

# Predicting the altitudinal distribution of an introduced phytophagous insect against an invasive alien plant from laboratory controlled experiments: case of *Cibdela janthina* (Hymenoptera:Argidae) and *Rubus alceifolius* (Rosaceae) in La Réunion

Alexandre Mathieu · Yves Dumont · Frédéric Chiroleu ·  
Pierre-François Duyck · Olivier Flores · Gérard Lebreton ·  
Bernard Reynaud · Serge Quilici

Received: 10 September 2013 / Accepted: 20 March 2014  
© International Organization for Biological Control (IOBC) 2014

**Abstract** The sawfly *Cibdela janthina* (Hymenoptera: Argidae) native to Sumatra was introduced on La Réunion (France, Indian Ocean) in 2007 to control the giant bramble *Rubus alceifolius* (Rosaceae), one of the most invasive plants on this island. We determined the influence of temperature on the development duration and survival of *C. janthina* preimaginal stages in controlled conditions in order to parameterize a survival model and to relate the predicted survival

with observed patterns of defoliation of the host plant at different altitudes. We adjusted the Régnière model to survival data, combined with the Lactin-2 model on development rate of the three preimaginal stages of *C. janthina*. This model adequately predicts the observed defoliation and the altitudinal limit of the biological control agent. Our results also show that studies on temperature-related constraints on the biology of an agent introduced for weed control should be emphasized both in the pre-release and the post-release phases of a biological control program to evaluate the potential success of the control programme.

Handling Editor: John Scott.

**Electronic supplementary material** The online version of this article (doi:10.1007/s10526-014-9574-y) contains supplementary material, which is available to authorized users.

A. Mathieu (✉) · F. Chiroleu · P.-F. Duyck ·  
O. Flores · G. Lebreton · B. Reynaud · S. Quilici  
CIRAD Pôle de Protection des Plantes, UMR C53  
PVBMT CIRAD-Université de la Réunion, 7 Chemin de  
l'IRAT, Ligne Paradis, 97410 Saint Pierre, La Réunion,  
France  
e-mail: alexandre.mathieu@cirad.fr

F. Chiroleu  
e-mail: frederic.chiroleu@cirad.fr

P.-F. Duyck  
e-mail: pierre-francois.duyck@cirad.fr

O. Flores  
e-mail: olivier.flores@cirad.fr

**Keywords** Biological control · Invasive plant ·  
Sawfly · Temperature-related development ·  
Altitude · Distribution area

G. Lebreton  
e-mail: gerard.lebreton@cirad.fr

B. Reynaud  
e-mail: bernard.reynaud@cirad.fr

S. Quilici  
e-mail: serge.quilici@cirad.fr

Y. Dumont  
CIRAD, UMR AMAP, TA A51/PS2, Boulevard de la  
Lironde, 34398 Montpellier Cedex 5, France  
e-mail: yves.dumont@cirad.fr

## Introduction

Biological invasions constitute one of the main threats to biodiversity after habitat destruction and fragmentation (Vitousek et al. 1996). By causing disruptions, invasive alien plants constitute a threat to the original structuration of plant communities in preserved habitats on oceanic islands (Macdonald et al. 1991; Strasberg et al. 2005).

In the Indian Ocean, the Malagasy region, including the Mascarene Islands (La Réunion, Mauritius and Rodrigues), is recognized as a biodiversity hotspot (Myers et al. 2000). In this region, La Réunion (21°06'S; 55°36'E; 2,512 km<sup>2</sup>), a small French island, is of major interest in terms of conservation, since about 30 % of the indigenous vegetation of the island is still preserved (Strasberg et al. 2005). However, since human settlement during the seventeenth century, this island has experienced a deep and fast transformation of its native ecosystems, mostly due to changes in land-use and biological invasions. Among the invasive alien plants, the giant bramble *Rubus alceifolius* Poiret (Rosaceae), introduced in the mid-nineteenth century, is considered to be among the most threatening species of undisturbed native ecosystems on La Réunion (Macdonald et al. 1991).

Native of Southeast Asia, *R. alceifolius* has been also accidentally introduced into other Indian Ocean islands (Madagascar, Mayotte, Mauritius) and in Queensland (Australia). This bramble is a perennial liana-like shrub which can multiply both by sexual and vegetative means (Baret et al. 2003). It currently grows in a wide range of habitats in the island from sea level to 1,700 m a.s.l (Macdonald et al. 1991; Baret et al. 2004). The vegetative multiplication by sprouts and rooting of stem tips occurs in all its distribution area (Amsellem et al. 2001; Baret et al. 2003), whereas flowering and seed production decrease with altitude up to 1,100 m a.s.l (Baret et al. 2004). Both the lack of its associated phytophagous insects on the island and its spread into intact tropical forest have led the authorities to support its control by a biological control agent.

A series of phytophagous insects and pathogens of *Rubus* species were collected between 1997 and 2001 in Southeast Asia, its native area. Among them, the sawfly *Cibdela janthina* Klug (Hymenoptera: Argidae) was selected as a potential biological agent

against *R. alceifolius* after host specificity tests and released a first time on the east coast of La Réunion in October 2007 (van Achterberg and Desmier de Chenon 2009; Le Bourgeois et al. 2013).

*Cibdela janthina* belongs to the Argidae, a family of sawflies, comprising almost 900 species worldwide. Although gregarious larvae of Argidae may be important defoliators, few species in this family have been considered for biological control of weeds (Regas-Williams and Habeck 1979; Badenes-Perez and Johnson 2007). To our knowledge *C. janthina* is the first insect used as biological control agent against an invasive plant in France.

