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ORIGINAL ARTICLE

Delayed development of the whitefly (*Bemisia tabaci*) and increased parasitism by *Encarsia bimaculata* in response to sublethal doses of piperonyl butoxide

Ming-Hui Qian[1], Qiong-Bo Hu[2], Shun-Xiang Ren[2], Nasser S. Mandour[3], Bao-Li Qiu[2] and P. A. Stansly[4]

[1]*Department of Forest Protection, Guangdong Academy of Forestry,* [2]*College of Natural Resources and Environment, South China Agricultural University, Guangzhou, China,* [3]*Department of Plant Protection, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt and* [4]*University of Florida – IFAS, Immokalee, Florida, USA*

Abstract Effects of sublethal piperonyl butoxide (PB) on parasitization of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) by *Encarsia bimaculata* Heraty et Polaszek (Hymenoptera: Aphelinidae) were evaluated both in cage and greenhouse experiments. When first, second and third instar *B. tabaci* nymphs were treated with PB, all but the first instar were significantly prolonged. Data indicated that sublethal PB could improve *E. bimaculata* parasitism rates without influencing parasitoid eclosion rates. Prolonged development increased rates of parasitism by *E. bimaculata*, from 17.6% to 24.7% in cages, presumably by increasing the duration of host exposure. Sublethal PB combined with *E. bimaculata* as an integrated approach to control *B. tabaci* was evaluated using life table parameters under greenhouse conditions. Indices of population trend (I) calculated from life tables were estimated at 4.6 for *B. tabaci* exposed to PB and parasitoids compared to 14.1 with parasitoids alone and 23.5 in untreated controls. The results showed that after PB was sprayed and parasitoids introduced, development of *B. tabaci* was delayed and the peak of each stage was postponed. The older nymphal stage had highest mortality, primarily due to mortality caused by parasitism by *E. bimaculata*.

Key words *Bemisia tabaci*, *Encarsia bimaculata*, parasitization, sublethal piperonyl butoxide

Introduction

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a polyphagous pest, occurring widely in both tropical and subtropical regions where it has become a limiting factor for the production of many vegetables, field crops and ornamentals (Oliveira *et al*., 2001). *B. tabaci* is attacked by insect parasitoids in the genera *Encarsia*, *Eretmocerus* (Aphelinidae) and *Amitus* (Platygastridae) (Gerling *et al*., 2001). Aphelinid parasitoids from *Encarsia* and *Eretmocerus* genera, lady beetles and lacewings in Coleoptera and Neuroptera were found to be the dominant arthropod predators of *B. tabaci* in China (Li *et al*., 2011). Among these, *Encarsia bimaculata* Heraty et Polaszek is found in Asia and Australasia as well as Israel, Mexico and the USA (Heraty & Polaszek, 2000; Schmidt *et al.*, 2001; Antony *et al*., 2004; Qian *et al.*, 2007) and is one of two most abundant parasitoids attacking *B. tabaci* in south China (Qiu *et al.*, 2004b). Field studies reported average levels of parasitism by *E. bimaculata* of 15%–22%, peaking at 87.3% on vegetables and

Correspondence: Ming-Hui Qian, Department of Forest Protection, Guangdong Academy of Forestry, Guangzhou 510520, China. Tel: +86 20 87035648; fax: +86 20 87031245; email: Susanqian842@hotmail.com

Shun-Xiang Ren, College of Natural Resources and Environment, South China Agricultural University, Guangzhou, China. email: rensxcn@yahoo.com.cn

ornamentals in southern China in 2002–2004 (Qiu *et al.*, 2004b).

Piperonyl butoxide (PB) is a synergist used with a wide variety of insecticides and was developed in 1947 using naturally occurring safrole as the key raw ingredient. It is used in conjunction with insecticides such as pyrethrins, pyrethroids, rotenone and carbamates (Tozzi, 1998). In field trials in Israel, sublethal PB alone resulted in a shift in age structure of *B. tabaci* toward a greater proportion of young instars compared to other treatments, facilitating a greater level of parasitism in PB-treated plots (Devine *et al.*, 1998b). Low rates of PB that killed a portion of exposed whitefly nymphs still allowed immature parasitoids to continue to develop successfully in surviving hosts. The hypothesis that slowing host growth rate might lead to increased parasitism of *B. tabaci* by *Eretmocerus mundus* Mercet was tested on large host populations in controlled temperature cabinets over two generations (Devine *et al.*, 2000). Highly significant increases in parasitism (7%–8%, $P < 0.000\,1$) were observed on host populations of comparable size. Devine's work provided unique evidence that host growth rates could be artificially manipulated to confer an advantage to parasitoids.

With these views in mind, the present work focused on effects of sublethal exposure of PB to *B. tabaci* on parasitization by *Encarsia bimaculata* both in cages and greenhouses. Moreover, a life table was constructed to quantify the impact of mortality caused by *E. bimaculata* to *B. tabaci* in cages and greenhouses.

Materials and methods

Plants, whiteflies and parasitoids

Hibiscus rosa-sinensis L. (Malvaceae) was used as the host plant for *B. tabaci* in this study. Hibiscus cuttings were planted in 18 cm diameter plastic pots maintained at 25°C. Plants were regularly watered, and provided with fertilizers and supplementary lighting. Plants were kept free of whitefly in a small greenhouse (4 × 3 × 3 m) until they produced 5–6 leaves, when they were used for experiments.

