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Trauma in the life of a *Nebuloxyla*, an Early Devonian basal euphyllophyte

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ABSTRACT. Anatomically preserved material from Lower Devonian strata of the Battery Point Formation (Gaspé Peninsula, Quebec, Canada) offers a rare opportunity to reconstruct the sequence of events in the life of a *Nebuloxyla mikmaqiana* plant (early euphyllophyte) that led to wounding and the plant's recovery after wounding. Using serial sections from cellulose acetate peels of the *Nebuloxyla* axis and volume renderings of the affected tissues, we show that the wound, centered around a branch and caused likely by an herbivore, resulted in removal of the extraxylary tissues of the branch starting from its very base, associated with bending of the xylem strand that supplied the branch. Downward bending of the branch xylem toward the base of the main axis led to the separation and displacement of a significant portion of it, leaving a short portion of the xylem strand at the base of the branch in its original position. The displaced strand of branch xylem, after stripping of the extraxylary tissues along with some of its own thickness, was left protruding outwards from the open wound surface. Subsequent development of wound response tissue filled the gap left by the wound around the protruding displaced xylem strand, surrounding it with wound periderm. This specimen provides evidence that plants as old as *Nebuloxyla* had the capacity to endure and survive traumatic events, and to deploy effective and sophisticated response mechanisms that had already evolved in the euphyllophyte clade by Emsian time. More broadly, this occurrence re-emphasizes the importance of permineralized fossils in documenting details of plant anatomy and development, even allowing glimpses into minutia of their daily life, such as interactions with their environment and immediate responses to these interactions.

KEYWORDS: anatomy, Devonian, early tracheophyte, euphyllophyte, fossil, trauma, wound response

INTRODUCTION

Nebuloxyla mikmaqiana Lalica et Tomescu (2023) is a small plant that was a component of Early Devonian floras whose diversity was an outcome of the first major tracheophyte radiation. The axes of *Nebuloxyla* are vascularized by a protostele consisting of tracheids with *Psilophyton*-type (P-type) wall thickenings (Lalica and Tomescu, 2023). P-type thickenings have only been documented to date in basal euphyllophytes (trimerophytes) and are considered plesiomophic in the group (Kenrick and Crane, 1997; Bickner and Tomescu, 2019). Their presence supports the placement of *Nebuloxyla* in Subdivision Euphyllophytina Kenrick et Crane (1997). Notably, in contrast to coeval euphyllophytes that have centrarch primary xylem maturation, the xylem of *Nebuloxyla* consists of mixed tracheids of variable size that lack a distinguishable pattern of maturation.

Another notable feature of *Nebuloxyla* is the capability of producing wound-response

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periderm (Lalica and Tomescu, 2023). The wounds documented in this plant are deeply concave, produced by removal of outer and inner cortex tissues. The wounds are filled with reaction tissue consisting of regular files of cells that represent periderm and indicate that the plant was alive at the time of wounding and actively responding to it.

Nebuloxyla represents one of the several occurrences of wound-response periderm reported from the Battery Point Formation, which represent the oldest records of periderm of any kind (Lalica and Tomescu, 2023). The other wound periderm occurrences in this rock unit have been reported by Banks (1981) and Banks and Colthart (1993) from trimerophyte specimens initially assigned to *Psilophyton* Dawson emend. Hueber et Banks (Hueber and Banks, 1967), some of which belong to the more recently described species *Kenrickia bivena* Toledo et Tomescu (Toledo et al., 2021).

