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The Petiole Mining Fly *Thrypticus* sp. (Diptera: Dolichopodidae), a New Agent for the Biological Control of Water Hyacinth (*Eichhornia crassipes*)

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Abstract

Since the beginning of the investigations on natural enemies of water hyacinth, *Thrypticus* has been mentioned among the top seven candidates for biological control. In 1995, a revived interest for biocontrol of water hyacinth directed the investigations to new agents and *Thrypticus* stood as a priority candidate. *Thrypticus* occurs in Central and South America, from Panama (probably introduced) to Argentina and it is probably the most prevalent phytophagous insect in the whole native range of water hyacinth. Until now, water hyacinth was the only host known in the family. We found five different species of *Thrypticus* attacking four plant species in the Pontederiaceae family: Eichhornia crassipes (Martius) Solm-Laubach (two species of *Thrypticus*), *E. azurea* (Sw.) Kunth , *Pontederia cordata* L. and *P. subovata* (Seub.) Lowden. Adults are 1.5 to 2 mm long, metallic green in color. Females lay eggs singly in petioles of *E. crassipes*, inserting the eggs into the tissues just above the water line. The larvae, which are only a few mm long, bore through the bases of the petioles. The horizontal tunnels, open at both ends, are blackish in color. When larvae are mature, the leaf is already senescent, and the adults emerge from petioles above or below water level and starting to rot. Although the effect of its damage on the demography of water hyacinth is unknown, other attributes of the fly like the apparent strict monophagy, ubiquity and abundance appear promising.

Keywords: water hyacinth, biological control, mining fly *Thrypticus*

Since the beginning of the investigations on natural enemies of water hyacinth, *Thrypticus* has been mentioned among the top seven candidates for biological control. In 1995, a revived interest for new agents for water hyacinth, directed the investigations to second priority candidates. Among these, Cordo (1996) recommended to study *Cornops, Palustra* and *Thrypticus* in merit of their predicted narrow host range. Due to the failure of *Palustra* and the studies of *Cornops* conducted at PPRI of South Africa, the research at the South American Biological Control Laboratory (SABCL) focussed on *Thrypticus*. We present here the literature review and early field and laboratory research at SABCL in Argentina during the growing season1997-1998. Since the South American *Thrypticus* are essentially unidentifiable (D. Bickel, Australian Museum, Sydney, Australia, personal communication) we call the species treated here by the host plant name.

Materials and Methods

Literature Review. A literature survey, conducted at the USDA, National Agricultural Library on *Thrypticus* and associated plants, produced 12 answers from which a literature review was conducted.

Research at SABCL. Damaged petioles were collected during four trips made to northeastern Argentina (Chaco region) in December 1997 and January, February, March and June 1998. When convenient, other plants of similar architecture to water hyacinth and located in the vecinities of plants attacked by *Thrypticus* were examined carefully in search of mines. Petioles with mines of *Thrypticus* were separated from the plants, cut above the damaged zone and brought to the lab in plastic bags kept in the ice box. Entire plants with good mine-density were also collected and brought to the lab, with their roots in water, in 30 liter buckets covered with plastic bags. For emergence of adults, groups of about 10-20 petioles were placed in emergence boxes in the rearing chamber at 28 °C. The petioles were first placed randomly in the emergence boxes and in age-categories later. Emergence boxes used were of two kinds, the standard 4-liter plastic container covered with aluminum foil with one vial on top and a second kind, constructed with different materials. The latter consisted of a black polyethilene bag with a clear acrylic cylinder in its opening attached with rubber bands. The cylinder's top end was covered with a fine nylon mesh with a hole, closed with a cotton swab. Adults emerged from the petioles in the black plastic bag were removed from the clear acrylic cylinder on top, through the hole in the mesh with a regular insect aspirator. Adults collected from emergence boxes were handled in a CO_2 box to separate sexes and transfered to clear 500 ml plastic cups where they were kept and fed until used for rearing trials or killed for preservation. An inverted vial with sucrose 10% and a cotton wick was used, screwed through the cup lid to feed the adults. Moist tissue paper at the bottom provided humidity. Water condensation on the cages' walls had to be avoided with good ventilation, otherwise the adults were trapped and killed, unable to escape from the condensed drops.

