



HAL
open science

Advances in understanding Lepidoptera cold tolerance

Hamzeh Izadi, Ross N. Cuthbert, Phillip Joschka Haubrock, David Renault

► To cite this version:

Hamzeh Izadi, Ross N. Cuthbert, Phillip Joschka Haubrock, David Renault. Advances in understanding Lepidoptera cold tolerance. *Journal of Thermal Biology*, 2024, 125, pp.103992. <10.1016/j.jtherbio.2024.103992>. <hal-04766773>

HAL Id: hal-04766773

<https://hal.science/hal-04766773v1>

Submitted on 9 Dec 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC 4.0 - Attribution - Non-commercial use - International License

Journal Pre-proof



Advances in understanding Lepidoptera cold tolerance

Hamzeh Izadi, Ross N. Cuthbert, Phillip J. Haubrock, David Renault

PII: S0306-4565(24)00210-9

DOI: <https://doi.org/10.1016/j.jtherbio.2024.103992>

Reference: TB 103992

To appear in: *Journal of Thermal Biology*

Received Date: 10 July 2024

Revised Date: 25 September 2024

Accepted Date: 30 September 2024

Please cite this article as: Izadi, H., Cuthbert, R.N., Haubrock, P.J., Renault, D., Advances in understanding Lepidoptera cold tolerance, *Journal of Thermal Biology*, <https://doi.org/10.1016/j.jtherbio.2024.103992>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Advances in understanding Lepidoptera cold tolerance

Hamzeh Izadi^{a*}, Ross N. Cuthbert^b, Phillip J. Haubrock^c, David Renault^d

^aDivision of Plant Protection, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran

^bInstitute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Belfast, BT9 5DL United Kingdom

^cFaculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, University of South Bohemia in České Budějovice, 389 25 Vodňany, Czech Republic

^dUniversité de Rennes, CNRS, ECOBIO [(Ecosystèmes, biodiversité, évolution)], 35000 Rennes, France

*Corresponding author: Hamzeh Izadi, E-mail: izadi@vru.ac.ir

1 ABSTRACT

2 Ambient thermal conditions mediate insect growth, development, reproduction, survival, and
3 distribution. With increasingly frequent and severe cold spells, it is critical to determine low-
4 temperature performance and cold tolerances of ecologically and economically essential insect
5 groups to predict their responses to global environmental change. This review covers the cold
6 tolerance strategies of 49 species of Lepidoptera (moths and butterflies), focusing on species that
7 are known as crop pests and crop storage facilities. We synthesize cold tolerance strategies of well-
8 studied species within this order, finding that diapause is a distinctive mechanism that has
9 independently evolved in different genera and families of Lepidoptera. However, the occurrence
10 of diapause in each life stage is specific to the species, and in most studied lepidopteran species,
11 the feeding stage (as larva) is the predominant overwintering stage. We also found that the onset
12 of diapause and the improvement of cold tolerance are interdependent phenomena that typically
13 occur together. Moreover, adopting a cold tolerance strategy is not an inherent, fixed trait and is
14 greatly influenced by a species' geographic distribution and rearing conditions. This review further
15 finds that freeze avoidance rather than freeze tolerance or chill susceptibility is the primary cold
16 tolerance strategy among lepidopteran species. The cold hardiness of lepidopteran insects
17 primarily depends on the accumulation of cryoprotectants and the depression of the supercooling
18 point. We highlight variations in cold tolerance strategies and mechanisms among a subset of
19 Lepidoptera, however, further work is needed to elucidate these strategies for the vast numbers of
20 neglected species and populations to understand broad-scale responses to global change.

21 *Keywords:* Environmental change, Butterflies, Moths, Economic pests, Supercooling point, Lethal
22 temperature, Diapause

23 1. Introduction

24 As ectothermic animals, the growth, development, reproduction, survival, and distribution of
25 insects highly depend on the temperature of their surrounding environment (Jaworski and
26 Hilszczański, 2014; Régnière et al., 2012). Animals have developed a range of responses and
27 strategies that allow them to handle adverse environmental conditions (Bale and Hayward, 2010).
28 These strategies have assisted insects' successful colonization of various habitats across the globe,
29 from the equator to the poles. An insect's ability to tolerate thermal variations and temperature
30 extremes relies on complex adaptations involving behavioral, physiological, and molecular
31 responses (Overgaard and MacMillan, 2016), but also depends on relative humidity and the
32 organism's nutritional status. Some insect species can also regulate their body temperature within
33 specific limits in their natural environment (Woods et al., 2003); this ability may be achieved
34 through behavioral (e.g., basking in the sun to gain heat or heliothermy, and shivering),
35 physiological (for instance evaporative cooling), or morphological (e.g., cuticular pigmentation)
36 processes (González-Tokman et al., 2020; Lahondère, 2023)

37 Seasonal adaptations of insects have been intensively studied over the past decades (Danks,
38 2007), with particular attention having been given to mechanisms allowing them to survive winter
39 periods, especially when temperatures fall below 0 °C, which places integral body fluids of insects
40 at freezing risks (Dillon and Lozier, 2019). The studies have significantly increased our
41 understanding of insect cold tolerance's behavioral, physiological, and molecular correlates (Teets
42 and Denlinger, 2013) and offer valuable information for estimating overwintering limits and
43 occurrence trends, particularly for pest species (Prasanna B., 2018). As climate change may offer
44 novel opportunities for insects to colonize new habitats and enable the expansion beyond their
45 current distributions, information on cold tolerance strategy and ability to survive extreme climatic

46 conditions valuably completes the knowledge on the basic vital requirements of insect species to
47 refine predictions of their potential distribution. Recent investigations have shown that thermal
48 fluctuations, and multiple occurrences of cold temperatures, can negatively impact the mounted
49 survival strategies (Colinet et al., 2015) with cascading consequences on reproduction and
50 phenology (Buckley et al., 2017; Colinet et al., 2015; Forrest, 2016). In this context, thermal
51 adaptations of agricultural pests, in particular lepidopterans, are increasingly attracting research
52 attention, given that the results can be used for predicting pest population dynamics and
53 management options (Mutamiswa et al., 2023; Subedi et al., 2023).

54 The order Lepidoptera comprises over 180,000 known species (Perveen and Khan, 2017) that
55 inhabit all terrestrial habitats and showcase a diverse range of characteristics such as size, shape,
56 biology, feeding habits, habitat, and behavior. Most adult moths and butterflies are plant
57 pollinators, and are thus highly beneficial insects providing ecosystem services; conversely, their
58 caterpillars are significant plant defoliators and are major agricultural and forest pests, thus posing
59 a significant threat to sustainable food production and food security (Subedi et al., 2023). As
60 outlined above, the importance of lepidopteran pest outbreaks is driven by the winter
61 environmental conditions and the strategies and ability of the insects to survive wintertime.

62 The ability of lepidopterans, as for other insect species, to withstand exposure to low
63 temperatures, whether for a short or extended period, is referred to as cold hardiness or cold
64 tolerance (Lee, 1991); it can greatly vary from one lepidopteran species to the next, or according
65 to developmental stage, age, sex, nutritional and physiological status, diapause development, or
66 geographic origin (Andreadis and Athanassiou, 2017; Pei et al., 2020). The cold tolerance
67 characteristics of lepidopterans have been examined in a large diversity of species, with

68 overwintering strategies belonging to one of the three main categories typically found in insects:
69 (i) freeze tolerance, (ii) freeze avoidance, and (iii) chill susceptibility (Sinclair et al., 2015).

70 To get a better overview of the overwintering strategies and cold tolerance of economically
71 important lepidopteran (moths and butterflies) pests, a literature search was conducted using
72 SCOPUS and Google Scholar with the following terms in the search strings (Title, abstract,
73 keywords): Lepidoptera', 'lepidopteran', 'butterfly', 'moth', 'cold tolerance', 'diapause',
74 'overwinter', 'supercooling', 'SCP', 'cryoprotectant', 'freeze tolerance', 'freeze intolerance',
75 'freeze avoidance' and 'chill intolerance'. In doing so, then examined (i) whether cold tolerance is
76 a part of the overwintering (diapause) syndrome, (ii) which strategy, freeze tolerance or freeze
77 avoidance, is more common, and (iii) if there are any phylogenetic and ontogenetic relationships
78 between lepidopteran species and their cold tolerance strategy. For this review, we included all
79 studied Lepidoptera species for which sufficient information was available (over 150 papers were
80 reviewed), intentionally excluding those with insufficient data, ending up with 49 species from 29
81 genera, belonging to 16 families.

83 **2. Cold tolerance strategies**

84 Insects have developed various strategies to survive exposure to cold temperatures. These
85 strategies can be categorized into three main types based on the survival of internal ice formation
86 (Colinet et al., 2015; Richard E. Lee, 2010; Sinclair et al., 2015; Storey, J. M., and Storey, 2004;
87 Toxopeus and Sinclair, 2018). Understanding these mechanisms is essential for predicting insect
88 behavior and population dynamics in response to climate change. Freeze-tolerant insects survive
89 freezing by producing heat-shock proteins and accumulating cryoprotectants (**Figure 1**). They
90 freeze at higher temperatures to prevent ice crystal formation and potential damage. Winter

91 temperatures do not affect the supercooling point in these insects, but repeated freezing can lower
92 this point and boost cryoprotectant levels. Surviving internal ice formation requires managing
93 cellular dehydration, mechanical damage, and metabolic restrictions. Freeze-tolerant insects
94 control the quality and quantity of ice, prevent cell damage, manage biochemical processes during
95 freezing and thawing, and restore physiological processes after thawing. The accumulation of
96 certain molecules facilitates freeze tolerance, and it is proposed that this ability evolved in insects
97 already adapted to low temperatures or desiccation (Toxopeus and Sinclair, 2018; Voituron et al.,
98 2002). Freeze-avoidant insects lower their supercooling point (SCP) by producing antifreeze and
99 heat-shock proteins, accumulating cryoprotectants, and removing ice nucleators. Lower SCPs are
100 associated with harsher winter temperatures; mortality can result if temperatures drop below SCP
101 (Colinet et al., 2015; Sinclair et al., 2015). Chill-susceptible insects cannot survive internal ice
102 formation and typically die before freezing due to injuries unrelated to freezing, such as damage
103 to tissues or organs, at low temperatures (**Figure 1**). Chill susceptibility can be further subdivided
104 into additional categories, with some insects being able to survive extensive chilling while others
105 do not (Colinet et al., 2015; Sinclair et al., 2015).

106 Many lepidopteran species utilize the freeze-avoidant or-tolerant strategy to survive in low
107 temperatures (Zachariassen, 1985). In the freeze avoidance strategy, most mortalities occur at a
108 temperature near the supercooling point (SCP) (**Table A1**). These insects can maintain a
109 supercooled state in their body fluids, which is achieved through the accumulation of compatible
110 osmolytes (Behroozi et al., 2012; Bemani et al., 2012a; Goto et al., 2003; Heydari and Izadi, 2014;
111 Košťál et al., 2007; Sadeghi et al., 2012; Storey and Storey, 2005). It is important to note that
112 insects can experience chill injuries at low temperatures due to ion and water balance disruption.
113 Cell membranes and epithelia typically rely on active transporters to regulate ions outside the cells

114 (Gerber and Overgaard, 2018; MacMillan et al., 2016b). However, when temperatures decrease,
115 the functionality of these ion pumps is compromised. Insects adjust to shifting environmental
116 conditions by accumulating osmolytes through various mechanisms. These small solutes aid in
117 preserving cell vacuolome. Unlike salt ions, most osmolytes are considered compatible, as they
118 do not interfere with macromolecules. Some osmolytes can even enhance stability in proteins and
119 ligand binding. Insects can produce and store osmolytes such as trehalose, sugar alcohols, glucose,
120 amino acids, and methylamines, in response to cold acclimation, rapid cold hardening (RCH), or
121 when experiencing stress (stress-induced osmolyte production). However, insects use glycogen to
122 produce trehalose, a disaccharide that acts as an osmolyte. This process involves the action of
123 glycogen phosphorylase, which supplies glucosyl residues for trehalose synthesis.(Chaudhuri et
124 al., 2017; Košťál et al., 2004; MacMillan et al., 2009; Olsson et al., 2016; Ransberry et al., 2011;
125 Yancey, 2001). Glycerol is a crucial component for insect cold tolerance. It helps protect insects
126 from freezing damage by preventing the formation of ice crystals within their bodies. Temperature
127 regulates glycerol accumulation and synthesis and involves specific genetic mechanisms (Kukal
128 et al., 1988). The study demonstrated that glycerol biosynthesis genes have a high expression level
129 to synthesize a high level of glycerol as a rapid cold hardiness factor (Vatanparast et al., 2022).
130 The diamondback moth, *Plutella xylostella* becomes cold-tolerant through rapid cold hardiness
131 (RCH) and glycerol accumulation in response to brief exposure to low temperatures (Park and
132 Kim, 2014).

133 Cryoprotective dehydration is the less common adaptation that freeze-avoidant insects may use
134 to survive cold. In this strategy, ice formation is inhibited by an increase in cell viscosity via the
135 removal of osmotically active water (Clark et al., 2009; Elnitsky et al., 2008; Sinclair et al., 2015;
136 Worland et al., 2010). So far, cryoprotective dehydration has not been described from Lepidoptera.

137 In the freeze-tolerance strategy, individuals are capable of surviving temperatures lower than
138 their SCPs (**Table A1**) (Costanzo and Lee, 2013; R.E. Lee, 2010a; Sinclair et al., 2015). The ability
139 of freeze-tolerant species to withstand extracellular ice varies among species and may depend on
140 factors such as cold acclimation, the accumulation of compatible osmolytes, here acting as
141 cryoprotectants, and the presence of ice-nucleating agents.

142 Among the 49 considered lepidopteran species, more than 75% of them adopted a freeze-
143 avoidant strategy (**Table 1**) (**Figure 2**), while 14 species are freeze-tolerant and only one species
144 is chill susceptible (**Table 1**). It is generally expected that insects from temperate latitudes of the
145 Southern Hemisphere rely on freeze-tolerance as their primary survival strategy, whereas those
146 inhabiting the North tend to use freeze avoidance as their primary approach (Sinclair et al., 2003a).
147 Consistently, around 77% of insect species in the Southern Hemisphere that can survive in cold
148 temperatures employ the freeze-tolerance strategy (Wharton et al., 2003). Interestingly, a few
149 species, follow the bimodal (both freeze avoidance and freeze tolerance) cold tolerance strategy
150 (**Table 1**) (Masoudmagham et al., 2021; Vrba et al., 2017; Williams et al., 2014). The different
151 climates in the two hemispheres have likely led to the independent evolution of freeze tolerance
152 for various reasons. The difference between the hemispheres is indicative of many larger variations
153 in ecological processes in the Northern and Southern regions (Sinclair et al., 2003a). In the face of
154 extremely low temperatures, freeze tolerance and freeze avoidance may exist as parallel and
155 alternative strategies (Sinclair, 1999). Using a combination of these strategies allows insects to
156 adapt to harsh environments, but it requires optimizing all factors related to both cold-hardiness
157 strategies. However, this mixed approach provides energy benefits in fluctuating environments,
158 helping insects endure difficult periods (Voituron et al., 2002).

159 Overall, more than 63% of the lepidopteran pests assessed here achieved cold hardiness through
160 the supercooling point (SCP) depression, but most of the species employed both adaptations
161 (cryoprotectant accumulation and SCP depression) to enhance their cold hardiness, while a few
162 species did not utilize any such adaptations. The cold tolerance of four species was enhanced by
163 expanding their SCP without accumulating cryoprotectants. Alternatively, cold hardening was
164 achieved solely by accumulating cryoprotectants in some species (**Table 1**). The consistency and
165 lack of variation in the SCP across different *Papilio* species suggest that specific lepidopteran
166 genera may possess a unique characteristic of increased cold tolerance, and thus improvement in
167 cold tolerance could be a genera-specific character of some Lepidoptera. However, the study of
168 cold tolerance in insects has been conducted across multiple species and genera, highlighting the
169 adaptational genetic variation in these traits. Additionally, the study shows that cold tolerance can
170 vary between closely related species, even within the same genus. This suggests that cold tolerance
171 is not a fixed trait specific to a particular genus but rather can evolve and vary across different
172 species within a genus (R.E. Lee, 2010a; MacMillan et al., 2016a; Wang et al., 2016; Zhao et al.,
173 2021). Freeze-avoidant insects cause a colligative depression of the SCP and stabilize cells'
174 membrane and protein structures at both freezing and non-freezing temperatures by accumulating
175 cryoprotectants. Freeze-tolerant insects stabilize the supercooled state by reducing the
176 hemolymph's freezing point (Andreadis and Athanassiou, 2017; Duman, 2015; Watanabe M and
177 Tanaka K, 1999). Freezing-point depression occurs when a non-volatile substance is added to a
178 solvent, causing the solvent's freezing point to decrease. This phenomenon is a colligative property
179 that depends only on the concentration of the solute and not on its chemical nature. The colligative
180 property of freezing-point depression is a result of the solute disrupting the formation of hydrogen

181 bonds between water molecules, making it more difficult for the solution to freeze (Danks, 1996;
182 Ditrich, 2018; Natalia G. Li, 2016; Toxopeus et al., 2019).

