Eur. J. Entomol. **95**: 359–366, 1998
ISSN 1210–5759

Facultative hyperparasitism in *Brachymeria pomonae* (Hymenoptera: Chalcididae)

ERNEST B. WHITE[1], JULIO S. BERNAL[2], DAN GONZÁLEZ[1] and SERGUEI V. TRIAPITSYN[1]

[1]Department of Entomology, University of California, Riverside, CA 92521, USA;
e-mail: ernwhite@gte.net

[2]Biological Control Laboratory, Texas A & M University, College Station, TX 77843-2475, USA

Chalcididae, *Brachymeria pomonae*, facultative hyperparasitism, biological control, quarantine, *Apanteles oenone*, *Cardiochiles nigriceps*, *Pectinophora gossypiella*, pink bollworm

Abstract. This report summarizes a study designed to uncover any tendency towards hyperparasitic behavior in *Brachymeria pomonae* (Cameron), a parasitoid of pink bollworm (PBW) (*Pectinophora gossypiella* Saunders) imported from Australia to California for biological control of the latter pest species. *Brachymeria pomonae* hyperparasitized both *Apanteles oenone* Nixon (ca. 10% of pupae exposed) and *Cardiochiles nigriceps* Viereck (ca. 23% of pupae exposed), and all hyperparasitic offspring of *B. pomonae* were males. However, *B. pomonae*'s aggressive primary parasitism of several lepidopterous hosts, together with the low hyperparasitism rates and the failure to produce hyperparasitic female offspring suggested that hyperparasitism is a facultative behavior in this parasitoid. *Brachymeria pomonae* caused substantial mortality in *A. oenone* and *C. nigriceps* as a result of ovipositional probing. Finally, it did not attack PBW nor *A. oenone* pupae if they were not enclosed in a PBW cocoon, but aggressively attacked the pupae of both when enclosed in PBW cocoons. The results are of significance because *B. pomonae* was a candidate for release against PBW in California. Because of its facultative hyperparasitic habit, no effort was made to release it from quarantine. The basis for this decision, including the uncertain impact that hyperparasitoids may have on biological control programs, is discussed.

INTRODUCTION

The quarantine phase is an important component of biological control projects involving exotic natural enemies. In the USA, exotic natural enemies enter the country under authorization of the federal government. All incoming material (which may include some hosts, host plant parts, etc.) must be handled under strict quarantine conditions in a federally approved facility. After a screening process in which undesirable organisms are excluded (particularly hyperparasitic and pest species), authorization may be requested to release the desired species from quarantine, either for release into the environment or for laboratory study. The request for release must include information concerning the biology of the natural enemy, including whether it is a primary or secondary parasitoid (i.e. hyperparasitic) in the case of parasitic species [see Ertle (1993) for additional discussion concerning the objectives and procedures of the quarantine phase].

Pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), is a major pest of cotton in the western USA (Anonymous, 1984). The genus *Pectinophora* contains three known species, *P. gossypiella*, *P. scutigera* (Holdaway) and *P. endema* (Common) and all three species occur together only in Australia (D. González, unpubl.). Thus, Australia is a prime area to search for natural enemies of pink bollworm (PBW). From cotton boll samples received from western Australia in June of 1993, we recovered two PBW parasitoids, *Apanteles oenone* Nixon (Hymenoptera: Braconidae) and

Brachymeria pomonae (Cameron) (Hymenoptera: Chalcididae). Both were propagated by D. Powell in the quarantine facility of the University of California, Riverside, and both appeared to be aggressive parasitoids of PBW.

Apanteles oenone is a larval parasitoid of PBW. The genus *Apanteles* includes several well known biological control agents with no reports of hyperparasitic activity (see Krombein et al., 1979). Thus, authorization was requested and received for its release from quarantine.

