Biology and host specificity of gall-inducing *Acythoepus burkhartorum* (Coleoptera: Curculionidae), a biological-control agent for the invasive weed *Coccinia grandis* (Cucurbitaceae) in Guam and Saipan

Anantanarayanan Raman, Zerlene T. Cruz, Rangaswamy Muniappan & Gadi V. P. Reddy

An introduced cucurbit *Coccinia grandis* (Linnaeus) Voigt has grown into problem proportions in Hawaii and the Pacific islands of Guam and Saipan. The biology of *Acythoepus burkhartorum* O’Brien, 1998, a potential biological-control agent of *C. grandis*, has been described. By inducing the gall – a sink for nutrients – and by deriving nutrition, *A. burkhartorum* places *C. grandis* under stress. Especially during the late larval stage, the weevil displays an unusual behaviour of shedding dry gall tissues with its mandibles to ‘prepare’ the ‘pupal case’ and using the shredded sclerenchyma fibres to fill the open and cut ends of the pupal case. This ability to ‘create’ such a pupal case is unique among weevils. Because *A. burkhartorum* is able to sever tender shoots of *C. grandis* at points where galls are induced, we consider that this weevil will be highly relevant in *C. grandis* management. Although non-gall-inducing species of baridine weevils have a wide host range, the known gall-inducing species are specific to their respective hosts, similar to the majority of gall-inducing insects. *A. burkhartorum* prefer consistently either petioles or stems, behave identically by tunnelling through soft tissues within the host organs, and induce galls. Because of the potential of *A. burkhartorum* in biological control of *C. grandis*, we tested its specificity against *C. grandis* and *Zehneria guamensis* (an endemic cucurbit of Guam and Mariana Islands), following ‘choice’ and ‘no choice’ test modes. No feeding hole or gall development occurred on *Z. guamensis* indicating a categorical response that *A. burkhartorum* is specific to *C. grandis*. This result encouraged field release of *A. burkhartorum* in Guam and Saipan.

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Introduction

Within Curculionoidea, high numbers of gall-inducers exist only in Ceurthorhynchinae, Baridinae, and Curculioninae (Korotyaev et al. 2005). Several species of Curculioninae are well-known gall inducers on plants belonging to Asteraceae and Caryophyllaceae (Anderson 1962; Kaplin 1981; Dieckmann 1988). Currently only five species of gall-inducing Baridinae are known, including *Acythoepus burkhartorum* O’Brien, 1998 (Table 1). Gall-inducing

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species of Baridinae show no noteworthy affinity either to particular plant families or to any related clusters of plant families, a trait, which is evident in the non-gall-inducing species of Baridinae (Pakaluk 1994; Korotyaev et al. 2001; Marvaldi 2003). While describing *A. burkhartorum* and *A. cocciniae* O’Brien, 1998 from Kenya, O’Brien & Pakaluk (1998) implicated them to be potential biological-control agents of *Coccinia grandis* (= *C. indica* Wight & Arnold), a native plant of the tropical and sub-tropical Ethiopian region. *C. grandis* was introduced by human action into the Hawaiian Islands and in the recent past its populations have grown into problem proportions. In the last two decades, *C. grandis* has emerged as an invasive weed in the islands of Guam and Saipan, where it is a problem plant both in managed gardens and natural areas (Pacific Island Ecosystems at Risk 2005). Although used extensively as a vegetable (e.g., Wasanvisut & Viriyapanich 2003) and a medicinal plant (e.g., Patel & Srinivasan 1997) in south-east and south Asian countries (e.g., India, Sri Lanka, Thailand), the Global Invasive Species Database (2005) lists *C. grandis* as a noxious weed. *Coccinia grandis* grows overwhelmingly in Hawaii, Guam, and nearby islands, and competes intensely with the native vegetation. It acts as a host for melon fly (*Bactrocera cucurbitae* Coquillet, 1899; Diptera: Tephritidae) and as a reservoir for several other insect pests and microbial pathogens (e.g., ring-spot virus) of cucurbitaceous crops.

