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Impact of Cold Exposure on the Mortality of *Tuta absoluta* Pupae

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ABSTRACRT

The tomato leafminer, *Tuta absoluta* is a devastating invasive pest that poses a serious threat to tomato crops worldwide. Its extensive global dispersion serves its capacity to adapt to variations in climate conditions. In this context, the pupa is the most resistant stage to prolonged exposure to cold temperatures. Therefore, indicators of cold resistance were studied in overwintering pupae collected from the field and pupae reared under two constant conditions, high temperature, and long day (25°C, 16:8 L:D and 65±5% RH), and low temperature and short day (15°C, 13:11 L:D and 65±5% RH). The results show that the super cooling point (SCP) significantly decreased in December (-20.5±1.2°C) and January (-20.26±0.78°C) with a decrease in temperature. In the laboratory, the decrease in temperature and photoperiod increased the tolerance of pupae to subzero temperatures. Lethal Temperature 50 (LT_{50}) and LT_{90} of pupae collected in the field were recorded at -13.70 to -10.23°C and -18.73 to -15.37°C, respectively. A comparison of lethal temperatures with the lowest ambient temperature in December and January indicated that *T. absoluta* has a high overwintering potential in Karaj, Alborz Province, Iran, and can easily survive cold winters.

Keywords: Cold hardiness, Lethal temperature, Supercooling point, Tomato leafminer.

INTRODUCTION

The tomato leafminer, *Tuta absoluta*, is a highly destructive pest that poses a significant threat to both cultivated and wild Solanaceous plants (Tumuhaise *et al*., 2016). The larvae of this pest cause extensive damage to the leaves, stems, terminal buds, and fruits of tomato crops, resulting in yield losses of up to 90% in both greenhouse and field conditions (Desneux *et al*., 2010, 2011; Bloem and Spaltenstein, 2011; Biondi *et al*., 2018). Since its first detection in South America, the pest has rapidly spread to other regions, with its first appearance in Europe in 2006 (Desneux *et al*., 2010; Allache *et al*., 2015; Duarte *et al*., 2015), however, it was first reported from Urmia, Iran, in 2010 (Baniameri and Cheraghian, 2012).

The global distribution of *T. absoluta* suggests that it is capable of adapting to low temperatures (Bloem and Spaltenstein, 2011; Biondi and Desneux, 2019). Therefore, the potential for overwintering in regions with cold winters should be taken into consideration. The invasion and establishment of *T. absoluta* highlights its ability to disperse and adapt to new environmental conditions (Bloem and Spaltenstein, 2011), owing to its high reproductive potential, broad host range, and thermal adaptation (Van Damme *et al*., 2015). In the native areas as well as in the areas where *T. absoluta* was initially established, temperature fluctuations were minimal. However, as the pest's distribution expanded, its ability to tolerate low temperatures has become important.

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Temperature plays a critical role in establishing new species (Renault *et al*., 2018; Denlinger and Lee, 2019; Lee *et al*., 2019). In this study, monitoring the last larval stages in the field showed that the insect spends unfavorable seasons as pupae in the soil cocoons. Therefore, pupae were considered for further study. To survive the adverse season, insects during winter may require cold hardiness (Bale, 1996). Many insects in response to subzero temperatures increase their super cooling capacity by lowering the supercooling points (SCPs) (Lee, 1991; Bale, 2002; Lee, 2010; Andreadis and Athanassiou, 2017). Several studies have examined the cold-hardiness of various populations of *T. absoluta* (Van Damme *et al*., 2015; Kahrer *et al*., 2019; Tarusikirwa *et al*., 2020a, b; Li *et al*., 2021). In Belgium, Van Damme *et al*. (2015) investigated the SCP and Lethal Temperatures (LTs) of *T. absoluta*. These experiments demonstrated that the insect exhibited cold hardiness in response to subzero temperatures. Given the diverse climate of Iran and the significance of tomato as a primary product, it is imperative to investigate its ability to withstand cold temperatures.

In the present study, the SCPs of overwintering pupae were measured in autumn and winter. In addition, the lower Lethal Temperatures (LTs) and survival rate of overwintering pupae were estimated at temperatures ranging from 0 to -20°C with an exposure time of 2 hours.

