Structural Resistance of Cashew (*Anacardium occidentale*) against Powdery Mildew (*Oidium anacardii*)

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Abstract

Studies were conducted in the laboratory at Wye College, University of London, to investigate some aspects of pre-infection structural resistance on cashew leaf and flower surfaces. Using the Scanning, Transmission Electron Microscopes and the light microscope, cashew leaf surfaces were found to be covered by a layer of epicuticular wax, which appeared to develop with age. Flower surfaces were found to be covered by hairs of varying shape and length which appeared to help trap tiny mildew spores. This appears to explain why flower organs are extremely susceptible to mildew infection. Using artificial inoculation, mildew infection was possible on young leaves, but infection did not occur on older leaves. Epicuticular wax layer observed on cashew leaf surface, as a pre-infection resistance factor, appeared to be responsible for inhibition of germination and development of ***O. anacardii*** *on older leaves.*

Key words: Structural resistance, Cashew, *Anacardium occidentale*, Powdery mildew, *Oidium anacardii*, Tanzania

Introduction

Cashew (*Anacardium occidentale*) a member of the Anacardiaceae family, is a tree of commercial importance. Cashew nut is one of the most important export crops in Tanzania. It predominates as the main source of cash income in the southern part of the country.

Among various problems facing cashew production in the country is the devastating attack of powdery mildew disease incited by *Oidium anacardii* Noack (Casulli, 1979). The disease may cause crop losses ranging between 70 to 100% (Sijaona and Shomari, 1987).

At present, the use of sulphur dust and organic fungicides such as Bayfidan (Triadimenol) and Anvil (Hexaconazole) is recommended for control of the disease (Sijaona and Shomari, 1987). However, Waller *et al.*, (1992), pointed out a range of problems associated with chemical controls some which include logistical, economical and application problems. In addition, the use of sulphur dust may have a long-term effect on soil acidity in areas where the cation exchange capacity of soils is already low. Thus, the use of fungicides is unlikely to be a sustainable option for control of powdery mildew in the long term, although it may be necessary in the short term until other control strategies are developed.

Field observations have shown that differences in susceptibility to powdery mildew exist among the cashew populations in Tanzania. This has been reflected by variability between clones in terms of disease severity scores as well as their final nut yields, under severe powdery mildew pressure (Sijaona, 1997). Immunity, which is often associated with single or

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major gene resistance has not been reported, but a range of partial resistance (possibly under polygenic control), has been suggested to occur (Sijaona, 1987) The occurrence of apparent partial resistance in local and exotic germplasm collections makes resistance among cashew genotypes the possible control strategy of choice in the long term.

During the course of the co-evolution, plants and pathogens have evolved a highly complex and intimate relationship. Pathogens have developed sophisticated offensive systems to parasitize plants. On the other hand, plants have in general exhibited a remarkable potential to defend themselves against infection by pathogens.

Some plants are capable of preventing certain pathogens from penetrating their cell walls. Others seem to unleash their defensive weapons shortly after cell wall penetration and invasion of few cells (Asher, 1982; Asher and Thomas, 1983; Bennet, 1981; Manners and Thomas, 1983).

Numerous studies on the mechanisms by which plants resist infection by fungi have been conducted. However, such plants have been limited to major crop plants (Ingram, 1973). Resistance to fungal infection in plants is often based on structural or chemical features and these are in turn subdivided into either pre-infection (passive or constitutive) or post-infection (active or induced) phenomena (Ingham, 1973).

Pre-infection resistance factors include those, which are present in plants prior to their contact with pathogens. They normally discourage entry of pathogens into the internal tissues and may resist their growth when ingress has been gained. This subject has been reviewed by a number of workers (Wood, 1967; Royle, 1976; Campell *et al.*, 1980; Akai & Fukutonii, 1980; Mansfield, 1983). Features often quoted, as examples of pre-infection resistance are thickness or hardness of cuticle, the size and shape of stomata and leaf waxiness and hairiness.

Sijaona (1997) reported that, a young expanding cashew leaf passers through a series of visually observed stages before reaching maturity. Initially, all parts of the young leaf appear shiny; however, the shining is gradually replaced by dullness (pale green) as the leaf ages. The development of a dull appearance of the leaf starts from the petiole and progresses towards the apex of the leaf. This change appears to be associated with cuticular wax formation. Other workers (Rao and Hassani, 1957; Ohler, 1979) reported similar observations.

The present study was conducted in order to investigate some aspects of pre-infection structural resistance factors on cashew against powdery mildew.

Materials and Methods

Examination of cashew leaf and flower surfaces

The surfaces of cashew leaves and flowers were examined by using the Scanning Electron Microscopy (SEM) and the internal structures were examined using Transmission Electron Microscopy (TEM) at Wye college, University of London, UK.

Scanning Electron Microscopy (SEM)

Using critical point drying approach, five stages were involved in preparation of tissue:

Squares (4 mm x 4 mm in size) of leaf or flower tissue were cut and fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.0. The specimens were placed in a refrigerator overnight before they were washed twice for 10-minutes in 0.1M phosphate buffer, pH 7.0.

Each specimen was loaded into a critical point drier specimen container then subjected to dehydration through an ethanol dilution series (50, 70, 80, 90, 3 x 100%) for 20 min at each step.

