J. Appl. Entomol. **130(1)**, 67–71 (2006) doi: 10.1111/j.1439-0418.2005.01023.x

SHORT COMMUNICATION

Susceptibility of source plants to *Sugarcane Fiji disease virus* influences the acquisition and transmission of the virus by the planthopper vector *Perkinsiella saccharicida*

K. Dhileepan[1], B. J. Croft[2], A. W. Ridley[1], A. P. James[3] and S. Raghu[4]
[1]CRC for Tropical Plant Protection and School of Integrative Biology, The University of Queensland, Brisbane, Qld, Australia; [2]BSES Limited, Woodford, Qld, Australia; [3]David North Plant Research Centre, BSES Limited, Brisbane, Qld, Australia; [4]Illinois Natural History Survey, Champaign, IL, USA

Ms. received: June 14, 2005; accepted: October 31, 2005

Abstract: Fiji leaf gall (FLG) caused by *Sugarcane Fiji disease virus* (SCFDV) is transmitted by the planthopper *Perkinsiella saccharicida*. FLG is managed through the identification and exploitation of plant resistance. The glasshouse-based resistance screening produced inconsistent transmission results and the factors responsible for that are not known. A series of glasshouse trials conducted over a 2-year period was compared to identify the factors responsible for the erratic transmission results. SCFDV transmission was greater when the virus was acquired by the vector from a cultivar that was susceptible to the virus than when the virus was acquired from a resistant cultivar. Virus acquisition by the vector was also greater when the vector was exposed to the susceptible cultivars than when exposed to the resistant cultivar. Results suggest that the variation in transmission levels is due to variation in susceptibility of sugarcane cultivars to SCFDV used for virus acquisition by the vector.

Key words: *Fijivirus*, host plant resistance, sugarcane, Tritrophic interactions, vector–virus–host plant interactions, virus transmission efficiency

1 Introduction

Fiji leaf gall (FLG) (formerly known as Fiji disease) caused by *Sugarcane Fiji disease virus* (SCFDV) (formerly known as Fiji disease virus) is one of the most important diseases of sugarcane in Australia and several sugar-producing areas of Asia and the Pacific region (Smith and Candy, 2004). SCFDV is a double-stranded RNA virus of the genus *Fijivirus*, family Reoviridae (Matthews, 1982). Sugarcane infected by SCFDV shows leaf galls and distortion, leading to the death of meristematic tissue and stunting, which results in severe yield reductions (Egan and Ryan, 1986). SCFDV is present in both gall (Hatta and Fracki, 1976) and non-gall tissues, but gall tissue contained more virus than the non-gall tissue (Ridley, unpublished data). There was no significant relationship between FLG resistance level and concentration of SCFDV in infected plants (Ridley, unpublished data).

Sugarcane Fiji disease virus is transmitted by planthoppers of the genus *Perkinsiella* (Hem., Delphacidae) in a persistent and propagative manner (Hughes and Robinson, 1961; Hutchinson and Francki, 1973; Francki et al., 1986). *Perkinsiella saccharicida* Kirkaldy is the only known vector of SCFDV in Australia (Mungomery and Bell, 1933; Francki and Grivell, 1972). The planthopper can acquire the virus only during its early nymphal stages (Daniels et al., 1969; Croft and Ryan, 1984), and once infected, the planthopper remains viruliferous for life (Hughes and Robinson, 1961). Less than 25% of *P. saccharicida* adults transmit the disease (Egan et al., 1989).

Fiji leaf gall is managed through the identification and exploitation of plant resistance (Ryan, 1988). Currently, all new Australian sugarcane cultivars are screened for resistance to FLG before they are approved for commercial release. However, due to low and fluctuating vector populations and reductions in the overall field incidence of FLG, consistent and reliable field resistance ratings have been difficult to achieve (Dhileepan et al., 2003; Croft et al., 2004). Screening for FLG resistance in the glasshouse can yield results more quickly (Hayes, 1972; Ledger and Ryan, 1977) and most glasshouse screening has involved inoculating individual test seedlings with viruliferous vectors in insect-proof cages (Egan et al., 1989). However, such resistance ratings do not always reflect resistance observed in the field (Reimers et al., 1982), and trials conducted over the years produced inconsistent transmission results (Egan, 1982). The factors responsible for the variation in

SCFDV transmission in the glasshouse are not known.

