Seasonal phenology of *Bactrocera minax* (Diptera: Tephritidae) in western Bhutan

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**Abstract**

The Chinese citrus fruit fly, *Bactrocera (Tetradacus) minax* (Enderlein), is one of the major citrus pests in Bhutan and can cause >50% mandarin (*Citrus reticulata* Blanco) fruit drop. As part of the development of a management strategy for the fly in mandarin orchards, population monitoring and experimental manipulations were carried out to determine: (i) adult emergence period; (ii) adult phenology patterns; (iii) period of crop susceptibility; and (iv) period from fruit drop to pupation. In western Bhutan, adult flies emerge from the overwintering pupal stage in late April/early May. Most flies are mature by the end of May and it is inferred that mating occurs at this time: from the beginning of June males rapidly disappear from the population and by mid- to late June are rare or absent from traps. Mature females are present in the mandarin crop at the beginning of June, but very little oviposition occurs until mid-June, while most damage has occurred by mid-July. Initiation of oviposition into mandarins is almost certainly linked to crop phenology. Adult flies disappear from the orchard system during August. After fruit drop, larvae were recorded leaving the fruit to pupate within 13 days. The use of early to mid-season protein bait sprays and/or targeted use of systemic insecticides during the one month oviposition period, plus the removal of fallen fruit once every 10 days, are recommended as control strategies.

**Keywords:** citrus, *Bactrocera minax*, Bhutan, control, fruit drop

**Introduction**

*Bactrocera minax* (Enderlein) (= *B. citri* Chen) (Diptera: Tephritidae) (Chinese citrus fruit fly) is considered unique among the tephritid sub-family Dacinae in being univoltine (van Schoubroeck, 1999), a trait more commonly associated with temperate fruit flies such as *Rhagoletis pomonella* Walsh (Fletcher, 1989). Recorded from southern China, India (West Bengal and Sikkim) and Bhutan (Drew, 1979; Wang & Luyi, 1995), the larval host range of *B. minax* is largely restricted to wild and cultivated species of *Citrus* (Allwood *et al.*, 1999), some of which are endemic to southern China and the eastern Himalayan region (Rieger, 2006), but has also been reported from *Fortunella crassifolia* (White & Elson-Harris, 1992) and *Lycium chinense* (Wang & Luyi, 1995).

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Bactrocera minax has been recognized as a serious pest of commercial citrus, especially in China, for more than half a century (Wang & Luyi, 1995). For the eastern Himalayan kingdom of Bhutan, local land-race varieties of mandarins (Citrus reticulata Blanco) are one of the primary cash crops, with fruit exported to neighbouring India and Bangladesh. Unfortunately, crop losses of between 35% and 75% to B. minax infestation are common in mid and high altitude orchards (>1100 m) and the fly is considered one of the major limiters of production (van Schoubroeck, 1999).

Relatively little research into the ecology and pest management of B. minax has been published (Zhang, 1989; Yang et al., 1994; Wang & Luyi, 1995; van Schoubroeck, 1999). In China, adult emergence is from late April to early May, with adults present in the field until September and oviposition occurring from mid-June through to mid-September, although this phenological pattern varies between provinces and appears to be dependent on local temperature conditions (Wang & Luyi, 1995). Adult emergence period in eastern Bhutan has also been identified as the April/May period, but other adult phenological patterns are unclear due to the lack of an efficient monitoring method (van Schoubroeck, 1999). There are statements in the literature that a delay in egg development occurs after oviposition, but there appears to be no formal data to support this and results are conflicting (Wang & Luyi, 1995; van Schoubroeck, 1999). Published information on pest management is also restricted, but includes a variety of strategies such as fruit and sugar based baiting (Wang & Luyi, 1995), application of the sterile male technique (Wang & Zhang, 1993), soil tillage and intercropping (Yang et al., 1994), crop hygiene (collection and removal of fallen fruit) and baiting with dry protein (van Schoubroeck, 1999).

In Bhutan, at the start of our project recommendations to growers included extensive protein bait sprays fortnightly for at least three months, or regular cover sprays, from spring through to the autumn harvest, and crop hygiene involving the collection of fallen fruit every three days (National Plant Protection Centre, Extension Leaflet, Ministry of Agriculture, Bhutan). The need for full season cover sprays was based on observations that young larvae could be found in fruit throughout the season, which implied that ovipositing adults were also present throughout the season. The phenology of adult populations was unknown, however, as the flies were not considered to respond to either cue lure or methyl eugenol (White & Elson-Harris, 1992), the standard lures used for trapping dacies, and other trapping techniques also did not work or had very low efficiency (van Schoubroeck, 1999). Recommendations for crop hygiene were based on those reported for other dacin fruit flies, where pre-pupal maggots leave the fruit within a few hours after it falls (Hulthen & Clarke, 2006). The application of long-term, regular cover spraying had little grower support due to cost, other commitments in the agricultural cycle, and religious beliefs. Similarly, picking up fallen fruit every three days was also considered impractical for most farmers (van Schoubroeck, 1999) and was rarely implemented.

