

Life history and adult dynamics of *Bactrocera dorsalis* in the citrus orchard of Nanchang, a subtropical area from China: implications for a control timeline

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ABSTRACT: *Bactrocera dorsalis* (Hendel) has invaded Jiangxi, a subtropical area of China and poses a serious threat to its host crops, especially citrus fruits. Thus far, the ecological characteristics of *B. dorsalis* in this area remain unclear, which has restricted prevention and control efforts. A study was carried out in citrus orchards in Nanchang, a region of north Jiangxi, between 2008 and 2014 to clarify the life history and adult dynamics of *B. dorsalis*, and then a management plan for *B. dorsalis* were recommended. The results showed that *B. dorsalis* exhibits mixed voltinism, between 4 and 5 generations per year, and overwinters at the fourth and fifth generation pupae stage. Adult stages are active from early May to mid-January of the following year with a distinct peak density between October and November. To control this pest, we suggested that trapping adults using methyl eugenol (ME) and hydrolyzed protein (HP) as lures and removing damaged citrus fruits should be carried out from early May to early December and from late August to mid-December, respectively. Turning soil to eliminate overwintering pupae should begin in late December and end in late February of the following year, and fruit bagging should be performed from mid-August to late October. Releasing parasitic wasps or chemical pesticides would be most effective from mid-August to early September. These results will be useful for citrus farmers and orchard managers as they provide a clear control timetable for applying various control measures, which can help prevent or limit the occurrence of *B. dorsalis* and its damage to citrus orchards.

KEYWORDS: *Bactrocera dorsalis*, life cycle, population dynamics, citrus orchards, control timeline

INTRODUCTION

Citrus are among the most widely cultivated types of fruit trees in the world. Global production is close to 123 million tons per annum¹. In China, citrus cultivation occurs in over 19 provinces, especially in regions south of the Yangtze River. Among these, Jiangxi province is a major citrus-producing region, with approximately 322 000 hectares of plantations, which, in 2014, produced 3 450 000 tons of citrus². Both Gannan navel orange (*Citrus sinensis* (L.) Osbeck) and Nanfeng honey orange (*C. reticulata* Blanco cv. Kinokuni) are the dominant varieties in this area. At the end of twentieth century, *Bactrocera dorsalis* (Hendel) invaded the Jiangxi region³ (Fig. 1) and posed a serious threat to local citrus production.

This pest is a highly invasive species of tephritid fruit fly that is endemic to Asia, but has been introduced to Hawaii, the Mariana Islands and sub-Saharan countries over the last century^{4,5}. After

introduction, *B. dorsalis* can easily disperse and severely damage as it has a high reproductive potential (between 400–1800 eggs per female), a short life cycle (more than 5 generations per year in most tropical regions), a rapid dispersal ability (diffusion range 50–100 km per year) and a broad host range (46 plant families and more than 250 types of fruits and vegetables)^{6,7}. Males of the species respond strongly to methyl eugenol (ME) and this is used for monitoring and estimating populations^{8,9}. Female adults lay eggs in clutches under the skin of fruits by piercing the tissue with their ovipositor. When the eggs hatch, the larvae feed on the flesh, causing fruits to rot and drop¹⁰. These damaged fruits include honey orange (*C. reticulata* Blanco cv. Kinokuni), navel orange [*C. sinensis* (L.) Osbeck cv. Newhall, *C. sinensis* (L.) Osbeck cv. Skaggs Bonanza, *C. sinensis* (L.) Osbeck cv. Washington], pomelo [*Citrus maxima* (Burm.) Merr.], mango (*Mangifera indica* L.), guava (*Psidium guajava* L.), papaya (*Carica papaya* L.), and carambola (*Aver-*

