Bulletin of Entomological Research (2001) **91**, 461–469 DOI: 10.1079/BER2001129

The biology of *Diadromus collaris* (Hymenoptera: Ichneumonidae), a pupal parasitoid of *Plutella xylostella* (Lepidoptera: Plutellidae), and its interactions with *Oomyzus sokolowskii* (Hymenoptera: Eulophidae)

Shu-sheng Liu*, Xin-geng Wang, Zu-hua Shi and F.B. Gebremeskel

Institute of Applied Entomology, Zhejiang University, 268 Kaixuan Road, Hangzhou 310029, China

Abstract

The ichneumonid *Diadromus collaris* (Gravenhorst) has been recorded in many parts of the world as an important parasitoid of the diamondback moth, *Plutella xylostella* (Linnaeus), a serious pest of brassica vegetable crops worldwide. Some aspects of its biology and its interactions with *Oomyzus sokolowskii* (Kurdjumov), another major parasitoid of the same pest, were studied in the laboratory. At 25°C, female wasps did not have mature eggs in their ovaries until about 12 h after emergence. Both males and females mated successfully 24–48 h after emergence, and females started to oviposit one to two days after emergence. Unmated females produced male progeny only; mated females produced progeny of both sexes. The development rate of the parasitoid increased linearly with temperature from 15 to 30°C, with an estimated low temperature threshold of 7.4°C and a thermal constant of 225.1 day-degrees for development from egg to adulthood. Rates of survival from larva to adulthood were about 90% between 20 and 28°C and decreased as temperature decreased or increased. No immatures survived to adulthood at 35°C. When provided with honey solution, the females lived on average 8.3, 11.5 and 7.0 days, and parasitized 26, 44 and 46 host pupae at 20, 25 and 30°C, respectively. Female wasps could be stored at 15°C for up to four weeks without detrimental effects on reproduction. Females of *D. collaris* attacked host pupae already parasitized by *O. sokolowskii*, inserting their ovipositor into the hosts at a similar frequency as into unparasitized host pupae, but they did not lay eggs inside the hosts.

Introduction

Diadromus collaris (Gravenhorst) (junior synonym: *Thyraeella collaris*) (Hymenoptera: Ichneumonidae) is a solitary, pupal endoparasitoid of the diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), a major insect pest of brassica crops worldwide (Waterhouse & Norris, 1987; Talekar & Shelton, 1993). The parasitoid has been recorded as one of the major parasitoids of *P. xylostella* from many parts of the world (Waterhouse & Norris, 1987; Mustata, 1992; Wakisaka *et al*., 1992; Ali & Karim, 1995; Kfir, 1997; Usha *et al*., 1997; Liu *et al*., 2000;). *Diadromus collaris* has also been successfully introduced to and established in several countries or regions for enhancing biological control

*Fax: 86 571 86049815
E-mail: shshliu@zju.edu.cn

https://doi.org/10.1079/BER2001129 Published online by Cambridge University Press

of *P. xylostella*, including Australia (Wilson, 1960; Goodwin, 1979; Hamilton, 1979), Barbados, Cook Islands, New Zealand (see Waterhouse & Norris, 1987; Beck & Cameron, 1990), and more recently Malaysia (Ooi & Lim, 1989; Ooi, 1992; Verkerk & Wright, 1996).

Despite its wide distribution and apparent significance in the biological control of *P. xylostella*, little has been reported on the biology of this parasitoid (Verkerk & Wright, 1996). To provide basic information for further research and evaluation of this parasitoid, laboratory studies were made of its biology, and in particular the following three aspects were observed in more detail. First, the mating behaviour and mode of reproduction were observed because there have been conflicting reports on this aspect of the parasitoid (Kfir, 1998; Liu *et al*., 2000). Second, the major population parameters of the parasitoid were examined in relation to temperature because temperature is usually one of the abiotic factors of overarching importance in both laboratory and field. Third, methods of cold storage of both the parasitoid and its host were investigated to provide techniques that will be useful in the rearing of this parasitoid. In addition, observations were made on the interactions between *D. collaris* and *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera: Eulophidae), another major parasitoid of *P. xylostella*. These two species of parasitoids overlap in parasitism of host stages and often occur together in the field (e.g. Kfir, 1997; Liu, *et al*., 2000). Knowledge on their interactions will be essential to the evaluation of both parasitoids.

Materials and methods

Parasitoid and host cultures, and host plants

Plutella xylostella was originally collected from brassica vegetable crop fields in the eastern suburbs of Hangzhou, China in 1995. Founding individuals of both *D. collaris* and *O. sokolowskii* were collected from parasitized *P. xylostella* from the same locality. Stock cultures of *P. xylostella*, *D. collaris* and *O. sokolowskii* were maintained in separate temperature-controlled rooms at 25–30°C with a photoperiod of 14L:10D and 60–80% rh.

The culture of *P. xylostella* was reared on potted common cabbage, *Brassica oleracea* var. *capitata* L. (Brassicaceae), and maintained using the procedures described by Wang *et al*. (1999).

