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SHORT COMMUNICATION (no. Words 2230 except Title, Abstract,Tables and captions)

Acceptance and suitability of *Cacyreus marshalli* (Lepidoptera: Lycaenidae) as host for three indigenous parasitoids

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Abstract:. Laboratory tests were conducted in Italy to evaluate the acceptance and suitability of the alien butterfly *Cacyreus marshalli* Butler as host for three indigenous parasitoids, *Trichogramma brassicae* (Bezdenko) *Exorista larvarum* (L.) and *Brachymeria tibialis* (Nees). Only *E. larvarum* and *B. tibialis* showed potential to adapt to *C. marshalli*. Their contribution to biological control appeared to be especially related to host mortality due to incomplete parasitoid development.

KeyWords: *Cacyreus marshalli*, native natural enemies, *Trichogramma brassicae*, *Exorista larvarum*, *Brachymeria tibialis*, biological control

The Geranium Bronze *Cacyreus marshalli* Butler, native of South Africa, has established in Italy and other European countries (Suffert 2012). In South Africa *Apanteles* sp., other unidentified braconids and small tachinids were reported as larval parasitoids of *C. marshalli* (Clark and Dickson, 1971). In Europe, two native parasitoids have been recorded as antagonists of this exotic pest of cultivated geraniums, namely the hymenopteran *Trichogramma evanescens* Westwood in Spain (Sarto i Monteys and Gabarra 1998) and the tachinid *Aplomya confinis* (Fallen) in Italy (Vicidomini and Dindo 2006).

With the aim of enhancing knowledge on the associations between *C. marshalli* and indigenous antagonists, laboratory tests were conducted to evaluate the acceptance and suitability of this alien species as host for three polyphagous parasitoids of Lepidoptera, all widespread in Italy and Europe (www.faunaeur.org accessed January 9 2013): the hymenopterous egg parasitoid *Trichogramma brassicae* (Bezdenko), the tachinid larval parasitoid *Exorista larvarum* (L.) and the hymenopterous pupal parasitoid *Brachymeria tibialis* (Walker).

Colonies of *C. marshalli*, maintained as described by Quacchia, Ferracini, Bonelli, Balletto, and Alma (2008), and *Ephestia kuehniella* Zeller, used as control, were supplied by DISAFA. Batches of parasitized *E. kuehniella* eggs were supplied by Koppert Biological Systems (The Netherlands).

Two experiments were carried out to test the acceptance and suitability of *C. marshalli* by *T. brassicae. Cacyreus marshalli* eggs were obtained in the first experiment by releasing twenty newly-emerged adults in a cage containing two *P. zonale* potted plants. After 24 hrs portions of leaves with eggs were cut off and exposed to twenty less than 24hrs-old mated and unfed *T. brassicae* adults with no previous contact with a host egg . After 48 hrs the leaf portions were individually isolated. Ten replicates (= 10 tubes with 5 eggs each for a total of 50 eggs) were carried out. In the second experiment, three healthy *P. zonale* potted plants were individually exposed to 50, 100, and 200 *C. marshalli* (males and females) for 48 hrs. They were singularly isolated and the number of eggs laid by the butterfly was recorded. *Trichogramma brassicae* adults were released in each of the three cages (100, 1,000 and 50,000, respectively). As control, ten replicates of five 72 h-old *E. kuehniella* eggs, distributed on a sticky strip, were performed. Each of these strips was exposed to twenty *T. brassicae* as described above. The eggs were checked daily and the parasitism rate and percentage of emergence for each treatment were estimated.

Exorista larvarum and *B. tibialis* were maintained on the factitious lepidopterous host *Galleria mellonella* (L.) as described by Dindo, Farneti and Baronio (2001) and Dindo, Marchetti and Baronio(2007). Females had already oviposited on/in *G. mellonella* larvae/pupae before the test. The acceptance and suitability of *C. marshalli* and *G. mellonella* (maintained as control) by the two parasitoids were tested under no-choice conditions.

