An assessment of the remodelling of bifurcations in hazel (*Corylus avellana* L.) in response to bracing, drilling and splitting

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Author Contribution Statement

Duncan Slater: initiator of this investigation into the remodelling of bifurcations of hazel, PhD student of Professor Ennos, first author of this paper. Work for this paper involved direct experimentation and collection of data, organisation of data, statistical analysis and the writing of this paper.

Prof. Roland Ennos: Supervisor to Mr. Duncan Slater, editor and reviewer of this manuscript.

Key Message:

This paper provides an insight into the ability of bifurcations in hazel trees to remodel themselves after bracing, drilling and splitting. The study uses evidence from field observations and testing the strength of these bifurcations using a universal testing machine alongside wood density tests. This work highlights the importance of the centrally-placed xylem at the apex of hazel forks in supplying tensile strength to the bifurcation. Additionally, it provides evidence that rod-braced bifurcations can atrophy in terms of their tensile strength, growth rate and wood density, suggesting that thigmomorphogenesis plays an important role in the development of a strong bifurcation.

Conflict of Interest:

The authors declare that they have no conflict of interest in reporting the findings of this study.

ABSTRACT

The ability of trees to remodel their woody structure after injury or strain to outer tissues greatly assists in their survival; however, this remodelling process is complex because it is influenced by many factors. The speed and extent of remodelling of branch junctions in trees around a mechanical flaw such as included bark will dictate to what extent and for how long the junction is mechanically weakened.

In this study, 100 normally-formed bifurcations in semi-mature hazel (*Corylus avellana* L.) were artificially modified by being rod-braced, drilled through the apex or split, and then left to grow in-situ. Two further groups: 120 normally-formed bifurcations and 70 bark-included bifurcations: were identified as controls. After two to four years these bifurcations were harvested and underwent tests of their bending strength. The bifurcations rigidly-braced over three growing seasons developed adverse taper in their branches and had only 70.5% of the bending strength of the normally-formed bifurcations. Bifurcations with the central 20% of the xylem drilled out of them were capable of recovering fully from this defect; in contrast, split bifurcations were found to be highly vulnerable to failure during wind-loading events.

This study concludes that a bifurcation may be considered compromised in its bending strength if its apex is compromised, but that semi-mature bifurcations in hazel do exhibit a good ability to remodel after injury. The role of thigmomorphogenesis in this remodelling process is assessed with reference to the rod-braced specimens that suffered no significant mechanical perturbation at their apices.

Keywords

Bark inclusion; bifurcation; biomechanics; bracing; *Corylus avellana* L.; remodelling; thigmomorphogenesis; tree crotch; tree fork

INTRODUCTION

In response to mechanical perturbation, plants undergo the process of thigmomorphogensis, whereby plant growth adapts in response to strains experienced by the plant's tissues (Jaffe and Forbes, 1993; Coutand, 2010; Telewski, 2012). Mechanosensing and subsequent adaption of plant growth is well-reported for plant height and form, the modification of the shapes of leaves, peduncles, petioles and the selective thickening of the branches and stems of plants (Whitehead, 1963; Jaffe, 1973; Grace, 1977; Biro *et al.*, 1980; Braam and Davis, 1990; Farnsworth and Niklas, 1995; Pruyn *et al.*, 2000; Telewski, 2006). It can be surmised that the majority of plant structures are likely to have this ability to respond to strain, including the junctions of the aerial parts of woody plants. Indeed, Jungnikl *et al.* (2009) found substantial adaptation to the tissues of junctions in Pinus using wide angled x-ray scattering to determine micro-fibril angle differences and CT scanning to uncover wood density differences. These analyses showed substantial modifications to the scanned branch junctions where stresses acting on these junctions would be heightened.

Thigmomorphogensis is triggered by the strain experienced by meristematic cells (Philipson *et al.*, 1971; Telewski, 2006; Monshausen and Haswell, 2013). In trees and other woody plants, thigmomorphogenesis can be a local phenomenon to parts of their structure, with secondary thickening occurring fastest where the highest mechanical strains are experienced (Steucek and Kellogg, 1972; Mattheck and Linnard, 1998). It is important, however, to note that remodelling within woody plants may be for a range of functions and that mechanical strain is only one potential influence upon how a plant's structure develops. In woody plants, sapwood serves a range of functions (Gartner, 1995; Badel *et al.*, 2015), not solely the structural support of the plant's stems and branches, and remodelling responses to a defect formed in the sapwood of a woody plant are potentially complex.

Junctions in the aerial parts of trees are considered to be potential failure points by arboriculturists (Shigo, 1981; Lonsdale, 1999), although scientific studies of the bending strength of such junctions have been restricted to static testing for practical reasons (Gilman, 2003; Kane *et al.*, 2008; Slater and Ennos, 2013). Static testing, in contrast to the dynamic movement of plants under natural loading, involves the application of a fixed load or a fixed rate of displacement in order to assess the strength of a component of a plant's structure, and results from such tests need careful interpretation when related back to 'real world' performance of such components. A greater understanding of the biomechanical behaviour of such junctions and their ability to remodel around a defect would assist in tree management and the prediction of tree failures.

An anatomical model for junctions in trees has been outlined by Slater *et al.* (2014) based upon visual observation of the grain patterns found at junctions of 20 tree and shrub species. This model was supported by CT scanning of bifurcations in common hazel (*Corylus avellana* L.) to observe the orientation of vessels, rays and fibres at the bifurcation apex. This anatomical model emphasises the importance of the xylem lying under the branch bark ridge as the main contributor to the bending strength of bifurcations, with the xylem tissues in this location typically being denser and exhibiting fewer vessels of a smaller diameter and shorter length when compared with adjacent xylem in the stem (Slater *et al.*, 2014).

Slater *et al.* (2014) also describe how the wood grain pattern formed at the bifurcation apex results in some degree of interlocking of the grain such that wood fibres need to be stretched axially or pulled out of the tissue matrix along their length in order to break the bifurcation apart (Fig. 1). In mature limbs of many temperate tree species, whirled grain can be found at the apex of junctions (Lev-Yadun and Aloni, 1990) as a subsequent development of this initial interlocking pattern (Fig. 1b).

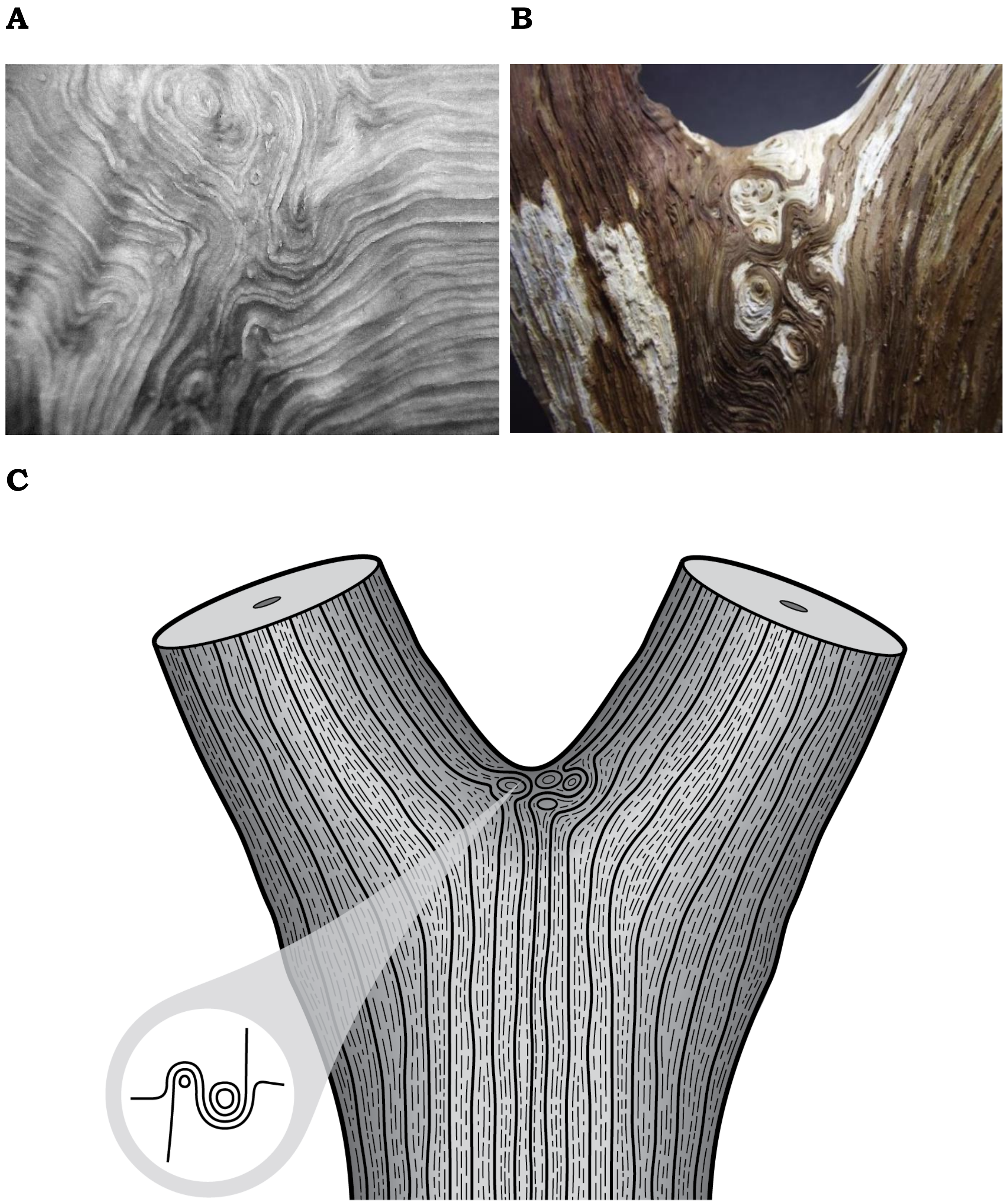

Figure 1: A: Interlocking wood grain pattern at the apex of a junction of common ash (*Fraxinus excelsior* L.), as exposed by de-barking. B: Wood grain pattern at the apex of a bifurcation of common oak (*Quercus robur* L.) incorporating whirled grain. C: Diagrammatic representation of interlocking wood grain in a normally-formed bifurcation in a woody plant, based upon the anatomical model of Slater *et al.* (2014) with inset displaying a basic interlocking pattern of wood grain incorporating whirled grain

It is a common occurrence, however, that bark is included in such bifurcations during their development. These bark-included bifurcations are weaker under static loading than normally-formed bifurcations (Kane *et al.*, 2008; Slater and Ennos, 2015). In addition, if the apex of a bifurcation consists of bark then that bark could act as a barrier to the future development of a normally-formed connection consisting of this denser tortuous sapwood.