For killing and preservation several methods were tried. Newly emerged adults were fed for 24 hours or more before killing them, to allow normal colour to develop and hardening of exoskeleton. They were killed with ethyl acetate or chloroform or in the freezer and placed in 70% or 80% ethanol or pinned. Some adults were fixed in boiling 70% ethanol and kept in the same fluid.

Rearing of adults was attempted in the laboratory, in the greenhouse and outdoors. In the laboratory, small cages made with clear plastic 2-liter bottles were used. A small water hyacinth plant was placed inside the bottle and a cotton lid moistened with 10% sucrose provided food. In the greenhouse, a fine 70 nylon mesh 61x61x61 cm cage was used. Water sugar solution was sprayed on water hyacinth leaves and moistened cotton swabs were also placed in different places of the cage. In the back yard, field-collected attacked plants were placed among non attacked plants in a 2 x 4 x 1.80 m walk-in cage and in concrete tanks.

Results and Discussion

Literature Review. *Taxonomy.* The genus *Thrypticus* (Diptera: Dolichopodidae), was erected by Gerstacker in 1864 and the type species is *T. smaragdinus* Gerstacker by monotypy. The body coloration of adults is usually bright metallic green and the eyes with short hairs between facets. The female oviscapt is blade like, narrow in dorsal view. *Thrypticus* is unique among the Dolichopodidae and represents a major evolutionary change because all known larvae are phytophagous stem-miners in the monocot families Cyperaceae, Poaceae and Juncaceae (Pontederiaceae not mentioned) whereas all other Dolichopodids larvae are free living and predatory (Dyte 1993). The sclerotized blade like oviscapt is designed for piercing and ovipositing within stems. The relatively small adults are generally taken in wet grassland or marsh habitat (Bickel 1986).

Thrypticus is almost cosmopolitan in distribution, with 71 described species, 39 from the New World, 4 afrotropical, 23 paleartic, 2 Oriental and 3 new Australian species. The genus seems to have radiated extensively in the neotropics (Bickel 1986).

Bennett and Zwolfer (1968) first mentioned this genus attacking water hyacinth in northern South America and Trinidad. They described and illustrated the damage and estimated insect density. However they gave no information on the host specificity nor on the effect of the attack on the growth rate of *E. crassipes* (Martius) Solms-Laubach. Later on, in 1976, Bennett attributed the species of *Thrypticus* reared by Bennett and Zwolfer (1968) to *Thrypticus insulanus* Van Duzee (misspelt as "*insularis*"). But according to Robinson (1975), *Thrypticus insulanus* is identical to *T. minutus* Parent and since this is an older name, *T. insulanus* must be regarded as a synonym of *T. minutus* (Dyte 1993). However, for unknown reasons, this epithet was subsequently not mentioned in the literature and the fly was referred to as *Thrypticus* sp.

Distribution. In America, Bennet and Zwolfer (1968) found *Thrypticus* in Trinidad, Guyana, Surinam and Brazil. Curiously, Bennett (1972) did not mention its presence in British Honduras, Jamaica, Barbados and St. Vincent and Trinidad. Mitchell and Thomas (1972) account for its existence in Argentina, Uruguay, Brazil, Guyana and Trinidad.

Life History. Cruttwell (1973) described preliminary investigations on the life cycle of *Thrypticus* sp. on water hyacinth, and it is the only account of this type available in the literature. Adults are 1.5 to 2 mm long, light brown in color. Females lay eggs singly in young stems of *E. crassipes*, inserting the eggs 0.5 to 1 mm into the tissues just above the water line. The eggs are yellow, 0.5 mm long and 0.17 mm diameter, curved with one end narrower than the other. Stems (petioles) are only suitable for oviposition just after separation from the leaf sheath, thus all tunnels in one stem are usually of the same age. The egg hatches in a few days and the first larval stage tunnels horizontally in the aerenchyma of the stem, making a second exit hole at the other end of the tunnel. The damaged tissues blacken and the tunnel first becomes visible on the seventh day after the egg is laid. Subsequently the larva continues to feed in the tunnel, enlarging and lengthening it by tunneling toward the center, and the tunnel and two exit holes become clearly visible.

