

**BUREAU OF SUGAR EXPERIMENT STATIONS
QUEENSLAND, AUSTRALIA**

FINAL REPORT

BSS132 - PLANT RESISTANCE TO CANEGRUBS

by

PG Allsopp, MC Cox and KA Nutt

SD02003

Principal Investigators

Dr Peter Allsopp and Dr Michael Cox

BSES

Private Bag 4

BUNDABERG DC Q 4670

Phone 07 4132 5200

Email: pallsopp@bses.org.au or

mcox@bses.org.au

Ms Kerry Nutt

BSES

PO Box 86

INDOOROOPILLY Q 4068

Phone 07 3331 3365

Email: knutt@bses.org.au

The Sugar Research and Development Corporation funded this project between the 1994-95 and 1999-2000 financial years. The research organisations are not partners, joint venturers, employees or agents of SRDC and have no authority to bind SRDC legally in any publication of substantive details or results of this project.

CONTENTS

Page No.

SUMMARY

1.0	BACKGROUND.....	1
2.0	OBJECTIVES	2
3.0	OUTCOMES.....	2
4.0	RESEARCH METHODOLOGY, RESULTS AND DISCUSSION	2
4.1	Pot-based screening trials.....	2
4.2	Field-based screening trials.....	3
4.3	Determination of chemical mechanisms.....	3
4.4	Protocol and strategy for improving resistance	4
5.0	RECOMMENDATIONS.....	7
6.0	PUBLICATIONS	7
7.0	ACKNOWLEDGMENTS	7
8.0	REFERENCES	8
APPENDIX 1	POT-BASED SCREENING TRIALS	11
APPENDIX 2	FIELD-BASED SCREENING TRIALS	32
APPENDIX 3	DETERMINATION OF CHEMICAL MECHANISMS	44

SUMMARY

The project sought to determine the range and types of resistances to feeding by canegrubs in sugarcane clones and their wild relatives. Resistance was tested in both pot trials and field trials and the importance of several biochemical factors in this resistance was investigated.

Field and pot trials showed that a range of tolerance and antibiosis resistance mechanisms to canegrub feeding exists within the current sugarcane genome and close relatives. Tolerance effects were apparent in the growth of tops, roots and stubble. Antibiosis effects were apparent on grub survival and grub growth. Some of these tolerance effects are partially correlated with the general vigour of clones, but there are a number of clones that depart from the general relationships for tolerance and antibiosis. These clones would be especially important in any future program to increase the levels of resistance within the breeding gene pool.

There was reasonable repeatability of pot-based tolerance levels between pot trials and with results derived from field trials. However, the type of canegrub and its underlying biology influenced the usefulness of field trials.

Biochemical investigations showed no clear relationships between any factor and antibiosis phenotypes. However, there were indications that the type of cell-wall sugars may influence resistance to canegrubs. In addition, the data clearly indicated that the quantity and type of phenolic compounds in the roots change following feeding by canegrubs.

These results would be best incorporated into the breeding program through a specific subprogram targeting grub resistance and using recurrent selection with rapid generation turnover. This would require the development of an appropriate screening system and may require two stages. The first would need to handle large numbers of clones to discard the least resistant. This could be followed by a more intensive screening (eg pot technique) to identify the most resistant clones. Those identified after the first cycle of selection would then be recombined to produce more progeny and the process repeated. It is likely that several cycles will be necessary to increase resistance to a level that provides an economic protection.

1.0 BACKGROUND

Melolonthine whitegrubs (canegrubs) are the most serious pests of Australian sugarcane (Allsopp *et al.* 1993) costing the industry each year >A\$10m (US\$7.8m) in insecticide and residual lost production. This is during a period when insecticides give reasonable control. Larvae damage the roots, reducing plant growth and in severe cases killing the plant. Root damage also makes the plant more susceptible to lodging and removal during harvest of the stubble which grows the following year's crop. Nineteen endemic species in the genera *Antitrogus*, *Dermolepida*, *Lepidiota* and *Rhopaea* (all Melolonthini) damage sugarcane in different areas of eastern Australia (Allsopp *et al.* 1993).

Since the late 1940s, control of whitegrubs in Australian sugarcane has depended heavily on synthetic insecticides. Currently, controlled-release chlorpyrifos (suSCon® Blue or suSCon® Plus) or the biopesticide BioCane™ is applied at or soon after planting and gives control of most species for 2-3 years. Alternatively, granular cadusafos (Rugby® 100G) or liquid imidacloprid (Confidor® 200SC) can be applied to the ratooning stubble after the annual harvest; these give control of larvae present at application, but require irrigation or rainfall to make them effective. Efficient use of insecticides is complicated by the long-term nature of the crop (about five years between replanting), the inaccessibility of the crop for much of the year, difficulties in sampling and treating subterranean insects, and by the one- or two-year life cycles of different species.

Host-plant resistance could provide excellent protection of sugarcane from feeding by whitegrubs. It would afford growers a low-input alternative to insecticides, without the necessity for sampling and application costs. Australian canegrowers regularly adopt new higher-yielding cultivars, so a whitegrub resistant line would have a good acceptance rate. Resistance to whitegrubs has been reported in sugarcane in Australia (Easterby 1926; Bell 1934, 1940; Mungomery and Buzacott 1940; McDougall 1940; Skinner 1941; Knust 1947; Anderson and Luckett 1960), Mauritius (Moutia and Mamet 1946; Williams and Moutia 1954), Tanzania (Jepson 1956) and India (Gupta and Avasthy 1957; David and Ananthanarayana 1982). Other grasses, such as maize, oats, wheat, barley and turf grasses, also show variability in tolerance (Bigger *et al.* 1939; Hoegemeyer 1941; Rodriguez del Bosque 1982; Crutchfield and Potter 1995) or in nonpreference (Crocker *et al.* 1990) to whitegrubs. In sugarcane, resistance has usually been attributed to tolerance, through different root architecture, tillering ability or different ability to reproduce roots rapidly during an attack or after the attack is substantially over (Mungomery and Buzacott 1940; Volp 1947; Jepson 1956; Evans 1964). Most of the Australian information is anecdotal, all comparisons are of only a few superseded cultivars, and some, like the status of POJ2878 (resistant (Mungomery and Buzacott 1940; McDougall 1940); very sensitive (Anderson and Luckett 1960)), is contradictory.

Integrated pest management (IPM) systems for canegrubs must comprise a combination of practices, including the judicious use of insecticides, crop rotation, field sanitation, natural enemies and inherently resistant plant cultivars. Plant resistance offers many advantages in economic and sustainable agriculture.

This project sought to determine the repeatability of the current screening procedure and screen more clones of *Saccharum* hybrids, related species and intergeneric crosses. It sought to determine the role of several known chemical mechanisms in resistance/tolerance. A technique for screening significant numbers of sugarcane clones

for canegrub resistance/tolerance was developed. A strategy to research and evaluate the inclusion of canegrub resistance into the breeding program and integration into an IPM program for canegrubs is proposed. The project tested resistance in southern Queensland and in the Burdekin (greyback canegrub).

2.0 OBJECTIVES

- Screen more clones of *Saccharum* hybrids, related species and intergeneric crosses for resistance to canegrubs.
- Determine the repeatability of the current screening procedure and how it reflects field resistance in both southern Queensland and the Burdekin.
- Determine the role of several known chemical mechanisms in resistance/tolerance.
- Develop a protocol for screening significant numbers of sugarcane clones for canegrub resistance/tolerance.
- Propose a strategy to research and evaluate the inclusion of canegrub resistance in the cane breeding and selection programs, and in integrated pest management of canegrubs.

3.0 OUTCOMES

- Confirmation from field and pot trials that a range of tolerance and antibiosis resistance mechanisms to canegrub feeding exists within the current sugarcane genome and close relatives.
- Determination that tolerance effects are partially correlated with the general vigour of clones.
- Determination of reasonable repeatability of pot-based tolerance levels between pot trials and with results derived from field trials.
- Determination of the presence of clones departing significantly from general relationships and therefore of significant interest in further breeding work.
- Indications that the quantity and type of phenolic compounds in the roots change following feeding by canegrubs.
- Indications that the type of cell-wall sugars may influence resistance to canegrubs.

4.0 RESEARCH METHODOLOGY, RESULTS AND DISCUSSION

4.1 Pot-based screening trials

Details of the methods used, results and statistical analyses are given in Appendix 1. The significance of the results is also discussed in the Appendix.

The main conclusions from the pot-based screening trials are listed below. Our trials indicate that there is significant variability among clones of sugarcane and their relatives in some of the components that would contribute to a 'grub-resistant' cultivar.

We have demonstrated variation in tolerance through:

- differences among clones in root loss and in flow-on tolerance to reduced top and stubble loss. These effects are partially correlated with general vigour of the clone, with the more vigorous clones retaining more roots and tops when attacked by grubs;
- presence of some clones that show significant departures from the general correlations - these clones will be of especial interest in future breeding;
- reasonable repeatability between pot trials (including use of different grub species) in the determination of effects on plant growth;
- reasonable degree of genetic determination of growth parameters in uninfested and infested plants.

We have also demonstrated variation in antibiosis through:

- differences among clones in the survival of grubs and the weight of surviving grubs, with some clones showing significant departures from overall trends;
- some correlation between the potential root growth of clones and the weight of surviving grubs;
- no consistent correlation between potential root growth of clones and the number of surviving grubs;
- no consistent correlation between grub survival and weight of surviving grubs;
- reasonable repeatability across pot trials of the effects on final weights of larvae, but not of effects on grub survival - this indicates that extrinsic factors may be influencing grub survival.

4.2 Field-based screening trials

Details of the methods used, results and statistical analyses are given in Appendix 2. The significance of the results is also discussed in the Appendix.

Overall, the field trials allow assessment of different resistance components than do the pot trials. Field trials assess not only the tolerance to yield reduction in the infested crop, but also yield reduction through stool damage and/or removal in the subsequent ratoon crop(s). However, they do not distinguish between antibiosis and antixenosis components (that cause different numbers of grubs), and control of infestation levels and timing can make results difficult to interpret. Pot trials do allow estimation of yield reduction in the infested crop, they allow differentiation of effects on tops, roots and stubble, and also allow estimates of antibiosis effects without compounding these with differences in antixenosis. Pot trials do not, however, allow estimates of the cumulative effects of damage on succeeding ratoon crops.

4.3 Determination of chemical mechanisms

Details of the methods of analyses, results and discussion are given in Appendix 3. The significance of the results is also discussed in the Appendix.

Our tests indicate that there are considerable differences among clones in the total amounts and types of total sugars, cell-wall sugars and phenolics. In addition, the amounts and types of phenolics varied significantly between uninfested and infested plants of the same clone. These factors are all putative defence mechanisms that could be involved in resistance.

However, we could show no correlation between these factors and the antibiosis groupings of the clones examined. Perhaps, this is not surprising. If antibiosis to canegrubs in sugarcane is multigenic, a number of defence factors may contribute to that type of resistance, and the contribution of each factor to overall antibiosis may vary among clones. Consequently, it would be expected that correlations between individual defence factors, and overall resistance would not be high. In other words, just because phenotypes are the same, it does not mean that the chemical basis is the same.

We could show no significant variation among clones in amounts of diterpenoids, triterpenes, phytosterols, gibberellans, sesquiterpene lactones, alkaloids, quaternary alkaloids, N-oxides, and fats and waxes that may have contributed to differences in antibiosis.

4.4 Protocol and strategy for improving resistance

Our work demonstrates the presence of all three components of resistance in plants to pests: tolerance, the ability of a plant to keep growing even though it is attacked; antibiosis, where a plant affects the survival or development of a pest that feeds on it; antixenosis or nonpreference, where the pest prefers one genotype of a plant over other genotypes. Each of these components has a role to play in improving resistance in commercial sugarcane to canegrubs.

Tolerance is most effective at lower pest densities and will 'break down' under high pest pressures because the plant simply fails to compensate for damage. Our studies suggest that tolerance in sugarcane to canegrubs is multigenic. In part, that tolerance is related to the 'vigour' of a clone, making screening and selection relatively easy. However, many aspects other than vigour could be involved, perhaps contradictorily, so simple selection for a few characters could be difficult. There are two main positives to this type of resistance: it is multigenic and does not directly impose selection pressures on the pest. These mean that the pest can not develop its own resistance to these traits.

Antibiosis is, at first consideration, the most attractive form of resistance; pests are killed directly or reproduction curtailed. It is analogous to the simple insecticide solution, 'the silver bullet', desired by many growers. Once a mechanism is identified, then screening and selection are relatively simple and can often proceed in the absence of the pest. However, our work suggests that clones with the same phenotype (low grub survival or low grub weight gains) may not have the same genetic basis, and secondly that the chemical basis may be difficult to identify. Each form of antibiosis is likely to be based on one or a few genes and, like any insecticide, it poses severe selection pressure on the target pest. Hence, the logical outcome of overuse is the development of resistance in the pest. It may be possible, however, to 'pyramid' such genes to enhance the level of resistance and make it more difficult for the pest to overcome the resistance. This has been done effectively for stem rust in wheat, but requires good genetic knowledge, which is clearly not available in sugarcane.

Antixenosis relies on giving the pest a choice between at least two different lines, although the line preferred by the pest might occupy a smaller proportion of the area planted than the non-preferred line. This is the same principle exploited in the manipulation of harvest and planting times to congregate greyback canegrubs into the least-valuable or otherwise protected crops. Unless combined with other controls, the mechanism relies on damage still occurring somewhere, but the economic impact of that infestation may be minimised. Antixenosis will only work with a pest species where the adult female actively selects oviposition sites, such as greyback canegrub; it will be useless or impractical in species such as Childers canegrub and southern one-year canegrub where the adult females move little and oviposit near where they emerge. Screening and selection for antixenosis are relatively simple, but selection is always relative to the other lines tested at the same time.

Plant breeding for resistance can follow two very different approaches (Robinson 1996). The more traditional approach is vertical resistance, which is generally based on one or a relatively small number of genes. Variation for resistance is discreet; for example, resistant or susceptible in the simplest case, with a limited number of states. As antibiosis is generally involved, it often results in very high levels of resistance. However, due to the high selection pressure and the simple genetic nature of the resistance, the pest or disease organisms may change to overcome the resistance. The second approach is horizontal resistance, which is polygenic and results in continuous variation for resistance. Levels of resistance are usually lower, resulting in less selection pressure, and the polygenic inheritance makes it virtually impossible for the organism to overcome the resistance.

Breeding and selection for vertical and horizontal resistance use very different strategies. Development of vertical resistance simply involves crossing parents with adequate resistance levels and selecting resistant progeny using an appropriate screening method. This is used successfully in sugarcane for many of our major diseases. Horizontal resistance usually involves population improvement or recurrent selection. This involves several cycles of crossing 'resistant' parents, selecting progeny for higher levels of resistance and further crossing. This results in populations with progressively higher levels of resistance.

The resistance we have found in our experiments appears to be horizontal. The advantages of horizontal resistance are striking (Robinson 1996):

- it is durable, operating against all strains of the pest;
- it can be complete resistance - the level of horizontal resistance can be changed by breeding, but each small increase is valuable making other aspects of crop management more effective, easier, cheaper and safer;
- no 'source' of resistance needs to be identified because recurrent mass selection simply involves changing gene frequencies;
- breeders can screen for more than one objective at the same time - high yield, good quality of product, good agronomic suitability, good health in the presence of the pest.

However, breeding for horizontal resistance requires a different breeding system than that for vertical resistance. Without recognising the importance of horizontal resistance, breeders can inadvertently erode the level of these resistances.

What approach should be taken to breed for resistance in sugarcane to canegrubs? The genetics of commercial sugarcane is complex and largely unpredictable and most desirable characters are multigenic, so breeding in sugarcane uses recurrent mass selection. Population improvement, using recurrent selection with rapid generation turnover has been used effectively to increase the frequency of genes for high early ccs in sugarcane in Australia (Cox *et al.* 1994). A similar system could be used in breeding for horizontal resistance to canegrubs. The only difference between breeding for, say, increased ccs and breeding for canegrub resistance is that the latter must be done in the presence of the pest, otherwise the overall level of resistance will decrease through 'host erosion'. Clones identified within the pot trials that should be included as parents within any such breeding are: tolerance to loss of tops - Q99, Q115, Q118, Q137, Q150, 81N301, 84N4308, 85N1056, 89A3434; tolerance to loss of roots - BN83-3120, BN81-1394, BN83-3074, NCo310, Q91, Q99, Q137, Q152, Q161, 79N439, 90C6006; tolerance to loss of stubble - Q99, Q123, Q136, Q139, Q144, Q152, Q158, Q161, Q180^b, 85S7186, 89C5003, 89C6015, 90S7073; reduced grub survival - NCo310, Pelorus, Q117, Q133, Q145, 85S7186, 92S54; reduced grub weight - NCo310, Q20, Q137, Q170^b, 84N2172, 89C5003, 93N6006. In addition, a range of *Erianthus arundinaceus* clones tested in pot-trial 3 showed poor survival of larvae.

In practice, no sugarcane-breeding program would risk the erosion of levels of valuable yield and quality traits by exposing the entire population being screened to selection by canegrubs. One option would be to develop a subprogram of population improvement for resistance to canegrubs using recurrent selection with rapid generation turnover. The base population would include clones identified in this project as having the best levels of resistance (tolerance and antibiosis). Progeny from crosses made within this population would be screened in the presence of canegrubs. This would require the development of an appropriate screening system and may require two stages. The first would need to handle large numbers of clones to discard the least resistant. This could be followed by a more intensive screening (eg pot technique) to identify the most resistant clones. Those identified after the first cycle of selection would then be recombined to produce more progeny and the process repeated. It is likely that several cycles will be necessary to increase resistance to a level that provides an economic protection.

This project also identified a number of clones of wild species (eg *Saccharum spontaneum* and *Erianthus arundinaceus*) that showed enhanced resistance, both tolerance and antibiosis. Thus another approach would be to develop an introgression program, which may also be effective in enhancing resistance to canegrub. However, this is extremely long term and would require appropriate funding. One concern with the *S. spontaneum* clones is that the tolerance shown is probably related to the stoloniferous nature of the species and that this resistance would disappear in the hybrids, which are not stoloniferous.

When considering possible breeding approaches, a number of technologies need to be monitored. Firstly, transgenic clones are showing considerable promise as a tool in integrated pest management of canegrubs. Should genetically modified (GM) foods be accepted, this technology is likely to be adopted. Using varieties developed through conventional breeding approaches outlined above in addition to genetic engineering may enhance resistance even further. Secondly, genomics may help to identify genes involved in resistance to canegrubs and this could eventually be used either in genetic engineering or in developing markers for selection for resistance.

5.0 RECOMMENDATIONS

- Commence a population improvement subprogram to enhance the levels of tolerance and antibiosis in the current breeding gene pool. This will require development of appropriate screening methods to handle large numbers of progeny.
- Further investigation of the importance of changes in amounts and types of phenolic compounds following feeding may improve knowledge of the reaction of sugarcane to feeding by canegrubs.
- Further investigation of the antibiosis components of *Erianthus arundinaceus* (and possibly *Erianthus-Saccharum* hybrids) appears warranted. Determination of the biochemistry of this resistance mechanism could be useful.

6.0 PUBLICATIONS

Allsopp, P.G. (1994) Projects target grub-resistant cane. *Australian Canegrower* 16(22):28.

Allsopp, P.G., McGhie, T.K., Smith, G.R., Ford, R. and Cox, M.C. (1995) Progress in the development of cane varieties with resistance to canegrubs. *Proceedings of the Australian Society of Sugar Cane Technologists* 17:97-105.

Allsopp, P.G., McGhie, T.K., Cox, M.C. and Smith, G.R. (1996) Redesigning sugarcane for resistance to Australian canegrubs: a potential IPM component. *Integrated Pest Management Reviews* 1:79-90.

Allsopp, P.G. and Manners, J.M. (1997) Novel approaches for managing pests and diseases in sugarcane. In Keating, B.A. and Wilson, J.R. (Eds) *Intensive sugarcane production: meeting the challenges beyond 2000*. pp. 173-188. CAB International, Wallingford.

Allsopp, P.G., Shepherd, K.M., Smith, G.R., Cox, M.C. and Robertson, S.K. (1997) Use of host-plant resistance as a component in IPM for canegrubs. In Allsopp, P.G., Rogers, D.J. and Robertson, L.N. (Eds) *Soil invertebrates in 1997*. pp. 102-105. Bureau of Sugar Experiment Stations, Brisbane.

Allsopp, P.G. (2001) Minimizing the impact of whitegrubs in Australian sugarcane - development of useful strategies. In A Meneses (Ed) *Memoria X Congreso Nacional de la Caña Azúcar. II Simposio Nacional de Plagas*. pp. 21-31. ATAGUA, Guatemala.

7.0 ACKNOWLEDGMENTS

Many people within BSES Bundaberg and BSES Burdekin contributed to this project; especially we thank Norm McGill, Peter Hansen, George Bade, Tim Fischer and Andrew Horsfield. Biochemical studies benefited from discussions with Tony McGhie and Kellie Shepherd.

8.0 REFERENCES

- Allsopp, P.G. (1992) Sugars, amino acids, and ascorbic acid as phagostimulants for larvae of *Antitrogus parvulus* and *Lepidiota negatoria* (Coleoptera: Scarabaeidae). *Journal of Economic Entomology* 85:106-111.
- Allsopp, P.G., Chandler, K.J., Samson, P.R. and Story, P.G. (1993) *Pests of Australian sugarcane*. Bureau of Sugar Experiment Stations, Indooroopilly.
- Anderson, J. and Lockett, E.J.R. (1960) Problems of grub control and varietal reaction to grub attack in the Isis area. *Cane Grower's Quarterly Bulletin* 24:30-31.
- Bacic, A., Harris, P.J. and Stone, B.A. (1988) Structure and function of plant cell walls. In: *The Biochemistry of Plants: a Comprehensive Treatise. Volume 14. Carbohydrates* (ed. J. Preiss) pp.297-371. Academic Press, San Diego.
- Bell, A.F. (1934) The resistance of the seedling S.J.4 to grub damage in north Queensland. *Cane Grower's Quarterly Bulletin* 2:9-10.
- Bell, A.F. (1940) Grub damage to the variety S.J.2. *Cane Grower's Quarterly Bulletin* 8:40.
- Berding, N. (1996) Sugarcane weevil borer resistance: breeding strategy development using survey data. *Proceedings of the Australian Society of Sugar Cane Technologists* 18:90-99.
- Bigger, J.H., Flint, W.P., Shropshire, L.H. and Farrar, M.D. (1939) Hybrid corn shows variation in resistance to white grubs. *Illinois Agricultural Experiment Station Annual Report* 50:161-162.
- Cox, M.C., Hogarth, D.M. and Hansen, P.B. (1994) Breeding and selection for high early season sugar content in a sugarcane (*Saccharum* spp. hybrids) improvement program. *Australian Journal of Agricultural Research* 45:1569-75.
- Crocker, R.L., Marshall, D. and Kubica-Breier, J.S. (1990) Oat, wheat, and barley resistance to white grubs of *Phyllophaga congura* (Coleoptera: Scarabaeidae). *Journal of Economic Entomology* 83:1558-1562.
- Crutchfield, B.A. and Potter, D.A. (1995) Tolerance of cool-season turf grasses to feeding by Japanese beetle and southern masked chafer (Coleoptera: Scarabaeidae) grubs. *Journal of Economic Entomology* 88:1380-1387.
- David, H. and Ananthanaryana, K. (1982) A technique for artificial infestation of sugarcane varieties for evaluating resistance to white-grubs in pot experiments. *Journal of Soil Biology and Ecology* 2:36-39.
- Easterby, H.T. (1926) Varieties of sugar cane in Queensland. *Bulletin, General Series, Queensland Bureau of Sugar Experiment Stations* no. 2.
- Evans, H. (1964) The root system of sugarcane - an evaluation of its salient features. *Indian Journal of Sugar Cane Research and Development* 8:160-171.

