bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

`

Behaviour and cold hardiness of the purple stem borer in winter, colonizing more northerly latitudes

Jianrong Huang [1,2*¶], Guoping Li[1¶], Haixia Lei[3¶], Chunbin Fan[4], Caihong Tian[1], Qi Chen[5], Bo Huang[1], Huilong Li[3], Zhaocheng Lu[3], Hongqiang Feng[1*]

[1] Henan Key Laboratory of Crop Pest Control, MOA's Regional Key Lab of Crop IPM in Southern Part of Northern China; Institute of Plant Protection, Henan Academy of Agricultural Sciences, Zhengzhou 450002, CHINA

[2] Centre for Ecology and Conservation, University of Exeter, Penryn, Cornwall TR10 9FE, UK

[3] Xinyang Academy of Agricultural Sciences, Xinyang 464000, CHINA

[4] Tianjing Beidagang Wetland Conservation Centre, Tianjing 300273, CHINA

[5] Luohe Academy of Agricultural Sciences, Luohe 462000, CHINA

* Corresponding author:

E-mail: hjr130705@126.com (JR) ; feng_hq@163.com (HQ)

¶These authors contributed equally to this work.

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

`

Abstract To escape or alleviate low temperatures in winter, insects have evolved many behavioral and physiological strategies. The rice pest insect, the purple stem borer, *Sesamia inferens* (Walker) is currently reported to be expanding their northern distributions and causing damage to summer maize in Xinxiang, China. However, their method of coping with the lower temperature in the new northern breeding area in winter is largely unknown. This paper investigates the overwinter site of *S. inferens*, and identifies the cold hardiness of larvae collected from a new breeding area in winter and explores a potential distribution based on low temperature threshold and on species distribution model, MaxEnt. The results show that the overwintering location of the *S. inferens* population is more likely to be underground with increasing latitude and,in the north, with the temperature decreasing, the larvae gradually moved down the corn stalk and drilled completely underground by February 18th. Those who were still above ground were all winterkilled. The cold hardiness test shows the species is a moderate freeze-tolerant one, and Supercooling Points (SCP), Freezing Points (FP) and mortality rate during the middle of winter (January, SCP: -7.653, FP: -6.596) were significantly lower than early winter (October) or late winter (March). Distribution in the new expansion area was predicted and the survival probability area was below N 35° for the Air Lower Lethal Temperature ($ALLT_{50}$) and below N 40° for the Underground Lower Lethal Temperature ($ULLT_{50}$), The suitable habitat areas for *S. inferens* with MaxEnt were also below N 40°. This study suggests the overwinter strategies have led to the colonization of up to a five degree more northerly overwintering latitude. This behavior of *S. inferens* could help maize producers to propose a control method to increase pest mortality by extracting the maize stubble after harvest.

Key words: *Sesamia inferens*; Behavioral adaptation; Cold hardiness; Overwintering distribution

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

`

1 Introduction

Temperature is the main abiotic factor that determines the growth and breeding of ectotherms. The distribution of most insects is directly related to extreme temperature (Sunday et al., 2012). The minimum temperature in winter which determines the survival rate of insect wintering populations is an important factor limiting the potential geographical distribution of insects (Bale, 1996; Stahl et al., 2006), particularly in ectotherms, setting northern range limits (Stahl et al., 2006; Gray, 2008; Calosi et al., 2010). The low temperature in winter constrains the behavioral strategies of colonial insects (Labrie et al., 2008), such as migration, drilling holes in refuges or making a thick cocoon, to avoid winterkilling. Insects also avoid chill injury by regulating physiological metabolism and accumulation of cold-tolerant substances. The warming of the global climate also leads to poleward distribution in some species' ranges, and may result in insects evolving new overwintering strategies in different breeding areas (Reynolds et al., 2015；Hu et al., 2015).

The purple stem borer, *Seramia inferens* Walker, belongs to the order Lepidoptera, Noctuidae, and *Sesamia*. They are polyphagous insects and their hosts are mainly gramineous crops or weeds such as rice, wheat, maize and they could live for a whole generation on rice, water shoots, and maize (Guo, 2017). With the changes in the farming system, crop distribution, and use of pesticides, *S. inferens* is gradually becoming a significant rice pest in many parts of China (Chen et al., 2015). *S. inferens* is distributed in rice-producing countries, mainly in Asia, the India Peninsula, Bangladesh, Myanmar, Thailand, Malaysia, Taiwan, Pakistan and Japan. In China, the species is distributed south of N 34 degrees latitude (Zhang, 1965), The ranges are consistent with research by Ezcurra et al., (1978). Based on the cold resistant characteristics and field experiments of *S. inferens*, the distribution area was also agreed to be south of N 34 degrees latitude by Gu (1985). However, adult *S. inferens* were first found by light trap and also caused maize plant damage in Xinxiang City, Henan Province, China (E: 113.696°, N: 35.021°) in 2014 (Huang and

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Feng, 2015). Further investigation illustrated that *S. inferens* can produce three generations in total and overwinter in local areas, following field surveys (Huang et al., 2017). They must have developed tolerance to cold to establish a population in the new northern breeding area in China in winter. Past research mainly focuses on overwintering in rice stubble in southern areas. It states that *S. inferens* overwinter as late instar larvae, and it is an exclusively freeze-tolerant insect (Guo et al., 2002; Sun et al., 2014). Significant damage to maize crops has also been reported in southern China (Xie et al., 1985). However, studies on overwintering strategies of the pest in the maize planting area and cold tolerance of *S. inferens* in the new expansion area remain inadequate.

In this study, the behavioral strategies were confirmed by investigating the overwinter site and survival of *S. inferens* in early-middle-late winter in the field. The diapause larvae were also collected and the cold hardiness was measured, and a regional distribution based on low temperature threshold and species distribution model MaxEnt were used to generate a potential distribution of *S. inferens*. This research aims to identify the methods of *S. inferens* larvae to tolerate cold temperatures and to study the northern distribution boundary in the expansion zone.

2 Materials and Methods

2.1 Latitudinal overwintering position of *S. inferens*

Field surveys were carried out in the late overwinter period on the 5th of February 2016 in Nanjing (NJ) (N 32.65°, E 119.43°) and the 5th of February 2016 in Changsha (CS) (N 28.47°, E 113.35°) in remaining rice stubble. *S. inferens* larvae were also investigated on the 18th of February 2016 in Xinxiang (XX) (N 35.02°, E 113.69°) in remaining maize stalks. The number of *S. inferens* at each location on the rice plants was recorded. Four vertical positions of the remaining rice or maize stalk from top to bottom were devised, including >20 cm above ground (AG), 10-20 cm AG, 0-10 cm AG and underground. The data of Xianyou (XY) (N 25.36°, E 118.69) samples cited in Gu (2014) were documented in a field survey on the 2nd and 11th of

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

February in 2013.

2.2 Underground-forward behavior on corn stalks

The vertical distribution of *S. inferens* larvae in maize plants was destructively sampled to determine survival of field conditions during the winters of 2015-2016 on the 6th of October, 2nd of November, 10th of December and 18th of February in XX. Numbers of living and dead *S. inferens* larvae and the position of those insects in the maize stalk were recorded. The mortality ratio at each location on the maize plant was calculated. Six vertical positions of the maize stalk, which remained completely in the field except the corn ear, from top to bottom are defined as positions 1-6. Position 1 represents the high maize ear section. Position 2 represents two sections of the maize ear. Position 3 represents two sections under the maize ear. Position 4 represents three sections above the soil surface. Position 5 represents the base of the maize stem and maize root under the soil surface. Position 6 represents the surrounding soil near the root.

2.3 Cold hardiness during winter

Wild *S. inferens* larvae were collected on remaining summer maize stalks in Xinxiang City (N 32°, E 119°) in different winter periods on October 31st and November 10th in 2015, January 27th, February 18th and March 30th in 2016. Each larva replicate was placed in a 0.5 ml plastic tube with a 1-2 mm diameter ventilation hole in the lid and labeled. A weight test was then carried out (Mettler Toledo ME204, Zurich, Switzerland). The Supercooling Points (SCP) and Freezing Points (FP) for each larval replicate were then measured by a small thermocouple thermometer (Liu et al., 2016) (Temp 20, Beijing Pengcheng Electronic Technology Center), which meant placing the thermometer into the plastic tube and touching the larvae bodies immobilized with cotton wool. Thermocouples were attached to a multichannel data logger (USB-TC, Measurement Computing, Norton, MA), and recorded and logged by using Tracer-DAQ software (Measurement Computing). The tubes were then placed in a -20℃ freezer for 30 minutes. The body temperature of the larvae was gradually reduced, so that all larval replicates could have SCP and FP recorded, as

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

in Leather (1993). After testing, death was assessed by the lack of mandibular and body movement after 6 hours recovery at 24℃ and a mortality rate was identified by the chill-coma recovery numbers. All sample larvae were then dried at 65℃ for 48 h in a drying oven and then weighed again, to determine the water content of each larva.

