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A new population of the biocontrol agent *Aphalara itadori* performs best on the hybrid host *Reynoutria* x *bohemica*

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HIGHLIGHTS

- In their exotic range *R. japonica* and *R. sachalinensis* hybridize into *R.* × *bohemica*.
- The Murakami population of *Aphalara itadori* performs best on *R.* × *bohemica*.
- This psyllid population causes most damage to *R.* × *bohemica* and *R. sachalinensis*.
- We advise releasing this biocontrol agent population against *R.* × *bohemica*.

GRAPHICAL ABSTRACT
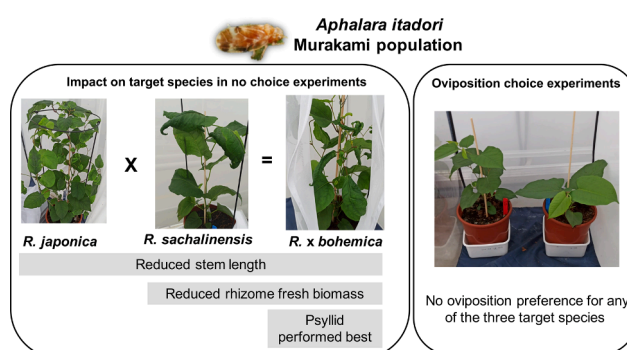
ARTICLE INFO

ABSTRACT

Aphalara itadori is a biological control agent of the invasive Asian knotweeds *Reynoutria japonica, Reynoutria sachalinensis* and their hybrid *Reynoutria* × *bohemica,* which emerged across different areas of their introduced range, including Europe, North America and Oceania. The performance of *A. itadori* on these three target plants differs between geographically distant psyllid populations. A petition to release a population of this psyllid freshly collected in Murakami (Japan) to control the three target species in the Netherlands was approved in 2020. In order to optimize a biocontrol program using this *A. itadori* population, we assessed the effect of the three knotweed species on its performance, impact on plant growth and oviposition preference. The results of no-choice experiments indicated that the Murakami population performed best on *R.* × *bohemica*, where juveniles developed the fastest and the number of emerged adults was twice as high as that recorded on the other two *Reynoutria* hosts. These differences in performance between hosts were associated with a lower acceptance of *R. sachalinensis* for oviposition and a higher juvenile mortality on *R. japonica*. Infestation with the Murakami population had an overall negative impact on final stem length, so that infested plants were around 8 % shorter than control plants, and it reduced final rhizome fresh biomass by circa 50 % in *R. sachalinensis* and 35 % in *R.* × *bohemica*. When subjected to two-choice tests, females of the Murakami population did not show an oviposition preference for any of the *Reynoutria* species. These results suggest that in the Netherlands *R.* × *bohemica* is the

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best host to optimize the rearing of the Murakami population, and field releases should target this host species to promote establishment in the field. Based on these results, the Murakami population is expected to have the largest impact in the field on *R.* × *bohemica* and *R. sachalinensis*.

1. Introduction

Three invasive knotweed species are listed among the world's worst invaders (IUCN, 2021). *Reynoutria japonica* (Houtt.; syn. *Fallopia japonica* [Houtt.] Ronse Decr.; Japanese knotweed) and *Reynoutria sachalinensis* (F. Schmidt Nakai; syn. *Fallopia sachalinensis* [F. Schmidt] Ronse Decr.; Giant knotweed) are both native to eastern Asia, whereas *Reynoutria* × *bohemica* (Chrtek & Chrtková; syn. *Fallopia* × *bohemica* [Chrtek & Chrtková] J.P. Bailey; Bohemian knotweed) results from their hybridization in the introduced range, so that it is present in Europe, North America and Oceania (Bailey and Wisskirchen, 2006; Schuster et al., 2011). The psyllid *Aphalara itadori* Shinji has been identified as the best candidate for classical biological control of invasive knotweeds to date (Shaw et al., 2009). Extensive testing showed that the three *Reynoutria* species are the only suitable hosts supporting development and population growth outside its native range (Shaw et al, 2009; Jones et al., 2013; Cortat et al., 2015). *Aphalara itadori* displays interpopulation variation in its performance on the three *Reynoutria* hosts. Specifically, a population of this psyllid collected in Hokkaido, in northern Japan, performed best on *R. sachalinensis*, the predominant knotweed species in that area (Grevstad et al., 2013), whereas a population from Kyushu, in southern Japan, did best on *R. japonica,* the only *Reynoutria* species of the three target species that occurs in that area (Shaw et al., 2009). Identification of the host on which a particular *A. itadori* population to be released performs best will allow to optimize the rearing of the colony, and it will increase the likelihood of field establishment, by targeting this host species in the release areas.

Furthermore, the performance of biocontrol agents may also differ among populations of the target plant species (Underwood and Rausher, 2000; Hodkinson, 2009; Sun et al., 2020), making it necessary to test the performance of the candidate biocontrol agent population on weed populations from the target area. For example, when tested on plants of the three *Reynoutria* species, the same *A. itadori* population from Kyushu was reported to perform best on *R. japonica* plants from the UK (Shaw et al., 2009), whereas in the Netherlands it did best on *R.* × *bohemica* (Jones et al., 2013).

To date, two different populations of *A. itadori* have been released for knotweed biocontrol in the United Kingdom, Canada and the United States (Shaw et al., 2011; Jones et al., 2020). These populations were collected from regions of Japan selected due to a dominant presence there of the knotweed species targeted in the area of biocontrol. Hence, a population collected in Kyushu has been released in regions where *R. japonica* is the main control target, whereas a population from Hokkaido has been used where *R. sachalinensis* is the most problematic knotweed species (Grevstad et al., 2018; Jones et al., 2020; Supplementary Fig. 1). Unfortunately, despite several years and rounds of releases, both populations have failed to establish in the field in any of the areas of release. Several hypotheses have been put forward to explain this, including a very lengthy rearing of the populations under controlled conditions prior to release that may have resulted in adaptation to rearing conditions (approx. 66 generations in six years in the UK; Fung et al., 2020) and a suboptimal climatic match between the native and introduced ranges (Constantine et al., 2015; C. Pratt, personal communication). To avoid these obstacles for successful establishment, a new population of *A. itadori* was collected in 2019 by CABI UK for the intended release in the UK and the Netherlands. With the permit for release approved in 2020 (Wnb.BB.2019.005), the Netherlands became the first country in continental Europe where release of *A. itadori* has been approved. One of the key elements for successful establishment of a biocontrol agent is a suitable climate in the target area, with similar parameters to those of the site of collection (Robertson et al., 2008). The new population was collected in Murakami, Niigata Prefecture (central Japan), a region selected for its high similarity in climate with Northwestern Europe, according to the results of a CLIMEX model (Constantine et al., 2015). Both *R. japonica* and *R. sachalinensis* occur in the Murakami area, although the abundance of both species has been reported to be lower in this area compared to other regions in their distribution range (GBIF, 2021a; 2021b; Supplementary Fig. 1), and a range of other *Reynoutria* species can also be found in this region.