- Gupta, B.D. and Avasthy, P.N. (1957) Observations on a new beetle pest of sugarcane crop in Bihar. *Indian Sugar* 7:587-593.
- Harborne, J.B. (1998) *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis* 3rd edition. Chapman and Hall, London.
- Hoegemeyer, L.C. (1941) An association of root injury by white grubs, *Phyllophaga* spp., and lodging of crossbred strains of corn. *Journal of the American Society of Agronomy* 33:1100-1107.
- Iiyama, K. and Wallis, A.F.A. (1990) Determination of lignin in herbaceous plants by improved acetyl bromide procedure. *Journal of the Science of Food and Agriculture* 51:145-161.
- Irvine, J.E. (1977) Composition of cane and juice. In: *Cane Sugar Handbook*, 10th edition (eds G.P. Meade and J.C.P. Chen) pp. 15-29. Wiley, New York.
- Jepson, W.F. (1956) The biology and control of the sugar-cane chafer beetles in Tanganyika. *Bulletin of Entomological Research* 47:377-397.
- Knust, H.G. (1947) Resistance of Eros to grub attack. *Cane Grower's Quarterly Bulletin* 10:117.
- Lawther, J.M., Sun, R. and Banks, W.B. (1996) Fractional characterisation of wheat straw lignin components by alkaline nitrobenzene oxidation and FT-IR spectroscopy. *Journal of Agricultural and Food Chemistry* 44:1241-1247.
- McDougall, W.A. (1940) Notes on the use of varieties in lessening grub damage to cane. *Cane Grower's Quarterly Bulletin* 8:48-49.
- McGhie, T.K. (1997) A survey of pre-formed and induced defence mechanisms associated with resistance of sugarcane to the fungal root pathogen *Pachymetra chaunorhiza*. PhD thesis, University of Queensland.
- Moutia, L.A. and Mamet, R. (1946) A review of twenty-five years of economic entomology in the island of Mauritius. *Bulletin of Entomological Research* 36:439-472.
- Mungomery, R.W. and Buzacott, J.H. (1940) Varietal resistance to cane grubs. *Cane Grower's Quarterly Bulletin* 8:45-47.
- Odulaja, A. and Nokoe, S. (1993) A maximum-minimum approach for classifying crop varieties into resistance groups based on yield potential and loss. *International Journal of Pest Management* 39:64-66.
- Ricaud, C. and Ryan, C.C. (1989) Leaf scald. In: *Diseases of sugarcane. Major diseases* (eds C. Ricaud, B.T. Egan, A.G. Gillaspie and C.G. Hughes) pp. 39-58. Elsevier, Amsterdam.
- Robinson, R.A. (1996) *Return to Resistance. Breeding Crops to Reduce Pesticide Dependence*. AgAccess, Davis, California.

- Rodriguez del Bosque, L.A. (1982) *Suceptibilidad de variedades de maiz al ataque de la gallina ciega, Phyllophaga crinita Burmeister*. M.S. thesis, Instituto Tecnológico y de Estudios Superiores de Monterrey, Division de Ciencias Agropecuarias y Maritimas.
- Salisbury, F.B. and Ross, C.W. (1992) *Plant Physiology* 4th edition. Wadsworth Publishing Company, California.
- Skinner, J.C., Hogarth, D.M. and Wu, K.K. (1987). Selection methods, criteria, and indices. In: *Sugarcane Improvement through Breeding* (ed. D.J Heinz) pp. 409-453. Elsevier, Amsterdam.
- Skinner, S.O. (1941) Resistance of new seedlings to frenchi grub attack. *Cane Grower's Quarterly Bulletin* 8:142-143.
- Summers, C.B. and Felton, G.W. (1994) Prooxidant effects of phenolic acids on the generalist herbivore *Helicoverpa zea* (Lepidoptera: Noctuidae): potential mode of action for phenolic compounds in plant anti-herbivore chemistry. *Insect Biochemistry and Molecular Biology* 24:943-953.
- Volp, P. (1947) Varietal resistance to greyback grubs in the Mulgrave area. *Cane Grower's Quarterly Bulletin* 10:176-177.
- Williams, J.R. and Moutia, L.A. (1954) Some aspects of sugarcane entomology in Mauritius. *Proceedings of the International Society of Sugar Cane Technologists* 8:570-573.

APPENDIX 1 POT-BASED SCREENING TRIALS

MATERIALS AND METHODS

We tested 405 sugarcane clones (including some wild relatives) for different resistance components in six trials (one trial was completed before the start of the formal project and is included here for completeness) at BSES Bundaberg (71 in trial 1; 102 in trial 2; 82 in trial 3; 104 in trial 4; 105 in trial 5; 90 in trial 6; some clones in more than one trial). In each trial, single-bud cuttings of the different clones (Table 1) were grown in white 20 L plastic pots in the open. Pots were filled with a sand soil (about 95% sand, 4% clay, 1% silt). Cuttings were obtained from disease-free plants or were given a long-soak hot-water treatment (Ricaud and Ryan 1989) to rid them of possible ratoon stunting disease or leaf scald infection. We germinated cuttings in a mixture of peat moss and vermiculite and planted them into the pots in each October. Pots were fertilised with a balanced blend of nutrients and were watered each 3-4 days during the growth of the plants.

In each trial, pots were arranged in a randomised split-plot complete-block design with three (trial 1) or four (trials 2-6) replications. The split was pairs of pots infested or not infested with whitegrubs. Plants were allocated to pairs within blocks in December on the similarity of the number of live shoots and the height of the plant. We placed five young third-instar *Lepidiota picticollis* Lea (trial 1) or six young third-instar or in one block of one trial old second-instar *Antitrogus consanguineus* (Blackburn) (trial 2-6) in the pots in the following February and harvested each trial in May. We harvested the tops at ground level, and washed out and separated the below-ground stubble and attached roots. Tops, stubble and roots were weighed after drying at 105°C for 2 days. Living larvae were counted and the mean live weight of larvae determined for each pot.

Tolerance is that component of host-plant resistance where the plant has an ability to withstand feeding by the pest. To test for differences in tolerance among clones within each trial, data by pot on dry weights of tops, stubble and roots were subjected to analysis of variance (ANOVA). Means were separated by least-significant-difference (LSD) tests, using the mean-squares obtained in ANOVA. Dry weights were first analysed by split-plot design (infested-uninfested) to determine if there were differences among clones in their reaction to canegrubs; separate analyses on data from infested and uninfested pots were then performed. We also calculated three new parameters for each of top, stubble and root weights. If Y_i and Z_i are the yields of infested or uninfested plants of clone i , then the percentage yield loss is:

$$P_i = 100(Z_i - Y_i) / Z_i.$$

The relative yield of each clone when infested (RY_i) compared to that of the best yielding clone when infested (Y_{max}) is:

$$RY_i = 100 Y_i / Y_{max},$$

and the relative percentage yield loss of each clone (RP_i) compared to that of the most susceptible clone (P_{max}) is:

$$RP_i = 100 P_i / P_{max}.$$

Table 1. List of clones tested in different trials; code numbers are those used in the figures. Commercial clones are those grown commercially in Australia at some time. *E.* = *Erianthus*; *S.* = *Saccharum*. Clones labelled as 'commercial' have been or are currently so in Australia.

Clone	Origin/type	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
BN74-4445	Australia commercial hybrid		1				
BN81-1394	Australia commercial hybrid					1	
BN83-3074	Australia hybrid					2	
BN83-3120	Australia hybrid					3	
BN83-36009	Australia hybrid		2				
Cassius	Australia commercial hybrid	1					
Co290	India commercial hybrid	2					
Co6602	India hybrid					4	
CP44-101	USA commercial hybrid	3					
CP51-21	USA commercial hybrid	4					
CP63-588	USA hybrid					5	
CP74-2005	USA commercial hybrid					6	1
CP78-1628	USA hybrid				1		
CP80-1743	USA hybrid		3				
CP81-1238	USA hybrid		4				
CP81-1254	USA hybrid		5				
CP81-1302	USA hybrid		6				
CP81-1383	USA hybrid		7				
CP82-1505	USA hybrid		8				
D1135	Guyana commercial hybrid		9				
Eos	Australia commercial hybrid		10			7	2
Eros	Australia commercial hybrid	5					
Galagah	<i>S. spontaneum</i>		11				
Galagah 1286	<i>S. spontaneum</i>			1			
Galagah WT	<i>S. spontaneum</i>			2			
HQ409	Australia commercial hybrid		12				
H56-752	Hawaii commercial hybrid	6					
H70-6957	Hawaii commercial hybrid					8	
H78-0878	Hawaii hybrid		13				
H79-6185	Hawaii hybrid		14				
IJ76-333	Java <i>E. arundinaceus</i>		15	3			
IJ76-334	Java <i>E. arundinaceus</i>			4			
IJ76-357	Java <i>E. arundinaceus</i>			5			
IJ76-358	Java <i>E. arundinaceus</i>			6			
IJ76-364	Java <i>E. arundinaceus</i>			7			
IJ76-365	Java <i>E. arundinaceus</i>			8			
IJ76-367	Java <i>E. arundinaceus</i>			9			
IJ76-374	Java <i>E. arundinaceus</i>			10			
IJ76-381	Java <i>E. arundinaceus</i>			11			
IJ76-387	Java <i>E. arundinaceus</i>			12			
IJ76-388	Java <i>E. arundinaceus</i>			13			
IJ76-389	Java <i>E. arundinaceus</i>			14			
IJ76-394	Java <i>E. arundinaceus</i>				2		
IJ76-397	Java <i>E. arundinaceus</i>			15			
IJ76-398	Java <i>E. arundinaceus</i>			16			
IJ76-400	Java <i>E. arundinaceus</i>			17			
IJ76-404	Java <i>E. arundinaceus</i>			18			
IJ76-406	Java <i>E. arundinaceus</i>			19			
IJ76-407	Java <i>E. arundinaceus</i>			20			
IJ76-408	Java <i>E. arundinaceus</i>			21			
IJ76-417	Java <i>S. robustum</i>			22			
IJ76-502	Java <i>E. arundinaceus</i>			23			
IK76-22	Kalamintan <i>E. arundinaceus</i>			26			
IK76-41	Kalamintan <i>E. arundinaceus</i>			27			
IK76-48	Kalamintan <i>E. arundinaceus</i>			28			
IK76-49	Kalamintan <i>S. spontaneum</i>			29			
IK76-63	Kalamintan <i>E. arundinaceus</i>		16	30			
IK76-67	Kalamintan <i>S. spontaneum</i>			31			
IK76-72	Kalamintan <i>S. spontaneum</i>			32			
IK76-79	Kalamintan <i>E. arundinaceus</i>			33			
IK76-88	Kalamintan <i>E. arundinaceus</i>				3		
IK76-95	Kalamintan <i>S. officinarum</i>					9	
IK76-101	Kalamintan <i>E. arundinaceus</i>			24			
IK76-103	Kalamintan <i>E. arundinaceus</i>			25			
IM76-229	Madura <i>S. robustum</i>			34			
IS76-126	Sumatra <i>E. arundinaceus</i>			35			
IS76-132	Sumatra <i>S. spontaneum</i>			36			
IS76-141	Sumatra <i>S. spontaneum</i>				4		

Clone	Origin/type	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
IS76-148	Sumatra <i>S. spontaneum</i>				5		
IS76-150	Sumatra <i>E. arundinaceus</i>		17	37			
IS76-158	Sumatra <i>E. arundinaceus</i>			38			
IS76-163	Sumatra <i>E. arundinaceus</i>			39			
IS76-172	Sumatra <i>E. arundinaceus</i>			40			
IS76-173	Sumatra <i>S. spontaneum</i>				6		
IS76-176	Sumatra <i>E. arundinaceus</i>			41			
IS76-180	Sumatra <i>S. spontaneum</i>				7		
IS76-186	Sumatra <i>S. spontaneum</i>			42	8		
IS76-191	Sumatra <i>E. arundinaceus</i>			43			
IS76-193	Sumatra <i>E. arundinaceus</i>			44			
IS76-202	Sumatra <i>E. arundinaceus</i>			45			
IS76-205	Sumatra <i>E. arundinaceus</i>			46			
IS76-226	Sumatra <i>S. spontaneum</i>				9		
Mandalay	<i>S. spontaneum</i>			47			
MQ85-201	Australia hybrid		18				
NCo310	South Africa commercial hybrid	7	19	48	10	10	3
NG28-101	New Guinea <i>S. spontaneum</i>			49			
NG28-288	New Guinea <i>S. officinarum</i>					11	
NG51-2	New Guinea <i>S. spontaneum</i>			50			
NG51-153	New Guinea <i>S. officinarum</i>				11		
NG57-56	New Guinea <i>S. robustum</i>					12	
NG57-142	New Guinea <i>S. officinarum</i>				12		
NG57-151	New Guinea <i>S. officinarum</i>			51			
NG57-208	New Guinea <i>S. robustum</i>				13		
Orpheus	Australia commercial hybrid	8					
Otamite	<i>S. officinarum</i>				14		
Pasoeroean	<i>S. spontaneum</i>			52			
Pelorus	Australia commercial hybrid	9	20	53	15	13	4
POJ2878	Java commercial hybrid	10					
Q20	Australia commercial hybrid	11					
Q28	Australia commercial hybrid	12					
Q68	Australia commercial hybrid	13					
Q77	Australia commercial hybrid	14					
Q83	Australia commercial hybrid	15					
Q87	Australia commercial hybrid	16					
Q91	Australia commercial hybrid	17					
Q96	Australia commercial hybrid		21				
Q98	Australia commercial hybrid	18	22	54		14	5
Q99	Australia commercial hybrid	19	23	55	16	15	6
Q103	Australia commercial hybrid	20					
Q110	Australia commercial hybrid	21					
Q113	Australia commercial hybrid	22					
Q114	Australia commercial hybrid	23					
Q115	Australia commercial hybrid	24					
Q116	Australia commercial hybrid	25					
Q117	Australia commercial hybrid	26	24	56	17	16	7
Q118	Australia commercial hybrid	27	25	57			
Q120	Australia commercial hybrid	28					
Q121	Australia commercial hybrid	29					
Q122	Australia commercial hybrid	30					
Q123	Australia commercial hybrid	31	26	58	18	17	8
Q124	Australia commercial hybrid	32	27	59	19		9
Q125	Australia commercial hybrid	33	28	60	20	18	10
Q126	Australia commercial hybrid	34					
Q127	Australia commercial hybrid	35	29		21		11
Q128	Australia commercial hybrid	36	30	61	22	19	12
Q130	Australia commercial hybrid	37					
Q131	Australia commercial hybrid	38					
Q133	Australia commercial hybrid	39				20	13
Q135	Australia commercial hybrid	40					
Q136	Australia commercial hybrid	41	31	62	23	21	14
Q137	Australia commercial hybrid	42	32	63	24	22	15
Q138	Australia commercial hybrid	43				23	16
Q139	Australia commercial hybrid	44					
Q140	Australia commercial hybrid	45					
Q141	Australia commercial hybrid	46	33	64	25	24	17
Q142	Australia commercial hybrid	47					
Q143	Australia commercial hybrid	48					
Q144	Australia commercial hybrid	49	34	65	26	25	18
Q145	Australia commercial hybrid	50	35	66	27	26	19
Q146	Australia commercial hybrid	51					
Q147	Australia commercial hybrid	52	36	67	28	27	20
Q149	Australia commercial hybrid	53			29		
Q150	Australia commercial hybrid	54	37	68	30	28	21

Clone	Origin/type	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
Q151	Australia commercial hybrid				31		
Q152	Australia commercial hybrid	55	38	69	32	29	22
Q153	Australia commercial hybrid	56					
Q154	Australia commercial hybrid	57	39	70	33	30	23
Q155	Australia commercial hybrid	58				31	
Q156	Australia commercial hybrid				34		
Q157	Australia commercial hybrid				35		
Q158	Australia commercial hybrid	59			36	32	24
Q159	Australia commercial hybrid	60					
Q160	Australia commercial hybrid				37		
Q161	Australia commercial hybrid				38		
Q162	Australia commercial hybrid				39		
Q163(d)	Australia commercial hybrid		40				
Q164	Australia commercial hybrid				40		
Q165(d)	Australia commercial hybrid				41	33	25
Q166(d)	Australia commercial hybrid				42		
Q167(d)	Australia commercial hybrid				43		
Q168(d)	Australia commercial hybrid					34	
Q170(d)	Australia commercial hybrid		87				
Q171(d)	Australia commercial hybrid		91			35	26
Q173(d)	Australia commercial hybrid				50		
Q174(d)	Australia commercial hybrid				44	36	
Q175(d)	Australia commercial hybrid				67		
Q176(d)	Australia commercial hybrid				98	37	27
Q177(d)	Australia commercial hybrid				100	81	28
Q178(d)	Australia commercial hybrid						29
Q179(d)	Australia commercial hybrid						30
Q180(d)	Australia commercial hybrid				101	38	31
Q181(d)	Australia commercial hybrid				84		32
Q182(d)	Australia commercial hybrid		68				
Q183(d)	Australia commercial hybrid					39	33
Q184(d)	Australia commercial hybrid				69		
Q185(d)	Australia commercial hybrid						52
Q186(d)	Australia commercial hybrid						34
Q187(d)	Australia commercial hybrid						35
Q188(d)	Australia commercial hybrid		56				
Q193(d)	Australia commercial hybrid				73		
Q813	Australia commercial <i>S. officinarum</i>	61					
RB76-5418	Brazil hybrid						36
RB80-5246	Brazil hybrid		41				
R80/882	Reunion hybrid		42				
SES100A	<i>S. spontaneum</i>				45		
SES151A	<i>S. spontaneum</i>			71			
SES181	<i>E. arundinaceus</i>			72			
SES300	<i>E. arundinaceus</i>			73			
SES341	<i>S. spontaneum</i>			74			
SES343	<i>S. spontaneum</i>			75			
SES402	<i>S. spontaneum</i>			76			
SES516	<i>S. spontaneum</i>				46		
Tabongo	<i>S. spontaneum</i>				47		
Triton	Australia commercial hybrid	62					
Trojan	Australia commercial hybrid					40	
TUC74-20	Argentina hybrid		43				
UM68-11	<i>S. officinarum</i>				48		
US56-8-2	<i>S. spontaneum</i>			77			
VMC69-490	Philippines hybrid		44				
77N1015	Australia hybrid		45				
77S1321	Australia hybrid				49		
78N193	Australia hybrid					41	
79N238	Australia hybrid				51		
79N439	Australia hybrid				52		
79N568	Australia hybrid					42	
79N1170	Australia hybrid					43	
79N1484	Australia hybrid					44	
80A188	Australia hybrid		46				
80A278	Australia hybrid		47				
80A346	Australia hybrid		48				
80C529	Australia hybrid					45	
80N177	Australia hybrid		49				
80N706	Australia hybrid					46	
80N739	Australia hybrid				53		
80N800	Australia hybrid					47	
80N927	Australia hybrid					48	
80N965	Australia hybrid		50				
80N1080	Australia hybrid					49	

Clone	Origin/type	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
81A208	Australia hybrid		51				
81A465	Australia hybrid		52				
81N77	Australia hybrid					50	
81N82	Australia hybrid					51	37
81N301	Australia hybrid					52	
81N314	Australia hybrid		53				
81N371	Australia hybrid					53	
81S483	Australia hybrid		54				
82N257	Australia hybrid		55				
82N1102	Australia hybrid					54	
83A135	Australia hybrid				54	55	38
83C328	Australia hybrid		57				
83C495	Australia hybrid		58				
83C521	Australia hybrid		59				
83C538	Australia hybrid		60				
83C624	Australia hybrid		61				
83C627	Australia hybrid					56	
83C1030	Australia hybrid		62				
83C1032	Australia hybrid		63				
83N730	Australia hybrid					57	
83N847	Australia hybrid					58	
83N943	Australia hybrid				55		
83N1021	Australia hybrid		64				
83N1038	Australia hybrid					59	
83N1170	Australia hybrid					60	
83N1203	Australia hybrid		65				
83N1493	Australia hybrid		66				
83S1236	Australia hybrid		67				
83S2070	Australia hybrid		69				
83S2103	Australia hybrid		70				
84C18	Australia hybrid		71				
84C43	Australia hybrid		72				
84C44	Australia hybrid		73				
84C458	Australia hybrid		74				
84C621	Australia hybrid		75				
84C627	Australia hybrid		76				
84N2172	Australia hybrid		77				
84N2241	Australia hybrid		78				
84N2479	Australia hybrid		79				
84N2531	Australia hybrid		80				
84N3882	Australia hybrid		81				
84N4308	Australia hybrid		82				
84N4538	Australia hybrid		83				
84S35	Australia hybrid				59		
84S303	Australia hybrid				58		
84S818	Australia hybrid		84				
84S1225	Australia hybrid		85				
84S1263	Australia hybrid				56		
84S2504	Australia hybrid				57		
84S2651	Australia hybrid		86				
85A2234	Australia hybrid					61	39
85A2540	Australia hybrid				60	62	40
85A2734	Australia hybrid				61		
85C864	Australia hybrid				62		
85N1056	Australia hybrid				63		
85N1205	Australia hybrid				64		
85N1538	Australia hybrid				65		
85N1802	Australia hybrid				66		
85S826	Australia hybrid				81		
85S1064	Australia hybrid				68		
85S1247	Australia hybrid				70		
85S1509	Australia hybrid				71		
85S1716	Australia hybrid				72		
85S2236	Australia hybrid				74		
85S2275	Australia hybrid				75		
85S7035	Australia hybrid				76		
85S7102	Australia hybrid				77		
85S7146	Australia hybrid					63	
85S7151	Australia hybrid				78		
85S7183	Australia hybrid					64	
85S7186	Australia hybrid					65	
85S7325	Australia hybrid		88				
85S7327	Australia hybrid		89				
85S7329	Australia hybrid				79		
85S7335	Australia hybrid		90				

