ANATOMICAL RESPONSE AND RECOVERY OF TWIGS OF *JUGLANS NIGRA* FOLLOWING OVIPOSITION INJURY INFLICTED BY THE TWO-SPOTTED TREEHOPPER, *ENCHENOPA BIONTATA*

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ABSTRACT

Ovipositor wounds on one-, two-, and three-year-old twigs of black walnut were examined. The treehopper's ovipositor makes a longitudinal wound, which curves tangentially through the bark splitting the tissues along the cambial zone of first year twigs. Following oviposition in August to early October, there is some proliferation of wound periderm and callus tissue surrounding the wound. Following nymph emergence in May, extensive callus formation and cambial activity closes the wound and reestablishes the continuity of the vascular cambium. Third-year growth produces normal secondary xylem; externally a corky scar remains.

Keywords: Juglans nigra, wound repair, compartmentalization, periderm formation, anatomy.

INTRODUCTION[4]

Oviposition by the Two-spotted Treehopper, *Enchenopa binotata* (Say) on first-year twigs of *Juglans nigra* L. is not considered serious. However, the oviposition activities can produce twigs that appear, at least superficially, to be severely mutilated. Split two-year-old twigs revealed dark inclusions in the secondary xylem that had a direct positional relationship with external ovipositor scars. While only the bark is usually considered to be injured during oviposition (Wood and Patton 1971), the dark inclusions indicated a more extensive injury that involved both the vascular cambium and its derivatives.

Based on this observation, an anatomical study of response and recovery of black walnut twigs to ovipositor injury was undertaken as part of ongoing comprehensive research into the wounding and recovery of black walnut. The purpose of this study was to determine the extent of injury, the anatomical response, and the effectiveness of any compartmentalization phenomena (Shigo 1969; McGinnes et al. 1977) as a barrier to invasion of decay microorganisms into the vascular system. Efficient management of black walnut, for both nuts and wood products, requires knowledge of all facets of environmental influences on growth quality.

MATERIALS AND METHODS

Twigs of *Juglans nigra* that had extensive leafhopper ovipositor scars were collected in September 1976 from a walnut plantation site in northwest Lafayette County, Missouri. The ovipositor injuries were examined both macroscopically
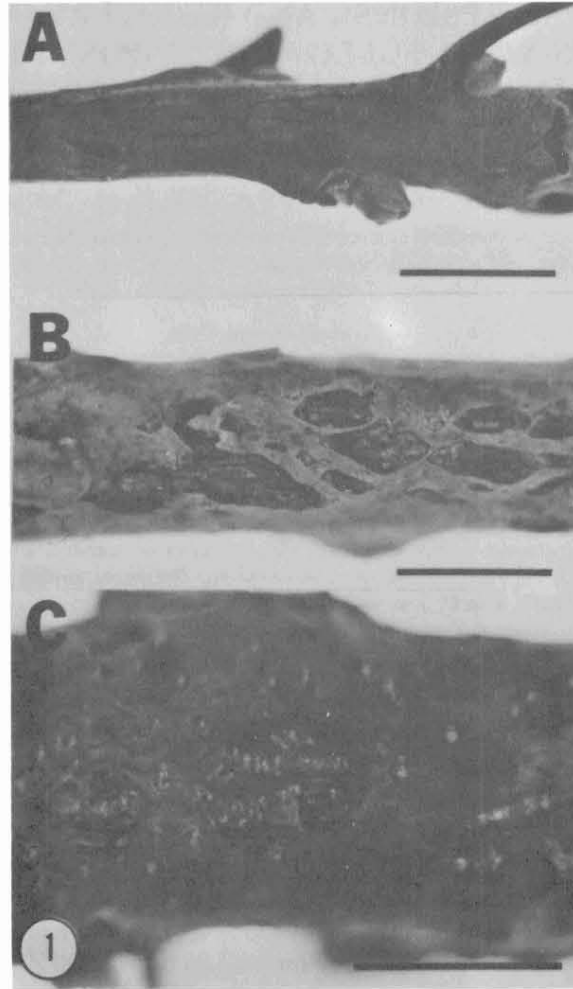
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FIG. 1. Treehopper ovipositor wounds and scars on young twigs of *Juglans nigra*. A. First-year twigs showing slit-like ovipositor wounds. B. Second-year twig showing scars from ovipositor wounds. C. Third-year twig showing two-year old scars. Bars equal 1 cm.