There are 3 larval instars with a total duration of 35 to 42 days, and the mature larva is about 4 mm long. Pupation occurs in an enlarged chamber below one existing hole, an epidermal window having been cut first. The adult emerges in 7 to 12 days. The adults rest in the *Eichhornia* stems, sometimes making short flights from stem to stem. Mating couples have been seen on stems between 9 and 12 a.m. There is a preoviposition period of unknown duration, and females lay a maximum of 50 eggs over a period of several days. No parasites have been encountered.

Host range. Few species of *Thrypticus* have been reared, but plant mining would appear to be characteristic of the genus since all known females of the genus have a sclerotized blade-like ovipositor for piercing the host plant. The seven genera of host plants listed are all Monocotyledons and represent four families: Cyperaceae (*Thrypticus sp. "*Michigan*"* on *Eleocharis palustris* (L.), *Scirpus americanus* Persoon and *Scirpus occi-*

dentalis (Muhl.); *T. fraterculus* (Nh.) on *Scirpus acutus* (Muhl.)); Juncaceae (*T. bellus* Loew and *Thrypticus* sp. "France" on Juncus sp.); Poaceae (*T. bellus* Loew and *T. smaragdinus* Gerst. on *Phragmites australis* (Cav.), *T. villaceous* V.D. on *Spartina alternifolia* Loisel., *T. muhlenbergiae* J. and C. *on Muhlenbergia sylvatica* (Tor.), *Thrypticus* sp. "California" and *Thrypticus* sp. "Louisiana" on aquatic grass) and Pontederiaceae (*T. minutus* Parent on *Eichhornia crassipes*). *Thrypticus* are often taken by sweeping emergent vegetation and adults of several paleartic species whose hosts are unknown have been recorded on *Scirpus*, *Juncus* or *Phragmites* (Dyte 1993).

As the larvae do not leave their tunnels to enter new stems, host specificity is entirely determined by selection by ovipositing females. In Trinidad, rice, yam and sweet potato plants in tins were partly immersed in outside concrete tanks containing breeding population of *Thrypticus* in *E. crassipes*. The adult, frequently seen resting on water hyacinth petioles, were never observed on the test plants and no tunnels appeared on the stems of the test plants over a period of several weeks. Insect-free *Eichhornia* plants put in as controls showed visible tunnels in 8 to 11 days, and increasing number of tunnels on further stems (petioles) developed (Cruttwell 1973).

Abundance. Thrypticus was one of the six species of natural enemies quantitatively sampled by Bennett and Zwolfer (1968) in their first survey in northern South America. Mitchell and Thomas (1972) noted that *Thrypticus* was fairly common throughout the survey area in Argentina, Uruguay, Brazil, Guyana and Trinidad.

Damage. Bennet and Zwolfer (1968) stated that *Thrypticus* of water hyacinth bore small tunnels in the base of petioles <u>below water level</u>; tunnels open at either end. Attacked plants are recognized by the small blackened tunnel orifices. Frequently, 30 or more tunnels occur on one stem. However, Mitchell and Thomas (1972) commented that the larvae bore through the bases of the stems just <u>above water level</u>. The horizontal tunnels, open at both ends, are blackish in color due partly to the presence of frass and partly to the roting caused by invasion of pathogens. The insect was found to cause considerable damage to a small patch of *E. crassipes* at Santos, Brazil. Nearly all the plants attacked had extensive roting at the bases of the stems, and in many cases the stems had collapsed completely.

As the larvae develop, the leaf ages and the stem gradually approaches the water. Eggs are laid above water level but all stages develop equally well below water surface. When larvae are mature, the leaf is already senescent, and the adults emerge from stems below water level and starting to rot. Consequently the effect on the plant is minimal, as the maximum damage occurs on already senescent stems and damage to young stems is negligible (Cruttwell 1973). However, the damage of *Thrypticus* may render plants more prone to being attacked by secondary organisms (Bennett 1968). In northern Argentina, Poi de Neiff *et al.* (1977) reported that significant attacks by *Thrypticus* were observed on *E. crassipes* with a maximum, in May 1976, when 79% of the petioles were damaged. The maximum insect density during the study period (Apr. 75 to Jul. 77) was 26 larvae per petiole. Sanders *et al.* (1982) mentioned that regardless of location of the plants in the Panama Canal, it was apparent that *Thrypticus* used erect, slender petioles more extensively than small, bulbous ones. They found that the mean number of oviposition holes per leaf during the study span (Jan. 78 to Jan. 80) was 21 (range 10-46).

