International Journal of Pest Management, July–September 2007; 53(3): 253–257

Effects of low temperatures on pupal survival of the stone leek leafminer *Liriomyza chinensis* (Diptera: Agromyzidae)

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Abstract

The cold-hardiness of *Liriomyza chinensis* pupae was measured in the laboratory by observing pupal mortality at low temperatures. Pupal mortality increased with decreasing temperature and with extended cold exposure time. No recently pupated pupae (6 h) were able to survive after 16 days of chilling at 0, 2.5 and 5°C, but 42.9% survival was observed at 10°C. Pupae at different developmental stages showed significant difference in mortality, with very low levels of mortality observed for older pupae (4 and 7 days) after exposure to 0°C for 16 days. The lethal time for 50% survival (LT_{50}) increased with increasing age of pupae. LT_{50} for 4-day-old pupae exposed to 0°C was 52.1 days. Analysis of mean temperatures at several localities indicated that *L. chinensis* is able to overwinter outdoors in southern regions, but is unable to overwinter in open fields in northern regions of Japan, suggesting that overwintering in these regions would only occur in greenhouses.

Keywords: *Leafminer,* Liriomyza chinensis, *low temperature, overwintering, cold survival*

1. Introduction

The stone leek leafminer, *Liriomyza chinensis* (Kato) (Diptera: Agromyzidae), is a major pest on *Allium* spp. in many countries including China, Malaysia, Singapore, Thailand (Spencer 1973, 1990; Chen et al. 2003), Korea (Hwang and Moon 1995), Vietnam (Andersen et al. 2002; Tran and Takagi 2005), Taiwan (Shiao 2004) and Japan (Yamamura 2004). The first record in Japan was in 1949 in Yamagata Prefecture where Japanese bunching onion (*Allium fistulosum* L.) and Chinese chive (*Allium bakeri* Regel) were attacked (Yamamura 2004). The species is now common throughout Japan, attacking Japanese bunching onion and common onion (*Allium cepa* L.) both outdoors and in greenhouses (Tokumaru and Okadome 2004; Yamamura 2004).

In Japan, *L. chinensis* population numbers have been observed to peak from August to September in Yawata City (34°N, 135°E), from late June to August in Kyoto City (35°N, 135°E) (Tokumaru and Okadome 2004) and May to September in Fukuoka Prefecture (33°N, 131°E) (Yamamura 2004). Population numbers declined during the winter until December and no specimens of *L. chinensis* were observed in the field from January to April (Tokumaru and Okadome 2004). During summer and autumn, the generations of the leafminer often overlap, and so all leafminer stages may be present in an area concurrently. All stages of *L. chinensis* can survive the winter in greenhouses. The first adult leafminers to appear in the field may be either offspring of the adults that overwintered in greenhouses or flies derived from overwintering pupae in the field. It is not yet clear whether there is a physiological diapause or just slow development of the leafminer pupae over winter.

Bale (1991) suggested that winter survival and cold hardiness are the primary factors governing subsequent population levels of some temperate insect species. While cold tolerance has been used effectively to measure the ability of pupae of *Liriomyza sativae* (Branchar) and *Liriomyza huidobrensis* (Branchard) to overwinter outdoors (Van de Linden 1993; Zhao and Kang 2000; Chen and Kang 2004, 2005a), there is limited published information available for *L. chinensis* (Tagae and Ohtomo 2002). The aim of this study was to investigate the effect of low temperatures on survival of *L. chinensis* pupae and to determine the overwintering potential of the leafminer in Japan.

2. Materials and methods

2.1. Insect rearing

Liriomyza chinensis used here came from a culture reared by the Fukuoka Agricultural Research Center, Fukuoka, Japan, since 2002 from flies collected from commercial onion fields in Fukuoka Prefecture. The leafminer was reared on Japanese bunching onion, *Allium fistulosum* L., as described by Tran and Takagi (2005). Seeds were sown in a tray (20 × 60 × 15 cm) in a potting soil (40% water content, pH 5.5–6.5,

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ISSN 0967-0874 print/ISSN 1366-5863 online © 2007 Taylor & Francis
DOI: 10.1080/09670870701472157

0.035%N, 0.123%P, 0.018%K). Two months after germination, single plants were transplanted into plastic pots (9 cm in diameter). Trays (32 × 44 × 6 cm) each containing 15 potted plants were placed in a small greenhouse at 20 ± 5°C and 60 ± 10% humidity.