For maintenance of the *D. collaris* cultures, pairs of male and female parasitoids, two to three days post-emergence, were each introduced into a clear, ventilated plastic rearing jar (1000 ml) containing ten young pupae of *P. xylostella* (0–24 h after pupation) for 24 h. Ten percent honey solution was provided as food for the wasps. At the end of each 24 h exposure, the exposed host pupae were collected and kept in the temperature-controlled room until emergence of adult parasitoids of the following generation. In each generation, 25–30 pairs of wasps, chosen randomly from 500–600 wasps of the current generation, were used to start the next generation.

Oomyzus sokolowskii is a larval-pupal parasitoid and oviposits into larvae of all four instars of *P. xylostella* (Wang *et al*., 1999). For convenience in maintaining the stock culture, host larvae of the third and fourth instars were exposed for oviposition by the parasitoid. As a standard procedure, 18 host larvae were placed on a cabbage leaf and exposed to six mated female wasps in an experimental container for 48 h. The container was made of a clear plastic box measuring 14.5 cm in height and 9 cm top diameter and 13 cm base diameter. The top was covered with a fine mesh for ventilation. The cabbage leaf carrying host larvae was fixed to the container base by inserting its petiole through a hole in the centre. The containers were each placed on top of a 500-ml glass bottle containing water, and the petiole of the cabbage leaf was plunged in water to maintain leaf freshness. Ten percent honey solution was provided as food for the wasps. After 48 h exposure, the wasps were taken out and the exposed larvae were reared on the leaves until two to three days after pupation. The pupae were then collected and placed in plastic rearing jars for emergence of adult parasitoids. In each generation, 30–40 female wasps, chosen randomly from over 500 wasps of the current generation, were used to start the next generation.

Observations on mating and reproduction of Diadromus collaris

Virgin males and females of known age were obtained by confining parasitized host pupae individually in small tubes and collecting the newly emerged adults of *D. collaris* every 24 h. Continuous observations were made on the mating process of 40 pairs of males and females, and also made on the oviposition behaviour of ten mated females. Female parasitoids, fed with 10% honey solution or water, were dissected at 0–1, 12–16, 24–28, 36–40 and 48–53 h after emergence to observe the status of ovaries and count the number of mature eggs.

In an attempt to determine the mode of reproduction of this parasitoid, 15 pairs of newly emerged males and females were each confined in a tube for 24 h. Meanwhile, another 30 virgin females were kept in isolation. All female wasps were then each provided with ten young host pupae for oviposition for 24 h. The host pupae exposed were reared to allow parasitoid development from egg to adult emergence of the next generation. All progeny produced by each female were subsequently counted and sexed. Rearing and observations were conducted at 25°C.

Because *D. collaris* has a strong preference for oviposition in young host pupae in which a high proportion of its progeny develop successfully (Lloyd, 1940; Wang & Liu, 1997), only pupae 0–24 h after pupation were used as hosts. Ten percent honey solution was provided as food for adult parasitoids unless otherwise specified.

Effect of temperature on population parameters

Parasitoids and their hosts were kept at nine constant temperatures from 15 to 35°C (± 0.5°C) in controlled 250 l environmental cabinets (see table 2). A photoperiod of 14L:10D in each of the cabinets was provided by eight vertically installed 30 watt fluorescent lamps on both sides and operated by time switches (06:00–20:00). Levels of relative humidity were maintained within the range of 60–80% by providing free water in trays in the cabinets when necessary. Temperature and relative humidity in each of the cabinets were recorded continuously with thermo-hygrographs.

Each temperature experiment was conducted using the following procedure. Two to three days post-emergence,

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mated female parasitoids bred at 25°C were used to start each test cohort. Five to ten female parasitoids were each introduced into a plastic rearing jar containing ten young pupae of *P. xylostella* and placed at the test temperature. Twenty-four hours later, all parasitoids were removed and the exposed host pupae were maintained at the test temperature until emergence of adult parasitoids or host moths. The exposed host pupae were observed twice a day, at 08:00 and 16:00, to record parasitoid emergence. Host pupae that failed to produce either adult parasitoids or moths were dissected to determine (by the presence of a dead parasitoid larva or pupa) whether they had been parasitized.

The relationship between temperature, T, and development rate, $V(T)$, which is defined as the reciprocal of mean development time was investigated using regression analysis. The logistic equation was chosen as the model (Liu & Meng, 1999) and the regression analysis was conducted with STATISTICA® of StatSoft, Inc. To facilitate use of the information, development time data within an appropriate temperature range were further analysed by linear regression between T and $V(T)$ to derive a notional low temperature threshold and a thermal constant (Campbell *et al*., 1974; Liu & Meng, 1999).

