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FINAL REPORT

The influence of beneficial soil invertebrates on soil function and productivity

CSE 105C

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Part 1 - Summary Details

REPORT

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Part 3 - Final Report

1. Background

Healthy soils are fundamental to the profitability and sustainability of cotton ecosystems. A recent soil health workshop convened by CRDC & Australian Cotton CRC (Dec. 2001) recognised several issues needing increased attention. They included the paucity of understanding of soil fauna and its role in improving soil structure and fertility, reducing disease and pest incidence and enhancing cotton production. In addition, the impact of management practices (e.g. pesticides, nitrogen application, irrigation, pupae busting) on the abundance and functional biodiversity of beneficial soil fauna is poorly understood.

Earthworms, sometimes referred to as “ecosystem engineers”, have long been regarded as flagships amongst soil fauna, because of their widely demonstrated potential to influence soils (including other biota) and plant production. The benefits earthworms can bring include improved aggregate stability, increased porosity, aeration and water infiltration, enhanced nutrient availability, retention of nutrients on-farm (through efficient burial of surface organic matter and fertilisers and prevention of leaching in surface water flow), deeper rooting of plants, and reductions in the incidence of root diseases.

There have been some surveys of surface-dwelling invertebrates in relation to management practices in cotton fields, but very few studies have considered the functional role of the soil fauna. Most notably, one earthworm, the native *Heteroporodrilus mediterreus*, has been linked to improved water movement through the profile and better water use by cotton plants under periods of reduced water availability. Surveys of earthworms in and near cotton fields appear not have been done, but extensive surveys of pastures and cereal crops throughout south-eastern Australia have shown that the earthworm fauna there is dominated by accidentally introduced, exotic species, particularly Lumbricidae from Europe (e.g. *Aporrectodea trapezoides*). The distributions of these species extend through northern NSW to southern Qld. Further north, other species of earthworms of exotic tropical origin (e.g. *Pontoscolex corethrurus*) become more common. Whether or not these common species have colonised cotton fields or could colonise them if given the chance is unknown. Cotton farming in the past has presented several potential hazards for soil fauna (e.g. heavy pesticide use, tillage), but recent trends in the industry such as less frequent use of and softer pesticides, reduced tillage and greater organic matter retention, would seem likely to open opportunities for population growth and (re)colonisation of soil fauna like earthworms – if locally available on-farm. Indeed, in reduced tillage systems, soil engineers such as earthworms take on more critical roles, replacing some of the benefits the plough brought (e.g. redistribution of organic matter through the soil profile).

Earthworms such as *A. trapezoides* and *P. corethrurus* can significantly enhance plant production (e.g. increases in pasture and wheat yield and N content) and programs to introduce them to farms have been undertaken in several countries. However, not all crop species respond to the presence of worms. For example, whilst wheat and oats grew and yielded more in field cages in SA which included *A. trapezoides*, lupins did not. In several tropical countries, field trials have shown that whilst grain biomass of sorghum and maize can be greatly increased by the addition of worms including *P. corethrurus*, yields of cowpea and peanuts are reduced. The implications for cotton production and quality through improved management of soil fauna such as earthworms remains unknown, but should be explored.

Recent research has shown that soil fungi are important food sources for earthworms. Earthworms have the capacity to reduce the incidence of fungal root diseases. Some species of *Fusarium* are highly preferred food sources for several common earthworm

species. *Fusarium* wilt is a major problem for cotton production in Australia. It would be sensible to check if a more robust soil fauna community, earthworms in particular, would have the potential to reduce the incidence of *Fusarium* in cotton.

Historically, there has been limited linkage between above and below ground ecological studies in cotton ecosystems in Australia. However, research elsewhere has shown that soil biota (e.g. earthworms and Collembola) can greatly influence the abundance and diversity of foliar feeding insect pests (e.g. aphids) through stimulation of plant growth and nutrient content. The quality (e.g. polyphenolic content) of plants can, as well as being harnessed by plant breeders to reduce above-ground herbivore attack, reduce rates of nutrient cycling below ground through the relative acceptance of plant litter as food for saprophagous organisms. Further work is needed to test for linkages between soil faunal activity, nutrient supply and foliar pest status in cotton systems.

2. Project Objectives

This project sought to initiate studies on :

1. The factors influencing the establishment and abundance of earthworm populations in cotton ecosystems, and
2. The potential of earthworms to improve soil health & plant production in cotton ecosystems.

The main objectives of this study were to :

(i) Conduct surveys of earthworm fauna in irrigated and dryland cotton, in other associated crops, in cotton season and during the winter – at ACRI and elsewhere in the Namoi Valley.

Substantially Achieved : When soils dry out, resident earthworms retreat deep into the soil profile, and are thus very difficult to sample effectively. The recent drought made planned, initial surveys in the Namoi Valley pointless in late winter – early spring 2002 [this time of year is appropriate for surveys in southern Australia, because mature earthworms (required for species IDs) are most common then. In the absence of any information to the contrary, we assumed the same would apply for the Namoi Valley]. These surveys were thus postponed until winter-spring 2003. We had originally planned to involve farmers and IDOs in additional surveys in the second year of the project, but the delay in the initial survey limited our options on this. Also, we deemed it better to focus ourselves tightly on selected sites, rather than risk frustration amongst “volunteers”, working more broadly in likely sub-optimal conditions (following drought conditions when earthworm numbers were likely to be reduced). See comments made in progress reports.

(ii) Conduct glasshouse (pot) experiments to determine earthworm effects on cotton growth and yield.

Achieved :

(iii) Conduct earthworm inoculations and field cage experiments to determine establishment and effects on cotton.

Not Achieved : Our original intention was to initiate inoculations and field cage (PVC pipe / mesh based) experiments to determine establishment of earthworms and their effects on cotton growth. However, because of the drought-related delays in surveys (and thus identification of appropriate earthworm species to use for such inoculations), low abundances of earthworms locally in the Namoi Valley and concerns of what might be introduced there accidentally (e.g. soil born plant diseases)

by introducing earthworms from elsewhere, we considered it better to concentrate on glass-house studies. See comments made in progress reports.

3. Methodology

(i) Field Surveys

A total of 64 cotton farms were surveyed for earthworms, in August and September 2003. Most sites were in the Namoi Valley, northern N.S.W. (e.g. from Breeza Plain south of Gunnedah, to Bellata north of Narrabri, and Burren Junction and Pilliga west of Wee Waa), plus a site in the Macintyre Valley near Goondiwindi, Qld. Soils were prepared for chemical analyses of key soil attributes by a routine analytical laboratory. A mix of dry-land and irrigated farms was surveyed, but the focus was mainly on the latter.

On most farms, two transects (each comprising 5 sample points, placed 10 m apart) were surveyed within one field that had recently been used for cotton production. The two transects were located on opposite sides of the field, thus several hundred metres apart. Habitats with relatively undisturbed soils were identified near to each transect in the cotton fields, and a similar transect of sampling points was surveyed there. These “undisturbed” habitats were commonly roadside verges, stock routes, and pastures. There were two exceptions to this methodology. At Breeza Station in the Upper Namoi Valley, 5 cropping soil transects and 4 undisturbed soil transects were sampled. At Macintyre Downs, Goondiwindi, 8 cropping soil transects and 7 undisturbed soil transects were sampled.

At each sampling point, a soil sample (0.1m² in area, 10 cm deep) was excavated by spade and hand-sorted for earthworms. The earthworms thus found were preserved immediately in 70% ethanol for later identification. The ethanol used in the field was replaced with fresh ethanol within 24 h for storage of the specimens. A handful of top soil from each hole dug for earthworms was retained for later soil characterisation analyses. These soil samples were bulked for each transect, mixed and then sub-sampled. Only the soils in the “cotton” fields have been analysed thus far. The following characteristics were measured : particle size (% coarse sand, fine sand, silt and clay), pH, electrical conductivity (E.C.), Organic C, Total N and Olsen P. These characteristics, along with rainfall, include some of the main factors found to correlate with earthworm abundance in surveys elsewhere in Australian agricultural soils. Local site descriptions were also recorded (e.g. current botanical composition – major species, recent agricultural use / practices as indicated by the farmer).

(ii) Glasshouse Experiments

a. Earthworm collections

Two earthworm species, with contrasting burrowing behaviours, were used throughout the experimental work; *Aporrectodea caliginosa* and *A. longa* (Lumbricidae). *A. caliginosa* is an endogeic species, feeding mostly within the mineral soil layer and with a predominantly horizontal burrowing behaviour. *A. longa* is an anecic species, feeding mostly at the soil surface and with a predominantly vertical burrowing behaviour. Both species were collected in late winter or early spring from pastures in north-western Tasmania where they are extremely numerous and can be obtained easily. Both species are exotic (European). Neither was recorded during the earthworm survey in the Namoi region – they were selected for the glasshouse experiments because of their contrasting behaviours and their ease of collection in large numbers. We aimed to use them as model systems (in particular when faced with collecting difficulties in cotton growing regions, due to drought).

The earthworms were usually used for experiments immediately upon returning with them from Tasmania. They were transported in moist sphagnum moss. In some cases, the earthworms were maintained in a constant temperature cabinet (15°C; 12:12 light dark regime) in moist, commercial, sandy loam prior to experimental use.

b. Earthworm Survival in Different Soils

Glasshouse experiments were conducted in Canberra, using contrasting cotton soils from Narrabri, ACRI (grey clay) and Hillston (red-brown earth), as well as a commercial sandy loam (“Gardener’s Choice”, from Garden World, Hume) (Table 1) to determine the relative survival and growth of *A. caliginosa* and *A. longa* in two soils used for cotton production and another (sandy loam) already

known to be acceptable to the earthworms. Plastic flower pots (approx 30 cm diam at top rim, free-draining with drain hole covered by fine mesh) were filled with soil (8-9 kg soil pot⁻¹). The soils were watered to 20-25% gravimetric soil moisture content (suitable for earthworm survival and growth). There were 10 replicate pots per soil treatment. The pots were maintained in a constant temperature cabinet at 15°C; 12:12 light - dark regime. Weights of the pots were checked regularly throughout the experiment and watered as needed to control water content at 20-25%. 200 g of finely chopped cotton trash was added to the soil surface in each pot (source of food, should it be selected).

Table 1. Physical and chemical characteristics of soils used in experiments

	Narrabri Grey Clay	Hillston Red-Brown Earth	Commercial Sandy Loam
Particle Size			
Coarse Sand (% w/w)	13.5	7.3	54.3
Fine Sand (% w/w)	14.4	30.5	23.4
Silt (% w/w)	17.0	10.3	8.5
Clay (% w/w)	51.0	48.3	8.5
pH (CaCl ₂)	7.5	7.4	6.9
E.C. (dS/m)	0.22	0.61	0.48
Organic C (% w/w)	1.00	0.88	3.4
Organic Matter (% w/w)	1.9	1.7	6.5
Total N (% w/w)	0.07	0.09	0.07
Olsen P (mg/kg)	14	27	29

Earthworms (large juveniles or adults) were maintained in moist sphagnum moss overnight, prior to washing in water, blotting dry, determining their fresh weights and then adding to the pots. This period in moss voided the earthworm's guts and enabled more exact body mass measurements. The weights of the worms were measured in groups of 10 individuals of the same species. Groups were selected such that their biomass varied little. A group of ten earthworms of each species was added to each pot and the tops of the pots were "sealed" with fine mesh to prevent earthworm escape.

After approx 10 weeks, the earthworms were hand-sorted from the soil in each pot, washed in water and maintained on moist filter paper overnight (to again void their gut contents) and then weighed fresh.

c. Soil Preferences of Earthworms

Preferences of earthworms (*A. caliginosa*) within a range of soil types were measured using short-term choice tests (1 week exposure). Four soil types were used : the soils from Narrabri, ACRI (grey clay), Hillston (red brown earth) and the commercial sandy loam ("Gardeners' Choice") referred to above, plus a grey clay from CSIRO Plant Industry's Ginninderra Farm in Canberra. Sixty plastic boxes (7 cm high x 12 cm wide x 21 cm long) were filled with equal portions of two different soil types, arranged at either end with a piece of paper tissue separating the soils in the middle of each box. A further 10 boxes contained a blend of equal portions of all four soils mixed together. There were thus 7 treatments :

1. 10 boxes with Narrabri and Hillston soils
2. 10 boxes with Narrabri soil and commercial sandy loam

3. 10 boxes with Narrabri and Ginninderra soils
4. 10 boxes with Hillston soil and commercial sandy loam
5. 10 boxes with Hillston and Ginninderra soils
6. 10 boxes with Ginninderra soil and commercial sandy loam
7. 10 boxes with a mix of all soils in both sides

The soils (approx 1.6 kg in total box⁻¹) were maintained at 20-25% gravimetric moisture content in a 15°C constant temperature room, in the dark. Ten earthworms of similar size (large juveniles or adults) were placed on the soil surface in the centre of each box after first being weighed fresh (see above). The top of each box was sealed with “Glad Wrap” film, which confined the worms and also helped maintain soil moisture. After a week, the soils in each box were hand-sorted for earthworms and their presence in a particular soil type was recorded. The worms were also weighed again.

The experiment was repeated using *A. longa*, except that only 6 earthworms were used box⁻¹ in this case, because of *A. longa*'s larger size).

d. Earthworm Influences on Cotton Growth (ACRI soil and Hillston soil)

Experiment 1. Cotton was grown during spring – summer in small pots made from PVC pipe (20 cm diam, 25 cm tall) to test the influences of earthworms on plant growth. These pots (n = 40) were filled with approx 4 kg of dry Narrabri (n = 20) or Hillston soil (n = 20), with water added to establish the soils at approx 20% gravimetric soil moisture. The pots were maintained in a water bath set at 15°C. The water bath was kept in a glasshouse in which air temperatures (shade) varied from approx 20 - 40°C. Five *A. longa* and 5 *A. caliginosa* (large juveniles or adults) were added to each of 20 of the pots (10 for each soil type), 2-3 weeks after soil establishment, and after the earthworms had been left on moist filter paper overnight to void their gut contents, washed in water, blotted dry and weighed fresh. No fertiliser was applied to the pots. Earthworms were confined within the pots by sealing the tops of the pots with fine mesh for 7 weeks after earthworm addition. Five cotton seeds (SICOT 189 QA) were sown into each pot, 7 weeks after adding the earthworms, and later thinned to 2 plants pot⁻¹ after germination. The pots were removed from the water bath at this time and then maintained on a bench in the glasshouse. The soils in the pots were watered as required to maintain original soil moisture content. After 21 weeks (since adding earthworms, i.e. 14 weeks since sowing cotton seeds), the plants were cut off at the soil surface, dried at 60°C for 20 h and weighed. Bolls and other plant material were treated separately. Earthworms were sought by hand-sorting the soil, but none were found. During the term of the experiment, various measures were made of plant height, number of leaves, plant colour, numbers of squares, flowers and bolls and evidence of earthworm casting on the soil surface. A leaf from the top of the 6th internode was taken from each plant and freeze-dried, 10 weeks after sowing, for N analysis (Kjeldahl technique). Insect pests (thrips and whitefly) were controlled by occasional sprays of Dichlorvos, Regent, Decis and Summer Oil.

