South African Avocado Growers' Association Yearbook 1999. 22:39-47

Reaction of avocado wood on injections into the trunk

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ABSTRACT

Sealing holes after injection of air or water did not decrease damage caused to avocado wood. The un-neutralized solutions of a 10%, as well as a 15% H_3PO_3 injection tended to cause less damage than the neutralized solutions while un-neutralized and neutralized solutions of a 20% H_3PO_3 injection caused similar levels of damage. Addition of $ZnSO_4$ or Solubor alone did not increase the damage to the wood.

Although avocado wood seems to have the ability to heal and form new functional wood where damage, due to injections occurred, minimum injections necessary to maintain tree health should be used in order to minimise damage to wood over the long term.

INTRODUCTION

Since Darvas and co-workers reported in the 1980's (Darvas *et al,* 1983) that Phytophthora root rot of avocados could be controlled by injecting trunks of diseased trees with Aliette, the method became general practice. At present avocado trees, depending on tree condition, are injected 1-3 times per year. An anatomical investigation into the effect of injections on avocado wood has not been done before. In this report however, wood of avocado trees after being injected once (two holes per tree) with different solutions, were inspected. Wood of a tree which was injected over a long period was also examined to determine the long term influence of injections.

MATERIALS & METHODS

Four year old Hass trees (on Duke *7* rootstock) at Westfalia Estate, to be removed due to tree crowding, were used for the trial. Trees were injected with different compounds and at different concentrations (Table 1). A 25 year old Fuerte tree (on Guatemalan seedling rootstock) which was injected over a long period was also examined. Most of the injection holes were drilled in a horizontal, radial direction, i.e. in the direction of the central core of the trunk. Two weeks after injection, the trees were cut down at the height where the injections were applied, and a disc of the wood (including the injection

hole) was cut from each trunk. Each disc of wood was fixed in FAA for preservation. The wood discs were then inspected anatomically at the University of Pretoria. A smaller sub-sample of the wood (including the discoloured tissue around the hole) was taken from each wood disc and split tangentially, so that tissue close to the bark and the core could be inspected separately. A rough sketch of each wood disc and sub-sample was made for easy interpretation of results (not shown). Transverse, radial and tangential sections (each replicated three times) were made of each sub-sample with a sliding microtome, as illustrated in Figure 1. The sections were stained with Safranin and Fast Green, (O' Brein & McCully, 1981) and mounted in Entellin.

Table 1. Injection treatments applied

TREATMENT*	NUMBER
Air + hole sealed	1a
Air	1b
Water + hole sealed	2a
Water	2b
10% H_3PO_3 unneutralized (pH = 0)	3a
10% H_3PO_3 neutralized with KOH (pH = 5.5)	3b
10% H_3PO_3 neutralized with NaOH (pH = 5.5)	3c
10% H_3PO_3 neutralized with K_2CO_3 (pH = 5.5)	3d
15% H_3PO_3 unneutralized (pH = 0)	4a
15% H_3PO_3 neutralized with KOH (pH = 5.5)	4b
20% H_3PO_3 unneutralized (pH = 0)	5a
20% H_3PO_3 neutralized with KOH (pH = 5.5)	5b
20% H_3PO_3 neutralized with NaOH (pH = 5.5)	5c
10% H_3PO_3 neutralized with KOH (pH = 5.5) + $ZnS0_4$ (6g/ℓ)	6
10% H_3PO_3 neutralized with KOH (pH = 5.5 + Solubor (30g/ℓ)	7
10% H_3PO_3 neutralized with KOH (pH = 5.5) + Solubor (30g/ℓ) + Vercene (6.8g/ℓ) + $ZnSO_4$ (6g/ℓ)	8
Wood of a tree, injected for several years (as treatment 8)	9

* All injection holes were left unsealed, unless otherwise specified (it was then sealed with silicone sealant immediately after injection)

RESULTS & DISCUSSION

The three dimensional structure of wood required all three planes of the wood to be inspected, and therefore three separate sections, namely transverse, radial and tangential sections were made (Fahn, 1982).

