
CHAPTER 12. ESTIMATING THE HOST RANGE OF THE TACHINID *TRICHOPODA GIACOMELLII*, INTRODUCED INTO AUSTRALIA FOR BIOLOGICAL CONTROL OF THE GREEN VEGETABLE BUG

M. Coombs

CSIRO Entomology, 120 Meiers Road, Indooroopilly, Queensland, Australia 4068
marc.coombs@csiro.au

BACKGROUND

DESCRIPTION OF PEST INVASION AND PROBLEM

Nezara viridula (L.) is a cosmopolitan pest of fruit, vegetables, and field crops (Todd, 1989). The native geographic range of *N. viridula* is thought to include Ethiopia, southern Europe, and the Mediterranean region (Hokkanen, 1986; Jones, 1988). Other species in the genus occur in Africa and Asia (Freeman, 1940). First recorded in Australia in 1916, *N. viridula* soon became a widespread and serious pest of most legume crops, cucurbits, potatoes, tomatoes, passion fruit, sorghum, sunflower, tobacco, maize, crucifers, spinach, grapes, citrus, rice, and macadamia nuts (Hely *et al.*, 1982; Waterhouse and Norris, 1987). In northern Victoria, central New South Wales, and southern Queensland, *N. viridula* is a serious pest of soybeans and pecans (Clarke, 1992; Coombs, 2000). Immature and adult bugs feed on vegetative buds, developing and mature fruits, and seeds, causing reductions in crop quality and yield. The pest status of *N. viridula* in Australia is assumed to be partly due to the absence of parasitoids of the nymphs and adults. No native Australian tachinids have been found to parasitize *N. viridula* effectively, although occasional oviposition and development of some species may occur (Cantrell, 1984; Coombs and Khan, 1997).

Previous introductions of biological control agents to Australia for control of *N. viridula* include *Trichopoda pennipes* (Fabricius) and *Trichopoda pilipes* (Fabricius) (Diptera: Tachinidae), which are important parasitoids of *N. viridula* in the southern United States (Jones, 1988). Neither species established in Australia (Waterhouse and Norris, 1987) and would not now be considered for introduction because of their apparent lack of host specificity. Both species have

a broad host range that reportedly includes species of Coreidae, Scutelleridae, Largidae, Mantidae, and Acrididae (Arnaud, 1978; Follett *et al.*, 1999). An additional tachinid, *Bogosia antinorii* Rondani, which is native to Kenya, was introduced but similarly failed to establish (Waterhouse and Sands, 2001). Several species of parasitoids of eggs (primarily Scelionidae) have been released, of which *Trissolcus basalis* (Wollaston) has contributed to the control of *N. viridula* in southeastern Australia (Waterhouse and Norris, 1987; Waterhouse and Sands, 2001). In certain regions of eastern Australia, particularly those that produce soybeans and nut crops, *N. viridula* has remained a significant pest (Clarke and Walter, 1993; Coombs 2000).

In South America, *Trichopoda giacomellii* (Blanchard) has been shown to regulate populations of *N. viridula* in soybeans in conjunction with *T. basalis* (Liljestrom and Bernstein, 1990; Ferreira *et al.*, 1991). Based on its performance in Argentina, *T. giacomellii* was identified as a promising potential agent for biological control of *N. viridula* in Australia (Figure 1).