*Cibdela janthina* is a multivoltine species and develops throughout the year without diapause. Eggs are laid in batches inside the main veins on the underside of terminal leaves. Larvae feed gregariously on the margins on the underside of leaves. When larval development is achieved, the larva drops on the soil and builds a cocoon in the litter, where the prepupa turns into a pupa. Females do not need to mate for oviposition to occur but unmated females produce only males (van Achterberg and Desmier de Chenon 2009; Le Bourgeois et al. 2013).

Since 2007, larvae of *C. janthina* have successfully defoliated large thickets of *R. alceifolius* in the lowlands, which lead the females to oviposit at higher altitudes where the plant still remains abundant. However, as eggs and larvae are restricted to the plant, they are more dependent on local environmental conditions than adults. Altitudinal limits of insects are mainly explained by thermal-related constraints on survival (Hodkinson 2005). Most studies of temperature-dependent process rates in insects concern insect pests of temperate regions (Hodkinson 2005) or mountainous tropical regions (Duyck et al. 2006; Crespo-Pérez et al. 2013), while few studies have dealt with phytophagous insects used in classical biological control of weeds (Byrne et al. 2002; Dhileepan et al. 2005).

The objectives of our study were to determine the influence of temperature on development duration and survival of *C. janthina* preimaginal stages in laboratory conditions in order to parameterize a survival model and, then, to compare the current effectiveness of the biological control against *R. alceifolius* along an altitudinal gradient with patterns expected from the predicted survival of the insect.

## Materials and methods

### Field observations of defoliation

The defoliation of *R. alceifolius* thickets was estimated along an altitudinal transect from sea level (21°06'S and 55°73'E) up to 1,570 m a.s.l. (21°17'S and 55°59'E) on the east coast of La Réunion at six different dates: in April and July 2010, January and August 2011 then January and July 2012. The area surveyed was about 70 km<sup>2</sup> and close to the initial release point of *C. janthina* in 2007 at the altitude of 220 m. The percentage of defoliation of *R. alceifolius* thickets was visually estimated by the same experimenter, using seven defoliation classes: 0, 1–10, 11–25, 26–50, 51–75, 76–99 and 100 %. All twigs were completely defoliated in the 100 % defoliation class. For analyses, the median of percentage ranges was used. The size of the thickets varied from single isolated plants to large monospecific thickets of *R. alceifolius* (>1,000 m<sup>2</sup>). The number of thickets recorded at each date, defined as different plots along the altitudinal transect, ranged from 260 to 408. To measure the evolution of the defoliation, both in time and in altitude, we made the assumption that the altitude at which the defoliation of thickets was estimated to be 50 % was a good gauge of the effectiveness of *C. janthina*.

### Experimental conditions in laboratory

All experiments were conducted with *C. janthina* adult females or eggs collected in *R. alceifolius* thickets in the south-eastern area of La Réunion (55°57'E and 21°33'S). Environmental chambers (Sanyo MLR-350; Sanyo, Osaka, Japan) were used for the experiments. They were programmed with a photoperiod of L:D 12:12 h under five constant temperatures (15, 20, 25, 30 and 35 ± 1 °C). The relative humidity was 80 ± 10 % (checked by a thermo-hygrometer HOBO; Onset, Pocasset, MA, USA). Water dishes were placed inside the chambers to maintain a high relative air humidity. Eggs or neonate larvae until the fourth instar were reared into 4 ml plastic boxes (20 × 20 × 10 mm), and fifth to seventh instars into 108 ml plastic boxes (90 × 60 × 20 mm). Humidity and sterility were maintained with a filter paper placed in rearing boxes and moistened every day with a 2 % solution of benzoic

acid (Nipagin-M), an antifungal solution often used in Hymenoptera or Lepidoptera rearings. Filter papers and rearing boxes were changed or cleaned in accordance with larval growth and feces production.

### Development of preimaginal stages

Adult females of *C. janthina* were collected in the field and released on a healthy undamaged potted *R. alceifolius* plants grown inside a cylindrical mesh-screened field cage (2.5 m in height by 3 m in diameter, Synthetic Industries, Gainesville, GA, USA). Leaves with eggs freshly inserted in nerves (<2 h) were isolated and batches of eggs were removed off the leaf but kept in the nerve and moved to 4 ml boxes. The egg survival and development duration were recorded using 50 eggs for each of the five temperatures tested. The number of hatched eggs was recorded twice a day.

To obtain a synchronized cohort of first instar larvae, eggs collected in the field were kept in a 4 l plastic container with moistened paper in an environmental chamber at 15 ± 1 °C during a few days then moved at 25 °C to initiate hatching. After egg hatching, eighty neonate larvae (<2 h old) were transferred into individual rearing boxes for each of the five temperatures tested. Larvae were fed with a piece of fresh healthy expanded leaf collected on potted *R. alceifolius* plant grown in outside insect-proof cages. Leaf pieces were renewed once a day at 15 and 20 °C and twice a day at 25, 30 and 35 °C. Survival and the presence of molts between each instar were recorded twice a day. We defined instar-type of larval development as the number of larval instars occurring before pupation.

A few hours before pupation, larvae stopped feeding and were removed into 108 ml boxes half-filled with loam and pozzolan gravels to allow them to build their cocoon. The pupae that survived from the larval experiment were used to record pupal development and survival. As the prepupa and the pupa are hidden and protected in an opaque cocoon buried in loam, the period for which individuals were in their cocoon was considered as one stage, called “pupal stage” in this study. Observations of pupal development were made daily until adult emergence. Upon emergence, sex of pupae was determined by examining the adult genitalia at the extremity of the abdomen.

