Bulletin of Entomological Research

cambridge.org/ber

Potential host range of *Cotesia vanessae* (Hymenoptera: Braconidae), a parasitoid new to North America and a possible biological control agent of noctuid pest species

V. A. D. Hervet[1], R. A. Laird[2] and K. D. Floate[3]

[1]Morden Research and Development Centre, Agriculture and Agri-Food Canada, 101 Rte 100 #100 Morden, Manitoba, Canada R6 M 1Y5; [2]Department of Biological Sciences, University of Lethbridge, 4401 University Drive W., Lethbridge, Alberta, Canada T1K 3M4 and [3]Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, 5403-1st Ave. S., Lethbridge, Alberta, Canada T1J 4B1

Research Paper

Cite this article: Hervet VAD, Laird RA, Floate KD (2023). Potential host range of *Cotesia vanessae* (Hymenoptera: Braconidae), a parasitoid new to North America and a possible biological control agent of noctuid pest species. *Bulletin of Entomological Research* **113**, 145–161. https://doi.org/10.1017/S0007485322000025

Received: 9 March 2021
Revised: 17 October 2021
Accepted: 4 January 2022
First published online: 21 December 2022

Keywords:
Biological control; cutworm; fitness parameters; Microgastrinae; Noctuidae; phylogenetic comparison of host species

Author for correspondence:
V. A. D. Hervet,
Email: vincent.hervet@agr.gc.ca

Abstract

The likelihood of parasitoids establishing in new geographic regions depends upon the availability of suitable host species. Identifying these hosts and the degree of their suitability is particularly important when they include species that are economically important as pests. In laboratory studies, we examined the suitability of 47 species of Lepidoptera as potential hosts of a parthenogenetic strain of the gregarious parasitoid *Cotesia vanessae* (Hymenoptera: Braconidae). Previously known from Eurasia and northern Africa, the first known recovery of *C. vanessae* in North America was in 2009. *C. vanessae* completed development in 34 species, of which three were known hosts (Noctuidae) and 31 (30 Noctuidae, 1 Nymphalidae) were not. Many of these noctuid species are economic pests. Parasitoid fitness was generally highest on species of Plusiinae (Noctuidae), measured as either percentage of successful parasitism, developmental time, or number and mass of F_1 progeny. Closely related species were generally similar in their suitability as hosts. In some cases, parasitoid eggs or larvae were killed by the immune system of the parasitized host, but the host eventually failed to excrete food waste, did not pupate, and ultimately died. Such cases reached up to 100% mortality depending upon the lepidopteran species. The suitability of many species of noctuid pests as hosts for *C. vanessae* suggests that this parasitoid will become established widely throughout North America and may help to suppress populations of some pest species.

Introduction

The likelihood of parasitoids establishing in new geographic regions depends upon the availability of suitable host species. Identifying these hosts and the degree of their suitability is particularly important when they include species that are economically important as pests. *Cotesia vanessae* (Reinhard) (Hymenoptera: Braconidae) is a multivoltine, gregarious parasitoid of lepidopteran species in the families Noctuidae and Nymphalidae (Nixon, 1974; Papp, 2011–2012). It has been commonly reported from the Palaearctic region (Hervet *et al.*, 2014), especially Europe and northern Africa, where populations may be either bisexual or parthenogenetic (Stefanescu *et al.*, 2012). The first report of *C. vanessae* in North America was made from parthenogenetic specimens reared from tomato looper, *Chrysodeixis chalcites* (Esper) (Noctuidae), and cabbage looper, *Trichoplusia ni* (Hübner) (Noctuidae), collected in southern Ontario, Canada, in 2009 (Hervet *et al.*, 2014). The occurrence of *C. vanessae* in North America is probably the result of an accidental introduction. Both *C. chalcites* and *T. ni* are pests of economic concern (see Lindgren and Green, 1984; Murillo *et al.*, 2013; Pacheco *et al.*, 2018 and references therein; Pacheco *et al.*, 2021). Given its reported host range, which includes pestiferous Noctuidae as well as some Nymphalidae, *C. vanessae* may potentially help suppress populations of pest species in North America. *C. vanessae* has previously been reported from species in the families Notodontidae, Lasiocampidae, Crambidae, and Pterophoridae but these records are old and possibly erroneous. Nixon (1974) reported the 'replacement of black by bright reddish yellow on the gaster' in some *C. vanessae* specimens. Recent molecular advances clarifying the taxonomy of Microgastrinae provide evidence that when such differences are observed between specimens they likely represent different species, and a number of accepted names in the genus *Cotesia* are, or are suspected to be, complexes of cryptic species (Fernández-Triana *et al.*, 2020).

