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Notes on the presence of *Aeolothrips intermedius* in northwestern Tuscany and on its development under laboratory conditions

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Abstract

Data obtained in 2005 and 2006 both from captures with yellow sticky traps and from periodic direct samplings on flowers showed that *Aeolothrips intermedius* Bagnall (Thysanoptera Aeolothripidae) is, among its congenera, the species most frequently present in phytocenoces of northwestern Tuscany. It shows a predatory behaviour, and completes in Tuscany 3-4 generations a year, with 3 peaks of presences, in June, in July and in August.

During 2006, by rearing this species under laboratory conditions (mean temperature in May 23 ± 2 °C and in June 27 ± 2 °C and natural photoperiod) it was found that the duration of embryonic development was 6.8 and 3.7 days respectively in May and in June; duration of the first larval instar was 3.2 days in May and 2.4 in June; duration of the second larval instar was 5.4 and 4.1 days and finally, duration of the pre-pupa and pupa was totally 6.2 days in May and 5.6 in June. Life cycle duration (egg-adult) takes 21.6 ± 2.2 days in May and 15.7 ± 1.6 days in June.

Key words: biology, predator, duration of development, Aeolothripidae, Italy.

Introduction

The Aeolothripidae family includes 240 species that live on flowers and, in many cases, species that prey on larvae of other Thysanoptera and of small Arthropods (Priesner, 1927; 1964; Mound and Marullo, 1993; zur Strassen, 1995). Therefore predators belonging to this family play an important role together with other beneficial agents in the biocontrol of thrips pests (Loomans *et al*., 1995).

Within the genus *Aeolothrips*, the species *Aeolothrips intermedius* Bagnall (Thysanoptera Aeolothripidae) is distributed throughout western and eastern Europe (Bournier *et al*., 1978; Marullo, 2004a) the middle East and India but now it can be considered cosmopolitan (Riudavets, 1995). It is regarded in Italy as the most common species of its genus (Marullo, 1993). The species is found constantly in Tuscany (Conti, 2002), but it is also common throughout the rest of the Italian peninsula (zur Strassen, 1987; Marullo and zur Strassen, 1995). *A. intermedius* is habitually floricolous. In fact it is common in many biocenoses of cultivated and wild plants (Riudavets, 1995) where, in the flowers, larvae exhibit primarily predatory behaviour while the adults are fed also on pollen (Marullo, 2004b). The adults of *A. intermedius*, which have the characteristic striped wings typical of the species of this genus, visit the flowers both of trees and herbaceous plants, with a clear preference for Leguminosae but also Graminaceae, Convolvulaceae, Brassicaceae, Caryophyllaceae, Malvaceae, Solanaceae, Umbelliferae, Rutaceae, Labiatae, Borraginaceae, Rubiaceae, Campanulaceae, Dipsacaceae, Compositae, Leguminosae, Scrophulariaceae (Bagnall, 1934; Bournier *et al*., 1978; Marullo, 1991; 1993).

A. intermedius has been studied by various authors, with focus on its feeding habits (Bournier *et al*., 1978; 1979; Lacasa *et al*., 1982). Comparative tests by the cited authors, using different types of prey (including various species of Thysanoptera), suggested that both the larvae and the adult females are generic predators, even though they present marked dietary preferences.

The aim of the present study was to determine the presence of *A. intermedius* and of possible other species of phytophagous Thysanoptera preyed by *A. intermedius* in natural and modified ecosystems of northwestern Tuscany. Additionally, laboratory tests were conducted in order to verify some biological data of the species.

Materials and methods

Open field investigations

a) Qualitative investigations in natural and modified ecosystems

During the years 2005 and 2006, starting from the early spring and continuing up to late autumn, biweekly sampling was conducted on flowers of both cultivated and wild Leguminosae, since the species belonging to this plant family are among the insect's preferred hosts. The sampled species included wisteria [*Wisteria sinensis* (Sims) DC.] collected from hedges in the area of Vicopisano (Pisa), alfalfa (*Medicago sativa* L.), clover (*Trifolium incarnatum* L.), French honeysuckle (*Hedysarum coronarium* L.) and sainfoin (*Onobrychis sativa* Lam.) grown in the experimental fields of the "E. Avanzi" Agro-Environmental Inter-Departmental Research Centre of the University of Pisa, and yellow sweet clover [*Melilotus officinalis* (L.) Pall.] collected from an uncultivated field on the outskirts of Pisa. Finally, open field and greenhouse cultivated roses (*Rosa* spp.) from an area near Pescia were sampled. Flowers were then subjected to shaking in the laboratory over a white paper surface in order to isolate adult forms of Thysanoptera, which were then gathered up and stored in a 70° alcohol

solution. The specimens were determined after mounting on a slide with Faure Liquid or Canada Balsam, according to the method proposed by Bournier (1983).

