



Timings of leaf abscission, nitrogen resorption, and autumn coloration across contrasting canopy positions in *Acer japonicum*

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Abstract

Key message Abscission zone development during late autumn is independent of a given light environment and anthocyanin-based coloration in a fullmoon maple, a seasonal deciduous species.

Abstract Similar to the decline in leaf function during autumn senescence, the timing of formation and development of the abscission zone (AZ) in the leaf petiole is crucial for leaf longevity. However, the link between AZ development and leaf function decline remains unclear. Autumn leaf coloration has been proposed as a potential consequence of AZ development, possibly resulting from sugar accumulation caused by AZ-induced phloem blockage, but evidence for impaired phloem transport is lacking. To clarify the relationship between AZ development, anthocyanin accumulation, and changes in leaf functional traits, we tracked anatomical changes in the AZ of adult fullmoon maple (*Acer japonicum* Thunb.), alongside dynamics of nitrogen (N), non-structural carbohydrates, and pigments estimated from reflectance spectra. Traits were compared between leaves under open and shaded conditions to assess the influence of leaf productivity. We found no relationship between AZ development and anthocyanin accumulation. In contrast, sugar accumulation, N resorption, and chlorophyll degradation synchronized with AZ cell degradation. N resorption continued until leaf shedding, suggesting phloem remains functional despite AZ progression. Although the seasonal pattern and absolute amount of anthocyanin accumulation exhibited clear light dependency, the seasonal pattern of AZ development was identical under both light conditions, suggesting genetic control of AZ development. This study provides the first direct evidence that AZ development, a key process in determining leaf longevity, occurs independently of light environment and anthocyanin-based coloration.

Keywords Abscission zone · Autumn coloring · Cell degradation · Light condition · Nitrogen resorption · Non-structural carbohydrate

Introduction

Leaf longevity and productivity are key factors in determining plant growth, with leaf productivity typically measured by photosynthetic performance. Carbon and nitrogen

resource limitations underpin the well-known trade-off between leaf longevity and productivity (Kikuzawa 1991; Reich et al. 1997; Wright et al. 2004; Shiklomanov et al. 2020). However, some flush-type emergent species have a genetically fixed leaf longevity, characterized by flushing leaves in spring and abscising them in autumn at roughly the same time each year (Kikuzawa 1988; Koike 1990; Koike et al. 2001). The abscission zone (AZ) consists of a separation layer located at the interface where the organ detachment occurs, and a protective layer that safeguards the underlying tissues during and after abscission (Addicott 1982; Sexton and Roberts 1982; Roberts et al. 2002). The development of the AZ is an energy-consuming process that is accompanied by cell division and the synthesis of hydrophobic compounds such as lignin or suberin. As environmental conditions become less favorable, such as during low temperatures in late autumn, the AZ in seasonal deciduous trees

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becomes increasingly active (Addicott 1982; Sexton and Roberts 1982; Roberts et al. 2002). Eventually, the AZ at the base of the petiole facilitates leaf detachment in autumn, marking the definitive end of leaf longevity. The development of the AZ, associated with the decline in leaf function, still needs further investigation to clarify its role in determining leaf longevity (Moline and Bostrack 1972; Schaberg et al. 2008; Primka and Smith 2019). Schaberg et al. (2008) demonstrated that the timing of AZ formation coincides with physiological changes in leaf function, such as reductions in photosynthetic activity and nutrient mobilization, particularly under stress conditions. Similarly, Primka and Smith (2019) highlighted the correlation between AZ formation and pigment degradation in several deciduous species. However, the reciprocal relationship between AZ anatomical development and the decline in leaf productivity remains unclear.

Since deciduous species in temperate zones shed their leaves at roughly the same time each year, AZ development in these species is also expected to follow a similar seasonal trajectory. During this period, leaf function declines sharply as key physiological processes, such as photosynthesis and pigment metabolism, progressively diminish (Koike 1990; Koike et al. 2001; Mattila and Tyystjärvi 2023; Kitao et al. 2024). Seasonal deciduous tree species must withdraw nitrogen from senescing leaves before leaf fall to support leaf emergence in the following season (Eckstein et al. 1999; Kitao et al. 2022, 2024). Abscission zone development, which involves tissue collapse, is generally assumed to cause physical blockage of the vascular transport system. Therefore, AZ development may reduce the efficiency of nitrogen resorption, given that the timing of leaf abscission closely correlates with the duration of nitrogen resorption (Niinemets and Tamm 2005).

The autumn leaf senescence and AZ development are regulated by interconnected hormonal signaling with gene expression changes beginning approximately one month before visible symptoms and coordinated by transcription factors (Sexton and Roberts 1982; McKim et al. 2008; Jin et al. 2015; Lihavainen et al. 2021; Li and Su 2024). The development of AZ may be a key factor in autumn leaf coloration, as it may block phloem loading and lead to increased leaf sugar accumulation. This hypothesis can be supported by the findings that girdling and stem cooling enhance sugar-induced anthocyanin synthesis (Murakami et al. 2008; Schaberg et al. 2017; Lihavainen et al. 2021). Since elevated leaf sugar concentrations serve as a signal of leaf senescence (Ono et al. 2001; Wingler et al. 2006; Kim 2019), AZ development may act as one of the factors promoting senescence, leading to a decline in photosynthetic activity and pigment degradation (Koike 1990; Koike et al. 2001; Mattila and Tyystjärvi 2023; Kitao et al. 2024).

Conversely, a novel putative function of anthocyanins, as secondary metabolites, has been proposed: they may serve as an alternative sink for photosynthates otherwise directed toward sugar and starch (Hernández and Van Breusegem 2010; Kitao et al. 2024), thereby potentially preventing early sugar-mediated leaf senescence (Ono et al. 2001; Landi et al. 2015; Lo Piccolo et al. 2018; Lo Piccolo and Landi 2021; Davies et al. 2022). In this context, two possible factors inducing sugar accumulation and activating anthocyanin synthesis are 1) blockage of phloem loading at the AZ as described above (Murakami et al. 2008; Schaberg et al. 2017), and 2) cessation of starch synthesis coupled with enhanced starch degradation to soluble sugars (Kim 2019; Kitao et al. 2024). We support the latter mechanism, as phloem transport must remain functional given that nitrogen was efficiently resorbed in a typical anthocyanic tree species, fullmoon maple (*Acer japonicum* Thunb.) (Kitao et al. 2024). However, no evidence has yet demonstrated whether phloem loading is blocked at the AZ. Detailed analysis, especially sequential anatomical observations, is needed to determine whether AZ formation is a cause or a consequence of leaf senescence.

