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

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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

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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 < 0.0001) 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 1600 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 LC25 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).
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).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>LC-P equations</th>
<th>( R^2 )</th>
<th>LC(_{25} ) (mg/L)</th>
<th>C1 (mg/L)</th>
<th>C2 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st instar</td>
<td>( y = 0.3658x + 0.6866 )</td>
<td>0.9309</td>
<td>186.43</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>2nd instar</td>
<td>( y = 0.3423x + 0.9446 )</td>
<td>0.9607</td>
<td>267.15</td>
<td>200</td>
<td>400</td>
</tr>
<tr>
<td>3rd instar</td>
<td>( y = 0.6066x + 0.5960 )</td>
<td>0.9857</td>
<td>1672.80</td>
<td>800</td>
<td>1600</td>
</tr>
</tbody>
</table>

\( R^2 \): coefficient of determination; \( R \): coefficient correlation; C1: sublethal concentration lower approach LC\(_{25} \); C2: sublethal concentration higher approach LC\(_{25} \).

**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: $x = \text{Age interval}$, $l_x = \text{Actual number entering stage during observation}$, $f_d = \text{Mortality factor}$, $d_x = \text{Actual number dying in course of development}$, and $100q_{lx} / l_x = \text{Percentage mortality}$, 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:

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

(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:

$$I = SE \times SL1 \times SL2 \times SL3 \times SL4 \times SP \times F \times P_\varphi \times PF$$

(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, $P_\varphi$ = the realized fecundity, and $PF$ = the proportion of the females in the generation. Values for $F$, $P_\varphi$ 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:

$$\text{IIPC} = I/I_{ck}$$

(3)

where $I_{ck}$ is the population trend of *B. tabaci* for a given treatment and $I$ 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:

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

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Egg</th>
<th>1st instar</th>
<th>2nd instar</th>
<th>3rd instar</th>
<th>4th instar</th>
<th>“Pupae”</th>
<th>Egg-adult</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check</td>
<td>7.98 ± 0.31 a</td>
<td>3.91 ± 0.29 a</td>
<td>3.02 ± 0.60 b/c</td>
<td>2.69 ± 0.43 e</td>
<td>2.56 ± 0.17 b</td>
<td>2.80 ± 0.24 b</td>
<td>22.95 ± 0.73 c</td>
<td>47.44 ± 3.56 c</td>
</tr>
<tr>
<td>1s100</td>
<td>7.96 ± 0.36 a</td>
<td>3.96 ± 0.34 a</td>
<td>3.90 ± 0.14 a</td>
<td>2.94 ± 0.50 de</td>
<td>2.83 ± 0.36 b</td>
<td>2.89 ± 0.20 b</td>
<td>24.47 ± 1.44 bc</td>
<td>64.94 ± 3.59 b</td>
</tr>
<tr>
<td>1s200</td>
<td>7.89 ± 0.56 a</td>
<td>4.12 ± 0.32 a</td>
<td>4.15 ± 0.61 a</td>
<td>3.19 ± 0.41 d/e</td>
<td>3.08 ± 0.21 b</td>
<td>2.91 ± 0.24 b</td>
<td>25.34 ± 0.64 ab</td>
<td>76.16 ± 2.59 a</td>
</tr>
<tr>
<td>2s200</td>
<td>7.93 ± 0.28 a</td>
<td>3.94 ± 0.25 a</td>
<td>3.64 ± 0.33 abc</td>
<td>4.31 ± 0.41 ab</td>
<td>3.86 ± 0.43 ab</td>
<td>2.93 ± 0.49 ab</td>
<td>26.60 ± 0.47 a</td>
<td>58.66 ± 3.86 bc</td>
</tr>
<tr>
<td>2s400</td>
<td>7.99 ± 0.53 a</td>
<td>3.87 ± 0.23 a</td>
<td>3.78 ± 0.18 ab</td>
<td>4.69 ± 0.42 a</td>
<td>4.08 ± 0.96 a</td>
<td>2.99 ± 0.39 ab</td>
<td>27.38 ± 0.42 a</td>
<td>78.53 ± 3.61 a</td>
</tr>
<tr>
<td>3x800</td>
<td>7.92 ± 0.49 a</td>
<td>3.96 ± 0.40 a</td>
<td>3.06 ± 0.42 bc</td>
<td>3.56 ± 0.37 b/c/d/e</td>
<td>4.39 ± 0.37 a</td>
<td>3.30 ± 0.41 ab</td>
<td>26.17 ± 0.76 ab</td>
<td>55.71 ± 4.04 bc</td>
</tr>
<tr>
<td>3s1600</td>
<td>7.82 ± 0.48 a</td>
<td>3.93 ± 0.35 a</td>
<td>2.93 ± 0.31 c</td>
<td>3.94 ± 0.36 abc</td>
<td>4.61 ± 0.64 a</td>
<td>3.55 ± 0.29 a</td>
<td>26.78 ± 1.46 a</td>
<td>62.45 ± 2.44 b</td>
</tr>
</tbody>
</table>

1 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 different (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.