and microscopically. Sections were prepared for microscopic observation using traditional histological techniques. Some specimens were sectioned unembedded using a sliding microtome. Other specimens were fixed in FAA, dehydrated, infiltrated, and embedded in Paraplast Plus prior to sectioning. Selected sections were stained with iron-alum safranin (Gray and Pickle 1956) and 1% fast green FCF in 95% ethanol, or Sudan IV. The terminology describing the vascular cambium and associated tissues follows the recommendations of Schmid (1976).

RESULTS

Numerous first-, second-, and third-year twigs had prominent ovipositor scars (Fig. 1A–C), that were produced during the current season, one year ago, and two years ago, respectively. Continued periderm development obscured the in-
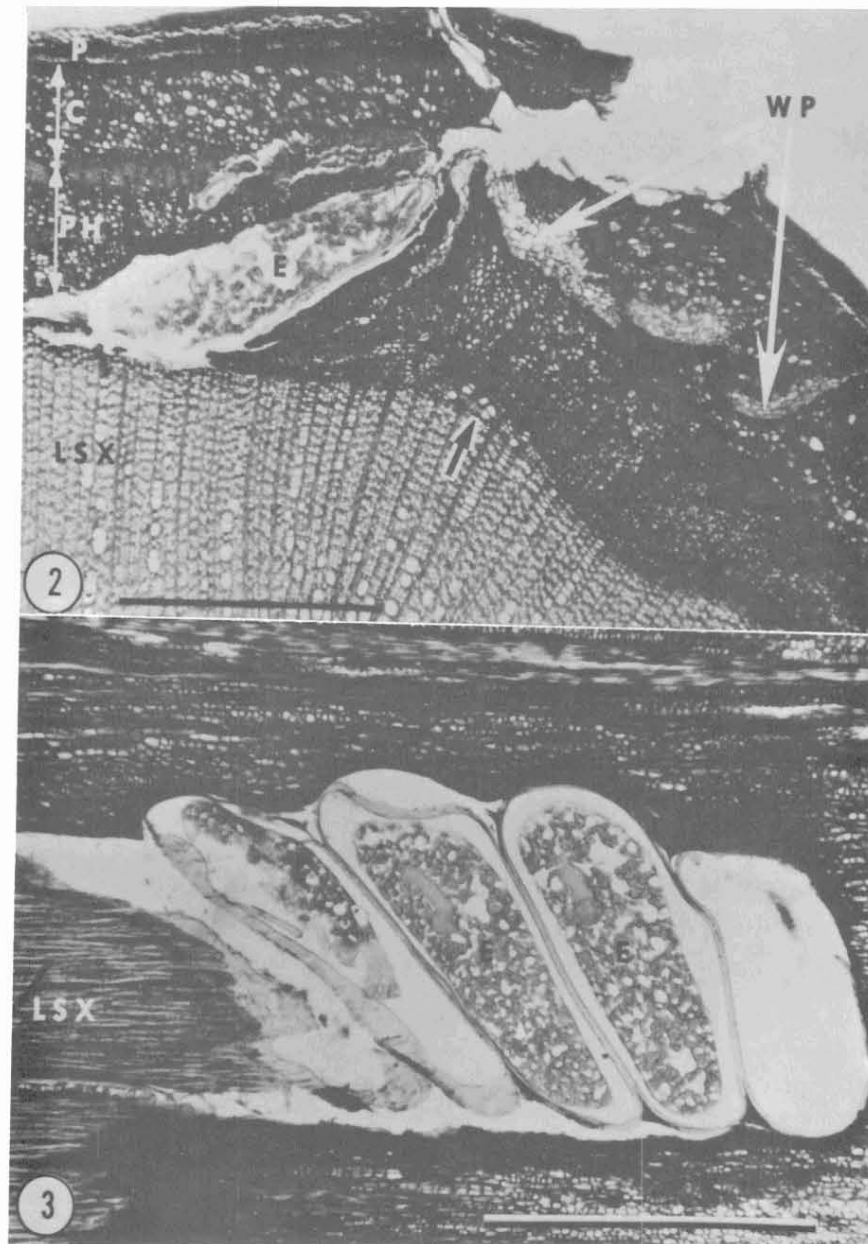
FIG. 2.   Transection of ovipositor wound in a first-year twig showing the placement of treehopper eggs and developing wound periderm. Note localized late cambial derivatives (black arrow). C-cortex, E-egg, LSX-late secondary xylem, P-periderm, PH-phloem, WP-wound periderm. Bar equals 1 mm.

