Studies in Argentina on two new species of *Thrypticus* (Diptera: Dolichopodidae) as agents for the biological control of water hyacinth, *Eichhornia crassipes*

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Summary

For about thirty years *Thrypticus* spp. (Diptera, Dolichopodidae) were considered as possible candidates for biocontrol of water hyacinth (*Eichhornia crassipes*) in its adventive range. Initially it was thought that there was only one species attacking plants within the Pontederiaceae. However, five new species of *Thrypticus* have been identified from water hyacinth. Due to their abundance and wide geographical distribution, *T. truncatus* and *T. sagittatus* (provisional names) were prioritized for further investigation. Both species have similar behaviour and appear to share the same similar ecological niche. The larvae bore a horizontal mine in the petiole, making a small incision in the vascular bundles. The larvae then feed mainly on the exuded sap. In the Southern Hemisphere, the flies reproduce from spring through to the end of summer. During autumn and winter, no oviposition was recorded, suggesting that both *Thrypticus* species spend the winter months as larvae in the petioles. One generation in summer requires about 7 weeks. Preliminary host-range testing, conducted in the laboratory and in the field by interspersing test plants among infested water-hyacinth plants, showed that none of the following plants were attacked: *Eichhornia azurea*, *Pontederia cordata* var. *cordata* and var. *lancifolia*, *P. rotundifolia*, *Echinodorus grandiflorus*, *Canna glauca*, *Myriophyllum aquaticum*, *Heteranthera reniformis*, *H. callifolia* and *Monochoria africana*. During field surveys, both species of flies were only reared from *E. crassipes*. These results indicate that both species warrant further studies on their biology and specificity.

Keywords: biological control, *Eichhornia crassipes*, *Thrypticus* spp., water hyacinth.

Introduction

Water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laub.) is a very damaging aquatic weed occurring on water bodies in more than 50 countries with warm climates (Mitchell & Thomas 1972). This weed has different strategies for growing in a broad range of environments, including sexual and vegetative reproduction, a very fast growth and dispersal rate in tropical regions, an ability to survive attack by a complex of natural enemies, and seeds which remain viable for long periods of time.

The control strategies include biological, chemical and integrated methods. Seven biological agents have been released in 33 countries (Julien & Griffiths 1998). While success has been achieved in some areas, results in other areas, including some areas of South Africa (Hill & Ockers 2001) have been less successful. Therefore, new agents are being considered for release around the world, including the petiole-boring flies in the genus *Thrypticus*. For about 30 years, *Thrypticus* spp. (Diptera, Dolichopodidae) have been considered as possible candidates for biocontrol of water hyacinth in its adventive range (Bennett 1968). These flies were suspected to have a wide host acceptance, but the studies being carried out at the South American Biological Control Lab (SABCL) suggested that they might be suitable (Cordo et al. 2000). This paper presents a summary of the advances in the knowledge of these
promising biological-control agents, including studies on the taxonomy, biology and host specificity.

**Materials and methods**

The surveyed area for *Thrypticus* on Pontederiaceae included the Paraná–Paraguay river catchments, southeast of Brazil, and the upper Amazon River near Iquitos in Perú. Adult flies were obtained by placing petioles of Pontederiaceae from the field into emergence boxes. The adult flies were kept in cold dishes to separate species and sexes. Microscope slides were prepared for descriptions of genitalia for taxonomic examination. After identification of the species, the two most abundant species on water hyacinth in Argentina were selected for further studies. We assigned them provisional names of *Thrypticus* sp1 and *Thrypticus* sp2. Observations on the behaviour of the larvae and adults were made in both the laboratory and the field. In the laboratory, observations were made with pure colonies of each species. In host-specificity tests, the appearance of mines was taken as evidence of oviposition due to the small size of the adults and their eggs. This implies that the female found the substrate suitable for egg laying, and that the larvae emerged and accepted the petiole for feeding. Consequently, the results of the specificity tests recorded both oviposition and larval development.

