Autumn Senescence in *Populus tremula*

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Abstract

The study that was conducted in this thesis confirmed that northern aspens enter into senescence earlier and the duration of their senescence period is shorter in general compared to the more southern growing aspen ecotypes. This indicates that finishing senescence faster has a high evolutionary value the further north a tree is located. The effects of girdling and the impact it had on autumn senescence were also studied. The loss of phloem transport in girdled trees resulted in that the tree accumulated sugars that promotes the tree into a much earlier senescence and also heavily increases the anthocyanin production during the senescence initiation. These effects seem to be a response to the sugar accumulation that takes place in the tree crown as a result of the girdling. Nitrogen levels were also lower in the girdled trees but the salvage efficiency of nitrogen in the leaves increased.

1. Background

1.1 Senescence

Senescence is the final developmental stage in a plant's organ and the function of senescence is to conserve valuable nutrients that are present in the organ. Senescence can be initiated by various triggers such as photoperiod, stress or shading. In annual plants senescence is often whole plant senescence and the process ends in the plant's death, the purpose of the senescence here is to reallocate nutrients to the seeds. In deciduous trees the aim of autumn senescence is to salvage valuable nutrients such as nitrogen and phosphorus from the leaf and store them in the bark and roots to promote next year’s growth.

The ability of which boreal trees achieve winter hardiness and accumulate and save nutrients from the poor soils in the northern forest is a crucial trait for their survivability. Senescence is a way for trees to salvage the nutrients that it has invested in the leaves during the summer. Senescence has many as yet unknown variables and the trigger mechanisms at a cellular level and are not yet fully understood, however it is known that the autumn senescence of boreal trees is under photoperiodic control and that it varies profoundly from trees at different latitudes. Also the speed of which trees can complete the process of salvaging its nutrients seems to vary between different ecotypes.

When leaf senescence occurs naturally it is typically coordinated at a whole leaf level. Senescence in some species starts in the margins or from the leaf tips and then moves towards the base of the leaf and its vascular network. Environmental factors such as stress can create uneven patterns of senescence; since it can speed it up once initiated or even trigger an earlier onset of senescence. (Lim PO et al., 2007)

When senescence is triggered the cell enters into a type of programmed cell death (PCD) pathway. The PCD pathway is a controlled degradation of the cell so it can be degraded in an ordered state. Even at a cell base level it seems that the cell death does not occur coherently although it starts with mesophyll cells and then moves on to the other cells. It may look like the cell death is coherent from
a macro perspective, but it is almost randomly initiated in the outer regions of the leaf and then get more coordinated as it propagates into whole leaf senescence (Jones et al., 1996). When the cells enter senescence it doesn’t do it at maximum speed but rather in a slow controlled degradation of the cells different organelles, this might be a strategy to effectively remobilize as much as possible of the nutrients and nitrogen in the leaf (van Doorn, 2005). The last parts of the cell to be degraded are the nucleus and the mitochondria, both are essential for gene expression and energy production. This shows that the cell requires functionality during the degradation process and degrades the different parts according to their value for the functionality of the process. This is a very good example for maximized effectiveness of the remobilization of nutrients within the cell (van Doorn et al., 2004).

When quantifying senescence for scientific purposes many methods and markers can be used; among these are chlorophyll content, photochemical efficiency, senescence–associated enzymes, membrane ion leakage and gene expression. Leaf chlorophyll can easily be estimated by its color that reflects the breakdown of mesophyll cells. The chlorophyll degradation pathway may be one of the more reliable indicator on the progress of senescence as it is the pace setting activity in senescence (Ougham, 2008). So by measuring the yellowing in the leaf one can get an indication on the progress of the senescence.

In European Aspen (Populus tremula) during autumn senescence about 80% (60% on a dry weight basis) of the total leaf nitrogen is remobilized (Keskitalo et al., 2005). The timing of leaf senescence in the northern latitudes is of great importance as entering into senescence too early will significantly shorten the already narrow growth period. Entering into senescence too late on the other hand can result in killing of the leaves by frost and then much of the nitrogen will be lost to the plant which may have large consequences on growth and survival in the next season. (Fracheboud et al., 2009)

Nitrogen is usually the limiting factor on growth in the forests of the northern latitudes which makes it such an important substance to recycle (Näsholm et al., 1998).

