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Understanding the mechanisms responsible for postharvest tip breakdown in asparagus: a biochemical approach

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Abstract

The UK asparagus season is relatively short (from April to June), resulting in large quantities of spears being imported to provide year-round supply. Currently, asparagus quality can only be maintained for one week, plus seven days shelf-life, using cold storage alone. A limiting factor, which contributes significantly towards commercial losses, is the development of the postharvest physiological disorder tip breakdown (also known as tip rot). The disorder is characterised by darkening tips followed by the presence of water-soaked bracts at the tip of the spear and a foul odour. However, the underlying causes of tip breakdown have not yet been fully elucidated. We hypothesise that fast growth, slow rate of sugar transport and localised cell death may be key components of the mechanism. To gain more insight into tip breakdown development, two asparagus cultivars with low and high susceptibility to tip breakdown ('Early California' and 'Aspalim', respectively) were grown under controlled conditions and subjected to warm (28/14 °C) and cold (18/8 °C) day/ night temperatures. Harvested spears were subjected to shelf-life storage, 14 days at 7 °C, to subjectively assess early symptoms and further development of tip breakdown. Spears were snap frozen for subsequent biochemical analysis (*viz.* non-structural carbohydrates and plant growth regulators [PGRs]). Results showed that 'Aspalim' spears grown at warmer temperatures had higher incidence of tip breakdown than 'Early California'. The relationship between incidence and severity of asparagus tip breakdown and the role of PGRs and sugar content during the season, as affected by genotype and growing conditions, are also discussed. The fundamental mechanistic understanding of asparagus tip breakdown will aid to implement optimum postharvest storage strategies to reduce food loss and the associated carbon footprint.

Keywords: *Asparagus officinalis*, abscisic acid (ABA), sugars, spear growth

INTRODUCTION

All-year-round supply of asparagus in the UK relies on large quantities of spears being imported. This is due to the UK's relatively short harvest season (from April to June) and the lack of suitable storage methods. Currently, maintaining asparagus quality using cold storage alone is limited to 1 week plus 7 days shelf-life (Anastasiadi et al., 2022). One of the main limiting factors, which contributes significantly towards commercial losses, is the development of the postharvest physiological disorder tip breakdown (also erroneously known as tip rot). This disorder is characterised by darkening tips followed by the presence of water-soaked bracts at the tip of the spear and a foul odour (Lill et al., 1996 Anastasiadi et al., 2022). Initial investigation into tip breakdown suggests that it is not pathological in origin but physiological (Beever et al., 1985; Carpenter et al., 1996), which develops in the rapidly dividing cells of the spear tip (Jermyn et al., 1999) during periods of hot weather (in-house data not yet reported).

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Fast growth and reduced levels of non-structural carbohydrates have been linked to a higher incidence of tip breakdown (Lill et al., 1994; Hurst et al., 1996; Lill et al., 1996; Lill and Borst, 2001). A recent study showed lower levels of the plant growth regulator abscisic acid (ABA) and ABA catabolites coincided with higher sugar content (possible sugar-ABA crosstalk) under controlled atmosphere (CA) storage to delay senescence (Anastasiadi et al., 2022). The main limiting factor affecting storage during this study was the development of tip breakdown. Furthermore, Anastasiadi and co-workers (2020) found genotype differences in susceptibility to tip breakdown, which was in agreement with others (Lill et al., 1994). It was noted that warm adapted genotypes ('Jaleo') had lower susceptibility to tip breakdown compared to cold adapted genotypes ('Gijnlim') (Anastasiadi et al., 2020). Despite previous research, the underlying causes of tip breakdown have not yet been fully elucidated.

Herein, we hypothesise that fast growth, slow rate of sugar transport and localised cell death may be key components of the mechanisms behind asparagus tip breakdown. To test this, cold acclimated ('Aspalim') *vs*. warm acclimated ('Early California') cultivars were grown under two different controlled temperature regimes (cold *vs*. warm). The development of tip breakdown was assessed during shelf-life conditions and the content of non-structural carbohydrates and plant growth regulators analysed.

