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SHORT COMMUNICATION

Cold tolerance of biological control agents *Amblydromalus limonicus* and *Iphiseius degenerans*

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Abstract

Knowledge about cold tolerance of non-native biological control agents is critical to avoid permanently establishing them in new temperate areas outside of their native range. The cold tolerance of the predatory mites, *Amblydromalus limonicus* and *Iphiseius degenerans*, was investigated in the laboratory to assess their establishment potential in northern Europe, particularly Sweden. The lethal time of *I. degenerans* (the number of days until 100% mortality was reached) declined steeply from 5°C to 0°C and was almost zero at −5°C. The lethal time of *A. limonicus* did not differ between 5°C and 0°C, but was reduced at −5°C. For both species, $LTime_{50}$ (the number of days until 50% of the mites died) was longer for fed than for unfed mites. The lethal temperature of *A. limonicus* (the temperature at which 100% mortality was reached) was −17.75°C, whereas most *I. degenerans* died at −8.5°C. $LTemp_{50}$ (the temperature at which 50% of the mites died) was lower for *A. limonicus* (−9.8°C) than for *I. degenerans* (−0.1°C). Collectively, these findings suggest that *I. degenerans* is unlikely to establish in Sweden but that *A. limonicus* is more cold tolerant. This highlights the risk associated with releasing *A. limonicus* in Sweden due to concerns about potential establishment.

KEYWORDS
environmental risk assessment, establishment potential, lethal temperature, lethal time, non-native species

1 | INTRODUCTION

Augmentative biological control involves the periodic release of mass-reared natural enemies to suppress the population of a specific pest organism without the aim of permanent establishment (Bale et al., 2008). While augmentative biological control is commonly practiced, especially in greenhouses, non-native species that can survive and establish outside the crops where they are released may have undesirable effects on the environment (Simberloff & Stiling, 1996). A notable example is that of the aphid predator ladybird (*Harmonia axyridis*) used in augmentative biological control in Europe, which has had adverse effects on non-target insects, humans and crops (Majerus et al., 2006). To prevent the introduction of non-native species with potential to become invasive, environmental risk assessments are carried out to examine their possible side effects (Delfosse, 2005). Environmental risk assessments of a candidate for augmentative biological control often include studies of host range, dispersal ability and cold hardiness. Testing for cold hardiness is important for species that are brought for use in greenhouses in temperate climates, where they shall be active at production temperatures but not survive the outside environment during winter (Allen, 2009; Van Lenteren et al., 2006).

Laboratory experiments are often used to assess a species' ability to survive low temperatures. Lethal time is the time required to

kill a population following long-term exposure to set temperatures (e.g., 5°C, 0°C and −5°C) (Bale & Walters, 2001). $LTime_{50}$ is the time it takes for 50% of the population to die when exposed to different constant temperatures. According to Bale and Walters (2001), there is a strong correlative relationship between the time needed to kill 50% of the sample population at 5°C and the duration of winter survival in the field in the UK. However, it is important to note that while 5°C is a common temperature in, e.g. the UK winter, 0°C or −5°C may be better suited for lethal time experiments in countries experiencing lower average temperatures (e.g. Sweden) (White et al., 2018). Lethal time is the most informative indicator of naturally occurring cold stress because it integrates cumulative mortality over longer periods (Bale, 2002). Lethal temperature is the temperature at which a population dies following a brief time exposure (e.g. 1 min), while $LTemp_{50}$ is the temperature at which 50% of the sample population dies after a short exposure. Lethal temperature is determined by exposing experimental samples to different low and sub-zero temperatures and then rewarming them to a favourable temperature (Allen, 2009; Bale & Walters, 2001).

Predatory mites of the family Phytoseiidae are being mass-reared and commercially used in augmentative biological control of spider mites, thrips and whiteflies in greenhouses (McMurtry & Croft, 1997). Two mite species used in augmentative biological control are *Amblydromalus limonicus* (Garman and McGregor) (Acari: Phytoseiidae) and *Iphiseius degenerans* Berlese (Acari: Phytoseiidae). *Amblydomalus limonicus* occurs naturally in temperate to subtropical regions of New Zealand, Australia, North and South America and is commercially used against thrips in Europe, e.g. in Spain and Austria (Chorąży et al., 2016; Knapp et al., 2013; Lee & Zhang, 2018; Walzer et al., 2017). *Iphiseius degenerans* occurs naturally in the Mediterranean part of Europe and Africa and is commercialized to control thrips and spider mites in, e.g. Belgium (CABI, 2022; McMurtry, 1977; Vantornhout et al., 2005). Despite being used in augmentative biological control in several European countries, the cold tolerance features of these two species have not yet been studied. We assessed the cold tolerance of *A. limonicus* and *I. degenerans* through assays of lethal time and lethal temperature in the laboratory and related the results with temperatures in northern Europe, specifically Sweden.