Experiment 2. A further experiment was run using the same pots made from PVC pipe (20 cm diam, 25 cm tall). These pots (n = 40) were filled with approx 4 kg dry Narrabri soil, with water added to establish the soil at approx 20% gravimetric soil moisture. The pots were maintained initially in a water bath set at 15°C. The water bath was kept in a glasshouse in which air temperatures (shade) varied from 20 - 40°C. Two *A. longa* and 4 *A. caliginosa* (large juveniles or adults) were added to each of 20 of the pots, after they had been washed in water, blotted dry and weighed fresh. All pots were fertilised with 2 g urea and 0.02 g ZnSO₄ in solution, applied evenly to the soil surface with a syringe. Earthworms were confined within the pots by sealing the tops of the pots with fine mesh for 4 weeks after earthworm addition. The pots were removed from the water bath, 5 weeks after adding earthworms to them, and maintained on a bench for the remainder of the experiment. Five cotton seeds (SICOT 189 QA) were sown into each pot 6 weeks after adding the earthworms, and later thinned to 1 plant pot⁻¹ after germination. The soils in the pots were watered as required to maintain original soil moisture content. After 12 weeks (since adding earthworms), plants were cut off at the soil surface, dried at 60°C for 20 h and weighed. Earthworms were hand-sorted from the soil and weighed fresh after first washing in water and blotting dry.

e. Earthworm Influences on Cotton Growth (ACRI soil)

Experiment 1. A glass-house experiment was established using 30 plastic pots (approx 30 cm diam x 30 cm tall) that were filled with approx 9 kg dry Narrabri soil pot⁻¹. The soils were watered to establish 20-25% gravimetric soil moisture content (suitable for earthworm survival and growth). There were three treatments : 10 pots with 20 *A. caliginosa* each, 10 pots with 10 *A. caliginosa* and 10 *A. longa* each, and 10 pots with no earthworms (control). The fresh weights of the earthworms were measured prior to their addition to the pots (after voiding their guts on moist filter paper for 24 h prior to weighing). The average earthworm weights pot⁻¹ were 8.73 ± 0.22 g ($\bar{x} \pm$ S.E.) (*A. caliginosa* only), 4.36 ± 0.08 g (*A. caliginosa* in pots with *A. longa*), and 17.77 ± 0.35 g (*A. longa* in pots with *A. caliginosa*). 200 g of finely chopped, wet cotton trash was added to the soil surface in each pot. Fine mesh lids were used to seal the tops of the pots and contain the earthworms initially (whilst they “settled in”). Any earthworms that did not burrow into the soil within a few hours were replaced. After 10 weeks, these lids were removed. The pots were initially maintained in a 15°C constant temperature room with 12 : 12 light-dark regime (for 10 weeks; synchronised with pots referred to in b) above) then moved to a naturally lit air conditioned glasshouse in which air temperatures (shade) fluctuated between 20-45°C during the experiment (air coolers set to operate at 25°C and above). At this time, 10 seeds of SICOT189 QA cotton were sown into each pot. No fertiliser was added to the pots. Seedlings were later thinned to 5 pot⁻¹. Weights of the pots were checked regularly throughout the experiment and the pots were watered as needed to control water content at 20-25% (all pots received similar amounts of water). The experiment ran during spring to summer. Pots were arranged at random in the glasshouse. Insecticides were occasionally applied to control two-spotted mite and whitefly on the developing cotton plants (Vertimec, Apollo, Summer Oil, Regent, Decis). Various plant attributes (plant height, number of leaves, plant colour) and evidence of earthworm casting on the soil surface were measured at intervals until 21 weeks after adding the earthworms (i.e. 11 weeks after sowing cotton seed), when the above ground vegetation was harvested and dried at 60°C for 20 h. Virtually no squares formed during the term of the experiment. The soil was then hand-sorted for earthworms, but very few were found in either earthworm treatment. [Note from the results from b) above, earthworm survival should have been high at the time of sowing the cotton seed].

Experiment 2. The previous experiment was of concern in that (i) no fertiliser was used, thus the cotton plants were operating at less potential than likely to be experienced in the field [but under such conditions perhaps any earthworm effects on plant growth might be more obvious ?], and (ii) most earthworms died during the experiment, thus providing a source of fertiliser disproportionate to that available in the control treatment. A further experiment was thus conducted with the following treatments, again using Narrabri soil (maintained at 20-25% soil moisture) and the same 30 cm diam. plastic pots. This experiment was also run from spring to summer.

1. 10 pots with Narrabri soil, fertiliser and no earthworms
2. 10 pots with Narrabri soil, fertiliser and 20 dead *A. caliginosa* (10.82 ± 0.12 g)
3. 10 pots with Narrabri soil, fertiliser and 20 dead *A. longa* (44.47 ± 0.80 g)
4. 10 pots with Narrabri soil, fertiliser and 20 live *A. caliginosa* (10.53 ± 0.10 g)
5. 10 pots with Narrabri soil, fertiliser and 20 live *A. longa* (48.28 ± 0.50 g)
6. 10 pots with Narrabri soil, fertiliser and 10 live *A. caliginosa* (5.39 ± 0.04 g) + 10 live *A. longa* (23.35 ± 0.35 g)
7. 10 pots with Narrabri soil, fertiliser and 20 live *A. caliginosa* (not sown with seed) (10.31 ± 0.13 g)
8. 10 pots with Narrabri soil, fertiliser and 20 live *A. longa* (not sown with seed) (48.22 ± 0.44 g)

Pots were prepared with moist soil, live earthworms (large juveniles or adults) were added as required, and the pots were initially placed in a 15°C constant temperature room with 12 : 12 light - dark regime. 200 g of finely chopped, wet cotton trash was added to the soil surface in each pot. Fine mesh lids were used to seal the tops of the pots and contain the earthworms. Any earthworms that did not burrow into the soil within a few hours were replaced. After 9 weeks, these lids were removed and the pots in treatments 1-6 were moved to a naturally lit air conditioned glasshouse in which air temperatures (shade) fluctuated between 20-45°C during the experiment (air coolers set to operate at 25°C and above). At this time, 10 seeds of SICOT189 QA cotton were sown into each pot. Seedlings were later thinned to 5 pot⁻¹. Weights of the pots were checked regularly throughout the experiment and watered as needed to control water content at 20-25% (all pots received similar amounts of water). Pots were

arranged at random in the glasshouse. Fertiliser (5 g of urea) and 0.05 g ZnSO₄ was added to each pot at the time of sowing the seed, as were the dead earthworms (placed in three 5 cm deep holes in the soil; other treatments received the same holes, but no dead earthworms). Dead earthworms were obtained by freezing similar numbers / biomass of earthworms at the same time as live earthworms were added to the pots 8 weeks earlier. A further 5 g of urea was added to each pot 4 weeks after sowing. The soil in all pots in treatments 7 & 8 was hand-sorted after the 9 weeks in the 15°C room to check survival of the earthworms. All earthworms were weighed fresh at the start and end of the experiment. The average biomass of earthworms added to each pot ($\bar{x} \pm \text{S.E.}$) is given above alongside the relevant treatment.

Insecticides were occasionally applied to control two-spotted mite and whitefly on the developing cotton plants (Vertimec, Apollo, Summer Oil, Regent, Decis). Various plant attributes (plant height, number of leaves, plant colour, numbers of squares, flowers and bolls) and evidence of earthworm casting on the soil surface were measured at intervals until 31 weeks after adding the earthworms (i.e. 22 weeks after sowing cotton seed). Bolls were harvested at 18 and 22 weeks after sowing the cotton seed, dried at 60°C for 20 h and weighed.

f. Earthworm Influences on Cotton and Wheat Growth (ACRI soil and Commercial Sandy Loam)

The earlier experiments which demonstrated little effect of *A. caliginosa* and *A. longa* on cotton growth in Narrabri soil raised the question of whether or not cotton is responsive to earthworm influences (at least in the short term) or if the earthworms are unable to influence cotton growth in Narrabri soil, but might influence it in a different soil type. From previous work, we knew that *A. caliginosa* and *A. longa* can increase wheat growth when it is grown in sandy loam. A glasshouse pot experiment was therefore devised in which both wheat and cotton were grown in Narrabri soil and sandy loam (“Gardiner’s Choice”) concurrently in the presence or absence of earthworms. There were 10 replicate pots for each treatment. The treatments were as follows, with 15 *A. caliginosa* and 15 *A. longa* (either large juveniles or adults) added to each pot requiring earthworms.

1. 10 pots with Narrabri soil, fertiliser, wheat and earthworms
2. 10 pots with Narrabri soil, fertiliser, wheat and no earthworms
3. 10 pots with Narrabri soil, no fertiliser, wheat and earthworms
4. 10 pots with Narrabri soil, no fertiliser, wheat and no earthworms
5. 10 pots with Narrabri soil, fertiliser, cotton and earthworms
6. 10 pots with Narrabri soil, fertiliser, cotton and no earthworms
7. 10 pots with sandy loam, fertiliser, cotton and earthworms
8. 10 pots with sandy loam, fertiliser, cotton and no earthworms
9. 10 pots with sandy loam, no fertiliser, cotton and earthworms
10. 10 pots with sandy loam, no fertiliser, cotton and no earthworms
11. 10 pots with sandy loam, no fertiliser, wheat and earthworms
12. 10 pots with sandy loam, no fertiliser, wheat and no earthworms

[The experiment was not fully factorial : e.g. we reasoned we knew from previous work that cotton would not grow adequately in Narrabri soil in this situation without fertiliser]

The pots used were the same as described above in section b) (i.e. approx 30 cm diam) and were filled with approx 9 kg dry soil pot⁻¹. The soils were watered to establish 20-25% gravimetric soil moisture content (suitable for earthworm survival and growth). The pots were maintained in a naturally lit air conditioned glasshouse in which air temperatures (shade) fluctuated between 20-40°C during the experiment (air coolers set to operate at 25°C and above). Weights of the pots were checked regularly throughout the experiment and watered as needed to control water content at 20-25% (all pots received similar amounts of water). The experiment ran during late winter to mid spring. Pots were arranged at random in the glasshouse.

The fresh weights of the earthworms were measured prior to their addition to the pots. Similar biomass of earthworms was added to each pot. Fine mesh lids were used to seal the tops of the pots and contain the earthworms initially (whilst they “settled in”). Any earthworms that did not burrow into the soil within a few hours were replaced. After 2 weeks these lids were removed.

Wheat (local variety used at ACRI) and cotton (SICOT 189 QA) seeds were sown into the pots a week after the earthworms were added. 12 wheat and 12 cotton seeds were planted per pot. These were later thinned to 3 seedlings per pot. Fertiliser (5 g of urea) and 0.05 g ZnSO₄ was added to each pot, as required by treatment, at time of seeding. Whilst the wheat germinated well, the cotton did not (probably due to cool temperatures prevailing). The cotton seedlings that did emerge were of poor quality. The seedling cotton plants were therefore removed and the same pots were reseeded 7 weeks after the addition of the earthworms.

Plant sizes were assessed at intervals over 9 weeks for wheat (from sowing) and 7 weeks for cotton (from second sowing), at which times plants were cut off at soil level, dried at 60°C for 20 hours and weighed. The soil within each pot was hand-sorted for earthworms at harvest and these were weighed fresh after washing to remove loose soil adhering to their bodies.

g. Effects of Pesticides on Earthworms

The survival and biomass of *A. longa* and *A. caliginosa* were measured following exposure to some commonly used pesticides (recommended rates; made with distilled water) in the cotton industry (Endosulfan, Spinosad, Abamectin, Deltamethrin, Methomyl, Chlorpyrifos, Dipel) – both under direct contact in moist tissue paper and through surface application to soil containing worms. The treatments in both experiments were :

a. Endosulfan	350g/L EC	recommended rate = 2.1 L/ha
b. Spinosad	480g/L SC	recommended rate = 0.15-0.2 L/ha
c. Abamectin	18g/LEC	recommended rate = 0.6 L/ha
d. Deltamethrin	5.5g/L EC	recommended rate = 2.7-3.5 L/ha
e. Methomyl	225g/L SL	recommended rate = 1.8-2.4 L/ha
f. Chlorpyrifos	300g/L EC	recommended rate = 1.75-5 L/ha
g. Dipel	597g/L SC	recommended rate = 1.0-4.0 L/ha
h. Control	Water only	

(i) Direct Contact (worms on moist tissue)

There were 10 replicates for each of the 8 treatments, for each earthworm species. Two *A. longa* and five *A. caliginosa* were used in each replicate (mostly large juveniles, a few adults). Each replicate consisted of a 9 cm diam plastic Petri dish, lined with Whatman filter paper, moistened with 1.5 ml distilled water. Pesticide solution or water (control) was added to the filter paper (1 ml). Earthworms were then added and the dishes were sealed and stored in the dark at 15°C. Survival was measured by gentle prodding and noting movement at 24, 48, 72, 144 and 168 h exposure.

(ii) Surface Application to Soil

There were 10 replicates for each of the 8 treatments, for each earthworm species. Two *A. longa* and five *A. caliginosa* were used in each replicate. Each replicate consisted of a 1 L plastic box (10 cm x 16 cm x 10 cm) filled with commercial sandy loam (“Gardener’s Choice”) which was maintained at approx 20% gravimetric soil moisture and kept in the dark at 15°C. The lids of the boxes were sealed with fine mesh. Earthworms were added after first being weighed fresh. One day later, when all earthworms had burrowed into the soil, solutions of pesticide were applied evenly to the soil surface using a syringe. After 28 days exposure, the earthworms were hand-sorted from the soil and weighed fresh after washing in water and gently blotting dry.

h. Effects of Flooding on Earthworms

The effect of temporary flooding on the survival of *A. caliginosa* and *A. longa* was tested in the laboratory using Narrabri soil. Forty two plastic boxes (750 ml) were filled with soil which was maintained initially at 25% gravimetric moisture and at 15°C in a dark room. Ten *A. caliginosa* and 5 *A. longa* (large juveniles or adults) were added to each box and the lids were covered with fine mesh to confine the earthworms. Three days later, half the boxes were then flooded with water to a depth of 2cm above the soil surface (for the duration of the experiment). The remaining boxes were maintained at 25% soil moisture. The soil in 3 flooded and 3 control boxes was hand-sorted for earthworms 2, 4, 7, 9, 11, 15 and 17 days after flooding occurred. The earthworms were weighed fresh, before and after the experiment.

4. Results

(i) Field Surveys

Earthworms were generally rare at the sites sampled. Earthworms were found at 38 transects in the cropping soils and 58 transects in the undisturbed soils. Somewhat unexpectedly (because tillage is known to be highly damaging to earthworm populations), earthworms were more common, when found, in the cropping soils than in the undisturbed soils (Figs 1-4). Perhaps, tillage effects are, at least in part, offset by greater water availability in the cropping soils in the Namoi region. Both the cropping and undisturbed soils tended to be dominated by either native or exotic species (i.e. there was rarely an even mix). None of the native species (all Megascolecidae) could be identified with certainty to genus or species. They were simply given coded names (Species A to L). All the exotic species were identifiable (Lumbricidae and Acanthodrilidae). Native species C and the European lumbricid, *Aporrectodea rosea*, were the most common in cropping soils. The cosmopolitan acanthodrilid, *Microscolex dubius*, predominated at one site in undisturbed land where total earthworm abundance was unusually high.