Potential for Biological Control. Bennett and Zwolfer (1968) stated that at least until the identity of the species has been established, priority should be given to the investigation of other species: *Neochetina, Acigona, Sameodes*, *Cornops* and *Orthogalumna*.

However, in the same year, Bennett (1968), affirmed that *Acigona* and *Sameodes* cause appreciable damage and appear adequately host specific to warrant further investigations; similarly, *Orthogalumna, Neochetina*, *Cornops* and *Thrypticus* merit further studies. Mitchell and Thomas (1972) felt that *Thrypticus* warranted a much more detailed investigation particularly, initially, in terms of its host specificity. They were probably impressed by the heavy damage observed at Santos in Brazil. Cruttwell (1973) considered that the damage of *Thrypticus* to water hyacinth was minimal, thus indirectly lowering the insect´s capabilities as an agent for biocontrol. In Argentina, Poi de Neiff *et al.* (1977) estimated that Cornops and *Thrypticus* were responsible for most of the decay of water hyacinth foliage, greatly increasing the number of rotten leaves. Although water hyacinth petioles were heavily used as oviposition sites by *Thrypticus* on some sites, the only potential effect of this species on water hyacinth was that oviposition scars could serve as entry points into the plant for facultative pathogens or secondary invaders. The result would be an increase in the rate of senescence of individual water hyacinth leaves (Sanders *et al.* 1982).

Research at SABCL. *Host Range*. In Argentina, we found *Thrypticus* sp. attacking the most common species of aquatic and semiaquatic Pontederiacea: *E. crassipes*, *E. azurea*, *P. cordata*. and *P. subovata*. *Thrypticus* was not found on *Pontederia rotundifolia* (formerly known as *Reussia rotundifolia*), another common Pontederiaceae in the country. Since we did not find species of *Heteranthera* (four species in Argentina) in our surveys, it is unknown if plants of this genus could host *Thrypticus*. Aquatic plants, other than Pontederiaceae, of same habitat and morphologic architecture were examined for *Thrypticus* damage. Hundreds of petioles or stems of the following plants were examined: *Typha* spp., *Echinodorus* spp., *Scirpus* spp., *Thalia* spp. and *Canna glauca*. No oviposition orifices nor larval tunnels were found.

Field occurrence. Most visited sites with *E. crassipes*, had petioles damaged by *Thrypticus*. Average presence (No. sites with *Thrypticus* per No. total sites examined) was 80% (16/20). Intensity of damage varied with sites but in general, only a few sites showed high density of mines in the petioles. In *E. azurea* presence was much lower, 31% (4/13), similarly *P. cordata*, 26% (6/23).

E. crassipes. Total number of petioles collected during the season (1,683) had a mean of 15 mines per petiole and a maximum of 56 mines per petiole. The mines were only in the basal part of the petioles, usually in the zone near or below water level, that is petiole insertion to 10 cm above. The best sites found with good and constant damage through the season were Estero near Río Bermejo in Chaco province and Palo Santo in Formosa province. Both places shared the presence of tall, slender and packed plants in a small channel crossing the road. Both patches were small about 10-15 m long by 5 m wide. The plants were about 1 m. tall, with 10 leaves. Petioles were thin (1.5 cm diameter at the base) with a hard, compact aerenchyma. The preference of *Thrypticus* for this kind of plant has been mentioned in the literature.

E. azurea. The 253 damaged petioles of this plant had a mean of 17 mines per petiole and a maximum of 64 mines per petiole. The fly seems to prefer the spongy tissues of the sheath of the floriferous petiole. Most of the mines were found in this organ although some few mines were observed on the harder tissues of the stems. *Thrypticus* attacks were more rare on *E. azurea* than on *E. crassipes*. Only three sites have been found, while in water hyacinth it is prevalent. It was noted that most of the attacked petioles were underwater or partially submerged.

