves. IT 2, ICE 16, NOR 17, NOR 16, NOR 13, which also had overwintered in Åland, were not significantly lower than NOR9 that had overwintered in Åland. The same situation happened on IT 8, NOR 13, NOR 9 that had overwintered in Lammi, and NOR 5, NOR 6 from the same origin with NOR 9. NOR 6 shows significant differences from ICE 2 (Iceland), ICE 4, NOR 29 that had overwintered in Åland.

Table. 4 Fv/Fm ratio statistical significance of woodland strawberry later formed summer leaf between 15 genotypes and 2 overwintering sites in post hoc Tukey tests.

Genotype Overwintering site			Genotype Overwintering site		
		Sig.			Sig.
NOR 6 Åland	ICE 2 Åland	0.008	NOR 9 Åland	ICE 2 Åland	0.000
	ICE 2 Lammi	0.006		ICE 2 Lammi	0.000
	ICE 4 Åland	0.002		ICE 4 Åland	0.000
	NOR 11 Åland	0.016		ICE 4 Lammi	0.005
	NOR 29 Åland	0.003		ICE 16 Lammi	0.005
	NOR 29 Lammi	0.025		NOR 17 Lammi	0.025
NOR 6 Lammi	ICE 2 Åland	0.028	NOR 16 Lammi	0.043	

	ICE 2 Lammi	0.022		NOR 11 Åland	0.001
	ICE 4 Åland	0.007		NOR 11 Lammi	0.004
	NOR 29 Åland	0.011		NOR 29 Åland	0.000
NOR 9 Åland	IT 2 Lammi	0.014		NOR 29 Lammi	0.001
	IT 8 Åland	0.014		NOR 27 Åland	0.009
	IT 12 Åland	0.006		NOR 27 Lammi	0.010
	IT 12 Lammi	0.047			

3.4 Two woodland strawberry spring phenology events

3.4.1 Flowering onset date of woodland strawberry

The flowering onset of woodland strawberry plants occurred from mid-May to early June. According to overwintering in Åland or Lammi, there is no significance differences between 15 genotypes of woodland strawberries on the date of flowering onset in this observation (Fig. 11). Although there were no significant differences, almost all genotypes showed an earlier date of flowering onset in the plants that had overwintered in Åland than in Lammi (except NOR 16, which was the opposite). However, genotypes NOR 13, NOR 5 and NOR 6, had significantly earlier flowering onset dates than ICE 2, NOR 29 and NOR 27. Overall, the dates of flowering onset of different genotypes all differed according to the place of origin, and genotypes from the same place of origin all had a high degree of similarity. The three groups of nine genotypes from Norway were also divided into two categories of earlier and later flowering onset dates based on a more subdivided origin. NOR 11, NOR 29, and NOR 27 from Tromso, Norway has closer flowering onset dates to plants from Italy and Iceland (Fig. 10).



Figure. 11 Date of woodland strawberry flowering onset in the spring 2021. The recording period begun on 9 May and ended on 8 June (2021). Significance level $\alpha = 0.05$; different letters indicate different levels of significance ($F=11.056$, $df=29$, $p<0.001$).

3.4.2 First stolon production date of woodland strawberry

The dates of first stolon production in woodland strawberry differed significantly depending on the genotypic origin (Fig. 12), in late May and early June, respectively. The stolon production dates of all genotypes were earlier in the plants overwintering in Åland, than in the plants overwintering in Lammi, with significant differences in IT 12 and NOR 17. There were also different levels of variability between stolon production dates for different genotypes from different origins. For example, NOR 5, NOR 6, NOR 9, NOR 17, NOR 16, NOR 13 from the Alta and Kåfjord regions of Norway produced stolons earlier than woodland strawberries from other regions. The stolon production dates were significantly earlier than IT 8, ICE 16 and NOR 27 (Fig. 12).

