Possible role of fungi in negatively affecting fruit-set in avocados

T.V. Thomas,*§ Albert Eicker§ and P.J. Robbertse†

§ Department of Botany, University of Pretoria, Pretoria 0002, Republic of South Africa

† Department of Plant Production and Soil Science, University of Pretoria, Pretoria 0002, Republic of South Africa

Received 15 February 1994; revised 20 June 1994

Poor fruit set continues to be a major problem in the avocado industry in South Africa. In the past this was estimated to have caused a loss of income of millions of rands. In this investigation the role of fungi on the pistils of avocado cultivars after pollination was examined. Pollinated pistils of avocado cultivars 'Pinkerton', 'Ryan', 'Hass', 'Fuerte' and 'Nabal' were removed twenty-four hours after the first anthesis (female stage), for detection and isolation of fungi. Germinating conidia were seen on the surface of stigma adjacent to the germinated pollen grains. A number of dematiaceous fungi were isolated from the pistils and the most important species were identified.

When a drop of the spore suspension of the most frequently isolated fungi was inoculated on the stigmas of emasculated flowers after hand pollination, a high rate of abscission was observed. Thus some of these fungi appear to have played a significant role which resulted in greater abscission of flowers.

Swak vrugset is steeds 'n groot probleem in die avokado-industrie in Suid-Afrika. In die verlede was dit die oorsaak van miljoene rande se verlies aan inkomste. Hierdie ondersoek handel oor die rol van fungi wat na bestuiwing op die stempels voorkom. Bestuifde stempels van die kultivars 'Pinkerton', 'Ryan', 'Hass', 'Fuerte' en 'Nabal' is 24 uur na antese (vroulike stadium) verwyder vir die opspoor en identifikasie van fungi. Ontkiemende konidia is op die oppervlak van die stempels tussen die ontkiemende stuifmeel waargeneem. 'n Aantal fungi wat op die stampers voorgekom het, is geïsoleer en die belangrikste spesies is geïdentifiseer.

Nadat stempels van handbestuifde, ge-emaskuleerde blomme met 'n druppel gesuspendeerde spore van die mees algemeen geïsoleerde fungi geïnokuleer is, is 'n groot persentasie blomme afgewerp. Dit wil dus voorkom asof sommige van die fungi 'n betekenisvolle rol by die afsnoering van blomme gespeel het.

Keywords: Avocado, flower abscission, pollen competition, fungi on stigma.

* To whom correspondence should be addressed.

Introduction

Avocado (*Persea americana* Mill) has been cultivated commercially in the Republic of South Africa since 1920 (Durand 1990). It has been observed that many healthy trees produce few fruits, even during favourable seasons. The most favourable temperatures for fertilization and fruit set in avocado cultivars are between 28 and 33°C during the day and between 20 and 25°C at night (Sedgley & Annells 1981; Bringhurst 1951, 1952; Peterson 1956; Berg & Whitsell 1974). The Lowveld region of the eastern Transvaal, home to a large number of avocado orchards, has suitable weather conditions for the production of avocados. In most of these orchards the two functionally different trees (Type A and Type B) which belong to different avocado cultivars (Bringhurst 1951) are planted side by side to enhance fruit set. In spite of all this, a high percentage of abscission of flowers and young fruits are common in most of the orchards. Goldring *et al.* (1987) reported that a high rate of self-pollination and defects in the pollen (pollen viability) are the causes of massive abscission of fruitlets in the avocado cultivar 'Hass'. Scarborough and Smith (1977) observed that abortion of flowers in some plants occurs with a variety of pathogens including virus, bacteria and fungi. Clay and Jones (1984) reported that the fungi *Atkinsonella hypoxylon* (PK) infecting *Danthonia spicata* cause abortion of most of the flowers.

The increased abscission of flowers and young fruits in avocados might be due to certain factors which might affect the development of the pollen tube and fertilization in the flower. In this study, we analysed the possible role of fungi at the time of germination of pollen grains on the stigma, growth of pollen tubes in the style and ovary, fertilization and fruit set in the flowers of different avocado cultivars.

