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Formation of Gum Ducts in *Ailanthus excelsa* in Response to Fungal Infection

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With 1 Figure

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Summary

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The formation of traumatic parenchyma followed by a lysigenous development of gum ducts in response to fungal infection was studied histologically in the secondary xylem of *Ailanthus excelsa* ROXB. (*Simaroubaceae*). Several layers of traumatic parenchyma cells developed instead of normal secondary xylem at some distance from the infection site. The lysis of these parenchyma cells resulted in formation of one or two tangential rows of gum ducts. Darkly stained epithelial cells bordered these cavities. After initiation of gum duct formation, more cells underwent lysis and eventually contributed to enlarge the gum ducts. Along with the formation of epithelial cells, traumatic parenchyma adjacent to these ducts became meristematic and cambiform. As lysis of epithelial cells progressed, the cambiform cells underwent repeated periclinal divisions and formed additional epithelial cells. These epithelial cells also accumulated phenolics and by their lysis released them into the ducts. In mature ducts, formation and lysis of epithelial cells ceased but divisions in the cambiform cells continued and daughter cells differentiated into axial parenchyma. Formation of parenchyma from cambiform cells, eventually led to a partial curing of these ducts. The injured portion of the secondary xylem was infested by fungal filaments that invaded all cell types of the secondary xylem.

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Zusammenfassung

RAJPUT K.S. & KOTHARI R.K. 2005. Die Bildung von gummiführenden Kanälen in *Ailanthus excelsa* als Antwort auf die Pilzinfektion. – Phyton (Horn, Austria) 45 (1): 33–43, 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

Die Bildung von verletzungsbedingtem Parenchym, an welches sich die lysogine Entwicklung von Genen mit Gummiinhalt anschließt wird histologisch im sekundären Xylem von *Ailanthus excelsa* ROXB. (*Simaroubaceae*) untersucht. Eine derartige Formation kann als Antwort auf eine Pilzinfektion gelten. In einiger Entfernung von der Infektionsstelle entwickeln sich einige Schichten von traumatischen Parenchymzellen anstelle von gewöhnlichem sekundärem Xylem. Das Auflösen dieser Parenchymzellen hat die Bildung von einem oder zwei tangentialen Gängen zur Folge. Dunkel gefärbte Epithelzellen begrenzen diese Höhlungen. Nach Beginn der Bildung der Gänge mit Gummiinhalt lösen sich immer mehr Zellen auf und tragen gelegentlich dazu bei, dass sich die Gänge erweitern. Gleichzeitig mit der Bildung der Epithelzellen werden auch an die Gänge angrenzende Parenchymzellen meristematisch und cambiform. Während die Auflösung der Epithelzellen fortschreitet, finden in den cambiformen Zellen wiederholte periclinale Teilungen statt, wodurch zusätzliche Zellen gebildet werden. Diese Epithelzellen akkumulieren auch phenolische Substanzen, welche sie bei ihrer Auflösung in die Gänge entleeren. In reifen Gängen wird die Bildung und Lyse von Epithelzellen beendet allerdings dauern die Teilungen in den Cambiformzellen weiter an und Tochterzellen differenzieren sich in axiales Parenchym. Die verletzten Bereiche des sekundären Xylems sind intensiv von Pilzhyphen befallen, welche in alle Zelltypen des sekundären Xylems eingedrungen waren.

Introduction

Gum and mucilage ducts commonly occur in vegetative organs of many angiosperm families. Ducts lined with characteristic parenchyma cells known as epithelial cells are found in many genera of Asteraceae, Apiaceae, Anacardiaceae, Fabaceae, Rosaceae and other families (FAHN 1979, MAKSYMOWYCH & LEDBETTER 1987). In addition to endogenously built ducts, gum ducts can be artificially induced in plant organs, by microorganisms, insects and mechanical injury (JOSEPH & al. 1988, SUBRAHMANYAM & SHAH 1988). Ducts developing in response to injury are termed traumatic ducts. Their development in the bark and/or wood of a few Indian species has already been studied (NAIR & al. 1980, 1983, 1985, JOSEPH & al. 1988, SUBRAHMANYAM & SHAH 1988).