P. cordata. Damage of *Thrypticus* on this plant was the least when examined as mines per petiole. The 170 petioles collected had a mean of 11 mines per petiole. However, the presence of the fly is higher than in *E. azurea*. Both varieties were found attacked although *P. cordata cordata* seems to show more damage than *P. cordata lancifolia*. This could be explained as var. *cordata* is more abundant in more tropical areas than var. *lancifolia* and the distribution of *Thrypticus* seems to be favored by high temperatures as well. This needs to be confirmed with more field data.

Two observations, about host plant range, were considered very interesting. In the first one, about 20 big 1 x 1 m *P. cordata cordata* plants located 50 m apart from one of the heaviest water hyacinth attacked populations found in the country had zero damage. It was curious that such vigorous, good looking, healthy plants were not attacked by flies abundant 50 meters away. The second case was *P. cordata lancifolia* which densely populated a pond of about 2,000 m2 with intermingled *E. azurea* vigorous plants (stems 2.5 cm diameter, pseudolamina 20 cm wide). Forty petioles of *E. azurea* heavily mined by *Thrypticus* were collected on 30 terminals examined, that is, almost every petiole had damage. However, in 300 petioles of *P. c. lancifolia* examined, only 10 damaged petioles were attacked very lightly with 5 mines per petiole.

Adults were observed for the first time in the field on *P. cordata cordata*. The plants were vigorous, 1.20 m high, with thick petioles. Water level was about 60 cm, that is, the plants were submerged from the middle down. Damage of *Thrypticus* was unusually high, 57 out of 60 petioles examined had mines. All of them were submerged about 30 cm below the water surface. Adults were resting or making short, jumping flights from petiole to petiole but very close to the water, less than 3-4 cm above. Fifteen adults were collected with the aspirator.

Pontederia subovata. This plant was found hosting *Thrypticus* only once. The mines were located in the submerged stems (internodes) rather than in the petioles as in the other three Pontederiaceae. The 14 stems collected had a mean of 8 mines per stem equal to 1-2 mines per internode.

Taxonomy and Mining Pattern. After handling hundreds of adults emerged from *E. crassipes*, *E. azurea*, *P. cordata* and *P. subovata* and studying the mining pattern in each host plant, it became apparent that more than one species of *Thrypticus* was involved. The preliminary study of male and female genitalia permitted the separation of five species from the four Pontederiaceae host plants. The specialist in Dolichopodidae flies, Dr. Dan Bickel of the Australian Museum of Sydney, Australia has offered to help in identifying and/or describing all species and arrangements are being made to accomplish this work. In the meanwhile, we arbitrarily called species “eichhorniae 1” and “eichhorniae 2” on *E. crassipes*, “azureae” on *E. azurea*, “pontederiae” on *P. cordata* and “reussiae” on *P. subovata*. In agreement with the morphological differences found, five distinctive mining patterns were identified in transversal cuts of the attacked petioles (stems in *P. subovata*). The species “eichhorniae 1” has a “U” shaped mine with one orifice at each end of the mine that is two orifices per mine; species “eichhorniae 2” has a “Y” shaped mine with two orifices; species “azureae” has a “C” shaped mine with two orifices; species “pontederiae” has a “C” shaped mine with more than two orifices, usually three to five and species “reussiae” an “O” shaped mine (totally surrounding the stem) with more than five orifices.

Emergence of Adults in Laboratory. Emergence of “eichhorniae” was very low. From

1,683 petioles with 25,245 mines, only 815 adults emerged; that is, 3.23 adults emerged from 100 mines. The rate was higher for "azureae", 6.36 adults/100 mines, and even higher for "pontederiae",12.03 adults/100 mines. Emergence rate for "reussiae" was 1.88 adults per 100 mines. The cause of this low emergence seems to be explained by the lack of larval development in the excised petioles. Only pupae or fully developed larvae in the cut petioles were able to complete development and produce adults.