Given the inter-population variability in performance on different target species displayed by *A. itadori*, determination of the performance of the Murakami population of *A. itadori* on plants of the three target species collected in the Netherlands, where they occur abundantly, is key to optimize the Dutch biocontrol program of invasive *Reynoutria* species. Assessing the impact of the Murakami population on plant growth on the three knotweeds will help predict the expected damage this *A. itadori* population could exert on these species. Additionally, establishing whether females display an oviposition preference may indicate which hosts *A. itadori* could spread to in the field, as juveniles are mostly sessile and female oviposition choice is the main determinant of population dispersal in this psyllid (Shaw et al., 2009). The aim of the present study was to assess 1) the performance of the Murakami population of *A. itadori* on the three invasive *Reynoutria* species, 2) the impact of feeding by the Murakami population on above and belowground growth of these *Reynoutria* spp., and 3) whether the Murakami population displayed an oviposition preference for any of the *Reynoutria* species.

2. Methods

2.1. Facilities and experimental conditions

All the experimental work took place in the greenhouse at Koppert Biological Systems (Berkel en Rodenrijs, Netherlands) from December 2020 to May 2021, with an ambient temperature of 21 ± 1 °C, a relative humidity of 51 ± 1 %, and a photoperiod of 14:10 LD (light:darkness).

2.2. Plant and insect material

Rhizomes of *R. japonica* (hereafter 'Rj'), *R.* × *bohemica* ('Rb') and *R. sachalinensis* ('Rs') were collected in 54 stands across the Netherlands between September and November 2020 (Supplementary Fig. 2). For each collection site, 10–30 rhizome fragments were collected in an area of 5–10 m^2 within the same *Reynoutria* stand. All rhizomes from each site were expected to be genetically uniform, given that in the three plant species reproduction is almost exclusively clonal via the rhizomes in Europe (Bailey et al., 2007).

Rhizomes were cleaned to remove soil and macrofauna, and stored at 4 °C until needed. To standardize plant material used in the experiments, rhizome fragments that were 2 to 8 cm long and contained a single bud were selected. These rhizome fragments were hydrated by placing them individually in containers with water for 24 h, weighed and transferred to 5 L pots containing 50 % cocopeat, 30 % Tuinturf and 20 % fine peat substrate. Plants sprouting from these rhizomes were used in the experiments four to five weeks after transfer. For further standardization, for each experiment we selected plants with a similar total leaf area based on a visual assessment, as this parameter may have an impact on psyllid oviposition choice, fecundity and performance.

The life cycle of *A. itadori* comprises five nymph stages prior to adult

emergence, and females, which can live over 50 days at 20–25 °C, can lay up to 700 eggs over their lifespan (Myint et al., 2012). Both adults and nymphs feed on the phloem of their host plants, and nymphs are regarded as the most damaging stage for the plants (Shaw et al., 2009). The *A. itadori* population used in these experiments (hereafter referred to as the 'Murakami population') was started from around 500 individuals collected in Murakami (coordinates of collection spot: latitude 38.44, longitude 139.51; Niigata Prefecture, Japan; Supplementary Fig. 1) in June 2019. This population was imported and reared under quarantine conditions on *R. japonica* for three generations and then on *R. × bohemica* for two generations at CABI Egham, UK (permit for importation and maintenance in quarantine conditions No. 51073/212997/5). In May and August 2020 the culture was moved to Koppert Biological Systems (Berkel en Rodenrijs, Netherlands) where it was reared on *R. × bohemica* for approximately seven generations until the start of the experiments. In the Netherlands, rearing took place first in a containment cell and then in the greenhouse.

To avoid potential variation due to adult age in the experiments, cohorts of *A. itadori* adults developing from eggs laid over a week were used in each experiment. Upon emergence, adults were transferred to a new cage and used 7–14 days later, to ensure they were past the pre-oviposition period (4.5 days at 20–25 °C, Myint et al., 2012).

2.3. Development study

Nymph development of the Murakami population was studied on 12 to 15 plants per species. Each plant was confined in a fine mesh sleeve (length 65 cm, diameter 26 cm, mesh size 0.1 mm; JoTech, United Kingdom) tightly wrapping the top of the pot and exposed to a single pair of adults for 24 h, thus limiting variation in egg age to a maximum of 24 h. When the 24 h period finished, the adults were removed from the cage and the number of eggs was recorded, so that only plants where at least one egg was recorded were considered as accepted for oviposition. If no eggs were recorded in a plant after a thorough examination, the plant was replaced by a new plant or re-used, and in both cases new pair of adults was introduced. All plants were infested over a period of six days. Nymph development was monitored and the number of adults emerging was recorded once a day until all individuals had either emerged as adults or died. The duration of the immature stage was calculated for each individual.

A degree-day (DD) model that estimates adult emergence of *A. itadori* by degree days accumulated was built for each of the *Reynoutria* species. For this purpose, temperature and relative humidity were recorded for the duration of the experiment inside 7 to 9 of the replicate cages of each *Reynoutria* species using dataloggers (LogTag Recorders ltd, New Zealand). The temperature in those replicates in which environmental conditions were not recorded was estimated as the average temperature in plants of the same species bearing a datalogger. The temperature recorded or estimated in those plants where adults emerged (15x*Rb*, 13x*Rj* and 6x*Rs*), along with the number of days it took each individual to complete the immature stage, was used to build the DD models. We used 10 °C as the lower developmental threshold temperature, as previously reported for *A. itadori* (Constantine et al., 2015).

2.4. Fecundity, juvenile survival and impact on plant growth

Fecundity and juvenile survival of the Murakami population on the three host plants as well as impact on plant growth were assessed in a single experiment using pairs of infested and uninfested plants of the three *Reynoutria* species.

For each collection site two plants were used. Each plant was confined in a fine mesh sleeve (see 2.3), and the pair of plants from the same site were placed side by side, to avoid effects of plant position due to environmental factors. One of the plants was then randomly assigned to the infestation treatment and infested with *A. itadori* (hereafter referred to as 'infested plant') whereas the other one remained free from

insects (hereafter referred to as 'control plant'). In five cases where only one plant from a collection site regenerated (3 *Rj* and 2 *Rs*), those plants were used for the infestation treatment.

