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Interactions among bethylid parasitoid species attacking the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae)

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Abstract

The question of whether biological control is most likely achieved by deploying single or multiple species of biological control agents is much debated. While utilizing several natural enemies may enhance control, there is also the potential for disruptive inter-specific interactions. Such interactions may be studied in the laboratory by focusing on the details of the interactions themselves and attempting to infer population level consequences from their sum, or by focusing more directly on the overall effects on natural enemy populations: we term these approaches 'reductionist' and 'holistic.' Here we conduct a holistic laboratory study on interactions between three species of parasitoid wasps that are parasitoids of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae): *Cephalonomia stephanoderis* Betrem, *C. hyalinipennis* Ashmead and *Prorops nasuta* Waterston (all Hymenoptera: Bethylidae). We find evidence for both intra- and inter-specific resource competition. Interactions between *C. stephanoderis* and *P. nasuta*, both indigenous to Africa, appear to be approximately symmetrical, while *C. hyalinipennis*, naturally found in the coffee plantations of Chiapas, Mexico, may exert a disruptive influence. *C. hyalinipennis* also has a low population growth rate. We now consider it to be a detrimental invader of the Mexican coffee agro-ecosystem that should not be encouraged by augmentative release or introduced into other regions. Overall, the most successful species, in terms of both emergence and female production, was *P. nasuta*. We compare these results with those from prior reductionist and holistic studies, and with observations on patterns of establishment of these bethylid species in the field. Given that it is increasingly clear that disruptive inter-specific interactions are generally common when multiple species are deployed in biological control, screening of potential agents should consider such interactions alongside the more 'traditional' focus on host specificity.
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Keywords: Bethylidae; *Cephalonomia stephanoderis*; *Cephalonomia hyalinipennis*; *Prorops nasuta*; Coffee berry borer; *Hypothenemus hampei*; Intra- and inter-specific resource competition; Multiple- versus single-species release

1. Introduction

There has been much debate as to whether better biological control is achieved by releasing single or multiple species of natural enemies (Collier et al., 2002; Denoth et al., 2002; Ehler, 1990; Hågvar, 1989; Murdoch and Briggs, 1996; Myers et al., 1989; Watt, 1965). Denoth

et al. (2002) recommend restraint in the introduction of multiple species due to detrimental interspecific interactions, such as intra-guild predation (Collier et al., 2002; Ferguson and Stiling, 1996; Holt and Polis, 1997; Hunter et al., 2002; Pérez-Lachaud et al., 2004; Rosenheim et al., 1993, 1995; Snyder and Ives, 2001) or that a more effective species, in terms of host suppression, may be competitively inferior to less effective natural enemies and thus fail to establish (Force, 1974; Hunter et al., 2002; Leveque et al., 1993; Turnbull and Chant, 1961). However, some theoretical and field investigations have

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indicated that multiple natural enemies can, under certain conditions, coexist and may be desirable in that they can additively suppress pest populations, for example by attacking different life-history stages of the host (Ehler, 1978; Gutierrez et al., 1988, 1998; Hassell, 1978; Huffaker et al., 1971; Kindlman and Ruzicka, 1992).

The above concerns apply to virtually all biological pest control programs because virtually all pests have more than one species of natural enemy (e.g., Mason and Huber, 2002) but because different pest species have different biologies and different arrays of natural enemies, pest control solutions need largely to be developed on a case-by-case basis. Here we address the issue of how many, and which, of three species of natural enemy, all bethylid wasps, are most likely to provide biological control of the coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae). Our study is an attempt to resolve an interpretational disparity between several prior studies that drew opposing conclusions from different types of experiments designed to evaluate inter-specific competition. We begin with a summary of the CBB control problem and of the prior studies concerning the coexistence of multiple natural enemies of the CBB.

1.1. Biological control of the CBB and coexistence of natural enemy species

The coffee berry borer is indigenous to Africa but has now spread throughout the coffee growing world and is considered the most serious pest of coffee worldwide (Baker, 1999; Baker et al., 2002; Damon, 2000). The CBB arrived in southern Mexico (Chiapas) in 1978 and now causes severe reductions in Mexican coffee production (Baker, 1984; Barrera et al., 1990b; Infante et al., 2001b). Control programs in Chiapas, incorporating Integrated Pest Management (IPM), have included the release of two African natural enemies of the CBB, the parasitoid wasps *Cephalonomia stephanoderis* Betrem and *Prorops nasuta* Waterston (both Hymenoptera: Bethylidae). Neither species has achieved economic control following a classical biological control strategy; *C. stephanoderis* has widely established in the Soconusco region of Chiapas but remains in low numbers (Barrera, 1994; Damon, 1999; Damon and Valle, 2002) and *P. nasuta* has not persisted for more than 15 months (Infante et al., 2001b, 2003). A further bethylid, *Cephalonomia hyalinipennis* Ashmead, which is indigenous to South and North America and Europe (Gordh and Móczár, 1990), has been discovered naturally attacking CBB in coffee plantations in the Soconusco region (Pérez-Lachaud, 1998). The biologies of each of these three species have been investigated in the context of their potential as agents of biological control (e.g., Abraham et al., 1990; Barrera et al., 1989, 1990a, 1993; Damon, 1999; Infante, 2000; Infante and Luis, 1993; Lauzière et al., 2001a; Pérez-

Lachaud and Hardy, 2001). Recent studies have focused on interactions between these species to evaluate whether they are likely to coexist in agro-ecosystems and the influence of interspecific interactions on host population suppression (Batchelor et al., 2005; Gutierrez et al., 1998; Infante et al., 2001a; Pérez-Lachaud et al., 2002, 2004).

The first laboratory evaluation of interspecific interactions was carried out by Infante et al. (2001a) using medium-term population trends in laboratory microcosms using coffee berries naturally infested by the CBB. Competition experiments were conducted between *C. stephanoderis* and *P. nasuta* (but not *C. hyalinipennis*) released at a range of densities (5 berries per parasitoid or 1, 2, 3 or 5 parasitoids per berry) and temperatures (18, 29 °C or an alternation between these temperatures) into 1 L jars containing 10–50 infested berries. Allospecific competitors were released either simultaneously or one species was released 10 days after the other. One month after the initial release, any adult parasitoids observed outside of the berries were collected, identified, and counted. Such collections ceased after a further 2.5 months. The outcome of any resource competition was thus determined according to progeny production, over a period that would allow between 2 and 5 parasitoid generations. When competitor species were released simultaneously at >18 °C *C. stephanoderis* generally produced more progeny than *P. nasuta*, but at 18 °C *P. nasuta* was more successful. When release was not simultaneous, the species that was released first generally produced more progeny, with the exception that at 18 °C *P. nasuta* out-produced *C. stephanoderis* irrespective of temporal priority. Infante et al. (2001a) found no evidence of interference competition from this experiment (chases and fights between adult females outside of the berries were never observed). They concluded that interspecific competition between *C. stephanoderis* and *P. nasuta* is mainly of the indirect, exploitative kind, and that the deployment of both species against the coffee berry borer is sound. For the purposes of the present study, we refer to this experimental approach as 'holistic' because the focus is on overall 'output' rather than on the mechanisms that lead to particular patterns on progeny production and, ultimately, coexistence.