Family Noctuidae (armyworms, cutworms, semi-loopers, and others) includes numerous species that are pests of horticultural and agricultural crops (Zhang, 1994; Zahiri, 2012; Floate and Hervet, 2017). Common examples include cotton bollworm (*Helicoverpa armigera* (Hübner)) (Sharma *et al.*, 2014), African armyworm (*Spodoptera exempta* Walker)

(Rose *et al.*, 1995), and fall armyworm (*Spodoptera frugiperda* (J. E. Smith)) (reviewed in Ramirez-Cabral *et al.*, 2017). Economic damage can be particularly severe during outbreaks, which are typically sporadic but may last for several years and affect large geographic regions. In the northcentral USA, outbreaks of pale western cutworm (*Agrotis orthogonia* Morrison) and army cutworm (*Euxoa auxiliaris* (Grote)) from ~1965 to 1975 caused annual production losses estimated at about US$ 120 million (in 2021 dollars) (USDA, 1977). Production losses and operation costs associated with outbreaks of pale western cutworm in western Canada from 1929 to 1932 were estimated at about US$ 290 million (in 2021 dollars) (McMillan, 1935). Reports of local outbreaks affecting agricultural crops in the Canadian prairie provinces are common and typically are associated with army cutworm, armyworm (*Mythimna unipuncta* (Haworth)), bristly cutworm (*Lacinipolia renigera* (Stephens)), dingy cutworm (*Feltia jaculifera* (Guenée)), glassy cutworm (*Apamea devastator* (Brace)), pale western cutworm, red-backed cutworm (*Euxoa ochrogaster* (Guenée)), and variegated cutworm (*Peridroma saucia* (Hübner)) (Floate, 2017).

The overall intent of this study was to examine the development of *C. vanessae* on different lepidopteran species present in North America with a twofold objective. First and foremost, we wanted to assess this parasitoid as a potential biological control agent of economic pest species. Second, we wanted to assess the risk that this adventive parasitoid might pose to native non-pest species of Lepidoptera. Fitness is ideally measured as lifetime reproductive success but obtaining this metric can be logistically difficult. In such cases, fitness can be measured indirectly using parameters that can include parasitism success, individual mass, size, and developmental time (Godfray, 1994; Roitberg *et al.*, 2001; Harvey, 2005).

To achieve this objective, we report results on the suitability of 47 species of Lepidoptera as potential hosts for *C. vanessae*, most of which are noctuid species that are pests of agricultural crops in North America. For species that supported parasitoid development, we also recorded successful parasitism rate, development time, brood size, brood mass, and individual parasitoid mass. Non-pest species and species in families other than Noctuidae were included to investigate the limits of *C. vanessae*'s host range. *C. vanessae* completed development in 34 species, of which three were known hosts (Noctuidae) and 31 (30 Noctuidae, 1 Nymphalidae) were not. The suitability of many lepidopteran species as hosts suggests that *C. vanessae* will become established throughout North America and contribute to the suppression of noctuid pest populations.

Materials and methods

Selection of species

We selected species using an approach similar to that of Wapshere (1974), in which species that are tested are increasingly more distantly related to the known hosts until the limit of the host range is reached. Previous work reported that *C. vanessae* had been reared from field-collected species in the subfamilies Plusiinae, Noctuini, Leucaniini, and Prodeniini (family Noctuidae), and in the tribe Nymphalini (family Nymphalidae) (Nixon, 1974; Shaw *et al.*, 2009; Stefanescu *et al.*, 2012; Hervet *et al.*, 2014). Thus, we sought to obtain a broad range of species from different subfamilies of Noctuidae, from the family Erebidae (closely related to Noctuidae) and from different subfamilies of Nymphalidae, including Nymphalinae (incl. tribe Nymphalini) plus more distantly related species whenever the opportunity arose. However, because of difficulties obtaining a range of species in a number of these taxonomic groups, most species tested were pests in the family Noctuidae, which were easier to procure.