183 In the pistachio fruit hull borer, *Arimania comaroffi*, the species exhibited the lowest SCP (-16
184 °C) and the highest level of cold hardiness in January when the ambient temperature was at its
185 lowest (Bemani et al., 2012b). In some other lepidopterans, cold tolerance may peak later through
186 diapause development and natural cold acclimation (e.g., *Ostrinia nubilalis*, *Ectomyelois*
187 *ceratoniae*, *Cydia pomonella*, *Kermania pistaciella*). In this particular group of insects, their
188 ability to tolerate cold temperatures is linked to their capacity to acclimate to such conditions
189 (Ahmadi et al., 2016; Heydari and Izadi, 2014; Khani and Moharramipour, 2010; Kojić et al.,
190 2018; Mollaei et al., 2016; Rozsypal et al., 2013; Stanic et al., 2004). In diapausing pupae of the
191 wild silkworm, *Antheraea pernyi*, the SCP and lower lethal temperature (LLT) are approximately
192 -20.0°C. This indicates that *A. pernyi* is a freeze-avoidant species (Liu et al., 2016). Furthermore,
193 in the case of the larch casebearer, *Coleophora laricella* Hübner (Coleophoridae), the lowest SCP
194 (-44.6 °C) and the lowest LT₅₀ (-40.8°C) coincided in January (Schebeck et al., 2022). Many
195 studies infer cold tolerance strategies by measuring separate metrics - the supercooling point (SCP)
196 and the median lethal temperature (LT₅₀). The interpretation of these metrics is as follows: If LT₅₀<
197 SCP, this indicates the organism has a freeze tolerance strategy, as it can withstand internal ice
198 formation. If LT₅₀=SCP, this suggests the organism has a freeze avoidance strategy, as it can
199 prevent internal ice formation. If LT₅₀>SCP, this implies the organism has chill susceptibility, as
200 it cannot tolerate low temperatures even without internal ice. This indirect method of inferring cold
201 tolerance is effective when there are substantial differences between SCP and the lower lethal
202 temperature (LLT)(Bemani et al., 2012a; Sinclair et al., 2015). Hence, comparing the LLT and
203 SCP is a reliable means of determining the cold tolerance strategy of these insects. However, it

204 becomes less accurate when SCP and LLT are closer in value (Sinclair et al., 2015). The South
205 American tomato pinworm, *Tuta absoluta* (Meyrick), has an SCP of -19.5°C , but its LT_{50} is -
206 9.4°C . This means the pest can survive exposure to temperatures near its SCP, but only for a limited
207 time. If the temperature remains below -9.5°C for an extended period, the South American tomato
208 pinworm cannot survive (Li et al., 2021). In the pupae of *Helicoverpa zea*, the LLT at -13.0°C is
209 significantly higher than the corresponding SCP at -19.0°C (Morey et al., 2012). Therefore, even
210 if the SCP changes, it may not significantly affect the cold tolerance of these species. From a
211 practical standpoint, predicting the pest population and identifying the timing of early-season
212 infestations are crucial elements of integrated pest management (IPM) programs. Furthermore,
213 comparing the SCP and LLT of a pest as an indicator of its cold tolerance strategy can aid in
214 predicting the magnitude of the pest population. In other words, this comparison can assist in
215 developing and validating models for more accurate pest population predictions (Sinclair et al.,
216 2015).

217

218 **3. Relationships to diapause and cold acclimation**

219 Diapause and cold acclimation are crucial for insects' survival in low temperatures, enabling
220 them to endure winter and other cold periods (Nazari et al., 2020). Diapause is a seasonal dormancy
221 that allows organisms to survive unfavorable conditions and optimizes the timing of reproduction
222 and growth (Denlinger, 2023; Roncalli et al., 2021). The winter-specific diapause is hormonally
223 regulated and responds to environmental cues, such as photoperiod, indicating the onset of harsh
224 conditions (Denlinger, 2022, 2002; Hand et al., 2016; Harsimran Kaur Gill et al., 2017; M. B.
225 Renfree and G. Shaw, 2000). Diapause entails a pause in growth or reproduction, enabling insects
226 to cope with adverse seasonal conditions through metabolic depression and physiological and

227 biochemical adaptations. Diapause can be classified as either facultative or obligatory. Facultative
228 diapause occurs when insect development is halted only in unfavorable environmental conditions
229 for growth and reproduction. This type of diapause is common in bi- and multivoltine species, and
230 numerous lepidopteran species exhibit facultative diapause (Denlinger, 2022, 2002; Denlinger
231 D.L, 2009; M. B. Renfree and G. Shaw, 2000). Three key agricultural pests, i.e., the potato tuber
232 moth, *Ph. operculella*, the rice stem borer, *Chilo suppressalis* Walker (Pyralidae), and the Indian
233 meal moth, *Plodia interpunctella* (Hübner) (Pyralidae) undergo facultative diapause (Cho et al.,
234 2005; Trivedi and Rajagopal, 1992; Tzanakakis, 1959). In obligatory diapause, which is a
235 characteristic of univoltine species e.g., the pistachio twig borer, *K. pistaciella*, and the eastern
236 spruce budworm, *Choristoneura fumiferana* (Clemens) (Tortricidae), moths undergo metabolic
237 depression (Milonas, 1999; Mollaei et al., 2017, 2016). During this type of diapause,
238 developmental processes are stopped at a predetermined time and specific life stage, independent
239 of any environmental factors (Denlinger, 1991; Hand et al., 2016; Moraiti and Papadopoulos,
240 2017). Although day length, or photoperiod, is the primary environmental factor that influences
241 this seasonal response, the temperature can also impact the initiation of diapause, in turn making
242 it more difficult to distinguish between photoperiod and temperature effects (Saunders, 2014).

243 In temperate and colder climates, the ability of many species to survive the winter is closely
244 related to the diapause state, which enhances cold tolerance before seasonal acclimatization to
245 temperature (Bale and Hayward, 2010). Out of the 49 lepidopteran species considered in the
246 present review, we found that adult diapause is rare; the majority (62%) overwinter as larvae, while
247 33% do so as a pupa and only 5% as an egg (**Figure 2**). Additionally, our study indicates that
248 overwintering is largely a family-specific trait, with a certain form of diapause prevailing within a
249 specific family of the order (**Table 2**). Therefore, the occurrence of diapause is not specific to one

250 family alone. For instance, in families such as Noctuidae, Pieridae, and Arctiidae, both pupal and
251 larval diapause can occur. Interestingly, the Papilionidae species overwinter in their pupal stage,
252 while the Satyridae, Pyralidae, Pieridae, and Noctuidae species enter the larval diapause during
253 winter. In certain lepidopteran species, diapause is a characteristic specific to the genus, and it
254 happens in the same life stage for various species within that genus. For instance, *Papilio* spp. have
255 pupal diapause, while *Colias* spp. exhibit larval diapause. This observation means that diapause
256 has evolved independently in various genera and families of Lepidoptera. Additionally, in a
257 specific genus, diapause can occur in different life stages (embryo, larva, pupa, and adult) in
258 different species (Hand et al., 2016; Harsimran Kaur Gill et al., 2017; Reznik, 2011).

259 Cold tolerance is often – but not always - a component of the diapause syndrome, and there is a
260 clear correlation between improving cold tolerance and the development of diapause (Denlinger,
261 1991). Consistently, *ca.* 87% of the considered lepidopteran species (14 out of 16 species with
262 sufficient data) exhibit cold tolerance as a component of diapause which improves with diapause
263 development (**Table 2**). The highest level of cold tolerance is often achieved when diapause is
264 well-established. Yet, in other species (**Table 2**), diapause does not necessarily make the insects
265 more cold-resistant than non-diapausing stages. In this case, the occurrence of diapause and
266 enhancement of cold tolerance seems to rely – at least partially – on two distinct processes (Chen
267 et al., 2014; Denlinger et al., 1992; Li et al., 2001; Schowalter and Ring, 2017; Xu et al., 2015). In
268 the fall webworm, *H. cunea* cold tolerance of the pupae can be decreased as diapause develops (Li
269 et al., 2001; Xu et al., 2015).

270 Acclimation involves cells or organisms adjusting to new environmental conditions by altering
271 their morphological and biochemical properties. The duration of this process varies among
272 organisms, ranging from hours to a lifetime, depending on their natural characteristics and the type

273 of adaptation required (Collier et al., 2019; Richard E. Lee, 2010; Marshall and Sinclair, 2015;
274 Mishra, 2019). Cold acclimation – a progressive and time-dependent process – is essential for
275 enhancing insect cold tolerance. Many temperate and polar insects increase their cold tolerance as
276 winter approaches. Short, cool autumn days initiate cold acclimatization (Clark and Worland,
277 2008; Richard E. Lee, 2010; Poikela et al., 2021).

278 Insects undergo significant biochemistry, cell function, and gene expression changes during cold
279 acclimation. These adaptations improve cell function and viability at low temperatures. In other
280 words, cold acclimation enhances insect cold tolerance through various physiological mechanisms.
281 For example, it can induce important transcriptional changes that enhance insect cold tolerance.
282 Survival under acute and chronic cold stress significantly improves with longer periods of cold
283 acclimation at slightly low temperatures (Enriquez and Colinet, 2019). Additionally, cold
284 acclimation boosts the depolarization resistance of insect neurons and muscles, increasing their
285 cold-induced depolarization and paralysis tolerance. This upgraded depolarization resistance
286 contributes to enhanced cold tolerance post-acclimation. Furthermore, cold acclimation raises the
287 metabolic rate in insects. The metabolic rate increase following cold acclimation correlates
288 positively with improved cold tolerance, indicating a physiological link regulated by common
289 genetic factors (Bayley et al., 2020). Cold acclimation enables insects to accumulate
290 cryoprotectants like glycerol and boost the production of ice-nucleating and heat-shock proteins.
291 These physiological modifications promote freeze-tolerance in insects after undergoing cold
292 acclimation. Furthermore, cold acclimation enhances the osmoregulatory capacity at low
293 temperatures, which is essential for maintaining extracellular ion balance and boosting cold
294 tolerance (Adamaki-Sotiraki et al., 2024; Collier et al., 2019; Isobe et al., 2013; Marshall and
295 Sinclair, 2015; Mishra, 2019; Poikela et al., 2021). However, cold acclimation plays a significant

296 role in the survival of diapausing lepidopterans (**Table 2**) (Ahmadi et al., 2016; Atapour and
297 Moharramipour, 2014, 2011, 2009; Heydari and Izadi, 2014; Izadi et al., 2019; Malekera et al.,
298 2022; Tarusikirwa et al., 2020a, 2020b). The overwintering larvae of the beet armyworm, *S.*
299 *exigua*, were classified as freeze-avoidant species. It has been revealed that acclimating to cold
300 temperatures below freezing can trigger the expression of a freezing tolerance strategy (Atapour
301 and Moharramipour, 2014; Kim, 2000; Zheng et al., 2014).

302 The extent of improvement in cold tolerance is determined by the duration and temperature of
303 acclimation. A longer acclimation period is directly associated with a higher increase in cold
304 tolerance. In other words, the duration of cold acclimation is important for enhancing insect cold
305 tolerance. Longer acclimation periods tend to increase cold tolerance, with the impact being
306 influenced by factors like sex and species (Collier et al., 2019; Enriquez and Colinet, 2019; Isobe
307 et al., 2013; R.E. Lee, 2010b; Mishra, 2019; Pei et al., 2023; Poikela et al., 2021). In cold-
308 acclimated diapausing larvae of *C. suppressalis* during freezing, water exited the cells into the
309 medium while glycerol simultaneously entered the cells from the medium. Thus, in the rice stem
310 borer larvae, both diapause and cold acclimation are crucial for glycerol accumulation to prevent
311 freezing damage. Since the cold-acclimated diapausing larvae of *C. suppressalis* are capable of
312 surviving temperatures below their SCP, they are considered to be freeze-tolerant (Atapour and
313 Moharramipour, 2009; Cho et al., 2005; Izumi et al., 2007, 2006). A study examined how cold
314 acclimation, cooling rate, and heat stress impact the cold tolerance of the potato tuber moth, *Ph.*
315 *operculella*. The study found that exposing the moth to cold temperatures (either 0°C or 5°C for 1
316 week) did not significantly change the supercooling point (SCPs). Still, it did lead to a significant
317 decrease in mortality for all life stages. The lower lethal temperature for 50% mortality (LT₅₀)

318 decreased after cold acclimation. It was also observed that slower cooling rates reduced mortality
319 rates (Hemmati et al., 2014).

320 Insects can also quickly improve their cold tolerance through rapid cold-hardening (RCH). This
321 process which occurs in response to short-term cold exposure lasting from minutes to hours, allows
322 insects to strengthen their cold tolerance rapidly, helping them adapt to sudden cold snaps and
323 daily temperature changes. However, there are still uncertainties about the specific mechanisms
324 involved (H. Colinet et al., 2018; Marshall and Sinclair, 2015; Teets and Denlinger, 2013). Rapid
325 cold hardening has also been shown to protect individuals from cold stress in various lepidopteran
326 species. By enabling physiological adaptations that preserve cell homeostasis and integrity under
327 unfavorable environmental conditions, RCH enhances the cold tolerance of moths and butterflies
328 (Andersen et al., 2016; Hayward et al., 2014; MacMillan et al., 2016a). The RCH treatment
329 enhanced cold tolerance in third and sixth-instar larvae of *S. frugiperda*, suggesting the importance
330 of the larval stage for cold tolerance in this species (Malekera et al., 2022). Furthermore, the
331 Monarch butterflies migrating to Mexico can quickly increase their cold resistance through
332 acclimation. Exposure to +4°C before -4°C enhances survival rates, showing rapid cold-hardening
333 ability. Longer acclimation to colder temperatures further boosts cold resistance. Wild-caught
334 monarchs are less tolerant to -4°C than those gradually acclimated in controlled conditions (Larsen
335 and Lee, 1994). The report (Malekera et al., 2022) suggests that RCH can potentially enhance the
336 cold tolerance of *S. exigua*. Furthermore, exposing larvae of the rice stem borer to 0 or 5°C for 2
337 or 4 hours before a critical temperature exposure resulted in increased survival rates compared to
338 control groups. The larvae exhibited the highest survival rate following 0°C treatment for 4 hours.
339 Therefore, it can be concluded that rapid cold hardening may improve the cold tolerance of the
340 rice stem borer larvae (QIANG et al., 2008).

341 In their native habitats, insects encounter daily temperature fluctuations linked to thermal
342 randomness. Consequently, insect species must be pre-adapted to regular and unforeseeable
343 thermal changes. This prior adjustment enables them to promptly overcome any chill damage that
344 could occur during unexpected cold conditions (Colinet et al., 2006; Hervé Colinet et al., 2018;
345 Enriquez et al., 2018; Marshall and Sinclair, 2015). At low temperatures, insects experience a
346 system-wide loss of homeostasis, which can be counteracted by intermittent periods of warmth.
347 These warm periods allow the insects to repair and recover physiologically, reducing the
348 cumulative damage caused by prolonged exposure to low temperatures. Exposure to fluctuating
349 thermal regimes (FTR) has enhanced insect survival in cold conditions. Researches indicate that
350 FTR helps insects combat the effects of low temperatures by promoting repair mechanisms to
351 maintain homeostasis. Furthermore, fluctuating thermal regimes have been linked to changes in
352 metabolic and lipid profiles, leading to increased levels of cryoprotectants and membrane
353 remodeling. These changes help the insects maintain their internal balance during cold exposure
354 (Colinet et al., 2015; H. Colinet et al., 2018; Grumiaux et al., 2019; Javal et al., 2016; Saeidi and
355 Moharramipour, 2017). Multiple studies have highlighted the benefits of fluctuating thermal
356 regimes in improving the cold tolerance of insects compared to constant low temperatures (H.
357 Colinet et al., 2018). For example, fluctuating thermal regimes enhanced the Bertha armyworm,
358 *Mamestra configurata* Walker (Noctuidae) adult emergence (Turnock, 1993). It has also been
359 observed that fluctuating thermal conditions have a positive impact on the survival and pupation
360 rates of the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Tortricidae) (Boardman et
361 al., 2013).

362

363 **4. Mechanisms of cold tolerance**

364 Insects employ several fascinating mechanisms involving biochemical, physiological, and
365 behavioral adaptations to tolerate cold temperatures. These mechanisms fall into three main
366 categories: avoidance, tolerance, and acclimation (**Figure 3**). Understanding these strategies
367 enhances our knowledge of insect survival and informs predictions about the impact of climate
368 change on insect populations and their distribution across ecosystems. Compatible osmolytes
369 (cryoprotectants) have been described as important components increasing the cold tolerance of
370 freeze-tolerant and freeze-avoidant insect species (Storey and Storey, 1988; Zachariassen, 1980).
371 Some of the most known compatible osmolytes include polyols, such as glycerol, sorbitol, and
372 myo-inositol; sugars, such as trehalose, glucose, and fructose; and amino acids, such as proline. In
373 insects, glycerol, trehalose, sorbitol, glucose, mannitol, xylitol, erythritol, ethylene glycol, and
374 myo-inositol (**Table A2**) are among the most extensively studied compatible osmolytes (Kathryn
375 A. Wurzel, 2005; Storey, J. M., and Storey, 2004; Toxopeus et al., 2019). These metabolites are
376 nontoxic, even when accumulated at high concentrations, and aid in stabilizing the functional state
377 of proteins by preventing denaturation of their native structures caused by harsh ambient
378 temperatures or freezing. Stabilizing cell membranes and soluble enzymes are also associated with
379 accumulating these metabolites during exposure to low temperatures (Grgac et al., 2022).

380 Lepidopteran species are no exception (**Table 3**), with glycerol and trehalose being accumulated
381 in wintertime in 39% and 30% of the lepidopterans considered in the present work (**Figure 2**).
382 Glycerol, a triol, is a viscous and hygroscopic liquid with moderate solubility in water. This non-
383 ionic polyol prevents the formation of ice by disrupting hydrogen bonds between water molecules
384 through the formation of strong hydrogen bonds with water molecules. Trehalose is a non-reducing
385 disaccharide composed of two glucose molecules (**Table 3**). It is derived from glycolytic
386 intermediates, specifically glucose-1-phosphate and glucose-6-phosphate. This disaccharide

387 serves as the main sugar circulating in insect hemolymph. Its levels fluctuate based on various
388 factors such as ambient temperature, the insect's physiological state, and its nutritional intake.
389 Trehalose plays several crucial roles, including serving as the predominant source of energy, acting
390 as a cryoprotectant to enhance the insect's cold tolerance, serving as an effective protein stabilizer
391 under stress conditions, and being a central product in a feedback mechanism that regulates insect
392 energy demands (Dou et al., 2019; Jain and Roy, 2010; Lee et al., 2002; Tang et al., 2018;
393 Thompson, 2003).