Leaf samples were then subjected to the critical point drying process involving replacement of acetone with liquid CO_2 for 1.5h, after which the specimens were kept dust free. Specimens were attached to metal stubs using araldite adhesive and coated with a very thin layer of gold using a Nanotech Semprep II Sputter Coater, to prevent build-up of electrical charge during

viewing. Leaf specimens were viewed using a Hitachi S40 Scanning Electron Microscope, with an accelerating voltage of 15KV. Photomicrographs were taken on 35 mm Ilford FP4 film.

Transmission Electron Microscopy (TEM)

Squares of 6 mm^2 area of infected leaf material were removed and immersed in drops of fixative (cold 2.5% glutaraldehyde in 0.1 phosphate buffer, pH 7.0) placed on sheets of dental wax. Tissue blocks (1mm^2) were cut while the material was in the fixative. The blocks were left overnight in the refrigerator. Specimens were washed twice for 10 min in phosphate buffer (0.1M, pH 7.0), followed by immersion in a second fixative (2% osmium tetroxide in phosphate buffer, 0.1M, pH 7.0) for 2 hr. The leaf samples were fixed in osmium tetroxide and washed twice in phosphate buffer for 10 minutes. Leaf samples were then dehydrated by immersion in 50% acetone for 10 minutes. Tissue blocks were stored in 70% acetone overnight in the refrigerator.

The dehydration series was completed with 10 minutes washes in 80% and 90% acetone followed by three washes of 1hr in dry (100%) acetone. The embedding process followed transition from acetone to Epon-Araldite Resin. The sequence used was 2:1 acetone: resin mixture for 2.5 hr, 1:1 mixture for 2.5 hr and 1:2 mixture overnight. Tissue blocks were then kept in fresh resin for four days prior to change into fresh resin overnight for two turns with vials uncovered. Blocks and labels were placed in moulds covered with fresh resin and baked in an oven at 60°C for 48h.

All sectioning was carried out on Reichert Ultracut E Ultramicrotone, using glass knives. Prior to sectioning for electron microscopy, 2μm sections were cut from tissue blocks and stained with toluidine blue for light microscopy. This allowed the selection of areas of interest suitable for preparation of ultrathin sections, which were normally cut at 130μm thickness. Silver-gold sections (70-80μm thick) were obtained by stretching the ultrathin sections on water with chloroform; these sections were then collected for staining on 300 mesh copper grids.

Grids bearing the ultrathin sections were immersed for 10 min in saturated solution of uranyl acetate in 70% ethanol. Five washes in distilled water removed surplus uranyl acetate prior to immersion in Reynolds lead citrate for 10 min. Viewing was done using the Hitachi HU-11A Transmission Electron Microscope. Photomicrographs were taken on 70-mm roll system using Agfa RA710P film.

Effect of leaf age on germination and development of *O. Anacardii*.

An inoculum of *O. anacardii* was collected from local cashew trees near Dar-Es-Salaam (Tanzania) and were packed between dry tissue papers and placed between sheets of old newspapers and delivered to Wye college. Cultures of *O. anacardii* were established at Wye College by inoculation on young tender leaves. All work on *O. anacardii* was conducted in a quarantine growth room at 25±2 °C, with 12 hr daylength. The culture was maintained on seedlings planted in pots. The fungus was routinely transferred to new shoots by leaf contact at weekly intervals.

Seven leaves from one flushing shoot were compared. The youngest expanded leaf was denoted as leaf number one and the rest were subsequently numbered down the shoot, with leaf number seven being the oldest leaf in the sample.

Leaf disks were cut from each of the test leaves starting from terminal end towards the petiole, in a zag fashion using a 19-mm diameter cork borer. The disks were also numbered following the same sequence, with the one near the terminal end denoted as number 1 and the one near the petiole as number 6. Leaf disks from similar positions of the leaf (or similar number) were set in one Petri dish to form a replicate so that each dish contained disks from all seven test leaf materials. These were replicated three times.

The disks were laid upper surface uppermost, on filter papers placed in sterile Petri dishes. The filter papers were moistened with 5 % D^+ glucose to saturation in order to provide a source of energy for the leaf disks. The moisture level was subsequently maintained by topping-up using a pasteur pipette, as was deemed necessary. Care was taken not to wet the surface of the leaf disks.

One day before inoculation, leaves bearing sporulating powdery mildew spores were tapped to dislodge old spores, thereby ensuring a supply of uniformly aged conidia for experimental inoculation (Edwards and Ayres, 1982; Hyde and Colhoun, 1975; Stanbridge *et al.*, 1971; Schnathorst, 1960). Inoculation was done using Camel hairbrush. Dry brushes were used to transfer inoculum from an infected host tissue onto the test leaf disks. The brush was gently rolled on a sporulating powdery mildew colony before it was brushed or tapped onto the test organs. After inoculation, the Petri dishes were covered and sealed with parafilm (3M), for the initial 12 hours after inoculation (HAI), to maintain high relative humidity. Incubation was done in a quarantine growth room at 25±2 ^{0}C, with 12 hr daylength.