The *B. tabaci* population used in this study was originally collected from hibiscus plants grown in the horticultural unit of South China Agricultural University (SCAU). Whiteflies (*B. tabaci*) and parasitoids (*E. bimaculata*) were reared following the methods of Qiu *et al.* (2007a, b) and Mandour *et al.* (2006, 2007a, b). Colonies were maintained on hibiscus plants housed in rearing cages (60 × 60 × 60 cm) in the laboratory for at least 10 generations before used in experiments.

The parasitoid *E. bimaculata* was originally collected from hibiscus plants in 2000 in Guangzhou, China and identified by Professor Jian Huang (Fujian Agricultural & Forest University, China). Voucher specimens were deposited in the Department of Entomology, SCAU, China. All cage experiments were conducted under laboratory conditions of 25 ± 2°C, 60% ± 10% RH. and 14 : 10 h L : D.

Chemical source

PB was obtained from Guangzhou Pesticide Company, Guangdong, China.

Selecting sublethal concentrations of PB

Twenty-four hibiscus plants were placed, four each, into six cages (60×60×60 cm) after cleaning each leaf with a soft brush. Approximately 50 pairs of whiteflies per leaf were introduced into the cages, and removed after an oviposition period of 4 h. All whiteflies were counted daily from the egg stage to adult emergence to estimate mortality. Nymphs were considered dead when they lost their normal yellow-green color, turgidity and smooth cuticle structure. Mortalities 24 h after treatments were used for analysis of lethal concentrations of PB (LC-P).

Because PB concentrations higher than 1 600 mg/L are inapplicable for field use, 100, 200, 400, 800 and 1 600 mg/L PB were chosen for selecting sublethal concentrations of PB. Infested leaves from potted plants were treated with one of five PB concentrations or a distilled water control when the whitefly nymphs reached first, second and third instar stages, respectively (Finney, 1971; Tang & Huang, 1982; Sithiprasasna *et al.*, 1996; Devine *et al.*, 1998b, 2000). One leaf per sample, each containing at least 200 whitefly nymphs, was immersed in different PB solutions or a water control for 5 s and allowed to dry at room temperature. Fourth instar nymphs and "pupae" were not treated with PB because they would not be suitable for *E. bimaculata* to parasitize 2 days after treatment (Qiu *et al.*, 2007b; Devine *et al.*, 2000). Each treatment was replicated four times.

Effects of sublethal PB on developmental periods and mortality of B. tabaci

Results from the above experiment were used to select two sublethal PB concentrations approaching the LC_{25} for each instar (Devine *et al.*, 1998b, 2000; Xu *et al.*, 1998; Mo *et al.*, 2002; Li *et al.*, 2003) to be sprayed on first, second and third instar whitefly nymphs (Table 1).

Table 1 Selected PB sublethal concentrations on different instars of *B. tabaci* in cages as determined by means of DPS software.

Treatments	LC-P equations	R^2	LC_{25} (mg/L)	C1 (mg/L)	C2 (mg/L)
1st instar	$y = 0.3658x + 0.6866$	0.9309	186.43	100	200
2nd instar	$y = 0.3423x + 0.9446$	0.9607	267.15	200	400
3rd instar	$y = 0.6068x + 0.5960$	0.9857	1672.80	800	1600

R^2: coefficient of determination; R: coefficient correlation; C1: sublethal concentration lower approach LC_{25}; C2: sublethal concentration higher approach LC_{25}.

Fifty whitefly eggs were counted on selected sample leaves and excess eggs removed with the aid of a fine paintbrush. A magnifying lens (17×) was used to count numbers of eggs, each nymphal instar and pupal stages daily until the emergence of adult whiteflies. Settled first instar nymphs were ringed and numbered using a permanent fine color pen to facilitate later identification of individuals. Each treatment was replicated four times.

Effects of sublethal PB on host-using rates by E. bimaculata

Hibiscus plants were pruned to five intact leaves per plant and placed four to a cage (60 × 60 × 60 cm) as a treatment. Adult *B. tabaci* were introduced in the middle of each cage at the rate of 50 adults per plant and allowed to oviposit for 2 days. Plants were shaken every 6 h to redistribute whiteflies and provide a uniform distribution of eggs among all caged plants. Two days later, adult whiteflies were removed with the aid of an aspirator and one leaf per plant was selected randomly and tagged as a sample leaf. The number of eggs on sample leaves was counted to total of 250 eggs per cage and excess eggs were removed. Each treatment was replicated four times with a total of 16 plants.

Following preliminary results and earlier studies (Cheng *et al*., 1989; Mandour *et al*., 2006, 2007a; Qiu *et al*., 2007a), plants were sprayed with 200 mg/L PB or distilled water as control midway through the second nymphal instar. Two days later, newly emerged adult *E. bimaculata* were released into the cages at two rates per host plant: D1 (10 females and 2 males) and D2 (5 females and 1 male) (Devine *et al.*, 2000).