Six potted plants at two to three-leaf stage were exposed to 50 mixed sex *L. chinensis* adults (about 1:1 sex ratio) in a plastic cage (45 × 30 × 25 cm) covered with a fine nylon mesh. After an exposure for 24 h, the flies were removed and these plants were maintained in an environmental chamber at a constant temperature of 25°C and a light:dark regime of 16:8 h until all leafminer larvae feeding on the plants emerged and pupated. Before incubation, the upper opening of each pot was covered with a piece of reversed funnel-shaped filter paper (11 cm in diameter) to prevent leafminer larvae from pupating in the soil. The pupae were transferred to Petri dishes (9 cm in diameter) lined with filter paper. These dishes were maintained at the same conditions and supplied with some drops of water for maintaining appropriate humidity (60–70%) for adult leafminer emergence.

2.2. *Effects of low temperature on survival of pupae*

The effects of low temperatures on pupal *L. chinensis* were examined using similar methods to Zhao and Kang (2000). Larvae were collected immediately after emergence from leaves and placed in plastic boxes (23 × 17 × 6 cm) containing humid tissue papers and covered with a fine nylon mesh. After pupation, they were kept in an incubator at 25°C for 6 h. Then, a group of five randomly selected pupae was placed in a Petri dish (6 cm in diameter) lined with filter paper and a 1.5-cm square of cotton soaked in water attached to the bottom of the dish. These dishes were exposed to low temperatures of 0, 2.5, 5, 7.5 or 10°C under a 16:8 h light:dark regime. The temperature was lowered at a rate of 0.5°C/min from the beginning (25°C) to the target temperatures (0, 2.5, 5, 7.5 and 10°C; temperature fluctuation of ±0.5°C). At each temperature treatment, groups of pupae were exposed for 1, 4, 7, 10, 13 and 16 days, respectively. After the required exposure, the temperature was increased at a rate of 0.5°C/min to 25°C and the pupae were then held until adult emergence. The control groups of pupae were kept continually in an incubator at 25°C, 16:8 h light:dark regime (i.e. without cold treatment). The numbers of emerged adults were counted daily. Checking adult emergence was ceased when no adult was emerged for 10 days. There were five replications (20 pupae in four Petri dishes per replicate) for each treatment.

2.3. *Effects of pupal age on low temperature survival*

Larvae were collected immediately after emergence from leaves and placed in a plastic box (23 × 17 × 6 cm) containing moistened tissue paper and covered with a fine nylon mesh. After pupation, they were kept in an incubator at 25°C, 16:8 h light:dark regime for 6 h, 1, 4 or 7 days. Then, 5 pupae were placed into a Petri dish (6 cm in diameter) lined with filter paper and a 1.5-cm square of cotton soaked in water attached to the bottom of the dish. These dishes were maintained at 0°C for 1, 4, 7, 13 and 16 days. The temperature was lowered at a rate of 0.5°C/min from the beginning (25°C) to 0°C (temperature fluctuation of ±0.5°C). After cold treatment, the temperature was increased at a rate of 0.5°C/min to 25°C and kept until adult emergence. The numbers of emerged adults were counted daily. Checking adult emergence was ceased when no adult was emerged for 10 days. There were five replications (20 pupae in four Petri dishes per replicate) for each treatment.

2.4. *Statistical analysis*

The survival and corrected percentage mortality of pupae were calculated as described by Zhao and Kang (2000). The percentages of mean mortality dependent temperature and duration of exposure time were arcsine-transformed and then analysed by two-way ANOVA, StatView ver. 5.0 (SAS Institute Inc. 1998). The time of median survival (LT_{50}) was estimated by probit analysis, SPSS ver. 12.0 (SPSS Inc. 2003).

3. Results

3.1. *Effects of low temperature on survival of pupal* L. chinensis

Low temperature and cold exposure time had significant effects on mortality of 6-h-old pupae (temperature: $F(3,72) = 18.014$, $P < 0.0001$; time: $F(5,72) = 74.434$, $P < 0.0001$). There were also significant temperature × time effects on mortality of the pupae ($F(15,72) = 4.936$, $P < 0.0001$). Low temperatures increased pupa mortality. After 4 days exposure, the pupal mortality was 57.5, 41.5, 24.6 and 24.4% at 0, 2.5, 5 and 10°C, respectively. Mortality of the pupae increased with the extended cold exposure time. When the pupae were exposed to 0, 2.5 and 5°C for 1 day, the mortality was 16.8, 10.9 and 13.2%, respectively. When the exposure at these temperatures was extended to 16 days or longer, almost all pupae were killed. However, when exposed to 10°C for 16 days, 42.9% of the pupae survived.