MATERIALS AND METHODS

1. Plant material and experimental design

'Aspalim' and 'Early California' (2 and 1 year-old-crowns, respectively), representing cold and warm acclimated cultivars, were planted in 35 L pots (45 cm [diameter] x 32.5 cm [height] - containing 33% sandy loam soil / 66% 'blueberry' compost mix). The pots were placed in two controlled light, relative humidity (RH) and temperature rooms (one crown per pot, 28 pots per room) simulating warm (28/14 °C) and cold (16/8 °C) day/night growing / harvesting conditions, for eight weeks. Both rooms were set to ten hours darkness followed by a steady rate increase of lighting up to 240 µmol m^{-2} s^{-1} within five hours, for the spears to green; the light was held for four hours and then decreased back to darkness within five hours. During darkness, RH was set at 95% for seven hours, reduced at a constant rate to 80% during the following eight hours, held for one hour at the end of the bright light period, and finally increased again during eight hours. The "warm room" was set to 14 °C for seven hours in line with the high RH; the temperature was then increased at a rate of 1.56 °C h^{-1} up to 28 °C, held for one hour synchronised with the RH and finally decreased back at the same rate. The "cold room" was programmed in a similar manner with the low temperature at 8 °C, the rate being 1.1 °C h^{-1} and the high temperature set at 18 °C. Soil moisture was checked periodically (Delta-T SM150T and HH2 reader on "organic" setting) and maintained at *ca*. 30% volumetric moisture content.

Prior to the start of the experiment, crowns had received the required number of chill units: one chill unit counted for each hour spent at ≤5 °C, with 900+ units for best vegetative response. Before harvest, spear growth rate was determined by measuring the length of individual spears for each treatment combination. Measurements of spears that were less than 5 cm at the beginning of the week were taken every two days for two or three weeks, from spear emergence to harvest date.

Spears were harvested three times a week during the harvest season and corresponded to early (weeks 2 to 3), mid- (weeks 3 to 5) and late (weeks 5 to 8) harvests. Once harvested, spears were cut to 16 cm length, packaged into shelf-life trays, and wrapped using commercial film (as described in Anastasiadi et al., 2020). The number of spears packaged into trays ranged between 2 and 6 depending on harvest availability. Spears were stored at 7 °C for up to 21 days. Sampling during shelf-life was performed at 14 and 21 days for analysis. The top 4 cm of each spear was individually snap frozen in liquid nitrogen and stored at -80 °C. Frozen material was subsequently freeze dried, pulverised, and stored at -40 °C prior to biochemical analysis.

2. Tip breakdown assessment

Spears were visually assessed for external quality according to Anastasiadi et al. (2022). The tip of each spear was then cut in half longitudinally to identify the presence of internal tip breakdown. Spears were classified into categories depending on the severity of tip breakdown observed: 0 = no tip breakdown, 1 = slight softening of the tip, 2 = slight darkening and softening of the tip, 3 = softening and darkening with slight watery tips but no tip disintegration, 4 = tip disintegration, 5 = severe tip disintegration.

3. Biochemical analysis

Non-structural carbohydrates (NSCs) were only extracted from 'Aspalim' samples. NSCs were extracted as previously described with modifications (Davis et al., 2007). Briefly, freeze–dried sample material (50 mg) was mixed with 1 mL of 62.5% (v/v) aqueous methanol solvent, placed in a shaking water bath at 55 °C for 15 min, vortexing the samples every 5 min to prevent layering, and then left to cool. The cooled samples were filtered through a 0.2 µm syringe driven filter unit (Fisher), and the clear extract was analysed. For the analysis of NSCs, 20 µL extract was injected into an Ashipak column (Shodex) with a guard column in an HPLC system (oven temperature 40 °C) coupled to an Evaporative Light-Scattering Detector (Agilent Technologies, UK). The mobile phase had a linearly increasing gradient of water in acetonitrile (% water): 0–15 min. 20–50 %; 15–20 min. hold at 50 %. The flow rate was 0.8 mL min^{-1} and presence and concentration of fructose, glucose and sucrose were calculated by comparing the peak areas in each sample to standards. Fructose, glucose and sucrose, standards were obtained from Sigma-Aldrich Co.

Abscisic acid (ABA) was extracted according to the method described in Garcia-Pastor et al. (2021a,b) with modifications. Freeze-dried powdered asparagus material (top 2cm from tip) was weighed (15 mg) and extracted with 1000 µL of precooled (4°C) methanol:water:formic acid (60:35:5 v/v/v). A labelled form of abscisic acid (d4-ABA) was added to the mixture as an internal standard. Extracts were analysed by injecting 10 µL onto a Hypersil Gold column 50 x 2.1 mm (1.9 µ particle size) with a guard column at 30°C. The mobile phases consisted of (A) water + 1mM ammonium formate + 0.1% formic acid and (B) methanol + 1mM ammonium formate + 0.1% formic acid, using an increasing gradient of B (5%, 40%, 95%, 5%) at a flow rate of 400 µL min^{-1}. Deuterated ABA was obtained from the National Research Council of Canada-Plant Biotechnology Institute (Saskatoon, SK, Canada). Non-deuterated (±) ABA was purchased from Sigma-Aldrich (Darmstadt, Germany).