Among diverse plant-feeding insects that are being explored currently for use in weed biological-control campaigns, Coleoptera are gaining relevance, especially because during their developmental stages they tunnel through axial organs of host plants, and their mandibulate larvae chew and consume large quantity of tissues of their herbaceous host plants besides fracturing vascular strands (May 1984; Pakaluk 1994; Bailey et al. 2002; Korotyaev & Gültekin 2003). Gall-inducing Coleoptera are particularly useful agents in weed management (Muniappan & McFadyen 2005), mainly because these insects are highly specific to their host plants (Raman 1996; Raman et al. 2005), and they induce modifications in their host plants – from fracturing of vascular strands and distorting nutrient transport (Raman & Dhileepan 1999; Raman et al. 2006) to reduced photosynthetic efficiency, stomatal conductance, and water potential (Florentine et al. 2005). In such a context, *A. burkhartorum* demonstrates traits of a reliable biological-control agent, because it is both a coleopteran and a gall inducer (Fig. 1). With *C. grandis* turning into an invasive plant in the Hawaiian Islands, the Hawaii Department of Agriculture searched for potential natural enemies for *C. grandis*. *Acythoeus burkhartorum* was one of the three organisms obtained from eastern African region and field-released in Hawaii in 1999.

Adults of *A. burkhartorum* live up to 24 months feeding on the leaves of *C. grandis*. Eggs inserted into tender shoots (petioles or tendrils or stems) of *C. grandis* hatch in 7–10 days. Five larval stages developing in 25±1 days have been reported in Hawaii (Murai et al. 1998). Mid-portions of galls, in the shape of barrels, adequate enough to enclose the pupae, severed from their respective plant axes and drop to the soil. Pupation occurs within the ‘dry’ severed gall portions, which act as pupal cases. Adults emerge in 25–30 days (Murai et al. 1998; personal observations R. Muniappan, G.V.P. Reddy, Z. Cruz).

Because only limited information is currently available on gall-inducing baridine weevils and *A. burkhartorum* is considered a potential candidate for the biological control of *C. grandis* in the Pacific Islands (Murai et al. 1998), in the present paper we provide details of the biology of *A. burkhartorum* in the context of its gall-inducing behaviour. This paper also includes details of gall growth and a

Figs 1–8. *Acythoeus burkhartorum* on *Coccinia grandis*. – 1, Swollen stem (mature gall) inhabited by fourth stage larva (Bar=1 cm); 2, egg (oblong outline with a distinct dark-brown solidified secretion at the proximal end; nucleus within the egg visible clearly below) deposited on petiole base (longitudinal-sectional view) (Bar=100 μm); 3, mature ‘paired’ galls induced both on basal tendril and adjacent petiole; vertically slit to show larval chambers (Bar =1 cm); 4, downward descent path (arrow) of a neonate larva. [note that the exposed gall is that of a second-stage larva]; LC – larval chamber (Bar=1 mm); 5, gall of a first-stage larva on the petiole (Bar=1 cm); 6, nutritive tissue of first-stage larva (cross sectional view); LC – larval chamber; arrows – damaged callus cells; intact callus cells bordering the larval chamber include dense cytoplasm and prominent nuclei (Bar=50 μm); 7, nutritive tissue of second-stage larva (cross-sectional view) with radial files of regenerating pith parenchyma cells; LC – larval chamber; arrow – callus cells damaged by larval feeding; cells away from the larval chamber include inclusions testing positively for starch (Bar=100 μm); 8, gall (cross-sectional view) of late third/early fourth larval stage showing regenerated parenchyma filling the damaged pith; arrows – polyphenolic substances (tested with diazotized-sulphanilic acid reaction) occluding the parenchyma cells; ph – phloem showing dilated elements; scl – inner cortical sclerenchyma; chl – outer cortical chlorenchyma (Bar=100 μm).
host-specificity test of *A. burkhartorum* conducted against *Zehneria guamensis* (Merry.) F. R. Forsberg (Cucurbitaceae), an endemic plant in Guam and nearby islands, and that of the introduction and establishment of *A. burkhartorum* in Guam and Saipan.

