J Plant Growth Regul (2002) 21:191–196
DOI: 10.1007/s003440010057

J P G R
Journal of Plant Growth Regulation

The Distance to Which Wound Effects Influence the Structure of Secondary Xylem of Decapitated *Pinus pinea*

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ABSTRACT

Decapitation of stems of annuals and trees for the study of vascular and fiber differentiation with or without hormonal application is a common procedure. There is controversy about whether wound effects play a role in such experiments, and to what distance from the point of decapitation. To examine this question, the distance from the point of decapitation at which apparent wound effects are obvious developmentally, was studied in decapitated 4-year-old *Pinus pinea* plants. The wound effects just below the cut included differentiation of many traumatic resin ducts, a parenchyma band instead of tracheids, more tracheid files, and a higher proportion of late wood. The increase in the number of resin ducts was still considerable and statistically significant 10 cm below the point of decapitation compared with the nondecapitated control. These results indicate that in pines, wound effects in the first 5 cm below the decapitation point (a common point for tissue examination) cannot be ignored in experiments on the regulation of vascular differentiation.

Key words: Decapitated stems; Hormonal regulation; *Pinus pinea*; Resin ducts; Vascular differentiation; Wound effects; Xylem

INTRODUCTION

The appearance of a vascular system is probably the most important single evolutionary change enabling the dominance of vascular land plants in many ecosystems and the existence of many land animals, including humans. Understanding the biology of vascular differentiation is of great theoretical and practical importance. For decades, decapitated young and mature trees and herbaceous plants have been used in experiments investigating the regulation of xylem differentiation with or without hormonal applications (see for example, Digby and Wareing 1966; Sachs 1981, 1991; Lev-Yadun and Aloni 1993; Aloni 1991, 2001). In such experiments, the upper parts of the shoot are removed, hormones (usually dissolved in lanolin) are applied to the cut, and the structure of the xylem one to several cm below the cut is studied. The control is usually a cut stem with lanolin application. Moreover, renewal of the cut surface and application of more lanolin may be done several times. Such experiments enabled the understanding of basic aspects of vascular differentiation, especially, issues related to the polar auxin transport or the involvement of other hormones in differentiation. In certain cases, the changes in patterns of differentiation were studied without external hormonal applica-

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Received: 9 October 2002/Accepted: 22 January 2002/Online publication: 24 May 2002

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tions. However, the role of wound effects in such experiments (and in related ones) was not always taken into consideration (see Lev-Yadun and Aloni 1995; Lev-Yadun 2000). Certain aspects of vascular differentiation, such as the number, size, and patterns of elements (tracheids, vessel members, fibers, parenchyma, and secretory tissues) known to be influenced by wound effects (Fahn 1988; Lev-Yadun and Aloni 1992, 1993, 1995; Lev-Yadun 1994a, b, 2001; Fink 1999) were referred to as if they were not influenced by wound effects.

The wood of *Pinus pinea* is characterized by large diameter, thin-walled early wood tracheids, and radially narrower thick-walled latewood tracheids. Resin ducts are scattered in both early- and latewood (Schweingruber 1990). The constitutive production of both axial and radial resin ducts is a basic aspect of wood formation in all pines (Jain 1976). In the genus *Pinus*, traumatic resin ducts are formed in addition to the constitutive ones (Fahn and Zamski 1970; Fahn 1988). Fahn (1988) concluded that ethylene regulates the differentiation of resin ducts. As wounding promotes ethylene production (Abeles and others 1992), it induces the development of traumatic resin ducts. A pine was chosen for this study because the formation of typical traumatic resin ducts following wounding enables an unequivocal study of the distance to which wounding affects differentiation.

It is shown here that considerable wound effects are histologically evident for at least 10 cm below the point of decapitation. Therefore, when using decapitation (with or without hormone applications) for studying xylem differentiation, the upper 10 cm (and probably even more) below the point of decapitation has a good chance of being structurally influenced by wound effects.

MATERIALS AND METHODS

Fifty four-year-old trees of *Pinus pinea* L. that were similar in height and general development were decapitated in the beginning of May when the cambium is active. Five of the decapitated plants and five untreated control plants were randomly sampled for histology 240 days later. Cross-sections, approximately 25 µm thick, were prepared with a Reichart sliding microtome from the decapitated plants just under the point of decapitation (distance 0) and 5, 10, and 40 cm below the cut. From the control plants, sections were prepared at the corresponding heights. The sections were stained with Safranin and fast green and mounted in Entellan New (E. Merck, D-6100 Darmstadt, Germany; Cat. no. 7961). Slides were examined under a bright field using a Leitz Dialux 20 microscope equipped with a Nikon FTN camera at a magnifications of ×63 to ×400. The number of resin ducts formed in the last growth ring was counted in all specimens. Because there is no anatomical marker for the beginning of the experiment in the control, the number of resin ducts in both control and decapitated plants was counted in the whole last growth ring. In addition, the average number of tracheids, the average radial diameter of the tracheids (along a radius) in the last growth ring, and the proportion (in %) of the part of the growth ring occupied by thick-walled tracheids (late wood) were also measured at the same as distances below the decapitation point and in corresponding levels in the control plants. The radius chosen for measurements was away from local wounds, branch junctions, or compression wood when such structures appeared.