Results of the probit analysis of survival of 6-h-old pupae to exposure time at 0, 2.5, 5 and 10°C (LT_{50}, slopes and intercepts of the time-mortality lines) are given in Table I. The time of median survival (LT_{50}) increased with the increasing temperatures exposed. LT_{50} was 3.7, 5.2, 6.8 and 11.6 days for an exposure at 0, 2.5, 5 and 10°C, respectively. The logistic regression of the survival of 6-h-old pupae at

0 ($\chi^2 = 18.63$, df = 4, $P = 0.001$), 2.5 ($\chi^2 = 12.06$, df = 4, $P = 0.017$) and 5°C ($\chi^2 = 35.804$, df = 4, $P \leq 0.0001$) as a function of exposure time was highly significant. The logistic regression of the survival of 6-h-old pupae at 10°C as a function of exposure time was not significant ($\chi^2 = 3.692$, df = 4, $P = 0.459$).

3.2. *Effects of pupal age on low temperature survival*

When exposed to 0°C, pupae of different stages had highly significantly different mortalities ($F(3,72) = 157.764$, $P < 0.0001$) and the mortality of each age group was significantly different with the extended exposure time at 0°C ($F(5,72) = 30.335$, $P < 0.0001$). The interaction between pupal age stages and exposure time at 0°C on mortality was also highly significant ($F(15,72) = 10.977$, $P < 0.0001$). Recently pupated and 1-day-old pupae had high mortality. When exposed for 10 days, the mortality of pupae aged 6 h, 1, 4 and 6 days was 87.7, 55.7, 10.6 and 6.4%, respectively . Mortality of 6-h-old and 1-day-old pupae increased with the extended cold exposure time. When the pupae were exposed for 4 days, the mortality of 6-h-old and 1-day-old pupae was 57.5 and 24.2%. When extended exposures 16 days or longer, the mortality reached 95.9 and 85.4% in 6-h-old and 1-day-old pupa groups. For other pupa stages aged 4 and 7 days, there was very low mortality after exposed to 0°C.

Probit parameters of time-response for different pupal stages exposed to 0°C are given in Table II. LT_{50} was increased with the increasing age of pupae. LT_{50} was 3.7, 7.8 and 52.1 days for pupae age 6 h, 1 and 4 days, respectively. For 7-day-old pupae, LT_{50} could not be calculated because most pupae survived at 0°C. The logistic regression of the survival of 6-h-old and 1-day-old pupae at 0°C as a function of exposure time was highly significant (6-h-old pupae: $\chi^2 = 18.63$, df = 4, $P = 0.001$; 1-day-old pupae: $\chi^2 = 30.57$, df = 4, $P < 0.0001$). The logistic regression of the survival of 4-day-old ($\chi^2 = 2.256$, df = 4, $P = 0.689$) and 7-day-old ($\chi^2 = 0.73$, df = 4, $P = 0.947$) pupae at 0°C as a function of exposure time was not significant.

4. Discussion

Low temperature mortality is an important factor governing the year-to-year abundance and distribution of agromyzid leafminers (Bale 1991; Chen and Kang 2005b). However, the results of this study indicate that low temperature is not a major factor limiting the distribution of *L. chinensis* in Japan, as most 7-day-old pupae of *L. chinensis* survived after being exposed to 0°C for 16 days, and LT_{50} of 4-day-old pupae exposed to 0°C was 52.1 days. This result is consistent with a previous study indicating that *L. chinensis* pupae could survive the winter in Iwate Prefecture, Northern Japan as about 90% of 37-day-old pupae were alive after exposure to 0°C for 60 days (Tagae and Ohtomo 2002).

Zhao and Kang (2000) reported that *L. sativae* could survive the winter in Nanjing and Shanghai at latitudes 30–32°N. *Liriomyza huidobrensis* is also well-adapted to overwinter at high latitudes (Parrella 1987; Van de Linden 1993; Chen and Kang 2004; Martin et al. 2005). The difference in geographic distribution and phenology between *L. huidobrensis* and *L. sativae* was found to be closely associated with their distinct levels of cold resistance (Chen and Kang 2004).