Brachymeria pomonae was originally described from a single specimen collected in Glen Innes, NSW, Australia, from *Cydia pomonella* (L.) (Bouček, 1988). It is a primary parasitoid of pupal PBW on cotton. In the laboratory, at 26 ± 2°C, males emerge in 16 to 19 days, and females in 18 to 21 days. Slightly more males than females are produced, and a preoviposition period of 4 days occurs under these conditions. Other species of Lepidoptera also serve as hosts for *B. pomonae*: *Amorbia cuneana* (Walsingham), *Galleria mellonella* (L.), *Trichoplusia ni* (Hübner) and *Spodoptera exigua* (Hübner). The latter host requires special handling to preserve the fragile cocoon which is almost non-existent in laboratory-reared *S. exigua.* Host size and adult parasitoid size are positively correlated; large hosts produce large parasitoids, but only within certain limits. Often, the food supply of a parasitoid developing in a large host is far from exhausted when it ceases feeding (E. White and D. Powell, unpubl.).

Because of the occurrence of hyperparasitism in the genus *Brachymeria* (Dowden, 1935; Clausen, 1940; Gordh, 1981), no attempt was made to obtain authorization for release of *B. pomonae* from quarantine until further tests could be conducted. Here we report the results of tests designed to determine the proclivity of *B. pomonae* to hyperparasitize those parasitoid hosts (i.e. secondary hosts) which it might encounter in a typical cotton field. In addition, we report on observations concerning host mortality caused by ovipositional probing by *B. pomonae*. Finally, we discuss the implications of our findings with respect to our biological control efforts against PBW in particular, and how the uncertain impact of hyperparasitism in biological control programs influences decisions pertaining to the release from quarantine and subsequent field release of exotic natural enemies.

MATERIAL AND METHODS

All tests were conducted in the quarantine laboratory in the Department of Entomology, University of California, Riverside. The ambient temperature during all tests was 26 ± 2°C. Relative humidity ranged from 22 to 56% with a mean near 40%. The normal diurnal light cycle was utilized through a large window with northern exposure. Overhead fluorescent lights supplemented the natural light from 8:00 to 17:00 h, five days per week. All tests were conducted between mid-June and mid-August during which time hours of daylight averaged near 14.

Pupae of parasitic Hymenoptera which occur in cotton or in the vicinity of a cotton field were exposed to *B. pomonae* females to check for potential hyperparasitism in this species. We exposed to parasitism the two parasitoid hosts, *A. oenone* and *Cardiochiles nigriceps* Viereck (Hymenoptera: Braconidae), that were available to us in a series of tests designed to uncover any hyperparasitic tendency in *B. pomonae.* The tests involving both parasitoid hosts were similar, but specific details are given below.

Tests on *A. oenone*

The same protocol was followed for each of two tests conducted on *A. oenone*. Parasitized PBW cocoons were isolated from the *A. oenone* culture into 3 ml shell vials that were closed with a cotton plug. Each was checked daily with the aid of transmitted light, until the *A. oenone* larva emerged from the host (within the host's cocoon). An additional 24 h were allowed for the parasitoid to spin its own cocoon and

begin pupation after which the parasitized PBW cocoons were combined into groups of three to six in 29 ml plastic vials and either set aside for control purposes or set up for exposure to *B. pomonae* females. In the first test, a control was not employed.

Exposure to *B. pomonae* was designed to be relatively intense in order to demonstrate facultative hyperparasitism in this species if that habit existed. We wanted each pupa to be exposed to as many different female *B. pomonae* as possible. Exposure to *B. pomonae* consisted of five consecutive 24 h periods, each employing a different pair of parasitoids. All of the *B. pomonae* females used in these tests were ≥ 5 d old, and were held with males from emergence until the ovipositional exposures were concluded – probably an unnecessary precaution in light of the single required copulation in other *Brachymeria* species (Dowden, 1935). Following exposure to *B. pomonae*, the pupae were inspected daily for evidence of change and emergence of parasitoids.