**Materials and methods**

**Plant and insect material**

Several shoot cuttings of *C. grandis* obtained from the natural areas that have been infested by *C. grandis* in the island of Guam (13°15’–13°30’N; 144°40’–144°55’E) were propagated in plastic pots (45 cm diameter) with commercial grade garden-potting mix. These pots were maintained in the greenhouse of the Agricultural Experiment Station (AES) at the University of Guam (UoG) (Mangilao, Guam) in controlled environmental conditions (L:D – 12:12, mean day temperature 32°C, mean night temperature 17°C, and relative humidity 65–80%). Fifty vigorously regenerating plants were quarantined to insect-proof cages (65×60×60 cm) and maintained in the greenhouse with the environmental conditions described earlier.

**Gall induction**

Fifty adults (equal number of males and females) of *A. burkhartorum* reared on *C. grandis* in the quarantine laboratory at the AES were released on the regenerated shoot cuttings of *C. grandis* at different growth stages and maintained in the insect-proof cages in January 2005.

Whenever mating pairs were observed, the plant that harboured them was separated for closer observations. A hand lens (20× magnification) was used to locate oviposition scars and egg locations, which were marked with a soft-tipped, permanent marker pen. Sequence of gall development was followed, using increase in stem girth as an indicator (i.e., gall growth), which was further supplemented by pen markings of egg locations. Age of the gall was determined by the inhabiting stage of the immature stage (larval stages 1–5, prepupa, pupa), which was noted by slitng the gall in the vertical median axis with a sharp razor blade, taking care not to damage the inhabitant. Several galls were slit to observe larval behaviour and gall development: stem tissue with oviposition scars: n=9; galls housing different developmental stages: n=39 (1st larval stage – 4, 2nd larval stage – 10, 3rd larval stage – 14, 4th larval stage – 7, 5th larval stage – 2, prepupal stage – 2), and galls including the pupal stage: n=23. Morphometric data of the dissected galls and the inhabiting larval stages were obtained using a calibrated ocular scale fitted in the stereo-binocular dissection microscope (Leica™ Zoom 2000, Leica, Wetzlar, Germany) and by measuring at the widest point. Mean and standard deviation were calculated using summarized content of variates in Genstat® for Windows® (2005). Morphometric parameters (length and width) of galls (in different growth stages) and inhabiting larval stages were analyzed with simple-linear regression with Genstat® for Windows® (2005); length and width parameters of the larvae were compared as explanatory variates of length and width of galls, respectively.

**Microscopy**

Galls of *C. grandis* with oviposition scars and those housing different stages of *A. burkhartorum* were

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**Table 1.** Gall-inducing Baridinae, host plants, and distribution.

<table>
<thead>
<tr>
<th>Weevil</th>
<th>Plant organ bearing the gall</th>
<th>Host</th>
<th>Host family</th>
<th>Distribution</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acythopecus burkhartorum</em></td>
<td>Stem</td>
<td><em>Coccinia grandis</em></td>
<td>Cucurbitaceae</td>
<td>Kenya</td>
<td>O’Brien &amp; Pakaluk 1998</td>
</tr>
<tr>
<td><em>Baris cordiae</em> Marshall, 1923</td>
<td>Petiole</td>
<td><em>Cordia obliqua</em></td>
<td>Boraginaceae</td>
<td>Peninsular India</td>
<td>Krishnamurthy et al. 1977; Mani 2000</td>
</tr>
<tr>
<td><em>Hollisiella crabro</em> (Faust, 1888)</td>
<td>Stem</td>
<td><em>Fissistigma polyanthum</em> (Hooker, f. &amp; Thomson.)</td>
<td>Annonaceae</td>
<td>Vietnam</td>
<td>Korotyaev et al. 2005</td>
</tr>
<tr>
<td><em>Thanius biennis</em> Prena &amp; Nishida, 2005</td>
<td>Stem</td>
<td><em>Psychotria marginata</em></td>
<td>Rubiaceae</td>
<td>Costa Rica</td>
<td>Prena &amp; Nishida 2005</td>
</tr>
<tr>
<td><em>Ulobaris loricata</em> (Boheman, 1836)</td>
<td>Root</td>
<td>Species indeterminate</td>
<td>Chenopodiaceae</td>
<td>Middle and Central Asia</td>
<td>Korotyaev et al. 2005</td>
</tr>
</tbody>
</table>
fixed in formal-acetic alcohol (FAA: 70% ethanol – 90 ml, 40% formalin – 5 ml, glacial acetic acid – 5 ml) for 72 h; each developmental stage of galls (oviposition scars, galls housing different immature stages) was stored in separate glass vials in 70% ethanol. FAA-fixed materials were cut at 5–7 μm in a rotary microtome (Leica™ 2245 [semi-motorized], Leica, Wetzlar, Germany). Most of the sections obtained were contrasted with 1% toluidine blue (dissolved in 1% aqueous borax solution) for use in bright-field microscopy; a few, randomly chosen sections of galls of known age, were prepared unstained for phase-contrast microscopy. All the stained (and contrasted in weak acid-alcohol solution) and the unstained sections were mounted in 5% aqueous glycerine on glass slides. From fresh galls, hand-cut sections were obtained for histochemical tests for starch (Periodic acid – Schiff’s reagent test; Feder & O’Brien 1968), lipids (Sudan black B reaction; Gomori 1952), and polyphenols (diazo-tized-sulphanilic acid reaction; Gahan 1984) All bright-field and phase-contrast microscopic observations and photographs were made in a Nikon™ Elipse 80 – i microscope fitted with a digital DS-L1 camera control unit (Nikon Corporation, Tokyo, Japan). Samples of vertically slit galls with the larvae were photographed in a stereo-binocular microscope (Leica™ Zoom 2000, Leica, Wetzlar, Germany).