Some of the wound periderm reported by Banks and Colthart (1993) is developed beneath the intact outer cortex, within inner cortex tissue. Banks and Colthart interpreted this type of periderm as a response to piercing-and-sucking herbivory based on the presence of small indentations – corresponding to penetration sites – on the surface of plant axes and of areas of lysed or hypertrophied cells in the outer cortex beneath the indentations. In other specimens, wound periderm proliferation fills the gap left in the tissues by removal of the epidermis, outer cortex and part of the inner cortex, similar to the occurrence documented in *Nebuloxyla* (Lalica and Tomescu, 2023). Banks and Colthart interpreted the wounds as arthropod surface feeding (chewing wounds), while also recognizing that abiotic causes could not be ruled out. Similarly, in *Nebuloxyla* Lalica and Tomescu (2023) interpreted the wounds as most likely produced by herbivores, due to their consistent concave geometry. The main line of reasoning supporting their interpretation was that arthropod feeding patterns form wounds of consistent shape, unlike the wounds produced by random events involving abiotic factors.

The largest wound documented in *Nebuloxyla* affects an area around a branching point in the axis and is associated with an intriguing pattern in vascularization that was not documented in the initial description (Lalica and Tomescu, 2023). Here, we document in detail the vascular anatomy in and around that wound area to make specific inferences on the dynamics of the event that produced the wound and the response of the living plant to this event.

MATERIAL AND METHODS

Nebuloxyla mikmaqiana is preserved by calcareous cellular permineralization in a sample collected in the 1960s by the late Dr Francis M. Hueber (Smithsonian Institution – US National Museum of Natural History) from the Battery Point Formation, on the southern shore of Gaspé Bay (Quebec, Canada) near Douglastown. The Battery Point Formation consists of rocks deposited in fluvial to coastal environments that host a broad diversity of plants. The age of this Gaspé flora is middle-to-late Emsian (McGregor, 1977; Hoffman and Tomescu, 2013), roughly 402–394 Ma (absolute age based on Cohen et al., 2013, updated 2020).

The material was studied in serial anatomical sections produced with the cellulose acetate peel technique (Joy et al., 1956) and mounted on slides with Eukitt (O. Kindler, Freiburg, Germany). The length of the axis was calculated by multiplying the number of acetate peels that section it by 22 µm (which is the average thickness of rock removed between two successive peels). Measurements of anatomical features were corrected for peel shrinkage using the coefficient of Long et al. (2022). The fossil was imaged using a Nikon Coolpix 8800VR digital camera mounted on a Nikon E400 compound microscope (Nikon, Tokyo, Japan), and an Olympus DP73 digital camera mounted on an Olympus SZX16 microscope (Olympus, Tokyo, Japan).

Images were processed using Adobe Photoshop (Adobe, San Jose, CA, USA). Each image was traced to highlight the position of the xylem, phloem and the external outline of the axis. The tracings were aligned manually using Adobe Photoshop and each tracing was saved as an independent jpg file. These files were uploaded to 3D Slicer (Fedorov et al., 2012) and the phloem, xylem and outline of the plant were segmented separately. Each of these segmentations was converted into a 3D model using 3D Slicer, which allowed for the 3D observation of the outline of the plant and its vascular system based on the sequential peels.

RESULTS

Below the branching point, the xylem strand of *Nebuloxyla* is terete, slightly elliptical in cross section as it widens in the plane of branching and is surrounded by a layer of phloem that is slightly thicker in the direction of branching (Fig. 1A). Above this level, a wound area filled with periderm (recognized by regular cell files) abuts the xylem strand, in the direction of branching (Figs 1B, C, 2, 3B). The cross-sectional profile of the xylem strand