A different, more mechanistic, approach to evaluating competition has been taken by several other studies that have explored factors influencing the outcomes of interspecific interactions. We refer to this as the 'reductionist' approach because the focus is on components of the competitive process; it is implicit that patterns of progeny production and coexistence can be inferred from their sum. Dyadic contests for host resources between adult females within a confined space representing an individual berry were studied by Pérez-Lachaud et al. (2002) and Batchelor et al. (2005). Interactions between and within *C. stephanoderis*, *C. hyalinipennis*, and

P. nasuta, with or without one individual having temporal priority ('ownership') or ovipositional experience, were observed at $28 \pm 2\,°C$ for 3 h (with subsequent daily inspection). Outcome of competition was determined according to behavior and mortality, rather than progeny production. These experiments showed that, in all three species, prior owners exhibit active resource defense against 'intruders' that attempt to take possession of the resource. In intraspecific contests, aggressive behaviors but no fatalities were observed. In interspecific interactions, losing females were killed in 69% of contests. *C. stephanoderis* was the most successful competitor in interspecific interactions as it nearly always won contests when given prior access to resources, usually defeated *C. hyalinipennis* owners when having ovipositional experience and generally defeated *P. nasuta* owners irrespective of experience. *P. nasuta* was the least successful competitor, usually losing contests, whether in the role of owner or intruder. The occurrence of fatal fighting between allospecifics but not conspecifics implies that interspecific competition is stronger than intraspecific competition, and thus that ecological coexistence of these species may be unlikely (e.g., Hassell, 2000, p. 120).

Further to these direct and competitive interspecific contest interactions, aspects of intra-guild predation have been reported between these bethylid species. Infante et al. (2001a) provided adult female *C. stephanoderis* and *P. nasuta* with developing allospecifics in the laboratory and observed limited interspecific ovicide and larvicide. *C. stephanoderis* has also been reported to feed on *C. hyalinipennis* larvae and *C. hyalinipennis* feeds on immature *C. stephanoderis* and *P. nasuta* (Pérez-Lachaud et al., 2004). Furthermore, *C. hyalinipennis* can also develop as a hyperparasitoid of *C. stephanoderis* and *P. nasuta* (Pérez-Lachaud et al., 2004).

While fighting, predation and facultative hyperparasitism constitute interspecific interference competition [species directly reduce each other's survival (Griffith and Poulson, 1993)], suggesting coexistence is unlikely, the importance of these interactions in the field remains unevaluated. Furthermore, there may be additional important yet unidentified aspects of interactions between these species. The reductionist approach by itself may thus not readily lead to robust recommendations concerning multi-species interactions in agro-ecosystems. In contrast, the holistic approach may point to likely outcomes but not to causality. In the case of CBB biocontrol strategy, the holistic approach has lead to the recommendation of multi-species release while reductionist studies have cautioned against this.

Here we conduct laboratory experiments that combine elements of both approaches. Inter- and intra-specific interactions are studied using naturally infested berries with outcomes evaluated in terms of progeny production (holistic). These interactions, however, occur between small numbers of females within a restricted number of berries, for a single generation, such that the outcomes may more readily be interpreted in terms of trophic and contest interactions (reductionist) than when larger numbers of berries and parasitoids are used in each experimental replicate.

2. Materials and methods

Our experiments used coffee berries naturally infested with the CBB. All berries used were *Coffea canephora* (robusta variety) and were collected from various localities around Tapachula (Chiapas, Mexico) between early January and early March in 2003 and 2004. Berries were selected for experiments on the basis of evidence of infestation by CBB and the presence of suitable host stages. Berries that contained suitable host stages were dark brown or black and had dark dust around holes originally bored by CBB adults on entry. To assess the likely content of berries thus selected, we dissected five samples of 20–50 berries, from throughout the experimental period, and recorded the presence and life-history stages of any CBB inside. Of the 130 berries dissected, all contained at least one adult CBB and 128 contained hosts at a suitable stage (eggs, larvae, prepupae, and pupae) for oviposition by bethylid parasitoids. There was, however, significant between-sample variation in the number of suitable CBB per berry (log-linear ANOVA corrected for overdispersion, $F_{4,125} = 12.64$, $P < 0.001$): the average number per sample ranged from 15.34 (SD ± 6.1, $N = 50$) to 38.2 (SD ± 19.3, $N = 20$). Even the lower of these host availabilities should provide abundant reproductive opportunities for the bethylid species studied. None of the dissected berries contained parasitoids: we thus assumed that there were no parasitoids already present in the berries used in experiments (prior data on field collections also support this assumption: Damon (1999) found parasitoids in only 1 in every 1136 CBB infested berries dissected).

Parasitoids were cultured at El Colegio de la Frontera Sur (Tapachula). The *P. nasuta* stock was derived from insects reared in Brazil in 1992 and the *C. stephanoderis* stock from insects collected in Togo in 1988. The *C. hyalinipennis* culture was initiated in 1997 from material collected in the Soconusco region (Mexico) (Pérez-Lachaud, 1998). Experiments were conducted during two periods: late January to mid-April 2003 and mid-January to mid-May 2004.

Each experimental replicate was carried out using a glass tube (1.5 cm diameter × 7.3 cm height) closed with nylon gauze held in place with a flexible plastic ring. The number of berries and the number and species of wasps placed into each tube was varied. A tube contained one, two or three berries and one, two or three 'foundress' female parasitoids (no male wasps were introduced into

any tubes). Some tubes with multiple foundresses contained only one species of parasitoid; we refer to these as intra-specific competition replicates. The remainder of the multiple foundress replicates evaluated inter-specific competition, and contained wasps belonging to either two or three of the bethylid species studied. In multiple-female replicates, parasitoids were introduced simultaneously to avoid priority effects (Batchelor et al., 2005; Infante et al., 2001a; Petersen and Hardy, 1996).

The initial combinations of berries and wasps used in intra-specific competition replicates are shown in Table 1, comprising nine possible combinations of parasitoids and berries for each species. There were at least 10 replicates of each combination (500 overall, Table 1); a high degree of replication was chosen due to the significant variation in the number of suitable CBB per berry in pilot samples (see above). The initial 12 combinations and numbers of replicates (195 overall) used to evaluate inter-specific competition are shown in Table 2. Taken together these

combinations allowed us to control for effects of density on parasitoid production and to compare between intra- and inter-specific competition replicates.