As part of a project developing an integrated pest management programme for B. minax in Bhutanese mandarin orchards, we studied the ecology and phenology of the fly. Specifically we wanted to address the questions of when mature adult flies were present in the orchard, at what stage of the crop cycle was the fruit susceptible, and was collection of fallen fruit every three days necessary for crop hygiene purposes. The aim was to determine if more focused recommendations could be made for B. minax control based on cover sprays, protein bait sprays, or other forms of population manipulation. As such, information is presented below on: (i) timing of adult emergence; (ii) timing of fly sexual maturation; (iii) oviposition period with respect to the mandarin crop; and (iv) the time taken for pre-pupal larvae to leave fallen fruit. Points one to three are relevant to monitoring and the timing of chemical controls, while point four is relevant to the development of crop hygiene recommendations. In addition to fly phenology, data on crop phenology are also presented. Based on laboratory and field data, van Schoubroeck (1999) demonstrated that mandarin fruit <11 mm diameter is not oviposited into by B. minax, as the fruit is physically too small for the fly to balance upon. Using the 11 mm figure, tests were conducted to see if the window for targeted control could be narrowed.

Materials and methods

Field sites

Unless otherwise stated, research was carried out in orchards within the Rimchu Valley, west from Punakha (89.87E/27.58N), western Bhutan between 2000 and 2005. The altitude is approximately 1300 m and the climate consists of a winter with subzero minimum temperatures and a mild summer with maximum temperatures of 30°C. The rainfall occurs in May to August, influenced by the monsoons. A smaller number of trials were carried out in the Tsirang district (90.12E/27.01N) of south-western Bhutan, at an altitude of 1500 m. Trial orchards were not sprayed for fruit fly, or other pests, during experimental periods.

Determination of adult emergence period

Experiment 1a

Cone-shaped pupal emergence traps constructed of fine wire mesh, with a base of approximately 0.1 m² and with an internal horizontal band of Tangle-Trap® Insect Trap Coating (The Tanglefoot Company, Michigan, USA), were used to determine the emergence period of adult flies (which overwinter as puparia in the soil). Thirty-two traps (four under each of eight trees) were placed in a 20-year-old mandarin orchard at Rimchu on 21 April 2000 and subsequently inspected weekly, beginning 26 April. Under the same trees, 15 × 1 m quadrats of soil not covered by the traps were sieved on 21 April 2000 to obtain an estimate of B. minax pupal density.

Experiment 1b

In mid-December 2004, using traps of a similar design but larger base (~0.2 m²), 12 traps (one per tree) were placed in the same orchard as used previously, under trees where there was natural fruit fall. To six of these traps, an additional 30 pieces of field-collected infested fruit per trap were placed to increase probable pupal numbers. The horizontal Tanglefoot band was applied to each trap on 12 April 2005, prior to the expected time of first emergence. The traps were again inspected weekly to count flies caught on the Tanglefoot band.
Seasonal phenology of adult flies

Experiment 2a

In 2000, fifteen McPhail (liquid lure) traps were set at Rimchu in 20-year-old mandarin trees and five traps set approximately 200 m away in a 7-year-old orchard. The traps were cleared weekly and the lure (~300 ml per trap) refreshed from mid-April 2000 until the end of October 2000. Three different types of lure: Australian Pinnacle Protein Insect Lure (Mauri Yeast Australia, Toowoomba, Australia) diluted 25 ml protein to 1 litre water; Indian protein hydrolysate powder (M/S International Drugs and Chemical Co., Mumbai, India) 5 g powder to 1 litre water; and orange-ammonia solution (750 ml orange juice, 25 g ammonium carbonate, 1 litre water, 0.2% potassium sorbate preservative) were used across the 20 traps as there was no prior experience to judge as to which lure(s) may have been more effective in attracting adult B. minax. All three lure types subsequently caught flies, with no seasonal differences in lure efficiency (authors’ unpublished data), and so the combined data is presented in this paper. Collected flies were sexed, counted and stored in 70% ethanol. The females were subsequently dissected for estimation of sexual maturity state and graded into three categories – immature (ovaries small and no eggs present), semimature (ovaries with eggs developing but not mature), mature (ovaries with mature eggs present) using a modified scoring system based on Fletcher et al. (1978). In 2002, seven traps using only Indian protein (concentration as above) were placed in the 20-year-old trees at Rimchu from mid-March to end of August.

Experiment 2b

Ten McPhail traps, five baited with Australian Pinnacle protein and five with orange-ammonia solution, were set in a mandarin orchard at Suntalay, Tsirang. The traps were set from the second week of April to the end of October 2000 and caught flies handled as per the Rimchu study.