## Analysis of preimaginal development rates

The relationship between temperature and development rates of eggs, larvae and pupae was described by the degree-day model and by the non-linear Lactin-2 model (Lactin et al. 1995). The degree-day model states that the relationship between development rate  $r(T)$  (1/development time in days) versus temperature can be described by a linear equation:  $r(T) = a + bT$ , where  $T$  is rearing temperature,  $a$  is the intercept and  $b$  is the slope of the linear function. The thermal constant  $K$  (i.e. the number of degree-days above the lower threshold required to complete development) was calculated from the linear equation. As the temperature of 25 °C was close to the optimum temperature for development and in a non-linear portion of the curve of development rates against temperature, the linear model was fitted on all development rates at 15 and 20 °C only to avoid unrealistic overestimates of the lower temperature threshold and the thermal constant.

In order to estimate the lower and upper temperature thresholds, and the optimum temperature for development of *C. janthina*, we used the Lactin-2 model (Lactin et al. 1995) defined as follow:  $r(T) = e^{\rho T} - e^{\rho T_L - (T_L - T)/\Delta T} + \lambda$ , where  $\rho$ ,  $T_L$ ,  $\Delta T$  and  $\lambda$  are parameters to be estimated.

## Analysis of preimaginal survival

The survival rates and their variances at each temperature for eggs, larval instars, larval and pupal stages were calculated using the Kaplan–Meier estimator. The stage-specific survival response,  $s(T)$ , to constant temperature  $T$  was modeled using a second-degree polynomial logistic function (Régnière et al. 2012; Crespo-Pérez et al. 2013). Following Régnière et al. (2012), we assume that survival probability is constant throughout a life stage and that cumulative daily survival during development at a given temperature is described by the following relationship:  $s(T, t) = \left(1 + e^{a+bT+cT^2}\right)^{-t}$ , where  $a$ ,  $b$ , and  $c$  are polynomial parameters to be estimated and  $t$  is the duration of exposure to temperature  $T$  during development. The data on daily cumulative survival of each life stage were fitted using non-linear least squares. Finally, the overall survival rate  $s(T)$  at a given temperature  $T$  is modeled by the equation:

$s(T) = \left(1 + e^{a+bT+cT^2}\right)^{-1/r(T)}$ , where  $a$ ,  $b$ , and  $c$  are parameters to be estimated and  $r(T)$  is the development rate.

## Relationship between survival and altitude

The mean monthly temperatures and the mean monthly maximum and minimum temperatures from 2002 to 2011 were collected from four weather stations at 40, 230, 1,025 and 1,550 m a.s.l along the surveyed altitudinal transect (Supplementary materials Appendix A). As minimum and maximum temperatures in tropical areas are punctual events, both in time and space, and variation of mean temperatures between winter and summer in La Réunion is relatively small ( $\sim 5$  °C), we therefore considered that mean temperature was sufficient to investigate altitude-related physiological limitations of *C. janthina* preimaginal stages. The relationship between mean yearly temperature and altitude was better described by a slightly decreasing exponential than by a linear function (Appendix A in supplementary material). The survival probability of eggs and larvae and of all preimaginal stages were thus predicted at different elevations by replacing the temperature by its function of altitude in  $s(T)$ . Predicted survival probabilities along the altitudinal transect were then compared to the observed pattern of defoliation, which is a good estimator of the presence of *C. janthina* larvae.

## Statistical methods

The effects of altitude, season and year of observation, and their interactions on percentage of defoliation of *R. alceifolius* along the altitudinal transect were determined using generalized linear models (GLM) with quasi-binomial error in order to account for overdispersion in the models. We used standard simplification procedures to eliminate non-significant terms from the model. The significance of each term was assessed through the change in deviance between models with and without that term. For each defoliation survey date, the altitude at which defoliation was 50 % was estimated using the calibrated GLM. Its range of variation was estimated using the Delta method.

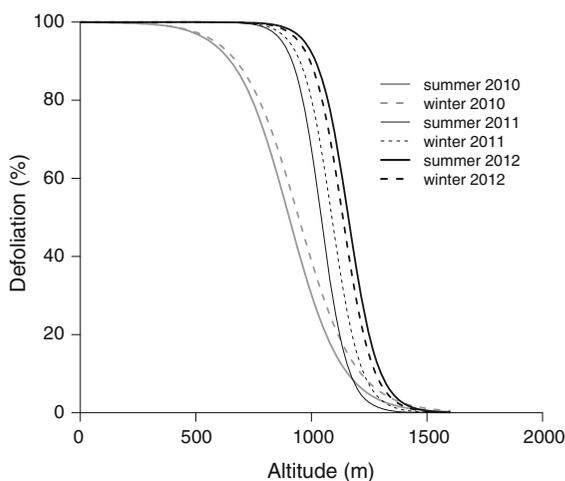
For larval instars and the pupal stage, differences in mean development durations among instar-types at

various constant temperatures were tested for significance by analysis of variance (ANOVA) followed by a Tukey–Kramer test. The Lactin-2 model and the Régnière model were fitted on observed development and survival rates against temperature, respectively. Parameters and their standards errors (SE) were provided by the ANOVA of the non-linear models. Standard errors of the lower ( $T_{min}$ ) and upper ( $T_{max}$ ) temperature thresholds, the optimal temperature for development ( $T_{opt}$ ), and the thermal constant ( $K$ ) were estimated using a bootstrap on residuals resampled 1,000 times. All statistical tests, models and charts were performed with R software (R Development Core Team 2012).