For *E. larvarum,* 60 last instar larvae of each lepidopterous species (Mellini, Gardenghi and Coulibaly 1994), were individually placed in a plexiglass cage containing about 25 parasitoid adult females and 25 adult males, which had emerged 5-6 days before (Dindo et al. 2007). *Cacyreus marshalli* and *G. mellonella* larvae were, respectively, 0.93±0.06 cm and 2.4 ± 0.03 cm long (mean±SE) when they were tested. The larvae were removed from the cage after 2 hrs and were individually transferred into Petri dishes with food until death, parasitoid puparium formation or host emergence. Each host larva was considered as a replicate. The larvae were deemed to have been “accepted” when at least one *E. larvarum* egg was found on their body. The results were evaluated in terms of the following traits: number and percentage of accepted larvae, eggs/accepted larva, number and percentage of suitable larvae (i.e. accepted larvae from which puparia formed), number and percentage (based on puparia) of emerged flies, number and percentage of dead larvae over accepted larvae, weights of the newly-formed puparia, development times from egg to puparium and from puparium to adult emergence.

For *B. tibialis*, *C. marshalli* or *G. mellonella* 2-day old pupae were individually exposed to about 10 parasitoid females and 10 males of mixed ages. The host pupae were removed from the cage as soon as a female pierced their body with the ovipositor and were considered as non-accepted if no ovipositor insertion was detected within 2 hrs. Upon removal from the cage, all pupae were individually kept in plastic Petri dishes until death, parasitoid or host emergence. Twenty-five pupae of each species were tested, each being considered as a replicate. Mean (±SE) pupal length was 0.8±0.02 cm for *C. marshalli* and 1.5±0.2 cm for *G. mellonella.* The number and percentage of accepted and suitable pupae (=accepted pupae leading to the emergence of a parasitoid adult) were calculated. The newly-emerged adults were sexed and their weights (in mg) and development times from egg to adult (in days) were separately recorded for males and females. The experiments with the three parasitoids were carried out at 26±1°C, 65±5% RH, L16:D8 photoperiod.

Trichogramma brassicae did not parasitize the eggs of the lycaenid, invariably failing to exhibit interest in the hosts. For this reason, no statistical analysis was performed. In the second experiment oviposition by *C. marshalli* occurred on all the tested plants. In particular 219, 421, and 630 eggs were recorded on the *P. zonale* plants exposed to 50, 100, and 200 *C. marshalli* adults, respectively. In both experiments no parasitism of *C. marshalli* eggs ever occurred, while in the control the parasitism rate by *T. brassicae* recorded on the factitious host *E. kuehniella* eggs reached a mean value of 92%, with a mean percentage of emergence of 89%. Thus, the commercially produced *T. brassicae* strain evaluated in this study did not prove a good candidate for use against *C. marshalli.* On the contrary, Groussier, Tabone, Coste, and Rizzo (2006) reported good parasitism of *C. marshalli* by *Trichogramma* spp., especially *T. chilonis* Ishii. In view of such positive results and given that many *Trichogramma* species are commonly used as biocontrol agents of various lepidopteran pests (Babendreier, Kuske, and Bigler 2003), the potential of other species different from *T. brassicae* and their role as to host acceptance and suitability deserve further investigation.

Conversely, although the mature larvae of *C. marshalli* were considerably undersized compared to the recorded host species of *E. larvarum*, including *G. mellonella* (Cerretti and Tschorsnig 2010), successful parasitism of the lycaenid occurred in the laboratory, but at very low rates (Table 1). *Cacyreus marshalli* larvae were poorly accepted by female flies, possibly due to different factors including their low mobility, an important cue for host acceptance by *E. larvarum* and other *Exorista* species (Stireman 2002; Depalo, Dindo, and Eizaguirre 2012). Suitability to *E. larvarum* was also lower for *C. marshalli* compared to *G. mellonella* (Table 1) and, similarly to parasitoid size and development times (reported in Table 2), it was probably affected by host size In this regard, Baronio, Dindo, Campadelli, and Sighinolfi (2002) showed that the development and size of *E. larvarum* were affected both by the amount of food and the vital space available to larvae. Independently of puparium formation, most of the accepted *C. marshalli* larvae died, at a not significantly different rate compared to *G. mellonella*, while the non-accepted ones pupated and emerged as adults (Table 1). As the number of flies obtained from the Geranium Bronze was very low, the puparium-to-adult development times were not subjected to statistical analysis. These times (means±SE) were 8.9±0.2 and 9.5±0.5 days for the flies respectively obtained from *G. mellonella* (n= 36) and *C. marshalli* (n=2), that is slightly longer in the latter host species.

