

Impact of cold exposure on the mortality of *Tuta absoluta* pupae

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Abstract

The tomato leafminer, *Tuta absoluta* (Meyrick, 1917), 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 supercooling 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 (LT₅₀) and LT₉₀ 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: Supercooling point, Tomato leafminer, Cold hardiness, Lethal temperature.

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.*,

31 2010; Allache *et al.*, 2015; Duarte *et al.*, 2015), however, it was first reported from Iran, Urmia in
32 2012 (Baniamери and Cheraghian, 2012).

33 The global distribution of *T. absoluta* suggests that it is capable of adapting to low temperatures
34 (Bloem and Spaltenstein, 2011; Biondi *et al.*, 2019). Therefore, the potential for overwintering in
35 regions with cold winters should be taken into consideration. The invasion and establishment of
36 *T. absoluta* highlights its ability to disperse and adapt to new environmental conditions (Bloem
37 and Spaltenstein, 2011), owing to its high reproductive potential, broad host range, and thermal
38 adaptation (Van Damme *et al.*, 2015). In the native areas as well as in the areas where *T. absoluta*
39 was initially established, temperature fluctuations were minimal. However, as the pest's
40 distribution expanded, its ability to tolerate low temperatures has become important. Temperature
41 plays a critical role in establishing new species (Renault *et al.*, 2018; Denlinger and Lee, 2019;
42 Lee *et al.*, 2019). In this study, monitoring the last larval stages in the field showed that the insect
43 spends unfavorable seasons as pupae in the soil cocoons. Therefore, pupae were considered for
44 further study. To survive the adverse season, insects during winter may require cold hardiness
45 (Bale, 1966). Many insects in response to subzero temperatures increase their supercooling
46 capacity by lowering the supercooling points (SCPs) (Lee, 1991; Bale, 2002; Lee, 2010; Andreadis
47 and Athanassiou, 2017). Several studies have examined the cold-hardiness of various populations
48 of *T. absoluta* (Van Damme *et al.*, 2015; Kahrer *et al.*, 2019; Tarusikirwa *et al.*, 2020a, b; Li *et al.*,
49 2021). In Belgium, Van Damme *et al.* (2015) investigated the SCP and lethal temperatures (LT)
50 of *T. absoluta*. These experiments demonstrated that the insect exhibited cold hardiness in response
51 to subzero temperatures. Given the diverse climate of Iran and the significance of tomato as a
52 primary product, it is imperative to investigate its ability to withstand cold temperatures.

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

56

57 **Materials and Methods**

58 **Insects**

59 The third and fourth instar larvae were collected from an infected tomato field in Karaj, Alborz,
60 Iran (35°7743 N, 50°9068 E) in October 2019. The insects were kept in transparent containers with
61 sand poured into the bottom, and a net was placed on the top for ventilation. Some tomato branches

62 wrapped with moistened cotton and a transparent plastic cover at the end of the stems were placed
63 in each container, and the larvae were transferred into these branches. The experimental containers
64 were placed in the field, and water was added to the cotton every two days to maintain leaf
65 moisture. After completion of the larval period, the *T. absoluta* pupated in the sand (Fig. 1). Until
66 the adult emergence in the field, pupae were carried out to the laboratory monthly to determine
67 SCP and survival. The insects used in laboratory experiments were reared on tomato leaves (var.
68 Super2270) under two different conditions; a long-day condition of 16:8 (Light: Darkness) at 25
69 °C, 65 ± 5% relative humidity (RH), and a short-day condition of 13:11 (L:D) at 15 °C, 65 ± 5%
70 RH.

71

72 **Supercooling point determination**

73 The SCP was measured with a thermocouple (NiCr-Ni probe) connected to an automatic
74 temperature recorder (Measurement Computing, USB-5203, USA). Samples were taped to a
75 thermocouple and placed in a programmable test chamber (Binder, MK53, Lenzkirch, Germany).
76 The temperature was decreased at a rate of 0.5 °C/min, starting at 25 °C and ending at -30 °C. The
77 temperature was recorded at 30-second intervals using Data Acquisition (DAQ) software. The SCP
78 was determined as the lowest temperature, after which latent freezing heat was released (Lee,
79 1991).