## Results

### Defoliation along an altitudinal transect

The percentage of defoliation was 100 % in lowland and gradually decreased with increasing altitude (GLM with altitude:  $F = 4094.7$ ,  $df = 1$ , 1948,  $P < 0.0001$ ) (Fig. 1). It slightly increased in altitude during successive years (year:  $F = 175.0$ ,  $df = 2$ , 1946,  $P < 0.0001$ ; interaction year  $\times$  altitude:  $F = 41.9$ ,  $df = 2$ , 1944,  $P < 0.0001$ ). In 2010 and 2011, the defoliation was higher in altitude during the winter season than during the summer season, but the inverse tendency was



**Fig. 1** Fitted curves of defoliation of *Rubus alceifolius* thickets in relation with altitude at two seasons during three years, using a quasi-binomial GLM

observed in 2012 (interaction year  $\times$  season:  $F = 8.1$ ,  $df = 1$ , 1941,  $P < 0.0001$ ) (Fig. 1). The highest site where defoliation was observed was at 1,557 m a.s.l during summer in 2012. The average altitudes ( $\pm$  SE) at which plant defoliation was estimated to be 50 % were  $902 \pm 13$  m a.s.l in summer 2010,  $944 \pm 33$  m a.s.l in winter 2010,  $1,041 \pm 5$  m a.s.l in summer 2011,  $1,088 \pm 6$  m a.s.l in winter 2011,  $1,159 \pm 3$  m a.s.l in summer 2012, and  $1,136 \pm 5$  m a.s.l in winter 2012.

### Preimaginal developmental time

The developmental times of *C. janthina* egg, larval and pupal stages according to temperature and instar-types are shown in Table 1. The number of molts showed that *C. janthina* males develop through five or six instars and females through six or seven instars. The number of instars was significantly higher for females than males (ANOVA,  $F = 68.0$ ,  $df = 1$ , 115,  $P < 0.0001$ ).

No embryonic development was observed at 30–35 °C. The developmental duration of eggs decreased significantly with increasing temperature from 15 to 25 °C (ANOVA,  $F = 990.3$ ,  $df = 1$ , 88,  $P < 0.0001$ ). The duration of the larval stage decreased with increasing temperature (ANOVA,  $F = 425.2$ ,  $df = 1$ , 104,  $P < 0.0001$ ) and was longer for females than for males (ANOVA,  $F = 20.9$ ,  $df = 1$ , 97,  $P < 0.0001$ ). The duration of pupal stage decreased with increasing temperature (ANOVA, temperature:  $F = 482.5$ ,  $df = 1$ , 96,  $P < 0.0001$ ) and was shorter for males than for females within a temperature (ANOVA, interaction temperature  $\times$  sex:  $F = 8.5$ ,  $df = 1$ , 96,  $P = 0.0044$ ). Time from egg hatching to adult emergence decreased with increasing temperature (ANOVA, temperature:  $F = 443.4$ ,  $df = 1$ , 96,  $P < 0.0001$ ) and was significantly shorter for males than for females (ANOVA, sex:  $F = 11.9$ ,  $df = 1$ , 96,  $P < 0.001$ ), also at given temperature (ANOVA, interaction temperature  $\times$  sex:  $F = 13.4$ ,  $df = 1$ , 96,  $P < 0.001$ ).

In Table 2, we present the parameterization of the linear and Lactin-2 models used to describe the effect of the temperature on the development of *C. janthina* preimaginal stages. The thermal constants  $K$  of egg stage, female and male larval stage and pupal stage were 163.2, 241.8, 188.7 and 217.5 DD, respectively. The lower temperature thresholds for development of the preimaginal stages ranged from 9.5 to 11.3 °C and from 10.4 to 11.5 °C according to the linear and the

**Table 1** Mean  $\pm$  SE developmental time (days) for egg, larval and pupal stages of *Cibdela janthina* according to instar-type at a range of four constant temperatures from 15 to 30 °C, 80  $\pm$  10 % RH and photoperiod of 12:12 L:D

Temp (°C)	Instar-type	<i>n</i> <sup>a</sup>	Egg	Larva	Pupa
15	VI	15	28.0 $\pm$ 0.1 a	52.8 $\pm$ 0.8 b	57.7 $\pm$ 1.9 a
	VII	3		55.2 $\pm$ 1.5 a	54.7 $\pm$ 3.7 a
20	V	14	15.8 $\pm$ 0.1 b	21.6 $\pm$ 0.3 d	26.7 $\pm$ 0.6 b
	VI	23		25.0 $\pm$ 0.3 c*	24.8 $\pm$ 0.4 b
25	V	12	10.4 $\pm$ 0.1 c	16.0 $\pm$ 0.2 f	16.5 $\pm$ 0.6 c
	VI	45		18.5 $\pm$ 0.1 e*	15.9 $\pm$ 0.2 c
	VII	3		20.6 $\pm$ 0.6 de	15.8 $\pm$ 0.2 c
30	VI	7	–	19.5 $\pm$ 0.2 de	–
	VII	9		21.6 $\pm$ 0.5 d	

<sup>a</sup> Number of specimens that reached the pupal stage

Developmental mean times of the same stage (column) followed by the same letter are not significantly different (ANOVA, Tukey–Kramer test,  $P < 0.05$ ). Asterisks denote significant differences in developmental times between sexes at the same temperature and instar-type ( $t$  test,  $P < 0.05$ )

**Table 2** Estimated values ( $\pm$  SE) of fitted coefficients and measured parameters determined by the degree-day and the Lactin-2 models used to describe the effect of temperature on the development of *Cibdela janthina* egg, larval and pupal stages

