

Potential biological control agents for management of cogongrass (Cyperales: Poaceae) in the southeastern USA

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Abstract

Cogongrass, *Imperata cylindrica* (L.) Palisot de Beauvois (Cyperales: Poaceae), is a serious invasive weed in the southeastern USA. Surveys for potential biological control agents of cogongrass were conducted in Asia and East Africa from 2013 to 2016. Several insect herbivores were found that may have restricted host ranges based on field collection data and life histories. Stemborers in the genus *Acrapex* (Lepidoptera: Noctuidae) were collected from cogongrass in Tanzania, Uganda, and Japan. In the Philippines, larvae of *Emmalocera* sp. (Lepidoptera: Pyralidae) and *Chilo* sp. (Lepidoptera: Crambidae) were found boring in cogongrass. Cecidomyiid midges were found in both Japan and Indonesia. A Japanese midge identified as a *Contarinia* sp. (Diptera: Cecidomyiidae) caused deformation of the stem, whereas the Indonesian midge *Orseolia javanica* Kieffer & van Leeuwen-Reijnders (Diptera: Cecidomyiidae) induced the formation of a basal stem gall. Previous research suggested that the host range of *O. javanica* was restricted to cogongrass.

Key Words: *Imperata cylindrica*; weed; exotic; invasive; natural enemy; biological control

Resumen

La cisca, *Imperata cylindrica* (L.) Palisot de Beauvois (Cyperales: Poaceae), es una maleza invasora grave en el sureste de los EE.UU. Se realizó un sondeo de las agentes potenciales de control biológico de plantas de cisca en Asia y África Oriental desde 2013 hasta 2016. Varios insectos herbívoros fueron encontrados que pueden tener un rango restringido de hospederos a partir de datos de recolección de campo e historias de vida. Se recolectaron barrenadores del tallo en el género *Acrapex* (Lepidoptera: Noctuidae) de plantas de cisca en Tanzania, Uganda y Japón. En las Filipinas, se encontraron larvas de *Emmalocera* sp. (Lepidoptera: Pyralidae) y *Chilo* sp. (Lepidoptera: Crambidae) barrenando plantas de cisca. Se encuentran jejénes cecidomyiidos tanto en Japón e Indonesia. Un jején japonés identificado como un *Contarinia* sp. (Diptera: Cecidomyiidae) causó la deformación del tallos, mientras que el jején de Indonesia, *Orseolia javanica* Kieffer & van Leeuwen-Reijnders (Diptera: Cecidomyiidae) indujo la formación de una agalla en la base del tallo. Las investigaciones anteriores sugieren que la gama de hospederos de *O. javanica* es restringida a plantas de cisca.

Palabras Clave: *Imperata cylindrica*; hierba; exótico; invasor; enemigo natural; control biológico

Cogongrass, *Imperata cylindrica* (L.) Palisot de Beauvois (Cyperales: Poaceae), is a federally listed noxious weed in the USA (USDA/NRCS 2016), and is considered a serious weed in several countries in Asia (Brook 1989; Kuusipalo et al. 1995; Garrity et al. 1997) and West Africa (Chikoye et al. 2000; Chikoye & Ekeleme 2001). The plant has an extensive Old World distribution including sub-Saharan Africa, southern Europe, most of Asia, and northern Australia (Hubbard et al. 1944). Cogongrass was introduced into the southeastern USA in the early

1900s as packing material and as a livestock forage grass, putatively from Japan and the Philippines, respectively (Tabor 1952), and has since spread throughout the southeastern USA from Texas to North Carolina (Burrell et al. 2015; EDDMapS 2016). A recent sequence-based molecular study, which included cogongrass samples from the USA, Japan, the Philippines, and Brazil, showed that cogongrass reproduces clonally. This work identified 4 clonal lineages of cogongrass in the southeastern USA, with 1 clone dominant in peninsular Florida, 1

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clone widespread throughout much of the Gulf coast, and 2 other less common types (Burrell et al. 2015). In addition, the congeneric species, *Imperata brasiliensis* Trinius (Cyperales: Poaceae), was included in the study with samples from southern Florida and multiple sites in Brazil. Previous morphological studies proposed synonymy of *I. brasiliensis* and *I. cylindrica* (Hall 1998; Ward 2004), and this supposition was supported by a molecular study (Lucardi et al. 2014). However, Burrell et al.'s (2015) study, based on 2,320 genome-wide markers, found that all *I. brasiliensis* samples grouped into a clonal lineage genetically distinct from *I. cylindrica*, providing strong evidence for separate species status for *I. cylindrica* and *I. brasiliensis*.