2 | MATERIALS AND METHODS

To investigate the cold hardiness of *A. limonicus* and *I. degenerans*, we measured their lethal time and lethal temperature in climate chambers (Aralab FitoClima Chambers). Additionally, we estimated the time and temperature at which 50% of the mites died ($LTime_{50}$ and $LTemp_{50}$). Samples of *A. limonicus* were obtained from Biobasiq (Koppert), while *I. degenerans* were obtained from Lindesro AB and stored at 10°C. An order of mites was made every week, and individual mites were tested after 24 h. The mites were delivered in one container (batch) per week, which might have introduced some variability in the conditions of the mites tested. To ensure consistency within our experiments, we selected larger, more active and motile mites. After exposure to a range of low and sub-zero temperatures, the mites were considered to be alive if they moved away when touched. Photosynthetic photon flux density (PPFD) during the experiments was 100 μmol/m^2/s.

2.1 | Measurement of lethal time

Lethal time was measured at constant temperatures of 5°C, 0°C and −5°C. For each temperature regime, 10 mites of each species were placed in individual transparent glass vials and fed (Nutrimite, Biobasiq), while another 10 mites had no access to food. A piece of moist filter paper was added to each glass vial at the start of the experiment, and fresh moist papers were added after recording survival. This procedure was replicated for three batches of *A. limonicus* and two of *I. degenerans*. A batch consisted of one container of mites ordered each week. In total, 180 individuals of *A. limonicus* and 120 of *I. degenerans* were tested. Mortality was recorded at 24-h intervals by examining the mites under a microscope (Nikon SMZ 1500) at room temperature. Before recording mortality, individual glass vials were warmed by rolling them between the palms of the hands for 1 min after being taken out of the climate chambers. Motile mites were considered alive, and non-motile mites were considered dead. The glass vials containing mites considered dead were maintained in the climate chambers for 24 h and examined again to confirm mortality.

2.2 | Measurement of lethal temperature

To measure lethal temperature, mites were exposed to a range of low and subzero temperatures: 5°C, 0°C, −5°C, −10°C, −15°C and −20°C. However, as the vials containing the mites provided some buffer against rapid change in temperature, the temperatures were adjusted to 3.88°C, 0.75°C, −3.88°C, −8.5°C, −13.13°C and −17.75°C, i.e. the actual temperature experienced in the vials. This adjustment was based on a study where temperatures inside vials were recorded (Kjellström, 2019). The mites were individually transferred to transparent glass vials and placed in climate chambers at an acclimation temperature of 10°C. The temperature was lowered by 0.3°C/min to the target temperatures and held there for 1 min. This cooling rate was used because rates ranging from 0.1 to 0.5°C/min are preferred as they ensure a balance between ecological relevance (cooling rates in nature) and the efficient use of time (Sinclair et al., 2015). The temperature was then raised again by 0.3°C/min to 10°C, which was maintained for 24 h. Mite mortality was assessed directly afterwards at room temperature. The glass vials were kept at the acclimation temperature of 10°C for an additional 24 h and examined again to confirm mortality. Batch was not considered in the lethal temperature experiment because not all temperatures could be tested in each batch due to some batches arriving with a delay. Groups of 20 mites were tested in five replications for each temperature regime, resulting in 700 tested mites of each species.

TABLE 1 Average, minimum and maximum temperatures from November to March (spanning 2010–2023), for seven localities ranging from northern to southern Sweden.