80

81 **Effect of low temperature on the survival of overwintering pupae**

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

89

90 **Statistical analysis**

91 The SCP and survival rates of overwintering pupae were analyzed using a one-way analysis of
92 variance (ANOVA). Tukey's honest significance test was conducted to compare means with a

93 significance level of $P < 0.05$. Statistical analysis was performed using SPSS version 26.0 software
94 (SPSS Inc., Chicago, IL, USA).

95 The pupal mortality data were subjected to a binary logistic regression model to calculate lethal
96 temperature 50 and 90 after a 2-h exposure to subzero temperatures. The lethal temperatures are
97 calculated by the following equation:

$$98 \quad Y = Ln \frac{p}{1-p}$$

$$99 \quad LT50 = \frac{Y - a}{b}$$

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

101

102 **Results**

103 **Supercooling points and lower lethal temperatures**

104 Supercooling point differed significantly between months ($F = 3.907$; df t, e = 4, 40; $P = 0.01$).
105 SCPs of individuals varied from -11.36 to -26.18 °C. Mean monthly SCPs varied from $-17.06 \pm$
106 1.14 °C in November to -21.78 ± 1.31 °C in October (Fig. 2).

107 The LT_{50} and LT_{90} of pupae under laboratory conditions were higher than those under field
108 conditions. The highest LT_{50} and LT_{90} recorded at 25 °C were -3.53 °C and -6.81 °C, and the LT_{50}
109 and LT_{90} at 15 °C were -8.40 °C and -13.21 °C, respectively. In the field, the LT_{50} ranged from -
110 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
111 measured in January. The LT_{50} was lower in November and December, increased in January, and
112 decreased in February. The lowest LT_{90} was observed in December (-18.73 °C) (Table 1).

113

114 **Survival rates in the field-collected and lab-reared pupae**

115 In field-collected pupae, at -5 °C, 20% mortality was observed only in December. At -10 C, the
116 lowest survival rate was observed in January. At -15 °C, less than half of the individuals survived
117 in all field samples, and the lowest survival was recorded in November. At -17 °C, the mortality
118 rate was over 90% in all months. None of the field samples survived at -20 °C. The survival rates
119 of pupae reared at 25 and 15 °C in 2 h exposure at -5 °C were 10 and 100%, and at -10 °C, it was
120 5 and 20%, respectively. None of the 25 °C pupae survived at -15 °C. At -17 °C and -20 °C, all
121 pupae at both rearing temperatures died. The observed and expected mortality rates at low
122 temperatures under laboratory and field conditions are shown in Fig. 3.

123

124 **Overwintering potential of *T. absoluta***

125 The average daily temperature in 2019 in Karaj, Alborz province, is shown in Fig. 4. The lowest
126 average daily temperature was $-2.37\text{ }^{\circ}\text{C}$, and the absolute minimum temperature of $-4.5\text{ }^{\circ}\text{C}$ was
127 recorded in February. The LT_{50} and LT_{90} of overwintering pupae were much lower than the lowest
128 daily temperature. In this experiment, the highest LT_{50} and LT_{90} were observed at a constant
129 temperature of $25\text{ }^{\circ}\text{C}$, which were -3.53 , and $-6.81\text{ }^{\circ}\text{C}$, respectively. Under constant conditions of
130 $25\text{ }^{\circ}\text{C}$, the LT_{50} level was slightly higher than the minimum ambient temperature.