The prepared tubes were then kept at ambient temperature ($27.5 \pm 3.5\,°C$) and $62.5 \pm 32.5\%$ relative humidity. They were kept in darkness for the first 18 days and then transferred to natural daylight timings (approx. 11:13 light:dark photoperiod). Each replicate was inspected daily from the time of parasitoid introduction until parasitoid emergence was thought to be complete. All emerging parasitoids were collected and identified to species and gender. There were usually several CBB adults found dead outside of the berries within a tube, but we did not record their numbers. Berries were dissected and inspected for unemerged parasitoids 14 days after the first parasitoid emerged from a given tube, except when emergence continued beyond 14 days in which case we dissected berries after the first two days with no parasitoid emergence. The production of female parasitoids of each species from each tube was calculated as the total unemerged plus emerged females minus the number of foundress females introduced. We focus exclusively on the production of females, rather than that of both sexes, since it is the number of female progeny that most influences host-parasitoid population biology (parameter c in classical host-parasitoid population dynamic models, e.g., Hassell (2000, p. 10), see also Pérez-Lachaud and Hardy (2001)).

2.1. Statistical analysis

Data were analyzed using generalized linear modeling (in Genstat version 7.2.0.208 and GLIM version 4.0), which allows parametric analysis of data with non-normally distributed error variances without prior transformation. Proportional data (replicates with emergences) were explored using logistic analyses assuming binomially distributed residuals (Crawley, 1993; Wilson and Hardy, 2002) and significance was assessed by change in deviance, G (which approximates χ^2). Count data (number of emerging females per replicate) were explored using log-linear analyses in which the dispersion parameter was estimated empirically to take overdispersion (variances greater than the mean leading to non-Poisson distributed residuals) into account, with significance assessed using F ratio tests (Crawley, 1993). G tests (log-likelihood ratio tests) were used to analyze data in contingency tables. Data on number of emerging females per foundress were square-root transformed prior to standard parametric analysis (that assume normally distributed residuals) to stabilize residual variances. Our general approach was thus to use, where possible, parametric analyses in which the assumed distribution of residuals was matched to the data rather than transforming data to fit standard assumptions. Assumed error distributions and variances were checked using residual and

Table 1
Initial conditions of, and outcomes from, replicates focussing on intra-specific interactions

Species	Initial conditions		Replicates	Outcomes	
	Number of foundresses	Number of berries		Replicates with emergence	
				Number	%
C. stephanoderis	1	1	19	11	57.9
	2	1	34	13	38.2
	3	1	21	10	47.6
	1	2	23	10	43.5
	2	2	21	10	47.6
	3	2	17	11	64.7
	1	3	21	10	47.6
	2	3	23	12	52.2
	3	3	26	11	42.3
Total			205	98	47.8
C. hyalinipennis	1	1	20	10	50.0
	2	1	23	10	43.5
	3	1	17	12	70.6
	1	2	23	10	43.5
	2	2	19	11	57.9
	3	2	19	11	57.9
	1	3	25	12	48.0
	2	3	17	13	76.5
	3	3	19	10	52.6
Total			182	99	54.4
P. nasuta	1	1	15	10	66.7
	2	1	13	11	84.6
	3	1	15	12	80.0
	1	2	14	10	71.4
	2	2	10	10	100.0
	3	2	13	12	92.3
	1	3	10	9	90.0
	2	3	13	11	84.6
	3	3	10	10	100.0
Total			113	95	84.1
Total			500	292	63.4

Table 2
Initial conditions of, and outcomes from, replicates focussing on inter-specific interactions

Initial conditions				Replicates	Outcomes				
C. stephanoderis	C. hyalinipennis	P. nasuta	No. of berries		Replicates with emergence	% Replicates with emergence			
						Any species	C. stephanoderis	C. hyalinipennis	P. nasuta
1	1	—	1	22	9	40.9	9.1	36.4	—
1	1	—	2	18	9	50.0	27.8	38.9	—
1	1	—	3	24	13	54.2	20.9	45.8	—
1	—	1	1	21	12	57.1	23.8	—	42.9
1	—	1	2	13	12	92.3	53.8	—	76.9
1	—	1	3	15	11	73.3	33.3	—	60.0
—	1	1	1	13	11	84.6	—	30.8	53.8
—	1	1	2	14	10	71.4	—	42.9	71.4
—	1	1	3	19	12	63.2	—	15.8	57.9
1	1	1	1	12	11	91.7	25.0	41.7	33.3
1	1	1	2	12	10	83.3	8.3	33.3	75.0
1	1	1	3	12	10	83.3	16.7	41.7	66.7
Total				195	130	70.4	24.3	36.4	59.8

normality plots (e.g., Wilson and Hardy, 2002). Further, we adopted the top–down approach (i.e., stepwise backward analysis) in which significance is assessed when explanatory variables are removed from an initially complex model (containing all main effects and interaction terms of interest) to obtain a parsimonious statistical description.

Possible Type I errors due to multiple comparisons (e.g., Quinn and Keough, 2002, pp. 48–50) were controlled for using the false discovery rate (FDR) procedure of Benjamini and Hochberg (1995), which retains more statistical power than Bonferroni-based methods. All statistical comparisons were treated as one 'family' of tests and the family-wide α-value adopted was 0.05. After using this procedure, all P values > 0.011 were no longer considered to represent genuine underlying relationships: possible Type I errors were thus exposed in 9 cases. However, because multiple comparisons methods, including the FDR, are inherently conservative (possibly generating Type II errors) we also indicate the initial interpretations in these cases.

Analysis of proportion of replicates with emergence included all replicates prepared for each experiment, but subsequent analyses of species emergence, number of emerging females per replicate and number of emerging females per foundress were undertaken on the subset of replicates from which parasitoids emerged.

3. Results

3.1. Emergence from berries

Parasitoid progeny emerged in 63.4% of intra-specific competition replicates and 70.4% of inter-specific competition replicates (Tables 1 and 2). Among intra-specific competition replicates, there was significant inter-specific variation in the proportion of replicates from which progeny emerged ($G = 25.11$, $df = 2$, $P < 0.001$, $n = 500$).

This was due to the relatively frequent emergence of *P. nasuta* (Table 1); the proportion of emergence of the two *Cephalonomia* species did not differ significantly ($G = 0.61$, $df = 1$, $P = 0.79$). The proportion of emergence was unaffected by the number of foundresses ($G = 1.54$, $df = 2$, $P = 0.215$) and the number of berries provided ($G = 0.53$, $df = 2$, $P = 0.588$).