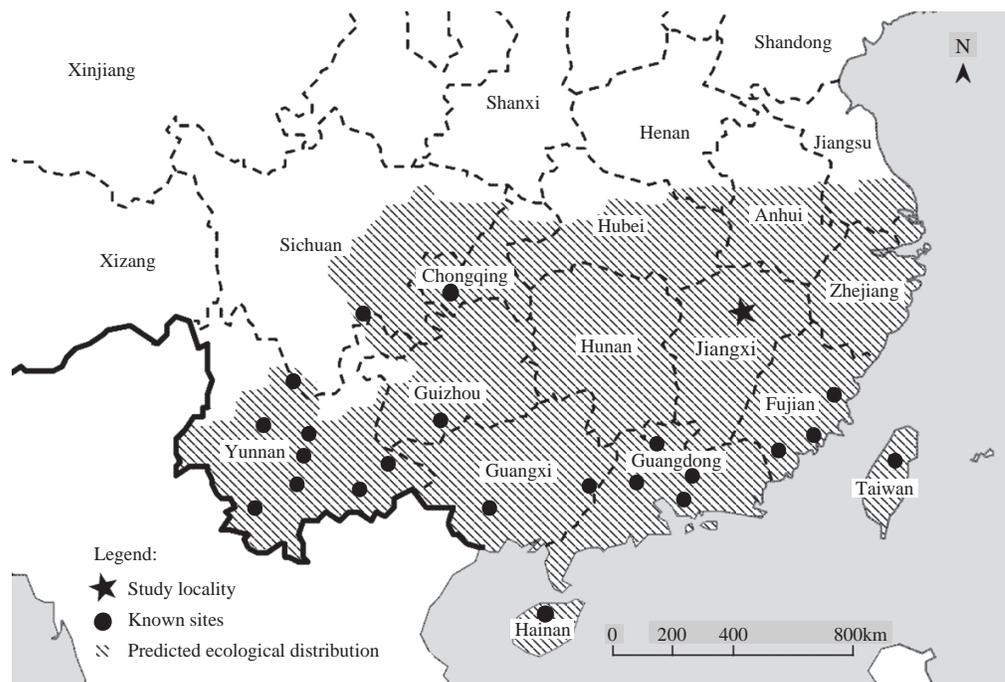


Fig. 1 Study locality and ecological distribution of *B. dorsalis* in China.

rhoa carambola L.)¹¹. In China, damage to fruits from *B. dorsalis* has caused serious economic losses for many years. For example, the citrus losses in Wuxi, Jiangsu province were estimated to range from 20–30% of the total crop in 2005¹², and a similar loss in Suzhou and Shanghai occurred in 2006¹³. In Fuzhou, Fujian province, *B. dorsalis* significantly impacted star fruit, and the highest catches in carambola orchards exceeded 900 adults per trap per week in 2008 and 2009¹⁴. Thus *B. dorsalis* is a destructive pest in areas where it occurs¹⁵.

Several control and monitoring measures have been employed in recent years to limit damage from *B. dorsalis*. These include (1) monitoring populations using traps with methyl eugenol (ME) or hydrolyzed protein (HP) in the Goa region, India and Peshawar, Pakistan¹⁶, (2) removing or destroying infested fruits in a timely manner, and (3) eliminating or reducing pupae by turning soil in Yunnan, China¹⁷, (4) bagging fruit to prevent female oviposition on fruit skins in Fujian, China, (5) releasing parasitic wasps for *Spalangia endius* Walker and *Pachycrepoideus vindemmia* (Rondani) in Hawaii and French Polynesia¹⁸, and (6) cover sprays using pesticides or baits in Rota, Mariana Islands and Hawaii¹⁹. Nonetheless, except for chemical control, these control measures have rarely been

used in many fruit and vegetable bases where it outbreaked in China. This is partly attributable to a traditional dependence on pesticides. In addition, the timing of when and how to perform other effective control measures is still unknown for most areas or habitats/crops. To improve the acceptance and use of non-chemical controls by farmers, it is necessary to further refine these control measures using guidelines based on the biology and ecology of *B. dorsalis* populations.

However, information on the occurrence parameters of *B. dorsalis* remains poorly understood in the Nanchang area. The objective of this study was initially to clarify its life history and population dynamics in this area, using common approaches that involved the rearing from 2009–2012 and trapping from 2008–2014. Then, an optimal control timeline on the five available control measures based on this information was developed to be used by farmers to prevent the occurrence, development and damage of this destructive fruit pest in citrus orchards of this area and even those with similar habitats or environmental conditions.