Cogongrass infests cattle pastures, golf courses, and lawns; it also thrives in poor soil conditions such as ditch banks, road side and railroad rights-of-way, and reclaimed phosphate mining areas (MacDonald 2009). The most significant economic losses occur in pine plantations, where cogongrass outcompetes native ground cover and increases the frequency and intensity of fires (Jose et al. 2002). It has also been shown to displace native vegetation in sandhill communities in Florida (Lippincott 2000) and decrease plant species richness and the abundance of endemic plant species in longleaf pine communities (Brewer 2008).

Control of cogongrass relies primarily on mowing and the application of chemical herbicides (Jose et al. 2002). In 2009, the state of Alabama dedicated \$6.3 million of federal stimulus funds exclusively for chemical control of this invasive weed (Barry 2009). Biological control using natural enemies from the native range of cogongrass has received little attention, and no biological control agents have been introduced anywhere in the world. Van Loan et al. (2002) reviewed the literature on natural enemies of cogongrass, both within and outside of the USA, and mentioned several insect herbivores and plant fungi from the native range, highlighting an Indonesian stem gall midge, *Orseolia javanica* Kieffer & van Leeuwen-Reijnders (Diptera: Cecidomyiidae), as the only insect examined for host specificity.

From 2013 to 2016, surveys were conducted at several locations in East Africa and Asia to identify potential biological control agents of cogongrass. East Africa was selected for surveys based on the lack of weediness of cogongrass in this region (Ivens 1983) and speculation of an East African center of diversity of cogongrass (Evans 1991; A. M. Burrell, unpublished data). Asia was targeted for exploration due to historical evidence that the provenance of the cogongrass introduced into the southeastern USA was Asia (Tabor 1952). The objective of this paper is to provide information on the insects discovered during the surveys that may have potential as biological control agents.

Materials and Methods

EXPERIMENTAL LOCATIONS

Surveys were conducted from 2013 to 2016 in Tanzania and Uganda in East Africa, and the Philippines, Japan, and Indonesia in Asia (Table 1). During initial surveys in each country, all herbivorous insects found associated with cogongrass were collected, whereas later surveys targeted specific insects that were thought to have potential as biological control agents due to the type of plant damage they caused and evidence of host specificity.

INSECT IDENTIFICATIONS

Insects were identified by taxonomic experts based on morphology and/or were barcoded at the cytochrome oxidase subunit 1 gene (*CO1*) at either Texas A&M University (for details see Takasu et al. 2014) or at the Canadian Centre for DNA Barcoding (<http://www.ccdb.ca/>).

REARING PROCEDURES

Selected insect species were hand-carried or shipped to a University of Florida biological control quarantine facility in Fort Pierce, Florida, under United States Department of Agriculture, Animal and Plant Health Inspection Service permit no. P526P-14-01076. Attempts were made to establish laboratory colonies by either placing appropriate insect stages on whole cogongrass plants, cut stems of cogongrass, or on an artificial diet (Frontier Agricultural Sciences, Product F9775B, Newark, Delaware) to which 10 g/L of freeze-dried cogongrass leaf powder had been added. This diet was selected due to the close phylogenetic relationship of cogongrass and sugarcane (Skendzic et al. 2007; Welker et al. 2015). Further details of methods used for specific insects are included in the sections below.