A second test was conducted to compare natural mortality in *A. oenone* pupae with mortality observed among pupae exposed to *B. pomonae*. Protocol was identical to that in the previous test except that a portion of the parasitized PBW cocoons were held without exposure to *B. pomonae*. Data obtained from the *A. oenone* pupae exposed to *B. pomonae* during both tests were pooled for analysis and were compared against the data obtained from the unexposed (i.e. control) pupae. Further, parasitized PBW larvae fail to cocoon on occasion, and thus parasitoid larvae are released freely into the environment. We had several *A. oenone* pupae available to us that were not contained within pink bollworm cocoons. Three of these "free" *A. oenone* cocoons were exposed in each of two vials to the 5 day sting protocol described above.

Tests on *C. nigriceps*

Pupae of *C. nigriceps* were supplied by B. Vinson (Texas A&M University, College Station, Texas). These pupae were exposed to *B. pomonae* in a second series of tests. A first group of 22 pupae was divided into two groups of 11 and each group was placed in a 38 ml plastic vial with a ventilated cover. A male and three female *B. pomonae* were added to each vial, along with honey for food. A 72 h ovipositional period ensued, following which the parasitoids in the vials were transferred to the opposite vial for an additional 24 h sting period.

A second group of 21 viable pupae was subjected to a protocol similar to that used in the first test. Groups of 11 and 10 pupae each were placed in 38 ml vials together with three females and one male *B. pomonae*. After the first ovipositional exposure (48 h in this case), the parasitoids were transferred from one vial to the other as before, but on this occasion, a "fresh" female (a mature female which had not been kept together with hosts for at least 48 h) was substituted for one of those completing the 48 h oviposition period. An additional 24 h were allowed before the ovipositional exposure was terminated. Similar to the case of *A. oenone* pupae, the data from both tests were pooled for analysis.

RESULTS

A. oenone tests

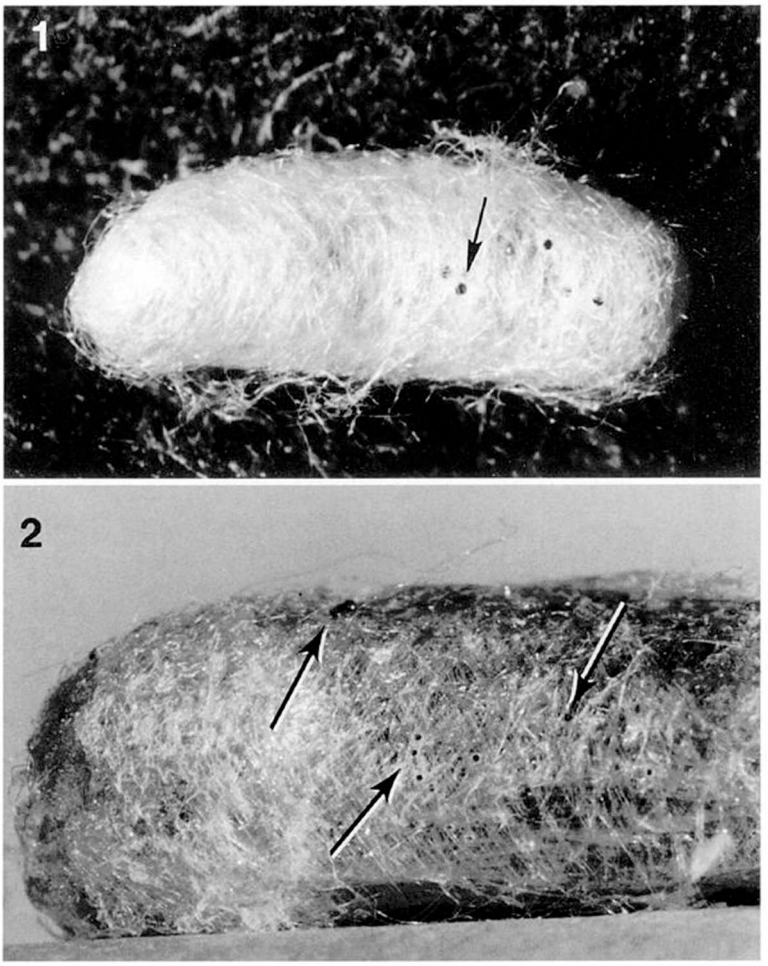
Mortality in the *A. oenone* pupae exposed to *B. pomonae* was ca. 49%, and was higher than that which occurred in the control pupae, ca. 11% ($P = 0.007$, $G = 7.330$, $n = 71$) (Table 1). This suggested that exposure to *B. pomonae* resulted in higher mortality rates in the host pupae, perhaps caused by ovipositional probing. This was confirmed when during dissection of pupae which had failed to emerge, minute punctures (wounds), visible at 10×, were evident in their cocoons (Fig. 1). The number of puncture-wounds per perforated cocoon ranged from 1 to 24 in the pupae exposed to *B. pomonae*, whereas no wounds were evident in the control pupae (Table 1). In addition, ca. 96% of the dead pupae and only ca. 21% of the live pupae showed ovipositional punctures ($P << 0.001$, $G = 27.078$, $n = 42$) (Table 1). The punctured individuals that survived apparently did so because probing occurred late in the developmental stage of the primary parasitoid which could either elude the thrusts of the ovipositor, or was impervious to the probing.