Insect sources and species verification

C. vanessae were obtained from a colony held at the Lethbridge Research and Development Centre (LRDC), Agriculture and Agri-Food Canada, in Lethbridge, AB, Canada. Source material for the colony was obtained in 2011 from H. Murillo Pacheco, who established it with adults reared from caterpillars of *C. chalcites* and *T. ni* collected in southern Ontario in 2009 and 2010 (Hervet *et al.*, 2014; Pacheco *et al.*, 2018). The colony was maintained on *T. ni* reared on a modified McMorran diet (McMorran, 1965; Grisdale, 1973; Hervet *et al.*, 2016). Eggs of *T. ni* and diet were purchased from Insect Production Service, Natural Resources Canada, Sault Ste., Marie, ON, Canada. Rearing methods are described in Hervet (2017) and Hervet *et al.* (2016). For clarity, in subsequent text, larval stages of *C. vanessae* are referred to as 'larvae', whereas larval stages of lepidopteran species are referred to as 'caterpillars'.

Lepidoptera were field-collected or purchased as described in table 3-1 of Hervet (2017). Some species were obtained as eggs recovered from gravid females collected in UV light traps operated at Lethbridge in the summers and autumns of 2012–2014. Other species were purchased as eggs from Benzon Research Inc., Carlisle, PA, USA (Canadian Food Inspection Agency Import Permits No. P-2011-04397, P-2013-02134, P-2014-02394). A smaller number of species were collected in the field as eggs or caterpillars. When numbers permitted (ca. 50+ individuals per species), field-collected material was used directly for host testing; i.e., *Apamea sordens* (Hufnagel), *Dargida diffusa* (Walker) (Noctuidae), *Aglais milberti* (Godart) (Nymphalidae), *Malacosoma disstria* Hübner (Lasiocampidae), and *Pieris rapae* (L.) (Pieridae). Otherwise, caterpillars were reared to produce a second generation that was used for host testing. Caterpillars of most species were successfully reared on a modified McMorran diet using methods described in Hervet *et al.* (2016).

Species' identifications were determined by their morphological traits and use of DNA barcoding. Species were initially identified using taxonomic keys (Eichlin, 1975; Eichlin and Cunningham, 1978; Schmidt, 2015) and with reference to preserved specimens in the main insect collection at the LRDC. Species identifications were subsequently verified with cytochrome c oxidase subunit 1 (COI) barcodes, using primers and general methods described in Hebert *et al.* (2003). DNA extractions and amplifications were done at the LRDC; sequencing was done at the University of Calgary (University Core DNA Services, Calgary, Alberta, Canada).

The sole exception to the above procedures was limited to parasitoids recovered by the senior author (VADH) in 2019 at Winnipeg, Manitoba from a caterpillar of the painted lady butterfly, *Vanessa cardui* (Nymphalidae), feeding on creeping thistle, *Cirsium arvense* (L.) Scopoli (Asteraceae). The morphology of these parasitoids was consistent with *C. vanessae*, but physical specimens of this species have not been reported in North America from outside of Ontario (but see Hervet *et al.*, 2014). To confirm this determination, DNA was extracted, amplified, and sequenced at the University of Manitoba in Winnipeg using the general methods and primers (LepF and LepR) described in Hajibabaei *et al.* (2006).

https://doi.org/10.1017/S0007485322000025 Published online by Cambridge University Press

Host testing

Host suitability was assessed in no-choice tests in which fourth-instar caterpillars were individually exposed to *C. vanessae* females. Preliminary studies showed fourth-instars to be most readily parasitized and to produce larger parasitoid broods relative to earlier instars. Use of later instars increased the risk that caterpillars might pupate before parasitoids were able to complete development. When fourth-instars were unavailable, fifth-instars typically were used instead. Most species were assessed for host suitability using 30–35 caterpillars, but numbers ranged from 1 (*Amphipyra tragopoginis* (Clerck) (Noctuidae)) to 75 (*Helicoverpa zea* (Boddie) (Noctuidae)) depending upon availability.