Clone	Origin/type	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
85S7341	Australia hybrid				80		
86A182	Australia hybrid					66	41
86A700	Australia hybrid					67	42
86C406	Australia hybrid				82		
86C451	Australia hybrid						43
86C501	Australia hybrid				83		
86N2168	Australia hybrid				85		
87A1413	Australia hybrid					68	44
87S7018	Australia hybrid				86		
87S7095	Australia hybrid				87		
87S7111	Australia hybrid				88		
87S7188	Australia hybrid				89		
87S7199	Australia hybrid				90		
87S7212	Australia hybrid					69	
87S7220	Australia hybrid					70	
87S7221	Australia hybrid				91		
87S7223	Australia hybrid					71	
87S7245	Australia hybrid				92		
87S7268	Australia hybrid				93		
87S7345	Australia hybrid				94		
87S7346	Australia hybrid		92				
87S7364	Australia hybrid		93				
87S7367	Australia hybrid				95		
87S7368	Australia hybrid					72	
87S7453	Australia hybrid					73	
87S7604	Australia hybrid					74	
87S7663	Australia hybrid					75	
87S8040	Australia hybrid					76	
87S8042	Australia hybrid					77	
87S8090	Australia hybrid					78	
87S8210	Australia hybrid					79	
88A1157	Australia hybrid				96		
88A1515	Australia hybrid				97	80	45
88A2095	Australia hybrid				99		
88B205	Australia hybrid		94				
88B206	Australia hybrid	63					
88B207	Australia hybrid	64	95	78			
88B209	Australia hybrid	65	96	79	102	82	46
88B216	Australia hybrid	66	97	80	103	83	
88B222	Australia hybrid	67	98	81	104	84	
88B230	Australia hybrid	68	99	82			
88B241	Australia hybrid	69					
88B244	Australia hybrid	70					
88B262	Australia hybrid	71					
88B279	Australia hybrid		100				
88B284	Australia hybrid		101				
88B295	Australia hybrid		102				
88C6002	Australia hybrid					85	
88C6015	Australia hybrid					86	
88C6017	Australia hybrid					87	
88S6003	Australia hybrid					88	
88S9095	Australia hybrid						47
88S9322	Australia hybrid						48
88S9323	Australia hybrid						49
89A3434	Australia hybrid					89	50
89A3567	Australia hybrid					90	51
89B24	Australia hybrid		103				
89B29	Australia hybrid		104				
89C5003	Australia hybrid		105				
89C6015	Australia hybrid					91	
89S7865	Australia hybrid					92	
89S8099	Australia hybrid					93	
89S8191	Australia hybrid					94	
90C6001	Australia hybrid					95	
90C6006	Australia hybrid					96	
90S7057	Australia hybrid						53
90S7063	Australia hybrid						54
90S7146	Australia hybrid					97	
90S7187	Australia hybrid						55
91S6012	Australia hybrid						56
91S7008	Australia hybrid					98	
91S7135	Australia hybrid						57
91S7172	Australia hybrid						58
91S7175	Australia hybrid						59
91S7179	Australia hybrid						60

Clone	Origin/type	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
91S7234	Australia hybrid						61
91S7266	Australia hybrid						62
91S7283	Australia hybrid						63
91S7347	Australia hybrid						64
91S7354	Australia hybrid						65
91S7376	Australia hybrid						66
92S50	Australia hybrid						67
92S54	Australia hybrid						68
92S206	Australia hybrid						69
92S234	Australia hybrid						70
92S250	Australia hybrid						71
92S251	Australia hybrid						72
92S260	Australia hybrid						73
92S286	Australia hybrid						74
92S469	Australia hybrid						75
92S566	Australia hybrid						76
92S605	Australia hybrid						77
92S622	Australia hybrid						78
92S636	Australia hybrid						79
92S639	Australia hybrid						80
92S694	Australia hybrid						81
92S767	Australia hybrid						82
92S803	Australia hybrid						83
92S853	Australia hybrid						84
92S929	Australia hybrid						85
92S972	Australia hybrid						86
92S981	Australia hybrid						87
92S2075	Australia hybrid						88
92S2343	Australia hybrid						89
93N6006	Australia hybrid					99	
93W6001	Australia hybrid					100	
93W6003	Australia hybrid					101	
93W6004	Australia hybrid					102	
93W6005	Australia hybrid					103	
93W6008	Australia hybrid					104	
93W6009	Australia hybrid					105	
94S5000	Australia hybrid						90

If RP_i (x axis) is plotted against RY_i (y axis), then the clones falling on the left side are most tolerant, those on the right side are most susceptible, those at the top are highest yielding in the presence of larvae, and those at the bottom are lowest yielding in the presence of larvae (Odulaja and Nokoe 1993). A desirable clone is one that provides high yield and low percentage yield loss; hence, clones in the upper left quadrant are the selection target.

Antibiosis is a second component of resistance where a resistant plant has an adverse effect on the pest's development and/or survival. We tested for this by analysing the number of surviving larvae and the mean weight of the surviving larvae within each trial using ANOVA and LSD. Numbers of surviving larvae were first transformed with the arcsin transformation. Care in interpreting these data is needed to separate antibiosis effects from starvation due to lack of root growth.

The repeatability of the effects of our screening was determined using the degree of concordance in rankings of yields of infested and uninfested tops, roots and stubble, their relative percentage yield losses, relative yields, number of surviving grubs and mean weight of surviving grubs. This was tested on the 17 clones in common to all six trials using Kendall's coefficient of concordance, where the value of the coefficient can vary between 1 (perfect concordance) and 0 (no concordance).

We considered the correlation of resistance effects with Spearman's rank correlation.

The degree of genetic determination on a repeated plot basis (g^2) (heritability in the broad sense; Skinner *et al.* 1987) is defined as the proportion of genotypic variance (σ^2_G) relative to the phenotypic variance (σ^2_P) (Skinner *et al.* 1987; Berding 1996) and it is important in understanding the selection process. We calculated it for the relative percentage yield loss for tops, roots and stubble and for grub survival and grub weights for each of the trials as

$$g^2 = \sigma^2_G / \sigma^2_P,$$

$$\text{where } \sigma^2_G = (MS_{\text{clone}} - MS_{\text{error}})/r$$

$$\text{and } \sigma^2_P = \sigma^2_G + (MS_{\text{error}}/r)$$

where MS is the respective mean squares taken from the ANOVA and r is the number of replicates.

RESULTS

Plant growth

In each trial there was a highly significant effect of infestation on the yield of tops and roots (Table 2) with significantly lower mean yields in infested plots. This is consistent with field experience of the effect of canegrub infestation and indicates that the pot-based technique is capable of detecting an effect of infestation. The effect of infestation on stubble yields is less clear. In four of the six trials there was a significant effect of infestation on stubble yields, with lower mean yields in infested pots. There was no such significant effect in trials 4 and 6 (Table 2). These differences may reflect the spectrum of clones in each trial, but they also indicate that there is potentially greater tolerance to grub feeding in growth of stubble than in growth of tops or roots.

Table 2. F value, degrees of freedom and associated probability of significance for effect of infestation on yields of tops, roots and stubble in each of the six pot trials.

Trial	Denominator degrees of freedom	F value, probability		
		Tops	Roots	Stubble
1	139	659.5, <0.0001	650.0, <0.0001	33.6, <0.0001
2	312	278.2, <0.0001	790.1, <0.0001	36.6, <0.0001
3	229	313.3, <0.001	413.9, <0.0001	155.8, <0.0001
4	307	416.7, <0.0001	1217.5, <0.0001	0.61, 0.44
5	309	371.4, <0.0001	1237.4, <0.0001	20.18, <0.0001
6	262	22.5, <0.0001	1143.0, <0.0001	0.03, 0.86

In each trial there was a significant difference among clones in the yield of tops, roots and stubble of infested plants (Table 3). This variation is consistent with field observations that some clones 'do better under grub infestation' and indicates that there is good potential for selection for tolerance.

Table 3. F value, degrees of freedom and associated probability of significance for variation of tops, roots and stubble weights of infested plants in each of the six pot trials.

Trial	Degrees of freedom	F value, probability		
		Tops	Roots	Stubble
1	70, 140	4.1, <0.0001	2.0, <0.0001	2.5, 0.0001
2	104, 312	1.3, 0.046	2.9, <0.0001	2.2, <0.0001
3	81, 220	5.2, <0.0001	3.8, <0.0001	5.3, <0.0001
4	103, 306	2.9, <0.0001	2.2, <0.0001	2.8, <0.0001
5	104, 310	1.7, <0.0001	2.5, <0.0001	1.7, <0.0001
6	89, 264	2.8, <0.0001	3.8, <0.0001	1.3, <0.0001

There were significant agreements in rankings between the six trials of the yield of infested and uninfested tops, roots and stubble of the 17 common clones (Table 4). This indicates that yield data are reasonably reproducible between trials.

In each trial there was considerable variation among clones in their relative yield when infested and relative percentage yield loss (Figure 1). Generally, there are many clones showing poor tolerance to loss of tops and loss of roots (those in the bottom right of each graph). However, the interesting clones are those towards the top left of each graph. Although there are not many, clones such as IS76-150 (17) in trial 2, Q137 (63) in trial 3, Q161 (38) in trial 4 and Q99 (6) in trial 6 that show considerable tolerance to root and top loss. The plots for stubble generally show a different pattern with a much wider spread of tolerances.

Table 4. Agreement in rankings (measured by Kendall's coefficient of concordance) of growth, resistance and grub parameters between the six pot trials.

Parameter	Kendall's coefficient of concordance	Probability of significance
Dry weight of uninfested roots	0.42	0.0007
Dry weight of infested roots	0.40	0.0015
Dry weight of uninfested tops	0.51	<0.0001
Dry weight of infested tops	0.36	0.0048
Dry weight of uninfested stubble	0.38	0.0021
Dry weight of infested stubble	0.38	0.0023
Relative percentage yield loss - roots	0.30	0.024
Relative yield – roots	0.41	0.0009
Relative percentage yield loss - tops	0.22	0.16
Relative yield - tops	0.36	0.0048
Relative percentage yield loss - stubble	0.22	0.17
Relative yield - stubble	0.38	0.0023
Number of surviving grubs	0.12	0.76
Mean weight of surviving grubs	0.40	0.0015

In tolerance to root loss, the potential size of the root mass (given by the dry weight of roots in uninfested plants) is a good predictor of relative yield of infested plants (Table 5; Figure 2) and is also usually significantly correlated with relative percentage yield loss (Table 5). This indicates that clones with a large root mass will still have large root masses and lose a smaller proportion of their roots following feeding. These are consistent with the type of feeding damage inflicted by canegrubs - roots are pruned, but individual larvae will consume similar amounts on each clone. Again, the outliers in this relationship are the ones of most interest.

Table 5. Correlation (r and probability of significance) of dry weight of uninfested roots with relative root yield of infested plants (RY) and relative percentage root yield loss of infested plants (RP) in each of the six pot trials.

Trial	n	RY	RP
1	71	0.50, 0.0001	-0.27, 0.022
2	105	0.72, <0.0001	-0.29, <0.0001
3	82	0.80, <0.0001	-0.31, 0.0042
4	104	0.66, <0.0001	-0.26, 0.0075
5	105	0.58, <0.0001	-0.19, 0.049
6	90	0.67, <0.0001	-0.18, 0.086

In tolerance to top loss and stubble loss, the potential size of the top or stubble mass (given by the dry weight of tops or stubble in uninfested plants) is also a good predictor of relative yield of infested plants; it is unreliable as a predictor of relative percentage yield loss (Table 6). This reflects the longer physiological pathways between root damage and yield of tops or stubble.

Table 6. Correlation (r and probability of significance) of dry weight of uninfested tops or stubble with relative top or stubble yield of infested plants (RY) and relative percentage top or stubble yield loss of infested plants (RP) in each of the six pot trials.

Trial	n	Tops		Stubble	
		RY	RP	RY	RP
1	71	0.69, <0.0001	-0.043, 0.72	0.58, <0.0001	0.27, 0.022
2	105	0.68, <0.0001	0.23, 0.021	0.72, <0.0001	0.086, 0.38
3	82	0.90, <0.0001	-0.37, 0.0007	0.85, <0.0001	-0.12, 0.29
4	104	0.57, <0.0001	0.11, 0.25	0.72, <0.0001	0.19, 0.057
5	105	0.65, <0.0001	0.27, 0.0062	0.55, <0.0001	0.24, 0.014
6	90	0.52, <0.0001	0.56, <0.0001	0.48, <0.0001	0.70, <0.0001

Canegrub survival and weight gain

In three of the six trials there were significant differences among clones in the survival of canegrubs (Table 7; Figure 3). Again, what is important are the outliers; Q145 (50) in trial 1, IJ76-334 (4), IJ76-404 (18) and IS76-191 (43) in trial 3, and IS76-226 (9) and NG57-208 (13) in trial 4 are good examples of clones where larval survival is poor (Figure 3).

Table 7. F value, degrees of freedom and associated probability of significance for variation in grub survival and weight of individual surviving grubs in each of the six pot trials.

Trial	Degrees of freedom	F value, probability	
		Grub survival	Grub weight
1	70, 139	1.70, 0.0042	2.26, <0.0001
2	104, 312	1.16, 0.17	1.28, 0.053
3	81, 220	1.57, 0.0054	Too many 0 survivals to allow analysis
4	103, 306	2.50, <0.0001	1.43, 0.011
5	104, 310	0.89, 0.76	1.64, 0.0006
6	89, 264	0.87, 0.79	1.68, 0.0009

In four of the six trials, there were significant differences among clones in the weights of surviving larvae; data in trial 3 could not be analysed because there were too many pots in which no grubs survived and trial 2 just failed to make significance at the 5% level (Table 7; Figure 3). Again, there are clones in each of the trials that show significantly low grub weights, such as Q20 (11), Q137 (42) and POJ2878 (10) in trial 1, 84N2172 (77) and 89C5003 (105) in trial 2, NCo310 (10) in trial 4, 93S2343 (99) in trial 5, and CP74-2005 (1) in trial 6 (Figure 3).

One hypothesis is that, where grub survival is high, then the weight of those surviving grubs will be low - a crowding/competition effect. Our data show no consistent correlation between the number of surviving grubs and their mean weights, with two trials having no significant correlation, two having low but significant positive correlation and two having low but significant negative correlation (Table 8; Figure 3).

Table 8. Correlation between the mean number of surviving grubs and the mean weight of individual surviving grubs in each of the six pot trials.

Trial	n	Correlation	Probability of significance
1	71	-0.399	0.00057
2	105	0.022	0.82
3	82	0.322	0.0033
4	104	0.260	0.0080
5	105	-0.112	0.26
6	90	-0.216	0.041

A second hypothesis is that grub survival and/or grub weights are correlated with the potential mass of food (roots) produced by the different clones (measured in our experiments by the weight of roots in uninfested pots). In four trials there was no significant correlation of grub survival with weight of uninfested roots and in two trials there was a significant, moderate positive correlation (Table 9, Figure 4). Grub weights, however, were significantly and positively correlated with weights of uninfested roots in five of the six trials (Table 9, Figure 4), indicating that grubs will tend to be heavier when feeding on clones with a large potential food supply. However, the variation explained by these correlations is low, accounting for less than 19% of the variation in larval weights. Again the outliers to these general relationships are of most interest, although there are no clones with large amounts of roots and very poor survival or very low weights in any of the trials (Figure 4).

Table 9. Correlation between the mean dry weight of uninfested plants and each of the mean number of surviving grubs and the mean weight of individual surviving grubs each of the six pot trials.

Trial	n	Grub survival		Grub weight	
		Correlation	Probability	Correlation	Probability
1	71	-0.019	0.88	0.326	0.0057
2	105	0.008	0.94	0.332	0.0006
3	82	0.479	<0.0001	0.432	0.0001
4	104	0.362	<0.0001	0.432	<0.0001
5	105	-0.070	0.48	0.193	0.049
6	90	0.018	0.87	0.056	0.60

There was significant agreement in rankings between the six trials of the weight of surviving grubs, but not of the number of surviving grubs under the 17 common clones (Table 4). This indicates that weight data are reasonably reproducible between trials, but survival data are not. Larvae used in each of the trials were collected from the field and could have been infected with pathogens that might have affected survival.

Degree of genetic determination

Table 10 shows that the degree of genetic determination (broad-sense heritability) for the crop-yield parameters is reasonably high, although variable between the different trials and their component clones. The degree of genetic determination for the effect on grub

weights is also significant, and is much more variable (and in two trials zero) for the number of surviving grubs. The latter probably reflects that other factors were influencing survival in at least trials 5 and 6.

Table 10. Degree of genetic determination on a repeated plot basis for each parameter in each of the six trials.

Parameter	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
Dry weight of uninfested roots	0.61	0.60	0.71	0.60	0.44	0.73
Dry weight of infested roots	0.49	0.65	0.74	0.55	0.60	0.74
Dry weight of uninfested tops	0.84	0.38	0.83	0.65	0.54	0.60
Dry weight of infested tops	0.76	0.23	0.81	0.66	0.41	0.64
Dry weight of uninfested stubble	0.62	0.57	0.85	0.69	0.32	0.50
Dry weight of infested stubble	0.59	0.54	0.81	0.64	0.43	0.24
Number of surviving grubs	0.41	0.14	0.36	0.60	0	0
Mean weight of surviving grubs	0.56	0.22	*	0.30	0.39	0.40

*ANOVA could not be completed.

CONCLUSIONS

Our pot trials indicate that there is significant variability among clones of sugarcane and their relatives in some of the components that would contribute to a 'grub-resistant' cultivar.

We have demonstrated variation in tolerance through:

- differences among clones in root loss and in flow-on tolerance to reduced top and stubble loss. These effects are partially correlated with general vigour of the clone, with the more vigorous clones retaining more roots and tops when attacked by grubs;
- presence of some clones that show significant departures from the general correlations - these clones will be of especial interest in future breeding;
- reasonable repeatability between pot trials (including use of different grub species) in the determination of effects on plant growth;
- reasonable degree of genetic determination of growth parameters in uninfested and infested plants.

We have also demonstrated variation in antibiosis through:

- differences among clones in the survival of grubs and the weight of surviving grubs, with some clones showing significant departures from overall trends;
- some correlation between the potential root growth of clones and the weight of surviving grubs;
- no consistent correlation between potential root growth of clones and the number of surviving grubs;
- no consistent correlation between grub survival and weight of surviving grubs;
- reasonable repeatability across pot trials of the effects on final weights of larvae, but not of effects on grub survival - this indicates that extrinsic factors may be influencing grub survival.

Figure 1. Relationship between the relative yield of each clone when infested compared to that of the best yielding clone when infested and the relative percentage yield loss of each clone compared to that of the most susceptible clone for each clone in each trial. Clones are identified by numbers given in Table 1. Clones falling on the left side are most tolerant, those on the right side are most susceptible, those at the top are highest yielding in the presence of larvae, and those at the bottom are lowest yielding in the presence of larvae.

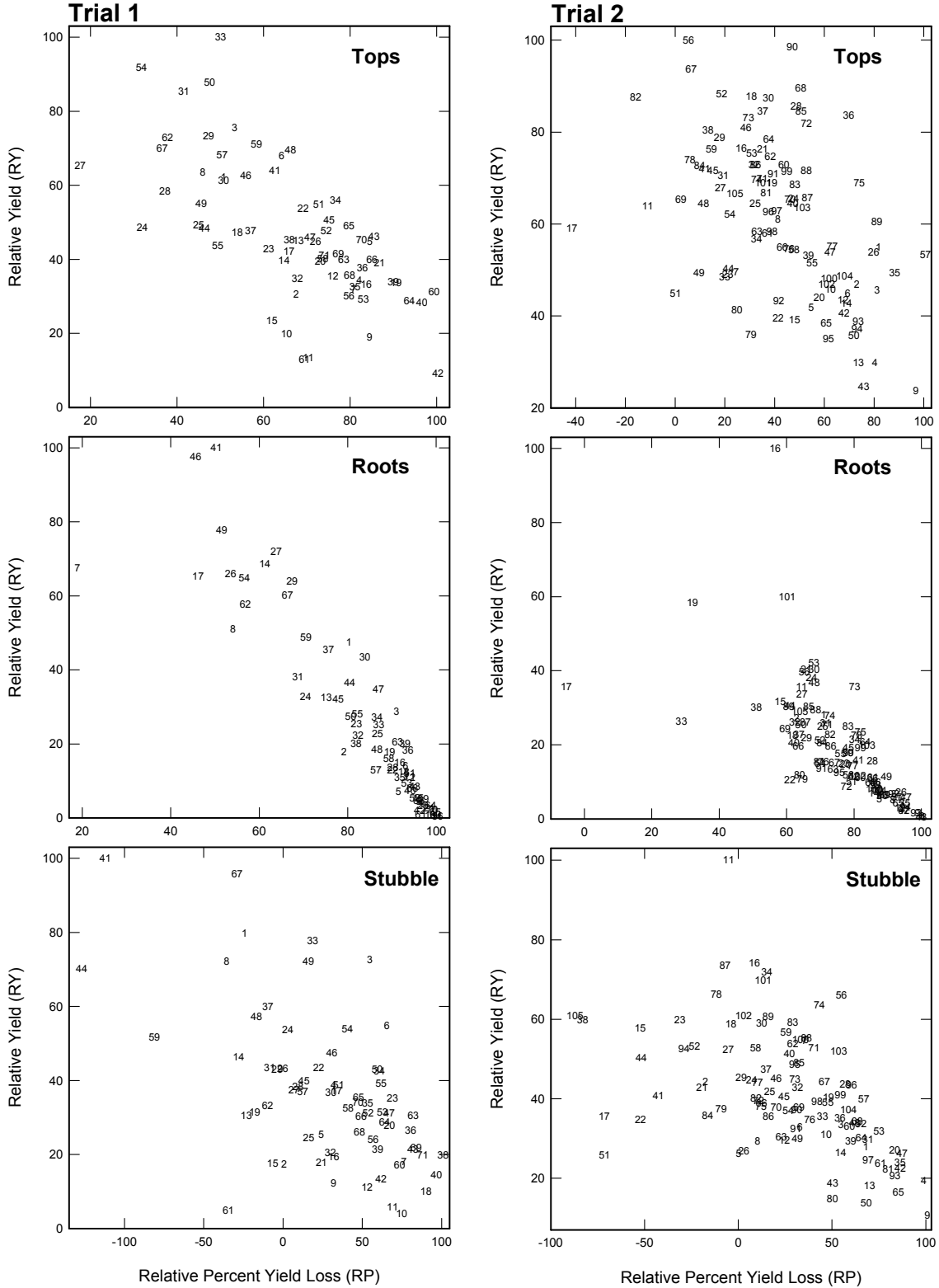


Figure 1 (cont.)