**Host-specificity test**

Adults of *A. burkhartorum* obtained from the Hawaii Department of Agriculture were reared on potted *C. grandis* plants maintained in the insect-proof cages in the quarantine laboratory of AES following the same procedures described under ‘Plant and insect materials’. Because Murai et al. (1998) have tested the host range of *A. burkhartorum* using different plant species belonging to Violales (Caricaceae, Flacourtiaceae, Passifloraceae, Violaceae, and Turneraeaceae), following centrifugal phylogenetic method (Wapshere 1974), we tested the specificity of *A. burkhartorum* using its established host *C. grandis* and an endemic species of *Cucurbita* in Guam and Mariana Islands, viz., *Zehneria guamensis*, following ‘choice’ and ‘no choice’ test modes. We focussed on testing the susceptibility of *Z. guamensis*, mainly because of the concern on the possibility of *A. burkhartorum* radiating on to *Z. guamensis* in Guam and neighbouring islands in 2002. Individuals of *Z. guamensis* were raised in the greenhouse from seeds (propagation methods similar to the methods followed for raising shoot cuttings of *C. grandis*) obtained from the limestone forests in Guam. Both *C. grandis* and *Z. guamensis* were raised to ca. 100 cm height bearing multiple branches and were used in tests by maintaining them in the insect-proof cages. ‘Choice’ experiments, including growing plants of both *C. grandis* and *Z. guamensis* in the same cage, and ‘no choice’ experiments, including growing plants of either *C. grandis* or *Z. guamensis* in one cage, were conducted by releasing the 20 mixed-sex pairs of adult weevils into each cage. All plants were adequately and regularly watered and maintained. Observations were made every day on the number of feeding holes cut by adult weevils on leaves and galls induced on either petioles or tendrils or stems.

**Field release**

After conducting host specificity tests, an environmental-assessment statement was prepared and submitted to Animal and Plant Health Inspection Service (APHIS) of US Department of Agriculture (USDA 2004). After securing permits from APHIS to field release this weevil in Guam and Saipan, adult weevils were released on *C. grandis* vines in selected areas on Guam and Saipan.

**Results**

**Oviposition**

Adult females oviposit frequently in either tender petioles or tendrils and less frequently in tender stems. The frequency of oviposition into petioles is usually higher than into tendrils and stems. In a sample of mature galls (*n=98*), the ratio of galls on petioles, tendril bases, and young stems was 3:1:1. Females dig pits with their rostrum and deposit one egg in each pit. After oviposition, the female covers the egg (400×220 μm) with a ‘secretion’, which solidifies upon exposure to air and turns brownish-black; no noteworthy change, either subcellular or growth, occurs in the cells surrounding the egg (Fig. 2). Usually one egg occurs at one node, which later results in a single gall at that node. However, paired galls at the same node arising from two eggs deposited in the petiole and tendril have also been observed (three in a population of 98) (Fig. 3).

