



cottons, *G. herbaceum* var. *africanum* (Prentice, 1972) and three of its ecospecies, was also evaluated under glasshouse conditions.

### Materials and methods

#### PATHOGENICITY OF *M. ACRONEA* TO COTTON CV. MAKOKA

Sixteen, 20 cm diameter, plastic, plant pots were each filled with steam-sterilised soil and planted with an acid-delinted, germinating seed of *G. hirsutum* cv. Makoka. 1500 second-stage juveniles of *M. acronea* in 10 ml of water were inoculated around the planting hole in each of eight treated pots. A 10 ml portion of nematode-free supernatant from the suspension of *M. acronea* was added to the soil in eight control pots. All the pots were arranged in 2 × 8 randomised rows and maintained in a heated glasshouse (25–36 °C) with regular watering and a 13 h day-length for 14 weeks.

The cotton plants were then lopped at soil level, their heights measured and any bolls that had been produced were harvested and weighed. In order to estimate the remaining population of *M. acronea*, the soil was bio-assayed by replanting the control and infested pots with single, one week-old cotton seedlings, cv. Makoka. These plants were carefully up-rooted four weeks later, their roots washed free of soil and stained in hot acid fuchsin in lactic acid and glycerol solution (Bridge *et al.*, 1981). The extent and type of root symptoms were described and the numbers of mature females of *M. acronea* per gram of root counted.

#### PATHOGENICITY OF *M. ACRONEA* TO COTTON CV. MAKOKA, UNDER WATER-STRESSED CONDITIONS

Twelve 0.5 m long tubes were cut from a 20 cm diameter, plastic drainpipe and the lower 15 cm of each was pushed into steam sterilized soil contained in a 25 cm diameter, plastic, plant pot (Fig. 1 A). The tubes were then part filled with more steam-sterilized soil. The remaining space in the top 30 cm of tube was filled with 6 dm<sup>3</sup> of soil which was infested with more than 14 000 *M. acronea* second-stage juveniles. The tubes for the control plants were filled with soil that had been similarly infested, before being autoclaved at 100 k pascals for 30 min, allowed to aerate for 6 weeks, then partially reconstituted with the addition of 100 ml of nematode-free supernatant, from an extract from the unsterilised, infested soil.

Three acid de-linted germinating cotton seeds, cv. Makoka, were planted into the top of each tube. The six infested and six control tubes were then randomised in two rows and maintained in the glasshouse at temperatures fluctuating between 25 and 34 °C, with a 12 h day-length. The soil in the tubes and the plant pot below were kept moist by top watering during the first week to establish the seedlings. After this time, in an attempt to

simulate the dry conditions that prevail in the upper soil layers in the field in southern Malawi, watering was done in the supporting plant pot only. The dry conditions that were thus created in the upper part of the tube were intended to stress the plants for water and stimulate the natural, positively geotropic movement of the tap-root in search of moisture.

The heights of the plants were noted at intervals and observations were made on the roots of any that had died. The date of the onset of square formation was noted and the experiment was terminated after 122 days, once the first bolls had begun to split. The plants were then carefully up-rooted and the final stem and tap-root lengths and the number and weights of the harvested bolls per plant were all noted.

#### INTRA-GENERIC SUSCEPTIBILITY OF *GOSSYPIMUM* TO *M. ACRONEA*

The following species and cultivars of *Gossypium* spp. were raised in 15 cm diameter pots of steam sterilized soil during the course of four separate susceptibility tests :

(i) *Comparative susceptibility between the original wild cotton genome and its derivatives*

- a) *G. herbaceum* var. *africanum*; wild, linted precursor of all modern cottons
- b) *G. arboreum*; Asian, Old World ecospecies derived from (a)
- c) *G. herbaceum*; Asian, Old World ecospecies derived from (a)
- d) *G. barbadense* var. *brasileense*; New World hybrid, derived from (c)
- e) *G. barbadense*; Egyptian, New World hybrid, derived from (d)
- f) *G. hirsutum*; cv. Makoka (control) New World hybrid, derived from (c).

(ii) *Comparative susceptibility of two New World, M. incognita-resistant cultivars*

- G. hirsutum*, cv. Makoka (control)
- and two *M. incognita*-resistant cvs of *G. hirsutum* from the U.S.A. : Clewewilt and Auburn 623 RNR.

(iii) *Comparative susceptibility of three Malawian cultivars*

- G. hirsutum*, cv. Makoka (control)
- and two other Malawian cvs of *G. hirsutum*, derived from Auburn 637 : ALA 54 and LA 70.16.