Model	Parameters	Egg	Larva	Pupa
Linear	<i>b</i>	0.0061 $\pm$ 0.0000	0.0046 $\pm$ 0.0000	0.0046 $\pm$ 0.0000
	<i>a</i>	–0.0580 $\pm$ 0.0002	–0.0503 $\pm$ 0.0005	–0.0521 $\pm$ 0.0003
	<i>T</i> <sub>min</sub>	9.5 $\pm$ 0.1	10.9 $\pm$ 0.0	11.3 $\pm$ 0.0
	<i>K</i>	163.2 $\pm$ 0.1	216.2 $\pm$ 9.7	217.5 $\pm$ 0.2
	R <sup>2</sup>	0.984	0.896	0.944
	RSS	6.6	7.6	12.8
Lactin-2	$\rho$	0.0062 $\pm$ 0.0000	0.0060 $\pm$ 0.0012	0.0042 $\pm$ 0.0000
	<i>T</i> <sub>L</sub>	33.3472 $\pm$ 0.0219	49.6400 $\pm$ 3.9043	31.9397 $\pm$ 0.4371
	$\Delta T$	1.5282 $\pm$ 0.0106	7.0901 $\pm$ 2.2062	0.7526 $\pm$ 0.1698
	$\lambda$	–1.0667 $\pm$ 0.0003	–1.0656 $\pm$ 0.0100	–1.0481 $\pm$ 0.0005
	<i>T</i> <sub>min</sub>	10.4 $\pm$ 0.0	11.5 $\pm$ 0.0	11.1 $\pm$ 0.1
	<i>T</i> <sub>opt</sub>	26.2 $\pm$ 0.0	26.3 $\pm$ 0.0	27.6 $\pm$ 0.0
	<i>T</i> <sub>max</sub>	30.0 $\pm$ 0.0	34.9 $\pm$ 0.0	30.0 $\pm$ 0.0
	R <sup>2</sup>	0.995	0.903	0.974
	RSS	10.0	20.8	12.8

SE of coefficients and parameters were calculated by model and obtained by 1,000 resampled bootstraps, respectively

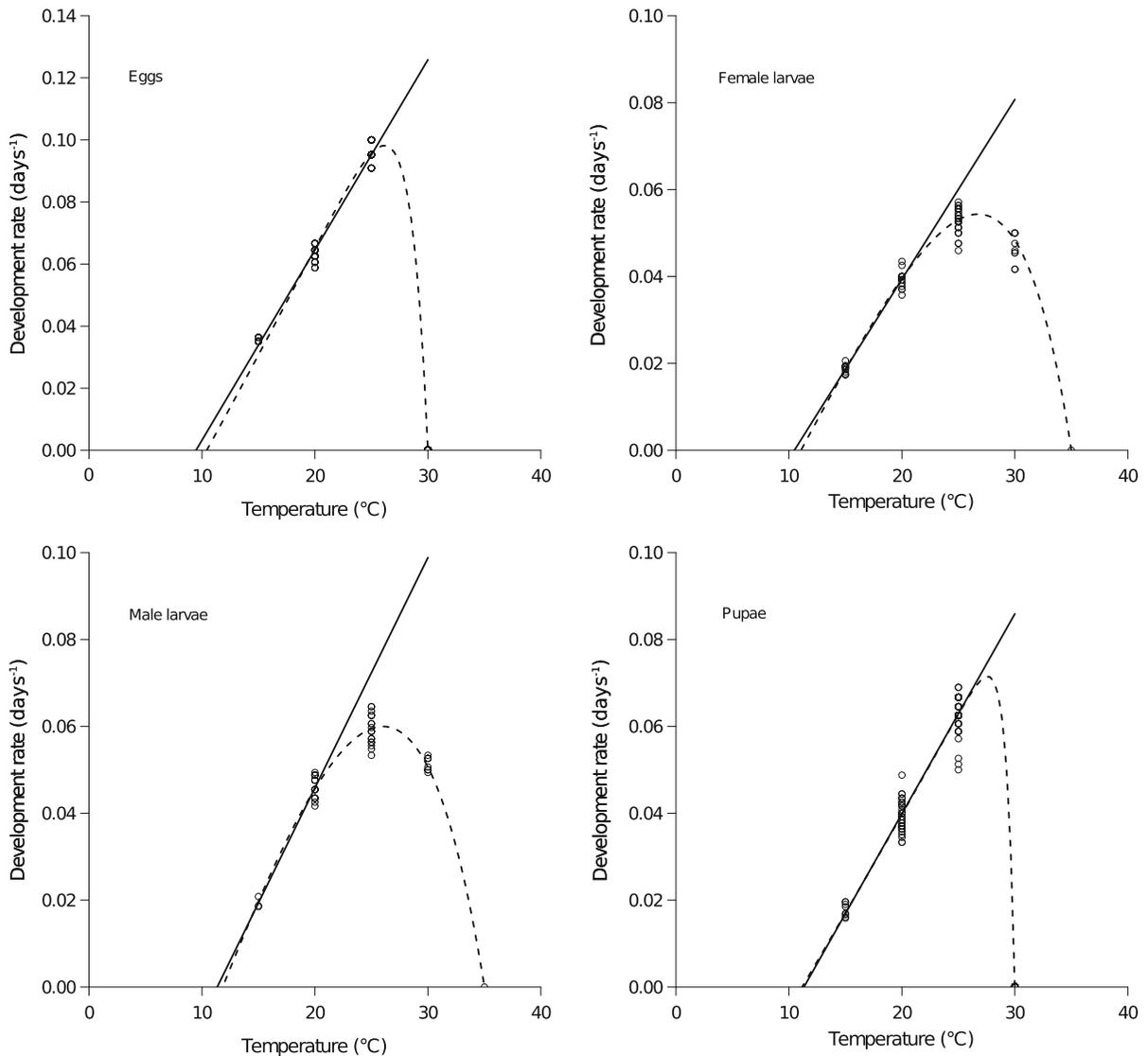
Residual sum of squares (RSS):  $\times 10^{-4}$

The coefficients of determination of linear models are adjusted

Lactin-2 models, respectively. The estimated optimal temperatures for development of the preimaginal stages ranged between 26.0 and 27.6 °C. Because there was no successful completion of eggs and pupae development over 30 °C, the development rates at these temperatures were assumed to equal zero (Fig. 2).