Materials and Methods

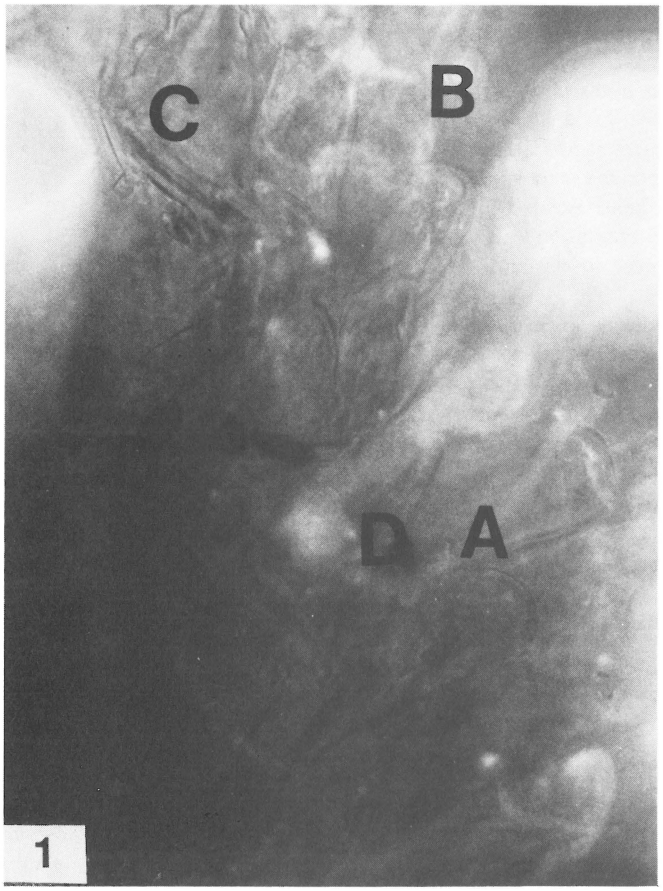
Ten trees of each of the cultivars 'Pinkerton', 'Nabal', 'Hass', 'Ryan' and 'Fuerte', were selected from orchards in the Lowveld region of the eastern Transvaal for investigation. As a control experiment, flowers from the selected trees of each of the avocado cultivars were covered with nylon bags before they opened. After the anthesis, stamens were removed from the flowers prior to the dehiscence of anthers. At the time of female anthesis the flowers were hand-pollinated and again covered with nylon bags. The number of flowers dropped were recorded and analysed.

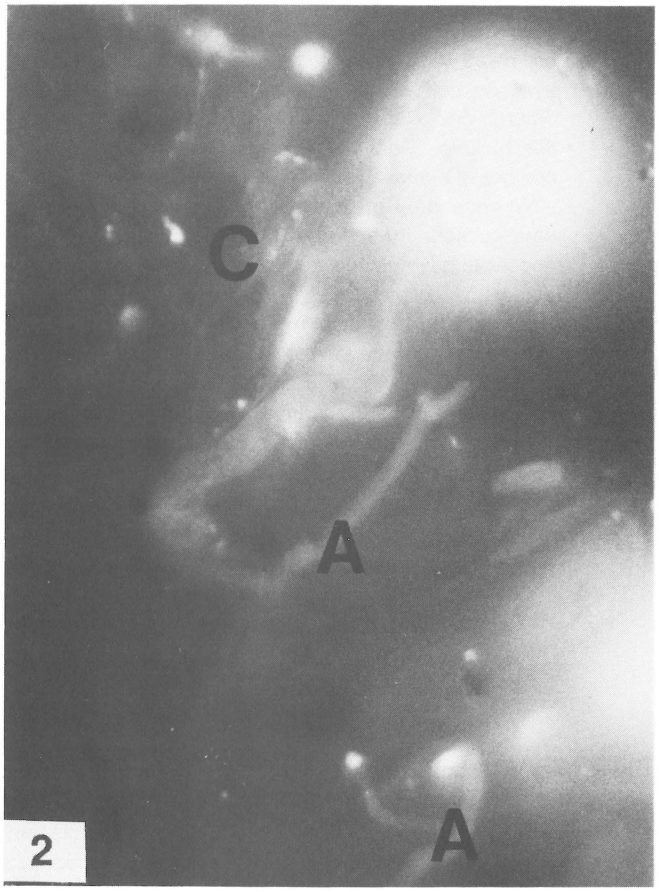
Hyphae detection on the stigma, style and ovary

