Background and Aims Physiological data suggest that autumn leaf colours of deciduous trees are adaptations to environmental stress. Recently, the evolution of autumn colouration has been linked to tree condition and defence. Most current hypotheses presume that autumn colours vary between tree individuals. This study was designed to test if within-tree variation should be taken into account in experimental and theoretical research on autumn colouration.

Methods Distribution of red autumn leaf colours was compared between partially dead and vigorous specimens of Norway maple (Acer platanoides) in a 3-year study. In August, the amount of reddish foliage was estimated in pairs of partially dead and control trees. Within-tree variation in the distribution of reddish leaves was evaluated. Leaf nitrogen and carbon concentrations were analysed.

Key Results Reddish leaf colours were more frequent in partially dead trees than in control trees. Reddish leaves were evenly distributed in control trees, while patchiness of red leaf pigments was pronounced in partially dead trees. Large patches of red leaves were found beneath or next to dead tree parts. These patches reoccurred every year. Leaf nitrogen concentration was lower in reddish than in green leaves but the phenomenon seemed similar in both partially dead and control trees.

Conclusions The results suggest that red leaf colouration and branch condition are interrelated in Norway maple. Early reddish colours may be used as an indication of leaf nitrogen and carbon levels but not as an indication of tree condition. Studies that concentrate on entire trees may not operate at an optimal level to detect the evolutionary mechanisms behind autumnal leaf colour variation.

Key words: Acer platanoides, Norway maple, branch die-back, coevolution hypothesis, leaf senescence, patchy distribution, red leaf pigments, tree condition, within-tree variation.

INTRODUCTION

Both yellow and red autumn leaf colours of deciduous trees are thought to be adaptations that prevent or decrease photooxidative damage, and to be an aid to recover nutrients during autumn senescence (Wheldale, 1925; Merzlyak and Gitelson, 1995; Hoch et al., 2001, 2003; Lee, 2002; Lee and Gould, 2002a). Yellow colour pigments, mainly carotenoids, are present in leaves throughout the growing season, but they are not visible before the breakdown of chlorophyll in autumn (Merzlyak and Gitelson, 1995; Ougham et al., 2005). Red colour pigments, mostly anthocyanins, are translocated to, or synthesized in, autumn leaves (Lee and Gould, 2002b; Lee et al., 2003; Gould, 2004). Autumn leaves also contain flavonoids and other phenolics that are perceived by many insects at ultraviolet wavelengths but that are invisible to the human eye (Matile, 2000; Briscoe and Chittka, 2001; Riipi et al., 2002; Salminen et al., 2004a; Manetas, 2006).

Archetti (2000) and Hamilton and Brown (2001) suggested that bright autumn leaf colours have evolved in a coevolutionary process where plants signal to herbivores how unsuitable as host plants they will be in the near future. It has further been assumed that tree condition correlates with autumn leaf colours. Lev-Yadun et al. (2004) suggested that bright leaf colouration has evolved in an "arms race" where plants aim to undermine insect camouflage. Sinkkonen (2006a) found a causal relationship between the reproductive investment of trees and the earliness of yellow autumn colouration in white birch (Betula pubescens). He proposed that a physiologically sub-optimal onset of autumn leaf colours might aid woody plants to protect their ongoing or upcoming sexual reproduction from sucking insects (Sinkkonen, 2006b). He further predicted that the onset of autumn colours is physiologically optimal if a plant does not reproduce greatly. Archetti (2000) and Hamilton and Brown (2001) suggested that leaf colouration is a costly signal to insect herbivores, but Sinkkonen (2006b) assumed that coevolution is based on a common interest. While the ideas of Lev-Yadun et al. (2004) and Sinkkonen (2006b) have received relatively little attention, the original coevolution hypothesis has been widely discussed (Wilkinson et al., 2002; Archetti and Brown, 2004; Schaefer and Wilkinson, 2004; Ougham et al., 2005; Chittka and Döring, 2007). Much of the debate has concentrated on details of the original ideas by Hamilton, Archetti and Brown. Interestingly, it has been suggested that herbivores utilize autumn leaf colours as a cue for nutrient availability or tree defences (Holopainen and Peltonen, 2002; Archetti and Brown, 2006; Schaefer and Rolshausen, 2006a, b), but that herbivores per se have not affected the evolution of autumnal leaf colouration. This has complicated the conclusions drawn from studies where aphid behaviour has depended on leaf colouration. Both the coevolution hypothesis and the camouflage hypothesis operate at the level of individual plants.
According to the original coevolution hypothesis of autumn leaf colours, the intensity or earliness of the signal reveals how well a tree can fight against its pests (Archetti, 2000; Hamilton and Brown, 2001; Archetti and Brown, 2004). Hagen et al. (2003, 2004) explored the relationship between autumn colouration and leaf fluctuating asymmetry (leaf FA = the random deviation of leaves from perfect symmetry), which they used to measure tree condition (see Van Valen, 1962; Möller, 1995; Martel et al., 1999; Lempiä et al., 2000). Hagen and colleagues found that trees with highly asymmetric leaves did not reveal as much early autumn leaf colours as other trees did, and suggested that the results proposed a positive causal relationship between relative autumn coloration and tree condition in a subspecies of white birch, *Betula pubescens* (Hagen et al., 2004). Since Kozlov (2003, 2004) has found that leaf fall is independent of leaf FA in white birch, and since these results call into question the use of leaf FA as a measure of sink strength and resource acquisition, other measures are needed to test whether woody plants can signal their current or upcoming vigour using autumn leaf colours.

