

**Biology, Field Release and Monitoring of the Rust Fungus
Puccinia spegazzinii (Pucciniales: Pucciniaceae),
a Biological Control Agent of *Mikania micrantha* (Asteraceae) in
Papua New Guinea and Fiji**

**M. D. Day¹, A. P. Kawi², J. Fidelis³, A. Tunabuna⁴, W. Orapa⁴, B. Swamy⁵,
J. Ratutini⁵, J. Saul-Maora³ and C. F. Dewhurst⁶**

¹Department of Employment, Economic Development and Innovation, Biosecurity Queensland, Ecosciences Precinct, GPO Box 267, Brisbane, Qld 4001, Australia

Michael.Day@deedi.qld.gov.au

²National Agriculture Research Institute, Island Regional Centre, PO Box 204, Kokopo, East New Britain, Papua New Guinea anna.kawi@nari.org.pg

³PNG Cocoa and Coconut Research Institute, PO Box 1846, Rabaul, East New Britain, Papua New Guinea

⁴Secretariat of the Pacific Community, Land Resource Division, 3 Luke Street, Nabua, Private Mail Bag, Suva, Fiji Islands

⁵Koronivia Research Station, Ministry of Primary Industries, PO Box-77, Nausori, Fiji Islands

⁶PNG Oil Palm Research Association Inc, P.O. Box 97, Kimbe, West New Britain Province, Papua New Guinea

Abstract

Mikania micrantha Kunth (Asteraceae), mikania or mile-a-minute, is a neotropical plant species now found in all lowland provinces of Papua New Guinea (PNG) and all major islands of Fiji. The weed invades plantations and cropping areas, thereby reducing productivity and threatening food security of rural communities. As part of an Australian Government-funded biological control program, the rust fungus *Puccinia spegazzinii* de Toni was imported into PNG and Fiji in 2008 and released. Life cycle studies were conducted in PNG and inoculation techniques were evaluated. Field releases were made in areas where *M. micrantha* was abundant and monthly sampling at three sites determined the impact of the rust on *M. micrantha* in the field. *P. spegazzinii* is a microcyclic, autoecious rust, with a life cycle of 15-21 days. The most efficient inoculation method was to place 3-4 week old plants under infected plants in a perspex inoculation chamber for 48 hours at 26±1°C and 98% relative humidity. The most efficient field release method was to transplant about five infected 3-4 week old plants in amongst *M. micrantha* growing densely under canopy or in gullies where there is adequate water and humidity. The rust fungus has now been released at nearly 560 sites in 15 provinces in PNG and over 80 sites on four islands in Fiji. In PNG, the rust has established at over 180 sites in 11 provinces, spreading up to 40 km from some sites; while in Fiji, it has established at 25 sites on two islands. Detailed field monitoring has shown that *P. spegazzinii* reduces mikania density and therefore has the potential to control this weed in many parts of both countries.

Introduction

Mikania micrantha Kunth (Asteraceae), mikania or mile-a-minute, is an aggressive neotropical vine. It has become a major weed throughout Asia and the South Pacific (Waterhouse and Norris, 1987; Waterhouse, 1994). It grows about 1 m/month and flowers profusely, producing thousands of light-weight barbed seeds. The seeds are spread by wind, by people on clothing and possessions, and by animals on their fur. *M. micrantha* is a particular problem on subsistence farms, where slash and burn agriculture is practiced. The plant can quickly invade cleared lands and smother crops. The main control method on subsistence farms is hand-pulling or slashing, as the use of herbicides is not economically feasible for resource-poor farmers (Day et al. 2011).

Plants can reshoot from broken stems and the vine's rapid growth rate means that land holders have to constantly clear land of the weed (Waterhouse and Norris, 1987; Holm et al., 1991). In subsistence farms, *M. micrantha* can grow over and kill crops such as taro, banana and papaw, while in plantations, it can smother cocoa, coconut seedlings or young oil palm trees, reducing flowering and productivity (Day et al., in press).