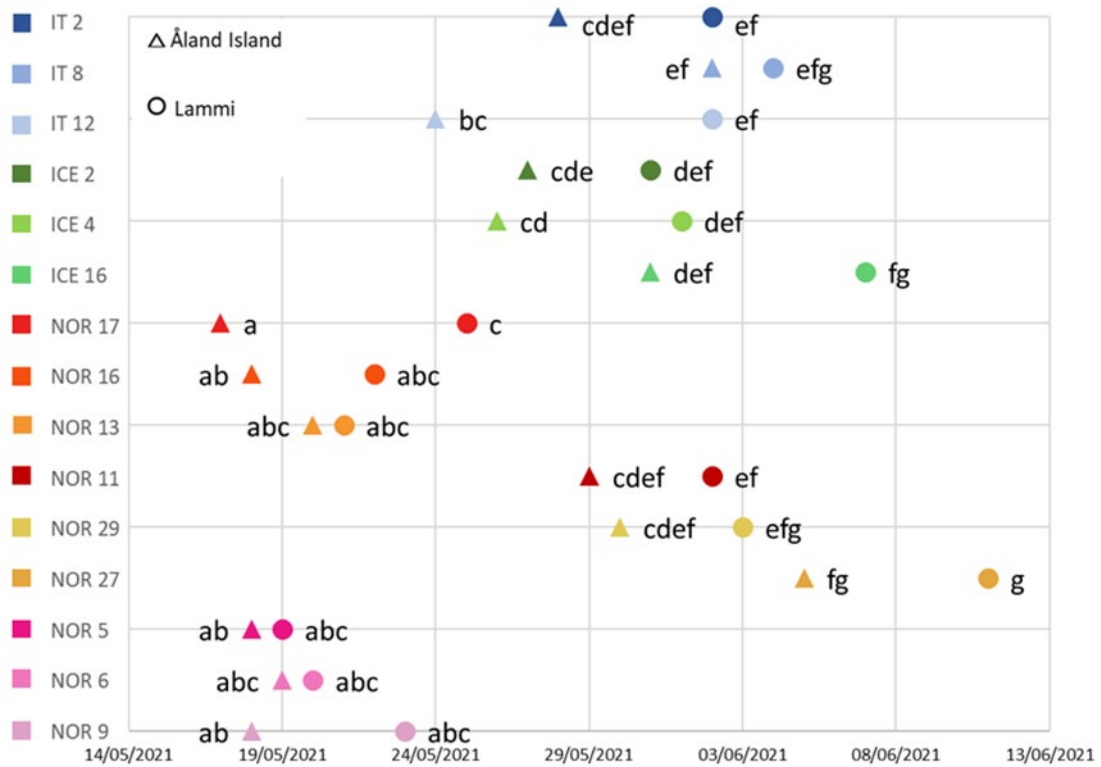


Figure.12 Date of woodland strawberry first stolon produced in the spring 2021. The recording period begun on 14 May and ended on 13 June (2021). Significance level $\alpha = 0.05$; different letters indicate different levels of significance ($F=23.003$, $df=29$, $p<0.001$).

4 Discussion

4.1 Chlorophyll fluorescence in three types of leaves: winter leaf, first summer leaf, later formed summer leaf

Chlorophyll fluorescence (F_v/F_m) are often referred to quantum yield of PSII by dark-adapted leaves, which is suitable for measuring the quantum efficiency of PSII in leaves, and an indicator of plant photosynthetic performance (Butler and Kitajima 1975; Kitajima and Butler 1975). The chlorophyll fluorescence is a quantitative mean of detecting PSII status, which is an indirect way of measuring capacity of the light reactions of photosynthesis, by measuring the electron transport through PSII. Measured values for healthy plants are generally between 0.79 and 0.83 (Baker and Rosenqvist 2004). The F_v/F_m ratios of the measured leaves of woodland strawberries in this experiment were below this interval at several times. The lower values for winter leaves until 20 April are presumed to be due to the exposure to high light in

combination with low temperatures in spring. The chlorophyll function in winter leaves needs a recovery process after overwintering (Körner and Larcher 1988). In contrast, the decline in values of winter leaves that started on 16 May is due to the start of the senescence process.

Interestingly, the first summer leaves had normal range of Fv/Fm, but only for two weeks before the senescence process began. The senescence was completed before the end of June. First summer leaves of woodland strawberry in spring have previously not been observed, and these results were unexpected. Their Fv/Fm ratios remained in the normal range for only two weeks before the senescence process began. And the senescence was completed before the end of June. The conjecture that can be made about this phenomenon is that this group of first summer leaves are leaves that are especially for the spring and also temporary leaves that will senesce in the spring.