2.4 Lethal low temperature experiment

Larvae were collected in Anyang (N 35.57, E 114.85) in the same field on the 5th of November 2015 and each larva was also placed in a separate 0.5 ml plastic tube as mentioned above. 20 cm of soil was placed in a container (L605×W410×H240 mm), along with moist cotton wool at the bottom of the container in order to guarantee the same humidity as the overwintering period. The tubes with larvae were then placed vertically into soil in the container. 2 cm of soil was placed on the tube to simulate the overwintering environment of *S. inferens* underground. Because the larvae were diapaused, there was no need for food to survive. The container was placed in an outdoor environment. Two microclimate sensors (TH11R，HHW) were installed in air (10 cm from the soil surface) with a shelf, and at 2 cm soil depth to record the temperature of both sites until March 30th 2016. Low-temperature exposure experiments were carried out to measure the thresholds for the long-term survival of *S. inferens* at a constant temperature, separated into 4 times (Nov 9th, Dec11th, Jan 28th, Feb 19th) and 7 gradient low temperatures (0℃, -5℃, -10℃, -15℃, -20℃, -25℃, and -30℃). 7×20 larval replicates were treated each time and each larva was also placed in a separate 0.5 ml plastic tube as mentioned above. All replicates were placed in a 0℃ freezer for a 12 hour cold treatment, and then 20 replicates were taken out and placed at ambient temperature for 24 hours to determine whether they were alive when moving or dead. The number of dead larvae was recorded. Those remaining replicates then experienced -5 ℃ for 12 hours by reducing the freezer to the next temperature (-10 ℃), and then 20 replicates were taken out to check if alive or dead, and so forth until all 7×20 larval replicates were tested. Ultimately, the mortality rate of different temperatures was identified. A low temperature threshold: Air Lower Lethal Temperature ($ALLT_{50}$), which is defined as the temperature where 50% of individuals

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

`

die in the low-temperature exposure experiments mentioned above, was calculated by a logistic linear regression (Andersen et al., 2015). In this study, because the sampled larvae on different dates showed similar mortality rates after exposure to different temperatures, the data of the sample dates were taken together to calculate the $ALLT_{50,}$ and the $ALLT_{50}$ value was -4.5 ℃.

2.5 The distribution based on low temperature threshold

Daily minimum temperatures in 47 different cities (Henan province, Hebei province, Tianjing city and Beijing city were included, and those cities formed a south-north belt in north plain of China, in order to illustrate the northern overwinter site of *S. inferens*) from 2007 to 2014 were collected from national weather stations (https://data.cma.cn/). Previous research shows the minimum temperatures above ground were linearly correlated with minimum temperatures underground (Parton and Logan, 1981; Horton, 2012). In this study, the winter temperatures from November to March were recorded in the air (10 cm from the soil surface) and underground (at 2 cm soil depth) by two microclimate sensors mentioned above and it was found that there is an obvious significant linear relationship between lowest daily air and lowest underground temperature (R^2=0.9161, p<0.001, n=100). Based on this linear model, the minimum daily air temperatures in different cities were translated into the minimum daily soil temperature at 2 cm underground because the underground data were not available at national weather stations and the landscape and soil composites of the 47 cities were very similar. Another low temperature threshold, Underground Lower Lethal Temperature ($ULLT_{50}$) was also translated by linear equation and the value was -9.7 ℃, i.e. when the air temperature was -9.7 ℃ the soil temperature postulated reached -4.5 ℃. 47 location survival rates of air and underground was speculated by predicting all larvae would die when the annual daily minimum temperature was lower than the $ALLT_{50}$ or underground, respectively, i.e. if the annual minimum temperature is lower than the $ALLT_{50}$ or $ULLT_{50}$, 0% of the population will survival. 100% will survival if the annual minimum temperature is higher than the $ALLT_{50}$ or $ULLT_{50.}$ The calculation of survival rates in different cities

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

from 2007 to 2014, resulted in identification of the total proportion of insect survival over the 8 years. Based on the proportion of each cities, a potential distribution based on annual minimum temperature threshold could be produced with ArcMap (version 10.2, ESRI, Redlands, CA, USA) (Hong et al., 2014).

2.6 The distribution based on MaxEnt

In order to calibrate the distribution mentioned above and make up for the deficiency of the limited field-investigation scale, a species distribution model MaxEnt (version 3.3.3k) was used to generate a potential district-level distribution map of *S. inferens*. 19 bioclimatic data layers from the WorldClimte dataset were obtained (Fick and Hijmans, 2017; http://www.worldclim.org/). 147 district (cities) occurrences were obtained from published articles or websites, including the new northern breeding region in China (Zhang et al, unpublished, S1 Table 1 and S1 Table 2). 60% of total district (city) occurrences were used for model calibration (training data: 98 districts) and the remaining for model validation (test data: 49 districts). GoogleEarth was used to generate approximate coordinates. The area under the ROC (receiver operating characteristic) curve metric was used to evaluate the model performance (Phillips et al., 2006; Kumar et al., 2014) (S1 Fig 1). Current and potential distribution maps were generated using ArcMap and three arbitrary categories were defined as low (<0.15), medium (0.15-0.53) and high (>0.53) based on predicted habitat suitability.

2.7 Statistics

Data were tested for normal distribution using the Shapiro-Wilk test and normally distributed data was compared by an independent samples t-test and Pearson's correction test.The differences between factors were evaluated using Tukey-Kramer groupings comparison in the least squares means and Non-normally distributed data were compared by nonparametric tests of group differences. The linear or nonlinear models were identified with $p<0.05$ for the significance, and all data were analyzed with the software program R 3.5.3 (R Core Team 2017).

3 Results

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

`

3.1 Geographical variation in overwinter position

The *S. inferens* population was closer to the ground with increasing latitude in late winter and 100% and 75% of overwinter individuals were underground in northern locations in XX and NJ, respectively. But in southern regions, CS and XY, they were mainly 0-10 cm AG (Fig 1).

Fig 1. Geographical variation in overwintering position. UG: Underground; AG: above ground, XX: Xingxiang(*n*=19), NJ: Nanjing(*n*=74), CS: Changsha(*n*=33); XY: Xianyou(*n*=13).

3.2 Overwintering behavioral in maize stalk

Before overwintering, on the 6th of October, 72.222% (n=18) of the living larvae were distributed into two sections of maize ear and two sections under the maize ear in the field (positions 2 and 3). No larvae were found on the underground part. On the 2nd of November, the location of the population distribution shifted to the ground. 48.276% (*n*=29) of the population was located at positions 2 and 3, meanwhile, 13.793% of the population was on the underground part at the base of the maize stalk (position 5). On the 10th of December, it was found that 75% (*n*=64) of the population was distributed in the underground part (position 5), and the number of insects at other locations was in total only 25%. On the 18th of February, 100% (*n*=19) of the population had drilled into the underground position 5 (Fig 2).

Fig 2. Underground-forward behavior of *S. inferens* in remaining summer corn stalks in winter.

The mortality rates which include above ground and underground *S. inferens* larvae were 25.5% (*n*=86) and 20.83% (*n*=25) on the 10th of December and the 18th of February in the field, respectively. 100% of total overwintering populations who remained AG in winter died, and only 1.802% of total overwintering populations who

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

drilled into the ground died (Fig 3).

Fig 3. Proportion of *S. inferens* dead larvae in remaining winter maize stalks.

3.3 Cold hardiness of *S. inferens*

There were significant differences in SCP and FP of *S. inferens* larvae over time (SCP: $F_{4,91}$=4.993, p= 0.003; FP: $F_{4,91}$=10.26, p<0.001). The mean SCP (±SE) of *S. inferens* larvae ranged from the highest (-5.731±0.405 °C) in March to the lowest (-7.653±0.952 °C) in January (Fig 4) and the mean and minimum value of the SCP first decreased and then rose with the cold temperature event. The lowest value -20.02 °C appeared on the 27th of Jan (Table 1). Similarly, the mean of the FP first decreased and then rose. The means of the FPs in middle winter (December: -5.49±0.447; January:-6.596±0.889) were significantly lower than early and end of winter (November, October and March). In contrast, there was no significant difference in the fresh weight of larvae during the cold temperature period ($F_{4,91}$=1.997, p=0.102), but there was a significant gradual decrease in dry weight ($F_{4,91}$=3.934, p=0.006) and water content of the larvae ($F_{4,87}$=3.19, p=0.017). Larvae dry weight showed a decreased tendency throughout the winter and the water content decreased at the beginning of winter and slightly increased at the end of winter (Table 1).