In this paper, a test is made as to whether tree condition affects the existence, prevalence and distribution of visible pigments in tree foliage (see Ramírez et al., 2008). Because aphids are known to be attracted by yellow colour (Holopainen and Penttonen, 2002; Chittka and Döring, 2007), a study was made of red leaf pigmentation, which is not preferred by aphids. Branch dieback was used as a measure of tree condition, and total nitrogen levels in reddish and green leaves was analysed. In addition an examination was also made as to whether entire trees are the most appropriate level to study the earliness of autumn leaf colours.

**MATERIALS AND METHODS**

Norway maple (*Acer platanoides* L.) was selected in order to investigate the connection between tree condition and red leaf colouration. Norway maple grows and reproduces vigorously in urban areas (Lehvävirta and Rita, 2002; Quigley, 2004). In this species, abiotic stress produces different leaf colour changes as compared to biotic stresses (Vollenweider and Günthardt-Goerg, 2005), and the earliness and onset of red leaf colouration is inversely correlated with the nutritional value of leaves (Schaberg et al., 2003; see also Baltzer and Thomas, 2005).

In August and September 2005, the pattern of colour change of maples was followed in parks of Pori, Finland. Some of the trees became partially reddish more than a month before leaf fall in August, and it was observed that branch condition and direct and indirect sunlight might affect the pattern of colour change. Once it became visible, the red colour did not disappear during the autumn; on the contrary, as autumn senescence proceeded, visual differences became more apparent. A similar pattern was observed in 2006 and 2007.

Fieldwork was carried out on 31 August 2005, at 1445–1600 h local time (approx. 18 °C, sunny weather). Before the fieldwork, the average temperature had been above 10 °C for 2 weeks, and no frosts had occurred during the summer. The fieldwork was repeated on 26 August 2006, when time of day and environmental conditions were similar to those in 2005, and on 28 August 2007. The number of trees evaluated were 32, 32 and 30 in 2005, 2006 and 2007, respectively.

Tree condition of mature Norway maples was estimated using two categories: control trees had all major branches alive in the outer canopy, and partially dead trees had at least one dead, leafless major branch (length >1 m, diameter >2.5 cm) in their outer canopy. Leaf colour was estimated using three categories: the proportion of visible reddish pigments in the leaves was either 0 %, between 0–1 %, or 1–50 %. The distinction between the second and the third category was straightforward: reddish pigments were visible either in a few leaves or in many leaves, depending on the tree. Patchiness of reddish leaf colours was classified as being present or not. An exceptional thunderstorm occurred in Pori (rainfall >100 mm in a few hours) 2 weeks before the fieldwork in 2007. As a result of the storm, moisture conditions were different from the two previous years during the onset of autumn pigmentation. Most of the trees were the same from year to year, but some of the original control trees had a leafless major branch in 2006 and 2007.