Plants of the infestation treatment were exposed to five pairs of adults over seven days. Upon adult removal, the total number of eggs laid on the plants was recorded and used to calculate the average fecundity per female and day. The final number of adults emerging from these eggs was assessed 49 days after the last day of oviposition, but due to time constraints this assessment was done in a subset of the plants. By the moment the experiment ended over 99 % of adults were expected to have emerged in all three *Reynoutria* species, according to the DD models. Therefore, any differences between species in the final number of adults would not be due to differences in development rate between hosts.

The impact of the Murakami population on plant growth was assessed in parallel to psyllid performance, in this case considering both the infested and the control plants in the plant subset in which the final number of adults was recorded. Impact of insect feeding was measured by comparing final stem length, final rhizome fresh biomass and final aboveground dry biomass between infested and control plants of each collection point. Infested and control plants of each *Reynoutria* species did not differ either in their initial stem length or their initial rhizome fresh biomass (Supplementary Fig. 3). Final aboveground dry biomass was determined at the end of the experiment after a drying period of 7–10 days at 60 °C. The length of the main stem was recorded in all plants at the start and end of the experiment. Likewise, rhizome fresh biomass was recorded upon rhizome transfer to pots and at the end of the experiment.

This experiment was blocked in five blocks, each of them comprising 1–5 replicates per *Reynoutria* species and set up in independent walk-in cages over a five-day period. Fecundity was studied in all five blocks (20 *Rb* plants, 18 *Rj* plants and 16 *Rs* plants), but, due to time constraints, only four blocks were considered to study final number of adults (15 *Rb*, 15 *Rj* and 11 *Rs* plants) and impact on plant growth (12 pairs of *Rj* plants, 15 pairs of *Rb* plants and 9 pairs of *Rs* plants). Temperature and relative humidity in each block were measured with dataloggers placed in each walk-in cage.

2.5. Oviposition preference

Potential oviposition preference for a particular *Reynoutria* species was assessed by means of a two-choice experiment in which the three two-species combinations were evaluated (*Rj*–*Rb*: JB; *Rj*–*Rs*: JS; *Rs*–*Rb*: SB). In each replicate one pair of adults was introduced in a mesh cage (60 × 60 × 90 cm) containing two plants of different *Reynoutria* species and the number of eggs laid on each species was recorded seven days later. The position of the plant species in the cage was randomly allocated in the different replicates, to avoid any potential effects of plant location. The two plants inside each cage did not differ in total leaf area, which was estimated by summing up the estimated area of every leaf in that plant (Supplementary Methods 1).

Plant pairs from which no eggs were recorded were replaced by a new pair of plants and adults, or reused and exposed to a new pair of adults. There was a total of 14 JB, 15 JS and 13 SB replicates in which eggs were laid.

2.6. Data analysis

In the development study, we tested whether *Reynoutria* species had a significant effect on plant acceptance for oviposition, fecundity, plant support of egg development to adulthood, juvenile survival, number of adults recorded per plant and number of degree-days to complete the immature stage. For the parameters fecundity, juvenile survival and number of adults recorded per plant, we considered two of the following different scenarios: 1) all the plant replicates set up, 2) only those replicates accepted for oviposition, or 3) only those replicates accepted for

oviposition where adults emerged. This approach allowed us to explore the reasons behind any potential difference in psyllid performance among plant species (i.e. differences in the final number of adults of the Murakami population among *Reynoutria* species could be due to differences in their odds to be accepted for oviposition, in the number of eggs it received once accepted or in its ability to support development to adulthood). We additionally assessed whether plant species significantly affected the duration of the immature stage, both in number of days and on degree-days accumulated at adult emergence, in both cases including plant replicate as a random effect to account for the effect of the particular plant that several individuals were developing on. To evaluate density-dependent effects, we assessed if the initial number of eggs laid on a plant had a significant effect both on the duration of the immature stage and on juvenile survival. Given that more than one plant per collection site were used in this experiment, site of collection was included in the models as a random factor. Additionally, we evaluated if the degree-days accumulated daily inside the plant cages differed among *Reynoutria* species, to account for a potential confounding effect of temperature on performance of the Murakami population (Table 1).

In the experiment that studied female fecundity, juvenile survival and impact on plant growth, we evaluated the effect of plant species on fecundity, juvenile survival and the number of adults recorded in a plant. We further explored if fecundity had a significant impact on juvenile survival to evaluate density-dependent effects. We tested whether final stem length, rhizome fresh biomass and aboveground dry biomass were significantly affected by plant species and infestation status, in the two first cases considering either initial plant stem length or initial rhizome fresh biomass as covariables to account for any potential effects of initial plant size. In these analyses, collection site was included as a random effect to allow for paired analyses between control and infested plants of the same origin. Additionally, in infested plants we assessed whether the number of eggs recorded on a plant and the number of adults were correlated to the final plant size parameters that proved to

be significantly affected by *A. itadori* infestation, in this case also considering initial plant size values as covariables (Table 1). In this experiment, we first explored whether the association between different parameters differed between the different blocks, so as to consider any potential variation owing to the blocked design of the experiment. Given that the variation within blocks was observed to exceed that between treatments, and that no differences were observed when block was included as a random factor or excluded from the analyses, the data of all blocks was pooled and analyzed together.

In the oviposition preference experiment, only replicates in which eggs were observed were considered as valid. Female preference to lay their eggs on one or the two plants present and on the plant in a particular position in the cage was evaluated. For each two-species combination we also studied the effect of *Reynoutria* species on the probability of a plant being accepted for oviposition and on the number of eggs it received. To rule out a potential confounding effect of total leaf area on the probability of a plant receiving eggs or the number of eggs laid on it, we tested whether total leaf surface was associated with the probability of a plant being accepted for oviposition, and if it was correlated with the number of eggs it received (Table 1).

We assessed significance of all factors included in the full models reported in Table 1 in a stepwise manner, removing factors from the model, and comparing models with and without these factors by an Analysis of Variance (ANOVA) on the model deviances. Tukey's HSD test for multiple comparisons was used to assess differences between factor levels of those factors that remained in the final reduced model. Interactions between factors were explored in all models that included more than one explanatory variable. In those analyses that included continuous explanatory variables with values on very different scales, these variables were normalized by z-scaling them, which implied subtracting the mean and dividing by the standard deviation (Becker et al., 1988).

As previously reported in other studies with *A. itadori*, owing to the

Table 1

Summary of the statistical tests performed on the data of the three experiments described in this paper.