TABLE 1. Hyperparasitism, mortality (% ± SE), and ovipositional puncturing (% ± SE) of secondary host pupae (*Apanteles oenone* and *Cardiochiles nigriceps*) by *Brachymeria pomonae*.

	Pupae (n)	Hyper-parasitized	Non-hyperparasitized		Punctured[1]	
			Mortality	(n)	Dead	Alive
A. oenone	59	6	0.49 ± 0.07	53	0.96 ± 0.04	0.21 ± 0.11
Control	18	0	0.11 ± 0.07	18	—	0.00 ± 0.00
C. nigriceps	43	10	0.76 ± 0.08	33	0.91 ± 0.05	—

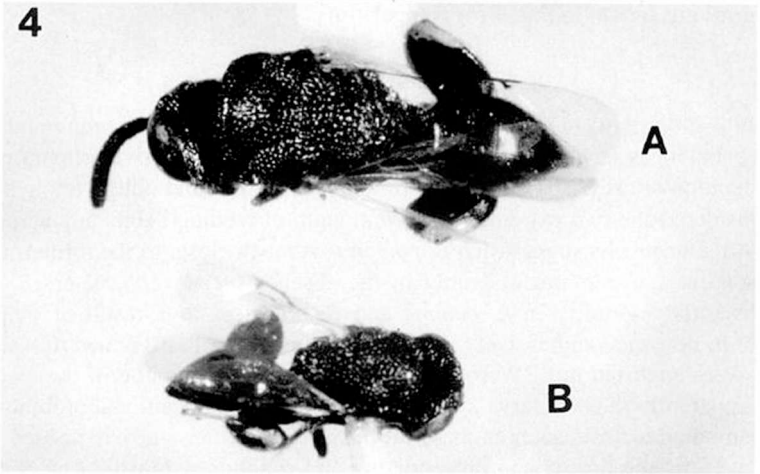
[1]Among "Non-hyperparasitized," next column left.

Six *A. oenone* pupae yielded *B. pomonae* offspring (Table 1). The host remains of these hyperparasitic individuals showed up to 8 ovipositional wounds. All hyperparasitic individuals were males.



Figs 1–2. 1 – *Apanteles oenone* pupa (23×) showing at least 5 punctures left by the ovipositional probing of *Brachymeria pomonae*. 2 – *Cardiochiles nigriceps* pupa (12×) with at least 11 punctures, two of which (upper arrows) are plugged by the clotted haemolymph of the host parasitoid.