Fig 4. SCP and FP of *S. inferens* larvae during the overwinter period. Same pattern bar with different letters on top shows a significant difference at p< 0.05.

After a 30 minute SCP test, 91.667% of the tested *S. inferens* larvae were dead when returned to 24 ℃ immediately after freezing on October 31st. 15.789% died in the January 27th SCP test, and 76.471% died in the March 31st test. The number of dead larvae after the test was significantly negatively correlated with the daily air mean temperature on the sampling date (r=-0.915, p =0.029) (Table 1).

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Table 1 Minimum SCP, fresh mass, dry mass, water content and mortality rate of *S. inferens* larvae after SCP test.

Date	*n*	Minimum SCP (℃)	Fresh mass (g)	Dry mass (g)	Water content (g)	Mortality rate after cold hardiness test (%)	Daily mean air temperature (℃)
Oct 31st	24	-10.38	0.19±0.015 a	0.069±0.007 ab	0.121±0.008 a	91.667	13.67
Dec 10th	20	-11.95	0.179±0.019 a	0.087±0.012 a	0.092±0.01 ab	30	2.18
Jan 27th	19	-20.02	0.146±0.016 a	0.051±0.007 bc	0.094±0.01 ab	15.789	-3.24
Feb 18th	12	-19.74	0.157±0.02 a	0.069±0.011 ab	0.088±0.01 b	37.5	3.03
Mar 31st	17	-17.9	0.14±0.011 a	0.037±0.005 c	0.103±0.007 ab	76.471	20.19

(Means flankedwith different letters at the same column are significantly different at $P<0.05$)

3. 4 Current and potential distribution of *S. inferens*

The $ALLT_{50}$ was -4.5 based on a nonlinear regression: $y=(1.03054)^x$ (y: Mortality rate, x: Temperature (℃), $R^2=0.878$, $p<0.001$, $n=24$) (Fig 5A). The $ULLT_{50}$ was -9.713℃ after being translated by a linear model between minimum daily air and underground temperature: $y=0.6628*x+2.6267$ (y: Underground, x: Air, $R^2=0.9161$, $p<0.0001$, $n=100$) (Fig 5B). The regional survival ratio was calculated with both $ALLT_{50}$ and $ULLT_{50}$ based on low temperature threshold. ≥50% probability survival regions were below N 35° due to the $ALLT_{50}$ and below N 40° due to the $ULLT_{50}$ (Fig 6A).

The MaxEnt model predicted potential distribution of *S. inferens* with a high accuracy test AUC value of 0.97 and training AUC value of 0.981(S1 Fig 2), and three temperature-variables: BIO6 (minimum temperature of coldest month), BIO10 (mean temperature of warmest quarter), and BIO2 (mean of monthly difference of maximum and minimum), which showed a top three permutation importance on the model (S1 Table 3). Model predictions closely matched the new overwinter site and also showed potentially suitable districts in north regions, and predicted suitable habitat areas of *S. inferens* below N 40°, and the most suitable areas were below N 38° (Fig 6B). This means that the underground overwinter temperature boundary defined by the $ULLT_{50}$ is reliable, and overwinter strategies overcoming the

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

`

304 winterkilling led to five latitude wider overwintering regions.

305

306 **Fig 5. (A) Mortality rate of *S. inferens* larvae after lethal low temperature**
307 **experiments and (B) the relationship between daily minimum air and UG**
308 **temperature during the overwinter period.**

309

310 **Fig. 6 Current and potential distribution of *S. inferens* in northern China.**
311 (A) Potential distribution based on the low temperature threshold, the size of the circle
312 in A means the survival probability, and the blue and purple circles are sourced from
313 $ALLT_{50}$ and $ULLT_{50}$, respectively. (B) Current overwinter sites and predicted suitable
314 habitat area modeling by MaxEnt, the darker color in B represents the better predicted
315 habitat suitability. The white points are the new northern overwinter sites.

316 # 4 Discussion

317 There are many studies which show the latitudinal pattern of insects facing cold
318 temperatures in winter. Higher latitude populations possess a stronger cold tolerance,
319 a shorter chill-coma recovery time and a higher survival rate (Hoffmann et al.,2002;
320 David et al., 2003; Chen et al., 2004; Rochefort et al.,2011; Tian et al., 2016). This
321 research shows the overwintering location of the *S. inferens* population was placed
322 closer to the ground with increasing latitude. In the new northern breeding area in
323 winter, with the temperature decreasing, *S. inferens* larvae gradually climbed down
324 and drilled into the base of the maize stalk under the soil surface before the coldest
325 temperature arrived. Larvae remaining above ground in winter were all winterkilled.
326 Compared to the year-round site at N 19° in the south (Zhou, 1985), it is an obvious
327 behavioral evolution strategy of *S. inferens* to survive, to find the warmer
328 microclimate of the overwintering site in the north in winter. Insects usually select
329 warmer micro-habitats to survive. *Coccinella septempunctata* L., *Ceratomegilla*
330 *undecimnotata* (Schneider), *Hippodamia variegata* (Goeze) *Harmonia axyridis*
331 (Pallas) and *Coccinella septempunctata* select human houses, isolated grass tussocks
332 or under covered stones as overwintering sites (Honek et al.,2007; Labrie et al., 2008;

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

`

Koštál et al., 2014). The underground-forward behavior of *S. inferens* is also to avoid the chill damage in the northern area. Similarly, *Sesamia nonagrioide*, the same family but another species, also show underground-forward behavior in winter (Gillyboeuf et al., 1996).

Physiological adaptation of insects for low temperatures is by partial dehydration, increasing body fluid osmolality, and the accumulation of a complex mixture of winter specific metabolites, which strengthen their cold hardiness (Rozsypal et al., 2013; Koštál et al., 2014). In this paper, the mean SCP of larvae which were collected from the field was -7.653±0.952℃ (lowest), and the mortality rate of the population was 15.789% (lowest) after a 30 minute cold hardiness test in January (middle winter). Meanwhile, the water contents gradually decreased and were lowest on Feb 18th which shows the diapaused insects transfer fluidity into antifreeze proteins and glycoproteins (Rozsypal et al., 2014). When insects begin to recover in spring the fluidity increases. In this research, insects survived when body temperature was under freezing point and showed an extreme low temperature tolerance capability meaning that the population of *S. inferens* larvae in the northern breeding area was a moderately freeze-tolerant one. These results correspond with Guo et al. (2002) and Sun et al. (2014) where the pest hosts on rice. Cold hardiness is also related to the host insects have fed on (Morey et al., 2016; Alford et al., 2016) and the difference in the cold tolerance mechanisms between maize and rice need to be further investigated in the new breeding region.

Minimum temperatures in the coldest month are linearly related to insect mortality (Hoffmann et al., 2002). $ALLT_{50}$ or the lethal temperature (LTe50) of insect cold exposure are the best predictors of cold distribution limits and are usually used to assess the potential distribution according to the annual minimum temperature (Ungerer et al., 1999; Andersen et al., 2015). This research used the same method to show a northern distribution considering the $ALLT_{50}$ in locations under N 35°. However, depending on the $ULLT_{50}$, the results showed a northerly distribution below N 40° when insects overwinter underground. This was also in agreement with the results of the MaxEnt modeling, which show below N 40° predicted suitable habitat

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

areas. Distribution of insects is highly impacted by climatic factors (temperature, moisture, humidity and their variations), especially the effects of temperature (Aregbesola et al., 2019). MaxEnt integrates insect occurrence records with climatic and other environmental variables and the MaxEnt model of this study shows a similar result to de la Vega (2015), that the minimum temperature of the coldest month was the important abiotic factor restricting their geographic distribution. This research examined the geographical distributions of *Triatoma infestans* and *Rhodnius prolixus*. The MaxEnt model also included many other important parameters such as the precipitation, which showed a high contribution in the model. It is usually used to predict potential distributions of insect pests (Kumar et al., 2014). Although artificial because the linear model based on the point data might not represent the actual regional patterns, the linear relationship between air and soil temperature has indeed been studied in different areas (Parton and Logan, 1981; Horton, 2012), Hong (2014) has taken advantage of this linear relationship from 96 weather stations in Shanxi, China, and classified the overwintering sites of the southern root-knot nematode. These results also show that the underground low temperature threshold defined by the ULLT50 could be extrapolated to other underground overwinter species.