In this study, we investigated the ability of bifurcations in hazel trees to remodel around artificially-induced defects. Previous work by Steucek and Kellogg (1972) in Norway spruce (*Picea abies* (L.) H. Karst.) identifies that trees remodel around such defects and discontinuities partially due to heightened stress levels at the location of the induced defect and partly due to the partial girdling that has occurred. Hazel (*Corylus avellana* L.) was selected as the test subject for this study because the authors have carried out a series of complementary investigations into the anatomy and biomechanical properties of bifurcations in this species.

For this study, we investigated the loss of bending strength to these bifurcations caused by artificial wounding, comparing them to both normally-formed and bark-included bifurcations grown in the same location. The three artificial defects studied were fixed-rod bracing of the two branches arising from bifurcations, the drilling out of the centrally-placed xylem at the apex of bifurcations and the splitting of the apex of the bifurcation by pulling the two branches apart from each other.

It was hypothesized that the braced bifurcations, in the absence of them experiencing mechanical perturbation at their apices, would become weaker over time. It was further hypothesized that the drilled-out and split bifurcations would remodel around their artificially-induced defects, recovering their bending strength over time. Overall the study aimed to provide evidence that mechanical loading was a key factor in the development of strength in these bifurcations, as well as identifying the typical pattern of anatomical remodelling that occurred around these defect types.

MATERIALS AND METHODS

Selection of hazel bifurcations

A wind-exposed semi-mature shelterbelt consisting of a mix of broadleaves species which contained semi-mature hazel trees was selected for this experiment. The planted area was on the southern boundary of the campus of Myerscough College, Lancashire, England – grid reference: SD497399 (Easting 349711, Northing 439982). The trees in this shelterbelt were planted as 3-year-old bare-rooted stock in 2004, making the hazel trees 13 years of age by the end of this study. All the bifurcations used for this experiment were formed less than two metres above ground level; this facilitated their modification by bracing, drilling or splitting and ensured that the age and diameters of these bifurcations were similar.

Bifurcation selection was biased towards choosing bifurcations with a high diameter ratio (80%+), as expressed by the percentage difference between the diameters of the thinner branch to the thicker branch arising from the bifurcation and as measured proximal to the bifurcation. Bifurcations were also selected so that both branches and the parent stem were ascending, all of them forming a relatively upright Y-shape, with no other significant branching to be found above or below 200 mm of the bifurcation apex. No more than three bifurcations were selected in the crown of any one hazel tree, which resulted in a random scattering of sample collecting along the 450 metre length of the shelterbelt.

Modifications to the hazel bifurcations

In December 2010 an initial experiment was devised whereby 50 hazel bifurcations had the centre of their apex drilled out and were left to develop over two to four years (Fig. 2b). The drill bit size was selected for each bifurcation so that 20% of the width of the apical tissues were removed (Table 1) based on a measurement of the parent stem perpendicular to the bifurcation and just below the bulge formed by the branch bark ridge (PS_2, Fig. 4).

This drilling scheme matches that carried out by Slater and Ennos (2013) on bifurcations of hazel that were tested to determine the contribution of the centrally-placed xylem to the bending strength of such bifurcations. However, in this experiment, these drilled bifurcations were left in-situ, attached as a component of the tree, to assess whether and how the bifurcations would re-model around the induced defect of the drill hole. Each drill hole made was filled with silicon sealant which facilitated identification of these modified bifurcations when they were mechanically tested, and each was sprayed with a standard fluorescent forestry marking paint so that they could be identified and harvested at a later date. In addition, 50 normally-formed bifurcations were also selected and spray-painted within the same wooded area, to act as a control of the bending strength of unmodified bifurcations.

Table 1: Determination of drill size for hazel bifurcations modified by drilling, based upon the diameter of the parent stem, measured just below the termination of the branch bark ridge and perpendicular to the bifurcation

Diameter of parent stem (mm) perpendicular to bifurcation	Drill size used upon bifurcation
Up to 22.5	4 mm
22.5 – 27.49	5 mm
27.5 – 32.49	6 mm
32.5 – 37.49	7 mm
37.5 – 42.49	8 mm
42.5 – 47.49	9 mm
47.5 +	10 mm

In December 2011 the replicate number and scope of this experiment was expanded. A total of 50 further hazel bifurcations were artificially altered; 25 bifurcations had a 3 mm diameter steel rod fixed by bolts and washers fitted through the centre of both branches approximately

70 mm above the bifurcation to conjoin these branches (Fig. 2a); a further 25 bifurcations were carefully split by hand so that a crack (approximately half the length of the branch bark ridge) was induced at the bifurcation apex by bending the two branches above the bifurcation away from each other (Fig. 2c). The braced bifurcations were typically of a larger size (as measured by the parent stem diameter) than the mean of all the bifurcations at the start of the experiment, because of the need for the two branches of the bifurcation to be thick enough to accept the bracing rod and remain intact.

It was also determined at this time to add a further 70 normally-formed bifurcations to the original 50 normally-formed bifurcations, and also to identify in this shelterbelt 70 bark-included bifurcations for rupture testing. All additions were also marked with colour-coded fluorescent forest marking paint to aid their re-identification upon harvesting.

It was considered that a greater number of normally-formed bifurcations were required, as some would be subsequently drilled immediately prior to mechanical testing to compare with those drilled bifurcations that were left in-situ to grow and had remodelled around their drill hole due to subsequent secondary growth. By increasing the replicates within the normally-formed group it was also hoped to reduce the variability in the mean breaking stress in that group, providing a better comparison between treatment types. The bark-included bifurcations were added as a group type to compare with the extent of any strength loss in the artificially modified bifurcations and thus give additional context to our results.

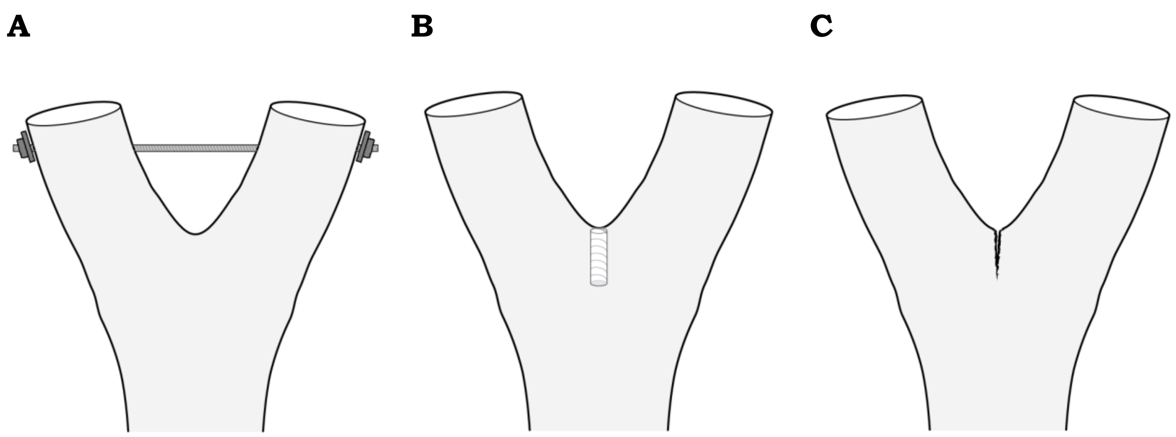

Figure 2: Artificially-modified bifurcations left to grow in-situ for two to four years: A: Diagram of rod-bracing created in 25 hazel bifurcations. B: Diagram of drill hole created in 50 hazel bifurcations. C: Diagram of split created in 25 hazel bifurcations

A summary of the different types of bifurcation investigated is provided in Table 2:

Table 2: Bifurcation types tested, research related to each type, numbers of replicates for each type, year of modification and associated growing seasons prior to mechanical testing

Name of bifurcation type	Description	Factor assessed	No. of replicates	Year of artificial modification	Growing seasons between modification and testing
Bark-included	Naturally-occurring bifurcations with bark found to be incorporated within the apex of the bifurcation (Fig. 6)	Effect of bark obstructing the normal anatomical connection at a bifurcation	70	Not modified	N/A
Braced	Normally-formed bifurcations modified by the conjoining of the two branches above the bifurcation with a 3 mm steel rod fitted through both branches, with a 7 mm washer and nut fitted at each end of the rod. These were left to grow within the tree's crown for three years prior to testing (Fig. 1a)	Effect upon remodelling by completely preventing mechanical perturbation at the apex of the bifurcation	25	2011	3
Newly-drilled	Normally-formed bifurcations drilled at their apices using a drill-size as defined in Table 1, immediately prior to	Effect of removing centrally-placed interlocking xylem at the	60	2015	0

	mechanical testing (Fig. 1b)	apex of the bifurcation			
Normally-formed	Naturally-occurring bifurcations with no flaws observed in morphology	To act as a benchmark for all other modifications	60	Not modified	N/A
Pre-drilled	Normally-formed bifurcations modified by drilling at their apices using a drill-size as defined in Table 1, and left to grow within the tree's crown for two or four years prior to testing (Fig. 1b)	Effect of remodelling after the removal of the centrally-placed interlocking xylem at the apex of the bifurcation	50	2010	2 and 4
Pre-split	Normally-formed bifurcations modified by carefully splitting the apex by hand, by bending away from each other the two arising branches. These were left to grow within the tree's crown for three years prior to testing (Fig. 1c)	Effect of remodelling after the cracking of the top part of the bifurcation	25	2011	3

Observations

Prior to harvesting of the bifurcations in 2013 and 2015, basic observations were recorded of the condition and morphology of the selected bifurcations, including any swellings associated with the artificially-modified bifurcations and also whether bifurcations had failed in-situ, prior to harvesting, within the shelterbelt.