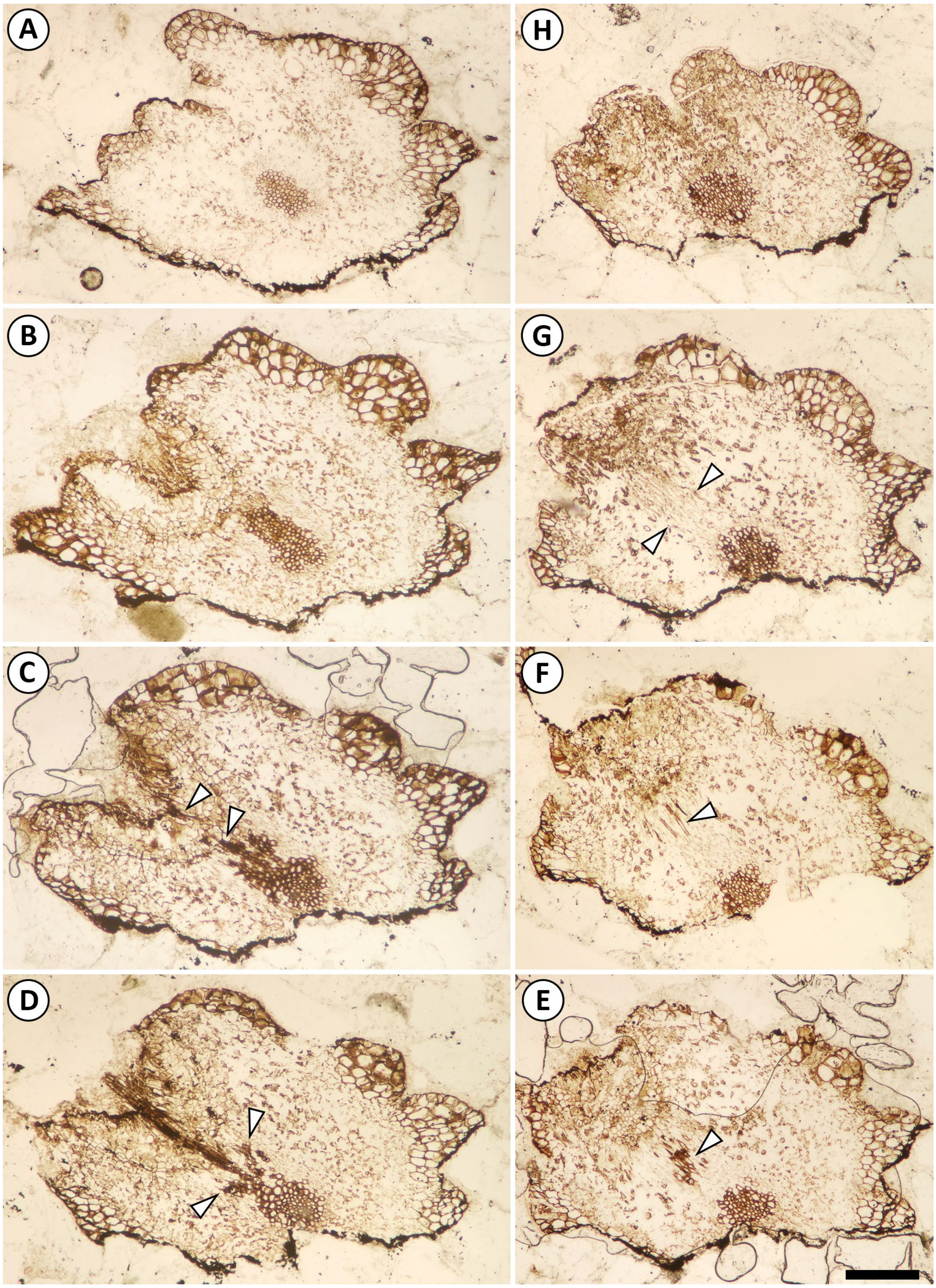

Figure 1. Selected serial transverse sections through *Nebuloxyla* axis (from bottom to top **A** to **H**). Radial elongation of xylem strand basal to branching point visible in **B** and **C**; section immediately basal to the separation of branch xylem from axis xylem in **D**, where the xylem of the branch is separated into a thin strand that is displaced and diverges from the axis horizontally, and the remaining branch xylem that is crescent-shaped (arrowheads; see also Fig. 2 for more serial sections); the thin strand of displaced xylem is partially visible in **C** (arrowheads); the remaining branch xylem left by the separation of the displaced xylem strand diverges from the axis xylem at a shallower angle, curves in a horizontal direction and tapers until it disappears (arrowheads in **E** and **F**); remaining phloem of the branch, oriented horizontally and parallel with the remaining branch xylem is visible in **F** (around the distalmost tracheids of the tapering remnants of the branch xylem, indicated by the arrowhead), and at a level above the tapered end of the remaining branch xylem in **G** (between arrowheads); see also Fig. 3 for a different representation of the same planes of section and Lalica and Tomescu (2023) for the extent and details of the wound response tissue that is present in B through G. Scale bar = 300 µm. From A to H, respectively: USNM 557790-1 Btop #108f, #86f, #70f, #68f, #52f, #48f, #44f, #24f

is progressively more elongated in the plane of branching (Figs 1B, C, 3B) and becomes constricted (Figs 1C, 3B), foreshadowing the two resulting xylem strands (Figs 1C, 2). The comparable size of the two strands, along with the interpretations presented here (see below) for the particular anatomical structures present at this branching point of the axis, are inconsistent with our initial interpretation of branching morphology in *Nebuloxyla* as anisotomous (Lalica and Tomescu, 2023). Consequently, we amend here our original description: branching in *Nebuloxyla* was roughly isotomous. However, for clarity of descriptions and discussions, we will refer to the xylem strand that is not affected