Restoration of tensile strength in bark samples of *Ficus benjamina* due to coagulation of latex during fast self-healing of fissures

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**Background and Aims** The functions of plant latex have been discussed for a long time. Today, many studies support a defence mechanism as being its main function. A role as a self-healing mechanism was never attributed to the coagulation of latex. In this study we quantified the contribution of the coagulation of *Ficus benjamina* (weeping fig) latex to a restoration of the mechanical properties of the bark after external lesions.

**Methods** Tensile tests of *F. benjamina* bark were conducted either immediately after injury or at various latency times after injury.

**Key Results** A significant increase in the tensile strength of bark samples until 30 min after injury was found, and this effect could be attributed to the coagulation of plant latex alone. The tensile strength remains nearly constant until several hours or days after injury. Then, very probably due to other mechanisms such as cell growth and cell proliferation, the tensile strength begins to increase slightly again.

**Conclusions** The coagulation of latex seals lesions and serves as a quick and effective pre-step of subsequent, more effective, long-lasting self-healing mechanisms such as cell growth and proliferation. Thus, a fast self-healing effect can be included in the list of functions of plant latex.

**Key words:** Self-healing, latex coagulation, tensile strength, external lesion, *Ficus benjamina*.

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**INTRODUCTION**

Latex is a milky plant sap that is exuded after tissue injury. It can be found in about 10% of all flowering plant species (>20,000 species from some 40 families), in both dicotyledonous and monocotyledonous plants (Lewinsohn, 1991; Hunter, 1994; Agrawal and Konno, 2009). A discussion on the functions of plant latex arose already more than a hundred years ago (James, 1887; Parkin, 1900). Today, many studies support the idea that latex mainly acts as a plant defence system (e.g. James, 1887; Parkin, 1900). Today, many studies support the idea that latex mainly acts as a plant defence system (e.g. Dussourd and Eisner, 1987; Agrawal and Konno, 2009), although this is also questioned by others (e.g. Hunter, 1994). Further possible functions of latex in plants, such as a nutrition or waste storage, a transport system, or as a water reservoir, are critically discussed in the literature (Hunter, 1994; Agrawal and Konno, 2009). Therefore, the question of whether the coagulation of plant latices may also contribute to repair of plant lesions is unanswered to this day. Upon external injury of a plant stem, the latex oozes out and quickly coagulates. For the plant it is important to seal lesions originating from external injury. Besides the prevention of the entry of pathogens (fungi, bacteria and viruses) into underlying tissues, the restoration of the mechanical properties is also a crucial factor. Therefore, in particular the recovery of the mechanical properties of the peripheral region (e.g. the bark) of a plant stem is important, as stresses in plant stems are largest in these regions of the stem (e.g. Archer, 1987; Gordon, 1989) and lesions are at risk of expanding due to such stresses. Mechanisms repairing lesions of the outer plant tissues comprise a complex sequence of swelling of cells, cell growth and proliferation (Speck *et al.*, 2004, 2006; Busch *et al.*, 2010). However, after a quick sealing, the time scale until these mechanisms have an effect on the mechanics of the injured plant stems typically amounts to a few to several days (e.g. Sussex *et al.*, 1972; Wilson and Grange, 1984). A faster mechanism, such as the coagulation of plant latices, enabling at least a partial restoration of the mechanical properties, could act as a first quick step of the complete recovery of these properties by cell and tissue growth in later repair phases. A recovery of the tensile strength of the outer region of a plant stem, i.e. the maximum stress during a tensile test, is suitable for a quantification of the self-repairing capabilities (for tensile tests on plant tissues, see Speck *et al.*, 1996; Spatz *et al.*, 1998; Rüeggeberg *et al.*, 2009).