Fullmoon maple, a typical anthocyanic tree species, accumulates anthocyanins, particularly in sun-exposed outer canopy leaves, to aid nitrogen resorption by preventing photoinhibition (Feild et al. 2001; Lee et al. 2003; Kitao et al. 2022, 2024). Leaves of fullmoon maple canopy flush simultaneously in spring and shed nearly simultaneously in autumn. This synchronicity allows assessment of light effects on leaf physiological traits without confounding age-dependent variation. A previous study (Kitao et al. 2024) found that although sun-exposed leaves contain higher amounts of sugars and anthocyanins compared to shade leaves, nitrogen resorption, the ratio of sugar to total non-structural carbohydrates, and chlorophyll degradation during autumn senescence remain consistent across light conditions within the canopy. This synchronization also enables the investigation of AZ formation concerning physiological changes across different light environments and providing insights into the role of AZ in leaf senescence.

We hypothesized that AZ formation does not entirely cause vascular blockage since nitrogen resorption remains unaffected even in sun-exposed leaves with high anthocyanin levels (Kitao et al. 2024). To test this hypothesis, we observed AZ development by light microscopy techniques in leaves of fullmoon maple grown under different canopy light conditions, corresponding to the time course of nitrogen content and autumn coloring. By comparing AZ anatomical features in leaves exposed to different light conditions, we aim to investigate the synchronicity of leaf dynamics between sun and shade leaves in seasonal deciduous species.

Materials and methods

Plant materials

An adult tree of fullmoon maple (*Acer japonicum* Thunb.) growing in an open site of the arboretum at the Hokkaido Research Center, Forestry and Forest Products Research Institute (43.0°N, 141.4°E; 180 m a.s.l.) was selected (Kitao et al. 2024). Leaves located in the outer canopy ('open') and inner canopy ('shade') on the south-facing side were chosen. Four leaves from each light condition were selected for measurement on the following dates: late summer (Aug 19), autumn (Sep 8, Sep 24; Oct 5, Oct 12), and late autumn (Oct 19, Oct 26; Nov 2). The time courses of light intensity in each light condition and of temperature are shown in the supplementary figure (Fig. S2) of Kitao et al. (2024). Briefly, the average integrated photosynthetically active radiation (PAR) during the sampling period was 18.5 and 1.3 mol m⁻² day⁻¹ for the open and shade conditions, respectively. The daily mean air temperature under the canopy at a height of 50 cm above the ground was 20.6 ± 1.4 °C, 15.8 ± 2.0 °C, and 8.0 ± 1.7 °C in late summer, autumn, and late autumn, respectively; mean temperatures on each measurement date were 17.9 °C (Aug 19), 17.3 °C (Sep 8), 15.5 °C (Sep 24), 15.0 °C (Oct 5), 12.1 °C (Oct 12), 8.0 °C (Oct 19), 10.8 °C (Oct 26), and 7.4 °C (Nov 2).

Reflective spectrum of leaves

Two shoots with two leaves were sampled, and the leaf blade was separated. For the evaluation of leaf coloring, the reflectance spectra of 400–1000 nm wavelength range from the upper side (adaxial side) of the sampled leaf blade were measured using a portable leaf spectrometer (CI-710, CID Bio-Science Inc., WA). In the case where coloration was heterogeneous, the area with the largest colored region was selected. The Anthocyanin Reflectance Index (ARI), the chlorophyll-based Normalized Difference Vegetation Index (cNDVI), and the Plant Senescence Reflectance Index (PSRI) from the obtained spectra were calculated following (Sims and Gamon 2002) as follows:

$$\text{ARI} = 1/R_{550} - 1/R_{700}$$

$$\text{cNDVI} = (R_{750} - R_{705}) / (R_{750} + R_{705})$$

$$\text{PSRI} = (R_{680} - R_{500}) / R_{750}$$

where R with a subscript denotes the reflectance at the corresponding wavelength (in nanometers).

N and NSC

Following the reflective spectrum measurement, leaf blades were oven-dried for 2 days at 65 °C and ground using a ball mill (TissueLyser II, QIAGEN K.K., Tokyo, Japan). The nitrogen content in the leaves was determined using a total nitrogen/carbon analyzer (Sumigraph NC-22, Sumika Chemical Analysis Service, Ltd., Tokyo, Japan). Approximately 3 mg of fine powder was extracted with 80% aqueous ethanol. The phenol–sulfuric acid method was employed to determine the sugar content in the supernatants. The residues were enzymatically degraded with amyloglucosidase (A9228, Sigma-Aldrich Japan K.K., Tokyo, Japan) following treatment with aqueous KOH solution, and starch content was determined by the mutarotase-glucose oxidase method (Glucose CII test, FUJIFILM Wako Pure Chemical Corp., Tokyo, Japan).

Anatomical observation

The site of the junction between the petiole and the shoot (Fig. 1a) was trimmed with a sharp razor. The specimens were fixed with a 5% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.2). After dehydration through an ethanol series and displacement with *n*-butyl glycidyl ether (QY-1), specimens were embedded in epoxy resin (Epon 812, TAAB Laboratories Equipment Ltd., UK). Embedded specimens were trimmed and sectioned to 1.0–1.5 µm thickness with glass knives on a microtome (HistoCore MULTICUT, Leica Biosystems, Nussloch, Germany). We confirmed that longitudinal sections contained the axial sections of the vascular bundle (Fig. 1b). Sections were prepared as stained and unstained samples. Stained sections were treated with 0.01% aqueous safranin, and both were observed using an optical and epifluorescence microscope (BX41, Evident Corp., Tokyo, Japan). Unstained sections were observed under UV excitation using a U-MWU2 mirror unit (excitation, 330–385 nm; emission, 420 nm).

Cells that accumulate hydrophobic material, such as lignin or suberin in the cell walls around the wounded tissue, are difficult for the solution to penetrate during the embedding process. In this study, tissues that collapsed during sectioning were considered to have collapsed cells due to the accumulation of hydrophobic material.