Autumn senescence in each Populus tremula tree initiates about the same date every year indicating a photoperiodic control (Keskitalo., 2005). The speed of which the aspen will finish the senescence cycle can vary much and is dependent on many factors. It has been shown that low temperature will accelerate the speed of senescence once it has been initiated (Fracheboud et al., 2009). The speed of the degradation when it is conducted under cold conditions can be up to 3 times faster in comparison to a warm year; a warmer year slow the degradation speed down. A correlation has also been shown between trees that enters senescence late and having a shorter time period of leaf senescence. It has also been hypothesized that the trees that has a short senescence duration might be less efficient in remobilizing nitrogen, this might be an adaptation to the northern latitudes were the yearly carbon gain and ensuring winter survival is of higher priority than salvaging all the nitrogen from the leaves (Fracheboud et al., 2009).

1.2 Effects of girdling

Girdling is a mechanical wounding of the stem and a method for removing the phloem and cambium in a circular area around the stem; as a consequence the phloem transport is disrupted. When the main stem is girdled the plant cannot transport sugars down to the roots. Xylem transport is still functional and water can be transported from the roots to the leaves unhindered. Girdled plants will experience a gradual increase of sugar concentration in the foliage and this will eventually have
several effects on the leaves. The elevated sugar levels trigger the expression of anthocyanins in the leaves during the senescence, the anthocyanin levels have been reported to be up to 5 times more than in ungirdled plants (Murakami et al., 2008). The root synthesized hormones such as cytokinin will also be affected, as cytokinin levels are influenced by nitrogen availability, the levels of cytokinins in the girdled trees may be less and thus promoting an earlier senescence (Sakakibara et al., 2008)

1.3 Anthocyanins
Anthocyanins are a large group of molecules produced in the leaf, their exact function in the leaf is still under debate. Several hypotheses have emerged where some state that the function of the anthocyanins in the leaves is to deter herbivores by the bright red color (Archetti, 2000). It has also been suggested that the accumulated anthocyanins in the leaf are a representation of the excretion process of toxins into the senescing leaves (Ford, 1986). The most common view of the role of anthocyanins is however that they are a nonfunctional byproduct of the leaf senescence (Mohr and Schopfer., 1994; Archetti., 2000; Matile., 2000). Anthocyanins can also be induced when the leaves have high carbohydrate levels as when they are senescing and photosynthetic proteins are being degraded (Matile., 2000). Although anthocyanins can be induced by tissue that has low carbohydrate levels shows that they have other roles as well. Anthocyanins could be synthesized in senescing leaves well before the chlorophyll breakdown has begun, and it has also been shown that intense light, cool temperature and mild drought induce synthesis of anthocyanins (Field., 2001). I.e. anthocyanins are often synthesized when the leaf is stressed. The leaf would certainly need some kind of measure to protect itself from the very reactive chlorophyll degradation products, so a plausible theory is that one of the roles of anthocyanins in senescing leaves are protecting the leaf from degrading chlorophyll and inhibit oxidative damage caused by these degradation products. If no measure was taken against reactive oxygen species (ROS) damage caused by the degrading chlorophyll it would most likely interfere with the nutrient reallocation process and reduce its effectiveness (Field., 2000).

1.4 Nitrogen and senescence
Gardner’s has known for a long time that if you fertilizing a deciduous plant in late summer or early autumn will cause it to continue to grow later into the autumn then it usually would. The tree will probably not enter senescence or delay the onset and may not develop the winter hardiness required to survive the cold of the winter. Interestingly alders (Alnus serrulata) do not undergo autumn senescence and stay green until frost damages their leaves enough for them to die and detach. This is a consequence of their symbiotic relationship with the bacteria Frankia alni, a nitrogen fixing bacteria gives the tree nitrogen in exchange for carbohydrates. This explains why the alders don’t have a need for salvaging nitrogen from their leaves as they will receive nitrogen from their symbiont instead, and is a nice demonstration of the importance of nitrogen status for autumnal senescence.
1.5 The SwAsp collection