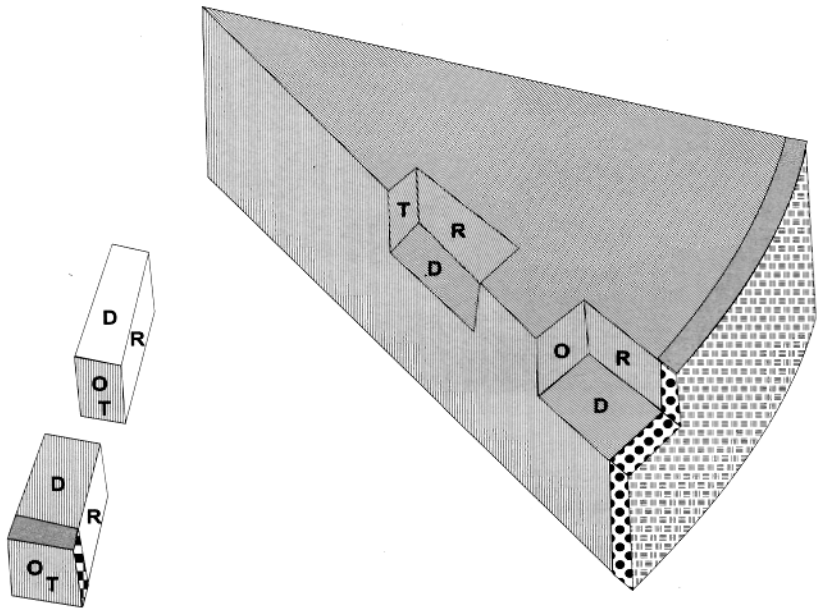

Figure 1: Schematic illustration of a wood disc indicating where the sub-samples were cut and how the sections were made. D = transverse section; R = radial section; T = tangential section

Transverse section (Fig. 2A)

The vessels were distributed as single vessels or in radial groups of two or three with radial wood rays consisting of two cell layers. Relatively small axial parenchyma cells bordered the vessels. Oil cells which had a slightly smaller diameter than the vessels, were also distributed in between wood vessels. Fibres were thick walled.

Tangential section (Fig. 2B)

Wood vessel elements (single cells of the compound wood vessels) contained single perforations, with alternately arranged rough pits. The axial wood parenchyma [cells] adjacent to the vessels were relatively few. Rays were biseriate heterocellular, with oil cells above and/or below. Oil cells sometimes occurred scattered between the rays. The oil in the oil cells gave a characteristic aroma to the wood, as in the case of related indigenous stinkwood (Kromhout, 1975). Fibres were thick-walled and septate.

Radial longitudinal section (Fig. 2C)

The radial view of axially elongated cells showed little variation from those in Fig. 2B, although the radially elongated ray cells were cut lengthwise. Three cell types could be distinguished in the rays, namely long cells, square cells and oil cells. The axial parenchyma cells and some fibres were filled with starch grains.