394 Some – but not all– lepidopteran species can accumulate several compatible osmolytes (**Table**
395 **3**). In the case of the pink maize stalk borer, *S. nonagrioides*, trehalose synthesis does not seem to
396 function as an osmolyte enhancing the pest's ability to survive in cold temperatures, while the
397 accumulation of glycerol does (Andreadis et al., 2011; Gillyboeuf et al., 1994). Overall, the
398 accumulation of compatible osmolytes is responsible for cold tolerance acquisition in more than
399 85% of lepidopteran pests considered here. Roughly 77% of the species studied use more than one
400 cryoprotectant (**Figure 2**).

401 Cold tolerance in insects encompasses a range of biochemical, physiological, and
402 endocrinological adjustments (Wang et al., 2016), with several enzymes playing a crucial role in
403 the process. In particular, the activity of antioxidant enzymes has been relatively studied as
404 biochemical mechanisms to prevent freezing injury and enhance insects' cold hardiness (Cheng et
405 al., 2016; Wang et al., 2016).(QIANG Cheng-kui and QIANG Cheng-kui, 2012). The levels of
406 three antioxidant enzymes—superoxide dismutase (SOD), peroxidase (POD), and catalase
407 (CAT)—were found to be increased in the overwintering larvae of the rice stem borer (QIANG
408 Cheng-kui and QIANG Cheng-kui, 2012). Prolonged exposure to cold and metabolic slowdown
409 during diapause is linked to reduced mitochondrial activity and key enzymes of cellular respiration

410 (Lebenzon et al., 2023; Štětina et al., 2020; Su et al., 2024). Interestingly, the number of
411 mitochondria in the brains of diapausing pupae of *H. armigera* is significantly lower than in non-
412 diapausing ones, suggesting metabolic depression during diapause (Su et al., 2024). Moreover,
413 trehalose-6-phosphate synthase (TPS) activity is significantly higher during *H. armigera* larval–
414 pupal development in diapausing individuals and is closely correlated with the changes in the
415 trehalose content. Upon diapause termination, trehalose levels decrease significantly, while
416 glucose levels rise rapidly, indicating the role of trehalose in the regulation of *H. armigera* cold
417 hardening during pupal diapause (Xu J1, Bao B, Zhang ZF, Yi YZ, 2009). Similarly, the European
418 spurge hawkmoth, *Hyles euphorbiae* (Lepidoptera, Sphingidae), has an up-regulated glycolysis
419 enzyme and a shift in gene expression patterns towards carbohydrate metabolism. This leads to
420 the production of cryoprotectants, mainly glycerol. Identifying several possible enzymes involved
421 in glucose, glycerol, myo-inositol, sorbitol, and trehalose synthesis indicates their role in this
422 process (Barth et al., 2018). In sum, several enzymes enhance cold tolerance through antioxidant
423 or metabolic activity. Antioxidant activity refers to the ability of enzymes to neutralize harmful
424 molecules whereas, metabolic activity refers to the overall chemical reactions that occur in an
425 organism to maintain life (Hayward and Colinet, 2023; Teets et al., 2023).

426 Insects exposed to stressors, such as temperature fluctuations, may respond by producing heat
427 shock proteins (HSPs). These proteins are crucial for insect survival under temperature stress, as
428 they help reduce oxidative damage and improve tolerance to extreme conditions (Wang et al.,
429 2024). In the larvae of the Carob moth, researchers studied various stressors and observed
430 increased levels of oxidative stress markers and higher antioxidant enzyme activity. Additionally,
431 they used Quantitative RT-PCR to confirm that increased heat and cold tolerance were linked to
432 the up-regulation of two heat shock proteins, HSP70 and HSP90. Interestingly, the study found

433 that HSP70 transcripts increased significantly with cold treatment, while HSP90 transcripts
434 showed a greater increase in response to high temperatures (Farahani et al., 2020). Heat shock
435 genes and proteins, particularly heat shock proteins, play a vital role in protecting *B. mori* from
436 increased heat stress within a certain range. However, beyond this range, the silkworm's biology
437 is impacted. The silkworm can adapt to heat by upregulating thermotolerance genes and proteins
438 but produces heat-resistant proteins at different temperatures (Ashraf and Qamar, 2023). In the
439 Asian corn borer, *O. furnacalis* experiences extreme thermal stress, which induces rapid
440 transcription of HSP70 or HSP90. This may be an adaptation to rapid and drastic temperature
441 changes (Quan et al., 2022). However, the upregulation of HSPs helps insects withstand the
442 prolonged cold exposure experienced during the overwintering diapause period. The magnitude
443 and timing of HSP induction can vary between different Lepidoptera species and developmental
444 stages, reflecting their unique cold tolerance strategies (Ashraf and Qamar, 2023; Farahani et al.,
445 2020; Quan et al., 2022; Wang et al., 2024).

446 Overall, the cold tolerance of insects results from a combination of physiological, biochemical,
447 and behavioral adaptations that allow them to survive in diverse and challenging environments.
448 These adaptations are finely tuned to balance the need to avoid freezing injury with the demands
449 of energy conservation and survival during periods of cold stress. However, the specific
450 mechanisms used by insects to tolerate cold will vary depending on the species and the
451 environment they live in. For example, research has shown that ambient temperature, rather than
452 body size, considerably impacts the cold hardiness of overwintering larvae of *Streltzoviella*
453 *insularis* (Pei et al., 2020).

454

455 **5. Cold tolerance plasticity**

456 Insect cold tolerance plasticity refers to insects' ability to adjust their physiological and
457 biochemical processes in response to temperature changes, allowing them to survive and function
458 in cold environments. This adaptability is vital for temperate and polar insects, where temperatures
459 vary widely. Insect cold tolerance shows impressive flexibility, adapting through different
460 mechanisms such as environmental conditions, morphological traits, behavioral adaptations,
461 diapause, genetic and epigenetic factors, cold acclimation, rapid cold hardening, developmental
462 stage, seasonal changes, geographical distribution and molecular strategies (**Figure 4**) (Bayley et
463 al., 2020; deJong and Saastamoinen, 2018; Jass et al., 2019; Rodrigues and Beldade, 2020; Sinclair
464 et al., 2015; Teets and Denlinger, 2013; Weaving et al., 2022). Cold tolerance traits, including
465 critical thermal minimum (CT_{min}), supercooling point (SCP), and survival after cold exposure,
466 often correlate with the distribution of insects. Therefore, the adoption of a cold tolerance strategy
467 is not an inherent, fixed trait for a species; rather, it can vary depending on the geographical
468 distribution of the species (Sinclair et al., 2015; Tremblay et al., 2021). For example, populations
469 of *C. suppressalis* from Japan (Izumi et al., 2006), Korea (Cho et al., 2005), and Iran (Atapour and
470 Moharramipour, 2009) are freeze-tolerant. In contrast, in China (QIANG Cheng-kui and QIANG
471 Cheng-kui, 2012), populations of this pest are freeze-avoidant. Similarly, specimens of *Cossus*
472 *cossus* (Cossidae) from Yakutia (Russia) with an SCP of $-5.05\text{ }^{\circ}\text{C}$ are considered freeze-tolerant
473 (N.G. Li, 2016) whereas, in North Europe, specimens with an SCP of $-17.70\text{ }^{\circ}\text{C}$, are freeze-
474 avoidant (N.G. Li, 2016; Sømme, 1982). However, the relationship between cold tolerance and
475 distribution is complex. A study on the giant swallowtail butterfly, *P. cresphontes*, found that low
476 temperatures during autumn are unlikely to limit its distribution, despite the larvae being chill-
477 tolerant or modestly freeze-avoidant at their northern range limit (Tremblay et al., 2021).
478 Furthermore, the growing conditions and climatic patterns of lepidopteran's habitat play a

479 significant role in shaping the evolution of its specific cold tolerance mechanisms, whether it be
480 freeze tolerance, freeze avoidance, or a combination of strategies (Bale and Hayward, 2010). For
481 instance, in natural conditions, *C. pomonella* primarily depends on the accumulation of compatible
482 osmolytes and the depression of the supercooling point to improve its ability to withstand cold
483 temperatures. Conversely, in laboratory conditions, the pest's cold tolerance can be improved by
484 removing ice-nucleating agents (Neven, 1999). The supercooling point of field-collected larvae of
485 *S. exigua* is significantly higher than that of laboratory-reared larvae (Kim and Kim, 1997), and
486 cold tolerance strategies depend on rearing conditions in *E. ceratoniae*. However, the rearing
487 conditions did not affect the cold tolerance of one of the major lepidopteran pests, the Indian meal
488 moth *P. interpunctella* (Carrillo et al., 2005). Different developmental stages of the potato tuber
489 moth, *Ph. operculella*, cannot tolerate temperatures below their SCPs. Therefore, this pest has been
490 categorized as a freeze-avoidant insect (Hemmati et al., 2017). Another research showed that
491 acclimation to cold temperatures improved survival in subzero conditions for immature and adult
492 stages of *Ph. operculella*. Pupae were the most cold-tolerant, while late instars were the least, with
493 pre-freeze mortality observed in all stages, making the pest chill-tolerant rather than freeze-
494 avoidant (Andreadis et al., 2016). The differences in results can be attributed to the acclimation
495 and overwintering status of this pest (Andreadis et al., 2016).

496 The climatic conditions in temperate zones do not allow insects to tolerate very low temperatures
497 for extended periods due to their sensitivity to cold temperatures. Therefore, most species in this
498 area adopt a strategy that is intolerant to freezing. By employing this strategy, certain insects can
499 exploit their environment and overcome biological limitations. For instance, they may migrate to
500 (micro)habitats with relatively warmer temperatures or seek sheltered environments (Bale and
501 Hayward, 2010; Richard E. Lee, 2010; Sinclair, 1999; Stockton et al., 2019). The monarch

502 butterfly, *Danaus plexippus*, and the painted lady, *Vanessa cardui*, are well-known examples of
503 species that undertake long-distance migrations to reach warmer habitats (Grant et al., 2022;
504 Kendrick and McCord, 2023; Shirey and Ries, 2023; Suchan et al., 2024).

505 Insects in regions with long, harsh winters, like high latitudes or alpine areas, often have more
506 developed cold tolerance strategies than those from gentler climates (Bale and Hayward, 2010).
507 The distinctive environmental conditions in the Southern Hemisphere are believed to be the
508 primary selection pressure that favored the evolution of the freeze-tolerance strategy (Sinclair et
509 al., 2003a, 2003b). Therefore, freeze-tolerant insects are more prevalent in areas with abrupt and
510 severe temperature drops, such as the southern hemisphere. This approach enables them to trigger
511 and control ice formation within their bodies promptly. Conversely, freeze-avoidant insects, which
512 avert internal ice formation via supercooling, thrive in regions with gradual seasonal cooling, like
513 the northern hemisphere. This allows them to steadily ready themselves for winter by eliminating
514 ice-nucleating agents from their bodies (Sinclair, 1999). A study examined cold tolerance variation
515 in the African sugarcane borer, *Eldana saccharina* (Pyralidae), revealing significant differences in
516 CT_{min} among geographic lines and a positive correlation with local climates (Kleynhans et al.,
517 2014). The pine white butterfly, *Neophasia menapia*, and the Mexican pine white, *N. terlooii*
518 (Pieridae), overwinter as eggs with supercooling points of $-29.0\text{ }^{\circ}\text{C}$, and $-21.8\text{ }^{\circ}\text{C}$, respectively.
519 Both species are freeze-avoidant and can survive down to their respective SCPs. Research suggests
520 that the colder winters in northern Arizona may play a role in the northern range limit for *N.*
521 *terlooii*. Overall, this study exhibits variations in the cold hardiness strategies, reflecting their
522 disjunct geographic ranges (Halbritter et al., 2018).

523 The ability of invasive pests to tolerate colds directly impacts their survival and success. These
524 pests adapt to cold conditions through various mechanisms, cold acclimation or rapid cold

525 hardiness, enabling them to thrive in new environments (ZHANG et al., 2021). For example, the
526 fall armyworm, *S. frugiperda*, is a crop pest native to tropical and subtropical regions of the
527 Americas which has now colonized most parts of the world (Palli et al., 2023; Sun et al., 2021).
528 Cold acclimation and rapid cold hardening may help this pest to rapidly enhance its cold tolerance
529 when facing sudden variations of the temperature that may occur during winter times in the
530 subtropical or temperate regions of Asian countries (Malekera et al., 2022). Moreover, the level of
531 cold hardiness directly determines its geographic distribution and allows the survival of the insect
532 during the winter periods in a vast number of regions (Zhang et al., 2021). The fall webworm, *H.*
533 *cunea*, is native to North America and has been unintentionally introduced to certain areas of
534 Europe and Asia. Cold acclimation in this species enhanced cold tolerance without the buildup of
535 sugars and polyols. This suggests that the insect's ability to adapt to various habitats is due to its
536 inherent capacity to enhance cold tolerance in summer after cold acclimation (Li et al., 2001).
537 Results of a study indicate that cold tolerance of early instar larvae of an invasive forest insect, *L.*
538 *dispar* is linked to regional climate, with individuals from cold climate populations recovering
539 faster from chill coma (Hafker et al., 2024).

540 Phylogenetic relationships in insect cold tolerance concern insect species' evolutionary history
541 and genetic relatedness regarding their capacity to endure freezing temperatures. Analyzing the
542 phylogenetic connections of insects with diverse cold tolerance abilities allows researchers to
543 understand the genetic foundation of cold tolerance and its evolutionary development. This
544 knowledge aids in comprehending the mechanisms behind cold tolerance in insects and may guide
545 strategies for controlling insect populations in shifting climates. Moreover, understanding
546 phylogenetic and ontogenetic differences in thermal limits is crucial for predicting species'
547 distributions and climate change responses (Bowler and Terblanche, 2008; Poikela et al., 2021;

548 Vernon and Vannier, 2002; Wang et al., 2016). A study examined phylogenetic and ontogenetic
549 variation in the plasticity of thermal tolerance in lepidopteran species. Results showed that
550 phylogenetic and ontogenetic factors may play roles in shaping the cold tolerance of lepidopteran
551 species, with closely related species often exhibiting similar cold tolerance traits (Kingsolver and
552 Buckley, 2020). In this context, the Papilionidae species studied are freeze-avoidant and improve
553 their cold tolerance through cold acclimation, but they do not experience supercooling point
554 depression. Conversely, various species of Pyralidae depend on cold acclimation and supercooling
555 point expansion to enhance their cold tolerance. This suggests that the evolution of cold tolerance
556 is not solely driven by environmental pressures but also by the genetic background of the species
557 (Kingsolver and Buckley, 2020). Ontogenetic relationships refer to the changes in cold tolerance
558 during an insect's life cycle. Studies have demonstrated that cold tolerance can vary significantly
559 across different life stages, with some stages being more tolerant to cold temperatures than others.
560 For instance, research on the fall armyworm, *S. frugiperda* has shown that adult stages are more
561 tolerant to moderately low temperatures than eggs, larvae, and pupae, indicating that cold tolerance
562 can change during development (ZHANG et al., 2021). Moreover, the cold tolerance of the Indian
563 meal moth, *P. interpunctella* (M A Carrillo C A Cannon, W F WilckeWilliam, D
564 HutchisonWilliam, 2005). and the wild silkworm, *Antheraea pernyi* (Saturniidae) are closely
565 linked to their developmental stage (Liu et al., 2016). Additionally, the cold tolerance of insects
566 can be influenced by factors such as age. Some studies suggest that younger insects may be more
567 susceptible to cold temperatures than older ones. A study on the cold hardiness of invasive fall
568 armyworms in China found that pupae and older larvae have higher survival rates than eggs and
569 younger larvae (Vatanparast and Park, 2022). This highlights the importance of considering the
570 ontogenetic stage when studying cold tolerance in insects. However, basal thermal tolerance and

571 acclimation responses (i.e. phenotypic plasticity) are strongly influenced by age and ontogeny of
572 the species (Ganesan et al., 2021; Papadopoulos et al., 2023). The overwintering stage of 859
573 lepidopteran species was reported in the LepTraits 1.0 database. This database was also used to
574 compare the data diapausing stage of each species (Shirey et al., 2022). However, the
575 overwintering stage of insects often exhibits the highest cold tolerance. This is supported by
576 various studies that highlighted the adaptations and physiological changes that occur during this
577 stage to enhance cold tolerance. The overwintering larvae of the codling moth, *C. pomonella*, with
578 the highest cold tolerance, are especially capable of surviving at subzero temperatures (Rozsypal
579 et al., 2013). The cold tolerance of diapausing larvae of the pistachio white leaf borer, *Ocneria*
580 *terebinthina* (Behroozi et al., 2012), the carob moth, *E. ceratoniae* (Heydari and Izadi, 2014)),
581 pupae of the European grapevine moth, *Lobesia botrana* (Masoudmagham et al., 2021) and
582 overwinter eggs of the pine white butterfly, *N. menapia*, and the Mexican pine white, *N. terlooii*
583 (Halbritter et al., 2018) was significantly higher than that of non-diapausing ones.