**Gall growth and larval activity**

From the peripheral region where oviposition occurred, neonate larvae move into the soft central core of the plant organ; in a few instances, neonate larvae move upwards especially when the eggs had been deposited at the bases of petioles or tendrils. The larvae move by chewing and feeding on the parenchymatous cells (Fig. 4). In three to four days after the emergence of the first larval stage, the
host-plant organ housing the larva, which could be either petiole or tendril or stem, shows a modest swelling as the visible expression of the gall (Fig. 5). Larval feeding on the pith parenchyma cells, coupled with its tunnelling behaviour (either upwards or downwards in the plant organ) induces the parenchyma cells to regenerate callus cells especially along the perimeter of the larval chamber (Fig. 6). The inhabiting larva derives its nutrition from these callus cells and the supplementing radial files of parenchyma cells, which include dense cytoplasm and prominent nuclei. Histochemical localization of starch and lipids indicated that the regenerated parenchymatous linings lining the larval chamber include lipidic inclusions, whereas parenchyma cells away from the larva include starch (Fig. 7). With maturation of the larva and intense feeding, the gall grows in length and in width, essentially by increment in the radially arranged pith-parenchyma cells. When the larva is in its late third larval stage of development, feeding extends up to the outer cortex of the host organ. In doing so, the larva consumes vascular tissues as well; however, regenerative callus parenchyma fills the bulk of the gall. As the larva matures to the fourth larval stage, gall cells accumulate polyphenolic materials in the callus parenchyma cells (Fig. 8).

As the larva turns into fifth stage, several notable changes occur in the gall rapidly and abruptly. The fifth-stage larva deposits frass at both extremities of the larval chamber (Fig. 9). Deposition of frass (probably because of its ‘toxic’ nature) at either end of the larval chamber triggers localized degeneration of gall tissue at those points (Fig. 10). Because the vascular strands are fractured by larval feeding, the gall dries. At this stage, the inhabiting mature larva shreds the cortical sclerenchyma using its mandibles and thus prepares a part of the gall as a ‘pupal case’ (Figs. 11, 12, 13). Matching with the dimensions of the larval chamber (10–15 mm in length), the prepupa fills the ends of the larval chamber (which subsequently becomes the pupal case) with shredded sclerenchyma fibres (Fig. 14). Degenerating extremities of the larval chamber, induced dryness, shredding of the scaffolding hard tissue (e.g., sclerenchyma) induce severing at both ends of this ‘portion’ of the gall (viz., the larval chamber) into a barrel-shaped pupal case with its ends closed with shredded fibres (Fig. 15, 16). The severed pupal case drops to soil and the adult emerges from it after 25–30 days.

Measured parameters such as the gall length and width, and larval length and width show a gradual and statistically significant increase during gall development (ANOVA: p<0.001 in all instances) (Fig. 17). Maximum gall growth (both in terms of length and width) occurs during the development of the first and second larval stages. Gall length, in particular, shows a drop, especially during the inhabitation time of the fifth larval stage, whereas gall width values remain at 4±1 mm throughout the gall growth period, including the inhabitation time of the fifth larval stage. A comparison of gall length vs. larval length presents a significant positive relationship (p<0.001) (Fig. 18), whereas that of gall width vs. larval width presents a less significant, negative relationship (p<0.015) (Fig. 19).

Host-specificity test
The number of days lapsed from the time of release of weevils into cages, the time taken by them to commence feeding, and the time that lapsed before galls became visible on the tested plants (C. grandis and Z. guamensis) are provided in Table 2. No feeding hole or gall development occurred on Z. guamensis either in the ‘choice’ or in the ‘no choice’ tests.

Field release
A total of 321 adults of A. burkhartorum was field released in various locations in Guam (Ordot Pump Station, Public Health, Tai area, Cross Island Road next to Baza Gardens, and UoG house #35) on 8 October 2004. Further 202 adults of A. burkhartorum were field released at different sites in Saipan (Kagman, Ben Cepada’s Ranch and near Capital Hill) on 9 February 2005.