MATERIALS AND METHODS

Study site

The study site was located at the Jiangxi Agriculture University Experiment Farm of Northwest



Fig. 2 Population rearing and adult trapping. Left, rearing box; Middle, egg cup; Right, trapping adult in citrus orchard.

Nanchang, Jiangxi (115°49' E, 28°46' N) (Fig. 1). The farm contains approximately 15 hectares of citrus (*C. reticulata*, *C. sinensis*) along with kumquat (*Fortunella margarita* Lour.), peach [*Prunus persica* (L.) Batsch], pear [*Pyrus pyrifolia* (Burm. f.) Nakai], grape (*Vitis vinifera* L.), and red bayberry [*Myrica rubra* (Lour.) Set Zucc.]. These crops and their planting areas did not change between 2008 and 2014. But due to the severe damage of *B. dorsalis* on citrus, since 2010 farmers removed infested fruits and used cover sprays (beta-cypermethrin and malathion) during periods of citrus fruit growth and ripening. The area has four distinct seasons, with a longer summer and winter and a shorter spring and autumn. The annual average temperature is approximately 17°C with summer maxima of 40°C and winter minima of -10°C. The seasonal differences cause large fluctuations in the occurrence and abundance of the pest and the damage it causes.

Life history

B. dorsalis population was initially collected from the farm, from 50–60 infested citrus fruits (*C. sinensis*), during October, 2009. The infested fruits were randomly placed in 6 plastic trays each with approximately 5 cm of soil as a pupation medium for mature larvae exiting the fruits. A total of about 280 overwintering pupae in the soil in the tray were obtained, which were used in experiments.

These overwintering pupae from infested fruits were kept together in a tray, and placed in a rearing cage. About 220 adults emerged from these pupae the next year. Perforated (approximately 1 mm holes) transparent plastic cups containing sliced mature citrus fruit and then covered with plastic film were used as egg cups (Fig. 2). Once every 2 days during the laying period, an egg cup every time was placed in the rearing cage for 24 hours, and eggs propagated from these adults were laid on the inner wall of egg cups. In order to clarify the generation of *B. dorsalis*, these eggs from the first

Table 1 Trapping period of *B. dorsalis* over a seven-year period at the Jiangxi Agriculture University Experiment Farm, Northwest Nanchang, Jiangxi.

Trapping period	Number of trap	
	Steiner	McPhail
28 April–17 December 2008	3	2
1 May–18 December 2009	3	2
8 May 2010–2 January 2011	3	2
28 April–18 December 2011	3	2
29 April–31 December 2012	4	3
30 April–28 December 2013	3	2
23 April–16 December 2014	3	2

and last period of adults (no count) were transferred to artificial larval diets with a little water to establish separate population. The same procedure was adopted for rearing each generation of *B. dorsalis* between 2010 and 2011. Adults were supplied with fresh citrus fruits, beer yeast, honey and water as food, and larvae were raised on artificial diets that included white sugar, beer yeast, water, wheat bran, methyl-p-hydroxybenzoate and potassium sorbate²⁰. During the rearing, we recorded the developmental stage and generation number in relation to the life cycle of this fruit fly.

All these processes were carried out in an insect rearing room with two windows (270 cm × 180 cm), about 300 m from the experiment farm. In order to simulate the four seasons as much as possible, the windows were always open for ventilation and daylighting, and heating, humidification and lighting equipments were not used, except for an exception with artificial light during our observations so as to observe more clearly. So, its life history observed could be a good reflection of what was happening outdoors.

Adult dynamics

The adult dynamics of *B. dorsalis* were determined by trapping with ME-baited (3 ml) Steiner traps and HP-baited (0.5 l, 24 g/l) McPhail traps in the experimental orchard from 2008–2014 (Fig. 2). In the trapping, we kept the same concentration and dose of the two lures when renewed each time. The trapping started in late April or early May (2009 and 2010) as *B. dorsalis* adults usually begin to occur in early May in Nanchang area²² and ended in mid-late December or early January the next year (2011) when no adults were captured (Table 1).