by the wound (see below) as the main axis xylem, and to the one affected by the wound as the branch xylem. The phloem layer is truncated by the wound from the level at which the xylem strand becomes elongated in the plane of branching all the way to a point above the branching point (Figs 1B–G, 3B); see also Lalica and Tomescu (2023) for extent and details of the wound response tissue on the corresponding sections.

The architecture of the xylem strand that would have supplied the branch is heavily altered – as compared to the typical xylem architecture of a dichotomous branching point – in the area affected by the wound, in two ways (Figs 1C–F, 3). On one hand, starting below its point of divergence from the main axis and up to a plane just above that divergence, the branch xylem is missing more than half of its portion that faces toward the epidermis of the axis, so that it is approximately crescent-shaped in cross section (Figs 1D, 2, 3A at asterisks, 3B top right). The remaining portion of the strand (that is crescent-shaped proximally) tapers in the apical direction, all the way to disappearance, as it bends laterally and diverges away from the xylem strand of the main axis (Figs 1D–F, 2, 3A at arrowheads). Along the same axis segment where the crescent-shaped branch xylem tapers and bends laterally, the phloem surrounding it changes orientation, paralleling the orientation of the xylem (Fig. 1E–G). On the other hand, a thin strand of xylem, only about five tracheids thick, diverges from the incomplete xylem strand of the branch at a much wider angle, which is what produces the crescent-shaped profile of the latter. The divergence of this strand is almost perpendicular to the direction of the main axis and the strand is slightly bent downwards distally (Figs 1C, D, 3), between the level where the axis xylem becomes elongated immediately below the branching point (Fig. 1C) and the level where the xylem strand of the branch diverges from that of the main axis. This thin xylem strand extends outwards through the wound response tissues, unaccompanied by a corresponding complement of phloem, and is truncated ~540 μm beyond the surface of the wound (Figs 1D, 3).

