

BACTERIAL CANKER OF AVOCADO

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Progress Report:

SUMMARY

*Five different bacterial strains, two of them belonging to the genus **Pseudomonas** were found associated with cankers on avocado trees. Koch's postulates were partly fulfilled with one of the isolates which exhibited streptomycin sensitivity. Avocado leaves and fruit were not parasitized by the **Pseudomonas** sp. and a typical Hr-reaction was not found on tobacco leaves.*

OPSOMMING

*Vyf verskillende bakteriese isolate is verkry uit kankeragtige letsels op avokado bome. Twee van hierdie isolate behoort tot die **Pseudomonas** groep. Koch se postulate kon tot 'n redelike mate bewys word met die een **Pseudomonas** sp. Hierdie isolaat vertoon streptomisien sen-sitiwiteit, is nie patogenies op avokado blare en vrugte nie en vorm geen Hr-reaksie nie.*

INTRODUCTION

In 1980 a canker of avocado (*Persea americana* Mill.) presumably caused by a bacterium, was reported from the North Eastern Transvaal (Myburgh & Kotzé, 1982). Subsequently a similar disease was observed in other avocado-growing areas of the Transvaal. Cankerosus lesions, typical of the disease, was originally observed only on stems and branches of the cultivar Hass, but occurs now also on Fuerte and Edranol. Cankers first appear at the base of the tree and then spread upwards and, to a lesser extent, downwards and side wards. Infected areas are slightly sunken and darker in colour than the surrounding healthy bark. Watery pockets with reddish brown tissue and fluid underneath the bark are associated with cankers. Narrow brown streaks extend into the tissue above and below the cankers. In spring and autumn a white exúdate surrounds the lesions (Fig. 1).



FIG. 1: Seven year old Hass tree with cankers at the base of the tree.

We have previously isolated various bacteria from the cankerous lesions and performed preliminary experiments to determine the pathogenicity of the various isolates (Myburgh & Kotzé, 1982). In this report evidence is presented on the Identity and pathogenicity of the causal organism, as well as on the *in vitro* effect of various antibiotics on the pathogen.

MATERIALS AND METHODS

Tissues from cankerous lesions and from healthy trees were fixed with glutaraldehyde and with osmium and then dehydrated with alcohol. Healthy and diseased pieces of tissue were gold plated and examined under the scanning electron microscope. Small pieces of the tissue 0,5 x 0,5 mm were embedded in Spurr's resin, sectioned and examined with the transmission electron microscope.

Small segments, 1x1 mm of infected tissue were cut from the thin brown streaks extending from the canker. The segments were macerated in drops of sterile distilled water and the suspensions were streaked onto nutrient agar and King B medium (King *et al*, 1954). Colonies that developed after 4 days (d) Incubation at 25°C were isolated and Identified according to Holding & Collee (1971).

Single cell isolates of the various bacteria were cultivated in nutrient broth at ambient temperature on a reciprocal shaker. After 24 h Incubation, the bacterial cells were centrifuged off at 500 r p m for 15 mm., washed three times with sterile distilled water and resuspended in sterile distilled water. One year old Hass trees were Inoculated with the various isolates by introducing the cell suspensions through slits cut in the branches. After inoculation, sterile cotton wool pads were placed on the slits and

taped onto the branches. Sterile distilled water was used as control and the inoculations were replicated five times. Results were recorded 4 weeks (w) after Inoculation.

The various bacterial isolates were evaluated for Hypersensitivity towards tobacco (*Nicotiana tabacum* L). Water suspensions containing 10^7 cells/ml were injected by means of a 16 mm syringe into the leaves of tobacco cultivar, White Burley. Sterile water was used as a control. The leaves were examined 48 hours after inoculation for the development of necrotic lesions. The *in vitro* effect on leaves and petioles of a fluorescent *Pseudomonas* sp. associated with avocado canker was determined. Detached young leaves and petioles were surface sterilized for 3 min. in 2,5% NaOCl, rinsed three times with sterile distilled water and placed on double layers of sterile moist filter paper in Petridishes.

Slits were made in petioles and leaves, a drop of the bacterial suspension was placed in each slit. A non-pathogenic *Pseudomonas* sp and sterile distilled water were used as controls. The leaves and petioles were examined regularly for disease development for a period of 7 w.

The same two isolates were evaluated for pathogenicity on avocado fruit. Fruit from the cv Hass, Fuerte and Edranol were surface sterilized for 3 min. with 2,5% NaOCl and rinsed three times with sterile distilled water. Holes were made on opposite sides of each fruit with a cork borer. A drop of bacterial suspension was placed in each hole and the holes were covered with sterile cotton wool. A control consisting of sterile distilled water was incorporated in the experiment. Half of the fruit was stored at ambient temperature (10° 22°C) and the rest at 25°C. Observations were made after 6 d.