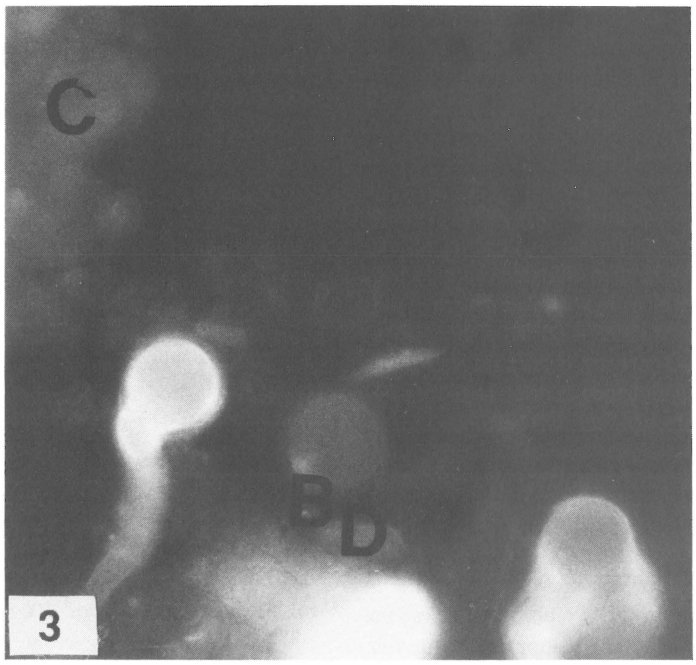
The pistils were treated according to the methods followed by Koske and Gemma (1989). The pistils of twenty-five flowers from each of the ten trees of the avocado cultivars 'Pinkerton', 'Nabal', 'Hass', 'Ryan' and 'Fuerte', were collected after the second day of their opening for detection of fungi. These pistils were fixed immediately in 50% ethyl alcohol for 6 h. For clearing the tissues, 3 g of fresh rinsed pistils were placed in 40 cm^3 of 2.5% aqueous solution of KOH (w/v) in 55 cm^3 test tubes (25 × 150 mm). The pistils and KOH were either heated in a water bath at 90°C for 30 min or autoclaved at 120°C for 3 min. After this treatment the pistils were washed in several changes of water. They were then acidified by soaking in 1% HCl for 12 h. The acidified pistils were then stained in acidic tryphan blue solution (500 cm^3 glycerol, 450 cm^3 H_2O, 50 cm^3 1% HCl containing 0.05% tryphan blue) and were again autoclaved at 120°C for 3 min. The tryphan blue solution was drained and the material was de-stained in acidic glycerol and kept at room temperature. The pistils were taken out and checked for fungi using a light microscope fitted with epi-fluorescence optics.

Fungal isolation

Isolation of fungi from avocado pistils was conducted according to methods of Fisher *et al.* (1986). Pistils of six flowers were removed from each of the ten selected trees of the avocado cultivars, 24 h after anthesis (female stage). The pistils were washed in running tap water for 10 min and then surface-sterilized by soaking in 20% commercial chlorax for 10 min, and finally rinsed four times in sterile water (Carroll & Carroll 1978; Petrini & Müller 1979). The pistils were cut into small fragments with a sterile knife. The fragments were then placed in 120-mm Petri dishes containing 2% malt extract agar, supplemented with 50 mg dm^{-3} oxytetracycline-hydrochloride. The Petri dishes were sealed and incubated at 20 – 25°C. The fragments were checked after the third day for fungal growth. Growing fungi were transferred into 60-mm Petri dishes containing 2% malt extract agar. The fungi were identified by the Mycology Department, Plant Protection







Figures 1 – 3 **1.** Light micrograph of germinating conidia on the avocado stigma after 24 h of female anthesis (×500). **2,3.** Light micrographs of hyphae on the tissues of avocado stigma after female anthesis (×500). A, Hyphae; B, Ungerminated pollen grains; C, Receptive tissues of stigma; D, Conidia; E, Hyphae growing into the tissues of the style.

Research Institute, Pretoria, and the Botany Department, University of Pretoria.

Spore suspension, preparation and inoculation

In order to ascertain the role of fungi after pollination the fungal spores were used to inoculate the stigmas of avocado flowers at the time of female anthesis. For this purpose the most frequently isolated fungi were selected and cultured in low-nutrient media to induce sporulation. Spores or conidia were collected in small vials containing 1 cm^3 of sterile water and stored at 6°C. Before inoculation the diluted spore suspension was heated in a water bath to 45°C. The heat shock was expected to promote spore germination on the stigma (Zaracovitis 1966). Prior to male anthesis, ten flowers of three selected trees of each cultivar were emasculated in the male phase and pollinated in the female stage by rubbing together the anthers taken from the neighbouring trees. After one hour the pollinated stigmas were inoculated with a drop of the spore suspension, using a sterile pipette. The number of flowers that dropped was recorded. Twenty-five of these dropped flowers from all cultivars were collected for the isolation of fungi, using the method mentioned previously.

The number of flowers dropped in all avocado cultivars which had been inoculated with the five different fungi after hand-pollination, was compared with the number of flowers dropped after hand-pollination only. The difference in the means between the two independent samples was statistically evaluated by Students *t*-test.

Results

Detection

The incidence of fungal invasion was high on open pollinated flowers collected from different trees of each avocado cultivar (Figure 4). The receptive tissues of many stigmas contained germinating conidia (Figure 1) and fungal hyphae with the pollen tubes of germinating pollen grains. The fungal hyphae were distinguished by their dark colour (Figures 2 & 3). The pollen tubes appeared to be short and hyaline in nature. Both ungerminated, and germinated pollen grains with short pollen tubes, were also seen on the surface of the stigmas of some of the pistils (Figures 1 – 3).