In this study, we examined the self-healing properties of the stem of the weeping fig (*Ficus benjamina*) after injury. This latex-bearing plant belonging to the fig family (Moraceae) is a common, easy to grow plant with a suitable amount of latex produced. Furthermore, the close relationship to *Ficus elastica*, for which many studies on its latex have been performed, makes it an ideal test material.

**MATERIALS AND METHODS**

One- to two-year old *Ficus benjamina* L. (weeping fig) plants were purchased in a plant nursery and afterwards grown in a greenhouse under semi-controlled conditions (i.e. plants were grown at ≥25 °C and watered on a regular basis) for 1 year.

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The bark of stem segments between 7.0 and 10.0 mm in diameter was injured horizontally (i.e. orthogonal to the orientation of fibres and laticifers and the stresses occurring during bending) using a prepared razor blade to obtain a defined depth of injury (0.4 ± 0.1 mm). The test samples were prepared in three steps (Fig. 1) before tensile tests were conducted in the laboratory. Bark was chosen as laticifers are most abundant in this tissue (authors’ observation). Tensile tests were performed because tensile stresses represent the predominant normal stresses that are caused in the plant stem during bending (e.g. caused by wind forces). An additional reason for performing tension tests is the fact that pre-tension of the bark and wood of *F. benjamina* was observed (data not shown). Thus, during bending, compressive stresses at the leeward side of the plant stem are reduced due to the pre-tension. In contrast, tensile stresses at the windward side of the plant stem are increased due to the pre-stresses (Mattheck and Kubler, 1997). Therefore, the highest normal stresses in the periphery of a stem, i.e. also in the bark, of *F. benjamina* due to bending will always be tensile stresses. Additionally, compression of the bark is less critical as it diminishes the size of the injuries, whereas tension expands the lesions. Thus, tensile tests are chosen for characterizing the most critical mechanical loads on the injured bark.

Bark samples were tested either immediately after injury or at various latency times after injury (10 min to 24 h). As a control, uninjured bark samples were also tested. To examine whether possible self-healing effects during these tests can be attributed to the coagulation of the latex, additional tensile tests were conducted with samples in which the uncoagulated latex was cleaned from the bark samples immediately after injury. Cleaned samples were tested both immediately after injury and after a latency time of 30 min. All tensile tests were conducted with a Hegewald & Peschke Inspekt retrofit material testing machine equipped with an Instron 2525 series drop-through static 1 kN load cell (accuracy ≥ 0.34 N). The tensile tests were performed with a speed of 0.5 mm min⁻¹ and an initial clamp distance of 6 mm (resulting in a strain rate of 0.14 % s⁻¹). Sample thickness averaged 0.8 ± 0.1 mm (no significant difference between any groups) and sample width was on average 5.2 ± 0.8 mm. The length of the samples was about 30 mm. In order to maintain comparable conditions for all tested groups, both the depth of injury and the sample thickness were kept constant in good approximation. To quantify the restoration of the most important mechanical properties, both the tensile strength and the apparent Young’s modulus were calculated for each sample. The term ‘apparent’ Young’s modulus was chosen to clarify that injured bark samples are inhomogeneous fibrous samples of a non-uniform shape, due to the reduction of the cross-sectional area after they were injured. Thus, even though the requirements for the calculation of a Young’s modulus (homogenous material of a uniform shape) are not met in our case, we were able to quantify the resistance to stretching of injured bark samples.

Additional tensile tests were conducted on the pure coagulated latex of *F. benjamina*. For that purpose, fresh uncoagulated latex was collected in silicone moulds. To ensure a complete coagulation of the samples, the latex was removed from the moulds after 72 h. All samples were cut into a rectangular shape (sample thickness averaged 0.3 ± 0.1 mm and sample width averaged 8.2 ± 0.4 mm) before testing.

**Statistics**

All mean values are given with standard deviation. The tensile strengths and apparent Young’s moduli were compared by *t*-tests with the software PASW Statistics 18.0-0 (SPSS Inc., Chicago, IL, USA). Sample thicknesses were compared by an analysis of variance (ANOVA) on ranks with the software SigmaStat for Windows Version 3-10-0 (Systat Software Inc., Chicago, IL, USA).