### Preimaginal survivorship

Survival of egg, larval and pupal stages were significantly affected by temperature (egg:  $\chi^2 = 131.8$ ,  $df = 4$ ,  $P < 0.001$ ; larva:  $\chi^2 = 124.4$ ,  $df = 4$ ,  $P < 0.001$ ; pupa:  $\chi^2 = 65.0$ ,  $df = 3$ ,  $P < 0.001$ ) (Table 3). All eggs died before hatching and no



**Fig. 2** Linear (solid line) and Lactin-2 (dashed line) fitted models of development rate (1/development duration) for *Cibdela janthina* eggs, female larvae, male larvae and pupae

evidence of development was observed at 30 and 35 °C. Mortality of the 1<sup>st</sup> larval instar reached 100 % at 35 °C. At this temperature, larvae did not even eat. Survival rates of the larval stage were not different at 15 and 30 °C ( $P = 0.86$ ), being 22.5 and 20.0 %, respectively, whereas survival rates of pupal stage at these temperatures were significantly different with 44.4 and 0.0 % ( $P < 0.0001$ ). At 20 and 25 °C, most pupae developed to the adult stage and the survival rate was above 90 %, whereas no pupae reached the adult stage at 30 °C. For all stages, higher survival rates were observed close to the optimum temperature

for development estimated by the Lactin-2 model. The temperatures at which survival rate was maximal varied between 20 and 25 °C for eggs, larvae and pupae.

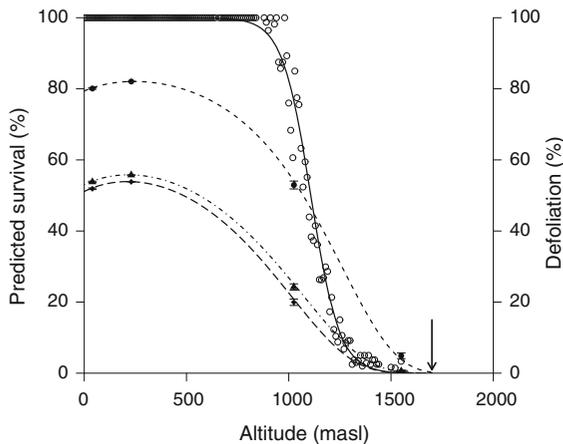
#### Relationship between survival and altitude

The predicted survival rates to larval and pupal stages and to adult emergence slightly increased from sea level to 250 m a.s.l, then decreased with altitude (Fig. 3). The Régnière model predicts survival at altitudes higher than the maximum observed altitude

**Table 3** Mean  $\pm$  SE survival rates of *Cibdela janthina* egg, larval and pupal stages at five constant temperatures

Stage	Temperature ( $^{\circ}$ C)				
	15	20	25	30	35
Egg	0.260 $\pm$ 0.062	0.800 $\pm$ 0.057	0.740 $\pm$ 0.062	0.000 $\pm$ 0.000	0.000 $\pm$ 0.000
Larva	0.225 $\pm$ 0.047	0.594 $\pm$ 0.059	0.750 $\pm$ 0.048	0.200 $\pm$ 0.045	0.000 $\pm$ 0.000
Pupa	0.444 $\pm$ 0.117	0.902 $\pm$ 0.046	0.917 $\pm$ 0.036	0.000 $\pm$ 0.000	– <sup>a</sup>

<sup>a</sup> The survival rate of the pupal stage at 35  $^{\circ}$ C is not available, as all larvae died at this temperature



**Fig. 3** Predicted survival rates in percentages of eggs (closed circles), eggs and larvae (closed triangles), and the three preimaginal stages of *C. janthina* (closed diamonds) by the Régnière's model near four weather stations located along an altitudinal gradient in La Réunion. Dashed lines represent the predicted survival rates using the relationship between mean yearly temperature and altitude (Appendix A in supplementary material). The solid line represents the fitted curve of the observed defoliation of *Rubus alceifolius* thickets in relation with altitude in 2011 and 2012 (open circles). Arrow represents the maximum observed altitudinal limit of *R. alceifolius* in La Réunion

for defoliation (i.e. 1,557 m a.s.l). Parameter values for Régnière's survival model can be found in Supplementary material (Appendix B in supplementary material).

## Discussion

The success of a biological control agent against a weed is commonly regarded as its capacity to reduce economic, social, or environmental impacts of the target organism to a level below which its threat is contained. However, there is little assessment of the

success rate of the agent beyond the establishment phase (McFadyen 1998). Monitoring of the effectiveness of agents in reducing weed populations is often neglected in weed biocontrol programmes (Fowler et al. 2000). Models are often used to predict the impact of biological control agents on key growth parameters of the target weed in the pre-release phase and to make relevant choices in the selection of potential biological control agents (Raghu et al. 2006; Buckley et al. 2003). But this tool has been rarely used in post-release evaluation of the impact on weed population dynamics (Buckley et al. 2005).

In the case of the biological control of *R. alceifolius* by *C. janthina* in La Réunion, no data on the potential impact of the agent on the weed were available before the release. Since the release in 2007, the biannually monitoring of the defoliation allowed us to follow the spread of *C. janthina* and its impact related to altitude. As *R. alceifolius* grows up to 1,700 m a.s.l and only produces seeds below 1,100 m a.s.l (Baret et al. 2003, 2004), our study focused on the prediction of the overlap of altitudinal ranges of the two species. With laboratory experiments on the effect of temperature on survival of the agent, our study allowed comparison between the observed distribution of *C. janthina*, indirectly measured by the observed defoliation of *R. alceifolius* thickets, and its predicted altitudinal limit.