FIG. 3.   Tangential section of a first-year twig showing the longitudinal orientation of the egg packet between the late secondary xylem and the phloem. Phloem derived callus tissue is immediately adjacent to the egg packet. Labels same as Fig. 2. Bar equals 1 mm.
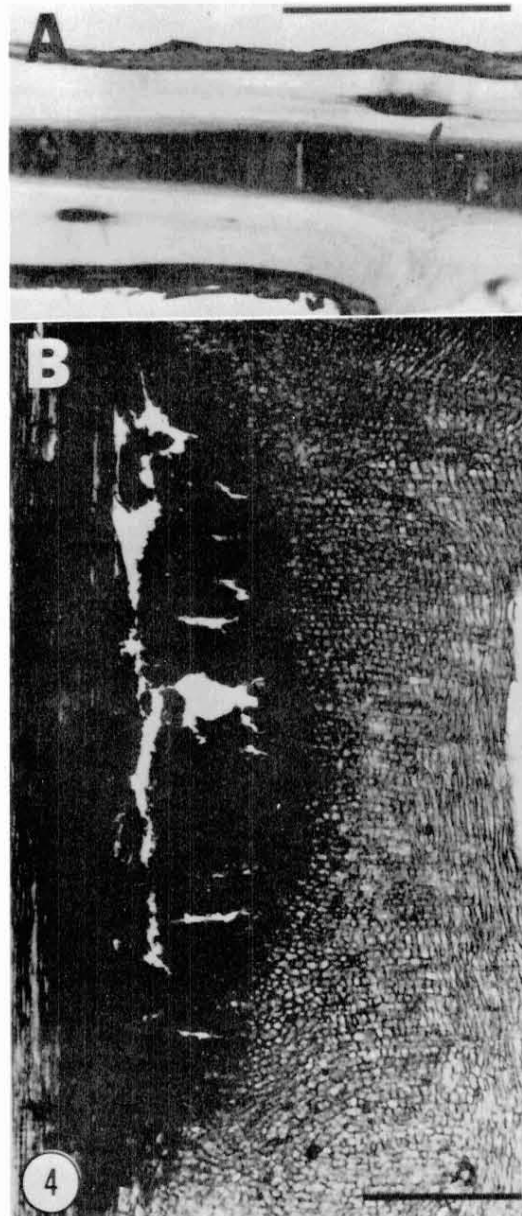
FIG. 4. A. Split two-year-old twig showing dark inclusions remaining from ovipositor wounds. Bar equals 1 cm. B. Microtomed longisection showing dark, occluded cells in the wound area and proximally (left) in the parenchyma cells of the late secondary xylem. Distally parenchymatous cambial derivatives form raylike tissue. Bar equals 1 mm.

jury scars on older twigs. The current year's ovipositor injuries were visible as longitudinal slits 5–7 mm long and generally oriented parallel to the long axis of first-year twigs. Occasionally ovipositor slits were found on petiole bases. A slight bulge beneath the slits indicated successful implantation of a packet of eggs. Ovipositor scars on second- and third-year twigs were larger, more prominent,

and displayed masses of corky tissue not unlike large lenticels (Fig. 1B–C). The ovipositor wounds angled tangentially, slicing through the cortex and phloem, then splitting tissues along the cambial zone (Fig. 2). In a few wounds, the ovipositor slits were sharply curved producing shallow wounds that did not penetrate beyond the bundles of primary phloem fibers. The packets of eggs were deposited deeply into the wounds such that the lower portions of the egg packet were in contact with the exposed face of the latewood (Figs. 2–3).