All *G. mellonella* and 52% *C. marshalli* pupae were accepted by *B. tibialis* females. Separate 2x2 contingency tables were used to test the independence of host species and number of accepted and suitable pupae. The difference in host acceptance was significant (Yates corrected χ2 =13.27; df=1; P= 0.0003), but the effect of pupal size on this parameter is doubtful since the recorded hosts of *B. tibialis* also include species of similar sizes as *C. marshalli* (Noyes 2012). The percentage of suitable pupae found for *C. marshalli* (=53.8) was lower compared to that recorded for *G. mellonella* (= 84), but the difference was not significant (Yates corrected χ2 =2.61; df=1; P= 0.11). All the accepted pupae of both host species died, whether successfully parasitized or not, while the non-accepted ones (only *C. marshalli*) emerged as adults. All *B. tibialis* which emerged from *C. marshalli* (=7) were males and, as expected, they were significantly undersized (weight=3.8±0.3 mg) compared to those (=15) obtained from *G. mellonella* (weight=8.4±0.1 mg) (one-way ANOVA, F= 340.96, df =1, 20; P= 0.0000001). Male development times in *C. marshalli* (=14.9±0.4 days) and *G. mellonella* (=15.7±0.3 days) were not significantly different (Kruskall-Wallis test, H= 1.46; N= 22; P= 0.23). The mean weights and development times of the six *B.*

tibialis females that emerged from *G. mellonella* pupae were 13.8±0.1 mg and 15.7±0.2 days, respectively.

The results obtained in the present study suggest that both *E. larvarum* and *B. tibialis* have potential to adapt to *C. marshalli* in nature. Their contribution to biological control appeared, however, to be especially related to host mortality due to incomplete parasitoid development and did not seem to be sufficient to decrease the populations of the target insect pest. A more effective strategy could be represented by classical biological control, with detection and importation of parasitoids of the Geranium Bronze from South Africa to the countries of introduction. A rather recent example of promising classical biological control in Italy is represented by the importation of *Torymus sinensis* Kamijo against *Dryocosmus kuriphilus* Yasumatsu (Quacchia, Moriya, Bosio, Scapin, and Alma 2008). This strategy is not however to be considered as alternative, but rather complementary to the exploitation of indigenous natural enemies. The parasitism of invading novel hosts by native natural enemies has already been reported for a number of various alien insect pests, including *D. kuriphilus* itself (Quacchia et al., 2012), and *Tuta absoluta* (Meyrick) (Ferracini et al. 2012). In this context, indigenous natural enemies, including *E. larvarum* and *B. tibialis*, may also play a role in the control of the Geranium Bronze in the countries of introduction.

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Figure captions

Table 1. Acceptance and suitability of *Cacyreus marshalli* and *Galleria mellonella* by *Exorista larvarum*: the 2x2 contingency tables for testing the independence of host species and number of A) accepted larvae, B) suitable larvae **(**= accepted larvae from which puparia formed), C) dead larvae (on accepted larvae); D) puparia which let a fly adult emerge. Yates corrected $\chi 2$ values are given (sample size < 100). Original number of larvae = 60 per species.

Table 2. Acceptance and suitability of *Cacyreus marshalli* vs. *Galleria mellonella* by *Exorista larvarum*: parasitoid eggs per accepted larva, puparial weights and development times from egg to puparium. Means±SE. Number of replicates (n) is given in parenthesis above the means. Means in a column followed by the same letter are not significantly different, $P > 0.05$; Kruskall-Wallis test.

TABLE 1

Parameter		Host species: *Galleria mellonella*	Host species: *Cacyreus marshalli*	χ2 (df=1)	P
A)	Accepted larvae (%)	60 (100)	36 (60)	27.55	0.00001*
	Non-accepted larvae (%)	0 (0)	24 (40)		
B)	Suitable larvae (%)	46 (76.7)	13 (36.1)	13.96	0.0002*
	Unsuitable larvae (%)	14 (23.3)	23 (63.9)		
C)	Dead larvae (%)	58 (96.7)	30 (83.3)	3.64	0.06
	Live larvae (%)	2 (3.3)	6 (16.7)		

TABLE 2

Host species	Eggs/accepted larva (no.)	Puparial weight (mg)	Time from egg to puparium (days)
Galleria mellonella	(60) 38.6±3.6a	(72) 30.2±1.6a	(72) 8.1±0.1a
Cacyreus marshalli	(36) 2.4±0.3b	(13) 9.1±0.9b	(13) 8.2±0.6a
H	64.9	28.4	0.15
N	96	85	85
P	0.00001	0.0001	0.69