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Wound reactions in stems of *Leonurus cardiaca*: a morphological, anatomical, and biomechanical study

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Short title: Wound reactions of *Leonurus cardiaca*

Abstract

During biological evolution, plants have evolved various types of wound reactions. The present study focuses on the self-healing of plant fibers in the model plant *Leonurus cardiaca*. In one of the square-shaped internodes of each plant, all four prominent strands of the collenchyma were damaged by a razor blade with a central incision in a longitudinal direction. After 20 to 24 days, the healed internodes were compared with undamaged controls with regard to morphology, anatomy, and biomechanics. Stem height increased highly significantly in healed and control plants, although the healed internodes were significantly shorter than the respective control internodes. Light microscopic examination of the wound region revealed permanent gaping of the wound and the formation of a distinct boundary layer. Four-point-bending tests showed a significantly lower flexural rigidity (self-healing efficiency: 44 %) and a highly significantly lower bending elastic modulus (self-healing efficiency: 66 %) of healed internodes compared with controls, although the axial second moments of area did not differ significantly (self-healing efficiency: 82 %). After a healing period of three weeks, recovery of morphological-anatomical characteristics and mechanical properties of healed internodes was incomplete compared with undamaged control samples. Nevertheless, the self-healing efficiency seemed to be sufficient to ensure the integrity of the stem during the entire growth period.

Keywords: plant fibers, collenchyma, self-healing efficiency, wound healing, boundary layer, four-point-bending test

Résumé : Durant l'évolution, les plantes ont développé plusieurs réactions de cicatrisations. La présente étude se focalise sur l'autoréparation des fibres végétales en utilisant la plante modèle *Leonurus cardiaca*. Pour chaque échantillon, un seul des entrenœuds de forme carrée fut incisé sur les quatre côtes de collenchyme de manière longitudinale avec une incision centrale par une lame de rasoir. Après 20 à 24 jours, les entrenœuds cicatrisés furent comparés aux plantes contrôles non blessées sur les plans morphologique, anatomique et biomécanique. La hauteur des tiges augmenta de manière très significative chez les plantes cicatrisées et les plantes contrôles, tandis que la longueur des entrenœuds cicatrisés fut significativement plus courte que celle des entrenœuds des plantes contrôles. Les analyses de microscopie des régions cicatrisées ont révélé une ouverture permanente de la blessure avec la formation d'un tissu cicatriciel bien distinct. Les tests de flexion quatre points ont montré une rigidité en flexion significativement diminuée (efficacité auto-réparatrice : 44 %) et un module élastique en flexion très significativement diminué (efficacité auto-réparatrice : 66 %) des entrenœuds cicatrisés comparés aux entrenœuds contrôles. Le second moment d'inertie ne montra pas de changement significatif (efficacité auto-réparatrice : 82 %). Après une période de cicatrisation de trois semaines, aucun rétablissement complet des caractéristiques morphologiques, anatomiques et des propriétés mécaniques des entre-nœuds cicatrisés n'a eu lieu comparé aux plantes contrôles. Cependant, l'efficacité d'autoréparation a été suffisante pour assurer l'intégrité de la tige durant toute la période de croissance.

Mots-clés : Fibres végétales, collenchyme, efficacité d'autoréparation, cicatrisation, tissu cicatriciel, test de flexion quatre points.

Introduction

During biological evolution, plants have evolved an amazing capacity to deal with various types of damage. Since plants can be considered as fiber-reinforced composite materials consisting of matrices (e.g., parenchyma, chlorenchyma) and reinforcements (e.g., vascular bundles, collenchyma, sclerenchyma), we can assume that the individual tissues also show diverse types of sealing and healing mechanisms. Comparative studies of artificially injured plant organs have revealed various wound reactions that can be subdivided into a fast self-sealing phase followed by a markedly slower self-healing phase, both being included under the umbrella term of self-repair (Harrington et al. 2015). Mechanical damage is immediately followed mainly by physical reactions causing rapid self-sealing that include: (i) tissue deformation in the wounded region (Anandan et al. 2018; Speck et al. 2018) and (ii) discharge of mucilage (Zimmermann and Granata 2002; Anandan et al. 2018). During the subsequent self-healing phase, chemical reactions and more complex biological responses dominate such as: (iii) coagulation of latex (Bauer and Speck 2012), (iv) local lignification of tissues (Paul-Victor et al. 2017), (v) formation of a (ligno-suberized) boundary layer (Ritteringer et al. 1987; Evert 2006), and (vi) development of a wound periderm (Evert 2006). All of the aforementioned mechanisms contribute at least partially to the modification of anatomical structures and the recovery of mechanical properties in the course of self-repair.