Conidial germination frequencies were assessed using the sellotape technique. A "sellotape strip" method as described by Butler & Mann (1959) was used to take leaf epidermal impressions for quantitative primary infection studies. A strip of clean sellotape was held by a pair of forceps, from which a piece (1cm^2) was cut using a pair of scissors. The piece was laid firmly on top of the leaf before applying a gentle pressure over it. The sellotape was eventually peeled-off together with surface fungal structural materials stuck to it. The piece was mounted on a slide in a drop of lactophenol cotton blue stain, which also stopped further growth of the fungus. After placing the cover slip, the preparation was gently warmed in order to expel air bubbles before it was observed under the light microscope.

Germination and fungal development values were usually determined for at least 200 conidia or germlings, from each of the three replicates. Production of a germ-tube with a length greater than its width was the criterion used to assess spore germination (Manners and Hossain, 1963). Time-course observations on germling development were recorded.

Results

Observations on leaf and flower surfaces

SEM

Plate 1 reveals a development stage of *O. anacardii* on a cashew leaf surface, on which an active germling had formed three primary

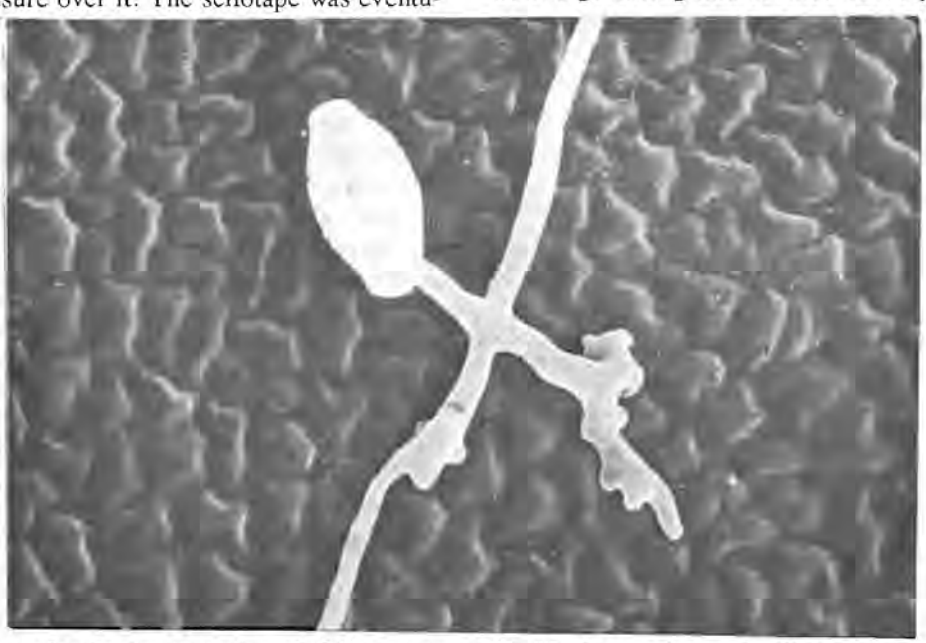

Plate1. A developing germling of *Oidium anacardii* with three primary hyphae on a cashew leaf surface. Note the coverage to epicuticular wax layer on the leaf surface (SEM at Magnification x5330)

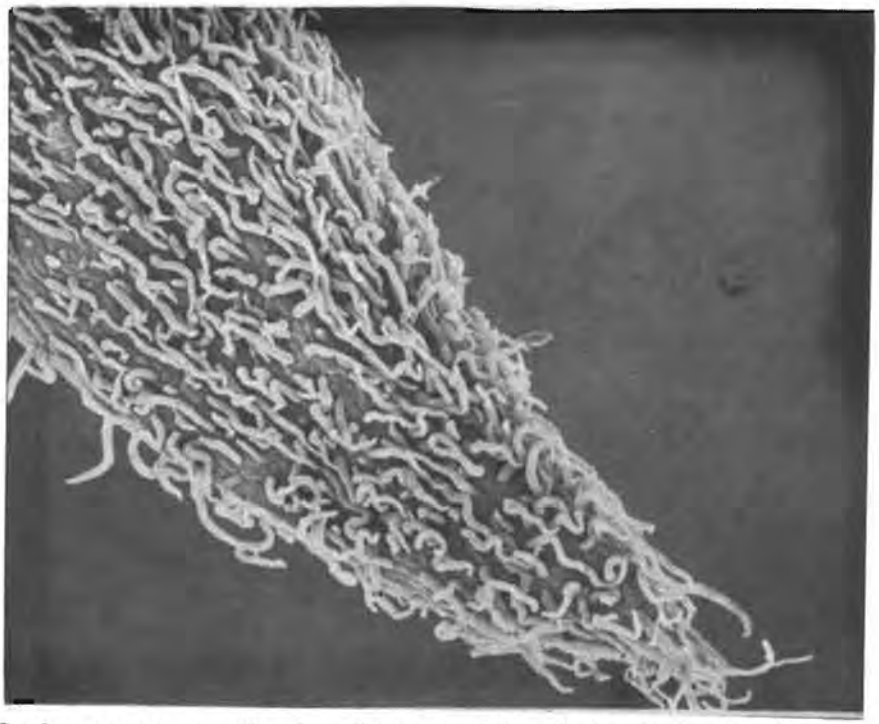

Plate 2. Surface appearance of cashew flower petal (terminal end) showing dense coverage of hairs. Note the dense and morphology of hairs. (SEM, Magnification x845)