The numbers of eggs, each nymphal instar and pupal stages were counted until the emergence of adult whiteflies and/or parasitoids every 3 days. First instar nymphs were ringed and numbered after settling and classified at each observation by instar and as dead, alive, disappeared or parasitized. (Hoddle *et al*., 1997; Hoddle & van Driesche, 1999; Selvakumaran *et al*., 2000; Mandour *et al.*, 2006, 2007a).

Greenhouse experiments

The experiments were carried out in six greenhouses (5 × 2 × 3 m) covered with nylon net on the sides and glass on top located in the Farming Practice Teaching Facility at SCAU. There were three treatments: PB (piperonyl butoxide + parasitoids), CKP (check with parasitoids) and CK (check without parasitoids). All treatments were replicated twice. CK greenhouses were 2 m away from greenhouses with parasitoids. Experiments were conducted during October to December and lasted 8 weeks. The temperature was 27.7 ± 6.1°C (20.5–35.2°C) in greenhouses.

Procedures were similar to those followed in cages. Hibiscus plants were lined up in each greenhouse in four rows of eight plants and given numbers from 1 to 32. When they produced 5–6 leaves, 50 adults of *B. tabaci* for each plant were collected from the stock colony using an aspirator and introduced into the greenhouse with a total of 1 600 whitefly adults per greenhouse. Plants were gently shaken after every 6 h to redistribute whiteflies over the plants. Ten hibiscus plants were chosen randomly as the sample plants and one leaf from each sample plant was selected randomly as a sample leaf. Selected leaves were tagged and labeled.

PB plants were sprayed with sublethal PB or distilled water as control midway through the second instar of *B. tabaci*. Adult parasitoids were introduced between the plants at the rate of 10 females + 2 males/plant 2 days after spraying. Adult *E. bimaculata* were collected from the stock colony, sexed and confined in glass tubes (10 × 2 cm) that were then distributed between the rows inside the greenhouse. Numbers of live, dead, missing and parasitized nymphs in all whitefly instars and stages were recorded at 3-day intervals using a 17× magnifying lens until emergence of all adult whiteflies and/or parasitoids (Mandour *et al.*, 2007b).

Life table for cage experiments

The column headings used by Southwood (1978) and Selvakumaran *et al*. (2000) were adopted in constructing

and arranging a life table for cage experiments with the data as follows: x = Age interval, l_x = Actual number entering stage during observation, fd_x = Mortality factor, d_x = Actual number dying in course of development, and $100qx/l_x$ = Percentage mortality, where the difference in l_x between egg stage and first instar represents d_x for the egg cohort, differences in l_x between first and second instar, second and third instar, third and fourth instar, fourth instar and pupal stage and pupal stage and successful emerged adults represent d_x for the first, second, third, fourth instar and "pupal" cohorts, respectively.

Rate of egg to adult survival per cohort was calculated as:

$$\text{Egg} - \text{adult survival}\,(\%) = \frac{\text{Total number of emerged whitefly adults}}{\text{The initial number of eggs}} \times 100 \quad (1)$$

Life table for greenhouse experiments

To assess the effect of different treatments on whitefly population increase, the population trend (I) of *B. tabaci* was calculated as described by Pang *et al.* (1992, 1995) as:

$$I = SE \times SL1 \times SL2 \times SL3 \times SL4 \times SP \times F \times P♀ \times PF, \quad (2)$$

where SE = the survival of the egg stage, SL1–SL4 = the survival of 1st–4th instars, SP = the survival of the pupal stage, F = the standard fecundity of whitely female, P♀ = the realized fecundity, and PF = the proportion of the females in the generation. Values for F, P♀ and PF were obtained from Qiu *et al.* (2004a), Huang *et al.* (2006) and Mandour *et al.* (2006). To compare the contribution of each treatment to control of *B. tabaci* in the presence of parasitoids, the Interference Index of Population Control (IIPC) proposed by Pang *et al.* (1992, 1995) was calculated as follows:

$$IIPC = I_t/I_{ck}, \quad (3)$$

where I_t is the population trend of *B. tabaci* for a given treatment and I_{ck} is the population trend of *B. tabaci* in the control treatment.

Data tabulation and statistical analysis

Host-use rate calculated as the parasitoid number reared from 100 host insects (Qiao *et al*., 2004), considered as the percentage of parasitism for *E. bimaculata* on *B. tabaci* in this study, was calculated using the following formula:

$$P\% = 100 \times P_L/(W_N+P_L), \quad (4)$$

where P% is percentage of parasitism, P_L is the number of parasitized *B. tabaci* nymphs and W_N is the number of unparasitized nymphs.

The LC-P equations and LC_{25} values for *B. tabaci* exposed to different rates of PB were evaluated by DPS software (Tang & Feng, 2002). Analysis of variance (ANOVA) was used to compare the rates of parasitism, instar duration and mortality among the conducted treatments (SAS Institute Inc., Cary, NC, USA). When *F*-values were significant, DMRT was used to separate the means (Qiu *et al.*, 2007a, b; Mandour *et al.*, 2006, 2007a,b).

Results

Selecting the sublethal concentrations of PB in cage experiments

Results of the analysis of the response of the first three instar nymphs exposed to five concentrations of PB (100, 200, 400, 800 and 1600 mg/L) is given in Table 1. The high and low confidence limits to the estimated LC_{25} given in Table 1 were selected as sublethal concentrations for each instar.