Results

JAPAN

Acrapex azumai Sugi (Lepidoptera: Noctuidae)

This stemborer was discovered in Itoshima, Fukuoka Prefecture, on Kyushu Island, Japan, in Aug 2013, when several plants in an undisturbed patch of *I. cylindrica* were observed with brown central tillers, a symptom often referred to as "dead-heart" in crop grasses. Further inspection revealed insect boring damage in the lower stem, and 1 or 2 circular holes (about 2–3 mm in diameter) from the stem tunnel to the exterior of the stem. Three mature lepidopteran larvae were found in tunnels, 2 of which were reared to adulthood and identified as *A. azumai* (Takasu et al. 2014). Previous to our finding, *A. azumai* had only been collected as an adult in Japan with no records of larval hosts. Because stemborers of gramineous crops (rice, sugarcane, sorghum, corn) have extensively been investigated in Japan (Kishino 1970; Kiritania 1990; Khan et al. 1991; Ishikawa et al. 1999; Kim 1999; Nagayama et al. 2004; Sallam 2006) and *A. azumai* had not previously been reported from these cultivated grasses, it may have a narrow host range. Larvae (56) of *A. azumai* were collected from the same location in Itoshima in Aug 2014. All larvae were approximately the same size and thought to be in the final instar, suggesting discrete generations. One pupa was found at the bottom of a stem tunnel near the soil level.

The larvae were placed in vials with 6- to 8-cm-long pieces of cogongrass stem and artificial diet, and hand-carried to the Fort Pierce quarantine facility. They continued to be reared in vials with both artificial diet and cut stem pieces, the latter of which were replaced every 2 to 3 d. The majority of larvae burrowed in the diet, whereas a few entered stems. Only 4 individuals (7%) completed development; 1 had deformed wings and 1 emerged several days after the first 3 to emerge had died. Two of the adults emerged on the same day and were placed together in a 4 L plastic container with cut cogongrass stems (about 20 cm) held upright in moist sand. The deformed adult that emerged was added 3 d later. The 3 adults died after 4 d, and the stems were removed and inspected. Five egg batches with 160 eggs in total were found in leaf sheaths. Eggs were held in Petri dishes (60 × 14 mm) on moistened filter paper and as they eclosed, neonates were transferred to whole plants ($n = 24$ neonates) or to 6- to 8-cm-long stem pieces ($n = 128$). Stem pieces were changed every 2 to 3 d, but no larvae developed beyond the 3rd instar. Whole plants that had been inoculated were dissected after 2 wk, but no larvae were recovered. Additional shipments of *A. azumai* larvae were received at the Fort Pierce quarantine in Nov 2015 (11 larvae) and Dec 2015 (6 larvae) but were not successfully colonized.

Table 1. Locations and dates of foreign surveys for insect herbivores of cogongrass.

Country	Dates	Areas visited
Tanzania	15–18 Feb 2013	Njombe District
Uganda	17–21 May 2015	Western Region, Masaka, Mbarara, Bushenyi, and Kabarole Districts
Philippines	23–27 Jul 2013	Laguna and Quezon Provinces
	16–19 Mar 2015	Laguna Province
Japan	22 Jul to 10 Aug 2013	Fukuoka and Saga Prefectures, Kyushu Region; Yamanashi Prefecture, Chubu Region; Kanagawa, Tokyo, Saitama, and Ibaraki Prefectures, Kanto Region
	3–12 Aug 2014	Fukuoka, Saga, and Kagoshima Prefectures, Kysuhu Region
	4–10 May 2015	Saga Prefecture
Indonesia	12–16 May 2015	Bogor and Cianjur Regencies, West Java
	9–13 Feb 2016	Cianjur Regency, West Java

Acrapex azumai also was collected from cogongrass on Ishigaki Island in far southern Japan in Jun 2015. A shipment of 30 *A. azumai* larvae from Ishigaki was received at the Fort Pierce biological control quarantine laboratory in Jun 2015, but the shipment was delayed in transit for several days and the larvae arrived in poor condition. Only 4 individuals pupated, from which 2 moths emerged. No mating was observed and no eggs were obtained.