Caterpillars were subjected to parasitism by holding them with soft metal forceps in a colony cage of adult *C. vanessae* in contact with a parasitoid's antennae. This usually resulted in the parasitoid immediately inserting its ovipositor into the caterpillar, typically for ~10–20 s, accompanied by the upward positioning of wings, and the middle and hind legs (see video entitled 'Parasitoid *Cotesia vanessae* parasitizing a cabbage looper (*Trichoplusia ni*) caterpillar' at https://www.youtube.com/watch?v=CvOe5dy0c1s). We interpreted this behaviour as parasitism; i.e., eggs being laid in the host. Occasionally, this response did not occur. In this case, the caterpillar was exposed to a different parasitoid for ~3 s. This was repeated until parasitism was observed. If parasitism was still not observed after exposure to about ten parasitoids, a parasitoid was aspirated into a small plastic container containing a caterpillar (BioQuip Products, Rancho Dominguez, California, USA, catalogue no. 1135A). This process usually triggered immediate parasitism. If parasitism was still not observed, exposures were repeated using older parasitoids available as we observed that parasitoids eclosed from cocoons for some time were more likely to parasitize certain lepidopteran species. Parasitoids used in experiments were typically 1–3 weeks old. Ultimately, all caterpillars of all lepidopteran species subjected to parasitism did experience parasitism, excluding *Spilosoma virginica* Fabricius (Erebidae: Arctiinae) caterpillars. Exposure of 30 individuals of this species failed to produce parasitism, possibly because of protection conferred by their dense pubescence.

During parasitism, the caterpillar and the attached parasitoid were carefully transfered from the *C. vanessae* colony cage to a translucent plastic 'test' cup (240 ml, Polar Plastic Ltd, Saint Laurent, QC). The parasitoid remained in this cup for 48 h to allow potential further parasitism (although this was rarely observed), then was removed. The same method was used for *S. virginica* although no parasitism was observed prior to placing a caterpillar in a cup with a parasitoid. The bottom of each cup contained a disc of filter paper (55 mm diameter) on which a block (7.3 ml) of artificial diet (modified McMorran diet; see Hervet *et al.*, 2016) was placed. Pieces of wax paper (3×3 cm^2) were placed in contact with the upper and lower surfaces of the diet block to reduce desiccation. To prolong parasitoid longevity during the 48 h that the parasitoid was retained in the cup with the host, the inside of the cup was lightly misted with water, and a drop of honey was placed on top of the upper piece of wax paper. Air holes (ca. 0.5 mm diameter) in the bottom and in the lid of the cup allowed for airflow and reduced moisture accumulation. On an approximate 5-day cycle (more often if required), diet blocks were replaced and frass within the cup was discarded. Foliage from host plants were used to rear species that did not develop on the artificial diet; i.e., *A. milberti*, *P. rapae*, *Habrosyne scripta* (Gosse) (Drepanidae), and *Hyles euphorbiae* (Linnaeus) (Sphingidae). Leaves attached to short sections of stem were placed onto a piece of wax paper to avoid contact with the filter paper and misted daily with water to reduce wilting. Foliage was replaced every 2–4 days (more often if required). Cups were held under conditions of 20 °C, 70–80% humidity, with a 12:12 h light:dark cycle. Rearing conditions were monitored with a data logger placed inside an empty cup in contact with test cups.

Parasitized caterpillars were held in test cups until either parasitoid larvae emerged (=successful parasitism; note the explicit distinction between 'parasitism' and 'successful parasitism'), the caterpillars died from other reasons, or until adult metamorphosis. Adult metamorphosis for almost all Lepidoptera species tested was observed, but required up to 8 months for *Mamestra configurata* Walker (Noctuidae). No adults emerged from apparently healthy pupae formed by *H. euphorbiae* and *H. scripta*, suggesting that an obligatory cold treatment was required to break pupal diapause.

Oviposition experiment

Although the caterpillars of some species readily supported egg-to-adult development of *C. vanessae* (e.g., *T. ni*), caterpillars of other species survived parasitism to metamorphose into adults (e.g., *H. zea*). We subsequently performed an oviposition experiment to determine if these latter cases reflected a lack of oviposition by the parasitoid or an immune response by the caterpillar that prevented parasitoid development. Caterpillars of *H. zea* ($n = 20$) and *T. ni* ($n = 20$) were exposed to *C. vanessae*, as previously described, until parasitism by adult parasitoids was observed. Five caterpillars of each species were then immediately dissected and examined for parasitoid eggs. The remaining caterpillars were held in individual cups, as previously described, with subsets dissected during the next 11 days for observations of parasitoid larvae and host immune reaction. For these dissections, the head and the last rear abdominal segments of each caterpillar were excised and the digestive system removed. Haemolymph was then squeezed from the haemocoel onto a microscope slide, diluted with one drop of saline solution, topped with a coverslip, and examined using a compound light microscope (400×). Caterpillars dissected immediately after parasitism had large fat bodies that clouded the haemolymph and hindered observations. Thereafter, caterpillars were starved for 2 days prior to dissection to shrink their fat bodies.