Mechanical testing

In January 2013, after two growing seasons, twenty-one of the bifurcations that were drilled in December 2010 and fifty of the normally-formed bifurcations were cut from the trees in order to carry out mechanical testing. The bifurcations were cut so that there was a minimum length of 220 mm of both branches and at least twice the length of the branch bark ridge of the parent stem on each bifurcations. The bifurcations were wrapped in individual plastic bags immediately after cutting to minimise sap loss, and were stored in a cold store kept at 2 °C prior to rupture testing.

Twenty-five of the normally-formed bifurcations had the centre of their apex drilled immediately prior to rupture testing, using the drill sizes as defined in Table 1.

A six millimetre hole was drilled perpendicular to the plane of the bifurcation in the middle of both branches of each bifurcation, approximately 200 mm from the bifurcation apex, and then it was bolted to the crosshead and base of an Instron™ universal testing machine (UTM) Model 4301 fitted with a 1 kN load cell (Fig. 3). The crosshead of the testing machine was then made to rise at a rate of 35 mm min^{-1} until each bifurcation was broken, whilst an interfacing computer recorded the displacement (in millimetres) and force applied (in Newtons) at a rate of 10 measurements per second.

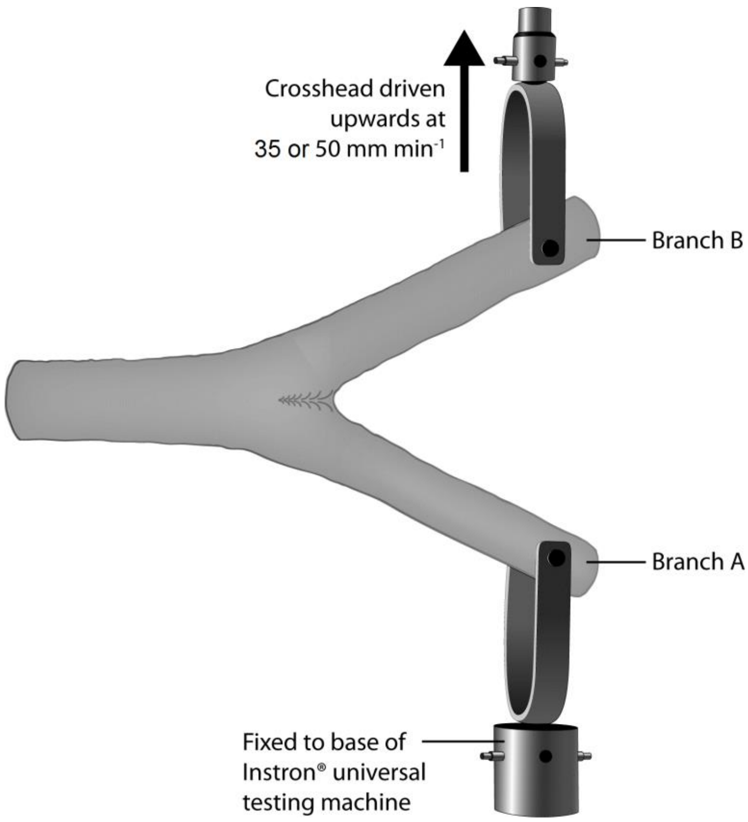

Figure 3: Diagram of the means of attachment of the bifurcations to the Instron™ Universal Testing Machine during the rupture tests

After this testing, careful observation was made by eye of the fracture surfaces of all bifurcations, in relation to their morphology and appearance.

In order to estimate their breaking stress, the following measurements were taken for each bifurcation: the diameter of both branches adjacent to the apex of the bifurcation perpendicular and in-line with the plane of the bifurcation (A_1, A_2, B_1 and B_2); the diameter of the parent stem just below the branch bark ridge, perpendicular and in-line with the plane of the bifurcation (PS_1 and PS_2); and the distances between the two drill holes in the two branches and between both drill holes and the apex of the bifurcation (*a*, *b* and *c*) (Fig. 4).

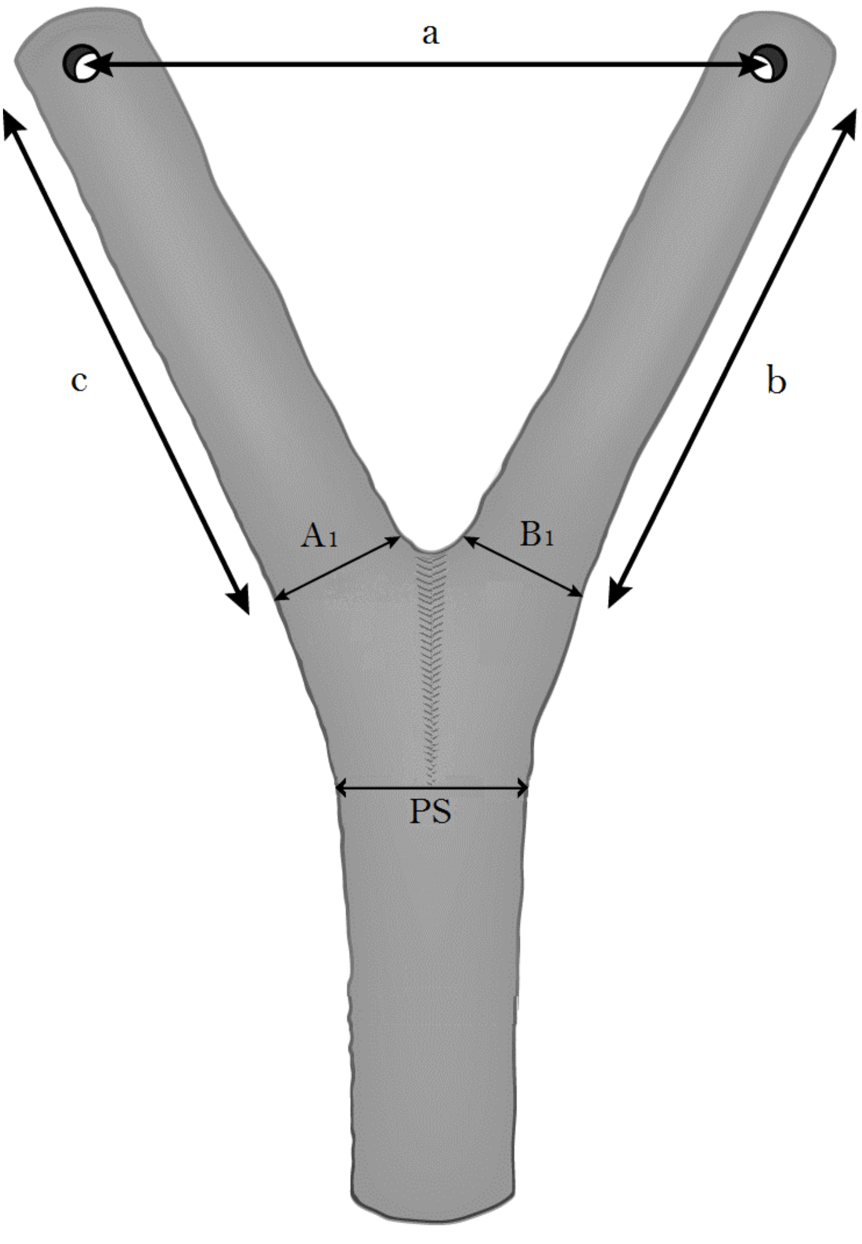

Figure 4: Measurements taken on each bifurcation in order to calculate its breaking stress: distances between the two drill holes and between each drill hole and the apex of the bifurcation (*a*, *b* and *c*) measured using a metal rule: diameters of the two branches just above the bifurcation apex, both in-line with the plane of the bifurcation (A_1 and B_1) and perpendicular to the plane of the bifurcation (A_2 and B_2 (not shown on 2D image)), and the diameter of the parent stem (*PS*) just below the branch bark ridge, both in the plane and perpendicular to the plane of the bifurcation measured using digital callipers

This method of rupture testing of hazel bifurcations was used by Slater and Ennos (2015), when they assessed the strength of hazel bifurcations containing bark-inclusions, and the same equations were used to estimate the breaking stress of the bifurcations as are reported in this previous paper.

To assist with comparing the relative strength of the bifurcations, three-point bending tests of the smaller diameter branch of the bifurcation were carried out, testing the yield strength of the middle of each branch whose structure had not been compromised by the rupture testing. The span for these branches was set at 215 mm for branches up to 20 mm in diameter and 275 mm for branches up to 23 mm in diameter, and the cross-head of the Instron, fitted with a semi-circular plastic probe, pressed down on the branch at a rate of 30 mm min^{-1} until the branch yielded substantially, with the interfacing computer recording force, displacement and calculating the yield strength of each branch tested. This procedure was used successfully in previous testing (Slater and Ennos, 2013; Slater and Ennos, 2015) Due to limitations of the testing machine in terms of the span length that could be used and the maximum load (900 kN) that could be applied, branches with a mid-diameter of over 23 mm could not be tested to their yield point. Careful observations of the yielding of each branch was undertaken, as these shorter spans could have resulted in shear failures (Vincent, 2012) which could have invalidated some of the test specimens; however, no shear failures were observed to occur in these test specimens.