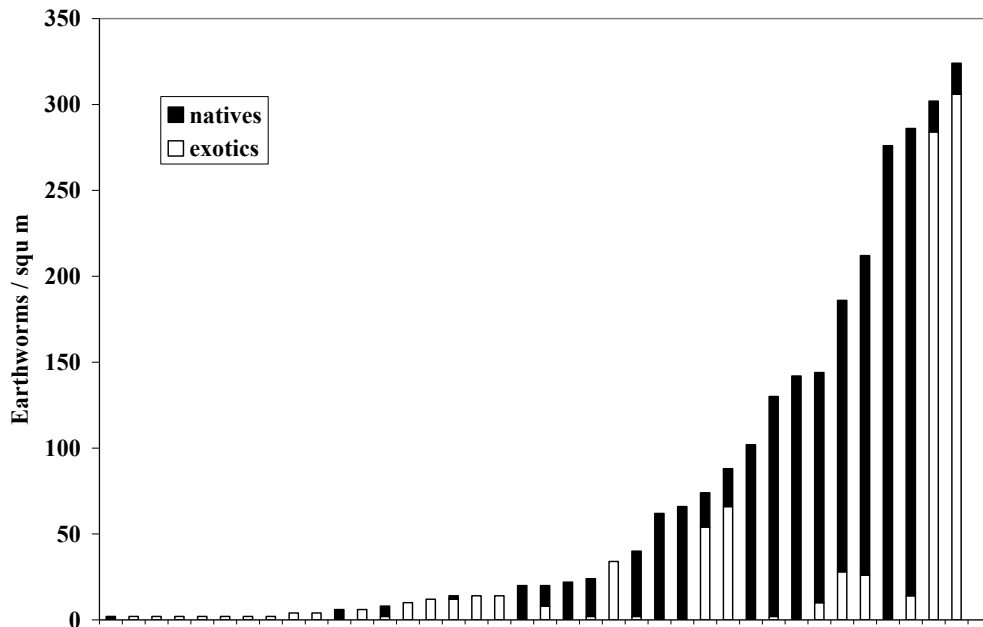


Fig. 1. Abundance of earthworms at individual transects in “cropping” soils, where > 0 earthworms were found. Sites are arranged in order of increasing total abundance.

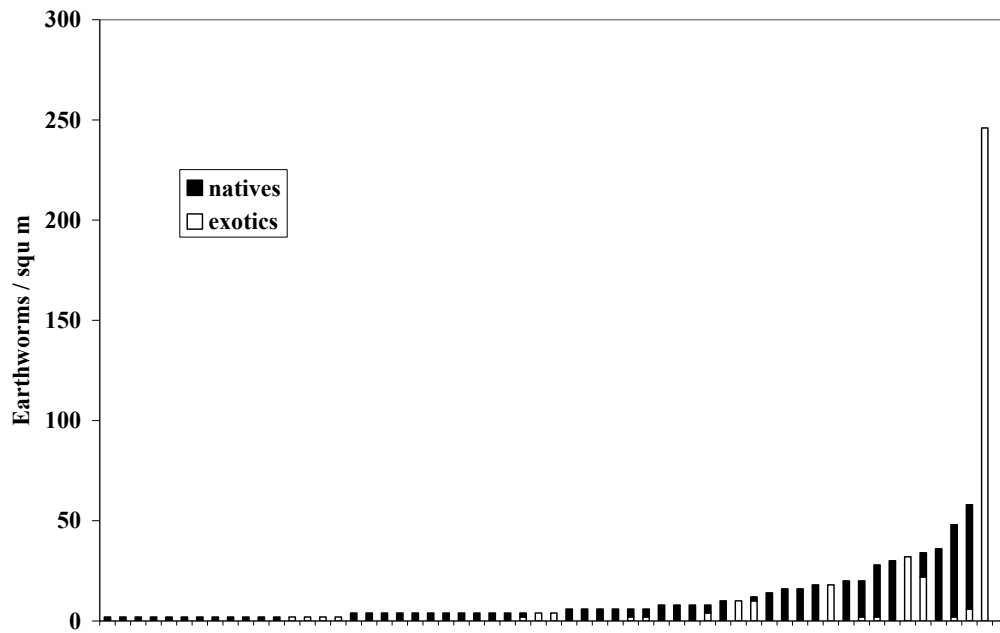


Fig. 2. Abundance of earthworms at individual transects in “undisturbed” soils, where > 0 earthworms were found. Sites are arranged in order of increasing total abundance.

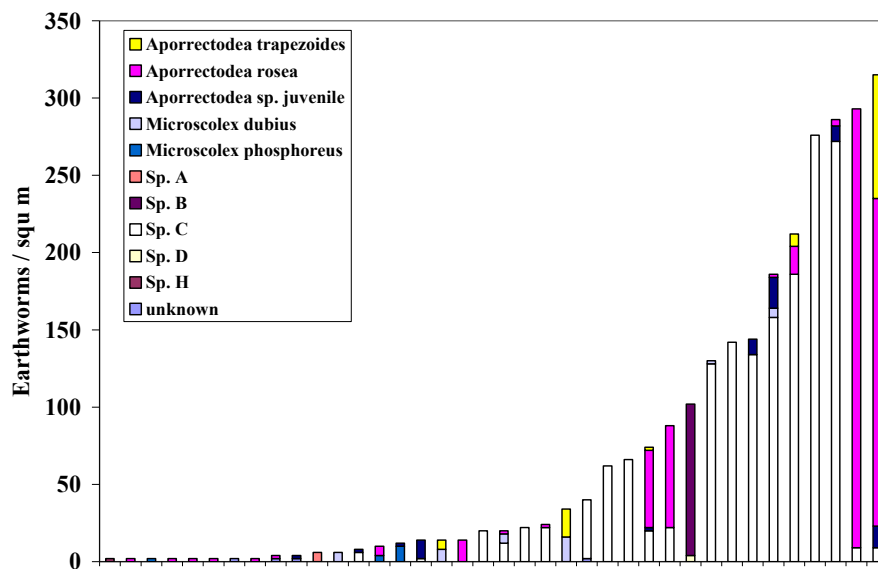


Fig. 3. Abundance of earthworms at individual transects in “cropping” soils, where > 0 earthworms were found. Sites are arranged in order of increasing total abundance.

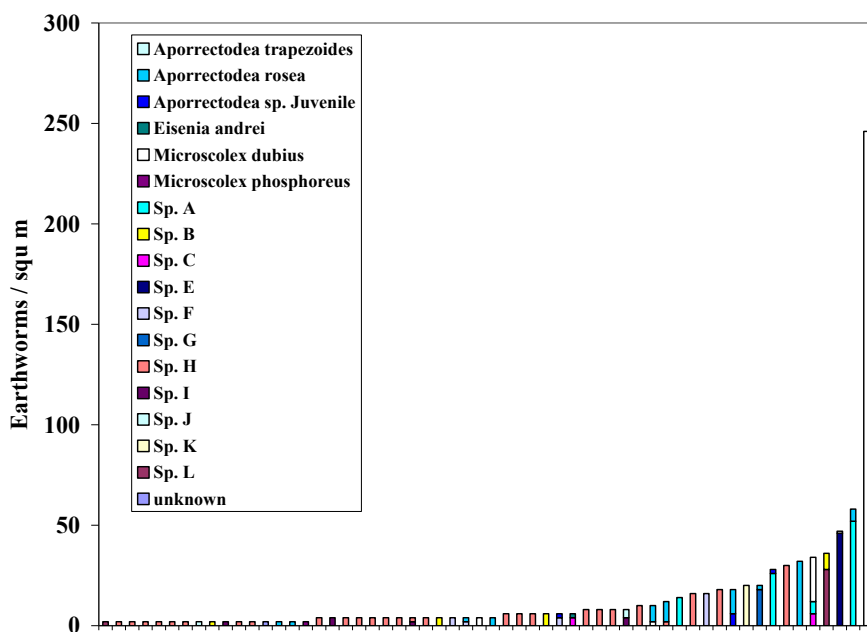


Fig. 4. Abundance of earthworms at individual transects in “undisturbed” soils, where > 0 earthworms were found. Sites are arranged in order of increasing total abundance.

The abundance of earthworms (all species combined) in the cropping soils was significantly correlated with particle size (coarse sand and silt content), E.C. and Total N (Table 2). Earthworm abundance was not correlated with organic carbon or organic matter content of the soils. The ten sites with > 100 earthworms m⁻² were mostly in the Upper Namoi (Table 3).

Table 2. Pearson Correlation coefficients (r) between total earthworm abundance and various soil characteristics for 137 transects in cropping soils

	r	Prob.	Mean	Range
Particle Size				
Coarse Sand (% w/w)	- 0.219	< 0.05	12.08	0.6 – 67.3
Fine Sand (% w/w)	- 0.174	< 0.05	18.20	6.04 – 49.4
Silt (% w/w)	0.317	< 0.05	19.92	3.0 – 36.5
Clay (% w/w)	0.137	> 0.05	44.47	3.5 – 69.0
pH (CaCl ₂)	0.109	> 0.05	7.46	4.4 – 8.4
E.C. (dS/m)	0.302	< 0.05	0.20	< 0.05 – 0.51
Organic C (% w/w)	- 0.012	> 0.05	1.27	0.6 – 2.4
Organic Matter (% w/w)	- 0.046	> 0.05	2.41	1.1 – 4.6
Total N (% w/w)	0.222	< 0.05	0.08	< 0.05 – 0.14
Olsen P (mg/kg)	0.065	> 0.05	15.83	2.0 – 64.0

Table 3. Locations of cropping sites with most earthworms.

Site	Earthworms m ⁻²	Use at Time of Sampling
1. Jangaree	324	Furrowed up, after wheat
2. Battery Hill	302	Furrowed up, after cotton
3. Kilmarnock (a)	286	Furrowed up, after cotton
4. ACRI – Leitch	276	In wheat, after cotton
5. Kilmarnock (b)	212	Furrowed up, after cotton
6. Breeza Station (b)	186	Not yet worked up, after cotton
7. Kurrawombi Downs	144	Furrowed up, after cotton
8. Oakville	142	Fallow, after wheat
9. Breeza Station (c)	130	Furrowed up, after cotton
10. Breeza Station (e)	102	In barley, after wheat

(ii) Glasshouse Experiments

a. Earthworm Survival in Different Soils

The earthworms in the Narrabri soil were distributed evenly throughout the pots when the soil was hand-sorted. The soil was riddled with macropores, indicating substantial earthworm activity. The earthworms in the Hillston soil were mostly confined to the top half of the pot, although macropores were observed in the soil lower down. Most earthworms were found at the interface between the plastic pot and the soil. The earthworms in the commercial sandy loam were confined to the top half of the pot. The soil in the lower half of the pots was noticeably much wetter than the top half (much more so than in the Narrabri and Hillston soil treatments), presumably indicating greater drainage in the sandy loam. Whilst the earthworms from the sandy loam were highly active when washed in water at the end of the experiment, they were less so when from the Narrabri soil, and much less so when from the Hillston soil.

Most earthworms survived in all treatments, but *A. caliginosa* suffered small, but significant mortality in the Narrabri soil (paired $t = 4.00$, $p < 0.05$) and sandy loam (paired $t = 2.45$, $p < 0.05$) (Fig. 5). There were no differences in survival across soil types. Very few earthworms were “curled up” (a sign of inactivity) in the sandy loam, but most were “curled up” in the other two soils (Fig. 6). The biomass of *A. caliginosa* increased during the experiment in the Narrabri soil (paired $t = 2.88$, $p < 0.05$) and the sandy loam (paired $t = 4.30$, $p < 0.05$), but decreased in the Hillston soil (paired $t = 3.05$, $p < 0.05$) (Fig. 7). The biomass of *A. longa* increased in the sandy loam (paired $t = 5.56$, $p < 0.05$), but decreased in the other two soils (paired $t = 6.25$, $p < 0.05$ for the Narrabri soil) (paired $t = 18.04$, $p < 0.05$ for the Hillston soil) (Fig. 8). Thus the cotton soils, whilst enabling the survival of earthworms in the short term, were in general less suitable to earthworm development than the commercial sandy loam.

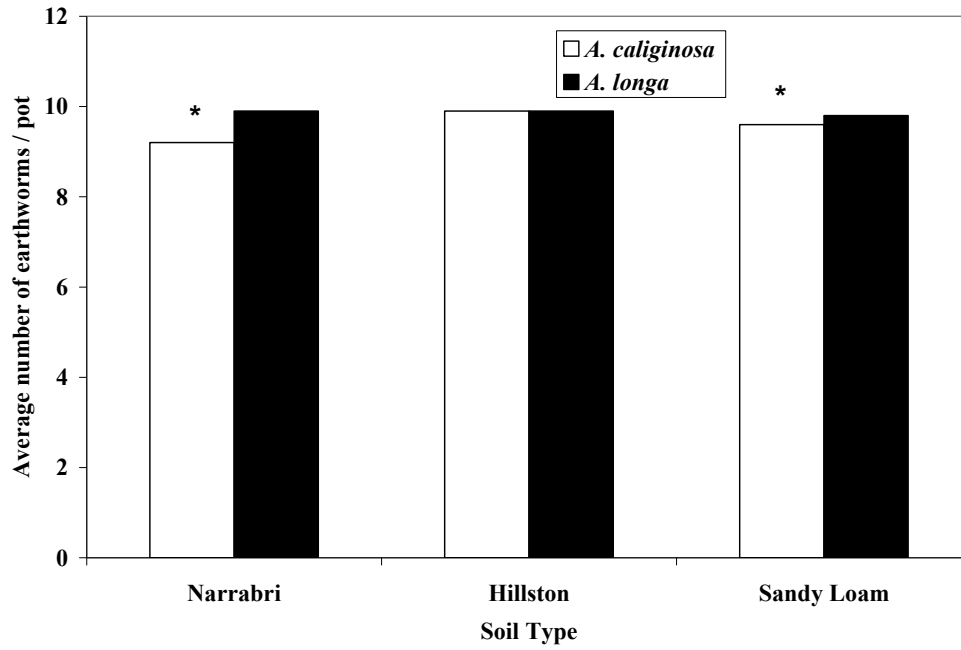


Fig. 5. Average number of *A. caliginosa* and *A. longa* after 10 weeks exposure to different soil types. * indicates significant mortality.

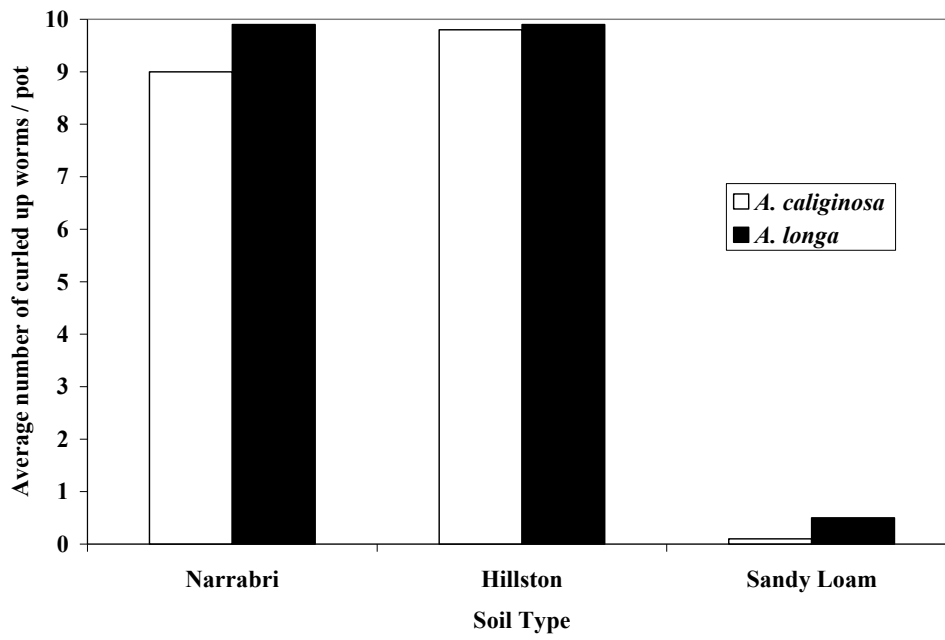


Fig. 6. Average number of "curled up" *A. caliginosa* and *A. longa* after 10 weeks exposure to different soil types.