A total of 3–4 Steiner and 2–3 McPhal traps were deployed each year for the duration of the

study (Table 1). The traps in section A and B were suspended from tree branches, hedges or metal poles approximately 1.0–1.5 m above the ground in locations not completely covered by foliage or in direct sunlight and 40–50 m apart to avoid interference. ME was changed monthly and HP semi-monthly¹⁰. The traps were usually checked weekly, but sometimes advanced or delayed for 1–2 days due to weather or human factors. We replaced in time some traps missed or impaired. Captured adults of *B. dorsalis* and other tephritid species were identified and counted according to the characteristics described by Drew and Raghu⁴.

Control timeline

Through the study for *B. dorsalis* in Nanchang, Jiangxi, we obtained information that included the overwintering stages of *B. dorsalis*, the number of generations per year, the duration of each generation and developmental stage, and the season and peak period of adult occurrences. Then, we, according to the information, formulated a specific control timeline for *B. dorsalis* on each control measure above-mentioned.

Statistical analysis

All the data were analyzed using SPSS 13.0. Relative trapping efficiency of the traps was compared using Wilcoxon signed ranks test for two related samples in nonparametric tests. Means and standard error of the mean (SEM) of adults collected using ME or HP lures each week were calculated using Explore procedures. Generalized linear models were developed, which accounted for the changing adult captures over the trapping period from 2008–2014.

RESULTS

Life history

In 2010 and 2011, number of *B. dorsalis* generations was similar, but the occurring time of different developmental stages in each generation was slightly different. For example, adults started occurring on May 1 in 2010 and the last adults died on December 21. In 2011, adults occurred on April 21 and died on January 10 the following year. But in both years, the first generation eggs and larvae appeared in late May. According to the developmental process of each stage in the two years, we incorporated the life history of *B. dorsalis* in the Nanchang area, Jiangxi.

Table 2 showed that *B. dorsalis* exhibits mixed voltinism of between 4 and 5 generations per year.

The fourth and fifth generation pupae overwinter in loose soil. Overwintering pupae emerge as adults beginning in late April each year. First generation eggs begin to occur in late May and end in early July, while second, third, fourth, and fifth generation eggs appear in early July, mid-August, late September, and early November, respectively. Correspondingly, the timing of larvae lagged behind that of eggs for 2–4 days (the duration of eggs) in each generation. There is some generation overlap, due to the longevity of adults and the long oviposition period (the average longevity is 84.8 days; and the oviposition period is more than 30 days). We did not observe eggs from fifth generation adults in any one year.

Seasonal dynamics

A total of 28 843 fruit flies were captured through the 37 traps. In addition to *B. dorsalis* (24 764), fruit fly species included *B. (Zeugodacus) tau* (Walker) (2707), *B. (Z.) scutellata* (Hendel) (1267), *B. (Z.) cucurbitae* (Coquillett) (100), *Dacus (Callantra) trimacula* (Wang) (5), and other non-tephritid dipterous insects (no count). As for *B. dorsalis*, trapping efficiency using ME as a lure was significantly better than trapping using HP as a lure in every year, based on a Wilcoxon signed rank test (Table 3).

From 2008–2014, the *B. dorsalis* adult captures with both types of lures exhibited large fluctuations at different dates. Generally, adults were first captured in early May, and kept low density levels till mid-August. Subsequently, they would gradually increase to a clear density peak between early September and late November. After December, adult catches were drastically reduced as the temperature decreased. The peak values of *B. dorsalis* adults also varied widely across the study years. The maximum peak value was 387 adults per trap per week (ME) and 56.5 adults per trap per week (HP) on November 14, 2010, while the minimum was 80.33 adults per trap per week (ME) on November 6, 2014, and 4.33 adults per trap per week (HP) on September 10, 2012. Comparatively, adult captures per trap per week were particularly high in 2010 and low in 2012 and 2014 at the experimental site (Fig. 3).

Annual fluctuation

In order to understand the year-to-year fluctuation, we calculated the average captures with ME and HP as lures in these years. The results showed that the average number was 42.10, 47.30, 54.75,

Table 2 Life history of *B. dorsalis* in Nanchang, Jiangxi.