4. Statistical assessment

Statistical analyses were carried out using Statistica for Windows version 10, 64-bit (Statistica Tulsa, OK 74104, USA). Analysis of variance (ANOVA) was used to identify significant differences ($p < 0.05$) between treatments followed by Fisher's post-hoc test.

RESULTS AND DISCUSSION

1. Growth rate

In both cultivars, growth rate was significantly reduced in spears grown under cold conditions compared to spears grown under warm conditions (Fig. 1). Furthermore, the increase in spear length appeared slower in Early California spears as shown by the slope of the graph, which coincided with lower mean spear weight compared to Aspalim (data not shown). However, it should be noted that Aspalim crowns were 1 year older and thus were more established.

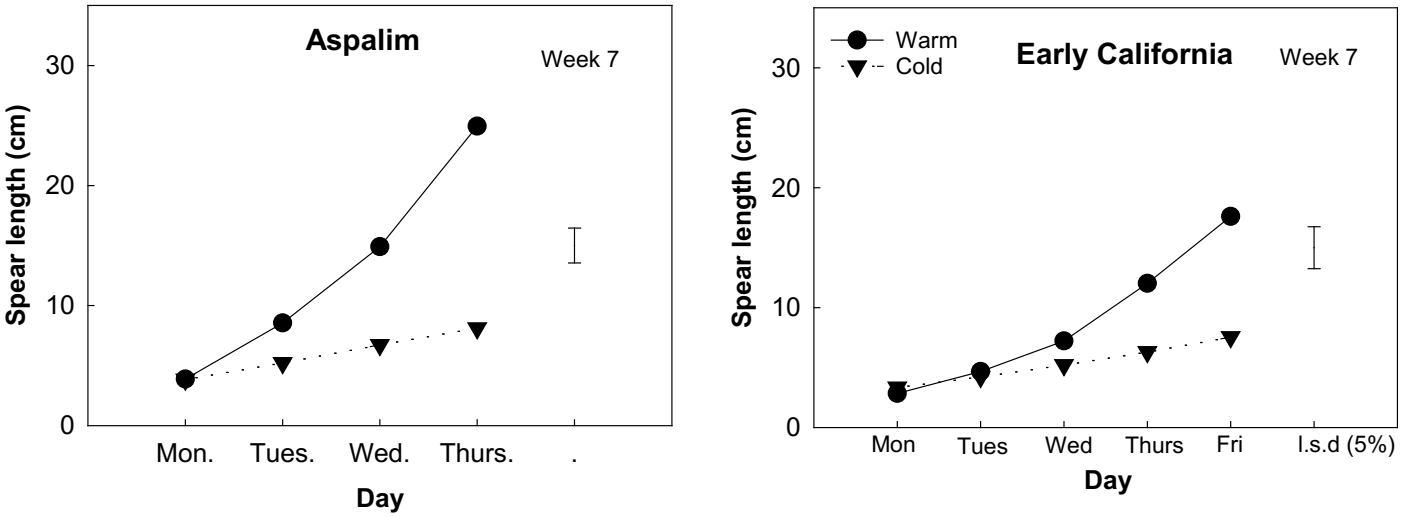


Figure 1 Spear growth rate for 'Aspalim' and 'Early California' under cold (18/8 °C) and warm (28/14 °C) day/night conditions. Each point in the graph represents the average of 10-14 spears. LSD bars ($P < 0.05$) are shown for the interaction Growing Conditions x Day was significant.

2. Tip breakdown assessment

Tip breakdown was not observed in spears grown under cold conditions. This agrees with Lill et al. (1996) who found that tip breakdown occurred under warm growing conditions. Our results also confirmed that a higher incidence of tip breakdown (3.4%) was observed in the highly susceptible cultivar, 'Aspalim', compared to the less susceptible genotype, 'Early California' (1.6%). However, tip breakdown incidence in 'Aspalim' spears was inconsistent during the course of the experiment (Fig. 2). This is in contrast with pers. commun. of a higher occurrence of tip breakdown later in the season. That said, in this experiment the spears were grown under constant 'warm' controlled conditions during an eight-week period, whereas the summer season in the UK is marked by a progressive and/or spiked increase in day and night temperatures, as well as light intensity.