The SwAsp collection contains over 100 genotypes of wild aspens collected from 12 different locations in Sweden (Ronneby, Simlång, Ydre, Vårgårda, Brunsberg, Uppsala, Älvdalen, Delsbo, Umeå, Dorotea, Luleå and Arjeplog). These ecotypes will represent some of the variation of the aspens in Sweden. The SwAsp common garden that is located in Sävar which lies about 15km outside Umeå. The garden is setup as grid systems were the clones are planted in four replicates in a randomized pattern. All the clones in the garden are of the same age and were planted in 2004. A web database exist that is linked to this garden so that when researchers conduct experiments in the garden they can include their experimental data into the database, large data sets exist with information from herbivores to senescence scoring for several years.

Fig 1. The geographic locations of the clones(1-Simlång, 2- Ronneby, 3- Vårgårda, 4-Ydre, 5-Brunsberg, 6-Uppsala, 7-Älvdalen, 8-Delsbo, 9-Dorotea, 10- Umeå , 11-Arjeplog, 12- Luleå)

1.6 Aim

The aim of this thesis is to investigate the effects of girdling during autumn senescence by measuring the chlorophyll and anthocyanin content during this period, and also the carbon and nitrogen content in the leaves. The variation in onset and duration of senescence in different Swedish aspen ecotypes will be examined.
2. Materials & Methods

2.1 Girdling.
For the girdling experiment 5 separate tree stands were chosen with 4 trees in each stand selected and labeled. They were all located relatively near each other and assumed to be the same clone/genotype. This assumption was later confirmed to be true by genotyping of the trees (data not shown). The location of the stands was relatively similar in light conditions as they were all positioned on the edge of a clearing or field. The age of the trees was probably 10-30 years. One tree in each stand was used as a control tree and got the number 1. One tree was girdled and marked as tree number 2. The third tree was fertilized and the fourth tree girdled and fertilized, this was done in all five stands.

The trees were then sampled with two initial samplings before bud set in June 15 and 18. Regular sampling of all the five stands took place between August 10 to October 15; with two sampling occasions each week taken around noon. Some trees could not be sampled to the end of the experiment as they had shed all their leaves earlier then October 15. The sampling was conducted by using a cutting pole that could be extended several meters as to reach higher when sampling to avoid shadowed leafs. From each tree 1 or 2 branches were cut down and from these 15 leaves were sampled and measured with a hand held spectrophotometer, measuring Anthocyanin and chlorophyll levels. These measurements were conducted in the field by a hand held Opti-Sciences™ CCM-200 chlorophyll content optical absorbance meter. This instrument will measure the optical absorbance of chlorophyll \( a \) at 665 nm and chlorophyll \( b \) at 642 nm and add the values. The anthocyanins were measured with the same instrument but set to measure at 530 nm; the instrument also has a leaf thickness compensator so that you get accurate comparable measurements regardless of the leaf thickness.

The samples were then divided into 3 containers with 5 leaves in each container and immediately frozen in liquid nitrogen. After the sampling was finished the stands 1-4 were then chosen for further analysis. The fifth stand was excluded from the dataset as it had a very severe mite infection; this had an effect on the trees as severe leaf damage by herbivores can promote a tree into senescence or at least severely alter their metabolism (Risley.,1993; Trumble et al.,1993). For analysis ten dates were chosen that should represent the whole senescence process. The dates were chosen from the chlorophyll degradation data where one could see a clear representation of the process. The same dates were used for analysis in all stands. The samples were then ground into a fine powder and stored in -80 for use for further analysis.

2.2 Nitrogen and carbon content analysis.
The powder from the ground leaves was put into small aluminum foil capsules and 3-3.5 mg of sample was used. The capsules were then put into a 96 well plate according to a premade pattern for analysis in a GC machine.

Leaf weight per area:
Leaves were also thawed from the samplings and 10mm circular cuts were made that was dried and weighed to get the leaf weight per area. Unfortunately not all samples could be analyzed since they had been crushed to powder for other analysis.