In this study we compared the SCFDV transmission trials conducted over a 2-year period in the glasshouse to understand the factors responsible for the erratic transmission results. This information will help to understand why glasshouse trials so far have failed, and allow us to develop a more reliable glasshouse-based resistance screening method.

2 Materials and Methods

2.1 Transmission trials

A series of choice and no-choice transmission experiments (table 1) were conducted in a glasshouse (5 m × 5 m) at the BSES Limited, Woodford, over a 2-year period (2000–2002) following the methods described in Dhileepan and Croft (2003). In choice trials, plants of each of the cultivars (16–40 plants, table 1) were placed in one of four blocks in the glasshouse and exposed to the vector. In no-choice trials, vectors were confined to individual test plants of each cultivar (10 plants, table 1) using a mesh cage. Within each trial, plants of similar height with a similar number of leaves were exposed for 2 weeks to vectors reared on SCFDV-infected plants.

The field resistance of different sugarcane cultivars to the virus is ranked on a scale of 1 (resistant) to 9 (susceptible) based on their susceptibility to the disease under field conditions (Croft et al., 2004). Sugarcane cultivars (WD1, Q110, Q87, Q90, Q124, NCo310 and Q102) with known field resistance ratings to FLG were used as source and test plants in all trials. The resistance scores for these cultivars are 1, 1, 2, 5, 6, 8 and 9 respectively (Croft et al., 2004). Six-week-old single-potted plants (3–4 leaf stage) were used in all trials, except in trial 2 where test plants were maintained in the field in a disease-free area, before use in the study.

Perkinsiella saccharicida populations were collected from sugarcane plants at Woodford in June 1999 and maintained on SCFDV-infected plants with characteristic gall symptoms in a glasshouse. As maintaining insect populations on susceptible plants (cultivar NCo310) is difficult because these plants succumb to FLG, some resistant plants (cultivar WD1) were also left among the susceptible plants to enable the maintenance of the colony for trial 1 (table 1). For all subsequent trials, rearing was only done on the resistant cultivar (WD1). Both adults and nymphs of unknown ages collected from SCFDV-infected plants were used in the transmission trials (table 1).

2.2 Post-transmission monitoring

After 2 weeks of exposure to the vector, planthoppers were counted and removed, and plant height (from soil level to emerging point of youngest leaf) and the total number of fully opened leaves were recorded. The test plants were then transferred to 20-cm pots in a different glasshouse, and sprayed with imidacloprid (250 mg active ingredient in 5 l of water) using an aerosol applicator (DynaFog®, Westfield, IN, USA) during the first and second weeks to kill any newly emerging nymphs. The test plants were monitored at 2-week intervals and the plants showing SCFDV gall symptoms were removed. After 6 months, all plants not showing symptoms were cut at soil level and allowed to regrow. The regrowth was monitored for disease symptoms for a further 4 months.

2.3 Virus incidence in vectors

Adults and nymphs of *P. saccharicida* collected from the mixture of SCFDV-infected susceptible (NCo310) and resistant (WD1) plants used in trial 1 in November 2000 ($n = 198$), from SCFDV-infected resistant (WD1) plants in December 2001 ($n = 199$), and from SCFDV-infected susceptible (NCo310) plants in May 2002 ($n = 96$) were stored at −20°C. A reverse transcriptase-polymerase chain reaction method developed for detecting SCFDV in sugarcane plant samples (Smith and Van de Velde, 1994; James et al., 1999; Ridley et al., 2006) was used to detect the presence of SCFDV in individual planthoppers.

2.4 Data analysis

The data from the choice trials (table 1) were analysed using a factorial analysis of variance with trial and recipient cultivar as factors. As trial 1A used a different source population for virus acquisition to that in the other trials (table 1), such an analytical approach allows the teasing apart of the roles of the source and recipient populations in virus transmission. A significant interaction term would allow us to focus on the differences on transmission of SCFDV among cultivars within a trial (to examine the effect of recipient cultivar), while a comparison of virus transmission between trials for a given cultivar would enable the examination of the effect of source plant used for virus acquisition by the vector. Differences among cultivars within trials in terms of the SCFDV transmission would not be surprising as these cultivars were chosen because of their differential susceptibility to FLG. Our primary interest in this study was to synthesize the results across trials to understand the role of the source plant in virus acquisition and transmission. Chi-square analyses were used to assess whether virus acquisition by different wing-forms and developmental stages of the vector differed in relation to exposure to