Experiment	Response variable	Fixed effects	Random effects	Model and family	Link function
Development study	Acceptance for oviposition	Sp	Site	GLM, QB	Logit
	Egg	Sp	Site	GLM, QP	Log
	Supports development to adulthood	Sp	Site	GLM, QB	Logit
	Ad	Sp	Site	GLM, QP	Log
	Juvenile survival	Sp * Egg	Site	GLM, QB	Logit
	Daily DD accumulated	Sp	Date	LMER	Identity
	Duration immature stage	Sp * Egg	Plant, Site	GLMER, PS	Log
	DD at adult emergence	Sp	Plant, Site	LMER	Identity
Fecundity, juvenile survival and impact on plant growth	Egg	Sp		GLM, QP	Log
	Ad	Sp		GLM, QP	Log
	Juvenile survival	Sp * Egg		GLM, QB	Logit
	Final stem length	Sp * Inf * SHI	Site	GLMER, GS	Log
	Final rhizome fresh biomass	Sp * Inf * RFWI	Site	GLMER, GS	Log
	Final aboveground dry biomass	Sp * Inf	Site	GLMER, GS	Log
	Final stem length (infested plants)	Sp * Ad/Egg * SHI		GLM, GS	Log
	Final rhizome fresh biomass (infested plants)	Sp *Ad/Egg * RFWI		GLM, GS	Log
Oviposition preference	Number of plants chosen for oviposition			χ^2 goodness of fit test	–
	Plant chosen for oviposition	Sp		χ^2 goodness of fit test	–
	Position of the plant with eggs in the cage			χ^2 goodness of fit test	–
	Acceptance for oviposition	Sp * TLA	Replicate	GLM, BI	Logit
	Egg	Sp		Paired Wilcoxon signed ranks test	–
	Egg	TLA		Spearman's correlation test	–

Sp = Plant species; Egg = Number of eggs; GLM = Generalized Linear Model; QP = Quasipoisson; Ad = Final number of adults; QB = Quasibinomial; GLMER = Generalized Mixed-Effects Model; DD = Degree-days; LMER = Linear Mixed-Effect Model; PS = Poisson; Inf = Infestation treatment; SHI = Initial stem height; Site = Plant collection site; GS = Gaussian; RFWI = Initial rhizome fresh weight; TLA = Total leaf area; BI = Binomial.

difficulty of accurately recording the total number of eggs laid in a non-destructive examination, the number of eggs recorded was considered as the best possible estimation (Fung et al., 2020). In several cases of the two experiments where adult number was recorded the number of adults surpassed the eggs recorded initially (development: 2x*Rj*, 6x*Rb*; fecundity and juvenile survival: 3x*Rb*, 1x*Rs*). In these cases, egg number was adjusted to reflect the number of adults in the survival analyses.

All statistical analyses were conducted in R (https://www.r-project.org), version 1.2.5033. The functions GLM and GLMER are included in package lme4 (Bates et al., 2015). A statistical significance threshold of 0.05 was used in all statistical tests.

3. Results

3.1. Development study

Acceptance of a plant for oviposition differed significantly between species, so that two thirds of *Rj* plants received eggs (68.2 %), which is nearly double but not significantly different than the 36.4 % acceptance on *Rs* plants (z = -2.27, p = 0.06). No difference was observed between acceptance for oviposition of *Rb* (60.0 %) and *Rj* plants (z = -0.58, p = 0.83). For plants accepted for oviposition, host species marginally affected female fecundity in 24 h (χ^2 = 5.99, p = 0.05). However, when considering all plants accepted and rejected, the differences between species in average female fecundity in 24 h were highly significant (χ^2 = 18.16, p < 0.001), so that an average of 6.2 eggs were laid per female and day on *Rs* plants, a significantly lower number than the average 17.7 eggs laid on *Rj* (z = -3.17, p = 0.004) and the 13.9 laid on *Rb* (z = -2.38, p = 0.040) (Table 2).

Development to adult was observed in all replicates of *Rb* and most of *Rj* (86.7 %), but only in 50 % of the *Rs* plants, so that the proportion of plants where individuals reached adulthood was significantly higher in *Rb* than in the other two species (*Rb*-*Rs*: z = 9.20, p < 0.001; *Rb*-*Rj*: z = -9.15, p < 0.001), but it did not differ between *Rj* and *Rs* (z = 0.32, p = 0.94). If we consider all plants that were accepted for oviposition, only around a third of the eggs laid on *Rs* plants developed into adults, a juvenile survival significantly lower than the 73 % recorded on *Rb* plants (z = -2.97, p = 0.008), but not significantly different from the 52 % recorded on *Rj* plants (z = 1.66, p = 0.22). No differences in juvenile survival were observed between plant species when only those replicates where adults were recorded are considered (χ^2 = 2.68, p = 0.26). The number of adults recorded per plant differed between *Reynoutria* species when we consider all accepted and rejected for oviposition (χ^2 = 8.12, p = 0.017), with an average of 2.2 adults recorded in *Rs*, a value significantly lower than the 9 adults per plant recorded in *Rj* (χ^2 = -2.47, p = 0.036) and the 12.9 recorded on *Rb* plants (z = -2.79, p = 0.014). Similarly, if only replicates accepted for oviposition are considered, less adults were recorded in *Rs* plants than in *Rb* (z = -3.69, p < 0.001) (Table 2).

The duration of the immature stage differed significantly between *Reynoutria* species (χ^2 = 15.26, p < 0.001), so that in *Rb* plants it was on average 39.8 ± 0.2 days, which was significantly shorter than the 43.2 ± 0.3 days it took individuals to reach adulthood in *Rj* plants (z = -4.13, p < 0.001), but not different from the 41.6 ± 0.3 days it lasted on *Rs*

plants (z = 1.60; p = 0.38) (Supplementary Fig. 4). A high variation in the duration of the immature stage was observed between both the juveniles developing on the same plant and juveniles developing on different plants of the same species (ranging 35–55 days on *Rj*; 33–50 days on *Rb*; 36–53 days on *Rs*; Supplementary Fig. 5).

We observed no density-dependent effects, as the number of eggs recorded on a plant did not impact the duration of the immature stage (χ^2 = 1.87, p = 0.17) or survival to adulthood on that plant (χ^2 = 0.56, p = 0.46).

The average temperature in the caged plants ranged from 19.6 to 20.5 °C, and it was significantly higher in *Rb* plants than in *Rj* (z = -6.28, p < 0.001) and *Rs* (z = -11.90, p < 0.001) plants throughout the period the experiment took place, with higher DD accumulated per day in *Rb* plants than on the other two species (Supplementary Fig. 6). An average of 452 ± 3 DD (range 366–565) was required by *A. itadori* immatures to complete development on *Rj* plants. On *Rb* the values were significantly lower (z = -9.34, p < 0.001), so that individuals completed the immature stage after accumulating an average of 419 ± 2 DD (range 359–528). As for *Rs* plants, adults emerged from these plants at an average accumulation of 428 ± 3 DD (range 363 to 545), which was significantly lower than *Rj* (z = -3.43, p = 0.002), but higher than *Rb* (z = -2.78, p = 0.014) (Fig. 1).