Biological control of *M. micrantha* was first attempted in the 1980s, with the introduction of the thrips *Liothrips mikaniae* (Priesner) (Thysanoptera: Phlaeothripidae) into the Solomon Islands in 1988 and Malaysia in 1990 (Cock et al., 2000). However, it failed to establish in either country (Julien and Griffiths, 1998; Evans and Ellison, 2005). The thrips was also sent to Papua New Guinea (PNG) in 1988 but the colony died out in quarantine before field releases could be conducted (Cock et al., 2000).

Renewed efforts into the biological control of *M. micrantha* commenced in 2005 with the release of the rust *Puccinia spegazzinii* de Toni (Pucciniales: Pucciniaceae) into India, mainland China and Taiwan, following exploration and host specificity testing by CABI Europe-UK (Evans and Ellison, 2005; Ellison et al., 2008; Ellison and Day, 2011). However, the rust appears to have established only in Taiwan (Ellison and Day, 2011).

Biological control of *M. micrantha* in the Pacific recommenced in 2006 following meetings of the Pacific Plant Protection Organization held in 2002 and 2004, where *M. micrantha* was rated as the

second most important weed in the region behind the vine *Merremia peltata* L. (Convolvulaceae) (Dovey et al., 2004). A project, funded by the Australian Government and managed by the Queensland Government, aimed to introduce into PNG and Fiji safe biological control agents that were effective elsewhere (Pene et al., 2007).

Two butterfly species, *Actinote antea*s (Doubleday and Hewitson) (Lepidoptera: Arctiidae) and *A. thalia pyrrrha* Fabr., were imported into Fiji in 2006 from Indonesia, where they were reported to aid the control of chromolaena, *Chromolaena odorata* (L.) King and Robinson (Asteraceae), and also damage *M. micrantha* (R. Desmier de Chenon pers. comm. 2006). However, colonies of both species died out before additional host-specificity testing could be completed. The rust *P. spegazzinii* was introduced into both PNG and Fiji in November 2008, following testing by CABI of additional plant species of concern to both countries. This paper reports on the biology, field release and monitoring of *P. spegazzinii* in PNG and Fiji.

Materials and Methods

Biological and culturing

Four small bare-rooted *M. micrantha* plants, each infected seven days prior with *P. spegazzinii* collected from Ecuador, were imported from CABI Europe-UK into quarantine facilities at the National Agriculture Research Institute, Island Regional Center, at Kerevat, East New Britain, PNG and the Secretariat of the Pacific Community, Fiji in November 2008. Plants were potted into clean pots with sterilized soil and held in a laboratory to aid recovery.

When the pustules on the imported plants reached maturity (about 15 days after inoculation), the infected plants were placed on a stand in a perspex inoculation chamber (60cm x 60cm x 45cm) within a quarantine laboratory (26±1°C, natural lighting). Healthy 3-4 week old *M. micrantha* plants, grown from 3cm long cuttings and containing 2-4 pairs of leaves, were placed under the infected plants. The chamber was sealed and the plants left for 48 hours for sporulation to occur and the fresh plants to be inoculated. After two days, the newly inoculated

plants were removed and placed under a light bank in the laboratory for 48 hours prior to being placed in a shade house for pustules to develop. Plants were watered as necessary.

A culture of *P. spegazzinii* was maintained by repeating the above steps, using plants with mature pustules to inoculate young healthy plants. The life cycle of the rust was determined through daily observations of developing pustules.

To facilitate an increased number of field releases, small 3-4 week old plants were placed for 4-5 days in a field site where the rust had been established previously. The plants were then returned to the shade house for the pustules to develop. Using this field inoculation technique, in contrast with the laboratory procedure, many more plants can be inoculated.

Field release and monitoring

Field releases of *P. spegazzinii* were conducted by transplanting infected potted plants amongst patches of actively growing *M. micrantha* such that the infected plants trailed over the field plants to encourage the inoculation process. Releases were generally conducted in areas where there was greater shade and damp soil to help keep the potted plants alive until infection of the field plants occurred. Release sites were inspected about three months after the rust was released, by which time the pustules had a chance to develop on the field plants and be more easily seen.