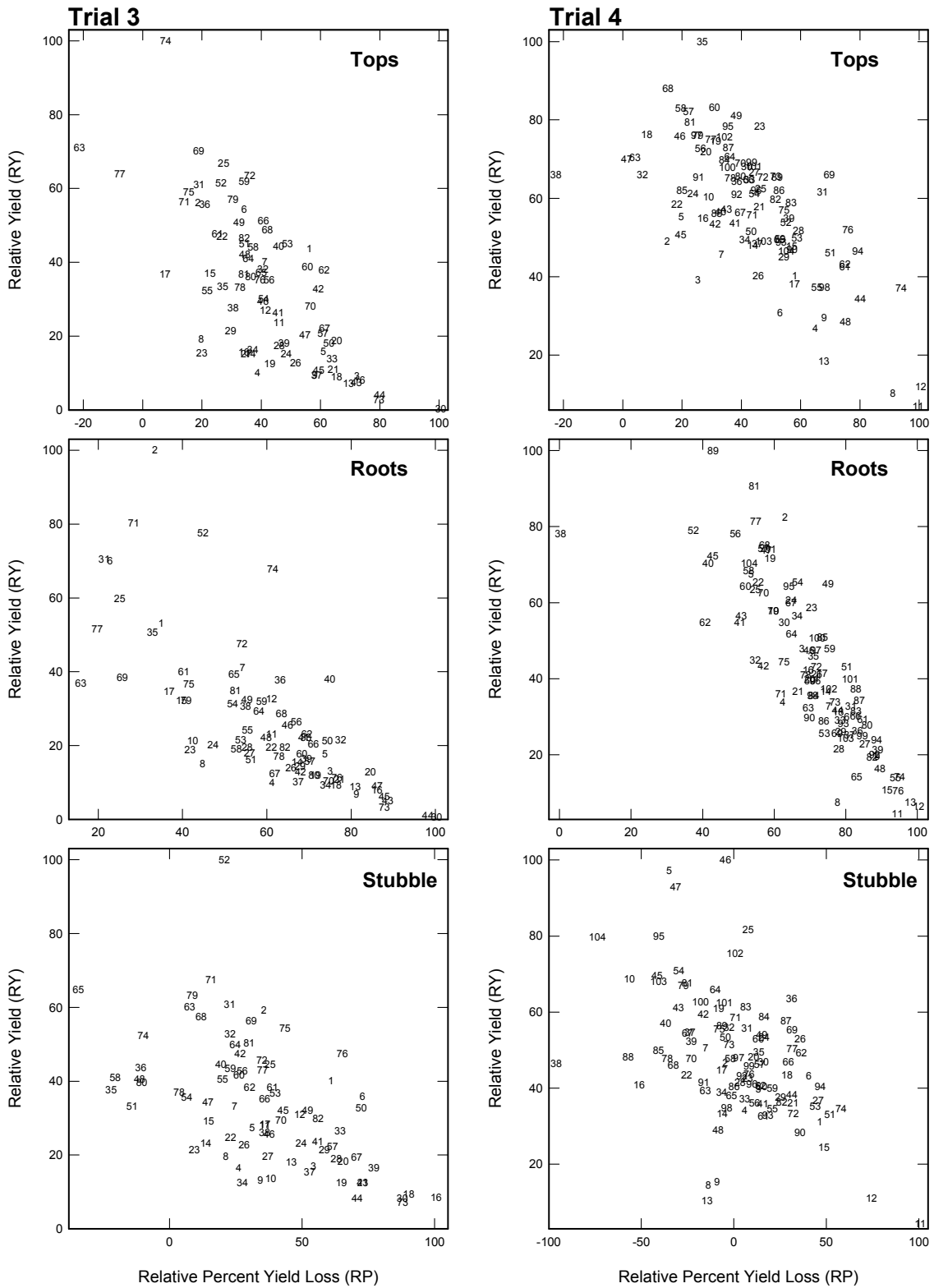


Figure 1 (cont.)

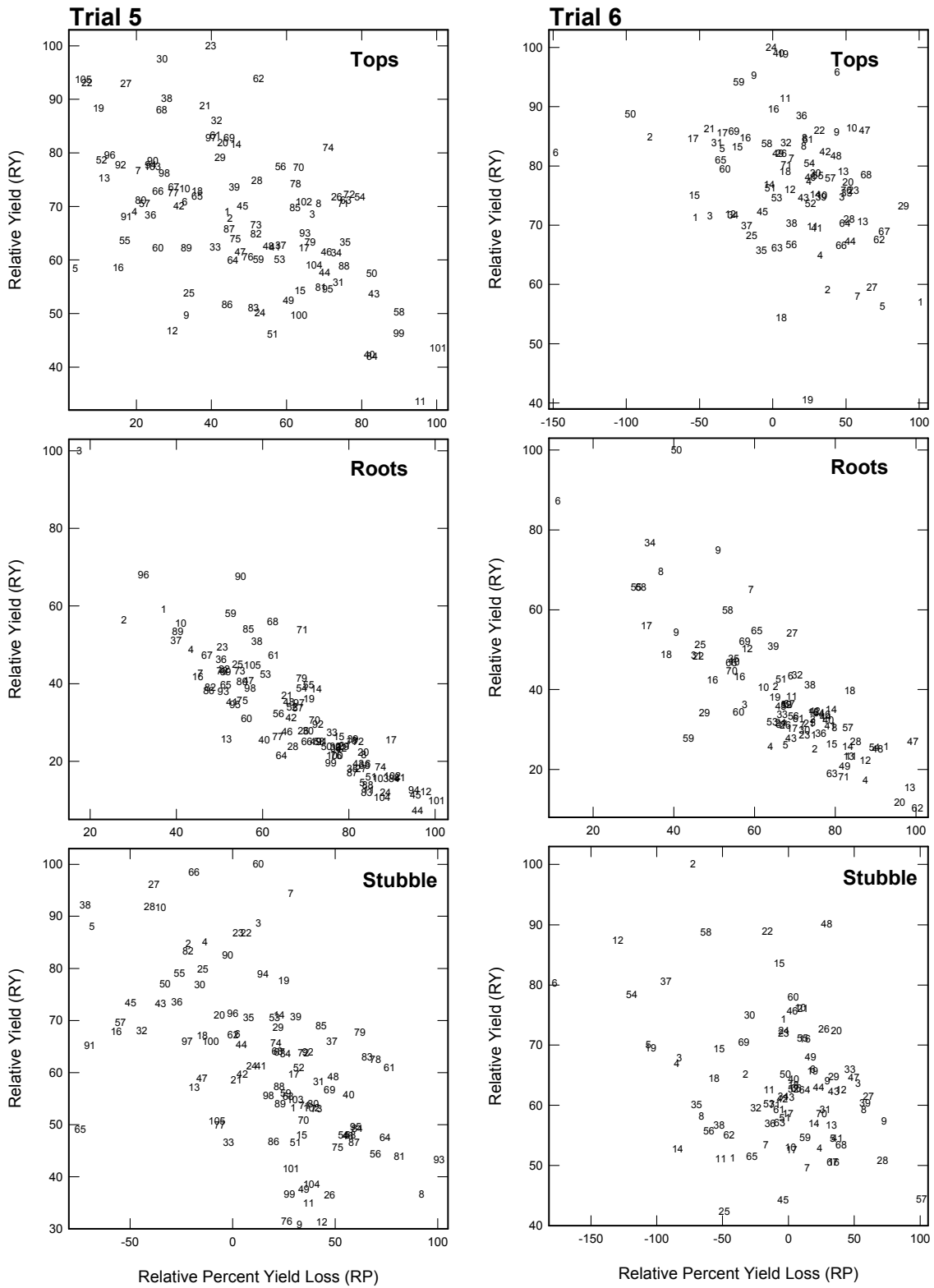


Figure 2. Relationship between the relative yield of roots of each clone when infested compared to that of the best yielding clone when infested and the yield of roots when uninfested for each clone in each trial. Clones are identified by numbers given in Table 1.

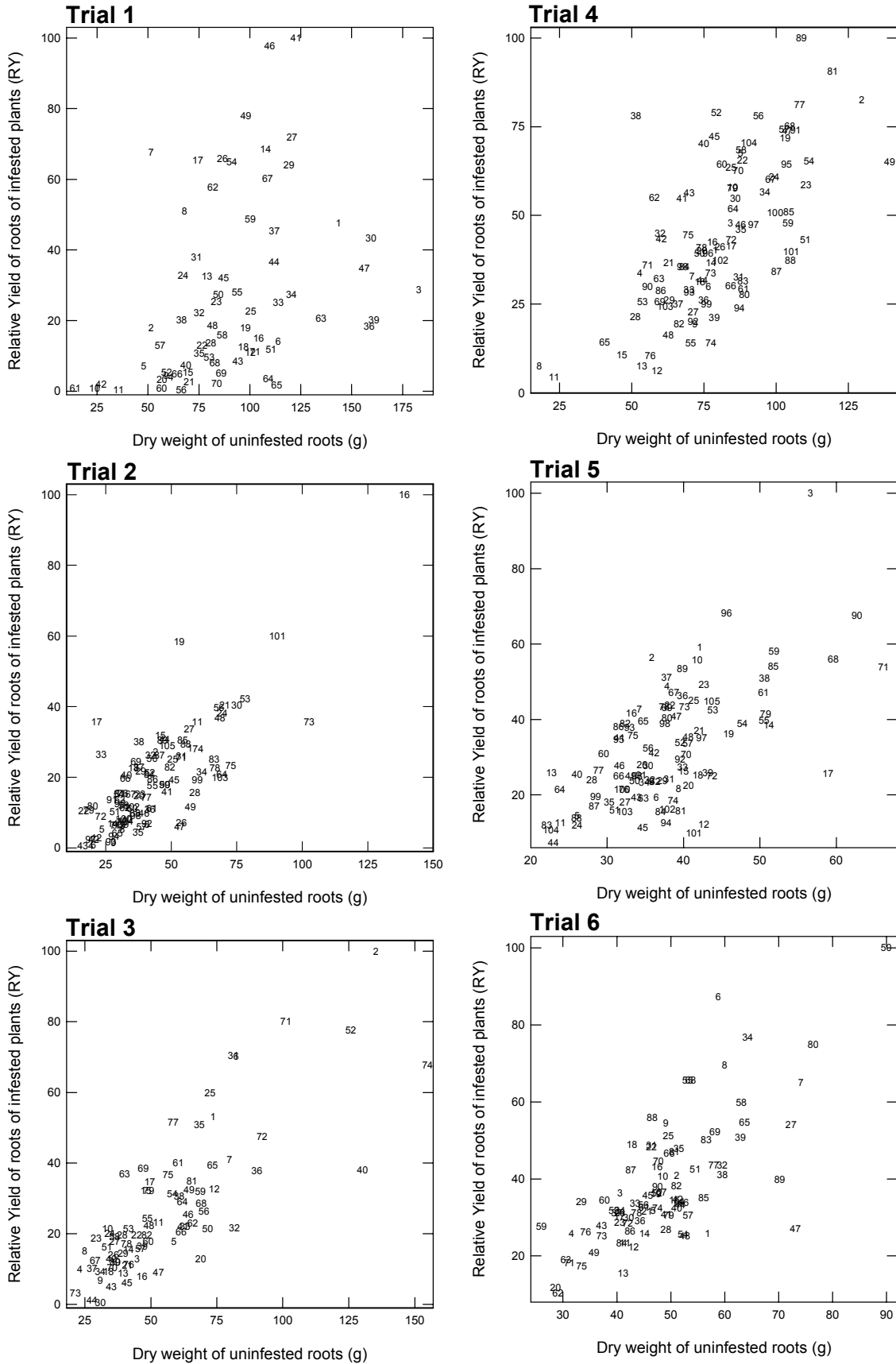


Figure 3. Relationship between the mean number of surviving larvae and the mean weight of surviving larvae for each clone in each pot trial. Clones are identified by numbers given in Table 1.

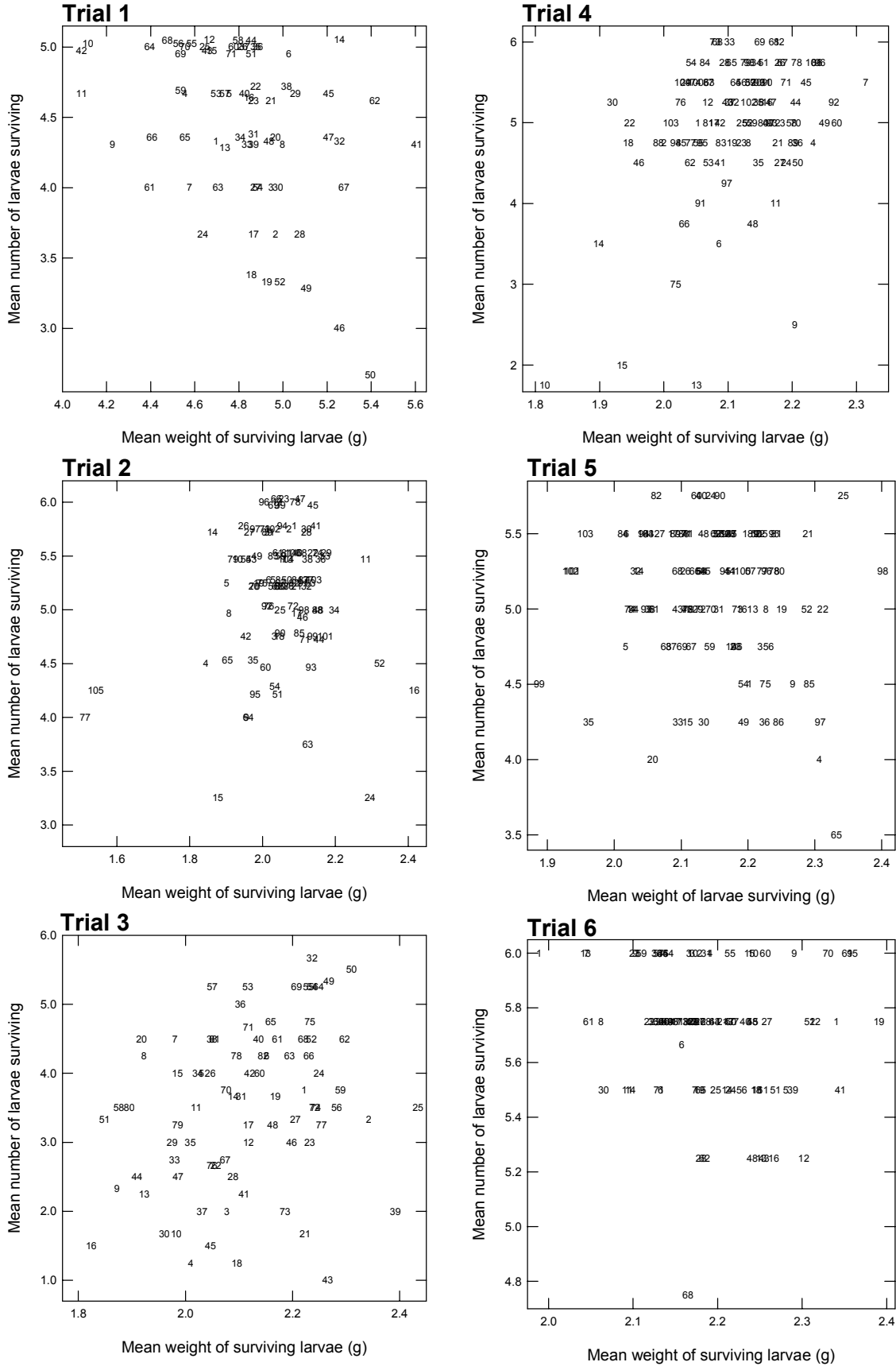


Figure 4. Relationship between each of the mean number of surviving larvae and the mean weight of surviving larvae versus the dry weight of uninfested roots for each clone in each pot trial. Clones are identified by numbers given in Table 1.

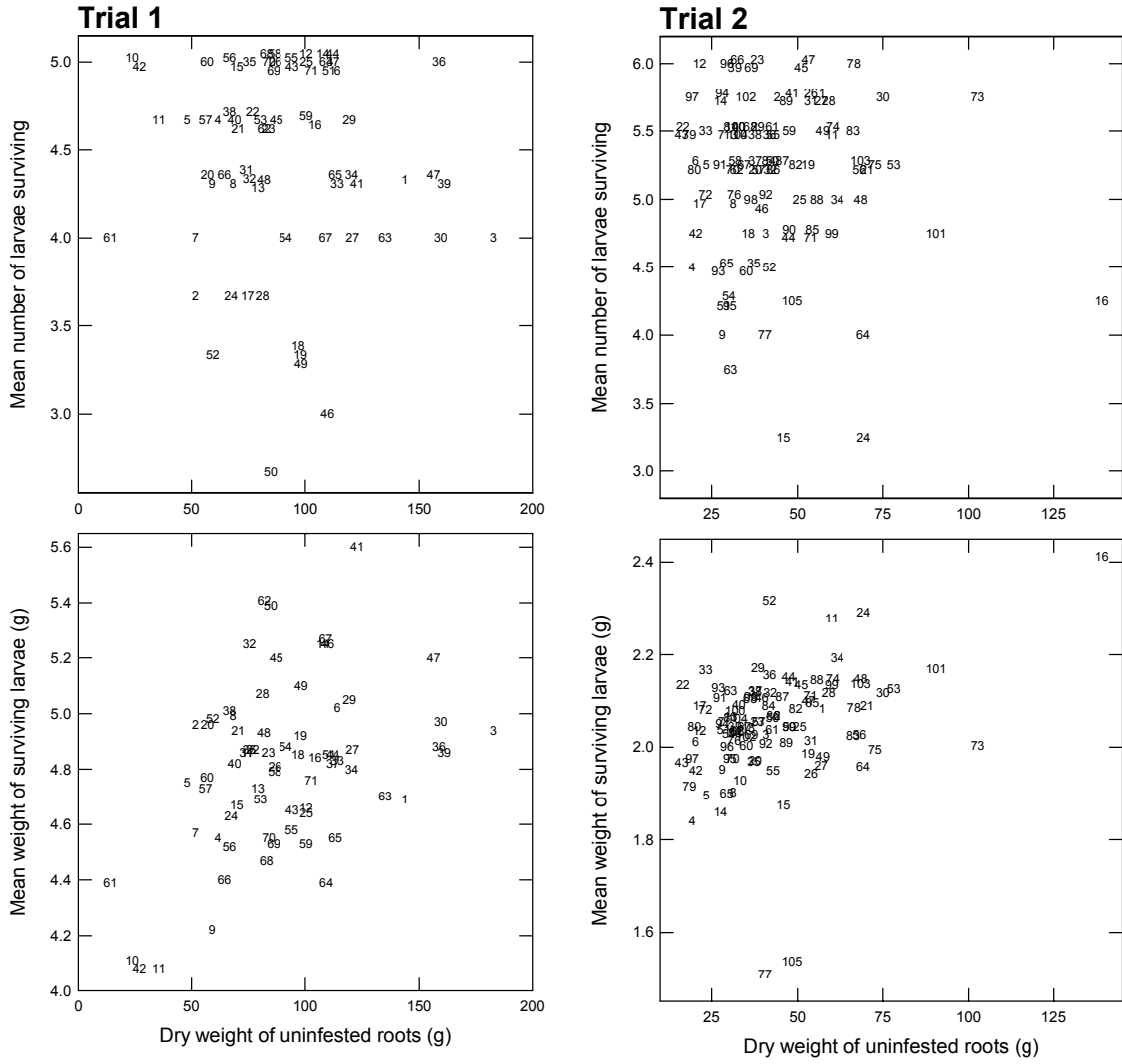


Figure 4 (cont.)