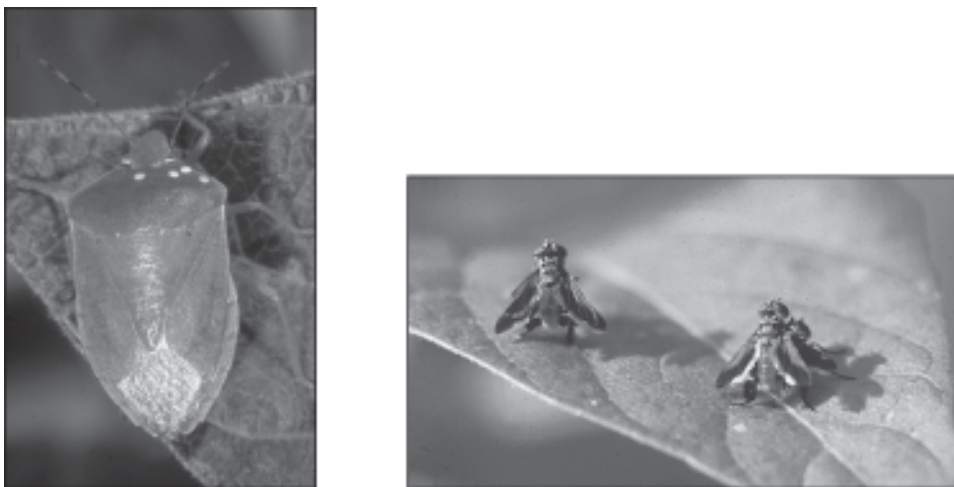


Figure 1. Adult *Nezara viridula* (L.) with parasitoid eggs attached (left) and mating pairs of *Trichopoda giacomellii* (Blanchard) (right). Photos: CSIRO. (UGA1295004 and UGA1295005)

DESCRIPTION OF AGENT PROPOSED FOR INTRODUCTION

Biology and life-history of agent Adults of *T. giacomellii* live from 4 to 15 days, and each female lays up to 275 eggs, which are deposited externally on the host thorax and abdomen (Coombs, 1997). Supernumerary parasitism is common (2-13 parasitoid eggs per host), although only one parasitoid completes development (La Porta, 1990). Pupariation occurs in nearby soil (La Porta, 1987). Death of the host is coincident with or occurs shortly after parasitoid emergence (Coombs and Khan, 1998). Field studies in the fly's native range indicate that 80 to 90% of host individuals may be parasitized, dependent on crop type and time of year (La Porta, 1990).

Hosts in the native range of agent In Argentina, *T. giacomellii* is an important, relatively specific parasitoid of adult and late-instar nymphs of *N. viridula* (La Porta, 1990; Liljestrom, 1991). Indigenous hosts of *T. giacomellii* in Argentina include *Acrosternum musiva* (Bergroth), *Acrosternum herbida* (Stål), *Acladra kinbergii* (Stål), *Edesia meditabunda* (Fabricius), and *Piezodorous guildinii* (Westwood) (Liljestrom, 1980; La Porta, 1987), whereas parasitism of

N. viridula represents a new association (*sensu* Hokkanen and Pimentel, 1984, 1989). *Nezara viridula* was first reported in Argentina in 1919 (La Porta and de Crouzel, 1984). Clearly, *T. giacomellii* has a demonstrated ability to expand its host range.

Source of agent Live specimens of *T. giacomellii* were imported to Australia from La Plata, Argentina (34° 58' S, 57° 53' W) in February 1994 (Sands and Coombs, 1999). Climate matching (Sutherst and Maywald, 1985) indicated the suitability of eastern mainland Australia for survival of *T. giacomellii* (D. P. A. Sands, unpublished data).

Other known hosts. A parallel biological control program for *N. viridula* in South Africa, using *T. giacomellii* originating from this project, reported inclusion of the South African native pentatomids *Bathycoelia natalicola* Schouteden and *Nezara pallidoconspersa* Stål as field hosts (M. van den Berg, pers. comm.). Both species are pests of macadamia, and any potential population suppression by *T. giacomellii* was viewed as desirable.

DESCRIPTION OF FAUNA IN AREA OF INTRODUCTION

AUSTRALIAN SPECIES RELATED TO THE TARGET PEST

No native insects in the genus *Nezara* occur in Australia Gross (1976) included *Glaucias amyoti* (White), *Alciphron glaucus* (Fabricius), and *Plautia affinis* Dallas, along with *N. viridula*, in a *Pentatoma* species group. Subsequent revision by Cassis and Gross (2002) placed *G. amyoti*, *G. sulcata*, and *A. glaucus* in the *Nezarini*, but *P. affinis* was transferred to the *Antestini*, which includes *Anaxilaus* (3 spp.), *Antestiopsis* (2 spp.), *Novatilla* (2 spp.), and *Plautia* (3 spp.). Compilation of the host test list pre-dated the Cassis and Gross (2002) revision and was based solely on the Gross (1976) species groupings. Additional species of *Plautia* and *Glaucias* and possibly other *Antestini* would likely have been included on the test list had the review of Cassis and Gross (2002) been available earlier.