NAIR & al. 1980, 1985 have studied the formation of gum ducts in response to the application of certain chemicals like ethephone (2-chloroethyl phosphonic acid) and paraquat in bark and sapwood of *Azadirachta indica* and *Ailanthus excelsa* (SHAH & BABU 1986). In untreated control samples of both the species, formation of gum ducts was observed only fifteen days after the injury and only a minor amount of gum exudation was observed while those trees treated with ethephone showed copious gum exudation for a prolonged period (NAIR & al. 1980). However, in gum ducts developed in response to an injury (by making an incisions with a chisel), only a few ducts were formed and remained active for a short period, while gum ducts formed after an injury immediately followed by a fungal infection were active for a longer time and formed several tangential rows (RAJPUT & al. 2004). In *Ailanthus excelsa*, the stem has normal gum ducts only in the pith (BABU 1985) and a mechanical injury does not induce an immediate response by formation of cavities in the wood. However, traumatic cavities and vascular occlusions develop in the secondary xylem of *Ailanthus* in response to injury and ethephone treatment (SHAH & BABU 1986).

In the present study, development of gum ducts in response to fungal infection is observed in differentiating xylem collected from naturally infected trees growing in the Gir Forest at Junagadh, Gujarat. This study is aimed at understanding whether the process of gum duct formation of naturally infected *Ailanthus excelsa* is the same as that of experimental studies. In addition, our results are compared with earlier studies on the induction of gum ducts in *Azadirachta* (NAIR & al. 1980, 1983, 1985).

Materials and Methods

As the bark of *Ailanthus* has anthelmintic, febrifuge, expectorant and antispasmodic properties, it is used against asthma, bronchitis, and dysentery (ANONYMOUS 1972). Therefore, *Ailanthus* trees are often debarked by Vaidyas (local people who prepare and provide herbal medicine) and, in the present study such plants showing exudation of gum were selected.

Samples of cambial tissue along with outer xylem and inner phloem were collected from such mechanically injured trunks (35–45 cm in diameter) from 17–20 years old non-infected and infected trees growing naturally in the Gir Forest of Junagadh. The trunks had been injured previously by local people due to the medicinal value of the non-functional phloem. Ten trees were sampled at breast height and four blocks were taken from each tree. Each block measured about 60 mmX20 mm in length and width, respectively. The samples were excised from two places, one exactly passing through the injured portion and other from an intact portion of the secondary xylem 15–20 cm away from the injury. The samples were fixed in FAA (BERLYN & MIKSCHE 1976) and aspirated to remove air from the tissue. Transverse and longitudinal sections of 12–15 μm thickness were cut with a sliding microtome and stained with a combination of tannic acid-ferric chloride-lacmoid (CHEADLE & al. 1953). After dehydration in an ethanol-xylene series, the sections were mounted in DPX. Temporary slides were also prepared and stained with ferric chloride to test for phenolics (JENSEN 1962).

The terms cambial zone and cambium have been used to include the entire population of ray and fusiform cambial cells between the xylem and phloem. The cambial activity was determined by counting the number of undifferentiated layers between xylem and phloem in transverse sections.