Two release sites near the research station at Kerevat were monitored in detail every month. At each site, ten 1m² quadrats were placed randomly and the percent plant cover by *M. micrantha* was recorded in each. The number of infected leaves, petioles and stems in each quadrat was recorded.

Results

Biology

P. spegazzinii has a life cycle of 15-21 days. Tiny white spots appear on the upper leaf surface about six days after inoculation and the pustules continue to develop and grow, turning yellow by 11 days. Mature pustules become brown by day

15-17 when they are ready to infect other plants.

Field release and monitoring

In PNG, the rust was released at over 560 sites in all 15 provinces infested with *M. micrantha*. Of the sites which were re-checked, the establishment rate was about 50%, with pustules being found at over 180 sites in 11 provinces. The rust established better in the wetter provinces of Oro (100% of release sites), Western (82%) and East New Britain (78%). Although the rust established at only three release sites out of seven checked in East Sepik Province, it spread up to 40 km in 16 months. The rust also spread widely in Oro and West New Britain provinces (Fig. 1).

Establishment was poor in Northern Solomons (4% of release sites), Sandaun (11%), Gulf (13%) and Madang (22%) provinces. To date, the rust has not established in Milne Bay, Morobe and New Ireland provinces, as well as around Port Moresby but recent release sites still need to be revisited (Fig. 1).

In Fiji, *M. micrantha* was found on all major islands. The rust was released at over 80 sites on the islands of Viti Levu, Vanua Levu, Taveuni and Ovalau. Pustules were found at 39% of sites checked on Viti Levu and 38% of sites on Vanua Levu (Fig. 2). Establishment was better on the wetter eastern side of Viti Levu than the drier western side of the island. On the eastern side of Viti Levu, the rust has begun to spread to other sites. No spread has been reported on Vanua Levu to date. Release sites on Taveuni and Ovalau are yet to be checked (Fig. 2).

In the field at one site near Kerevat, PNG, *P. spegazzinii* suppressed the growth of *M. micrantha*, allowing the growth of other plants (such as clycine *Glycine wightii*) over the *M. micrantha*, further reducing its cover. During a subsequent long dry season in 2010, the rust was not detected and *M. micrantha* began to increase again. However, the rust re-appeared after rains in early 2011 and the growth of *M. micrantha* is again beginning to be checked (Fig. 3).

At Tavilo, East New Britain (Fig. 4), the rust is suppressing *M. micrantha*, with cover decreasing from 100% to 40% following the release of the rust. Monitoring at both sites in PNG is continuing.

Discussion

M. micrantha is considered a major weed in most wet lowland areas of both PNG and Fiji (Day et al., in press). The rapid growth rate of *M. micrantha* and its ability to smother crops and reduce productivity is a concern for land holders. Conventional control methods such as hand-pulling and slashing is time-consuming and the plant can re-shoot from the broken fragments left behind (Waterhouse and Norris, 1987; Holm et al., 1991). Biological control therefore is seen as a viable strategy.

Following additional host specificity testing by CABI, *P. spegazzinii* was approved for release in both countries. Laboratory trials suggested that the rust has the ability to reduce the growth rate of *M. micrantha*, which should reduce its competitiveness and limit its ability to smother crops (Day et al., 2011). Field monitoring at two sites confirmed that plant density has decreased since the release of the rust.

The rust has been widely released in PNG and has established in most provinces, where it is beginning to disperse from the release sites. At sites where it is currently in low abundance due to being