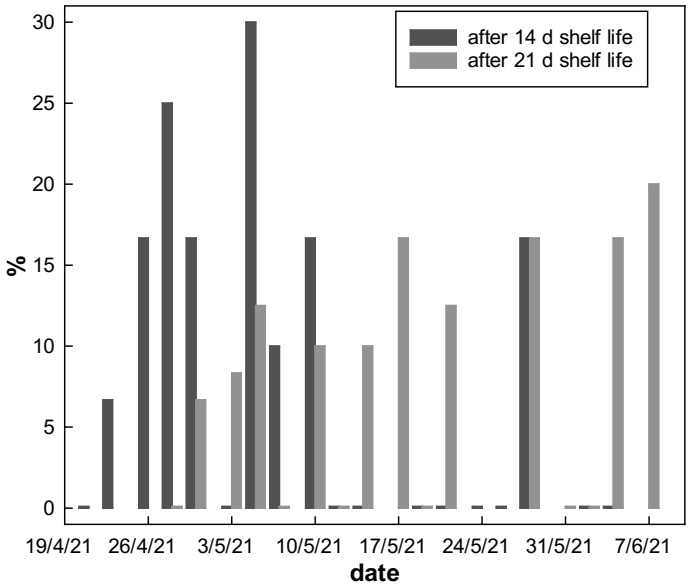


Figure 2 Percentage of 'Aspalim' spears with tip breakdown. Spears were grown under warm (28/14 °C) day/night temperature conditions and assessed after 14 and 21 days of shelf-life (7 °C), during an eight-week harvest period.

3. Non-structural carbohydrates

'Aspalim' spears grown under cold conditions had overall higher sugar content (fructose and glucose) at harvest compared to warm grown spears (34.4 vs. 20.6 mg g^{-1} dry weight [DW] and 24.9 vs. 12.3 mg g-1 DW, respectively). These results support previous studies where asparagus grown in more favourable field growing conditions (cooler temperatures) resulted in spears with higher sugar content and better quality (e.g., ascorbic acid content) during storage (Anastasiadi et al., 2020)

Fructose concentrations were overall lower in spears identified with tip breakdown (10.9 mg g^{-1} DW) compared to healthy spears (25.4 mg g^{-1} DW). However, no significant differences were observed for glucose and sucrose despite mid and late season spears showing lower concentrations in tip breakdown spears (Fig. 3). Further experiments will be needed to validate whether the decreasing trend in sugar content towards the end of the harvest season can be related with a predisposition to tip breakdown. Additionally, transcriptomic studies are being carried out to help elucidate the potential role of non-structural carbohydrates in asparagus tip breakdown disorder.