Whether individual fibers or fiber bundles can heal after damage has not yet been investigated in plants. With regard to this scientific question, stems of *Leonurus cardiaca* are particularly suitable plant models, since the square-shaped hollow internodes of Lamiaceae have four pronounced strands of collenchyma fibers in a peripheral position at the corners (Leroux 2012; Kaminski et al. 2017). The ultimate aim of the present study has been an analysis of healing approximately three weeks after longitudinal damage of collenchyma fibers at the four edges of one internode compared with undamaged control plants. Three main aspects

were addressed in this comparative study: (1) morphometric characteristics of entire plants and individual internodes, (2) anatomical changes in the wound region compared to intact internodes, and (3) the self-healing efficiency of bending rigidity in the wounded internodes.

Materials and methods

Overall experimental design

Morphological, anatomical, and mechanical characteristics of a control group and an intervention group “healed” were compared, whereby the intervention consisted of the mechanical damage of the samples and a healing period of 20 to 24 days. The control group did not receive this intervention. Preliminary tests and investigations on other plants have shown that wound healing is completed after about 3 weeks (Paul-Victor et al. 2017; Anandan et al. 2018).

Test plants

Motherwort (*Leonurus cardiaca* L.) is a perennial subshrub of the Lamiaceae family. Test plants were bought from local garden centers and cultivated in pots outdoors in the Botanic Garden of the University of Freiburg, Germany. Only stems with a height of more than 20 cm and/or five or more internodes were included in the experiment. The assignment of pot and treatment (no intervention / intervention) was performed by using the randomization function of Excel 2010 (Microsoft Corporation). Four to eleven stems per pot were included in the analyses and underwent the same treatment.

Mechanical damage

With the help of a razor blade (Derby Extra), a 3.6 cm long and approx. 400 µm deep central incision in the longitudinal direction was made on all four edges of the fifth internode (counted from the apex). If this internode was too short (less than 4.2 cm), the adjacent fourth

internode was damaged instead. A specially designed holder (Technical Research Group Biology II, University of Freiburg) that made it possible to determine the width of the protruding blade was used to standardize the cutting depth and to ensure that mainly the collenchyma was cut.

Four-point-bending test

Four-point-bending tests were performed as shown in Fig. 1 on internodes from the control group and from the intervention group “healed” at 20 to 24 days after the day of damage. Flexural rigidity was measured by using a custom-made bending apparatus consisting of a frame to which two cylindrical steel supports for the sample with variable distance L were attached (Rowe and Speck 1996; Rowe et al. 2006). The sample supports were always located within the nodes defining the tested internode, and the supports of the mass holder were placed outside the nodes. In addition, the samples were placed in such a way that the sample supports did not touch the area of damage. The distance a between the support of the mass holder and the specimen holder was chosen so that it was, on average, one third of L . Preliminary tests had shown that a ratio of the distance L to the edge length of the sample of 20 to 40 was reasonable for experimental testing to minimize shear influence. In the main experiment, a median aspect ratio of 22 was chosen. A force F was applied manually by the addition of weights (m) in cumulative steps. At one minute after a weight was applied to the sample, its deflection was measured by means of the measuring eyepiece of a dissecting microscope (Leitz Wetzlar 12.5 x). Each square-shaped internode was measured in two orthogonal directions (A and B) with respect to the cross-section, by turning the sample by 90° after the first measurement. If a stable positioning of the sample was only possible in one direction, the measurement was not repeated in the second direction. In order to prevent the samples from drying out, they were cut immediately before the bending test, and both sample ends were immersed in liquid paraffin.

With the help of the software ImageJ (version 1.47) and the BoneJ plug-in (version 1.3.10) (Doubé et al. 2010), the axial second moment of area was calculated from microscopic images of the entire cross-sections of the internodes. Since the alignment of the cross-sections could not be clearly assigned to the bending directions A and B, the axial second moments of area of all four theoretically possible alignments were first calculated, and then the arithmetic mean of these values (I_m) was used for the subsequent calculations.