Similar patterns were found for inter-specific competition replicates: emergence from replicates with two allospecific foundresses was unaffected by the number of berries ($G = 1.24$, $df = 2$, $P = 0.29$) but varied according to species composition ($G = 5.26$, $df = 2$, $P = 0.005$, $n = 159$), again due to the relatively frequent emergence from replicates containing *P. nasuta* (comparison of replicates with and without *P. nasuta*: $G = 10.39$ $df = 1$, $P = 0.001$). Emergence of each species was not affected by the identity of the other species present (*P. nasuta*: $G = 0.02$, $df = 1$, $P = 0.884$, $n = 95$; *C. hyalinipennis*: $G = 1.29$, $df = 1$, $P = 0.256$, $n = 110$; *C. stephanoderis*: $G = 4.23$, $df = 1$, $P = 0.04$, $n = 113$, note that while *C. stephanoderis* females emerged less often from replicates that also contained *C. hyalinipennis* than from those containing *P. nasuta*, this result is interpreted as non-significant following correction for FDR). Emergence rates of the three species were unaffected by the number of berries in a replicate.

3.2. Progeny production from berries with emergence

3.2.1. Intra-specific competition

For *C. stephanoderis*, production of female progeny per replicate increased with increasing number of berries ($F_{2,89} = 8.91$, $P < 0.001$, $n = 98$, Fig. 1A) but there was no significant influence of foundress number ($F_{2,89} = 0.19$, $P = 0.830$) or a berry number × foundress number interaction ($F_{4,89} = 0.21$, $P = 0.934$). In terms of female production per foundress, there was an increase with increasing number of berries ($F_{2,89} = 8.43$, $P < 0.001$,
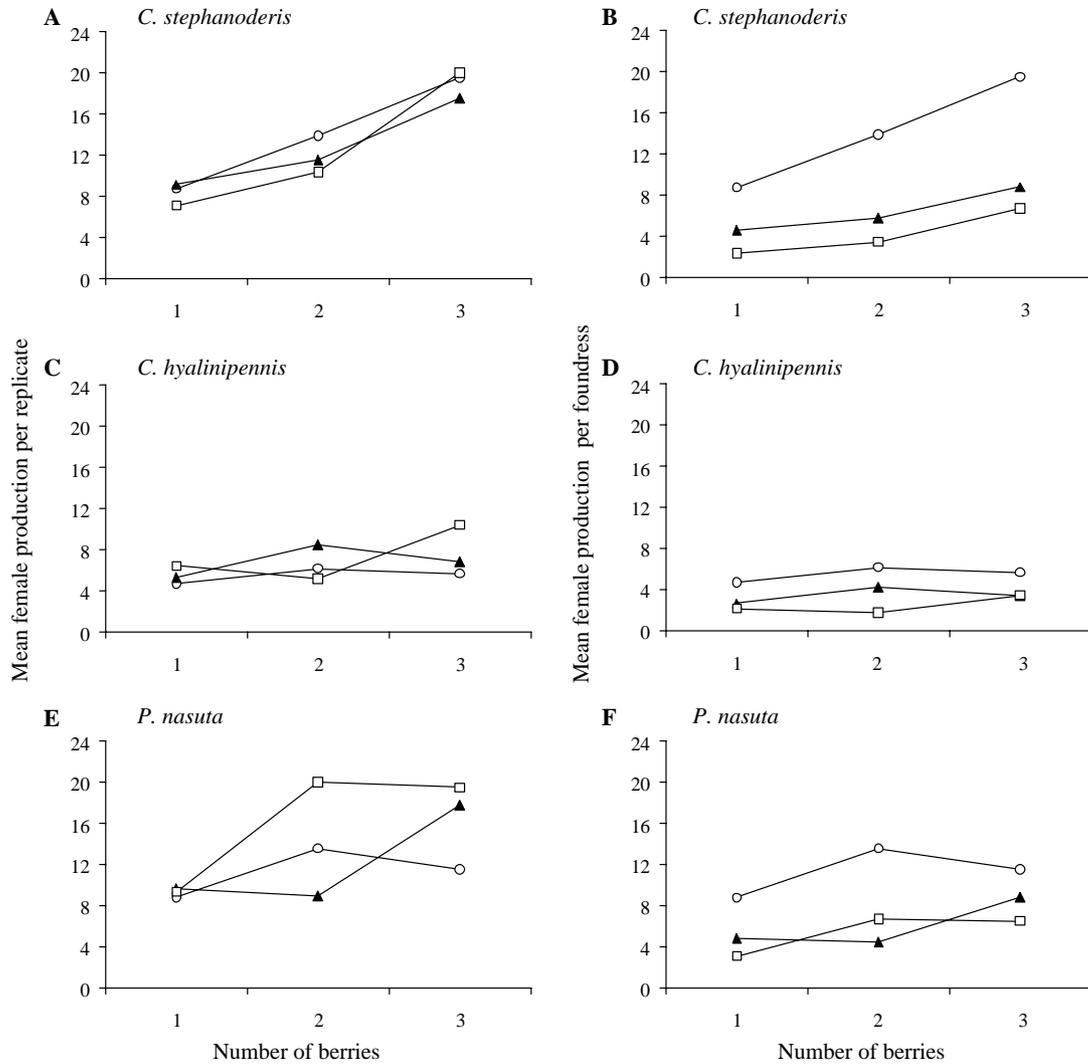
Fig. 1. Intraspecific competition. Mean female production per replicate (A, C, and E). Mean female production per foundress (B, D, and F). 1 foundress, (○); 2 foundresses, (▲); 3 foundresses, (□).

Fig. 1B), but a decrease with increasing number of foundresses ($F_{2,89} = 25.34$, $P < 0.001$). There was no significant berry number × foundress number interaction ($F_{4,89} = 0.59$, $P = 0.672$). The effect of foundress number was due to a qualitative distinction between single and multiple foundress cases: the resultant reduction in fit of the statistical model when the 2- and 3-foundress data were combined [i.e., aggregation of factor levels, Crawley (1993, p. 190)] was not significant after correction for FDR ($F_{1,92} = 4.35$, $P = 0.04$).

For *C. hyalinipennis*, female production per replicate (Fig. 1C) was not influenced by number of berries ($F_{2,90} = 0.98$, $P = 0.379$, $n = 99$) or foundress number ($F_{2,90} = 0.92$, $P = 0.402$) and there was no significant berry × foundress interaction ($F_{4,90} = 1.27$, $P = 0.288$). Production per foundress decreased as foundress number was increased but not significantly after correction for FDR ($F_{2,90} = 3.91$, $P = 0.024$, Fig. 1D). No significant effect of number of berries on female production per foundress

was detected ($F_{2,90} = 0.86$, $P = 0.426$) and there was no berry × foundress interaction ($F_{4,90} = 0.51$, $P = 0.730$).