The population behavior of animals which escape cold weather usually by large-scale migration into a warmer habitat, will lead to relocated populations regionally, but not all individuals can successfully fly to suitable places. *S. inferens* have a weak flight tendency and capability in the field (Han et al., 2012). This paper shows another behavioral strategy of *S. inferens* larvae by locally searching for a warmer place by drilling into the ground in early winter. The microclimate of the overwintering site could result in the *S. inferens* colonizing more northerly latitudes and the insect's physiological and biochemical changes may be a factor favoring this northern expansion leading to a higher winter survival chance. Past studies have shown that *S. inferens* are distributed below N 34° degrees north latitude. This study also documents a new location for *S. inferens*: a northern distribution record of N 40°. A large amount of research has shown that the overwintering boundary of many insects has been reportedly spreading poleward due to global warming (Wheatley et al., 2017). It

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

cannot be overlooked that the warmer changes in northern China in these two centuries have driven the change of animal redistribution (Wu, 2016), but the strategy of behavioral or physiological changes in this research to overcome winter will certainly help to colonize a five degree more northerly latitude. For maize producers, ploughing the maize stubble in autumn after harvesting will lead to an increased mortality of *S. inferens* larvae due to cold exposure. This study allows us to propose a simple non-pollutant pest control method in northern China.

Data Acessability

All data used in this manuscript is displayed in this context or provided as supporting information in the file: Supplementary Material 1.

Author Contributions

JR and HQ conceived and designed the experiments. GP, HX and CB performed the overwinter investigation. JR analyzed the raw data. JR, GP, HX, CB, CH, QC, BH, HL, ZC, and HQ finalized the manuscript. All authors read and approved the final manuscript.

Funding

J.H.'s visiting scholarship to the University of Exeter was funded by the Youth Exchange Funding of Henan Academy of Agricultural sciences. This research was supported by the National Natural Science Foundation of China (Grant No. 31401731) and Youth Funding of Henan Academy of Agricultural sciences (No.2020YQxx), Supported by the national key research and development program of China (Grant Nos. 2016YFD0300705, 2018YFD0200602, and 2018YFD0200605).

Acknowledgements

We thank Chao Han and Mingyong Ma for carrying out the overwinter investigation

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

on Nanjing and Changsha, respectively. We thanks those who contributed to discussions of the ideas presented in this paper. Instructive comments from the anonymous reviews will greatly improved this manuscript.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

`

References

Alford L, Androdias A, Franco T, Pierre JS, Burel F, van Baaren J. Potential host manipulation by the aphid parasitoid *Aphidius avenae* to enhance cold tolerance. PloS one. 2016; 11(12): e0168693

Andersen JL, Manenti T, Sørensen JG, MacMillan HA, Loeschcke V, Overgaard J. How to assess Drosophila cold tolerance: chill coma temperature and lower lethal temperature are the best predictors of cold distribution limits. Funct Ecol. 2015; 29(1): 55-65

Aregbesola OZ, Legg JP, Sigsgaard L, Lund OS, Rapisarda C. Potential impact of climate change on whiteflies and implications for the spread of vectored viruses. J Pest Sci, 2019; 92(2): 381-392.

Bale J S. Insect cold hardiness: a matter of life and death. Eur J Entomol 1996; 93(3): 369-82

Calosi P, Bilton DT, Spicer JI, Votier SC, Atfield A. What determines a species geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). J Anim Ecol. 2010; 79(1): 194-204

Chen B, Kang L. Variation in cold hardiness of Liriomyza huidobrensis (Diptera: Agromyzidae) along latitudinal gradients. Environ Entomol. 2004; 33(2): 155-164

Chen XJ, Lu DH. Study Advances in Occurrence and Control of the Polyphagous Pink Stem Borer *Sesamia inferens*. China Agric Sci Bull. 2015; 31(25): 171-175. Chinese

David JR, Gibert P, Moreteau B, Gilchrist GW, Huey RB. The fly that came in from the cold: geographic variation of recovery time from low-temperature exposure in *Drosophila subobscura*. Funct Ecol. 2003;17(4): 425-430

de la Vega GJ, Medone P, Ceccarelli S, Rabinovich J, Schilman PE. Geographical distribution, climatic variability and thermo-tolerance of Chagas disease vectors. Ecography. 2015; 38(8):851-860

Ezcurra E, Rapoport EH, Marino CR. The geographical distribution of insect pests. J Biogeogr.1978; 5(2): 149-157

Fick SE, and Hijmans RJ. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Int J Climatol. 2017; 37(12): 4302-4315

Gillyboeuf N, Anglade P, Lavenseau L, Peypelut L. Cold hardiness and overwintering strategy of

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

the pink maize stalk borer, *Sesamia nonagrioides* Lef (Lepidoptera, Noctuidae). Oecologia. 1994; 99(3-4), 366-373.

Gray DR. The relationship between climate and outbreak characteristics of the spruce budworm in eastern Canada. Clim Change. 2008; 87(3-4): 361-383

Gu L. Characteristics of rice pest in Xianyou county [dissertation], Fujian: Fujian Agriculture and Forestry University. 2014

Gu NH. Preliminary study on the characteristics of hibernation of pink rice borer. Acta Ecol Sin. 1985; 5(1): 64-70. Chinese

Guo H F, Li Q, Feng J C, Zhang H. Comparison of cold tolerance of three specie of overwintering rice stem borers in Nanjing area. J Jiangsu agric Sci. 2002; 18(2):85-88. Chinese

Guo YY. Crop pests and diseases in China (The 3nd press). Beijing: China Agricultural Press. 2017.

Han LZ, Peng YF, Wu KM. Studies on larval dispersal ability in the field and adult flight capacity of the pink stem borer, *Sesamia inferens*. Plant Prot. 2012; 38(4): 9-13. Chinese

Hoffmann AA, Anderson A, Hallas R. Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. Ecol Lett. 2002; 5(5): 614-618

Honek A, Martinková Z, Pekár S. Aggregation characteristics of three species of Coccinellidae (Coleoptera) at hibernation sites. Eur J Entomol. 2007; 104(1): 51

Hong B, Zhang F, Li YM, Zhang SL, Chen ZJ, Gao F, Liang YL. GIS-based regional classification for overwintering of southern root-knot nematode in Shanxi Province. Acta Ecol Sin. 2014; 34(16): 4603-4611

Horton B. Models for estimation of hourly soil temperature at 5 cm depth and for degree-day accumulation from minimum and maximum soil temperature. Soil Res. 2012; 50(6): 447-454

Hu CX, Hou ML, Wei GS, Shi BK, Huang JL. Potential overwintering boundary and voltinism changes in the brown planthopper, *Nilaparvata lugens*, in China in response to global warming. Clim Change. 2015; 132(2): 337-352

Huang JR, Feng HQ. Preliminary report on damage of summer corn seedlings by the purple stem borer, *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae) in northern Henan province, Plant Protect,2015, 41(2):231-233. Chinese.

Huang JR, Huang B, Li GP, Tian CH, Feng HQ. Preliminary study on the occurrence of pink stem

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

`

488 borer, *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae) in northern Henan Province. Plant
489 Protect. 2017; 43(2):156-162. Chinese.
490 Kleynhans E, Mitchell KA, Conlong DE, Terblanche JS. Evolved variation in cold tolerance
491 among populations of *Eldana saccharina* (Lepidoptera: Pyralidae) in South Africa. J Evol
492 Biol. 2014; 27(6): 1149-1159
493 Koštál V, Miklas B, Doležal P, Rozsypal J, Zahradníčková H. Physiology of cold tolerance in the
494 bark beetle, *Pityogenes chalcographus* and its overwintering in spruce stands. J Insect
495 Physiol. 2014; 63(1): 62-70
496 Kumar S, Graham J, West AM, Evangelista PH. Using district-level occurrences in MaxEnt for
497 predicting the invasion potential of an exotic insect pest in India. Comput Electron Agric.
498 2014; 103(2): 55-62
499 Labrie G, Coderre D, Lucas E. Overwintering strategy of multicolored Asian lady beetle
500 (Coleoptera: Coccinellidae): cold-free space as a factor of invasive success. Ann Entomol
501 Soc Am. 2008; 101(5): 860-866
502 Leather SR, Walters KFA, Bale JS. The ecology of insect overwintering. Cambridge: Cambridge
503 University Press; 1993.
504 Liu YQ. Zheng XX, Ma HF, Xia R X, Li YP, Zhang QR. Supercooling capacity and cold
505 tolerance of the wild silkworm, *Antheraea pernyi* (Lepidoptera: Saturniidae). J Econ Entomol.
506 2016; 109(4): 1619-1627
507 Morey AC, Venette RC, Nystrom Santacruz EC, Mosca LA. Hutchison WD. Host-mediated shift
508 in the cold tolerance of an invasive insect. Ecol Evol. 2016; 6(22): 8267-8275
509 Parton WJ, Logan JA. A model for diurnal variation in soil and air temperature. Agric Meteorol.
510 1981; 23(3): 205-216
511 Phillips SJ, Anderson RP, Schapire RE. Maximum entropy modeling of species geographic
512 distributions. Ecol Model. 2006; 190 (3-4): 231-259
513 Reynolds DS, Reynolds WC, Brandenburg RL. Overwintering, oviposition, and larval survival of
514 hunting billbugs (Coleoptera: Curculionidae) and implications for adult damage in north
515 carolina turfgrass. J Econ Entomol. 2015; 109(1): 240-248.
516 Rochefort S, Berthiaume R, Hébert C, Charest M, Bauce é. Effect of temperature and host tree on
517 cold hardiness of hemlock looper eggs along a latitudinal gradient. J Insect Physiol. 2011;