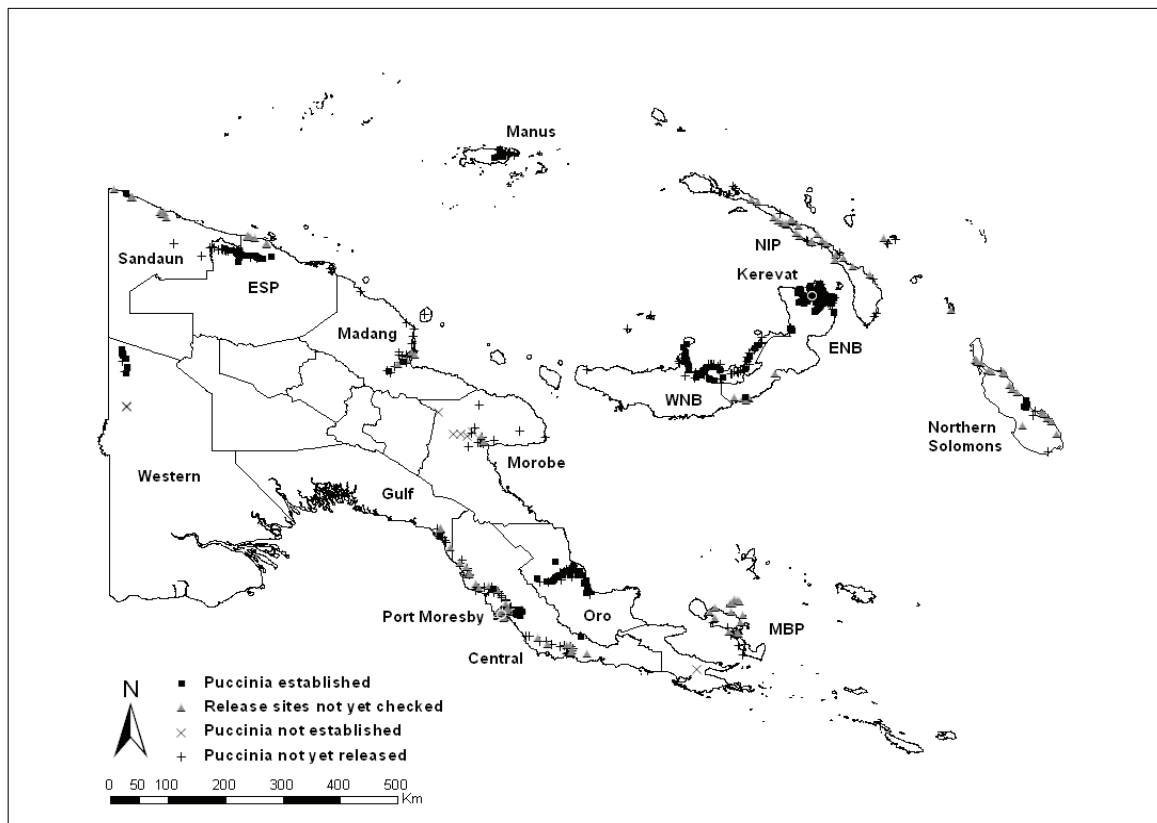


Figure 1. The distribution of *M. micrantha* in Papua New Guinea (all symbols); and the sites where *P. spegazzinii* is established, released but establishment unconfirmed, not established, and not yet released. ESP = East Sepik Province, ENB = East New Britain Province, MBP = Milne Bay Province, NIP = New Ireland Province and WNB = West New Britain.