	November			December			January			February			March		
	Average	Max	Min	Average	Max	Min	Average	Max	Min	Average	Max	Min	Average	Max	Min
Site (N to S)															
Skellefteå	−1.1	2.9	−7.0	−4.9	−0.4	−13.2	−7.8	−1.2	−12.3	−6.9	−0.7	−14.1	−2.6	0.6	−8.3
Hudiksvall	1.5	4.0	−3.0	−2.2	1.8	−10.0	−4.1	2.1	−9.6	−3.2	0.9	−9.4	0.5	3.8	−4.2
Stockholm	4.3	6.9	0.3	0.7	4.1	−6.6	−0.6	4.0	−4.2	−0.2	2.9	−4.2	2.2	4.5	−2.2
Jönköping	3.4	5.9	−0.3	−0.1	3.3	−8.0	−1.8	3.2	−7.7	−1.5	1.9	−5.6	1.1	3.8	−4.4
Visby	5.3	7.5	2.8	2.0	4.7	−3.6	0.0	4.2	−4.9	0.0	3.1	−4.1	1.8	3.9	−3.0
Kalmar	5.1	7.2	2.5	1.4	4.8	−6.5	0.1	4.6	−5.0	0.2	3.6	−3.1	2.7	5.2	−2.3
Malmö	6.3	8.0	3.3	3.0	6.2	−4.4	1.4	5.3	−3.6	1.5	5.0	−1.6	3.8	5.8	−1.1

2.3 | Swedish meteorological and hydrological data

We obtained monthly temperature data for 2010–2023 from the Swedish Meteorological and Hydrological Institute (SMHI) for seven localities ranging from North to South Sweden. We then calculated the average, minimum and maximum temperatures for each month (Table 1).

2.4 | Statistical analysis

Generalized linear models (GLMs) were used to analyse the effects of temperature (5°C/0°C/−5°C), treatment (fed/unfed) and the interaction between them on lethal time (number of days) of both species. The lethal time of *A. limonicus* was modelled with a Poisson error distribution. A negative binomial distribution was used for *I. degenerans* due to overdispersion in the data. Batch was also included as a fixed effect in the models to control for batch-specific effects. When a significant effect of temperature or the interaction between temperature and treatment was detected, we performed post hoc pairwise comparisons using the package "emmeans" (Lenth, 2023). To assess lethal temperature, we used a binomial GLM to relate the temperature regimes (10°C/3.88°C/0.75°C/−3.88°C/−8.5°C/−13.13°C/−17.75°C) to mite mortality. Post hoc pairwise comparisons of mite mortality between temperature regimes were performed. The two lowest temperatures (−13.13°C/−17.75°C) were excluded from the *I. degenerans* analysis because none of the mites survived.

To estimate $LTime_{50}$, we constructed mortality curves using binomial GLMs with a probit link function. In the models, we related mite mortality (response variable) and time (log-transformed number of days; explanatory variable) for each combination of temperature and treatment (5°C – fed and unfed; 0°C – fed and unfed; −5°C – fed and unfed). The $LTime_{50}$ values were then estimated with the *dose.p* function in R package MASS (Brian et al., 2023). $LTime_{50}$ values for *I. degenerans* at 0°C and −5°C were not estimated because the mites only survived 1–2 days at those temperatures. For $LTemp_{50}$, binomial GLMMs with an observation-level random effect were used to relate mite mortality and temperature (converted to a continuous variable). $LTemp_{50}$ was then estimated using an adaptation of the *dose.p* function for GLMM. Analyses were performed using R software version 4.2.1 (R Core Team, 2022).

3 | RESULTS

3.1 | Lethal time

The lethal time of *I. degenerans* declined steeply from 5°C to 0°C (Figure 1a). It was influenced by an interaction between temperature and treatment (Table 2), where unfed mites had a shorter lethal time than those that were fed, except at −5°C where the lethal time of both groups were almost zero, and thus did not differ (Figure 1a). The lethal time of *A. limonicus* did not change between