To confirm the position of the abscission surface within the petiole, winter buds were sampled from the same node under two light conditions on Dec 10, 2025. The longitudinal hand sections of the leaf scar were prepared with a sharp razor blade and examined using a digital microscope (Dino-lite Edge AM7915MZT, AnMo Electronics Corp., Taiwan). The distance between the leaf abscission surface

Fig. 1 Overviews of the anatomical observation of the abscission zone (AZ) of a fullmoon maple in this study. **a** The specimens were sampled from the junction between the petiole and the shoot, indicated by the solid square in the panel. A typical overview of a specimen sampled on Sep 24 is shown. **b** A microscopic image of the longitudinal section corresponding to the position indicated by the solid square in panel (a). c: cortex; Vb: vascular bundle; b: bud. Scale bars = 100 μ m. A representative overview of a section from an open-condition leaf sampled on Sep 24 is shown. Scale bars = 100 μ m. **c** Structural differences of the cortex of the petiole between the abaxial and adaxial sides. The cells in the cortex on the abaxial side have a larger diameter than those on the adaxial side. A representative overview of a section from an open-condition leaf sampled on Aug 19 is shown. Scale bars = 100 μ m. **d** A typical tangential longitudinal section (a plane parallel to the vascular bundle but not containing the vascular bundle itself) of the petiole from an open-condition leaf sampled on Aug 19. Although the size of cortical cells gradually decreased from the leaf blade direction toward the petiole base direction around the junction of the petiole and the twig, the representative AZ (defined as the area composed of smaller, more isodiametric cells (e.g., Addicott (1982)) was difficult to clearly distinguish. Instead of identifying AZ, the area within which leaf detachment could occur was outlined, as indicated by the dotted square. The dotted square is centered on the mean position of the abscission surface, and its upper and lower edges represent +SD and -SD, respectively. The dotted circle denotes the petiole base, the site at which secondary abscission is potentially initiated (see Fig. S1). Scale bars = 100 μ m. **e** Longitudinal section of the petiole collected in December. The dotted square indicates the abscission surface, and the petiole base by the dotted circle. To estimate the position of the abscission surface, the remaining length of the petiole (double-headed arrow) was measured. Wb: winter bud; P: petiole; T: twig. Scale bar = 1 mm

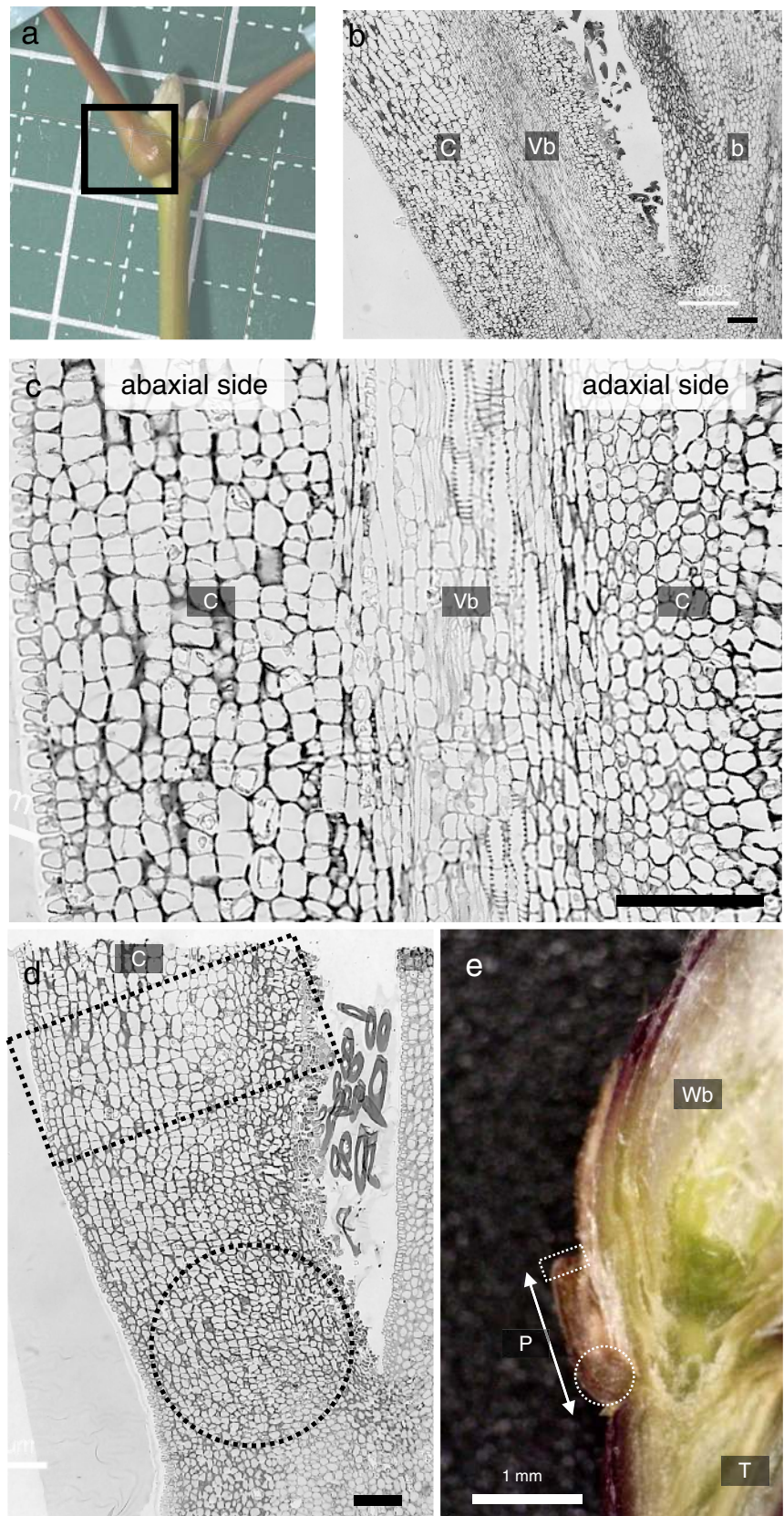
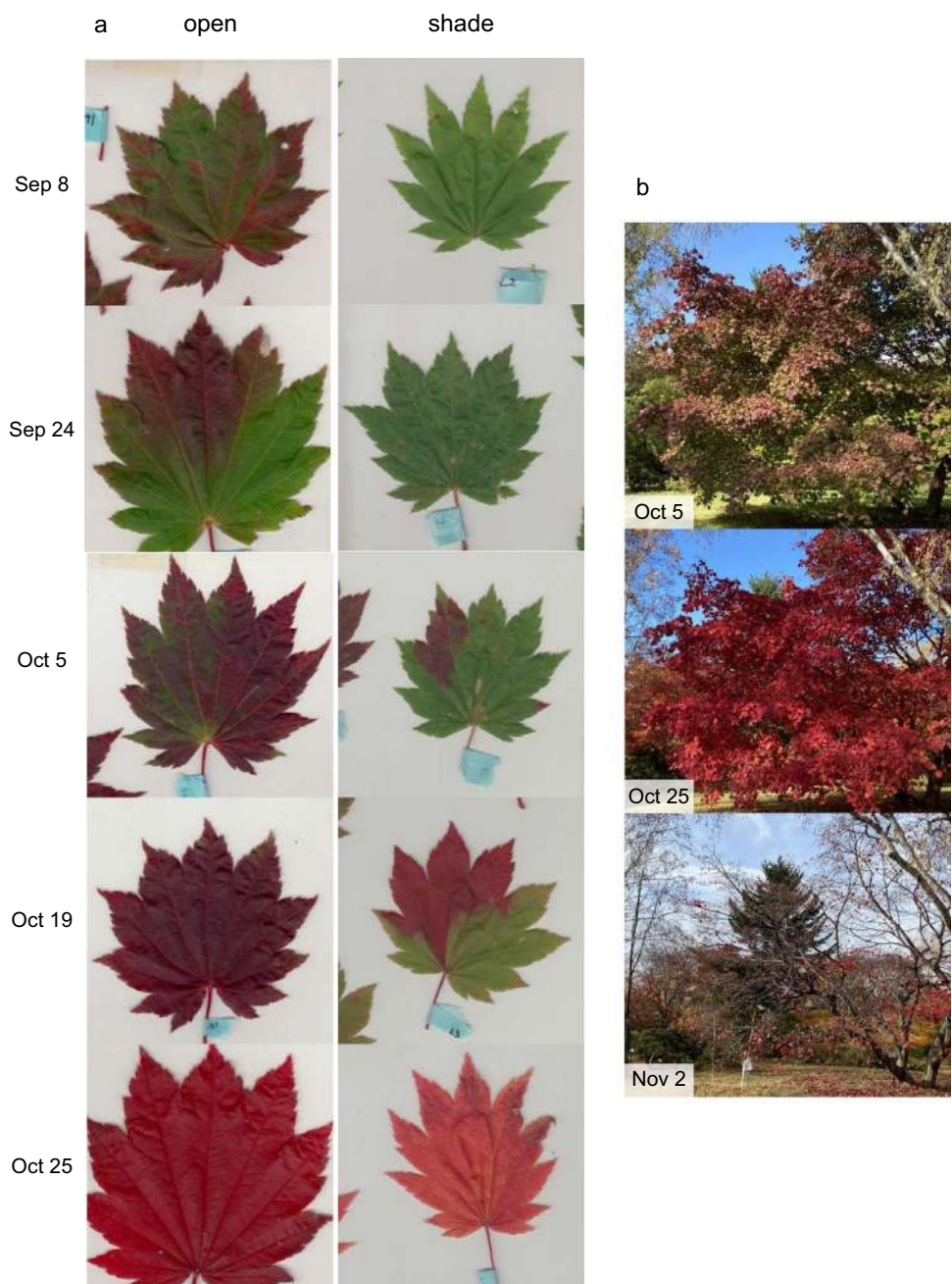