In February 2015, after four growing seasons for the original set of drilled bifurcations and three growing seasons for the braced and split bifurcations, all the remaining bifurcations were cut from the hazel trees and subjected to the same method of bagging, storage and rupture testing. A different Instron™ testing machine (Model 3344) had to be used for this second set of mechanical tests, as the original UTM had suffered a breakdown in the two year period between these two tests. The parameters of the rupture tests were the same in nearly all respects; however, the rate of displacement was increased to 50 mm min^{-1}, due to the large number of bifurcations that had to be processed. This higher rate of displacement for this second set of tests did not make any discernible difference to the kinematics of failure.

The bifurcations with bark included within them were classified after testing in terms of the relative occlusion of the bark into the bifurcation, giving rise to three types of bark inclusion: embedded, cup-shaped and wide-mouthed (Fig. 5). This classification of bark-inclusions was used by Slater and Ennos (2015), who identified significant differences in breaking stress between these three morphological types of bark-included bifurcation in hazel. For each braced bifurcation tested, bolt cutters were used to cut the steel rod that conjoined their two branches in two places prior to testing.

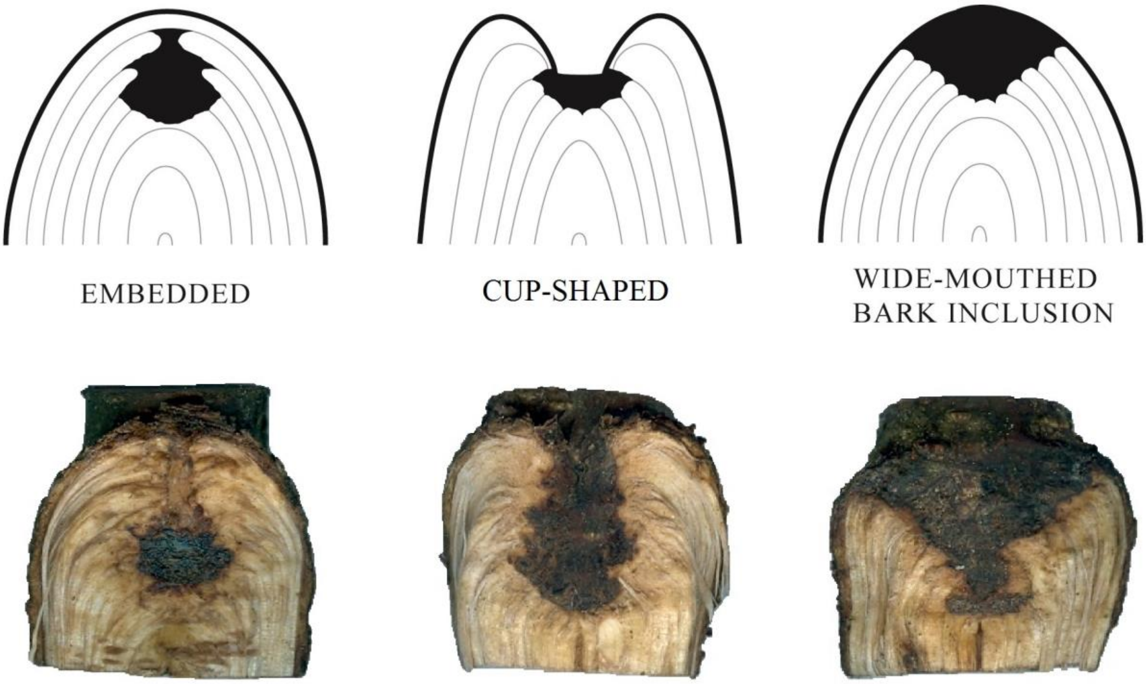

Figure 5: Diagrams and images defining three morphological types of bark-included junctions in hazel, based on observations of the fracture surfaces of bifurcations. Embedded bark is surrounded entirely by xylem, the bark having been occluded into the junction. A cup-shaped bark inclusion has sapwood formed around included bark which lies at the centre of the join – there is sapwood at the apex of the bifurcation rather than bark. A wide-mouthed bark inclusion has a substantial width of included bark at the apex of the bifurcation, situated above any connecting sapwood

Wood density testing

Wood density tests were carried out on small samples of the xylem excised from the apices, from the side of the bifurcations adjacent to their apices and from the parent stems of all the bifurcations tested in 2015. The purpose of this testing was to ascertain if the remodelling around the induced defects also affected the mechanical qualities of the new wood being laid around these defects. Both braced and normally-formed bifurcations could provide xylem from all three locations, whereas the drilled or split bifurcations and those with included bark could only supply xylem samples from the side of the bifurcation apex and the stem (Fig.s 2b, 2c and 6). Samples were cut using a pull saw and billhook blade, their fresh weight taken and their volume calculated by measuring the displacement weight when each sample was immersed in distilled water on a weighing scales. The mean volume of these samples for this wood density test was 444.4 mm^3 ± 8.4 SE (standard error).

The samples were then oven dried for 96 hours at 60 °C and their dry weight recorded. Given the small size of the samples, this length of drying time was considered sufficient. Wood density was calculated by dividing the dry weight of each sample by the volume of the sample (Hughes, 2005).

Statistical analysis

All statistical tests were carried out using MiniTab® version 17.

A X^2 test was used to assess whether there were differences in modes of failure for the bifurcation types.

For comparisons between bifurcation types, and for sub-sets within each bifurcation type, General Linear Model (GLM) ANOVAs were used to find differences in mean breaking stress, with the parent stem diameter (PS_1) and the diameter ratio of the bifurcations as covariates where appropriate, in combination with a post-hoc Tukey test at a 5% confidence level. Residuals were assessed for the normality of their distribution using the Anderson-Darling test. For the ANOVA assessing the bending strength of all types of bifurcations (Fig. 7), residuals of the transformed data satisfied the Anderson-Darling test for normality (AD_{299} = 0.695; p = 0.07). Likewise, for the ANOVA assessing the bending strength of different types of bark-included bifurcations, residuals satisfied the Anderson-Darling test for normality (AD_{66} = 0.359; p = 0.441). For the ANOVA assessing the pre-drilled bifurcations (Fig. 9) the residuals satisfied the Anderson-Darling test for normality (AD_{94}= 0.698; p = 0.066).

To determine if the branches of the braced bifurcations exhibited adverse taper a paired t-test comparing the diameter of the branches above the fitted steel brace and at the apex of the bifurcation was carried out.

To determine differences between the wood density of samples extracted from the apices and sides of bifurcations and the adjacent stem wood, a GLM ANOVA with sample volume as a covariate was used, in combination with a post-hoc Tukey test at a 5% confidence level. Residuals were assessed for the normality of their distribution using a Kolmogorov-Smirnov test as the Anderson-Darling test gave a marginal result. Residuals from the ANOVA assessing differences in wood density satisfied the Kolmogorov-Smirnov test for normality (KS_{372}= 0.046; p = 0.059). For assessing the difference between wood density in normally-formed and braced bifurcations, residuals from this ANOVA satisfied the Anderson-Darling test for normality (AD_{100}= 0.512; p = 0.191).

RESULTS

Specimen losses and mean specimen dimensions

Over the four years of this experiment, a number of the selected bifurcations (20 out of the total of 290 bifurcations) were lost prior to the mechanical testing. Fourteen of the bifurcations were removed from this study in 2012 as a length of the shelterbelt's edge was accidently flailed when a neighbouring hedgerow was pruned; the remaining six bifurcations which were lost could not be found in 2015 due to the bio-degradability of the forestry marker paint used, as it was concluded that the paint had weathered away.

In addition, two types of the modified bifurcations suffered replicate losses for other reasons. Seven of the braced bifurcations grew over the three years to a size that was too large for the testing machine to break them (having started at the upper end of the parent stem diameter sizes chosen), which reduced this group's size to 14 testable replicates. Twenty of the twenty-five split bifurcations suffered wind-induced mechanical failure over the three years they were in-situ. For this latter group, observations were subsequently made of these failures and of the morphology of the five bifurcations that remained.

The mean parent stem diameter (PS_1) for the remaining 243 bifurcations was 30.35 mm ± 0.37 SE, the mean diameter of the smaller branch of the bifurcation just above its point of attachment (b_1) was 21.23 mm ± 0.26 SE and the mean diameter ratio for these bifurcations was 80.98 ± 0.75% SE.

Observations of bifurcations prior to testing

Bark-included bifurcations

Ten of the normally-formed bifurcations were found to contain embedded bark, so the data generated from these 10 bifurcations was moved to the bark-included group for analysis. To compensate for the reduction in the group size of the normally-formed bifurcations, the number of replicates allotted to the newly-drilled group was reduced to obtain a roughly equal number of replicates within these two groups. The categorisation of the remaining 58 bark-included bifurcations resulted in 36 being identified as wide-mouthed bark inclusions and 22 identified as cup-shaped bark inclusions (Fig. 5).