Effects of sublethal PB on developmental periods and mortality of B. tabaci

Development of first instars was not prolonged by applications of 100 and 200 mg/L rates of PB sprayed on first instar nymphs, but development of the second instar was significantly delayed by applications of 100 and 200 mg/L PB on first instars (Table 2). The greatest effect was seen on the third instars in response to 200 and 400 mg/L PB (2s200 and 2s400) sprayed on the second nymphal instar. These treatments delayed third instar development from 2.69 ± 0.43 days to 4.31 ± 0.41 and 4.69 ± 0.42 days, respectively. A significant increase in the overall development time from egg to adult existed. Greatest increases in mortality, from 47% in the control to greater than 75%, were seen from the 200 mg/L rate sprayed on first instars and the 400 mg/L rate sprayed on adults (Table 2). Significant differences existed among PB treatment and control in term of rates of mortality.

Effects of sublethal PB on host-using rates and eclosion rates of E. bimaculata

Host use rate as determined by percentage parasitism of *B. tabaci* nymphs treated with 200 mg/L PB midway

Table 2 Effects of sublethal PB on developmental periods of *B. tabaci* in cages (days ± SE).

Treatment†	Egg	1st instar	2nd instar	3rd instar	4th instar	"Pupae"	Egg–adult	Mortality
Check	7.98 ± 0.31 a	3.91 ± 0.29 a	3.02 ± 0.60 bc	2.69 ± 0.43 e	2.56 ± 0.17 b	2.80 ± 0.24 b	22.95 ± 0.73 c	47.44 ± 3.56 c
1s100	7.96 ± 0.36 a	3.96 ± 0.34 a	3.90 ± 0.14 a	2.94 ± 0.50 de	2.83 ± 0.36 b	2.89 ± 0.20 b	24.47 ± 1.44 bc	64.94 ± 3.59 b
1s200	7.89 ± 0.56 a	4.12 ± 0.32 a	4.15 ± 0.61 a	3.19 ± 0.41 cde	3.08 ± 0.21 b	2.91 ± 0.24 b	25.34 ± 0.64 ab	76.16 ± 2.59 a
2s200	7.93 ± 0.28 a	3.94 ± 0.25 a	3.64 ± 0.33 abc	4.31 ± 0.41 ab	3.86 ± 0.43 ab	2.93 ± 0.49 ab	26.60 ± 0.47 a	58.66 ± 3.86 bc
2s400	7.99 ± 0.53 a	3.87 ± 0.23 a	3.78 ± 0.18 ab	4.69 ± 0.42 a	4.08 ± 0.96 a	2.99 ± 0.39 ab	27.38 ± 0.42 a	78.53 ± 3.61 a
3s800	7.92 ± 0.49 a	3.96 ± 0.40 a	3.06 ± 0.42 bc	3.56 ± 0.37 bcde	4.39 ± 0.37 a	3.30 ± 0.41 ab	26.17 ± 0.76 ab	55.71 ± 4.04 bc
3s1600	7.82 ± 0.48 a	3.93 ± 0.35 a	2.93 ± 0.31 c	3.94 ± 0.36 abc	4.61 ± 0.64 a	3.55 ± 0.29 a	26.78 ± 1.46 a	62.45 ± 2.44 b

†Instar of whitefly when treated with different concentrations (mg/L), e.g., 1s100 means 1st nymphal instar sprayed with 100 mg/L PB.
Means followed by the same letter are not significantly difference ($P < 0.05$) by DMRT.

Table 3 Percentage parasitism and parasitoid emergence from PB-treated and untreated *B. tabaci* nymphs following releases of 10 or 5 female *E. bimaculata* per plant in cages.

Treatment	10 *E. bimaculata*/plant		5 *E. bimaculata*/plant	
	Host-use (%)	Adult eclosion	Host-use (%)	Adult eclosion
Untreated	17.6 ± 2.3	74.3 ± 10.3	9.5 ± 1.5	73.9 ± 11.8
200 mg/L PB	23.9 ± 3.3*	78.2 ± 11.6	11.3 ± 2.1	75.7 ± 14.5

Means followed by * indicate significant difference ($P < 0.05$, *t*-test).

through the second nymphal instar was significantly greater than for untreated nymphs when the release rate was 10 female *E. bimaculata* per plant (Table 3). A similar trend was seen with the lower (5 parasitoids/plant) release rate although the difference was not statistically significant at $P < 0.05$. Emergence rates were not affected by the PB treatment at either release rate. Furthermore, the 200 mg/L PB significantly increased *E. bimaculata* parasitism rates with no effect on parasitoid adult eclosion rates.

Life tables for B. tabaci *after sublethal PB combined with* E. bimaculata

Data for each treatment were pooled to construct the life table (Table 4). Rates of mortality due to unknown cause (including predation-like mortality by wasp host feeding) across all immature cohorts were highest in the third instar and first instar cohorts and lowest in egg and pupal cohorts. Also, the loss rates in cohorts of all instars/stages due to disappearance were very low and did not exceed 1%. Mortality rates in fourth instar cohorts were consistently higher in all treatments compared to younger stages due to parasitism. The highest mortality rates in the fourth instar cohorts due to parasitization by *E. bimaculata* were recorded in PBD1 treatment (45.52%); while the lowest rates were recorded in the CKD2 treatment (15.91%, Table 4).