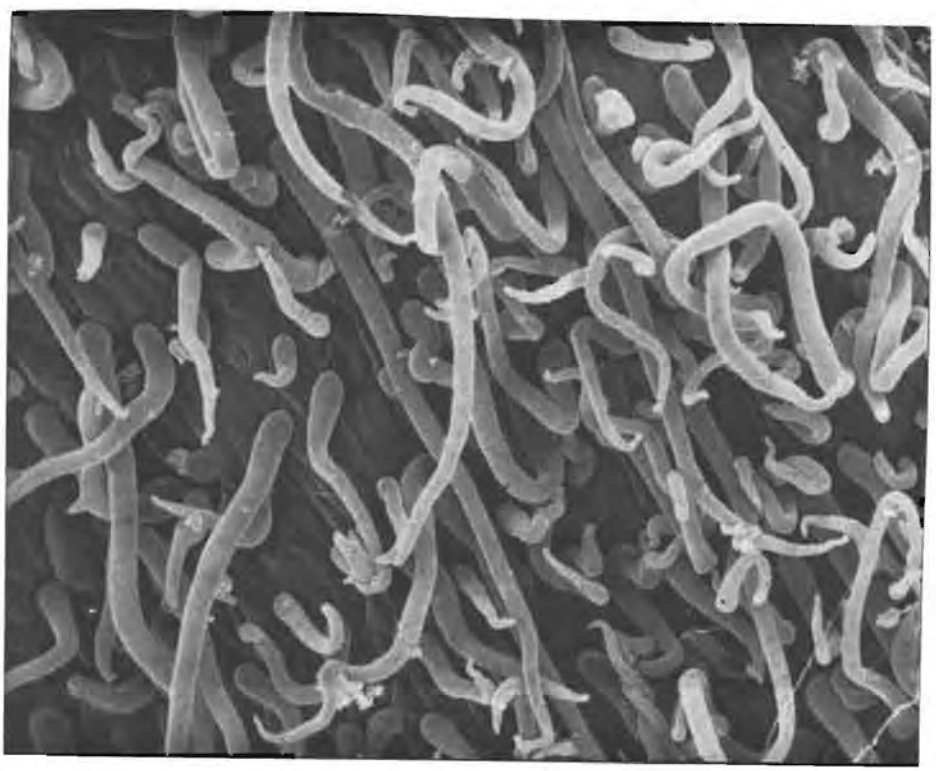

Plate 3. Surface appearance of cashew flower bract showing dense coverage of hairs. Note the dense and morphology of hairs. (SEM, Magnification x1,650)