Figs 9–16. *Acythopeus burkhartorum* on *Coccinia grandis* – 9, gall (cross-sectional view) of fifth larval stage showing deposition of frass (f) at the one of the larval chamber (LC) terminals (Bar=100 μm); 10, gall of the fifth larval stage with a ring of degenerating tissue (arrows) preparing the ‘pupal case’ to be severed from the remainder of the gall (Bar=1 mm); 11, gall of the fifth larval stage (opened to expose the larva) showing the larva shredding the dry inner cortical sclerenchyma and outer cortical chlorenchyma (Bar=1 mm); 12, shredded tissue including sclerenchyma fibres, chlorenchyma cells, and parenchyma cells with polyphenolic (dark-brown) inclusions (Bar=100 μm); 13, ‘pupal case’ showing the ‘thin’ outer rim of fibrous and chlorenchymatous cortex. * – a portion of larval head, (Bar=100 μm); 14, pupal case severed from the gall (Bar=1 cm); 15, pupal case (slit with a scalpel) showing the pupa within (Bar=1 cm); 16, end of the pupal case showing the ‘stuffing’ of shredded dry tissue (Bar=0.5 cm);
Discussion

Host relations of gall-inducing Baridinae
Although the Baridinae are a large subfamily of the Curculionidae, little is known about host associations and life histories of both gall-inducing and non-gall-inducing species of Baridinae. Available information does not indicate any definite pattern of host-plant use by gall-inducing species on either related plant families or related plant genera (Prena 2001, 2003). The known species of gall-inducing Baridinae (Table 1) feed on diverse and unrelated host plants belonging to large clades of Angiosperms (sensu Angiosperm Phylogeny Group 2003): *Acythopeus burkhartorum* feeds only on some Cucurbitaceae, belonging to ‘Rosids: Eurosids I’ [Core Eudicots]; *Hollisiella crabro* feeds on one species of Annonaceae, belonging to the basal angiosperm clade Magnoliids; *Baris cordiae* and *Thanius biennis* feed on species of Boraginaceae and Rubiaceae, belonging to ‘Asterids: Euasterids I’ [Core Eudicots]; *Ulobaris loricata* feeds on one species of Chenopodiaceae, belonging to Caryophyllales [Core Eudicots]. Nonetheless, what is consistent at least among *B. cordiae*, *T. biennis*, and *A. burkhartorum* is that they prefer either petioles (that are similar to stems in primary structure) or stems, behave identically by tunnelling through soft tissues within the host organs, and induce galls. Although non-gall-inducing species of baridine weevils feed on more than one host plant (Korotyaev et al. 2001), the known gall-inducing species are specific to their respective hosts showing a strong level of fidelity to their host plants (Raman 1996, Abrahamson et al. 1998, Raman et al. 2005). In such a context, the role of *A. burkhartorum* in biological-control campaigns against *C. grandis* gains in relevance.

Unique characteristics of *A. burkhartorum*
The interaction between growth stages of *A. burkhartorum* and *C. grandis* exhibits high levels of synchronization. By depositing one egg per node on tender shoots, gravid females of *A. burkhartorum* display...
resource-partitioning behaviour by either minimizing intra-specific competition within larval populations or eliminating it completely. Oviposition behaviour of *A. burkhartorum* is different, when compared with other gall-inducing weevils, such as *Conotrachelus albocinereus* Fiedler, 1940 (Curculionidae: Curculioninae) living on *Parthenium hysterophorus* (Asteraceae) (Florentine et al. 2002) and *Ceutorhynchus napi* Gyllenhal, 1837 (Curculionidae: Ceutorhynchinae) living on *Brassica napus* var. *oleifera* Lineaus (Brassicaceae) (Le Papé & Bronner 1987), although all the three compared weevils deposit only one egg at each oviposition site. *Conotrachelus albocinereus* and *C. napi*, while ovipositing on their respective host plants, push eggs deep into mature stem tissue, whereas, *A. burkhartorum* oviposits by sinking eggs superficially into tender shoot (e.g., petioles, tendrils, stems). By inserting eggs deep into mature stems, *C. albocinereus* and *C. napi* minimize movement for the neonate larvae within the cortical tissue of their respective host plants, because the egg is enclosed in a proteinaceous sheath and the egg and the secretion together induce gall growth (Le Papé & Bronner 1987, Florentine et al. 2002). On the contrary, the egg of *A. burkhartorum* is not enclosed in a proteinaceous sheath and the egg, *per se*, does not trigger any gall growth. The neonate larvae of *A. burkhartorum* gnaw their way into the soft cortical parenchyma until they reach the point where they settle eventually and the gnawing initiates gall growth in *C. grandis*. We speculate that the mode of insertion of eggs – either superficially or deeply – depends on the softness of tender shoots or hardness of mature shoots of the chosen host-plant site and in high probability this behaviour is an adaptive strategy of the species concerned. During oviposition, *C. albocinereus* covers the sites of egg deposition with chewed plant materials, whereas, *A. burkhartorum* and *C. napi* (Le Papé & Bronner 1987) cover egg sites with a ‘secretion’, which solidifies upon exposure to air. Covering of the egg site with a solidifying secretion is currently known also in two non-gall-inducing species of Baridinae (Korotyaev & Gültekin 2003) and the possible reason for such behaviour is to protect the egg.