A DD model for each *Reynoutria* host was estimated based on the recorded cumulative adult emergence by DD accumulated on each host species. The cumulative percentage of adult emergence (*p*) by DD accumulated can be estimated from the following equations for *Rj* [1], *Rb* [2] and *Rs* [3], where DD indicates cumulative degree days.

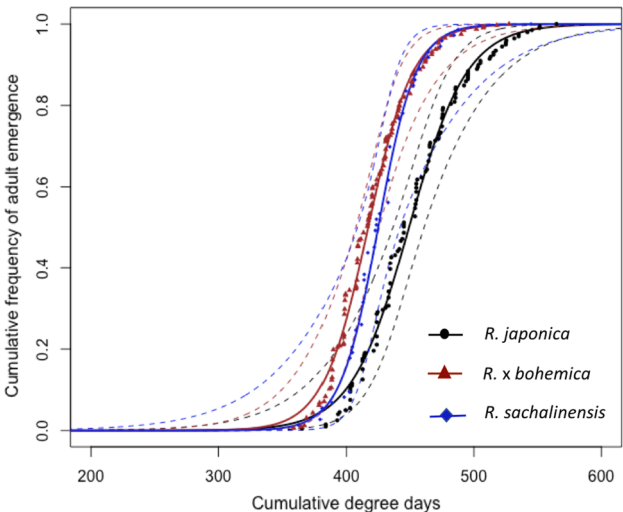$$0.044816DD - 20.122866 = \log\frac{p}{1-p} \tag{1}$$

Fig. 1. Models of cumulative frequency of adult emergence by degree days, per *Reynoutria* species. Points represent the observed values, and dashed lines delimit the 95 % confidence interval of the models. The confidence interval of *R. sachalinensis* overlaps with those of *R. × bohemica* and *R. japonica*.

Table 2
Performance of the Murakami population of *A. itadori* on the three *Reynoutria* species in the development study (mean ± SE).

Species	Replicates started	Number of eggs			Juvenile survival (%)		Number of adults		
		% Replicates with eggs (N)	In all replicates	In accepted replicates	In accepted replicates	In replicates with adults	% Replicates with adults (N)	In all replicates	In accepted replicates
R. japonica	22	68.2b (15)	17.7 ± 3.1b	26.0 ± 2.3 a	52.2 ± 9.2 ab	60.3 ± 8.6 a	86.7 a (13)	9.0 ± 2.2b	13.3 ± 2.5 ab
R. × bohemica	25	60.0 ab (15)	13.9 ± 2.9b	23.1 ± 3.1 a	73.1 ± 8.0b	73.1 ± 8.0 a	100.0 a (15)	12.9 ± 3.0b	21.5 ± 3.4b
R. sachalinensis	33	36.4 a (12)	6.2 ± 1.8 a	17.5 ± 2.7 a	33.1 ± 10.3 a	66.2 ± 5.6 a	50.0 a (6)	2.2 ± 0.9 a	6.1 ± 2.2 a

"All replicates" indicates the sum of replicates accepted and rejected for oviposition.
Different lettering indicates significant differences between species in that parameter (p < 0.05).

Table 3

Performance of the Murakami population of *A. itadori* on the three *Reynoutria* species in the experiment that assessed fecundity, juvenile survival and impact on plant growth (mean ± SE).

Species	Plants for fecundity assessment (N)	Average N eggs per female and per day	Plants for adult and juvenile survival assessment (N)	Adults emerged by day 49 (%)[1]	Eggs developed into adults by day 49 (%)	Average N of adults per female and per day on day 49
R. japonica	18	19.2 ± 1.2b	15	99.4	40.3 ± 4.4 a	7.5 ± 0.8 a
R. × bohemica	20	19.9 ± 1.1b	15	100.0	76.9 ± 4.1b	15.2 ± 1.2b
R. sachalinensis	16	9.6 ± 1.7 a	11	100.0	61.5 ± 10.0b	7.0 ± 1.9 a

Different lettering indicates significant differences between host species in that parameter (p < 0.05).

[1] Estimated by the DD model, assuming all eggs laid on the middle day of oviposition.

$$0.057388DD - 23.930665 = \log\frac{p}{1-p} \qquad (2)$$

$$0.06760DD - 28.77822 = \log\frac{p}{1-p} \qquad (3)$$

3.2. Fecundity and juvenile survival

The maximum number of adults per female and per day of oviposition emerged from *Rb* plants, with an average of 15.2 ± 1.2 per plant, whereas only around half that number developed on both *Rj* (7.4 ± 0.8, z = 3.89, p < 0.001) and *Rs* (7.0 ± 1.9, z = -3.71, p < 0.001). This was a result of the effect of plant species on both female fecundity ($\chi^2 = 30.71$, p < 0.001) and on the proportion of eggs that developed into adults ($\chi^2 = 34.15$, p < 0.001). Specifically, the highest numbers of eggs per female per day were recorded on *Rb* and *Rj*, with an average of around 20 eggs per female and per day, nearly doubling the number found on *Rs* (*Rb-Rs*: z = -4.91, p < 0.001; *Rj-Rs*: z = -4.57, p < 0.001). In contrast, the proportion of eggs developing into adults within 49 days was significantly higher on *Rb* (76.9 % ± 4.1) and *Rs* (61.5 ± 10.0) than on *Rj* (40.3 ± 4.4; *Rb-Rs*: z = 5.46, p < 0.001; *Rj-Rs*: z = 3.15, p = 0.005) (Table 3).

Additionally, there was no indication of density-dependent effects in this experiment, given that the percentage of eggs that had successfully completed the immature stage at the end of the experiment was not affected by the initial number of eggs in the plant ($\chi^2 = 0.26$, p = 0.61).

3.3. Impact on plant growth

Infestation with *A. itadori* had an overall negative impact on final stem length across plant species ($\chi^2 = 5.20$, p = 0.02; Fig. 2A), so that the average final stem length of infested plants was around 8 % lower than that of control plants. Infestation further caused a significant reduction in final rhizome fresh biomass in both *Rb* (35 % lower biomass than control plants; z = -3.60, p = 0.004) and *Rs* (50 % lower biomass than control plants; z = -5.32, p < 0.001), but not in *Rj* (z = 0.98, p = 0.92) (Fig. 2B). *Aphalara itadori* infestation had no effect on aboveground dry biomass ($\chi^2 = 0.02$, p = 0.89) (Fig. 2C).