All plants used were obtained from seeds or collected as small plants from the field. The trials were conducted with non-clonal plants. Water hyacinth was cultured in pools (2 × 1.4 × 0.6 m) with water and 15 cm of soil in the bottom as source of nutrients. The identifications of the species of plant used were based on Castellanos (1959), Cabrera (1969), Eckenwalder et al. (1986) and Horn (1987).

**Host-range testing**

Two type of tests were conducted to determine the host range of selected species of *Thrypticus*.

**Field-based host-specificity trials**

Field trials were conducted in a canal (200 × 10 m) connected to Carabelas Grande River (34°4.98'S; 58°48.6'W), Buenos Aires Province. This river belongs to the delta of Paraná River and is representative of the temperate environment of this catchment basin. The water hyacinth mat covered the whole canal and supported a natural population of *Thrypticus* sp1 and *Thrypticus* sp2. Five sites were marked 15 m apart along the canal. At each site, one plant of each of the test-plant species and one non-infested water-hyacinth plant (control) were interspersed. The test species used were: Pontederiaceae – *Eichhornia azurea* (Swartz) Kunth, *Pontederia cordata* L.; *Echinochloa crus-galli* (L.) P. Beauv.; *Echinochloa var. lanccifolia* (Muhl.) Torrey, *P. rotundifolia* (L.f.); Alismataceae – *Echinodorus grandiflorus* (Chamisso et Schlechtendahl) Micelli.; Cannaceae – *Canna glauca* L.; and Haloragaceae – *Myriophyllum aquaticum* (Veloso) Verdecourt.

These species were selected because they possess aerenchyma. This tissue is important in the development of these *Thrypticus* species. The plants were in position for a mean duration of 14 days. After the exposure, the test-plant species and the water-hyacinth control were removed and returned to the laboratory to record the development of larval mines. This experiment was repeated five times during the summers of 2001 and 2002.

**Laboratory-based host-specificity trials in garden pools**

The oviposition tests, to establish the host range of two selected species of *Thrypticus*, were carried out in two walk-in cages each containing a plastic garden pool (2 × 1.4 × 0.6 m). The water-hyacinth culture, started 5 months before the experiment, had 90 plants per pool during the period of the trials. Each pool was divided into 30 quadrats. Each quadrat was assigned at random to the test plants or water-hyacinth control plants. Five plants of each of five test-plant species plus five water hyacinth controls were exposed, one plant per quadrat, simultaneously to the flies. It was necessary to maintain the pools filled with water hyacinth as the canopy they formed was necessary to prevent the *Thrypticus* flying toward the mesh of the cage when released. The test plants (all Pontederiaceae) were from the species: *Eichhornia azurea*; *Pontederia cordata*, *P. cordata* var. *lancifolia*; *Heteranthera reniformis* Ruiz & Pavon; *H. callifolia* Rchb. ex. Kunth; *Monochoria africana* (Solms-Laub.)N.E. Brown. Test plants were kept in pots with soil in the pools.

Tests with *Thrypticus* sp1 and *Thrypticus* sp2 were performed separately. From 23 to 30 January 2002, 99 females + 76 males of *Thrypticus* sp1 were released in one cage. From 14 to 30 January 2002, 444 females + 366 males of *Thrypticus* sp2 were released in the other cage. The mean temperature inside the cages for the period of the trials was 22.9°C, with a maximum of 41.1°C and a minimum of 12.5°C.

**Results**

**Taxonomy**

The taxonomy of the group of species that utilize water hyacinth and other Pontederiaceae as host plants was studied by Dr Daniel Bickel (Australian Museum, Sydney) and M.C. Hernández (SABCL, Argentina). They described five new species from water hyacinth: *Thrypticus truncatus* Bickel & Hernández, *T. sagittatus* Bickel & Hernández, *T. yanayacu* Bickel & Hernández, *T. chanophallus* Bickel & Hernández and *T. circularis* Bickel & Hernández.
Thrypticus as agents for control of water hyacinth

Thrypticus truncatus (provisional name: Thrypticus sp1) and T. sagittatus (Thrypticus sp2) were selected for further studies because they are the most abundant species on water hyacinth in Argentina. Both species are mostly metallic green with silvery dust, but each with a particular distribution of this colour. Additionally, the shape of the abdomen differs in dorsal view; Thrypticus sp1 is oval shape, while Thrypticus sp2 is more conical.