Following oviposition in August through September, some wound periderm and callus tissue were formed. Wound periderm formation was initiated in the vicinity of the primary phloem fibers. Differentiation of the wound phellogen continued extending beneath the proximal surface of the wound outward through the cortex and inward through the phloem (Fig. 2). Distally the wound phellogen became continuous with the normal stem periderm, and proximally with the vascular cambium. Limited cambial activity in the vicinity of the wound produced both parenchymatous callus derivatives and more normal cambial derivatives (Fig. 2, dark arrow). Small amounts of callus tissue may be formed by the ray parenchyma on the exposed surface of the secondary xylem. Wound periderm formation along the distal surface of the wound was delayed, although there was callus tissue formed by phloem parenchyma cells. Some occluded axial and ray parenchyma cells were found near the exposed surface of the secondary xylem. A few tylosed latewood vessels may be present.

Second-year twigs (Fig. 1B) exhibited dark inclusions at the increment boundary between the first- and second-year's secondary xylem (Fig. 4A). The inclusions were radially internal to ovipositor scars. Longitudinal sections through the inclusions showed dead and occluded cells and a portion of the injury recovery tissue (Fig. 4B). Proximally the first-year's latewood contained occluded ray and axial parenchyma cells and tylosed vessels. Occluded callus-like cells formed the bulk of the dark inclusion. Distally anomalous cambial derivatives gradually were reorganized into normal secondary xylem derivatives (Fig. 4B, right). No material of insect origin was ever observed or recognized in the wound area following nymph emergence.

In transverse sections, the fissure between the first-year's and second-year's secondary xylem shows the extent of cambial disruption. The wound was closed by a proliferation of callus tissue derived from extant cells in the cortex, phloem, and cambial zone. Wound periderm differentiated immediately adjacent to necrosed tissues on the inner exposed surfaces of the wound (Figs. 5, 6). The wound periderm cells lacked conspicuous contents and had relatively thin walls. These cells did have the characteristic birefringence and positive Sudan IV staining indicative of the deposition of suberin and other waxy substances on the cell walls (Fig. 7, cf. Figs. 5, 6). Distinct lamellae were not visible indicating that there were relatively few layers of suberin deposited on the cell wall.