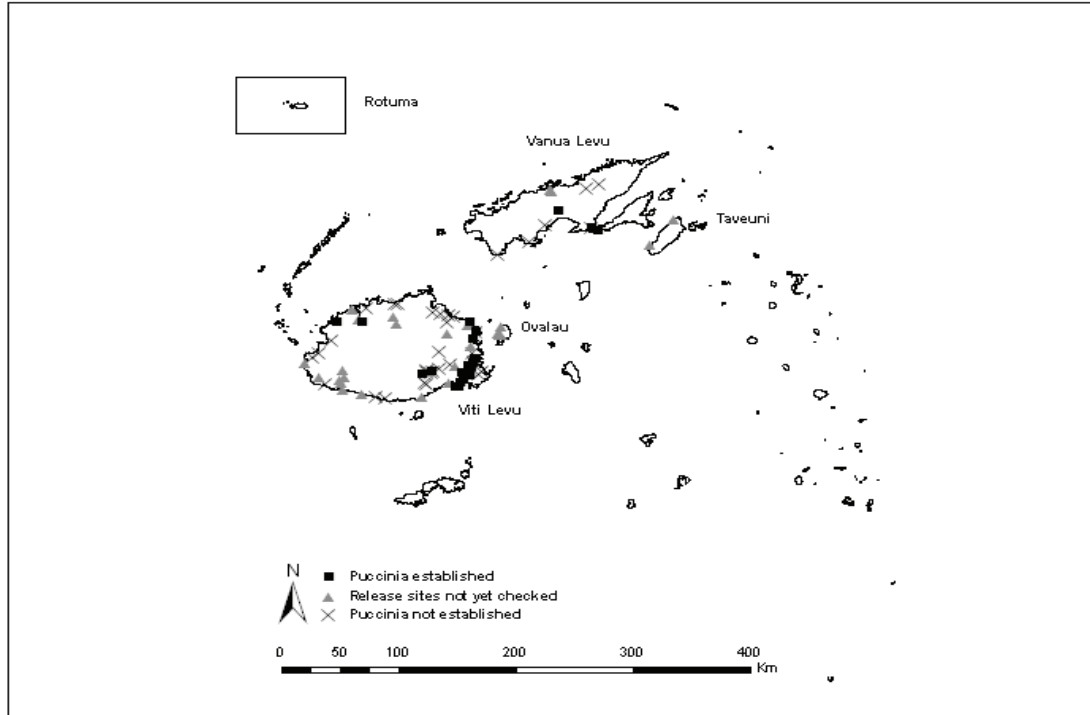


Figure 2. Sites in Fiji where *P. spegazzinii* is established, released but establishment unconfirmed, and not established.

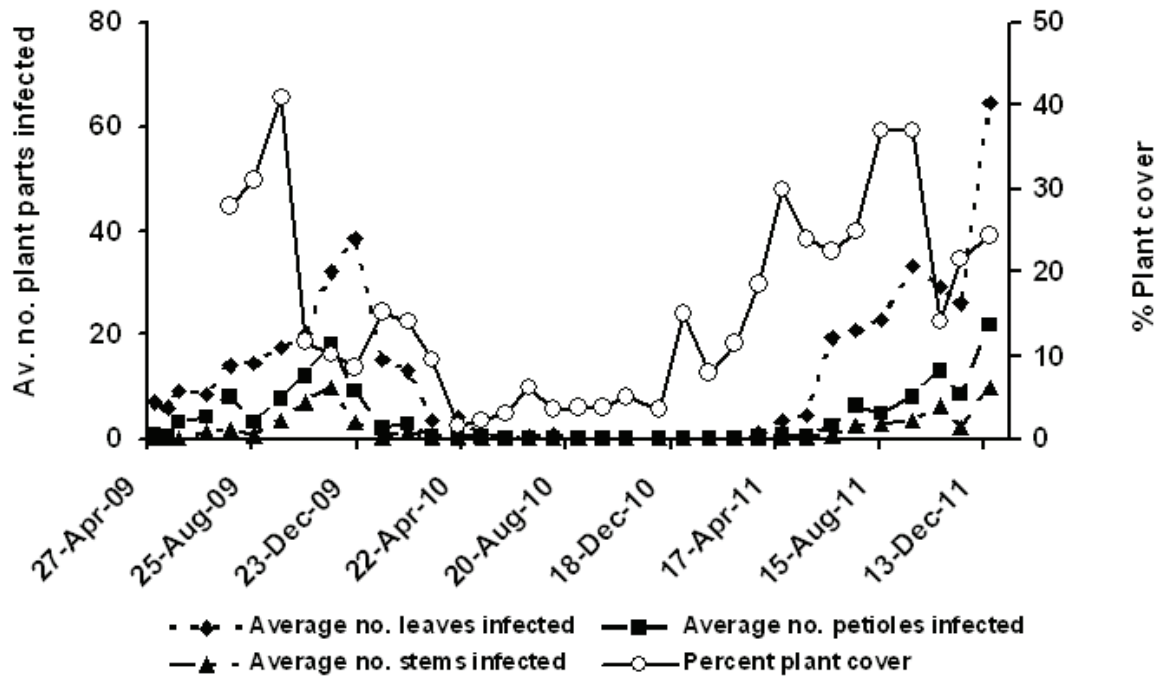


Figure 3. The mean number of leaves, petioles and stems of *M. micrantha* infected by *P. spegazzinii* per 1 m² and the percent plant cover of *M. micrantha* at site 1, Kerevat, East New Britain, PNG (release date: 10 January 2009).