Our analysis of mean winter temperature data (Table III) and cold tolerance results revealed that *L. chinensis* could overwinter outdoors in most areas of Japan. The successful survival after cold chilling of *L. chinensis* pupae in the laboratory and possible increasing survival due to fluctuating low temperatures in the field suggests that the leafminer is likely to

Table I. Probit parameters of survival of 6-h-old pupae of *L. chinensis* to exposure time at different low temperatures.

Temperature (°C)	Intercepts[a]	Slope[a]	LT_{50}[b]	χ^2[c]
0	0.634 (0.112)	−0.172 (0.015)	3.7 (0.37–5.9)	18.63 (4, 0.001)
2.5	0.759 (0.109)	−0.147 (0.013)	5.2 (2.3–7.1)	12.06 (4, 0.017)
5	1.356 (0.12)	−0.199 (0.014)	6.8 (3.3–9.7)	35.804 (4, <0.0001)
10	0.974 (0.109)	−0.084 (0.011)	11.6 (10.3–3.3)	3.692 (4, 0.459)

[a]Standard error; [b]95% fiducial limit; [c]df, *P*. LT_{50} is defined as the time of median survival.

Table II. Probit parameters of survival of different pupa stages of *L. chinensis* to exposure time at 0 C.

Stage	Intercepts[a]	Slope[a]	LT_{50}[b]	χ^2[c]
6 h	0.634 (0.112)	−0.172 (0.015)	3.7 (0.37–5.9)	18.63 (4, 0.001)
1 day	1.218 (0.114)	−0.156 (0.012)	7.8 (4.1–11.1)	30.57 (4, <0.0001)
4 days	1.301 (0.13)	−0.025 (0.013)	52.1 (30.5–2773.6)	2.256 (4, 0.689)
7 days	1.147 (0.127)	0.004 (0.013)		0.73 (4, 0.947)

[a]Standard error; [b]95% fiducial limit; [c]df, *P*. LT_{50} is defined as the time of median survival.

Table III. The mean winter temperatures (°C) of selected localities in Japan[a].

Locality	Latitude	Longitude	December	January	February	March
Sapporo	43°N	141°E	−1.1	−3.8	−3.5	0.2
Sendai	38°N	141°E	4.3	1.5	1.8	4.6
Nigata	38°N	139°E	5.3	2.5	2.6	5.5
Nagoya	34°N	137°E	6.7	4.3	4.8	8.3
Tokyo	35°N	140°E	8.4	5.9	6.1	9.0
Hiroshima	34°N	132°E	7.1	4.9	5.3	8.6
Osaka	35°N	136°E	8.3	5.8	6.0	9.1
Fukuoka	33°N	131°E	8.7	6.5	6.9	10.0
Kagoshima	32°N	130°E	10.0	7.9	8.9	12.2
Takamatsu	34°N	134°E	7.5	5.3	5.5	8.5
Naha	26°N	128°E	18.5	16.6	16.7	18.6

[a]The data were calculated during 1971 – 2003 (National Astronomical Observation 2004).

be able to overwinter outdoors in southern regions of Japan. The field population of the stone leek leafminer in the southern areas of Japan would be derived from the overwintering population which recolonizes the field early in the growing season. In these areas, leafminer management in the spring must be focused on destroying leafminer pupae in the soil by field sanitation or soil fumigation as well as by controlling the leafminer populations in the greenhouses.

Although there is currently no information on cold survival ability of different geographic populations of *L. chinensis* in Japan, it appears to be very difficult for *L. chinensis* to overwinter in the northern region surrounding Sapporo, as most *L. chinensis* pupae were killed after exposed to 5°C for 16 days and the mean temperature of the areas was sub-zero during the winter. We conclude that in northern Japan winter survival of the leafminer is possible only in heated greenhouses, and that the first adult generation of the leafminer in the field must be offspring of the adults that overwintered in the greenhouses. Since the leafminer cannot overwinter in the northern region, the control strategy in this region must be to prevent the spread of the leafminer from heated greenhouses in the growing season (e.g. by avoiding transplanting infested seedlings).

Acknowledgement

We thank Mr Hiroyuki Takemoto, Fukuoka Agricultural Research Centre, Japan for providing *L. chinensis*. We gratefully acknowledge the constructive comments given by Dr Peter Ridland, Victoria, Australia to an early version of the manuscript. This work was supported in part by a Grand-in-Aid from the Japanese Society for the Promotion of Science and Technology (No. 15208007).

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