More generally, the Australian subfamily Pentatominae contains 321 species in 113 genera, of which 303 species and 83 genera are endemic to Australia. In the family Pentatomidae as a whole, the Australian fauna includes 360 species in 135 genera, of which 330 species and 94 genera are endemic to Australia.

LOCAL SPECIES OF VALUE AS BIOLOGICAL CONTROL AGENTS

Members of the predatory Asopinae (8 genera and 11 species) are of value as native biological control agents. *Oechalia schellenbergii* (Guèrin-Mèneville) and *Cermatulus nasalis* (Westwood) are common in agricultural habitats co-occurring with *N. viridula* and *P. affinis*.

LOCAL SPECIES OF MARKED CONSERVATION VALUE

No Australian pentatomids are currently recognized as endangered, nor do any have icon status (Clarke and Spier-Ashcroft, 2003). Given the high degree of endemism of the fauna (70% of the genera and 90% of the species) most Australian pentatomids represent evolutionarily unique and valuable species or groups of species.

THE TESTING PLAN: ANALYSIS OF METHODS

SELECTION OF SPECIES FOR THE TEST LIST

Species selected The list of species tested is given in Table 1.

Of the three species closely allied to *N. viridula*, *P. affinis* is a pest of agricultural and horticultural crops often found in close association with *N. viridula*, *G. amyoti* is a forest-adapted species with occasional records as a minor pest of horticultural crops; and *A. glaucus* is confined to rainforest habitats in coastal eastern Australia. All three species are native to Australia. Representative species from the families Scutelleridae, Tessaratomidae, and Coreidae were included in the host test list because the related tachinids *T. pennipes* and *T. pilipes* have some hosts in these groups. *Trichopoda giacomellii* is not known to attack these groups in its native range.

Table 1. Homoptera selected for host specificity studies with *Trichopoda giacomellii*.

Pentatomidae	
<i>Glaucias amyoti</i> (White)	Close relative of <i>N. viridula</i>
<i>Plautia affinis</i> (Dallas)	Close relative of <i>N. viridula</i>
<i>Alciphron glaucus</i> (Fabricius)	Close relative of <i>N. viridula</i>
<i>Biprorulus bibax</i> Breddin	Pest species
<i>Piezodorous hybneri</i> (Gmelin)	Pest species
<i>Cuspicona simplex</i> Walker	Pest species
<i>Cuspicona forticornis</i> Breddin	Locally available, added for good measure
<i>Anaxarchus pardalinus</i> (Stål)	Locally available, added for good measure
<i>Oechalia schellenbergii</i> (Guèrin-Mèneville)	Beneficial predator, agric. importance
<i>Cermatulus nasalis</i> (Westwood)	Beneficial predator, agric. importance
Scutelleridae	
<i>Lampromicra senator</i> (Fabricius)	Pest species
<i>Tectocoris diophthalmus</i> (Thunberg)	Pest species
Tessaratomidae	
<i>Musgraveia sulciventris</i> (Stål)	Pest species
Coreidae	
<i>Amblypelta nitida</i> Stål	Pest species
<i>A. lutescens lutescens</i> (Distant)	Pest species

Species that could not be tested Little or no information is available for most Australian pentatomids other than the collection records associated with physical specimens held in museum collections. Though desirable in principle, testing other poorly known genera or tribes of Australian pentatomids was impractical because of difficulty in locating such species and establishing viable laboratory cultures. Practicality dictated that host test species be selected from species about which some biological information was available, often because they were associated with agriculture as pests or beneficial species. The species selected for tests with *T. giacomellii* were, for the most part, well studied species for which we could locate detailed information on geographic distribution, habitat, host plant associations, seasonality, and in some cases, rearing methods.