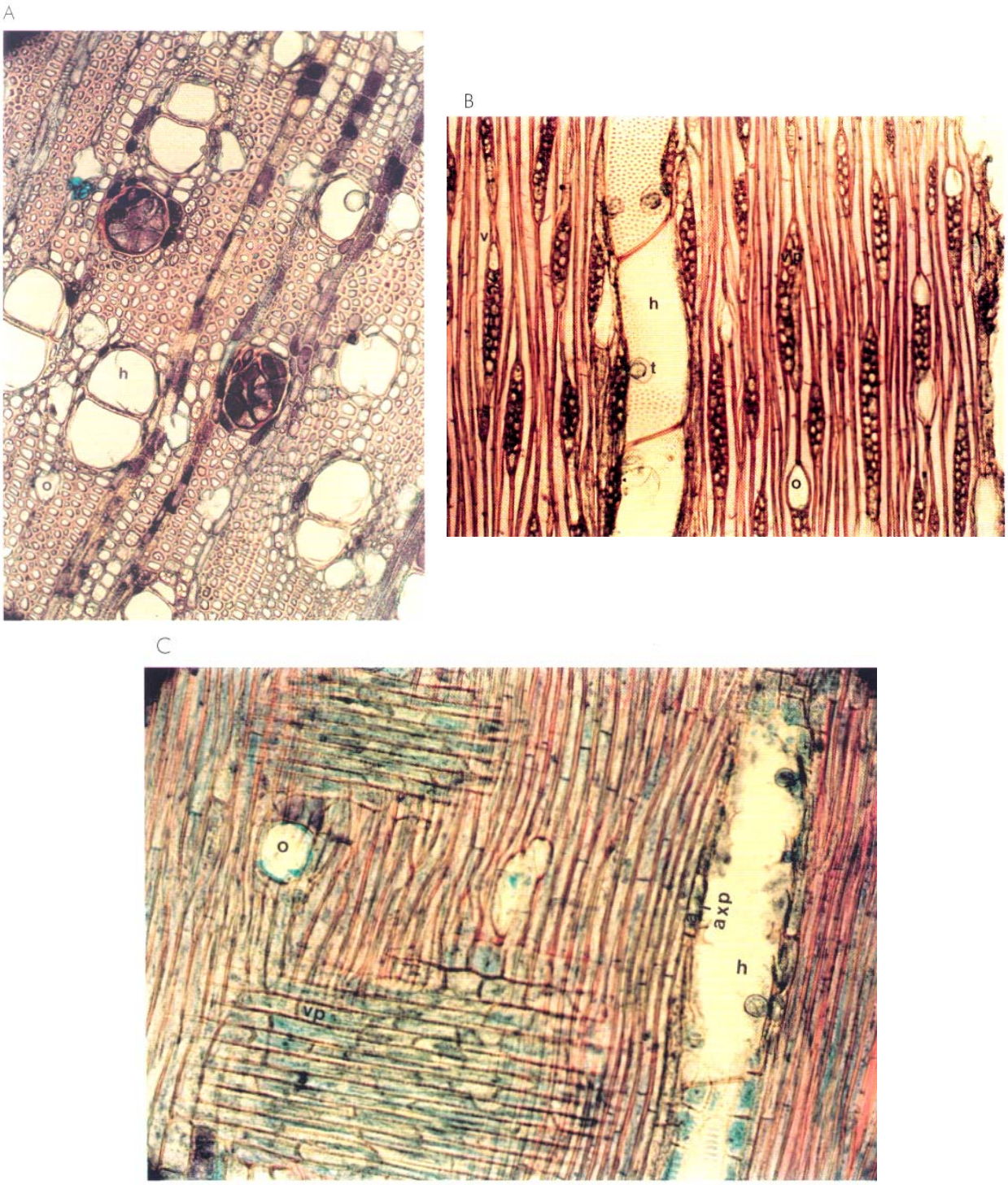

Figure 2. Three light micrographs indicating the structure of avocado wood. A = transverse section; B = tangential section; C = radial longitudinal sections. h = wood vessel; v = fibre; axp = axial xylem (wood) parenchyma; vp = ray parenchyma.

In all three views, single tyloses (vesicles) could be seen in the vessels. The tyloses were formed after wood vessels lost their function and internal pressure, with the result

that the contents of the neighboring axial parenchyma cells "inflated" the pit membranes into the vessels (Fig. 3). Pits normally function as valves through which liquids and solutes from the wood vessels, move to the axial parenchyma cells. After formation of tyloses, the vessels are blocked, resulting in both vessels and the adjacent parenchyma losing their function.

Effect of injections on the wood anatomy

After the injection, wood surrounding the hole turned brown. In a section, perpendicular to the injection hole (tangential), brown discolouration was visible at the top, bottom and both sides of the hole. On either side of the hole, the brown discolouration ended in a darker brown line. A similar line occurred at the top and bottom, as well as at the end of the hole. The brown stained area around the hole was more or less wedge-shaped with a darker border. The following observations and conclusions were made from wood around the injection hole:

All the cells (vessels, fibres, axial wood parenchyma, as well as oil cells) of the tissue directly bordering the hole (in other words the lighter coloured area), were dead with little or no cell contents. The exception was the ray parenchyma, of which some were discoloured to slightly purple (Fig. 4A). Due to the fact that the tissue directly around the hole was slightly stained (from now on referred to as **washed-out area),** the automatic camera used for taking photographs, over compensated with the result that Fig. 4 A was over exposed. The darker brown border where the browning ends, is referred to as the **reaction zone,** because this is the area where the wood reacted to the injection compound and limited its spread. In the first wood vessels of this area, closest to the injection hole, tyloses were formed. The live parenchyma cells apparently were killed before complete blocking could occur, and therefore the blocking (tyloses formation) in adjacent wood vessels was more obvious (Fig. 4B and 4C). The axial wood parenchyma (Fig. 5A) and ray parenchyma in the reaction zone (Fig. 5B) showed mixed staining due to phenolic compounds which were formed in reaction to the injection compound. Gum secretions also formed in the vessels and ray cells (Fig. 5C). Further away from the hole, the tissue was apparently not affected much, and only scattered tyloses in the vessels were observed. Due to the large number of sections which were examined and in an effort to enhance the objectivity, a numerical grading system was used for evaluations. For each section examined, the following ratings were done:

a. The **intensity of stained cells** in the reaction zone was rated on a scale from 1 -5.

   Explanation: Safranin mainly stains lignified cell walls red and cytoplasm a slight pink-red colour, while the green dye stains cellulose cell walls green and undamaged cytoplasm, light green. Phenolic compounds are formed in the vacuoles of the affected cells. These compounds stain more intensely and can be blue, green or orange. Damage is thus also related to the intensity of staining.

b. The **starch content** of the parenchyma cells and fibres was rated on a scale of 1 -5.

Explanation: Reserve carbohydrate is stored in the form of starch grains in the live parenchyma cells and sometimes in live fibre cells. Where damage to the tissue was to the extent that cell membranes disintegrated, starch grains were washed out and little or no starch remained in the cells.

c. **Gum secretions** in wood vessels were rated on a scale of 1-5.