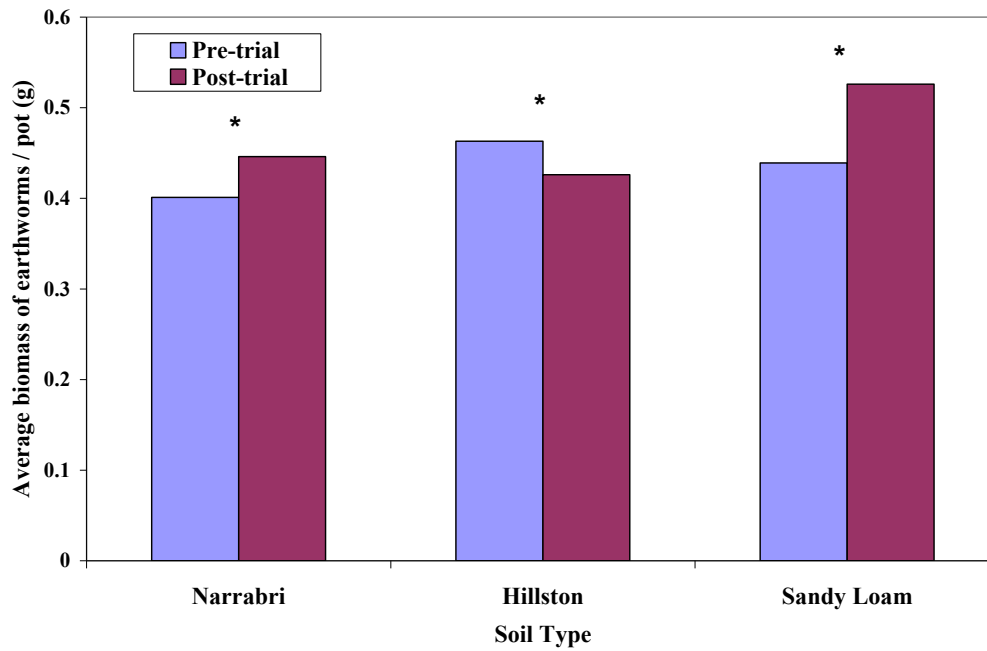


Fig. 7. Average biomass of *A. caliginosa* in different soil types. * indicates significant difference between pre-trial and post-trial biomass.

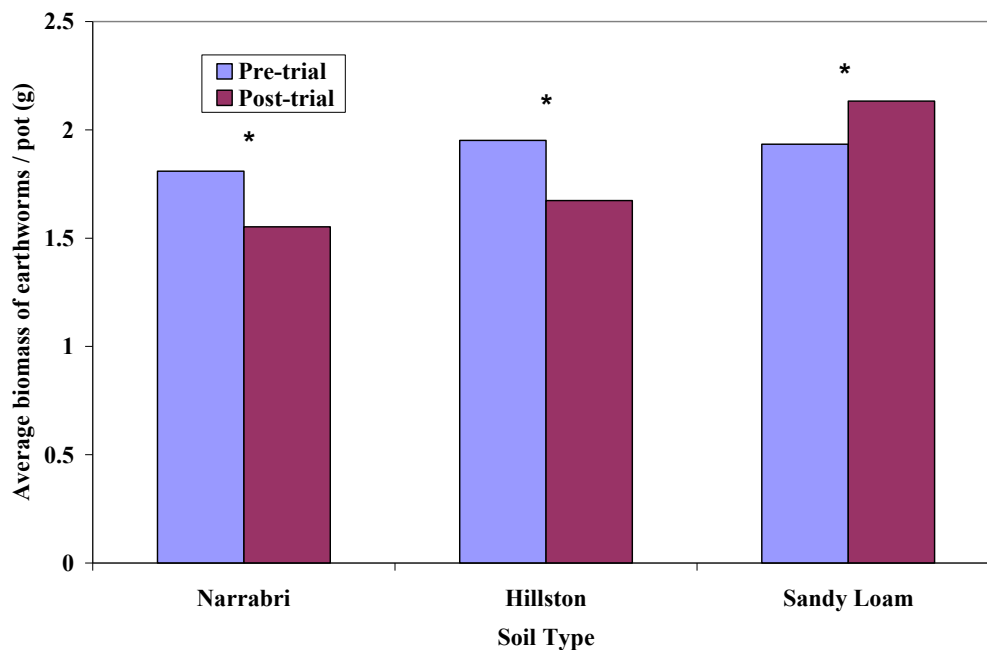


Fig. 8. Average biomass of *A. longa* in different soil types. * indicates significant difference between pre-trial and post-trial biomass.

b. Soil Preferences of Earthworms

All earthworms survived for the week in the preference tests. The total numbers of *A. caliginosa* and *A. longa* found in the different soil types at the end of the experiment are given in Table 4 [Data for individual comparisons between pairs of soils are not presented here, but are available from G. Baker. Note there were no differences between recoveries of earthworms from the two ends of the boxes which contained the mixed soils at both ends]. Both earthworm species generally preferred the heavier soils to the commercial sandy loam. The biomass of *A. caliginosa* was generally higher at the end of the experiment (one week) than at the start, but the biomass of *A. longa* tended to be less (Table 5).

Table 4. Frequencies of earthworms in different soil types when given equal choices

Earthworm Species	Soil Type				Chi squared	Prob.
	Narrabri	Hillston	Sandy Loam	Ginninderra		
<i>A. caliginosa</i>	181	114	109	186	34.31	< 0.05
<i>A. longa</i>	83	110	54	110	24.58	< 0.05

Table 5. Average pre-trial and post-trial biomass (g) of individual earthworms in soil preference tests

Earthworm & Soil Type	Pre-trial	Post-trial	Paired t	Prob.
<i>A. caliginosa</i>				
Narrabri	0.39	0.45	4.05	< 0.05
Hillston	0.40	0.38	1.76	> 0.05
Sandy Loam	0.39	0.48	1.25	> 0.05
Ginninderra	0.41	0.62	3.93	< 0.05
Mix	0.41	0.51	8.54	< 0.05
<i>A. longa</i>				
Narrabri	1.73	1.69	0.66	> 0.05
Hillston	1.77	1.66	3.05	< 0.05
Sandy Loam	1.76	1.64	1.36	> 0.05
Ginninderra	1.66	1.69	0.85	> 0.05
Mix	1.58	1.48	1.89	> 0.05

c. Earthworm Influences on Cotton Growth (ACRI soil and Hillston soil)

Experiment 1. Whilst the growth of the cotton plants was inevitably poor (limited soil volume, no fertiliser added), earthworms increased the biomass of the few bolls grown from both Narrabri and Hillston soils ($F = 18.43$, $p < 0.05$) (Fig. 9). The biomass of the rest of the cotton plants was unaffected ($F = 1.38$, $p > 0.05$) (Fig. 9), but the % N in leaves was significantly higher in the presence of the earthworms in both soil types ($F = 122.91$, $p < 0.05$) (Fig. 10). The relative heights of plants growing in the two soil types varied in time (Fig. 11). There was a suggestion in the data that the cotton plants flowered earlier and more in the presence of earthworms (Fig. 12).

No earthworms were recovered from the pots at the end of the experiment (cause of mortality unknown, but probably the high temperature in the glasshouse was important). However, prior to sowing the cotton seed, numerous earthworm castings were found on the soil surface (both soil types, but especially the Narrabri soil), indicating substantial activity.

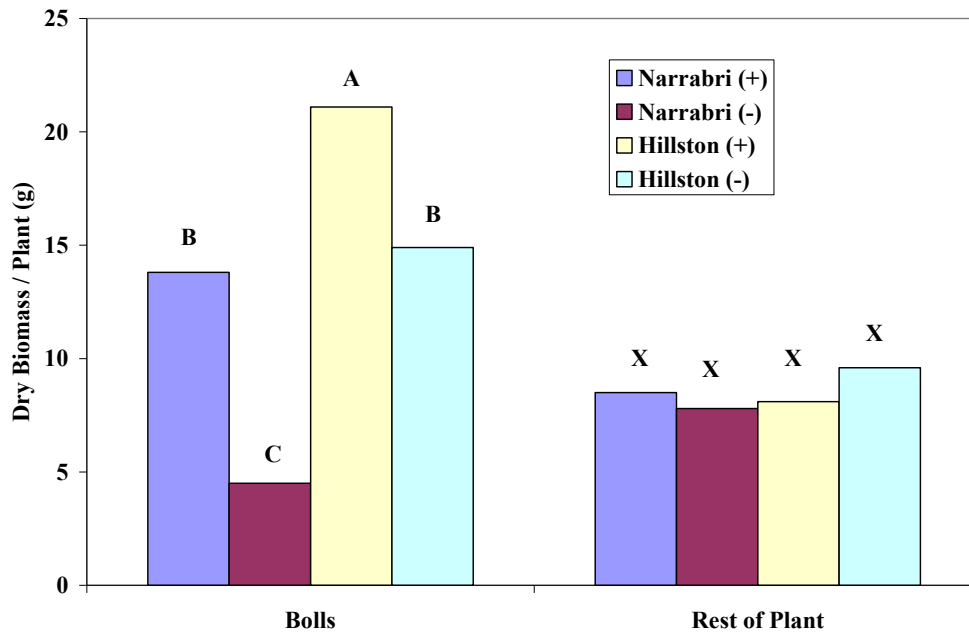


Fig. 9. Average dry biomass of bolls plant⁻¹ and the dry biomass of the remainder of the plants at harvest where cotton plants (SICOT 189) were grown in Narrabri and Hillston soils in pots in a glasshouse, in the presence (+) or absence (-) of earthworms. Different letters above the bars indicate significant differences between treatments, within a plant part.

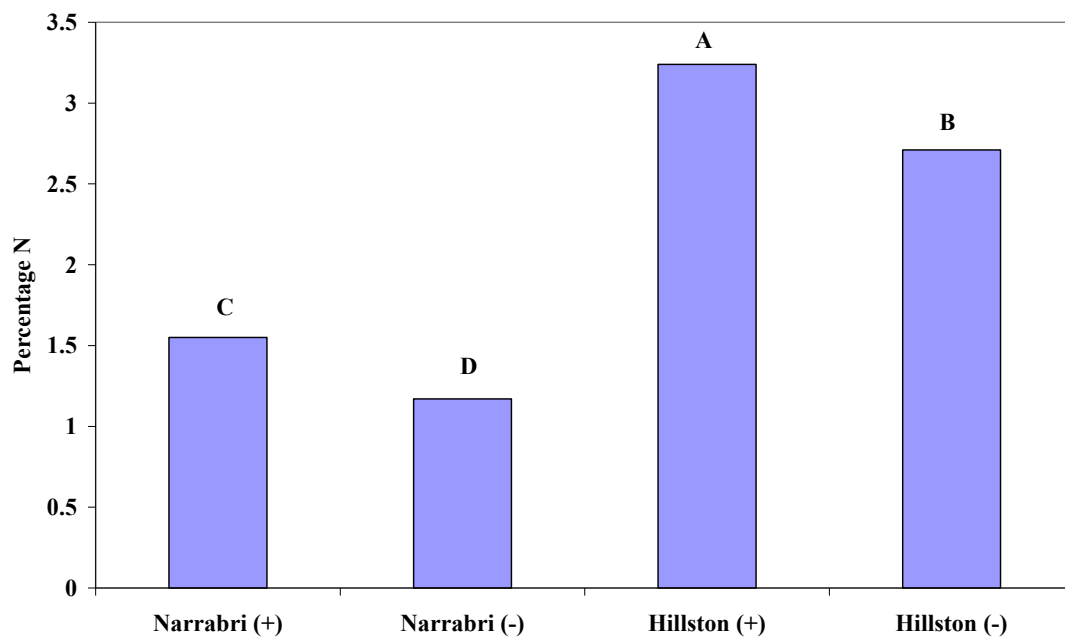


Fig. 10. Average nitrogen content (%) of cotton leaves where cotton plants (SICOT 189) were grown in Narrabri and Hillston soils in pots in a glasshouse, in the presence (+) or absence (-) of earthworms. Different letters above the bars indicate significant differences between treatments

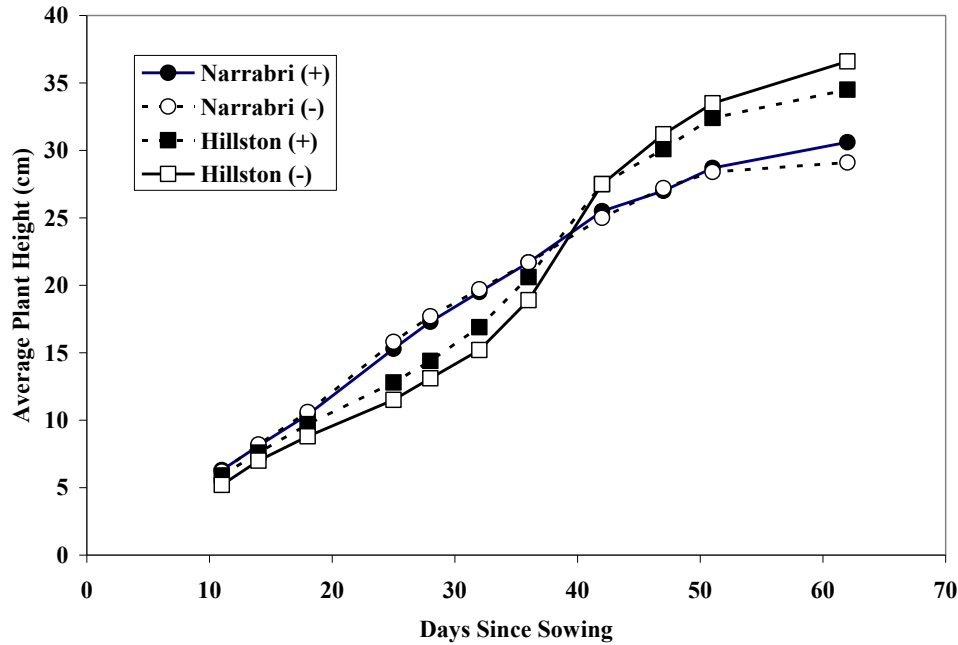


Fig. 11. Average heights (cm) of cotton plants (SICOT 189) grown in Narrabri and Hillston soils in pots in a glasshouse, in the presence (+) or absence (-) of earthworms.

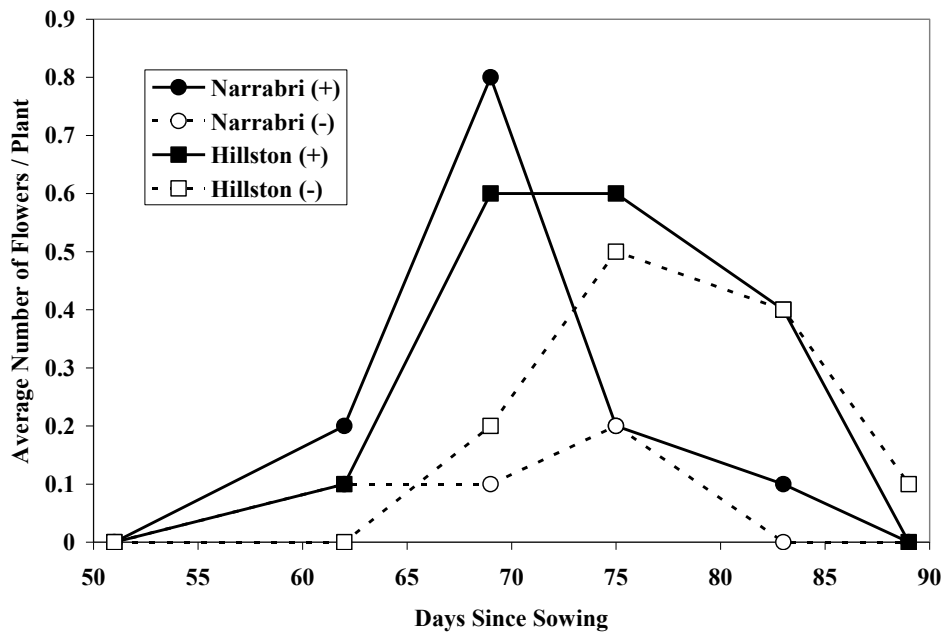


Fig. 12. Average numbers of flowers on cotton plants (SICOT 189) grown in Narrabri and Hillston soils in pots in a glasshouse, in the presence (+) or absence (-) of earthworms.