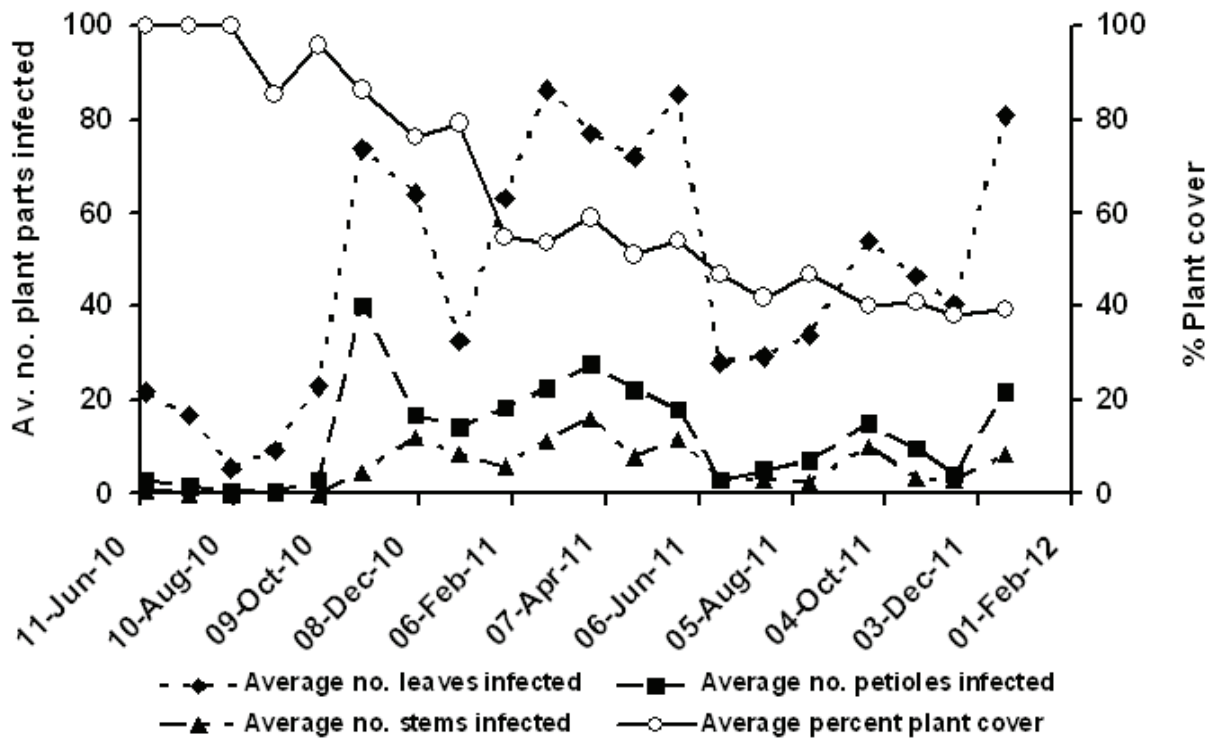


Figure 4. The mean number of leaves, petioles and stems of *M. micrantha* infected by *P. spegazzinii* per 1 m² and the percent plant cover of *M. micrantha* at site 2, near Kerevat, East New Britain, PNG (release date: 31 March 2010).

recently released, it is anticipated that populations will increase with time and it is expected that *P. spegazzinii* will have similar impacts on *M. micrantha* at these sites as at the monitoring sites. However, at some sites which are drier, such as in parts of Gulf and Morobe provinces and around Port Moresby, the impact of the rust may be less.

There are still many release sites that need to be checked and still a few sites where the rust has not yet been released, especially in more remote regions. The release and monitoring program will therefore continue in an effort to get the rust established in all parts of PNG where *M. micrantha* occurs.

In Fiji, *P. spegazzinii* has not been as widely released. There were lengthy delays in obtaining approval to release the rust; and many sites, particularly those away from the main islands of Viti Levu and Vanua Levu, are difficult and costly to reach. The rust has established well in the eastern areas of Viti Levu, which are much wetter than the western part of the island. It has also established

at several sites on Vanua Levu. Field monitoring continues, with a slight decrease in plant density observed following the release of the rust.