Lateral to the wound proper, the vascular cambium produced abnormal derivatives for a short period of time. Starting at the lateral edges of the wound and gradually continuing toward the center, a vascular cambium differentiated from the callus tissue continuous with the intact vascular cambium. In Figs. 5 and 6 the vascular cambium has not differentiated across the darkly stained parenchyma cells in the center of the wounded area (Fig. 6, arrows). Externally large numbers of parenchyma cells were produced beneath the oviposition slit. In all wounds
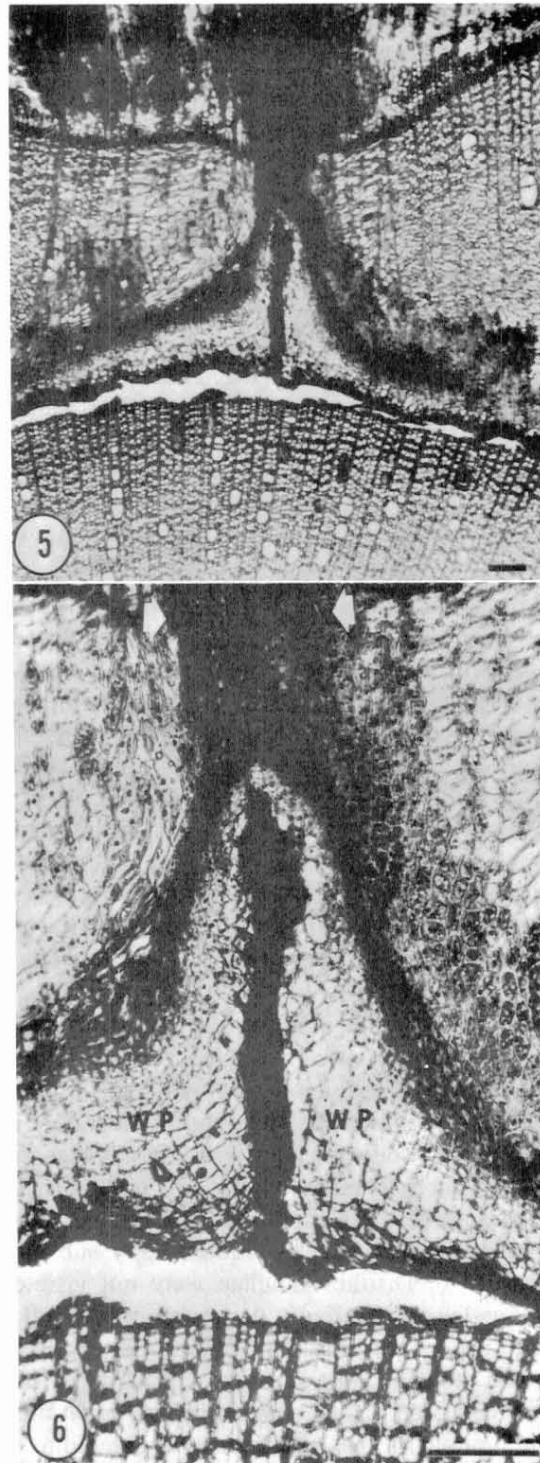
FIG. 5. Transection showing wound closure, extent of cambial zone disruption, and occluded parenchyma cells and tylosed vessels of the late-formed secondary xylem. Bar equals 100 micrometers.
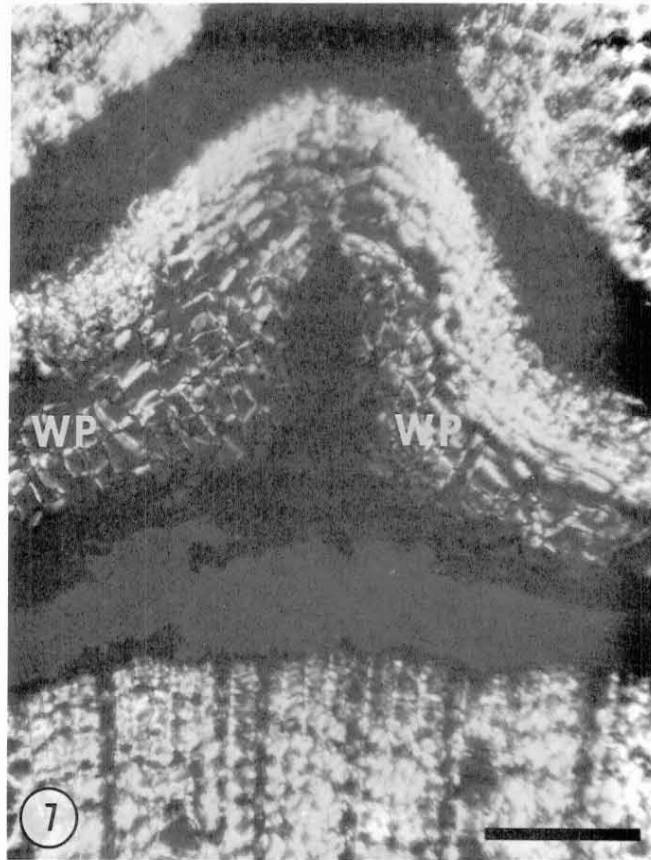
FIG. 7.   Same transection as Figs. 5–6 viewed through crossed polarizing filters, showing birefringence of the thin-walled wound periderm (WP). Bar equals 100 micrometers.

examined, the vascular cambium was complete or nearly complete across the wounded area by the end of the second-year's growth, about four months after the emergence of the nymphs. Subsequent growth produced normal secondary xylem and phloem (Fig. 8), although externally a prominent scar remained. A new stem phellogen differentiated in the proximal portions of the wound-induced parenchyma (arrows).

DISCUSSION

Most of the ovipositor wounds examined showed severely disrupted activity of the vascular cambium in the immediate vicinity of the wound. Less severe wounds may represent probing and unsuccessful oviposition attempts. Typical treehopper ovipositor behavior produced a slit completely through the bark, com-