Explanation: Gum usually is not present in undamaged tissue, because it is not formed in special cells. Gum secretions in wood vessels are therefore an indication of damaged axial wood parenchyma (Fig. 5).

d. **The number of vessel elements per section containing tyloses,** but not blocked by tyloses (Fig. 3), were counted and expressed as a percentage.

Explanation: As already mentioned, tyloses are extrusions of the pit axial parenchyma. Tyloses are only formed in vessels which have lost their function, but where the xylem parenchyma could still react. The wood vessels with tyloses usually occurred some distance away from the injection hole. This area of the wood usually stained brown in fresh wood. Directly adjacent to the hole, the tissue was damaged to such an extent that no cell contents were present and therefore no tyloses could be formed. Tyloses therefore were formed in the reaction zone, which lies some distance away from the hole where the parenchyma cells were not damaged to the extent that they could no longer react (Fig. 4).

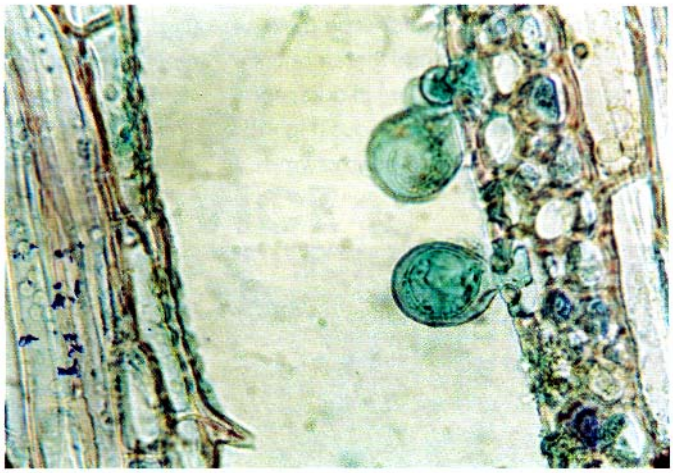

Figure 3. Enlargement of a portion of a wood vessel and bordering tissue showing tyloses.

e. The **number of vessel elements that were blocked with tyloses** was counted (Fig. 4)

Explanation: Some wood vessels in the reaction zone were totally blocked with tyloses, which probably was the result of drastic reaction by the wood to the injections

For the criteria where ratings were done (scale 1-5), ratings of the three replicates of each sub-sample were added (resulting in a maximum possible value of 15). The

average values of the evaluations are given in Figures 6, 7 and 8.