Measurement of oviposition period in mandarin

Experiment 3a

In mid-April 2001, at the younger mandarin orchard at Rimchu, 160 fruit-bearing branches (approx 3–4 branches per plant and at least five fruit per branch) were enclosed with white muslin bags to protect the fruit from ovipositing flies. Fruit was still extremely small, <10 mm diameter, and had not been previously oviposited into. Beginning on 12 June, cohorts of 16 different bagged branches, selected without conscious bias, were exposed for a two-week period and then enclosed again, for a total of ten fortnightly intervals. The last exposure period was 21 August to 4 September. The logic of the experiment was that the protect-expose-protect design would mean that any of the subsequently harvested fruit, if found to be infested, could only have been infested during the fortnight exposure period. On the day of first exposure, the diameters of 80 fruits were measured for each cohort. The trial was harvested in October 2001 and the numbers of fruit fly infested fruit recorded.

Experiment 3b

In parallel with experiment 3a, on 12 June 2001, 16 previously unprotected branches were enclosed and not subsequently exposed, to determine if there had been any oviposition prior to that date. Similarly, additional cohorts of 16 previously unenclosed branches were enclosed at monthly intervals on 10 July, 7 August and 4 September. These trials were harvested along with experiment 3a.

Experiment 3c

On 21 May 2004, the fruit bagging experiment was repeated in a mandarin orchard at Botokha, Punakha. Six cohorts of seven branches each were enclosed and then one cohort at a time subsequently exposed for two-week periods, beginning on 4 June and ending on 27 August. For both experiments 3a and 3c controls (fruit bagged and never uncovered) were run: subsequent infestation rates were so low (<1%) that no corrections were made to the primary data. As for experiment 3a, the diameters of fruits were measured. In this case, 20 fruits for each cohort were measured from 21 May to 22 October, 2004.

Experiment 3d

In parallel with experiment 3c, seven cohorts each of seven branches were enclosed and not subsequently exposed, beginning with the first cohort on 21 May and continuing at two-week intervals. Experiments 3c and 3d were harvested in late October 2004.

Measurement of period from fruit drop to puparia development

Experiment 4

On 16 November 2001, 40 fruits that had dropped to ground in the previous 24 h were collected from the field and set on clean, heat sterilized sand in the laboratory. The sand was subsequently sieved daily and any puparia formed in the preceding 24 h removed and counted. On
30 November 2001 a second cohort of 70 fruits that had dropped over a period of 7 days (the ground having been cleared of all fallen fruit on 23 November 2001), were collected and handled in an identical fashion.

Results

Determination of adult emergence period

Very few flies were trapped in spring 2000, with two and three females collected respectively on 10 and 17 May. Three newly emergent flies, two with their ptilinums still expanded, were observed resting on the trunks of mandarin trees on 26 April. Based on quadrat sampling, pupal density under the trees was estimated at 3.5 m$^{-2}$ and low catch is a probably a simple reflection of the low probability of having placed a trap over puparia. In 2005, 60 flies were trapped, all most equally between seeded and unseeded traps (27 and 33 respectively). The first flies were collected on 29 April, while the last flies were collected on 20 May (fig. 1). Sex ratio was near unity (male:female, 1.14:1) and there was no obvious difference in the emergence patterns of the sexes. The two seasons’ results are consistent in that, in the Rimchu district, emergence occurs from late April to mid-May.

Seasonal phenology of adult flies

Seasonal phenology patterns at Rimchu for 2000 and 2002 were largely consistent (fig. 2a,b). Adults were first

![Fig. 2. Mean (+ SE) seasonal catches of male (●) and female (▲) Bactrocera minax in MacPhail traps in mandarin orchards in western Bhutan: (a) Rimchu Valley 2000, (b) Rimchu Valley 2002, (c) Suntalay (Tsirang) 2000.](image)

![Fig. 3. Pattern of seasonal ovarian development of female Bactrocera minax caught in MacPhail traps in a mandarin orchard at Rimchu Valley, western Bhutan in 2000 (a) and 2002 (b). □, Immature; □, semi-mature; □, mature.](image)
caught in traps at the end of April/beginning of May, peaked in June and July, and then declined into August. Significantly fewer males were trapped in both seasons than females and males had a different seasonal pattern than females. Male catches in traps peaked in late May and then declined rapidly during June. This is consistent with a pattern seen in other univoltine insect species, where the females mate only once in their life and the males die off after the mating period (e.g. Shohet & Clarke, 1997). At Suntalay in 2000, the season was significantly reduced over that observed at Rimchu. Emergence was again at the very start of May, but populations peaked in early May and then declined sharply thereafter (fig. 2c). Continuous rainfall was recorded at Tsirang from May to mid-July 2000 and may have significantly influenced trap efficacy.

