Effects of the physical properties of two tropical cotton soils on their permanent wilting point and relative humidity, in relation to survival and distribution of *Meloidogyne acronea* [1]

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SUMMARY

The distribution of *Meloidogyne acronea*, an important pest of cotton in southern Malawi, was found to be restricted by soil-type. Sampling had shown that this nematode is present in large numbers in an alluvial soil while being almost entirely absent in vertisol from the same field. A preliminary experiment indicated that *M. acronea* is able to complete its life cycle on cotton equally well in both soil-types during the growing season. *In vitro* studies showed that it survives dry conditions as dormant eggs within egg masses at a relative humidity of 97.7% but not at 96 or 100% in the absence of a host. Investigations of the two soils at permanent wilting point (p.w.p.) showed that there are significant differences in their relative humidities and rates of infiltration of water. Other studies showed that shrinkage occurs on drying in the vertisol but not in the alluvium. In temperate soils p.w.p. is rarely reached under natural conditions whereas in tropical soils it is a regular occurrence. The dry season in Malawi often lasts up to seven months. The differences in physical properties between the two soil-types not only affects their relative humidities at p.w.p. but also the level of soil moisture that is subsequently lost to the atmosphere as the dry season progresses. The vertisol has a relative humidity of less than 96% at p.w.p. and further moisture is lost during the dry season due to the shrinkage and cracking of the soil. This ensures the death of all *M. acronea* eggs and thus limits its distribution to alluvial soils which have a high waterholding capacity.

RÉSUMÉ

Effets des propriétés physiques de deux sols tropicaux cultivés en cotonnier sur leur point de flétrissement permanent et leur humidité relative mis en relation avec la survie et la répartition de Meloidogyne acronea.

Il a été observé que le type de sol influence la distribution de *Meloidogyne acronea* Coetzee, 1956, un nématode très nuisible au cotonnier, dans le sud du Malawi. L'étude des échantillons a montré que le nématode est présent en grand nombre dans un sol alluvial mais presque totalement absent par contre dans un vertisol du même champ. Une expérience préliminaire a montré que *M. acronea* est capable d'achever son cycle biologique, pendant la période de croissance du cotonnier, indifféremment sur les deux types de sol. Des études *in vitro* ont montré que *M. acronea* survit aux conditions de sécheresse, à l'état d'œufs dormants, au sein d'une masse d'œufs, à une humidité relative de 97,7%, mais ne survit pas à 96% ou 100% en l'absence d'un hôte.

Des recherches, faites sur les deux sols au point de flétrissement permanent, ont révélé l'existence de différences significatives au niveau de l'humidité relative et de la vitesse d'infiltration de l'eau. D'autres études ont montré qu'une contraction se produit au cours du dessèchement du vertisol, ce qui n'est pas le cas du sol alluvial. Dans les sols tempérés, le point de flétrissement permanent n'est généralement pas atteint dans les conditions naturelles tandis que dans les sols tropicaux c'est un phénomène fréquent. Au Malawi la saison sèche dure souvent jusqu'à sept mois. Les différences dans les propriétés physiques entre les deux types de sols n'affectent pas seulement leur humidité relative au point de flétrissement permanent mais également le niveau d'humidité dans le sol, laquelle est par la suite perdue dans l'atmosphère au cours de la saison sèche. Le vertisol au point de flétrissement, présente une humidité relative inférieure à 96% à laquelle s'ajoute une perte d'humidité supplémentaire durant la saison sèche, due à la contraction et à la fissuration du sol. Ceci cause la mort de tous les œufs de *M. acronea* et limite ainsi la répartition du nématode aux sols alluviaux qui ont une plus grande capacité de rétention d'eau.

Cotton, *Gossypium hirsutum* L., is the main cash crop for small-holders in the lowland areas of southern Malawi, where rainfall is unreliable and scant and dry seasons are long. A new pest of cotton, identified in 1975 as *Meloidogyne acronea*, Coetzee, 1956, was suspected of causing serious damage to cotton in this area (Bridge, Jones & Page, 1976) and recent work has shown that this nematode can cause delay in flowering, and a significant yield loss in cotton and sorghum, (Page & Bridge, unpub.).

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A survey of the cotton-growing areas of Malawi early in 1975, (Bridge & Page, 1975) and early 1977 (Bridge & Page, 1977) showed that *M. acronea* is restricted to two sites in the lower Shire Valley : the village of Mafunga to the east of the Shire river and Ngabu village on the west bank, which includes Ngabu experimental farm attached to the Shire Valley Development Project. Two soil types, an alluvium and a vertisol (Mitchell, 1973) occur at Ngabu Farm. Extensive sampling on the farm showed *M. acronea* restricted to the southern part, on the alluvial soil, and nearly or completely absent in the vertisol north of the watercourse that bisects the farm.