Five of the six "free" *A. oenone* (those without PBW cocoons) exposed to *B. pomonae* yielded healthy *A. oenone* adults, and the sixth contained a dead *A. oenone* male. Wound marks were not evident in any of the six "free" cocoons. When given smaller hosts such as *A. oenone* and PBW, *B. pomonae* consistently ignored exposed pupae (those not contained in the primary host's cocoon). It appears that *B. pomonae* requires the host cocoon to facilitate its extensive probing when ovipositing in smaller hosts.



Figs 3–4. 3 – *Cardiochiles nigriceps* pupa (approx. 7.5×) mortally wounded by the ovipositional activity of *Brachymeria pomonae* but not parasitized. Ovipositional punctures are revealed by the necrotic spots. 4 – (A) *Brachymeria pomonae* males (22×) demonstrating size difference between a primary male of average size reared on *Pectinophora gossypiella* and (B) a secondary (i.e. hyperparasitic) male of average size reared on *Apanteles oenone*.

C. nigriceps tests

Mortality in the *C. nigriceps* pupae exposed to *B. pomonae* was ca. 76% (Table 1), higher than in the case of *A. oenone* pupae. As in the case of *A. oenone* pupae, this high rate of mortality can be attributed to ovipositional probing as well. Similar to *A. oenone* pupae, minute necrotic spots reveal the ovipositor penetrations in dead *C. nigriceps* pupae (Fig. 3). The pupa shown in Fig. 3 was not parasitized, but shows mortal wounding caused by ovipositional probing by *B. pomonae*. In addition, 91% of the dead *C. nigriceps* pupae showed ovipositional punctures (Table 1), and up to 48 wounds were evident in these pupae. Ovipositional wounds were evident in one of the 2 live pupae which were examined for punctures.

Ten *C. nigriceps* pupae yielded *B. pomonae* offspring (Table 1). The host remains of these hyperparasitic individuals showed up to 12 ovipositional wounds. As in the case of *A. oenone* pupae, all hyperparasitic individuals were males.

Facultative hyperparasitism

The rates of facultative hyperparasitism were relatively low, ca. 10% and 23% in *A. oenone* and *C. nigriceps* pupae, respectively. All hyperparasitic offspring were males, and were deposited in the Entomology Research Museum, University of California, Riverside. The average length of hyperparasitic males emerging from *C. nigriceps* was 4.2 mm (range 3.6–4.5 mm, n = 8). Hyperparasitic males from *A. oenone* measured ca. 1.9 mm (range 1.8–2.1 mm, n = 3). Finally, primary males from the *B. pomonae* source culture (on PBW) measured ca. 3.2 mm (range 2.6–3.8 mm, n = 31). All measurements were made on point-mounted specimens. A middle-size male (2.9 mm) from the series of 31 primary males obtained from the source culture and a middle-sized hyperparasitic male from *A. oenone* (1.9 mm) are shown in Fig. 4 for comparison.

DISCUSSION

Our results show that: (1) *B. pomonae* hyperparasitized both *A. oenone* and *C. nigriceps*. This behavior is facultative in light of the parasitoid's aggressive primary parasitism of several lepidopterous hosts. Obligatory primary parasitism and obligatory hyperparasitism are considered the two extremes on a continuum of feeding habits among parasitoids (Ehler, 1990). Our results suggest that *B. pomonae* is much closer to the former, and that it probably acts as a hyperparasitoid only in the absence of any choice. (2) *B. pomonae* caused substantial mortality in *A. oenone* and *C. nigriceps* as a result of ovipositional probing. (3) *B. pomonae* aggressively attacked the pupae of both PBW and *A. oenone*, only when they were enclosed in PBW cocoons, and it did not attack either if they were not so enclosed. Apparently the host larva's cocoon is essential to ovipositional probing by *B. pomonae* when smaller hosts such as these are involved. Further study is needed to clarify the role played by the host cocoon in initiating and enabling oviposition by *B. pomonae*. Finally, (4) all hyperparasitic offspring of *B. pomonae* were males. The apparent production of males only as hyperparasitoids in this species resembles the habit of alloparasitic aphelinids (Walter, 1983); in the present case, however, alloparasitism is facultative, not obligatory. Our evidence is preliminary, and therefore further studies are called for since to our knowledge the development of male larvae only as hyperparasitoids is not known outside the Aphelinidae. Alternatively, the exclusive emergence of males from secondary