DISCUSSION AND CONCLUSIONS

The anatomy described above is best interpreted as resulting from a wounding event that entailed a number of processes occurring concomitantly or sequentially (Fig. 4). (1) Removal by mechanical force of tissues that formed a concave cavity in the area around the branching point of the axis. The tissues removed went as deep as part of the phloem layer but did not affect the xylem of the main axis (Fig. 4A–E). (2) The removal process also bent the branch and its xylem in a basal direction (Fig. 4A–C). (3) This led to separation of the branch xylem along a longitudinal-oblique plane (Fig. 4B, C); the separation started on the adaxial side of the branch xylem in a point distal to the separation of the axis and branch xylem strands (Fig. 4B), and propagated more or less longitudinally, due to continued exertion of downward pull on the branch by the wounding agent, primarily along connections between the lateral walls of tracheids, toward the base of the strand, reaching below the level of separation between the axis and branch xylem (Fig. 4C); this process (i) left a small apically tapering portion of the branch xylem in its original position and (ii) displaced most of it laterally and downwards. (4) Tissues around (and possibly part of) the branch xylem were stripped away from around the downward displaced strand of branch xylem during tissue removal in the wounding process (Fig. 4D). This left a naked strand of branch xylem protruding outwards from the wound surface that was also truncated some distance from the wound surface (Fig. 4E). (5) The process of wound healing consisted of production of periderm (Lalica and Tomescu, 2023) which filled the gap left by the wound, around the protruding remnant of the branch xylem (Fig. 4F).