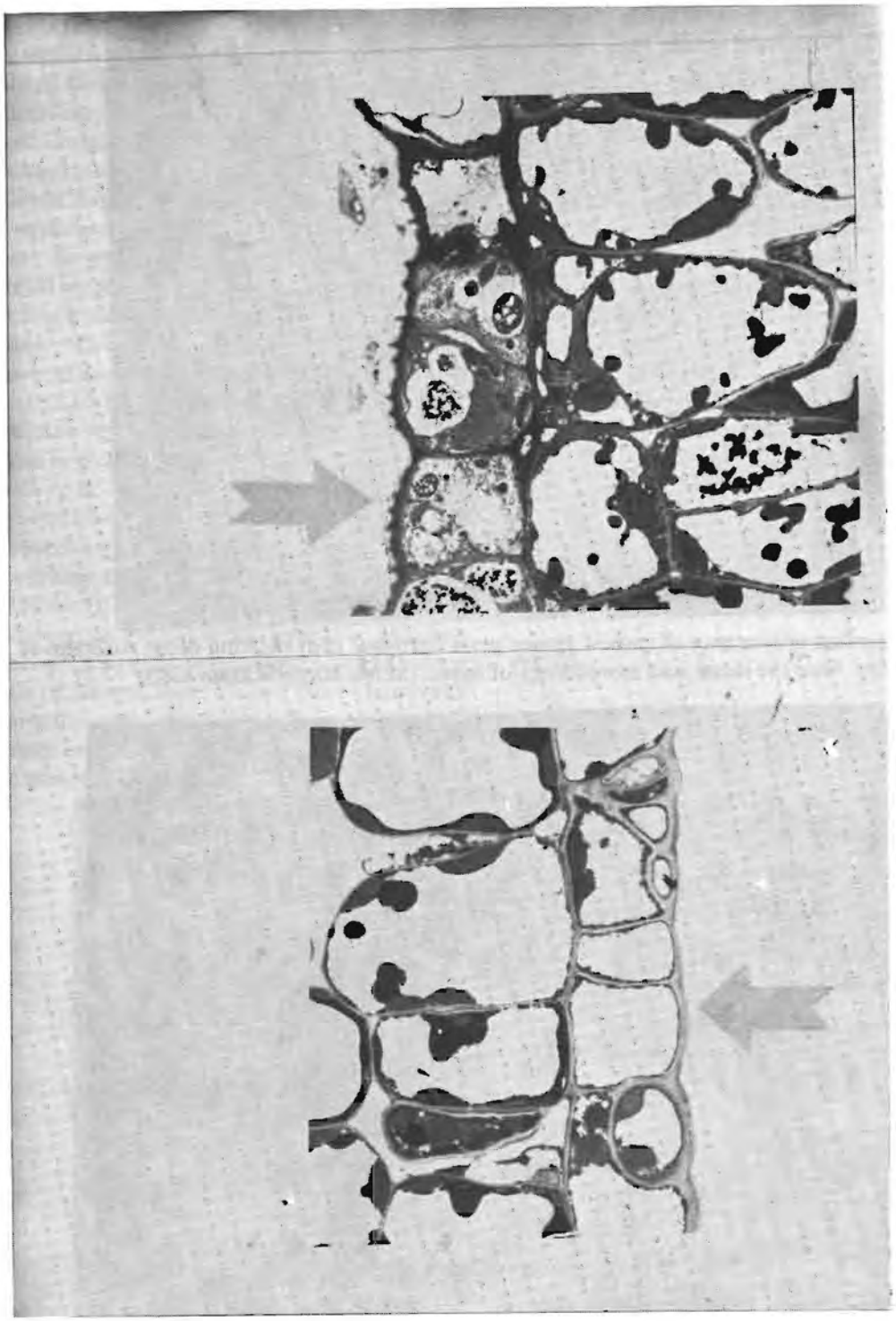

Plate 4. TEM photomicrographs of transverse section of mildew infected cashew leaf showing (A) Upper (B) Lower leaf surface (arrowed). [Magnification x2,400 and 2,600 respectively]

hyphae, 18 HAI. An important feature of the host revealed by SEM was the coverage of a layer of epicuticular wax on the surface of the leaf. The wax layer was thick and raised above the general level to give the appearance of a corrugated iron sheet, lying almost parallel to the leaf axis.

Plates 2 and 3 reveal the surface appearance of cashew flower bract and petal, respectively. It was interesting to note massive numbers of hairs of different morphology on the surface of these flower parts. Some hairs were straight, some were curled at varying positions and differences in length were also evident.

TEM

Cashew leaves comprised of single - celled epidermal and palisade mesophyll layers with three or four layers of packed spongy mesophyll cells (Plate 4). The outer wall of the upper epidermis was covered by an electron-dense layer presumed to be the cuticle (Plate 4 A). This layer was much thinner and smooth on the lower epidermis (Plate 4.B). Mesophyll cells were packed with dark bodies presumed to be phenols or tannins.

Effect of leaf age on germination and development of *O. Anacardii*

Figure 1 shows disease development on discs from leaves of different age. After six days of incubation there were clear variations in colony formation on leaves of different age. Only leaf number one was heavily infected by mildew. Some infection was observed on leaves of intermediate age, but no colonies were formed on leaf six or seven.

Figure 2 presents the percentage germination of *O. anacardii* recorded on leaf disks from leaves of different age. There were striking differences in spore germination. High spore germination levels were observed on young leaves 1, 2 and 3, intermediate levels of germination occurred on leaves 4 and 5. However, there

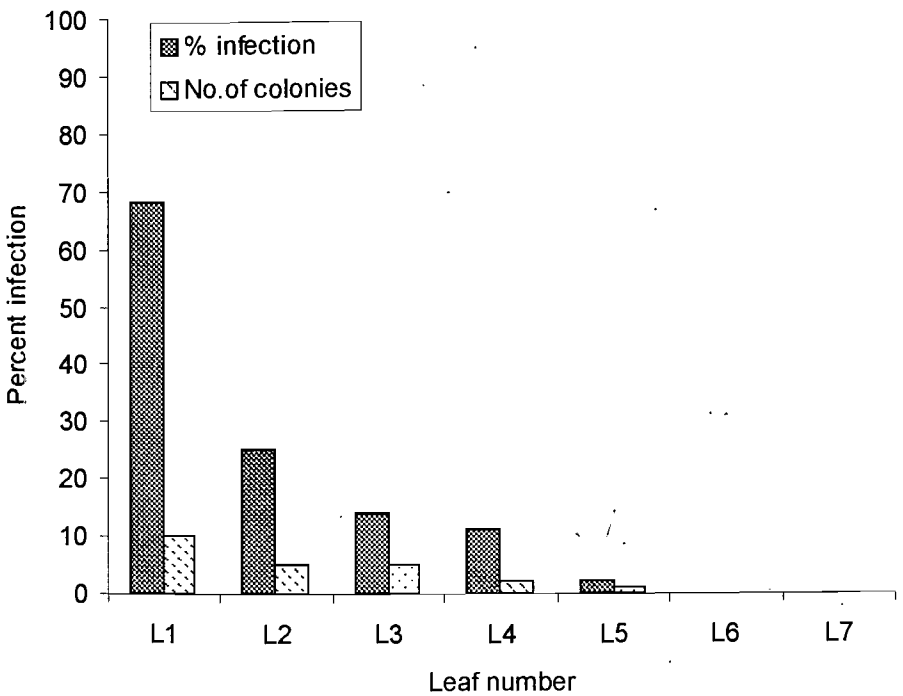

Figure 1. Formation of macroscopic colonies of *O.anacardii* on leaves of different age, six days after inoculation.