Female *P. nasuta* production per replicate increased with an increase in both the number of berries and the number of foundresses, but neither result was significant after correction for FDR (respectively, $F_{2,86} = 3.66$, $P = 0.030$, $n = 95$, Fig. 1E; $F_{2,86} = 4.07$, $P = 0.021$, Fig. 1E). There was no significant berry × foundress interaction ($F_{4,86} = 1.85$, $P = 0.127$). Female production per foundress decreased as the number of foundresses was increased ($F_{2,86} = 10.19$, $P < 0.001$, Fig. 1F): single-foundress replicates had significantly higher female production per foundress than those with two or three foundresses (aggregating factor levels, as above, lead to no significant reduction in fit, $F_{1,89} = 0.01$, $P = 0.905$). The number of berries had no significant effect on female production per foundress ($F_{2,86} = 2.50$, $P = 0.088$) and there was no significant berry × foundress interaction ($F_{4,86} = 1.65$, $P = 0.168$).

3.3. Comparisons of intraspecific competition

A comparison of female progeny production among species revealed that, as the number of berries increased, the number of females emerging per replicate also increased ($F_{2,265} = 13.09$, $P < 0.001$, $n = 292$). There were significant differences in female production per replicate between the three species ($F_{2,265} = 23.58$, $P < 0.001$), which could be attributed to lower female production in *C. hyalinipennis* replicates than those which contained *P. nasuta* or *C. stephanoderis* (aggregating factor levels lead to no significant reduction in fit, $F_{1,274} = 1.64$, $P = 0.105$).

Female production per foundress also increased significantly as the number of berries was increased ($F_{2,265} = 8.11$, $P < 0.001$, $n = 292$). Conversely, as the number of foundresses per replicate increased, the number of emerging females per foundress decreased ($F_{2,265} = 33.82$, $P < 0.001$). The three species differed significantly in female production per foundress ($F_{2,265} = 24.26$, $P < 0.001$); *C. hyalinipennis* had significantly lower female production per foundress than *C. stephanoderis* or *P. nasuta* (aggregation of factor levels lead to no significant reduction in fit $F_{1,284} = 2.49$, $P = 0.060$).

3.3.1. Inter-specific competition
3.3.1.1. Species emergence.
In this section, we analyze results in terms of whether or not species were represented among the progeny emerging from replicates (patterns in the numbers of individuals emerging are explored in the following section). Since laboratory-observed contests commonly lead to the loser's death (Batchelor et al., 2005; Pérez-Lachaud et al., 2002), we first examined the frequency with which more than one species of progeny emerged from inter-specific competition replicates (Table 3). Multi-species emergence was observed in 34/130 replicates: the frequency of multi-species emergence from single berry replicates (4/43) was significantly lower than from replicates containing two or more berries (30/87) ($G = 10.7$, $df = 1$, $P < 0.01$), suggesting that contest interactions could be occurring in single berry replicates when individuals are forced to share or compete for resources. We further explored whether being forced to interact within a berry affected the frequency of emergence of each species by comparing emergence from single berry replicates with emergence from replicates with multiple berries (where contests could potentially be avoided) among the replicates with two foundress species (i.e., dyadic replicates). The emergence rates of each of the species when competing with allospecifics were not significantly different when a single berry or multiple berries were provided (*C. stephanoderis*, $G = 1.43$, $df = 1$, $P > 0.1$; *C. hyalinipennis*, $G = 0.01$, $df = 1$, $P > 0.1$; *P. nasuta* $G = 3.71$, $df = 1$, $P > 0.05$).

Turning to the combinations of species that emerged, *P. nasuta* and *C. hyalinipennis* never emerged together from single-berry replicates ($n = 22$, Table 3) but did so significantly more often from replicates that contained multiple berries (13/42 occasions, Table 3, $G = 12.63$, $df = 1$, $P < 0.001$). *C. stephanoderis* and *P. nasuta* emerged together from 10/43 replicates containing multiple berries and from 3/23 single berry replicates (Table 3, $G = 1.04$, $df = 1$, $P > 0.1$). Both *Cephalonomia* species emerged from 1/20 single-berry replicates and from 6/42 multiple-berry replicates (Table 3, $G = 1.32$, $df = 1$, $P > 0.1$). From the 32 replicates with all three species of foundresses, all three species of progeny emerged only once, from a three-berry replicate (Table 3).

Considering the frequency of emergence of each species from interspecific replicates, *P. nasuta* emerged most often (77/99 replicates), whether competing against either or both of the *Cephalonomia* species (Table 3). *C. stephanoderis* emerged least often from interspecific replicates (35/97 replicates), and had particularly low

Table 3
Species composition of female progeny emerging from inter-specific competition replicates

Foundress species combination	Number of berries	*C. stephanoderis* only	*C. hyalinipennis* only	*P. nasuta* only	*C. stephanoderis* and *C. hyalinipennis*	*C. stephanoderis* and *P. nasuta*	*C. hyalinipennis* and *P. nasuta*	*C. stephanoderis*, *C. hyalinipennis* and *P. nasuta*
C. stephanoderis and	1	1	7	—	1	—	—	—
C. hyalinipennis	2	2	4	—	3	—	—	—
	3	2	8	—	3	—	—	—
C. stephanoderis and	1	3	—	7	—	2	—	—
P. nasuta	2	2	—	5	—	5	—	—
	3	2	—	6	—	3	—	—
C. hyalinipennis and	1	—	4	7	—	—	0	—
P. nasuta	2	—	0	4	—	—	6	—
	3	—	1	9	—	—	2	—
C. stephanoderis,	1	2	5	3	0	1	0	0
C. hyalinipennis,	2	0	1	5	0	1	3	0
and *P. nasuta*	3	0	2	4	0	1	2	1

emergence from replicates initially containing all three species (6/31 replicates, Table 3). *C. hyalinipennis* emerged from 53/95 interspecific replicates, achieving highest success in dyadic replicates containing *C. stephanoderis* ($n = 31$, *C. hyalinipennis* emerged from 26 replicates and *C. stephanoderis* from 12, Table 3).

Taken together, the above data on patterns of species emergence suggest that *P. nasuta* was the most and *C. stephanoderis* the least successful, and that interspecific competition occurs.

3.4. Number of emerging females per replicate

We first examine the influence of interspecific competition on female production per species by comparing between 2-foundress (dyadic) replicates in which foundress density is constant. We then re-analyze these data with the addition of replicates containing all three species of foundresses (triadic interactions).