MATERIALS AND METHODS

Insects

The third and fourth instar larvae were collected from an infected tomato field in Karaj, Alborz Province, Iran (35° 7743 N, 50° 9068 E) in October 2019. The insects were kept in transparent containers with sand in the bottom, and a net was placed on the top for ventilation. Some tomato branches wrapped with moistened cotton and a transparent plastic cover at the end of the stems were placed in each container, and the larvae were transferred to these branches. The experimental containers were placed in the field, and water was added to the cotton every two days to maintain leaf moisture. After completion of the larval period, the *T. absoluta* pupated in the sand (Figure 1). Until the adult emergence in the field, pupae were carried out in the laboratory monthly to determine SCP and survival. The insects used in laboratory experiments were reared on tomato leaves (var. Super2270) under two different conditions: a long-day condition of 16:8 (Light: Darkness) at 25°C, 65±5% Relative Humidity (RH), and a short-day condition of 13:11 (L:D) at 15°C, 65±5% RH.

Figure 1. Pupation of *T. absoluta* in the soil cocoons. Before pupation, the larvae in the soil cover the soil particles on the cocoon and pupate inside it.

Supercooling Point Determination

The SCP was measured with a thermocouple (NiCr-Ni probe) connected to an automatic temperature recorder (Measurement Computing, USB-5203, USA). Samples were taped to a thermocouple and placed in a programmable test chamber (Binder, MK53, Lenzkirch, Germany). The temperature was decreased at a rate of 0.5°C min^{-1}, starting at 25°C and ending at -30°C. The temperature was recorded at 30-second intervals using Data Acquisition (DAQ) software. The SCP was determined as the lowest temperature, after which latent freezing heat was released (Lee, 1991).

Effect of Low Temperature on the Survival of Overwintering Pupae

Overwintering pupae and pupae under constant conditions of 25°C, 16:8 L:D, and 15°C, 13:11 L:D, with 65±5%RH were placed in a programmable test chamber. (Binder GmbH, MK53, Tuttlingen, Germany). The temperature was decreased at a rate of 0.5°C min^{-1}, starting at 25°C and ending at 0, -5, -10, -15, -17, and -20±0.5°C. After 2 hours of exposure to the desired treatment temperature, it returned to the initial temperature at 0.5°C min^{-1}. After the treatment, pupae were maintained at rearing temperature to determine the survival rate. Pupae that were darker and dry and from which no adult emerged were considered dead. The 50 and 90% lethal temperatures were calculated.

STATISTICAL ANALYSIS

The SCP and survival rates of overwintering pupae were analyzed using a one-way Analysis Of Variance (ANOVA). Tukey's honest significance test was conducted to compare means with a significance level of P< 0.05. Statistical analysis was performed using SPSS version 26.0 software (SPSS Inc., Chicago, IL, USA).

The pupal mortality data were subjected to a binary logistic regression model to calculate lethal temperature 50 and 90°C after a 2-hour exposure to subzero temperatures. The lethal temperatures were calculated by the following equation:

$$Y = Ln\frac{p}{1-p}$$

$$LT50 = \frac{Y-a}{b}$$

Where, p is the survival rate; a and b are constant and temp in the logistic regression results.

RESULTS

Supercooling Points and Lower Lethal Temperatures

Supercooling point differed significantly between months (F= 3.907; df t, e= 4, 40; P= 0.01). SCPs of individuals varied from -11.36 to -26.18°C. Mean monthly SCPs varied from -17.06±1.14°C in November to -21.78±1.31ºC in October (Figure 2).

The LT_{50} and LT_{90} of pupae under laboratory conditions were higher than those under field conditions. The highest LT_{50} and LT_{90} recorded at 25°C were -3.53 and -6.81°C, and the LT_{50} and LT_{90} at 15°C were -8.40 and -13.21°C, respectively. In the field, the LT_{50} ranged from -13.70 to -10.23°C, and the LT_{90} ranged from -18.73 to -15.37°C. The highest LT_{50} and LT_{90} were measured in January. The LT_{50} was lower in November and December, increased in January, and decreased in February. The lowest LT_{90} was observed in December (-18.73°C) (Table 1).

Survival Rates in the Field-Collected and Lab-Reared Pupae

In field-collected pupae, at -5°C, 20% mortality was observed only in December. At -10°C, the lowest survival rate was