To observe the effect of temperature and adult food on level of parasitism and reproduction of the parasitoid, recently emerged female wasps kept at 20, 25 and 30°C were caged with males for 24 h to ensure mating. At each test temperature, single wasps were each provided with ten young host pupae in a rearing jar every 24 h until death. A proportion of females from each of the temperatures was fed with 10% honey solution, while the remainder were fed with water only (see table 3). All exposed host pupae were maintained at their respective test temperatures until emergence of adult parasitoids or moths. Progeny of wasps that resulted from sequential daily exposures were subsequently counted and sexed. Host pupae that failed to produce either adult parasitoids or host moths were dissected to determine whether they had been parasitized.

Effect of storage

Parasitism by adult wasps after cold storage

In a preliminary observation, it was found that female parasitoids kept at 15°C for two to three weeks performed better than those kept at 4°C for the same period of time. An experiment was therefore conducted to assess the performance of female parasitoids after various periods of storage at 15°C. Parasitoids reared at 25°C were kept in rearing jars and moved to 15°C one day after emergence (assuming the females had mated). After various periods of time (see table 4), a sub-sample of females was moved out, and each of the female wasps was provided with ten young host pupae at 25°C for 24 h. At the end of the 24 h exposure to host pupae, the females were dissected to count the number of mature eggs and observe the status of the ovaries. Exposed host pupae were reared at 25°C to determine the number of host pupae parasitized. For comparison, identical observations were made of 15 females kept at 25°C for two days after emergence when tested.

Parasitism by Diadromus collaris *of host pupae after cold storage*

Young pupae of *P. xylostella* that had developed over the temperature range of 20–30°C, were kept at 4°C for various periods of time before they were exposed to female wasps of *D. collaris* for parasitism (see table 5). Two to three days post-emergence, mated female wasps reared at 25°C were used for study. In all trials, host pupae were exposed in groups of ten in rearing jars to single female parasitoids at 25°C for 24 h. The exposed host pupae were reared at 25°C until emergence of host moths or parasitoid wasps. Host pupae that failed to produce either wasps or moths were dissected to determine whether they were parasitized.

Interspecific interactions with Oomyzus sokolowskii

Oviposition response of Diadromus collaris *to young host pupae parasitized by* Oomyzus sokolowskii

Second and late fourth instar *P. xylostella* larvae were collected from the stock culture and placed singly into vials (1 cm diameter × 7.5 cm height). Three mated *O. sokolowskii* female parasitoids were collected from the stock culture one to two days after their emergence and were released into each vial for oviposition. Direct observation was made to determine the deposition of eggs by one of the female wasps. The parasitized host larvae were then transferred onto cabbage leaves placed in experimental containers and maintained at 26°C. The host larvae were observed three times a day at 08:00, 14:00 and 20:00 to collect newly formed pupae.

Newly formed host pupae, which contained either 6- to 24-h-old eggs of *O. sokolowskii* (i.e. hosts parasitized by *O. sokolowskii* in the fourth instar) or six- to seven-day-old immatures of *O. sokolowskii* (i.e. hosts parasitized in the second instar), were collected and placed singly in glass vials. Mated *D. collaris* females (three days after emergence) were then released individually into the glass vials to parasitize the host pupae for 1 h. Meanwhile, *D. collaris* females were exposed singly to young, unparasitized pupae of *P. xylostella* as control treatments. The oviposition activities of *D. collaris* female wasps were recorded by direct observation. Host pupae attacked by *D. collaris* were removed immediately after the wasps had left them. Of the 60 hosts that were parasitized by *O. sokolowskii* at the fourth instar and then exposed to *D. collaris* as young pupae, 15 were dissected 48 h and another 15 were dissected 72 h after oviposition by the former to detect the presence of eggs or larvae of either parasitoids. The remaining 30 were maintained at 26°C until emergence of parasitoid. The number of ovipositor insertions, parasitoid progeny and mortality of parasitoid pupae were recorded. Host pupae that failed to produce parasitoid wasps were dissected to determine whether they had been parasitized by either of the parasitoids.

Oviposition response of Diadromus collaris *to parasitized vs. unparasitized host pupae*

Two young pupae of *P. xylostella*, one unparasitized and the other containing *O. sokolowskii* eggs deposited 24 h previously, were offered to single *D. collaris* females in glass vials. Direct and continuous observations were then made to record the oviposition activities and host pupae attacked by each female. Host attack was determined when a female parasitoid inserted her ovipositor into a host pupa. Each host pupa attacked was removed from the vial as soon as the female had left. In all, 50 female parasitoids were observed.

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The sequence of attack on the two categories of host pupae, the time required from ovipositor insertion to withdrawal, and the subsequent number of parasitoid progeny were recorded.

In a similar experiment, observations were made to determine the effect of the presence of parasitized host pupae on oviposition by *D. collaris* females into unparasitized host pupae. Eight unparasitized host pupae and five host pupae parasitized by *O. sokolowskii* 24 h previously were mixed and placed in a rearing jar. Mated *D. collaris* females (four days post-emergence) were individually released into each of the jars for oviposition. In the control treatment, eight young, unparasitized host pupae were offered to each *D. collaris* female. Twenty-four hours later, female wasps were removed and all exposed host pupae were maintained at 26°C until emergence of parasitoids. Twenty replicates were conducted for both the treatment and the control. The number of host pupae parasitized and subsequently the number of parasitoid progeny produced were recorded.