Damage to Plants. The damage that *Thrypticus* produces to its host plants is due to the mining activities of the larvae when they develop inside the aerenchyma tissues of the petioles. The female punctures a hole in the petiole where it lays one egg. After the larva hatches, it begins building the horizontal mine and making a second hole at the end. So, shortly after hatching, the mine already has two holes at both ends of the mine. During its development, the larva moves from one end of the mine to the other, enlarging the holes gradually and following a course that is different for each species of *Thrypticus*. The mines are characterized by their distinctive pattern, the diameter of the orifices, the distance between orifices and the diameter of the window through which the adult emerges. This window is partially cut by the fully developed larvae before pupation, but it remains closed until adults push it open for emergence. The two mines parameters that seem to be specific for each *Thrypticus* species are the distance between orifices and the diameter of the emergence window. They apparently respond to the mining pattern and the different size of the adults, that in the case of the window diameter, appears linearly correlated to the adult´s size. Following this reasoning, "reussiae" with 0.813 mm diameter windows has the largest adult followed by "pontederiae", 0.705 mm, then "eichhorniae 1 and 2", 0.630 mm and last "azureae" with 0.480 mm window.

Rearing. Several rearing trials were attempted to produce a laboratory colony but all failed. In the laboratory, adults (5-20 males and females) placed in 2-liter clear plastic bottles and fed with sucrose 10%, survived about 20 days without showing any signs of courtship or mating. Temperature and/or light of laboratory were not high enough to elicit sexual behavior. Adults in the bottles were attracted by intense light when incandescent or fluorescent lamps were put close to the bottles. Outdoor trials were equally unproductive. Adults, supposedly emerging from field-collected attacked plants, were unable to oviposit on healthy caged and uncaged plants. Best results were obtained in the greenhouse, here 356 adults (145 males and 211 females) produced 285 mines on 43 petioles of 43 plants. However, none of the mines produced adults because the rapid growing rate of water hyacinth in the greenhouse caused a quick leaf turnover and all mines were lost. The exception was one sole adult that emerged 52 days after its parents were released in the cage. Overall adult survival had maximum and mean values of 25 and 10-15 days respectively.

Curious adult behavior, interpreted as courtship and mating, was observed more than once in the greenhouse, noon and early afternoon, when temperature and light had maximum daily values. Immediately after more than 100 newly emerged adults were released in a cage, they started to show sexual activities. The male approached the female, that was resting on the middle and high part of the petiole, by very short, quick flights, that resembled jumps more than flights. After several approaching jumps back and forth, over the female, the male jumped and landed very near the females posterior end. Then the male jumped to the female's back, copulated for 30 to 50 sec to finally fly away. The described behavior was observed about 15 times from 12:00 to 13:00 hrs. After that, approaching jumps were seen several times but without mating.

Preservation of Adults. Most techniques and fluids tried were unsuccessful in preserving the shape and color of the adults. Colors are apparently good characters used for identification. Thus, preservation of real natural adults' colors was essential for taxonomic purposes. Adults pinned with minute pins immediately after being killed with ethyl acetate, chloroform or freezer, collapsed badly after a couple of days. The exoskeleton shrinking was so severe that in cases it was difficult to tell what kind of fly was on the pin. Adults preserved in ethanol 70% did not collapse but started to disintegrate after several months. Fixing the adults in boiling ethanol 70% did not make any improvement. However, ethanol 80% or higher avoided the disintegration and collapsing although some of the colors changed. Adults killed and dried in the freezer for 2-3 months, yielded collapsed although rather acceptable adults.

General Discussion. The consequences of the mine's damage on the plant's overall health were not attempted to be determined. Visual observation of attacked plants have not shown signs of weakening or early senescence. However, close monitoring of selected plant populations were not carried out because of the long distances to the sites where the best *Thrypticus* populations occur. The research was focused on laboratory studies for obtaining necessary information for rearing and future testing. Although the effect of its damage on the demography of water hyacinth is obscure, other attributes of the fly like the presumed strict monophagy, ubiquity and abundance appear promising. The tiny tunnels produced by the larvae, often very abundantly, were judged trivial by some authors and auspicious by others. Despite this polarity, whatever degree of damage *Thrypticus* inflicts to the plant, it will possibly enhance the overall stress produced by other agents. The high specialization of *Thrypticus* on members of the plant family Pontederiaceae, virtually assures that at least one of the species will soon become a new agent for biocontrol of water hyacinth.

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