Nonreproductive killing

For nearly all host species tested, several caterpillars died following parasitism but without parasitoid emergence. A few caterpillars used in tests were field collected and could have died of diseases but most caterpillars used in tests were reared to at least one generation in the lab and came from healthy colonies. We therefore suspected that exposure to parasitism somehow induced the death of most caterpillars that died without parasitoid emergence – a phenomenon that has been referred to by previous authors as 'nonreproductive killing', which is part of parasitoids 'nonreproductive effects' (Abram *et al.*, 2019). This phenomenon was particularly evident for *S. frugiperda* for which all caterpillars subjected to parasitism in host trials died without producing parasitoids. To test if nonreproductive killing took place, fourth-instar *S. frugiperda* were haphazardly removed from a colony cage and at this 'initial time' (T_0) they were either subjected ($n = 35$ caterpillars) or not subjected ($n = 35$ caterpillars) to parasitism. The caterpillars were then reared individually using methods previously described and monitored to record time to death, time to

https://doi.org/10.1017/S0007485322000025 Published online by Cambridge University Press

pupation, and (or) time to adult emergence. To better understand the cause of this phenomenon, the haemolymph of *S. frugiperda* caterpillars was observed with a compound light microscope (400×) immediately after their death.

Host suitability

Species for which no caterpillars that experienced parasitism produced parasitoids were classified as non-hosts; those that did produce parasitoids were classified as hosts.

We measured the suitability of Lepidoptera species for *C. vanessae* using a suite of up to seven fitness parameters. The first of these was percentage of successful parasitism (parameter 1). This was defined as the percentage of caterpillars that experienced parasitism from which parasitoid larvae emerged (excluding caterpillars that died without producing parasitoids). Third-instar *C. vanessae* larvae typically emerge en masse from the host to immediately begin spinning cocoons in which they pupate and subsequently emerge as adults (see video entitled 'Parasitoid larvae (*Cotesia vanessae*) emerging from their caterpillar host (*Trichoplusia ni*)' at https://www.youtube.com/watch?v=JCDbJMLE2JU) (Hervet *et al.*, 2014). Thus, we measured the following six additional parameters: parameter 2 = time for egg + larval development (i.e., the number of days from parasitism to emergence of larvae); parameter 3 = time for pupal development (i.e., the number of days from larval emergence from the host to emergence of adults from the cocoon mass); parameter 4 = time for egg-to-adult development (i.e., parameter 2 + parameter 3); parameter 5 = brood size (i.e., the number of adult parasitoids from an individual host, excluding adults that failed to emerge from cocoons); parameter 6 = average adult mass per brood (i.e., for each brood, the average mass of individual parasitoids dried at 20 °C and 20% relative humidity for at least 1 month); parameter 7 = total adult mass per brood (i.e., parameter 5 × parameter 6). For parameter 6, average adult mass was initially calculated by averaging the mass of ten individual adults under pristine conditions weighed separately. When this process proved to be too time consuming, average adult mass was calculated by weighing 30 adults simultaneously and then dividing by 30. The bulk mass approach was used for *Abagrotis reedi* Buckett, *Actebia balanitis* (Grote), *Agrotis ipsilon* (Hufnagel), *Anagrapha falcifera* (Kirby), *Anaplectoides prasina* (Denis & Schiffermüller), *A. devastator*, *Apamea lignicolora* (Guenée), *Caradrina morpheus* (Hufnagel), *Chrysodeixis includens* (Walker), *D. diffusa*, *Feltia herilis* (Grote), *H. zea*, *Heliothis virescens* (Fabricius), *Lacanobia grandis* (Guenée), *M. unipuncta*, and *Spaelotis clandestina* (Harris) (Noctuidae).