The mean flexural rigidity of each sample could be calculated by equation (1) (Rowe et al. 2006):

$$(1) \quad EI_m (Nm^2) = \frac{1}{2} \left(\frac{L^2 a}{16b_A} + \frac{L^2 a}{16b_B} \right)$$

where E (MPa) is the bending elastic modulus, I_m (m⁴) is the mean axial second moment of area (calculated as described above), L (m) is the distance between the centers of the sample holders, a (m) is the distance between the centers of the sample holder and the mass holder, and b_A (m/N) and b_B (m/N) are the slopes of the displacement-force curves of the measurements in directions A and B, respectively.

Self-healing efficiency

The geometrical data (e.g., axial second moment of area) and mechanical properties (e.g., elastic bending modulus and flexural rigidity) of virgin (= undamaged control) and healed samples can be used to calculate a dimensionless self-healing efficiency η expressing the percentage recovery of the respective characteristic according to equation (2) (Lucas et al. 2016; Cohades et al. 2018):

$$(2) \quad \eta (\%) = \frac{\text{healed characteristic}}{\text{virgin characteristic}} * 100$$

Morphometric measurements

In addition to the mechanical test, morphometric measurements were performed on all samples at the start of the study (one day before the damage or on the day of the damage, but immediately before wounding of the internodes belonging to the intervention group “healed”) and at the end of the experiment after 20 to 24 days. The following morphological parameters were determined in the test plants and internodes: height and number of internodes of the stem, length, and sequential number of the examined internode (counted from the apex). Height and length were measured with a ruler. Raw data, means $\pm$ standard deviations, medians and interquartile ranges, minimum and maximum values, and numbers of samples are given in supplementary Table S1.

Anatomical studies

Segments of the undamaged and healed internodes were placed in distilled water and stored in the refrigerator immediately after the bending test was performed. Within one week, hand cuts of all samples were made by using a razor blade (Derby Extra). The sections were first bleached for 7 minutes in 20 % Eau de Javel (Floreal Haagen GmbH), then stained for 1 minute with fuchsin-chrysoidine-astra blue (FCA according to Mulisch and Welsch 2010), rinsed with distilled water, and finally mounted in distilled water. Lignified cell walls appeared in various shades of red from yellow-red (xylem) to purple (sclerenchyma), cutinized cell walls were highlighted in yellow, and non-lignified cells appeared in blue (e.g., collenchyma). In addition, fresh cross-sections were made by using the hand-held microtome MT.5503 (EUROMEX Microscopes, Holland) and stained with 3%w/v aqueous Acridine Orange (ACO), a fluorescence dye highlighting lignified tissues in bright yellow. Cross-sections stained with FCA or ACO were examined by means of a light microscope (Olympus BX61, Olympus Corporation, Tokyo, Japan) and photographed with a DP71 camera module by using CellP imaging software (Version 2.8, Olympus Soft Imaging Solutions GmbH). Other fresh sections were stained with phloroglucinol–hydrochloric acid (PHA) (5 g

phloroglucin dissolved in 100 ml of 92% ethanol) for 5 min after having been bleached for 7 minutes in 20 % Eau de Javel (Floreal Haagen GmbH) to discriminate between non-lignified and lignified tissues, the latter being stained red. These sections were photographed with a single lens reflex (SLR) (Nikon D 50, lens: 60 mm).

Statistical analysis

Calculations and diagram creation based on raw data were performed in Excel 2007 (Microsoft, Redmond, Washington, USA). Statistical analyses and box plots were carried out with Gnu R 2.15.2 (R Core Team, 2012). Unless otherwise specified, data are given as medians and interquartile ranges (IQR) or means $\pm$ standard deviations. Data were tested for normal distribution by use of the Gnu R Shapiro test and for homogeneity of variances by the Gnu R F-test. For comparison of paired data sets, the Gnu R paired Wilcoxon-test was used when the data were not normally distributed. The paired t-test function was used if the data were normally distributed. For a comparison of unpaired data sets, either the unpaired Wilcoxon-test (data were not normally distributed) or the unpaired t-test (data were normally distributed and variances were equal) was used. Levels of significance were $p > 0.05$: not significant (n.s.); $p \leq 0.05$: significant (*); $p \leq 0.01$: very significant (**); $p \leq 0.001$: highly significant (***)

Results

Morphometric data

The macroscopic examination of the healed internodes on the day of the bending tests showed an opening of the edges in the wound area, whereas such an opening was not visible in the controls (Fig. 2). A buckling or collapsing of the stems at the healed internodes was never observed.