b) Captures with yellow sticky traps

During the summer 2006, in a field of alfalfa of 3 ha of extension in the surroundings of Pisa (San Piero a Grado) 9 rectangular (40 x 25 cm) yellow sticky traps were positioned and replaced weekly. For each trap, all specimens of the genus *Aeolothrips* captured on the trap were counted, and those present on 10 randomly chosen 20 cm^2 (5 x 4 cm) rectangles (equivalent to 10% of the surface area of the given trap) were then detached with solvents and mounted on a slide with Faure Liquid or Canada Balsam as described previously. Subsequently, presences of *A. intermedius* were first calculated as a percentage of the specimens of the *Aeolothrips* genus obtained from the given rectangles, and then as a percentage of the total number of *Aeolothrips* spp. captured.

Laboratory investigations

During the summer 2006, pairs of adult specimens (of both sexes) of *A. intermedius*, collected directly from flowers, were isolated in Huffaker cages (Huffaker, 1948) with a rose petal as vegetal support, yellow sweet clover sprigs for oviposition and alfalfa flowers in order to induce oviposition after mating and to allow subsequent observation of the development cycle. Cages were maintained under laboratory conditions at room temperature (registered in May 23 ± 2 °C and in June 27 ± 2 °C) and natural photoperiod (43°42'43"N, 10°24'46"E).

Ovipositions and emergences were checked every 24h by microscope observation, and 30 newly emerged larvae were singularly isolated (to avoid cannibalism) in the same Huffaker cages. Larvae were fed with a small quantity of alfalfa pollen and with larvae of *Thrips tabaci* Lindeman and/or of *Frankliniella occidentalis* (Pergande) obtained respectively from alfalfa flowers and from a seasonal rearing set up at the same time in our laboratory. The life cycle has been therefore controlled until to obtain the adult.

Results and discussions

Results of open-field investigations

Table 1 lists the flower species sampled and the composition of the Thysanoptera fauna that habitually live on such species. The table demonstrates, firstly, that on the sampled flowers *A. intermedius* was by far the most frequent among its congenera. The table also shows that *A. intermedius* was found associated with *Frankliniella intonsa* (Trybom) and frequently with *T. tabaci*, suggesting a trophic dependence of *A. intermedius* larvae on *F. intonsa* and *T. tabaci* larvae. This suggestion is in agreement with observations by Bournier *et al.* (1978, 1979), who found that *T. tabaci* larvae were the preferred prey of *A. intermedius* because they permit the greatest fecundity. These findings are also in agreement with the data given by Strapazzon (1998), who detected the coexistence of *A. intermedius* with *F. intonsa.*

The most interesting result from these data was the presence of *A. intermedius* both among populations of *F. occidentalis* alone and also among populations of other Thysanoptera that include the latter species. This indicates that the predator is also characterized by a non occasional trophic dependence on *F. occidentalis*, in line with statements by Riudavets *et al*. (1993) and Zegula *et al*. (2003).

The results of sampling in the lucerne field, where the presence of *A. intermedius* was detected with yellow sticky traps, are shown in figure 1. The graph indicates that presence of the first adults of the species was recorded at the beginning of April, and the last at the end of September. As laboratory data indicate that a generation has a duration of about 16-22 days, it is reasonable to assume that the species completes in Tuscany 3-4 scalar generations/year, with a peak of adult presences in mid June, a second peak in mid July and a third in mid August. This finding is in agreement with the data reported by Bournier *et al.* (1978) for the area of Montpellier, where the two first peaks of presences were recorded in the same periods but the third is not present in France due probably to different thermal conditions. The same authors indicated as well, for the area of Montpellier, 3-4 probably generations/year.