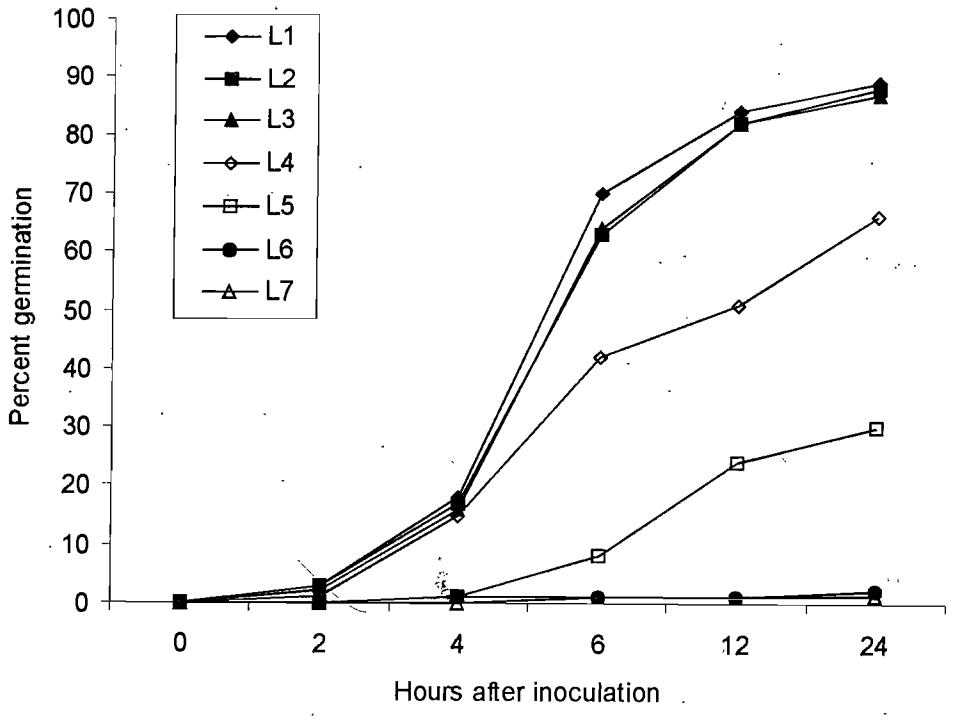

Figure 2. Percent germination of *O. Anacardii* on leaves of different age

was little spore germination (<4%) on the older leaves (6 and 7).

Figure 3 shows that young leaves, particularly the first leaf supported a large proportion of germlings with more than 1 and 5 primary hyphae 48 HAI. No hyphae were produced on leaves 6 and 7.

Discussion

Results from TEM studies indicate that epicuticular wax form ridged and thick sheet on the upper leaf surface and a comparatively smooth and thin layer on lower surface. There was no any evidence in variation of mildew infection on upper and lower leaf surfaces of cashew. However, it would be interesting to compare and relate cuticle formation on shiny and dull cashew leaf portions in relation to infection by *O. anacardii* among the susceptible and resistant genotypes.

Use of SEM has revealed interesting morphological features on the surface of flower bracts and petals. Dense coverage of hairs of different shape and size, were observed. Working on Erysipheae of wheat in Japan, Homma (1937) concluded that, morphological characters such as fewer surface hairs and fewer thick – walled epidermal cells, were associated with susceptible wheat varieties. Although Nashaat and Moore (1991) reported that neither profuse epidermal hairs nor leaf cuticle contributed directly to resistance in *Triticum timopheevii,* these hairs on cashew flower parts may account for high susceptibility levels observed in the field in Tanzania (Sijaona, 1997). The tiny mildew spores of *O. anacardii* become trapped within the hairs and provide a conducive environment for powdery mildew disease development. In future, it will be worthwhile to compare these morphological characters among different cashew genotypes, which have varying levels of susceptibility or resistance to infection by *O. anacardii.*

Powdery mildew infection studies on cashew leaves of different age have revealed fun-