584 Epigenetics, studying how environmental conditions influence gene expression. Populations of
585 the same species living in different climates may have genetic differences influencing their cold
586 tolerance. These genetic variations can be naturally selected over generations. Environmental
587 stresses, including freezing temperatures, can lead to epigenetic changes that affect gene
588 expression related to cold tolerance (Banerjee et al., 2017). Furthermore, parental exposure can
589 influence offspring traits. Parental exposure can shape insects' cold adaptations, affecting the
590 survival of future generations. In a study with *Drosophila melanogaster*, researchers examined the
591 impact of maternal cold stress on offspring cold tolerance. The findings revealed that maternal
592 cold stress significantly affected the survival rate of offspring exposed to cold stress. Certain
593 offspring groups displayed higher survival rates, while others exhibited lower tolerance to cold

594 exposure (Jacob Welch and Christopher Marks, 2023). However, there is no direct information
595 connecting epigenetics and cold tolerance in Lepidoptera based on the provided search results.

596

597 **9. Conclusion**

598 The ability to tolerate cold temperatures is not an inherent, unchanging trait but a highly adaptable
599 one, influenced significantly by factors such as the overwintering stage, geographic location, and
600 rearing conditions. A key factor in lepidopteran insects' ability to withstand cold temperatures is
601 the accumulation of cryoprotectants, which helps lower their supercooling point. However, this
602 association is often questionable, as the accumulation is too low in many cases to have measurable
603 effects. Our review also shows a strong correlation between diapause and increased cold tolerance
604 in most lepidopteran species. Despite this correlation, both phenomena are complex, and their
605 mechanistic relationship remains largely untested. Additionally, freeze avoidance is identified as
606 the primary mechanism by which these insects tolerate cold temperatures. However, there is a lack
607 of information for many species and contexts, highlighting the need for future research to fill these
608 gaps and better understand how traits and context-dependencies influence cold tolerance strategies
609 in Lepidoptera.

610

611

612

613

614

615 **References**

- 616 Adamaki-Sotiraki, C., Abenaim, L., Mannucci, A., Rumbos, C.I., Bedini, S., Castagna, A., Conte,
617 G., Tognocchi, M., Dolianitis, V., Athanassiou, C.G., Conti, B., 2024. Performance of
618 *Hermetia illucens* (Diptera: Stratiomyidae) larvae reared on organic waste diets and pupal
619 chitin and chitosan yield. Environmental Science and Pollution Research.
620 <https://doi.org/10.1007/s11356-024-33545-x>
- 621 Ahmadi, B., Moharramipour, S., Sinclair, B.J., 2016. Overwintering biology of the carob moth
622 *Apomyelois ceratoniae* (Lepidoptera: Pyralidae). Int J Pest Manag 62, 69–74.
623 <https://doi.org/10.1080/09670874.2015.1102984>
- 624 Andersen, M.K., Folkersen, R., MacMillan, H.A., Overgaard, J., 2016. Cold acclimation improves
625 chill tolerance in the migratory locust through preservation of ion balance and membrane
626 potential. J Exp Biol 220, 487–496. <https://doi.org/10.1242/jeb.150813>
- 627 Andreadis, S.S., Athanassiou, C.G., 2017. A review of insect cold hardiness and its potential in
628 stored product insect control. Crop Protection 91, 93–99.
629 <https://doi.org/10.1016/j.cropro.2016.08.013>
- 630 Andreadis, S.S., Poulia, Y., Noukari, S., Aslanidou, B., Savopoulou-Soultani, M., 2016. Cold
631 hardiness attributes of a field population of the potato tuberworm, *Phthorimaea operculella*.
632 Entomol Exp Appl 160, 109–116. <https://doi.org/10.1111/eea.12465>
- 633 Andreadis, S.S., Vryzas, Z., Papadopoulou-Mourkidou, E., Savopoulou-Soultani, M., 2011. Cold
634 tolerance of field-collected and laboratory reared larvae of *Sesamia nonagrioides*
635 (Lepidoptera: Noctuidae). Cryo-Letters 32, 297–307.
- 636 Ashraf, H., Qamar, A., 2023. A review on heat stress altering the insect life history strategies and
637 underlying mechanisms: Special reference to an economically important Lepidoptera,
638 *Bombyx mori* (Linnaeus, 1758) (Lepidoptera: Bombycidae). SHILAP Soc Hispano Luso Am
639 Lepid 51, 339–357. <https://doi.org/10.57065/shilap.468>
- 640 Atapour, M., Moharramipour, S., 2014. Cold hardiness process of beet armyworm larvae ,
641 *Spodoptera exigua* (Lepidoptera : Noctuidae). J Crop Prot 3, 147–158.
- 642 Atapour, M., Moharramipour, S., 2011. Changes in supercooling point and glycogen reserves in
643 overwintering and lab-reared samples of beet armyworm, *Spodoptera exigua* (Lep.:
644 Noctuidae) to determining of cold hardiness strategy. Appl Entomol Phytopathol 78, 199–
645 216.
- 646 Atapour, M., Moharramipour, S., 2009. Changes of cold hardiness, supercooling capacity, and
647 major cryoprotectants in overwintering larvae of *Chilo suppressalis* (Lepidoptera: Pyralidae).
648 Environ Entomol 38, 260–265. <https://doi.org/10.1603/022.038.0132>

- 649 Bale, J.S., Hayward, S.A.L.L., 2010. Insect overwintering in a changing climate. *J Exp Biol* 213,
650 980–994. <https://doi.org/10.1242/jeb.037911>
- 651 Banerjee, A., Wani, S.H., Roychoudhury, A., 2017. Epigenetic control of plant cold responses.
652 *Front Plant Sci* 8. <https://doi.org/10.3389/fpls.2017.01643>
- 653 Barth, M.B., Buchwalder, K., Kawahara, A.Y., Zhou, X., Liu, S., Krezdorn, N., Rotter, B., Horres,
654 R., Hundsdoerfer, A.K., 2018. Functional characterization of the *Hyles euphorbiae*
655 hawkmoth transcriptome reveals strong expression of phorbol ester detoxification and
656 seasonal cold hardiness genes. *Front Zool* 15, 20. [https://doi.org/10.1186/s12983-018-0252-](https://doi.org/10.1186/s12983-018-0252-2)
657 2
- 658 Bayley, J.S., Sørensen, J.G., Moos, M., Košťál, V., Overgaard, J., 2020. Cold acclimation increases
659 depolarization resistance and tolerance in muscle fibers from a chill-susceptible insect,
660 locusta migratoria. *Am J Physiol Regul Integr Comp Physiol* 319, R439–R447.
661 <https://doi.org/10.1152/ajpregu.00068.2020>
- 662 Behroozi, E., Izadi, H., Samih, M.A.A., Moharamipour, S., 2012. Physiological strategy in
663 overwintering larvae of pistachio white leaf borer, *Ocneria terebinthina* Strg. (Lepidoptera:
664 Lymantriidae) in Rafsanjan, Iran. *Ital J Zool* 79, 44–49.
665 <https://doi.org/10.1080/11250003.2011.592152>
- 666 Bemani, M., Izadi, H., Mahdian, K., Khani, A., Amin samih, M., 2012. Study on the physiology
667 of diapause, cold hardiness and supercooling point of overwintering pupae of the pistachio
668 fruit hull borer, *Arimania comaroffi*. *J Insect Physiol* 58, 897–902.
669 <https://doi.org/10.1016/j.jinsphys.2012.04.003>
- 670 Boardman, L., Sørensen, J.G., Terblanche, J.S., 2013. Physiological responses to fluctuating
671 thermal and hydration regimes in the chill susceptible insect, *Thaumatotibia leucotreta*. *J*
672 *Insect Physiol* 59, 781–794. <https://doi.org/10.1016/j.jinsphys.2013.05.005>
- 673 Bowler, K., Terblanche, J.S., 2008. Insect thermal tolerance: what is the role of ontogeny, ageing
674 and senescence? *Biol Rev* 83, 339–355. <https://doi.org/10.1111/j.1469-185X.2008.00046.x>
- 675 Buckley, L., Arakaki, A., Cannistra, A., Kharouba, H., Kingsolver, J., 2017. Insect development,
676 thermal plasticity and fitness implications in changing, seasonal environments. *Integ Comp*
677 *Biol* 57. <https://doi.org/10.1093/icb/icx032>
- 678 Carrillo, M.A., Cannon, C.A., 2005. Supercooling point variability in the Indian meal moth, *Plodia*
679 *interpunctella* (Hübner) (Lepidoptera: Pyralidae). *J Stored Prod Res* 41, 556–564.
680 <https://doi.org/10.1016/j.jspr.2004.10.002>
- 681 Carrillo, M.A., Cannon, C.A., Wilcke, W.F., Morey, R. V., Kaliyan, N., Hutchison, W.D., 2005.
682 Relationship between supercooling point and mortality at low temperatures in indianmeal

- 683 moth (Lepidoptera: Pyralidae). J Econ Entomol 98, 618–625.
684 <https://doi.org/10.1093/jee/98.2.618>
- 685 Chaudhuri, P., Rashid, N., Thapliyal, C., 2017. Osmolyte System and Its Biological Significance,
686 in: Cellular Osmolytes. Springer Singapore, Singapore, pp. 1–34.
687 https://doi.org/10.1007/978-981-10-3707-8_1
- 688 Chen, C., Wei, X.T., Xiao, H.J., He, H.M., Xia, Q.W., Xue, F. Sen, 2014. Diapause induction and
689 termination in *Hyphantria cunea* (Drury) (Lepidoptera: Arctiinae). PLoS One 9.
690 <https://doi.org/10.1371/journal.pone.0098145>
- 691 Cheng, W., Li, D., Wang, Y., Liu, Y., Zhu- Salzman, K., 2016. Cloning of heat shock protein
692 genes (hsp70, hsc70 and hsp90) and their expression in response to larval diapause and
693 thermal stress in the wheat blossom midge, *Sitodiplosis mosellana*. J Insect Physiol 95, 66–
694 77. <https://doi.org/10.1016/j.jinsphys.2016.09.005>
- 695 Cho, J.R., Lee, J.S., Kim, J.J., Lee, M., Kim, H.S., Boo, K.S., 2005. Cold hardiness of diapausing
696 rice stem borer, *Chilo suppressalis* Walker (Lepidoptera: Pyralidae). J Asia Pac Entomol 8,
697 161–166. [https://doi.org/10.1016/S1226-8615\(08\)60087-4](https://doi.org/10.1016/S1226-8615(08)60087-4)
- 698 Clark, M.S., Thorne, M.A.S., Purać, J., Burns, G., Hillyard, G., Popović, Ž.D., Grubor-Lajšić, G.,
699 Worland, M.R., 2009. Surviving the cold: Molecular analyses of insect cryoprotective
700 dehydration in the Arctic springtail *Megaphorura arctica* (Tullberg). BMC Genomics 10, 1–
701 19. <https://doi.org/10.1186/1471-2164-10-328>
- 702 Clark, M.S., Worland, M.R., 2008. How insects survive the cold: Molecular mechanisms - A
703 review. J Comp Physiol B. <https://doi.org/10.1007/s00360-008-0286-4>
- 704 Colinet, H., Renault, D., Hance, T., Vernon, P., 2006. The impact of fluctuating thermal regimes
705 on the survival of a cold-exposed parasitic wasp, *Aphidius colemani*. Physiol Entomol.
706 <https://doi.org/10.1111/j.1365-3032.2006.00511.x>
- 707 Colinet, Hervé, Rinehart, J.P., Yocum, G.D., Greenlee, K.J., 2018. Mechanisms underpinning the
708 beneficial effects of fluctuating thermal regimes in insect cold tolerance. J Exp Biol 23;221.
709 <https://doi.org/10.1242/JEB.164806>
- 710 Colinet, H., Sinclair, B.J., Vernon, P., Renault, D., 2015. Insects in fluctuating thermal
711 environments. Annu Rev Entomol 60, 123–140. <https://doi.org/10.1146/annurev-ento-010814-021017>
- 713 Collier, R.J., Baumgard, L.H., Zimbelman, R.B., Xiao, Y., 2019. Heat stress: physiology of
714 acclimation and adaptation. Animal Frontiers 9, 12–19. <https://doi.org/10.1093/af/vfy031>
- 715 Costanzo, J.P., Lee, R.E., 2013. Avoidance and tolerance of freezing in ectothermic vertebrates. J
716 Exp Biol 216, 1961–1967. <https://doi.org/10.1242/jeb.070268>

- 717 Danks, H.V., 2007. The elements of seasonal adaptations in insects. *Can Entomol* 139, 1–44.
718 <https://doi.org/10.4039/n06-048>
- 719 Danks, H. V., 1996. The wider integration of studies on insect cold-hardiness. *Eur J Entomol.* 93,
720 383-403
- 721 deJong, M., Saastamoinen, M., 2018. Environmental and genetic control of cold tolerance in the
722 Glanville fritillary butterfly. *J (Basel)* 31. <https://doi.org/10.1111/jeb.13247>
- 723 Denlinger, D.L., 2023. Insect diapause: from a rich history to an exciting future. *J Exp Biol* 226.
724 <https://doi.org/10.1242/jeb.245329>
- 725 Denlinger, D.L., 2022. *Insect Diapause*. Cambridge University Press.
726 <https://doi.org/10.1017/9781108609364>
- 727 Denlinger D.L, 2009. Diapause, in: Resh V. H. and Carde R.T (Ed.), *Encyclopedia of Insects*.
728 Elsevier Inc, pp. 267–271.
- 729 Denlinger, D.L., 2002. Regulation of Diapause. *Annu Rev Entomol* 47, 93–122.
730 <https://doi.org/10.1146/annurev.ento.47.091201.145137>
- 731 Denlinger, D.L., 1991. Relationship between Cold Hardiness and Diapause, in: *Insects at Low*
732 *Temperature*. pp. 174–198. https://doi.org/10.1007/978-1-4757-0190-6_8
- 733 Denlinger, D.L., Lee, R.E., Yocum, G.D., Kukal, O., 1992. Role of chilling in the acquisition of
734 cold tolerance and the capacitation to express stress proteins in diapausing pharate larvae of
735 the gypsy moth, *Lymantria dispar*. *Arch Insect Biochem Physiol* 21, 271–280.
736 <https://doi.org/10.1002/arch.940210404>
- 737 Dillon, M.E., Lozier, J.D., 2019. Adaptation to the abiotic environment in insects: the influence of
738 variability on ecophysiology and evolutionary genomics. *Curr Opin Insect Sci* 36, 131–139.
739 <https://doi.org/10.1016/j.cois.2019.09.003>
- 740 Ditrich, T., 2018. Supercooling point is an individually fixed metric of cold tolerance in
741 *Pyrrhocoris apterus*. *J Therm Biol* 74, 208–213.
742 <https://doi.org/10.1016/j.jtherbio.2018.04.004>
- 743 Dou, M., Lu, C., Sun, Z., Rao, W., 2019. Natural cryoprotectants combinations of L-proline and
744 trehalose for red blood cells cryopreservation. *Cryobiology* 91, 23–29.
745 <https://doi.org/10.1016/j.cryobiol.2019.11.002>
- 746 Duman, J.G., 2015. Animal ice-binding (antifreeze) proteins and glycolipids: An overview with
747 emphasis on physiological function. *J Exp Biol* 218, 1846–1855.
748 <https://doi.org/10.1242/jeb.116905>

- 749 Elnitsky, M.A., Hayward, S.A.L., Rinehart, J.P., Denlinger, D.L., Lee, R.E., 2008. Cryoprotective
750 dehydration and the resistance to inoculative freezing in the Antarctic midge, *Belgica*
751 *antarctica*. J Exp Biol 211, 524–530. <https://doi.org/10.1242/jeb.011874>
- 752 Enriquez, T., Colinet, H., 2019. Cold acclimation triggers major transcriptional changes in
753 *Drosophila suzukii*. BMC Genomics 20. <https://doi.org/10.1186/s12864-019-5745-7>
- 754 Enriquez, T., Ruel, D., Charrier, M., Colinet, H., 2018. Effects of fluctuating thermal regimes on
755 cold survival and life history traits of the spotted wing *Drosophila* (*Drosophila suzukii*).
756 Insect Sci. <https://doi.org/10.1111/1744-7917.12649>
- 757 Farahani, S., Bandani, A.R., Alizadeh, H., Goldansaz, S.H., Whyard, S., 2020. Differential
758 expression of heat shock proteins and antioxidant enzymes in response to temperature,
759 starvation, and parasitism in the Carob moth larvae, *Ectomyelois ceratoniae* (Lepidoptera:
760 Pyralidae). PLoS One 15, e0228104. <https://doi.org/10.1371/journal.pone.0228104>
- 761 Forrest, J.R., 2016. Complex responses of insect phenology to climate change. Curr Opin Insect
762 Sci 17, 49–54. <https://doi.org/10.1016/j.cois.2016.07.002>
- 763 Ganesan, L., Fields, P.G., Jayas, D.S., Jian, F., 2021. Effects of developmental stage, cold
764 acclimation and diet on the cold tolerance of three species of Cryptolestes (Coleoptera:
765 Laemophloeidae). J Stored Prod Res 91, 101773. <https://doi.org/10.1016/j.jspr.2021.101773>
- 766 Gerber, L., Overgaard, J., 2018. Cold tolerance is linked to osmoregulatory function of the hindgut
767 in *Locusta migratoria*. J Exp Biol 221. <https://doi.org/10.1242/jeb.173930>
- 768 Gillyboeuf, N., Anglade, P., Lavenseau, L., Peypelut, L., 1994. Cold hardiness and overwintering
769 strategy of the pink maize stalk borer, *Sesamia nonagrioides* Lef (Lepidoptera, noctuidae).
770 Oecologia 99, 366–373. <https://doi.org/10.1007/BF00627750>
- 771 González-Tokman, D., Córdoba-Aguilar, A., Dáttilo, W., Lira-Noriega, A., Sánchez-Guillén,
772 R.A., Villalobos, F., 2020. Insect responses to heat: physiological mechanisms, evolution and
773 ecological implications in a warming world. Biol Rev 95, 802–821.
774 <https://doi.org/10.1111/brv.12588>
- 775 Goto, M., Li, Y.-P., Honma, T., 2003. Changes of diapause and cold hardiness in the Shonai
776 ecotype larvae of the rice stem borer, *Chilo suppressalis* Walker (Lepidoptera: Pyralidae)
777 during overwintering. Appl Entomol Zool 36, 323–328. <https://doi.org/10.1303/aez.2001.323>
- 778 Grant, T.J., Fisher, K.E., Krishnan, N., Mullins, A.N., Hellmich, R.L., Sappington, T.W.,
779 Adelman, J.S., Coats, J.R., Hartzler, R.G., Pleasants, J.M., Bradbury, S.P., 2022. Monarch
780 butterfly ecology, behavior, and vulnerabilities in North Central United States agricultural
781 landscapes. Bioscience 72, 1176–1203. <https://doi.org/10.1093/biosci/biac094>