Table 1. *Descriptions of* Sugarcane Fiji disease virus *(SCFDV) transmission trials conducted in the glasshouse during 2000–2002*

Trial no.	Trial method	Season	Source cultivars for virus acquisition	Vectors (per glasshouse or plant)	% proportion of nymphs	Vector wing-form (% proportion)	Replication (plants × cultivars × block)
1A	Choice	October 2000	NCo310 + WD1	>4000	55 ($n = 1283$)	Macropterous (95)	240 (10 × 6 × 4)
1B	No-choice	October 2000	NCo310 + WD1	10/plant	50 ($n = 570$)	Macropterous (95)	60 (10 × 6)*
2	Choice	February 2001	WD1	>6000	23 ($n = 5876$)	Macropterous (88)	96 (4 × 6 × 4)
3A	Choice	May 2001	WD1	480	50 ($n = 480$)	Brachypterous (78)	120 (5 × 6 × 4)
3B	Choice	May 2001	WD1	2669	14 ($n = 1188$)	Brachypterous (78)	120 (5 × 6 × 4)
4	No-choice	December 2001	WD1	15/plant	53 ($n = 170$)	Macropterous (97)	60 (10 × 6)*

*Plants × cultivars.

the different source plants. Spearman's correlation coefficient was used to investigate the effects of recipient plant vigour and vector population density on virus transmission.

3 Results and Discussion

In choice trials, the SCFDV transmission was erratic and varied from 0% to 52.5% (fig. 1). ANOVA revealed a significant interaction effect ($F_{15,72} = 2.22$, $P = 0.013$) indicating that virus transmission differed significantly among trials for a given recipient cultivar. There appeared to be a consistent pattern associated with the resistance status of source plant used for virus acquisition by the vector. Comparison of virus transmission within cultivars between trials revealed that, with the exception of cultivar Q110 (resistance rating 1), the transmission of the virus was significantly higher when the vectors acquired the virus from a mixed population of susceptible (NCo310) and resistant (WD1) cultivars than from resistant (WD1) cultivars alone (fig. 1). In no-choice trials, virus transmission was only observed when the vectors acquired the virus from the mixed source populations containing susceptible (NCo310) and resistant (WD1) cultivars (Q110–0%, Q87–17%, Q90–30%, Q124–25%, NCo310–0% and Q102–60%).

Polymerase chain reaction analyses revealed that this was because of the pattern of virus acquisition by the vectors in relation to the resistance status of source plants. The incidence of the virus in the vector populations was much higher in insects that were exposed to the susceptible (NCo310) cultivar and lowest when the insects were exposed to the resistant (WD1) cultivar, while those exposed to mixed stand of susceptible and resistant cultivars showed an intermediate incidence of SCFDV (fig. 2). These patterns were consistent for the different wing-forms and developmental stages of the vector (macropterous male: $\chi^2 = 79.75$, d.f. = 2, $P << 0.001$; macropterous female: $\chi^2 = 53.11$, d.f. = 2, $P << 0.001$; brachypterous female: $\chi^2 = 20.24$, d.f. = 2, $P << 0.001$; nymph: $\chi^2 = 38.33$, d.f. = 2, $P << 0.001$; fig. 2).

Vigour of the recipient cultivar (plant height, $R^2 = 0.02$; $P = 0.97$; number of leaves, $R^2 = 0.06$; $P = 0.63$) was not correlated with the transmission of the virus. Only when the virus was acquired from a mixture of susceptible (NCo310) and resistant (WD1) cultivars, the density of adults and nymphs was positively correlated with transmission (adults: Spearman's rho = 0.598, $P = 0.002$; nymphs: Spearman's rho = 0.647, $P = 0.001$).

The results suggest that sugarcane cultivars used as source plants for virus acquisition by the vector affect virus acquisition (fig. 2) and transmission efficiency (fig. 1). A re-examination of previous transmission trial results indicates that the transmission efficiency of *P. saccharicida* was higher (72%) when the nymphs were reared on SCFDV-infected susceptible NCo310 cultivar than on SCFDV-infected resistant Q87 (18%) and CP44–101 (15%) cultivars (BSES 1981, unpublished data). Furthermore, in the last decade, replacement of field populations of the susceptible cultivar (NCo310) with the resistant cultivar (WD1) as source plants for the virus and the vector has resulted in low disease transmission (DHILEEPAN et al., 2003). It appears that the variations in transmission levels in previous screening trials over the years could be due to varying levels of resistance to SCFDV in source plants used for virus acquisition by the vector. A similar effect of host plant resistance on the acquisition and transmission of *Tomato yellow leaf curl virus* by its whitefly vector has been reported (LAPIDOT et al., 2001).