Although the biology and behaviour of *A. burkhartorum* up to the pupal stage are similar to other weevils (e.g., see Gültekin 2004 for several citations), the ability of *A. burkhartorum* to ‘create’ a pupal case by severing a portion of the gall makes it exceptional, since such an ability is unknown in any other Curculionidae. However, the ability of *Cleonidius poricollis* Mannerheim, 1843 (Curculionidae: Cleoninae) to construct ‘sand tubes’ with root tissues of its host plant (*Descurainia pinnata* (Walter) Britton [Brassicaceae]) and particles of sand (O’Brien and Marshall 1987) needs to be viewed in conjunction here. *A. burkhartorum*’s behaviour stands out in being able to utilize *C. grandis* for nutrition, and especially during late larval stages, when it is able to shred the drying plant tissues to ‘prepare’ the pupal case, sever the barrel-shaped pupal case from the gall, and use shredded sclerenchyma fibres to close the ends of the pupal case.

### A. burkhartorum in the biological control of *C. grandis*

Gall-inducing Coleoptera and Lepidoptera are favoured presently in weed management campaigns, because these arthropods either are monophagous or have a narrow host range (Muniappan & McFadyen 2005). Their capability to re-canalize the host-plant’s developmental processes and metabolic activity, to place their host plants in stress by draining nutrients and other key metabolic products to the inducing larva, and to induce anoxic conditions within the host plant render them as candidates of choice in weed-biological control programmes (Florentine et al. 2001, 2002, 2005, Raman et al., 2006). The usefulness of *A. burkhartorum* in the management of *C. grandis* is simple, yet elegant; it demonstrates its usefulness in the management of *C. grandis* by severing parts tender stems, tendrils, and petioles (viz., by creating the pupal case).

### A. burkhartorum

<table>
<thead>
<tr>
<th>'Choice' test</th>
<th>'No choice' test</th>
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<tbody>
<tr>
<td>Adult feeding holes/leaf</td>
<td>63.8±10.4</td>
</tr>
<tr>
<td>Days taken to initiate galls</td>
<td>13</td>
</tr>
<tr>
<td>Days lapsed to start feeding</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>32.5±13.1</td>
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*Table 2. Host specificity tests for Acythopeus burkhartorum on Coccinia grandis and Zehneria guamensis.*
several species of plants; in our ‘choice’ and ‘no-choice’ host-specificity test, we used only one endemic species of Cucurbitaceae (*Z. guamensis*), which has yielded a categorical response that *A. burkhartorum* is specific to *C. grandis*. In the context of such a definitive outcome, an environmental assessment was prepared, which was approved by APHIS, USDA; consequently field-release of *A. burkhartorum* on the islands of Guam and Saipan was authorized. Data pertaining to establishment in the field at the release sites are being accrued; predation of the pupae of *A. burkhartorum* – in spite of being confined to the ‘pupal case’ – by ants appears to be a hurdle in the successful establishment of *A. burkhartorum* in Guam and Saipan. Monitoring of horizontal spread from the released sites is ongoing.

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**References**