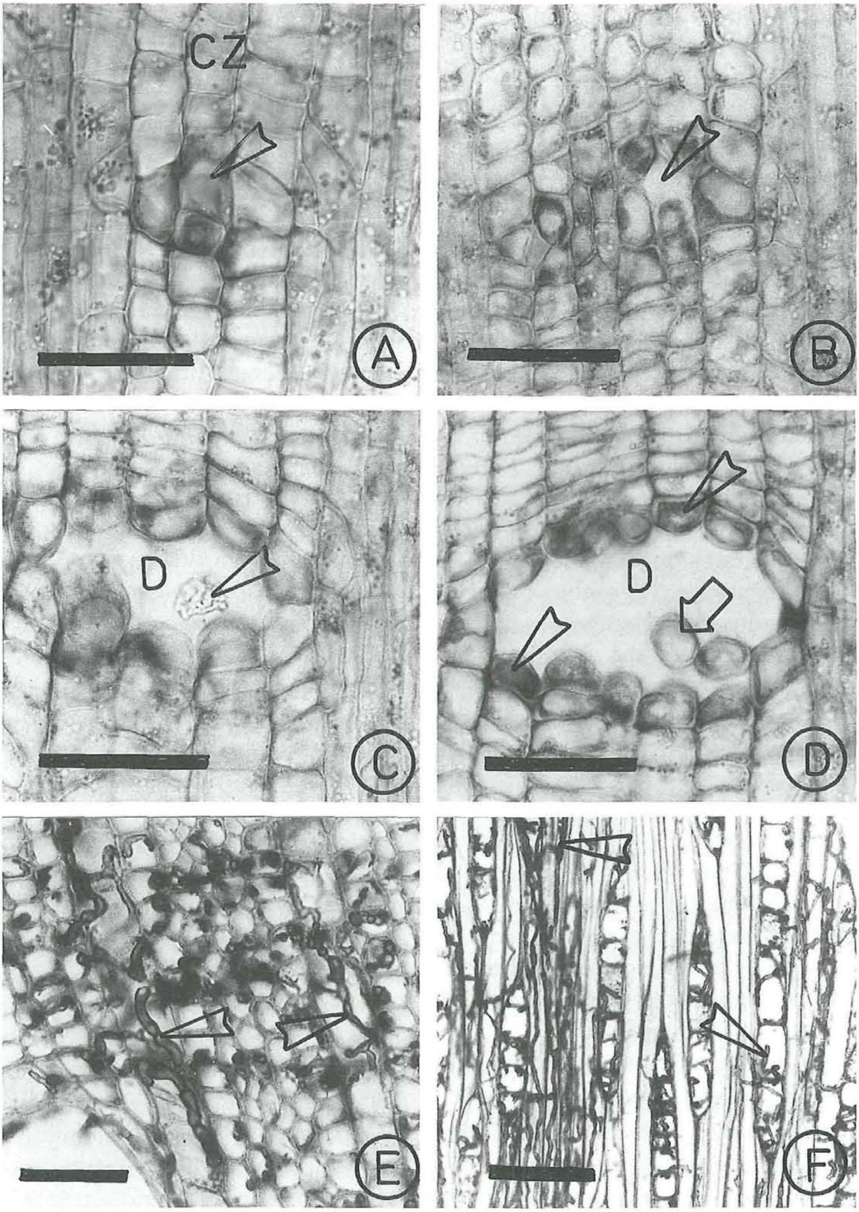
Results

Formation of Gum Ducts

In initial stages of duct development, one or two darkly stained undifferentiated xylem cells (which are indistinguishable from the cambial cells) very close to the cambial zone show dense cytoplasm and large nuclei (Fig. 1A). These cells undergo periclinal and anticlinal divisions to form a group of more or less isodiametric cells. From these cells, centrally situated ones undergo autolysis, thus initiating a lysigenous vertical duct (Fig. 1A, B). Cells surrounding the cavity reorient themselves to form densely stained epithelial cells (Fig. 1D). The lysis of epithelial cells continues even after the cambium is located some distance from the ducts, which are then surrounded by mature xylem. As lysis of epithelial cells progresses further, traumatic parenchyma cells surrounding the epithelial cells become meristematic and appear cambiform (Fig. 1C, D). If lysis of epithelial cells proceeds rapidly, peripheral cambiform cells divide anticlinally and transversely to form additional epithelial cells, leading to a tangential and radial enlargement of the cavity/duct. However, the xylem rays limit their tangential widening and rays remain like bridges amidst the ramifying cavities. When lysis of epithelial cells proceeds rapidly, autolysis of cells also involves both lysigenous and schizogenous activity (Fig. 1D).