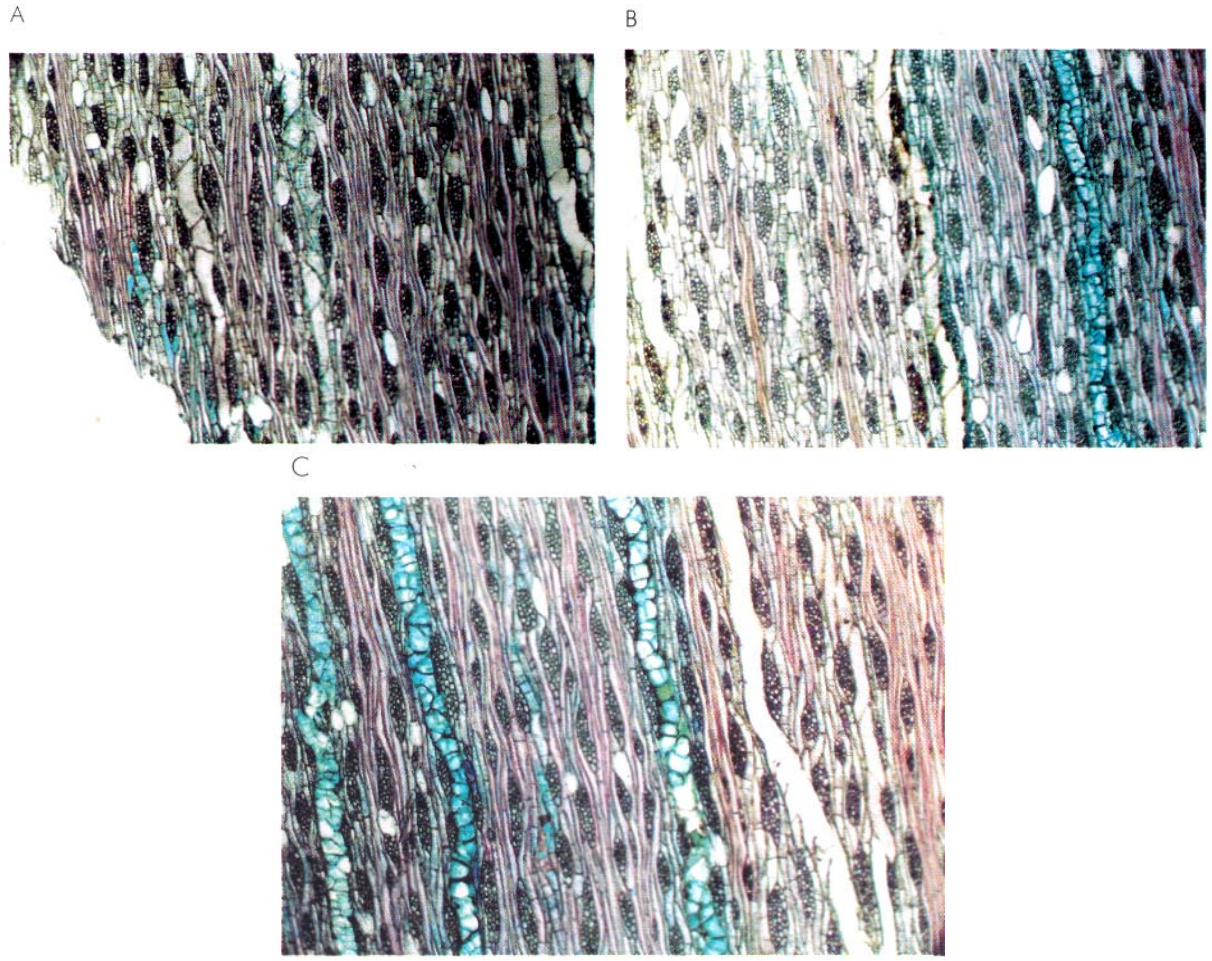

Figure 4. Three light micrographs (A.B.C taken of wood from sample 3a) in tangential view indicating the damage of the tissue close to and further a way from the hole, as well as showing the ray parenchyman.

Tissues from trees injected with different compounds more or less showed the same reaction, namely washed out tissue around the hole followed by a reaction zone of blocked wood vessels and wood vessels with tyloses and stained wood parenchyma. Outside the reaction zone they were obtained from samples where holes were drilled exactly radially and where the tangential sections were then made perpendicular to the hole. The sections should be made exactly tangential, radial or transverse to enable identification of the cells. In cases where the holes were drilled skew, the sections also had to be made skew which made interpretation more difficult. It was not physically possible to cut all the sections to the same thickness, and thicker sections stained slightly darker than thin sections, resulting in differences in interpretation. However, the bar graphs serve to indicate differences. Approximately 10% of the vessels were blocked in treatments 3b and 3d, while 8-10% were blocked in treatments 2b, 3c, 4b, 5a, 5b and 8. In the rest of the treatments, the percentage blocked vessels were approximately 6%. In most treatments there was a fair relationship between the percentage blocked vessels and vessels with tyloses, except for treatments 3b and 9, where the percentage vessels with tyloses were much lower than the percentage

blocked vessels. The percentage vessels containing gum secretions varied from approximately 4% (treatments 1b and 9) to just below 6%. Only treatment 8 had 7% of the wood vessels containing gum secretions.