Perkinsiella saccharicida is an inefficient vector with <25% of the individuals carrying the virus actually transmitting it (EGAN et al., 1989). This combined with the low rate of virus acquisition when the source plant

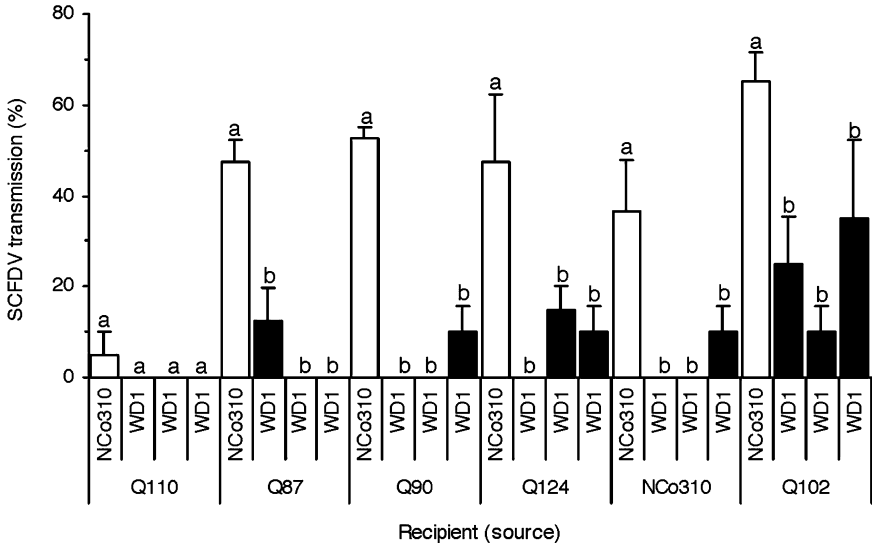

Fig. 1. *Variation in* Sugarcane Fiji disease virus *(SCFDV) transmission by* Perkinsiella saccharicida *in resistant (Q110 and Q87), moderately resistant (Q90 and Q124) and susceptible (NCo310 and Q102) cultivars in choice trials in relation to different source plants for virus acquisition. Solid bars represent trials using a mixture of susceptible (NCo310) and resistant (WD1) cultivars as source plants. Clear bars represent trials using a resistant (WD1) cultivar as source plants. Vertical bars represent standard error*