The later formed summer leaves were formed at the end of May, with Fv/Fm ratios remaining in the normal range for two weeks. After 14 June, Fv/Fm of all later formed summer leaves remained below 0.7, despite temperatures were in the range most suitable for photosynthesis in woodland strawberries (Chabot and Chabot 1977). In particular, the NOR 5, NOR 6, and NOR 9 genotypes from Alta in Norway showed the greatest decrease due to senescence, falling to 0.1-0.4 by the end of the measurement period.

When comparing the effects of different overwintering conditions on the Fv/Fm ratio of leaves, winter leaves did not behave in the same way as summer leaves. In early April, before the winter leaves adapted to Viikki environmental conditions, the Fv/Fm of plants that had overwintered at Lammi was higher and remained around 0.6. The Fv/Fm of plants that had overwintered in Åland during the same period showed a more pronounced upward trend from 0.3 to 0.6. However, first summer leaves and late formed summer leaves did not exhibit significant numerical differences for the different overwintering sites. The Fv/Fm ratio of summer leaves was generally correlated with leaf senescence and was not significantly influenced by other factors.

In summary, different overwintering conditions had a significant effect on the Fv/Fm ratio of winter leaves at the beginning of spring. Warmer winter conditions would

make the winter leaves less functional. Another explanation is that the winter leaves on Åland have been exposed to high light for longer time during spring with low temperatures, which makes the leaves stressed and have lower photosynthetic capacity. However, after adaptation to spring environmental conditions, the differences became less pronounced. As for the summer leaves, the different overwintering conditions did not have a particular effect.

4.2 The phenology of first summer leaf development in spring

Spring plays a very important role in plant growth processes and nutrient cycling in the ecosystem. Menzel *et al.* (2006) have observed earlier spring phenological events and longer growing seasons at mid and high latitudes due to global climate change. Studies on the phenology among different species have been conducted using continuous data sets to observe the relationships or correlations between plant phenology and environmental variables in different seasons. Recently, studies of autumn phenology have been gradually expanded and are no longer limited to a few species or small areas (Liu *et al.* 2016).

The woodland strawberry plants used in this thesis sprouted two sets of summer leaves in the spring. There are a few morphological differences between them: the first summer leaf is smaller, closer to the ground and has shorter petiole than the second summer leaf. However, since this was beyond what was expected when the experiment was designed, no further anatomical observations were made. No specific leaf size measurements were made either, only a rough description. According to previous studies, woodland strawberries have extensively dimorphic leaves, as evidenced by shorter winter leaf petioles, smaller leaf area, and thinner epidermal layer and fewer air spaces as anatomical features. Such characteristics allow winter leaves to be more adapted to cold winter conditions (Åström *et al.* 2015). The first summer leaves are morphologically between the winter and later formed summer leaves and can be presumed to be transitional leaves produced to adapt to the spring environment. However, the basis for this speculation is not sufficient, and it can only be observed from their chlorophyll fluorescence that most of the first summer leaves senesce by the end of June. Another reason is that woodland strawberry plants are

planted in pots that are not exactly natural conditions. Since plants were not fertilized in the spring, nutrients washed away by rainfall could lead to nutrient deficiencies and affect leaf longevity.

Since only the production of first summer leaves was recorded, and for the later formed summer leaves it can only be determined that they develop and mature by the end of May. So, the conclusion about the effect of different overwintering conditions on the development of summer leaves is not comprehensive. The only conclusion that warmer overwintering conditions will affect earlier formation of first summer leaf.

4.3 Winter leaf senescence in spring

The two main function of the leaves in winter is to provide the plant with the ability to photosynthesize under the snow and to maintain the plant's physiological activity in winter. Another main function of winter leaves is for the plants to be able to begin growth early in the spring without first having to produce new leaves nor risking newly developing leaves for frost damages, because the winter leaves are cold hardy and photosynthesizing when the snow cover is thin enough (Åström *et al.* 2015; Tolvanen 2020). Therefore, the winter leaves are fully functional and suitable for spring conditions without snow. Those winter leaves reached optimal F_v/F_m in mid-May, and remained high until early-mid June. The winter leaves naturally senescence in late spring and are replaced with summer leaves, which are better adapted to the summer environment.