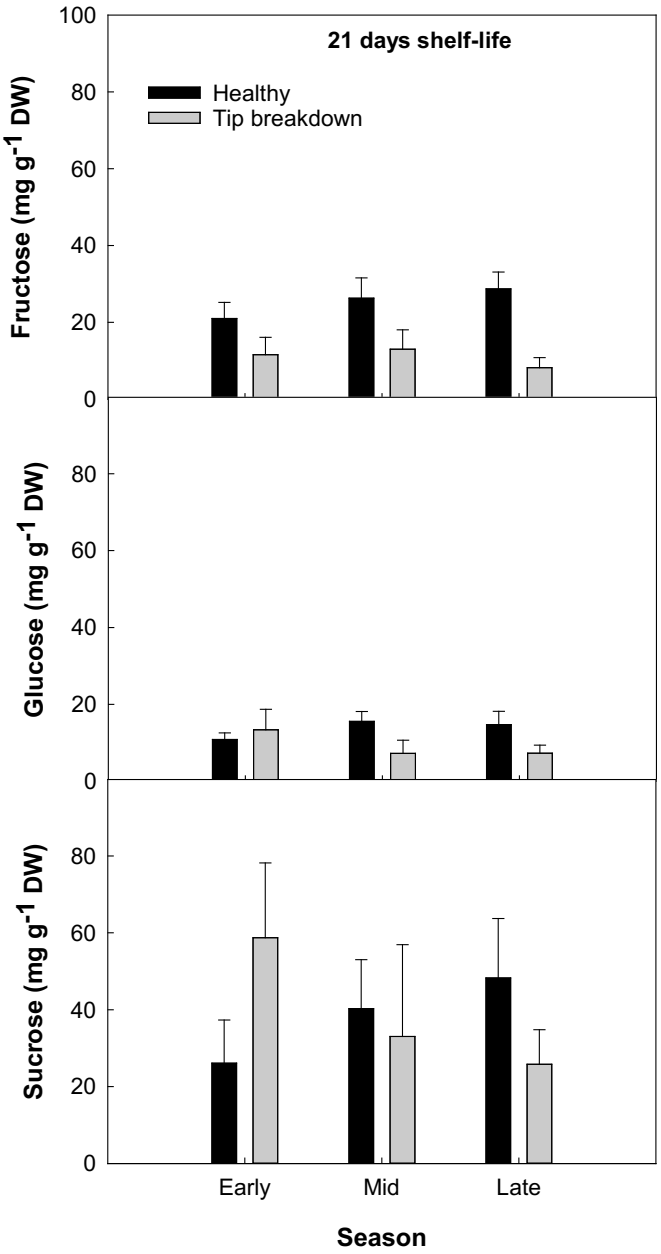


Figure 3. Fructose, glucose, and sucrose concentrations (mg g^{-1} DW) in 'Aspalim' healthy *vs.* tip breakdown spear tips grown under cold (16/8 °C) and warm (28/14 °C) day/night temperature conditions, at harvest and after 21 days at 7°C. Standard error bars shown. The interaction Season x Spear State (healthy *vs*. tip rot) was not significant (P > 0.05).

4. Abscisic acid

ABA concentrations after 14 days shelf-life were overall significantly lower in spears identified with tip breakdown (1573.1 ng g-1 DW) compared to healthy ones (3260.9 ng g-1 DW). Although this trend was observed throughout the harvest season (early, mid, and late), the significant difference in ABA concentration was mainly observed in early-season spears, which had *ca*. 2.5-fold higher ABA content than healthy spears. Higher ABA content in asparagus tips has been previously associated with spears with lower quality and early senescence (Anastasiadi et al., 2022). Therefore, we can hypothesise that the postharvest tip breakdown disorder and asparagus senescence are two independent processes which may be governed by different molecular pathways.

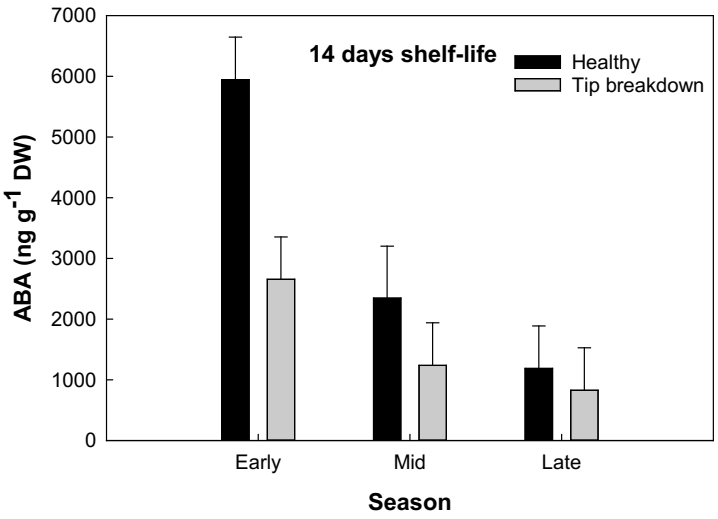


Figure 5. Abscisic acid (ABA) concentrations (ng g^{-1} DW) in 'Aspalim' healthy *vs*. tip breakdown spear tips grown under cold (16/8 °C) and warm (28/14 °C) day/night temperature conditions, at harvest and after 14 days at 7°C. Standard error bars shown. The interaction Season x Spear State (healthy *vs*. tip rot) was not significant ($P > 0.05$).

CONCLUSION

The cold acclimated 'Aspalim' spears were more susceptible to postharvest tip breakdown (higher incidence) than 'Early California', when grown under warm (28/14 °C) day/night conditions. 'Early California', a warm acclimated cultivar, seemed to grow at a lower rate compared to 'Aspalim'. Abscisic acid accumulation was higher in healthy spears compared to those with tip breakdown after 14 days of shelf-life suggesting that asparagus tip breakdown develops independently to senescence processes. A more detailed hormonal study, where cytokinins and auxins are quantified, alongside a transcriptomic analysis, will aid to fully elucidate the role of plant growth regulators in the incidence and development of postharvest tip breakdown in asparagus spears.

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