Effects of sublethal PB combined with E. bimaculata *on developmental periods of* B. tabaci *in greenhouse experiments*

Developmental periods of second, third, fourth instar *B. tabaci* nymphs and the overall development from egg to adult were significantly prolonged in response to 400 mg/L PB applied to second instars combined with *E. bimaculata* (Table 5). Also, development of third instar nymphs of whiteflies exposed to *E. bimaculata* alone was significantly longer than unexposed B. *tabaci* nymphs.

Index of population trends following sublethal PB combined with E. bimaculata

To simplify the results within the table, data of first and second nymphs were put together as "younger nymphs", while those of third and fourth nymphs were put together as "older nymphs" (Qiu *et al.*, 2004a) (Table 6). Results indicated that lowest survivorship was seen in older nymphs. The primary cause for death was parasitism by *E. bimaculata*. The Indexes of Population Trend (I) of *B. tabaci* were 4.60 in PB spots, while 14.12 in parasitoid control spots and 23.54 in no parasitoid control spots. Furthermore, the 400 mg/L PB had no effect on parasitoid adult eclosion rates, being 73.68% ± 9.74% in CK, 71.43% ± 10.07% in CKP and 75.74% ± 15.41% in PB.

Table 4 Life table parameter for *B. tabaci* after treatement with sublethal PB (200 mg/L) or distilled water (CK) combined with exposure to *E. bimaculata* at a rate of 10 females (D1) or 5 females (D2) per plant in cages.

Stage (x)	No. entering stage (l_x)				Mortality factor (fd_x)	No. dead by factor (d_x)				Mortality percent (100 qx/l_x)			
	CKD1	CKD2	PBD1	PBD2		CKD1	CKD2	PBD1	PBD2	CKD1	CKD2	PBD1	PBD2
Egg	1000	1000	1000	1000	Unknown	75	81	79	84	7.5	8.10	7.90	8.40
1st instar	925	919	921	916	Unknown	132	125	143	129	14.27	13.60	15.53	14.08
					Disappearance	4	8	7	6	0.43	0.87	0.76	0.66
2nd instar	789	786	771	781	Unknown	95	87	112	103	12.04	11.07	14.53	13.19
					Disappearance	3	5	2	1	0.38	0.64	0.26	0.13
3rd instar	691	694	657	677	Unknown	106	93	129	118	15.34	13.40	19.63	17.43
					Disappearance	3	4	3	2	0.43	0.58	0.46	0.30
4th instar	582	597	525	557	Unknown	68	65	85	79	11.68	10.89	16.19	14.18
					Disappearance	5	4	1	2	0.86	0.67	0.19	0.36
					Parasitized	176	95	239	113	30.24	15.91	45.52	20.29
Pupa	333	433	200	363	Unknown	24	32	21	29	7.21	7.39	10.5	7.99
					Disappearance	1	2	2	1	0.3	0.46	1	0.28
Adult	308	399	177	333									

D1 (10 females) and D2 (5 females) *E. bimaculata* for each host plant.

Table 5 Effects of sublethal PB with *E. bimaculata* (PB) compared to an untreated check (CK) and a check plus parasitoid (CKP) on developmental periods (days ± SE) of *B. tabaci* in greenhouses.

	Egg	1st instar	2nd instar	3rd instar	4th instar	Pupal	Egg–adult
CK	6.55 ± 0.34 a	3.05 ± 0.13 a	2.43 ± 0.22 b	2.22 ± 0.18 c	2.28 ± 0.17 b	2.35 ± 0.21 a	18.85 ± 0.37 b
CKP	6.50 ± 0.22 a	2.93 ± 0.17 a	2.40 ± 0.18 b	3.10 ± 0.34 b	2.83 ± 0.15 b	2.43 ± 0.17 a	20.18 ± 0.66 b
PB	6.45 ± 0.21 a	2.95 ± 0.21 a	2.95 ± 0.26 a	4.35 ± 0.21 a	3.60 ± 0.26 a	2.53 ± 0.17 a	22.83 ± 0.19 a

Means followed by different letters indicate the significant difference ($P < 0.05$) by DMRT.

Table 6 Life table parameters for *B. tabaci* after sublethal PB cooperating with *E. bimaculata* in greenhouses.

Developmental stages	Mortality factor	Egg–adult survivorship		
		CK	CKP	PB + P
Egg	Unhatched	0.9624	0.9575	0.9607
	Unknown	0.9408	0.9456	0.9633
Younger nymph	Unknown	0.6991	0.6901	0.6909
Older nymph	Unknown	0.7069	0.6819	0.5222
	Parasitized	0.9872	0.6219	0.2583
Adult	Rate of female to male	0.5431	0.5431	0.5431
	Standard eggs/female	400	400	400
	Rate of standard eggs	0.2453	0.2453	0.2453
I		23.54	14.12	4.60
IIPC		1	0.5999	0.1952

CK, control without parasitoid; CKP, control with parasitoids; PB + P, PB treatment plus parasitoid; I, Index of population trend; IIPC, Interference index of population control.