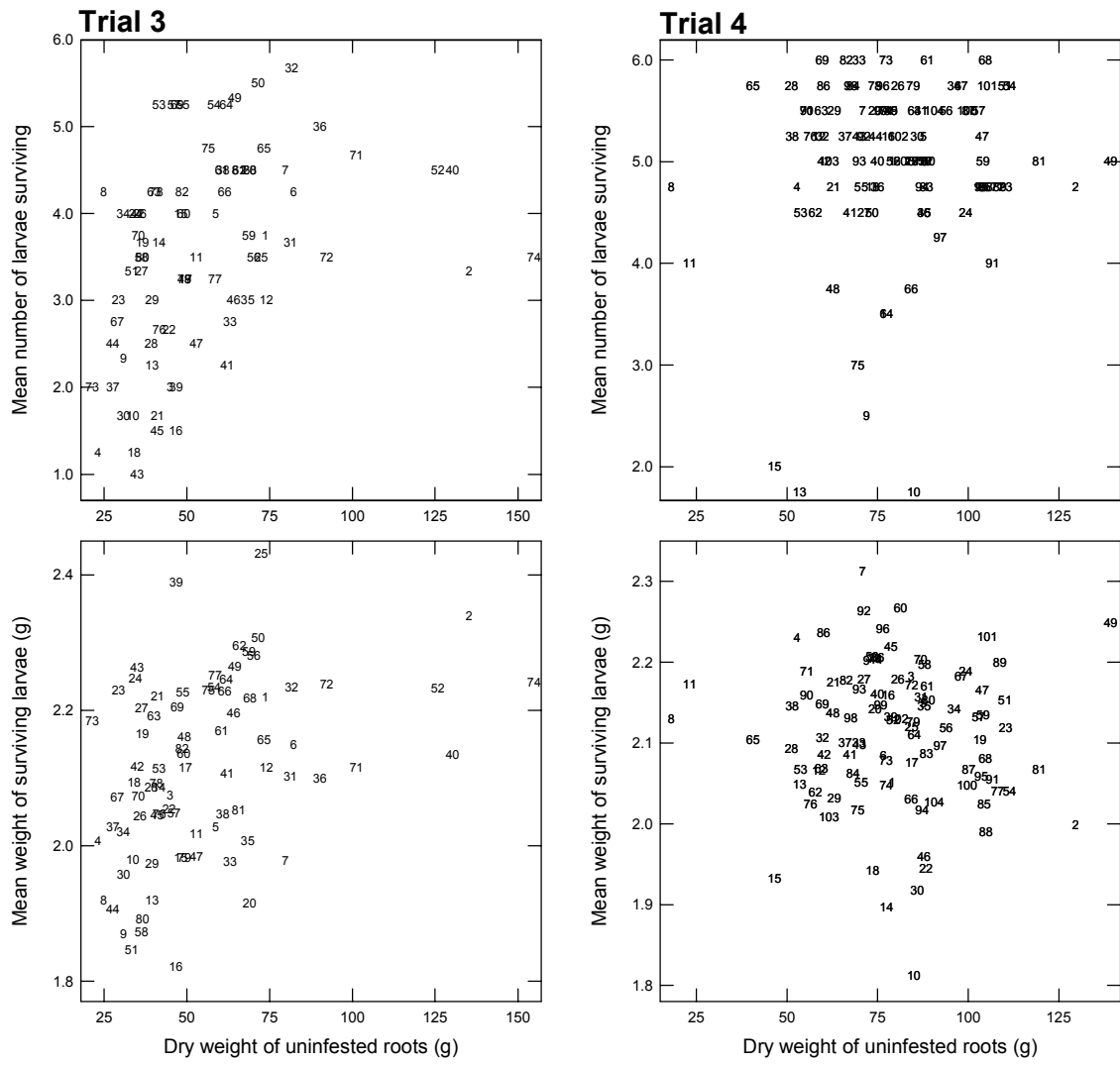
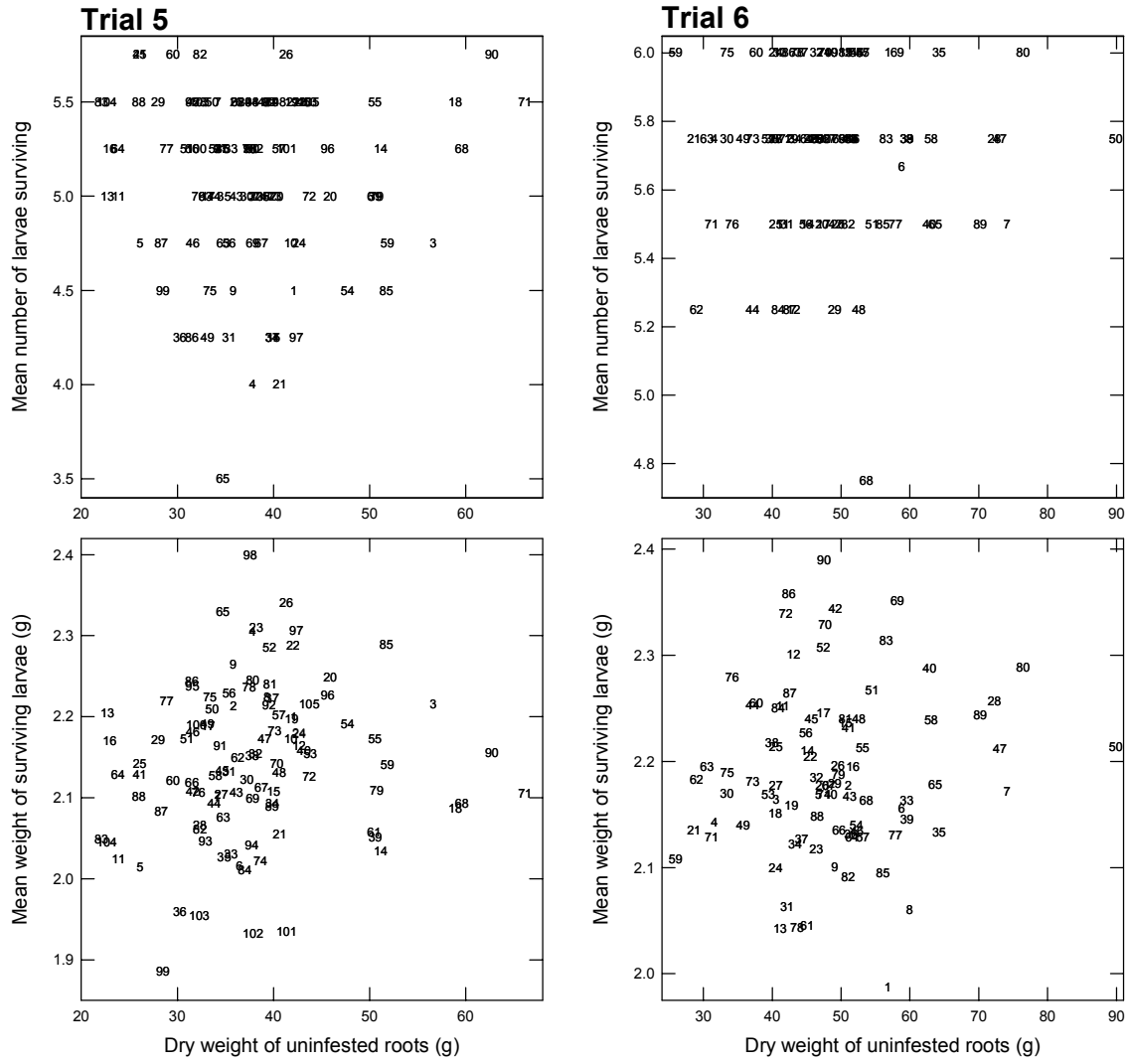


Figure 4 (cont.)



APPENDIX 2 FIELD-BASED SCREENING TRIALS

We screened a range of clones for different components of resistance in four field trials. These trials were arranged differently depending on the location.

Two trials in the Bundaberg area tested for resistance to southern one-year canegrubs (*Antitrogus consanguineus*), the species used in five of the six pot trials. This species is very susceptible to chlorpyrifos (the active constituent in the controlled-release insecticide suSCon® Blue), so we used a split-plot design (suSCon-treated; no suSCon applied) to test the reaction of clones under grub infestation and without significant grub infestation. We monitored the numbers of grubs in the untreated portions to estimate the combined outcomes of any antibiosis and antixenosis (non-preference) effects, although, given the restricted dispersal of adult females of southern one-year canegrub and the absence of apparent preferences for crops of various heights, etc., in these females, we considered that differences in grub numbers would mainly reflect differences in antibiosis. We monitored cane yields in the insecticide-treated and untreated subplots and used differences in these to estimate tolerance effects.

Two trials in the Burdekin area tested for resistance to greyback canegrubs (*Demolepida albohirtum*). This species is less susceptible to chlorpyrifos and, when the trials were established, suSCon® Blue was largely ineffective against greyback canegrub due to enhanced degradation of chlorpyrifos in the high pH soils of the Burdekin. Our experimental approach was to establish two trials using the same range of clones, one in an area of expected grub infestation and another in an area that was not expected to be infested. Differences in numbers of larvae among clones would reflect a combined outcome of antibiosis and antixenosis, because adults of greyback canegrubs show strong preference for oviposition in the tallest cane available during the November-December flight period. Differences in crop yield would reflect the outcome of differences in grub numbers and tolerance of the clones to grub feeding.

MATERIALS AND METHODS

Trial 1 - McCarthy

In this trial, we tested 15 clones (Table 1) planted in a randomised block design of six replicates at Farnsfield, south of Bundaberg, an area prone to infestation only by southern 1-year canegrub (*Antitrogus consanguineus*). Each plot was four rows wide and 20 m long, with half of this length treated with suSCon® Blue at 28 kg/ha and half left untreated. The trial was planted in September 1995 and harvested each September in the plant crop, first ratoon and second ratoon. Grub numbers were determined in the first and second ratoons in March 1997 and March 1998, respectively; no significant grub infestation was present in the plant crop in March 1996. From each untreated subplot, we took four standard 30 cm³ samples and counted the number of third instar larvae. Data were analysed by ANOVA and the means separated by the protected LSD test; numbers of larvae were first transformed by $x^{0.4}$ to stabilise differences in variance. We also calculated three new parameters for yields. If Y_i and Z_i are the yields of infested or uninfested plants of clone i , then the percentage yield loss is:

$$P_i = 100(Z_i - Y_i) / Z_i.$$

The relative yield of each clone when infested (RY_i) compared to that of the best yielding clone when infested (Y_{max}) is:

$$RY_i = 100 Y_i / Y_{max},$$

and the relative percentage yield loss of each clone (RP_i) compared to that of the most susceptible clone (P_{max}) is:

$$RP_i = 100 P_i / P_{max}.$$

The repeatability of the effects on yields was determined using the degree of concordance in rankings of relative percentage yield losses and relative infested yields. This was tested on the plant-crop, first-ratoon, second-ratoon and total three-crop yields for the 15 clones and for these parameters and the yield of tops in pot trial 1 for the 13 clones in common. We used Kendall's coefficient of concordance, where the value of the coefficient can vary between 1 (perfect concordance) and 0 (no concordance).

Trial 2 - Rasmussen

In this trial, we tested 25 clones (Table 5) planted in a randomised block design of four replicates at Sharon, west of Bundaberg, an area prone to infestation only by southern 1-year canegrub (*Antitrogus consanguineus*). Each plot was two rows wide and 20 m long, with half of this length treated with suSCon® Blue at 28 kg/ha and half left untreated. The trial was planted in March 1996 and harvested as a plant crop in August 1997 and a second ratoon in July 1999; the first ratoon was harvested by the operator without notification. Grub numbers were determined in the first and second ratoons in March 1998 (three replicates only) and March 1999, respectively; no significant grub infestation was present in the plant crop in March 1997. From each untreated subplot, we took four standard 30 cm³ samples and counted the number of third-instar larvae. Data were analysed as in trial 1.

Trial 3 – Pilchowski and Trial 4 - Todeschino

These two trials each tested 25 clones (Table 9) planted in randomised-block designs in the Burdekin. History suggested that trial 4 was likely to be infested by greyback canegrub (*Dermolepida albohirtum*), whereas trial 3 had not previously been infested. Each plot was four rows wide and 10 m long and there were three replicates in trial 3 and six replicates in trial 4. Both trials were planted in April 1997 and harvested as plant crops in October 1998 and first ratoons in October 1999. Trial 3 was not appreciably infested with grubs in March 1998, but was sampled for grubs in March 1999. Trial 4 was sampled for grubs in four of the replicates (those infested) in March 1998, but was not appreciably infested in March 1999. To sample grubs, we counted the number of young third instars in four 30 cm³ samples dug from the rows of each plot. Data were analysed as in trial 1, except that in both trials numbers of larvae in the four samples from each plot were combined and, in trial 3, transformed with $\ln(x+1)$ before analysis.

RESULTS

Trial 1 - McCarthy

Analysis of the grub numbers over the first and second ratoons showed significantly higher numbers of larvae in the second ratoon ($F = 31.77$, $df = 1,345$, $P < 0.0001$), no significant clone*year interaction ($F = 1.51$, $df = 14,345$, $P = 0.11$) and no significant

differences among clones in the number of larvae ($F = 1.36$, $df = 14,70$, $P = 0.0.20$) (Table 1). This indicates no significant difference among clones in combined antibiosis or antixenosis. This is not consistent with the grub survival data from pot trial 1 that tested 13 of these clones (Q151 and Q170^{db} were not included in that trial). In that trial, grub survival was significantly lower on Q141 and Q145 than on seven of the other clones (Table 1).

Table 1. Mean number of larvae per sample in each of the first and second ratoons of trial 1 compared with number of surviving larvae in pot trial 1.

Clone	Larvae per sample in field trial 1 (untransformed)		Number of surviving larvae in pot trial 1*
	First ratoon	Second ratoon	
CP51-21	1.6	2.3	4.7 ab
Q110	2.5	4.0	4.7 ab
Q124	1.9	2.8	4.3 abcd
Q135	1.8	2.3	4.7 ab
Q136	1.6	4.3	4.3 abc
Q138	3.4	4.3	5.0 a
Q141	3.1	3.3	3.0 de
Q144	1.5	3.0	3.3 cde
Q145	2.0	1.8	2.7 e
Q147	1.3	3.4	3.3 bcde
Q150	1.2	2.9	4.0 abcde
Q151	1.3	3.2	-
Q154	2.3	3.5	4.7 ab
Q155	1.9	1.2	5.0 a
Q170 ^{db}	1.2	2.9	-

*Numbers followed by the same letter are not significantly different (ANOVA on transformed data).

Analysis of crop yields (Table 2) over the three years showed significant suSCon-crop ($F = 39.54$, $df = 2,328$, $P < 0.0001$) and crop*clone ($F = 8.36$, $df = 28,328$, $P < 0.0001$) interactions, indicating that yields from each crop should be analysed separately. In each crop, there were significant clone and suSCon effects, but no significant suSCon*clone interaction (Table 3); however, in the first ratoon there was a strong trend to differences in the interaction ($P = 0.07$).

Table 2. Yields (t cane/ha) of suSCon-treated plots of 15 cultivars and percentage reduction in yield in untreated plots in trial 1.

Clone	Plant crop		First ratoon		Second ratoon	
	Yield	% reduction	Yield	% reduction	Yield	% reduction
CP51-21	76.2	5.6	97.8	13.2	76.2	32.0
Q110	74.5	4.5	100.2	27.8	49.1	56.8
Q124	105.4	10.4	127.9	18.9	101.2	39.1
Q135	89.6	6.6	109.2	24.8	70.1	48.7
Q136	66.6	2.9	115.1	26.7	50.8	42.0
Q138	109.7	1.5	124.7	19.6	105.3	31.7
Q141	90.5	5.6	110.0	42.7	50.7	57.5
Q144	47.9	9.1	78.8	26.3	57.0	48.0
Q145	113.3	3.7	143.2	14.4	85.5	35.0
Q147	89.4	3.1	105.4	17.4	59.7	45.3
Q150	106.5	0.3	130.1	21.0	85.2	24.5
Q151	105.5	3.6	106.4	16.0	58.1	35.2
Q154	93.6	-0.1	121.8	10.5	84.2	22.0
Q155	47.6	4.3	85.8	35.0	47.1	57.6
Q170 [Ⓛ]	109.8	4.5	118.5	14.3	67.8	43.7

Table 3. F value, degrees of freedom and associated probability of significance for effects of clone, suSCon application and the suSCon*clone interaction for each crop in trial 1.

Effect	Degrees of freedom	F value, probability of significance		
		Plant crop	First ratoon	Second ratoon
Clone	14, 70	14.93, <0.0001	7.19, < 0.0001	9.14, < 0.0001
suSCon	1, 75	27.57, < 0.0001	196.28, < 0.0001	253.93, < 0.0001
Clone*suSCon interaction	14, 75	0.95, 0.51	1.72, 0.070	0.74, 0.73

The relative yields of infested plants (RY) and relative percentage yield loss of infested plants (RP) (Table 4) each show significant concordance in rankings over the three crops and with the total three-crop yields (RY - coefficient of concordance = 0.91 , $P < 0.0001$; RP - coefficient of concordance = 0.73 , $P = 0.0002$). For the 13 clones in common with pot trial 1, there was also significant concordance in rankings over the three crops, the total three-crop yields and the yield of tops in the pot trial (RY - coefficient of concordance = 0.72 , $P < 0.0001$; RP - coefficient of concordance = 0.59 , $P = 0.0004$).

Table 4. Relative yield of infested plants (RY) and relative percentage yield loss of infested plants (RP) of each clone in each crop in trial 1.

Clone	Plant crop		First ratoon		Second ratoon		Combined crops	
	RY	RP	RY	RP	RY	RP	RY	RP
CP51-21	65.8	54.1	69.2	31.0	71.9	55.5	72.6	50.6
Q110	65.2	43.1	59.0	65.0	29.5	98.7	57.3	80.4
Q124	86.5	100	84.6	44.1	85.6	67.9	90.4	68.0
Q135	76.6	63.4	66.9	58.1	50.0	84.6	70.2	76.2
Q136	59.2	27.3	68.9	62.4	40.9	72.9	62.1	70.7
Q138	98.9	14.7	81.8	45.9	100	54.9	97.5	53.3
Q141	78.3	53.3	51.4	100	29.9	99.8	59.1	98.5
Q144	39.9	87.1	47.4	61.6	41.1	83.4	45.7	87.0
Q145	100	35.4	100	33.6	77.2	60.8	100	48.7
Q147	79.4	29.3	71.0	40.6	45.3	78.7	71.8	57.6
Q150	97.3	3.1	83.8	49.2	89.3	42.6	95.1	46.0
Q151	93.1	35.0	73.0	37.3	52.2	61.1	79.6	46.5
Q154	85.8	-1.1	88.9	24.7	91.2	38.2	93.4	31.8
Q155	41.7	41.6	45.4	82.0	27.8	100	42.2	100
Q170 ^d	96.1	42.9	82.8	33.5	53.0	75.8	85.1	53.0

Trial 2 - Rasmussen

Analysis of numbers of larvae (Table 5) showed no significant difference among clones in either the first or second ratoon crops (first ratoon - $F = 1.17$, $df = 24,48$, $P = 0.32$; second ratoon - $F = 1.60$, $df = 24,72$, $P = 0.067$). In both crops, grub numbers were too low for useful comparison with survival in pot trials (mean per sample in trial 1: 0.69, trial 2: 0.44).

Analysis of crop yields (Table 6) over the plant and second-ratoon crops showed significant suSCon*crop ($F = 9.51$, $df = 1,174$, $P = 0.0024$) and crop*clone ($F = 3.62$, $df = 24,174$, $P < 0.0001$) interactions, indicating that yields from each crop should be analysed separately. In each crop, there were significant clone and suSCon effects, but no significant suSCon*clone interaction (Table 7). The suSCon effect was small in the plant crop, 2.44 t cane/ha, although larger in the second-ratoon crop, 13.5 t cane/ha.

Table 5. Mean number of larvae per sample in each of the first and second ratoons of trial 2.

Clone	Larvae per sample	
	First ratoon	Second ratoon
CP51-21	0.50	0.44
Q110	1.42	0.13
Q118	0.17	0.00
Q120	1.00	0.19
Q124	0.17	0.31
Q135	0.83	0.44
Q136	1.33	2.00
Q137	2.50	1.38
Q138	0.67	0.82
Q141	1.08	0.13
Q144	0.42	0.75
Q145	0.00	0.00
Q146	1.42	0.38
Q147	0.67	0.82
Q150	0.25	0.25
Q151	0.67	0.00
Q153	0.92	0.56
Q154	0.25	0.25
Q155	0.42	0.19
Q159	0.25	0.44
88B207	0.25	0.06
88B209	0.67	0.19
88B216	0.42	0.13
88B222	0.58	0.63
88B230	0.33	0.44

Table 6. Yields (t cane/ha) of suSCon-treated plots of 25 clones and percentage reduction in yield in untreated plots in trial 2.

Clone	Plant crop		Second ratoon	
	Yield	% reduction	Yield	% reduction
CP51-21	80.3	5.6	58.2	12.0
Q110	83.5	-0.2	58.7	23.6
Q118	56.7	-19.1	57.3	7.6
Q120	67.5	-5.4	66.3	23.6
Q124	114.0	7.9	96.2	20.3
Q135	90.8	-2.0	61.0	28.7
Q136	97.8	-2.0	63.3	23.9
Q137	95.8	-0.5	61.3	48.1
Q138	117.0	3.0	88.5	-3.2
Q141	89.7	-5.4	80.8	15.9
Q144	61.7	5.1	47.2	20.8
Q145	106.8	-3.7	84.0	17.9
Q146	86.3	4.4	56.0	41.1
Q147	87.8	13.9	73.5	20.4
Q150	110.0	11.6	90.7	6.2
Q151	86.0	-0.8	87.3	25.0
Q153	75.0	14.7	41.8	13.9
Q154	92.3	2.5	108.7	10.0
Q155	71.3	13.3	64.3	22.5
Q159	91.5	1.3	56.8	22.3
88B207	59.5	5.9	62.2	8.6
88B209	84.7	6.1	89.2	14.6
88B216	47.0	0.7	32.7	25.0
88B222	70.0	6.2	65.8	8.6
88B230	85.2	3.9	74.0	17.3

Table 7. F value, degrees of freedom and associated probability of significance for effects of clone, suSCon application and the suSCon*clone interaction for each crop in trial 2.

Effect	Degrees of freedom	F value, probability of significance	
		Plant crop	Second ratoon
Clone	24,72	10.16, < 0.0001	5.72, < 0.0001
suSCon	1,75	5.11, 0.027	58.38, < 0.0001
Clone*suSCon interaction	24,75	1.09, 0.37	0.72, 0.82

The relative yields of infested plants (RY) and relative percentage yield loss of infested plants (RP) (Table 8) each show no significant concordance in rankings over the two crops (RY - coefficient of concordance = 0.72, P = 0.079; RP - coefficient of concordance = 0.34, P = 0.88). For the 24 clones in common with pot trial 1, there was significant concordance in rankings over the two crops and the yield of tops in the pot trial for RY

(coefficient of concordance = 0.56 , P = 0.022), but not for RP (coefficient of concordance = 0.35 , P = 0.40).

Table 8. Relative yield of infested plants (RY) and relative percentage yield loss of infested plants (RP) of each clone in each crop in trial 2.

Clone	Plant crop		Second ratoon	
	RY	RP	RY	RP
CP51-21	66.8	38.2	52.3	25.0
Q110	73.7	-1.4	45.8	49.0
Q118	59.5	-130.3	54.2	15.7
Q120	62.7	-37.0	51.8	49.1
Q124	92.5	53.8	78.4	42.2
Q135	81.6	-13.8	44.5	59.6
Q136	88.0	-13.9	49.2	49.8
Q137	84.9	-3.6	32.5	100
Q138	100	20.4	93.4	-6.7
Q141	83.3	-36.8	69.5	33.0
Q144	51.5	35.0	38.2	43.3
Q145	97.6	-25.5	70.5	37.1
Q146	72.7	30.3	33.7	85.4
Q147	66.7	94.4	59.8	42.4
Q150	85.5	78.8	86.9	13.0
Q151	76.4	-5.3	66.9	52.0
Q153	56.4	100	36.8	28.0
Q154	79.3	17.2	100	20.7
Q155	54.5	90.8	50.9	46.9
Q159	79.6	8.7	45.1	46.3
88B207	49.3	40.1	58.1	17.8
88B209	70.0	41.6	77.9	30.3
88B216	41.1	4.8	25.0	52.0
88B222	57.9	42.2	61.5	17.9
88B230	72.1	26.7	62.5	36.1

Trials 3 and 4 - Pilchowski and Todeschino

In the first ratoon of trial 3, there were no significant differences among clones in the number of larvae (F = 1.42, df = 24,48, P = 0.15) despite what appears to be large differences among untransformed means (Table 9). This indicates a high level of intra-block variation in grub distribution, probably compounded by the large area need to accommodate the number of plots (clones) in each block relative to the patches of more uniform grub distribution.