In the beginning of cavity/duct formation, epithelial cells are compactly arranged but later their walls become dark and the cells separate from each other (Fig. 1D). At the time of lysis, senescing epithelial cells show dark cytoplasm due to accumulation of phenolics and eventually the cells undergo lysis. Lysis of these cells releases phenolic compounds into the ducts which are then stained dark. However, lysis of epithelial cells is not sequential since intact cells are often left amidst lysing cells. After a stage of active secretion, all traumatic parenchyma cells, except the cambiform cells, undergo lysis and thereafter the cavity/duct remains inactive. In the beginning of cavity/duct formation, the accumulation of phenolics is minimal (Fig. 1D), but as the ducts become inactive, more phenolics accu-

Fig. 1. Transverse (A–E) and tangential (F) view of cambium and xylem of *Ailanthus excelsa* showing various stages of gum duct/cavity development. A: Initiation of duct formation. Note the group of darkly stained undifferentiated traumatic parenchyma cells of xylem very close to the cambial zone (arrowhead). B: Lysigenous developing duct/cavity from the cambium. Arrowhead indicates lysis of a single cell from the group of darkly stained epithelial cells. C: Rapid lysis of epithelial cells in differentiating xylem enlarges the duct/cavity in radial and tangential direction. Arrowhead indicates cytoplasmic debris of the cell that underwent autolysis. D: A large active duct. Note the accumulation of phenolic substance in epithelial cells at the edge of the duct/cavity (arrowheads). Arrow shows loosely arranged cells embedded



in cavity showing schizogeny. E: Severely infected xylem showing ray and axial parenchyma and fibres occluded with fungal hyphae (arrowheads). F: Severely infected xylem showing all cell types occluded with fungi. Arrowheads indicate fibres, axial and ray parenchyma with fungal mycelium. CZ = Cambial Zone; D = Duct. Figs. 1A–F: Scale Bar = 75 μm.

mulate thus the ducts stain dark black. In the latter stage although the duct/cavity remains inactive and there is no further lysis of cells, the cambiform cells divide continuously and help in the healing of these cavities/ducts. As long as the duct/cavity is active, differentiating xylem cells around the duct remain thin-walled and unlignified but as the ducts become inactive these cells undergo lignification and become thickwalled.

Structure of Xylem and Fungal Infection

Sections of wounds showing exudation of gum revealed unidentified fungi in all cell types of the secondary xylem and in several rows of gum ducts embedded in the traumatic parenchyma formed in the outer sapwood. In infected trees, the xylem has several tangential rows of gum ducts/cavities. Ray cells in the outer and middle sapwood separate rows of adjacent cavities/ducts while normal xylem derivatives are present between rows of cavities/ducts. Fungal hyphae are present in all cell types of the xylem near the cavities/ducts (Fig. 1E, F). Filamentous fungi represent the most conspicuous form of infection. Fungal hyphae of different diameters ramify on the outer part of the injury and gradually enter into vascular tissues. There they infect all cell types viz. fibres, vessel elements, axial and ray parenchyma cells (Fig. 1E, F). Among these cell types, parenchyma cells are the only living ones at maturity and are therefore killed after infection and/or wounding. When infection becomes severe, lumens of all cell types have been noticed to be filled with fungal mycelium. Similarly, vessel elements are also completely blocked with fungal filaments. Several vessels near the cavities/ducts are also found plugged with gum-like material and tyloses. When the development of cavities/ducts is in progress, the newly formed xylem is exclusively composed of parenchyma cells and often lacks fibres and vessels. After inactivation of these ducts, differentiating xylem is normal consisting of all xylem cell types. After the inactivation of the ducts/cavities, unlignified thin-walled parenchyma cells formed around the cavities undergo secondary wall thickening and become thick-walled and lignified as previously mentioned.