Isolation

From the pollinated flowers of 10 trees each of 5 different avocado cultivars, 305 isolations were made for identification. Nineteen different fungal taxa were identified (Figure 5), but the isolated mycoflora mainly consisted of deuteromycetes and ascomycetes. It was observed that some of the fungi were frequently present in all cultivars. Also more than one fungal taxon was isolated from a tree.

Inoculation

The inoculation of stigmas with the spore suspension of the frequently isolated fungi *Nigrospora oryza* (Berk & Br.), *Fusarium oxysporum* (Link ex. Fr.), *Pithomycis graminicola* (Roy & Rai), *Alternaria alternata* (Fr.) Keissler and *Cladosporium cladosporioides* (Fresen) de Varies, resulted in a high rate of abscission in all cultivars. All the inoculated fungi were isolated again when the pistils of the dropped flowers were cultured. It was found that most pistils of the dropped flowers contained the inoculated fungi (Figure 7). *Alternaria alternata* and *Cladosporium cladosporioides* were regained from 80 to 100% of the inoculated flowers that dropped. In the control experiment, where flowers were hand-pollinated only, 50 – 65% of flowers dropped. The results of the inoculation experiment and those of the hand pollination were analysed statistically using Students *t*-test (Fisher & Yates 1963). Critical values for *t* (probability level at 0.05 and 0.01, the limit >2.31 and >3.36) were found to be high. High significance was observed in all cases where flowers dropped after hand pollination (mean 65.4), and was compared with that of flowers that dropped after hand pollination and inoculation with different fungi (*Alternaria alternata*, 17.84; *Cladosporium cladosporioides*, 17.84; *Fusarium oxysporium*, 10.57; *Nigrospora oryza*, 5.49; *Pithomycis graminicola*, 17.84.).

Discussion

The results of detection of fungi (Figures 1 – 4) and isolation of fungi (Figure 5) from the fresh pistils of avocado cultivars 24 h after anthesis (female stage), showed a high percentage of fungal invasion on stigma and style. In addition, since nominal pollination took place, many ungerminated pollen grains as

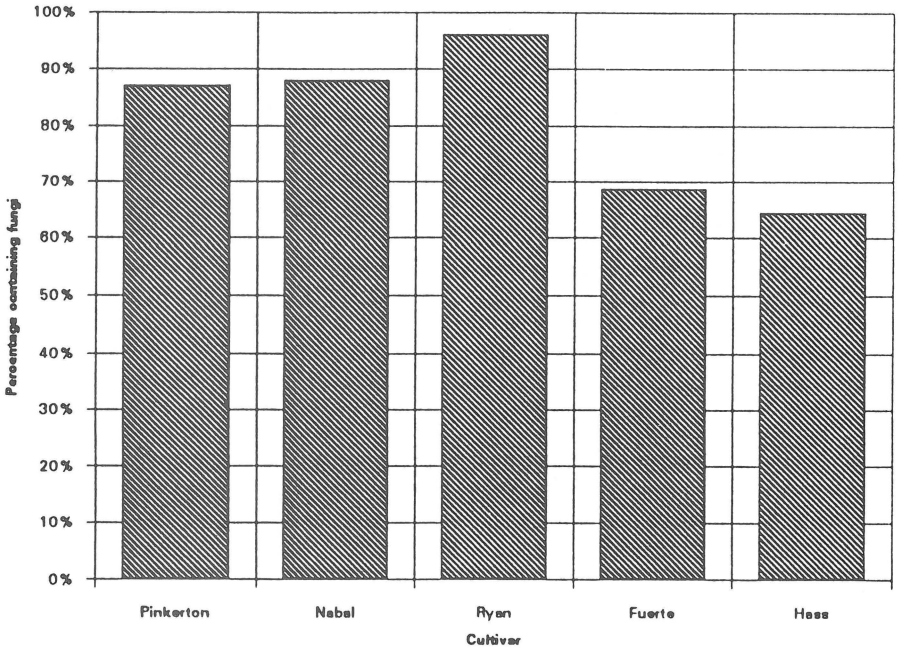


Figure 4 Summary of detection of fungi from avocado cultivars. The percentages of pistils on which the fungi were found in each cultivar, are given.