Experiment 2. Mortality of the earthworms added to the PVC pipe pots was high (66% recovery of *A. caliginosa* and 45% recovery of *A. longa*). Many of the worms that were recovered appeared to be in poor condition. This probably reflects the high temperatures the earthworms would have been exposed to in the small volumes of soil in the PVC pipe pots, when placed on the bench in the glasshouse. The initial fresh biomass of earthworms added to the pots was 1.81 ± 0.05 g for *A. caliginosa* and 4.21 ± 0.14 g for *A. longa* ($\bar{x} \pm$ S.E.). The final biomass of earthworms was 0.85 ± 0.10 g for *A. caliginosa*

and 0.91 ± 0.20 g for *A. longa* ($\bar{x} \pm$ S.E.). The mortality of the earthworms would therefore have added some, unknown quantity, of fertility to the earthworm enriched pots.

The dry biomass of the cotton plants did not vary significantly between treatments ($t = 1.47$, $p > 0.05$) (mean dry biomass with earthworms and without earthworms = 0.61 ± 0.05 g and 0.72 ± 0.06 g respectively). The heights of the plants, also did not differ significantly (Wilcoxon Signed Rank test, Normal approximation with continuity correction = 0.915 , $p > 0.05$: non-parametric stats were used because of unequal variances) (height of plant at base of stem of 2nd true leaf with and without earthworms = 74.1 ± 1.8 mm and 76.4 ± 2.8 mm respectively).

The sizes of leaves (largest of 2nd sets of true leaves for each plant) were measured at harvest on a scale from very small (< 1.5 cm) to very, very large (> 5.5 cm) in length.(Fig. 13). There was a slight tendency, as with the biomass and plant height data, for treatments with earthworms to produce smaller leaves.

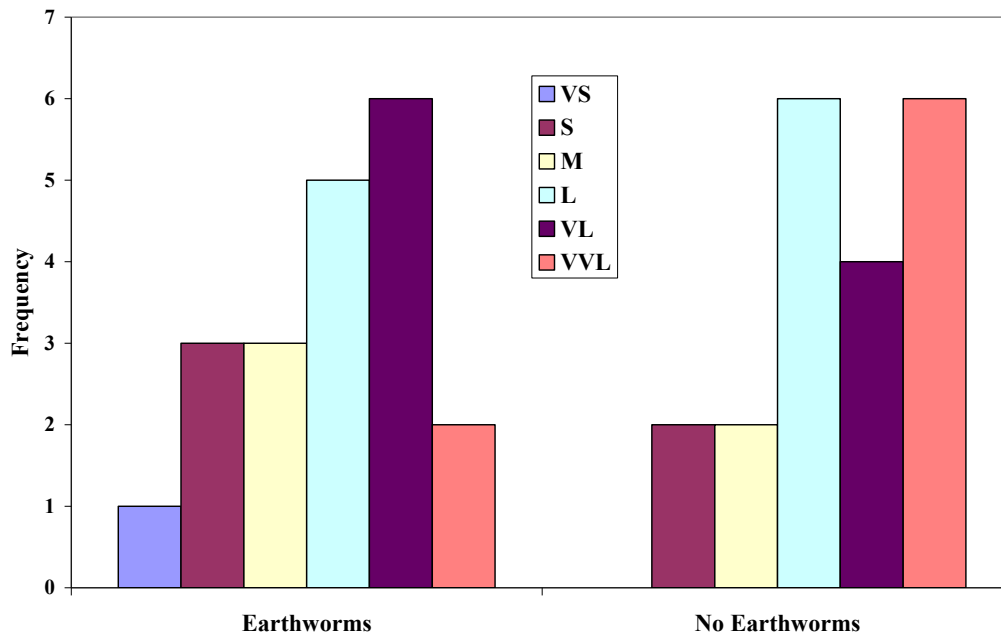


Fig. 13. Frequencies of lengths of the largest of the second true leaves for each cotton plant growing in Narrabri soil, with or without earthworms. VS = < 1.5 cm, S = 1.5-2.5 cm, M = 2.5-3.5 cm, L = 3.5-4.5 cm, VL = 4.5-5.5 cm, and VVL > 5.5 cm in length.

d. Earthworm Influences on Cotton Growth (ACRI soil)

Experiment 1. Again, the growth of the cotton plants was poor (no fertiliser was added), but the presence of earthworms increased the biomass of cotton harvested at the end of the experiment ($F = 6.30$, $p < 0.05$) (Fig. 14). These results were reflected in the heights of the cotton plants (Fig. 15).

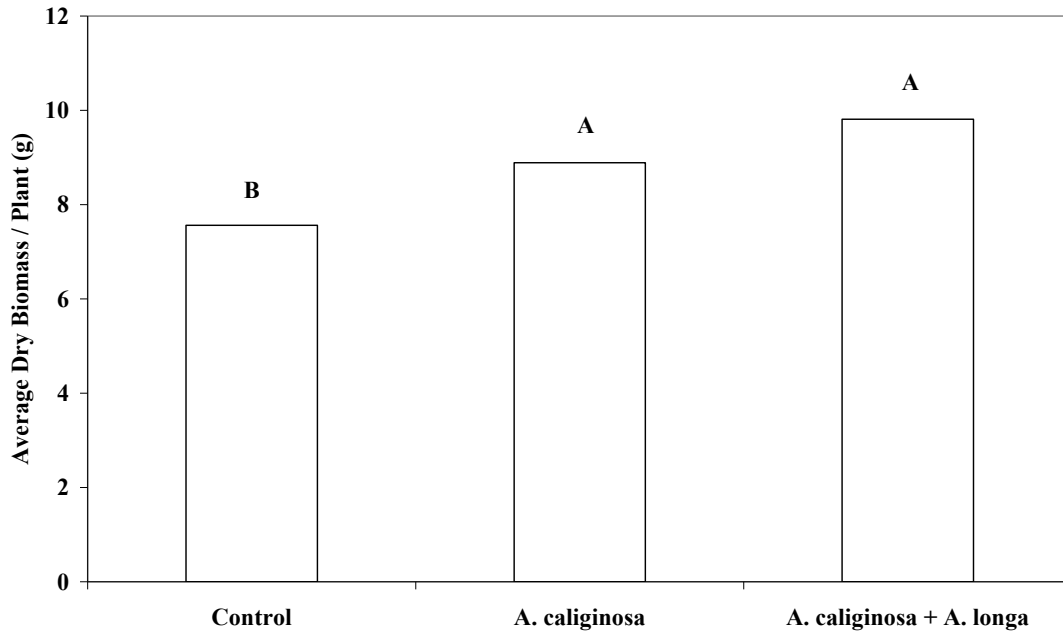


Fig. 14. Average dry biomass of plants at harvest, where cotton plants (SICOT 189) were grown in Narrabri soil in pots in a glasshouse, in the presence or absence of earthworms. Different letters above the bars indicate significant differences between treatments.

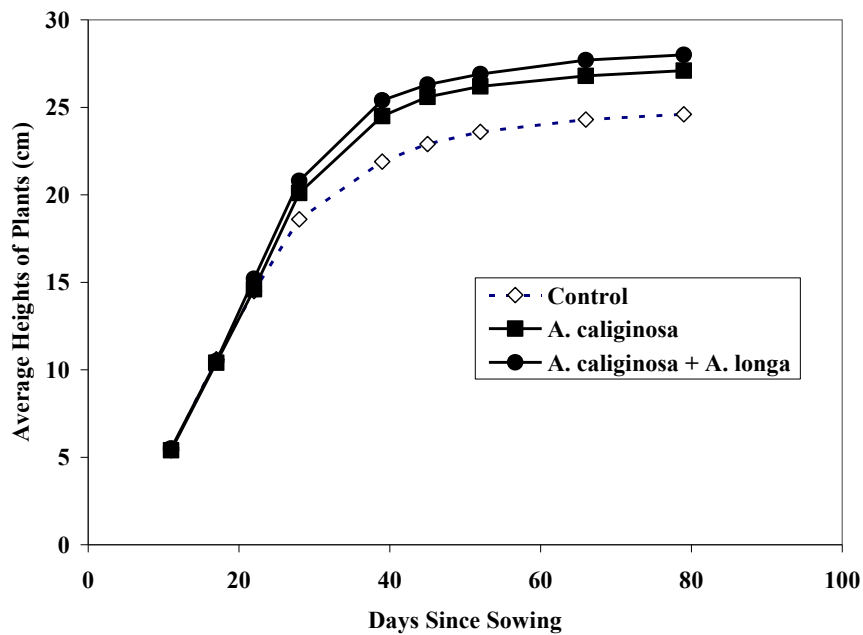


Fig. 15. Average height of cotton plants (SICOT 189) grown in Narrabri soil in pots in a glasshouse, in the presence or absence of earthworms.

Experiment 2. Most earthworms were recovered when soils were hand-sorted after 9 weeks (84.5 % recovery for *A. caliginosa* in Treatment 7 and 82.5 % recovery of *A. longa* in Treatment 8). The fresh biomass of the earthworms at this time ($6.83 \pm 0.16 \text{ g pot}^{-1}$ for *A. caliginosa* and $22.80 \pm 0.89 \text{ g pot}^{-1}$ for *A. longa*) was however substantially less than at the start of the experiment ($10.31 \pm 0.13 \text{ g pot}^{-1}$ for *A. caliginosa* and $48.22 \pm 0.44 \text{ g pot}^{-1}$ for *A. longa*). Only a small proportion of the earthworms added to the pots were recovered 31 weeks later, when the cotton plants were cut and dried (21.5 % recovery

for *A. caliginosa* in Treatment 4, 24.5 % recovery of *A. longa* in Treatment 5, and 19.0% recovery of *A. caliginosa* and 43.0% recovery of *A. longa* in Treatment 6).

There was no significant difference between treatments in boll biomass plant⁻¹ at 18 weeks after sowing, but there was at 22 weeks after sowing ($F = 1.24$, $p > 0.05$ for 18 weeks; $F = 2.62 < 0.05$ for 22 weeks ; data transformed to logs for analyses) (Fig. 16). Boll biomass was greatest in the presence of *A. longa* (at high densities), whether the earthworms were alive or dead, and least in the controls (no earthworms). There was also a significant difference in plant height between treatments at 14 weeks after sowing, when height was last measured ($F = 5.03$, $p < 0.05$) (Fig. 17). In this case, plant height was greatest in the presence of dead *A. caliginosa* and least in the presence of live *A. longa*. The numbers of leaves plant⁻¹ varied between treatments, ($H = 19.71$; $p < 0.05$; non-parametric stats done because transformations were inadequate for parametric stats analyses), with most leaves being produced in the control treatment and least in the presence of both earthworm species.

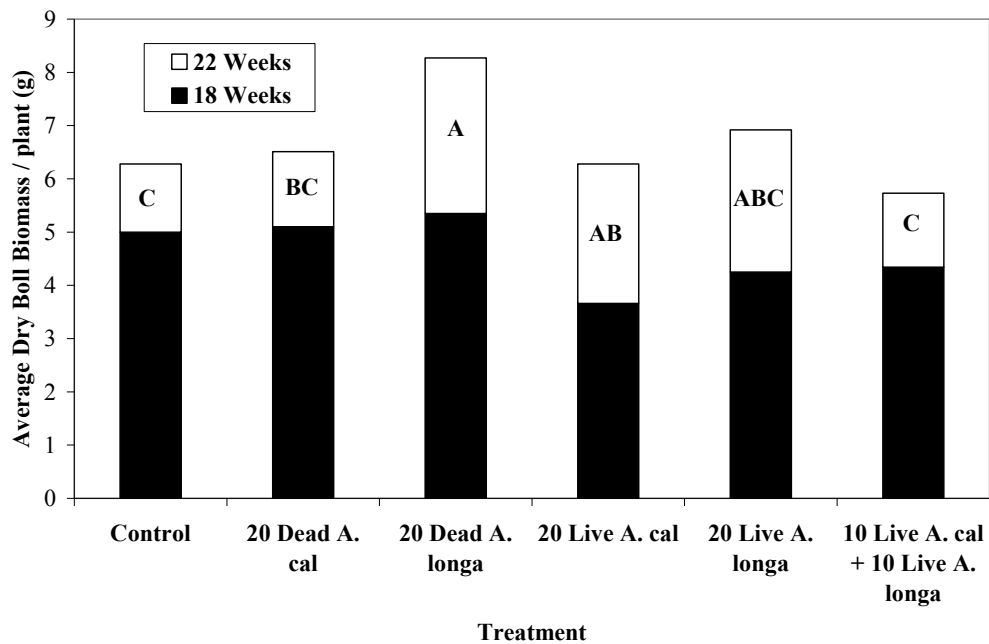


Fig. 16. Average dry boll biomass plant⁻¹ for cotton grown in Narrabri soil in the glasshouse, at 18 and 22 weeks after sowing. There were no differences between treatments when bolls were harvested at 18 weeks. Different letters within bars indicate significant differences between treatments at 22 weeks.

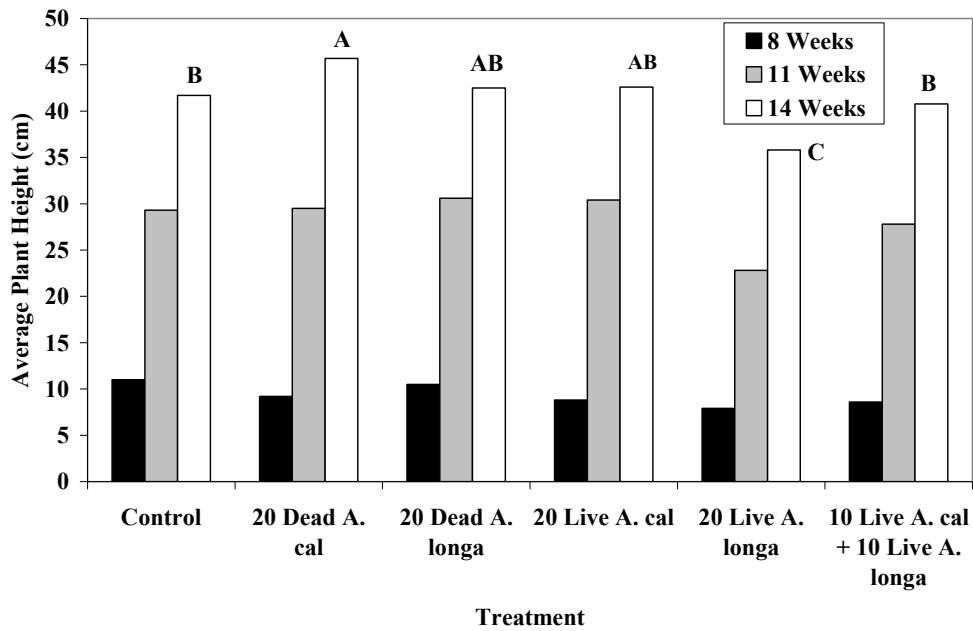


Fig. 17. Average plant height for cotton grown in Narrabri soil in the glasshouse, at 8, 11 and 14 weeks after sowing. Different letters above bars indicate significant differences between treatments at 14 weeks.

e. Earthworm Influences on Cotton and Wheat Growth (ACRI soil and Commercial Sandy Loam)