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

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.

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Table 4  Life table parameter for *B. tabaci* after treatment 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.

<table>
<thead>
<tr>
<th>Stage (x)</th>
<th>No. entering stage ((l_x))</th>
<th>Mortality factor ((d_x))</th>
<th>No. dead by factor ((d_x))</th>
<th>Mortality percent ((100\ q_x/l_x))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CKD1</td>
<td>CKD2</td>
<td>PBD1</td>
<td>PBD2</td>
</tr>
<tr>
<td>Egg</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>1st instar</td>
<td>925</td>
<td>919</td>
<td>921</td>
<td>916</td>
</tr>
<tr>
<td>2nd instar</td>
<td>789</td>
<td>786</td>
<td>771</td>
<td>781</td>
</tr>
<tr>
<td>3rd instar</td>
<td>691</td>
<td>694</td>
<td>657</td>
<td>677</td>
</tr>
<tr>
<td>4th instar</td>
<td>582</td>
<td>597</td>
<td>525</td>
<td>557</td>
</tr>
<tr>
<td>Pupa</td>
<td>333</td>
<td>433</td>
<td>200</td>
<td>363</td>
</tr>
<tr>
<td>Adult</td>
<td>308</td>
<td>399</td>
<td>177</td>
<td>333</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Developmental stages</th>
<th>Egg</th>
<th>1st instar (±\ SE)</th>
<th>2nd instar (±\ SE)</th>
<th>3rd instar (±\ SE)</th>
<th>4th instar (±\ SE)</th>
<th>Pupal (±\ SE)</th>
<th>Egg–adult (±\ SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>6.55 ± 0.34 (^a)</td>
<td>3.05 ± 0.13 (^a)</td>
<td>2.43 ± 0.22 (^b)</td>
<td>2.22 ± 0.18 (^c)</td>
<td>2.28 ± 0.17 (^b)</td>
<td>2.35 ± 0.21 (^a)</td>
<td>18.85 ± 0.37 (^b)</td>
</tr>
<tr>
<td>CKP</td>
<td>6.50 ± 0.22 (^a)</td>
<td>2.93 ± 0.17 (^a)</td>
<td>2.40 ± 0.18 (^b)</td>
<td>3.10 ± 0.34 (^b)</td>
<td>2.83 ± 0.15 (^a)</td>
<td>2.43 ± 0.17 (^a)</td>
<td>20.18 ± 0.66 (^b)</td>
</tr>
<tr>
<td>PB</td>
<td>6.45 ± 0.21 (^a)</td>
<td>2.95 ± 0.21 (^a)</td>
<td>2.95 ± 0.26 (^a)</td>
<td>4.35 ± 0.21 (^a)</td>
<td>3.60 ± 0.26 (^a)</td>
<td>2.53 ± 0.17 (^a)</td>
<td>22.83 ± 0.19 (^a)</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Developmental stages</th>
<th>Mortality factor</th>
<th>Egg–adult survivorship</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CK</td>
</tr>
<tr>
<td>Egg</td>
<td>Unhatched</td>
<td>0.9624</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>0.9408</td>
</tr>
<tr>
<td>Younger nymph</td>
<td>Unknown</td>
<td>0.6991</td>
</tr>
<tr>
<td>Older nymph</td>
<td>Unknown</td>
<td>0.7069</td>
</tr>
<tr>
<td></td>
<td>Parasitized</td>
<td>0.9872</td>
</tr>
<tr>
<td>Adult</td>
<td>Rate of female to male</td>
<td>0.5431</td>
</tr>
<tr>
<td></td>
<td>Standard eggs/female</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>Rate of standard eggs</td>
<td>0.2453</td>
</tr>
<tr>
<td>I</td>
<td>23.54</td>
<td>14.12</td>
</tr>
<tr>
<td>IIIPC</td>
<td>1</td>
<td>0.5999</td>
</tr>
</tbody>
</table>

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

Acknowledgments

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