2.3 Sävar SwAsp tree collection senescence scoring
The SwAsp common garden that is located in Sävar was scored for senescence two times a week between August 20 and October 9. The trees were scored visually according to a senescence score card from 0-7. Where 0 was totally green leafs to 7 that is an almost clean stem without leafs or only dead leafs, the score 3 were used as a marker for onset of senescence, the score indicated the appearance of the first yellow color. The scores were then summed up for all the trees and the number of days to complete senescence was also calculated (the amount of days between score 3 to score 7). The senescence score card can be viewed in Appendix D.
3. Results and Discussion

3.1 Northern ecotypes of aspens enter senescence earlier than southern.

To investigate if there was any difference between trees from different populations in the onset and duration of senescence a senescence scoring was conducted based on the foliage color.

![Summed senescence score graph](image)

Fig 3. The average score of the different clone groups from the year 2009 and 2007 summed up. Southern clones are to the left and northern clones are to the right. The data from 2007 was collected by Fracheboud Y and Björkén L (unpublished data).

When the score are summed up the trees that have acquired a high score will be those that enter into senescence early. As apparent in the graph (fig 3) northern aspens differ from southern aspens with a much higher score. Since they have grown in the same conditions and climate northern aspens have a genetic makeup that make them enter into senescence earlier than the southern aspens. This is consistent with previous data (Fracheboud Y, 2009). The difference between the 2007 and 2009 scoring is due to the longer scoring period in the year 2007, the scoring was conducted until the 25 of October which is almost 20 more days of scoring then 2009 where a severe frost killed all of the leaves in the beginning of October. This longer scoring period will shift all the scores to a higher value in 2007, but the same trend can be seen in both years that the northern aspens finish senescence faster and earlier than those from the south.
Fig 4. The year day of score 3 was the day set as onset of senescence. The measurements from the year 2007 were compared to the same measurements done in 2009. Northern aspen ecotypes are to the right in the graph and the southern to the left. The size of the bubble indicates number of trees in the clone group that acquired the score 3.

In fig 4 we can see that the majority of the northern aspens acquire a score of 3 much earlier than the southern aspens. This indicates that they are initiating their senescence earlier than the southern aspens. We can also see a pattern that the majority of the trees in clone group 1-8 seems to initiate their senescence coherently as many of them acquire a score of 3 on the year day 250 (September 7)
Fig 5. The year-day of onset of senescence for each clone compared between 2 years.

The onset of senescence does have a natural variation from year to year that can be seen in fig 5. The variation will shrink with later dates of onset. A linear correlation can be seen when all the clones within the clone groups are plotted, showing a shift of onset with each clone group.

3.2 The time to complete senescence differed between the southern and northern clones.

Fig 6. The average number of days for each clone group to complete senescence compared to measurements taken 2007. The data from 2007 was collected by Fracheboud Y and Björkén L (unpublished data).
The days between the score 3 and 7 was defined as the duration of senescence. Also here there was a difference between population, the four northern populations finish senescence faster. There was a difference of 8 days between the most southern ecotypes and the most northern ecotypes that indicated that there probably is a genetic component that influences how fast the tree can complete senescence. The same aspens have been measured in the year 2007 as well, as indicated from fig 6 data correlates to this year’s measurements. If compared to the same data measured in the greenhouse 2006 the trend of a faster speed in senescence of the northern aspens cannot be seen, in fact the northern aspens take longer time to finish senescence then the southern (Table 1). This might indicate that the northern aspens are more prone to speed up their senescence process in colder climate then the southern aspens that seldom have the same harsh conditions or cold autumns and therefore haven’t acquired this trait as it is probably of a lower evolutionary priority in the south.

Table 1. Days to complete senescence of the SwAsp trees grown in a greenhouse and outside in the Sävar garden.

<table>
<thead>
<tr>
<th>Senescence speed (days)</th>
<th>Northern (8-12)</th>
<th>southern (1-4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006 (GH)</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>2007(Sävar)</td>
<td>24</td>
<td>33</td>
</tr>
<tr>
<td>2009 (Sävar)</td>
<td>24</td>
<td>29</td>
</tr>
</tbody>
</table>

The scoring of senescence in the SwAsp garden in 2009 was relatively straightforward, a complication was however the heavy yellow fungal infections making the visual scoring harder. The fungal infections might have altered the senescence since many of the trees had leaves that rather than turning yellow turned black. The yellow fungi also made the trees harder to score since the yellowing of leaves is also the sign of senescence.
3.3 Girdling will affect senescence duration and onset and anthocyanin production.