Results of laboratory investigations

The species, as well the others in the order, presents hemimetabolic development, with two instars that show a predatory behaviour, and two aphagous pupal stages (prepupa and pupa) within the protection of a "silken" cocoon (Bailey, 1940; Lewis, 1973). Our biological observations confirm that reproduction is normally amphigonic (figure 2a), but as it is haplodiploid it presents arrhenotokous parthenogenesis as the mechanism for determination of male sex (Bournier *et al.*, 1978). The female lays isolated eggs, inserting them into plant tissue (leaf veins or tender shoots) of various herbaceous plants (figure 2b).

During the hatching the larva emerges from the pole of the egg that protrudes out of the oviposition substrate (figure 3a), and immediately begins to search for preys on which to feed, pricking and sucking them (figure 3b).

The second instar larva, initially highly mobile, feeds in the same manner and undergoes rapid growth. After reaching maturity it seeks a suitable place (in Huffaker cage generally at the bifurcation of two veins of the leaf, along the edge of the glass covering the cage or along the bottom edge) to spin a cocoon (figure 4a) with an anal secretion produced by Malpighian tubes (Conti *et al*., 2009). The next moult in prepupa takes place inside the cocoon. Subsequent two moults take place as well inside the same cocoon, with the development of the pupa and finally the adult. The adult remains within the cocoon for some hours, sometimes for more than a day, after which it emerges through an opening made with the aid of the hooks of the anterior tarsi. In open field, in the cocoon the species pass the winter in the ground. Characteristic it is the fact that, likewise to other insects as *Leucopis palumbii* Rondani (Diptera Chamaemyiidae) (Raspi, 1988) while the summer cocoon is white and it appears silky, the one produced for the overwintering it is rigid and brown (figure 4b).

Table 1. List of Thysanoptera detected on spring-summer flowers during 2005 and 2006 in the Province of Pisa and the area surrounding Pescia. The first column lists sampling dates, the second column shows flower species sampled, the third the *Aeolothrips* species detected and the fourth the other Thysanoptera species.

Date	Plants	*Aeolothrips* spp.	Others Thysanoptera
14-4-2005	*Wisteria sinensis* (Sims) DC.	*Aeolothrips ericae* Bagnall	*Thrips flavus* Schrank *Thrips tabaci* Lindeman *Thrips meridionalis* (Priesner)
20-5-2005	*Medicago sativa* L.	*Aeolothrips intermedius* Bagnall	*Limothrips cerealium* Haliday *Odontothrips confusus* Priesner *Frankliniella intonsa* (Trybom) *Thrips tabaci* Lindeman
21-5-2005	*Trifolium incarnatum* L.	*Aeolothrips intermedius* Bagnall	*Haplothrips aculeatus* (F.) *Frankliniella intonsa* (Trybom) *Thrips tabaci* Lindeman
26-5-2005	*Melilotus officinalis* (L.) Pall.	*Aeolothrips intermedius* Bagnall	*Odontothrips confusus* Priesner *Frankliniella intonsa* (Trybom) *Thrips tabaci* Lindeman *Kakothrips robustus* (Uzel)
10-6-2005	*Rosa* spp.	*Aeolothrips intermedius* Bagnall	*Frankliniella intonsa* (Trybom)
16-7-2005	*Medicago sativa* L.	*Aeolothrips intermedius* Bagnall	*Frankliniella intonsa* (Trybom) *Thrips tabaci* Lindeman *Frankliniella occidentalis* (Pergande)
18-7-2005	*Medicago sativa* L.	*Aeolothrips intermedius* Bagnall	*Odontothrips confusus* Priesner *Frankliniella intonsa* (Trybom)
21-7-2005	*Medicago sativa* L.	-	*Odontothrips confusus* Priesner *Frankliniella intonsa* (Trybom) *Limothrips cerealium* Haliday
22-7-2005	*Medicago sativa* L.	*Aeolothrips intermedius* Bagnall	*Odontothrips confusus* Priesner *Frankliniella intonsa* (Trybom)
29-7-2005	*Rosa* spp.	*Aeolothrips intermedius* Bagnall	*Frankliniella intonsa* (Trybom) *Frankliniella occidentalis* (Pergande)
4-8-2005	*Medicago sativa* L.	*Aeolothrips intermedius* Bagnall	*Odontothrips confusus* Priesner *Frankliniella intonsa* (Trybom) *Thrips tabaci* Lindeman *Haplothrips aculeatus* (F.)
20-8-2005	*Rosa* spp.	*Aeolothrips intermedius* Bagnall	*Frankliniella intonsa* (Trybom) *Thrips tabaci* Lindeman *Thrips major* Uzel
27-8-2005	*Rosa* spp.	-	*Frankliniella intonsa* (Trybom) *Thrips tabaci* Lindeman
2-9-2005	*Medicago sativa* L.	-	*Frankliniella intonsa* (Trybom) *Thrips tabaci* Lindeman
3-9-2005	*Rosa* spp.	-	*Frankliniella intonsa* (Trybom) *Thrips tabaci* Lindeman
7-5-2006	*Onobrychis sativa* Lam.	*Aeolothrips intermedius* Bagnall	*Frankliniella intonsa* (Trybom) *Thrips tabaci* Lindeman
8-5-2006	*Hedysarum coronarium* L.	*Aeolothrips intermedius* Bagnall	*Kakothrips robustus* (Uzel) *Thrips tabaci* Lindeman
13-5-2006	*Medicago sativa* L.	*Aeolothrips intermedius* Bagnall	*Thrips tabaci* Lindeman *Frankliniella intonsa* (Trybom)
31-8-2006	*Rosa* spp. (glasshouse)	*Aeolothrips intermedius* Bagnall	*Frankliniella occidentalis* (Pergande)