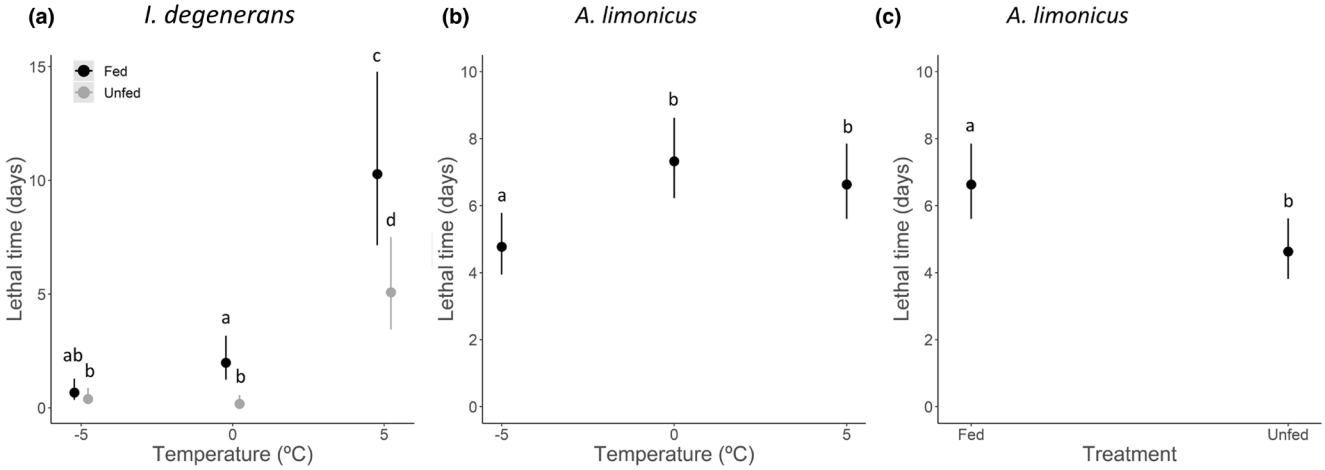

FIGURE 1 Effect of (a) the interaction between temperature and treatment on lethal time of *I. degenerans* and of (b) temperature and (c) treatment on lethal time of *A. limonicus*. A shared letter indicates no significant differences ($p < 0.05$). The dots represent the model estimates and the lines are 95% confidence intervals.

TABLE 2 Effects of temperature (5°C/0°C/−5°C), treatment (fed/unfed), and their interaction, on the number of days until mites died (lethal time), and the effect of temperature (10°C/3.88°C/0.75°C/−3.88°C/−8.5°C/−13.13°C/−17.75°C) on mite mortality rate (lethal temperature).

		I. degenerans			*A. limonicus*		
Response variable	**Predictor**	χ^2	**df**	***p* Value**	χ^2	**df**	***p* Value**
Number of days (Lethal time)	Treatment	8.61	1	**0.003**	9.84	1	**0.002**
	Temperature	86.98	2	**<0.001**	15.86	2	**<0.001**
	Batch	0.98	1	0.322	10.78	2	**0.005**
	Treatment × Temperature	9.02	2	**0.011**	1.53	2	0.467
Mortality rate (Lethal temperature)	Temperature	200.63	4	**<0.001**	357.76	6	**<0.001**

Note: Because different batches of mites were used in the lethal time experiment, we included batch as a fixed effect in the lethal time models. Shown are the chi-square (χ^2) values, degrees of freedom (df) and *p* values. Bold denotes $p < 0.05$.

5°C and 0°C, but declined at −5°C (Table 2, Figure 1b). Unfed mites had a shorter lethal time than mites that were fed (Table 2, Figure 1c). There was no interaction between temperature and treatment (Table 2). The lethal time of *A. limonicus* was influenced by batch and was lower in batch 3 than in batches 1 and 2 (Table 2, Figure S1). For both species, $LTime_{50}$ was longer for fed than for unfed mites (Figure 2).

3.2 | Lethal temperature

The mortality rate of *I. degenerans* increased from 10°C to −8.5°C. It was less than 25% at 10°C between 25% and 50% at 3.88°C, 0.75°C and −3.88°C and increased to almost 100% at −8.5°C (Table 2, Figure 3a). The mortality rate of *A. limonicus* increased from 10°C to −17.75°C, remaining low between 10°C and −8.5°C (<25%), then increasing sharply to approximately 60% at −13.13°C and to almost 100% at −17.75°C (Table 2, Figure 3b). $LTemp_{50}$ was lower for *A. limonicus* (−9.8°C) than *I. degenerans* (−0.1°C) (Figure 4).

3.3 | Swedish meteorological and hydrological data

In Northern Sweden (Skellefteå), the average temperature during the coldest months (November–March) varies between −1.1°C and −7.8°C, with minimum temperatures ranging from −7°C to −14.1°C and maximum temperatures from 2.9°C to −1.2°C. In Central Sweden (Stockholm), the average temperatures in the same months range from 4.3°C to −0.6°C, with minimum temperatures ranging from 0.3°C to −6.6°C and maximum temperatures ranging from 6.9°C to 2.9°C. In Southern Sweden (Malmö), the average temperature varies between 6.3°C and 1.4°C, with minimum temperatures ranging from 3.3°C to −4.4°C and maximum temperatures ranging from 8.0°C to 5.0°C (Table 1).