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FIG. 6.   Detail from Fig. 5 showing wound periderm (WP), closure of the wound, and gradual reorganization of the vascular cambium. Continuity of the vascular cambium will be reestablished following differentiation of the parenchyma cells between the arrows. Bar equals 100 micrometers.
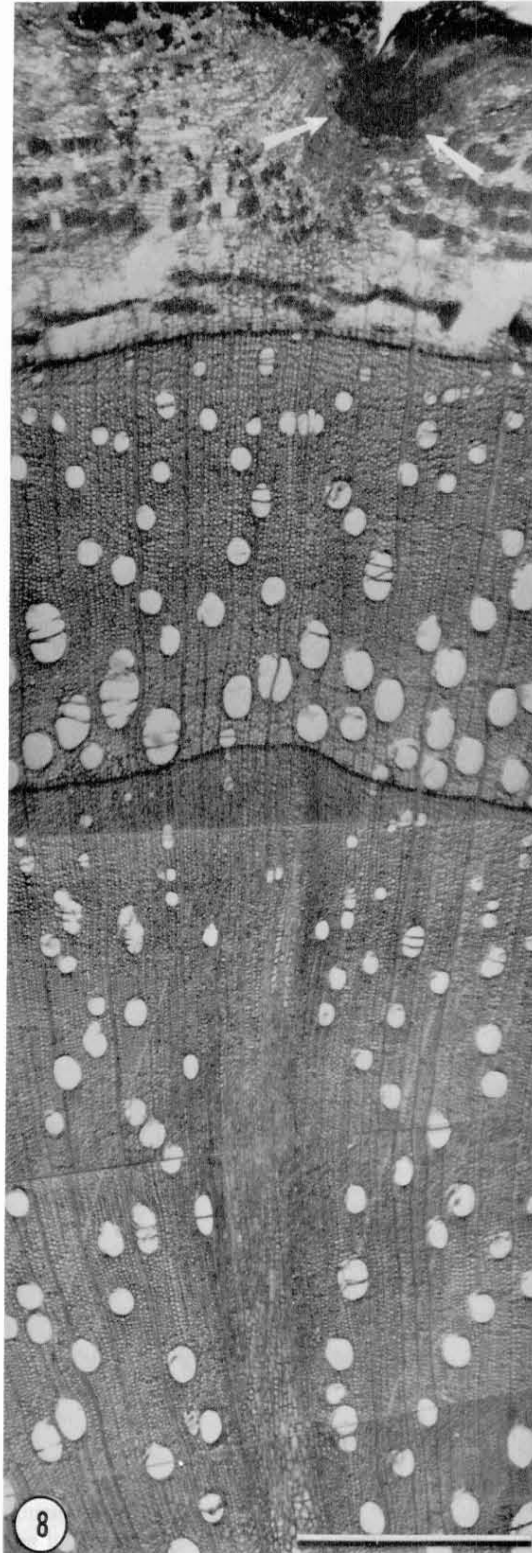
posed of the cortex, phloem and, externally, a small amount of periderm, in these juvenile twigs. The bark was split tangentially, away from the wood, along the cambial zone. The wedgelike action of the ovipositor would be expected to split tissues along a path of least resistance, offered in this case by the thin-walled cells of the vascular cambium and its recent derivatives.

Anatomical evidence showed that the wounds are well compartmentalized and recover quickly, usually in one growth season. This is undoubtedly because of the large proportion of live parenchyma cells present in the tissues of juvenile stems and their ability to react to injury. The proximal wound compartmental wall is formed by occluded parenchyma cells and tylosed vessels (Figs. 4A–B, 5). The distal compartmental wall is composed of occluded parenchyma cells and wound periderm cells. The compartmentalization and periderm production observed in this study agree with the pattern of nonspecific wound responses described in coniferous species (Mullick 1977). There was no evidence of any pathogenic microorganisms introduced through these wounds even though SEM observations confirmed the presence of bacteria among the egg packets.

CONCLUSIONS

In general, while not massive, the ovipositor wounds are less of a superficial feature than previously considered. This is best illustrated by the prominent dark inclusions remaining in the secondary xylem (Fig. 4A). The large corky scars may persist for a year or two after the wound has been compartmentalized and normal cambial activity reestablished. Sparse treehopper activity would seem to have insignificant detrimental effects on black walnut. However, extensive activity (Fig. 1B) would certainly result in a loss of vigor and growth rate, especially in seedlings, because of the total wound repair effort.

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FIG. 8. Transection of a three-year-old stem showing a full year's growth following complete wound recovery. Superficial scar remains, radially aligned with raylike wound recovery tissue. New stem phellogen is differentiating proximal to the wound scar (arrows). Bar equals 1 mm.