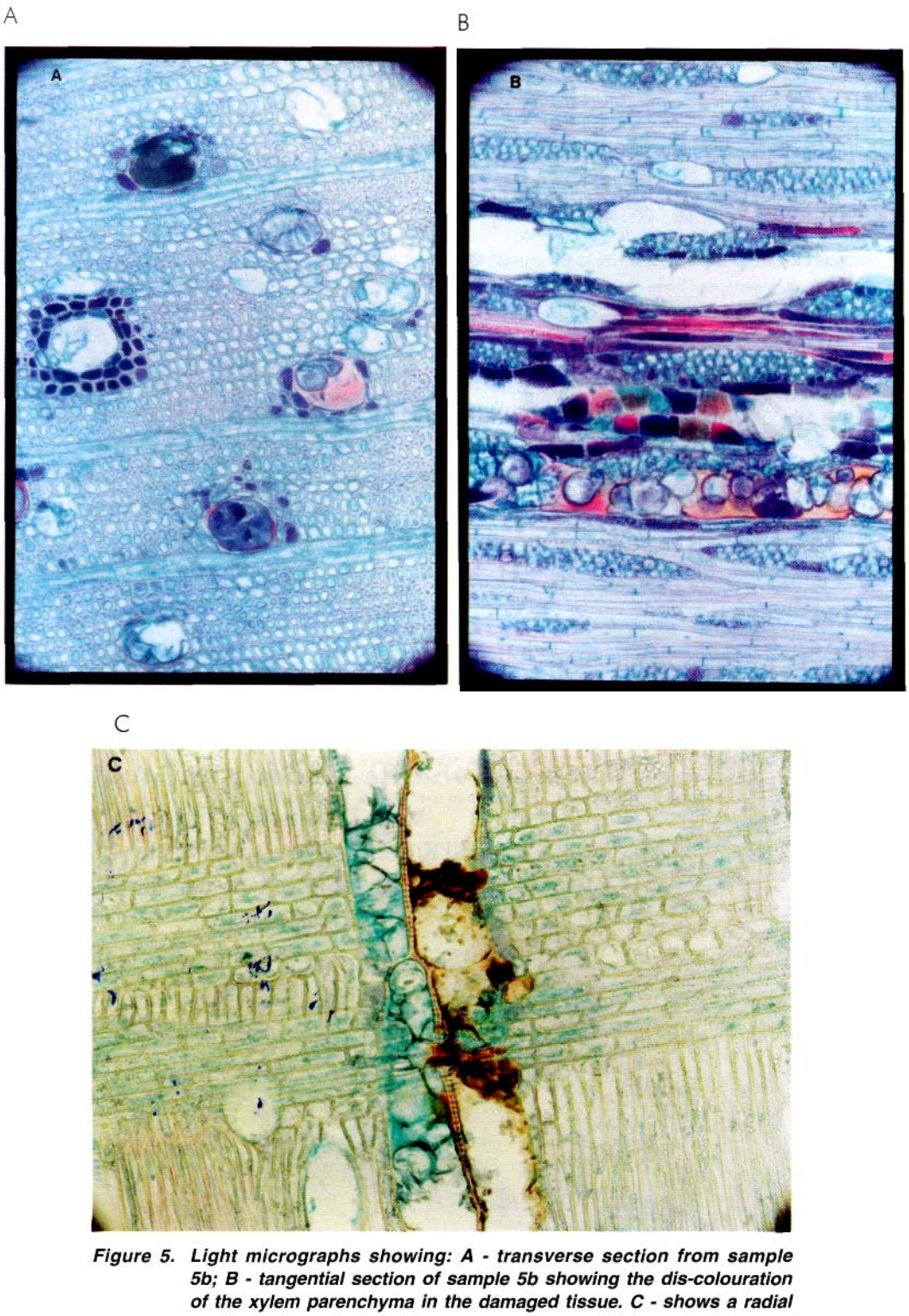

Figure 5. Light micrographs showing: A - transverse section from sample 5b; B - tangential section of sample 5b showing the dis-colouration of the xylem parenchyma in the damaged tissue. C - shows a radial section of sample 3a with gum secretions and tyloses in the wood vessels.

As mentioned before, starch grains are stored products that occur in live cells and one would expect a decrease in starch grains in damaged tissue. In fig.6, 7 and 8 it is shown that there was a slight negative correlation between the starch content of the tissue, and the percentage wood vessels containing tyloses and number of vessels blocked with tyloses. Treatment 1b which had the lowest percentage wood vessels with tyloses, also had the highest percentage cells containing starch grains.

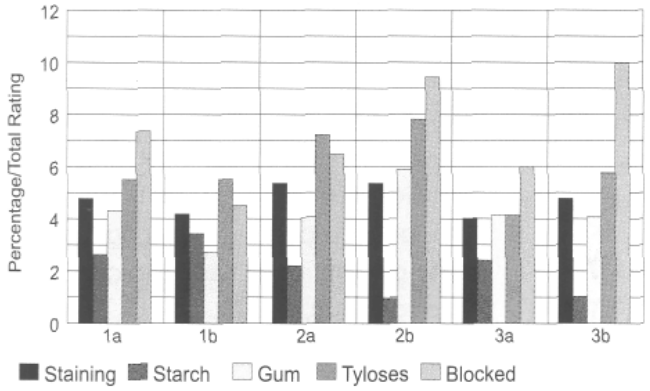

Figure 6. Damage to wood according to different parameters

It is important to mention that interpretation of the results was made without knowing what the treatments were. It could be seen that some samples were more damaged, e.g. treatment 3b, in which it appears that the reaction zone was not formed effectively resulting in the washed-out appearance of cells also beyond the reaction zone. In treatment 3b the reaction zone was also exceptionally wide, which could indicate that reaction to the injection compound took place over a longer period, or be an indication of an exceptionally detrimental effect of the injection compound.

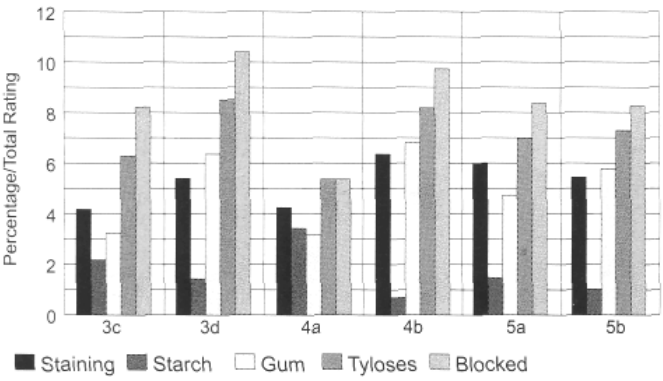

Figure 7. Damage to wood according to different parameters (continue)