4 | DISCUSSION

We observed that the predatory mite *A. limonicus* was more cold tolerant than *I. degenerans*. The lethal time of *A. limonicus* declined

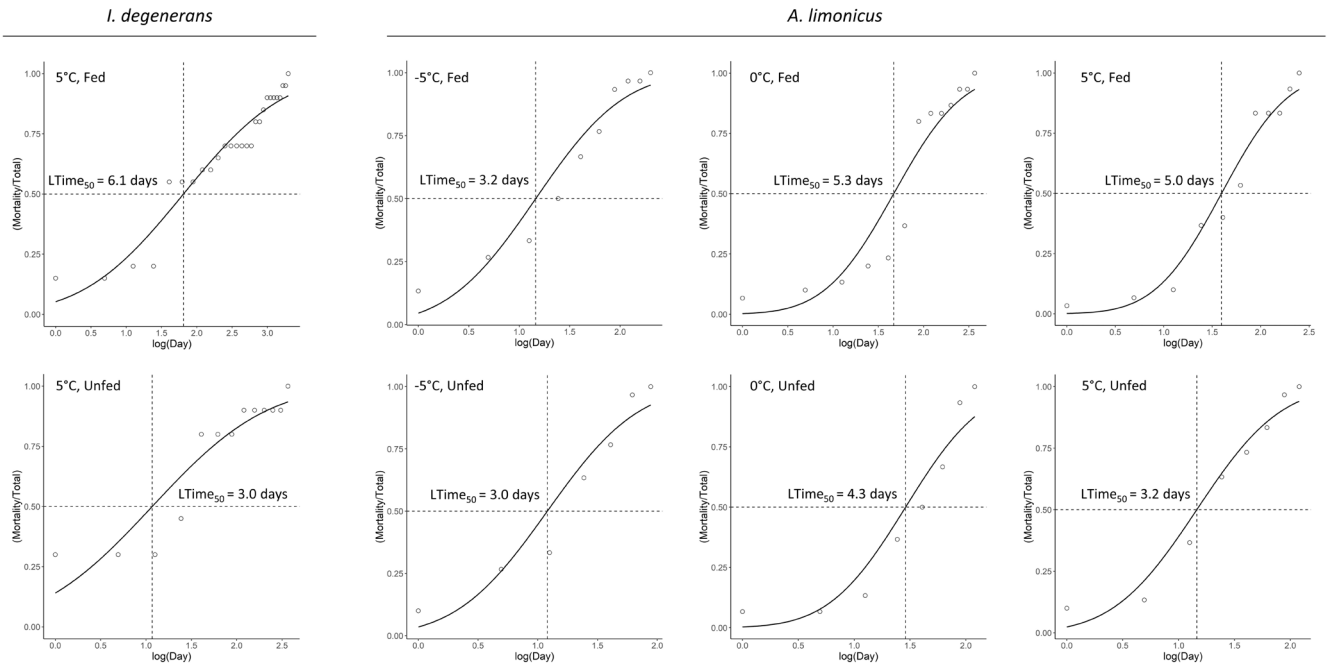

FIGURE 2 The number of days at which 50% of *I. degenerans* and *A. limonicus* individuals died ($LTime_{50}$) at −5°C, 0°C and 5°C. As the variable "Day" was log-transformed, the corresponding $LTime_{50}$ values in days are indicated in the plots. $LTime_{50}$ of *I. degenerans* at 0°C and −5°C are not presented because the individuals only survived 1–2 days at those temperatures.