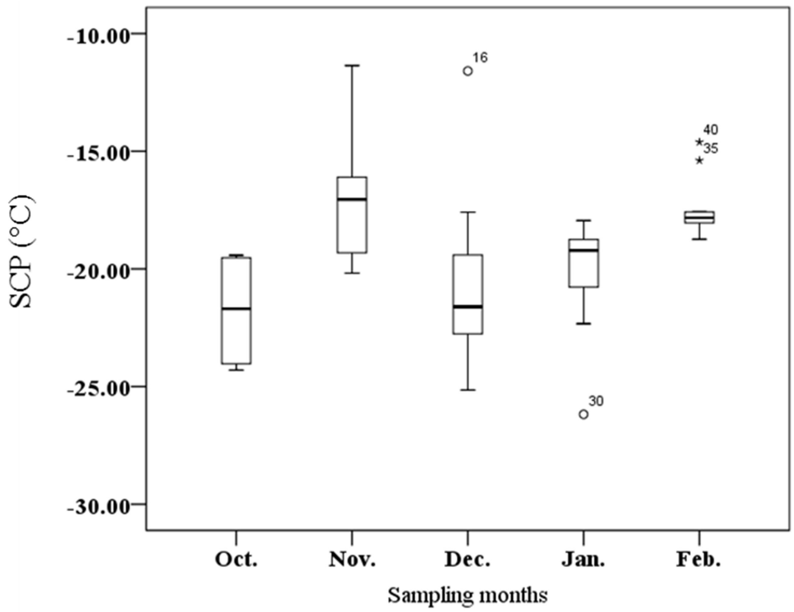

Figure 2. Supercooling points of field-collected pupae of *Tuta absoluta* in 2019.

Table 1. The LT_{50} and LT_{90} of *Tuta absoluta* pupa under field and laboratory condition.[a]

Location	Month	Temp	LT_{50} (°C)	95% CL (°C)	LT_{90} (°C)	95% CL (°C)
Field collected	November		-12.53	-12.38, -10.66	-15.77	-17.09, -11.18
	December		-12.32	-14.29, -11.14	-18.73	-20.49, -17.23
	January		-10.23	-10.25, -9.82	-15.37	-17.34, -10.37
	February		-13.70	-15.07, -12.75	-16.62	-17.05, -15.31
Lab reared		25 °C	-3.53	-3.42, -2.58	-6.81	-9.46, -2.89
		15 °C	-8.40	-9.53, -6.74	-13.21	-14.59, -10.05

[a] LT: Lethal Temperature, CL: 95% Lower and upper Confidence Limits.

observed in January. At -15°C, less than half of the individuals survived in all field samples, and the lowest survival was recorded in November. At -17°C, the mortality rate was over 90% in all months. None of the field samples survived at -20°C. The survival rates of pupae reared at 25 and 15°C in 2 hours exposure at -5°C were 10 and 100%, and at -10°C, it was 5 and 20%, respectively. None of the 25°C pupae survived at -15°C. At -17 and -20°C, all pupae at both rearing temperatures died. The observed and expected mortality rates at low temperatures under laboratory and field conditions are shown in Figure 3.

Overwintering Potential of *T. absoluta*

The average daily temperature in 2019 in Karaj, Alborz Province, is shown in Figure 4. The lowest average daily temperature was -2.37°C, and the absolute minimum temperature of -4.5°C was recorded in February. The LT_{50} and LT_{90} of overwintering pupae were much lower than the lowest daily temperature. In this experiment, the highest LT_{50} and LT_{90} were observed at a constant temperature of 25°C, which were -3.53, and -6.81°C, respectively.