Oviposition response of Oomyzus sokolowskii *to young host pupae containing eggs of* Diadromus collaris

Single young pupae of *P. xylostella* that had been parasitized by *D. collaris* 1 h previously were exposed to three females of *O. sokolowskii*. Single unparasitized young host pupae were offered to three *O. sokolowskii* females as a control. Twenty-five replicates were conducted for both the treatment and the control. Direct and continuous observations were made for 4 h with each replicate.

Results

Mating and reproduction of Diadromus collaris

As in other ichneumonids, the development cycle of *D. collaris* consists of an egg, a larva and a pupa inside the host and then a free-living adult stage. The two ovaries of a female wasp contained six ovarioles. No mature eggs were present on emergence. When adults were fed with 10% honey solution at 25°C, mature eggs, as judged from their well-formed spindle shape, started to appear < 12h after emergence. From 24 h post-emergence onwards, the females had on average 5–6 mature eggs in their ovaries (table 1). Honey solution taken by the adults promoted the development of eggs, as females provided with honey had nearly twice as many mature eggs as those fed with water only (table 1).

Table 1. Number of mature eggs in ovaries of female *Diadromus collaris* at different ages post-emergence at 25°C, fed with either 10% honey solution or water.

Age in h post-emergence	Honey-fed		Water-fed	
	n	Mean ± S.D.	n	Mean ± S.D.
0–4	20	0	20	0
12–16	20	1.5 ± 1.9		
24–28	20	4.7 ± 1.0	20	2.7 ± 1.4
36–40	20	5.0 ± 2.0		
48–52	20	5.8 ± 1.3	20	3.3 ± 1.6

Both males and females mated successfully within 24–48 h of emergence. Only males actively searched for mates. Copulation lasted 20–60 s, with a mean duration of 36 ± 7 s (n = 40). During a 24 h period, the 30 virgin females produced on average 2.9 wasps for the next generation (all males), while the 15 mated females produced on average 3.5 wasps (59% females).

Effect of temperature on population parameters

Dissection of dead, parasitized pupae allowed detection of the corpses of parasitoids from the second instar onwards. This, coupled with the records of the number of parasitoids that emerged, meant that the data could be used to estimate parasitoid survival from the second instar to adult emergence.

The rates of survival from larva to adult emergence were about 90% between 20 and 28°C, and then declined as temperature either decreased or increased (table 2). The survival decreased to 13% at 15°C and none of the parasitoids survived to adult emergence at 35°C.

Mean development times decreased as temperature increased from 15 to 30°C, and then remained more or less unchanged as temperature further increased to 32.5°C. At 15°C, females took 1.12 times longer to develop to adulthood than males. However, the difference between females and males decreased as temperature increased up to 27.5°C, and then disappeared at higher temperatures. For simplicity, the weighted development times for both males and females were combined for regression analysis of the relationships between temperature, T , and development rate, $V(T)$. The data were described satisfactorily (R^2 = 0.999) by the following logistic equation,

Table 2. Rates of survival and development times from oviposition to adult emergence of *Diadromus collaris* at constant temperatures.

Temp. (°C)	Survival from larva to adult emergence		Development time in days			
			Male		Female	
	n	%	n	Mean ± S.D.	n	Mean ± S.D.
15.0	77	13.3	5	29.1 ± 0.89	5	32.5 ± 1.64
17.5	22	75.0	5	22.1 ± 0.99	11	23.4 ± 1.00
20.0	72	91.2	35	17.5 ± 1.06	30	18.2 ± 1.47
22.5	32	90.9	18	14.3 ± 0.46	11	14.8 ± 0.40
25.0	119	94.9	71	11.9 ± 0.82	42	12.3 ± 0.82
27.5	44	86.7	18	11.1 ± 0.85	20	11.1 ± 0.67
30.0	68	72.7	21	10.1 ± 1.23	28	10.4 ± 0.76
32.5	54	44.3	11	10.4 ± 0.82	13	10.0 ± 0.54
35.0	46	0.0	–		–	