**RESULTS**

The tensile strength of uninjured bark samples showed the largest values of all measurements, with an average of 25.1 ± 3.8 MPa (Fig. 2). Immediately after injury the latex spread over the entire lesion or even ran off the lesion. The tensile strength of bark samples immediately after injury (10.6 ± 1.3 MPa) decreased to 42 % of the values obtained for uninjured bark. Up to a latency time of 30 min the tensile strength increases to a significantly higher value (13.7 ± 3.8 MPa), representing 55 % of the value obtained for uninjured bark, and remains nearly constant for a considerable time, until 150 min after injury. Not before several hours or days after injury does the tensile strength begin to increase again slightly. When the uncoagulated latex was cleaned off the samples immediately after injury, the tensile strength was significantly lower than in the case of uncleaned samples, and no increase in tensile strength could be found up to 30 min after injury. Instead, the tensile strength remained the same immediately after injury (8.2 ± 2.7 MPa) and at 30 min after injury (8.3 ± 2.6 MPa) and thus even after 30 min remained below the values found for non-cleaned bark immediately after injury. Representative stress–strain curves of tensile tests of uninjured bark, recently injured bark and bark 30 min after injury are shown in Fig. 3. In contrast to the tensile strengths, apparent Young’s moduli do not show a constant increase after injury following the decrease caused by injury. Apparent Young’s moduli do not differ significantly after injury up until a latency time of 30 min after injury (Fig. 4). As can be seen in Fig. 3, tensile test curves immediately after injury and 30 min after injury exhibit a similar slope during the linear elastic range (from 0 to 2 % strain), yielding similar apparent Young’s moduli. Differences can only be
observed in the behaviour after the linear elastic range, resulting in different tensile strengths.

The tensile strength of the pure coagulated latex averaged $0.5 \pm 0.3$ MPa, thus representing only about 2% of the tensile strength of the uninjured bark. With an average of $0.05 \pm 0.03$ GPa, the Young’s modulus of the pure coagulated latex amounted to about 9% of the apparent Young’s modulus of the uninjured bark.

**DISCUSSION**

Although plant latex is known for its sticky properties (Agrawal, 2009) and is used to repair technical materials such as, for example, cement (see Chung, 2004 for an overview), a self-repairing function of the latex within the plant itself has never been studied in detail. The significant increase in tensile strength until 30 min after injury indicates a considerable contribution to the self-healing process of *F. benjamina* bark and stems. Hence, besides a plant defence system (e.g. Dussourd and Eisner, 1987; Agrawal and Konno, 2009) and possible further functions such as a nutrition or waste storage, a transport system, or as a water reservoir (Hunter, 1994; Agrawal and Konno, 2009), a contribution to the self-healing system of the plant can be included in the list of functions of latex. A horizontal cut with a razor blade to injure the plant samples is a very artificial type wounding. However, such a reproducible horizontal cut severs plant fibres completely and thus reduces the tensile strength to a greater extent than any other (artificial or natural) wounding scenario. Therefore, such an injury represents the worst case for the plant. Thus, its impact is more severe than most of the more realistic wounding scenarios such as, for example, injuries due to wind-induced contact with neighbouring plants or injuries due to feeding or boring animals.