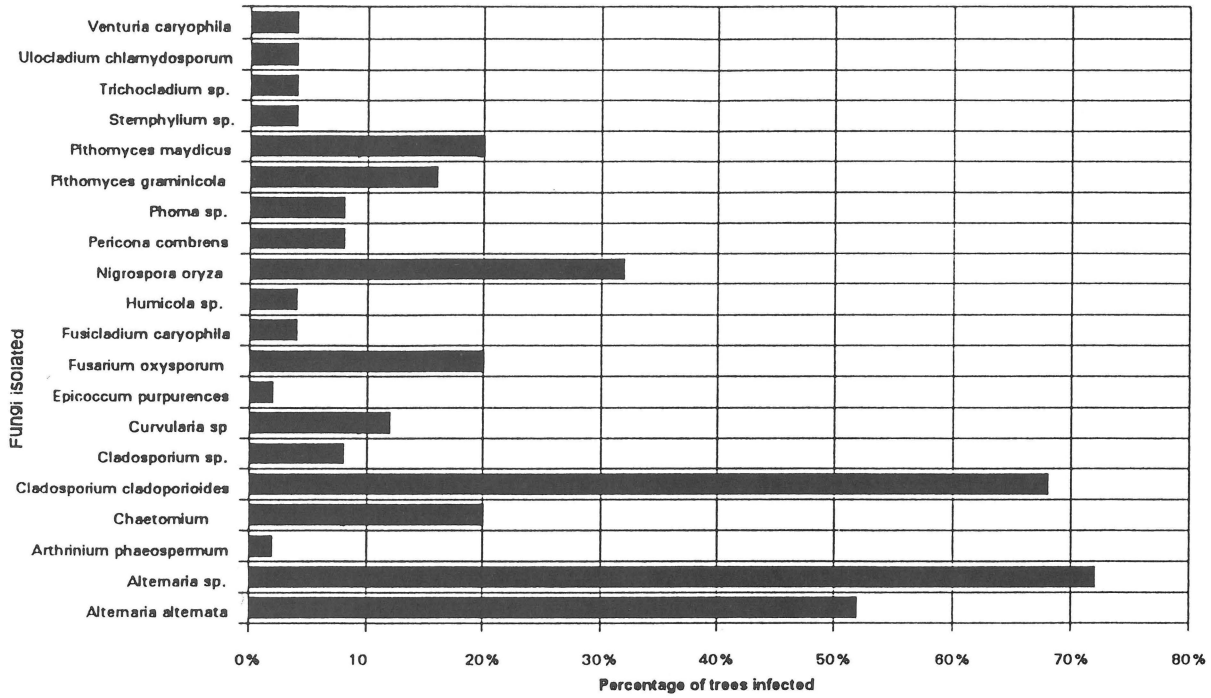


Figure 5 Percentage occurrence of fungi isolated from 50 trees of five different avocado cultivars.

well as germinated pollen with short pollen tubes were seen on such pistils.

Miller and Richard (1974), Macdougall (1968) and Rovira and Boven (1976) reported that fungal spores can germinate on fresh and soft tissues of plants in less than 24 h if favourable conditions are available. Diehl (1950) stated that natural infection can occur when conidia or ascospores germinate on stigma like the pollen grains and penetrate the tissues of the ovule. Gottleib (1978) found that osmotic factors, pH and stimulatory substances promote the germination of fungal spores and growth of mycelium. According to Callow (1983), the interaction of the fungi with the host tissue depends on the receptive tissues of the stigma and various endogenous and exogenous factors. Barash *et al.* (1964) and Kosuge and Hewitt (1964) noticed that exudate from the stigma of the host stimulates the pathogen, resulting in rapid germination of conidia and elongation of germ tubes. Strobel and Hewitt (1964) found that spores of *Diplodia* sp. germinate on and infect the stigmatic portion of the flower. The mycelia develop later with rising of pH level, resume growth and rot the berry. Nishimura and Kohmoto (1983) observed that colonized fungi produce the host specific toxin (HST) in advance of invasion, which stimulates the growth and uptake of nutrients. The key role of this substance is the disfunction of the plasma membrane, which induces the host-susceptible state of fungal invasion. McClellan & Hewitt (1973) reported that in the early '*Botrytis* rot' of grapes the fungus invades the stigma and remains attached to the developing grapes. In developing apricots, *Botrytis* spp. in the style, which adhere to fruit or the developing ovule, produce chemical substances which cause abortion of fruits.

These findings more or less agree with results of detection and isolation of fungi from the pistils of avocado cultivars 24 h after anthesis (female stage) of the flower. The stigma of a newly opened avocado flower with various osmotic factors and stimulatory substances, is a 'suitable substrate' for germination of pollen grains and fungal spores. These substances were shown to promote germination of pollen grains and fungal spores, as well as spreading of fungal hyphae on avocado stigma. It is possible that growing fungi present on the receptive tissues of stigma and style can affect the growth of the pollen tubes towards the style and ovary. Latency of infection has been reported in avocados attacked by *Colletotrichum* sp. (Binyamini & Schiffman-Nadel 1972). In these fruits, fungus forms appressoria within the wax layers, growing and penetrating into the fruit at maturity. According to Mayer (1989), fungal invasion may lead to alteration of structure, cell permeability and depletion of supply of phosphate ions. Kolattukudy (1985) and Mayer (1989) showed that fungal exudates contain enzymes which cause the alteration of plant cells. The increased activity of these enzymes causes the cell wall component to be dissolved, the pollen grains to be aborted, the level of sucrose to be reduced and results in localized changes in cellular metabolism and hormonal relationship on the stigma. Inouye (1962) and Lister and Murant (1967) observed that the pathogen also decreased the vigour of the pollen and caused pollen abortion. This involves the disintegration of cytoplasm followed by the collapse of the pollen grain. In avocados this explains the presence of large numbers of ungerminated pollen grains and pollen grains with short pollen tubes on the stigma and style (Figures 1 – 3).