Fig. 2 Time course of the coloration of the fullmoon maple. **a** Comparison of leaf coloration between open and shade light conditions. **b** Seasonal changes in the overview of the surveyed tree



and the petiole base was then measured in twelve samples for each treatment.

Statistical analysis

Two-way ANOVA was performed for each parameter to determine the effects of date, light condition, and their interaction. Tukey's multiple comparisons test was employed to assess differences between dates within each light condition. All statistical analyses were conducted using R version 4.4.2 (R Core Team 2024).

Results

Anatomical features of the abscission zone of fullmoon maple

The cell shape in the cortex of the petiole was heterogeneous between the abaxial and adaxial sides at the base of the petiole of fullmoon maple (Fig. 1c and d). Although the area consisting of smaller, isodiametric cells around the petiole base appeared to represent a typical separation layer in the AZ according to the definition by Addicott (1982), a distinct

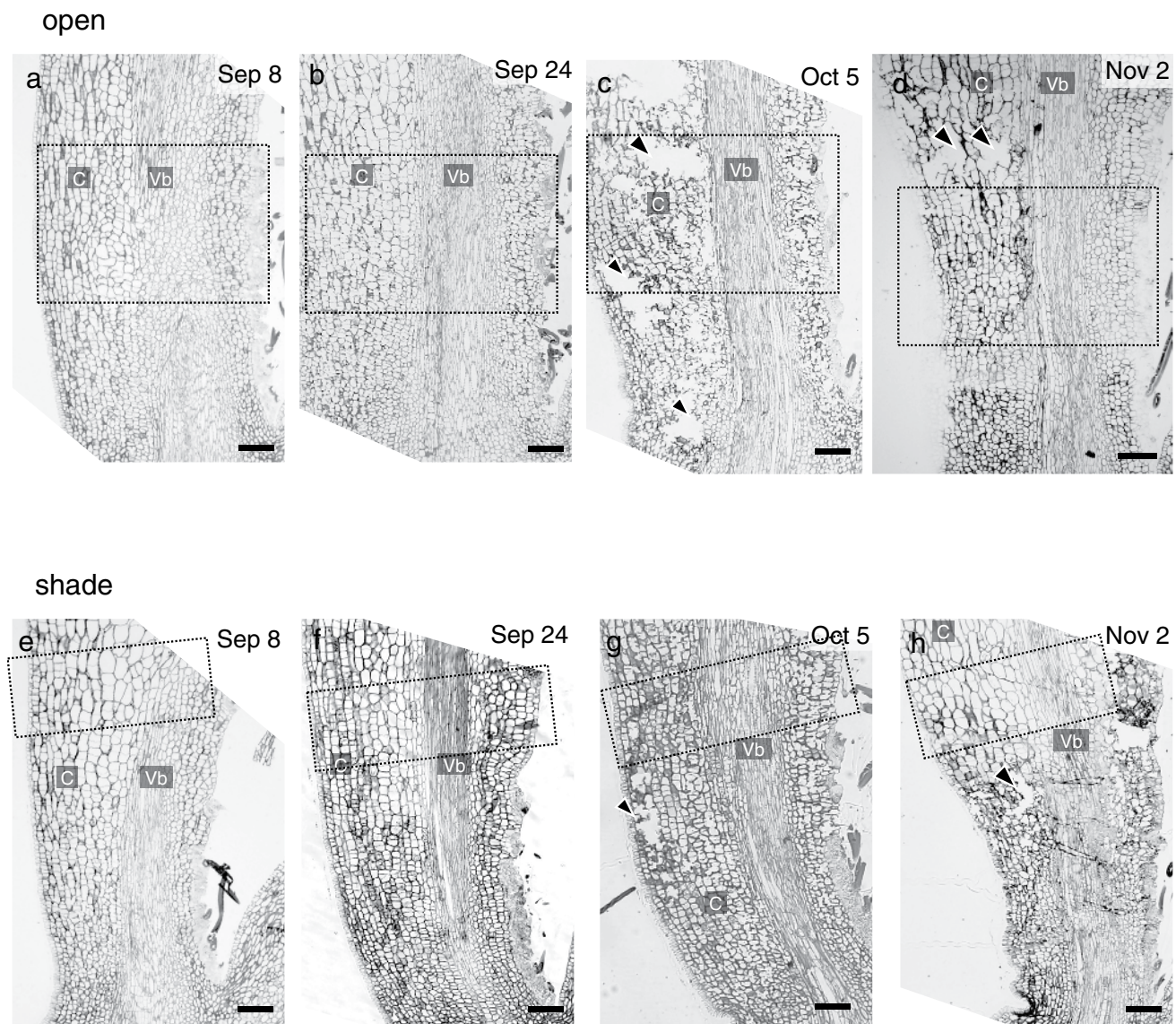


Fig. 3 Time course of abscission zone (AZ) development compared between open (a–d) and shade (e–h) light conditions. Arrowheads in panels c, e, g, and h indicate cell degradation. The dotted squares denote the area within which leaf detachment could occur. The dotted

square is centered on the mean position of the abscission surface, and its upper and lower edges represent +SD and –SD, respectively. c, cortex; Vb, vascular bundle. Scale bars=100 μ m

separation layer-like structure was not clearly distinguishable in the tangential longitudinal section (a plane running parallel to the vascular bundle without cutting through it) (dotted circle in Fig. 1d). In addition cell collapse or cytolysis first occurred at a more distal position, rather than within the smaller cells around the petiole base, encompassing the region that correspond to the abscission surface (dotted square in Fig. 1d), as described in a later section. The putative position of the abscission surface, estimated from winter samples that preserved the abscission plane (Fig. 1e), was located 0.59 ± 0.23 mm and 0.93 ± 0.11 mm (mean $\pm$ SD) distal to the petiole base under open and shade conditions,

respectively. Under the open condition, the distance from the petiole base and the abscission surface was significantly reduced ($P < 0.001$, two-sample t-test comparing open and shade conditions).

Changes in the abscission zone (AZ) and leaf coloring compared with open and shade conditions