Biology

Adults

Both species, Thrypticus sp1 and Thrypticus sp2, reproduce in the same habitat. They coexist in the protected microenvironment under the water-hyacinth canopy, where they are very elusive insects. The individuals remain in the basal part of the petioles where they make short flights up and down or between the petioles. They walk backwards, descending the petiole toward the water surface. The adults emerge around noon and mating takes place in the warmer part of the day. Before copulation, the male moves near the female and jumps repeatedly over her, up and down. In some of these jumps, he alights on the female for an instant. If the female remains in the same place for a following jump, mating occurs. Mating lasts from 1 to 2 min. Thrypticus truncatus adults live for about 5–9 days and their complete development (egg to adult) takes 7 weeks in summer.

Larvae

The larvae of Thrypticus sp1 and Thrypticus sp2 have no evident morphological differences and show similar behaviour. The first instar larva mines across the septa of the aerenchyma joining the vascular bundles spread in the tissue. The larva scrapes a small portion in each bundle. Although the larva eats the tissues to dig the mine, it feeds mainly on the sap that exudes from the damaged bundles. There is some doubt whether it is true phytophagy or if the larvae are feeding on bacteria, yeasts or fungi in the plant wound (D. Bickel, pers. comm.). Inside the mine, the larva moves back and forth re-visiting the damaged bundles and enlarging the mine to accommodate its increasing diameter. The larva does not leave the mine although there are openings at each end. Moreover, the larvae do not survive out of the mine nor do they have the ability to form another new mine if transferred to a new petiole.

Pupa

The late-instar larva cuts an epidermal operculum near one of the orifices in the petiole and digs a chamber. After sealing the chamber, pupation occurs.

Reproductive period

In the southern part of its distribution, near Buenos Aires, both species of Thrypticus reproduce from spring to the end of summer. New mines were not recorded in autumn or winter. They spend the cold season as larvae in the basal part of the petioles. These parts remain alive during regular winters even when freezing temperatures kill the laminae and distal part of the petioles.

Host-range tests

Field-based host-specificity trials with Thrypticus sp1 and Thrypticus sp2.

Mines of Thrypticus were produced only on water-hyacinth control plants. Mines were not recorded in any of the test plants. The larvae did not complete their development in the water-hyacinth controls because the petioles decayed rapidly when the plants were transported back to laboratory conditions.

Laboratory-based host-specificity trials in garden pools enclosed in walk-in cages

Both species of Thrypticus produced mines on water hyacinth, but not on any of the test plant species. Forty-five days after the first release, all the test plants and water-hyacinth controls were examined for mines. The mean number of mines per plant in controls of Thrypticus sp1 was 1.4 (SD 0.89). For Thrypticus sp2, the mean number of mines per plant in controls was 2.2 (SD 3.27). With the methodology used, the development time of the larvae is prolonged and the petioles deteriorate before they can complete their development.

Discussion

This study has achieved several objectives. The taxonomy of this group has now been revised and will be published shortly. Most aspects of the biology have been quantified. However, further studies are required to quantify the impact of the flies on water hyacinth. The most promising aspect of this study is that both the laboratory and the field-based host-specificity trials concur with the field surveys in Argentina that both Thrypticus sp1 and Thrypticus sp2 are monophagous on water hyacinth. According to current evidence, both species are safe for use as biocontrol agents of water hyacinth around the world.

Acknowledgements

We thank Alejandro Sosa for his valuable contribution and support in field works and trips. Also thank Daniel Gandolfo and Arabella Bugliani for reading and comments.

References