3.5. Dyadic interactions

While more female *C. stephanoderis* emerged in two-berry than one-berry replicates the difference was not significant after correction for FDR ($F_{2,60} = 3.95$, $P = 0.024$, $n = 66$, Fig. 2). Female production was not significantly influenced by the identity of the competing species ($F_{1,60} = 1.19$, $P = 0.279$, $n = 66$) and there was no significant berry number × foundress species combination interaction ($F_{2,60} = 0.98$, $P = 0.383$, $n = 66$). For *C. hyalinipennis*, female production was uninfluenced by the number of berries provided ($F_{2,58} = 0.46$, $P = 0.635$, $n = 64$) but was significantly higher in replicates containing *C. stephanoderis* than in those containing *P. nasuta* ($F_{1,58} = 15.81$, $P < 0.001$, $n = 64$, Fig. 2). There was no berry × species interaction ($F_{2,58} = 0.55$, $P = 0.582$, $n = 64$). Production of *P. nasuta* females was not significantly influenced by the identity of the competing species ($F_{1,62} = 1.88$, $P = 0.175$, $n = 68$) or the number of berries provided ($F_{2,62} = 0.19$, $P = 0.828$, $n = 68$), nor was there a berry × species interaction ($F_{2,62} = 0.02$, $P = 0.984$, $n = 68$) (Fig. 2).

Comparison of overall female production per replicate, irrespective of species identities, indicated that production was higher in replicates containing *C. stephanoderis* and *P. nasuta* rather than *C. hyalinipennis*, but this difference was not significant after correction for FDR ($F_{2,90} = 3.68$, $P = 0.029$, $n = 99$, Fig. 3). Increasing the number of berries available had no significant affect on overall female production per replicate ($F_{2,90} = 2.91$, $P = 0.059$) and there was no significant berry × species interaction ($F_{4,90} = 0.51$, $P = 0.727$).

3.6. Dyadic and triadic interactions

Analysis of female production per replicate using both dyadic and triadic replicates generated the same
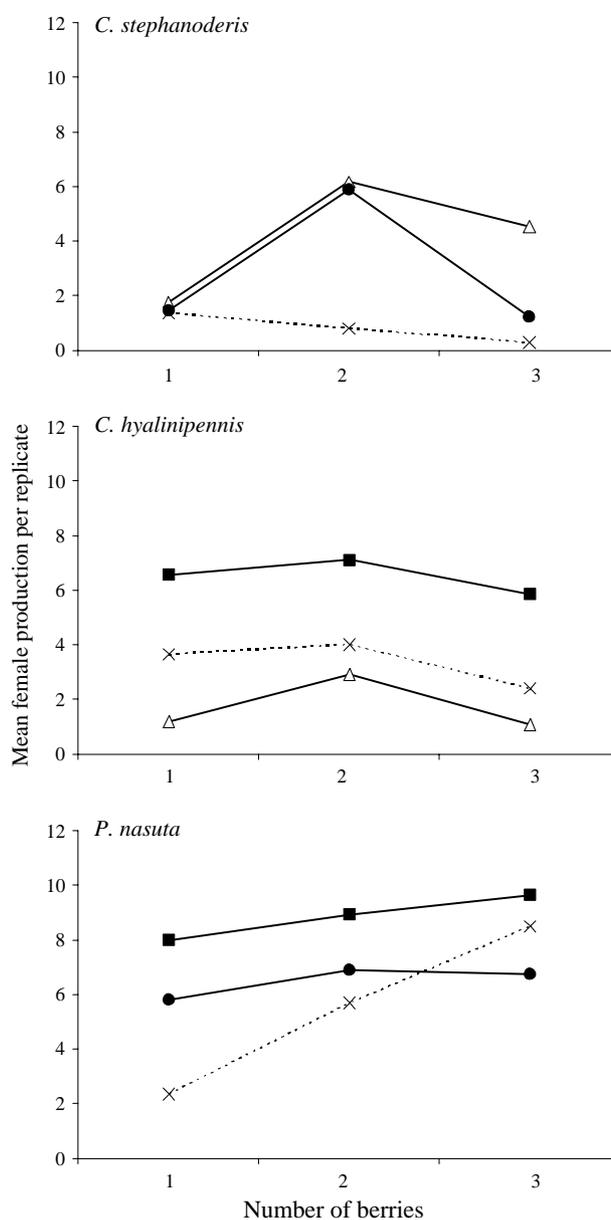

Fig. 2. Interspecific competition. Mean female production per foundress for each species when present with *C. stephanoderis* (■), *C. hyalinipennis* (●), *P. nasuta* (△), or all three species present –×–.

qualitative conclusions as above, with the exception that for *C. stephanoderis* there was an effect of competing species identity: production was significantly higher in dyadic replicates containing *P. nasuta* than in triadic replicates ($F_{2,88} = 5.88$, $P = 0.004$, $n = 97$, Fig. 2).

3.6.1. Comparison between intra- and inter-specific replicates

We compared overall female production, irrespective of species identities, per replicate under intra- and inter-specific competition. Production was influenced by the combination of foundress species present ($F_{6,209} = 2.99$, $P = 0.008$, Fig. 3) and positively influenced by the num-
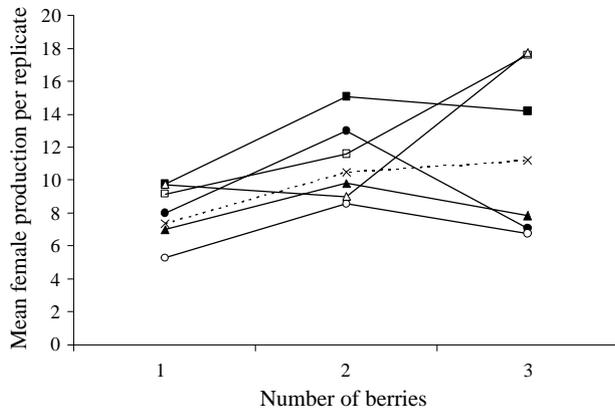
Fig. 3. Intra- and inter-specific competition. Mean female production per replicate, regardless of which species emerged. Foundress species; *C. stephanoderis* and *C. stephanoderis* (□), *C. hyalinipennis* and *C. hyalinipennis* (○), *P. nasuta* and *P. nasuta* (△), *C. stephanoderis* and *C. hyalinipennis* (●), *C. stephanoderis* and *P. nasuta* (■), *P. nasuta* and *C. hyalinipennis* (▲), all three species –×–.