Phylogeny of lepidopteran species

To measure the genetic relatedness of the Noctuidae and Erebidae species that we tested in our study, we generated COI sequences. These sequences were supplemented with additional sequences from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) (Benson *et al.*, 2005) and Barcode of Life Data Systems (BOLD) (https://www.boldsystems.org/) (Ratnasingham and Hebert, 2007) for species that are considered reliable hosts for *C. vanessae* (Hervet *et al.*, 2014), but which we did not test. A neighbour-joining tree (Kimura two-parameter distance) was generated to assess the host quality of different clades of Noctuidae according to the maximum likelihood of evolutionary distance among species based on their COI sequences (Kimura, 1980), and to predict the suitability of non-tested species as hosts for *C. vanessae* (Supplementary fig. 1).

Data analyses

A Fisher's exact test was used to determine whether the percentage of *S. frugiperda* caterpillars that died without producing parasitoids was contingent on *C. vanessae* parasitism. A Welch's two-sample *t*-test was used to compare time to death of the parasitized group with the time to emergence of adult moths of the non-parasitized group.

A χ^2 contingency test was used to test whether the percentage of successful parasitism differed among host species, followed by post-hoc comparisons using Fisher's exact tests with Bonferroni corrections (parameter 1). Because of uncertainty regarding its cause for most species, data associated with caterpillars dying without producing parasitoids were excluded from this analysis and subsequent analyses. Caterpillars dying without producing parasitoids may have been a consequence of *C. vanessae* parasitism; i.e., nonreproductive killing. However, it may have instead or additionally reflected infection by pathogens or, in the case of field-collected material, parasitism by other parasitoid species. Excluding these data from consideration may have inflated our estimates of percentage of successful parasitism. For example, if 30 caterpillars of a given species were parasitized and ten each either survived to become adults, produced adult parasitoids (i.e., successful parasitism), or died without producing parasitoids, percentage of successful parasitism would be calculated as 50% (10/20) rather than as low as 33% (10/30; i.e., if caterpillar deaths were a result of nonreproductive killing, rather than other incidental, unrelated causes not caused by parasitism). In any case, this did not affect our determination of whether a given test species was a host for *C. vanessae*.

Prior to examining the effect of host species on the remaining fitness parameters 2 through 6, data were assessed for assumptions of normal distribution and homoscedasticity using residual-by-predicted plots. These assumptions were met for measures of time for pupal development (parameter 3), brood size (parameter 5), and average adult mass per brood (parameter 6). The effect of host species on these parameters was therefore examined using one-way analysis of variance (ANOVA). When a host effect was detected, Tukey's range tests were performed to identify statistically significant differences among host species. Assumptions of normal distribution and homoscedasticity were not fully met for measures of time for egg + larval development (parameter 2), time for total development (parameter 4), or estimated total brood mass (parameter 7). The effect of host species on these latter three parameters was therefore examined using Kruskal–Wallis tests. When a host effect was detected, post-hoc tests were performed using Dunn tests (pairwise multiple comparisons using rank sums, corrected according to Bonferroni) to identify the species between which differences occurred.

Degrees of freedom sometimes varied among statistical tests depending upon the parameter being measured. For example, a lack of data on development time for a specific brood did not impede the measurement of its mass.

All analyses were conducted in R version 3.3.0 (Fox and Weisberg, 2011; Dinno, 2016; R Core Team, 2016). Unless otherwise stated, all values are reported as means ± SEMs.

Results

With one exception, the determination of species examined in the current study was unambiguous and supported by both morphological characters and COI barcodes. For the exception, the

https://doi.org/10.1017/S0007485322000025 Published online by Cambridge University Press

morphology of *Abagrotis* sp. was most similar to *Abagrotis orbis* (Grote), but had closest genetic similarity to the morphologically similar *Abagrotis baueri* McDunnough (Noctuidae). COI sequences obtained in the current study for test species as potential hosts were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under accession numbers as reported in table 1. The COI sequence obtained for a parasitoid recovered in Winnipeg confirmed its morphological determination as *C. vanessae* (accession no. MW417940).

Results identified egg-to-adult development of *C. vanessae* on 34 of the 47 lepidopteran species tested, including 31 species not previously reported to be hosts (table 1). Previous reports of *C. vanessae* developing in *Anarta trifolii*, *A. sordens*, and *T. ni* (Tobias, 1971, 1976, 1986; Hervet *et al*., 2014) were confirmed. For species on which *C. vanessae* did not develop (table 1), the parasitized hosts either survived to complete development or died prior to pupation. At one extreme, 27 of 29 parasitized individuals of *Spodoptera exigua* (Hübner) survived to become adults and two died without producing parasitoids, whereas at the other extreme only 19 of 58 parasitized *Trichordestra lilacina* (Harvey) survived (Supplementary table 1).