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$$V(T) = \frac{0.1067}{1 + e^{(3.8706 - 0.2012T)}} \quad (1)$$

where e is the base of natural logarithms (fig. 1). Linear regression between T and $V(T)$ with the data from 15 to 30°C resulted in the following equation (r = 0.995),

$$V(T) = 0.4443T - 3.2662 \quad (2)$$

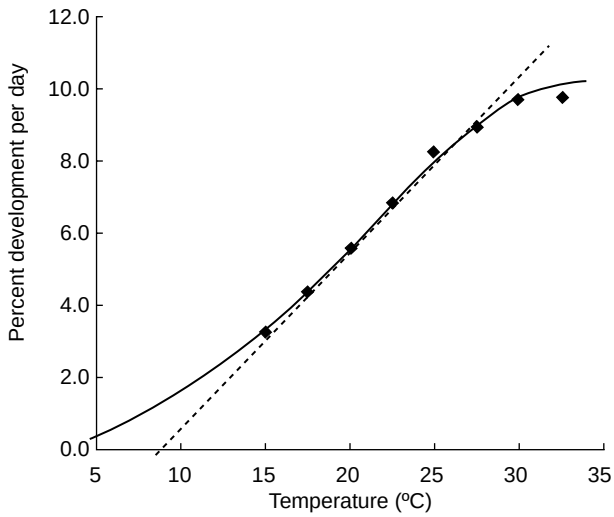

Fig. 1. The relationship between temperature and per cent development per day from egg to adult emergence in *Diadromus collaris*. The data point at 32.5°C was excluded from the linear regression. ◆, Observed; ——, logistic; -------, linear.

From this equation, the low temperature threshold was estimated to be 7.4°C, and the thermal constant to be 225.1 day-degrees (fig. 1).

The data on the performance of adults and their progeny at 20, 25 and 30°C are summarized in table 3. When 10% honey solution was provided as food for the female wasps, they had the longest mean oviposition period of 11.5 days and each parasitized on average 43.7 host pupae at 25°C. The mean numbers of host pupae parasitized were similar between 25 and 30°C, but decreased by about one-third at 20°C. When the females were provided with water only as food, both the oviposition period and the number of host pupae parasitized were significantly reduced at all three temperatures tested. As observed with their parents, the rates of survival from larva to adulthood of the progeny were about 90% at 20 and 25°C but declined to about 70% at 30°C. There were differences in the sex ratio between temperatures. Provision of honey for adult females significantly increased the proportion of females in their progeny at all three temperatures tested, but did not influence progeny survival.

Effect of storage

Parasitism by adult wasps after cold storage

When provided with honey solution, female wasps could be stored at 15°C for up to three to four weeks without any apparent detrimental effects on their reproduction (table 4). A storage period of longer than 28 days not only reduced their parasitism potential but also impaired the development of eggs in the ovaries.

Table 3. Levels of parasitism of *Plutella xylostella* pupae by *Diadromus collaris* in relation to food and temperature.

Temp. (°C)	Food for adult	n	No. of host pupae parasitized (Mean ± S.D.)	Oviposition period in days (Mean ± S.D.)	% survival from larva to adult emergence (Mean ± S.D.)	% females in progeny (Mean ± S.D.)
20	Honey	5	26.0 ± 5.7 b[1]	8.3 ± 2.7 b	91.0 ± 5.2 a	68.3 ± 7.3 a
25	Honey	15	43.7 ± 5.2 a	11.5 ± 1.8 a	95.8 ± 4.0 a	56.8 ± 5.8 b
30	Honey	5	45.5 ± 4.2 a	7.0 ± 2.3 b	74.5 ± 4.4 b	48.1 ± 5.6 c
20	Water	5	6.5 ± 3.6 c	4.0 ± 1.6 c	91.7 ± 6.0 a	43.9 ± 6.4 c
25	Water	5	6.0 ± 3.2 c	4.3 ± 1.6 c	90.9 ± 4.6 a	25.0 ± 7.2 d
30	Water	5	9.0 ± 3.3 c	4.5 ± 1.6 c	70.4 ± 5.6 b	22.2 ± 6.1 d

[1] Means in each column followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test.

Table 4. Level of parasitism and status of ovaries of *Diadromus collaris* adult females after various periods of storage at 15°C.

Days in storage	n	No. of host pupae parasitized per female in 24 h at 25°C (Mean ± S.D.)	No. of mature eggs left in ovaries (Mean ± S.D.)	Status of ovaries, compared to those of females 24–48 h post-emergence
0	15	4.8 ± 0.7 b	3.8 ± 1.6 a	
12	6	5.0 ± 0.5 b[1]	4.7 ± 1.5 a	Healthy, with developing eggs
20	5	6.0 ± 0.5 a	4.5 ± 1.5 a	As above
25	5	4.0 ± 1.0 b	4.8 ± 1.5 a	As above
28	5	3.0 ± 1.0 c	4.7 ± 1.5 a	Healthy, but very few developing eggs
32	5	3.6 ± 1.2 bc	1.4 ± 1.2 b	Somewhat deformed, no developing eggs
36	6	3.0 ± 1.7 c	1.3 ± 0.8 b	Deformed, no developing eggs
40	6	1.8 ± 1.3 c	1.0 ± 1.3 b	As above

[1] Means in each column followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test.