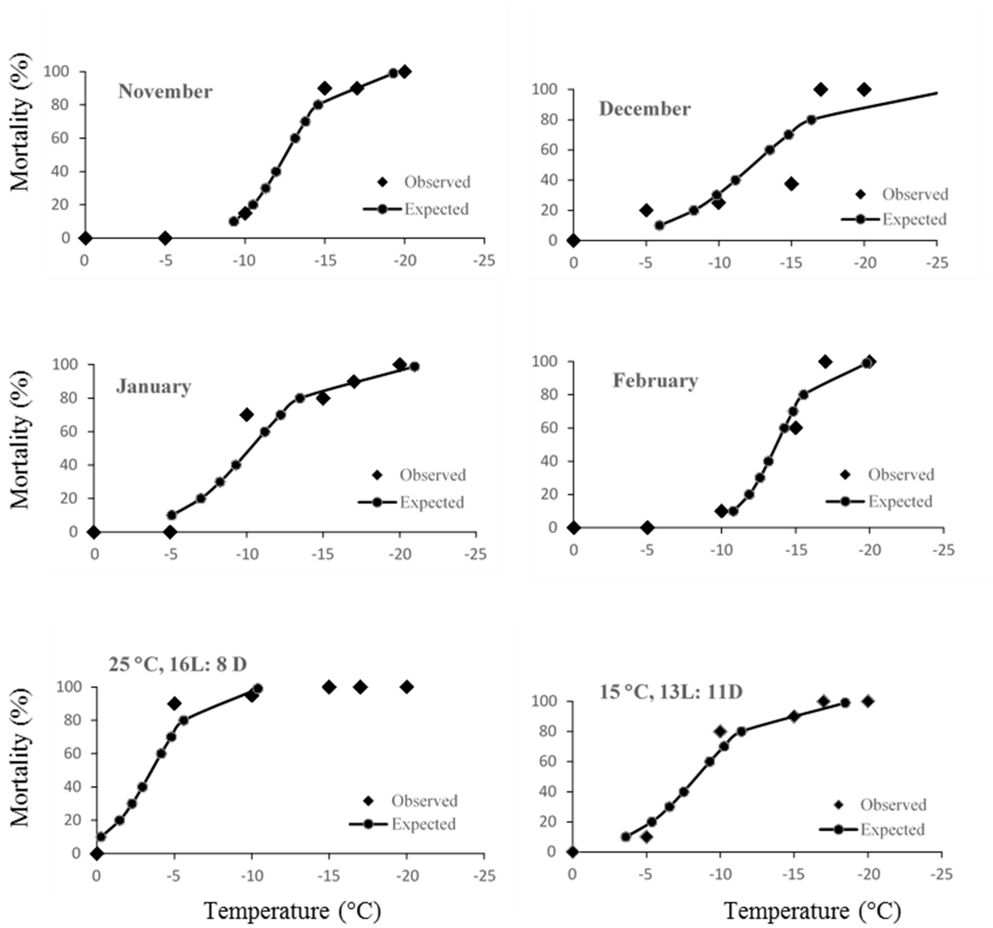

Figure 3. The observed and expected mortality rates at low temperatures under field and laboratory conditions.

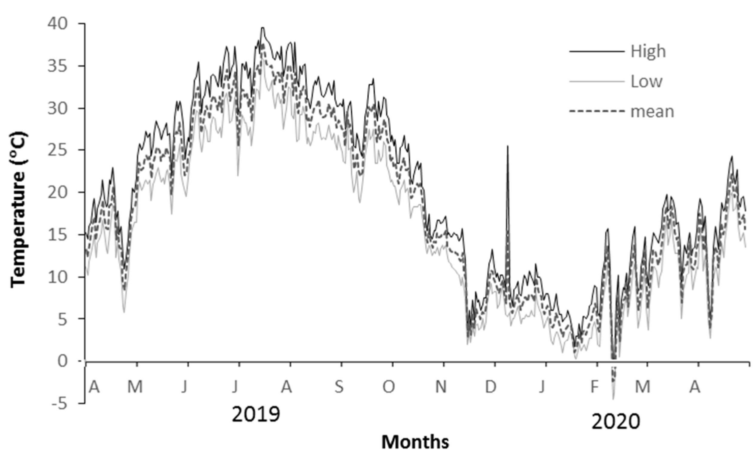

Figure 4. Changes in daily ambient temperature in 2019 and 2020 in Karaj, Alborz Province.

Under constant conditions of 25°C, the LT_{50} level was slightly higher than the minimum ambient temperature.

DISCUSSION

Several studies indicated that *T. absoluta* can spend the winter as an egg, pupa, or adult (EPPO, 2005; Sannino and Espinosa, 2010; Cuthbertson *et al*., 2013; Illakwahhi and Srivastava, 2017; Han *et al*., 2018). However, several studies have highlighted the overwintering of this pest as a pupal form (Sannino and Espinosa, 2010; Han *et al*., 2018; Kahrer *et al*., 2019; De Campos *et al*., 2021). We found that the overwintering pupae were cold hardy insects. The SCPs varied significantly between October and February. The SCPs of overwintering pupae decreased from the beginning of fall to winter, and increased with the rising ambient temperatures, indicating adaptation to environmental conditions. The decrease in the SCP of *T. absolute* during the cold months could be due to the accumulation of cryoprotectants. Insects regulate their supercooling capacity through factors such as accumulation of low molecular mass cryoprotectants, which lower the SCP of body fluids (Somme, 1982; Lee, 2010). The most common accumulated substances are sugars and polyhydric alcohols such as glycerol, sorbitol, and trehalose, sometimes referred to as low molecular weight antifreeze substances (Zachariassen, 1985; Hahn and Denlinger, 2007; Zhao *et al*., 2022). Therefore, further investigation is necessary to determine the synthesis of cryoprotectants in this insect under cold conditions.