Drilled bifurcations

Observations of the pre-drilled bifurcations showed a range of remodelling responses to the initial drilling of the hole at their apices. In general, despite some initial dysfunction caused to adjacent tissues after drilling, additional sapwood had grown around the induced defect (Fig 6a). Three of these bifurcations had fully embedded the silicon, surrounding it with new sapwood after four years of growth, and many more had started to cover over the top of the drill hole. In the majority of these bifurcations a general swelling in the location of the branch bark ridge was evident. For thirteen of these bifurcations, however, the drill-hole had initiated the development of included bark at the apex or a larger extent of associated dysfunction around the original drill-hole had resulted in a failure to occlude the drill-hole. No significant volume of decayed xylem was found in any of these bifurcations. This difference in development allowed the pre-drilled bifurcations to be classified into three sub-categories to match the bark-included ones: i) that the silicon in the drill-hole had become embedded; ii) that the bifurcation was forming a cup-shape around the drill-hole; or iii) that the drill-hole was still wide open at the bifurcation's apex.

Split bifurcations

For the pre-split bifurcations, the high number of replicate losses through wind-induced failure was investigated. It was observed that for the five split bifurcations that had persisted for three years and been subjected to rupture testing, all had split further down the stem since the initial splitting was carried out in 2013, and the split had been halted either by encountering a substantial knot in the parent stem (for four of them) or a substantial bend in the parent stem (in one case only). The twenty bifurcations that had mechanically failed had done so due to natural wind-induced movement and subsequent propagation of the original split down the parent stem, with the split at some point deviating to the edge of the stem, causing one branch to fall away from the tree.

The propagation of these splits and the failure of so many of this type of bifurcation meant that this group had to be excluded from any statistical analysis relating to the breaking stresses of the bifurcations. An image of the typical surviving pre-split bifurcation is provided in Figure 7b.

Braced bifurcations

It was evident that the installation of the steel rod in 2011 had resulted in abnormal swelling of the branches at the point of drilling the 3 mm hole needed to fit the brace (Fig. 6c). All braced bifurcations exhibited some level of occlusion of the rod, nuts and washers and some had wholly occluded the nuts and washers. Measurements were taken of the diameter of the branches just above each braced bifurcation's apex, as with all other bifurcations, but also the branch diameters were measured at the point above the bracing rod and its associated swelling, to determine if the bracing had resulted in the branches developing adverse taper.

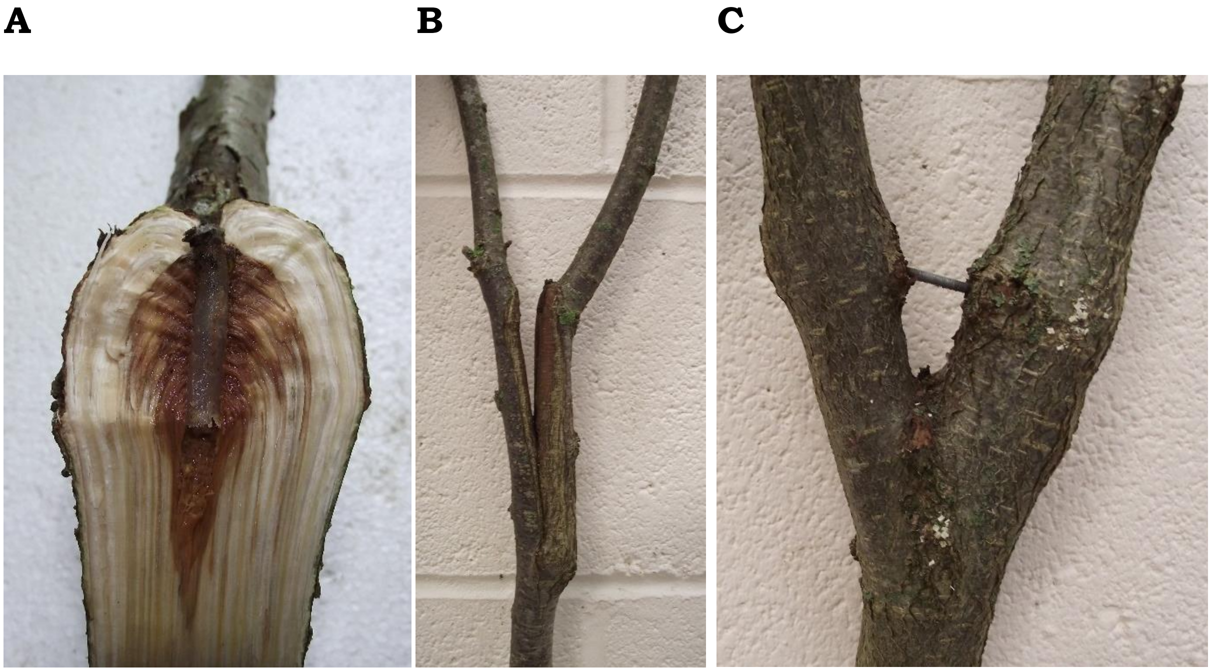

Figure 6: A: Fracture surface of a pre-drilled bifurcation after two growing seasons, showing the silicon inserted into the initial drill-hole, dysfunction induced in the sapwood around the drill hole (discoloured area) and the remodelling of the sapwood to form a cup-shaped union; B: Typical deformation of a pre-split bifurcation, where the crack had subsequently propagated to a knot in the parent stem and then been arrested; C: Typical deformation of the branches of a braced bifurcation around the implanted steel rod, after three years of growth, showing adverse taper in the smaller branch

Mechanical testing

All bifurcation types

Twelve of the tested bifurcations suffered branch failure, rather than failing at the bifurcation itself. To assess bifurcation strength, all those bifurcations that suffered branch failure were excluded from this part of the data analysis.

A statistical comparison was made of the mean breaking stresses of the main five bifurcation types and the yield stress of the smaller branches, using a GLM ANOVA and post-hoc Tukey test after a natural log transformation of the data. It was found that there were significant differences between groups ($F_{5,293}$ = 61.54; R^2 = 51.23%; p < 0.001); pairwise comparisons identified that the branches yielded at the highest mean stress, and the bark-included and newly-drilled bifurcations broke at the lowest mean stress (Fig. 7).

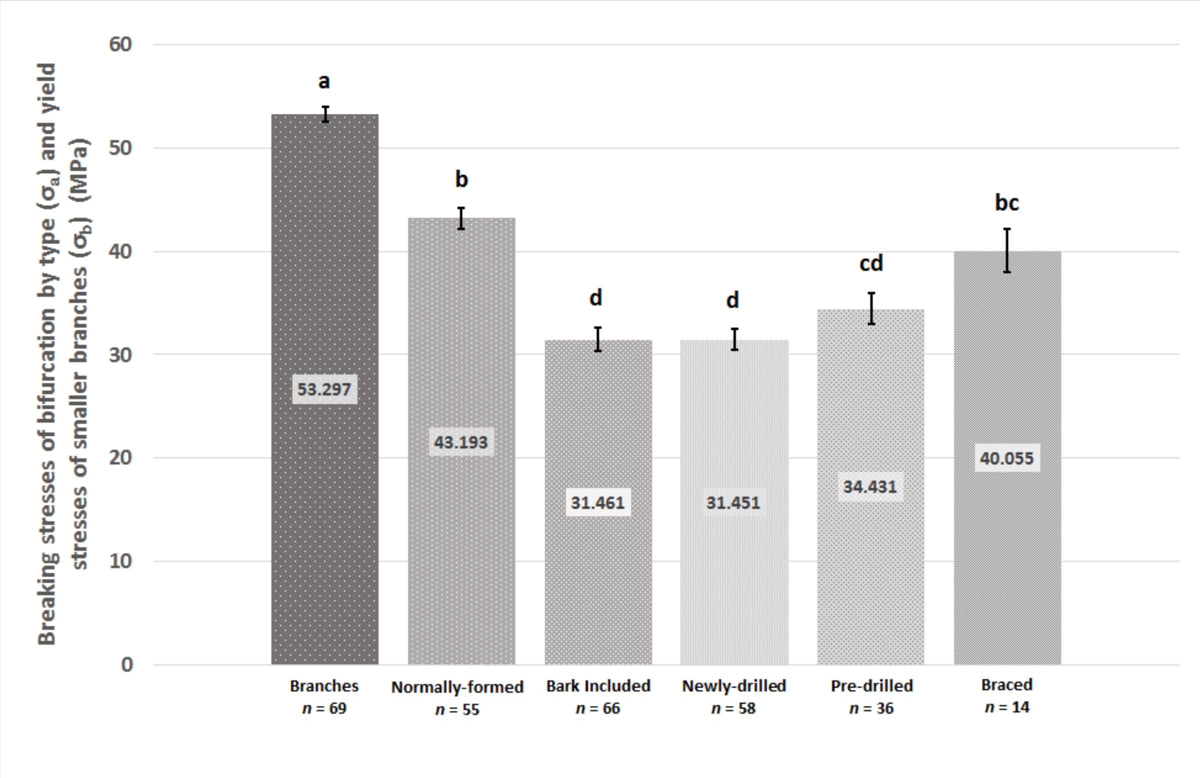

Figure 7: Mean breaking stresses for the main bifurcation types tested (excluding branch failures) and the mean yield stress of the smaller branches as found by three-point bending. The pre-split type is not included as its replicate number was too small to be statistically analysed (*n* = 2). Error bars represent standard error. Letters above bars identify significant differences between groups by using a GLM ANOVA and post-hoc Tukey test at a 5% confidence limit

Bark-included bifurcations

A comparison between the three sub-types of the bark-included bifurcations in relation to their mean breaking stress is provided in Figure 8. A GLM ANOVA ($F_{2,61}$ = 10.44; R^2 = 38.93%; p < 0.001) with diameter ratio and the diameter of the parent stem as covariates found that there were significant differences between these groups and a post-hoc Tukey test identified that the wide-mouthed bark-inclusions broke apart at a lower stress than the other two types. The diameter ratio was a significant covariate (p < 0.001) in that a higher diameter ratio resulted in a lower breaking stress, but the parent stem diameter was not a significant covariate (p = 0.381).