Generation	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
	F S L	F S L	F S L	F S L	F S L	F S L	F S L	F S L	F S L	F S L	F S L	F S L
Overwintering	•••	••										
1st	+	+++	+++	+								
		••	•••	•••								
		*	**	**								
2nd			++	+++	+++	+++						
			••	•••	•••	•••						
				*	*	*						
3rd				+	+++	+++	+++	+++	+			
					••	•••	•••	•••	••			
					*	*	*	*	*			
4th						••	•••	•••	•••	+		
						••	•••	•••	•••	•••	•••	•••
						*	*	*	*	*	*	*
5th							••	•••	•••	•••	•••	•••
							+	+++	+++	+++	+++	+++

+ adult; o egg; * larva; • pupa. F, S, and L represent the first, second, and last 10-day period of a month, respectively.

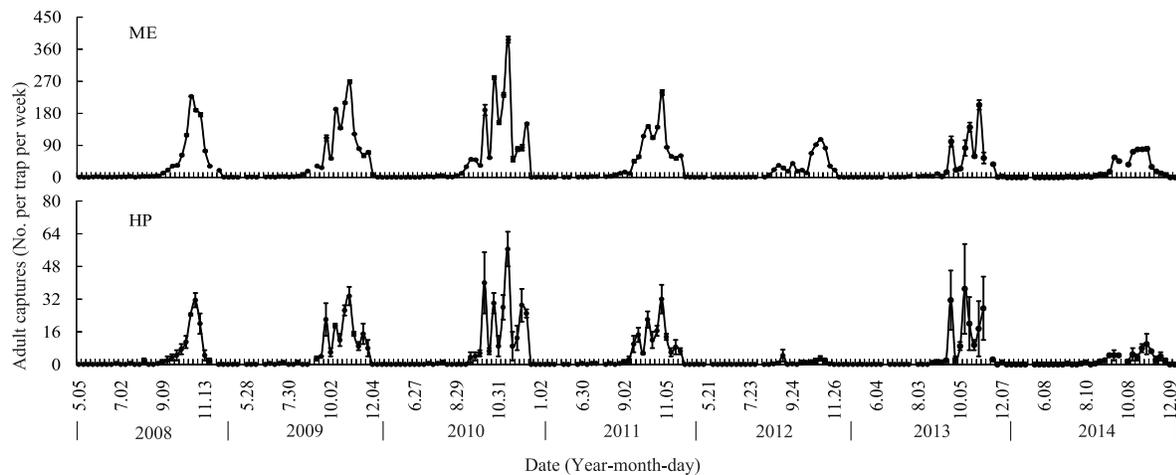


Fig. 3 Average catches (mean ± SEM) of *B. dorsalis* adults with ME and HP as lures on specific dates between 2008 and 2014 in Nanchang, Jiangxi.

39.04, 18.22, 25.01, and 17.54 adults per trap per week (ME), and was 3.44, 5.60, 7.28, 4.92, 0.49, 4.97, and 1.85 adults per trap per week (HP) from 2008–2014, respectively. Generalized linear models indicated that the average captures with the two lures slightly declined across these study years. The two linear equations were $y = 8701.56 - 4.31x$ ($R^2 = 0.62, p < 0.01$) for ME and $y = 1024.24 - 0.51x$ ($R^2 = 0.51, p < 0.05$) for HP (Fig. 4).

Suggestions for optimal control scheduling

Based on the life history and population dynamics of *B. dorsalis* established in this study, the citrus

growth stage in this region, we incorporated the six available measures for *B. dorsalis* control into a management plan. Table 4 suggests that efforts to trap adults with ME or HP (control measure A) and to remove injured fruits in a timely manner (control measure B) should be conducted from early May to early December and from late August to mid-December, respectively. Turning soil to eliminate pupae (control measure C) should begin in late December and end in late February. Fruit bagging to prevent oviposition (control measure D) should be performed from mid-August to late October. The most effective period for releasing parasitic wasps