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Parasitism by Diadromus collaris *of host pupae after cold storage*

The rates of survival from larva to adulthood of the parasitoid were little affected when the host pupae were stored at 4°C for up to 25 days prior to exposure for parasitism (table 6). If it is assumed that the rates of survival from egg to the first instar were also little affected, the data on rates of parasitism in table 5 would suggest that the acceptability of host pupae to oviposition by the parasitoid decreased as the duration of pupal storage at 4°C increased. As the duration of storage at 4°C increased, the proportion of host pupae that developed into moths decreased, despite the fact that the corresponding proportion of unparasitized pupae increased (table 5).

By contrast, the survival of the parasitoid from larva to adulthood and the sex ratio of the progeny were not significantly affected by the cold storage of host pupae prior to parasitism (table 6). The reduction in the number of progeny produced with the increase of cold storage duration (table 6) was a result of the corresponding reduction in parasitism, associated with the decrease of acceptability of host pupae to parasitoid oviposition (table 5).

Interspecific interactions with Oomyzus sokolowskii

Oviposition response of Diadromus collaris *to young host pupae parasitized by* Oomyzus sokolowskii

Females of *D. collaris* attacked similar numbers of unparasitized host pupae and host pupae parasitized by *O. sokolowskii* 6–24 h previously (i.e. the host pupae contained *O. sokolowskii* eggs) (table 7). However, when host pupae parasitized by *O. sokolowskii* six to seven days previously (i.e. the host pupae contained *O. sokolowskii* larvae) were provided, the number attacked was significant reduced (table 7). Subsequent observations of the resultant parasitoids indicated that the secondary attacks by *D. collaris* females did not affect the survival of *O. sokolowskii*, but did not produce any progeny of *D. collaris* either (table 7). Dissection of another 30 host pupae, that were parasitized in the fourth instar by *O. sokolowskii* and attacked by *D. collaris* females at the pupal stage, showed that *D. collaris* females did not lay any eggs into the host pupae that had already been parasitized by *O. sokolowskii* (table 8).

Oviposition response of Diadromus collaris *to parasitized vs. unparasitized host pupae*

When *D. collaris* females were exposed to an unparasitized host pupa and a host pupa parasitized by *O. sokolowskii* 24 h previously, the frequency of ovipositor insertions into both types of hosts was similar ($\chi^2 = 0.087$, d.f. = 1, $P > 0.05$) (table 9). However, the durations from ovipositor insertion to withdrawal from parasitized host pupae were 3–5 s, apparently shorter than those (5–8 s) from unparasitized host pupae. Subsequent observation of the resultant parasitoids indicated that no eggs were deposited by *D. collaris* females into host pupae already parasitized by *O. sokolowskii* (table 9). The presence of pupae already parasitized by *O. sokolowskii* was shown to reduce the level of parasitism of unparasitized host pupae by *D. collaris* (table 10).

Table 5. Survival of *Plutella xylostella* pupae after various periods of storage at 4 ± 0.5°C and their susceptibility to parasitism by *Diadromus collaris*.

Storage duration in days	n[1]	% parasitism by *D. collaris* (Mean ± S.D.)	% host pupae that developed to adult moth (Mean ± S.D.)	% dead host pupae[2] (Mean ± S.D.)
0	20	40.4 ± 15.0 a[3]	49.3 ± 26.0 ab	10.3 ± 11 c
5	20	31.3 ± 14.0 b	55.0 ± 20.0 a	13.7 ± 20 c
10	20	25.4 ± 12.7 bc	38.7 ± 11.0 bc	36.0 ± 14 b
15	20	23.2 ± 11.6 bc	32.3 ± 10.0 bc	44.2 ± 18 ab
20	20	20.3 ± 10.5 c	29.4 ± 9.5 cd	50.1 ± 15 a
25	20	19.8 ± 10.0 c	26.7 ± 10.0 d	53.5 ± 11 a

[1] Number of replicates.
[2] Host pupae that were dead without evidence of being parasitized.
[3] Means in each column followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test.

Table 6. Production of progeny by *Diadromus collaris* exposed to pupae of *Plutella xylostella* stored at 4°C for various periods.

Storage duration (days)	n[1]	% survival from larva to adult emergence (Mean ± S.D.)[2]	No. of progeny produced in 24 h (Mean ± S.D.)	% females in progeny (Mean ± S.D.)
0	19	88.0 ± 9.4 a	3.6 ± 1.30 a	42.5 ± 7.6 a
5	19	79.5 ± 16.5 a	2.6 ± 1.20 b	40.8 ± 5.9 a
10	18	81.4 ± 11.7 a	2.4 ± 0.78 bc	45.1 ± 4.7 a
15	19	72.4 ± 14.3 a	1.8 ± 0.79 bc	47.3 ± 6.9 a
20	20	76.5 ± 14.9 a	1.5 ± 0.77 c	38.3 ± 8.3 a
25	19	79.0 ± 15.2 a	1.7 ± 0.78 c	48.5 ± 9.2 [a]

[1] Numbers of replicates that produced parasitoid progeny (20 replicates observed for each storage duration).
[2] Means in each column followed by the same letter are not significantly different at 5% level as determined by Duncan's multiple range test.