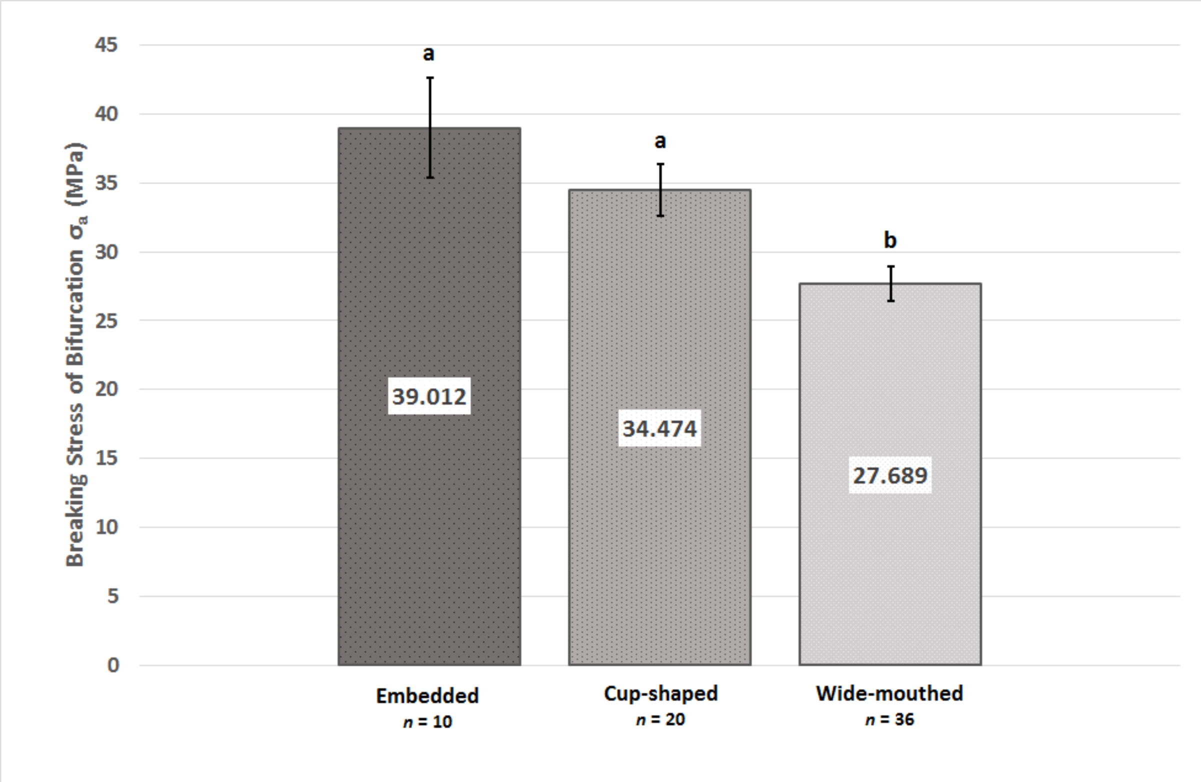

Figure 8: Mean breaking stresses of the three types of bark-included bifurcation tested. Error bars represent standard error. Letters above bars identify significant differences between groups by using a GLM ANOVA and post-hoc Tukey test at a 5% confidence limit

The difference in bending strength between these three types of bark-included bifurcations and the normally-formed bifurcations was a reduction of 9.7% in bending strength for those with embedded bark, a reduction of 20.2% for cup-shaped bifurcations and a reduction of 35.9% for wide-mouthed bark-included bifurcations.

Drilled and pre-drilled bifurcations

The mean breaking stress of the newly-drilled bifurcations was 31.45 MPa ± 1.01 SE, whereas for the pre-drilled bifurcations that were allowed to grow for two growing seasons it was 32.85 MPa ± 1.85 SE, and for the pre-drilled bifurcations that remodelled around the drill holes for four growing seasons it was 36.64 MPa ± 2.35 SE.

It was observed that growth responses in the pre-drilled bifurcations were mixed, with some bifurcations suffering more xylem and cambial dysfunction than others, and some growing rapidly around the drill-hole with little to no dysfunction evident. As a consequence, the pre-drilled bifurcations were placed into three groups corresponding to the classification of the bark-included group in this study: 3 of the pre-drilled bifurcations had occluded the drill-hole and were categorised as 'embedded', 20 more bifurcations had partly occluded the drill-hole and were categorised as 'cup-shaped' and the remaining 13 bifurcations in the pre-drilled group exhibited no evidence of occlusion and had suffered dieback related to the drill-hole; these were categorised as 'wide-mouthed'. A statistical comparison between these groups and the newly-drilled bifurcations is provided in Figure 9.

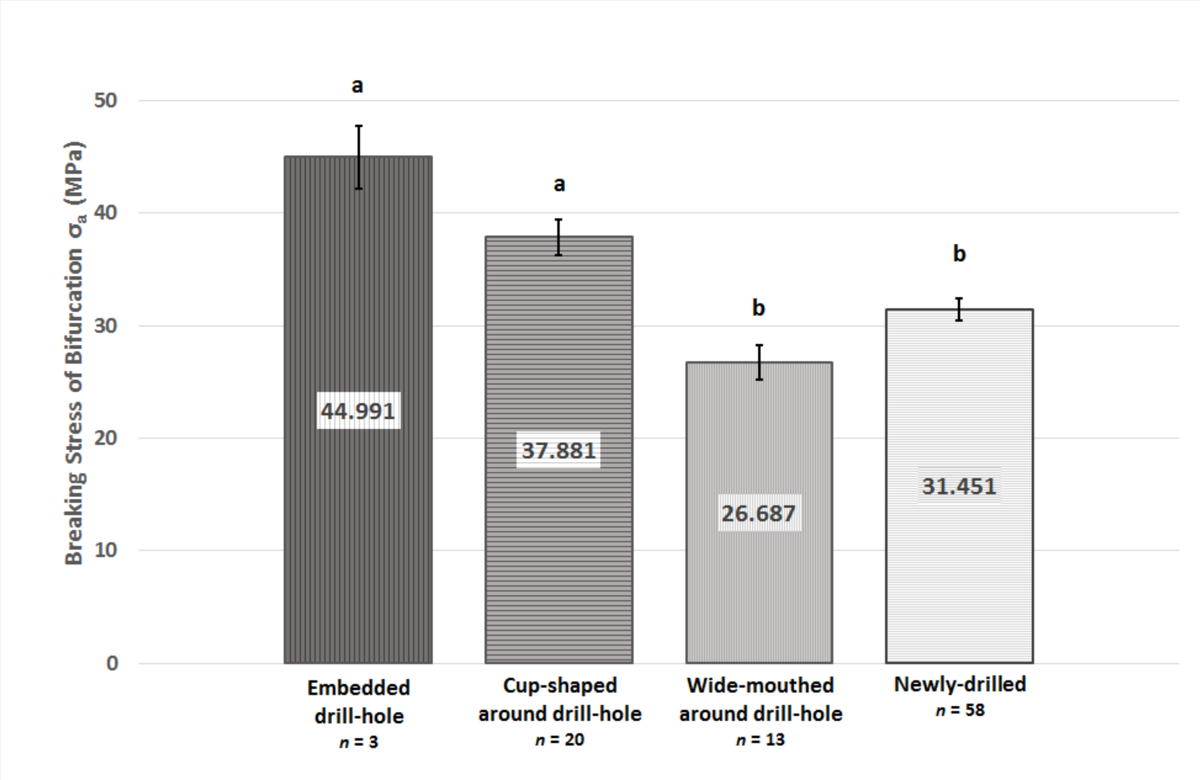

Figure 9: Mean breaking stresses of the three types of pre-drilled bifurcation tested. Error bars represent standard error. Letters above bars identify significant differences between groups through using a GLM ANOVA and post-hoc Tukey test at a 5% confidence limit ($F_{3, 88}$ = 5.70; R^2 = 42.34%; p = 0.001). The diameter ratio was a significant covariate (p < 0.001) and the parent stem diameter was not significant (p = 0.909)

Bifurcations in the pre-drilled group that showed the most regrowth around the drill-hole (embedded or cup-shaped) had a higher strength than those where regrowth had not occurred (wide-mouthed), which had similar strength to the newly-drilled bifurcations (Fig. 9).

Braced bifurcations

For the braced bifurcations the mean diameter of the branches arising from the bifurcations was 24.59 mm ± 1.01 SE, but the mean diameter of the branches just above the bracing rod was 28.40 mm ± 1.01 SE. A paired T-Test identified that a significant adverse taper had developed in the branches ($T_{1, 13}$ = 4.75; p < 0.001). Data was normally distributed (AD_{28} = 0.643; p = 0.084). This adverse branch taper was not exhibited by any other bifurcation type.

Further to this observation, the breaking stress of these braced bifurcations was additionally calculated based on the section modulus of the smaller branch just above the steel rod brace and its associated swelling. This further assessment takes into account the larger branch that would actually have to be borne by the bifurcation if the brace was not in place. The mean breaking stress of the braced bifurcations using the section modulus of the smaller branch at the bifurcation apex was 40.06 MPa ± 2.08 SE (Fig. 7), but when taking into account the section modulus of that same branch above the brace, the equivalent breaking stress reduced to only 30.47 MPa ± 1.44 SE. These two mean breaking stresses were compared with the mean breaking stresses of the normally-formed bifurcations (Fig. 10).