Fieldwork was done along three boulevards (Kalevanpuisto, Raathiuhoneenkatu and Ratakatu) that had a low traffic density (see Supplementary Information, available online). In 2005, all partially dead maples that had a major dead branch in the outer canopy were included in the study, except for five cases (see below). If a tree had minor (length <1 m, diameter <2 cm) but no major leafless branches, or if a tree had leafless branches only in shade inside its canopy, the tree was classified as a control tree. Partially leafless trees were excluded if the distance to the closest possible control tree was more than 25 m, or if the tree was in shade during the fieldwork. For these reasons, four partially dead trees that had reddish leaves had to be excluded, and one partially dead tree that had totally green foliage. No reddish leaves were observed in the neighbouring trees of the five trees excluded. In the following years, the same five trees were excluded. Note that the purpose of this work was not to find all the reasons for early autumn colour changes, but to study if tree condition, branch die-back and leaf nitrogen levels are connected to early autumn leaf colours. Note also that fieldwork was done 2 weeks before the onset of yellow autumn leaf colours.

For carbon and nitrogen analyses, undamaged leaves from six trees were sampled during the fieldwork in 2007. All trees that had a large reddish patch within 4 m above the soil surface were selected for study, together with their neighbouring trees. In addition, a tree that was growing separately in a sunny place along the street Kalevanpuisto was sampled. The tree had no dead major branches and it was evaluated independently by two people as being seemingly vigorous. Reddish pigments were not patchily distributed in this extra tree. All sampled leaves were sun leaves i.e. they received direct sunlight for several hours per day. Sampling was done in one location of the canopy of green trees and in two locations in the canopy of partially reddish trees. In every partially dead tree, leaves were taken from a large reddish patch and from a nearby green...
location. In the reddish, vigorous tree, leaves were sampled from one green and one reddish location. Within tree pairs, the distance above the soil surface was equal for all leaves sampled. In every location, at least ten leaves were sampled; thus at least 20 leaves were sampled from partially reddish trees, and at least ten from green trees. Immediately after sampling, leaves were frozen and stored at $-18^\circ$C until dried in a vacuum drier for 24 h.

Leaf total carbon and nitrogen were analysed with a Leco CNS-2000 carbon and nitrogen analyser (Leco Corporation, USA). Four-to-six leaves out of ten were selected randomly from every sampling location for the analyses. These leaves were divided into two or three C and N samples that consisted of one or two whole leaves, i.e. approx. 200 mg of crushed dry material. Every C and N sample was analysed in two separate vials and the mean of the two analyses was used in statistical comparisons. As a result, the data set consisted of 13 reddish and 19 green leaf samples.

The results were analysed using Mann–Whitney $U$ and McNemar tests that are suitable for ordinal and categorical data (Agresti, 2002). Spearman correlation coefficients were calculated between leaf colour and total C and N concentrations, and Student’s $t$-tests were used to compare separate effects of branch and tree condition on C and N concentrations. The appropriate branch-based and tree-based mean values were used in the latter analyses.

**RESULTS**

In 2005, red pigmentation was more widespread in the foliage of partially dead maples than in control trees (Table 1; Fig. 1). When pairs of two neighbouring trees were compared, the partially dead tree had redder foliage in 14 out of 16 pairs (Table 1). Red was patchily distributed in the foliage of seven partially dead trees, while patchy distribution was absent in the control group ($U = 56.0$, $n = 32$, $P < 0.0005$). Whenever red was patchily distributed, patches occurred beside or below leafless branches (see Supplementary Information, available online).

In 2006, red leaf colouration was more abundant in partially dead trees than in the control group (Table 1; Fig. 1), and partially dead trees had redder foliage in 13 out of 16 pairs. As in 2005, patchiness of red colouration was absent in the control group, but patches of red leaves occurred beside or below leafless branches in the leaf canopy of 11 partially dead trees ($U = 40.0$, $n = 32$, $P < 0.0005$).

In 2007, red leaf colouration was more common in partially dead trees than in the control trees (Table 1; Fig. 1). Reddish foliage was absent in four tree pairs. Among the remaining pairs, the partially dead tree had redder foliage in eight and the control tree in two pairs. Patchiness of red colouration was absent in the control group, but patches of red leaves occurred beside or below leafless branches in the canopy of ten trees ($U = 37.5$, $n = 30$, $P < 0.0005$).