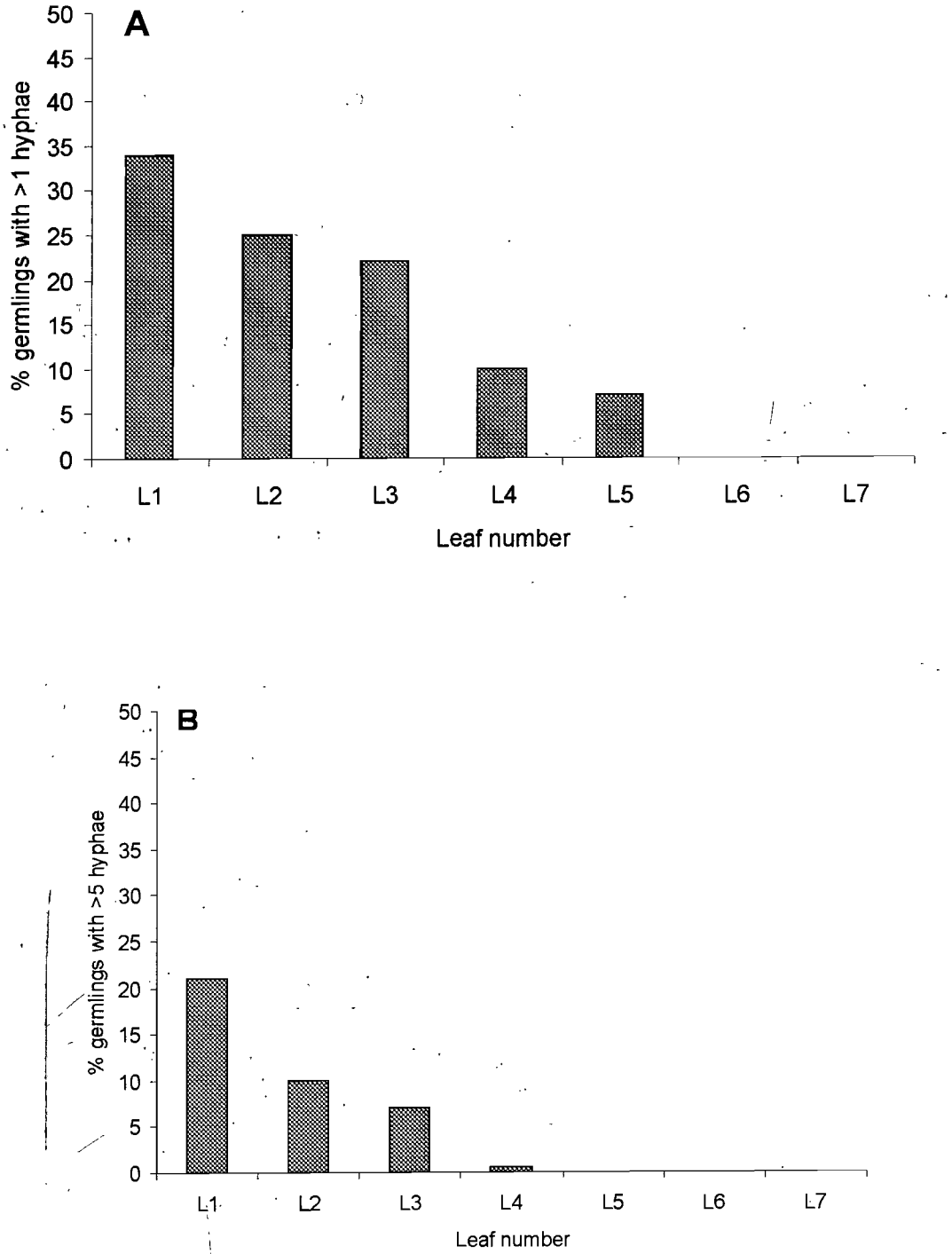

Figure 3. Production of (A) > 1 hypae and (B) > 5 hyphae during development and colonization of *O.anacardii* on leaves of different age, 48 hours after inoculation.

damental differences between young and old leaves. It was revealed that a young expanding cashew leaf passes through a series of visually observed stages before reaching maturity. Initially, all parts of the young leaf appear shiny, with time; the shining is gradually replaced by dullness (pale green). The development of dull appearance starts from the petiole and progresses towards the apex of the leaf (Sijaona, 1997). These changes appear to be associated with cuticular wax formation (Rao and Hassani, 1957; Ohler, 1979). Thus, young leaves appear to have less waxy layer and thus more susceptible to powdery mildew infection, than older leaves. The main differences in this study appeared to be the relative ease with which the fungus penetrated the host cuticle and epidermal wall. The fungus penetrated young leaves easily, thus causing infection; however, it failed to infect older leaves. Thus, the infection process on older leaves appeared to be arrested during the penetration stage.

A number of workers have reported similar observations that young leaves were highly susceptible to powdery mildew, but become resistant with age (Mence and Hilderbrandt, 1966; Peries, 1962; Schnathorst, 1959; Godwin, 1985). These results explain why there is generally no powdery mildew infection on older or mature leaves in the field.

Mence and Hiderbrandt, (1966), working on resistance to powdery mildew on rose reported that thickness of cuticle and outer epidermal cell wall, increased with age. They also observed that increase in resistance with age of leaves was paralleled by increase in cuticle thickness. Thickness of the cuticle and upper epidermal wall, has been positively correlated with resistance in a number of hosts (Schnathorst, 1959). Sometimes the upper leaf surface may be resistant while the lower surface may be susceptible to powdery mildew infection due to variations in cuticle thickness (Jhooty and Mckeen, 1965, Carver *et al.*, 1990). Thus, epicuticular wax layer observed on cashew leaf surface, as pre-formed inhibitors, appear to be responsible for inhibition of germination and development of *O. anacardii* on older cashew leaves.

Conclusion

Variations in powdery mildew infection on different organs and age of a cashew tree are sometimes caused by variations in the surface structure. Presence of epicuticular wax on mature leaves and dense hairs on cashew flower organs, have shown to play a major role as resistance structures against powdery mildew infection on cashew.

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References

Akai, S. and Fukutomi, M., 1980. Preformed internal defenses. In: *Plant disease: An advanced treatise* (Horsfall, J.G. and Cowling, E.B., eds.). Vol. 5, pp. 139-159. Academic Press New York.

Asher, M. J.C., 1982. The expression of partial resistance to powdery mildew in barley seedlings In: *Barley Genetics IV.* pp.466-471. Proceedings of the fourth International Barley Genetics Symposium, Edinburgh, 22 - 29 July 1981.

Asher, M.J.C. and Thomas, C.E., 1983. The expression of partial resistance to *Erysiphe graminis* in spring barley. *Plant Pathology*, **32**:79-89.

Bennet, F. G. A., 1981. The expression of resistance to powdery mildew in winter wheat cultivars. II Adult plant resistance. *Annals of Applied Biology*, **98**:305 - 317

Butler, E.E. and Mann M.P., 1959. Use of cellophane tape for mounting and photographing phytopathogenic fungi. *Phytopathology*. **49**:321- 324.