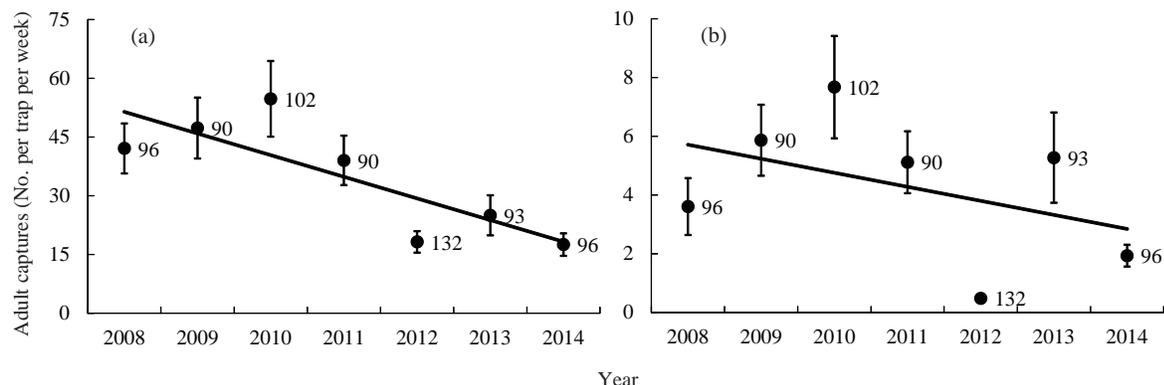


Fig. 4 Annual fluctuations of *B. dorsalis* adult captures in Nanchang, Jiangxi from 2008–2014. (a) Relation between the average captures of *B. dorsalis* adult using ME as a lure (●) and years of trapping. Linear model of catches (black line), $y = 8701.56 - 4.31x$, $F = 12.60$, $df = 1676$, $p < 0.001$, $R^2 = 0.62$. (b) Relation between the mean catch of *B. dorsalis* adult using HP as a lure (●) and years of trapping. Linear model of catches (black line), $y = 1024.24 - 0.51x$, $F = 5.42$, $df = 1460$, $p = 0.020$, $R^2 = 0.51$. Data are mean \pm SEM. Values beside the black spots (●) are the sampling numbers for each year.

Table 3 Comparison on the trapping efficiency between ME and HP from 2008–2014 (adults/trap/week) based on nonparametric tests.

Year	Species	ME mean \pm SE	HP mean \pm SE	Wilcoxon test Z	p
08	<i>B. dorsalis</i>	32.10 \pm 6.39	3.61 \pm 0.97	-4.71	<0.001
	<i>B. tau</i>	2.16 \pm 0.30	3.29 \pm 0.66	-1.77	0.077
	<i>B. scutellata</i>	0.45 \pm 0.04	1.75 \pm 0.48	-3.74	<0.001
	<i>B. cucunbitae</i>	0.10 \pm 0.04	0.16 \pm 0.06	-0.58	0.564
09	<i>B. dorsalis</i>	47.30 \pm 7.79	5.87 \pm 1.21	-4.63	<0.001
	<i>B. tau</i>	1.97 \pm 0.29	3.02 \pm 0.53	-2.35	0.019
	<i>B. scutellata</i>	0.49 \pm 0.11	1.85 \pm 0.40	-3.62	<0.001
	<i>B. cucunbitae</i>	0.07 \pm 0.03	0.19 \pm 0.07	-1.47	0.142
10	<i>B. dorsalis</i>	54.75 \pm 9.65	7.68 \pm 1.74	-4.29	<0.001
	<i>B. tau</i>	2.32 \pm 0.36	3.78 \pm 0.73	-2.23	0.026
	<i>B. scutellata</i>	0.60 \pm 0.14	2.43 \pm 0.62	-3.84	<0.001
	<i>B. cucunbitae</i>	0.10 \pm 0.04	0.13 \pm 0.06	-0.58	0.564
11	<i>B. dorsalis</i>	39.04 \pm 6.29	5.12 \pm 1.05	-4.46	<0.001
	<i>B. tau</i>	1.61 \pm 0.26	2.63 \pm 0.41	-3.94	<0.001
	<i>B. scutellata</i>	0.46 \pm 0.11	2.02 \pm 0.51	-3.67	<0.001
	<i>B. cucunbitae</i>	0.04 \pm 0.03	0.07 \pm 0.04	-0.26	0.792
12	<i>B. dorsalis</i>	18.21 \pm 2.76	0.48 \pm 0.14	-3.92	<0.001
	<i>B. tau</i>	1.64 \pm 0.22	2.44 \pm 0.35	-1.93	0.053
	<i>B. scutellata</i>	0.33 \pm 0.07	1.53 \pm 0.30	-4.46	<0.001
	<i>B. cucunbitae</i>	0.05 \pm 0.02	0.06 \pm 0.03	-0.25	0.803
13	<i>B. dorsalis</i>	25.01 \pm 5.11	5.27 \pm 1.53	-4.11	<0.001
	<i>B. tau</i>	1.54 \pm 0.21	3.19 \pm 0.58	-2.66	0.008
	<i>B. scutellata</i>	0.49 \pm 0.11	2.42 \pm 0.55	-3.38	0.001
	<i>B. cucunbitae</i>	0.04 \pm 0.03	0.14 \pm 0.06	-1.47	0.140
14	<i>B. dorsalis</i>	17.54 \pm 2.87	1.93 \pm 0.37	-4.29	<0.001
	<i>B. tau</i>	1.55 \pm 0.24	3.02 \pm 0.58	-2.96	0.003
	<i>B. scutellata</i>	0.36 \pm 0.10	2.12 \pm 0.54	-3.79	<0.001
	<i>B. cucunbitae</i>	0.05 \pm 0.02	0.11 \pm 0.05	-0.83	0.405