- 782 Grgac, R., Rozsypal, J., Des Marteaux, L., Štětina, T., Košťál, V., 2022. Stabilization of insect cell
783 membranes and soluble enzymes by accumulated cryoprotectants during freezing stress.
784 Proceedings of the National Academy of Sciences 119.
785 <https://doi.org/10.1073/pnas.2211744119>
- 786 Grumiaux, C., Andersen, M., Overgaard, J., Colinet, H., 2019. Fluctuating thermal regime
787 preserves physiological homeostasis and reproductive capacity in *Drosophila suzukii*. J
788 (Basel) 113. <https://doi.org/10.1016/j.jinsphys.2019.01.001>
- 789 Hafker, P., Thompson, L.M., Walter, J.A., Parry, D., Grayson, K.L., 2024. Geographic variation
790 in larval cold tolerance and exposure across the invasion front of a widely established forest
791 insect. Insect Sci. <https://doi.org/10.1111/1744-7917.13358>
- 792 Halbritter, D.A., Teets, N.M., Williams, C.M., Daniels, J.C., 2018. Differences in winter cold
793 hardiness reflect the geographic range disjunction of *Neophasia menapia* and *Neophasia*
794 *terlooii* (Lepidoptera: Pieridae). J Insect Physiol 107, 204–211.
795 <https://doi.org/10.1016/j.jinsphys.2018.03.005>
- 796 Hand, S.C., Denlinger, D.L., Podrabsky, J.E., Roy, R., 2016. Mechanisms of animal diapause:
797 Recent developments from nematodes, crustaceans, insects, and fish. Am J Physiol Regul
798 Integr Comp Physiol 310, R1193–R1211. <https://doi.org/10.1152/ajpregu.00250.2015>
- 799 Harsimran Kaur Gill, Gaurav Goyal, Gurminder Chahil, 2017. Insect Diapause: A Review. J Agric
800 Sci Technol A 7. <https://doi.org/10.17265/2161-6256/2017.07.002>
- 801 Hayward, S. AL, Colinet, H., 2023. Metabolomics as a tool to elucidate biochemical cold
802 adaptation in insects. Curr Opin Insect Sci 58, 101061.
803 <https://doi.org/10.1016/j.cois.2023.101061>
- 804 Hayward, S.A.L., Manso, B., Cossins, A.R., 2014. Molecular basis of chill resistance adaptations
805 in poikilothermic animals. J Exp Biol 217, 6–15. <https://doi.org/10.1242/jeb.096537>
- 806 Hemmati, C., Moharramipour, S., Talebi, A.A., 2017. Diapause induced by temperature and
807 photoperiod affects fatty acid compositions and cold tolerance of *Phthorimaea operculella*
808 (Lepidoptera: Gelechiidae). Environ Entomol 46, 1456–1463.
809 <https://doi.org/10.1093/ee/nvx167>
- 810 Hemmati, C., Moharramipour, S., Talebi, A.A., 2014. Effects of cold acclimation, cooling rate and
811 heat stress on cold tolerance of the potato tuber moth *Phthorimaea operculella* (Lepidoptera:
812 Gelechiidae). Eur J Entomol 111, 487–494. <https://doi.org/10.14411/eje.2014.063>
- 813 Heydari, M., Izadi, H., 2014. Effects of seasonal acclimation on cold tolerance and biochemical
814 status of the carob moth, *Ectomyelois ceratoniae* Zeller, last instar larvae. Bull Entomol Res
815 104, 592–600. <https://doi.org/10.1017/S0007485314000364>

- 816 Isobe, K., Takahashi, A., Tamura, K., 2013. Cold tolerance and metabolic rate increased by cold
817 acclimation in *Drosophila albomicans* from natural populations. *Genes Genet Syst* 88, 289–
818 300. <https://doi.org/10.1266/ggs.88.289>
- 819 Izadi, H., Mohammadzadeh, M., Mehrabian, M., 2019. Cold tolerance of the *Tribolium castaneum*
820 (Coleoptera: Tenebrionidae), under different thermal regimes: impact of cold acclimation. *J*
821 *Econ Entomol* 112, 1983–1988. <https://doi.org/10.1093/jee/toz089>
- 822 Izumi, Y., Sonoda, S., Tsumuki, H., 2007. Effects of diapause and cold-acclimation on the
823 avoidance of freezing injury in fat body tissue of the rice stem borer, *Chilo suppressalis*
824 Walker. *J Insect Physiol* 53, 685–690. <https://doi.org/10.1016/j.jinsphys.2007.04.005>
- 825 Izumi, Y., Sonoda, S., Yoshida, H., Danks, H. V., Tsumuki, H., Tsumuki, H., 2006. Role of
826 membrane transport of water and glycerol in the freeze tolerance of the rice stem borer, *Chilo*
827 *suppressalis* Walker (Lepidoptera: Pyralidae). *J Insect Physiol* 52, 215–220.
828 <https://doi.org/10.1016/j.jinsphys.2005.11.001>
- 829 Jacob Welch, Christopher Marks, 2023. The effect of parental cold stress on offspring cold
830 tolerance in *Drosophila melanogaster*, in: Mid-East Honors Association . Ohio.
- 831 Jain, N.K., Roy, I., 2010. Trehalose and protein stability. *Curr Protoc Protein Sci.*
832 <https://doi.org/10.1002/0471140864.ps0409s59>
- 833 Jass, A., Yerushalmi, G., Donini, A., Davis, H., MacMillan, H., 2019. An impressive capacity for
834 cold tolerance plasticity protects against ionoregulatory collapse in the disease vector *Aedes*
835 *aegypti*. *J (Basel)* 222. <https://doi.org/10.1242/jeb.214056>
- 836 Javal, M., Renault, D., Colinet, H., 2016. Impact of fluctuating thermal regimes on *Drosophila*
837 *melanogaster* survival to cold stress, in: *Animal Biology*. [https://doi.org/10.1163/15707563-](https://doi.org/10.1163/15707563-00002510)
838 00002510
- 839 Jaworski, T., Hilszczański, J., 2014. The effect of temperature and humidity changes on insects
840 development their impact on forest ecosystems in the expected climate change. *For Res Pap*
841 74, 345–355. <https://doi.org/10.2478/frp-2013-0033>
- 842 Kathryn A. Wurzel, 2005. Glycerol. *Encyclopedia of Toxicology*.
- 843 Kendrick, M.R., McCord, J.W., 2023. Overwintering and breeding patterns of monarch butterflies
844 (*Danaus plexippus*) in coastal plain habitats of the southeastern USA. *Sci Rep* 13, 10438.
845 <https://doi.org/10.1038/s41598-023-37225-7>
- 846 Khani, A., Moharramipour, S., 2010. Cold hardiness and supercooling capacity in the
847 overwintering larvae of the codling moth, *Cydia pomonella* . *J Insect Sci* 10, 1–12.
848 <https://doi.org/10.1673/031.010.8301>

- 849 Kim, Y., 2000. Effect of thermoperiod and photoperiod on cold tolerance of *Spodoptera exigua*
850 (Lepidoptera: Noctuidae). Environ Entomol.
- 851 Kim, Y., Kim, N., 1997. Cold hardiness in *Spodoptera exigua* (Lepidoptera: Noctuidae). Environ
852 Entomol 26, 1117–1123. <https://doi.org/10.1093/ee/26.5.1117>
- 853 Kingsolver, J.G., Buckley, L.B., 2020. Ontogenetic variation in thermal sensitivity shapes insect
854 ecological responses to climate change. Curr Opin Insect Sci 41, 17–24.
855 <https://doi.org/10.1016/j.cois.2020.05.005>
- 856 Kleynhans, E., Mitchell, K.A., Conlong, D.E., Terblanche, J.S., 2014. Evolved variation in cold
857 tolerance among populations of *Idana saccharina* (Lepidoptera: Pyralidae) in South Africa.
858 J Evol Biol 27, 1149–1159. <https://doi.org/10.1111/jeb.12390>
- 859 Kojić, D., Popović, Ž.D., Orčić, D., Purać, J., Orčić, S., Vukašinović, E.L., Nikolić, T. V.,
860 Blagojević, D.P., 2018. The influence of low temperature and diapause phase on sugar and
861 polyol content in the European corn borer *Ostrinia nubilalis* (Hbn.). J Insect Physiol 109,
862 107–113. <https://doi.org/10.1016/j.jinsphys.2018.07.007>
- 863 Košťál, V., Vambera, J., Bastl, J., 2004. On the nature of pre-freeze mortality in insects: Water
864 balance, ion homeostasis and energy charge in the adults of *Pyrrhocoris apterus*. J Exp Biol
865 207, 1509–1521. <https://doi.org/10.1242/jeb.00923>
- 866 Košťál, V., Zahradníčková, H., Šimek, P., Zelený, J., 2007. Multiple component system of sugars
867 and polyols in the overwintering spruce bark beetle, *Ips typographus*. J Insect Physiol 53,
868 580–586. <https://doi.org/10.1016/j.jinsphys.2007.02.009>
- 869 Kukal, O., Serianni, A.S., Duman, J.G., 1988. Glycerol metabolism in a freeze-tolerant arctic
870 insect: an in vivo ¹³C NMR study. J Comp Physiol B 158, 175–183.
871 <https://doi.org/10.1007/BF01075831>
- 872 Lahondère, C., 2023. Recent advances in insect thermoregulation. J Exp Biolo 226.
873 <https://doi.org/10.1242/jeb.245751>
- 874 Larsen, K.J., Lee, R.E., 1994. Cold tolerance including rapid cold-hardening and inoculative
875 freezing of fall migrant monarch butterflies in Ohio, J. Insect Ph.vsiol.
- 876 Lebenzon, J.E., Overgaard, J., Jørgensen, L.B., 2023. Chilled, starved or frozen: insect
877 mitochondrial adaptations to overcome the cold. Curr Opin Insect Sci 58, 101076.
878 <https://doi.org/10.1016/j.cois.2023.101076>
- 879 Lee, K.Y., Chang, Y.D., Kim, Y.G., 2002. Trehalose, a major sugar cryoprotectant of the
880 overwintering rice water weevil, *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae). J
881 Asia Pac Entomol 5, 35–41. [https://doi.org/10.1016/S1226-8615\(08\)60130-2](https://doi.org/10.1016/S1226-8615(08)60130-2)

- 882 Lee, Richard E., 2010. A primer on insect cold-tolerance, in: *Low Temperature Biology of Insects*.
883 <https://doi.org/10.1017/CBO9780511675997.002>
- 884 Lee, R.E., 2010a. A primer on insect cold tolerance, in: Denlinger, D.L., Lee, R.E.J. (Ed.), *Low*
885 *Temperature Biology of Insects*. Cambridge University Press, Cambridge, pp. 3–34.
- 886 Lee, R.E., 2010b. A primer on insect cold tolerance: Low temperature biology of insects. pp. 3–
887 34.
- 888 Lee, R.E., 1991. Principles of insect low temperature tolerance, in: Lee, R.E. Jr., D.D.L. (Ed.),
889 *Insects at Low Temperature*. Chapman and Hall, New York, pp. 17–46.
890 https://doi.org/10.1007/978-1-4757-0190-6_2
- 891 Li, Natalia G., 2016. Cryoprotectant systems and cold tolerance of insects inhabiting central
892 Yakutia (Russian Far East). *Eur J Entomol* 113, 537–541.
893 <https://doi.org/10.14411/eje.2016.073>
- 894 Li, N.G., 2016. Strong tolerance to freezing is a major survival strategy in insects inhabiting central
895 Yakutia (Sakha Republic, Russia), the coldest region on earth. *Cryobiology* 73, 221–225.
896 <https://doi.org/10.1016/j.cryobiol.2016.07.007>
- 897 Li, X. wei, Li, D., Zhang, Z. jun, Huang, J., Zhang, J. ming, Hafeez, M., Wang, L. kun, Guo, W.
898 chao, Lu, Y. bin, 2021. Supercooling capacity and cold tolerance of the South American
899 tomato pinworm, *Tuta absoluta*, a newly invaded pest in China. *J Pest Sci* (2004) 94, 845–
900 858. <https://doi.org/10.1007/s10340-020-01301-y>
- 901 Li, Y.P., Goto, M., Ito, S., Sato, Y., Sasaki, K., Goto, N., 2001. Physiology of diapause and cold
902 hardiness in the overwintering pupae of the fall webworm *Hyphantria cunea* (Lepidoptera:
903 Arctiidae) in Japan. *J Insect Physiol* 47, 1181–1187. [https://doi.org/10.1016/S0022-1910\(01\)00099-3](https://doi.org/10.1016/S0022-1910(01)00099-3)
- 905 Liu, Y.-Q., Zheng, X.-X., Ma, H.-F., Xia, R.-X., Li, Y.-P., Zhang, Q.-R., 2016. Supercooling
906 capacity and cold tolerance of the wild silkworm, *Antheraea pernyi* (Lepidoptera:
907 Saturniidae). *J Econ Entomol* 109, 1619–1627. <https://doi.org/10.1093/jee/tow121>
- 908 M A Carrillo C A Cannon, W F Wilcke William, D Hutchison William, D.H., 2005. Relationship
909 between supercooling point and mortality at low temperatures in Indianmeal moth
910 (Lepidoptera: Pyralidae). *J Econ Entomol* 98, 618–625.
- 911 M. B. Renfree and G. Shaw, 2000. DIAPAUSE. *Annu Rev Entomol* 62, 353–75.
- 912 MacMillan, H.A., Guglielmo, C.G., Sinclair, B.J., 2009. Membrane remodeling and glucose in
913 *Drosophila melanogaster*: A test of rapid cold-hardening and chilling tolerance hypotheses.
914 *J Insect Physiol*. <https://doi.org/10.1016/j.jinsphys.2008.11.015>

- 915 MacMillan, H.A., Knee, J.M., Dennis, A.B., Udaka, H., Marshall, K.E., Merritt, T.J.S., Sinclair,
916 B.J., 2016a. Cold acclimation wholly reorganizes the *Drosophila melanogaster* transcriptome
917 and metabolome. *Sci Rep* 6. <https://doi.org/10.1038/srep28999>
- 918 MacMillan, H.A., Schou, M.F., Kristensen, T.N., Overgaard, J., 2016b. Preservation of potassium
919 balance is strongly associated with insect cold tolerance in the field: a seasonal study of
920 *Drosophila subobscura*. *Biol Lett* 12, 20160123. <https://doi.org/10.1098/rsbl.2016.0123>
- 921 Malekera, M.J., Acharya, R., Hwang, H.S., Lee, K.Y., 2022. Effect of cold acclimation and rapid
922 cold-hardening on the survival of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera:
923 Noctuidae) under cold stress. *J Asia Pac Entomol* 25.
924 <https://doi.org/10.1016/j.aspen.2021.101862>
- 925 Marshall, K.E., Sinclair, B.J., 2015. The relative importance of number, duration and intensity of
926 cold stress events in determining survival and energetics of an overwintering insect. *Funct*
927 *Ecol* 29, 357–366. <https://doi.org/10.1111/1365-2435.12328>
- 928 Masoudmaghani, A., Izadi, H., Mohammadzadeh, M., 2021. Expanded supercooling capacity with
929 no cryoprotectant accumulation underlies cold tolerance of the European grapevine moth. *J*
930 *Econ Entomol* 114, 828–838. <https://doi.org/10.1093/jee/toab005>
- 931 Milonas, P.G., 1999. Cold hardiness in diapause and non-diapause larvae of the summer fruit
932 tortrix.pdf. *Eur J Entomol* 96, 183–187.
- 933 Mishra, S.K., 2019. Acclimatization, in: *Encyclopedia of Animal Cognition and Behavior*.
934 Springer International Publishing, Cham, pp. 1–2. [https://doi.org/10.1007/978-3-319-47829-](https://doi.org/10.1007/978-3-319-47829-6_1035-1)
935 [6_1035-1](https://doi.org/10.1007/978-3-319-47829-6_1035-1)
- 936 Mollaei, M., Izadi, H., Moharrampour, S., Behroozi Moghadam, E., 2017. Physiology of
937 hibernating larvae of the pistachio twig borer, *Kermania pistaciella* Amsel (Lepidoptera:
938 Tineidae), collected from akbari cultivar of *Pistacia vera* L. *Neotrop Entomol* 46, 58–65.
939 <https://doi.org/10.1007/s13744-016-0438-2>
- 940 Mollaei, M., Izadi, H., Šimek, P., Košťál, V., 2016. Overwintering biology and limits of cold
941 tolerance in larvae of pistachio twig borer, *Kermania pistaciella*. *Bull Entomol Res* 106, 538–
942 545. <https://doi.org/10.1017/S0007485316000237>
- 943 Moraiti, C.A., Papadopoulos, N.T., 2017. Obligate annual and successive facultative diapause
944 establish a bet-hedging strategy of *Rhagoletis cerasi* (Diptera: Tephritidae) in seasonally
945 unpredictable environments. *Physiol Entomol* 42, 225–231.
946 <https://doi.org/10.1111/phen.12206>