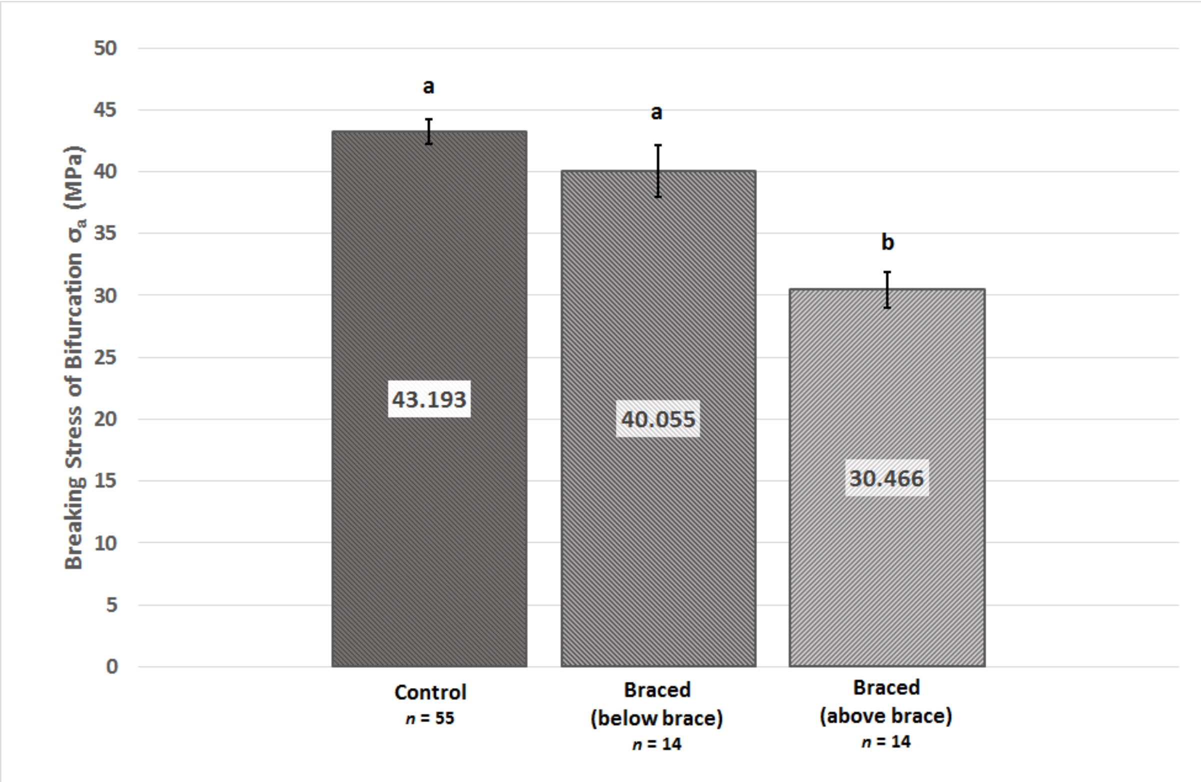

Figure 10: Mean breaking stresses of the normally-formed bifurcations and the two estimates of the breaking stresses of the braced bifurcations, taking into account the section modulus of the smaller branch either below or above the brace rod. Error bars represent standard error. Letters above bars identify significant differences between groups by using a GLM ANOVA and post-hoc Dunnett test at a 5% confidence limit ($F_{3, 77}$ = 19.32; R^2 = 35.59%; p < 0.001). The diameter ratio was a significant covariate (p = 0.017), with an increasing diameter ratio resulting in a lowering of breaking stress; the parent stem diameter was not found to be a significant factor (p = 0.631)

Wood density at hazel bifurcations

The results of the wood density testing are provided in Table 3. Statistical analysis of the data found that all the samples excised from under the branch bark ridge were significantly denser than those excised from the adjacent stem. Overall, samples from the side of the bifurcation apex (n = 161) were 27.1% denser than the samples from the stem and the highest mean density was found at the apex of the normally-formed bifurcations.

	Mean wood density (± standard error) of extracted sample (Kgm^{-3}), by location		
Bifurcation type	**Apex**	**Side**	**Stem**
Normally-formed	644.9 ± 4.3 **A** *n* = 36	632.8 ± 5.8 **A** *n* = 36	493.0 ± 6.9 **C** *n* = 36
Bark Included	N/A	628.6 ± 5.3	490.9 ± 5.2

		AB $n = 57$	**C** $n = 57$
Newly-drilled	N/A	628.9 ± 5.8 **AB** $n = 30$	488.7 ± 7.6 **C** $n = 30$
Pre-drilled	N/A	595.0 ± 4.7 **B** $n = 19$	494.8 ± 12.2 **C** $n = 19$
Pre-split	N/A	614.6 ± 5.7 **AB** $n = 5$	503.2 ± 22.5 **C** $n = 5$
Braced	619.9 ± 8.3 **AB** $n = 14$	619.9 ± 11.0 **AB** $n = 14$	480.0 ± 6.7 **C** $n = 14$

Table 3: Wood density of samples taken from different bifurcation types tested, by location. Letters (A, AB, B and C) below the mean in each entry identify differences between these means across bifurcation type and location of xylem extraction, as identified by a GLM ANOVA with the sample volume as a covariate and post-hoc Tukey test at a 5% confidence limit ($F_{13,357}$ = 93.94; R^2 = 78.09%; p < 0.001). . Sample volume was not a significant factor in the differences found in wood density between groups (p = 0.509)

A significant difference in the wood density of normally-formed and braced bifurcations was also identified using a GLM ANOVA ($F_{3,\ 96}$ = 3.16; R^2 = 8.99%; p = 0.028) and post-hoc Tukey test (Fig. 11). The samples from the apices of the normally-formed bifurcations were 4% denser than the samples from the braced bifurcations.

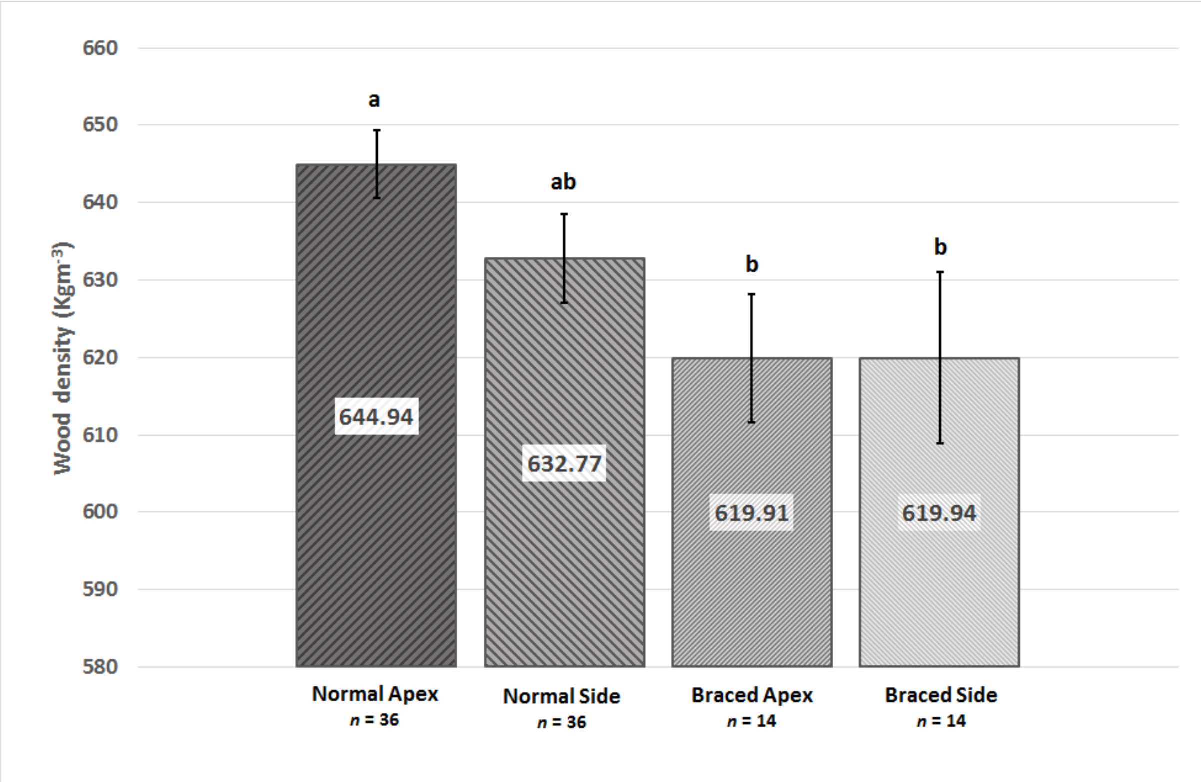

Figure 11: Mean wood density of samples excised from the apices and sides of normally-formed and braced bifurcations. Letters above bars identify differences between groups by using a GLM ANOVA and post-hoc Tukey test at a 5% confidence limit

DISCUSSION

This study has successfully identified the extent by which both the natural and experimentally-induced defects weakened these hazel bifurcations and that remodelling can potentially overcome these defects due to changes in growth probably caused by mechanical strain.

Bark-included bifurcations

The findings from this assessment of bark-included bifurcations support those of Slater and Ennos (2015), in that bifurcations with wide-mouthed bark inclusions were significantly weaker than those with a cup-shaped morphology and that overall the bark-included bifurcations had only 72.8% of the strength of the normally-formed bifurcations. Those bifurcations with embedded bark can be considered as ones that have remodelled successfully to occlude the bark which would otherwise have weakened them substantially.

Drilled and pre-drilled bifurcations

Interestingly, the bifurcations that were drilled at the point of testing were found to have the same mean breaking stress as the bark-included bifurcations. Both of these bifurcation types lack the interlocking wood grain pattern at the apex of the bifurcation, as found using CT scanning by Slater *et al.* (2014), the former type by having it drilled out, the latter type by failing to develop it sufficiently.