Response of Wood to Fungal Infestation

Filamentous fungi penetrate all cell types of the xylem, but parenchyma cells are the only living cells after maturity and therefore they are the only ones that respond to infection. If appropriate temperature and moisture conditions are available, fungal infection spreads rapidly through the cell walls and the empty lumens of fibres and vessels. The penetration of fungal hyphae into fibres, vessels and into axial and ray parenchyma is seen in three ways: 1) fungal hyphae penetrate through pit membranes and apertures of associated pit pairs; 2) they enter through perforation plates and pits of vessel walls thus passing from one vessel member to another; 3) the hyphae bore holes through cell walls. All fibres, ray and axial parenchyma cells of xylem contain one or more hyphal strands (Fig. 1F) and the lumens filled with multiple hyphae is not an uncommon feature. Similarly, vessels blocked with fungal mycelia and tyloses are also observed frequently.

Discussion

Formation of gum ducts in *Ailanthus excelsa* is not a regular feature in its wood, but mechanical injuries induce them after a certain period of time and not as an immediate response (SHAH & BABU 1986). However, trees were found exuding gum from the decayed portion of the stem and through old blazes. That occurrence developed a curiosity as to why only certain wounds exuded. Therefore, in the present study samples that showed exudation of gum were collected from infected trees of *Ailanthus*, which showed exudation of gum.

In many plants, formation of gum is a natural phenomenon, whereas in some others it is not a natural phenomenon but they do produce ducts in response to ageing, stress, wounding, and injury by insects or other pathogens, or to treatment with certain chemicals (BABU 1985). The ducts developing in response to injuries are also termed traumatic ducts. The development of traumatic ducts in the bark and wood of a few tree species has been studied by earlier workers (FAHN & ZAMSKI 1970, KIATGRAJAI & al. 1976, FAHN 1979, NAIR & al. 1981a,b, 1983). Many have reported certain chemicals like ethephone (2-chloroethyl phosphonic acid) to be agents inducing traumatic gum or gum resin ducts (HILLIS 1975, GREENWOOD & MOREY 1979, NAIR & al. 1980, GEDALOVICH & FAHN 1985). Such ducts are also developed in response to microbial infection (BABU 1985, BABU & SHAH 1987, BABU & al. 1987, GEDALOVICH & FAHN 1985).

In *Citrus* (GEDALOVICH & FAHN 1985) and *Bombax ceiba* (BABU & SHAH 1987) fungal invasion induces the formation of gum ducts. A sharp increase in ethylene concentration in stem segments of *Citrus* artificially inoculated with *Phytophthora citrophthora*, is correlated with the formation of gum ducts, and it has been assumed that ethylene produced by *Phytophthora* in the infected tissue directly influences the development of the ducts (GEDALOVICH & FAHN 1985). It has been inferred that higher levels of ethylene trigger a change in the differentiation pattern of the cambium from production of normal xylem elements to gum secreting cells (GEDALOVICH & FAHN 1985). Ethylene acts by inducing synthesis of hydrolytic enzymes in xylem mother cells. These enzymes dissolve the middle lamella between cells and this process leads to formation of the duct lumen. In the present study, the development of gum ducts is noticed from the cambium as well as from xylem derivatives close to the cambial zone of *Ailanthus*. A large number of microorganisms in culture are reported to produce ethylene and many plant infections are associated with production of ethylene (MAHADEVAN 1984). Thus, earlier investigators (GEDALOVICH & FAHN 1985, BABU & SHAH 1987) concluded that development of traumatic parenchyma and gum ducts/cavities in infected trees is mediated by ethylene. Formation of ducts/cavities in *Ailanthus* may be associated with the formation of ethylene in response to fungal infection. Traumatic parenchyma cells and adjacent rays remained unlignified though ethylene (GRISEBACH 1981, HIGUCHI 1981, MILLER & al. 1985) and infection (COLLENDAVELLOO & al. 1982, 1983) are supposed to promote lignification in trees. However, there are few reports that ethylene also inhibits lignification (GOODWIN 1978, RADEMACHER & al. 1984, BABU & al. 1987). It has been observed that in etiolated *Pisum*, ethylene inhibits xylogenesis and fibre lignification and the latter is resumed once ethylene is withdrawn. Similar observations are reported for *Acer saccharum* (RADEMACHER & al. 1984), in which concentration of lignin in wood parenchyma formed after wounding was lower than in those cells formed before wounding. It is also suggested that virulent pathogens can inhibit development of lignin barriers by diverting lignin precursors into other metabolic pathways (GRISEBACH 1981). It appears true in the case of *Ailanthus*, when epithelial cells and ducts/cavities are active in gum secretion, traumatic parenchyma cells do not undergo lignification and parenchyma cells show lignified walls only when the ducts are functionally inactive.