Discussion

There are few published reports on PB used as an insect growth regulator (IGR) to increase development time. Devine *et al.* (1998b) found sublethal PB alone resulted in a shift in age structure of *B. tabaci* toward a greater proportion of young instars compared to other treatments in field trials in Israel. Devine and Denholm (1998a) suggested PB potential use as IGR for the management of the *B. tabaci* in cotton. Devine *et al.* (1999) tested the response of pyriproxyfen-resistant and susceptible *B. tabaci* to IGRs pyriproxyfen and fenoxycarb alone and in combination with PB. They showed that PB was antagonistic to pyriproxyfen, but had no effect on the toxicity of fenoxycarb. Devine *et al.* (2000) proved that low rates of PB could slow host growth rate, lead to increased parasitism of *B. tabaci* by *Eretmocerus mundus* Mercet in controlled temperature cabinets.

It was hypothesized that parasitoids released on hosts whose development had been prolonged by an earlier application of sublethal PB would have more time to find and parasitize whitefly nymphs at the appropriate stage (Devine *et al.*, 1998b). Thus, whitefly populations exposed to PB should exhibit greater percentage parasitism than unexposed populations. This hypothesis was supported and extended by the results of this study. The development of *B. tabaci* was indeed delayed by both the 200 and 400 mg/L rates of PB in the third instar, the preferred host stage for *E. bimaculata*. Furthermore, the 200 mg/L rate, which caused no significant whitefly mortality, significantly increased *E. bimaculata* parasitism rates with no effect on parasitoid eclosion rates. The results were consistent with that reported by Devine *et al.* (1998b, 2000) for the parasitoid *Eretmocerus mundus* and demonstrate that the principal can be applied to different parasitoid species, and host plants and under different environmental conditions.

Since 400 mg/L PB delayed third instar development (4.69 ± 0.42 days) as compared to that of 200 mg/L (4.31 ± 0.41 days) sprayed on the second nymphal instar, 400 mg/L rate of PB was used in greenhouse experiments. Due to the high mortality at 400 mg/L, the 200 mg/L rate applied to second instars was used for the cage experiment to take advantage of prolonging developmental time while minimizing whitefly mortality.

High release rates of the parasitoid *Encarsia bimaculata* (10 females/plant) is usually accompanied with high parasitism rates with the subsequent high mortality rates due to unknown causes (host feeding by parasitoid). This trend of increased mortality factor due to known causes was observed in cage and greenhouse experiments.

Life table analysis showed for the first time that the principal mortality factor impacting older nymphs under these conditions was successful parasitism by *E. bimaculata.* Furthermore, the greatest loss due to unknown causes, primarily due to host feeding by the parasitoid (Arno *et al*., 2010), was recorded in the younger instar cohort. The application of PB prolonged the development of *B. tabaci* nymphal instars that in turn increased their exposure to searching parasitoids, thereby increasing mortality. These findings, both in cages and greenhouses, are in concert with those reported for *B. tabaci* in the presence of *E. eremicus* (Hoddle & van Driesche, 1999; Driesch, 1999), *Eretmocerus.* sp. nr. *furuhashii* (Mandour *et al.*, 2006) and *E. bimaculata* (Mandour *et al*., 2007a; Qiu *et al.*, 2007a,b). Hoddle and van Driesche (1999) attributed the successful control of *B. tabaci* on poinsettia to the higher mortality rates caused by host feeding of searching wasps rather than higher parasitism rates.

Indices of population trend (I) calculated from greenhouse life tables were estimated at 4.6 for *B. tabaci* exposed to PB plus parasitoids compared to 14.1 for parasitoids alone and 23.5 in untreated controls. These results quantified for the first time the extent to which PB spray followed by parasitoid introduction can significantly decrease whitefly population growth compared to a control. Nevertheless, an I value greater than 1 indicates that sublethal PB combined with *E. bimaculata* alone cannot solve the whitefly problem. Other means such as predator release and application of entomopathogenic fungi will be necessary to reduce the I value to less than 1 in biologically managed systems.

This research confirms earlier results of Devine *et al.* (1998b) and extends them to the greenhouse environment using a different parasitoid. Our results also showed that sublethal PB did not significantly reduce parasitoid adult emergence rates, indicating that the treatment was innocuous to *E. bimaculata*. We chose a widely used ornamental *Hibiscus rosa-sinensis* L. typically propagated in greenhouses as a model for other greenhouse-grown ornamentals and vegetables subject to attack by *B. tabaci*. We showed that use of sublethal PB on young whitefly nymphs would not only minimize risk of collateral damage to the parasitoid population but also increase parasitism rates, thereby enhancing its contribution to whitefly management. Sublethal PB could thus increase the benefit derived from parasitoids while also reducing environmental risk and selection for insecticide resistance by decreasing the number of insecticide treatments needed for whitefly control.

This study is the first report from China showing sublethal PB functioning as an IGR. Our results suggest that

sublethal PB could function as an IGR by prolonging the development times of *B. tabaci*, which in and of itself would decrease pest population growth rates. Furthermore, the combination of sublethal PB and *E. bimaculata* provides the potential system of enhanced mortality either through host feeding or parasitism by parasitoids such as *E. bimaculata*, which could further limit whitefly population growth and serve as a useful tool in integrated management systems.