ber of berries provided but the latter trend was not significant after correction for FDR (log-linear analysis, $F_{2,209} = 4.09$, $P = 0.018$, Fig. 3). There was no significant berry × species combination interaction ($F_{12,209} = 0.80$, $P = 0.655$). We then attempted to combine, in a stepwise manner, with order determined by inspection of parameter estimates (Crawley, 1993), the seven different combinations of foundress species, assessing the significance of the change in statistical fit after each simplification of the statistical model. All aggregations involving *C. hyalinipennis* did not lead to significant changes (P ranged between 0.7 and 0.13) and were thus retained. Species combinations not involving *C. hyalinipennis* also did not differ significantly and could be aggregated (P ranged between 0.93 and 0.32). Combining combinations with and without *C. hyalinipennis*, however, lead to a highly significant reduction in statistical fit ($F_{1,227} = 14.32$, $P < 0.001$). Combinations with *C. hyalinipennis* had
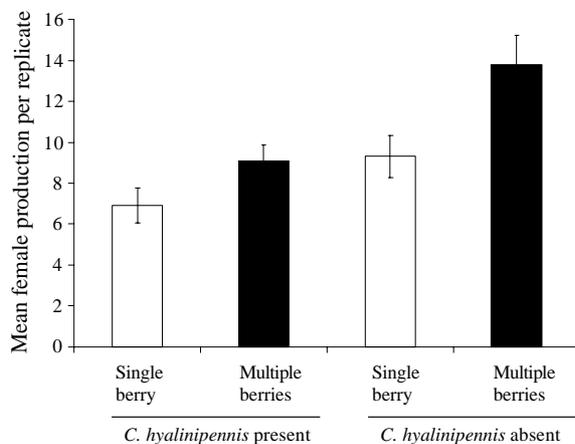

Fig. 4. Mean production per replicate when there were single- or multiple-berries and *C. hyalinipennis* was present or absent from the replicate.

lower production of females than those in which *C. hyalinipennis* was not present (Fig. 4).

4. Discussion

Overall, the most successful species, in terms of both emergence and female production, was *P. nasuta*. *C. stephanoderis* was the species that emerged least often but *C. hyalinipennis* had the lowest production of female progeny.

Our results constitute evidence for both intra- and inter-specific resource competition: emergence and female production were often higher when multiple berries were provided. Inter-specific competition was sufficiently severe to make multiple-species emergence from single berries uncommon. Because berries generally contained sufficient hosts for more than one foundress to reproduce, the low frequency of multi-species emergence from single berries indicates that interference competition, rather than simply exploitative competition, occurred. In contrast, Infante et al. (2001a) found no evidence for interference competition between *C. stephanoderis* and *P. nasuta*. We consider that our results differ because we used single-berry replicates, which are likely to force an interaction between foundresses. Nonetheless, our results suggest that reproduction by *C. stephanoderis* and *P. nasuta* are equally influenced by intraspecific and inter-specific competition against each other and this accords with Infante et al.'s (2001a) conclusion that these two species may be able to coexist ecologically.

Another important difference between our study and that of Infante et al.'s (2001a) is that we included *C. hyalinipennis*. *C. hyalinipennis* may have had a negative influence: from among replicates from which there was some female emergence, those containing *C. hyalinipennis* generally had low production. Although competitive interactions are a candidate explanation (Batchelor et al., 2005; Pérez-Lachaud et al., 2002, 2004), because the overall production (given that there was some emergence) of *P. nasuta* and *C. stephanoderis* in interspecific replicates was uninfluenced by the presence of *C. hyalinipennis*, it seems more likely that low production was due to a lower reproductive rate of *C. hyalinipennis* than the other species. *C. hyalinipennis* may be less effective at reproducing within coffee berries than the other two species because its association with the CBB is relatively recent [the CBB was first recorded in Mexico in 1978 (Baker, 1984)]. Preliminary work investigating the time taken by each of the three bethylid species to enter an infested coffee berry in the laboratory indicates that *C. hyalinipennis* is slower than *P. nasuta* and *C. stephanoderis* (T.P.B unpublished data).

These results derive from a relatively 'holistic' (albeit laboratory) experiment, with an emphasis on overall

outcome rather than the processes by which outcomes are achieved and, taken in isolation, would imply that the most effective biological control agent would be *P. nasuta* since it emerged most often and had the highest production of female offspring. However, more 'reductionist' studies, that have examined aspects of the life-histories of the parasitoids and their interactions, have had different implications as to which of the three species would be the most effective biological control agent.

Population dynamic theory suggests that increasing the production of female offspring per host attacked will lead to greater host suppression (Heimpel, 2000; Murdoch and Briggs, 1996). All three species feed on some of the hosts they attack but *P. nasuta* and *C. stephanoderis* do so destructively while *C. hyalinipennis* frequently both feeds and oviposits on the same host individual (e.g., Lauzière et al., 2001b; Pérez-Lachaud and Hardy, 2001). Furthermore, *P. nasuta* and *C. stephanoderis* almost always lay only one egg per host, while *C. hyalinipennis* lays 1–3 eggs (mean = 1.62) on CBB (Pérez-Lachaud and Hardy, 2001). In all three species, the probability of an egg surviving to adulthood is about 0.6–0.77, under the environmental conditions used in our study, and progeny sex ratios are broadly similar (proportion male ∼0.11–0.25) (Barrera et al., 1993; Hardy and Mayhew, 1998; Infante, 2000; Infante et al., 1992; Pérez-Lachaud and Hardy, 2001). The reproductive biologies of these species, as estimated separately in 'reductionist' laboratory studies, therefore imply that *C. hyalinipennis* would be expected to produce the most emerging females per host and best suppress CBB populations. Additionally, due to having the greatest fundamental per capita rate of increase it might be expected to displace *C. stephanoderis* and *P. nasuta* from the agro-ecosystem, other factors being equal (e.g., Heimpel, 2000).

Additional 'reductionist' studies have investigated interspecific interactions between *C. hyalinipennis*, *C. stephanoderis*, and *P. nasuta*. Adult females of all three species have been observed to feed upon the immature stages of their allospecific bethylid competitors (Infante et al., 2001a; Pérez-Lachaud et al., 2004). *C. hyalinipennis* has further been shown to be a facultative hyperparasitoid of the immature stages of *P. nasuta* and *C. stephanoderis* in the laboratory (Pérez-Lachaud et al., 2004). The 'advantage of facultative hyperparasitism' again implies that *C. hyalinipennis* might eventually displace *C. stephanoderis* and *P. nasuta*. Intra-guild predation and hyperparasitism are generally thought to be detrimental to host population suppression in biological control programmes (e.g. Pérez-Lachaud et al., 2004; Rosenheim et al., 1995).

A further aspect of interspecific interactions is that adult females may also interact via direct behavioural, and often fatal, contests for host resources. Laboratory studies found *C. stephanoderis* to be the most, and *P. nasuta* the least, successful competitor and that interspecific competition is stronger than intraspecific competition because intraspecific interactions are non-fatal (Batchelor et al., 2005; Pérez-Lachaud et al., 2002). Taken in isolation, such results would imply that *C. stephanoderis* would displace the other species from the agro-ecosystem.