(iv) *Comparative susceptibility of four African cultivars*

- G. hirsutum*, cv. Makoka (control)
- and three other African cvs of *G. hirsutum*, derived from Auburn 637 : UK 64, Coker 201 and AL-HG 9/238.

Each species/cultivar was replicated four times in tests (i), (iii) and (iv), and six times in experiment (ii). The pots were arranged in randomised rows and 1000 second-stage juveniles of *M. acronea* were inoculated around the

roots of each seedling. The plants were maintained in a heated glasshouse at temperatures fluctuating between 25 and 33 °C for 56 days. After this time the plants were carefully uprooted, their roots washed free of soil, symptoms of *M. acrona* noted and the numbers of mature females counted in random samples of root, stained in a solution of acid fuchsin in lactic acid and glycerol.

#### EFFECT OF GOSSYPOL ON *M. ACRONEA* JUVENILES

Second-stage *M. acrona* juveniles were exposed, in batches of 25, to aqueous solutions of gossypol, at concentrations of 0, 100, 1000 or 10 000 ppm, in glass cavity blocks at room temperature and observed at intervals over a 72 h period.

## Results

#### PATHOGENICITY OF *M. ACRONEA* TO COTTON CV. MAKOKA

The initial growth of the cotton plants infested with *M. acrona* was observed to be slower than that of the controls. After 14 weeks, the mean height of the infested plants had reached 65.7 cm while that of the controls was 102.1 cm ( $p < 0.01$ ). The mean fresh weight of the control plants was more than twice that of the infested plants ( $p < 0.01$ ). While all of the control plants were flowering normally and all but one had produced bolls by the end of the experiment, five out of eight of the infested plants had failed to flower ( $p < 0.05$ ) and only one had been able to produce bolls by the time the experiment was terminated (Table 1).

**Table 1.** Pathogenicity of *Meloidogyne acrona* to cotton cv. Makoka, under a normal watering regime.

	Control	Infested
Days to flowering	14.6	> 33.8 <i>a</i>
Final plant height (cm)	102.1	65.7 <i>b</i>
Fresh weight of stem and leaves (g)	15.3	6.4 <i>b</i>
Fresh weight of green bolls (g)	3.9	0.2 <i>b</i>

Means of eight replicates. *a*: significant at 5% (Chi-squared, Null hypothesis). *b*: significant at 1% (ANOVA).

All infested plants had extensive root proliferation. Slight swellings were visible where *M. acrona* had recently invaded root tips, due to hypertrophy of the surrounding cortical cells. Two or more lateral roots originated from each infection site. In many cases this had been accompanied by a cessation in apical growth of the old lateral. Slight swelling around the head of the swollen females was evident, mainly due to hyperplasia in the stele. Mature females had erupted through the cortical tissue and epidermis, to adopt an endoparasitic habit.

This prolific root condition contrasted with the fine dichotomously branched root system which was present in the control plants.

The bio-assay revealed that the test plants growing in infested soil harboured a mean of 69.5 mature females per gram of root.

#### PATHOGENICITY OF *M. ACRONEA* TO COTTON CV. MAKOKA UNDER WATER-STRESSED CONDITIONS

The growth of the cotton plants in *M. acrona*-infested soil was severely retarded during the first 8 weeks of the experiment. Within the first 4 weeks, five of these infested plants wilted and died, having failed to grow beyond the seedling stage (Fig. 1 A). On uprooting these plants and comparing them with non-infested plants, it was noted that the roots of the infested plants were severely distorted: The tap-root had failed to develop, while the secondary roots were severely stunted and confined within the top 4 cm of dry soil. Healthy tap-roots had reached lengths of 20 cm, or more, in the same time period (Fig. 1 B).