Initial number of eggs did not have a significant impact on final rhizome fresh biomass ($\chi^2 = 0.09$, p = 0.76) or stem length ($\chi^2 = 1.0$, p = 0.32). However, a higher number of *A. itadori* adults was associated with a lower final rhizome fresh biomass ($\chi^2 = 6.65$, p = 0.010), whereas adult number was not correlated with stem length ($\chi^2 = 0.59$, p = 0.44).

Additionally, high levels of leaf curling were observed in both *Rb* and *Rs* plants, especially in the young shoots, whereas *Rj* plants showed much lower levels of this leaf deformation.

3.4. Oviposition preference

Females laid their eggs on only one of the two plants in a cage significantly more often than distributing them between the two plants ($\chi^2 = 21.1$, p < 0.001). In this line, in all replicates of the combination JB
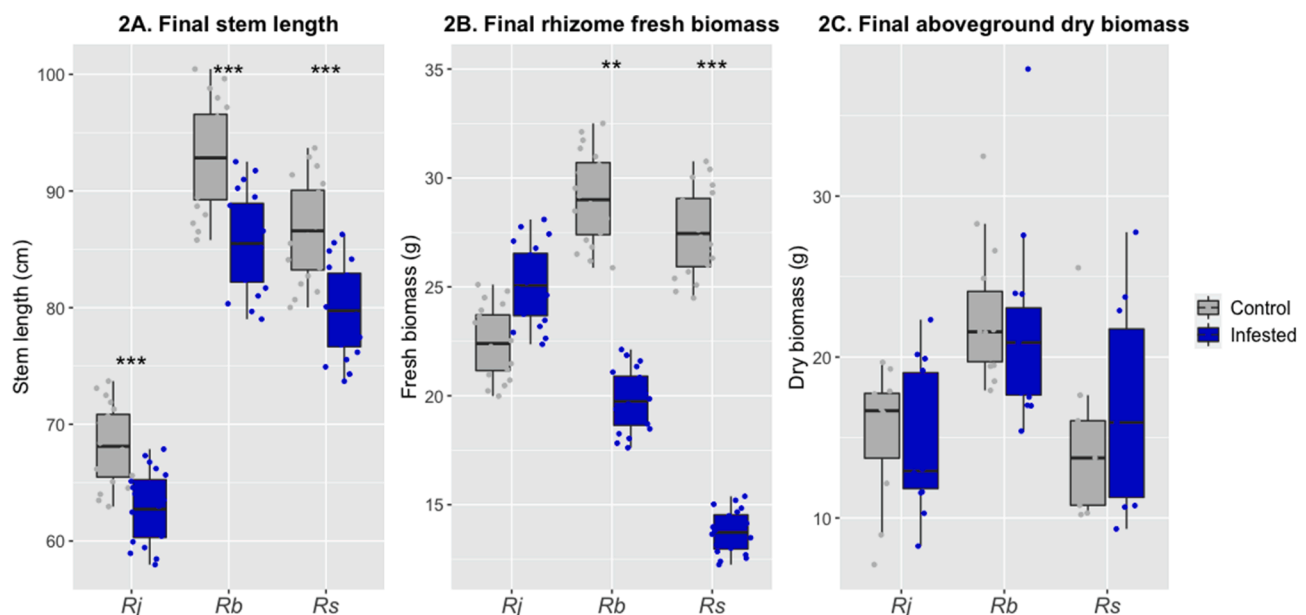

Fig. 2. Box-plots showing the final plant stem length, rhizome fresh biomass and aboveground dry biomass by *Reynoutria* species and infestation treatment, in the experiment assessing impact of *A. itadori* on plant growth. The lower and upper boxes limit the 25th and 75th percentiles, respectively; the horizontal line dividing the boxes indicates the median, and the vertical line indicates the minimum and maximum values, excluding outliers. In 2A and 2B the data indicate values predicted by the models including initial plant size as a covariable for the range of initial sizes shared by the three species, whereas in 2C it indicates observed values. Asterisks denote significant differences between infestation treatments. *Rj* = *R. japonica*, *Rb* = *R. × bohemica* and *Rs* = *R. sachalinensis*.
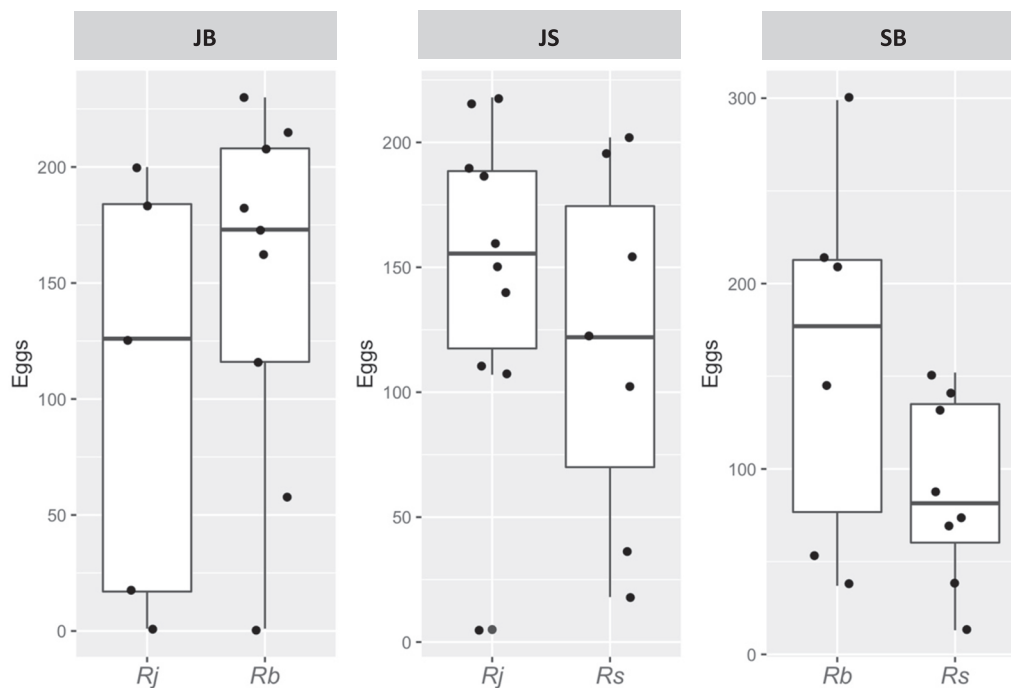
Fig. 3. Box-plot showing the number of eggs laid in each *Reynoutria* species per plant species combination in the oviposition choice experiment. Only cases with more than one egg are shown. The lower and upper boxes limit the 25th and 75th percentiles, respectively; the horizontal line dividing the boxes indicates the median, and the vertical line the minimum and maximum values, excluding outliers. *Rj = R. japonica, Rb = R. × bohemica* and *Rs = R. sachalinensis.*