Contarinia sp. (Diptera: Cecidomyiidae)

This gall midge causes a dead-heart symptom similar to that produced by *Acrapex* species. The midge was collected from the Ito campus of Kyushu University and several other locations in Fukuoka and Saga prefectures on Kyushu Island in late Jul to early Aug 2013. Dissection of plants revealed a spiraling deformity of the central tiller 4 to 15 cm above ground level. In several stems, a small pinkish larva was found near the deformity. No adults were reared, but larvae were identified as a *Contarinia* sp. Plant deformity in response to feeding by members of the genus *Contarinia* has been reported (Bardner et al. 1971; Brewer et al. 1994), and in sunflowers is thought to be associated with elevated levels of auxin (Brewer et al. 1994). As many cecidomyiid midges have narrow host ranges (Gagné 1989; Yukawa 2000; Gagné 2004), the midge found in cogongrass may warrant further investigation as a potential biological control agent.

INDONESIA

Orseolia javanica Kieffer & van Leeuwen-Reijnders (Diptera: Cecidomyiidae)

A previous study in West and Central Java, Indonesia, identified the stem gall midge *O. javanica* as a potential biological control agent of cogongrass (Mangoendihardjo 1980). Limited host range testing with cultivated rice, 2 wild *Oryza* species., corn, sorghum, and 2 other non-cultivated grasses, *Paspalum conjugatum* P. J. Bergius (Cyperales: Poaceae) and *Penisetum polystachyon* (L.) Schultes (Cyperales: Poaceae), suggested a narrow host range with midges surviving only in cogongrass. According to Mangoendihardjo (1980), midges held in cages with cogongrass plants laid 98% of their eggs on the soil and 2% on plant stems. Most eggs were viable, but very few larvae were able to successfully enter the plant. Once inside stems, larval feeding induced the formation of a stem gall, in which 1 larva developed and pupated. After gall formation, infested stems ceased development and senesced after the emergence of adult midges. During visits to West Java in May 2015 and Feb 2016, stem galls were abundant along bunds separating rice paddies in Cianjur District. Galls, along with stem crowns, roots,

and pieces of rhizome, were removed from the soil and placed in clear acrylic tubes (5.08 cm in diameter, 30 cm in length) sealed with plastic caps and transported inside coolers to the Fort Pierce quarantine laboratory. The 2015 collection was maintained in 8 groups of approximately 30 galls each, held in acrylic plastic containers (19 × 14 × 10 cm).

Of 247 galls collected, only 3 midges (1%) emerged, 2 females and 1 male. The females died several days before the male emerged so there was no opportunity for mating and colony establishment. Three species of parasitoids emerged from galls, including *Platygaster orseoliae* Buhl (Hymenoptera: Platygastridae), *Prospicrosctus mirificus* Girault (Hymenoptera: Pteromalidae), and an *Aprostocetus* sp. (Hymenoptera: Eulophidae) (Buhl & Hidayat 2016). In 2016, midges emerged from 14 of 215 galls (6.5%) collected in Feb, including 6 that emerged and died during transport. The other 8 adults (2 females and 6 males) emerged within the first 2 d in quarantine and were placed together in a cage with cogongrass plants. No mating or oviposition was observed and all midges died within 24 h of emergence. Plants were held for 6 wk but no galls formed, indicating a failure to obtain progeny. From this collection, a subset of galls ($n = 71$) was held individually to allow calculation of parasitism. *Aprostocetus* sp., *P. orseoliae*, and an unidentified eupelmid emerged from 26 (37%) galls.

PHILIPPINES

Emmalocera sp. (Lepidoptera: Pyralidae)

This stem borer was collected at 1 location in Laguna Province and 1 location in Quezon Province during a survey in Jul 2013. Its closest match in GenBank was *E. latilimbella* (Ragonot), but with only a 93% sequence similarity and an “E” value of 0.0. *Emmalocera latilimbella* is only known from Australia and Papua New Guinea (BOLDSYSTEMS 2016; Global Information System on Pyraloidea 2016), and a literature search revealed no records of larval host plants. With a 93% similarity, it seems unlikely that the borer is *E. latilimbella*, but based on similarity among other Lepidoptera (Kim et al. 1999; Cognato 2006), it is probably in the genus *Emmalocera*.