131

132 **Discussion**

133 Several studies indicated that *T. absoluta* can spend the winter as an egg, pupa, or adult (EPPO,
134 2005; Sannino and Espinosa, 2010; Cuthbertson *et al.*, 2013; Illakwashhi and Srivastava, 2017;
135 Han *et al.*, 2018). However, several studies have highlighted the overwintering of this pest as a
136 pupal form (Sannino and Espinosa, 2010; Han *et al.*, 2018; Kahrer *et al.*, 2019; De Campos *et al.*,
137 2021). We found that the overwintering pupae are cold hardy insects. The SCPs varied
138 significantly between October and February. The SCPs of overwintering pupae decreased from the
139 beginning of fall to winter and increased with the rising ambient temperatures, indicating
140 adaptation to environmental conditions. The decrease in the SCP of *T. absolute* during the cold
141 months could be due to the accumulation of cryoprotectants. Insects regulate their supercooling
142 capacity through factors such as the accumulation of low molecular mass cryoprotectants, which
143 lower the SCP of body fluids (Somme, 1982; Lee, 2010). The most common accumulated
144 substances are sugars and polyhydric alcohols such as glycerol, sorbitol, and trehalose, sometimes
145 referred to as low molecular weight antifreeze substances (Zachariassen, 1985; Hahn and
146 Denlinger, 2007; Zhao *et al.*, 2022). Therefore, further investigation is necessary to determine the
147 synthesis of cryoprotectants in this insect under cold conditions.

148 In this research, we demonstrated that the field-collected pupae are cold hardy insects. The cold
149 hardiness potential of *T. absoluta* has received considerable attention from other researchers.
150 According to Van Damme *et al.* (2015), the SCPs of pupae in Belgian populations were $-16.7\text{ }^{\circ}\text{C}$.
151 Li *et al.* (2021) reported a SCP of $-18.11\text{ }^{\circ}\text{C}$ for pupae of the Chinese population. However, the
152 SCP values determined from our field samples were lower than those of the above studies. The
153 LT_{50} and LT_{90} values in the field ranged from -10.23 to $-13.70\text{ }^{\circ}\text{C}$ and -15.37 to $-18.73\text{ }^{\circ}\text{C}$ in
154 December and January, respectively. Our findings showed that the lower lethal temperature values

155 under constant conditions in the laboratory were higher than those at 15 °C and in the field,
156 indicating that the changes in temperature and photoperiod improve cold hardiness under natural
157 field conditions. In this study, the LT₅₀ values of overwintering pupae were lower than those
158 reported by Li et al. (2021). In other Gelechiidae, LT₅₀ values for overwintering pupae were
159 reported in a range of -5.49 to -19.05 °C (Ahmadi *et al.*, 2017) and -11.4 to -18.0 °C (Ganji and
160 Moharramipour, 2017). In this study, the SCP values were found to be higher than LT₅₀, indicating
161 that the insect cannot tolerate temperatures below its SCP. This finding has also been proved by
162 Li et al. (2021) showing that the LT₉₀ was slightly higher than the SCP. Comparing LT₉₀ with the
163 minimum ambient temperature revealed that pupae of *T. absoluta* can survive the winter. The
164 extremely low temperatures during fall and winter were above the LT₅₀ and LT₉₀ of overwintering
165 pupae, indicating that larvae that complete their feeding produce pupae that can survive the winter
166 in the field. In laboratory samples, a short photoperiod and low temperature reduced the levels of
167 LTs, suggesting that this insect strengthens its cold resistance by receiving environmental signals
168 to overcome winter cold.

169 In conclusion, our observations have demonstrated that pupae form in plant debris and soil
170 cocoons, where a higher temperature than the ambient air temperature protects them from direct
171 exposure to cold and moisture, thus promoting their survival. The adult insects emerge as soon as
172 the weather warms and have the potential to treat greenhouse tomato farming during autumn and
173 winter. Because the adult population could able to tolerate cold weather and migrate into their
174 hosts in greenhouses. The lethal temperatures and SCP values of the overwintering pupae were
175 below the minimum ambient temperatures, indicating that the pupae can survive the winter in the
176 Alborz province. Lethal temperatures were lower under field conditions than in laboratory
177 samples, suggesting that pupae may be better adapted to adverse conditions when exposed to
178 temperature and humidity fluctuations, as well as changes in photoperiod. Pupae reared at low
179 temperatures and shorter photoperiods survived the cold better than those reared at high
180 temperatures and longer photoperiods, indicating that temperature and photoperiod are influential
181 factors in the adaptation of this insect to adverse environmental conditions (Beck, 1980). However,
182 comprehensive studies should be conducted to investigate the effects of temperature and
183 photoperiod, as well as the physiological mechanisms that are effective in low-temperature
184 tolerance. This information will be valuable in gaining a deeper understanding of the overwintering
185 mechanism of this pest and in developing effective management strategies.