DESCRIPTION OF TESTS RUN AND WHY THOSE TESTS WERE CHOSEN

Host tests were conducted as sequential, paired no-choice experiments. In each test, groups of naive *T. giacomellii* adults (n = 8-10 pairs) were exposed for 2 hours to a non-target test species (n = 10-15 adults), followed by exposure for 2 hours to the target pest, *N. viridula* (n = 15 adults). This process was replicated three times for each non-target/*N. viridula* comparison. Tests thus took the form of: NT, T, NT, T, NT, T; where NT = non-target species and T = target species. Testing a given non-target/target combination required 12 hours to complete. All tests were carried in daylight hours under a 14:10 (L:D) photoperiod. *Trichopoda giacomellii* adults oviposit throughout daylight hours (M. Coombs unpublished data). At the completion of each 2 hour test period, all bugs were recovered and the numbers of parasitoid eggs per bug were recorded. For test species that attracted oviposition, appropriate food was provided in mesh screened cages until parasitoid development took place or the bugs died. All host tests were carried out in large (1.0 x 1.0 x 1.4 m) mesh screened cages constructed from aluminium frames fitted with fine cotton gauze. *Trichopoda giacomellii* adults that emerged from non-target hosts were held to record fecundity and longevity by exposing them to *N. viridula* adults in gauze cages measuring 30 x 30 x 30 cm (Sands and Coombs, 1999). Cage construction (size and material colour) did not influence parasitoid oviposition behavior.

No-choice tests were used because in choice experiments oviposition behavior of *T. giacomellii* triggered by the presence of the target host might have resulted in inadvertent oviposition on otherwise non-acceptable hosts (i.e., a false positive due to priming). This observation, however, was not tested experimentally. No-choice tests determine physiological acceptance of a particular host, and in that regard negative results are very robust, given appropriate positive controls with the target pest.

TEST RESULTS AND INTERPRETATIONS

RESULTS, SETBACKS, PROBLEMS, AND THEIR SOLUTIONS

Three native pentatomid bugs, in addition to the target pest, were identified as supporting complete development of the agent. These were *P. affinis*, *G. amyoti*, and *A. glaucus*. All three species are closely allied to *N. viridula* (Gross, 1976) and were found in the laboratory to be of comparable attractiveness to *N. viridula* for attack by *T. giacomellii*. Other species, including predatory Asopines, either failed to attract oviposition by the parasitoid or, when oviposition

occurred, parasitoid larvae failed to develop (Sands and Coombs, 1999). Oviposition, but no development, was recorded for the pentatomids *Cuspicona forticornis* Breddin and *Anaxarchus pardalinus* (Stål). In both cases, larvae of *T. giacomellii* died as first instars while attempting to penetrate the hosts' integument. Representative examples of host test results are shown in Table 2. Approval for release of *T. giacomellii* was granted, acknowledging that some attack and development on native pentatomids might occur in the field. It was deemed that, if these nontarget hosts were encountered by *T. giacomellii* in the field, any impacts would be minor. Furthermore, any potential non-target impacts would be significantly less important than damage to crops caused by failure to control the target organism throughout Australia.

Table 2. Representative examples of sequential no-choice host tests to determine the specificity of *Trichopoda giacomellii*. Test results are presented for comparisons of *Cermatulus nasalis*/*Nezara viridula* and *Glaucias amyoti*/*Nezara viridula* exposed to *T. giacomellii* in alternating 2-hour time periods.

	Hours, Eastern Standard Time					
	0630-0830	0831-1030	1031-1230	1231-1430	1431-1630	1631-1830
Test species	<i>C. nasalis</i>	<i>N. viridula</i>	<i>C. nasalis</i>	<i>N. viridula</i>	<i>C. nasalis</i>	<i>N. viridula</i>
	(n =15)	(n=15)	(n=15)	(n =15)	(n=15)	(n=15)
Ave number of parasite eggs per host	0	2.2 ± 0.9	0	2.6 ± 1.4	0	2.3 ± 1.2
	0645-0845	0846-1045	1046-1245	1246-1445	1446-1645	1646-1845
Test species	<i>G. amyoti</i>	<i>N. viridula</i>	<i>G. amyoti</i>	<i>N. viridula</i>	<i>G. amyoti</i>	<i>N. viridula</i>
	(n =10)	(n=15)	(n=10)	(n =15)	(n=10)	(n=15)
Ave number of parasite eggs per host	0.9 ± 1.2	1.7 ± 0.7	1.1 ± 0.9	1.9 ± 1.2	0.8 ± 1.1	1.9 ± 1.1