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References

Antony, B., Palaniswami, M.S., Kirk, A.A. and Henneberry, J.T. (2004) Development of *Encarsia bimaculata* (Heraty and Polaszek) (Hymenoptera: Aphelinidae) in *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) nymphs. *Biological Control*, 30, 546–555.

Arno, J., Gabarra, R., Liu, T.-X., Simmons, A.M. and Gerling, D. (2010) Natural enemies of *Bemisia tabaci*: Predators and parasitoids. *Bemisia: Bionomics and Management of a Global Pest* (eds. P.A. Stansly & S.E. Naranjo), pp. 385–421. Springer Dordrecht-Heidelberg-London-New York.

Devine, G.J. and Denholm, I. (1998a) Potential of piperonyl butoxide for the management of the cotton whitefly, *Bemisia tabaci*. *Piperomyl Butoxide: The Insecticide Synergist* (ed. D.G. Jones), pp. 227–237. Academic San Diego. CA.

Devine, G.J., Ishaaya, I. and Horowitz, A.R. (1998b) Effects of piperonyl butoxide on *Bemisia tabaci:* mortality, development, parasitism and predation in Israeli cotton fields. *Crop Protection*, 17, 717–726.

Devine, G.J., Ishaaya, I., Horowitz, A.R. and Denholm, I. (1999) The response of pyriproxyfen-resistant and susceptible *Bemisia tabaci* Genn (Homoptera: Aleyrodidae) to pyriproxyfen and fenoxycarb alone and in combination with piperonyl butoxide. *Pesticide Science*, 55, 405–411.

Devine, G.J., Wright, D.J. and Denholm, I. (2000) A parasitic wasp *(Eretmocerus mundus* Mercet) can exploit chemically induced delays in the development rates of its whitefly host (*Bemisia tabaci* Genn.). *Biological Control*, 19, 64–75.

Cheng, H.K., Tian, Y.Q., Wei, B.C., Zhao, J.H. and Ye, C.Q. (1989) Technique for commercial-scale production of *Encarsia Formosa*. *Chinese Journal of Biological Control*, 5, 178–181.

Finney, M. (1971) *Probit Analysis*, 3rd edn. Cambridge University Press, Cambridge, UK.

Gerling, D., Alomar, S. and Arno, J. (2001) Biological control of *Bemisia tabaci* using predators and parasitoids. *Crop Protection*, 20, 779–799.

Heraty, J.M, and Polaszek, A. (2000) Morphometric analysis and descriptions of selected species in the *Encarsia strenua* group (Hymenoptera: Aphelinidae). *Journal of Hymenoptera Research*, 9, 142–169.

Hoddle, M.S. and van Driesche, R.G. (1999) Evaluation of inundative releases of *Eretmocerus eremicus* and *Encarsia formosa* Beltsville strain in commercial greenhouses for control of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on poinsettia stock plants. *Journal of Economic Entomology*, 92, 811–824.

Hoddle, M.S., van Driesche, R.G. and Sanderson, J.P. (1997) Biological control of *Bemisia argenitifolii* (Homoptera: Aleyrodidae) on poinsettia with inundative releases of *Encarsia formosa* Beltsville strain (Hymenoptera: Aphelinidae): can parasitoid reproduction augment inundative release? *Environmental Entomology*, 90, 910–924.

Huang, Z., Ren, S.X. and Wu, J.H. (2006) Combined effects of *Paecilomyces fumosoroseus* (Deuteromycotina: hyphomycetes) and imidacloprid for managing *Bemisia tabaci* (Homoptera: Aleyrodidae). *Acta Ecologica Sinica*, 26, 3250–3257.

Li, M., Sun, Z.W., Wang, J.X. and Mu, L.Y. (2003) Effects of sublethal dosages of eleven insecitcides on larval cocooning and adult emergence of *Chrysopa sinica* (Jieder). *Natural Enemies of Insects*, 25, 20–23.

Li, S.J., Xue, X., Ahmed, M.Z., Ren, S.X., Du, Y.Z., Wu, J.H., Cuthbertson, A.G.S. and Qiu, B. L. (2011) Host plants and natural enemies of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in China. *Insect Science*, 18, 101–120.

Mandour, N.S., Ren, S.X., Qiu, B.L. and Olleka, A. (2006) Effects of different kairomonal sources on the performance of *Eretmocerus* sp. near furuhashii (Hymenoptera: Aphelinidae) against *Bemisia tabaci* on cucumber: I – In cages. *Agricultural Research Journal, Suez Canal University*, 6, 207–211.

Mandour, N.S., Ren, S.X. and Qiu, B.L. (2007a) Effect of *Bemisia tabaci* honeydew and its carbohydrates on search time and parasitization of *Encarsia bimaculata*. *Journal of Applied Entomology*, 131, 645–651.

Mandour, N.S., Ren, S.X., Qiu, B.L. and Wäckers, F.L. (2007b) Effects of different kairomonal sources on the performance of *Eretmocerus* sp. near *furuhashii* (Hymenoptera: Aphelinidae) against *Bemisia tabaci* on cucumber: II – In greenhouse. *Journal of Plant Protection Research*, 47, 289–298.