hosts suggests that these hosts are unsuitable for development of female *B. pomonae*; either female parasitoids avoid laying female eggs in secondary hosts or extreme differential mortality precludes the full development of female larvae in these hosts. The prevalence of host quality-dependent sex allocation patterns among idiobiont parasitoids (King, 1989) such as *B. pomonae* lends support to the former explanation, i.e. female eggs are not laid in secondary hosts. However, we cannot completely discount the possibility of extreme differential mortality with the data available to us.

The female *B. pomonae* used in this study were subjected to intense ovipositional pressure under no-choice conditions. This may have resulted in more hyperparasitism than might occur under natural conditions. However, the hyperparasitism displayed by *B. pomonae* is important with regard to introduction of this species for biological control in that it could pose a risk to other parasitoids that attack a common or unrelated host. Also of significance is the host mortality caused by ovipositional probing. Host destruction by related hyperparasitic species was noted by earlier workers. One team related it to host feeding and concluded that "this habit of the secondaries must be regarded as increasing their power of destruction" (Muesebeck & Dohanian, 1927), a statement that was substantiated by our observations.

The facultative hyperparasitic habit displayed by *B. pomonae* increases its already broad host range. For these two reasons, we did not attempt to obtain authorization for its release from quarantine. We believe our decision illustrates long-existing standards that may not be obvious to critics of biological control. One of the legal responsibilities of a quarantine facility is to contain and exclude from release all hyperparasitic species received in conjunction with primary species. Hyperparasitic species are routinely detected and destroyed during usual quarantine operations. These events are rarely published. The present case is important for it documents the examination of an aggressive primary parasitoid that shows both a broad host range and facultative hyperparasitic tendencies, and was for these reasons rejected as a candidate biological control agent.

The impact of facultative hyperparasitoids on biological control interactions is complex and poorly understood. Some have suggested that hyperparasitoids may not be all bad (Flanders, 1963), and that they may serve a useful purpose in some situations (Ehler, 1979; Bennett, 1981; Luck et al., 1981). Rosenheim et al. (1995) reviewed the available evidence concerning intraguild predation and concluded that facultative hyperparasitoids may play a major role in biological control, but that the direction of their impact, whether positive or negative, was uncertain. Similarly, recent theoretical analyses of intraguild predation dynamics showed that intraguild predation could lead to unstable parasitoid-host dynamics, even when individual pairwise interactions were stable (Holt & Polis, 1997). However, Holt and Polis also suggested that stage-restricted intraguild predation (e.g., hyperparasitism) may provide a refuge which could be stabilizing.

Rosen & Kfir (1983) opposed the introduction of facultative hyperparasitoids, and Luck et al. (1981) contended that "because their impact on biological control remains uncertain, a conservative policy of excluding all hyperparasitoids must remain in effect". As discussed above, the impact of facultative hyperparasitism on biological control remains unpredictable, and therefore in the spirit of erring on the side of caution, we concur with Luck et al. (1981).

ACKNOWLEDGEMENTS. We acknowledge the substantial contributions during various phases of the work of J. Altmann (Loxton, S. Australia), D. Powell (University of California, Riverside), B. Vinson (Texas A&M University, College Station), M. Waggoner (UCR), and K. McGiffen (UCR). K. Hagen (since deceased) reviewed the manuscript and made many useful suggestions, and D. Rosen (since deceased) provided helpful literature citations. Partial support for foreign exploration was provided by the University of California in 1991–92 and 1995, by US/AID in 1991–93, and by USDA/OICD (foreign exploration grants) in 1993–95.