Chilo sp. (Lepidoptera: Crambidae)

During a survey in the Philippines in Mar 2015, crambid larvae were found boring in the stems of cogongrass near Binan, Laguna Province, causing damage similar to that described above for *Acrapex* species. Based on the *CO1* gene, the closest species in GenBank was *Chilo partellus* (Swinhoe), a notorious pest of corn and sorghum in southern Asia and East and southern Africa (Kfir et al. 2002; Sharma et al. 2007) that is not known to occur in the Philippines. However, the sequence similarity was only 92%

(E value = 0.0), suggesting that the insect was not *C. partellus*. This was confirmed by morphological identification as a *Chilo* sp., but taxonomically distinct from *C. partellus*. A collection of 30 larvae was sent to the Fort Pierce quarantine facility, where they were placed on cut stems of cogongrass. Although all the larvae appeared to be mature when collected, several did not pupate until late May and early Jun, suggesting that they may have been in aestivation at the time of collection, which was during the dry season in Luzon. Two individuals reached the adult stage in mid-Jun but efforts to establish a colony were unsuccessful.

EAST AND SOUTHERN AFRICA

Acrapex yakoba Le Ru (Lepidoptera: Noctuidae)

Le Ru and colleagues have been investigating lepidopteran stem-borers associated with native grasses in East and Central Africa for the past 13 yr (Le Ru et al. 2006), and they have discovered 4 species in the genus *Acrapex* that have only been collected from cogongrass (Le Ru et al. 2014). In Feb 2013, 580 larvae of *A. yakoba* were collected from several patches of cogongrass in the Njombe region of southwestern Tanzania. Of the total collection, 490 larvae were taken to the laboratories of the International Center of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya, whereas 90 larvae were hand-carried to the Fort Pierce quarantine laboratory. In Fort Pierce, 27% of larvae pupated and of those, 10 produced adults, which were placed in a 4 L plastic cage with cut stems of cogongrass. No mating was observed, but 206 eggs were laid in leaf sheaths in groups of 1 to 64. In total, 21 eggs eclosed and neonate larvae were placed on an artificial diet. All larvae died before molting to the 2nd instar. In Nairobi, 25% of larvae pupated, 16 adults emerged and laid 336 eggs, of which 170 eclosed (46%). Neonate larvae were placed on an ICIPE-developed diet (Onyango & Ochieng' Odero 1994), but none completed development.

Acrapex syscia Fletcher (Lepidoptera: Noctuidae)

In May 2014, *A. syscia* larvae were collected from cogongrass during a survey in southwestern Uganda. Larvae were either hand-carried to ICIPE laboratories in Nairobi, Kenya (482 larvae), or to the University of Florida biological control quarantine laboratory in Fort Pierce, Florida (88 larvae). At ICIPE, 452 larvae were placed on the same artificial diet as used for *A. yakoba* and the remaining 30 were placed on live cogongrass plants. Larval consumption of the artificial diet was poor compared with the feeding of *A. yakoba* and less than 9% pupated. A few adults (22 females and 21 males) emerged but several were malformed, and few emerged at the same time. Only 2 couples were paired, but no eggs were produced. Out of the 30 larvae placed on cogongrass plants, there was no evidence of colonization after 2 to 3 wk; after 7 to 8 wk, the plants were dissected and no larvae were recovered.

In Florida, 88 larvae were received alive in quarantine, of which 10 were placed on each of 4 whole plants whereas the remaining larvae were placed in vials on artificial diet (Frontier Agricultural Sciences, Product F9775B). From the larvae placed on whole plants, only 2 adults emerged, whereas 5 adults successfully developed on artificial diet. All adults were placed in one 4 L plastic cage with 4 to 6 cut stems of cogongrass (about 20 cm long) held upright in wet sand. Stems were inspected daily and replaced. In total, 158 eggs were collected in 4 batches and placed in Petri dishes with moistened filter paper. None of the eggs hatched, possibly due to lack of fertilization.