In this research, we demonstrated that the field-collected pupae are cold hardy insects. The cold hardiness potential of *T. absoluta* has received considerable attention from other researchers. According to Van Damme *et al*. (2015), the SCPs of pupae in Belgian populations were -16.7°C. Li *et al*. (2021) reported a SCP of -18.11°C for pupae of the Chinese population. However, the SCP values determined from our field samples were lower than those of the above studies. The LT_{50} and LT_{90} values in the field ranged from -10.23 to -13.70°C and -15.37 to -18.73°C in December and January, respectively. Our findings showed that the lower lethal temperature values under constant conditions in the laboratory were higher than those at 15°C and in the field, indicating that the changes in temperature and photoperiod improve cold hardiness under natural field conditions. In this study, the LT_{50} values of overwintering pupae were lower than those reported by Li *et al*. (2021). In other Gelechiidae, LT_{50} values for overwintering pupae were reported in a range of -5.49 to -19.05°C (Ahmadi *et al*., 2017) and -11.4 to -18.0°C (Ganji and Moharramipour, 2017). In this study, the LT50 values were found to be higher than SCP, indicating that the insect cannot tolerate temperatures below its SCP. This finding has also been proved by Li *et al*. (2021) showing that the LT_{90} was slightly higher than the SCP. Comparing LT_{90} with the minimum ambient temperature revealed that pupae of *T. absoluta* can survive the winter. The extremely low temperatures during fall and winter were above the LT_{50} and LT_{90} of overwintering pupae, indicating that larvae that complete their feeding produce pupae that can survive the winter in the field. In laboratory samples, a short photoperiod and low temperature reduced the levels of LTs, suggesting that this insect strengthens its cold resistance by receiving environmental signals to overcome winter cold.

In conclusion, our observations have demonstrated that pupae form plant debris and soil cocoons, where a higher temperature than the ambient air temperature protects them from direct exposure to cold and moisture, thus promoting their survival. The adult insects emerge as soon as the weather warms and have the potential to threaten greenhouse tomato farming during autumn and winter. Because the adult population are able to tolerate cold weather and migrate into their hosts in greenhouses.

The lethal temperatures and SCP values of the overwintering pupae were below the minimum ambient temperatures, indicating that the pupae can survive the winter in the Alborz Province. Lethal temperatures were lower under field conditions than in laboratory samples, suggesting that pupae may be better adapted to adverse conditions when exposed to temperature and humidity fluctuations, as well as changes in photoperiod. Pupae reared at low temperatures and shorter photoperiods survived the cold better than those reared at high temperatures and longer photoperiods, indicating that temperature and photoperiod are influential factors in the adaptation of this insect to adverse environmental conditions (Beck, 1980). However, comprehensive studies should be conducted to investigate the effects of temperature and photoperiod, as well as the physiological mechanisms that are effective in low-temperature tolerance. This information will be valuable in gaining a deeper understanding of the overwintering mechanism of this pest and in developing effective management strategies.

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تاثیر قرار گرفتن در معرض سرما بر مرگ و میر شفیره های*Tuta absoluta*

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چکیده

شب پره مینوز گوجه فرنگی *Tuta absoluta* (Meyrick, 1۹۱۷) یک آفت مهاجم و تهدیدی جدی برای تولید گوجه فرنگی در سراسر جهان به شمار می رود.. پراکنش جهانی این آفت نشان دهنده ظرفیت قابل توجه آن برای انطباق با تغییرات شرایط آب و هوایی است. از آنجا که مرحله شفیرگی مقاوم ترین مرحله در مواجهه طولانی مدت با سرما است، لذا ، شاخص‌های مقاومت به سرما در شفیره‌های زمستان‌گذران جمع‌آوری‌شده از مزرعه و شفیره‌های پرورش‌یافته در دو شرایط ثابت، دمای بالا و روز طولانی (۲۵ درجه سلسیوس، L : D ۱۶:۸ و RH 65±5%) و دمای پایین و روز کوتاه (۱۵ درجه سلسیوس ، L:D ۱۳:۱۱ و RH 65±5%) مورد بررسی قرار گرفت. نتایج نشان می دهد که نقطه انجماد (SCP) با کاهش دما در ماه های آذر (۱/۲ ± ۲۰/۵- درجه سلسیوس) و دی (۰/۷۸ ± ۲۰/۲۶- درجه سلسیوس) به طور قابل توجهی کاهش یافت. در آزمایشگاه، کاهش دما و دوره نوری باعث افزایش تحمل شفیره ها به دمای زیر صفر شد. دمای کشنده ۵۰ (LT50) و LT90شفیره های جمع آوری شده در مزرعه به ترتیب در ۱۳/۷۰- تا ۱۰/۲۳- درجه سلسیوس و ۱۸/۷۳- تا ۱۵/۳۷- درجه سلسیوس ثبت شد. مقایسه دمای کشنده با کمترین دمای محیط در آذر و دی ماه نشان داد که *T. absoluta*در استان البرز پتانسیل زمستان گذرانی بالایی دارد و به راحتی می تواند در زمستان های سرد زنده بماند.