A fluorescent *Pseudomonas* sp. isolated from cankerous tissue was tested *in vitro* for sensitivity towards bacitracin, ampiclox, oxytetracycline and streptomycin. Antibiotic assay discs containing the various antibiotics were placed on nutrient agar plates seeded with the *Pseudomonas* sp. After 48 hrs. incubation at 25°C the inhibition zones were recorded.

RESULTS & DISCUSSION

Electron Microscopical examination revealed that the cankerous tissues are associated with lots of gummosis, talls, starch and oil droplets which block most of the cortical cells (Fig. 2).

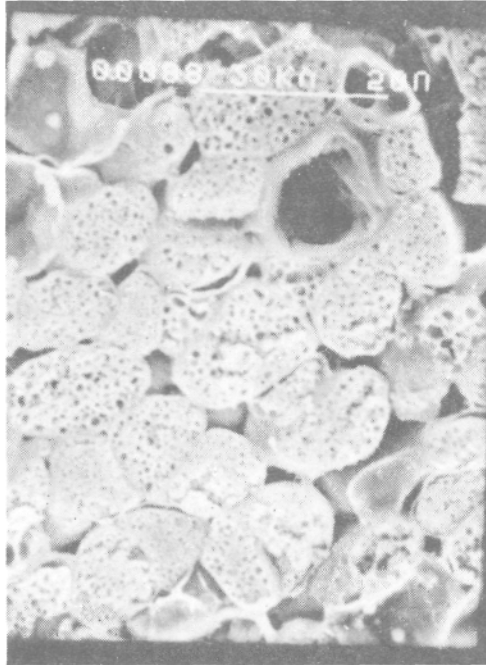


FIG 2: Cankeros tissue from a seven year old Hass tree showing the cortical cells blocked.

Five different bacterial strains, two of them belonging to the genus *Pseudomonas* were found associated with the cankers. Only one of the *Pseudomonas* spp. consistently produced cankerous lesions on avocado stems when inoculated artificially. This organism was reisolated from the artificially induced cankers and proved to be identical to the original isolate, when compared according to the API 20E identification system. Further investigations are at present being undertaken to establish the identity and pathovar of the isolate. Neither this isolate, nor any of the other produced necrotic lesions so far on tobacco leaves. All phytopathogenic *Pseudomonas* spp. thus far described give a hypersensitive reaction when inoculated on tobacco (Klement, Farkas & Lourekovich, 1964). The inability of the avocado isolate to produce lesions on tobacco seems to indicate that it is either an exception to the rule, that it is not a primary pathogen or that it has lost its pathogenicity. However, this experiment will have to be repeated with a fresh isolate before any definite conclusions can be made.

Avocado leaves, petioles and fruit were not parasitized by the *Pseudomonas* sp. This is in accordance with the situation in the field where the disease occurs essentially on stems and branches.

Streptomycin and oxytetracycline both inhibited growth of the avocado *Pseudomonas*, but a greater inhibitory action was exhibited by streptomycin than oxytetracycline (Fig. 3). Field studies are presently in progress to establish whether control of the disease can be achieved by these antibiotics.

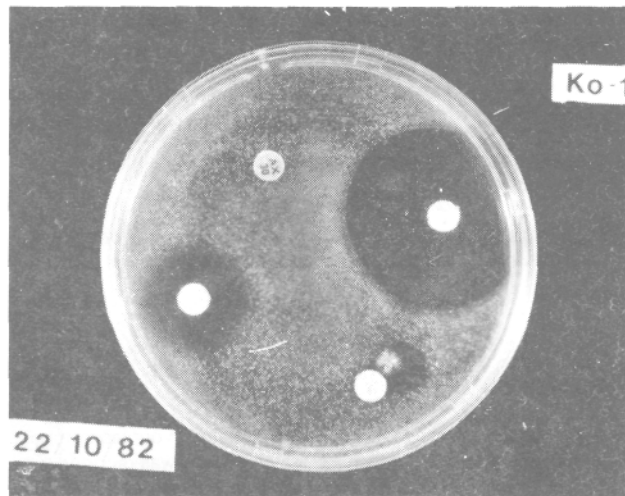


FIG. 3: The avocado *Pseudomonas* showing inhibitory action to Streptomycin (S10), oxytetracycline (T30), ampiclox (AX) and bacitracin (B10) *in vitro*.

REFERENCES

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