The three-year seasonal monitoring of the defoliation of *R. alceifolius* thickets by *C. janthina* larvae along an altitudinal gradient illustrated the expansion of the insect population and its impact on the weed in altitude until 2012. In 2012, a seasonal equilibrium between the insect and weed populations seems to have been reached. Further surveys to be conducted in summer and winter 2013 will enable us to confirm this altitudinal equilibrium.

Altitudinal limits of insects are mainly explained by temperature-related survival (Hodkinson 2005). As phytophagous biocontrol agents are released from

their natural enemies in the introduced range, they are mostly affected by abiotic factors and particularly by temperature. We thus investigated the influence of constant temperature on both development duration and survival of preimaginal stages of *C. janthina*.

Temperature strongly affects both developmental time and survival of all preimaginal stages of *C. janthina*. It also affects the development duration of larval instars and their number differently according to sex, with a tendency towards an additional instar at extreme temperatures. Adding an instar at these temperatures increases the time spent in the larval stage and thus could contribute to increase the probability for the larva to die during this stage, for instance through a longer exposure to predation and to deleterious temperature (Régnière et al. 2012).

The main objective of our study was to predict the altitudinal range and limits of the biological control agent. We were thus particularly interested in accurately estimating the lower development threshold, as the altitudinal limit should be found in places where temperatures likely approach lethal extremes. Both the linear degree-day and the non-linear Lactin-2 models produced similar estimates of the lower development threshold.

The *C. janthina* populations were still spreading in 2013 and their altitudinal distribution has probably not yet been stabilized all around the island. van Achterberg and Desmier de Chenon (2009) only found *C. janthina* up to 1,100 m a.s.l and *R. alceifolius* up to 1,200 m a.s.l in Sumatra, where it was replaced by other *Rubus* species above. Climate data from the upper zone of distribution of the insect collected during the years 2001–2003 in Sumatra (Parapat geophysical station at 1,061 m a.s.l, mean yearly temperature of 22.8 °C, mean minimum temperature of 18.5 °C and mean maximum temperature of 27.0 °C) showed warmer temperatures than those recorded in La Réunion (Appendix A in supplementary material). Although temperatures are similar or higher in Sumatra, the expected distribution range of *C. janthina* was higher in altitude in La Réunion than in its native range. This difference in altitudinal limits of *C. janthina* between the native (1,100 m a.s.l) and introduced ranges (1,550 m a.s.l) is likely to be explained by the limitation of available food in altitude.

Our study suggests that similar climatic conditions between the native range and the area of introduction do not necessarily involve similar altitudinal

distribution ranges for the biological control agent. Both the great phenotypic plasticity of *R. alceifolius* in La Réunion (Amsellem et al. 2001; Baret et al. 2004) and the absence of natural enemies of *C. janthina* could explain these higher altitudinal limits of the two species in La Réunion.

In 2012, the observed defoliation remained constant in altitude between winter and summer, which may indicate that the altitudinal equilibrium between the biological control agent and its host plant has been reached. However, our model predicted survival at altitudes higher than the maximum altitudes where defoliation occurred. Our study also shows that defoliation monitoring combined with laboratory experiments on the influence of temperature could be a good and quicker gauge of survival probability than field monitoring of survival or abundance of insects which requires substantial investments both in time and human resources. Despite *C. janthina* being able to completely defoliate and kill *R. alceifolius* in the altitudinal range where the weed produces flowers and seeds (i.e. below 1,100 m a.s.l), the bramble still remains a threat on native forests up to 1,700 m a.s.l (Baret et al. 2006).

In a context of global climate change, data on thermal requirements both for development and survival of biological control agents, but weeds also, could be useful to predict changes in distribution range (Bale et al. 2002). A small rise of mean temperatures could indeed cause different situations with negative or positive effects on the fitness of the two species, modifying the biological control agent's effectiveness in controlling the weed and the equilibrium of the system. Furthermore, similar studies on the thermal requirements of biological control agent against weeds could be favoured in a post-release phase both to evaluate the current effectiveness of the control and to improve the response of the system in a context of global warming.

Adding data on the effect of temperature on larval consumption, adult fecundity, plant growth rate and data on migration patterns could help to build a predictive model of the dynamics of the biocontrol system in the altitudinal range of *R. alceifolius* in La Réunion. These models should also provide a useful tool for practitioners in their future decisions on *R. alceifolius* management.

**Acknowledgments** The authors wish to thank S. Glénac for maintaining *Rubus alceifolius* cultures. We are grateful to John

Scott and two anonymous reviewers for helpful comments that improved the manuscript. This work was funded by CIRAD and the Direction de l'Environnement, de l'Aménagement et du Logement de La Réunion