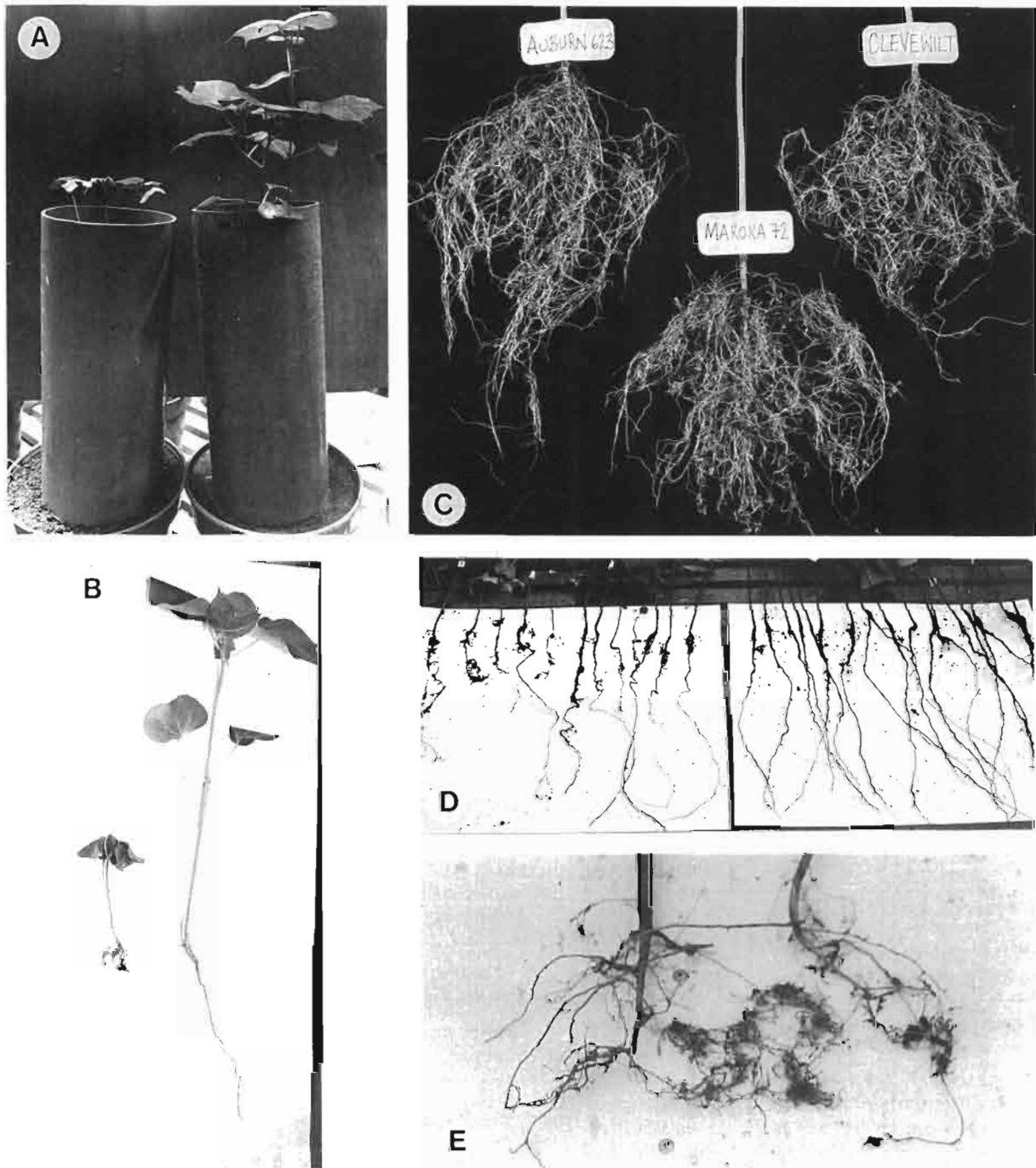
The mean height of the remaining infested plants was 104.2 cm after 21 days, while the mean height of the controls was 204.4 cm ( $p < 0.01$ ). By the 62nd day the mean heights of the remaining infested plants had increased to 118.0 cm, while the height of the controls had more than doubled to 446.8 cm ( $p < 0.001$ ). By the end of the experiment the remaining infested plants had reached a mean height of 342.2 cm, while the controls had reached a mean height of 570.6 cm ( $p < 0.05$ ).

Although square formation had been initiated in all the controls by day 56, the first squares were not produced by the infested plants until day 98. Consequently, the mean number of cotton bolls produced by each infested plant by the end of the experiment was 0.3, with a mean weight of 1.0 g, compared to a mean of 1.9 bolls, with a mean weight of 18.7 g, in the case of the control plants ( $p < 0.001$ ). A yield loss of 94.3% was calculated from the difference in total yields between the infested and the control plants (Table 2).

**Table 2.** Pathogenicity of *Meloidogyne acrona* to cotton cv. Makoka, under water-stressed conditions.

	Control	Infested
Plant height after 21 days (cm)	204.4	104.2 <i>b</i>
Plant height after 62 days (cm)	446.8	118.0 <i>c</i>
Plant height after 122 days (cm)	570.6	342.2 <i>a</i>
Tap-root length after 122 days (cm)	473.5	246.7 <i>b</i>
No. of bolls per plant	1.9	0.3 <i>c</i>
Weight of split bolls (g)	18.7	1.0 <i>c</i>

Means of six × three replicates  
*a*: significant at 5% (ANOVA)  
*b*: significant at 1% (ANOVA)  
*c*: significant at 0.1% (ANOVA).