The statistical test used to examine whether there was a difference between the number of resin ducts in the control and the decapitated plants at each distance was the non-parametric Mann–Whitney U Test.

RESULTS

In nondecapitated plants (Figure 1, part 1, Figure 2), the average number of resin ducts in the last growth ring was 16 at the point corresponding to the height of decapitation. Fifteen resin ducts were formed at 5 cm beneath it, at 10 cm, 9.8; and at 40 cm, 13.4 (13.8 resin ducts in the last growth ring on average, for all distances) (Figure 1, part 2, Figure 2). The average number of tracheids in the last growth ring was 51.2 at the point corresponding to the height of decapitation, 59 at 5 cm beneath it; 56 at 10 cm; and at 37.6 at 40 cm (50.95 on average, for all distances). The average radial diameter of the tracheids in the last growth ring was 17.8 µm at the point corresponding to the height of decapitation, 17.6 µm at 5 cm beneath it; 18.0 µm at 10 cm; and 19.9 µm at 40 cm (18.3 µm on average, for all distances). The proportion of late wood tracheids in the last growth ring was 40.8% at the point corresponding to the height of decapitation, 34.9% at 5 cm beneath it; 39.8% at 10 cm, and at 38.8% at 40 cm (38.6% on average, for all distances).

All decapitated plants formed several branches from suppressed buds just below the point of decapitation (Figure 1, part 1). In the decapitated plants, the histological picture is clearly different from the control. Immediately under the point of decapitation, the new xylem formed after decapi-

