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Response of the Cambial Zone in Conifers to Wounding

By

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S u m m a r y

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The sequence and character of histological changes in response of the cambial zone in *Abies alba*, *Picea abies, Pinus sylvestris* and *Larix decidua* to wounding were studied. Trees were wounded by removing square of bark from the wood on 7 April 1993 and response investigated 7, 14, 21, 28, 35, 42, 49, 56, 84, 112, and 140 days later. The sequence of changes was the same in all species. Callus developed at the wound edge by hypertrophy and hyperplasia of cells of the cambial zone. More tangential, increased frequency of transverse divisions was observed in fusiform cells of the cambial zone. On the surface of the callus a ligno-suberised layer formed. Internal to it new periderm developed only at the exposed part of the callus. A ligno-suberised layer represents durable protection at the ventral part of the callus, where new periderm did not form. A new cambium differentiated within the callus, giving rise to wound wood and bark. Tangential from the callus, a layer of lignified parenchyma cells formed at first, followed by a layer of short tracheids and a series of traumatic resin canals. The layer of lignified parenchyma cells and traumatic resin canals corresponded to the wall 4 of the CODIT concept. There existed little variation in timing of response between investigated species. Protective function of histological changes is discussed.

I n t r o d u c t i o n

The concept of compartmentalization of decay in trees (SHIGO 1986) tries to explain the spatial orientation of pre-existing and newly formed boundaries that limit the spread of dysfunction, infection and decay in xylem (LIESE & DUJESIEFKEN 1996, SHORTLE & al. 1996). The compartmentalization wall 4, a distinctive tissue that prevents a spread of infection from the injured wood into the

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wood formed after wounding, is laid down by the affected cambium (SHIGO 1986). Cambial response also results in the formation of the callus tissue, and subsequent regeneration of the vascular tissue at a wound edge (LARSON 1994). Lignification, accumulation of polyphenolics and suberisation of cell walls, which are part of the defence reactions in woody plants (PEARCE 1996), were detected in callus (SPANOS & WOODWARD 1997, BIGGS & BRITTON 1988). Knowledge of the formation of a protective zone in the vascular tissues during early stages of cambial response is necessary in research of effects of wound treatment in forest and urban trees. The objective of this pilot study was to reveal the sequence and character of histological changes following wounding of the cambial zone in fir, spruce, pine and larch.

Material and Methods

A single experimental tree of fir (*Abies alba* Mill.), spruce (*Picea abies* (L.) Karst.), Scots pine (*Pinus sylvestris* L.) and larch (*Larix decidua* Mill.) was used. The trees were 50 years old, healthy, and codominant. The cambial zone of these trees was wounded by removing a square (3 x 3 cm) of bark from the wood. Eleven wounds were set in semi-helical pattern along the stems on April 7, 1993. Distance between wounds was 15 cm. Wounded and unaffected tissues were collected after 7, 14, 21, 28, 35, 42, 49, 56, 84, 112, and 140 days. After fixation (formaldehyde, acetic acid, ethanol) and embedding (Polyethylenglicol 1500), 20 µm sections were prepared for light microscopy. Fluorescent microscopy was used in combination with a polychromatic stain Acridin Red-Chrysoidin/Astra blue (ACA) and the quenching autofluorescence for detection of lignin and suberin. An Olympus BH2 (HBO 100W, U(DM-400+L-420)) was used for conventional light microscopy and observations at UV excitation. Abbreviations: ligno-suberised layer = L-S layer; necrophylactic periderm = NP.

Results

The sequence of histological changes was the same in all conifers investigated. The cells at the wound surface were dead on day 7. Hypertrophy and hyperplasia of ray initials and xylem mother cells of rays took place under necrotic tissue. Callus formed at the wound edge by progressive hypertrophy and hyperplasia of all cambial cells as well as of phloem parenchyma (Table 1). Increased frequency of transverse divisions was observed more tangentially in fusiform cells of the cambial zone.

The first changes in the callus tissue occurred at the place where the NP of the bark was already formed. Exposed cells of the callus became thickened and lignified. Subsequent suberisation of these cells indicated the beginnings of the L-S layer formation. Suberisation was detected on day 49 in fir, spruce and pine, and on day 42 in larch. A new phellogen was observed under the L-S layer by day 49 in spruce and pine, by day 56 in fir and, by day 42 in larch. The formation of a L-S layer proceeded towards the inner (ventral) part of the callus (Table 1). Brown deposits, probably polyphenolics, occurred in suberised cells. NP developed only on the outer surface of the callus by the end of the experiment (Table 1).