**Fig. 1.** Effect of *Meloidogyne acronea* on the growth of cotton. *A*: Comparative growth of *M. acronea*-infested (left) and non-infested (right) cotton plants cv. Makoka, after four weeks under water-stressed conditions; *B*: Two cotton plants uprooted from *A*, showing severely distorted root system of the infested plant (left) compared to that of the control plant (right); *C*: Root proliferation in cotton cvs Auburn 623, Makoka and Cleveville, induced by *M. acronea*; *D*: Comparative growth of cotton tap-roots under water-stressed conditions, infested (left) and non-infested (right); *E*: Cotton roots, naturally infested with *M. acronea*, showing stunting and proliferation, collected from the field at Ngabu, Malawi.

Careful removal of all the remaining cotton roots from the tubes revealed that those growing in infested soil were showing proliferation of the lateral roots, symptoms typical of those caused by *M. acronea*. On closer examination, it was observed that the tap-roots of the infested plants were not only stunted, compared to the controls, but had been replaced by a series of lateral roots, giving each one a turned aside or zigzag appearance (Fig. 1 D). The mean length of these substitute tap-roots was 246.7 cm, while the mean length of the true tap-roots which were present on the control plants was 473.5 cm ( $p < 0.01$ ).

#### INTRAGENERIC SUSCEPTIBILITY OF *GOSSYPIUM* TO *M. ACRONEA*

All species and cultivars of *Gossypium* tested showed the same root symptoms as had previously been observed in *G. hirsutum* cv. Makoka infested with *M. acronea* (Fig. 1 C). There was no significant difference between any of the *Gossypium* species or cultivars in their susceptibility to *M. acronea*; almost all were equally, or more susceptible, to this nematode than the control cultivar. *G. hirsutum* cv. Clewewilt having the lowest infection level, was parasitised by a mean of 64.0 mature females per gram of root and *G. barbadense* the highest, was parasitised by a mean of 297.8 mature females per gram of root (Table 3).

**Table 3.** Susceptibility of *Gossypium* herbaceum var. africanum and its derivatives to *Meloidogyne acronea*.

Gossypium species/cultivar	No. of mature females of <i>M. acronea</i> (g of root)			
	Test (i)	test (ii)	Test (iii)	Test (iv)
<i>G. herbaceum</i> var. africanum	214.0			
<i>G. arboreum</i>	144.0			
<i>G. barbadense</i>	297.8			
<i>G. barbadense</i> var. brasiliense	203.5			
<i>G. herbaceum</i>	164.0			
<i>G. hirsutum</i> cv. Makoka	123.0	84.8	130.8	48.5
<i>G. hirsutum</i> cv. Auburn 623		84.3		
<i>G. hirsutum</i> cv. Clewewilt		64.0		
<i>G. hirsutum</i> cv. ALA 54			119.5	
<i>G. hirsutum</i> cv. L 470.16			103.3	
<i>G. hirsutum</i> cv. UK 64			30.5	
<i>G. hirsutum</i> cv. Coker 201				32.5
<i>G. hirsutum</i> cv. AL-HG 9/238			69.0	

Means of four replicates for tests (i), (iii) and (iv); means of six replicates for test (ii).

#### EFFECT OF GOSSYPOL ON *M. ACRONEA* JUVENILES

The *M. acronea* juveniles remained active throughout the 72 h test period, in water and at all three concentrations of gossypol.

## Discussion

It is clear from this work that *M. acronea* is able to cause significant damage to cotton, cv. Makoka, thus confirming the field observations made in Malawi by Bridge *et al.* (1976).

*M. acronea* invades its host via root-tips, curtailing root elongation and stimulating two or more lateral roots to be initiated close to the feeding site, possibly as a result of increased auxin levels. The resultant reduction in plant vigour leads to stunting, delayed flowering and a significant yield loss. The consequences of abnormal root development, induced by *M. acronea*, was even more pronounced, sometimes with lethal effects, when the plants were stressed for water. In this case, overall plant growth was delayed, as apical growth of the tap-root ceased, due to nematode invasion. The hydro-trophic function of the tap-root was then taken over by a succession of young lateral roots, until each, in turn, had its growth checked by nematode invasion.