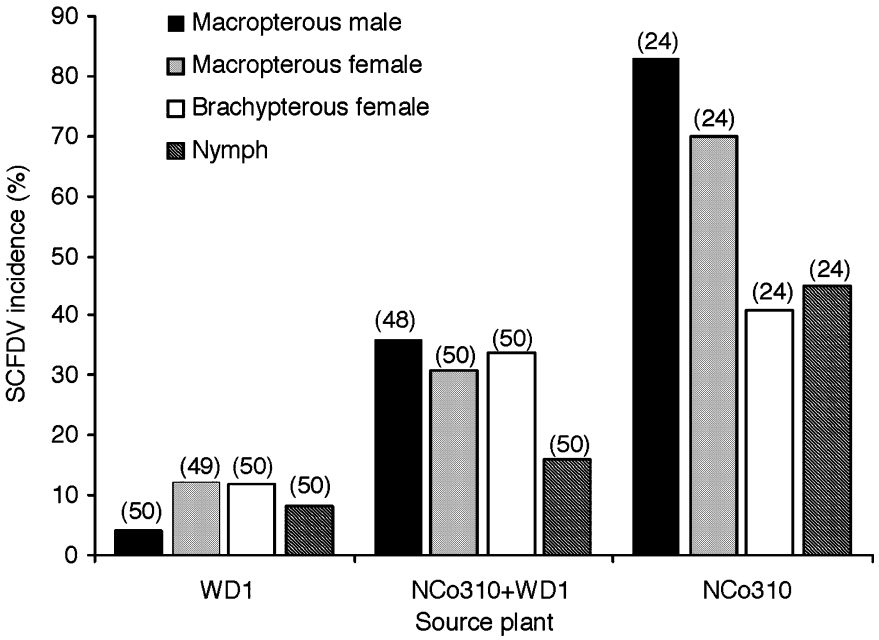

Fig. 2. *Incidence of* Sugarcane Fiji disease virus *(SCFDV) in macropterous males, macropterous females, brachypterous females and nymphs, raised on populations of the resistant (WD1) cultivar, a mixed population of susceptible (NCo310) and resistant (WD1) and on the susceptible (NCo310) cultivar. Numbers in brackets represent sample size*

is resistant, could further diminish its ability to transmit the disease. In the light of the results presented here, one possible explanation for these patterns could be the differential susceptibility of the source plants to the virus. Variation in the transmission efficiency of *P. saccharicida* reared on different virus source plant cultivars could either be due to variation in SCFDV titre between cultivars or variation in the ability of the vector to acquire the virus from different cultivars. Though it has been suggested that the susceptibility of sugarcane cultivars is related to the proportion of time spent on phloem feeding by *P. saccharicida* (Chang and Ota, 1978), recent electronic penetration graph studies suggest that there is no difference in the duration of phloem feeding between susceptible and resistant cultivars (B.J. Croft, unpublished data). Furthermore, there was no difference in the concentration of SCFDV detected between resistant and susceptible cultivars (Ridley, unpublished data). However, the proportion of leaf area galled was significantly greater in susceptible cultivars than in resistant cultivars (Ridley, unpublished data). This suggests that higher proportion of vectors acquire the virus from susceptible cultivars, possibly due to increased probability of the vector feeding on a gall where the virus is concentrated (Ridley, unpublished data).

Acknowledgements

Help from Kim Messenger, Mati Ronkainen and Andrew Greet, and facilities from BSES Woodford are gratefully acknowledged. Gimme Walter, Peter Allsopp and Myron Zalucki provided constructive comments on transmission experiments and earlier versions of the manuscript.

References

Chang, V. C. S.; Ota, A. K., 1978: Feeding activities of *Perkinsiella* leafhoppers and the Fiji disease resistance of sugar cane. J. Econ. Entomol. **71,** 297–300.

Croft, B. J.; Ryan, C. C., 1984: Acquisition of Fiji Disease Virus by *Perkinsiella saccharicida* Kirk. and Observations on its Instars. Brisbane, Australia: Bureau of Sugar Experiment Stations Internal Report.

Croft, B. J.; James, A. P.; Ridley, A.W.; Smith, G. R., 2004: Developing methods to screen sugarcane varieties for resistance to Fiji leaf gall. Proc. Aust. Soc. Sugar Cane Technol. **26,** 1–11.

Daniels, J.; Husain, A. A.; Hutchinson, P. B.; Wismer, C. A., 1969: An insectary method for testing sugarcane varieties for resistance to Fiji disease. Proc. Int. Soc. Sugar Cane Technol. **13,** 1100–1106.

Dhileepan, K.; Croft, B. J., 2003: Resistance to Fiji disease in sugarcane: role of cultivar preference by planthopper vector *Perkinsiella saccharicida* (Homoptera: Delphacidae). J. Econ. Entomol. **96,** 148–155.

Dhileepan, K.; Greet, A.; Ridley, A.; Croft, B. J.; Smith, G. R., 2003: Fiji disease resistance in sugarcane: relationship to cultivar preference in field populations of the planthopper vector *Perkinsiella saccharicida*. Ann. Appl. Biol. **143,** 375–379.

Egan, B. T., 1982: Monitoring the Fiji Disease Epidemic in the Bundaberg District. Internal Project Report. Brisbane, Australia: Bureau of Sugar Experiment Stations, 46 pp.

Egan, B.T.; Ryan, C. C., 1986: Predicting disease incidence and yield losses in sugarcane in a Fiji disease epidemic. In: Plant Virus Epidemics: Monitoring, Modeling and Predicting Outbreaks. Ed. by Mclean, G. D.; Garrett, R. G.; Ruesink, W. G. Sydney, Australia: Academic Press, 443–457.