- 947 Morey, A.C., Hutchison, W.D., Venette, R.C., Burkness, E.C., 2012. Cold hardiness of
948 *Helicoverpa zea* (Lepidoptera: Noctuidae) pupae. *Environ Entomol* 41, 172–179.
949 <https://doi.org/10.1603/en11026>
- 950 Mutamiswa, R., Mbande, A., Nyamukondiwa, C., Chidawanyika, F., 2023. Thermal adaptation in
951 Lepidoptera under shifting environments: mechanisms, patterns, and consequences.
952 *Phytoparasitica* 51, 929–955. <https://doi.org/10.1007/s12600-023-01095-6>
- 953 Nazari, P., Poorjavad, N., Izadi, H., Chan, B.K.K., 2020. Simultaneous occurrence of diapause and
954 cold hardiness in overwintering eggs of the apple oystershell scale, *Lepidosaphes malicola*
955 *borchsenius* (Hem.: Diaspididae). <https://doi.org/10.6620/ZS.2020.59-25>
- 956 Neven, L.G., 1999. Cold hardiness adaptations of codling moth, *Cydia pomonella*. *Cryobiology*
957 38, 43–50. <https://doi.org/10.1006/cryo.1998.2140>
- 958 Olsson, T., MacMillan, H.A., Nyberg, N., Staerk, D., Malmendal, A., Overgaard, J., 2016.
959 Hemolymph metabolites and osmolality are tightly linked to cold tolerance of *Drosophila*
960 species: A comparative study. *J Exp Biol* 219, 2504–2513.
961 <https://doi.org/10.1242/jeb.140152>
- 962 Overgaard, J., MacMillan, H.A., 2016. The integrative physiology of insect chill tolerance. *Annu*
963 *Rev Physiol* 79, 187–208. <https://doi.org/10.1146/annurev-physiol-022516-034142>
- 964 Palli, S.R., Biondi, A., Desneux, N., Du Plessis, H., Le Goff, G., Volkoff, A.-N., 2023. The fall
965 armyworm: recent advances in biology and management. *J Pest Sci* (2004) 96, 1341–1343.
966 <https://doi.org/10.1007/s10340-023-01688-4>
- 967 Papadopoulos, A.G., Koskinioti, P., Zarpas, K.D., Papadopoulos, N.T., 2023. Differential cold
968 tolerance on immature stages of geographically divergent *Ceratitis capitata* populations.
969 *Biology* 12, 1379. <https://doi.org/10.3390/biology12111379>
- 970 Park, Y., Kim, Y., 2014. A specific glycerol kinase induces rapid cold hardening of the
971 diamondback moth, *Plutella xylostella*. *J Insect Physiol* 67, 56–63.
972 <https://doi.org/10.1016/j.jinsphys.2014.06.010>
- 973 Pei, J., Li, C., Ren, L., Zong, S., 2020. Factors influencing cold hardiness during overwintering of
974 *Streltzoviella insularis* (Lepidoptera: Cossidae). *J Econ Entomol*.
975 <https://doi.org/10.1093/jee/toaa032>
- 976 Pei, Y., Jin, J., Wu, Q., Liang, X., Lv, C., Guo, J., 2023. Cold acclimation and supercooling
977 capacity of *Agasicles hygrophila* adults. *Insects* 14, 58.
978 <https://doi.org/10.3390/insects14010058>
- 979 Perveen, F.K., Khan, A., 2017. Introductory Chapter: Lepidoptera, in: *Lepidoptera*. InTech.
980 <https://doi.org/10.5772/intechopen.70452>

- 981 Poikela, N., Tyukmaeva, V., Hoikkala, A., Kankare, M., 2021. Multiple paths to cold tolerance:
982 the role of environmental cues, morphological traits and the circadian clock gene *vriille*. *BMC*
983 *Ecol Evol* 21, 117. <https://doi.org/10.1186/s12862-021-01849-y>
- 984 Prasanna B., H.J.E., E.R., P.V.M., 2018. Fall armyworm in Africa: a guide for integrated pest
985 management. USAID; CIMMYT.
- 986 QIANG, C., DU, Y., YU, L., CUI, Y., ZHENG, F., LU, M., 2008. Effect of rapid cold hardening
987 on the cold tolerance of the larvae of the rice stem borer, *Chilo suppressalis* (Walker). *Agric*
988 *Sci China* 7, 321–328. [https://doi.org/10.1016/S1671-2927\(08\)60072-3](https://doi.org/10.1016/S1671-2927(08)60072-3)
- 989 QIANG Cheng-kui, QIANG Cheng-kui, 2012. Overwintering physiology of the rice stem borer
990 larvae, *Chilo suppressalis* Walker (Lepidoptera: Pyralidae): Roles of glycerol, amino acids,
991 low-molecular weight carbohydrates and antioxidant enzymes. *Afr J Biotechnol* 11, 13030–
992 13039. <https://doi.org/10.5897/ajb11.1692>
- 993 Quan, Y., Wang, Z., Wei, H., He, K., 2022. Transcription dynamics of heat shock proteins in
994 response to thermal acclimation in *Ostrinia furnacalis*. *Front Physiol* 13.
995 <https://doi.org/10.3389/fphys.2022.992293>
- 996 Ransberry, V.E., MacMillan, H.A., Sinclair, B.J., 2011. The relationship between chill-coma onset
997 and recovery at the extremes of the thermal window of *Drosophila melanogaster*. *Physiol*
998 *Bioch Zool* 84, 553–559. <https://doi.org/10.1086/662642>
- 999 Régnière, J., St-Amant, R., Duval, P., 2012. Predicting insect distributions under climate change
1000 from physiological responses: Spruce budworm as an example. *Biol Invasions* 14, 1571–
1001 1586. <https://doi.org/10.1007/s10530-010-9918-1>
- 1002 Reznik, S.Y., 2011. Ecological and evolutionary aspects of photothermal regulation of diapause in
1003 Trichogrammatidae. *J Evol Biochem Physiol* 47, 512–523.
1004 <https://doi.org/10.1134/S0022093011060020>
- 1005 Rodrigues, Y.K., Beldade, P., 2020. Thermal plasticity in insects' response to climate change and
1006 to multifactorial environments. *Front Ecol Evol*. <https://doi.org/10.3389/fevo.2020.00271>
- 1007 Roncalli, V., Cieslak, M.C., Castelfranco, A.M., Hopcroft, R.R., Hartline, D.K., Lenz, P.H., 2021.
1008 Post-diapause transcriptomic restarts: insight from a high-latitude copepod. *BMC Genomics*
1009 22, 409. <https://doi.org/10.1186/s12864-021-07557-7>
- 1010 Rozsypal, J., Košťál, V., Zahradníčková, H., Šimek, P., 2013. Overwintering strategy and
1011 mechanisms of cold tolerance in the codling moth (*Cydia pomonella*). *PLoS One* 8.
1012 <https://doi.org/10.1371/journal.pone.0061745>

- 1013 Sadeghi, R., Izadi, H., Mahdian, K., 2012. Energy allocation changes in overwintering adults of
1014 the common pistachio psylla, *Agonoscena pistaciae* Burckhardt & Lauterer (Hemiptera:
1015 Psyllidae). *Neotrop Entomol* 41, 493–498. <https://doi.org/10.1007/s13744-012-0077-1>
- 1016 Saeidi, M., Moharramipour, S., 2017. Physiology of cold hardiness, seasonal fluctuations, and
1017 cryoprotectant contents in overwintering adults of *Hypera postica* (Coleoptera:
1018 Curculionidae). *Environ Entomol* 46, 960–966. <https://doi.org/10.1093/ee/nvx089>
- 1019 Saunders, D.S., 2014. Insect photoperiodism: Effects of temperature on the induction of insect
1020 diapause and diverse roles for the circadian system in the photoperiodic response. *Entomol*
1021 *Sci.* <https://doi.org/10.1111/ens.12059>
- 1022 Schebeck, M., Dobart, N., Ragland, G.J., Schopf, A., Stauffer, C., 2022. Facultative and obligate
1023 diapause phenotypes in populations of the European spruce bark beetle *Ips typographus*. *J*
1024 *Pest Sci* (2004) 95, 889–899. <https://doi.org/10.1007/s10340-021-01416-w>
- 1025 Schowalter, T.D., Ring, D.R., 2017. Biology and management of the fall webworm, *Hyphantria*
1026 *cunea* (Lepidoptera: Erebididae). *J Integr Pest Manag* 8, 7.
1027 <https://doi.org/10.1093/jipm/pmw019>
- 1028 Shirey, V., Larsen, E., Doherty, A., Kim, C.A., Al-Sulaiman, F.T., Hinolan, J.D., Itliong, M.G.A.,
1029 Naive, M.A.K., Ku, M., Belitz, M., Jeschke, G., Barve, V., Lamas, G., Kawahara, A.Y.,
1030 Guralnick, R., Pierce, N.E., Lohman, D.J., Ries, L., 2022. LepTraits 1.0 A globally
1031 comprehensive dataset of butterfly traits. *Sci Data* 9, 382. <https://doi.org/10.1038/s41597-022-01473-5>
- 1032
- 1033 Shirey, V., Ries, L., 2023. Population dynamics and drivers of the eastern monarch (*Danaus*
1034 *plexippus*) across its full annual cycle: a cross-scale synthesis of a model migratory species.
1035 *Curr Opin Insect Sci* 60, 101132. <https://doi.org/10.1016/j.cois.2023.101132>
- 1036 Sinclair, B.J., 1999. Insect cold tolerance: How many kinds of frozen? *Eur J Entomol* 96, 157–
1037 164.
- 1038 Sinclair, B.J., Addo-Bediako, A., Chown, S.L., 2003a. Climatic variability and the evolution of
1039 insect freeze tolerance. *Biol Rev Camb Philos Soc* 78, 181–195.
1040 <https://doi.org/10.1017/S1464793102006024>
- 1041 Sinclair, B.J., Coello Alvarado, L.E., Ferguson, L. V., 2015. An invitation to measure insect cold
1042 tolerance: Methods, approaches, and workflow. *J Therm Biol* 53, 180–197.
1043 <https://doi.org/10.1016/j.jtherbio.2015.11.003>
- 1044 Sinclair, B.J., Vernon, P., Klok, C.J., Chown, S.L., 2003b. Insects at low temperatures: An
1045 ecological perspective. *Trends Ecol Evol* 18, 257–262. [https://doi.org/10.1016/S0169-5347\(03\)00014-4](https://doi.org/10.1016/S0169-5347(03)00014-4)
- 1046

- 1047 Sømme, L., 1982. Supercooling and winter survival in terrestrial arthropods. *Comp Biochem*
1048 *Physiol A Physiol* 73, 519–543. [https://doi.org/10.1016/0300-9629\(82\)90260-2](https://doi.org/10.1016/0300-9629(82)90260-2)
- 1049 Stanic, B., Jovanovic-Galovic, A., Blagojevic, D.P., Grubor-Lajsic, G., Worland, R., Spasic, M.B.,
1050 2004. Cold hardiness in *Ostrinia nubilalis* (Lepidoptera: Pyralidae): Glycerol content, hexose
1051 monophosphate shunt activity, and antioxidative defense system. *Eur J Entomol* 101, 459–
1052 466. <https://doi.org/10.14411/eje.2004.065>
- 1053 Štětina, T., Des Marteaux, L.E., Košťál, V., 2020. Insect mitochondria as targets of freezing-
1054 induced injury. *Proceedings of the Royal Society B: Biological Sciences* 287, 20201273.
1055 <https://doi.org/10.1098/rspb.2020.1273>
- 1056 Stockton, D., Wallingford, A., Rendon, D., Fanning, P., Green, C.K., Diepenbrock, L., Ballman,
1057 E., Walton, V.M., Isaacs, R., Leach, H., Sial, A.A., Drummond, F., Burrack, H., Loeb, G.M.,
1058 Fanning, P., Isaacs, R., Diepenbrock, L., Stockton, D., Wallingford, A., Sial, A.A., Walton,
1059 V.M., Drummond, F., Rendon, D., Loeb, G.M., Ballman, E., Green, C.K., Fanning, P., Green,
1060 C.K., Diepenbrock, L., Ballman, E., Walton, V.M., Isaacs, R., Leach, H., Sial, A.A.,
1061 Drummond, F., Burrack, H., Loeb, G.M., 2019. Interactions between biotic and abiotic factors
1062 affect survival in overwintering *Drosophila suzukii* (Diptera: Drosophilidae). *Environ*
1063 *Entomol* 48, 454–464. <https://doi.org/10.1093/ee/nvy192>
- 1064 Storey, J. M., and Storey, K.B., 2004. Cold hardiness and freeze tolerance, in: *Functional*
1065 *Metabolism*. pp. 473–503.
- 1066 Storey, J.M., Storey, Kenneth B., 2005. Cold Hardiness and Freeze Tolerance, in: Storey, K.B.
1067 (Ed.), *Functional Metabolism*. John Wiley & Sons, Inc, pp. 473–503.
1068 <https://doi.org/10.1002/047167558x.ch17>
- 1069 Storey, K.B., Storey, J.M., 1988. Freeze tolerance: constraining forces, adaptive mechanisms. *Can*
1070 *J Zool* 66, 11-22–1127.
- 1071 Su, X.-L., Su, Z.-R., Xu, W.-H., 2024. The protease Lon prolongs insect lifespan by responding to
1072 reactive oxygen species and degrading mitochondrial transcription factor A. *Biochimica et*
1073 *Biophysica Acta (BBA) - Molecular Cell Research* 1871, 119648.
1074 <https://doi.org/10.1016/j.bbamcr.2023.119648>
- 1075 Subedi, B., Poudel, A., Aryal, S., 2023. The impact of climate change on insect pest biology and
1076 ecology: Implications for pest management strategies, crop production, and food security. *J*
1077 *Agric Food Res* 14, 100733. <https://doi.org/10.1016/j.jafr.2023.100733>
- 1078 Suchan, T., Bataille, C.P., Reich, M.S., Toro-Delgado, E., Vila, R., Pierce, N.E., Talavera, G.,
1079 2024. A trans-oceanic flight of over 4,200 km by painted lady butterflies. *Nat Commun* 15,
1080 5205. <https://doi.org/10.1038/s41467-024-49079-2>

- 1081 SUN, X., HU, C., JIA, H., WU, Q., SHEN, X., ZHAO, S., JIANG, Y., WU, K., 2021. Case study
 1082 on the first immigration of fall armyworm, *Spodoptera frugiperda* invading into China. *J*
 1083 *Integr Agric* 20, 664–672. [https://doi.org/10.1016/S2095-3119\(19\)62839-X](https://doi.org/10.1016/S2095-3119(19)62839-X)
- 1084 Tang, B., Wang, S., Wang, S.G., Wang, H.J., Zhang, J.Y., Cui, S.Y., 2018. Invertebrate trehalose-
 1085 6-phosphate synthase gene: Genetic architecture, biochemistry, physiological function, and
 1086 potential applications. *Front Physiol* 9. <https://doi.org/10.3389/fphys.2018.00030>
- 1087 Tarusikirwa, V.L., Mutamiswa, R., Chidawanyika, F., Nyamukondiwa, C., 2020a. Cold hardiness
 1088 of the South American tomato pinworm *Tuta absoluta* (Lepidoptera: Gelechiidae): both
 1089 larvae and adults are chill-susceptible. *Pest Manag Sci*. <https://doi.org/10.1002/ps.6006>
- 1090 Tarusikirwa, V.L., Mutamiswa, R., English, S., Chidawanyika, F., Nyamukondiwa, C., 2020b.
 1091 Thermal plasticity in the invasive south American tomato pinworm *Tuta absoluta* (Meyrick)
 1092 (Lepidoptera: Gelechiidae). *J Therm Biol* 90. <https://doi.org/10.1016/j.jtherbio.2020.102598>
- 1093 Teets, N.M., Denlinger, D.L., 2013. Physiological mechanisms of seasonal and rapid cold-
 1094 hardening in insects. *Physiol Entomol* 38, 105–116. <https://doi.org/10.1111/phen.12019>
- 1095 Teets, N.M., Marshall, K.E., Reynolds, J.A., 2023. Molecular Mechanisms of Winter Survival.
 1096 *Annu Rev Entomol* 68, 319–339. <https://doi.org/10.1146/annurev-ento-120120-095233>
- 1097 Thompson, S.N., 2003. Trehalose - The Insect “Blood” Sugar. *Adv In Insect Phys* 31, 205–285.
 1098 [https://doi.org/10.1016/S0065-2806\(03\)31004-5](https://doi.org/10.1016/S0065-2806(03)31004-5)
- 1099 Toxopeus, J., Košťál, V., Sinclair, B.J., 2019. Evidence for non-colligative function of small
 1100 cryoprotectants in a freeze-tolerant insect. *proceedings of the royal society B*.
 1101 <https://doi.org/10.1098/rspb.2019.0050>
- 1102 Toxopeus, J., Sinclair, B.J., 2018. Mechanisms underlying insect freeze tolerance. *Biological*
 1103 *Reviews* 93, 1891–1914. <https://doi.org/10.1111/brv.12425>
- 1104 Tremblay, P., MacMillan, H.A., Kharouba, H.M., 2021. Autumn larval cold tolerance does not
 1105 predict the northern range limit of a widespread butterfly species. *Ecol Evol* 11, 8332–8346.
 1106 <https://doi.org/10.1002/ece3.7663>
- 1107 Trivedi, T.P., Rajagopal, D., 1992. Distribution, biology, ecology and management of potato tuber
 1108 moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae): A review. *Tropical Pest*
 1109 *Management* 38, 279–285. <https://doi.org/10.1080/09670879209371709>
- 1110 Turnock, W.J. and B.R.P., 1993. The reversal of cold injury and its effect on the response to
 1111 subsequent cold exposures. *Cryo-Letters* 12, 251–256.
- 1112 Tzanakakis, M.E., 1959. An ecological study of the Indian-meal moth *Plodia interpunctella*
 1113 (Hübner) with emphasis on diapause. *Hilgardia*. <https://doi.org/10.3733/hilg.v29n05p205>