The results of laboratory tests are shown in table 2.

It can be seen from the table that:

a) under laboratory conditions (mean temperature in May 23 ± 2 °C and in June 27 ± 2 °C and natural photoperiod) the duration of the embrionic development of *A. intermedius,* when it oviposits in yellow sweet clover sprigs, is 6.8 ± 0.6 days in May and 3.7 ± 0.9 days in June. Bournier *et al*. (1978) recorded a mean duration of 6 ± 1 days in incubators at constant 26 °C and photoperiod 16:8 (L:D);

b) duration of the first larval instar was on average 3.2 ± 0.6 days in May and 2.4 ± 0.5 in June, while duration of the second was 5.4 ± 0.8 days in May and 4.1 ± 0.7 in June. In contrast, Bournier *et al.* (1978), in incubators in the above-described conditions, recorded 2 days for the first instar larva and 6 days for the second instar larva;

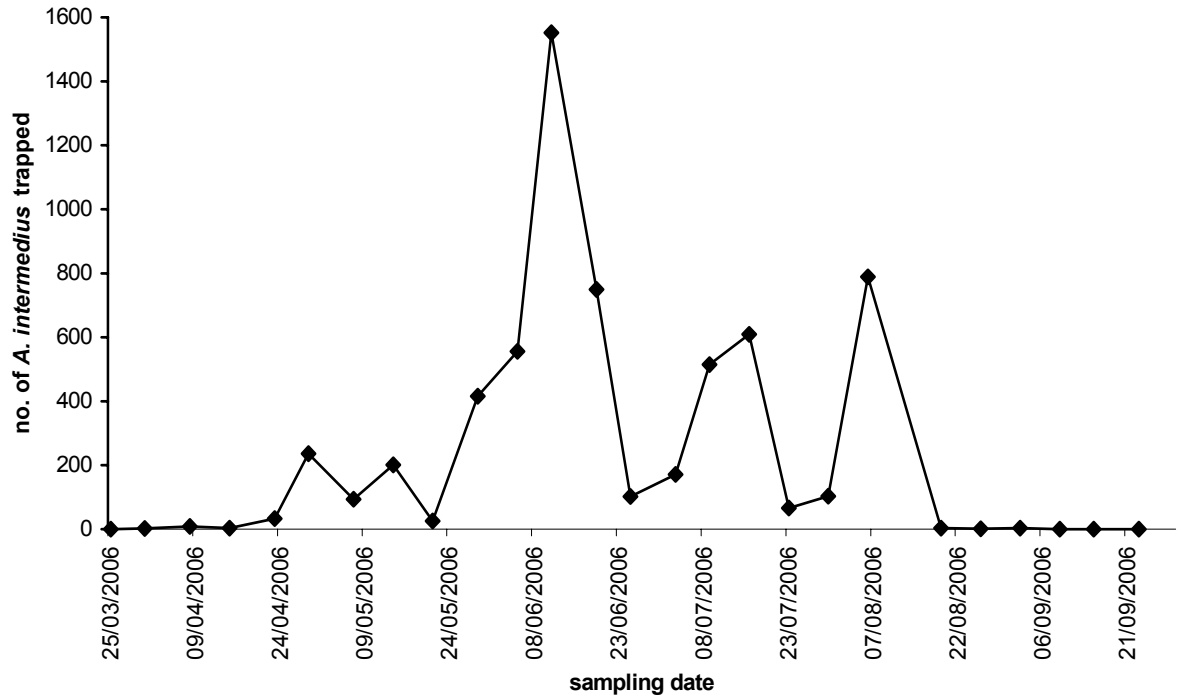

Figure 1. Trend of yellow sticky trap captures of adults in a field of alfalfa in the surroundings of Pisa (San Piero a Grado) during 2006.

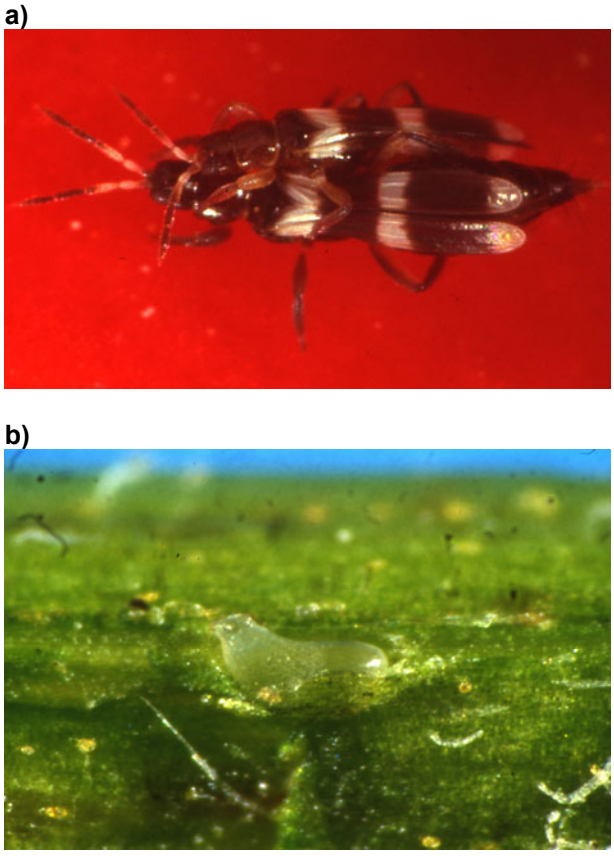

Figure 2. *A. intermedius*: a) mating adults; b) sweet clover twig, carefully opened to show the egg protruding from the twig.
(In colour at www.bulletinofinsectology.org)

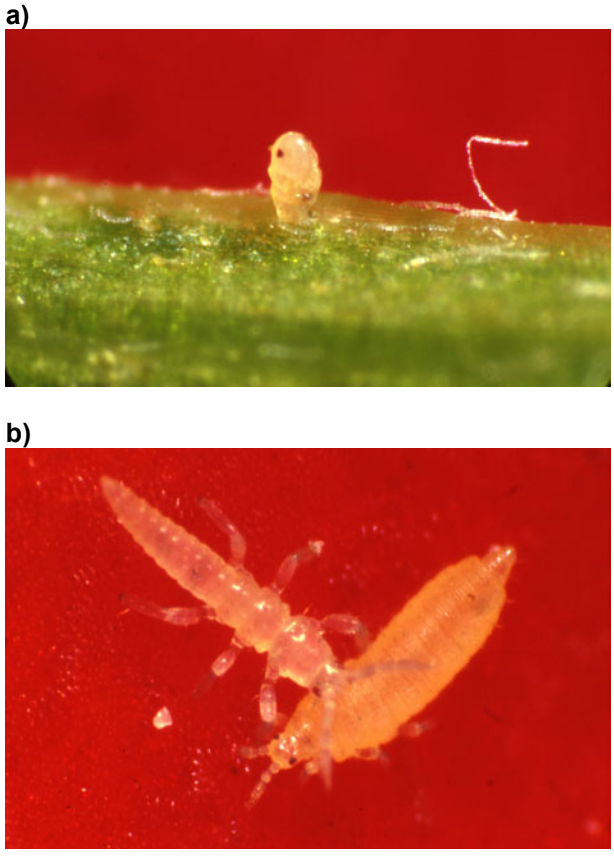

Figure 3. *A. intermedius*: a) hatching larva; b) first instar larva feeding on a larva of *F. occidentalis*.
(In colour at www.bulletinofinsectology.org)