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Table 7. Oviposition and parasitism by *Diadromus collaris* of host pupae that had been parasitized by *Oomyzus sokolowskii* during the second or fourth instar.

Host stage at oviposition by *O. sokolowskii*	n	No. of host pupae exposed to *D. collaris*[1]	No. of host pupae attacked by *D. collaris*	No. (%) of host pupae producing adults of	
				D. collaris	*O. sokolowskii*
Second instar	30	30	16 b[2]	0	13 (81.3 a)[3]
Fourth Instar	30	30	25 a	0	20 (80.0 a)[3]
Fourth Instar	30	–	–	–	25 (83.3 a)
Unparasitized pupae	30	30	28 a	24 (85.7)	–

[1]Each pupa was exposed for a fixed period of 1 h.
[2]Figures in the same column followed by the same letter are not significantly different at 5% level as determined by χ^2 test.
[3]Only the host pupae that received the second attack by *D. collaris* were included.

Table 8. Parasitism status of host pupae observed receiving ovipositor insertion first by *Oomyzus sokolowskii* females and then by *Diadromus collaris* females.

Time (h) at dissection[1]	n	Number of host pupae containing		
		O. sokolowskii eggs	*O. sokolowskii* larvae	*D. collaris* egg or larva
48	15	4	11	0
72	15	0	15	0

[1]Time from ovipositor insertion by *O. sokolowskii* females to dissection of host pupae, and the time from ovipositor insertion by *D. collaris* to dissection would be 12–24 h shorter than those listed in the table.

Table 9. Oviposition response of *Diadromus collaris* to *Plutella xylostella* pupae parasitized by *Oomyzus sokolowskii* vs. unparasitized host pupae, and subsequent survival and emergence of parasitoids.

Status of host pupae	n	No. of pupae receiving the 1st ovipositor insertion	No. of pupae receiving the 2nd ovipositor insertion	Total no. that received ovipositor insertion	No. (%) of pupae[2] producing adults of	
					D. collaris	*O. sokolowskii*
Parasitized	50	19 (+6)[1]	22	47	0 (0.0)	39 (83.0)
Unparasitized	50	22 (+3)	19	44	38 (86.4)	–

[1] The figures in brackets show the number of pupae in those observations where the female parasitoid inserted her ovipositor into either only the parasitized or the unparasitized pupa within the 4 h period of observation.
[2] Only those pupae that received ovipositor insertion by *D. collaris* were included in the calculations.

Table 10. Effect of the presence of host pupae parasitized by *Oomyzus sokolowskii* on the oviposition capacity of *Diadromus collaris* in unparasitized host pupae.

Host treatment	n	% parasitized (Mean ± S.D.)	No. of progeny per female[3] (Mean ± S.D.)	% of female progeny[2]
Parasitized + unparasitized	20	33.8 ± 15.2[1] a[2]	2.5 ± 1.1 a	43.4 a
Unparasitized only	20	51.3 ± 21.4 b	3.4 ± 1.4 b	41.8 a

[1] % parasitism of initially unparasitized pupae.
[2] Figures in each column followed by the same letter are not significantly different at 5% level as determined by Student-*t* test.
[3] Figures for *D. collaris* only.

Oviposition response of Oomyzus sokolowskii *to young host pupae containing eggs of* Diadromus collaris

In all, 75 *O. sokolowskii* females were observed each for 4 h for their response to host pupae parasitized by *D. collaris*, and another 75 females were observed for their response to unparasitized host pupae. In both cases, no act of ovipositor insertion was observed.

Discussion

Observations on the mating and reproduction of *D. collaris* in this study showed that the Hangzhou population of this parasitoid is arrhenotokous. *Diadromus collaris* has been reported to be arrhenotokous in France (Kalmes & Rojas-Rousse, 1988) and South Africa (Kfir, 1998), and recently for a population from Malaysia (Zhang *et al*., 1998),

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although the observation of *D. collaris* in France was associated with another plutellid moth as the host. A population of *D. collaris* in Queensland, Australia and a population of the parasitoid from Lishan, Taiwan, have been observed by us to be arrhenotokous (unpublished data). The populations of *D. collaris* in Australia and Malaysia were originally introduced from England via New Zealand (Wilson, 1960; Waterhouse & Norris, 1987; Ooi, 1992). The population in Taiwan may be native to the island because there has been no record of introduction of the parasitoid to Taiwan (N.S. Talakar, Asia Vegetable Research and Development Centre, personal communication, 1999) and because the parasitoid is widely distributed on the mainland of China (He, 1998; Liu *et al*., 2000). All these records and observations suggest that *D. collaris* is arrhenotokous over its wide geographic distributions in Europe, Asia and South Africa. It is thus confusing to see that Kfir (1998) states that *D. collaris* is arrhenotokous in South Africa but thelytokous in Europe, and then speculates that the parasitoid may have evolved from South Africa and dispersed to Europe.