The question still remained how the wood would react to repeated injections. To evaluate this, wood of a tree (trunk diameter approximately 34 cm), which was commercially injected over a period of several years, was also examined (treatment 9). The position of the injection holes and distribution of discoloured wood is shown in Fig. 9 and 10. The discolouration of wood tissue around the injection hole more or less corresponded with that of younger trees as described above. The discolouration extended to approximately 5 mm from the injection hole, but with older holes it extended to about 10-15mm. Where new holes were drilled in the vicinity of old holes, the browned areas coalesced. Interestingly, due to secondary growth in the thickness of the trunk, new healthy wood was formed over the injection holes and browned wood. The structure of the wood that covered the wounds is shown in Fig. 11. Directly above the wound, cambium developed from the wound callus and joined the original cambium, producing new healthy wood tissue. The fan-like bending of the radial wood rows, emphasizes the "effort" of the cambium to seal the wound with healthy tissue.

As can be seen in Fig. 12 the wood directly underneath and next to the old holes is darkly stained and not functional. The dark colouration is the result of tannins which formed in the tyloses of the wood parenchyma. This browning is not continuous, but is

limited to "islands" of stained wood progressing into unstained, functional wood further away from the injection hole. In fig. 9 and 10, it is shown that the brown coloured wood constitutes more or less one third of the diameter of the trunk. The rest of the wood is undamaged and probably still functioning.

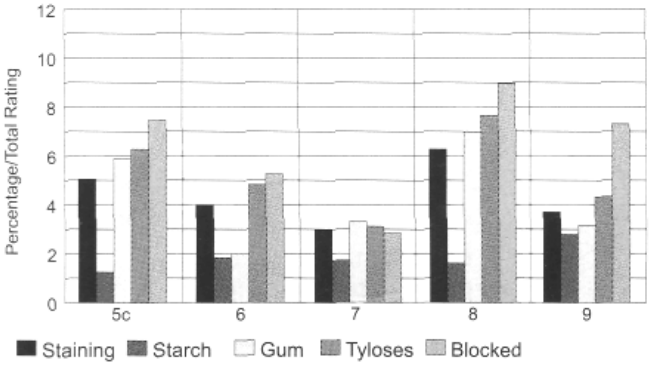

Figure 8. Damage to wood according to different parameters (continue)

In most types of trees the core wood (duramen) is not functional, while only the sap wood underneath the bark is functional. However, Fig. 9 and 10 indicate that the avocado wood examined, had not developed core wood. Figure 13, showing the sections made from the central part of the trunk, also shows that this central wood had not lost its function as transport tissue. Judging from Fig. 9 and 10, sufficient volume of newly formed and undamaged wood remained in the trunk to allow the tree to remain fully functional.

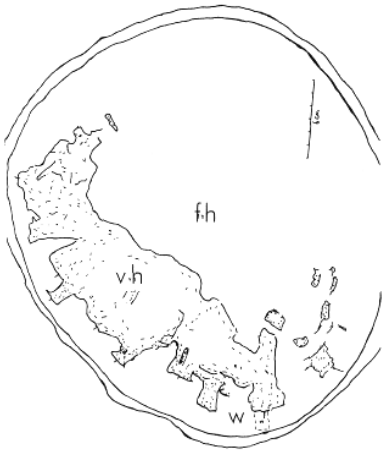

Figure 9. Diagram showing the top side of a disk cut from the trunk of a tree and repeatedly injected over a long period, indicating the distribution of discolored wood in a cross section: vh = discoloured, non-functional wood; fh = discolored, functional wood.

Figure 10. Diagram showing the bottom side of a disk cut from the trunk of a tree and repeatedly injected over a long period, indicating the distribution of discolored wood in a cross section: vh = discoloured, non-functional wood; fh = discolored, functional wood.

SUMMARY

Sealing the holes after injection of air or water did not decrease the damage caused to the wood. The unneutralized solutions of 10%, as well as a 15% H_3PO_3 tended to cause less damage than the neutralized solutions while unneutralized and neutralized solutions of a 20% H_3PO_3 injection caused similar levels of damage. Addition of $ZnSO_4$ or Solubor alone did not increase the damage to the wood, while addition of $ZnSO_4$ and

Solubor (and Vercene) tended to increase damage (this was not the case in the wood of the old tree). This difference between the reaction of the young and old tree may have been influenced by the differences in tree age, rootstock, and the scion cultivar.