**Fig. 1.** The frequency of reddish leaves in partially dead and control maples (*Acer platanoides*) in (A) August 2005, (B) 2006, and (C) 2007. Trees with a dead major branch had significantly more reddish leaves (percentage of total) than the control trees (all major branches alive). See Table 1 for statistics.

**Table 1.** Mann–Whitney U-test statistics for leaf colour differences, the dominance of reddish leaf pigmentation within tree pairs (control vs. partially dead trees), and the number of trees with patchy distribution of red. Patchiness was absent in the control group.

<table>
<thead>
<tr>
<th>Year</th>
<th>U</th>
<th>n</th>
<th>P</th>
<th>Control</th>
<th>No within-pair difference</th>
<th>Partially dead</th>
<th>Patchy</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>52.0</td>
<td>32</td>
<td>&lt;0.01</td>
<td>1</td>
<td>1</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>2006</td>
<td>47.5</td>
<td>32</td>
<td>0.001</td>
<td>2</td>
<td>1</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>2007</td>
<td>55.0</td>
<td>30</td>
<td>0.009</td>
<td>2</td>
<td>5</td>
<td>8</td>
<td>11</td>
</tr>
</tbody>
</table>
Branch condition was related to total nitrogen (N) and to total carbon (C) in leaves (Fig. 2). Similar correlations occurred between leaf colour and leaf N and C concentrations (Fig. 2). Branch condition affected both N concentration \( (t = -2.93, \text{d.f.} = 8, P = 0.019) \) and C concentration \( (t = 2.82, \text{d.f.} = 8, P = 0.022) \), but this may be a result of colour differences. Tree condition did not affect N concentration \( (P > 0.1) \) or C concentration \( (P > 0.1) \) in green leaves.

**DISCUSSION**

Based on this study, partially dead Norway maples and severely damaged tree parts reveal early red leaf colours more frequently than control trees and healthy tree parts. Leaf nitrogen concentration was lower in red leaves and ailing tree parts than in green leaves and in vigorous tree parts, respectively. There are several possible reasons for the patterns observed. The most likely reason is that foliar nitrogen deficiency per se is the key determinant of colour pigment accumulation in certain maple branches. Low levels of foliar nitrogen have been associated with early and high levels of leaf pigments, such as anthocyanins (Sauveste et al., 1993; Schaberg et al., 2003; Baltzer and Thomas, 2005). In this scenario, a plausible reason for varying nitrogen levels is competition between different branches and modules within a single tree (Koivunen et al., 2004; Stuefer et al., 2004; Vesk and Westoby, 2004). Nitrogen deficiency at the whole-tree level is an unlikely reason, since it should affect foliar colours all over the tree. Allocation of resources to reproduction is one of the reasons that could lead to low levels of foliar nitrogen in certain branches whilst the levels stay high in others (Sinkkonen, 2006b), but reproductive effort was not measured in this study. Because tree condition did not affect N concentration in green leaves, it seems unlikely that leaf colours can be used as an indication of tree vigour or of the defensive commitment of maple trees. Instead, they could be utilized as an indicator of the nutrition of tree modules and branches. Nutrient competition between branches was not measured in this study, but ailing branches seemed to compete strongly with neighbouring branches.

An alternative view is that poor nutrition induces certain branches to ensure nitrogen retranslocation under harsh environmental conditions, which results in the synthesis of anthocyanins and non-visible phenolic compounds. However, not all partially dead trees showed patchiness or red colour. Certain branches may have been dead for several years, and the effect of branch die-back may have ceased but the branch had not fallen down. It is also possible that the nutrient explanation does not hold for those partially dead trees that did not reveal patchiness or early leaf colour changes. Such trees may synthesize anthocyanins in their sun leaves as certain control trees did. There may also be different reasons why branches die. Intriguingly, the proportion of patchiness seemed to increase from 2005 to 2007. Environmental conditions, such as differences in precipitation, or a slight difference in the onset of leaf pigmentation may explain the between-year variability. It is possible that different weather conditions affected nitrogen dynamics in the trees. Note, however, that no signs of abiotic damage were found during field work (see Sager et al., 2005; Vollenweider and Günthardt-Goerg, 2005).