Leaf coloring occurred earlier in open-grown leaves than in shaded leaves (Fig. 2a). Leaf shedding began to be noticeable on Nov 2 under open light conditions, whereas some shaded leaves remained attached (Fig. 2b). The

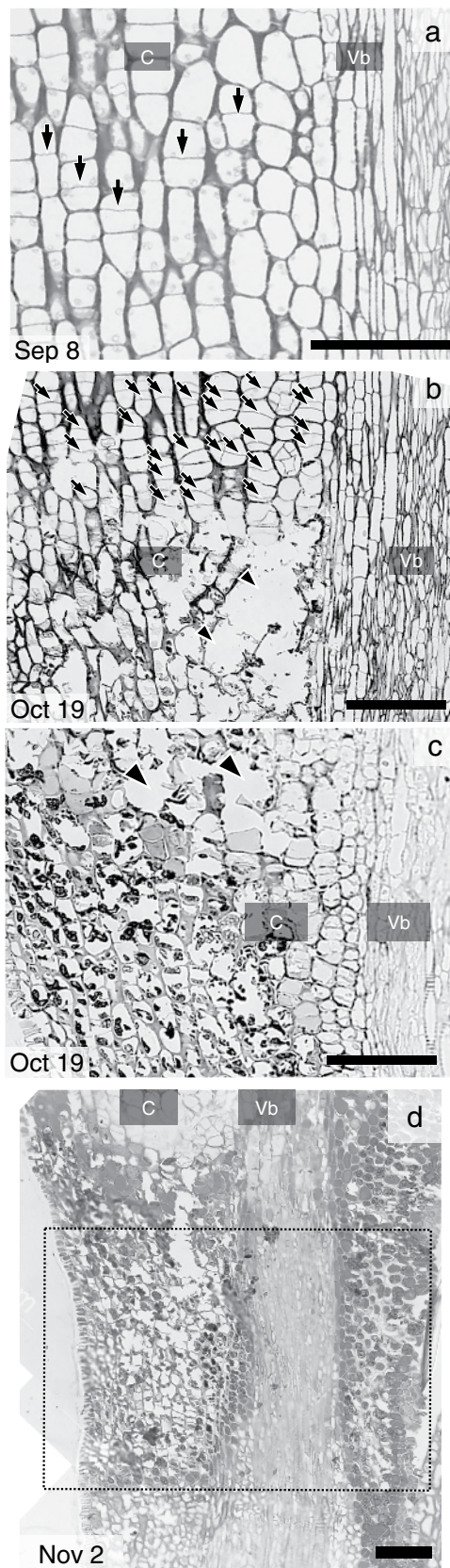


Fig. 4 Time course of cortical cells in the adaxial side of the open leaves sampled on Sep 8, Oct 19, and Nov 2. **a** Thin cell walls resembling the division plane of meristem cells are observed on Sep 8 (arrows). **b** The number of cell division planes appears increased (arrows), and cell degradation (arrowhead) is observed simultaneously on Oct 19. **c** Cellular contents in many cortical cells appear to aggregate, associated with cell degradation (arrowhead). **d** Micrographs just before shedding on Nov 2. The dotted square indicates the region that could include the abscission surface. The dotted square is centered on the mean position of the abscission surface, and its upper and lower edges represent +SD and -SD, respectively. Scale bars = 100 μm

time course of anatomical changes in the cortex is shown in Fig. 3, with panels a–d representing the open condition and panels e–h representing the shade condition (see also Fig. S2 for all samples examined in this study). Decomposition of cells in the AZ cortex was evident on Oct 5 (Fig. 3c and g) in both light conditions, regardless of leaf coloring stage. This decomposition occurred around the site of the abscission surface, especially in the open condition (dotted squares in Fig. 3). On the other hand, the vascular bundle maintained its structure until Nov 2 (Fig. 3d and h), when many leaves had already fallen (Fig. 2b). Typical changes in cortical cells under open conditions are shown in Fig. 4. Many cells exhibited thin, division-plane-like cell walls (Fig. 4a) on Sep 8, which corresponded to the early phase of leaf coloring. The cell division plane became more apparent on Oct 19 (Fig. 4b), accompanied by tissue collapse and degradation of cell contents (Fig. 4c). At the time of shedding on Nov 2, deteriorated cortical cells surrounded the vascular bundle with an axial length of approximately 200–400 μm (Fig. 4d), located at the abscission surface (Fig. 3). Typical changes in lignification/suberization, detected by the autofluorescence intensity in the AZ are shown in Fig. 5 (see also Fig. S3 for all samples examined in this study). Following tissue collapse on Oct 5 (Fig. 3c), lignification/suberization became more pronounced around the collapsed tissues on Oct 12 (Fig. 5c). The lignified and/or suberized area expanded axially, mainly on the abaxial side (Fig. 5d).