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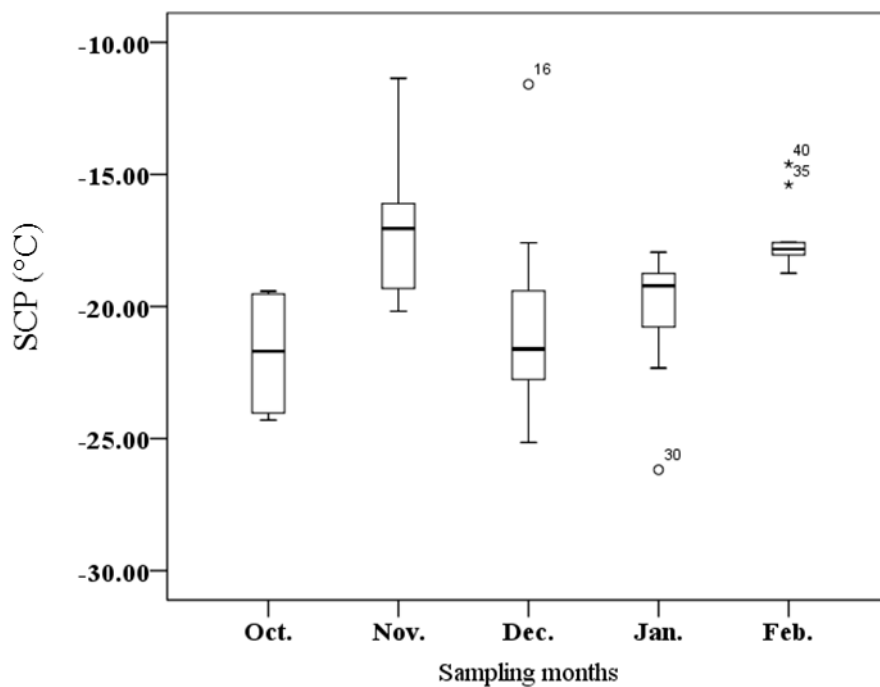
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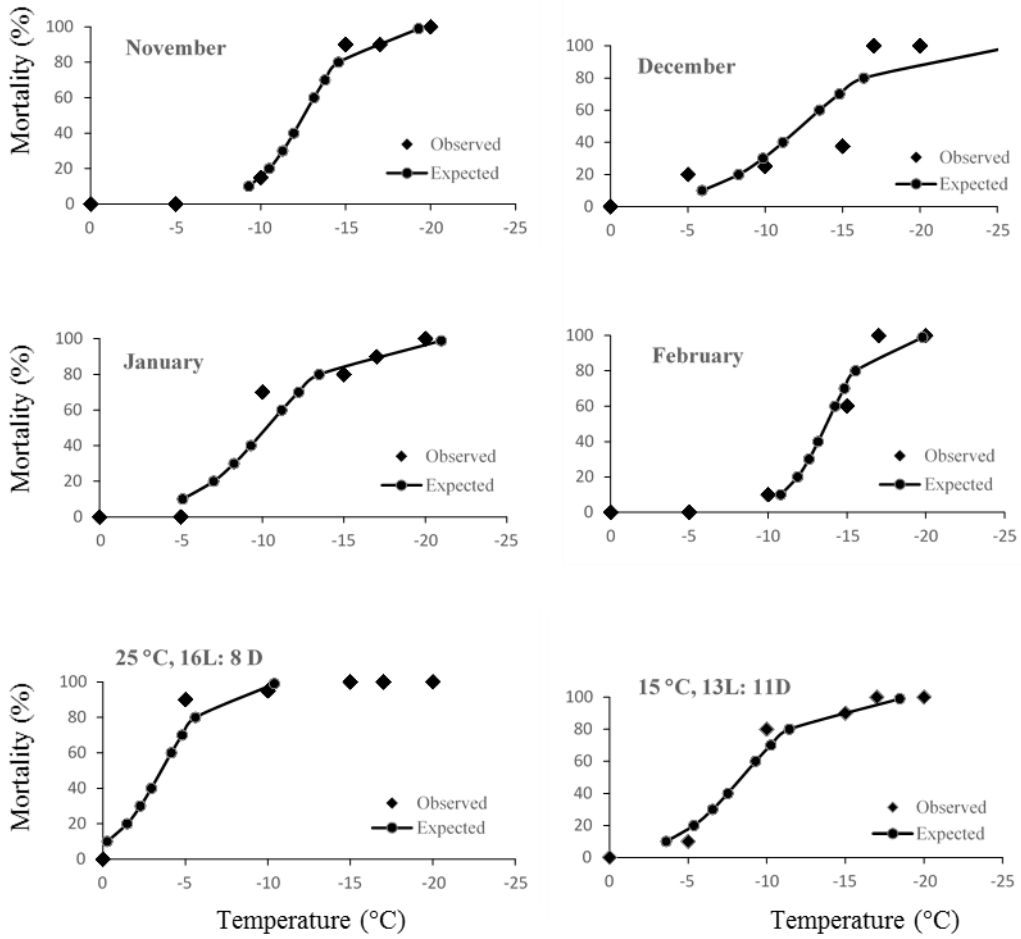
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Figure 1. Pupation of *T. absoluta* in the soil cocoons. Before pupation, the larvae in the soil cover the soil particles on the cocoon and pupate inside it.