Oviposition experiment

Results of the oviposition experiment showed that all caterpillars that experienced parasitism contained parasitoid eggs. The ability of caterpillars to survive and become moths was therefore attributed to the effectiveness of the host's immune system, rather than due to a lack of parasitism. Eggs and (or) larvae were observed in each of the *T. ni* and *H. zea* caterpillars that experienced parasitism (images in Hervet *et al*., 2018). Eggs were observed for up to 4 days post-parasitism with the appearance of larvae starting 5 days post-parasitism. Encapsulation of parasitoid eggs and larvae by the host's haemocytes is a normal immune response of lepidopteran larvae to parasitism (Lavine and Strand, 2002). In *T. ni*, a host with a high percentage of successful parasitism, encapsulation of eggs or larvae was not observed. In *H. zea*, a host with a low percentage of successful parasitism, encapsulation of eggs was observed 4 days after parasitism. All *H. zea* caterpillars dissected 11 days post-parasitism contained numerous dead first-instar larvae encapsulated by haemocytes. Only one dissected *H. zea* contained non-encapsulated live larvae alongside dead encapsulated smaller larvae, indicating that some parasitoid larvae evaded the host's immune response while others didn't within the same caterpillar.

Nonreproductive killing

In the experiment that tested the association between caterpillars that died not producing parasitoids and parasitism, all 35 parasitized *S. frugiperda* died not producing parasitoids, whereas all 35 non-parasitized *S. frugiperda* survived to become adults. These two outcomes were significantly different (Fisher's exact test, $P < 0.001$), confirming a case of parasitoid nonreproductive killing. Caterpillars from the parasitized group matured to their last instar, but did not pupate. Instead, they stopped excreting frass, became bloated until their integuments were tightly stretched, and eventually slowly turned dark and shrunk. The time taken for parasitized individuals to die (50 days ± 1.97 after T_0) was significantly longer than that required for non-parasitized individuals to metamorphose to adults (Welch's two-sample *t*-test, $t = -7.45$, $df = 40.35$, $P < 0.001$) (pupated in 18 days ± 0.34 after T_0, and emerged as moths in 37 days ± 0.61 after T_0). Dissection of parasitized individuals at time of death showed that their gut was full of food and was so bloated it nearly occupied the entire haemocoel. Their bodies were nearly depleted of haemolymph, which contained dead encapsulated first instar parasitoids.

Host suitability

An effect of host species was detected for each of the seven fitness parameters measured for *C. vanessae*. Across host species, percentage of successful parasitism (parameter 1) ranged from <10% to close to 100% ($\chi^2_{32} = 451.98$, $P < 0.001$; fig. 1). Effects of host species were detected for eggs + larval development time (parameter 2) (Kruskal–Wallis test, $\chi^2_{24} = 415.94$, $P < 0.001$), pupal development time (parameter 3) (ANOVA, $F_{24,\ 474} = 7.08$, $P < 0.001$), and egg-to-adult development time (parameter 4) (Kruskal–Wallis test, $\chi^2_{24} = 383.41$, $P < 0.001$; fig. 2). Parasitoids developing in *F. herilis* ($n = 5$) exhibited the longest times observed for both egg + larval development (32.7 ± 1.4 days) and for pupal development (11.1 ± 0.1 days). Brood size (parameter 5) showed a strong effect of host species (ANOVA, $F_{24,\ 445} = 19.5$, $P < 0.001$; fig. 3), but varied considerably even within a host species. For example, the size of broods emerging from *A. trifolii* ranged from 1 to 250 individuals, this latter brood being the largest observed in the study. Host species also affected the average mass of individual parasitoids (parameter 6) (ANOVA, $F_{24,\ 484} = 13.13$, $P < 0.001$; fig. 4), and the total mass of individuals in the same brood; i.e., brood mass (parameter 7) (Kruskal–Wallis test, $\chi^2_{24} = 310.24$, $P < 0.001$; fig. 5). Within broods, however, the mass of individual parasitoids was relatively constant. For example, the maximum difference in mass for parasitoids of the same brood developing in *T. ni* ranged between 0.02 mg (0.11 vs. 0.13 mg, $n = 10$) and 0.12 mg (0.14 vs. 0.26 mg, $n = 10$). Across broods, however, the observed maximum difference in individual mass was 0.25 mg (0.08 vs. 0.33 mg, $n = 251$ individuals combined across 26 broods from *T. ni*). Perhaps not surprisingly, the three host species that produced the heaviest broods also produced the largest broods; i.e., *A. trifolii*, *Noctua pronuba*, and *A. prasina*.