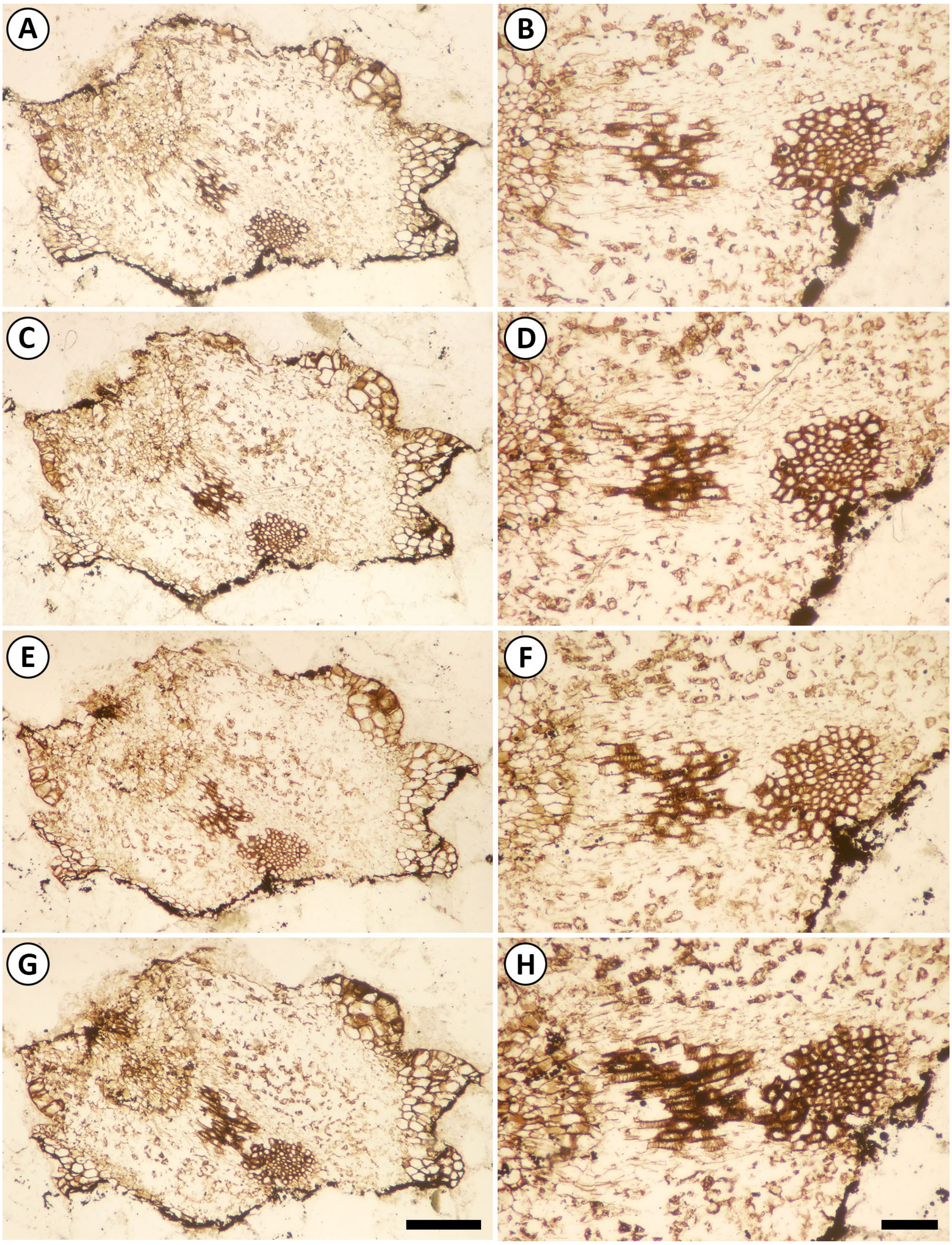

Figure 2. Serial transverse sections through *Nebuloxyla* axis (**A**, **C**, **E**, **G**) and details of their xylem and phloem (**B**, **D**, **F**, **H**). The sections, basalmost at the bottom (**G** and **H**) to distalmost at top (**A** and **C**), detail the anatomy of the xylem between the sections in Fig. 1D and 1E and show more clearly (higher magnification) the phloem layer that surrounds the xylem. The serial sections show the progressive distal curving of the branch xylem and phloem from closer to vertical proximally (**G** and **H**) to closer to horizontal distally (**A** and **C**). Scale bar = 300 µm in A, C, E, G, and 100 µm in B, D, F, H. From A to G and B to H, respectively: USNM 557790-1 Btop #53f, #54f, #56f, #59f

There are two possible alternative interpretations of the xylem architecture we describe here. One is that the two xylem strands that diverge at the same level from the main axis could represent a natural configuration of a new type not previously encountered in Early Devonian plants. In studying this *Nebuloxyla* specimen we were initially excited by this possibility, due to its close similarity with axillary branching, a feature known only