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

`

57(6): 751-759

Rozsypal J, Koštál V, Zahradníčková H, Šimek P. Overwintering strategy and mechanisms of cold tolerance in the codling moth (*Cydia pomonella*). PLoS One. 2013; 8(4): e61745

Rozsypal J, Koštál V, Berková P, Zahradníčková H, Šimek P. Seasonal changes in the composition of storage and membrane lipids in overwintering larvae of the codling moth, *Cydia pomonella*. J Therm Biol. 2014; 45: 124-133

Stahl K, Moore RD, McKendry IG. Climatology of winter cold spells in relation to mountain pine beetle mortality in British Columbia. Canada Clim Res. 2006; 32(1):13-23

Sun M, Tang XT, Lu MX, Yan WF, Du YZ. Cold tolerance characteristics and overwintering strategy of *Sesamia inferens* (Lepidoptera: Noctuidae). Fla Entomol. 2014; 97(4): 1544-1553

Sunday JM, Bates AE, Dulvy NK. Thermal tolerance and the global redistribution of animals. Nat Clim Chang. 2012; 2(9): 686-690

Tian B, Xu L, Zhang M, Feng Y, Zong S. Supercooling capacity and cryoprotectants of overwintering larvae from different populations of *Holcocerus hippophaecolus*. CryoLetters, 2016; 37(3): 206-217.

Ungerer MJ, Ayres MP, Lombardero MJ. Climate and the northern distribution limits of *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae). J Biogeogr. 1999; 26(6):1133-1145

Wheatley CJ, Beale CM, Bradbury RB, Pearce-Higgins JW, Critchlow R, Thomas CD. Climate change vulnerability for species-Assessing the assessments. Glob Chang Biol. 2017;23(9): 3704-3715

Wu J. Detection and attribution of the effects of climate change on bat distributions over the last 50 years. Clim Chang. 2016; 134(4): 681-696.

Xie DP, Cai RX, Zhao FZ, Li TC, Xie TY. A study of the bionomics of the pink stem borer of maize, *Sesamia inferens* walker and its control by insecticides. J Sichuan Agric C. 1985; 3(1): 33-40

Zhang SM. Discussion the boundary line between the ancient North and the Eastern regions in the east of the Qinling Mountains in China from the distribution of some agricultural insects. Acta Entomol Sin.1965; 14(4): 411-419. Chinese.

Zhou CB, Chen AF. Observation on the biology and wintering situation of *Sesamia inferens* in

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

548 northern Hainan province. China J Appli Entomol. 1985; 5(1):199-201. Chinese

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

`

Supporting information

S1 Fig 1. The receiver operating characteristic (ROC) curve of the MaxEnt model.

S1 Fig 2. AUC of different environmental variables based on results of jackknife tests in the MaxEnt model.

S1 Table 1. The live number of *S. inferens* larvae at northern overwinter sites.

S1 Table 2. The worldwide distribution of *S. inferens*

S1 Table 3. The permutation importance and **relative contributions of the environmental variables in the MaxEnt model.**