In this thesis, the senescence of woodland strawberry winter leaves in spring was correlated with different overwintering conditions. Winter leaves that had overwintered in Lammi remain functional for a longer period. Late phases of senescence tend to appear later than for strawberries overwintering in the Åland islands. When different genetic origins are compared, winter leaves from NOR 6 and NOR 9 in Alta, Norway, entered later phases of senescence earlier. These phenomena are different from the leaf senescence that occurs in autumn. Leaf senescence that occurs in the fall is usually delayed by warmer temperatures (Estiarte and Peñuelas

2015). In contrast, the winter leaves of woodland strawberries senesce slightly earlier due to warmer winter conditions, which included higher temperatures and thinner snowpack (Finnish Meteorological Institute, data from October 2020 to April 2021, 2022). Based on this conclusion, a larger difference is to be expected if the overwintering conditions were more different from each other than Lammi and Åland.

This is related to the different functionality of the winter leaves of woodland strawberries and the leaves of winter deciduous plants. Leaf senescence of winter deciduous plants occurs in the fall is aimed to cut the nutrient overhead in the winter (Estiarte and Peñuelas 2015; Peng *et al.* 2021). In contrast, the winter leaves of woodland strawberries produce summer leaves earlier in the spring when faced with higher temperatures. This also means that winter leaves are no longer playing the most important role as a primary producer in spring.

4.4 Flowering in spring

The dates of flowering occurred during mid-May to early June for the 15 genotypes of woodland strawberry covered in this thesis. Depending on the overwintering conditions (Åland and Lammi), the opening of the first flower differed, although no statistical differences were observed. Overall, warm overwintering conditions brought forward the flowering date of most genotypes of woodland strawberries, except for NOR 16. This is comparable to the conclusion reached by Bock *et al.* (2014). However, according to the findings of Bock *et al.* (2014), they focused more on warmer spring weather than mild winter conditions. This makes the changes of woodland strawberry flowering date after overwintering in different winter conditions, to explore the relationship between flowering date and winter condition in different species.

The similar changes in the timing of flowering could occur with the strawberry cultivar, garden strawberry (*Fragaria × ananassa*). In the case of the garden strawberry, the date of flowering is a phenological phenomenon that may have economical implications. The early flowering date may cause the garden strawberry to enter the reproductive state before the plant has accumulated sufficient nutrients (Davide *et al.* 2012). Therefore, further investigation of the effect of changes in winter conditions

on the flowering date of woodland strawberries will not only broaden the comprehensive study of woodland strawberry spring phenology. It also provides a reference for spring phenology study of garden strawberry.

4.5 First stolon production in spring

Woodland strawberry, possesses the ability to reproduce asexually by stolons. The stolon production starts in spring, so they are part of the spring phenology of the woodland strawberry. Among the woodland strawberry genotypes covered in this paper, the genotypes from Alta and Kåfjord in Norway produce stolons earlier than the genotypes from other regions. This may be due to the fact that the genotypes from these two regions have a different altitude and growing environment than the other regions. Unfortunately, this could not be confirmed in this paper due to the lack of data on the altitude of the native habitat. Leaving aside their place of origin, for the condition of different overwintering sites, it is still the origins that overwinters in warmer areas and that produces stolons earlier. This is similar to the flowering phenology. It can be interpreted that warmer overwintering conditions lead to earlier reproduction in woodland strawberries.

5 Conclusions

This study shows that different overwintering conditions have an effect on the spring phenology of woodland strawberry. These effects were present in all the 15 different genotypes of woodland strawberry. This was shown in the present study that summer foliage formation, flowering and first stolon formation in woodland strawberries are earlier after overwintering in higher winter temperatures and thinner snowpack. The senescence process of winter leaves of woodland strawberries is also advanced due to warmer overwintering conditions.

In other words, in the future, under the background of global climate change, the spring phenology of woodland strawberries may occur gradually earlier. The functionality of winter leaves in spring will gradually decrease and be replaced by the first summer leaves earlier. This will provide some reference for ecological and

phenological studies on woodland strawberries. Because this thesis only focused on the first summer leaves by the chlorophyll fluorescence (Fv/Fm ratio), it did not analyze the anatomical structure and synthetic substances that make them different from the later formed summer leaves. The first summer leaves may in the future replace the role that winter leaves would otherwise play in the spring. Perhaps in the future, studies could be conducted on the anatomical structure of the first summer leaves.

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