In other countries where *P. spegazzinii* has been released, establishment has been patchy. The rust has appeared to have established in Taiwan but does not appear to have established in mainland China or India (Ellison and Day, 2011). Following the promising results seen in PNG, plans are underway to introduce *P. spegazzinii* into Vanuatu and Guam and re-introduce it into mainland China.

Acknowledgements

The authors wish to acknowledge the Australian Center for International Agricultural Research for funding the project and provincial agricultural personnel in PNG and Fiji for assisting with field releases of the rust. CABI Europe-UK conducted the host specificity testing and supplied *P. spegazzinii*

to PNG and Fiji. Drs. Dane Panetta and Bill Palmer provided valuable comments on earlier versions of the manuscript.

References

- Cock, M.J.W., Ellison, C.A., Evans, H.C. & Ooi, P.A.C. (2000) Can failure be turned into success for biological control of mile-a-minute weed (*Mikania micrantha*)? In Proceedings of the X International Symposium on Biological Control of Weeds (ed Spencer, N.R.), pp. 155–167. Bozeman, Montana, USA.
- Day, M.D., Kawi, A., Tunabuna, A., Fidelis, J., Swamy, B., Ratutuni, J., Saul-Maora, J., Dewhurst, C.F. & Orapa, W. (2011) The distribution and socio-economic impacts of *Mikania micrantha* (Asteraceae) in Papua New Guinea and Fiji and prospects for its biocontrol. In Proceedings of the 23rd Asian-Pacific Weed Science Society Conference, pp 146–153. Asian-Pacific Weed Science Society.
- Day, M.D., Kawi, A., Kurika, K., Dewhurst, C.F., Waisale, S., Saul Maora, J., Fidelis, J., Bokosou, J. Moxon, J., Orapa, W. and Senaratne, K.A.D. (in press) *Mikania micrantha* Kunth (Asteraceae) (mile-a-minute): Its distribution and physical and socio economic impacts in Papua New Guinea. Pacific Science.
- Dovey, L., Orapa, W. & Randall, S. (2004) The need to build biological control capacity in the Pacific. In Proceedings of the XI International Symposium on Biological Control of Weeds (eds Cullen, J.M., Briese, D.T., Kriticos, D.J., Lonsdale, W.M., Morin, L. & Scott, J.K.), pp. 36–41. CSIRO Entomology Canberra, Australia.
- Ellison, C. & Day, M. (2011) Current status of releases of *Puccinia spegazzinii* for *Mikania micrantha* control. Biocontrol News and Information **32**, 1N.
- Ellison, C.A., Evans, H.C., Djeddour, D.H. & Thomas, S.E. (2008) Biology and host range of the rust fungus *Puccinia spegazzinii*: A new classical biological control agent for the invasive, alien weed *Mikania micrantha* in Asia. Biological Control **45**, 133–145.
- Evans, H.C. & Ellison, C.A. (2005) The biology and taxonomy of rust fungi associated with the neotropical vine *Mikania micrantha*, a major invasive weed in Asia. Mycologia **97**, 935–947.
- Holm L.G., Plucknett, D.L., Pancho, J.V. & Herberger, J.P. (1991) The World's Worst Weeds: Distribution and Biology. Kreiger Publishing Company, Malabar, Florida.
- Julien, M.H. & Griffiths, M.W. (1998) Biological Control of Weeds. A World Catalogue of Agents and their Target Weeds. Fourth Edition. CABI Publishing, Wallingford, U.K. 223p.
- Pene, S., Orapa, W. & Day, M. (2007) First fungal pathogen to be utilized for weed biocontrol in Fiji and Papua New Guinea. Biocontrol News and Information **28**, 55N–56N.
- Waterhouse, D.F. (1994) Biological Control of Weeds: Southeast Asian Prospects. ACIAR, Canberra. 302 pp.
- Waterhouse, D.F. & Norris, K.R. (1987) Biological Control: Pacific Prospects. Inkata Press, Melbourne. 355–372 pp.