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

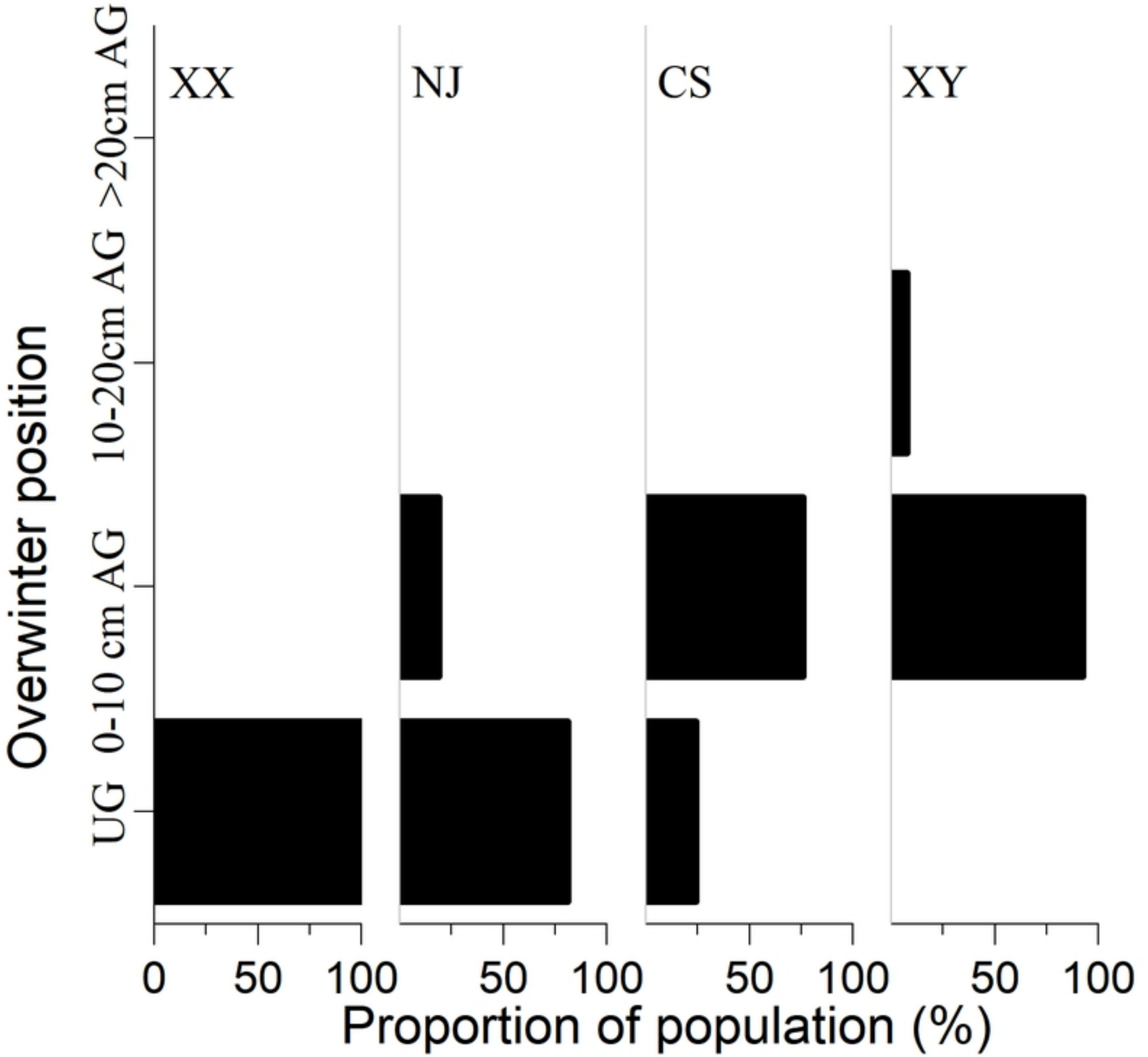

Fig 1

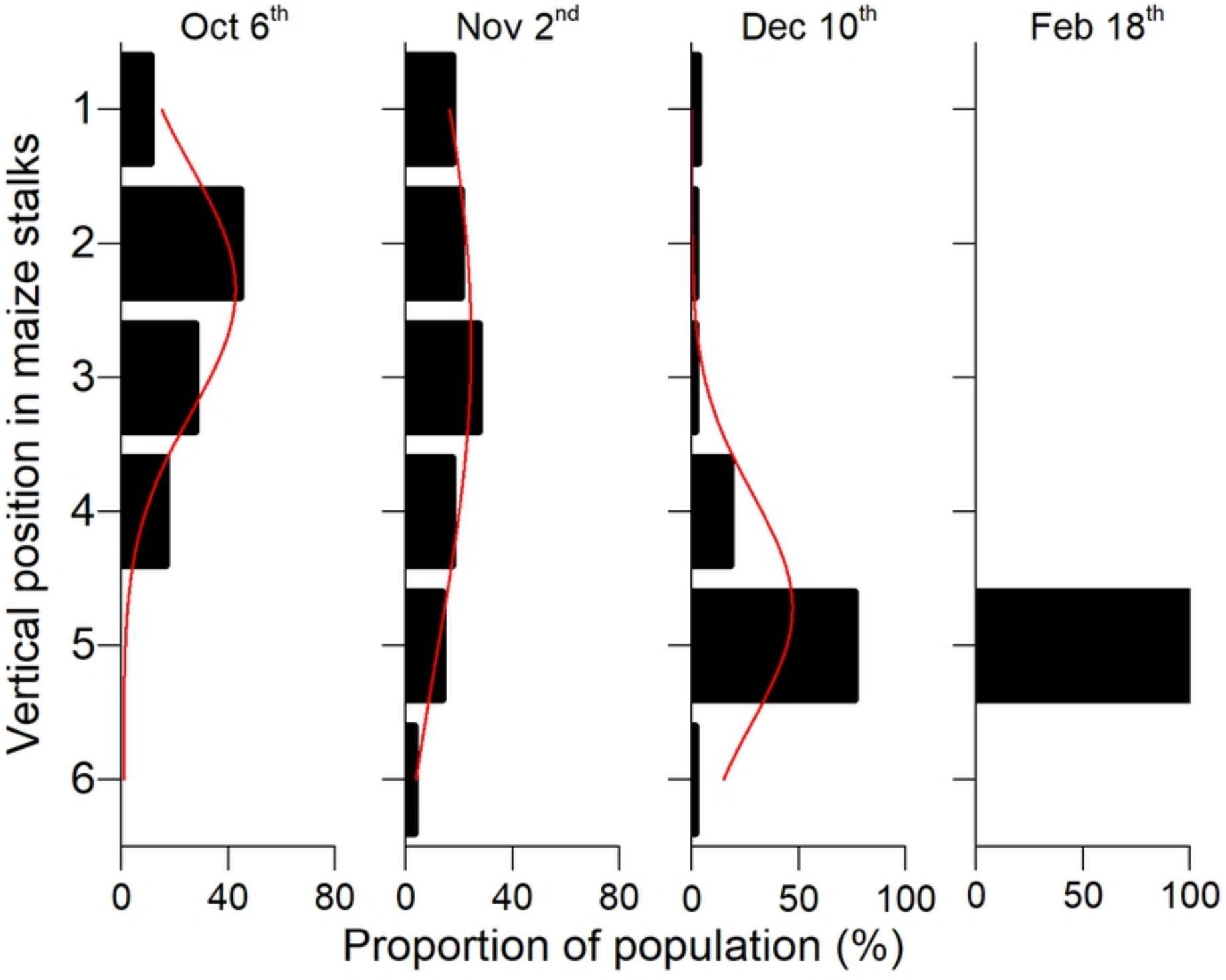
Oct 6th
Nov 2nd
Dec 10th
Feb 18th
Vertical position in maize stalks
1
2
3
4
5
6
0
40
80
0
40
80
0
50
100
0
50
100
Proportion of population (%)

Fig 2

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

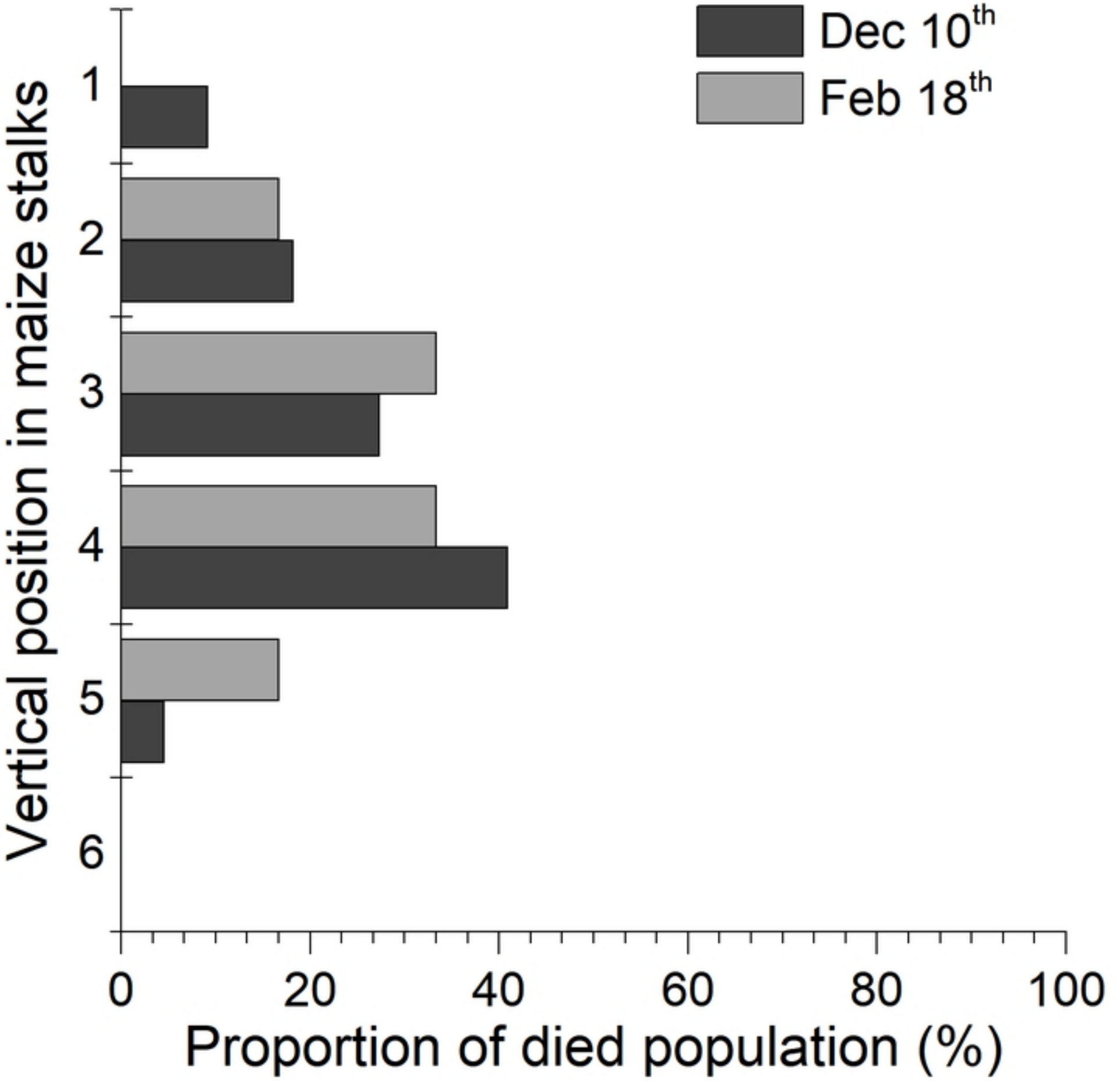

Fig 3

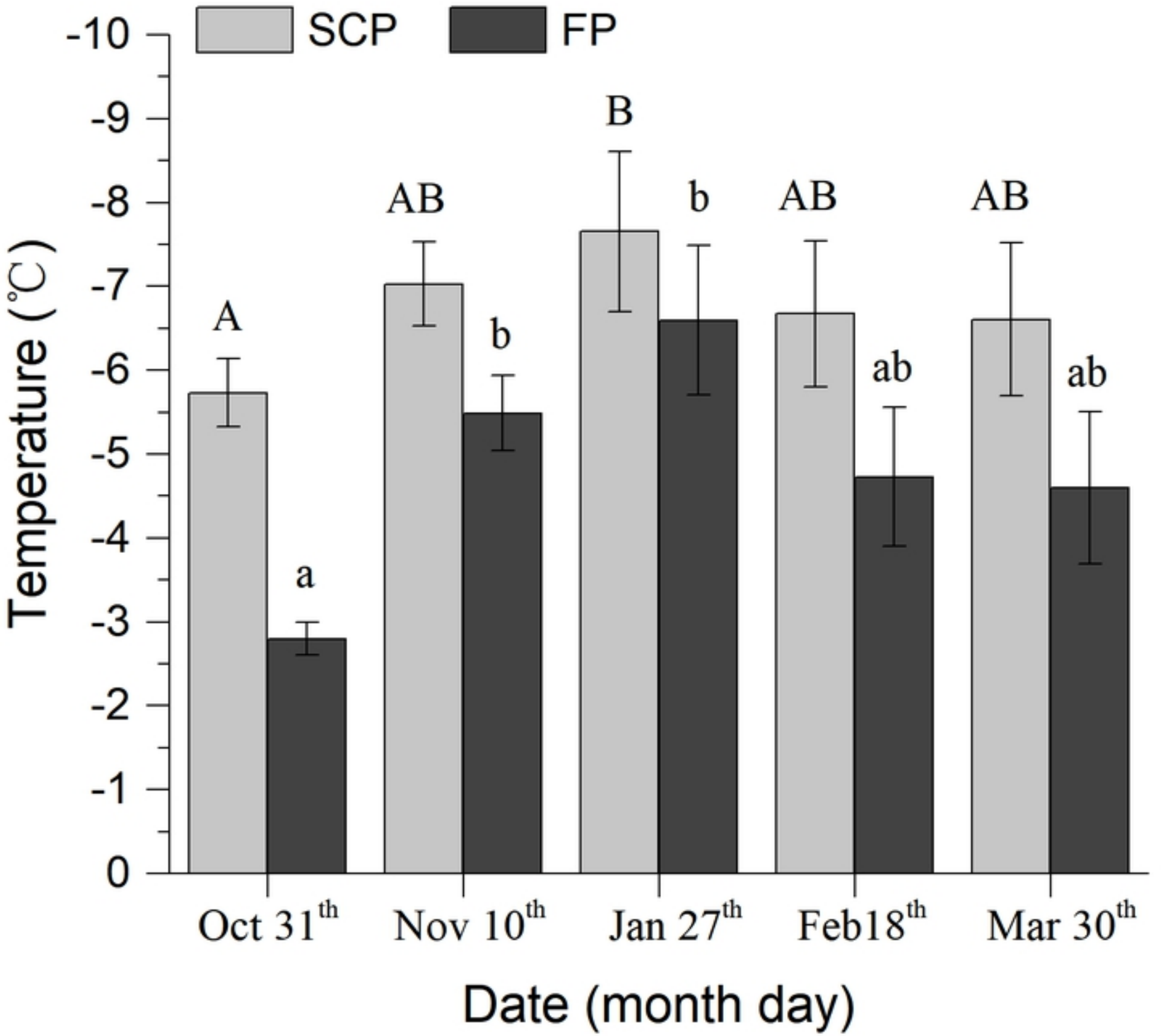
SCP
FP
-10
-9
-8
-7
-6
-5
-4
-3
-2
-1
0
Temperature (℃)
A
a
AB
b
B
b
AB
ab
AB
ab
Oct 31th
Nov 10th
Jan 27th
Feb18th
Mar 30th
Date (month day)