REFERENCES

ANONYMOUS 1984: *Integrated Pest Management for Cotton in the Western Region of the United States.* Division of Agriculture and Natural Resources, University of California, Oakland, 144 pp.

BENNETT F.D. 1981: Hyperparasitism in the practice of biological control. In Rosen D. (ed.): *The Role of Hyperparasitism in Biological Control: A Symposium.* Division of Agricultural Sciences, University of California, Berkeley, pp. 43–49.

BOUČEK Z. 1988: *Australian Chalcidoidea (Hymenoptera). A Biosystematic Revision of Genera of Fourteen Families, with a Reclassification of Species.* CAB International, Wallingford, 832 pp.

CLAUSEN C.P. 1940: *Entomophagous Insects.* Hafner, New York, 688 pp.

DOWDEN P.B. 1935: Brachymeria intermedia (Nees), a primary parasite and B. compsilura (Cwfd.), a secondary parasite of the gypsy moth. *J. Agric. Res.* **50**: 495–523.

EHLER L.E. 1979: Utility of facultative secondary parasites in biological control. *Environ. Entomol.* **8**: 829–832.

EHLER L.E. 1990: Introduction strategies in biological control of insects. In Mackauer M., Ehler L.E. & Roland J. (eds): *Critical Issues in Biological Control.* Intercept, Andover, Hants, pp. 111–134.

ERTLE L.R. 1993: What quarantine does and what the collector needs to know. In Van Driesche R.G. & Bellows T.S. Jr. (eds): *Steps in Classical Arthropod Biological Control. Proceedings Thomas Say Publications in Entomology.* Lanham, Maryland, pp. 53–65.

FLANDERS S.E. 1963: Hyperparasitism, a mutualistic phenomenon. *Can. Entomol.* **95**: 716–20.

GORDH G. 1981: The phenomenon of insect hyperparasitism and its taxonomic occurrence in the Insecta. In Rosen D. (ed.): *The Role of Hyperparasitism in Biological Control: A Symposium.* Division of Agricultural Sciences, University of California, Berkeley, pp. 10–18.

KING B.H. 1989: Host-size dependent sex ratios among parasitoid wasps: Does host growth matter. *Oecologia* **78**: 420–426.

HOLT R.D. & POLIS G.A. 1997: A theoretical framework for intraguild predation. *Am. Nat.* **149**: 745–764.

KROMBEIN K.V., HURD P.D. JR., SMITH D.R. & BURKS B.D. 1979: *Catalog of Hymenoptera in America North of Mexico.* Smithsonian Institution Press, Washington, D.C., 1198 pp.

LUCK R.F., MESSENGER P.S. & BARBIERI J.F. 1981: The influence of hyperparasitism on the performance of biological control agents. In Rosen D. (ed.): *The Role of Hyperparasitism in Biological Control: A Symposium.* Division of Agricultural Sciences, University of California, Berkeley, pp. 34–42.

MUESEBECK C.F.W. & DOHANIAN S.M. 1927: A study of hyperparasitism, with particular reference to the parasites of Apanteles melanoscelus (Ratzeburg). *Bull. U. S. Dep. Agric.* No. 1487, 35 pp.

ROSEN D. & KFIR R. 1983: A hyperparasite of coccids develops as a primary parasite of fly puparia. *Entomophaga* **28**: 83–88.

ROSENHEIM J.A., KAYA H.K., EHLER L.E., MAROIS J.J. & JAFFEE B.A. 1995: Intraguild predation among biological control agents: Theory and Evidence. *Biol. Control* **5**: 303–335.

WALTER G.H. 1983: "Divergent male ontogenies" in Aphelinidae (Hymenoptera: Chalcidoidea): A simplified classification and a suggested evolutionary sequence. *Biol. J. Linn. Soc.* **19**: 63–82.

Received June 6, 1997; accepted March 10, 1998