The xerophytic nature of cotton depends on its ability to extend its tap-root down to the underlying, moist, soil horizons in order to survive the frequent droughts that prevail in its natural environment. This tap-root may reach depths in excess of 2 m in the field (Prentice, 1972) and any damage which impedes this downward extension will have a deleterious effect on the overall growth of the cotton plant, particularly during drought conditions. Although normal growth of the majority of water-stressed, infested cotton plants was resumed once the series of substitute tap-roots had reached the moisture below, flowering had been delayed and this could lead to a significant yield loss in the field, where the rainy season is little more than 4 months long. Yield losses in *M. acronea*-infested cotton has been reported to be up to 50% at Ngabu, Malawi (Emlyn Jones, pers. comm.). The twisted or zigzag appearance of the substitute tap-roots closely resembled those which were collected from *M. acronea*-infested plants in the field (compare Figs 1 D and 1 E) which, originally, were said to have been turned aside by a weak plough-pan in the soil (Mitchell, 1973).

The cotton root symptoms caused by *M. acronea* contrast markedly with those caused by *M. incognita*. This latter gall-inducing species has little effect on the downward extension of the tap-root (Atkinson, 1892; Sasser, 1972). *M. incognita* infestation of the lateral roots also results in fewer feeder roots being produced by the cotton plant (Reynolds, 1973). Furthermore, in terms of its status as a cotton parasite, *M. incognita* is probably less well adapted to this host than *M. acronea*, because *M. incognita* thrives best in more mesophytic regions of the tropics and sub-tropics, for which cotton has only recently been bred.

The high degree of resistance that exists in cv. Auburn 623 RNR to *M. incognita* populations does not necessarily imply co-existence between these two orga-

nisms, as postulated by Shepherd (1983). The phytoalexin activity which is responsible for this resistance may be present in the seeds, leaves, flower buds, stem and root cortices of the cotton plant (Ingham, 1972) and is unlikely to have evolved specifically to counter nematode invasion but rather in response to attack from a range of pests. For example, gossypol and related terpenoid aldehydes are antifungal phytoalexins (Veech, 1981) while gossypol alone is the most important allelochemical that provides resistance to insects in cotton (Parrot, 1990).

The ability of *M. acronea* to successfully parasitize *G. herbaceum* var. *africanum* and its derivatives, including the *M. incognita*-resistant cultivars, Cleve-wilt and Auburn 623, together with the sustained mobility of the juveniles in the presence of gossypol, suggests that there has been co-existence and even some degree of co-evolution between *M. acronea* and this precursor of all modern cottons. This suggestion is plausible since isolated occurrences of *M. acronea* have been found in two areas of semi-arid savannah in southern Africa, namely the lower Shire valley of Malawi (Bridge *et al.*, 1976) and Vryburg Province in South Africa (Coetzee, 1956) which form part of the centre of origin for *G. herbaceum* var. *africanum*. It is ironic that *M. acronea* may have survived on indigenous hosts in southern Africa, since early Tertiary times; meanwhile the *G. herbaceum* var. *africanum* genome speciated in Asia, floated across the Atlantic (Purse-glove, 1984), hybridized with a New World lintless species of *Gossypium*, speciated into *G. hirsutum*, was bred into annual cultivars such as cv. Auburn 637 (Prentice, 1972) and subsequently into Makoka, the cultivar destined for peasant farmers in the lower Shire valley, where it was reunited with this parasite once again.

*M. acronea* has, at present, a very restricted distribution, being found only in southern Africa (Page, 1985). In common with most nematode pests, *M. acronea* has recently achieved pest status through the introduction of modern intensive cultivation practices (Page & Bridge, 1993). In the wild it is likely that surviving infested perennial cotton plants would tolerate invasion by *M. acronea*, once the substitute tap-root had made contact with the water-table and may develop resistance as the root systems become protected by VA mycorrhizal fungi (Page, 1983, 1985). Where soil moisture is high *M. acronea* may go into decline as adults and juveniles become infected with *Pasteuria penetrans* (Page & Bridge, 1985). Unfortunately, the practice of continuous monocropping with annual cottons repeatedly exposes plants to infection at their most vulnerable stage and this leads to an unnaturally large build-up of nematodes in the roots. Up to 3762 mature *M. acronea* females per gram of cotton root were found in the field at Ngabu Experiment Station (Bridge *et al.*, 1976). However, the distribution of this nematode appears to be restricted to soils with a high water-holding capacity, which allow the eggs

to survive over the 6-7 month-long dry season (Page, 1984). Therefore, it is unlikely that *M. acronea* poses a serious threat to small-holder cotton while it continues to be grown under non-intensive and rainfed conditions.

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