Several explanations for such localised occurences of *M. acronea* were investigated. The suggestion that *M. acronea* was a recently introduced pest was dismissed due to the seclusion of Mafunga. Chemical analyses of the alluvial soil and the vertisol and the role of micro-organisms in the two soil types showed no appreciable differences. To determine whether a difference in physical properties between the two soils was a factor limiting the distribution of the nematode, it was necessary first to establish whether *M. acronea* could survive and reproduce in moist vertisol in the presence of a host. To determine what effects the dry season could have on the soil environment in relation to the survival of *M. acronea* the evaporation rate, moisture content and especially the permanent wilting point (p.w.p.) within both the vertisol and the alluvium were measured.

Development of *M. acronea* on cotton roots in two soil types in field conditions

Vertisol and alluvial soil from Ngabu farm were autoclaved at 15 p.s.i. (100 k Pa) for 20 min and left to aerate for several days. Plastic pots of 18 cm diameter were filled with each of these soils. The pots were then partially immersed in 35 cm pots of sterilised soil, to provide a barrier to natural infection when they were sunk into the earth at Ngabu farm. Each soil type was replicated five times and a germinating cotton seed was planted in all of the small pots. 10 000 juveniles of *M. acronea*, plus the supernatant from an extract of unsterilised soil, were inoculated around the roots of each cotton seedling. The whole experiment was maintained under field conditions during the growing season, except that all pots were watered daily. Eight weeks later the cotton plants were all lifted, and their roots washed free of soil and stained in boiling acid fuchsin/lactic acid/glycerol solution for 3 min (Bridge, Page & Jordan, 1981). Mature females of *M. acronea* were counted and their numbers per gram of root estimated.

Mean numbers of 89.0 $\pm$ 49.6 and 88.8 $\pm$ 50.5 females of *M. acronea* per gram of root were recovered from the cotton plants growing in the vertisol and the alluvium, respectively, indicating that *M. acronea* was able to invade, develop and reproduce equally well in both soil types.

Determination of soil moistures at p.w.p. in the two soil types

Cotton plants were grown to maturity in 12 $\times$ 20 cm pots, six containing vertisol and six alluvial soil, in a heated glass-house, 25-33°. Watering was then stopped and the soil was left to dry until all the plants had wilted irrecoverably, indicating that p.w.p. had been reached. Small clods of soil were taken from the centre of each pot and the percentage moisture content measured by drying to constant weight. The moisture content of the alluvial soil at p.w.p. ranged from 10.91 to 16.53%, (mean 13.16% $\pm$ 0.8), while that of the vertisol was lower, 8.19 to 11.89%, (9.87% $\pm$ 0.5), ($p = < 0.01$).

Determination of infiltration rates of water in the two soil types

Plastic beakers, 3.5 cm diameter, with their bases removed, were pushed 1 cm into the surfaces of vertisol and alluvial soil at p.w.p., in 23 cm plastic pots. The beakers were then all filled with 50 ml of water and the time taken for it to drain away was recorded. This was repeated three times in each pot. The infiltration rate of the vertisol was much higher than that of the alluvium. It took a mean of 125 $\pm$ 7.6 secs for 50 ml of water to pass trough a 9.6 cm^2 area of the vertisol but a mean of 6,385 $\pm$ 222.0 secs for the same volume of water to pass through the same area of alluvial soil.

Survival of *M. acronea in vitro* at different relative humidities

Aqueous solutions of sulphuric acid or glycerol at concentrations adjusted to provide the following range of relative humidities, 5, 10, 30, 50, 80, 90, 93, 95, 96, 97.7 and 100% (at 25°) were placed in desiccators, (Solomon, 1951 ; Simons, 1973). Two watch glasses, one containing ten females of *M. acronea*, each full of eggs, plus attached egg-masses and the other containing 100 juveniles of *M. acronea* were placed inside each dessicator. The sealed dessicators were maintained at 25° for nine weeks, and after this time the different stages of *M. acronea* were removed, allowed to re-hydrate in Petri dishes of water at room temperature and observed at intervals over a period of fifteen days.

At 100% relative humidity, some second stage juveniles of *M. acronea* were still alive after nine weeks, though highly vacuolated. Juveniles had hatched from within females and attached egg-masses maintained at 100% relative humidity; many of these juveniles were still active, though they too were vacuolated. No juveniles had remained alive at humidities less than 100%. At 97.7% relative humidity the females and attached egg-masses remained intact after nine weeks and juveniles began to hatch only after ten days' rehydration and this continued for a further five days until 61% of the eggs had hatched. The remaining unhatched eggs had been invaded by a fungus (identified by C. Booth, Commonwealth Mycology Institute, as *Fusarium moniliforme* Sheld). Re-hydrated eggs that had been kept at any relative humidity less than 97.7% did not hatch.