In our laboratory, similar experiments to determine the effect of temperature on population parameters have also been conducted with *O. sokolowskii* (Wang *et al*., 1999) and *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) (Shi & Liu, 1999). Some broad comparison of temperature responses may be attempted between these three parasitoid species of *P. xylostella*. The rates of survival from larval to adult at 15°C and 35°C, the lowest and the highest constant temperatures examined for the three species, were 13% and 0% for *D. collaris*, 0% and 9% for *O. sokolowskii*, and 27% and 20% for *C. plutellae*, respectively (table 2; Shi & Liu, 1999; Wang *et al*., 1999). The data indicate that *C. plutellae* can survive and develop over a wider range of temperatures than either *D. collaris* or *O. sokolowskii*. The amplitude of temperature range for survival of *D. collaris* is similar to that of *O. sokolowskii*, but the temperatures are lower for *D. collaris* than for *O. sokolowskii*. Thus, areas with moderate temperatures, such as highland areas in the tropical regions, would be more suitable for the population development of *D. collaris* than areas with high temperatures. This inference seems to be supported by the observations in Hangzhou, China, an area of high summer temperatures up to 35–40°C, where *D. collaris* has been less abundant than both *C. plutellae* and *O. sokolowskii* (Liu *et al*., 2000).

The current results showed that *D. collaris* females emerge with no mature eggs in their ovaries, and egg maturation occurs only after emergence. Thus this species is synovigenic-anautogenous in egg development and maturation (Jervis & Kidd, 1996). Provision of food for adult females was shown to be critical for the promotion of egg development and parasitism in this parasitoid (tables 1 and 3). Manipulation of crop systems to provide sufficient nectar for *D. collaris* adults may be essential in the augmentation of this parasitoid for the control of *P. xylostella* in the field.

The observations on the interactions between *D. collaris* and *O. sokolowskii* showed that females of the former can discriminate between unparasitized host pupae and host pupae parasitized by the latter, apparently by internal examination with their ovipositor, and do not oviposit in already parasitized hosts. Lloyd (1940) reported that *D. collaris* discriminated between unparasitized prepupae of *P. xylostella* and prepupae parasitized by *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae). Females of *O. sokolowskii* did not oviposit into pupae of *P. xylostella* that had already been parasitized by *D. collaris*. In fact, *D. collaris* females rarely attempted to oviposit into pupae of *P. xylostella* even if the latter were unparasitized, as was shown in this study as well as in earlier studies by Wang & Liu (1997) and Wang *et al*. (1999). These observations indicate that direct competition between *D. collaris* and *O. sokolowskii* through parasitism of the same host individual would very rarely, if ever, occur, especially in the field where the parasitoids can move freely. Thus, coexistence of both *D. collaris* and *O. sokolowskii* in the same crop system can be expected to be beneficial to the biological control of *P. xylostella*. Introduction of *D. collaris* into new areas to supplement control of *P. xylostella* by *O. sokolowskii* can be safely recommended. However, introduction of *O. sokolowskii* into new areas to supplement control of *P. xylostella* by *D. collaris* requires careful consideration, because *O. sokolowskii* has a less restricted host range and has been shown to be a facultative hyperparasitoid of *P. xylostella* (Verkerk & Wright, 1996; Liu *et al*., 2000).

Acknowledgements

This study forms part of an Australian–China cooperative project 'Improvement of Integrated Pest Management of Brassica Vegetable Crops in China and Australia' (ACIAR CS2/1992/013), funded principally by the Australian Centre for International Agricultural Research and also by the China National Natural Science Foundation (Project No. 39870505). We are grateful to Dr Myron Zalucki and Dr Mike Furlong, Department of Zoology and Entomology, the University of Queensland, Australia for comments on an earlier version of the article.

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(Accepted 22 July 2001)
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https://doi.org/10.1079/BER2001129 Published online by Cambridge University Press

The Convention on Biological Diversity and Product Commercialisation in Development Assistance Projects: A Case Study of LUBILOSA

D Dent, *CABI Bioscience, Ascot, UK* and **C Lomer**, *Royal Veterinary and Agricultural University, Copenhagen, Denmark and formerly of the International Institute of Tropical Agriculture, Cotonou, Benin*

Biopesticides Series, No. 3

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£17.95 (US$30.00)

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The LUBILOSA (Lutte Biologique contre les Locustes et Sauteriaux) Programme was initiated in 1989 and has been successful in developing a bioinsecticide for the biological control of locusts and grasshoppers. The efficacy of the product named Green Muscle has been clearly demonstrated in Africa, and provides an environmentally friendly alternative to chemical insecticides. Although it predates the Convention on Biological Diversity, LUBILOSA has been conducted in accordance with the benefit sharing and related provisions of the Convention.

This book provides a review of the programme in order to demonstrate how such research and product commercialisation can be accomplished in the context of a development assistance project. In particular it shows how the provisions of the Convention can be fulfilled with respect to:

- Equitable sharing of research results and benefits
- Access to and transfer of technology
- Exchange of information
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