Most earthworms appeared to survive throughout the experiment, in fact in several instances, more large *A. caliginosa* were found during hand-sorting of the soils in the pots than were initially added to them ($n = 15 \text{ pot}^{-1}$) (Fig. 18). Small juvenile *Aporrectodea* spp. (most probably *A. caliginosa*, but conclusive identifications were not always possible) and cocoons were found in addition to the larger earthworms in several pots, which indicated that reproduction had occurred. There were no significant differences between treatments for the numbers of *A. longa* recovered from the pots ($F = 1.71, p > 0.05$). However, more, large *A. caliginosa* were recovered from the pots with sandy loam and cotton, than from the pots with Narrabri soil or sandy loam and wheat ($F = 9.47, p < 0.05$). Most recruitment of small earthworms occurred in the pots with sandy loam and cotton. [Note : Remember, the pots with cotton growing in them were maintained for a few more weeks than those with wheat – thus more time in the latter for recruitment of young]

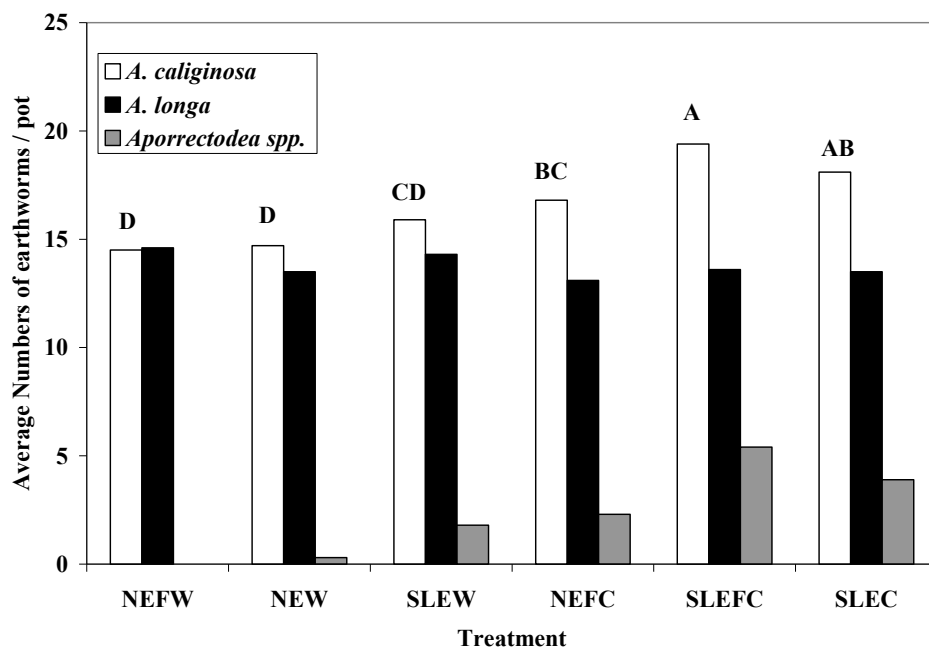


Fig. 18. Average numbers of large *A. caliginosa* and *A. longa* and small juvenile *Aporrectodea* spp. hand-sorted from flower pots after growing wheat (W) or cotton (C) in sandy loam (SL) or Narrabri (N) soil, with (F) or without (no symbol) fertiliser. Different letters above the bars for *A. caliginosa* indicate significant differences between treatments (large earthworms only). There were no differences between treatments involving *A. longa*.

The biomass pot⁻¹ for both *A. caliginosa* and *A. longa* decreased during the experiments (Fig. 19). For both species, biomass remained highest in the sandy loam with wheat and no fertiliser and was lowest in Narrabri soil with cotton and fertiliser, at the end of the experiment (F = 4.04, p < 0.05 for *A. caliginosa*; F = 11.10, p < 0.05 for *A. longa*).

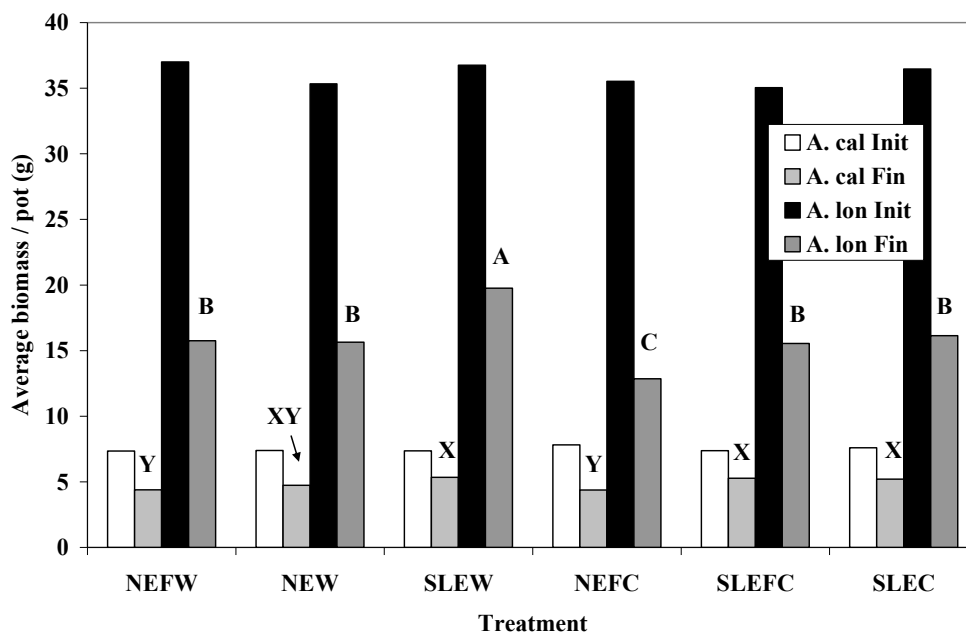


Fig. 19. Average biomass (initial and final) of *A. caliginosa* (*A. cal*) and *A. longa* (*A. lon*) (small juvenile *Aporrectodea* spp. assumed to be *A. caliginosa*) hand-sorted from flower pots after growing wheat (W) or cotton (C) in sandy loam (SL) or Narrabri (N) soil, with (F) or without fertiliser (no symbol). Different letters above the bars for final biomass (*A. caliginosa* and *A. longa* dealt with

separately) indicate significant differences between treatments. There were no differences between treatments for initial biomass, within each species.

For simplicity, only the sizes (and some other characteristics) of plants at harvest are included here. Data collected in an on-going way during the experiment are available from G. Baker. The dry biomass of wheat plants harvested from the pots varied between treatments (Kruskal-Wallis $H = 21.45$, $p < 0.05$), with greatest biomass in the treatment with sandy loam, earthworms and no fertiliser and least in the treatments with sandy loam or Narrabri soil, and no earthworms and no fertiliser (Fig. 20). Most notably, earthworms appeared to increase wheat biomass in sandy loam without fertiliser, but whilst there was a trend in this direction in Narrabri soil (with or without fertiliser) it was not significant.

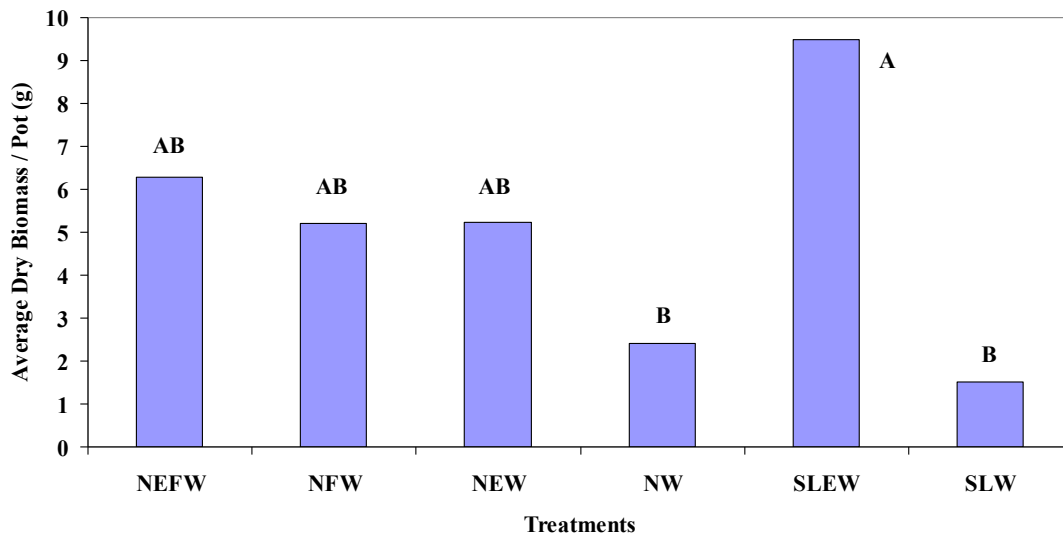


Fig. 20. Average dry biomass of wheat plants (W) growing in sandy loam (SL) or Narrabri (N) soil, with earthworms (E) or without (no symbol) earthworms, and with (F) or without (no symbol) fertiliser. Different letters above the bars indicate significant differences between treatments.

The dry biomass of cotton plants harvested from the pots varied between treatments ($H = 40.79$, $p < 0.05$), with greatest biomass in the treatment with sandy loam, no earthworms and fertiliser and least in the treatments with sandy loam, earthworms or no earthworms and no fertiliser (Fig. 21). There was a tendency for treatments containing earthworms to produce less cotton biomass, but this was not significant for any of the three relevant pairings of treatments in this regard (Fig. 21). The sizes of leaves appeared to vary between treatments, so a scale from very small (< 1.5 cm) to very large (> 4.5 cm) in length was devised and measured for the largest of the first and second sets of true leaves for each plant. Data are presented here for the first set of true leaves at harvest (Fig. 22). The sizes of the leaves reflected the dry biomass of the total plants. Again there was a tendency for treatments with earthworms to produce smaller leaves.

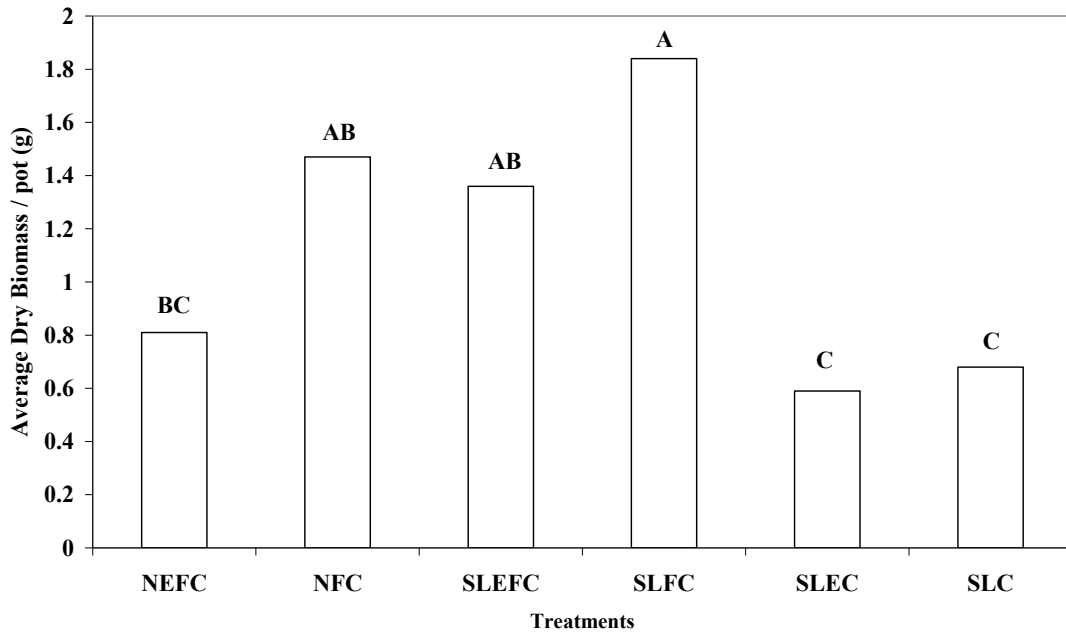


Fig. 21. Average dry biomass for cotton plants (C) growing in sandy loam (SL) or Narrabri (N) soil, with earthworms (E) or without (no symbol) earthworms, and with (F) or without (no symbol) fertiliser. Different letters above the bars indicate significant differences between treatments.

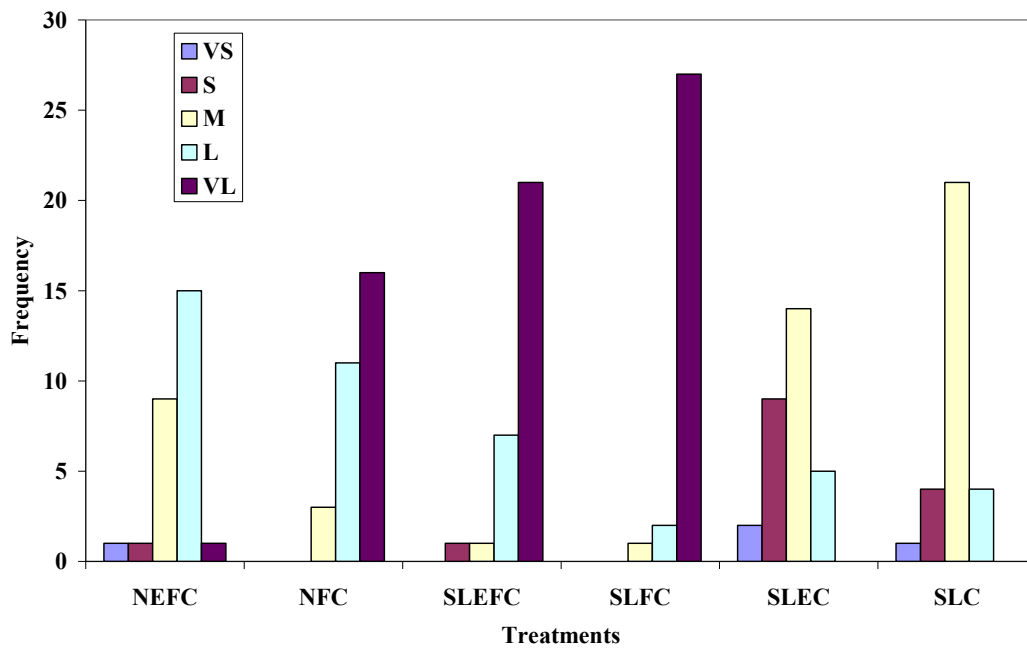


Fig. 22. Frequencies of lengths of the largest of the first true leaves for each cotton plant (C) growing in sandy loam (SL) or Narrabri (N) soil, with earthworms (E) or without (no symbol) earthworms, and with (F) or without (no symbol) fertiliser. VS = < 1.5 cm, S = 1.5-2.5 cm, M = 2.5-3.5 cm, L = 3.5-4.5 cm, and VL = > 4.5 cm in length.

In addition, the colour of the plants seemed variable between treatments. Prior to harvest, the plants in each pot were assessed as either light green in colour, medium green or dark green (Fig. 23). The plants were darkest green in colour in the pots with Narrabri soil and fertiliser and lightest green in the

pots with sandy loam and no fertiliser. There was no obvious difference between treatments with and without earthworms.