In the plant crop of trial 4, there were significant differences among clones in the number of larvae (F = 2.24, df = 24,72, P = 0.0046) (Table 9).

There was no significant concordance between rankings of grub numbers in the first ratoon of trial 3, those in the plant crop of trial 4 and those surviving in pot trial 5 for the

23 clones in common ($P = 0.49$) and no significant concordance for any of these pairs ($P > 0.15$).

Table 9. Mean number of larvae per plot in the first ratoon of trial 3 and plant crop of trial 4.

Clone	Trial 3 - first ratoon	Trial 4 – plant crop*
CP74-2005	14.0	7.3 defg
Eos	10.0	8.3 bcdefg
Q96	7.0	5.3 fg
Q117	4.0	4.3 g
Q124	4.3	10.0 abcdefg
Q127	6.3	7.8 cdefg
Q133	2.0	8.0 bcdefg
Q138	8.3	8.0 bcdefg
Q158	3.0	11.8 abcdef
Q165 ^{db}	10.3	9.5 abcdefg
Q171 ^{db}	12.0	15.5 a
Q176 ^{db}	3.0	14.3 abc
Q177 ^{db}	16.3	13.3 abcde
Q180 ^{db}	11.7	12.5 abcde
Q183 ^{db}	8.3	14.5 ab
81N82	10.0	5.0 g
83A135	6.0	4.0 g
85A2234	5.3	13.8 abcd
85A2540	13.3	7.5 defg
86A182	3.3	8.0 bcdefg
86A700	2.3	13.3 abcde
87A1413	3.3	7.0 efg
88A1515	5.7	7.8 cdefg
89A3434	3.7	7.3 defg
89A3567	12.3	3.8 g

*Means followed by the same letter are not significantly different at the 5% level.

Cane yields in both the plant crop and first ratoon of trial 3 differed significantly among clones (plant crop, $F = 3.05$, $df = 24,47$, $P = 0.0005$; first ratoon $F = 3.21$, $df = 24,48$, $P = 0.0003$) (Table 10).

Similarly, cane yields in both the plant crop and first ratoon of trial 4 differed significantly among clones (plant crop, $F = 2.24$, $df = 24,72$, $P = 0.0046$; first ratoon $F = 1.94$, $df = 24,72$, $P = 0.010$) (Table 10).

There was no significant concordance in the rankings of yields in the two infested crops (first ratoon of trial 3, plant crop of trial 4) ($P = 0.20$). This may be a reflection of the different crop ages and/or a genotype*environment interaction between the two farms.

There was also no concordance in the rankings of yields in either of the infested crops and with the yields of tops in infested pots of pot trial 5 (trial 3, $P = 0.40$; trial 4, $P = 0.21$). However, the rankings of the yields in the first ratoon of trial 4 showed significant concordance with the yields of infested plants in pot trial 5 (Kendall's coefficient of concordance = 0.84, $P = 0.025$). This indicates that pot trials better reflect the residual effect of grub damage on field yield (through debilitation and removal of stools) than they reflect the direct effect of in-field feeding.

There was significant concordance between the rankings of yields in the infested plant crop of trial 4 and the uninfested first ratoon of the same trial (Kendall's coefficient of concordance = 0.77, $P = 0.046$). The deviation from perfect concordance may reflect effects first-ratoon yields through differential stool removal or damage at the plant-crop harvest and/or 'normal' genotype*crop effects.

Table 10. Yields (t cane/ha) of the plant crop and first ratoon in trials 3 and 4.
Means within a column followed by the same letter are not significantly different at the 5% level.

Clone	Trial 3		Trial 4	
	Plant crop	First ratoon	Plant crop	First ratoon
CP74-2005	150.7 defg	137.6 bcd	126.2 abcd	101.3 abc
Eos	168.7 abcde	133.3 bcde	135.8 abcd	87.9 bcde
Q96	133.6 g	100.2 fg	128.7 abcd	94.9 abcd
Q117	162.0 bcdef	124.2 bcdef	129.9 abcd	83.2 bcde
Q124	155.8 cdefg	118.5 cdefg	124.3 abcd	90.4 abcde
Q127	169.3 abcde	110.0 efg	130.8 abcd	84.2 bcde
Q133	189.1 a	145.8 ab	116.0 bcd	86.9 bcde
Q138	148.7 efg	122.7 bcdefg	136.0 abcd	106.7 ab
Q158	164.2 bcdef	111.1 efg	101.8 d	95.9 abcd
Q165 [♠]	163.1 bcdef	113.1 defg	139.8 abcd	92.9 abcd
Q171 [♠]	166.6 abcdef	144.2 ab	112.3 cd	84.1 bcde
Q176 [♠]	144.2 fg	124.7 bcdef	117.2 abcd	72.2 de
Q177 [♠]	160.0 bcdef	122.0 bcdefg	133.3 abcd	90.7 abcde
Q180 [♠]	176.7 abc	124.2 bcdef	140.5 abcd	113.7 a
Q183 [♠]	176.2 abcd	144.7 ab	128.7 abcd	90.1 abcde
81N82	155.6 cdefg	140.2 bc	134.2 abcd	89.2 abcde
83A135	154.5 cdefg	146.9 ab	156.8 ab	88.2 bcde
85A2234	157.3 cdef	118.0 cdefg	143.3 abc	103.0 abc
85A2540	180.9 ab	166.0 a	130.2 abcd	113.4 a
86A182	170.5 abcde	116.4 cdefg	124.5 abcd	81.2 cde
86A700	155.3 cdefg	141.1 abc	150.0 abc	113.2 a
87A1413	188.2 a	137.6 bcd	158.0 a	107.2 ab
88A1515	134.0 g	97.3 f	115.5 cd	90.2 abcde
89A3434	155.1 cdefg	121.3 bcdefg	116.0 bcd	66.8 e
89A3567	151.8 efg	137.6 bcd	141.8 abcd	103.9 abc

Assuming that genotype*environment interactions across the two sites were small, we used the plant crop of trial 3 and the plant crop of trial 4 as the uninfested and infested crops, respectively, to calculate relative yields of infested plants (RY) and relative percentage yield loss of infested plants (RP) (Table 11). These showed no significant concordance in rankings with the values determined in pot trial 5 for the 23 clones in common (RY, $P = 0.26$; RP, $P = 0.17$).

Table 11. Relative yields of infested plants (RY) and relative percentage yield loss of infested plants (RP) using plant crop data from trial 3 (uninfested) and trial 4 (infested).

Clone	RY	RP
CP74-2005	79.9	42.1
Eos	85.9	50.4
Q96	81.5	9.5
Q117	82.2	51.3
Q133	73.4	100
Q138	86.1	22.1
Q158	64.4	98.3
Q165 ^{db}	88.5	37.0
Q171 ^{db}	71.1	84.3
Q176 ^{db}	74.2	48.4
Q177 ^{db}	84.4	43.2
Q180 ^{db}	88.9	53.0
Q183 ^{db}	81.5	69.7
81N82	84.9	35.6
83A135	99.2	-3.8
85A2234	90.7	23.0
85A2540	82.4	72.5
86A182	78.8	69.8
86A700	94.9	8.8
87A1413	100	41.5
88A1515	73.1	35.7
89A3434	73.4	65.2
89A3567	89.7	17.0

CONCLUSIONS

Trial 1

This trial demonstrates five points:

- the split-plot insecticide-treated design does allow an estimate of the resistance of clones to canegrubs in terms of yields of tops. However, even with six replicates, the differentiation of clonal responses is not sharp; this may reflect the underlying patchy distribution of relatively sedentary species of canegrub with subsequent intra-block variation in canegrub pressure and variation in measured yield responses;
- the design does not allow estimates in terms of roots or stubble, but both of these are likely to contribute to differentiation of top yields. Responses increase

between successive crops, possibly reflecting the cumulative damage to roots and stubble;

- there was significant concordance between relative tolerance measures of yields of tops among the three crops and the combined yields, and between these and the tolerance measures for top yields from pot trial 1;
- there was no detectable difference in numbers of canegrubs among clones, unlike the difference seen in pot trial 1;
- the use of insecticides may also suppress damage by pests other than canegrubs (eg wireworms) and affect growth, giving spurious results - indicated by the growth responses in the plant crop in the absence of significant canegrub populations.

Trial 2 demonstrated:

- numbers of canegrubs in field trials can not be controlled and may not be high enough to adequately differentiate clones; numbers do not always increase in successive crops;
- the use of insecticides may also suppress damage by pests other than canegrubs (eg wireworms) and affect growth, giving spurious results - indicated by the growth responses in the plant crop in the absence of significant canegrub populations;
- under the conditions of the test, there was concordance between field responses and pot trial 1 for relative yield of infested plants but not for relative percentage yield loss.

Trials 3 and 4 demonstrated:

- numbers of canegrubs in field trials can not be controlled and may not be high enough to adequately differentiate clones; numbers do not always increase in successive crops;
- 'uninfested' and 'infested' sites may not always remain so;
- data from uninfested and infested trials at different locations are difficult to interpret given the different farming environments;
- resistance to greyback canegrub (a species that feeds later in the growth of each crop) may be better assessed by the yield in the following ratoon crop than by a yield reduction in the infested crop.

Overall, the field trials allow assessment of different resistance components than do the pot trials. Field trials assess not only the tolerance to yield reduction in the infested crop, but also yield reduction through stool damage and/or removal in the subsequent ratoon crop(s). However, they do not distinguish between antibiosis and antixenosis components (what causes different numbers of grubs), and control of infestation levels and timing can make results difficult to interpret. Pot trials do allow estimation of yield reduction in the infested crop, they allow differentiation of effects on tops, roots and stubble, and also allow estimates of antibiosis effects without compounding these with differences in antixenosis. Pot trials do not, however, allow estimates of the cumulative effects of damage on succeeding ratoon crops.

APPENDIX 3 DETERMINATION OF CHEMICAL MECHANISMS

Material for examination of chemical mechanisms was collected in 1995 from a small pot trial containing 15 clones. Clones exhibited, in pot trial 1, one of three antibiosis phenotypes, with regard to canegrub response: poor grub survival (Q98, Q99, Q141, Q144, Q145); low grub weight gain (Q152, Q158, 88B207, 88B209, 88B244); and good grub growth and survival (Triton, Q77, Q124, Q136, Q140). Three replicates of each clone were tested, in pots both infested and not infested with canegrub.

Plant samples were examined for a selection of chemicals known to be involved in plant-insect interactions. Phytochemicals were extracted using either a specific method (described in relevant section), or as fractions of a total phytochemical extraction method (Harborne 1998).

SUGARS

Sugars can stimulate canegrub feeding (Allsopp 1992), with fructose and sucrose the most effective stimulants. Sucrose, glucose and fructose are the most abundant sugars in sugarcane (Irvine 1977), with reported sucrose concentrations in roots of cultivar CP65-337 of 0.026 M and in below-ground stubble of 0.45 M. When roots are severed by canegrubs, sugar levels in the part attached to the plant may rise because of the downward movement of these compounds. This may make these portions even more attractive.

We examined the total sugars present in roots of the nine clones, as well as the amount of cell-wall sugars that contribute to the structural rigidity of plants.

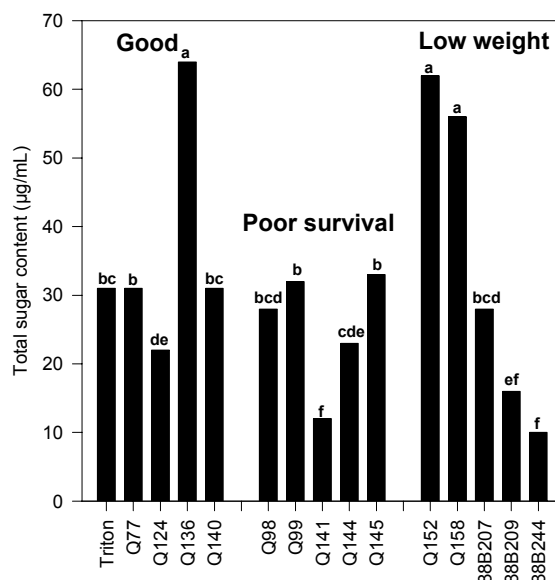
Total sugars

These analyses sought to determine the total amounts of sugars (including cell-wall sugars) present in roots using the phenol/sulphuric acid technique. Roots were taken from each of the three replicates of the uninfested plants of the pot trial and frozen for transport to Brisbane. Frozen material was weighed into 2 mg lots and mixed in 200 μ L of water in a test tube. To this were added 200 μ L of 5% phenol, followed by 1 mL of concentrated sulfuric acid. The test-tube was allowed to sit to cool for 10 minutes and then vortexed vigorously. After 60 minutes, the sample was vortexed again and transferred to 1.5 mL disposable cuvettes. Absorbance was read at 490 nm on a Perkin Elmer spectrophotometer. Standard concentration curves were prepared from a glucose base.

Analysis of total amounts of sugars showed significant differences among clones (ANOVA $F = 35.35$, $df = 14, 28$, $P < 0.0001$) (Fig. 1), but not among replicates ($F = 0.33$, $df = 0.33$, $P = 0.72$). Overall analysis of the five clones in each of the three antibiosis groups of hybrids showed no significant differences among groups (ANOVA $F = 1.74$, $df = 2, 42$, $P = 0.19$), but there was significant variation among clones within groups.

There was no significant correlation among mean total sugar content of each hybrid and grub weight ($r_s = 0.14$, $P = 0.61$) or grub survival ($r_s = 0.24$, $P = 0.38$) in the pot trial.

Figure 1. Mean total sugar content in 15 clones belonging to three antibiosis groups (clones with the same subscript letters are not significantly different at $P = 0.05$).



Cell-wall sugars

Cell walls contain polysaccharides, which in association with lignin are responsible for the structural rigidity of plants (Harborne 1998). Following sequential solvent extraction of root tissue to obtain cell-wall residue, cell-wall monosaccharides for three samples (one from each group) were examined by gas chromatography.

Table 1. Relative amounts of cell-wall sugars of three clones.

Constituent	Clone					
	Q140 'Good'		88B207 'Low weight'		Q98 'Poor survival'	
	Total	% of sugars	Total	% of sugars	Total	% of sugars
Arabinose	15459	21.1	6823	21.7	5654	22.7
Xylose	47117	64.2	19167	60.9	15633	62.8
Glucose	4953	6.7	2405	7.6	1840	7.4
Galactose	5900	8.0	3083	9.8	1752	7.0
Total cell-wall sugars	73429	-	31478	-	-	-
Xylose:Arabinose	3.05		2.81		2.77	
Glucose:Xylose	0.11		0.13		0.12	

The data (Table 1) suggest that grubs grow better when there are higher amounts of cell-wall sugars and higher ratios of xylose to arabinose and lower ratios of glucose to xylose.

The xylose:arabinose ratio indicates the amount of xylan branching, with a higher ratio indicating that the hemicelluloses are more linear. However, the significance of these results needs to be examined further by testing more samples.

Monosaccharides are insufficiently volatile for direct examination by gas chromatography. The presence of free hydroxyl (-OH) groups causes monosaccharides to irreversibly bind to the stationary phase; therefore, the -OH groups need to be protected by derivatisation. The most common types of derivatives for monosaccharides are trimethylsilyl ethers and acetates. However, the major shortcoming in the use of silylation for sugar derivatisation comes from the multiple reaction products produced from natural sugars due to anomers (α and β). Each single sugar usually gives rise to five tautomeric forms. The aldono-nitrile acetate reaction produces a straight chain derivative of the monosaccharide, and consequently anomeric isomers do not exist, and the monosaccharide is characterised by a single peak on the gas chromatograph. Aldono-nitrile acetate derivatisation is very lengthy and labour intensive, and was therefore unsuitable for examining a significant number of samples. A more efficient method was sought; however, a suitable method was not identified.

PHENOLIC COMPOUNDS

The term phenolic compound embraces a wide range of plant substances, which possess in common an aromatic ring bearing one or more hydroxyl substituents (Harborne 1998). Phenolic compounds are important because they are converted into several derivatives including phytoalexins (antimicrobial), coumarins (oral anticoagulants), lignin (cell-wall strength) and various flavonoids (Salisbury and Ross 1992). Flavonoids are the largest group of phenolic compounds, and some are known to have a bitter and repellent taste (flavonols, flavones and flavanones) and thus are thought to act as feeding deterrents. The complex flavonoids, or isoflavones, are known to be powerful natural insecticides (Harborne 1998).

We attempted to analyse for phenolic compounds by three methods: capillary electrophoresis (CE); high-performance liquid chromatography (HPLC); and three-dimensional fluorescence spectroscopy. CE is more sensitive than HPLC, but the latter allows possible identification of compounds using a combination of retention time, and UV absorbance spectra. Three-dimensional fluorescence spectroscopy does not allow direct identification of compounds but identifies classes of compounds without having to perform chromatographic separations.

Flavonoid/phenolic analysis by capillary electrophoresis

These analyses sought to determine the amounts of different flavonoids and phenolics extracted by methanol, using the capillary electrophoresis method developed in BS74S. Roots were taken from the pot trial and frozen for transport to Brisbane. Frozen material was weighed into 10 g lots and each placed in a 100 mL screw-top bottle containing 50 mL of flavonoid extracting solution (MeFES - 80% methanol, 19% water, 1% acetic acid). To each bottle, we added 1 mL of 200 $\mu\text{m}/\text{mL}$ apigenin as an internal standard for the analysis. Bottles were capped and shaken overnight on a flat-bed shaker at 28°C in the dark. After the sediment settled, the solution was filtered through Whatman #1 filter paper and stored at 4°C until analysis.

The analysis used an Applied Biosystems Capillary Electrophoresis System model 270A-HT with an uncoated LC Packings high-sensitivity capillary 122 cm long (100 cm from autosampler to detector) by 75 μm internal diameter. The running buffer was 30 mM borate in 20% methanol at pH 9.5 and detection was with a 395 nm tungsten lamp with applied voltage of 30 kV. The injection was for 1 second at 5" Hg and the run time was 60 minutes. The first flush cycle was 2 minutes with 1.0 M NaOH, the second was 2 minutes with 0.1 M NaOH, and the third was 3 minutes with buffer. Electropherograms were recorded on the Water Millennium data system.

We detected 32 different flavonoid/phenolic compounds (Table 2). There were significant differences among clones in the overall amounts of these compounds (Friedman Non-parametric ANOVA $T = 110.8$, $P < 0.0001$) and significant differences among the compounds in the overall amounts of each ($T = 316.3$, $P < 0.0001$). Analysis of total amounts of these compounds in each of the three antibiosis groups of clones showed no significant differences (ANOVA $F = 1.27$, $df = 2,12$, $P = 0.32$) (Fig. 2). Principal components analysis was used to summarise data on amounts of the different compounds in each clone. The first vector accounted for 30.6% of the variation, and appears to be a scaling parameter. The second to fifth vectors accounted for 14.3, 12.1, 9.0 and 7.6% of the variation, respectively, and the remaining vectors accounted for no more than 5.8% each; in total, the first five vectors accounted for 73.6% of the variation. All of vectors 2-5 showed strong contrasts among compounds, but there was no compound in common within these contrasts. Plots of vectors 2-5 against the preceding vector showed a clustering of hybrids in the 'poor survival' group from vectors 2 and 3 (Fig. 3), but this appears to be from low amounts of some of the compounds, not high amounts.

Figure 2. Total flavonoid/phenolic content in 15 clones belonging to three antibiosis groups.

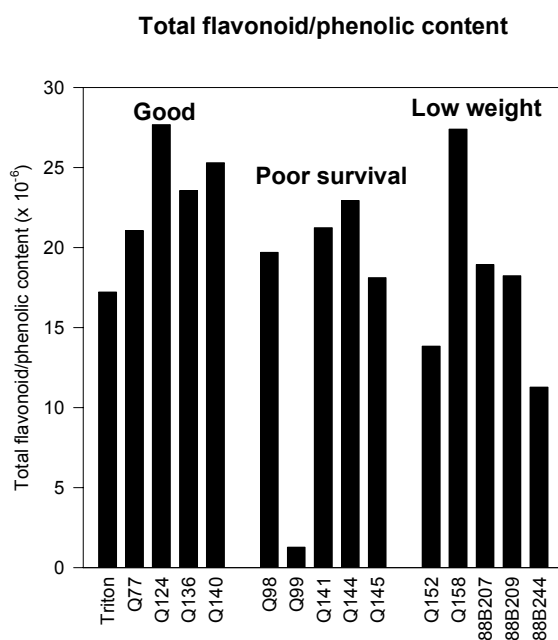
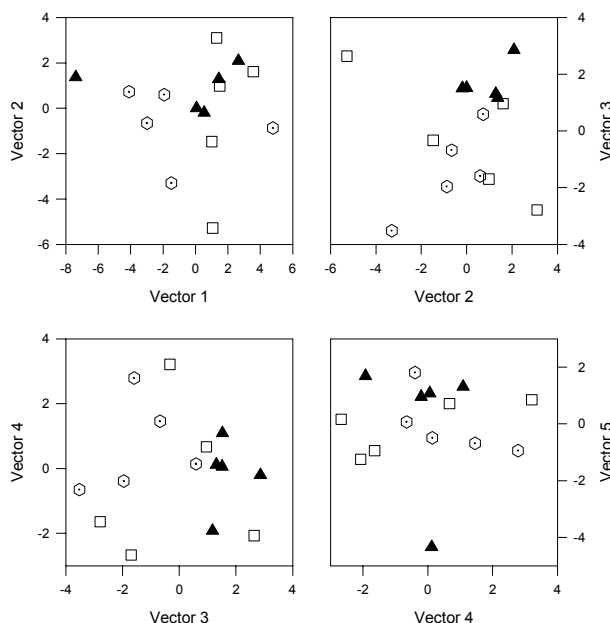


Figure 3. Plots of principal components vectors for 15 clones from three antibiosis groups (open squares = good; closed triangles = poor survival; open hexagons = low weight).



Multiple regression analysis (both forward and backward) showed that the contents of two compounds (22 and 29) explained 52% of the variation in grub weight ($F = 6.48$, $df = 2,12$, $P = 0.12$). However, these findings are counter-intuitive, because all coefficients were positive, indicating higher weights with higher flavonoid/phenolic contents. Similar analysis for grub survival showed that the contents of compounds 10, 28, 6 and 31 explained 86% of the variation in grub survival ($F = 15.46$, $df = 4,10$, $P = 0.0003$). Coefficients for the first two compounds were negative, indicating that survival was lower when the content of these compounds was higher; compounds 6 and 31 showed the reverse, counter-intuitive relationship.