Female dissection demonstrated that until at least mid-May most females trapped were still immature. Fully mature females first appeared in traps in mid- to late May and by mid- to late June all females trapped were fully mature (fig. 3). No immature flies were collected after late June, confirming that *B. minax* has only one generation per year. The pattern of female maturation was seven to 10 days earlier in 2002 compared to 2000 and most likely reflects across seasonal temperature variation. The peak of the male population in traps matched the period when the female population was becoming sexually mature.

**Measurement of oviposition period in mandarin**

Bagged fruits exposed for two weeks during June or the first half of July suffered infestation from *B. minax*, but in both 2001 and 2004 new infestation ceased or was extremely low from mid-July onwards (fig. 4a,b). Similarly, exposed fruit which was subsequently protected, showed very low infestation rates if protected from early to mid-June onwards (fig. 4c,d). In combination, these trials suggest that the oviposition period of *B. minax* at Rimchu is restricted from mid-June to mid-July. In 2001, mean mandarin size at Rimchu did not reach van Schoubroeck’s (1999) critical 11 mm diameter until the last week of June (fig. 5a), although 14\% of measured fruit had reached this threshold by mid-June. If van Schoubroeck’s minimum threshold measurement holds in the field, then the period of peak crop damage at Rimchu in 2001 was further restricted from very late June to mid-July. In 2004 at Botokha, the critical 11 mm fruit diameter was reached at the beginning of July, approximately one week later than in 2001 at Rimchu (fig. 5b).
Measurement of period from fruit drop to puparia development

In the first cohort of 40 fruits sampled within 24 h of fall, the first puparia were collected on day 18 and the last on day 32, after fruit fall (fig. 6a), with a high synchronization rate of first emergence. In the second cohort of 70 fruits, collected within 7 days of fall, puparia were first collected within 6 days of collection (maximum of 13 days after fall), with approximately 14% of all puparia developing in this period. While time to pupariation of this cohort followed an approximately normal distribution (fig. 6b), there was a very long tail, with the final puparium sieved 25 days after field collection of fruit.

Discussion

The unique univoltine life-cycle of B. minax results in clear delineation of the different components of the life cycle, something which is rarely seen in the tropical Dacinae. For western Bhutan these are presented in fig. 7. Puparia remain in the soil for 5 to 7 months, from an undetermined and probably variable period during autumn, through the winter, to begin emergence in late April. Emergence of adult flies from the soil occurs over a four-week period from late April to late May, after which they can be trapped using protein and fruit based lures. Some female flies remain within the orchard until September, but by that stage individuals are rare, with peak populations occurring from late April/early May to mid/late July. Based on their rarity in traps after late May, it is likely that males begin to die after what is almost certainly a short mating ‘window’ from mid to late May. The first sexually mature, egg-laying females appear in early June, just prior to the first mandarin fruit reaching 11 mm diameter, the first stage susceptible to fruit fly oviposition (van Schoubroek, 1999). One hundred percent of the female fly population reaches sexual maturity by late June. The oviposition period occurs from mid June to late July coinciding with the development to sexual maturity of the female population. Up to 35% fruit infestation was recorded in the branch enclosure experiments with increases in infestation rates occurring with longer periods of exposure. In commercial orchards, infestation rates are considerably higher, reaching 70–80% (authors’ unpublished data). After fruit fall, there is a long period, 18–52 days,
before larvae leave the fruit and pupate in the soil. This lengthy pre-pupal period is as unique among the Dacinae as is univoltinism and suggests that substantial larval development occurs after fruit fall. Our phenological summation for \textit{B. minax} in western Bhutan is broadly consistent with that proposed for \textit{B. minax} in eastern Bhutan (van Schoubroeck, 1999, p. 156), which gives us confidence that the model is applicable throughout the country.

This understanding provides opportunities for field pest management:

1. The majority of flies collected at the liquid lure (protein) traps, responded between the beginning of May and mid/late July; while post-teneral flies would be seeking protein food from late April to late May. Fortnightly protein bait spray applications from early May to late July would reduce the fly population markedly and prevent oviposition in developing fruit.

2. Because the oviposition period is well defined, from mid June to late July, a systemic insecticide cover spray in early July followed up by a second treatment in late July, would provide high mortality of eggs in fruit. These treatments would not damage pollinators, as they would be applied some weeks after fruit set.

3. The extended period between fruit fall and pupation, at least 18 days, would provide opportunity for cultural control practices. Collection of fallen fruits only once every ten days would prevent the development of puparia and thus reduce the fly population for the next year.

In an integrated pest management programme, all these options could be considered, depending on the physical location of the orchard. For some regions, with limited road and transport services, protein bait may become too expensive, especially as it has to be imported from India or further afield. In general, sound crop hygiene and occasional systemic cover sprays would be most economical.

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**References**


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