The partial recovery of the tensile strength takes place in a time period when other changes such as the changes in latex transparency also occur (Bauer et al., 2009), indicating that the coagulation is at a final stage. Hence, and as no increase in tensile strength could be observed when the latex was cleaned off the injured plant samples, this recovery can be attributed to the coagulation of the plant latex alone. The slight decrease in tensile strength when the latex is cleaned from the bark samples indicates that the uncoagulated, liquid latex also makes a small contribution to the self-healing mechanism. This might be due to adhesion, cohesion and capillary effects of the wet latex. Besides the well-known theory of latex coagulation in *Hevea brasiliensis* (Gidrol et al., 1994; d’Auzac et al., 1995), results from further plant species indicate diverse coagulation mechanisms among latex-bearing plants, some with even much shorter time scales compared with those of *F. benjamina* or *H. brasiliensis* (Bauer et al., 2010). Here
the question arises of why the latex coagulation is fast or even very fast for all latex-bearing plants. The rapidity of the latex coagulation of *F. benjamina* is advantageous for an efficient self-healing mechanism as the full healing effect due to latex coagulation only develops when coagulation is complete (i.e. after 30 min). In the case of the other functions mentioned above, the rapidity of latex coagulation plays a minor role or no role at all. In *F. benjamina* the laticifers are most numerous in the bark. Also in other species belonging to the genus *Ficus* laticifers were reported to be found most frequently or exclusively in the bark (Rachmilevitz et al., 1982; Mahlberg, 1993; Kang et al., 2000). The abundance of laticifers in the bark supports the thesis that the latex mainly acts as a plant defence system, but also provides the basis for an effective usage of the latex as a sealing and fast self-healing agent after external lesions. Furthermore, the effectiveness of these systems is increased by a wide spreading of the latex over the entire lesion right after injury.

As expected, the kind of artificial lesion dissecting about half of the samples’ cross-section has pronounced influences on its mechanical properties (Helliday et al., 2001). The apparent Young’s modulus is less reduced by the cut (reduction by approx. 40 % immediately after injury) than is the tensile strength (reduction by approx. 60 % immediately after injury). The more pronounced reduction of the tensile strength can be explained by the fact that the artificial lesion represents a notch initiating a fatal crack under tension loads. The reduction of approx. 40 % in apparent Young’s modulus immediately after injury fits in well with the percentage reduction of load-bearing cross-sectional area of the samples by injuring the samples that amounts to about 50 % of the original cross-section of the sample. Additionally – in contrast to the tensile strength – the apparent Young’s modulus seems not to be affected by the coagulation of the latex as no changes were observed from the time of injury until 30 min after injury. This is not disadvantageous for the plant as an alteration in this mechanical property alone would not influence the recovery of the stem’s resistance to sustain stresses that appear, for example, during bending. On the other hand, an increased resistance to fatal (normal) bending stresses is established by the observed increase in the tensile strength.

How the coagulation of the plant latex contributes in detail to the observed increase in tensile strength cannot be solved by our approach, but several hypotheses can be discussed. The tensile strength and the Young’s modulus of the coagulated latex are very small compared with those of the intact bark. This may explain why the apparent Young’s modulus of the samples is not increased significantly during latex coagulation. On the other hand, the observed increase in tensile strength of the whole bark taking place during the first 30 min after injury cannot be caused by an additional absorption of stresses by the coagulated latex alone. Thus, we propose a mechanism that can be compared with the mechanism of wet adhesion and/or the adhesion supported by a glue between two surfaces (Packham, 2005; Brockmann et al., 2009): in the case of both the uncoagulated and the coagulated, but still sticky, latex, the contact surfaces (i.e. the two sides of the lesion) remain in close contact with each other and are only separated by a thin layer of the uncoagulated (liquid) or coagulated latex (“glue”). We hypothesize that the latex acts as a ‘crack stopper’ hindering the crack propagation in the lesion and thus increasing the tensile strength (but not the Young’s modulus). Cross-linking taking place during coagulation increases this ‘crack-stopping’ function but will not increase the Young’s modulus significantly. Although the described repair effect may restore the mechanical properties of the plant stem only partially, the coagulation of the latex seals the lesion and reduces the crack propagation under tensile loading. Additionally, it serves as a quick and effective pre-step of subsequent, more effective but much longer lasting mechanisms such as cell growth and proliferation.

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LITERATURE CITED