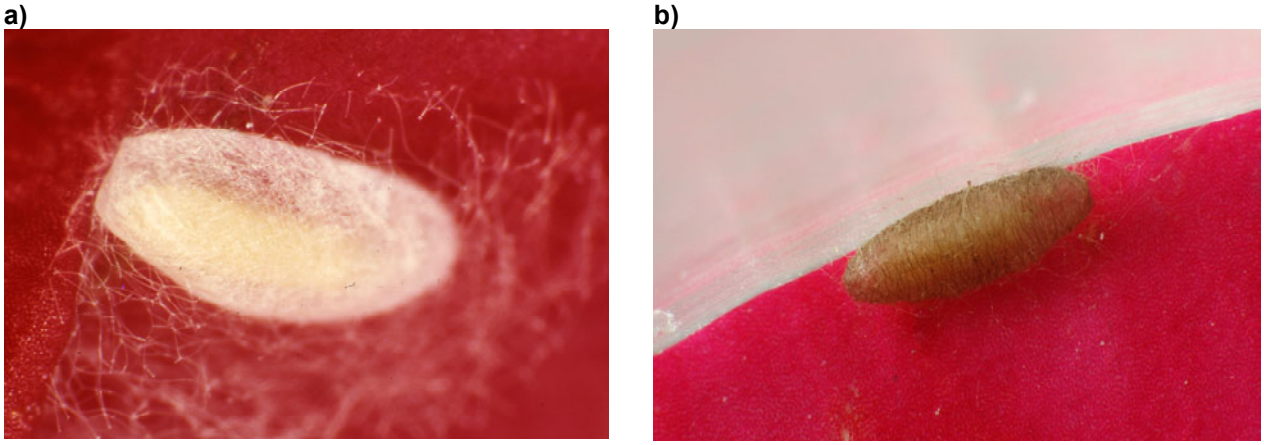

Figure 4. *A. intermedius*: a) summer cocoon; b) overwintering cocoon. (In colour at www.bulletinofinsectology.org)

Table 2. *A. intermedius*. Duration of development in days.

Stage	Duration in days ± SD May 2006	Duration in days ± SD June 2006
Egg	6.8 ± 0.6	3.7 ± 0.9
Larva 1st	3.2 ± 0.6	2.4 ± 0.5
Larva 2nd	5.4 ± 0.8	4.1 ± 0.7
Prepupa + pupa	6.2 ± 1.1	5.6 ± 0.7
Total	21.6 ± 2.2	15.7 ± 1.6

c) total duration of the prepupal and pupal instar was 6.2 ± 1.1 days in May and 5.6 ± 0.7 in June versus 6.5 recorded by Bournier *et al*. (1978) at constant 26 °C.

d) life cycle duration (egg-adult) takes 21.6 ± 2.2 days in May and 15.7 ± 1.6 days in June;

e) mortality, probably due to difficulties in manipulation of so small insects was high: about 50% for 1st instar larvae, 30% for 2nd instar larvae and 10% for the instars inside the cocoon.

Conclusions

On the basis of the data obtained both from samplings performed on flowers and from captures with sticky traps, it can be stated that *A. intermedius* is the most frequent species of this genus found in cultivated and wild flowering plant cenoses of northwestern Tuscany. Investigations conducted showed that the first adults are found starting from the beginning of April, subsequently disappearing completely after the end of September. In *A. intermedius*, duration of the life-cycle, from egg to adult, was 16-27 days, depending on temperature, and the species appears to be capable of completing in Tuscany 3-4 scalar generations a year. Three peaks of presences were recorded, one in mid-June, the second in mid-July and the last in mid-August.

On numerous flowering plants *A. intermedius* was detected in association with various different phytophagous Thysanoptera, which included *T. tabaci* but also frequently *F. occidentalis*.

Given its predatory behaviour, the abundant captures obtained by means of yellow sticky traps suggest that *A. intermedius* is not only ubiquitous but is also capable of playing a role of control of many other phytophagous Thysanoptera species.

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