## References

- Amsellem L, Noyer JL, Hossaert-McKey M (2001) Evidences for a switch in the reproductive biology of *Rubus alceifolius* (Rosaceae) towards apomixis, between its native range and its area of introduction. *Am J Bot* 88:2243–2251
- Badenes-Perez FR, Johnson MT (2007) Ecology, host specificity and impact of *Atomacera petroa* Smith (Hymenoptera: Argidae) on *Miconia calvescens* DC (Melastomataceae). *Biol Control* 43:95–101
- Bale JS, Masters GJ, Hodkinson ID, Awmack C, Bezemer TM, Brown VK, Butterfield J, Buse A, Coulson JC, Farrar J, Good JEG, Harrington R, Hartley S, Jones TH, Lindroth RL, Press MC, Symrnioudis I, Watt AD, Whittaker JB (2002) Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biol* 8:1–16
- Baret S, Nicolini E, Le Bourgeois T, Strasberg D (2003) Developmental patterns of the invasive bramble (*Rubus alceifolius* Poiret, Rosaceae) in Réunion Island: an architectural and morphometric analysis. *Ann Bot* 91:39–48
- Baret S, Maurice S, Le Bourgeois T, Strasberg D (2004) Altitudinal variation in fertility and vegetative growth in the invasive plant *Rubus alceifolius* Poiret (Rosaceae), on Réunion island. *Plant Ecol* 172:265–273
- Baret S, Rouget M, Richardson DM, Lavergne C, Egoh B, Dupont J, Strasberg D (2006) Current distribution and potential extent of the most invasive alien plant species on La Réunion (Indian Ocean, Mascarene islands). *Austral Ecol* 31:747–758
- Buckley YM, Briese DT, Rees M (2003) Demography and management of the invasive plant species *Hypericum perforatum*. I. Using multi-level mixed-effects models for characterizing growth, survival and fecundity in a long-term data set. *J Appl Ecol* 40:494–507
- Buckley YM, Rees M, Sheppard AW, Smyth MJ (2005) Stable coexistence of an invasive plant and biocontrol agent: a parameterized coupled plant-herbivore model. *J Appl Ecol* 42:70–79
- Byrne MJ, Currin S, Hill MP (2002) The influence of climate on the establishment and success of the biocontrol agent *Gratiana spadicea*, released on *Solanum sisymbriifolium* in South Africa. *Biol Control* 24:128–134
- Crespo-Pérez V, Dangles O, Régnière J, Chuine I (2013) Modeling temperature-dependent survival with small datasets: insights from tropical mountain agricultural pests. *Bull Entomol Res* 103:336–343
- Development Core Team R (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
- Dhileepan K, Treviño M, Raghu S (2005) Effect of temperature on the survival of *Aconophora compressa* Walker (Hemiptera: Membracidae): implications for weed biocontrol. *Aust J Entomol* 44:457–462
- Duyck PF, David P, Quilici S (2006) Climatic niche partitioning following successive invasions by fruit flies in La Réunion. *J Anim Ecol* 75:518–526
- Fowler SV, Syrett P, Hill RL (2000) Success and safety in the biological control of environmental weeds in New Zealand. *Austral Ecol* 25:553–562
- Hodkinson ID (2005) Terrestrial insects along elevation gradients: species and community responses to altitude. *Biol Rev* 80:489–513
- Lactin DJ, Holliday NJ, Johnson DL, Craigen R (1995) Improved rate model of temperature-dependent development by arthropods. *Environ Entomol* 24:68–75
- Le Bourgeois T, Baret S, Desmier de Chenon R (2013) Biological control of *Rubus alceifolius* (Rosaceae) in La Réunion Island (Indian Ocean): from investigations on the plant to the release of the biological control agent *Cibdela janthina* (Argidae). Proceedings of the XIII international symposium on biological control of weeds, 11–16 September 2011. Waikoloa, Hilo, USA, pp 153–160
- Macdonald IAW, Thébaud C, Strahm WA, Strasberg D (1991) Effects of alien plant invasions on native vegetation remnants on La Réunion (Mascarene Islands, Indian Ocean). *Environ Conserv* 18:51–61
- McFadyen REC (1998) Biological control of weeds. *Annu Rev Entomol* 43:369–393
- Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature* 403:853–858
- Raghu S, Wilson JR, Dhileepan K (2006) Refining the process of agent selection through understanding plant demography and plant response to herbivory. *Aust J Entomol* 45:308–316
- Regas-Williams KA, Habeck DH (1979) Life history of a poison-ivy sawfly *Arge humeralis* (Beauvois) (Hymenoptera: Argidae). *Fla Entomol* 62:356–363
- Régnière J, Powell J, Bentz B, Nealis V (2012) Effects of temperature on development, survival and reproduction of insects: Experimental design, data analysis and modeling. *J Insect Physiol* 58:634–647
- Strasberg D, Rouget M, Richardson DM, Baret S, Dupont J, Cowling RM (2005) An assessment of habitat diversity and transformation on La Réunion Island (Mascarene Islands, Indian Ocean) as a basis for identifying broad-scale conservation priorities. *Biodivers Conserv* 14:3015–3032
- van Achterberg C, Desmier de Chenon R (2009) The first report of the biology of *Proterops borneoensis* Szépligeti (Hymenoptera: Braconidae: Ichneutinae), with the description of a new species from China. *J Nat Hist* 43:619–633
- Vitousek PM, d'Antonio CM, Loope LL, Westbrooks R (1996) Biological invasions as global environmental change. *Am Sci* 84:468–478

**Alexandre Mathieu** is an agronomist. His PhD project focuses on modeling the population dynamics of *Cibdela janthina* and *Rubus alceifolius* in La Réunion.

**Dr. Yves Dumont** is an applied mathematician. He develops and studies deterministic models in epidemiology, ecology and plant sciences.

**Dr. Frédéric Chiroleu** is a biostatistician. He is mainly involved in the analyses of plant protection data.

**Dr. Pierre-François Duyck** studies insect bio-ecology, with particular interest in the study of relationships between invasive and indigenous species.

**Dr. Olivier Flores** is an ecologist working on diversity patterns, species distributions and population dynamics.

**Gérard Lebreton** is an engineer involved in studies on the conservation of native ecosystems in La Réunion.

**Dr. Bernard Reynaud** is an entomologist. He is director of UMR PVBMT and head of Plant Protection Center (3P).

**Dr. Serge Quilici** is an entomologist working on behavior, ecology and biocontrol of various fruit crop pests, particularly fruit flies.