At the beginning of the experiments, no significant differences were noted in the height of stems, number of internodes, or length of internodes between the samples of the control group and the samples of the intervention group “healed”. However, with the exception of the length of the healed internodes, which only increased by 0.1 %, the above-mentioned morphometric data of both the control samples and the healed samples increased very or highly significantly from the day of damage to the day of the bending test (Tab. A1). Figure 3 shows comparative boxplot and whisker diagrams displaying the height of stems (Fig. 3A) and the length of the examined internodes (Fig. 3B) and comparing control and healed samples at the day of damage (start) and the day of the bending test (end). Because of continued growth, the number of internodes of the control plants increased highly significantly from 10 to 12, and that of the healed plants very significantly from 9 to 11 (Tab. A1).

Anatomical studies

Intact cross-sections of *L. cardiaca* showed the typical bauplan (= basic body plan of plants; Drost et al. 2017) of Lamiaceae (Fig. 4A-B). The square hollow internodes consisted of prominent non-lignified collenchyma strands at the four corners and large areas of vascular tissue that lay directly underneath and that were connected at the periphery by a continuous ring of lignified tissue with directly adjacent parenchyma (Kaminski et al. 2018).

Approximately three weeks after the longitudinal incisions had been made in the collenchyma strands, the wounds were still gaping, and the strands remained split (Fig. 4C-E). In the wound region, the formation of a boundary layer of lipophilic compounds was observed in and/or on the cell walls (Fig. 4E). The slight red coloration, which could be seen in some places, was probably attributable to the sclerenchyma cells that were also seen in undamaged internodes in the area near the edges. In the wound region, we found local reinforcement in terms of additional impregnation and lignification of the boundary layer.

Biomechanics

Figure 5 presents the results of the four-point-bending tests carried out with control and healed internodes. Compared with the control samples, the flexural rigidity (Fig. 5A) of the healed internodes was restored to 44 % on average and was significantly lower ($p < 0.05$). The healed incisions on the four edges of the square cross-section reduced the average axial second moment of area (Fig. 5B) to 82 % of the controls. However, the statistical test showed no significant differences as to the axial second moments of area ($p > 0.05$). The bending elastic modulus (Fig. 5C) of the healed internodes was highly significantly lower than that of the undamaged controls ($p < 0.001$). The bending elastic modulus of the healed internodes was restored to an average of 66 % compared with the intact samples. More information can be found in Tab. A2.

Discussion

Since the experiments were carried out in May and June 2013, the *Leonurus* plants had formed several leaves. Nevertheless, all stems continued to grow, built new internodes, and formed flowers and fruits in the apex area in autumn.

Because collenchyma is well-known as the main supporting tissue of growing plant organs undergoing primary growth (Leroux 2012), investigations into the effects of previously injured and then healed collenchyma strands are of great interest. In the case of Lamiaceae, these strands contribute significantly to the second moment of area, because of their peripheral position, and thus to flexural rigidity (Kaminski et al. 2017). Although a variety of test protocols are possible with regard to the direction of the damage (longitudinal, transversal), the number of damaged fiber bundles (one to four) and all resulting combinations, the four strands in this case were cut longitudinally, which is the equivalent to a splitting of the strands with a large number of collenchyma fibers not being damaged. Nevertheless, the longitudinal incisions of all four fiber bundles is a major intervention in the integrity of the internode and thus also of the plant and, additionally, gives us the possibility

to observe different wound reactions at different “corners” of one and the same sample. The results show that the intact control plants and the test plants with one healed internode do not differ significantly in their height growth and in the formation of new internodes during the test period of approximately 3 weeks. Only the length growth of the internodes with the split collenchyma strands was significantly lower, as they had in fact stopped growing after injury. Strikingly, the collenchymatous edges were permanently gaping as a result of the incision. This could be an indication for pre-stresses in the epidermis, the cortex parenchyma, and the intact collenchyma fiber strands. The main driving force for the separation of the collenchyma fiber bundles is probably the pre-tension of the epidermis in the circumferential direction pulling the two halves of the fiber bundle apart. In addition, these processes may generally be supported by the dehydration of the individual tissues and, in particular, by the decrease of turgor in the cortical parenchyma that was pre-stressed under compression in the intact structure. Although the axial second moment of area of the healed cross-sections is on average 18 % smaller than those of the intact cross-sections, they do not differ significantly.