Table 2. Relative amounts of different flavonoids/phenolics ($\times 10^{-5}$) in 15 different clones belonging to three antibiosis groups.

Flavonoid/ Phenolic	Antibiosis group														
	Good					Poor survival					Low weight				
	Triton	Q77	Q124	Q136	Q140	Q98	Q99	Q141	Q144	Q145	Q152	Q158	88B207	88B209	88B244
1	6.61	1.70	4.48	5.16	1.14	4.42	0.60	2.35	3.64	4.71	3.59	2.68	2.10	0.76	2.54
2	2.63	0.60	0.87	1.29	0.65	0.90	0.09	0.82	1.26	1.10	1.04	1.51	0.87	1.11	0.77
3	8.77	1.51	2.52	4.16	0.87	2.25	0.00	2.12	2.20	3.20	2.56	1.77	0.72	2.73	1.55
4	3.95	4.77	6.19	7.22	2.90	5.93	1.26	6.78	4.84	5.94	3.41	8.05	4.03	1.21	3.51
5	1.13	1.17	1.70	0.84	1.25	0.67	0.00	0.00	1.96	1.95	0.00	0.92	1.08	0.96	1.27
6	5.87	11.36	6.53	7.28	5.37	3.38	1.20	6.55	9.82	7.12	8.10	8.51	12.37	5.61	6.88
7	1.83	1.37	2.01	3.78	2.65	2.44	0.57	1.86	0.00	2.83	2.90	4.94	3.13	4.04	0.66
8	11.98	8.46	7.74	12.17	6.61	10.12	0.63	14.54	6.16	8.10	3.82	11.50	6.53	4.32	3.84
9	1.94	2.38	1.95	2.23	2.47	2.64	0.00	2.00	1.94	2.10	2.28	2.77	1.23	2.51	0.57
10	3.69	2.10	5.10	4.06	3.06	5.56	0.56	7.53	7.60	2.91	3.77	3.16	5.81	3.77	2.67
11	2.22	2.48	2.72	7.73	0.00	0.00	0.00	3.12	2.06	3.35	3.95	11.00	2.50	7.82	0.00
12	5.38	15.31	49.17	60.30	46.33	43.42	1.76	20.79	39.65	19.71	14.31	34.03	38.13	29.42	22.38
13	1.23	2.01	4.88	0.96	4.11	3.52	0.00	7.66	3.88	3.57	3.08	4.04	0.00	1.43	0.52
14	10.11	3.99	2.08	4.02	1.43	6.83	0.00	0.00	3.18	3.08	1.90	1.71	3.29	6.54	2.59
15	12.16	9.46	10.48	4.61	8.64	1.58	1.32	11.98	17.44	7.55	6.11	12.27	6.11	9.98	3.77
16	2.83	5.00	2.16	1.44	1.69	3.04	0.00	0.00	0.00	2.18	1.34	6.64	3.53	6.65	0.00
17	3.29	7.36	2.77	4.32	6.27	4.69	0.00	0.00	9.10	9.35	23.96	6.30	9.17	5.97	6.40
18	12.38	23.62	38.09	29.99	37.23	31.74	1.40	29.95	31.40	20.34	12.16	33.35	34.15	23.45	15.81
19	3.01	3.11	2.26	1.60	2.90	1.57	0.00	3.76	4.78	0.00	1.32	2.01	0.00	0.00	0.00
20	1.75	1.73	0.86	0.00	0.93	0.00	0.00	0.00	0.00	0.00	0.00	2.04	0.00	1.59	0.00
21	0.00	3.37	4.48	1.77	4.39	0.00	0.00	3.64	4.66	2.56	0.00	6.46	0.00	0.00	0.00
22	4.70	11.30	5.37	0.00	13.04	2.93	0.29	3.40	0.00	1.90	3.58	0.00	6.62	2.79	0.08
23	19.94	4.49	16.43	8.51	6.34	8.97	1.25	17.51	21.40	11.97	4.10	20.40	5.58	10.30	3.45
24	13.87	30.67	29.31	9.38	31.17	13.94	1.05	20.17	20.57	16.95	6.65	30.24	12.67	12.23	11.86
25	8.53	16.85	25.33	13.28	26.52	14.71	0.59	18.15	15.18	13.63	5.88	22.56	11.59	12.13	9.92
26	2.84	2.37	3.85	1.84	0.00	1.77	0.00	3.13	0.00	2.39	0.00	3.36	0.00	0.00	0.00
27	3.04	3.01	3.71	1.46	0.00	1.92	0.00	3.44	0.00	2.75	0.00	3.25	0.00	0.00	0.00
28	0.00	2.21	6.74	0.00	0.00	5.07	0.00	5.05	0.00	6.60	0.00	0.00	0.00	0.00	0.00
29	5.22	10.43	7.21	20.19	13.92	4.09	0.00	5.02	7.64	4.59	10.41	11.28	0.00	12.26	6.21
30	2.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.60	0.00
31	4.09	4.74	15.01	11.96	5.92	4.50	0.00	4.79	2.87	2.58	8.12	6.47	18.05	5.01	4.46
32	4.79	11.68	4.72	3.98	15.06	4.29	0.17	6.14	6.12	6.23	0.00	10.71	0.00	0.00	1.14
Total	172.19	210.59	276.71	235.52	252.85	196.88	12.72	212.23	229.32	181.25	138.35	273.94	189.26	182.22	112.84

Soluble phenolics analysed by HPLC

These analyses sought to further purify the polar compounds, for more detailed analysis of these compounds, in particular the phenolics, using high-pressure liquid chromatography (HPLC).

A homogeneous sample of the frozen root material was prepared by grinding in a Waring blender with some dry ice. The tissue was then lyophilised and stored at -20°C. 250 mg of the dried and ground tissue were weighed into a 20 mL I-CHEM vial. The tissue was extracted overnight on an orbital shaker (150 rpm) in the dark at 28°C, with 10 mL methanol-water (4:1), containing apigenin at 200 µg/µL as the internal flavonoid standard. The sample was then filtered through Whatman 41 paper into 11 mL (16x100 mm) borosilicate glass test-tubes, using vacuum tap attachment. The filtrate was evaporated to 1 mL in a Speedivac without heat, and then sulfuric acid added to a final concentration of 2 M. The sample was then extracted with chloroform three times. The chloroform extract was evaporated to dryness and redissolved in 100% methanol before HPLC analysis.

HPLC analysis was through a Novapak C18 Column, on the Shimadzu Class-VP HPLC System. The samples were eluted from the column using two solvents; A (95% Milli Q water, 5% methanol, 1 mL/2L acetic acid) and B (5% Milli Q water, 95% methanol, 1 mL/2L acetic acid). The column began in solvent A, and compounds were eluted with a gradient to 80% solvent B over 60 min, at a flow rate of 1 mL/min. The concentration of solvent B was then brought up to 100% over 5 minutes. The column was washed with solvent B for a further 5 min, before re-equilibrating in solvent A for 10 min. Chromatographs were recorded and processed using the Shimadzu data analysis software.

In addition to apigenin, the internal standard, which does not occur naturally in sugarcane, we detected 33 phenolics in the samples of roots. UV spectra of the compounds indicate that the ones occurring in the highest concentrations are probably oxidation products of *o*-dihydroxy phenolic compounds such as chlorogenic acid and caffeic acid, with spectra resembling cinnamic acids (ferulic and *p*-coumaric acids). The compounds can be categorised into five major groups according to their UV spectra:

- single major maximum at approximately 280-300 nm (compounds 4, 6, 7, 12A, 13, 15, 18, 19);
- spectra resembling ferulic acid with maxima at approximately 325 nm and shoulder at approximately 290 nm (compounds 3, 9, 10, 16, 20, 21A, 22, 24, 25, 26, 27, 28, 29, 31, ferulic acid);
- spectra resembling flavone or anthocyanidin flavonoids with maxima at approximately 350 or 480 nm (compound 21);
- spectra with maxima at approximately 280 nm and shoulder at approximately 310 nm (compounds 1, 5, 8, 14, 23, 24A, *p*-coumaric acid);
- single major maximum at approximately 250 nm (compounds 2 and 17).

ANOVA (following $\ln(x+1)$ transformation) over all compounds except apigenin showed no significant effect of antibiosis group ($P = 0.81$), but a significant grub*phenolic effect ($F = 23.59$, $df = 32,1408$, $P = 0.0013$). In general, the clones that we tested responded to canegrub attack by producing compounds 8, 13, 15, 21, 24, 24A, 26, 27 and 31, increasing the amounts of compounds 4 ($P = 0.027$), 6 (0.020), *p*-coumaric acid (0.0063), 14 (0.026), 20 (<0.0001), 22 (< 0.0001), 28 (0.0004) and 29 (0.0014), and decreasing the amounts of

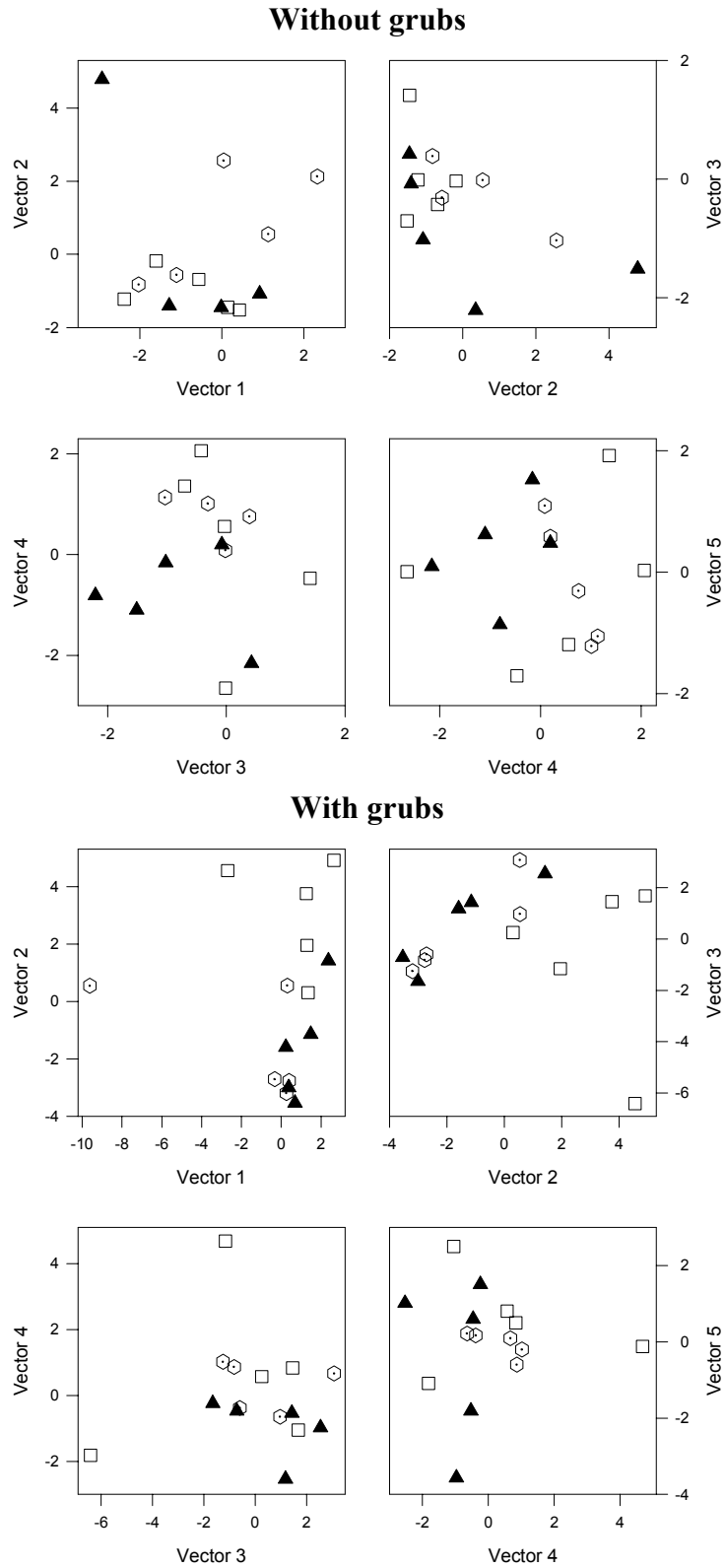
compounds 7 (0.031) and 23 (0.0007) (Table 3). The compounds that decreased are possibly precursors of some that increased. Phenolic 5 showed a significant grub*clone interaction ($P = 0.026$), with only Triton significantly increasing the amount of phenolic 5 when attacked by grubs. Many of the additional or increased compounds are ones with longer retention times (~25-60 min) in the HPLC extraction (the higher the code number, the longer the retention time). McGhie (1997) found that a general feature of all cultivars that he tested (not grub-damaged) was that most of the UV-absorbing compounds detected had low retention times (< 25 mins), indicating that the compounds are relatively more polar, water soluble and could be phenolic glycosides. The more lipophilic compounds with longer retention times, which would include the flavone aglycones and glycosides, were not seen in McGhie's samples.

Principal components analysis was used to summarise data on amounts of the different compounds in each clone, uninfested and infested. In plants without grubs, the first vector accounted for 33.3% of the variation, the second 19.8%, the third 16.3%, the fourth 9.7% and the fifth 6.7%. In plants with grubs, the first vector accounted for 26.9% of the variation, the second 25.9%, the third 16.4%, the fourth 8.5% and the fifth 6.4%. However, there is no obvious clustering of clones of the same antibiosis group in any of the plots of vectors 1-5 (Fig. 4).

The protective effects of phenolics are thought to be due to their ability to form complexes with proteins via both covalent and non-covalent interactions, which may result in impaired enzymatic functions, reduced protein digestibility and reduced availability of amino acids (Summers and Felton 1994). *O*-dihydroxy phenolics (eg caffeic acid and chlorogenic acid) are oxidised to quinones, a process enhanced by alkaline pH or plant oxidative enzymes (eg polyphenoloxidase, PPO), resulting in a direct oxidative challenge to the digestive system of the actively feeding insect (Summers and Felton 1994). The midguts of canegrubs are slightly alkaline with a pH range of 7.7-8.3. In this environment, the auto-oxidation of *O*-dihydroxyphenolics should proceed at an accelerated rate. The biological consequences of oxidative stress may be impaired growth and development, reduced reproductive potential and decreased adult longevity (Summers and Felton 1994).

13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.64	4.20	5.97	23.34	0.00	5.17
14	0.00	0.00	0.00	0.00	2.30	0.00	0.00	0.00	0.00	0.00	3.73	3.34	16.14	15.17	0.00	4.81
15	18.86	0.00	43.36	30.15	0.00	49.40	0.00	0.00	6.60	10.71	33.39	175.71	43.42	779.34	34.19	
16	0.00	0.00	0.00	0.00	0.00	8.88	0.00	0.00	0.00	0.00	0.00	8.03	0.00	0.00	0.00	
17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.28	0.00
18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	22.80	0.00
19	0.00	0.00	0.00	0.00	0.00	3.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20	13.15	6.78	8.98	8.11	5.13	8.10	8.39	1.97	2.91	8.70	4.68	10.83	13.39	3.95	11.29	
21	13.70	7.43	13.91	11.23	5.59	5.24	7.10	2.84	4.90	10.37	4.01	15.56	24.01	12.36	13.83	
21A	0.00	0.00	0.00	0.00	0.00	43.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.50	0.00
22	0.00	0.00	0.00	0.00	0.00	10.06	0.00	0.00	0.00	0.00	7.84	0.00	0.00	0.00	7.63	0.00
23	10.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	5.47	30.68	0.00	15.06	13.38	41.57	46.08	25.73	23.49	0.00	33.86	20.12	11.77	60.62	13.26	
24A	17.52	0.00	44.59	22.54	0.00	0.00	0.00	0.00	0.00	33.69	0.00	4.39	37.15	0.00	10.34	
25	9.32	8.12	0.00	7.23	0.00	21.44	8.07	3.66	5.67	9.48	22.80	19.75	27.78	46.55	12.48	
26	4.83	0.00	0.00	0.00	0.00	10.42	0.00	0.00	0.00	3.47	0.00	2.63	6.40	9.00	0.00	
27	5.57	0.00	0.00	0.00	0.00	21.33	0.00	0.00	0.00	9.33	0.00	5.77	0.00	5.57	0.00	
28	46.09	18.16	66.82	65.01	35.37	59.75	51.84	44.20	43.13	35.92	30.19	38.75	28.65	25.84	42.49	
29	32.78	10.47	13.12	14.94	10.00	45.44	44.08	12.74	12.51	20.76	4.10	11.85	12.19	20.25	19.34	
31	76.96	40.32	106.84	118.69	76.28	55.74	78.06	80.16	73.91	28.38	39.37	97.66	61.89	0.00	53.45	

Figure 4. Plots of principal components vectors for 15 clones from three antibiosis groups (open squares = good; closed triangles = poor survival; open hexagons = low weight) based on phenolics detected by HPLC.



Soluble phenolics analysed by 3-dimensional fluorescence spectroscopy

Roots from six clones of sugarcane (Q124 and Q140 - good grub survival and weight gain; Q141 and Q145 - poor grub survival; Q152 and Q158 low weight gain) were taken from infested and uninfested plants grown in a small pot trial at Bundaberg. Frozen root tissue was extracted using the first solvent extraction step of Harborne (1998), and provided to Dr Gerald Smith (Biomaterials, Industrial Research Limited, New Zealand) for analysis using 3-dimensional fluorescence spectroscopy. This technique has been used successfully to distinguish and identify classes of fluorophores present in mixtures without having to perform chromatographic separations. In particular, green fluorescence is emitted by proteins containing the Ser-Tyr-Gly motif. The tyrosine residue dehydrogenates (oxidises) to give a 4-OH cinnamoyl residue and concurrently this tripeptide sequence can undergo non-enzymatic cyclization to give an imine (N=C bond).

All uninfested roots exhibited similar fluorescence spectra with at least five different fluorophores present, although in different proportions. Examples of these 3-D fluorescence spectral contour maps covering different wavelength ranges are on file at BSES Bundaberg.

Fluorophores with maxima $\lambda_{em} = 425$ nm (emission wavelength) and $\lambda_{ex} = 305-320$ nm (excitation wavelength) are attributed to phenol propenyl entities, including the lignin precursors ferulic acid and sinapic acid, which have previously been identified in sugarcane roots. The fluorophores with maxima $\lambda_{em} = 410$ nm and $\lambda_{em} = 395$ nm and with $\lambda_{ex} = 340-360$ nm are attributes to flavonols and/or 5-hydroxyflavonols (5-hydroxyflavones are non-fluorescent). The fluorophore with $\lambda_{em} \sim 480$ nm and $\lambda_{ex} \sim 390-410$ nm is consistent with emission from the singlet state of the phenolate form of caffeic acid. The broad emission observed in the region $\lambda_{em} \sim 520$ nm and $\lambda = 80-400$ nm is similar to that observed from some aquatic organisms, and has been identified as the enamine oxidation (dehydrogenation) product of coumaroyl amino acid residue.

The tests suggest a trend towards more phenol propenyl fluorophores (eg the lignin precursors ferulic and sinapic acid) than flavonoids in the low-grub-weight clones compared with susceptible clones, which indicates greater polyphenoloxidase activity and less phenylalanine lyase (PAL) activity. This is a similar result to that of McGhie (1997), where *Pachymetra chaunorhiza* infected roots of highly resistant cultivars generally showed a decrease in PAL activity, while susceptible cultivars generally showed an increase. Polyphenoloxidase catalyses the oxidation of phenolics into large uncharacterised quinones (cause darkening). Chlorogenic acid and related compounds are readily oxidised into plant defence compounds. Phenylalanine lyase is involved in phenolic biosynthesis. PAL activity is believed to control the biosynthetic flux through the phenylpropanoid pathway; hence, higher PAL activity has the potential to produce greater concentrations of phenolic compounds in plant tissues (McGhie 1997).

Further, small-scale studies will be conducted by Dr Smith.

CELL WALL BOUND PHENOLICS

To analyse phenolic compounds covalently bound to the cell wall (implicated in cell-wall strength), the residue remaining after filtration of the initial solvent extraction (see Soluble

phenolics analysed by HPLC section above) requires treatment with sodium hydroxide to hydrolyse ester linkages.

The residue was extracted three times with 5 mL ethyl acetate to remove fats and waxes. After removing the ethyl acetate extract by filtering through Whatman #41 paper, the residue was scraped from the filter paper into a 20 mL I-CHEM vial and allowed to dry overnight. Approximately 50 mg of residue were transferred to a 4 mL glass vial, to which 1 mL of 1 M sodium hydroxide was added. Hydrolysis was initiated by heating at 70°C for 1 hour, and then allowed to continue overnight at 27°C. The samples were then acidified by adding 800 µL 1.5 M acetic acid. The sample was filtered through a 0.45 µm syringe filter to remove remaining residue. The filtrate was collected and stored at -20°C, while the remaining residue was discarded.

HPLC analysis was the same as for the soluble phenolics analysed by HPLC (see section above).

We detected 22 phenolics in the root samples (Table 4). The dominant phenolic compounds in all samples were the cinnamic acids, p-coumaric acid and ferulic acid (identified by comparison of retention time and UV-spectra with authentic compounds). The compounds can be categorised into five major groups according to their UV spectra:

- main peak at ~250 nm with shoulder at ~280 nm (compounds 1, 4, 8, 9);
- main peak at ~280 nm with shoulder at ~310 nm (compounds 2, 6, 22, p-coumaric acid);
- single peak at ~250 nm (compounds 3, 5);
- single peak at ~280-300 nm (compound 7);
- main peak at ~300 nm with shoulder at ~350 nm (compound 10);
- broad peak from ~250 nm (compound 12);
- main peak at ~325 nm with shoulder at ~290 nm (chlorogenic acid/ferulic acid type profile) (compounds 15, 16, 17, 18, 19, 20, 21, ferulic acid);
- single peak at ~320 nm (compound 14).

This corresponds well to the report of (Lawther *et al.* 1996) which indicates that in the Gramineae the majority of the cell-wall-bound phenolics are the cinnamic acids, p-coumaric and ferulic acid. Ferulic and p-coumaric acids are believed to function as bridges between the various components of the cell wall (Bacic *et al.* 1988), in particular between arabinoglucans (hemicellulose) and lignin (Iiyama and Wallis 1990; Lawther *et al.* 1996).