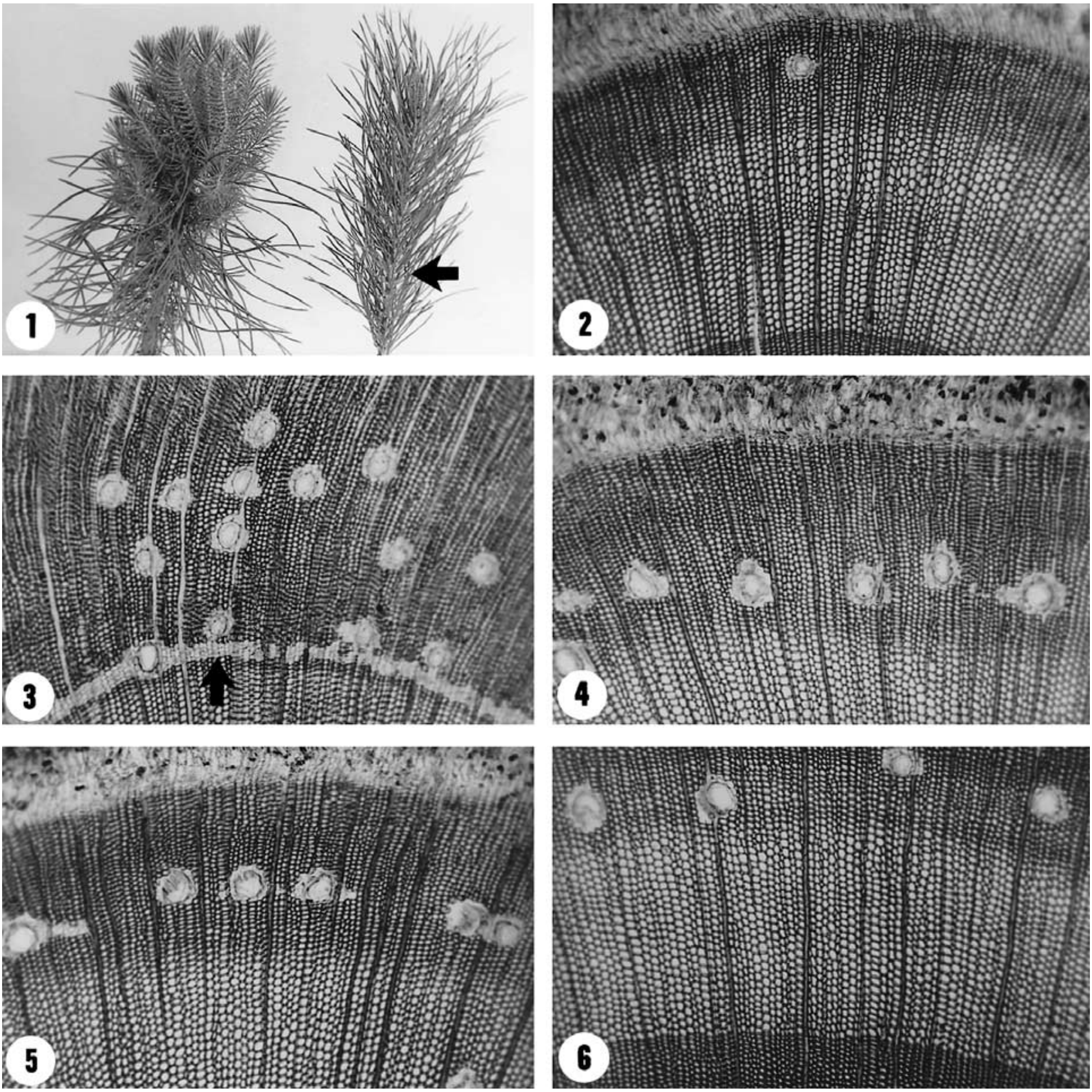

Figure 1. (**parts 1–6**) **Part 1.** Decapitated 4-year-old plants (left) 240 days after decapitation, with new branches carrying juvenile needles that appear in *Pinus pinea* under various types of stress. The intact (control) plants (right) have mature needles. The arrow marks the point where the section for Figure 2 was done.

Part 2. The xylem formed in the last growth ring in control plants at a point corresponding to 5 cm below the point of decapitation in treated plants. The previous growth ring border is seen at the lower center. The average number of resin ducts was 15 (Bar = 200 μm).

Part 3. The xylem formed in the last growth ring just beneath the point of decapitation, characterized by many (traumatic) resin ducts and a band of parenchyma (arrow) instead of tracheids. The average number of resin ducts was 104.6 (Bar = 200 μm).

Part 4. The xylem formed in the last growth ring 5 cm beneath the point of decapitation was still characterized by many (traumatic) resin ducts, although less than just beneath the cut. The average number of resin ducts was 46.6 (Bar = 200 μm).

Part 5. The xylem formed in the last growth ring 10 cm beneath the point of decapitation was still characterized by many (traumatic) resin ducts, although fewer than just beneath the cut. The average number of resin ducts was 41.4 (Bar = 200 μm) .

Part 6. The xylem formed 40 cm beneath the point of decapitation had a somewhat higher number of (traumatic) resin ducts than just beneath the cut, although the difference was not statistically significant. The dark late wood border of the previous growth ring is seen at the lower part. The average number of resin ducts in the last growth ring was 20.0 (Bar = 200 μm).

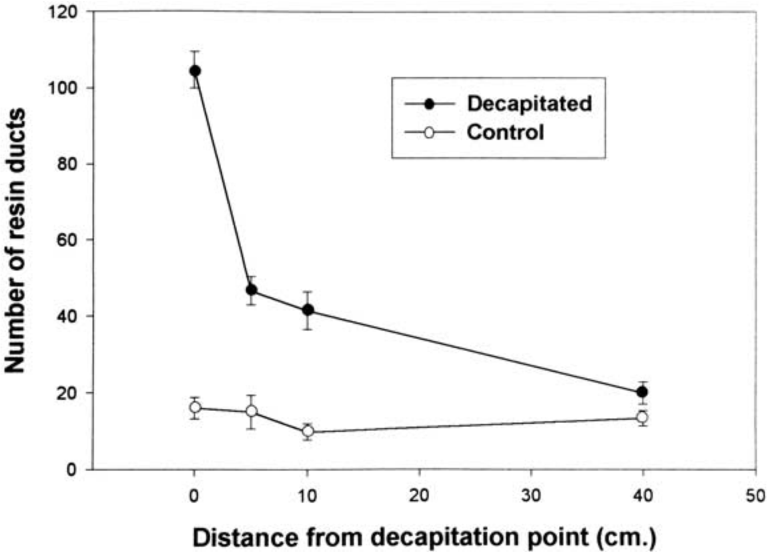

Figure 2. The number of resin ducts (± S.E.) in the last growth ring in decapitated and intact (control) plants.

tation was characterized by many more resin ducts (average 104.6) than in the control (average 16) (Figure 1, part 3, Figure 2). In addition, a band of parenchyma, several cell files wide, rather than tracheids was formed (Figure 1, part 3), more radial files of tracheids and a higher proportion of late wood. Five cm beneath the decapitation, the number of resin ducts (average 46.6) was still much higher than the control, but no parenchymatic band was formed (Figure 1, part 4, Figure 2). Ten cm beneath the decapitation, the number of resin ducts (average 41.4) (Figure 1, part 5, Figure 2) was still much higher than the control. Forty cm beneath the decapitation, the number of resin ducts (average 20.0) (Figure 1, part 6, Figure 2) was still higher than in the control, although the increase in resin duct number was less prominent (Figure 2). The average number of tracheids in the last growth ring was 110.6 at the point of decapitation, 70.4 at 5 cm beneath it; 61.8 at 10 cm; and 41 at 40 cm (70.95 on average, for all distances). The average radial diameter of the tracheids in the last growth ring was 17.6 µm at the point of decapitation, 16.9 µm at 5 cm beneath it; 18.5 µm at 10 cm; and 19.7 at 40 cm (18.2 µm on average, for all distances). The propotion of late wood tracheids in the last growth ring was 56.4% at the point of decapitation, 41.7% at 5 cm beneath it; 35.3% at 10 cm; 35.4% and at 40 cm (42.2% on average, for all distances).