- 1114 Vatanparast, M., Park, Y., 2022. Cold tolerance strategies of the fall armyworm, *Spodoptera*
1115 *frugiperda* (Smith) (Lepidoptera: Noctuidae). *Sci Rep* 12. [https://doi.org/10.1038/s41598-](https://doi.org/10.1038/s41598-022-08174-4)
1116 022-08174-4
- 1117 Vatanparast, M., Sajjadian, S.M., Park, Y., 2022. Glycerol biosynthesis plays an essential role in
1118 mediating cold tolerance the red imported fire ant, *Solenopsis invicta*. *Arch Insect Biochem*
1119 *Physiol* 109. <https://doi.org/10.1002/arch.21861>
- 1120 Vernon, P., Vannier, G., 2002. Evolution of freezing susceptibility and freezing tolerance in
1121 terrestrial arthropods. *C R Biol* 325, 1185–1190. [https://doi.org/10.1016/S1631-](https://doi.org/10.1016/S1631-0691(02)01536-6)
1122 0691(02)01536-6
- 1123 Voituron, Y., Mouquet, N., de Mazancourt, C., Clobert, J., 2002. To freeze or not to freeze? an
1124 evolutionary perspective on the cold-hardiness strategies of overwintering ectotherms. *Am*
1125 *Nat* 160, 255–270. <https://doi.org/10.1086/341021>
- 1126 Vrba, P., Nedvěd, O., Zahradníčková, H., Konvička, M., 2017. More complex than expected: Cold
1127 hardiness and the concentration of cryoprotectants in overwintering larvae of five *Erebia*
1128 butterflies (Lepidoptera: Nymphalidae). *Eur J Entomol* 114, 470–480.
1129 <https://doi.org/10.14411/eje.2017.060>
- 1130 Wang, J., Zhang, R.R., Gao, G.Q., Ma, M.Y., Chen, H., 2016. Cold tolerance and silencing of
1131 three cold-tolerance genes of overwintering Chinese white pine larvae. *Sci Rep* 6, 1–17.
1132 <https://doi.org/10.1038/srep34698>
- 1133 Wang, Y., Zhao, Y., Zhang, J., Li, Z., 2024. Heat shock protein genes affect the rapid cold
1134 hardening ability of two invasive Tephritids. *Insects* 15, 90.
1135 <https://doi.org/10.3390/insects15020090>
- 1136 Watanabe M, Tanaka K, 1999. Cold tolerance strategy of the freeze-intolerant chrysomelid,
1137 *Aulacophora nigripennis* (Coleoptera: Chrysomelidae), in warm-temperate regions. *Eur. J.*
1138 *Entomol.* 96: 175-181
- 1139 Weaving, H., Terblanche, J.S., Pottier, P., English, S., 2022. Meta-analysis reveals weak but
1140 pervasive plasticity in insect thermal limits. *Nat Commun* 13. [https://doi.org/10.1038/s41467-](https://doi.org/10.1038/s41467-022-32953-2)
1141 022-32953-2
- 1142 Wharton, D.A., Goodall, G., Marshall, C.J., 2003. Freezing survival and cryoprotective
1143 dehydration as cold tolerance mechanisms in the Antarctic nematode *Panagrolaimus davidi*.
1144 *J Exp Biol* 206, 215–221. <https://doi.org/10.1242/jeb.00083>
- 1145 Williams, C.M., Nicolai, A., Ferguson, L. V., Bernards, M.A., Hellmann, J.J., Sinclair, B.J., 2014.
1146 Cold hardiness and deacclimation of overwintering *Papilio zelicaon* pupae. *Comp Biochem*
1147 *Physiol A Mol Integr Physiol* 178, 51–58. <https://doi.org/10.1016/j.cbpa.2014.08.002>

- 1148 Woods, H.A., Makino, W., Cotner, J.B., Hobbie, S.E., Harrison, J.F., Acharya, K., Elser, J.J., 2003.
1149 Temperature and the chemical composition of poikilothermic organisms. *Funct Ecol* 17, 237–
1150 245. <https://doi.org/10.1046/j.1365-2435.2003.00724.x>
- 1151 Worland, M.R., Grubor-Lajšić, G., Purać, J., Thorne, M.A.S., Clark, M.S., 2010. Cryoprotective
1152 dehydration: Clues from an insect, in: *Topics in Current Genetics*. pp. 147–163.
1153 https://doi.org/10.1007/978-3-642-12422-8_9
- 1154 Xu J1, Bao B, Zhang ZF, Yi YZ, X.WH., 2009. Identification of a novel gene encoding the
1155 trehalose phosphate synthase in the cotton bollworm, *Helicoverpa armigera*. *Cryobiology* 19,
1156 250–7.
- 1157 Xu, X.R., Zhu, M.M., Li, L.L., Zhang, G.C., Zheng, Y., Li, T., Sun, S.H., 2015. Cold hardiness
1158 characteristic of the overwintering pupae of fall webworm *Hyphantria cunea* (Drury)
1159 (Lepidoptera: Arctiidae) in the northeast of China. *J Asia Pac Entomol* 18, 39–45.
1160 <https://doi.org/10.1016/j.aspen.2014.11.003>
- 1161 Yancey, P.H., 2001. Water Stress, Osmolytes and proteins. *Am Zool* 41, 699–709.
1162 <https://doi.org/10.1093/icb/41.4.699>
- 1163 Zachariassen, K.E., 1985. Physiology of cold tolerance in insects. *Physiol. Rev.* 65, 799–832.
- 1164 Zachariassen, K.E., 1980. The role of polyols and nucleating agents in cold-hardy beetles. *J Comp*
1165 *Physiol* 140, 227–234. <https://doi.org/10.1007/BF00690407>
- 1166 ZHANG, D. dan, ZHAO, S. yuan, WU, Q. lin, LI, Y. yan, WU, K. ming, 2021. Cold hardiness of
1167 the invasive fall armyworm, *Spodoptera frugiperda* in China. *J Integr Agric* 20, 764–771.
1168 [https://doi.org/10.1016/S2095-3119\(20\)63288-9](https://doi.org/10.1016/S2095-3119(20)63288-9)
- 1169 Zhao, D., Zheng, C., Shi, F., Xu, Y., Zong, S., Tao, J., 2021. Expression analysis of genes related
1170 to cold tolerance in *Dendroctonus valens*. *PeerJ* 9, e10864.
1171 <https://doi.org/10.7717/peerj.10864>
- 1172 Zheng, X.-L., Zhou, L.-J., Lu, W., Xian, Z.-H., Yang, Z.-D., Lei, C.-L., Wang, X.-P., 2014. Cold-
1173 Hardiness Mechanisms in Third Instar Larvae of *Spodoptera exigua* Hübner (Lepidoptera:
1174 Noctuidae) . *African Entomology* 22, 863–871. <https://doi.org/10.4001/003.022.0401>
- 1175

Table A1: Main traits associated with insects' cold tolerance strategies.

Cold tolerance strategy	Traits						
	Supercooling capacity	Lower lethal temperature	Internal ice formation	Ice-nucleating agents	Cryoprotectants	Supercooled state	Mortality occurrence
Freeze-tolerant	Limited	<SCP*	Yes	Present	Present	No	Beyond the SCP
Freeze-avoidant	Expanded	\geq SCP	No	Absent	Present	Yes	Before - or at - the SCP
Chill susceptible	Moderate	> SCP	No	Absent	Present	Yes	Before the SCP

*SCP= Supercooling point

Table A2. Formulae of the most common compatible solutes in insects

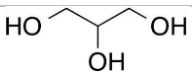
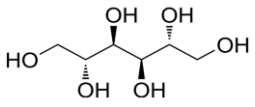
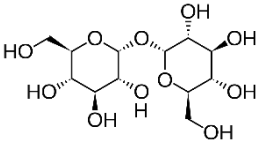
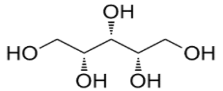
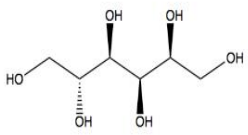
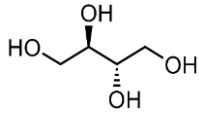
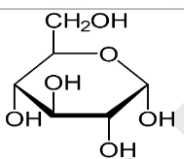
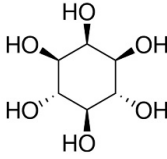

Cryoprotectant	Formula	Cryoprotectant	Formula
Glycerol		Mannitol	
Trehalose		Xylitol	
Sorbitol		Erythritol	
Glucose		Myo-inositol	
Ethylene glycol			

Table 1. Cold tolerance strategies of the species of Lepidoptera included in this review across their respective families and development stages.

Family	Species	DS	Cold tolerance strategy			Enhancement of cold tolerance		SCP (°C)
			CS	FA	FT	CA	SCPD	
Tortricidae	<i>Cydia pomonella</i>	Larva	-	*	-	*	*	-13.4 to -22.0
	<i>Lobesia botrana</i>	Pupa	-	*	*	-	*	-22.6 to -23.7
	<i>Choristoneura fumiferana</i>	Larva	-	-	*	*	*	-23.0 to -42.0
Tineidae	<i>Kermania pistaciella</i>	Larva	-	*	-	*	*	-19.4 to -22.7
Gelechiidae	<i>Phthorimaea operculella</i>	Pupa	-	*	-	-	*	-18.5 to -22.6
Pyralidae	<i>Ectomyelois ceratoniae</i>	Larva	*	*	-	*	*	-12.0 to -17.0
	<i>Arimania comaroffi</i>		-	*	-	*	*	-13.5 to -15.1
	<i>Plodia interpunctella</i>		-	*	-	*	*	-14.0 to -16.0
	<i>Chilo suppressalis</i>		-	-	*	*	*	-10.8 to -15.3
	<i>Ostrinia nubilalis</i>		-	-	*	*	*	-20.4 to -24.0

Diapausing stage: DS, Cold tolerance strategy: Chilling susceptibility (CS); Freeze avoidance (FA); Freeze tolerance (FT)

Enhancement of cold tolerance: Cold acclimation (CA); Supercooling point depression (SCPD), SCP= Supercooling point

Table 1. (Cont.)

Family	Species	LS	Cold tolerance strategy			Enhancement of cold tolerance		SCP (°C)
			CS	FA	FT	CA	SCPD	
Noctuidae	<i>Spodoptera exigua</i>	Larva	-	*	-	*	*	-6.0 to -12.0
	<i>Sesamia inferens</i>		-	-	*	*	-	-6.8
	<i>S. nonagrioides</i>		-	*	-	*	*	-5.0 to -8.0
	<i>S. cretica</i>		-	*	-	-	*	-15.0 to -17.2
	<i>Helicoverpa armigera</i>	Pupa	-	*	-	*	-	-18.1 to -19.5
	<i>H. zea</i>		-	*	-	-	*	-16.3 to -19.4
Pieridae	<i>Pieris brassicae</i>	Pupa	-	*	-	*	*	-17.7 to -25.5
	<i>Aporia crataegi</i>	Larva	-	-	*	*	-	-9.7 to -12.9
	<i>Colias palaeno</i>		-	*	-	*	*	-17.0 to -29.3
	<i>C. hyale</i>		-	*	-	*	*	-17.5 to -27.7
	<i>C. alfacariensis</i>		-	*	-	-	-	-18.6
	<i>C. phicomone</i>		-	*	-	-	-	-13.8
Gelechiidae	<i>Scrobipalpa ocellatella</i>	Larva	-	-	*	-	*	-6.0 to -25.0

Diapausing stage: DS, Cold tolerance strategy: Chilling susceptibility (CS); Freeze avoidance (FA); Freeze tolerance (FT)

Enhancement of cold tolerance: Cold acclimation (CA); Supercooling point depression (SCPD), SCP= Supercooling point

Table 1. (Cont.)

Family	Species	DS	Cold tolerance strategy			Enhancement of cold tolerance		SCP (°C)
			CS	FA	FT	CA	SCPD	
Papilionidae	<i>Papilio memnon</i>	Pupa	-	*	-	*	-	-20 to -25
	<i>P. xuthus</i>		-	*	-	*	-	
	<i>P. bianor</i>		-	*	-	*	-	
	<i>P. maackii</i>		-	*	-	*	-	
	<i>P. machaon</i>		-	*	-	*	-	
	<i>P. glaucus</i>		-	*	-	*	-	
	<i>P. canadensis</i>		-	*	-	*	-	
	<i>P. zelicaon</i>		-	*	*	*	-	
	<i>Parnassius bremeri</i>	Larva	-	*	-	*	*	-9.8 to -20
Plutellidae	<i>Plutella xylostella</i>	mixed	-	*	-	*	*	-10.1 to -23.2
Lymantriidae	<i>Lymantria monacha</i>	Egg	-	*	-	*	-	-28.6 to -30.1
	<i>L. dispar</i>		-	*	-	*	-	-23.6 to -28.9

Diapausing stage: DS, Cold tolerance strategy: Chilling susceptibility (CS); Freeze avoidance (FA); Freeze tolerance (FT)

Enhancement of cold tolerance: Cold acclimation (CA); Supercooling point depression (SCPD), SCP= Supercooling point

Table 1. (Cont.)

Family	Species	DS	Cold tolerance strategy			Enhancement of cold tolerance		SCP (°C)
			CS	FA	FT	CA	SCPE	
Satyridae	<i>Erebia medusa</i>	Larva	-	-	*	*	*	-8.0 to -22.0
	<i>E. pronoe</i>		-	*	-	*	*	
	<i>E. cassioides</i>		-	*	-	*	*	
	<i>E. pluto</i>		-	*	-	*	*	
	<i>E. aethiops</i>		-	*	*	*	*	
Lasiocampidae	<i>Dendrolimus tabulaeformis</i>	Larva	-	*	-	*	*	-5.9 to -14.1
	<i>D. superans sibiricus</i>		-	-	*	-	-	-40.7 to -11.6
	<i>Malacosoma disstria</i>	Egg	-	*	-	-	-	-26.8 to -40.3
Arctiidae	<i>Pyrrharctia isabella</i>	Larva	-	-	*	*	*	-5.0 to -10.0
	<i>Hyphantria cunea</i>	Pupa	-	*	-	*	*	-23.5
Cossidae	<i>Streltzoviella insularis</i>	Larva	-	-	*	*	*	-3.3 to -5.9
	<i>Cossus cossus</i>		-	-	*	-	-	-5.0
Saturniidae	<i>Antheraea pernyi</i>	Pupa	-	*	-	-	*	-15.6 to -20.1
Coleophoridae	<i>Coleophora laricella</i>	Larva	-	-	-	-	*	-30.9 to -44.6

Diapausing stage: DS, Cold tolerance strategy: Chilling susceptibility (CS); Freeze avoidance (FA); Freeze tolerance (FT)

Enhancement of cold tolerance: Cold acclimation (CA); Supercooling point depression (SCPD), SCP= Supercooling point

Table 2. Lepidopteran species included in this review are categorized by diapausing stage and their relationship between cold tolerance and diapause.

Diapause stage and strategy	No of species	Species
Egg	3	<i>Lymantria monachal</i> , <i>L. dispar</i> , <i>Malacosoma disstria</i> ,
Larva	30	<i>Cydia pomonella</i> , <i>Choristoneura fumiferana</i> , <i>Kermania pistaciella</i> , <i>Ectomyelois ceratoniae</i> , <i>Arimania comaroffi</i> <i>Plodia interpunctella</i> , <i>Chilo suppressalis</i> , <i>Ostrinia nubilalis</i> , <i>Spodoptera exigua</i> , <i>Sesamia inferens</i> , <i>S. nonagrioides</i> , <i>S. cretica</i> , <i>Aporia crataegi</i> , <i>Colias palaeno</i> , <i>C. hyale</i> , <i>C. alfacariensis</i> , <i>C. phicomone</i> , <i>Scrobipalpa ocellatella</i> , <i>Parnassius bremeri</i> , <i>Erebia medusa</i> , <i>E. pronoe</i> , <i>E. cassioides</i> , <i>E. pluto</i> , <i>E. aethiops</i> , <i>Dendrolimus tabulaeformis</i> , <i>D. superans sibiricus</i> , <i>Pyrrharctia Isabella</i> , <i>Hyphantria cunea</i> , <i>Streltzoviella insularis</i> , <i>Cossus cossus</i> , <i>Coleophora laricella</i>
Pupa	16	<i>Lobesia botrana</i> , <i>Phthorimaea operculella</i> , <i>Helicoverpa armigera</i> , <i>H. zea</i> , <i>Pieris brassicae</i> , <i>Papilio Memnon</i> , <i>P. Xuthus</i> , <i>P. bianor</i> , <i>P. maackii</i> , <i>P. machaon</i> , <i>P. glaucus</i> , <i>P. canadensis</i> , <i>P. zelicaon</i> , <i>Antheraea pernyi</i> , <i>Phyllonorycter ringoniella</i>
Mixed	1	<i>Plutella xylostella</i>
Cold tolerance is a component of diapause	14	<i>Cydia pomonella</i> , <i>Kermania pistaciella</i> , <i>Phthorimaea operculella</i> , <i>Ectomyelois ceratoniae</i> , <i>Arimania comaroffi</i> , <i>Sesamia nonagrioides</i> , <i>Helicoverpa armigera</i> , <i>Pieris brassicae</i> , <i>Plodia interpunctella</i> , <i>Chilo suppressalis</i> , <i>Ostrinia nubilalis</i> , <i>Pyrrharctia isabella</i> , <i>Lobesia botrana</i> , <i>Phyllonorycter ringoniella</i>
Cold tolerance is not a component of diapause	2	<i>Lymantria dispar</i> , <i>Hyphantria cunea</i>
Cold acclimation enhances cold tolerance	7	<i>S. frugiperda</i> , <i>T. absoluta</i> , <i>S. exigua</i> , <i>E. ceratoniae</i> , <i>P. interpunctella</i> , <i>C. suppressalis</i> , <i>Ph. operculella</i> .