Despite the intended standardization of the incisions with a special blade holder, the investigation of the cross-sections showed that the cut could not always be centered perfectly within the collenchyma strand, and that the depth of cuts varied. In some cases, the vascular tissues were partially incised in addition to the fiber bundles. This leads to different wound patterns, as the individual tissues obviously differentiate differently. Directly adjacent to the wounded collenchyma fibers and the cells of the cortex (including several sclerenchyma cells), a boundary layer was formed consisting of cells that stained brown and red with FCA and PHA. Further staining to clarify the chemical substances that caused the brown coloration did not produce any clear result. Thus, the brown color might be caused by impregnation with tannins, because evidence of lignin as tested by PHA and ACO was negative. Additionally, the presence of suberin as tested by Sudan IV or Sudan III could not be established. The red-

stained cell layer within the boundary layer seems to consist of cells with lignified cell walls, as has been verified by PHA and FCA and is supported by the yellow coloration after staining with ACO. These cells may represent the sclerenchyma cells of the cortex, and/or the staining might be a consequence of the deposition of lignin in the previously non-lignified cell walls of the parenchyma cells from the cortex and of collenchyma fibers.

Interestingly, no boundary layer is found when the cut exposes the xylem. This indicates that the formation of the boundary layer requires the presence of living divisible and physiologically active cells. These cells could have arisen by the transdifferentiation of the parenchyma and collenchyma cells adjacent to the wound. This assumption is supported by Leroux (2012) who has suggested that collenchyma cells have the ability to transdifferentiate and therefore are capable of undergoing wound healing or of initiating periderm formation and, thus, tissue regeneration. Even though the deposition of lipophilic substances on and in the cell walls in the wound area might contribute to increasing stiffness (Paul-Victor et al. 2017), the main task of a boundary layer is to prevent the intact tissues from dehydration and to serve as a protection against pathogens (Rittinger et al. 1987; Evert 2006).

In contrast to the axial second moment of area, which does not change significantly following the healed incisions of the collenchyma strands, the values for the elastic modulus are highly significantly lower by a factor of 0.66 compared with the intact internodes. Furthermore, within three weeks of healing, the flexural rigidity is restored to 44 % compared with the controls. Despite this significant difference, no buckling of the damaged internodes has been observed during the later formation of flowers and fruits, which corresponds to an additional top load. This indicates that the self-healing efficiency of young plants (before flowering) is sufficient to prevent the stems from collapsing, even if parts of the main supporting collenchyma fibers are damaged by longitudinal wounding. Kaminski et al. (2017) have shown that the formation of flowers and fruits leads to ontogenetic adaptive changes in

anatomy and mechanics. From June before flowering to September after fruit formation, the area ratio of the vascular tissue to the collenchyma continuously increases to become finally higher by a factor of approximately 2, which means that the bending rigidity in later stages is mainly carried by the lignified strengthening tissue. Furthermore, analyses by Kaminski et al. (2018) indicate that the area sum of the vascular tissues ($\alpha = 1.098$) and of the parenchyma ($\alpha = 1.071$) have moderate positive allometric scaling (i.e., a proportionally larger increase), whereas the area sum of collenchyma fibers is characterized by clear negative allometric scaling ($\alpha = 0.721$) (i.e., growth at a slower rate than the other tissues of the internode). This can be interpreted, at least partially, as a transition of the main stabilizing function during ontogeny from (stretchable) collenchyma fibers that are predominant in the young, still longitudinally, growing internodes to (non-stretchable) vascular tissues (e.g., sclerenchyma fibers, tracheids, vessels) at older ontogenetic stages, if no (or very limited) length growth takes place, and if additional top loads have to be carried. This, together with the (almost) cessation of growth in the length of the healed internodes, might explain the lack of stem failure after the healing of severe damage in the longitudinal direction. In a further test series, we plan to study the way that transverse cuts placed at the four corners of the stems of *L. cardiaca* are healed and their influence on the mechanical properties of healed stems.