Egan, B. T.; Ryan, C. C.; Francki, R. I. B., 1989: Fiji disease. In: Diseases of Sugarcane – Major Diseases. Ed. by Ricaud, C.; Egan, B. T.; Gillaspie, A. G.; Hughes, C. G. Amsterdam, The Netherlands: Elsevier, 263–287.

Francki, R. I. B.; Grivell, C. J., 1972: Occurrence of similar particles in Fiji disease virus-infected sugarcane and insect vector cells. Virology **48,** 305–307.

Francki, R. I. B; Ryan, C. C.; Hatta, T.; Rohozinski, J.; Grivell, C. J., 1986: Serological detection of Fiji disease virus antigens in the planthopper *Perkinsiella saccharicida* and its inefficient ability to transmit the virus. Plant Pathol. **35,** 324–328.

Hatta, T.; Fracki, R. I. B., 1976: Anatomy of virus-induced galls on leaves of sugarcane infected with Fiji disease virus and the cellular distribution of virus particles. Physiol. Plant Pathol. **9,** 321–330.

Hayes, A. G., 1972: Fiji disease resistance trials at Condong Mill, New South Wales. Proc. Qld. Soc. Sugar Cane Technol. **39,** 185–190.

Hughes, C. G.; Robinson, P. E., 1961: Fiji disease. In: Sugarcane Diseases of the World, Vol. 1. Ed. by Martin, J. P.; Abbott, E. V.; Hughes, C. G. Amsterdam, The Netherlands: Elsevier, 389–405

Hutchinson, P. B.; Francki, R. I. B., 1973: Sugarcane Fiji Disease Virus. Description of Plant Viruses, Vol. 119. Wallingford, UK: CAB International.

James, A. P.; Fowler, A. R.; Hekmeijer, S.; Smith, G. R.; Whittle, P. J. L., 1999: Indexing quarantined sugarcane for Fiji disease virus using RT-PCR. In: 12th Biennial Asia-Pacific Plant Pathology Conference Canberra: Australian Plant Pathology Society, 51.

Lapidot, M.; Friedmann, M.; Pilowsky, M.; Ben-joseph, R.; Cohen, S., 2001: Effect of host plant resistance to *Tomato yellow leaf curl virus* (TYLCV) on virus acquisition and transmission by its whitefly vector. Phytopathology **91,** 1209–1213.

Ledger, P. E.; Ryan, C. C., 1977: Screening of sugarcane varieties for resistance to Fiji disease in Queensland: the insectary-glasshouse method. Proc. Qld. Soc. Sugar Cane Technol. **44,** 79–82.

Matthews, R. E. F., 1982: Classification and nomenclature of viruses. Fourth Report of the International Committee on Taxonomy of Viruses. Intervirology **17,** 1–199.

Mungomery, R. W.; Bell, A. F., 1933: Fiji Disease of Sugar Cane and its Transmission. Bulletin No. 4. Brisbane, Australia: Bureau of Sugar Experiment Stations, Division of Pathology.

Reimers, J. F.; Hall, P.; Hogarth, D. M., 1982: The relationship between Fiji disease susceptibility and yield. Proc. Aust. Soc. Sugar Cane Technol. **4,** 103–110.

Ridley, A. W.; Dhileepan, K.; Hohnson, K. N.; Allsopp, P. G.; Nutt, K. A.; Walter, G. H.; Croft, B. J., 2006: Is the distribution of Fiji leaf gall in Australian sugarcane due to variation in vector *Perkinsiella saccharicida*? Australas. Plant Pathol. **35** (In press).

Ryan, C. C., 1988: Epidemiology and control of Fiji disease virus of sugarcane. In: Advances in Disease Vector Research, Vol. 5. New York: Springer-Verlag, 163–176.

Smith, G. R.; Candy, J. M., 2004: Improving Fiji disease resistance screening trials in sugarcane by considering virus transmission class and possible origin of *Fiji disease virus*. Aust. J. Agric. Res. **55,** 665–672.

Smith, G. R.; Van de Velde, R., 1994: Detection of sugarcane mosaic virus and Fiji disease virus in diseased sugarcane using the Polymerase Chain Reaction. Plant Dis. **78,** 557–561.

Author's address: Dr K. Dhileepan (corresponding author), Alan Fletcher Research Station, Department of Natural Resources & Mines, PO Box 36, Sherwood, Qld 4075, Australia. E-mail: k.dhileepan@qld.gov.au