All the fungi isolated were from the sterilized tissues of the avocado pistils. It is therefore evident that these fungi have established themselves in the tissues. The isolated mycoflora from the stigmas of avocado cultivars (Figure 5) consists mainly of dematiaceous fungi. High temperature and humidity in the orchards and favourable conditions on the stigma at the time of pollination promote the fungal invasion. Fungi *Alternaria* sp., *Fusarium* sp., *Cladosporium* sp., *Nigrospora* sp. and *Pithomyces* sp. were present on most of the trees selected for studies. *Cladosporium* spp. are good nutrient competitors and utilize host energy for defensive reaction (Riesen 1985), and may increase the infection rate (Blackeman & Fokkema 1985). *Alternaria* sp. and *Cladosporium* sp. (Figure 6) are well adapted to the tissues of pistils of all avocado cultivars. *Alternaria* spp. are less sensitive to dry conditions (Riesen 1985). O'Donnell and Dickinson (1980) considered *Cladosporium cladosporioides* as an accidental endophyte. These fungi might be residents with a parasitic phase (Leben 1965). Fungi considered here have in common a number of features, including their pathogenic nature, ability to utilize nutrients present on

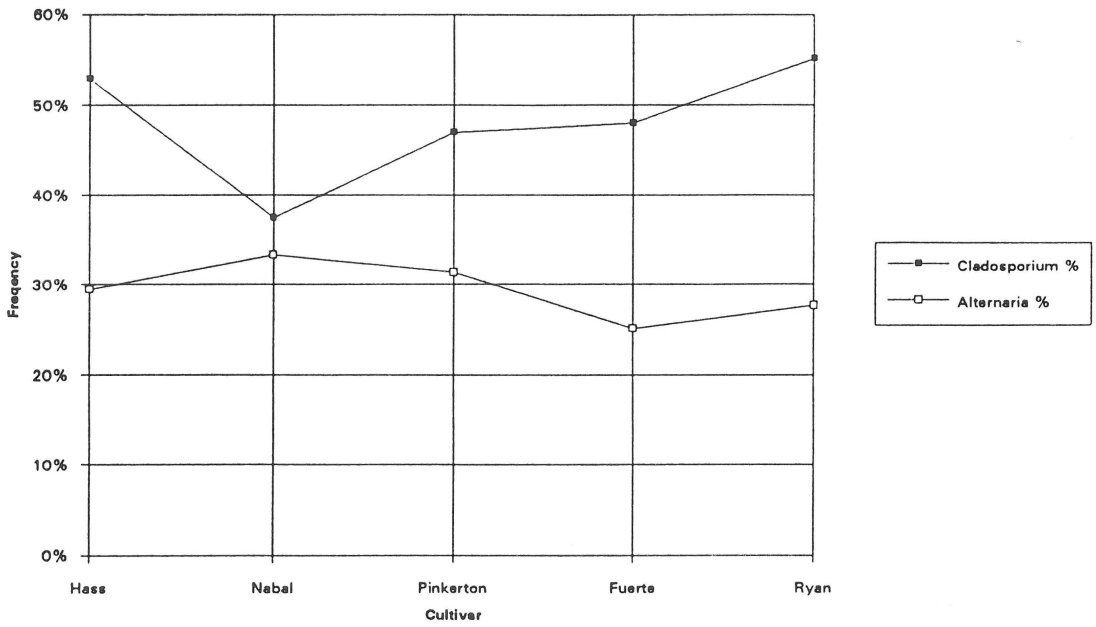


Figure 6 Frequencies of the commonly isolated fungi *Alternaria* sp. and *Cladosporia* sp. in different avocado cultivars.