Table 1. Formation of new tissues in the course of the experiment. Numbers = days after wounding. Location: A = wound edge, B = distal and tangential from the wound edge.

		Abies alba	*Picea abies*	*Pinus sylvestris*	*Larix decidua*
A	callus	42	49	35	35
	continuous ligno-suberised layer	84	56	84	49
	necrophylactic periderm	112	112	112	56
	occurrence of new cambium	84	56	84	49
B	layer of lignified parenchyma	112	112	84	112
	traumatic resin canals	112	84	84	49

A new cambium started to differentiate within the callus (Table 1) in the continuation of the original (affected) cambium. A woundwood began to form by divisions of the new cambium and differentiation of its derivatives. It comprised tracheoids, short and reoriented tracheids, and increased number of solitary resin canals. Woundwood grew over exposed wood towards the centre of the wound. Lignification and accumulation of brown deposits occurred in callus cells.

A layer of parenchyma cells differentiated at first tangentially from the callus. Starch grains were visible in the lumina. Thickening and lignification of the parenchyma cell walls were detected by day 56 in fir and pine, by day 84 in spruce, and by day 49 in larch. This process resulted in the formation of a layer of lignified parenchyma cells (Table 1). Brown deposits occurred in the lumina as well. After the parenchyma a distinctive layer of short tracheids, and finally a tangential series of traumatic resin canals formed (Table 1). Several stages in the differentiation of resin canals were seen on the same section. With ongoing response lumina of resin canals were occluded by tylosoids. The tylosoids underwent lignification accompanied by accumulation of brown deposits in their lumina. Hence, epithelial cells died and lost the capability to produce resin. Timing of tissue changes revealed that larch responds earlier and faster than the other three species (Table 1).

Discussion

Our results showed an identical response in fir, spruce, pine and larch. Formation of the callus at the wound edge is necessary for redifferentiation and regeneration of the lateral meristems (cf.: LARSON 1994). This always happened only after the formation of the continuous L-S layer on the entire surface of the callus in our experiment (Table 1). We assume that the L-S layer is necessary for differentiation of new phellogen internal to it as well as new cambium within the callus. Neither of the meristems occurred before L-S layer was formed (Table 1).

Structural responses in callus largely paralleled those found in wounded bark (OVEN & TORELLI 1994, OVEN & al. 1999). Formations of L-S layer in bark is considered to be prerequisite for differentiation of new phellogen internal to it (OVEN & al. 1999). The L-S barrier in living bark is then replaced by permanent

periderm (OVEN & al. 1999). Our results showed that the L-S layer represents a permanent protection barrier at the ventral part of the callus, where new periderm did not form. Relatively late formation of the L-S barrier at the ventral part of callus was observed in our experiment (Table 1). This supports the suggestion that this location is especially susceptible to disruption of pathogenic fungi in early stage of tissue response (cf.: BLANCHETTE & BIGGS 1992). Changes on the surface of the callus were reported to be stimulated by fungal challenge (WOODWARD & PEARCE 1988, SPANOS & WOODWARD 1997). The presence of pathogens was not investigated in our experiment.

Distally and tangential of the callus, layers of parenchyma cells and traumatic resin canals were formed in all investigated species. Resin canals are absent in normal wood of *Abies alba.* A layer of lignified parenchyma and traumatic resin canals have been frequently described as compartmentalization wall 4 in conifers (LIESE & DUJESIEFKEN 1996, SHIGO 1986, BANGERTER 1984, TORELLI & al. 1990). Lignification of cell walls and production of accessory substances were associated with both types of wall 4 barrier. Faint autofluorescence in cell walls of lignified parenchyma as well as in walls of tylosoids in traumatic resin canals suggested the presence of suberin.

The ligno-suberised layer and the compartmentalization wall 4 seem to have important role in protection of tissues formed after wounding. The more rapidly the barrier tissue develops, the lower the chances of a pathogen invading the new tissues (cf.: BLANCHETTE & BIGGS 1992). The data obtained from these studies indicate at roughly little variation in timing of response between investigated species. Further work is needed to examine the speed of response in different times of the year.

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