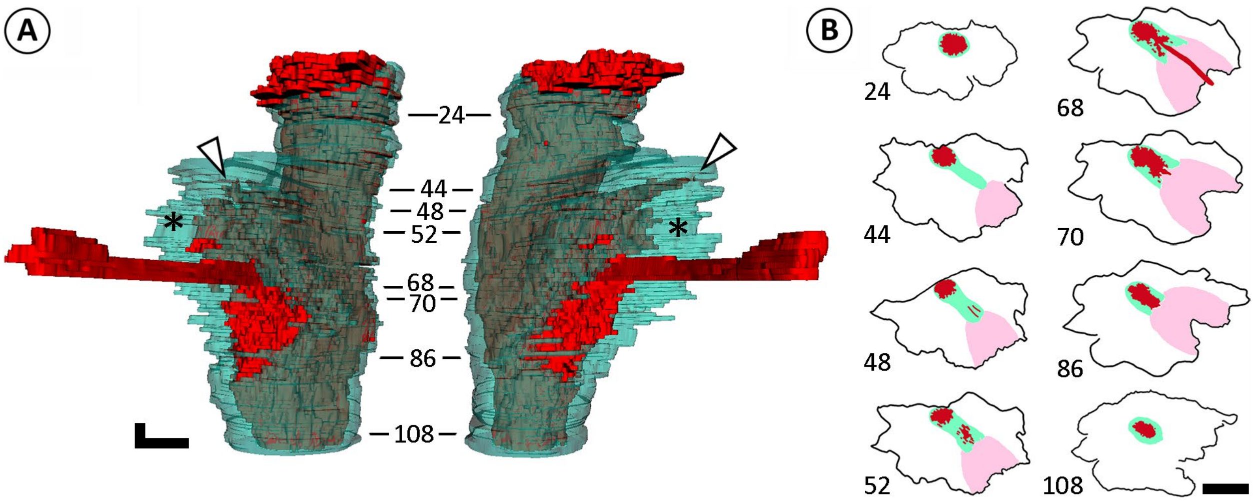

Figure 3. **A**. Two views of a volume rendering of the xylem (red) and phloem (green) of *Nebuloxyla* in the wound area; note that the portions of the xylem that are covered by the phloem layer appear dark brown-gray under the translucent green phloem and only the areas of xylem that are not covered by phloem (such as those that are directly in contact with wound response tissues) are colored red. The thin displaced xylem strand unaccompanied by phloem protrudes toward the viewer; the crescent-shaped remnants of the branch xylem left by the separation of the thin strand are in the area marked by asterisks; the tapering tip of the remaining branch xylem, surrounded by branch phloem, is indicated by the arrowheads; the section numbers, corresponding to those of the transverse sections in Figs 1 and 3B, are shown at their exact locations of those sections along the axis. Scale bars (horizontal and vertical) = 150 µm. (the upper area of xylem not covered by phloem – in red – corresponds to other wounds that are not discussed here); **B**. Tracings of the transverse sections marked in Fig. 3A and depicted in Fig. 1 showing the xylem (red), phloem (green), and wound response tissue (pink); the tracings have the same orientation as the volume rendering at right in Fig. 3A and are rotated 180 degrees compared to their orientation in Fig. 1. Scale bar = 0.5 mm

in representatives of the seed plant lineages that are much younger than *Nebuloxyla*. The second alternative interpretation is that we are looking at another possible natural configuration – a trace to a lateral that divides in a radial plane to form two strands, proximal to entering the base of the lateral. When considering these alternative interpretations, detailed examination of our specimen led us to reject them: both these interpretations are inconsistent with the strand of xylem that tapers in an apical direction all the way to disappearing, without exiting the cortex of the main axis. Instead, all the anatomical features of the *Nebuloxyla* specimen provide evidence that converges toward the interpretation we offered above. The geometry of the vascular tissues above and below the wound area provides unequivocal evidence that the wound is centered around a branching point (Figs 1, 3B). The xylem strand of the axis clearly divides to produce two strands, both of which have a complement of surrounding phloem (or whatever is left of it). The wounding (Fig. 4A) was most probably inflicted by an herbivore, as argued by (Lalica and Tomescu, 2023). The orientation of the strand of branch xylem that crosses the wound response tissue at a sharp angle from both the xylem of the axis and the remnants of branch xylem is evidence for bending of the branch immediately prior to or during tissue removal (Fig. 4B–D). The downward separation of the branch xylem along an oblique-longitudinal plane due to its bending (Fig. 4C, D) is not surprising given that lateral connections between tracheids are surfaces of minimum resistance in the structure of xylem strands. The tapering end of the branch xylem left in its original position reflects the original angle of branching. The almost horizontal orientation of the tracheids in the most distal portion of these xylem remnants (Figs 1F, 4C–F) indicates that the typical architecture of branch divergence from the axis involved arching laterally and outwards. This is also confirmed by the almost horizontal orientation of the remaining phloem around those xylem remnants (Fig. 1E–G). The fact that the bent strand of branch xylem that goes across the wound response tissues has no other regular tissues around it (Figs. 1D, 3) demonstrates that such tissues were stripped from around it (Fig. 4D–E), probably during the wounding process. Finally, the development of significant wound response tissue that filled the gap left by the wound and immobilized the protruding bent remnants of the branch xylem in its displaced position (Figs 1D, 3B at #68, 4F) is consistent with the growth responses of a living plant.