Fig 4

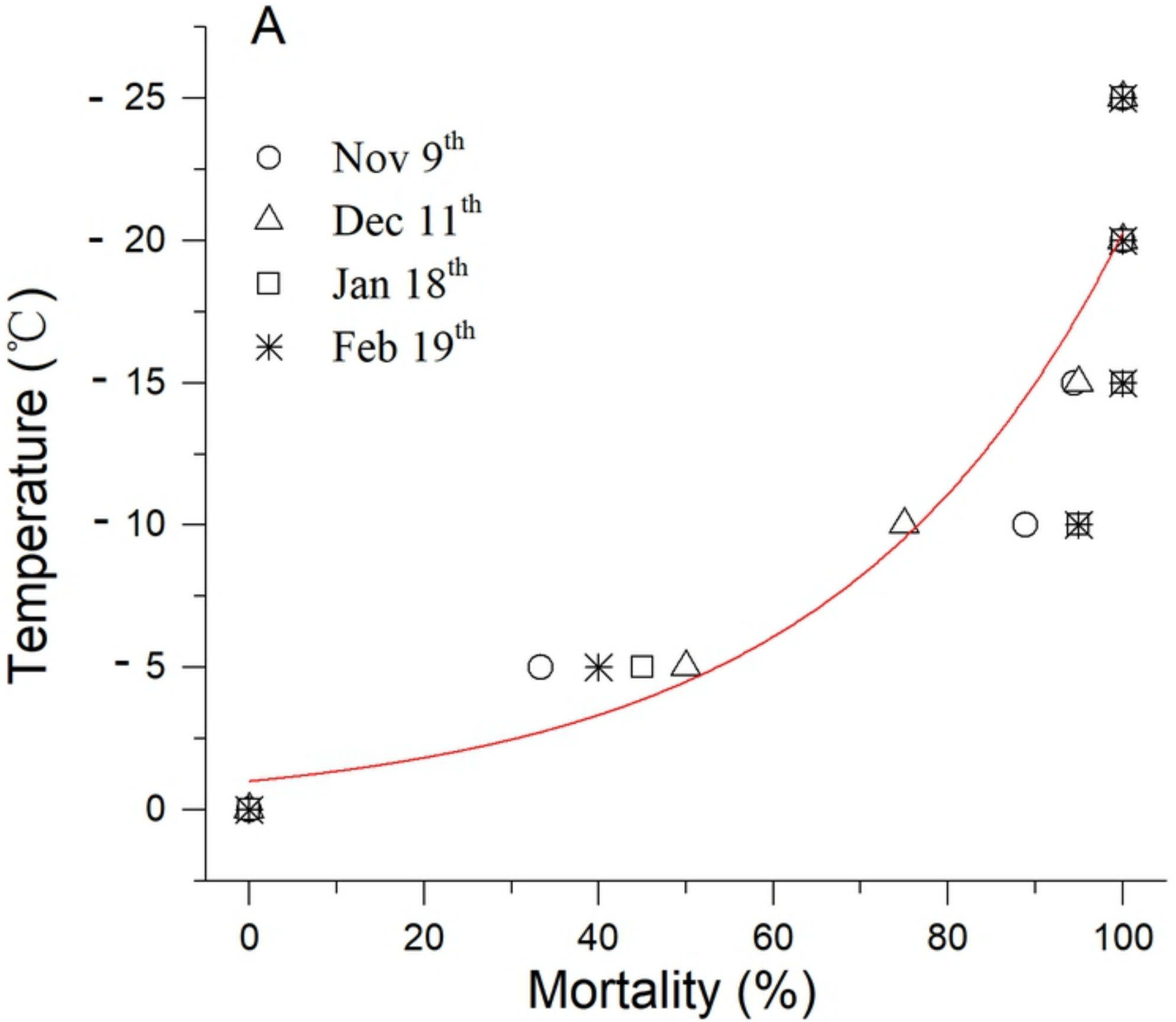
A
Nov 9th
Dec 11th
Jan 18th
Feb 19th
Temperature (°C)
- 25
- 20
- 15
- 10
- 5
0
0
20
40
60
80
100
Mortality (%)

Fig 5A

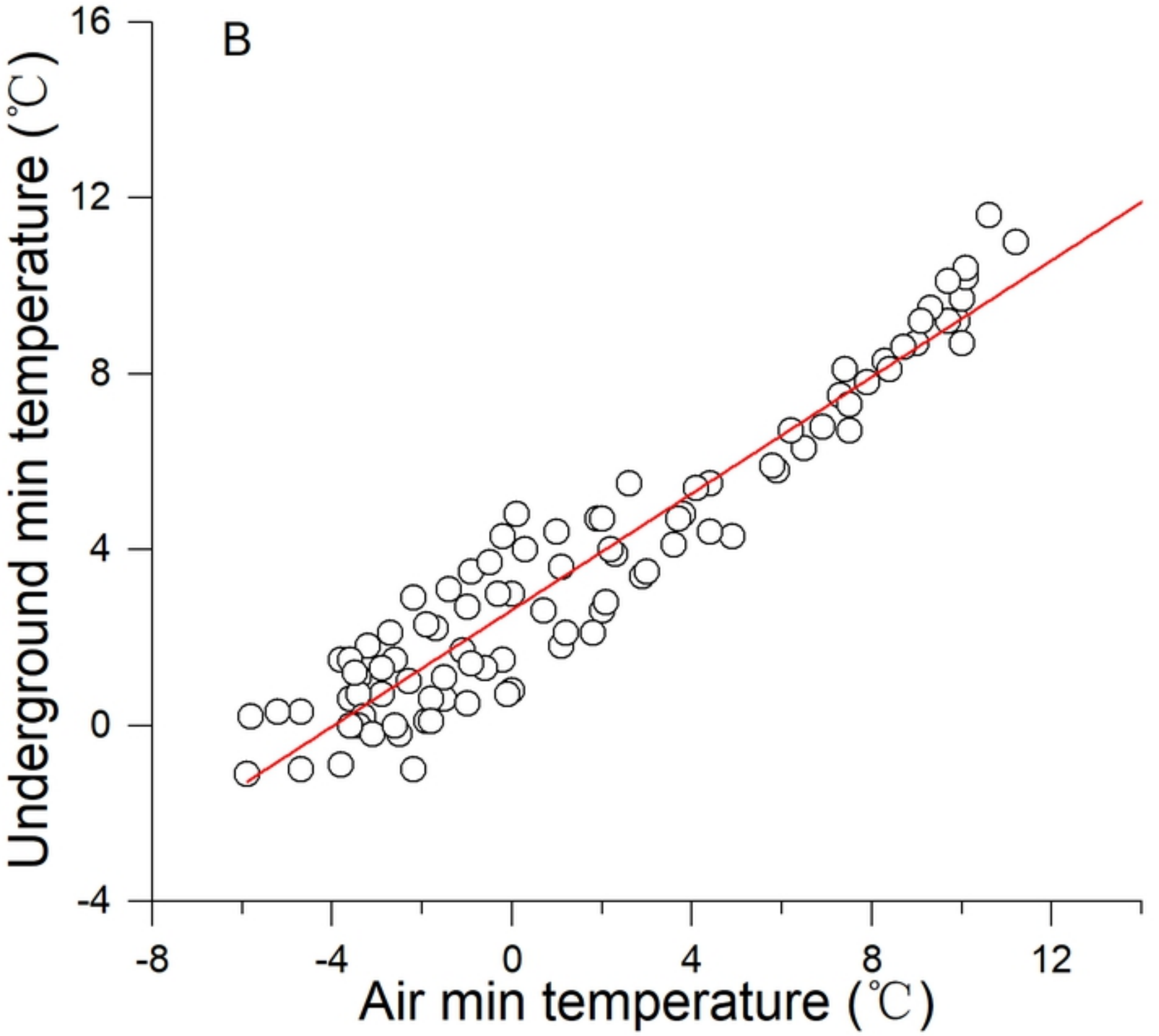
B
Underground min temperature (℃)
16
12
8
4
0
-4
-8
-4
0
4
8
12
Air min temperature (℃)