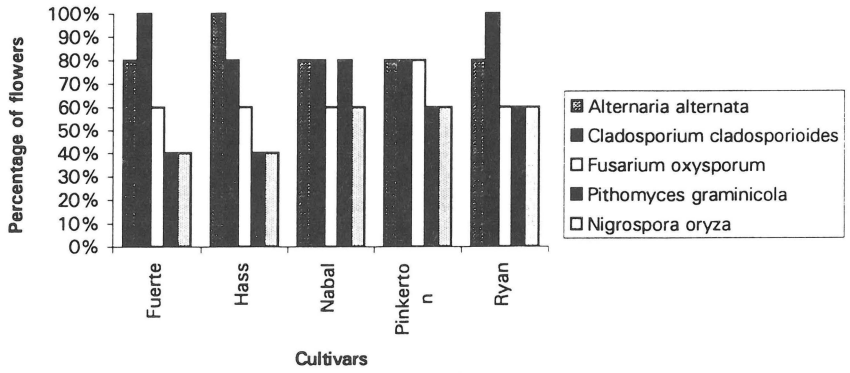


Figure 7 Percentage of flowers belonging to different cultivars from which the inoculated fungi were isolated.

the stigma and production of toxins. In the inoculation experiment, around 90% of flowers were dropped from all avocado cultivars, while in the control experiment where flowers had been hand-pollinated, only 51 – 65% of flowers dropped from the trees. According to the results of the isolation of fungi from the pistils of the dropped flowers which had been hand-pollinated and inoculated (Figure 7), it was confirmed that fungal hyphae were growing inside the tissues. It is evident from the observations that there is a strong interaction between germinating fungal spore, growing mycelium and pollen germination on the stigma. In *in vivo* pollination experiments in avocados it was found that conidia of the inoculated fungi germinated on the stigma and then continued growing with the pollen tube towards the ovary. Also in *in vitro* experiments it was observed that germination percentage and growth of the pollen tube decreased when the inoculated fungal spores germinated and invaded the growth media. These experiments will be continued to confirm the findings reported in this paper and to extend our knowledge regarding the role of fungi during the pollination and fruit set. Students *t*-test showed that the mean of the total flowers dropped in all cultivars after hand pollination is highly different ($P < 0.01$) from that of flowers dropped after hand pollination and inoculation of fungi.

Although the critical values obtained were different for each group where different fungi were inoculated, all the values obtained are statistically significant ($0.05 < P < 0.01$). From this it is evident that these fungi were greatly involved in the abscission of flowers in the avocado cultivars.

In conclusion, the association of fungi on the stigma at the time of pollination is widespread in all avocado cultivars. There might still be other, as yet unrecorded parasites or endophytes in avocado cultivars which affect the pollination process. Clearly, some of these fungi play a significant role on the stigma after pollination, by inhibiting the germination of pollen grains or growth of the pollen tube. Infected flowers are not fertilized and this leads to abscission of flowers and poor fruit set. Intensive surveys in orchards in representative parts of the country are therefore required to evaluate the status of the fungi, their pathogenicity and their role in fertilization and fruit set.

Acknowledgements

We thank Dr B.Q. Manicom of the Agricultural Research Council, Nelspruit, for helpful discussions and for providing the laboratory facilities.

References

BARASH, I., KLISIEWIEZ, J.M. & KOSUGE, T. 1964. Biochemical factors affecting pathogenecity of *Botrytis cinerea* on Safflower. *Phytopathol.* 54: 923 – 927.

BERG, B.O. & WHITSELL, R.H. 1974. Self-pollinated 'Hass' seedling. *Calif. Avocad. Soc. Yrb.* 51: 118 – 126.

BINYAMINI, N. & SCHIFFMAN-NADEL, M. 1972. Latent infection in avocado fruit due to *Colletotrichum gloeosporioides*.

Phytopathol. 62: 592 – 594.

BLACKEMAN, J.P. & FOKKEMA, N.J. 1985. Potential for biological control of plant diseases on the phylloplane. *Ann. R. Pl. Pathol.* 20: 167 – 192.

BRINGHURST, R.S. 1951. Influence of glass house conditions on flower behavior of 'Hass' and 'Anheim avocados. *Calif. Avocad. Soc. Yrb.* 36: 164 – 168.

BRINGHURST, R.S. 1952. Sexual reproduction in avocado. *Calif. Avocad. Soc. Yrb.* 37: 210 – 214.

CALLOW, J.A. 1983. Biochemical plant physiology. John Wiley & Sons, New York.

CARROLL, G.C. & CARROLL, F.E. 1978. Studies on the incidence of coniferous needle endophytes in the Pacific North West. *Can. J. Bot.* 56: 3032 – 3043.

CLAY, K. & JONES, J.P. 1984. Transmission of the fungus *Atkinsonella hypoxylon* (Clavicipitaceae) Cleistogamous seed of *Danthonia spicata* (Gramineae). *Can. J. Bot.* 62: 2893 – 2898.

DIEHL, W.W. 1950. *Balancia* and *Balanciae* in America, Agric. Monograph 4, pp. 1 – 82. U.S.D.A; Washington, DC.