![Chlorophyll Estimates, Stand 4.1](image)

Fig 8: Dates chosen for further analysis from chlorophyll measurements on tree 4.1. The dates marked with red are the 10 that were picked out for metabolomics, carbon and nitrogen analysis.

Chlorophyll levels were measured on all trees, a diagram of the chlorophyll levels in tree 4.1 is shown in Fig 8. Based on these curves, we selected 10 dates where samples were taken for further analysis. The dates were chosen from the 4.1 tree since it had the most clear and interpretable chlorophyll content graph. In the analysis of the data the fifth stand was excluded because of a heavy infection of mites damaging the leaves and resulting most likely in an altered senescence.

![Chlorophyll average](image)

Fig 9 Average chlorophyll content in the leaves of stand 1-4.
The chlorophyll levels in the girdled trees were about the same as the controls trees. The girdled trees chlorophyll levels then starts to decline constantly in the beginning of August until October. The control trees behaved as expected and maintained chlorophyll levels into the middle of September with some “wear and tear” damage that lowers the chlorophyll content somewhat. In September chlorophyll levels starts to rapidly decline as they begin senescence and start to degrade the chlorophyll. The girdled trees started their degradation process much earlier than the controls and when the controls enters into senescence the girdled trees have already lost more than half its total chlorophyll. Notably, no increase in the rate of chlorophyll degradation was noted in the girdled trees at the time when control trees initiated autumn senescence.

![Anthocyanin average graph](image)

**Fig 10** Average anthocyanin content in the leaves from August to September in stand 1-4.

When the girdled trees starts the degradation process of chlorophyll the anthocyanin levels stay at the same levels as the control trees up until the beginning of September.

**Table 2. The starting date of anthocyanin production in the girdled trees.**

<table>
<thead>
<tr>
<th>Stand</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3-Sep</td>
</tr>
<tr>
<td>2</td>
<td>31-Aug</td>
</tr>
<tr>
<td>3</td>
<td>5-Sep</td>
</tr>
<tr>
<td>4</td>
<td>3-Sep</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>2-Sep</strong></td>
</tr>
</tbody>
</table>

Anthocyanin production is most likely increased to protect the leaf from phototoxic damage from partially degraded chlorophyll that has started to rise to concentrations that could potentially damage the leaf. The girdled trees have an earlier onset of senescence; this seems to indicate that the salvage of nutrients is of a higher priority than maintaining functional leaves for an extended period of time. The duration of the senescence period is also extended and the degradation slowed down. The girdled trees might sense a nitrogen shortage as they are cut off from the mycorrhiza...
symbiont in the roots that delivers nitrogen. Alternatively, higher carbohydrate levels above are responsible for both earlier senescence and decreased anthocyanin accumulation. The tree therefore take more time to complete senescence to make sure it can salvage as much as possible from the leaves as the nitrogen would now be one of the higher priorities for the trees survival. The increased anthocyanin level supports this theory as their function most likely is to safeguard the salvage process and increase its efficiency.

Fig 11. Chlorophyll content with 3 linear regression lines with the slope of the green line representing the speed of senescence and the duration of senescence from the intersection of the green and red line to the intersection of the green and blue line.

We calculated the senescence period by making several linear models within the graph that represented the 3 stages (before, during and after senescence) and we could then extract the onset and end of senescence as these intersects. The difference in onset of senescence could then be seen.

Table 3. Senescence start and end date for stand 2-4.