Fig 5B

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

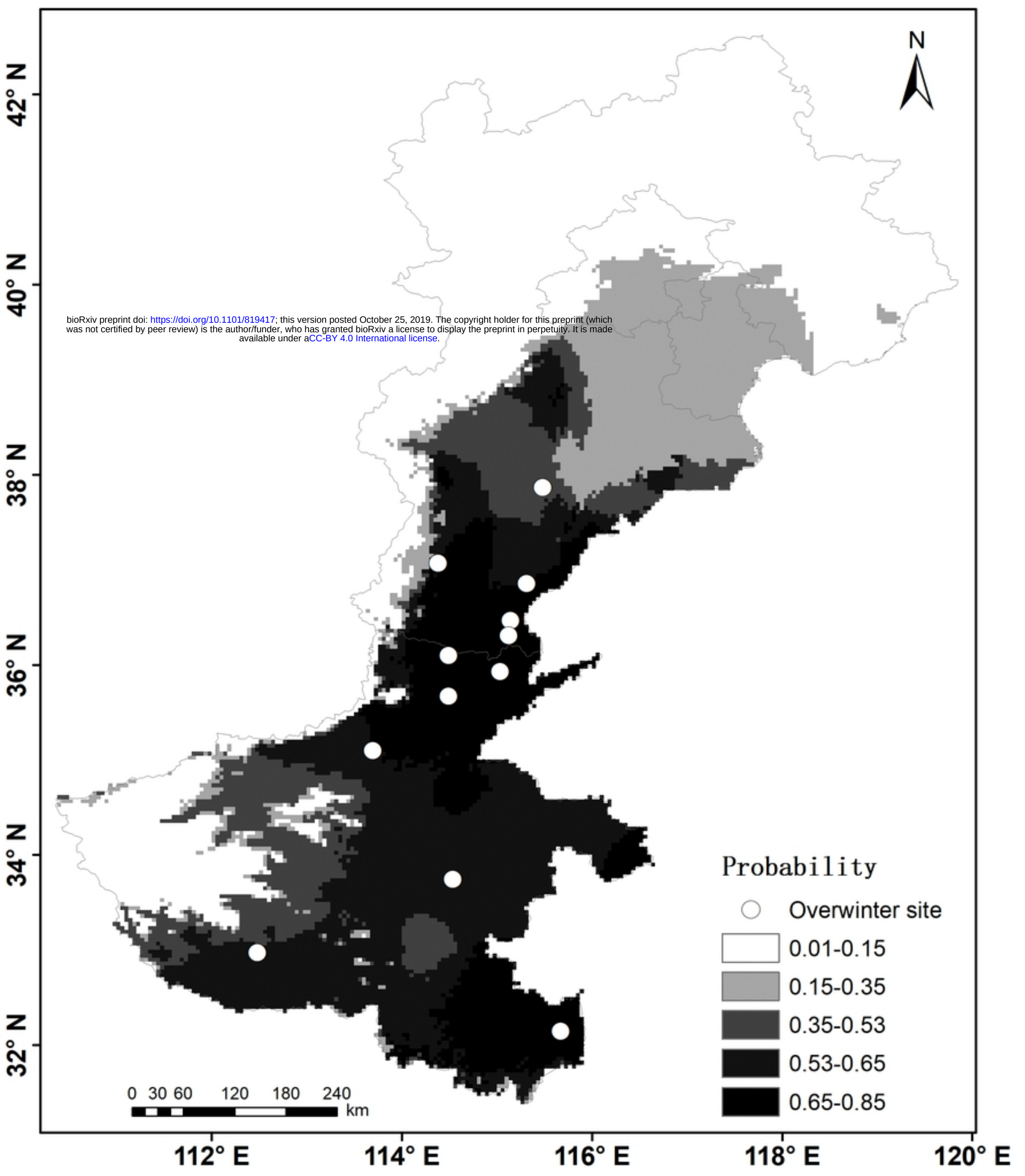

Fig 6B

bioRxiv preprint doi: https://doi.org/10.1101/819417; this version posted October 25, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

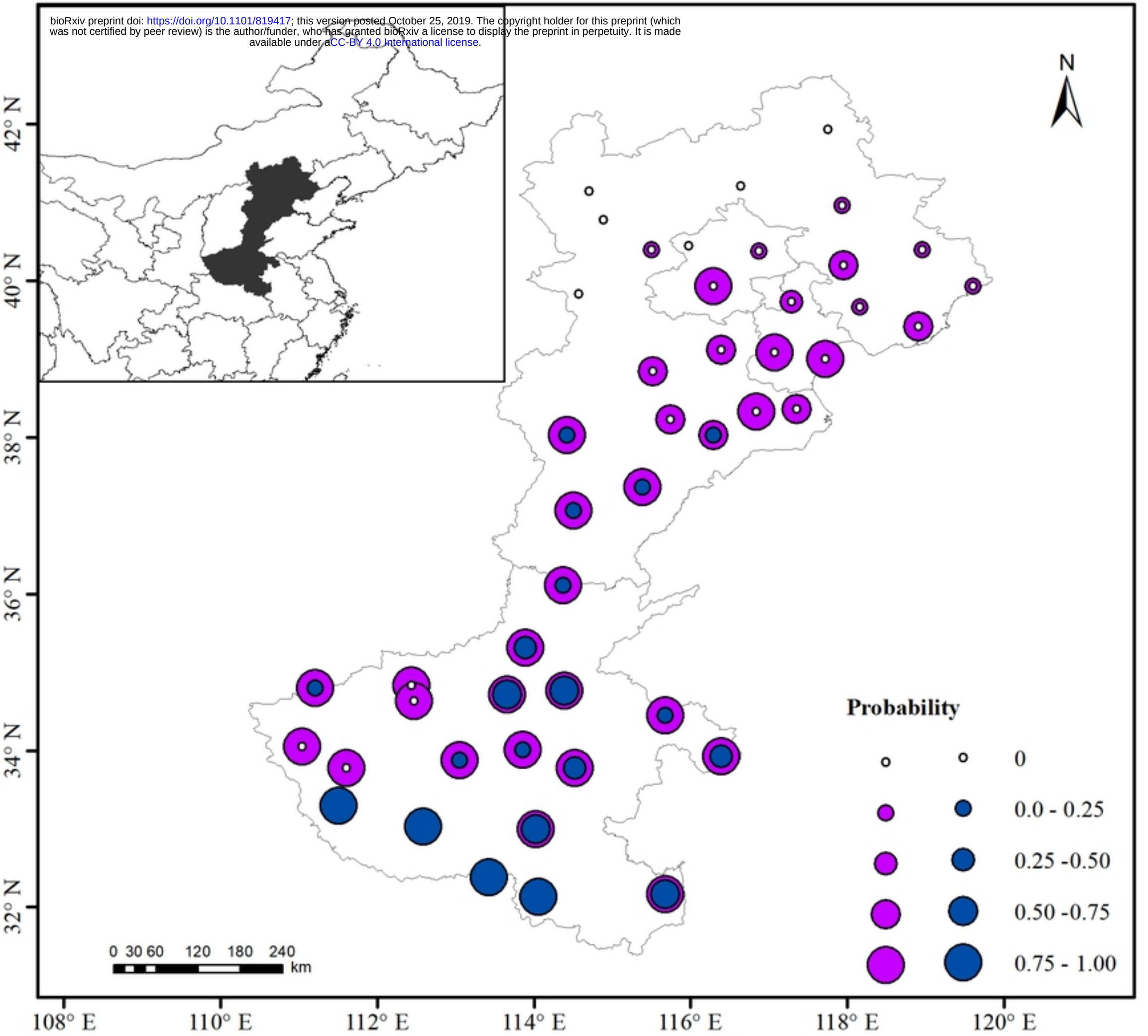

Fig 6A