The comparative effect of relative humidity on the moisture content in the two soil types

A number of 3 cm³ clods were taken from pots of both vertisol and alluvial soil and placed undisturbed at the same relative humidities as above. This soil had previously been allowed to settle with regular watering over a period of six months to give a stabilised structure. After nine weeks at a constant temperature of 25° the moisture contents of the soil cores were measured. The moisture contents of the vertisol were consistently lower than those of the alluvium at each relative humidity tested (Fig. 1).

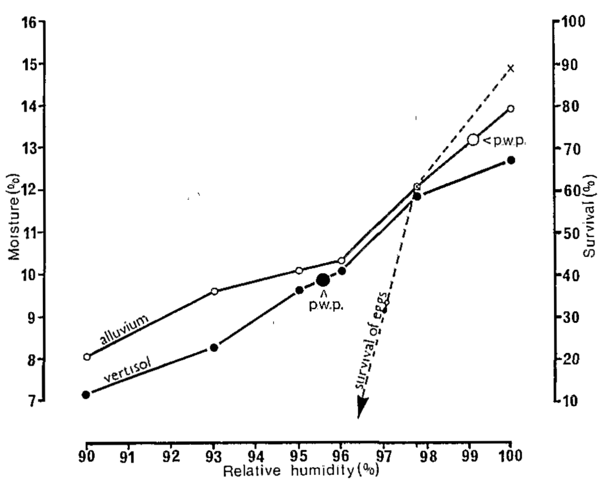

Fig. 1. Comparison of permanent wilting point (p.w.p.) in vertisol and alluvial soils at different conditions of moisture and relative humidity in relation to survival of *M. acronea* eggs at 25°.

The comparative effect of relative humidity on the specific volume in the two soil types.

A number of clods approx 2 cm³ of vertisol and alluvial soil, having retained their natural structure and pore space as above, were allowed to reach equilibrium at 95% relative humidity and field capacity over a period of two weeks. The dry clods were then infiltrated with paraffin in an evacuated chamber and the volumes of all the clods were determined using a method devised by Currie (1966). The specific volumes were calculated from a mean of two results for both soil types, and the changes in volume determined. The specific volume of the vertisol was 0.700 at field capacity and 0.612 at 95% relative humidity, a shrinkage of 14.5% on drying. The alluvium had a specific volume of 0.696 at field capacity and 0.764 at 95% relative humidity, a swelling of 8.9%.

Discussion

Conditions were apparently equally suitable for the development and reproduction of *M. acronea* in both the vertisol and the alluvium during the growing season, suggesting that it is soil conditions during the dry season, once p.w.p. had occurred, which restrict its survival. The eggs of *M. acronea* are the survival stage and dormancy occurs when the relative humidity is between 96 and 100%. Outside this range the eggs are killed or hatched nematodes die in the absence of a host.

Couch and Bloom (1960) found that eggs of *M. hapla* hatched equally well at field capacity and p.w.p., but at p.w.p. hatched nematodes were unable to invade due to higher moisture stress. Eggs of an isolate of *M. javanica* (Treub) Chitwood from Zimbabwe remained unaffected after twenty days exposure to 100% relative humidity (Daulton & Nusbaum, 1962). At 100% relative humidity, juveniles of *M. acronea* remained active and eggs hatched continually over a nine-week period, though in the absence of a host it is likely that death would have followed. Godfrey, Oliveira and Gittel (1933) and Tyler (1933) suggested that *M. javanica* populations are decreased more rapidly in wet soil than dry soil because eggs continually hatch and the juveniles are continuously active resulting in a rapid depletion of their energy reserves (Conrad

& Veihmeyer, 1929 ; Daulton & Nusbaum, 1962). Once the relative humidity falls below 100% little emergence occurs and the eggs of *Meloidogyne* spp. are protected by the gelatinous matrix of the eggs sac (Wallace, 1966, 1968). Further hatching is then inhibited due to moisture stress (Dropkin & Martin, 1957). In *M. acronea* some eggs are retained within the body of the dead female and are protected by the cuticle, (Bridge, Jones & Page 1976).

At 97.7% relative humidity, the juveniles of *M. acronea* did not survive but the eggs remained dormant for nine weeks ; further development and hatching began only after ten days rehydration.. De Guiran & Demeure (1978) and de Guiran (1980) have demonstrated a diapause in *M. incognita* eggs. A diapause was not observed in *M. acronea* eggs as the remaining unhatched eggs were invaded by a fungus, reflecting what may happen in nature. By 96% relative humidity, both eggs and juveniles of *M. acronea* had died after nine weeks. Godfrey and Hosking (1932) and Godfrey, Oliveira and Gittel (1933), found that at 90% humidity eggs in egg masses die within nine hours.