all eggs were on just one of the plants, and the same happened in 93 % of JS replicates and 92 % of SB replicates (Supplementary Fig. 7A). No significant differences were observed in any of the combinations in either the number of times a species was chosen for oviposition (JB: $\chi^2 =$ 1.14, p = 0.29; JS: $\chi^2 = 0.53$, p = 0.47; SB: $\chi^2 = 0.29$, p = 0.59; Supplementary Fig. 7B), or in the number of eggs laid per species (JB: V = 31.5, p = 0.20; JS: V = 77.0, p = 0.36; SB: V = 51.5, p = 0.70; Fig. 3).

Total leaf area of a plant was not associated with its probability of receiving eggs in any of the combinations (JB: $\chi^2 = 0.014$, p = 0.97; JS: $\chi^2 = 0.011$, p = 0.92; SB: $\chi^2 = 0.003$, p = 0.95). Furthermore, this parameter was not correlated with the number of eggs laid on that plant, neither when the replicates of all species were pooled together (R = -0.01, p = 0.93) nor when each species was considered independently (*Rj*: R = -0.54, p = 0.24; *Rb*: R = 0.38, p = 0.36; *Rs*: R = -0.52, p = 0.13). Additionally, the position of the plant in the cage did not affect the probability that it was chosen for oviposition, so that in no combinations it differed from the expected 50 % (JB: $\chi^2 = 0$, p = 1; JS: $\chi^2 = 0.53$, p = 0.47; SB: $\chi^2 = 0.29$, p = 0.59).

4. Discussion

We studied the performance, oviposition preference and impact on plant growth of the Murakami population of *A. itadori* on plants of its three target species collected as part of the knotweed biocontrol program in the Netherlands. Our study shows that host plant species greatly influences the oviposition rate, juvenile development and juvenile survival of *A. itadori*. This has important implications for the optimization of biological control of invasive knotweeds.

The Murakami population performs better on *R. × bohemica* than on the parental species of this hybrid, *R. japonica* and *R. sachalinensis*. Specifically, a higher number of emerged adults was recorded on *R. × bohemica*, where the juveniles also had the fastest development. The lower performance observed on *R. sachalinensis* derives mainly from the lower acceptance of this species for oviposition and to a lesser extent to a higher juvenile mortality. We acknowledge that lack of oviposition on a plant could also result from infertility in one or the two adults of the pair

or from their failure to mate, and not only from a lack of acceptance of the host plant. However, we would not expect the likelihood of these events to be associated with the plant species the pair was placed on. The lower acceptance of *R. sachalinensis* plants for oviposition could be related to the oviposition preference shown by *A. itadori* for young small leaves (Jones et al., 2021), which are more abundant in both *R. japonica* and *R. × bohemica* compared to the larger leaves of *R. sachalinensis*. The poorer performance of the Murakami population on *R. japonica*, in contrast, resulted from a high juvenile mortality. The tougher leathery consistency of the leaves in this species could make them less palatable or digestible than the softer and thinner leaves of the other two *Reynoutria* species (Fürstenberg-Hägg et al., 2013; Békési-Kallenberger et al., 2016). This could have contributed to the longer duration of the juvenile stage recorded on this host and to the higher nymph mortality. Faster development could also be associated with the higher average temperature recorded in plants of the hybrid throughout the experimental period in comparison with the parental species. However, the differences in temperature alone would not be enough to account for the significantly faster development on *R. × bohemica* in comparison with *R. japonica*, and no difference in development rate were observed between the hybrid and *R. sachalinensis* in spite of the significantly higher temperature recorded on *R. × bohemica*. The results of the performance experiments are in line with those of a preliminary study carried out at CABI UK, where the Murakami population performed best on *R. × bohemica* when plants collected from a single location in the Netherlands of each of the three target *Reynoutria* species were tested in a choice study (Supplementary Study 1; Supplementary Table 1).

Different *A. itadori* populations have been reported to perform better on the most abundant species in their area of collection (Shaw et al., 2009; Grevstad et al., 2013), and similar results have been observed in other specialist herbivores used as weed biocontrol agents (Goolsby et al., 2005), as well as on other herbivores that did better on geographically co-occurring host populations than on non-native populations of the same host species (Lambert and Casagrande, 2007). However, the results of our study indicated the population collected in Murakami performed better in *R. × bohemica*, a host presumed not to be

present in Japan. Even though *R.* × *bohemica* is generally considered to be absent in Japan (R. Shaw, personal communication), a range of species closely related to *R. japonica* and *R. sachalinensis* occur in the region where the Murakami population was collected (Bailey et al., 2007). This could have led *A. itadori* populations from this area to adapt to perform best on *Reynoutria* species with morphological traits intermediate between *R. sachalinensis* and *R. japonica*, just like those present in *R.* × *bohemica*. Alternatively, *R.* × *bohemica* could be more vulnerable to herbivore damage than the parental species due to hybrid susceptibility, in which the hybrid displays traits relating for instance to its chemical resistance or phenology that makes it more susceptible to herbivory (Whitham, 1989; Fritz, 1999). Additionally, the fact that *R.* × *bohemica* control plants registered the highest average stem length, rhizome fresh biomass and aboveground dry biomass at the end of the experiment points to hybrid vigor, in which hybrid species display increased growth performance than their parental species (Birchler et al., 2006). Since *R.* × *bohemica* is deemed to have emerged and be present exclusively in the exotic range of its parental species, this species would have never been exposed to the specialist herbivores that plague *R. japonica* and *R. sachalinensis* in their native range, and there might have been a tradeoff in this hybrid so that more resources are directed towards growth and less towards defense against herbivory. However, if this was the case, we would expect the other populations of *A. itadori* collected in different areas in Japan to also perform better on *R.* × *bohemica*, whereas the *A. itadori* populations collected in Hokkaido and Kyushu have been reported to perform best on *Reynoutria* species other than the hybrid.