Chemical composition of the leaves corresponding to the development of leaf abscission

Sugar content rapidly increased, and starch content started to decrease about the same time or right after the cellular collapse on Oct 5 (Fig. 6a and b). In contrast to the dependence of sugar and starch variation on the changes in AZ, ARI, as an index of anthocyanin accumulation, increased over time prior to the cellular collapse in AZ in open leaves (Fig. 6c). In shaded leaves, although ARI tended to increase

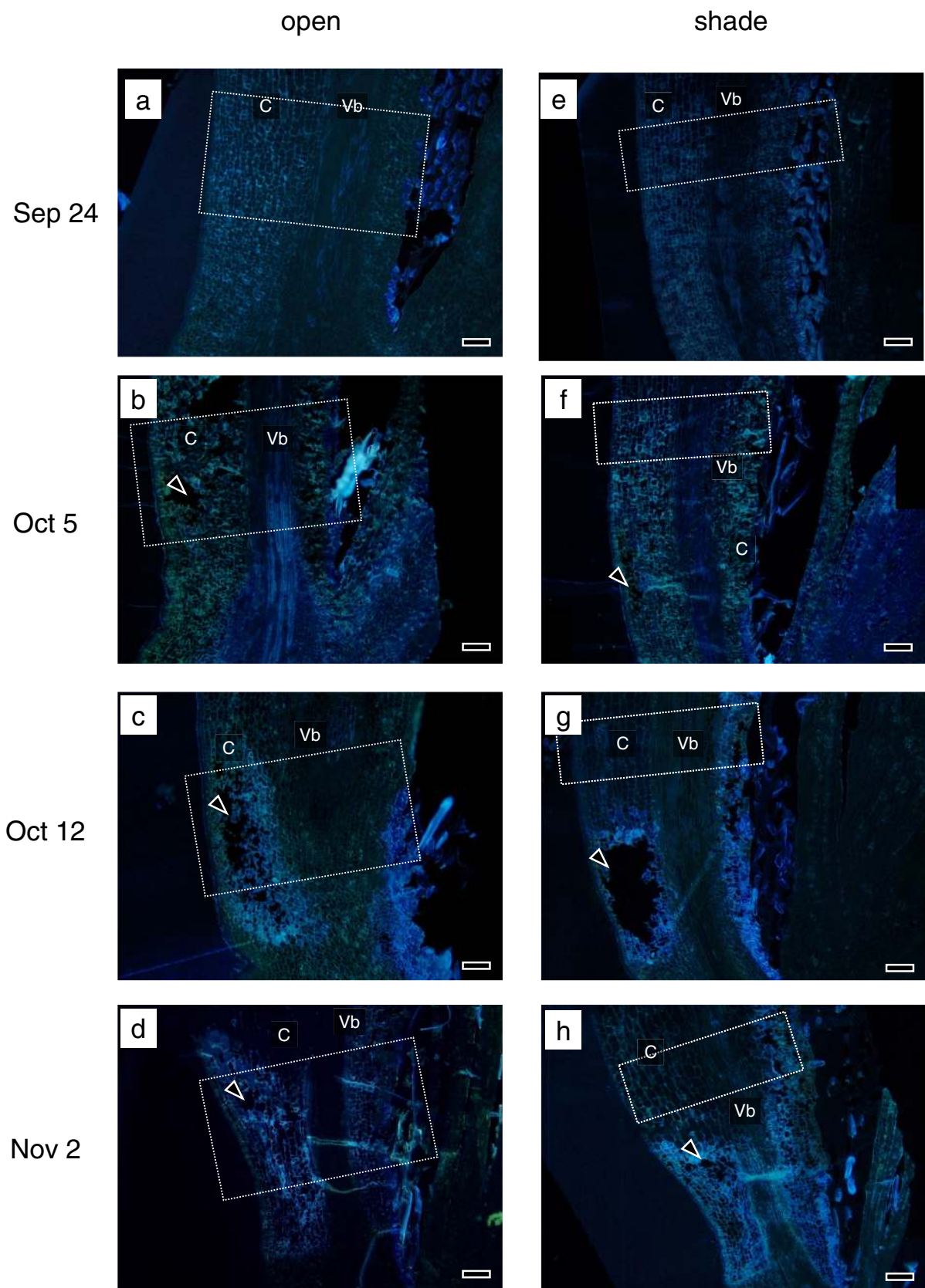


Fig. 5 Time course of the lignin/suberin accumulation on the cortical cells of the petiole under open (a–d) and shade (e–h) light conditions. Autofluorescence of lignin/suberin was observed using an epifluorescence microscope with a U-MWU2 mirror unit (excitation, 330–385 nm; emission, 420 nm). The dotted square indicates the region that could include the abscission surface. The dotted square is centered on the mean position of the abscission surface, and its upper and lower edges represent +SD and –SD, respectively. **a, e** Low lignin/suberin accumulation on Sep 24. **b, f** Low lignin/suberin accumulation with onset of cell degradation (arrowhead) on Oct 5. **c, g** Onset of lignin/suberin accumulation on Oct 19. **d, h** Expansion of lignin/suberin accumulation area on Nov 2. Scale bars=100 μ m

with date following the cellular collapse in AZ, no statistical significance was found. The cellular collapse in AZ was followed by decreases in N and cNDVI (a spectral index corresponding to chlorophyll content) in both light conditions (Fig. 6d and e), and these decreases continued from the onset of cell collapse to leaf shedding (Fig. 2b). PSRI, as an index of leaf senescence, calculated from the ratio of carotenoids to chlorophyll, spiked at the end of October, associated with the decrease in cNDVI (Fig. 6e and f). The change in PSRI was identical regardless of light condition.

Discussion

How does the abscission zone develop?