Reductionist studies have thus implied that, of the bethylids examined, either *C. stephanoderis* or *C. hyalinipennis* is likely to be the most successful biological control agent of the CBB and that *P. nasuta* is unlikely to be effective. In contrast, 'holistic' investigations have suggested that *P. nasuta* is likely to achieve greatest success (this study) or that *C. stephanoderis* and *P. nasuta* are able to coexist, with *C. stephanoderis* being more effective under the environmental conditions studied (Infante et al., 2001a). These contrasting conclusions all derive from laboratory studies, each attempting to evaluate the suitability of these species, alone or in combination, as biological control agents. Such predictions can be compared with the establishment of each species in coffee agro-ecosystems. In southern Mexico *C. hyalinipennis* is regionally indigenous and is present but rare in coffee plantations, (Pérez-Lachaud, 1998; Pérez-Lachaud and Hardy, 1999) while *C. stephanoderis* and *P. nasuta* have been released since 1989 in a classical biological control programme (Barrera et al., 1990b; Murphy and Moore, 1990). Observations suggest that *C. stephanoderis* persists in low numbers after release (Barrera, 1994; Barrera et al., 1990b; Damon, 1999; T.P.B pers. obs.) whereas *P. nasuta* has consistently failed to establish (Infante et al., 2001b, 2003).

The high success of *P. nasuta* in holistic laboratory studies suggests that its failure to establish in Mexico is not likely to be due to competitive interactions with *C. stephanoderis* and *C. hyalinipennis*. The population densities of the two *Cephalonomia* species in the agro-ecosystem are lower than in our study and also than in the holistic study of Infante et al. (2001a) (Damon, 2000; Damon and Valle, 2002) and thus contests and other forms of interference competition are less likely to occur in the field than in the laboratory. In contrast to observations in Mexico, but not to our laboratory results, *C. stephanoderis* and *P. nasuta* are reported to coexist in Togolese coffee plantations, where both species are indigenous (Moore and Prior, 1988; Vega et al., 1999). However, our finding that *P. nasuta* is more prevalent than *C. stephanoderis* is the opposite of field observations in Togo (Moore and Prior, 1988; Vega et al., 1999). Climatically, African and Mexican coffee growing regions are broadly similar, being classified as 'tropical continental' or 'rainforest,' and close to the equator with relatively invariant day length and sunlight. Within its native range, *P. nasuta* is more prevalent at higher altitude, particularly in East Africa (Barrera, 1994; Infante et al., 2001a). The failure of *P. nasuta* to

establish in Mexico may in part be due to predation by other arthropods (Infante et al., 2003), yet predation also affects *C. stephanoderis* (Henaut et al., 2001) and might be similarly expected to affect *C. hyalinipennis*, and remains unexplained.

The problems we must face are that different laboratory approaches to evaluating biological control agents and their interactions have generated different implications for best pest control practice and that there is no simple correspondence between laboratory results and (regionally inconsistent) field evidence. There are numerous reasons why such inconsistencies may arise. The parasitoids we have studied derive from medium to long-term laboratory cultures periodically augmented by field-caught individuals, and we consequently cannot be certain that differences in performance are due to genuine species differences rather than differences in culture size and genetic diversity. 'Reductionist' laboratory studies tend to focus on one or a few aspects of parasitoid life-history at a time and may overemphasise the importance of the aspects studied for the overall performance of the species or the strength of an interspecific interaction. Even 'holistic' laboratory studies are unlikely to take into account all of the aspects that influence the performance of a biological control agent in the field. For instance, the relative foraging abilities of *C. stephanoderis*, *C. hyalinipennis* or *P. nasuta* have not been assessed as hosts were always provided within small tubes or jars, and further species of parasitoids and predators were not present.

Despite the varied expectations concerning the suitability and coexistence of *C. stephanoderis* and *P. nasuta*, the weight of evidence suggests *C. hyalinipennis* will not be beneficial to CBB control, due to a low population growth rate and/or negative affects on other biological control agents (contests, hyperparasitism). We have previously warned against the introduction of *C. hyalinipennis* into regions in which it is not indigenous because of its possible non-target effects on both herbivores and parasitoids outside the coffee agro-ecosystem (Pérez-Lachaud et al., 2004). The results of the present study lead us to further recommend that *C. hyalinipennis* should not be encouraged, for example through mass rearing, within its native range as it now appears to be an ineffective, and possibly detrimental, invader of the Mexican coffee agro-ecosystem.

While it is widely accepted that screening is an important component of the evaluation of potential biological control agents to reduce non-target effects (e.g., Hopper, 2001; Waage, 2001) screening usually focuses on evaluations of host specificity. In the case of CBB biological control using bethylids, the agents are either effectively monophagous (*C. stephanoderis* and *P. nasuta*, Pérez-Lachaud and Hardy, 2001) or native (Pérez-Lachaud, 1998) and the focus of 'screening' we have carried out has been on exploring potentially disruptive inter-agent interactions. It seems increasingly clear that intra-guild predation and other disruptive inter-specific interactions

are generally common when more than one species are deployed in biological control programmes, yet at present there has been scant attention paid to this problem (Rosenheim et al., 1995). Interspecific interactions are likely to be complex and multifaceted and untangling them may require a combination of 'reductionist' and 'holistic' approaches. Even so, there may not be a simple correspondence between laboratory studies and the outcomes of actual biological control programmes and this remains a concern.

4.1. Conclusions and recommendations

Our study is an attempt to evaluate inter-specific competition, and coexistence, within the context of the biological control of a pest with multiple-natural enemies. In the laboratory, despite 'losing the battles,' in terms of offspring production per host, trophic interactions, and direct contests, *P. nasuta* appears to 'win the war' in terms of the most crucial attribute: recruitment of female offspring to the next generation. Yet, in the field it has failed to establish where *C. stephanoderis* has established (Mexico) or coexists with *C. stephanoderis* but at lower densities (Togo). Since *C. stephanoderis* also performs well in holistic laboratory studies and in dyadic contests, and is apparently more successful than *P. nasuta* in the field where they coexist, it seems that *C. stephanoderis* will often be the more effective natural enemy. Turning to *C. hyalinipennis*, this species was recently discovered to occur naturally in the Mexican coffee agro-ecosystem and initial evaluations of its biology suggested it might be a promising agent for biological control. Investigations of its interactions with *C. stephanoderis* and *P. nasuta* have, however, demonstrated the potential for intra-guild predation, hyperparasitism and fatal contests. Such interactions do not in themselves suggest that *C. hyalinipennis* should not be encouraged within its native range because negative interactions may be uncommon in the field and *C. hyalinipennis* may still be a more effective biological control agent than the other two bethylids. The present study has, however, indicated that *C. hyalinipennis* has a low rate of offspring production and that its presence may have a negative effect on recruitment of female offspring to *C. stephanoderis* populations. Current evidence thus suggests that *C. hyalinipennis* should not be encouraged as a member of the guild of natural enemies of the CBB in Mexico or in other regions.

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