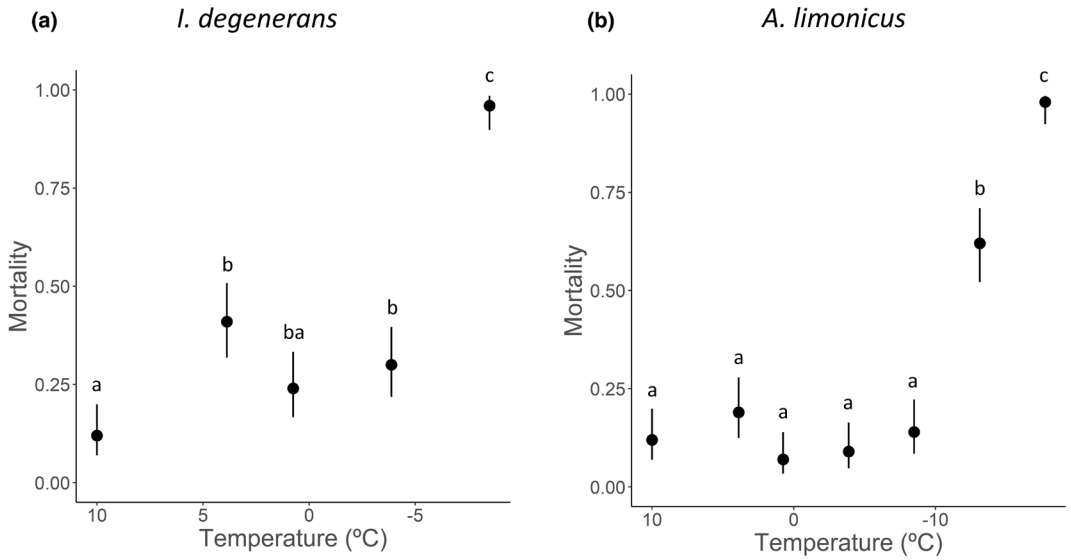

FIGURE 3 Effect of temperature on the mortality rate of (a) *I. degenerans* and (b) *A. limonicus*. A shared letter indicates no significant differences ($p < 0.05$). The dots represent the model estimates and the lines are 95% confidence intervals.

between 0°C and −5°C, whereas the lethal time of *I. degenerans* was reduced already between 5°C and 0°C. Furthermore, in the lethal temperature experiments, temperatures as low as −17.75°C were required to kill the majority of *A. limonicus* individuals, while most *I. degenerans* individuals died already at −8.5°C. Mites from both species survived longer when they had access to food, and some feeding was, therefore, apparent.

From November to March, temperatures vary significantly across Sweden, with northern localities such as Skellefteå experiencing the coldest temperatures (averages ranging from −1.1°C to −7.8°C) and central localities such as Stockholm ranging from 4.3°C to −0.6°C. Our results suggest that *I. degenerans* will quickly die after exposure to temperatures north of Stockholm, as individuals declined rapidly at 0°C, and the majority died at −8.5°C after a short

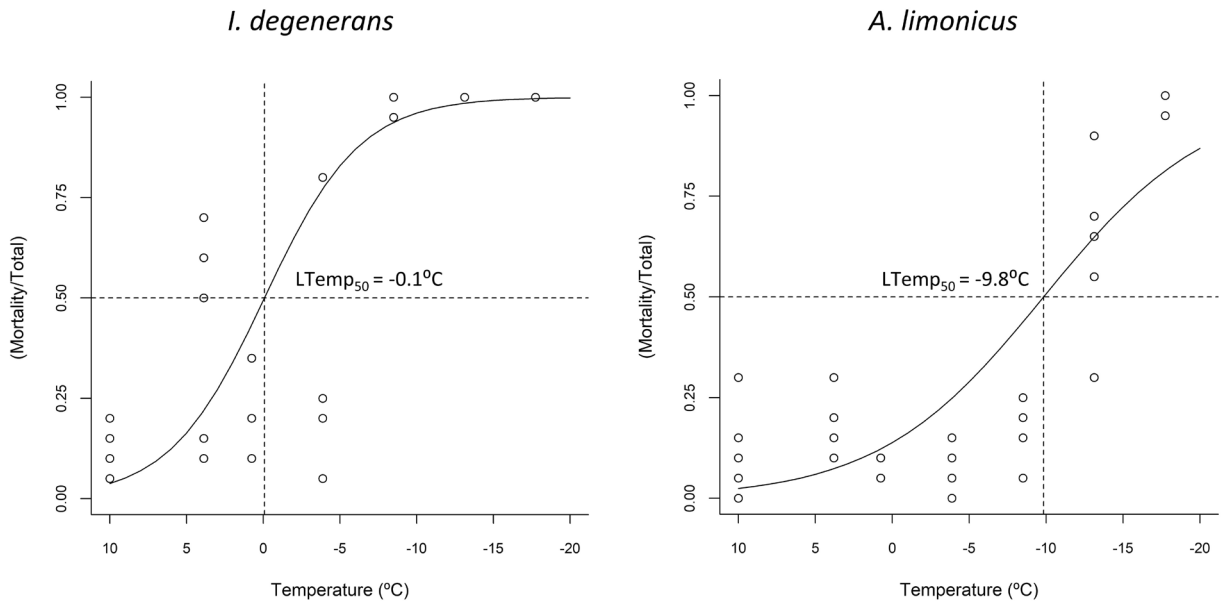

FIGURE 4 The temperature at which 50% of *I. degenerans* and *A. limonicus* mites died ($LTemp_{50}$).