Acrapex subalbissima Berio (Lepidoptera: Noctuidae)

During surveys in the Kipengere and Uzungwa mountains of Tanzania, Le Ru et al. (2014) found *A. subalbissima* only in cogongrass. They

stated that it is a "markedly hygrophilous species found along banks of rivers and streams." No attempt was made to initiate a laboratory colony of *A. subalbissima*.

Acrapex mitawa Le Ru (Lepidoptera: Noctuidae)

Le Ru et al. (2014) discovered *A. mitawa* in the Ruvuma region of Tanzania and only found it in cogongrass, despite sampling several other grasses. Like *A. subalbissima*, it was found in cogongrass growing in moist areas along river and stream banks and in marshes. No attempt was made to establish a laboratory colony of this species.

Discussion

Grasses have not often been targeted for biological weed control (Winston et al. 2014). Major concerns include the potential for non-target risks to economically important grasses (Grevstad et al. 2003) and the belief that grasses may have a paucity of specialized insect herbivores due to architectural simplicity (Lawton 1983) and decreased defensive chemistry (Tscharntke & Greiler 1995). However, several studies have demonstrated that insect herbivores may exhibit a high degree of specificity towards grasses (Tewksbury et al. 2002; Grevstad et al. 2003; Goolsby & Moran 2009; Goolsby et al. 2009). Our surveys in Africa and Asia strongly suggest the availability of specialized insect herbivores of cogongrass. Several *Acrapex* species stem-borers from East Africa appear to be specialist feeders on cogongrass (Le Ru et al. 2014), as well as *A. azumai* from Japan (Takasu et al. 2014). Limited host range testing of the gall midge *O. javanica* suggests that it may be monophagous, and based on field observations, it is highly damaging. These, and the other above-mentioned herbivores, may have value for classical biological control of cogongrass in the southeastern USA.

Although attempts were made to rear several insects, including the *Acrapex* species from Japan and Africa, a *Chilo* sp. from the Philippines, and *O. javanica* from Indonesia, no insects were successfully colonized. The lack of success with *O. javanica* is not surprising considering the low numbers of adult midges that emerged in quarantine. Once galls are recognizable in the field, larvae may be nearing maturity or have already pupated, allowing little time to transport live insects from Indonesia to a quarantine laboratory in the USA. Moreover, parasitism was very high, further diminishing chances for colony establishment. Efforts are currently underway at Bogor Agricultural University to develop laboratory rearing procedures, which would allow the shipment of large numbers of parasitoid-free individuals to Florida for colony establishment.

The failures to colonize the lepidopteran stem-borers are more difficult to explain, as fairly large numbers of larvae (≥ 30) were used on 7 occasions to try and initiate colonies. The lack of success in Florida could possibly be due to genetic differences between Florida cogongrass and the plants from which the insects were collected. Molecular studies have identified several clonal lineages of cogongrass in the USA (Burrell et al. 2015), and these are more closely related to Asian cogongrass types than cogongrass from East Africa (A. M. Burrell, unpublished data). Failure of herbivorous insects to complete development due to intraspecific differences between host plant populations has been reported in several systems (e.g., Garcia-Rossi et al. 2003; Dray et al. 2004; Goolsby et al. 2006; Manrique et al. 2008), including grasses (Tscharntke & Greiler 1995). Rearing the stem-borers on artificial diets was also attempted, as diets have successfully been used for many lepidopteran stem-borers (e.g., Shanower et al. 1993; Ng-Song et al. 1995, Le Ru et al. 2006). The artificial diet used in Fort Pierce was developed for *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae), a Neotropical pest of sugarcane. At ICIPE, the *Acrapex* species were pro-

vided a diet developed for *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) (Onyango & Ochieng' Odero 1994), a stemborer that primarily feeds on corn and sorghum (Kfir et al. 2002; Sezonlin et al. 2006). Apparently, neither of the diets was suitable for the cogongrass borers. Future efforts could evaluate other commercially available diets, but it may be more productive to place greater emphasis on developing rearing procedures using live plants at laboratories in the native ranges of candidate biological control agents, where differences in host plant genotypes can be eliminated as a possible cause of failures, and additional insects can more readily be collected in the field.

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