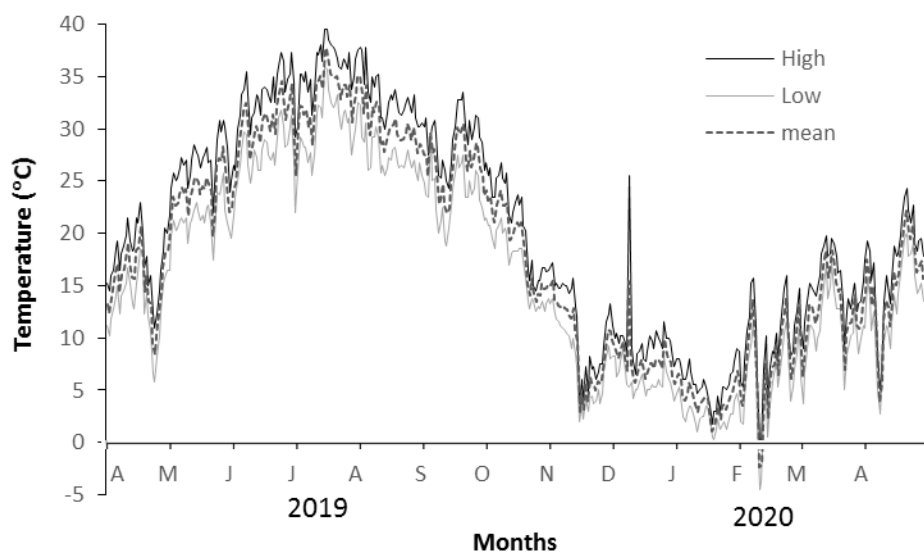


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Figure 2. Supercooling points of field-collected pupae of *Tuta absoluta* in 2019.



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



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298 **Figure 4.** Changes in daily ambient temperature in 2019 and 2020 in Karaj, Alborz province.
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301 **Table 1.** The LT_{50} and LT_{90} of *Tuta absoluta* pupa under field and laboratory condition.

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

302 LT: Lethal temperature.

303 CL: 95% lower and upper confidence limits.

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