exposure to low and subzero temperatures. $LTemp_{50}$ of *A. limonicus* was −9.8°C, the majority of the mites died at −17.75°C and their lethal time did not differ between 5°C and 0°C, although it was reduced at −5°C. Despite exhibiting higher cold tolerance, *A. limonicus* appears unlikely to persist for long around Skellefteå but could potentially persist around Stockholm. In southern Sweden (Malmö), where average temperatures vary between 6.3°C and 1.4°C, it is likely that *I. degenerans* will not withstand prolonged exposure compared to *A. limonicus* as individuals of *I. degenerans* rapidly died between 5°C and 0°C. On the other hand, our study indicates that *A. limonicus* might persist for a longer time in southern Sweden (Table 1). Survival during the entire winter is uncertain though, given the scarcity of food sources and unlikely reproduction. As such, for a complete picture of the cold tolerance of *A. limonicus*, as well as that of *I. degenerans*, it is necessary to test survival in field conditions (Hatherly et al., 2004).

Our study aligns with the previously described cold tolerance of *A. limonicus* (Dittmann et al., 2016) and suggests that the species is in the mid-range of cold tolerance among commercial phytoseiids. For example, $LTime_{50}$ of *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) and *Phytoseiulus longipes* Evans (Acari: Phytoseiidae) at 5°C, used to assess survival in the UK, ranges from approximately 40 to 60 days and 12.3 to 34.9 days, respectively (Allen, 2009; Hart et al., 2002). We found considerably lower $LTime_{50}$ values for *A. limonicus* at 5°C (3.2 and 5 days in the respective treatments). Furthermore, $LTemp_{50}$ of *A. limonicus* (−9.8°C) is higher than that of *N. californicus* (approximately −14 to −18°C) and *P. longipes* (approximately −8.0 to −13.1°C). On the other hand, $LTime_{50}$ of *A. limonicus* is longer than that of *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) (1.1 to 3.9 days; Allen, 2009) and *Phytoseiulus macropilis* Banks (Acari: Phytoseiidae) (1.8 to 2.6 days; Coombs & Bale, 2014) at 5°C. The $LTemp_{50}$ values of *A. swirskii* (−1.0 to −4.7°C) and *P. macropilis* (−3.9 to −5.7°C) for all life stages and treatment groups are also higher compared to *A. limonicus*. While we found that *I. degenerans* exhibited $LTime_{50}$ values at 5°C (3.0 and 6.1 days) similar to those of *A. limonicus*, its much higher $LTemp_{50}$ (−0.1°C) suggests it is in the lower range of cold tolerance.

Although we recommend further experimentation to confirm these findings, our work suggests that it is unlikely that *I. degenerans* will establish in northern Europe and is a relatively safe biological agent for augmentative releases in Sweden. *A. limonicus*, on the other hand, exhibits high cold tolerance and has been reported to be an aggressive predator of native predatory mites in Austria (Walzer et al., 2017). This raises concerns about the risks of its establishment as it may compete with native natural enemies and prevent more specialized predators from effectively controlling pest populations (Palevsky et al., 2013). Further research on the risks and side effects of the establishment of *A. limonicus* is thus needed.

To ensure sustainable and responsible augmentative biological control practices, we advocate a comprehensive approach that integrates rigorous risk assessments before releasing non-native species to avoid environmental disasters witnessed in the past. Assessment of cold tolerance should be a major part of such risk assessments in temperate regions.

AUTHOR CONTRIBUTIONS

Samuel Musyoka Mbaka: Investigation; formal analysis; visualization; writing – original draft; writing – review and editing; validation; data curation; software. **Sasha Vasconcelos:** Formal analysis; visualization; writing – review and editing; software; data curation. **Mohammad Hosein Rezai:** Investigation; data curation. **Miriam Frida Karlsson:** Conceptualization; methodology; supervision; funding

acquisition; project administration; resources; writing – review and editing. **Mattias Jonsson:** Conceptualization; methodology; funding acquisition; project administration; resources; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the Dryad Digital Repository and can be accessed using the following link: https://doi.org/10.5061/dryad.mgqnk9964.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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