Formation of gum ducts from fully differentiated parenchyma cells of mature secondary xylem of *Azadirachta indica* and *Ailanthus excelsa* in response to chemical (ethephone) treatment was noted by NAIR & al. 1980, 1983, 1985 using histological and ultrastructural methods. In their studies, development of gum ducts/cavities is reported from the fully differentiated parenchyma cells of the xylem. Although the pattern of duct formation was the same, in the present study the gum ducts are developed from traumatic parenchyma cells of the xylem very close to the cambium.

In standing trees decay often occurs after wounding which creates an entry for the decay organisms (SHIGO 1984). Fungi may enter via broken branch stubs associated with natural or intentional pruning, via logging wounds, insect damage and so forth (MALOY & MURRAY 2001). In the present study it appears that the fungal mycelium enters the xylem through wounding. The occurrence of vessels occluded with tyloses and of fungal mycelia in all cells types of the xylem frequently noticed in infected trees. Compartmentalisation including the formation of a barrier zone in infected trees is the defense mechanism by which boundaries are formed to isolate injured tissues and thus resist spread of a pathogen. Living trees often react to limit (stop) pathogens by compartmentalising the infection site. This zone is characterised by non-conducting tissues that have few vessels and an abundance of axial and ray parenchyma while vessels are occluded with tyloses and gum. This barrier zone is a key boundary in the trees to stop an invasion. Compartimentalisation has two parts: (a) chemical boundaries are formed within tissues present at the time of injury and infection (b) chemical and anatomical boundaries are formed after infection as the living cambium responds to form a barrier zone between tissues present at the time of infection and new tissues (SHIGO & MARX 1977). In woody plants, gums, resin and tannin-like substances are often produced and deposited in the tissue, and may serve as a barrier against subsequent pathogen infection. Formation of these substances is correlated with a number of factors including injury, pathogen attack or climatic conditions (MALOY & MURRAY 2001). In the present investigation deposition of tannin (phenolics) in parenchyma cells and vessel blockage due to gum and tyloses in infected xylem are reported while no such deposition is seen in the xylem of normal trees. Wound responses causing vascular blockage are mediated by ethylene (FUJINO & al. 1983, MORROW & DUTE 1999, DUTE & al. 2002) while in the absence of ethylene there is no formation of wound material. For example, wounded rhizomes of *Botrychium dissectum* when incubated with an ethylene inhibitor (silver thiosulphate) did not develop wound material (MORROW & DUTE 1999). In the present study, formation and blockage of vessels due to gum also may be associated with the formation of ethylene in response to wounding. Trees once invaded by vascular pathogens such as *Fusarium oxysporum* initiate various resistant mechanisms such as tyloses or gum formation to impede vertical spread of the fungal propagules (TIPPETT & SHIGO 1981, SHIGO 1984).

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