P.w.p. is considered to occur when the suction in the soil reaches 15 atmospheres or pF = 4.19, although for temperate zone plants it is a range of between 8 and 30 atmospheres (pF 3.9-4.45), (Loveday, 1974). The two soil types from the lower Shire Valley have different physical properties which cause water to be held in the alluvium at higher water tensions than in the vertisol and lead to an important difference in pF value at p.w.p. The cotton plants reached p.w.p. in the alluvium when the moisture content corresponded to a mean relative humidity of 98.9% (pF = 4.2 at 25°) and in the vertisol when the moisture content corresponded to a mean relative humidity of 95.5% (pF = 4.8 at 25°). A relative humidity of less than 97.7% would not be conducive to the survival of *M. acronea* eggs (Fig. 1). The pF value at p.w.p. therefore, should not be regarded as a constant but rather as a mean, as suggested by Slayter (1967).

In temperate soils p.w.p. is rarely reached under natural conditions and pore size is of importance only in determining the degree of aeration and the amount of space available for movement of nematodes within the soil (Jones, Larbey & Parrott, 1969). In the Tropics long periods of drought are often the norm and it is soil pore size and structure, in addition to aeration and movement, which determine the amount of water that will be retained after p.w.p. has been reached. The dry season in the lower Shire Valley lasts six to seven months. The alluvial soil from this part of Malawi is a highly porous clay with a top soil that slakes in water, whereas the vertisol is a montmorillonite clay (Mitchell, 1973). Montmorillonite has a considerable capacity for swelling on wetting, (Norrish, 1954 ; Quirk & Aylmore, 1960). Shrinkage occurs in the vertisol during the dry season and cracks more than 1 cm wide appear, penetrating at least to the middle of the soil profile, (Mitchell, 1973). This shrinkage was demonstrated during this work. (The apparent increase in volume that occurred in the alluvium on drying was probably due to its slaking property).

During the dry season, in the absence of roots of growing plants, soil below the immediate surface layer will lose its moisture only at the extremely slow rate induced by thermal effects, (Rostmistrov, 1913). In Senegal soil at a depth of 20-40 cm remained at p.w.p. throughout the nine-month long dry season (Demeure, 1976) and in the Ivory Coast at a depth of 25 cm for more than seven months in absence of a host (de Guiran & Germani, 1980). The slaking of the alluvial topsoil along with its narrow pore space, contribute to its high water-holding capacity (Mitchell, 1973) even when surface temperatures reach 60°, as dry season records for this part of Malawi confirm. By contrast the rapid infiltration rate of the vertisol demonstrated during this work suggests that its evaporation rate will be correspondingly high. This would lead to further drying out from the cracks within the vertisol as the dry season progresses (Adams & Hanks, 1964 ; Adams *et al.*, 1969). As the p.w.p. of the vertisol occurs when the relative humidity has already fallen below the level required for the survival of *M. acronea* eggs, any further drying out of the soil will ensure their complete destruction (Fig. 1). It is thus the soil's inability to retain moisture during the dry season which restricts the survival and thus the distribution of *M. acronea* within the Shire Valley. Previous work has shown that soil structure can affect the survival of nematodes within it. *Trichodorus* and *Longidorus* spp. occur in significant numbers only in soils with 80% or more coarse particles (Jones, Larbey & Parrott, 1969). *Heterodera glycines* Ichinohe, 1952 was said to survie longer in a clay soil type, similar to that in which rice might be grown (Slack, Riggs & Hamblen, 1972). Infestation by *Meloidogyne javanica* was extremely light in *Sesbania exaltata* (Raf) grown in fine-textured or clay loam soil and very heavy in a coarse textured or loamy sand soil this suggests a fairly close correlation between particle size and root-knot infection (Sleeth & Reynolds, 1955). Martin (1967) when working on tobacco in Zimbabwe, noted that the calcic hydromorphic black heavy clay or black vlei soil, was a less favourable medium for *Meloidogyne javanica* than red or sandy soils and suggested that the soil type itself was a contributory factor.

In Malawi alluvial soils with a high waterholding capacity occur along the present flood plains of the many tributaries that emerge from the hills and flow towards the Shire river. Vertisols cover a much larger part of the lower Shire Valley (Mitchell, 1973), but they are more difficult to work especially as they are baked hard prior to the onset of the rains, when most of the tillage has to be done. Both soil types are suitable for the cultivation of cotton as it is not particularly specialised in its requirements (Prentice, 1972). *M. acronea* has so far been identified at just three sites in the lower Shire Valley (Bridge & Page, 1977), infestation at these sites can be minimised at present by moving the susceptible crops to areas of vertisol. Irrigation is planned for the Shire Valley and once this is installed there will be a general increase in soil moisture, especially in the vertisols ; so, the annual fall in relative humidity to less than 96% may no longer occur, the limiting factor to the survival of *M. acronea* eggs during the dry season will be lost, and this nematode is then likely to spread throughout the valley.

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