It could be argued that ailing tree parts might have been actively defending themselves against pests. Certain plant species accumulate anthocyanins as defence compounds in infected tissues (Hipskind et al., 1996; Kangatharalingam et al., 2002). Partially dead trees might have been coloured patchily as a consequence of defensive actions that included production of defence compounds, such as anthocyanins and non-visible phenols. In earlier studies, Norway maple has been observed to change leaf colour to red in tree parts that are under pathogen attack (Vollenweider and Günthardt-Goerg, 2005). However, pathogen or herbivore attacks were unlikely since many large red patches reoccurred every autumn, and no new patches were observed during 2006 and 2007. Further, no signs or symptoms of pathogen attack were found in partially dead trees.

Red leaf colours are known to become more prevalent in sun leaves than in shade leaves. The traditional way to explain this is that the sole purpose of autumn tree colouration is to minimize the risk of photo-oxidative damage. In the maples observed, the phenomenon occurred independently of harsh temperatures and was thus hard to explain in terms of the photoprotection hypothesis. Therefore, an alternative view is proposed that red leaf pigments are produced in sun leaves because these are probable target sites of insect mass arrival. At least three facts support this possibility: herbivorous insects visually sense red surfaces as dull and uninteresting objects (Chittka and Döring, 2007); UV-visible phenols provide a more effective sun screen than anthocyanins (Manetas, 2006); and leaf nitrogen levels are lower in red than in green leaves. The possibility

![Figure 2](https://academic.oup.com/aob/article-abstract/102/3/361/228227)

**Fig. 2.** Leaf N and C concentrations (% dry weight) correlated with branch condition (N: \( r_N = 0.58, P < 0.001 \); C: \( r_C = -0.44, P = 0.011 \)) and leaf colour (N: \( r_N = -0.84, P < 0.001 \); C: \( r_C = 0.74, P < 0.001 \)) in Norway maple.
that red leaf colours are a true signal of below-average nutrient reserves should be investigated thoroughly (Karegeorgou and Manetas, 2006).

This study presents the first direct, intraspecific observation of a relationship between autumn leaf colours and tree condition, and the findings have implications for the coevolution hypotheses of autumn leaf colours. This is one of the first studies where timing of a potential autumn colour signal matches with the search for winter-hosts in host-alternating aphids (see Wilkinson et al., 2002; Archetti and Leather, 2005). In addition, it is evident that branch die-back cannot signal anything else but poor tree condition, which contradicts the original coevolution hypothesis (Archetti, 2000; Hamilton and Brown, 2001; Hagen et al., 2004). However, this does not mean that coevolution between deciduous woody plants and insects could not be based on nutrient signalling (Archetti and Brown, 2004; Sinkkonen, 2006b). Further, because reddish pigmentation tended to concentrate around dying branches, a potential signal to herbivores may not have evolved at the level of entire trees. This may be true in the case of many deciduous species that change leaf colour patchily, as Norway maples do (Vollenweider and Günthardt-Goerg, 2005). Although Norway maple grows vigorously in urban areas (Schwets and Brown, 2000; Lehvävirta and Rita, 2002; Quigley, 2004), autumn leaf pigments of the species should be studied in rural environments to find out how stressed branches change colour in natural plant communities. If early colour changes are found regularly in natural habitats, and if aphids or other insects respond to the changes, a colour pigment indicator might have evolved as a signal to herbivores, or even to the predators of insect herbivores.

In short, the results of this study suggest that Hamilton was partially correct (Hamilton and Brown, 2001). Vigour affects autumn leaf colours in Norway maple, which is a key component of the autumn colour show in many parts of Europe. However, colour pigment differences of Norway maple seem to be linked to branch condition and die-back. The probable reason is that leaves on ailing branches have low nitrogen levels.

SUPPLEMENTARY INFORMATION

Supplementary information is available online at http://aob.oxfordjournals.org/ and illustrates examples of partially dead trees together with their control trees, and a general view of the sampling area.

ACKNOWLEDGEMENTS

Thanks are expressed to the Superior Gardener Ismo Ahonen of Pori for searching for signs of pathogen infections in the study area, to Mirva Nykänen and Erika Heikkinen for performing the critical part in carbon and nitrogen analysis, and to two anonymous reviewers for critical comments.

LITERATURE CITED


Manetas V. 2006. Why some leaves are anthocyanic and why most anthocyanic leaves are red? *Flora* **201**: 163–177.