ANOVA (following $\ln(x+1)$ transformation) over all compounds showed no significant effect of antibiotics group ($P = 0.36$), but a significant grub*phenolic effect ($F = 2.99$, $df = 21,294$, $P < 0.0001$). In general, the clones that we tested responded to canegrub attack by significantly increasing the amounts of compounds 5 ($P = 0.0063$) and 7 (< 0.0001), trending towards higher amounts of p-coumaric acid (0.087), compound 14 (0.054) and compound 22 (0.077), significantly decreasing the amounts of compounds 3 (0.0052), 4 (0.012) and 15 (0.0063) and trending towards lower amounts of compound 6 (0.052) (Table 4). Phenolic 17 showed a significant grub*clone interaction ($P = 0.0036$); when attacked by grubs, the amount significantly increased in Q124, decreased in Q98, Q141, Q144 and 88B244, and did not vary in the remaining clones.

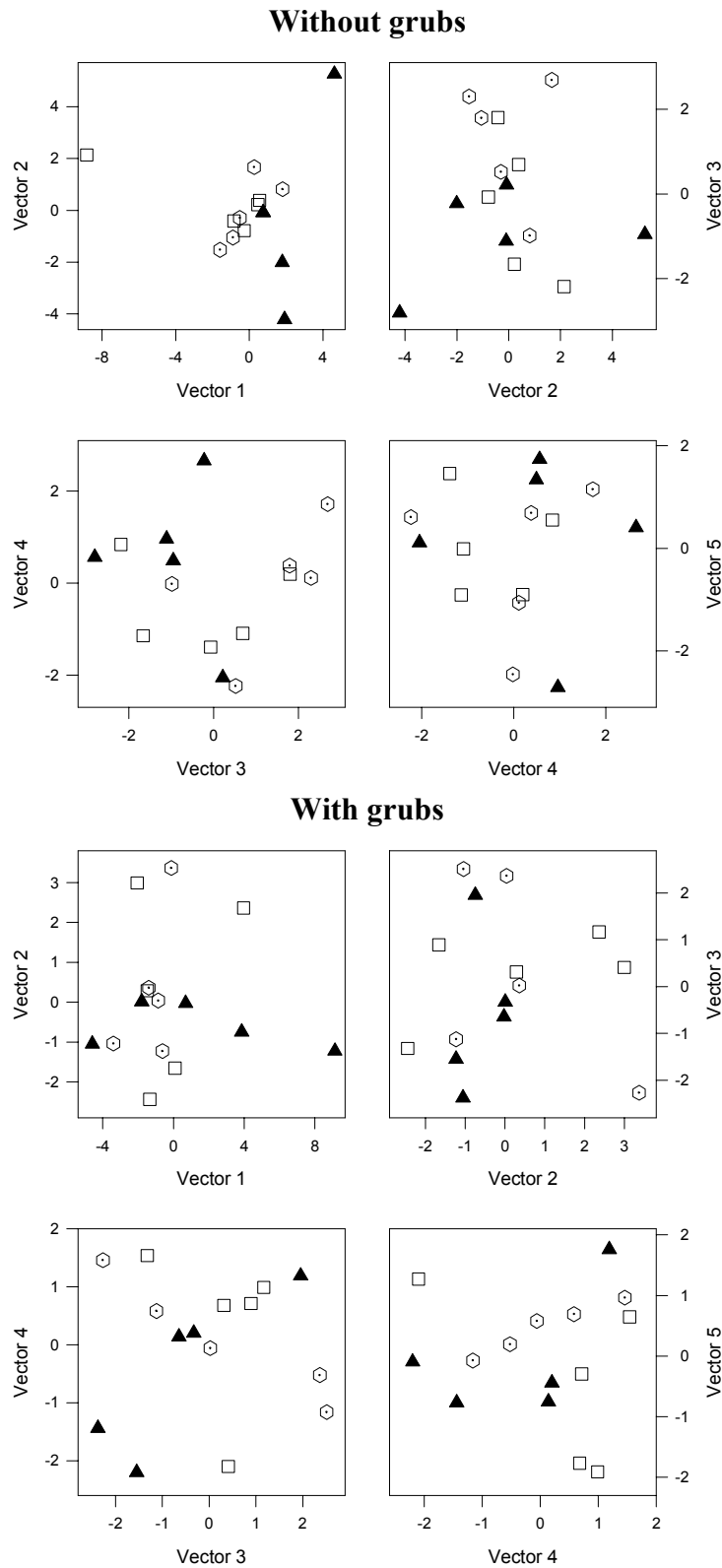
An explanation consistent with these data is offered by McGhie (1997). Both *p*-coumaric and ferulic concentrations may increase after challenge, but the ferulic acid participates in a peroxidase-mediated cross-linking reaction to produce polymers which strengthen the cell wall. Cross-linked ferulic acid-based polymers are likely to be more resistant to the alkali hydrolysis conditions used in these experiments, and an increase of these polymers would account for the apparent reduction of cell wall bound ferulic acid.

Principal components analysis was used to summarise data on amounts of the different compounds in each clone, uninfested and infested. In plants without grubs, the first vector accounted for 37.6% of the variation, the second 20.18%, the third 12.5%, the fourth 8.5% and the fifth 8.4%. In plants with grubs, the first vector accounted for 52.5% of the variation, the second 13.2%, the third 11.3%, the fourth 6.9% and the fifth 4.9%. However, there is no obvious clustering of clones of the same antibiosis group in any of the plots of vectors 1-5 (Fig. 5).

Table 4. Relative amounts of cell-wall phenolics in roots of uninfested and infested plants of 15 clones.

Phenolic	Clone														
	Q98	Q99	Q141	Q144	Q145	Q152	Q158	88B207	88B209	88B244	Q77	Q124	Q136	Q140	Triton
Uninfested															
1	29.15	24.76	42.92	25.06	18.43	26.87	21.24	25.89	22.49	31.20	24.42	25.09	22.39	28.30	20.78
2	8.29	8.34	8.92	11.54	16.32	7.91	8.02	10.73	7.55	7.40	8.64	4.92	9.33	9.77	8.44
3	20.91	18.22	30.51	18.24	13.39	19.74	16.24	17.23	23.34	19.03	18.50	18.18	21.56	16.02	18.14
4	24.77	19.73	34.08	19.32	14.49	21.84	18.17	21.71	19.18	25.99	21.18	21.49	20.92	25.16	18.14
5	24.27	23.05	23.23	21.94	25.71	26.33	10.99	13.72	13.75	13.93	10.47	8.24	15.38	13.79	15.57
6	0.00	11.48	10.84	14.44	11.41	4.78	11.58	12.56	8.75	11.97	7.99	26.72	14.12	10.44	6.26
7	56.58	86.28	101.81	112.67	102.15	73.69	85.90	75.48	94.90	93.31	70.89	42.40	64.21	84.01	76.27
8	30.44	29.01	33.75	29.33	39.33	28.30	29.48	33.32	27.57	28.91	28.24	36.12	29.70	29.43	39.10
9	77.90	83.67	89.38	105.64	95.98	76.99	79.59	83.98	73.50	78.06	81.28	40.67	89.27	88.04	80.30
10	42.14	34.84	39.24	35.59	32.05	35.02	31.99	30.53	26.90	29.09	27.79	2161.53	33.75	29.10	37.60
p-coumeric acid	3878.05	4145.47	5159.13	5540.51	4990.16	4590.51	4709.07	3918.79	4334.54	4618.99	4369.65	1734.76	3298.99	4551.03	3907.94
12	46.06	47.72	51.50	52.53	49.63	48.85	49.05	35.53	35.98	48.49	35.93	1000.56	49.02	39.10	47.44
ferulic acid	2260.69	1958.68	2924.01	2076.39	2320.13	2813.99	2039.97	1858.39	1713.92	2214.26	2007.09	961.95	2232.98	2213.65	2138.89
14	14.61	21.03	31.37	14.07	12.31	22.64	13.52	23.12	14.14	23.64	18.23	10.18	21.17	18.29	20.04
15	60.88	51.01	62.83	57.07	56.21	63.41	42.29	46.82	44.50	40.50	46.17	45.38	63.66	50.58	44.80
16	74.85	67.30	70.87	71.96	74.49	86.94	56.30	65.87	64.82	47.19	65.53	65.93	88.57	70.97	61.05
17	97.04	99.70	129.18	114.00	107.24	96.55	104.55	78.61	79.61	119.22	92.93	67.63	111.95	95.63	105.97
18	58.81	54.92	93.76	62.03	56.76	70.03	69.62	47.82	43.01	67.70	56.44	107.99	71.04	53.06	68.28
19	160.55	160.99	221.70	178.72	178.06	186.41	169.11	133.61	138.08	171.51	151.49	91.98	168.68	134.69	168.39
20	30.26	0.00	0.00	33.23	0.00	25.72	28.66	0.00	40.31	30.91	38.76	33.41	0.00	0.00	0.00
21	12.15	39.93	32.73	0.00	11.50	15.90	15.06	24.94	9.50	0.00	9.38	1.00	14.09	21.33	17.48
22	11.21	0.00	0.00	7.65	12.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Infested															
1	11.47	27.83	33.55	18.67	50.40	24.13	16.13	24.21	17.69	12.04	9.13	14.79	39.87	16.60	16.60
2	6.19	8.97	8.88	7.26	17.91	6.06	8.23	8.10	6.54	7.94	8.31	8.88	9.17	2.54	7.25
3	7.98	19.47	22.47	13.31	33.37	15.57	11.71	17.15	13.44	8.00	5.60	7.92	29.19	18.29	12.14
4	9.89	22.62	26.81	15.98	39.74	19.11	12.03	19.63	16.08	6.53	7.94	10.03	34.19	21.33	14.09
5	22.33	45.05	38.80	26.99	56.66	25.02	23.33	29.57	27.80	24.43	29.62	29.13	36.58	18.26	32.23
6	4.08	10.09	5.11	5.78	23.77	1.93	3.30	11.40	5.82	13.27	7.48	7.44	17.37	11.12	10.59
7	83.25	182.37	112.54	98.03	195.98	87.82	114.14	135.07	124.08	126.19	113.52	69.39	105.52	121.07	146.73
8	22.59	38.21	31.47	24.86	52.80	24.01	32.86	28.41	24.33	22.82	29.33	27.88	30.91	8.60	31.14
9	61.99	103.60	79.11	74.64	138.47	67.41	79.37	75.31	72.13	73.67	87.15	65.40	88.52	81.66	84.10
10	20.63	55.44	32.98	28.45	36.69	28.75	32.51	27.98	23.89	21.78	33.16	29.64	38.95	25.44	34.69
p-coumeric acid	4113.35	5721.90	5256.44	4383.64	6781.75	4643.20	5515.09	4446.91	4787.48	4499.86	5472.41	3860.86	4401.80	4862.71	4937.20
12	17.28	55.24	41.07	41.11	63.92	50.14	45.07	36.06	41.93	30.92	47.35	40.91	47.19	31.16	44.31
ferulic acid	1408.38	1600.36	1932.15	1567.08	2272.85	2133.64	1587.43	1471.45	1605.17	1254.88	1534.32	1791.16	2087.61	1750.64	1573.74
14	0.00	28.83	12.79	12.75	7.94	26.36	13.77	33.88	21.28	21.46	15.99	9.95	27.11	8.73	13.09
15	55.98	44.57	36.38	40.07	61.18	58.71	43.82	31.63	41.39	30.04	39.50	35.89	39.00	41.82	32.95
16	65.06	55.80	59.83	61.30	78.43	93.05	70.72	53.42	66.49	37.81	59.24	58.09	66.22	73.73	47.46
17	58.20	107.47	85.71	83.06	115.49	97.87	80.79	70.11	82.27	57.23	78.26	90.00	123.63	80.49	97.57
18	32.02	73.11	58.59	52.36	77.99	70.62	51.61	50.80	52.39	50.90	53.38	65.95	63.69	63.78	63.78
19	109.63	200.27	164.92	147.57	231.66	184.50	159.79	145.50	148.32	121.28	159.75	156.63	223.57	121.52	170.91
20	0.00	31.73	25.82	28.03	0.00	83.44	0.00	50.79	43.07	49.39	0.00	25.77	26.71	119.85	0.00
21	22.12	0.00	19.05	12.60	11.69	15.15	18.48	3.60	10.99	0.00	42.35	3.66	4.77	0.00	18.97
22	0.00	11.93	0.00	0.00	18.05	0.00	10.42	9.35	5.49	0.00	0.00	9.10	9.38	5.54	8.40

Figure 5. Plots of principal components vectors for 15 clones from three antibiosis groups (open squares = good; closed triangles = poor survival; open hexagons = low weight) based on cell-wall phenolics.



MONO- AND SESQUITERPENOIDS

Monoterpenoids are aromatic compounds with boiling points of 140-180°C. They attract insects towards flowers or to other plant parts, and have commercial utility as perfumes and food flavours. Sesquiterpenoids have a bitter or pungent taste and can be allergenic (bp > 200°C). These are the largest class of terpenoids, and they include insect antifeedants, and insect juvenile hormones and insect pheromones. Sesquiterpenoids also include plant growth regulators (eg abscissic acid) and related substances (Harborne 1998).

The chloroform extracts prepared for the Soluble phenolics analysed by HPLC were examined for the presence of mono- and sesquiterpenoids, using gas chromatography (GC). Compounds were separated using an Alltech Heliflex AT-1 Column, 30 m x 0.25 mm, 25 µ ID, on the Varian 3300 GC System. A 5 µL neat sample was injected into the column at 250°C, with a split ratio of 20:1. The carrier gas used was helium, with an inlet pressure 6 psi, a linear velocity of 12 cm/sec, and a split flow of 17.3. The column temperature was held at 40°C for 2 minutes, increased at 5°C/min to 300°C, and then held at that temperature of 15 mins. Chromatographs were recorded and processed using Waters Millennium data analysis software.

Initial investigations of methanol-soluble compounds revealed only low levels of such compounds, but there appeared to be some differences among the clones examined. Further purification of extracts (Harborne 1998) and the use of a column more specific for mono-/sesquiterpenoid separation revealed that the terpenoids were present in trace amounts, with larger peaks occurring in the elution temperature region that would indicate diterpenoids. Terpenoid compounds are known to be very volatile, and the tissue extracted was quite old, thus it is possible that the mono/sesquiterpenes had atomised, and hence only trace amounts were left. The method used for analysis did not produce repeatable results between replicate samples of the same clone, but it was still possible to perform a qualitative assessment. Our analysis revealed that for all clones, the total amount of terpenoid compounds appeared to decrease when the plants are infested with grubs. This may indicate that the plants are shifting their biochemical energy to other areas. Therefore, we believe that mono-/sesquiterpenoids and diterpenoids are not likely to be responsible for any resistance to canegrubs in sugarcane.

THIN LAYER CHROMATOGRAPHY (TLC) SCREENING

The range and number of discrete molecular structures produced by plants are huge; for example, it has been estimated that there are now over 10 000 known plant alkaloids. Therefore, thin layer chromatography was employed as a qualitative method for initial screening for the presence of possibly relevant classes of compounds.

Diterpenoids

Diterpenoids have the widest range of biological activity, including anti-feeding of insects (Harborne 1998).

Diterpenoids were extracted in the chloroform extracts prepared for the Soluble phenolics analysis by HPLC. 5 µL of extract were spotted onto silica gel plates, and the plates developed in *n*-hexane-ethyl acetate (17:3). Diterpenoids were detected by spraying the

plates with concentrated sulfuric acid and heating at 100°C for 10 mins, or by spraying with 5% antimony chloride in chloroform, and heated at 100°C for 2-5 mins. Plates were examined under daylight and ultraviolet light for spots in a range of colours.

No differences among clones could be detected using TLC.

Triterpenes and Phytosterols

Triterpenoids are generally found in waxy coatings and are believed to serve a protective function in repelling insect and microbe attack, they also have important taste properties, particularly bitterness. The most interesting phytosterols, from an insect perspective, are the insect moulting hormones (ecdysones). Insects have no capacity for steroidogenesis and are completely dependent upon their diet as a source of cholesterol, which is used for cell membranes and for the synthesis of the important steroid hormone, 20-hydroxyecdysone (Harborne 1998).

Triterpenes and phytosterols were extracted in the chloroform extracts prepared for the Soluble phenolics analysis by HPLC. 5 µL of extract were spotted onto silica gel plates and developed in hexane-ethyl acetate (1:1). Detection of triterpenes and phytosterols was accomplished by spraying plates with Carr-Price reagent (20% antimony chloride in chloroform), and heating at 100°C for 2-5 minutes, or by spraying with a mixture of 1 mL concentrated sulfuric acid, 20 mL acetic anhydride and 50 mL chloroform, and heating at 85-90°C for 15 minutes. Plates were examined under daylight and ultraviolet light for spots in a range of colours. To separate triterpenoids according to the number of isolated double bonds present in the molecule, TLC was performed on 5% silver nitrate-silica gel plates developed in hexane-ethyl acetate (1:1). Carr-Price reagent was again used to detect relevant compounds.

The thin layer chromatography was not very reliable in our tests, because there were differences between the replicates of samples, and the spray reagent did not detect the standard ecdysone.

Gibberellins

Gibberellins are tetracyclic diterpenoids, which are plant growth hormones (Harborne, 1998). We chose to investigate gibberellins because they may have influenced root growth.

Gibberellins were extracted in the chloroform extracts prepared for the Soluble phenolics analysis by HPLC. 5 µL of extract were spotted onto glass-backed silica gel plates, which were then developed in benzene-*n*-butanol-acetic acid (14:5:1). Detection involved spraying plates with concentrated sulfuric acid-water (7:3) and heating at 120°C for 10 mins, and viewing under ultraviolet light (gibberellins appear as fluorescent yellow-green spots).

TLC did not detect any differences among clones.

Sesquiterpene lactones

Sesquiterpene lactones are known to have anti-feedant properties and can cause survival reduction in insects (Harborne 1998).

Sesquiterpene lactones were extracted in the chloroform extracts prepared for the Soluble phenolics analysis by HPLC. 5 μ L of extract were spotted onto silica gel plates, developed in chloroform-diethyl ether (4:1). Sesquiterpene lactones were detected as brown spots, after placing plates into a sealed chamber containing iodine crystals. A second set of plates was sprayed with concentrated sulfuric acid and heated at 100-110°C for 5 minutes, to detect lactones as green, brown, yellow, red or blue spots.

We could not detect any differences among clones using TLC.

Alkaloids

Alkaloids are toxic to humans and have a dramatic physiological effects (eg nicotine). They are also known to have a bitter taste. Plants containing certain alkaloids are avoided by grazing animals and insects, while others are used as substrates for the synthesis of courtship pheromones (Harborne 1998). However, relatively few monocots have alkaloids.

Following removal of moderately polar compounds by chloroform extraction (see Soluble phenolics analysis by HPLC), 700 μ L ammonium hydroxide were added to each sample, to basify the remaining aqueous layer to pH10. The samples were then extracted two times with chloroform-methanol (3:1), each time transferring the lower chloroform-methanol layer to an 11 mL test-tube. The basic aqueous layer was then extracted once with chloroform, before transferring the lower chloroform layer to the test-tube containing the chloroform-methanol extract. The solvent was allowed to evaporate to dryness, and the samples were resuspended in chloroform for TLC analysis.

5 μ L of extract were spotted onto glass-backed silica gel plates, and then developed in methanol-concentrated ammonium hydroxide (200:3). Detection of alkaloids involved examination of plates under ultraviolet light for fluorescence, spraying with Dragendorff reagent (orange-brown spots on a yellow background), spraying with iodoplatinate reagent (range of colours), or spraying with Marquis reagent (1 mL formaldehyde in 10 mL concentrated sulfuric acid) (yellow to purple spots).

No alkaloids could be detected in our sugarcane samples by thin layer chromatography.

Quaternary alkaloids and N-oxides

Quaternary alkaloids and N-oxides are the non-basic forms of alkaloids.

The basic aqueous layer remaining after extraction of the alkaloids was evaporated to dryness (without heat) in a Speedivac. The residue was extracted with 1 mL 100% methanol overnight at 4°C. The methanol extract was removed to a storage vial, and the solvent allowed to evaporate. The samples were resuspended in methanol for TLC analysis. Thin layer chromatography for quaternary alkaloids and N-oxides used the same method as for alkaloids.

Using the iodoplatinate spray reagent, Q99 and Q144 of replicate G1 appeared to have more of a compound detected at RF 63-68 than other samples, while Q152 and 88B209 (replicate G1) appeared to have more of the same compound again.

Fats and waxes (polyacetylenes)

If acetylenes have an overall function, it is most likely as toxins in either plant-animal or plant-plant interactions. Some are highly poisonous, for example those found in the roots of water drop wart, *Oenanthe crocata* and fool's parsley, *Aethusa cunapium* (Harborne 1998).

Polyacetylenes were extracted from the residue remaining after filtration of the initial solvent extraction (see Soluble phenolics analysis by HPLC). 5 mL ethyl acetate were added to the residue, and allowed to extract for 5 mins. The sample was then filtered through Whatman 41 filter paper into 20 mL I-CHEM vials. The residue was extracted twice more and then the solvent allowed to evaporate to dryness. The samples were resuspended in ethyl acetate for TLC analysis. 5 μ L of extract were spotted onto silica gel plates and developed in chloroform-methanol (9:1). Polyacetylenes were detected by spraying with 0.4% isatin in concentrated sulfuric acid and heat (acetylenes appear as brown or green spots), or by spraying with 1% potassium permanganate in 2% aqueous sodium carbonate (yellow spots on a purple background).

Q158 in both replicate N1 and G1 responded positively to the isatin reagent with a single spot at RF 82.9, that was present in all other samples. The same result for 88B244 was detected using the potassium permanganate reagent. Using UV absorbance and HF₂₅₄ fluorescent indicator plates, Q124 and Q140 replicate G1 both revealed spots at RF 72.5, which were not present in other samples.

CONCLUSIONS

Our tests indicate that there are considerable differences among clones in the total amounts and types of total sugars, cell-wall sugars and phenolics. In addition, the amounts and types of phenolics varied significantly between uninfested and infested plants of the same clone. These factors are all putative defence factors that could be involved in resistance.

However, we could show no correlation between these factors and the antibiosis groupings of the clones examined. Perhaps, this is not surprising. If antibiosis to canegrubs in sugarcane is multigenic, a number of defence factors may contribute to that type of resistance, and the contribution of each factor to overall antibiosis may vary among clones. Consequently, it would be expected that correlations between individual defence factors, and overall resistance would not be high. In other words, just because phenotypes are the same, it does not mean that the chemical basis is the same.

We could show no significant variation among clones in amounts of diterpenoids, triterpenes, phytosterols, gibberellans, sesquiterpene lactones, alkaloids, quaternary alkaloids, N-oxides and fats and waxes that may have contributed to differences in antibiosis.