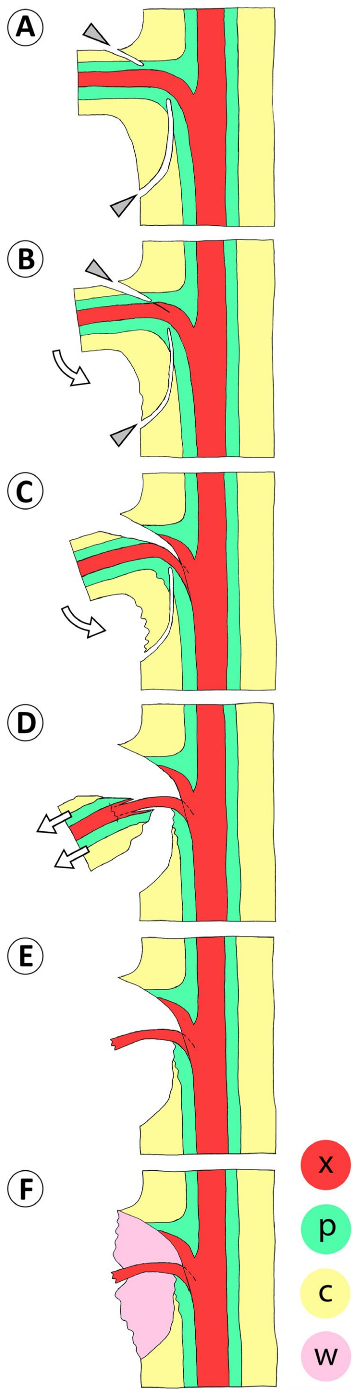

Figure 4. A time-lapse rendition (**A** through **F**) of the traumatic event that affected the *Nebuloxyla* axis and its aftermath. **A**. Wounding agent (probably an herbivore) starts severing the branch at its base (arrowheads); **B**. Branch severing (arrowheads) is also associated with a downward pull (arrow) bending the branch and inducing tearing between tracheids close to the base of the branch, on the top side of the branch xylem; **C**. Continued downward bending (arrow) leads to separation of a displaced strand of xylem from undisturbed, apically tapering remnants of the branch xylem; **D**. Outward pull on the severed branch tissues by the wounding agent (arrows) strips extraxylary tissues, along with some tracheids, off the displaced strand of branch xylem, which is severed at a level above the surface of the wound; **E**. The remnants of the displaced strand of branch xylem are left exposed and protruding outwards from the wound area; **F**. Wound response builds periderm that fills the gap left by the wound around the protruding strand of branch xylem. x = xylem, p = phloem, c = cortex, w = wound response tissue

It is rare to be able to piece together in detail an individual event in the life of a plant that lived in the past – even more so in the case of a punctual event (and its aftermath) that occurred in the life of a plant that lived 400 million years ago. *Nebuloxyla* demonstrates that even some of the smallest early tracheophytes were resilient and capable of recovery after trauma that affected tissues deep inside their axes. This is possible due to the good preservation of anatomy in the specimen documented here and re-emphasizes the value and importance of permineralized fossils in understanding the evolution of plant development.

The abundance of fossil specimens preserving wound responses in the Battery Point Formation indicates that plants of the Emsian communities experienced frequent traumatic events during their lifespans and had evolved mechanisms for responding to such events. This is corroborated by evidence for even older wound responses in the Pragian plants that are preserved in the Rhynie chert (Kidston and Lang, 1921; Kevan et al., 1975). The traumatic factors acting on plants during the Early Devonian were probably as diverse as those that affect the plants in modern ecosystems, comprising both abiotic and biotic agents. In the case of *Nebuloxyla* and several other Battery Point Formation plants, the evidence available points to biotic factors – herbivores – as the most likely causes of trauma (Banks and Colthart, 1993; Lalica and Tomescu, 2023). While the specific nature of the wound perpetrator in this *Nebuloxyla* plant remains shrouded in mystery, the frequency of wounds in the plant communities represented in the Battery Point Formation fossil assemblage indicates a level of intensity of plant-animal interactions in the Early Devonian that had not been gleaned previously.

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ADDITIONAL INFORMATION

CONFLICT OF INTEREST. None declared.

ETHICAL STATEMENT. None necessary.

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