Mo, J.C., Yang, T.C., Cheng, J.A. and Song, X.G. (2002) Lethal and sublethal effects of acetamiprid on the larvae of *Culex Pipiens* Pallens. *Entomologia Sinica*, 9, 45–49.

Oliveira, M.R.V., Henneberry, T.J. and Anderson, P. (2001) History, current status, and collaborative research projects for *Bemisia tabaci*. *Crop Protection*, 20, 709–723.

Pang, X.F., Liang, G.W., Zeng, L. and Wu, W.J. (1992) Population system and population system approach to rice brown planthopper. *Transactions of the Ecological Society for Chinese Youths*, 2, 1–9.

Pang, X.F. and Liang, G.W. (1995) *Pest Population System Control*. Guangdong Science and Technology Press, Guangzhou.

Qian, M.H., Ren, S.X. and Qiu, B.L. (2007) The reproductive model and development of *Encarsia bimaculata* (Hymenoptera: Aphelinidae) in *Bemisia tabaci* (Homoptera: Aleyrodidae). *Chinese Bulletin of Entomology*, 44, 397–401.

Qiao, X.R., Han, Y.S., Xu, D.H., Zhang, Y.L., Pen, J.Y. and Tian, Q.K. (2004) Study on the artificial propagation and use of Chouioia cunea Yang. *Hebei Forestry Science and Technology*, 3, 1–7.

Qiu, B.L., Ren, S.X. and Xiao, Y. (2003) Distribution and control of B biotype of *Bemisia tabaci* (Homoptera: Aleyrodidae) in China. *Entomological Journal of East China*, 12, 27–31.

Qiu, B.L., Ren, S.X., Lin, L. and Wang, X.M. (2004a) Effects of release density of *Eretmocerus* sp. (Hymenoptera: Aphelinidae) on the control effectiveness of *Bemisia tabaci* (Homoptera: Aleyrodidae). *Entomological Journal of East China*, 13, 27–30.

Qiu, B.L., Ren, S.X., Lin, L. and Wang, X.M. (2004b) Species and dynamics of aphelinid parasitoids of *Bemisia tabaci* in Guangdong area. *Entomological Knowledge*, 41, 18–22. (in Chinese)

Qiu, B.L., Ren, S.X., Xu, C.X. and Musa, P.D. (2007a) Developmental delay and mortality of *Bemisia tabaci* (Hemiptera: Aleyrodidae) parasitized by two aphelinids, *Encarsia bimaculata* and *Eretmocerus* sp. nr. *furuhashii* (Hymenoptera: Aphelinidae). *The Pan-Pacific Entomologist*, 83, 235–243.

Qiu, B.L., De Barro, P.J., He, Y.R. and Ren, S.X. (2007b) Suitability of *Bemisia tabaci* (Hemiptera: Aleyrodidae) instars for the parasitization by *Encarsia bimaculata* and *Eretmocerus* sp. nr. *furuhashii* (Hymenoptera: Aphelinidae) on glabrous and hirsute host plants. *Biocontrol Science and Technology*, 17, 823–839

Schmidt, S., Naumann, I.D. and De Barro, P.J. (2001) *Encarsia* (Hymenoptera: Aphelinidae) parasitoids of *Bemisia tabaci* and *Trialeurodes vaporariorum* in Australia and the Pacific. *Bulletin of Entomological Research*, 91, 369–387.

Selvakurmaran, A., Vatantharaj, B. and Kumarsen, D. (2000) Life table for the cardamom whitefly, *Kanakarajella cardamomi* (Devid and Subramaniam), a pest on cardamom. *Journal of Entomological Research*, 24, 311–317.

Sithiprasasna, R., Luepromchai, E. and Linthicum, K.J. (1996) Effects of sublethal dosages of methoprene on *Anopheles dirus* species A and B. *Journal of American Mosquito Control*, 12, 483–486.

Southwood, T.R.E. (1978) *Ecological Methods: with Particular Reference to the Study of Insect Population*. Chapman and Hall, London.

Starks, K.J., Munniapan, R. and Eikenbary, R.D. (1972) Interaction between plant resistance and parasitism against greenbug in barley and sorghum. *Annals of the Entomological Society of America*, 65, 650–655.

Tang, Z.H. and Huang, G. (1982) *The Resistance of Agricultural Pests to Insecticides*. China Agriculture Press.

Tang, Q.Y. and Feng, M.G. (2002) DPS Data Processing System for Practical Statistics. Science Press, Beijing, pp. 188–201.

Tozzi, A. (1998) A brief history of the development of piperonyl butoxide as an insecticide synergist. *Piperomyl Butoxide: The Insecticide Synergist* (ed. D.G. Jones), pp. 1–5. Academic San Diego. CA.

Weseloh, R.M. and Andreadis, T.G. (1982) Possible mechanism for synergism between *Bacillus thuringiensis* and the gypsy moth (Lepidoptera, Lymantriidae) parasitoid, *Apanteles melanoscelus* (Hymenoptera, Braconidae). *Annals of the Entomological Society of America*, 75, 435–438.

Xu, X.N., Wang, G. and Gao, S.P. (1998) Effects of sublethal concentration of fenpyroximate on reproduction of *Tetranychus viennensis* Zacher. *Journal of Anhui Agricultural University*, 25, 352–355.

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