(control measure E) or applying chemical pesticides (control measure F) ranges from mid-August to early and mid-September. Overall, coordinating the

periods of citrus fruit enlargement and fill with selection and implementation of the control measures can be crucial in reducing populations and limiting the damage of *B. dorsalis* in citrus orchards.

DISCUSSION

B. dorsalis was first recorded in Taiwan in 1912²² but has now spread to most of the countries in the Asia-Pacific region including India, Pakistan and Thailand^{23–25}. Due to its wide distribution and ability to cause serious damage to various crops, *B. dorsalis* is considered to be a major pest that urgently requires efficient management tactics²⁶. Many types of control measures have been developed to ensure the safe production of *B. dorsalis* host crops. However, these have had little success in minimising damage, largely due to the lack of information about when to implement the control measures in specific areas or for specific crops. To improve control, we proposed a control timeline for *B. dorsalis* in conjunction with the growth stages of citrus for Nanchang, Jiangxi. This work not only provides some basic information about *B. dorsalis* outbreaks and damage but also provides a control timetable usable directly by farmers for reducing or eliminating this fruit fly in the specific habitat of citrus orchards.

Before incorporating the control timeline, our rearing and trapping for *B. dorsalis* suggested its occurrence pattern in the Nanchang area of China; a population can complete 4–5 generations per year, and overwinter in loose soil as pupae. Adults begin

study, especially in winter due to the low survival rate of pupae (below 10% in Wuhan, Hubei which have the similar low temperature with Nanchang, Jiangxi)²⁶. This might explain why there was a low density of *B. dorsalis* adults from May to mid-August the following year. Additionally, the control timetable we have developed is relevant for the Nanchang area and may also be applicable in areas where *B. dorsalis* encounters similar habitats or environmental conditions, including citrus orchards in Shanghai, Zhejiang, Hunan, Hubei, Sichuan, and Chongqing, China. As for the other regions, they need to be rescheduled according to the occurrence rule of *B. dorsalis* in local.

Currently, *B. dorsalis* is still spreading around the world^{16,25}. This, except linked with the biology and ecology of *B. dorsalis* and the change of global climates and circumstances^{35,36}, was also related to people's control measures, especially the control timeline of them. However, here we did not differentiate which of these control measures, or all them used in combination, were more efficient and economical. It is possible that *B. dorsalis* would expand further or cause outbreaks at our experimental location or in citrus orchards when no suitable control measures were adopted. But, through this study, we believe that the implementation of these control measures according to a suitable control timeline were to some extent efficient in reducing the occurrence, development and damage of *B. dorsalis* in most fruit and vegetable bases, either singly or combined. Thus, our efforts would be still focused on the control measure of *B. dorsalis*, in particular the associations between these control measures and its biology and ecology.

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