<table>
<thead>
<tr>
<th>Senescence</th>
<th>start</th>
<th>end</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>Girdled</td>
</tr>
<tr>
<td>1</td>
<td>7-Sep</td>
<td>(19-Sep)</td>
<td>30-Sep</td>
<td>6-Oct</td>
</tr>
<tr>
<td>2</td>
<td>9-Sep</td>
<td>23-Aug</td>
<td>30-Sep</td>
<td>22-Sep</td>
</tr>
<tr>
<td>3</td>
<td>27-Aug</td>
<td>21-Aug</td>
<td>24-Sep</td>
<td>27-Sep</td>
</tr>
<tr>
<td>4</td>
<td>10-Sep</td>
<td>22-Aug</td>
<td>2-Oct</td>
<td>2-Oct</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>5-Sep</strong></td>
<td><strong>22-Aug</strong></td>
<td><strong>29-Sep</strong></td>
<td><strong>29-Sep</strong></td>
</tr>
</tbody>
</table>

The girdled trees started senescence about 14 days earlier then the control trees and took about 2 weeks longer time to complete senescence and they degraded chlorophyll at half the speed compared to the controls. The girdled tree from Stand 1 was excluded from the onset of senescence calculation since it was not representative for other wild growing aspen.
Autumn senescence in *Populus tremula*

Author: Erik Olofsson

UPSC 2009

3.4 Nitrogen remobilisation efficiency is different in girdled trees

When the Nitrogen content of the leaves was analyzed we found that the control trees have more nitrogen then the girdled trees in August (fig 13). Apparently girdling affected the trees nitrogen levels. The girdled trees seem to remobilize more nitrogen in g/cm² compared to the control trees; but if we consider the fraction of the nitrogen that has been salvaged the girdled trees reutilizes more nitrogen. The total percentage of nitrogen that was removed from the leaves (fig 15) when compared to the studies performed by Keskitalo et al 2005 that indicated an aspen tree could retrieve up to 80% of the nitrogen stored in their leafs. The total carbon content (fig 14) is higher in the girdled trees and is probably an effect of the sugar accumulation that has taken place because of the girdling

The measurements of nitrogen content only showed the percentage of nitrogen in the leaf. As seen in the supplementary data, all the girdled trees contain less nitrogen per weight unit and more

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**Fig 12.** Chlorophyll values of the control and girdled trees both in June and in August, with the control to left and the girdled tree to the right in each column pair.

The amount of chlorophyll in leaves can naturally be very variable, as can be seen in Fig 12. This makes comparisons between the different trees hard. The locations of the trees should have been relatively similar, in terms of light and water so that environmental factors shouldn’t impact too much on the experiment. The age of the trees could also have been a contribution to the variation as trees of different sizes were chosen. There could also be a difference in the nitrogen content in the soil. From the Chlorophyll estimates graph (Fig 12) we can see that the chlorophyll levels before any girdling took place were relatively similar. In August the chlorophyll levels between the girdled and ungirdled trees have changed and we can see that the girdling had a negative impact on the total amount of chlorophyll the tree can produce in the leaves. Stand 1 differs from the other stands as the girdled tree did not have less chlorophyll then the control, the elevated chlorophyll levels indicate that the amount of nitrogen that is available for stand 1 is much higher than the other stands.
carbon per weight unit. Control trees can perhaps fill their nitrogen reserves during the summer and doesn’t need to regulate the remobilization as strictly as the girdled tree that perceives a lack of nitrogen. Interestingly the chlorophyll levels in mid august is about the same level for both girdled and control trees before they start to drop as the girdled starts the degradation.

As observed the girdled trees can’t transport carbohydrates to the roots and the amount of carbohydrates (fig 14) in the leaves will increase and thereby the percentage of nitrogen will appear less. The girdled trees might sense the lower concentration of nitrogen and initiate nitrogen remobilization earlier. It has been observed that a girdled tree will increase synthesis of bark storage proteins (BSP) these BSP proteins will enhance the girdled trees ability to store more nitrogen (Cooke and Weih.,2005). To clarify some of these observations a leaf area/weight measurement was conducted to compare the control and girdled trees regarding nitrogen content.
3.5 The nitrogen remobilization percentage is higher in girdled trees but the amount is the same as in the control trees.

![Nitrogen Content per Leaf Area](image1)

Fig 13. The amount of nitrogen before and after senescence per leaf area from stand 1-4 summed up.