Two recurring problems were encountered throughout the study, and both related to the location and provision of nontarget species for host testing. Invariably, despite access to detailed location data, habitat, and host plant records, considerable time was spent locating and collecting sufficient numbers of individuals required to undertake tests. Even reportedly common species were difficult to find in some instances. In most cases, we were able to collect sufficient test individuals of a given species from the field. These individuals were exposed to *T. giacomellii* and subsequently discarded following tests if no parasitoid attack occurred. When parasitoid attack did occur, test individuals needed to be kept alive long enough to allow full development of the parasitoid (requiring approximately 14-16 days). As most pentatomid bugs are fruit feeders, adults could be kept alive by provision of appropriate fruit for that species. Substitute foods, such as freshly sliced green apple or dried raisins, were found to be suitable as a food source for the adults of several species. When few individuals of a given species were available from remote localities, rearing procedures were needed to provide sufficient adults for

testing. Egg laying and successful development of immature pentatomid bugs often required provision of species-specific food plants (the identity of which was not always known). For *G. amyoti* and *A. glaucus*, which originated from rainforest habitats in far north Queensland, this was overcome by simultaneously providing adults with a wide range of fruiting rainforest plants. Adults were allowed to self select plant species for oviposition. Newly emerged nymphs then either remained on these plants to feed or moved by themselves to other plant species as appropriate. Host plants suitable for adult egg laying and immature development were identified using this methodology for both *G. amyoti* and *A. glaucus*.

PREDICTED VERSUS REALISED FIELD HOST RANGE OF *T. GIACOMELLII*

Release and establishment studies for *T. giacomellii* in Australia were centred on a 1400-acre pecan plantation located at Moree, New South Wales (29° 29' S, 149° 53' E). Since its establishment, *T. giacomellii* has had a sustained impact on the abundance of *N. viridula*, reducing peak abundances to 15-35% of pre-establishment densities for the years 1999 to 2002 (Coombs and Sands, 2000; Coombs, 2003). Anecdotal evidence indicates that *N. viridula* numbers have declined further as of early 2004, and it is no longer regarded as a pest in the establishment area.

Nine other pentatomid and two scutellerid species were recorded as co-occurring with *N. viridula* in the establishment area, seven species of which were included in the pre-release host test list. Two pentatomid species, in addition to *N. viridula*, were recorded as field hosts for *T. giacomellii* at Moree (Coombs, 2003). These were *P. affinis* and *G. amyoti*, both of which were predicted to be potential hosts based on the pre-release quarantine evaluation (see above). Percent parasitism of *P. affinis* ranged from 1% to 45% on the introduced weeds *Ligustrum lucidum* Aiton and *Solanum nigrum* L., respectively. *Glaucias amyoti* was recovered only from *L. lucidum*, for which parasitism averaged less than 1%. Parasitism of *N. viridula* ranged from 9% to 70% on the same two host plants. There was no evidence of parasitism by *T. giacomellii* of the other seven species of pentatomids or two scutellerids present at the release site. Thus, no unpredicted host use was detected during the study.

The other non-target species identified as a potential host (*A. glaucus*) does not occur at the establishment site, being restricted to rainforest habitats in coastal eastern Australia. In its native range, *T. giacomellii* is apparently restricted to open rangeland and is not reported to attack pentatomids in closed-forest habitats (La Porta, 1990). Thus, habitat separation may exclude *A. glaucus* from becoming a host for *T. giacomellii* in the field.

SUMMARY

The parasitoid/host system of *T. giacomellii* and *N. viridula* gave no particular problems with regard to assessing parasitoid attack. Parasitoid eggs are attached externally to the host and easily observed. In addition, parasitoid development time is relatively short (about 2 weeks), allowing non-target species to be tested and assessed relatively quickly. The use of no-choice sequential tests appeared to give unambiguous results about which species were not hosts (i.e., negative results were robust and positive controls were obtained in controls), and test results were later conclusively supported by post-release field studies. Those species predicted to be hosts also proved to be so under field conditions, although the level of attack on *P. affinis* and,

in particular, *G. amyoti* was lower than expected. Laboratory results indicated that *P. affinis*, *G. amyoti*, and *N. viridula* were of equal attractiveness to *T. giacomellii* for oviposition. The tests employed made no prediction about the effects of host plant and/or habitat on parasitoid behaviour.

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