Campbell, C.L.; Huang, J. and Payne, G.A., 1980. Defense at the perimeter: The outer walls and the gates. In: *Plant diseases: An advanced treatise* (Horsfall, J.G. and Cowling, E.B., eds.), Vol. 5, pp. 103-120. Academic Press New York.

Carver, T.L.W.; Thomas, B.J.; Ingerson-Morris, A.S.M. and Roderick, H.W., 1990. The role of the abaxial leaf surface waxes of *Lolium spp* in resistance to *Erysiphe graminis*. *Plant Pathology*, **39**: 573 - 583.

Carver, T.L.W.; Thomas, B.J.; Ingerson-Morris, A.S.M. and Roderick, H.W., 1990. The role of the abaxial leaf surface waxes of *Lolium spp* in resistance to *Erysiphe graminis*. *Plant Pathology*, **39:** 573 - 583.

Casulli, F., 1979. Il mal bianco dell' anacardio in Tanzania. *Rivista di Agricoltura Subtropicale e Tropicale*, **73**: 241-248.

Edwards, M.C. and Ayres, P.G., 1982. Seasonal changes in resistance of *Quercus petrea* (sessile oak) leaves to *Microsphaera alphitoides*. *Transactions of the British Mycological Society*, **78**: 569-571.

Godwin, J.R., 1985. Resistance to powdery mildew disease in hops. *Ph.D. Thesis*, Wye College, University of London.

Homma, Y., 1937. Erysipheae of Japan. *Review of Applied Mycology*, **16,** 633.

Hyde, P.M. and Colhoun, J., 1975. Mechanisms of resistance of wheat to *Erysiphe graminis* f. sp. *tritici*. *Phytopathologische Zeitschrift*. 17: 314-335.

Ingham, J.L., 1973. Disease resistance in higher plants: The concept of pre-infectional and post-infectional resistance. *Phytopathologische* Zeitschrift. 17: 314-35.

Jhooty, J.S. and Mckeen, W.E., 1965. Studies on powdery mildew of strawberry caused by *Sphaerotheca macularis*. *Phytopathology*, **55**:281-285.

Manners, J.G. and Hossain, S.M.M., 1963. Effects of temperature and humidity on conidial germination in *Erysiphe graminis* . *Transactions of the British Mycological Society*, **46**: 225 - 234.

Manners, J.G. and Thomas, C.E., 1983. The expression of partial resistance to *E. graminis* in spring barley. *Plant Pathology*, **32**:79-89.

Mansfield, J.W., 1983. Antimicrobial compounds. In: *Biochemical Plant Pathology*, (Callow, J.A., ed.), pp.237-265. John Wiley & Sons Ltd.

Mence, M.J. and Hildebrandt, A.C., 1966. Resistance of powdery mildew in rose. *Annals of Applied Biology*, **58**:309-320.

Nashaat, N.I. And Moore, K., 1991. The expression of components of seedling resistance to *Erysiphe graminis* f. sp. *tritici* in *Triticum timopheevi* and a hexaploid derivative. *Plant Pathology*, 40: 495-502.

Ohler, J.G., 1979. Cashew. Koninklijk Instituunt Voor de Tropen. Amsterdam.

Peries, O.S., 1962. Studies on strawberry mildew, caused by *Sphaerotheca macularis* (Wallr.ex Fries) Jaczewski. II. Host- parasite relationships on foliage of strawberry varieties. *Annals of Applied Biology*, **50**:225-233.

Rao, V. N. M. and Hassan, M.V., 1957. Preliminary studies on the floral biology of cashew. *Indian Journal of Agriculture Science*, **27**: 277-288.

Royle, D.T., 1976. Structural features of resistance to plant diseases. In: *Biochemical Aspects of Plant Parasite Relationship* (Friend, J. and. Threlfall, D.R., eds.), pp.161-193. Academic Press New York.

Schnathorst, W.C., 1959. Resistance in lettuce to powdery mildew related to osmotic value. *Phytopathology*, **49:** 562-571.

Schnathorst, W.C., 1960. Effects of temperature and moisture stress on the lettuce powdery mildew fungus. *Phytopathology*, **50**: 304-308.

Sijaona, M.E.R. and Shomari, S.H., 1987. The powdery mildew disease of cashew in Tanzania. *TARO Newsletter*, **11**(3): 4-5.

Sijaona, M.E.R., 1997. Studies on aspects of cashew resistance to powdery mildew (*Oidium anacardii* Noack). Ph.D. Thesis, Wye College, University of London.

Stanbridge, B.; Gay, J. L. and Wood, R. K. S., 1971. Gross and fine structural changes in *Erysiphe graminis* and barley before and during infection. In *Ecology of leaf surface Micro-Organisms* (Preece, T. and Dickinson, C., eds.), pp. 367-379. Academic Press, London.

Waller, J.M.; Nathaniels, N. Q.R.; Sijaona, M.E.R. and Shomari, S.H., 1992. Cashew powdery mildew (*Oidium anacardii* Noack) in Tanzania. *Tropical Pest Management*, **38**(2): 160-163.

Wood, R. K. S., 1967. Physiological Plant Pathology. Blackwell, Oxford and Edinburgh.