Previously, we considered that the zone consisting of isodiametrically flattened cells near the junction of the petiole and the main stem is the typical ‘separation layer’ (Addicott 1982; Sexton and Roberts 1982). However, at least in the case of fullmoon maple, tissue degradation occurred at a locus distal from the petiole base, spanning a certain range of axial width (Fig. 3). Generally, organ abscission is induced by the degradation of cells in the separation layer (Addicott 1982; Sexton and Roberts 1982; Roberts et al. 2002). Therefore, the petiole of fullmoon maple should have a wide cell layer that induces abscission around the abscission surface. Especially, the boundary between lignified/suberized and non-lignified/non-suberized tissues in the cortex of the petiole (Fig. 5d, h) appears to roughly correspond to the position of the abscission surface, i.e., the putative location of the separation layer. Further clarification of the changes in cell-wall composition would be necessary to distinguish the

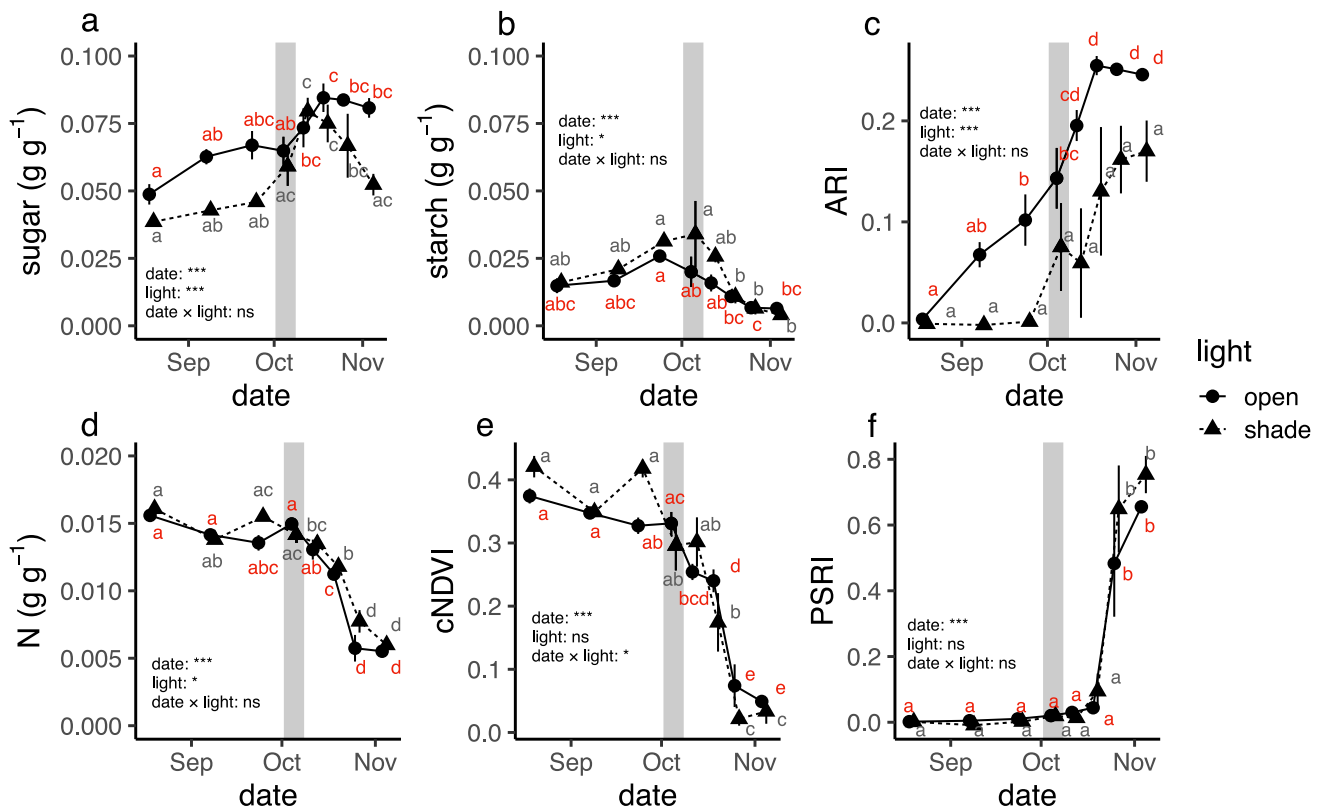


Fig. 6 Time course of non-structural carbohydrate (sugar (a) and starch (b)), Anthocyanin Reflectance Index (ARI) (c), nitrogen (N) (d), chlorophyll-based Normalized Difference Vegetation Index (cNDVI) (e), and Plant Senescence Reflectance Index (PSRI) (f) in leaves under open and shade conditions. The shaded area (gray bar) indicates the date of onset of cell degradation (Oct 5). Solid lines with circles and dotted lines with triangles indicate open and shade light conditions, respectively.

Asterisks in each panel indicate the results of two-way ANOVA (***, $P < 0.001$; *, $P < 0.05$; ns, not significant). Letters above symbols indicate the results of Tukey's multiple comparison. The same letter indicates no statistically significant difference at the $P < 0.05$ level between dates within each light condition. Error bars indicate the standard error of the mean

structure of the AZ more reliably. Because the preliminary observations indicated that the abscission surface formed in the previous year was shorter in height (Fig. S1), full moon maple appears to possess a secondary abscission layer (Addicott 1982). The actual degradation area is difficult to distinguish by cell shape, although cells in the AZ should be predeterminedly differentiated (Sexton and Roberts 1982; Roberts et al. 2002). The cells in the separation layer have been considered to have a specific sensitivity to hormonal signals or gene expression, triggering the local degradation of cells or cell wall breakdown (Taylor and Whitelaw 2001; Roberts et al. 2002). The results of our study indicate that the wide range of cells in the cortex might respond to the trigger of leaf abscission.

No deformation of the vascular bundle was found during the observation periods in this study, whereas the last samples were collected during the period of shedding of many leaves (Figs. 2 and 3). Therefore, the vascular bundles appear to play a role in supporting leaf attachment, because lignification/suberization, which can protect the fracture surface after shedding, occurred following cellular degradation (Fig. 5).

The site of the actual abscission surface of fullmoon maple showed a division plane that was activated during the degradation of cells (Fig. 4). Cell division in AZ is considered to be activated during AZ development (Berkley 1931; Addicott 1982). It has been reported that the activation of cell division and expansion during the developing AZ is needed to generate internal force to separate the organs in herbaceous species, which have very short leaf lifespans (Sexton and Redshaw 1981). Our observation did not find any notable cell expansion during the AZ development (Figs. 3 and 4). Thus, we conclude that fullmoon maples reduce the supporting force by tissue collapse in the phloem and are eventually shed by external forces such as wind.