The differences in resin duct numbers in the last growth ring at the point of decapitation and 5 and 10 cm below it were significantly different from the control (U1 = 0.000, U2 = 25.000, Z = 2.611, $p < 0.01$). The difference was not significant 40 cm below the cut (U1 = 6.000, U2 = 19.000, z = 1.357, $p=0.17$). At all points, more cell files were formed by the cambium in decapitated plants than in the control but the difference was statistically significant only just beneath the point of decapitation (Z = 2.29; $p = 0.02$).

The radial diameter of the tracheids in the growth rings seems not to be influenced by the wounding although the band of tracheids with thick secondary walls became significantly thicker just beneath the point of decapitation.

DISCUSSION

The goal of this study was to examine the question of how far from the point of decapitation wound effects would influence the histological structure of differentiating tissues. This issue is currently under debate (Lev-Yadun 2000; Aloni 2001) because if wound effects still occur at the structural level in the upper 5 cm distal to the point of decapitation, as proposed by Lev-Yadun 2000, the interpretation of vessel and fiber size, number, and patterns in some critical studies on the hormonal regulation of fiber and vascular differentiation may need reconsideration. Possible changes in interpretation may be necessary because wound effects may alter the polar auxin flow and increase ethylene effects and probably alter other developmental signals.

The results of the present study show clear and statistically significant evidence that at least in a pine, wound effects are still strong 10 cm distal to the point of decapitation. At 40 cm the effect is statistically insignificant. Thus, the distance at which wound effects were not strong enough to change tissue structure significantly is some where between 10 and 40 cm distal to the point of decapitation. The outcome of the present results cast doubt on Aloni's (2001) strong emphasis that at 5 cm distal to the point of decapitation there are no wound effects. The results of some of the experiments reviewed in Aloni (2001) should thus be reconsidered in view of the results presented here, with all the possible implications of the interpretations. In any case, it might well be that in certain plant species wound effects really cease at distances less than 10 cm. This possibility, however, has not been examined yet in depth and should be examined carefully in the model species.

What are the consequences of the clear developmental changes induced by wounding on xylem structure more than 10 cm beneath the point of decapitation? The accumulating data from external application of auxin transport inhibitors (Gälweiler and others 1998; Mattsson and others 1999; Sieburth 1999; Berleth and others 2000), mutants of hormonal physiology, a mutant of the gene *MONOPTEROS* in *Arabidopsis* that suffered from reduced auxin transport capacity (Przemeck and others 1996), a mutant in the gene *LOP1* defective in basipetal IAA transport and vascular patterning in *Arabidopsis* (Carland and McHale 1996), the ethylene-insensitive mutant Never *ripe* in tomato (Aloni and others 1998), a mutant in the gene *AtPIN1* in *Arabidopsis* (Gälweiler and others 1998), and a mutant in the gene *PINOID* in *Arabidopsis* (Christensen and others 2000) that suffered from diminished polar auxin transport, plants suffering from tumors (Wächter and others 1999) and transgenes with up to 19-fold reduction in IAA levels (Romano and others 1991) indicate that the general, qualitative effects of plant hormones (growth regulators) in fiber and vascular differentiation, proposed following classical wounding and application studies (Digby and Wareing 1966; Sachs 1981, 1991; Lev-Yadun and Aloni 1995; Aloni 2001) are correct. However, quantitative aspects, such as the number of vascular elements (Aloni and Zimmermann 1983; Aloni 1991), their size and pattern within the tissue (Lev-Yadun and Aloni 1993; Lev-Yadun 1994a, b, 2001), fiber numbers (Aloni 1987; Lev-Yadun and Aloni 1993; Lev-Yadun 1994a) and, of course, resin and gum ducts (Fahn 1988) are influenced by wounding (see Fink 1999; Lev-Yadun and Aloni 1993, 1995; Lev-Yadun 1994a, b, 2000, 2001). Therefore, wound effects must be taken into account in studies of decapitation. Certain classical published results of studies employing decapitation should thus be evaluated with caution concerning their quantitative aspects even if their general, qualitative aspects remain valid.

ACKNOWLEDGMENTS

The author thanks two anonymous reviewers for their important suggestions.

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