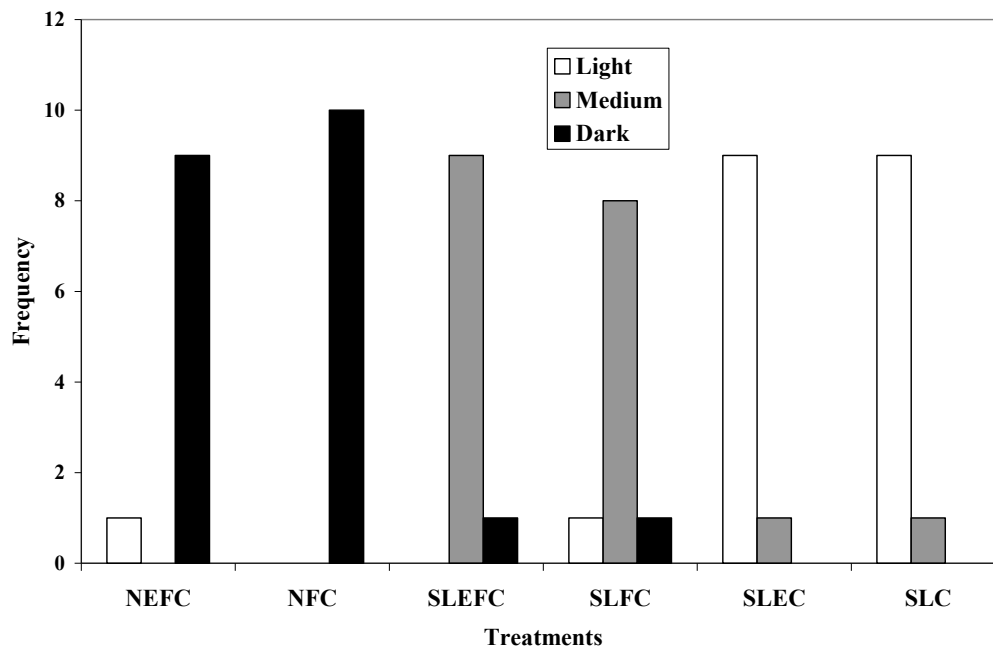


Fig. 23. Frequencies of colour classes for cotton plants (C) (measured overall for each pot) growing in sandy loam (SL) or Narrabri (N) soil, with earthworms (E) or without earthworms (no symbol), and with (F) or without fertiliser (no symbol).

f. Effects of Pesticides on Earthworms

(i) Direct Contact (worms on moist tissue)

For *A. caliginosa*, 24 h after treatment, there was a significant reduction in the survival of earthworms treated with Endosulfan, Methomyl and Chlorpyrifos compared with controls (Fig. 24). Spinosad, Abamectin, Deltamethrin and Dipel treatments all had 100% survival after 168 h. All the *A. caliginosa* were killed by Endosulfan and Methomyl within 48 h, and nearly all by Chlorpyrifos within 168 h.

For *A. longa*, 24 h after treatment, there was a significant reduction in the survival of earthworms treated with Methomyl compared with controls (Fig. 25). Spinosad, Abamectin, Deltamethrin, Chlorpyrifos and Dipel treatments all had 100% survival after 168 h. After 48 h, all *A. longa* treated with Endosulfan and Methomyl were dead.

[Additional Notes : After 24 h, *A. caliginosa* exposed to Endosulfan were still moving slightly, but did not retract their heads when prodded, and were therefore counted as dead. Both earthworm species responded quickly to exposure to Deltamethrin by producing copious amounts of mucus. Blistering of the skin was common in both species when exposed to Methomyl and movements were noticeably reduced of the control treatment. Bleeding was noticed in some of the *A. caliginosa* exposed to Chlorpyrifos.

After 48 h, *A. longa* movement was noticeably reduced when exposed to Deltamethrin. *A. longa* were blistered and some individuals had broken into fragments when exposed to Methomyl. Several *A. longa* were writhing within the dishes in the presence of Chlorpyrifos.]

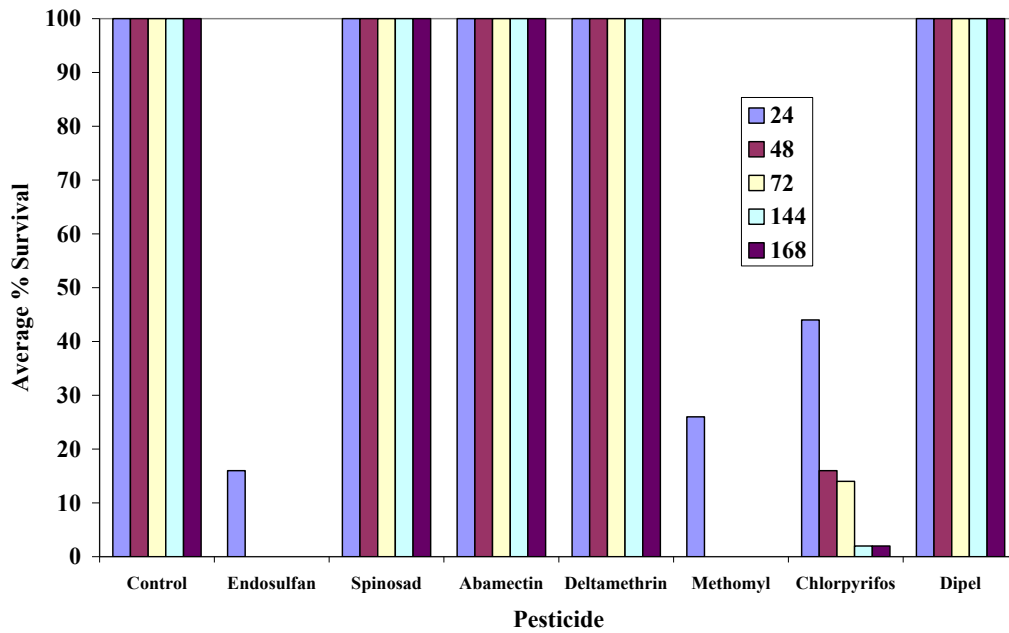


Fig. 24. Average survival of *A. caliginosa* after direct exposure to various pesticides in Petri dishes for up to 168 h.

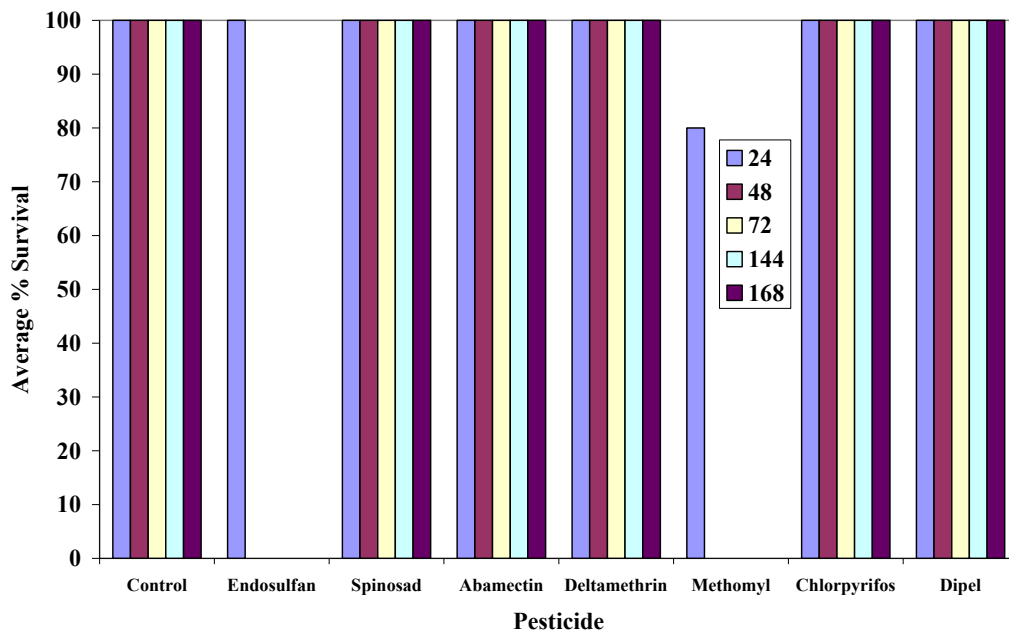


Fig. 25. Average survival of *A. longa* after direct exposure to various pesticides in Petri dishes for up to 168 h.

ii) Surface Application to Soil

All but one *A. caliginosa* survived in the control treatment. Similarly, virtually all the *A. caliginosa* in the pesticide treatments survived (2 died in each of the Spinosad and Deltamethrin treatments and 3 died in the Methomyl treatment). All the *A. longa* survived (all treatments).

The biomass of *A. caliginosa* was reduced during the experiment in most treatments, including the control (Fig. 26). *A. longa* lost weight only in the Endosulfan and Abamectin treatments (Fig. 27).

There was no difference in the biomass of the earthworms in the pesticide and control treatments at the end of the experiment, for either species. Small numbers of both species were “curled up” (i.e. inactive) (means of 0 – 0.5 worms box⁻¹ overall), but there was no obvious difference across treatments. These curled up worms were immediately active when washed in water prior to weighing, suggesting that their state of inactivity was quite recent.

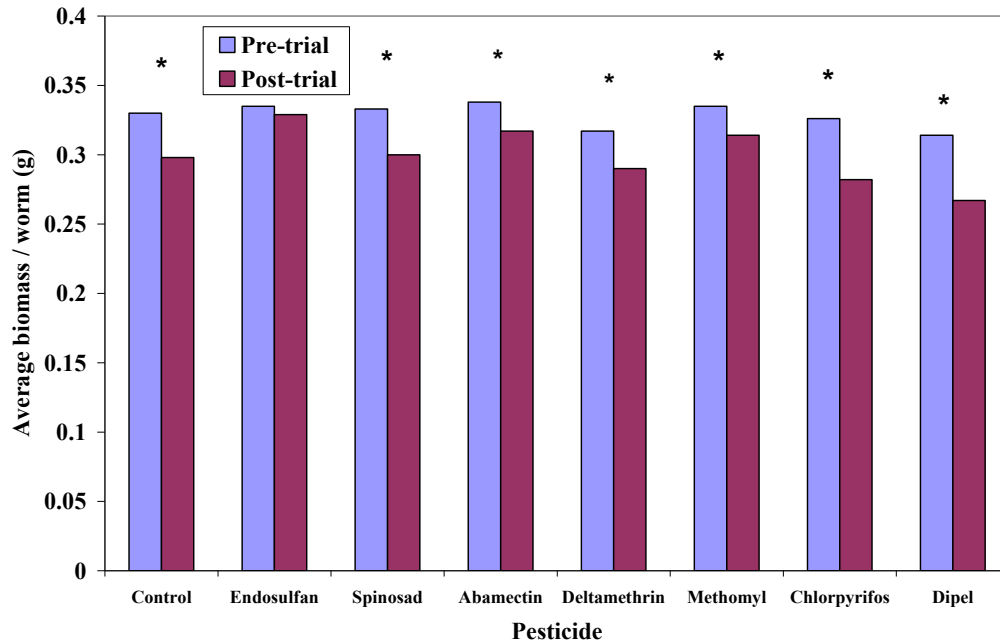


Fig. 26. Average biomass of individual *A. caliginosa* before and after exposure to various pesticides applied to the surface of soil. * indicates significant differences between pre-trial and post trial biomass.

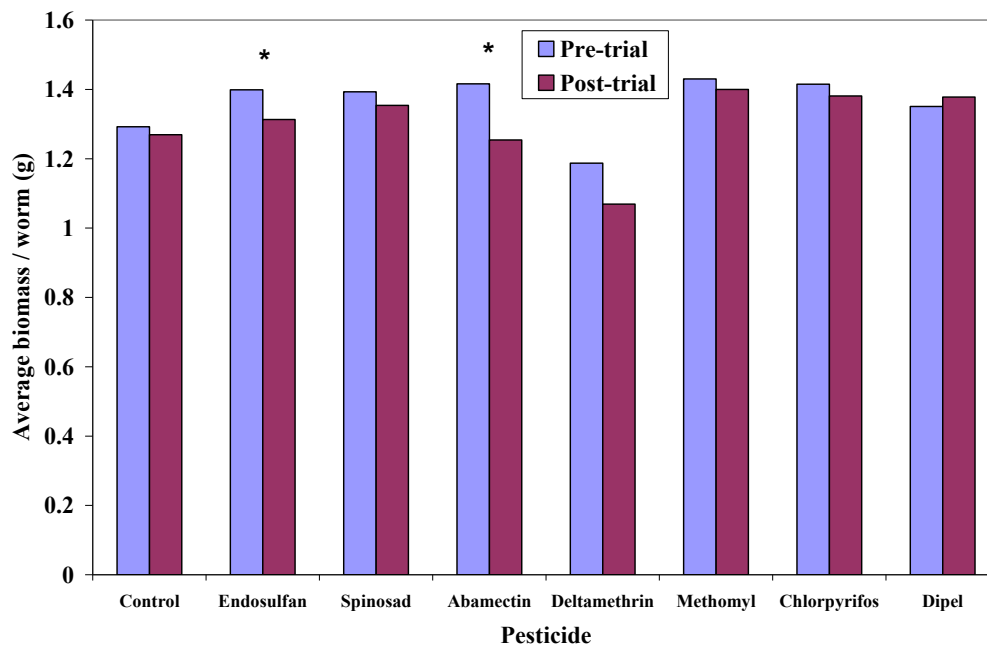


Fig. 27. Average biomass of individual *A. longa* before and after exposure to various pesticides applied to the surface of soil. * indicates significant differences between pre-trial and post trial biomass.

g. Effects of Flooding on Earthworms

All the *A. longa* and virtually all the *A. caliginosa* (93.3 to 100% for different days of observation) survived the flooding. Similarly, all the *A. longa* and most of the *A. caliginosa* (93.3 to 100%) survived in the control treatment. By chance, the earthworms used for the control treatments were initially smaller than those used in the flooded treatments. There was little evidence of a change in biomass per se during the course of the experiment for either earthworm species, but there was a significant interaction in an ANCOVA suggestive that both species tended to gain weight in the flooded boxes and lose weight in the control boxes (Tables 6 & 7).

Table 6. Average pre-trial and post-trial biomass (g) of individual earthworms in flooding experiment. * indicates significant difference between pre-trial and post-trial biomass.

Earthworm & Day	Control		Flooded	
	Pre-trial	Post-trial	Pre-trial	Post-trial
<i>A. caliginosa</i>				
Day 2	0.30	0.29	0.43	0.46
Day 4	0.30	0.30	0.44	0.47
Day 7	0.32	0.31	0.45	0.49
Day 9	0.29	0.28*	0.42	0.44
Day 11	0.29	0.28	0.43	0.46
Day 15	0.26	0.25	0.37	0.40*
Day 17	0.33	0.29	0.36	0.40
<i>A. longa</i>				
Day 2	1.74	1.67	1.73	1.99*
Day 4	1.67	1.85*	1.77	1.97*
Day 7	1.39	1.23	1.83	1.98
Day 9	1.45	1.34	1.98	2.13
Day 11	1.46	1.32*	1.58	1.68
Day 15	1.46	1.32	1.58	1.71
Day 17	1.47	1.38	1.65	1.90

Table 7. Summary of Analyses of Covariance for biomass of *A. caliginosa* and *A. longa* in flooding experiments

Source	<i>A. caliginosa</i>			<i>A. longa</i>	
	DF	F	Prob.	F	Prob.
Day (A)	6	4.67	< 0.05	4.22	< 0.05
Flood (B)	1	312.93	< 0.05	53.89	< 0.05
Pre/Post Trial (C)	1	1.30	> 0.05	1.27	> 0.05
A*B	6	2.13	> 0.05	2.89	< 0.05
A*C	6	0.11	> 0.05	0.36	> 0.05
B*C	1	8.92	< 0.05	6.90	< 0.05
A*B*C	6	0.14	> 0.05	0.21	> 0.05
Residual	56				
Total	83				

5. Conclusions – Take Home messages

Earthworms were more common in cropping soils than in less disturbed soils nearby. This was somewhat surprising, in view of well-known deleterious effects of tillage on earthworm populations. Possibly, greater availability of soil moisture in irrigated fields contributed to the result. Organic matter is also known to be a major driver of earthworm abundance from other studies, but no association between organic carbon / organic matter and earthworm abundance was found in this study. Earthworm abundance was most strongly correlated with measures of particle size (% silt) and electrical conductivity. Highest earthworm abundance tended to be recorded from farms in the Upper Namoi. Species richness was greater in the undisturbed soils.