Infestation of the Murakami population of *A. itadori* had a negative impact on knotweed growth of the three target species, so that it reduced the stem length in all three *Reynoutria* species and it also reduced the rhizome biomass in *R.* × *bohemica* and *R. sachalinensis*. A decrease in rhizome biomass could contribute to reduce the spread rate of knotweed, given that in their introduced range knotweed reproduction and spread is mainly by rhizome fragments (Bailey et al., 2007). Furthermore, this could influence their competitive ability. The impact observed in *R. sachalinensis* and *R.* × *bohemica* could be associated with the high incidence of leaf curling caused by psyllid infestation in these hosts, in comparison with the lesser foliar deformation observed in *R. japonica*. Leaf curling reduces the plants' total leaf area, which can result in a lower photosynthetic potential and compromise plant growth (Kadioglu and Terzi, 2007; Abd Allah, 2009). Juvenile development inside galls formed by rolled-up leaves has been reported in psyllid species, whose feeding induces metabolic changes in the leaf that favor juvenile growth (Hodkinson, 2009). Leaf curling has not been reported in the two other *A. itadori* populations previously used in knotweed biocontrol, that were collected in Kyushu and Hokkaido, and which caused a significant reduction in the total biomass of both *R. sachalinensis* and *R.* × *bohemica* (Grevstad et al., 2013). Further research would help determine the mechanism behind this growth deformation and its potential association with growth reduction. More specifically, further steps should be taken aimed to understand which development stage of *A. itadori* cause leaf curling and the number of individuals necessary to produce it, whether feeding by other *A. itadori* populations can also result in this phenomenon, the physiological mechanism behind it, and whether leaf curling and growth reduction are directly correlated.

Both in the no choice experiments and in the preliminary study, where groups of females of the Murakami population of *A. itadori* were exposed to plants of the three host species, *R. sachalinensis* received fewer eggs than *R. japonica* and *R.* × *bohemica*. Additionally, in the development study *R. sachalinensis* was accepted for oviposition less often than the other two host species. These results contrast with those of the two-choice assays with individual pairs of adults. In this assay, even though individual females showed a clear preference to lay all their eggs on one of the two available plants, no consistent choice for a particular host species was observed across females, neither in the

number of times a plant species was chosen nor in the number of eggs it received. This could be explained if the suitability for development of the Murakami population differed between *R. sachalinensis* plant stands, since no differences in the number of eggs received were observed between host species between those plants accepted for oviposition. This hypothesis would be supported by the moderate to high genotypic variation reported in *R. sachalinensis* plants collected in central-western Europe (Mandák et al., 2005; Krebs et al., 2010), which could result in variation in the suitability of the different genotypes for *A. itadori* development. Interestingly, the six *R. sachalinensis* plants that did not support development to adulthood in the development study came from the same three locations (two plants from each location), and plants from these locations had the lowest egg counts in the other no choice study and received no eggs in the two-choice assay. A high variation in the suitability of *R. sachalinensis* plants could also explain the lower number of eggs received by *R. sachalinensis* in the preliminary study, if the *R. sachalinensis* plants used in that study, which all originated from the same single stand in the Netherlands, had a low suitability for the Murakami population. This could be due to lingering interpopulation phenotypic variation as a result of exposure to different environmental conditions in the different collection sites, since the plants used in the experiments had only grown for one generation under the same conditions. Additionally, the differences in the experimental design of the preliminary assay and the two-choice assay, with groups of adults used in the former study and individual pairs in the latter, could partially account for the different results. Given the suitability of the three target species as hosts for development of the Murakami population, oviposition preference for or avoidance of a particular *Reynoutria* species might only show under a scenario where other conspecific females are present. For instance, the presence of conspecific individuals on a particular plant could act as an indication of the higher suitability of that plant as a host, and attract other females to lay their eggs there. In this line, previous olfactometer assays revealed a preference of *A. itadori* gravid females for the odor of other conspecific psyllids, especially when the host plant was present. These assays further revealed a non-significant pattern of oviposition preference towards plants where conspecific eggs had been laid (R. Shaw, personal communication). These findings suggest the role of an *A. itadori*-produced chemical, such as a pheromone, that could act as an attractant or aggregation compound for conspecific females. Further research to identify the potential compounds that could mediate female aggregation could confirm the role of olfactory cues in the oviposition avoidance of *R. sachalinensis* observed in assays with groups of adults of the Murakami population of *A. itadori*.

In case both hypotheses hold true – *R. sachalinensis* from different locations differ in their suitability as a host for the Murakami population and an oviposition preference only shows when other conspecific individuals are present –, we would expect females of the Murakami population to spread indistinctly to *R.* × *bohemica*, *R. japonica* and suitable *R. sachalinensis* plants in the field, and to avoid those *R. sachalinensis* stands not suitable for development.

In summary, when tested on plants of the three target plant species in the Netherlands, the Murakami population of *A. itadori* showed a superior performance on the hybrid *R.* × *bohemica*, but it did not display an oviposition preference among the three hosts in choice assays with individual pairs of adults. This psyllid population significantly reduced growth of the three *Reynoutria* species, with *R.* × *bohemica* and *R. sachalinensis* showing a higher susceptibility to psyllid damage than *R. japonica*, which was less impacted by *A. itadori* infestation. This is the first study on the performance of an *A. itadori* population from central Japan used in knotweed biocontrol.

The results presented here can help fine-tune the biocontrol program of invasive knotweeds in the Netherlands. The superior performance of the Murakami population on *R.* × *bohemica* suggests that plants of this hybrid species can best be used to optimize the rearing of this population for biocontrol in the Netherlands. Use of this host species would allow breeding programs to obtain the largest number of adults and to produce

the highest number of generations per year. Likewise, field releases should initially target *R. × bohemica* stands, to maximize the likelihood of juvenile survival, establishment and ultimately population growth. Upon successful field establishment, this population is expected to spread to and reduce growth in all three target species, with a greater impact on *R. × bohemica* and *R. sachalinensis*. The superior performance of the Murakami population on *R. × bohemica* renders it particularly suitable for knotweed biocontrol in the Netherlands, given that, together with *R. japonica, R. × bohemica* is the most widely spread knotweed species in the Netherlands, and is commonly considered the most difficult to manage. Release of the Murakami population for knotweed biocontrol has been recently approved in the UK and North America. Given that performance of a biocontrol agent on its host plants depends on the geographic origin of both the natural enemy and the host plant, our results suggest that further studies should examine whether the superior performance shown by the Murakami population on *R. × bohemica* in this greenhouse study is also true in field populations from those regions. In the broader context, the results presented here emphasize the importance of assessing the performance of biocontrol agents on a population level and considering host plants from the area targeted for release. This will aid to better address the specific biocontrol needs of each area and increase the chances of success of biological control programs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Disclosure statement

The positions and opinions presented in this article are those of the authors alone and are not intended to represent the views/any official position or scientific works of the European Food Safety Authority (EFSA).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2022.105007.

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