Table 3. Enhancement of cold tolerance through the accumulation of compatible cryoprotectants in different species of Lepidoptera (the units of mg and μg are based on gram body weight).

Species	Compatible osmolytes						Reference
	Trehalose	Glycerol	Glucose	Fructose	Myo- inositol	Sorbitol	
<i>Ectomyelois ceratoniae</i>	0.5 mg/g	-	-	-	0.07 mg/g	-	(Heydari and Izadi, 2014)
<i>Arimania comaroffi</i>	8.6 mg/g	-	-	-	5.3 mg/g	2.6 mg/g	(Bemani et al., 2012)
<i>Spodoptera exigua</i>	3.9 mg/g	2.17 mg/g	-	-	1.7 mg/g	-	(Atapour and Moharramipour, 2014)
<i>S. frugiperda</i>	-	~60 nmol/ml	-	-	-	-	(Vatanparast and Park, 2022)
<i>Sesamia inferens</i>	20.5 mg/g	359.8 $\mu\text{g/g}$	377.3 $\mu\text{g/g}$	69.8 $\mu\text{g/g}$	59.6 $\mu\text{g/g}$	-	(Sun et al., 2014)
<i>S. nonagrioides</i>	4.23 $\mu\text{g/g}$	4.54 mg/100 mg	-	-	-	-	(Andreadis et al., 2011; Gillyboeuf et al., 1994)
<i>Helicoverpa armigera</i>	1.8mg/100mg	-	0.8 mg/100mg	-	-	-	(Izumi et al., 2005)
<i>Pieris brassicae</i>	~11.0 mg/g	-	-	-	6.5 mg/g	40.0 mMolal	(PULLIN et al., 1991)
<i>Chilo suppressalis</i>	-	30 nmol/mg and 16.33 $\mu\text{g/mg}$	-	-	-	-	(Izumi et al., 2007; QIANG Cheng-kui and QIANG Cheng-kui, 2012)
<i>Ostrinia nubilalis</i>	4600.0 $\mu\text{g/g}$	~40.0 mg/g and 100.0 mg/100mg	-	-	-	-	(Andreadis et al., 2008; Kojić et al., 2018; Stanic et al., 2004)
<i>Cydia pomonella</i>	18.4 mg/g	1.5 mg/g	5.9 mg/g	*	-	-	(Khani et al., 2007; Rozsypal et al., 2013)
<i>Aporia crataegi</i>	-	0.8 M	-	-	-	-	(Li, 2012)
<i>Choristoneura fumiferana</i>	-	0.2 μM	-	-	-	-	(Marshall and Sinclair, 2015)
*Percent	70	70	23	7.7	38.5	15.4	

Roughly 77% of the species studied use more than one cryoprotectant.

*The percentage of lepidopteran species included in this review that utilize the osmolyte.

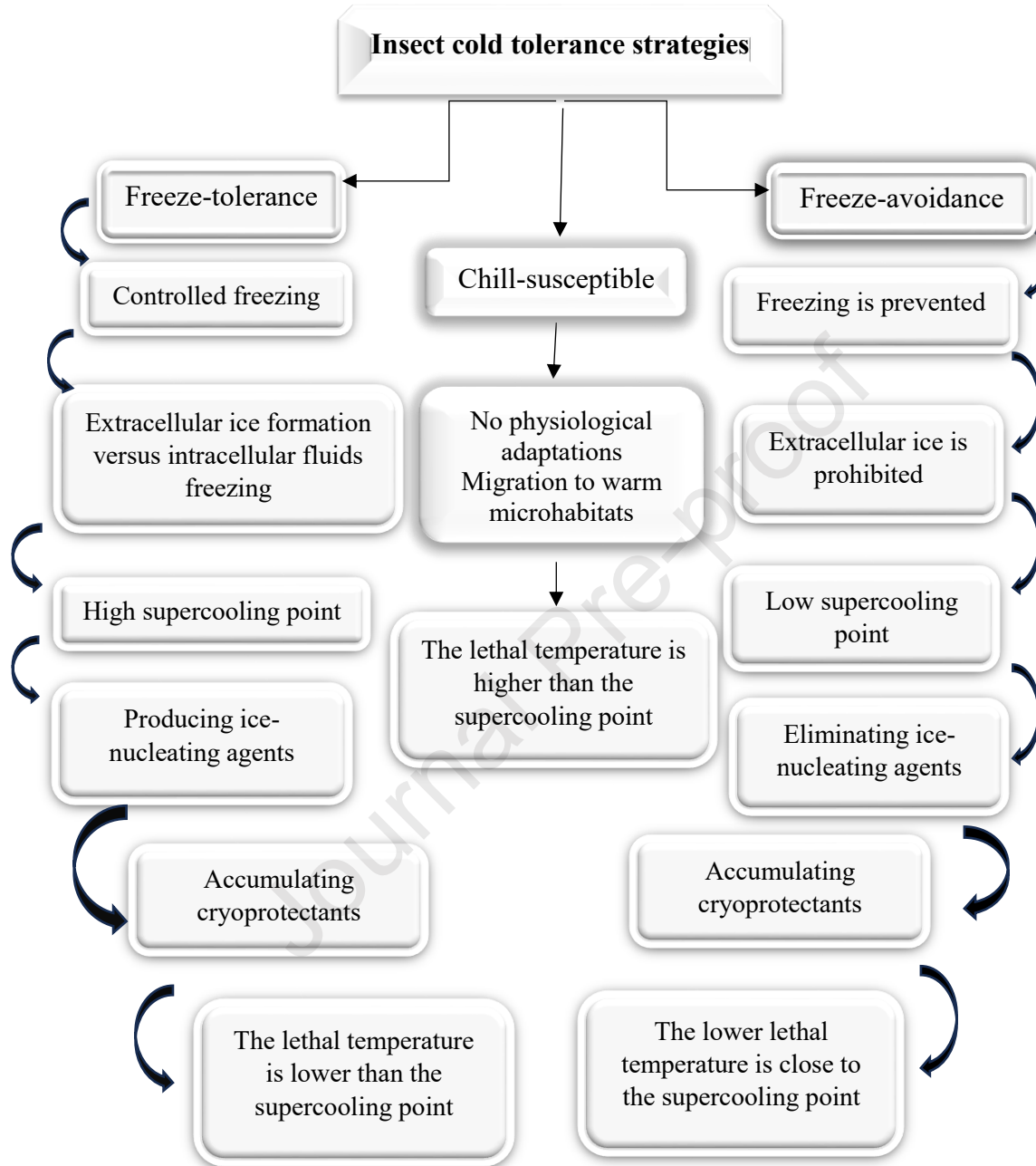


Fig 1. The primary features of insects' cold tolerance strategies

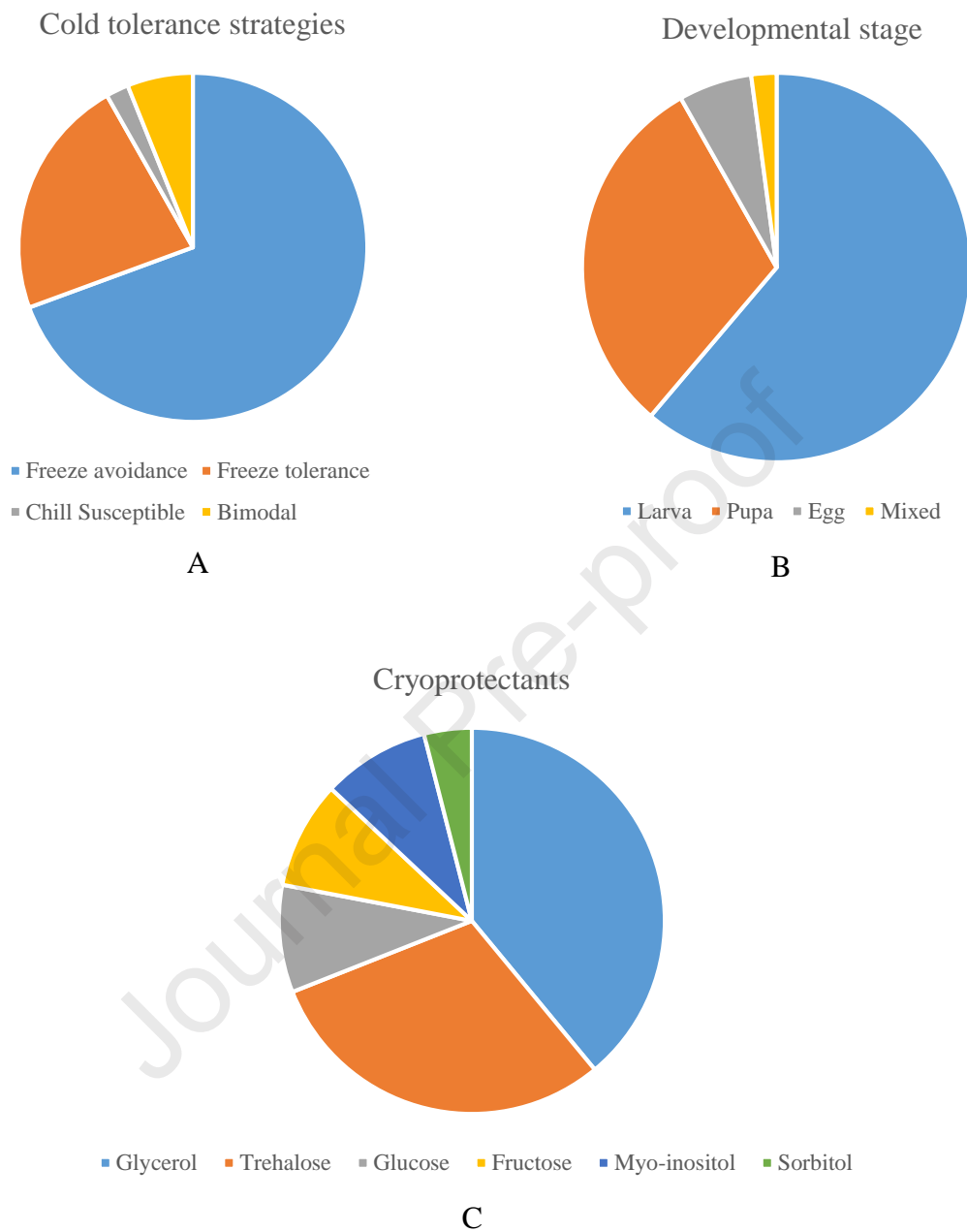


Figure 2. The rate of cold tolerance strategy (A), cryoprotectants (B), and occurrence of diapause (C) in Lepidoptera species assessed in this review.

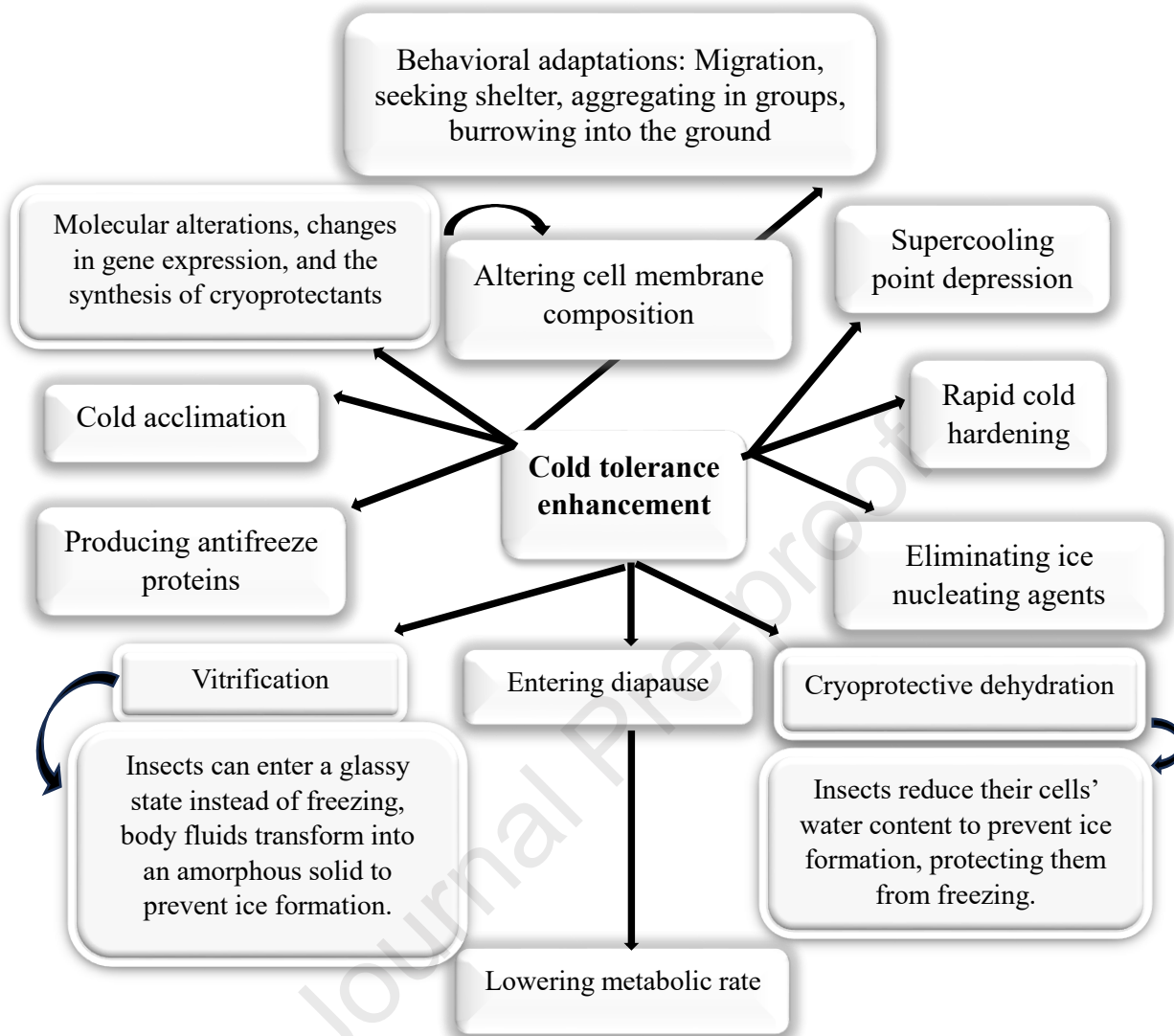


Fig 3. The primary mechanisms of insects' cold tolerance enhancement

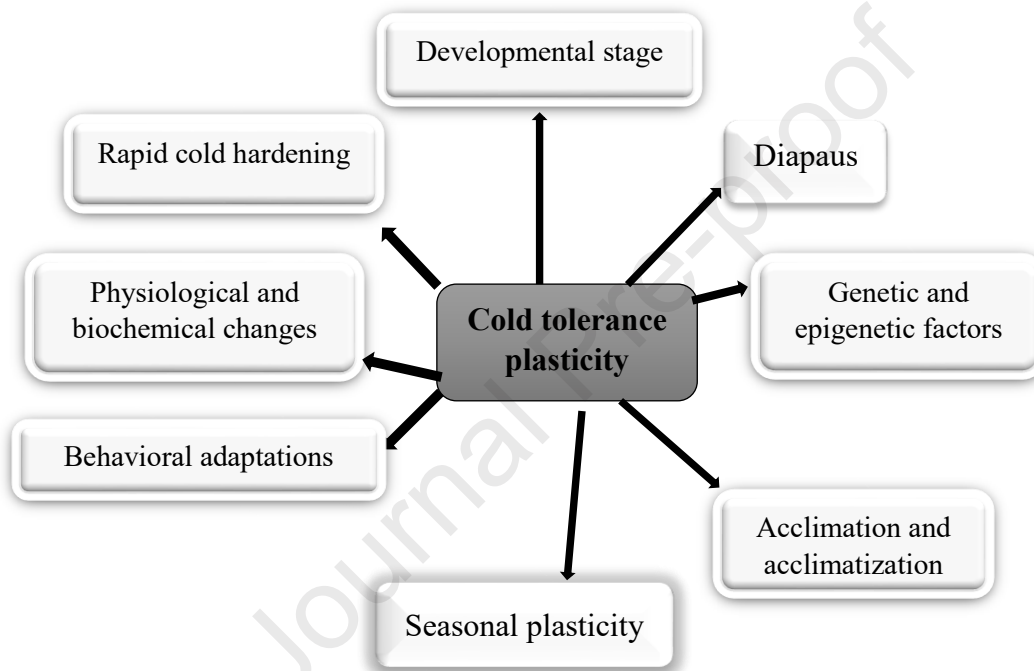


Fig 4. The primary mechanisms in insects' cold tolerance plasticity

Highlights

- 1- Ecologically and economically important species of Lepidoptera were reviewed for their low-temperature performance and cold tolerances.
- 2- Diapause was identified as a unique mechanism that has evolved independently in different Lepidoptera genera and families.
- 3- Diapause onset and cold tolerance improvement are interdependent.
- 4- The adoption of a cold tolerance strategy is greatly influenced by a species' geographic distribution and rearing conditions and is not an inherent, fixed trait.
- 5- Freeze intolerance is the primary cold tolerance strategy among lepidopteran species.

Data availability statement

Through our comprehensive review of the literature, we were able to critically evaluate and synthesize the information presented in these various sources. Based on our analysis, we have concluded and gained insights that enhance our understanding of the research topic. This paper summarizes the findings to offer a valuable overview of the current state of knowledge on the subject. While our study does not present new data, it is a valuable resource for researchers and practitioners seeking to explore further and contribute to this field of study. However, we have no unpublished data to share.

Journal Pre-proof

Funding

No external funding supported this study.

Journal Pre-proof

Declaration of competing interest

The authors declare no competing interest

Journal Pre-proof