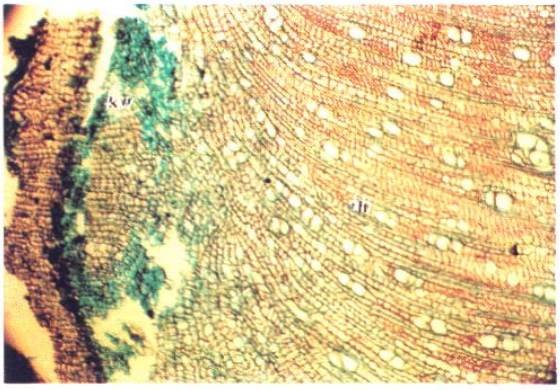

Figure 11. Light micrograph of functional wood (fh) - from the area marked w in Figure 9) that formed over the injection hole; kw - callus tissue.

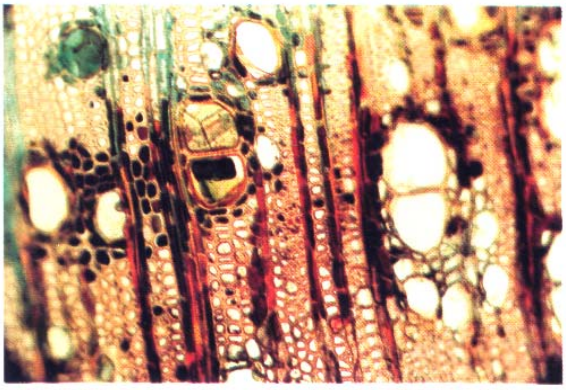

Figure 12. Light micrograph of a section of discoloured wood as shown in figures 9 and 10.

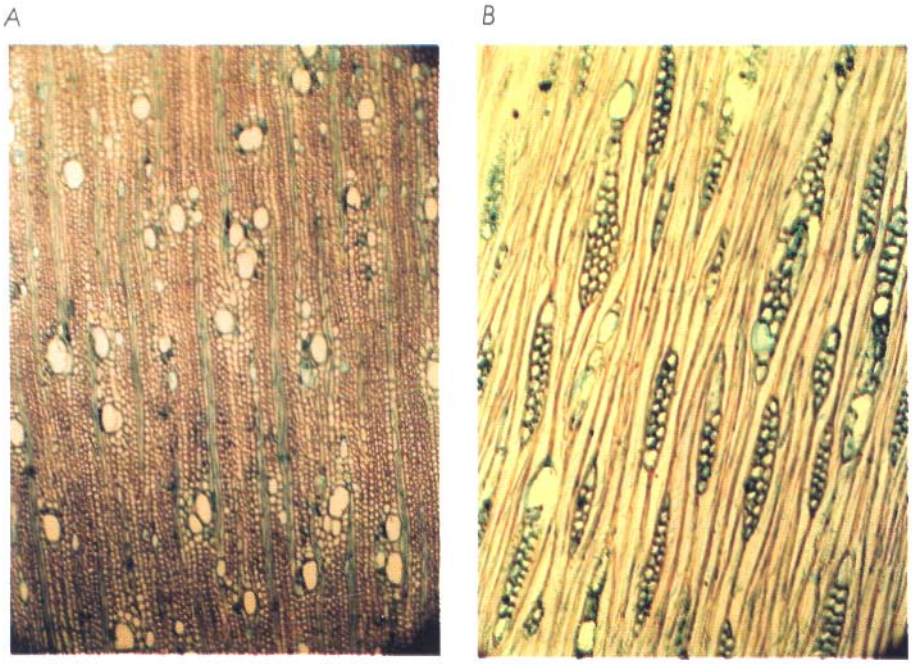

Figure 13. Light micrograph of functional wood from the central part of the old tree trunk (A Transverse section, B = Tangential section)

Although avocado wood seems to have the ability to heal and form new functional wood, the number of injection rounds applied per year should be limited to avoid excessive damage to the wood, thereby decreasing its effectivity. The minimum injections necessary to maintain tree health should be used in order to minimise damage to wood over the long term, so as to give optimum benefit to overall tree condition.

ACKNOWLEDGEMENTS

The authors wish to thank the management of Westfalia Estate for the use of experimental trees.

REFERENCES

DARVAS, J.M. TOERIEN, J.C. and MILNE, D.L. 1983. Injection of established avocado trees for the effective control of *Phytophthora* root rot. *Citrus and Subtropical Fruit Journal* 591:7-10

FAHN, A. 1982. Plant Anatomy. Pergamom Press, Oxford.

KROMHOUT, C.P. 1975. 'n Sleutel vir die uitkenning van die vernaamste inheemse houtsoorte van SuidAfrika. Bull. 50. Dept. of Forestry, Pretoria.

O'BREIN,T.P. & McCULLY, M.E. 1981. The study of plant structure, Principles and selected methods, Termarcarphi Pty Ltd. Wantirna. Australia.