Conclusion

The starting point of this project was the scientific question as to whether damaged plant fibers can be healed. In this context, the stems of *L. cardiaca* with their pronounced strands of collenchyma fibers are particularly suitable models for the investigation of this question. The results show that compared with the undamaged samples of the control group, longitudinally damaged collenchyma fibers cannot fully heal, and that therefore a complete recovery of the structural integrity and mechanical properties of the internode and the stem cannot be

achieved. However, the self-healing efficiency is sufficient to prevent the stem from buckling or collapsing in the wound area.

These results are all the more interesting because this question also arises with regard to technical fiber composite materials, and because engineers are searching for solutions in which damaged fibers/fiber bundles show self-repairing functions. On the basis of all the investigations carried out to date on the self-repair of plants, no biological solutions for the repair of technical fibers in fiber-reinforced composites can be transferred, as the fibers cannot be fully healed, even in plants. Rather, the loss of mechanical performance is compensated by the modification of the parenchyma (= matrix), for example by lignification of the existing tissue or the formation of callus tissue in the wound area.

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Figure captions

Fig. 1. Experimental set-up of four-point-bending tests. Each internode was measured in two directions by turning the sample by 90° after the first measurement.

Fig. 2. Control internode (A) and healed internode (B) of *Leonurus cardiaca* approximately three weeks after wounding. i: internode, n: node, ue: undamaged edge, he: healed edge. Scale bars equal 1 cm.

Fig. 3. Statistical analyses of morphometric data. Boxplot and whisker diagrams show (A) the height of the stems and (B) the length of the examined internodes as a function of control samples (gray boxes) and healed samples (white boxes) at the day of damage (start) and the day of the bending test (end). There were 20 to 24 days between the start and the end points. Different letters (a, b, c) indicate significant differences.

Fig. 4. Cross-sections of *Leonurus cardiaca* internodes. (A) Overview and (B) detail of an undamaged corner of a control sample. (C, E) Overview of a healed sample wounded at the four corners in the longitudinal direction and (D, F) detail of one healed corner. Staining: (A-D) FCA, (E) PHA, (F) ACO. B: boundary layer, C: collenchyma, Ct: cortex, E: epidermis, P: pith cavity, Pa: parenchyma, Ph: phloem, S: sclerenchyma, T: trichome, W: wound region, X: xylem.

Fig. 5. Statistical analyses of data from the mechanical tests. Boxplot and whisker diagrams show (A) flexural rigidity, (B) axial second moment of area, and (C) bending elastic modulus as a function of control samples (gray boxes) and healed samples (white boxes). Significant differences are indicated. Control samples $n = 19$, healed samples: $n = 16$.

Table captions*Appendix A1 Table. Morphometric data and statistics*

Tab. A1. Morphometric data given as medians and interquartile ranges (IQR). The statistical analyses refer to the differences between the data determined at the day of damage (start) and at the day of the bending test (end). There were 20 to 24 days between the start and the end points. Factor $f_{e/s}$ compares median data of end and start, whereas factor $f_{h/c}$ compares median data of healed and control samples. Control samples: $n = 19$, healed samples: $n = 16$.

Appendix A2 Table. Mechanical data and statistics

Tab. A2. Data derived in the framework of the four-point-bending tests. The self-healing efficiency is calculated according to equation (2), where 100 % corresponds to full recovery in relation to the intact samples of the control group. Control samples: $n = 19$, healed samples: $n = 16$.

Fig. 1. Experimental set-up of four-point-bending tests. Each internode was measured in two directions by turning the sample by 90° after the first measurement.

338x190mm (100 x 100 DPI)

Fig. 2. Control internode (A) and healed internode (B) of *Leonurus cardiaca* approximately three weeks after wounding. i: internode, n: node, ue: undamaged edge, he: healed edge. Scale bars equal 1 cm.

85x54mm (300 x 300 DPI)

Fig. 3. Statistical analyses of morphometric data. Boxplot and whisker diagrams show (A) the height of the stems and (B) the length of the examined internodes as a function of control samples (gray boxes) and healed samples (white boxes) at the day of damage (start) and the day of the bending test (end). There were 20 to 24 days between the start and the end points. Different letters (a, b, c) indicate significant differences.