DURAND, B.J. 1990. Inleiding tot die kweek van avokado's in Suid-Afrika. Boerdery in Suid-Afrika. *Avokado A.* 1/1990.

FISHER, P.J., ANSON, A.E. & PETRINI, O. 1986. Fungal endophytes in *Ulex europaeus* and *Ulex galli*. *Trans. Br. mycol Soc.* 86: 153 – 156.

FISHER, R.A. & YATES, F. 1963. Stastical Tables for Biological and Agricultural Research, 6th ed. Oliver & Boyd. Ltd, Edinburgh.

GOLDRING, A., GAZIT, S. & DEGANI, C. 1987. Isozyme analysis of mature avocado embryos to determine out-crossing rate in 'Hass' plot. *J. Am. S. Hort.* 112: 389 – 392.

GOTTLEIB, D. 1978. The germination of fungal spore. Meadow Field Press, Durham, England.

INOUYE, T. 1962. Studies on barley stripe mosaic in Japan. Special print in: Berichte des Ohara Instituts für Landwirtschaftliche Biologie, Okayama Universität, Boncl. XI, No. 4, March 1962, Kuraschiki, Japan.

KOLATTUKUDY, P.E. 1985. Enzymatic penetration of the plant cuticle by fungal pathogens. *Ann. R. Phytol.* 23: 223 – 250.

KOSKE, R.E. & GEMMA, J.N. 1989. A modified method for staining roots to detect VA micorrhizas. *Mycol. Res.* 92: 486 – 505.

KOSUGE, T. & HEWITT, W.B. 1964. Exudates of grape berries and their effect on germination of conidia of *Botrytis cinerea*. *Phytopathol.* 54: 167 – 172.

LEBEN, C. 1965. Epiphytic microorganisms in relation to plant diseases. *Ann. R. Phytol.* 3: 209 – 330.

LISTER, R.M. & MURANT A.F. 1967. Seed transmission of nematode-borne viruses. *Ann. Appl. Biol.* 59: 49 – 62.

McCLELLAN, W.D. & HEWITT, W.B. 1973. Early Botrytis rot of grapes. Time infection and latency of *Botrytis cinerea* Pers. in *Vitis viniferea* L. *Phytopathol.* 63: 1151 – 1157.

MACDOUGHALL, B.M. 1968. The exudation of C^{14} labelled substance from roots of wheat seedlings. Transactions of the ninth international congress of soil science, No. 3, p. 647.

MAYER, A.M. 1989. Anti fungal interaction: A plant physiologist view point. *Phytochemistry* 28: 311 – 317.

MILLAR, C.S. & RICHARD, G.M. 1974. Cautionary note on the collection of plant specimens for micological examination. *Trans. Br. mycol. Soc.* 63: 607 – 612.

NISHIMURA, S. & KOHMOTO, K. 1983. Role of toxin in plant pathogenesis. In: Toxin and Plant pathogenesis, eds. J.M. Daly & B.J. Deverall, pp. 127 – 143. Academic Press, Sydney, New York.

O'DONNELL, J. & DICKINSON, C.H. 1980. Pathogenecity of *Alternaria* and *Cladosporium* isolates on *Phaseolus*. *Trans. Br. mycol. Soc.* 74: 335 – 342.

PETERSON, P.A. 1956. Flowering types in the avocado with relation to fruit production. *Calif. Avocad. Soc. Yrb.* 40: 174 – 179.

PETRINI, O. & MÜLLER, E. 1979. Pilze als Endophyten von grünen Pflanzen. *Naturwissenschaften* 66: 262.

ROVIRA, A.D. & BOVEN, G.D. 1976. Translocation and loss of phosphate along roots of wheat seedlings. *Planta* 93: 15 – 25.

RIESEN, T.K. 1985. Endophytic fungi in winter wheat (*Triticum aestivum* L). A comparison between four wheat cultivars with different resistance to *Phaeosphaeria nodorum* (Muller) Hedjaroude. PhD thesis, ETh., Zurich 7689.

SCARBOROUGH, B.A. & SMITH, S.H. 1977. Effects of tobacco and tomato ring spot viruses on the reproductive tissues of *Pelargonium x hortorum*. *Phytopathol.* 67: 292 – 297.

SEDGLEY, M. & ANNELLS, C.M. 1981. Flowering and fruit set response to temperature in avocado cultivar 'Hass'. *Sci. Horta A* 14: 27 – 33.

STROBEL, G.A. & HEWITT, W.B. 1964. Time of infection and latency of *Diplodia viticola* in *Vitis vinifera* var. Thompson seedless. *Phytopathol.* 54: 636 – 639.

ZARACOVITIS, C. 1966. The germination *in vitro* of conidia of powdery mildew fungi. In: The Fungus Spore, ed. M.F. Madelin, pp. 273 – 286. Butterworth Publ., London.