The formation of AZ is known to be promoted by ethylene when its level and cellular sensitivity predominate over that of auxin, to which it acts antagonistically (Taylor and Whitelaw 2001; Roberts et al. 2002). In fullmoon maple of the same individual used in this study, the expression of some genes encoding an auxin polar mobilization enhancer (BIG), the enzyme that catalyzes the synthesis of ethylene from its precursor (ACS10), a senescence marker (SAG12), enzymes promoting cell wall reconstruction (xyloglucan endotrans glucosylase/hydrolase, XTR4), and a precursor-synthesizing gene of ABA (NSY) began to increase from late September to mid-October (Kitao et al. 2024) (Fig. S4). Because fullmoon maple shows similar leaf phenology from 2020 (this study) to 2023 (see Fig. S1 of Kitao et al. (2024)), AZ development in 2021 (based on the gene expression analysis of Kitao et al. (2024)) was assumed to follow a similar time course in this study

(denoted by gray vertical bars in Fig. S2). Thus, we propose that AZ development and cell degradation may be regulated, at least in part, by the levels of hormones and enzymes, or by the sensitivity of tissues to hormones associated with the expression of these genes.

Does developing the abscission zone affect the accumulation of sugars involved in the anthocyanin synthesis?

Blocking of phloem loading should cause the accumulation of assimilates in the organ (Murakami et al. 2008). Thus, disruption of the vascular bundle, including phloem, accompanying AZ development in autumn (Addicott 1982) is often assumed to be a trigger of sugar accumulation, which subsequently promotes anthocyanin synthesis, even though this has not yet been experimentally demonstrated (Zhang et al. 2013; Savage 2020). We could not find any microscopic changes indicative of physical blockage of the phloem in the vascular system during leaf fall. Significant nitrogen resorption during late October and early November (Fig. 6d) further supports continued phloem function, because nitrogen from degraded chloroplasts is relocated to the stem via the phloem (Estiarte et al. 2023). Therefore, sugar accumulation appears to be independent of the AZ development, although cellular degradation appears to coincide with the increase in sugar content (Fig. 6a). Kitao et al. (2024) suggested that anthocyanins can serve as an alternative sink for sugars once starch synthesis ceases in autumn. Several studies have also reported a similar role of anthocyanins in dissipating excess energy under conditions unfavorable for carbohydrate biosynthesis (Hernández and Van Breusegem 2010; Lo Piccolo et al. 2018; Mattila and Tyystjärvi 2023). Leaves must cope with rising sugar levels because excess sugar can accelerate leaf senescence (Wingler et al. 2006; Kim 2019). The increase in anthocyanin, shown by the changes in ARI before cell collapse, especially in open light conditions (Fig. 6c), also supports the independence of anthocyanin accumulation from AZ development.

We assumed that anthocyanin synthesis might correspond to an excess of sugars, determined by the balance between supply (photosynthetic carbon gain and starch-to-sugar conversion) and the energy required for phloem loading (Kim 2019). Photosynthetic activities assessed by gas exchange and chlorophyll fluorescence have been shown to decline toward the end of October (Kitao et al. 2024), and starch-to-sugar conversion was largely completed by that time. Consequently, sugar supply decreased as leaf shedding approached. However, the energy demand for phloem loading may also have diminished because N resorption was completed by late October. Therefore, even though leaf sugar concentration per se declined at the end of October,

some excess sugar, likely driving anthocyanin synthesis, probably persisted as a result of this shifting balance between supply and energy demand.

Is the decline of leaf productivity a trigger of leaf abscission?

In contrast to anthocyanin accumulation, nitrogen resorption could be accompanied by the AZ development, as indicated by the decline in N content following cell collapse (Fig. 6d), corresponding to the decrease in cNDVI, a proximal indicator of chlorophyll content (Fig. 6e). Because leaf longevity is believed to be determined to maximize the single-leaf productivity during its lifespan, a decrease in leaf productivity could be considered as a trigger of the leaf abscission (Kikuzawa 1991). Based on the seasonal changes in net photosynthetic rate and electron transport rate in leaves of fullmoon maple, leaf productivity appeared to decline after 19 October 2021 (Kitao et al. 2024). Cortical cell degradation appeared to precede the decline in leaf productivity (Fig. 3). Therefore, the timing of AZ development is likely not regulated by the potential of leaf productivity in fullmoon maple. While single-leaf productivity is strongly affected by growth light conditions through leaf structural development, the onset of AZ development and lignification was identical regardless of the light conditions (Figs. 3 and 5). This indicates that the potential leaf longevity of fullmoon maple could be inherently determined, followed by the deterioration of photosynthetic capacity involved with the nitrogen resorption.

The significant increase in sugar content following the decomposition of AZ cells appeared to be associated with AZ development involving cortical cell division, cell wall decomposition, and lignification/suberization (Figs. 3, 4, 5, 6 and S2–S3). Various processes involved in AZ development are regulated by numerous enzymes, including those involved in cell wall modification and lignin biosynthesis, as well as by coordinated gene expression (Sexton and Roberts 1982; Agustí et al. 2008). These metabolically demanding processes require substantial energy expenditure from carbohydrates, as the respiratory rate in the AZ has been reported to increase two to threefold (Sexton and Roberts 1982). The decrease in sugar content from Oct 18 may also be associated with cell division and the onset of lignification/suberization in the AZ.

In a previous study, a hypothesis was postulated that the outer leaves of the crown of fullmoon maple might protect inner leaves from strong light by shading until N resorption was completed (Kitao et al. 2022, 2024). Both open and shade leaves appear to exhibit similar physical toughness in terms of detachment due to the simultaneous formation of the AZ (Fig. 3), which supports this hypothesis theoretically, as open leaves at least persist as long as shade leaves.

Conclusion

The anatomical observation of the time course of AZ formation is a classical theme of plant scientists. While the subject of factors determining leaf longevity has fascinated many plant ecologists, the anatomical aspects have been often omitted due to the time required. This work focused on the development of the AZ involved in leaf function decline and revealed the possibility that AZ development is regulated independently of the leaf productivity determined by light environments, resulting in the consistent timing of defoliation in seasonal deciduous species.

The structure of the leaf abscission zone may vary among species and conditions. While abiotic and biotic stresses, such as drought or disease, induce shedding, anatomical observation of AZ development remains scarce. For a better understanding of leaf dynamics, systematic anatomical observation of abscission zones among species is needed.

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Data availability The data of the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interest The authors have no relevant financial or non-financial interests to disclose.

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