The pre-drilled bifurcations showed progressive recovery of their bending strength by remodelling around the initial drill holes created and the dysfunction in adjacent tissues (Fig. 6a; Fig. 9). The level of recovery varied substantially: among other factors, this may have been due to the different positions that these bifurcations had within the crowns of the hazel trees. Given the widely accepted principle of thigmomorphogenesis in plants and the evidence of atrophy in the braced bifurcations in this study, the bending moments experienced by these bifurcations when growing in-situ are likely to be linked to the extent of their remodelling around these drill holes; however, to verify this, such bifurcations would need to be the subject of a more detailed analysis using tilt meters or accelerometers to assess them for differences in movement under dynamic wind loading. This remodelling will be related both to the initial wounding and the subsequent additional mechanical strain under dynamic loading (Steucek and Kellogg, 1972).

Split bifurcations

It is clear from the observations of wind-induced failure in 80% of these modified bifurcations that their factor of safety was compromised by initially inducing the splits in their apices. The five bifurcations that remained intact had remodelled lower down the parent stem, after the propagating crack had been arrested by a major change in wood grain pattern and direction. This finding strongly suggests that the interlocking and denser wood at the apex of a hazel bifurcation is much-needed to prevent the initiation of cracks which would result in them splitting apart under the loading imposed by normal conditions. It should be noted that no other bifurcation type was observed to exhibit any wind-induced failures over the four year period of this experiment. This implies that the bark-included and drilled bifurcations in this study had a factor of safety high enough that they could persist under the loading conditions which resulted in 80% of the split bifurcations failing over three growing seasons.

Braced bifurcations

Despite the reduced number of replicates for this bifurcation type, the strength of the bifurcations and the wood density of the bifurcations' apices, when compared with normally-formed bifurcations, strongly suggests that the effect of the rod bracing was that these bifurcations atrophied in terms of their mechanical development. In contrast to the drilled bifurcations, the effect of putting in place a rigid brace will have prevented the braced bifurcations from experiencing mechanical strains at their apices.

The atrophying effect found was significant but could be argued not to be very substantial if the diameter of the smaller branch at the bifurcation apex was used to assess the breaking stress. This result implies that that mechanical loading is not the sole inducer of further sapwood developing in a given location: new layers of sapwood are needed for the provision of new tracheal elements through each component part of a tree's crown, even though some components may not experience substantial strains. However, for arboriculturists considering installing a rod brace in a tree, they should take into account the subsequent development of branches with adverse taper, the associated decline in the strength of the braced bifurcation and its increasing reliance upon the brace over time (Smiley *et al.*, 2000). In this study, if the braced bifurcations were required to support the arising branches once the brace was removed, then these bifurcations had only 70.5% of the strength of the normally-formed group and their factor of safety would have been substantially eroded.

Wood density

All the xylem formed under the branch bark ridge was substantially denser than that found in the adjacent parent stem, for all bifurcation types. A heightened wood density at the bifurcation is likely to result in a higher breaking stress for this component (Slater and Ennos, 2013), although it is only one factor amongst many that will affect the breaking stress of any given bifurcation. The mean density of the wood formed at the apices of the braced bifurcations was 4% less dense than the wood at the apex of the normally-formed bifurcations, suggesting that wood quality had atrophied in response to bracing.

Limitations of the study

It is important to acknowledge that this study is based upon data collected from semi-mature bifurcations in hazel trees, which gives rise to limitations in the scope of the subsequent findings. This study is part of a series that has examined bifurcations in this particular species to provide anatomical and mechanical models which could then be compared and contrasted to the bifurcations of other woody species by further study. The physiological pathways to this remodelling process were not examined as part of this study and could also be usefully examined in further research.

Conclusions

The denser xylem formed at the apex of bifurcations in hazel (and in other tree species) plays a key function in preventing failure at the junction (Slater and Ennos, 2013). Although the role of this modified xylem is important in supplying a higher bending strength, its absence does not necessarily result in bifurcation failure: connections formed either side of the bifurcation apex can clearly be adequate to give four years' longevity or more to the juvenile bifurcations tested in these semi-mature hazel trees.

From the pre-drilled bifurcations in this study, it is clear that they can satisfactorily remodel around an induced injury or defect and recover their full bending strength over time. This compliments the analysis of Slater and Ennos (2015) that remodelling around included bark can also fully recover the strength of bifurcations in hazel. This process of repair was not uniform amongst the bifurcations in this study, and further research could seek to find key factors that relate to the rate of repair of such bifurcations. In contrast, if the hazel bifurcation is split at its apex, although it has the potential to remodel, it is much more likely that it will fail completely under further wind-loading due to the initial crack propagating further down the stem. If a rod brace is installed above a hazel bifurcation, then development of the bifurcation will atrophy, identifying that thigmomorphogensis plays an important role in the mechanical development of bifurcations.

These findings help to measure the extent and degree of the remodelling of such bifurcations with different treatments, and could assist in determining a factor of safety for this component of a tree's crown. Further modelling needs to be extended beyond static rupture tests, to investigate the movement behaviour of bifurcations under dynamic wind loading, which is considered to be a key factor in the impetus for bifurcations to remodel after injury or occlude a naturally-occurring mechanical flaw, such as a bark-inclusion.

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REFERENCES

BADEL E, EWERS F W, COCHARD H and TELEWSKI F W (2015) Acclimation of mechanical and hydraulic functions in trees: impact of the thigmomorphogenetic process; *Frontiers in Plant Science* **6**, 266.

BIRO R L, HUNT E R, ERNER Y and JAFFE M J (1980) Thigmomorphogenesis: Changes in cell division and elongation in the internodes of mechanically perturbed or ethrel treated bean plants; *Annals of Botany* **45**, 655-664.

BRAAM J and DAVIS R W (1990) Rain-, wind- and touch-induced expression of calmodulin and calmodulin-related genes in *Arabidopsis*; *Cell* **60**, 357-364.

COUTAND, C (2010) Mechanosensing and thigmomorphogenesis, a physiological and biomechanical point of view; *Plant Science* **179**, 168-182.

FARNSWORTH K D and NIKLAS K J (1995) Theories of optimization, form and function in branching architecture in plants; *Functional Ecology* **9**, 355-363.

GARTNER, B L (1995) Patterns of xylem variation within a tree and their hydraulic and mechanical consequences; *In:* GARTNER G. L. (ed.) *Plant stems; physiological and functional morphology*; Academic Press, New York.

GILMAN E F (2003) Branch to stem diameter affects strength of attachment; *Journal of Arboriculture* 29, 291-294.

GRACE J (1977) *Plant Responses to Wind*; Academic Press, London.

HUGHES S W (2005) Archimedes revisited: a faster, better, cheaper method of accurately measuring the volume of small objects: *Physics Education* **40** (5), 468-474.

JAFFE M J and FORBES S (1993) Thigmomorphogenesis: the effect of mechanical perturbation on plants; *Plant Growth Regulation* **12**, 313-324.

JAFFE M J (1973) Thigmomorphogenesis: The response of plant growth and development to mechanical stimulation; *Planta* **114**, 143-157.

JUNGNIKL K, GOEBBELS J, BURGERT I and FRATZL P (2009) The role of material properties for the mechanical adaptation at branch junctions; *Trees: Structure and Function* **23,** 605-610.

KANE B, FARRELL R, ZEDAKER S M, LOFERSKI J R and SMITH D W (2008) Failure mode and prediction of the strength of branch attachments; *Arboriculture & Urban Forestry* **34**, 308-316.

LEV-YADUN S and ALONI R (1990) Vascular differentiation in branch junctions of trees: circular patterns and functional significance; *Trees: Structure and Function* **4**, 49-54.

LONSDALE D (1999) *The Principles of Tree Hazard Assessment and Management*; TSO, London.

MATTHECK C and LINNARD W (1998) *Design in Nature*; Springer, Berlin.

MONSHAUSEN G B and HASWELL E S (2013) A force of nature: molecular mechanisms of mechanoperception in plants; Journal of Experimental Botany 64, 4663-80.

PHILIPSON W, WARD J M and BUTTERFIELD B G (1971) *The vascular cambium: its development and activity;* Chapman & Hall Ltd., London.

PRUYN M L, EWERS B J III and TELEWSKI F W (2000) Thigmomorphogenesis: changes in the morphology and mechanical properties of two *Populus* hybrids in response to mechanical perturbation; *Tree Physiology* **20**, 535-540.

SHIGO A L (1991) *Modern Arboriculture*; Shigo and trees, associates, Durham New Hampshire US.

SLATER D and ENNOS A R (2013) Determining the mechanical properties of hazel forks by testing their component parts; *Trees: Structure and Function* **27** (6), 1515-1524

SLATER D, BRADLEY R S, WITHERS P J and ENNOS A R (2014) The anatomy and grain pattern in forks of hazel (*Corylus avellana* L.) and other tree species; *Trees: Structure and Function* **28** (5), 1437-1448.

SLATER D and ENNOS A R (2015) The level of occlusion of included bark affects the strength of bifurcations in hazel (*Corylus avellana* L.); *Journal of Arboriculture and Urban Forestry* **41** (4), 194-207.

SMILEY E T, GRECO C M and WILLIAMS J G (2000) Brace rods for co-dominant stems: Installation location and breaking strength; *Journal of Arboriculture* **26**, 170-176.

STEUCEK G L and KELLOGG R M (1972) The influence of a stem discontinuity on xylem development in Norway spruce (*Picea abies*); *Canadian Journal of Forest Research* **2**, 217-222.

TELEWSKI F W (2006) A unified hypothesis of mechanoperception in plants; *American Journal of Botany* **93**, 1466-76.

TELEWSKI F W (2012) Is windswept tree growth negative thigmotropism?; Plant Science 184, 20-28.

VINCENT J (2012) *Structural biomaterials*; 3rd Edition; New Jersey, USA, Princeton University Press.

WHITEHEAD F H (1963) Experimental studies of the effect of wind on plant growth and anatomy; *New Phytologist* **62**, 80-85.