![Carbon Content per Leaf Area](image2)

Fig 14. Carbon content before and after senescence per leaf area summed up for stand 1-4.
The nitrogen content before senescence is higher in the control trees compared to girdled trees. The decrease in nitrogen content in the girdled trees could be an effect of cut off internal nitrogen cycling through the root system (Millard and Thomson, 1989). The girdled trees remove more nitrogen from the leaves than control trees (fig 13), and since the girdled trees started with less nitrogen they actually remove a higher percentage of the total nitrogen available in the leaf (fig 15).

4. Concluding remarks and outlook

The scoring of the aspens in the SwAsp garden confirmed that there must be a genetic control on both onset and the speed of senescence. Finding the gene(s) that control the speed of senescence would be of great interest for understanding the mechanisms behind this process. It is possible that photoperodic control is more important in trees growing in zones where the daylengths varies more profoundly. Trees that grow further south might rather rely on temperature than on the daylengths as their seasonal indicator. The good correlation between the experimental data to the previous years results tells us that this is a robust system that is regulated by environmental factors such as light conditions and temperature. The initiation of senescence is highly light regulated but as Fig 4 & 5 indicate that we do have variability from year to year. But there are many factors that can make the tree enter senescence prematurely, such as fungal infections which was the case in 2007. This might have shifted the onset of senescence. And we do indeed see a difference of the onset as the northern aspens start senescence earlier.

The theories why the tree produce anthocyanins as a result of the girdling induced senescence is still not proven but it seems likely that the function is to increase the efficiency of the remobilization machinery, perhaps by providing protection from photooxidative damage. One should note however that even though the girdled trees undergo senescence the process that happens in these trees is probably not identical to “regular” autumn senescence, as the girdled trees have a massive stress factor influencing the whole process in many levels.
The girdled trees do not start the anthocyanin production in conjunction to their onset of senescence, the accumulation starts about 10 days after the girdled trees has entered senescence. At this date the girdled trees have already degraded half of the chlorophyll, this might be some threshold for the anthocyanins to be synthesized. Also when the control trees enter senescence around the 5th September no increase in the degradation rate of chlorophyll is observed in the girdled trees. It is likely that as a consequence of the girdling the trees have become insensitive to the regular photoperiodic control of autumn senescence.

Regarding the nitrogen remobilization it is hard to draw any conclusion without conducting more experiments we would for example like to know: why do the girdled trees have less total nitrogen content before senescence? It might be an effect of the high sugar concentration or of the disruption of phloem transport that inhibits nitrogen recycling. This experiment should have had more replicates as 5 appeared to be too few to factor out the natural variation and environmental factors as a large contributing reason for the noise in the data. It might also be interesting to analyze were the nitrogen gets stored and how much. The salvaged nitrogen can’t be transported down to the roots, but aspens are known to store the bulk of its nitrogen in the bark. We still don’t know if the tree increases its storage capability in the bark as a response to the girdling, although we know that the BSP protein does increase as a response to the girdling.

A metabolomics analysis will hopefully give facts that answers to what carbon species are being salvaged in the girdled trees (fig 15) and which carbohydrates that are important to salvage. This may also give insight to what nutrients that will have altered levels during senescence in the girdled trees.

Aknowledgement

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5. References


6. Appendix

6.1 Appendix A: chlorophyll and anthocyanin values from all the stands. X.3 +X.4 is the fertilized trees and x.2 + x.4 is the girlled trees.
Autumn senescence in *Populus tremula*  
Author: Erik Olofsson  
UPSC 2009

### Anthocyanin Estimates, Stand 4
- CCM Value
- 2009/08/10, 2009/09/03, 2009/09/28

### Chlorophyll Estimates, Stand 5
- CCM Value
- 2009/08/10, 2009/09/03, 2009/09/28

### Anthocyanin Estimates, Stand 5
- CCM Value
6.2 Appendix B: Carbon content for each individual tree

![Carbon content chart]

6.3 Appendix C: Nitrogen content in leaves before and after senescence and the amount nitrogen remobilized from the leaf during senescence.

![Nitrogen content chart]
6.4 Appendix D: Supplementary figure 1: Senescence score card with relative chlorophyll content.