The abundance of native species in the cropping soils in the Namoi Valley is surprising, given that other surveys in cropping soils in S.A., Victoria and southern N.S.W. (mostly wheat) have rarely found native species (these tend to be more common in pastures and other less disturbed habitats). The overall abundance of earthworms in the cropping fields in the Namoi Valley (0 – 324 earthworms m⁻²) is comparable with densities found in other cropping regions in southern Australia (e.g. a survey of pasture – cereal rotations in S.A. yielded 0 - 366 earthworms m⁻², see Baker 2004 for references).

This survey of course only focussed on one cotton production valley. The abundance (and potential role) of the earthworm fauna in other regions, with different soils etc, remains to be determined.

Nothing is known of the biology of the native earthworms found in the Namoi Valley survey, nor of their roles in soil health. The exotic lumbricid, *A. rosea*, which was occasionally common has rarely been shown to provide agronomic benefits (at least in the short term) in previous studies in grain and pasture systems in southern Australia. On the other hand, another exotic lumbricid, *A. trapezoides*, which was found occasionally in the survey, is known to provide substantial benefits through improved soil properties and plant production (grains and pasture species) (see Baker 2004 for references). The exotic acanthodrilid, *M. dubius*, was very abundant at one undisturbed site in the survey. This was not surprising – the site was unusually damp and had very large amounts of surface organic matter available – ideal for this epigeic species.

We used (for various practical reasons – see text above), the exotic *A. longa* and *A. caliginosa* as “model” species, involving two very different burrowing and feeding behaviours, to explore potential influences of earthworms on cotton (and wheat) production, in the early growth stages of the plants.

We also used these earthworm species to investigate tolerances to some of the environmental factors likely to prevail in cotton production systems. The earthworms survived in the “cotton” soils but overall didn’t respond (in terms of weight gain) as well in these soils as in a commercial sandy loam. However, when given choices, the earthworms preferred to locate themselves within the cotton soils.

The pesticides, Endosulfan, Methomyl and Chlorpyrifos, rapidly reduced survival of earthworms when applied directly. However, there was no evidence of significant mortality or weight loss for the earthworms due to these pesticides when applied to the soil surface in the short term (i.e. after 4 weeks). The actual pesticide exposure the worms experienced in the soil is of course unknown. Additional, simulated rainfall might have washed the pesticides more into the body of the soil and thus brought them closer to the earthworms. But soil mixing by the earthworms in the boxes was quite obvious and did suggest that the pesticides would have been mixed throughout much of soil mass.

Flooding (up to 2 weeks) had no effect on earthworm survival and did not reduce earthworm biomass. It should be remembered however that a natural earthworm response to flooding is to locate on or near the soil surface (to overcome oxygen deprivation). This can increase risk of predation in the field.

In the absence of fertiliser, earthworms increased cotton boll biomass, but not other vegetative biomass, in very poorly growing plants in small pots with Narrabri grey clay soil or a Hillston red-brown earth. The nitrogen content of the cotton plants was also increased by earthworms in this situation. Some contribution to these trends could have come from dead earthworms providing a fertilising effect within the soil. In larger pots (Narrabri soil only), earthworms increased the vegetative biomass of young cotton plants, as well as their heights. Again earthworm mortality could have contributed to these growth differences.

In the presence of fertiliser, there was no significant effect of earthworms on vegetative biomass or height when young cotton plants were grown in Narrabri soil in small pots. There was in this situation a tendency for plants to have smaller leaves in the presence of earthworms. In larger pots, earthworms (*A. longa*) had a small positive influence on cotton boll biomass, but this effect was found with dead earthworm additions as well as live earthworm additions (many of the latter died during the course of the experiment). Plant heights and numbers of leaves were also influenced by earthworms, but generally in a slightly negative way.

With these results re earthworm influences on cotton growth in mind, and already knowing that the same earthworm species can greatly enhance wheat growth and grain yield in sandy loams (in the absence of fertiliser), we were left wondering if cotton is simply not particularly responsive (at least in the short term) to earthworm effects, or if the soil type mostly used for the experiments (Narrabri grey clay) was driving the limited response. We thus conducted an experiment to measure the influence of earthworms on wheat growth in Narrabri soil and cotton growth in sandy loam, with back up treatments of earthworms and wheat in sandy loam and cotton in Narrabri soil. Fortunately, earthworm survival was high in this experiment, thus not providing a confounding effect of “unplanned” fertilisation.

As expected, the growth of wheat was generally enhanced by earthworms in the sandy loam (in the absence of fertiliser). There was no significant influence of earthworms on wheat growth in Narrabri soil, although there was a strong tendency for plants to be smaller in the absence of earthworms. Cotton growth tended to be greater in both Narrabri soil and the sandy loam in the absence of earthworms, but this was not significant. This trend was reflected in the sizes of leaves on the cotton plants, but not in their colour.

Overall, the results are thus somewhat confusing regarding the influences of earthworms on cotton growth. But in general it would appear that earthworms do not enhance cotton growth greatly – at least in the short term, for young plants. Mostly positive effects were noted in the absence of fertiliser, but these experiments were confounded with earthworm deaths that could have provided some additional nutrients for the plants. Some crop plants respond very positively to earthworms (e.g. sorghum, maize, rice) whilst others respond negatively (e.g. cowpea, peanuts) (see overseas literature cited in Baker 2004). Cotton may be positioned between these two extremes.

Key reference :

Baker, G.H. (2004). Managing earthworms as a resource in Australian pastures. In : Edwards, C.A. (Ed.). “Earthworm Ecology”. CRC Press, Boca Raton, U.S.A. pp 263-286.

6. Research in Relation to CRDC's 3 Outputs : Economic, Environmental & Social

The project addressed key CRDC outputs by seeking to improve profitability and sustainability of cotton production through harnessing soil fauna to improve soil health (structure & fertility) and cotton yield. The project also seeks in the longer term to assist farmers and extension staff in better recognising soil fauna on-farm and their beneficial role.

7. Summary

a) Technical Advances

This project is essentially the first to consider soil macrofauna, in particular earthworms, as a resource in the Australian cotton industry. It is an initial step in evaluating the abundance and composition of the fauna (albeit in only one cotton valley) and developing an understanding of the potential of these animals to influence cotton production.

b) Other Information Developed

Some information was gathered on the influences of soil type, flooding and pesticide use on the abundance of earthworms in cotton systems.

c) Changes to Intellectual Property Register

None required.

8. Future Plans

a) Further Development of Project Technology

Whether or not earthworms might influence cotton growth over longer time periods and in more ideal conditions (e.g. greater soil volumes and capacity for plants to grow more vigorously, several years of prior exposure of soils to earthworms before growing the cotton – i.e. additive effects of earthworm benefits) needs to be evaluated. Designs for field evaluation trials need to be developed for the common earthworm species in cotton fields (probably these can be adapted from previous research in grain production systems) and extended to consider other soil fauna groups. See also c) below.

b) Presentation and Dissemination of Project Outcomes

We intend to prepare scientific manuscripts, based on the data presented here, in the next few months.

Findings from the research will be further extended to industry through participation in field days, talks to farmer groups, articles in grower magazines & booklets and presentations and posters at workshops and conferences. We intend to present research results in novel fora for the cotton industry (e.g. Aust. Ecological Soc. Annual Conf). Scientific papers will be submitted to internationally recognised journals..

c) Future Research

This project essentially involved only one cotton production valley (following a significant drought when field populations of earthworms are likely to have been lower than usual), used earthworms found outside the cotton production system as earthworm “models” for experiments (enforced by the drought conditions), focussed only on glasshouse experiments and involved only one cotton soil type in the bulk of such experiments. The work simply considered outputs in the form of cotton growth and did not measure changes in soil properties, be they chemical (e.g. soil nutrient availability) or physical (e.g. aggregate structure, porosity – with links to water movement and root penetration). The plants were only occasionally grown to boll production. The work also did not consider in any detail the general improvements that might accrue to a cotton farming enterprise through enhanced production of other crops (e.g. wheat and other grains) that are grown in rotation with cotton. Such benefits could be direct through enhanced production from such crops, as well as longer term through resultant improved soil health (e.g. increased soil organic matter). The results of the present study therefore need to be regarded as preliminary and treated

with caution. There is an obvious need to gather more robust data covering these weaknesses in the current data set.

The biology and role of the several native earthworm species found, in this case in just the one cotton production valley, is basically unknown. This serves to highlight how we need to have a firmer understanding of these topics, in order to properly manage soil health in the industry.

Why were earthworms more abundant on some farms, especially in the Upper Namoi Valley, than others? The influences of various soil management practices, including the use of various cotton varieties (transgenic and conventional) and rotational practices, on the soil fauna in general is poorly understood in the Australian cotton industry.

As indicated in the Background section of this report, there is evidence from overseas research that some earthworms can have significant influences on the abundance of root diseases such as *Fusarium*. The capacity for earthworms to assist in the reduction of plant diseases (through more effective management of earthworms as a resource) should be explored. A protocol for developing such experimental work was developed during this project (not included here).

The Background section of this report also indicated the growing interest worldwide in the ecological interactions that can occur between above and below ground biota. For example, it is becoming evident that soil fauna can stimulate marked changes in the dynamics of above ground herbivorous pests and their natural enemies, mediated through changes in the crop plant. Further work is warranted in the Australian cotton production system as we strive to develop sustainable practices, including advances in soil health.

9. Publications Arising and Planned

Various aspects of the work were presented by G. Baker to consultants at the CCA AGM meeting in Goondiwindi in May 2003 (Paper entitled : Influence of beneficial soil fauna on cotton production and pests and diseases). An article is being drafted for submission to Aust Cotton Grower for its next issue. The work was incorporated into talks given by G. Baker at the Intern. Invasive Earthworm Workshop (Univ. Georgia, USA; Nov 2003) and the Intern Soil Zool. Colloq. (Rouen, France; Sept 2004). Some aspects of the work were included in a recent presentation by O. Knox et al (Underground cotton research and soil health) at the 2004 CRDC / Cotton CRC Farming Systems Forum on Nutrition.

The following scientific publication is in preparation :

Baker, G.H. The influence of earthworms (Lumbricidae) on the early growth of cotton (*Gossypium hirsutum*) and wheat (*Triticum aestivum*) in glasshouse experiments. Intended for Appl. Soil Ecol.

[Other publications will follow. G. Baker has a proven track record of scientific publication (> 150 papers, & on Edit. Board of leading int'l journals : Soil Biol. Biochem.; Appl. Soil Ecol.; Biol. Fertil. Soils) and extension of his research through the electronic and printed media.]

10. Online Resources

None have been developed for this small project.

11. Impact of Project Results and Conclusions for Cotton Industry

Interest in improving soil health is widespread in the cotton industry. Whilst this proposal is targeted at the CRDC's soils research program, it seeks to build linkages in time to other programs (e.g. Insect Management, Diseases) and thus integrate research across a range of disciplines. The project seeks to determine the value of improved management of earthworms in cotton ecosystems in Australia at a time when changing management practices (e.g. pesticide reduction) seemingly offer improved windows of opportunity for increases in their abundance.

12. Staffing

Several staff were employed or assisted in significant ways with this project. Donna Jones, Fiona Rayner and Paul Williams conducted the earthworm survey in the Namoi region under the local supervision of Colin Tann. Colin also kindly transported the Narrabri soil to Canberra, whilst Scott Hardwick did likewise for the Hillston soil. Briony Cowper and Graham Teakle carried out the lab and glasshouse experiments in Canberra. The efforts of these staff are greatly appreciated.

Part 4 - Final Report Executive Summary

Healthy soils are fundamental to the profitability and sustainability of cotton ecosystems. However, whilst soil fauna such as earthworms have been widely shown to be capable of markedly influencing soil structure and fertility (e.g. porosity, nutrient cycling, retention of nutrients on-farm), disease and pest incidence, and plant production and quality in other agricultural ecosystems, very little is known of their biology and functional role under cotton.

Cotton farming has presented several potential hazards for soil fauna (e.g. heavy pesticide use, tillage), but recent trends in the industry such as reduced (& softer) pesticide use, less tillage and retention of organic matter would seem likely to open opportunities for population growth and (re)colonisation of soil fauna such as earthworms. This project aimed to survey the status of the current earthworm fauna in and near to cotton fields in the Namoi Valley and to determine some of the major factors influencing its abundance there. Preliminary glasshouse experiments were conducted to evaluate the potential of earthworms to improve cotton production. The research was conducted in a period of drought in northern N.S.W. The results need to be considered in light of this (e.g. the field abundance that was observed may have been unusually low as a result of prolonged, low soil moisture).

Earthworms were more common in cropping soils used in recent times for cotton production than in less disturbed soils nearby. This result ran counter to expected, in that tillage is well known to reduce earthworm abundance. Possibly, greater availability of soil moisture in irrigated crops contributed to this result. Earthworm abundance within the cropping soils was most strongly correlated with measures of particle size (especially % silt) and electrical conductivity. Species richness was greatest in the undisturbed soils.

The abundance of the earthworms in the cropping soils in the Namoi Valley (0-324 earthworms m⁻²) was comparable with that reported previously for earthworm communities in other cropping systems in southern Australia (e.g. used for wheat production). However, the high frequency of native earthworm species in the Namoi soils was unusual. The biology of these native species is virtually unknown. Their role in improving soil health and cotton production remains unstudied.

Two exotic species of earthworms, *Aporrectodea caliginosa* and *A. longa*, with contrasting burrowing and feeding behaviours, and which could be obtained easily in large numbers during the study, were used to evaluate the suitability of two common "cotton" soils (a grey clay from ACRI, near Narrabri and a red-brown earth from near Hillston) for earthworm survival and development. [These earthworms were not found within the survey region]. The earthworms survived in these "cotton" soils but their growth was generally less than in a commercial sandy loam. Curiously however, both earthworm species preferred the Narrabri and Hillston soils to the sandy loam when given choices. Some commonly used pesticides (e.g. Endosulfan) killed the earthworms when placed in direct contact with them, but no influence on survival or growth was detected over 4 weeks when exposure was via soil application. Short term flooding in Narrabri soil was tolerated well by the earthworms.

In the absence of fertiliser and using Narrabri soil, earthworms increased cotton (SICOT 189) growth, but the plants generally grew poorly and the earthworm effects that were recorded were confounded with mortalities of earthworms that would have contributed some additional nutrients to the soils, compared with the control treatments. In the presence of fertiliser, no convincing, positive influences of earthworms on cotton growth were demonstrated (for young plants) using both the Narrabri soil and the commercial sandy loam. If anything, the cotton plants seemed slightly retarded in the presence of the earthworms. The same two earthworm species had a strong, positive influence on the growth of wheat plants in the commercial sandy loam and a similar (but not significant) effect on wheat growth in the Narrabri soil. Overall, the results suggest that these two earthworm species, in the short term at least, do not enhance early cotton growth, in contrast to some other crop plants.

This research project was a preliminary foray into the importance of one component of the soil biota, earthworms, in soil health and cotton production. It focused on surveying just one cotton production valley. It primarily used one soil type for the evaluation of earthworm effects on cotton production and only considered earthworm influences in the short term. More extensive research, in particular considering the most common earthworm species within cotton fields, earthworm influences on soil

physical and chemical properties, plant diseases and the dynamics of other pest and beneficial species in the cotton production system is recommended. Earthworm abundance varied greatly between farms in the Namoi Valley. The environmental factors (including agricultural management practices) that drive these differences need to be further understood.