181x65mm (300 x 300 DPI)

Fig. 4. Cross-sections of *Leonurus cardiaca* internodes. (A) Overview and (B) detail of an undamaged corner of a control sample. (C, E) Overview of a healed sample wounded at the four corners in the longitudinal direction and (D, F) detail of one healed corner. Staining: (A-D) FCA, (E) PHA, (F) ACO. B: boundary layer, C: collenchyma, Ct: cortex, E: epidermis, P: pith cavity, Pa: parenchyma, Ph: phloem, S: sclerenchyma, T: trichome, W: wound region, X: xylem.

159x236mm (300 x 300 DPI)

Fig. 5. Statistical analyses of data from the mechanical tests. Boxplot and whisker diagrams show (A) flexural rigidity, (B) axial second moment of area, and (C) bending elastic modulus as a function of control samples (gray boxes) and healed samples (white boxes). Significant differences are indicated. Control samples n = 19, healed samples: n = 16.

182x56mm (300 x 300 DPI)

Appendix A1 Table. Morphometric data and statistics

Tab. A1. Morphometric data given as medians and interquartile ranges (IQR). The statistical analyses refer to the differences between the data determined at the day of damage (start) and at the day of the bending test (end). There were 20 to 24 days between the start and the end points. Factor $f_{e/s}$ compares median data of end and start, whereas factor $f_{h/c}$ compares median data of healed and control samples. Control samples: $n = 19$, healed samples: $n = 16$.

	Start	End	$f_{e/s}$	Statistics	Test
Height of stems (mm)					
Control samples	371.00 (159.00)	518.00 (255.50)	1.40	$V = 0$ $p = 3.815 \cdot 10^{-6}$	[1] ***
Healed samples	328.50 (72.50)	426.50 (146.00)	1.30	$t = -8.8179$ $df = 15$ $p = 2.545 \cdot 10^{-7}$	[2] ***
$F_{h/c}$	0.89	0.82			
Statistics	$W = 194.5$ $p = 0.1642$	$W = 206.5$ $p = 0.07372$			
Test	[3] n.s.	[3] n.s.			
Number of internodes (/)					
Control samples	10.00 (1.50)	12.00 (2.50)	1.22	$V = 0$ $p = 1.174 \cdot 10^{-4}$	[1] ***
Healed samples	9.00 (2.25)	11.00 (1.00)	1.20	$V = 7$ $p = 2.593 \cdot 10^{-3}$	[1] **
$F_{h/c}$	0.90	0.92			
Statistics	$W = 163.5$ $p = 0.709$	$W = 200$ $p = 0.1075$			
Test	[3] n.s.	[3] n.s.			
Length of examined internodes (mm)					
Control samples	86.00 (34.00)	95.00 (37.50)	1.10	$V = 18.5$ $p = 6.334 \cdot 10^{-3}$	[1] **
Healed samples	69.50 (17.00)	70.00 (17.00)	1.01	$V = 27.5$ $p = 0.6526$	[1] n.s.
$F_{h/c}$	0.81	0.74			
Statistics	$W = 195$ $p = 0.1585$	$W = 227$ $p = 0.01354$			
Test	[3] n.s.	[3] *			

Note: [1] = paired Wilcoxon-test, [2] = paired t-test, [3] = unpaired Wilcoxon-test.

Appendix A2Table. Mechanical data and statistics

Tab. A2. Data derived in the framework of the four-point-bending tests. The self-healing efficiency is calculated according to equation (2), where 100 % corresponds to full recovery in relation to the intact samples of the control group. Control samples: $n = 19$, healed samples: $n = 16$.

Parameter	Control samples (end)	Healed samples (end)	Self-healing efficiency	Statistics	Test
Flexural rigidity EI_m (Nmm ²)	26,870 (29,231)	11,930 (8,685)	44 %	$W = 222$ $p = 0.01997$	[1] *
Axial second moment of area I_m (mm ⁴)	13.13 (12.18)	10.83 (5.67)	82 %	$W = 188$ $p = 0.2432$	[1] n.s.
Bending elastic modulus E (MPa)	2,013 (615)	1,324 (407)	66 %	$t = 6.1121$ $df = 32.952$ $p = 6.979 \cdot 10^{-7}$	[2] ***

Note: [1] = unpaired Wilcoxon-test, [2] unpaired t-test.