Many suitable hosts were identified in families Noctuidae and Nymphalidae in the current study and from previous reports in the literature (table 1). For example, of 18 species tested in the current study for tribe Noctuini (subfamily Noctuinae), only *Xestia c-nigrum* was identified as a non-host. However, membership in closely related tribes was not necessarily an indicator of host suitability. None of the three species of *Spodoptera* (tribe Prodeniini: subfamily Noctuinae) tested in the current study supported *C. vanessae* development.

Discussion

Host range and host suitability

Our overall results show that *C. vanessae* has a broad fundamental host range with many host species in families Noctuidae and Nymphalidae – a finding consistent with previous reports of host associations for this parasitoid. In the current study, we documented egg-to-adult development of *C. vanessae* on 31 species of Lepidoptera (30 Noctuidae, 1 Nymphalidae) for which this information was previously not known, and confirmed three (Noctuidae) previously reported host records. The literature identifies a further 30 host species in eight lepidopteran families; i.e., Noctuidae (15), Nymphalidae (7), Notodontidae (2),

https://doi.org/10.1017/S0007485322000025 Published online by Cambridge University Press

Table 1. Host suitability of lepidopteran species exposed to parasitism by *C. vanessae*

Taxonomy [common name]	GenBank accession no.[a]	Host suitability (n[b])	References[c]
Family Crambidae			
Subfamily Pyraustinae			
Loxostege sticticalis (L.) [N/A[d]]		Host	Meyer (1927)
Family Drepanidae			
Subfamily Thyatirinae			
Habrosyne scripta (Gosse) [scribe moth]	KX281205	Non-host (n = 30)	This study
Family Erebidae			
Subfamily Arctiinae			
Spilosoma virginica (Fabricius) [yellow woolly bear]	KX281216	Non-host (n = 30)	This study
Subfamily Eulepidotinae			
Anticarsia gemmatalis Hübner [velvetbean caterpillar[d]]	KX281227	Non-host (n = 45)	This study
Family Lasiocampidae			
Subfamily Lasiocampinae			
Lasiocampa trifolii Denis & Schiffermüller [grass eggar]		Host	Tobias (1971)
Malacosoma neustria (L.) [lackey moth]		Host	Özbek and Çoruh (2012)
Family Noctuidae			
Subfamily Acontiinae			
Tribe Acontiini			
Acontia lucida (Hufnagel) [N/A]		Host	Papp (2011–2012)
Subfamily Acronictinae			
Tribe Acronictini			
Simyra dentinosa Freyer [N/A]		Host	Karimpour *et al*. (2001)
Subfamily Amphipyrinae			
Tribe Amphipyrini			
Amphipyra tragopoginis (Clerck) [mouse moth]	KX281225	Excellent (n = 1)	This study
Subfamily Oncocnemidinae			
Calophasia opalina (Esper) [N/A]		Host	Papp (1988)
Subfamily Hadeninae			
Tribe Apameini			
Apamea devastator (Brace) [glassy cutworm[d]]	KX281186	Excellent (n = 9)	This study
Apamea lignicolora (Guenée) [wood-coloured quaker]	KX281195	Excellent (n = 35)	This study
Apamea sordens (Hufnagel) [rustic shoulder-knot[d]]	KX281217	Excellent (n = 30); host	This study; Tobias (1971, 1976, 1986)

Tribe Hadenini			
Anarta trifolii (Hufnagel) [clover cutworm[d]]	KX281194	Excellent ($n = 35$)	This study, Tobias (1986)
Dargida diffusa (Walker) [wheat head armyworm[d]]	KX281228	Poor ($n = 33$)	This study
Lacanobia grandis (Guenée) [grand arches moth]	KX281207	Poor ($n = 30$)	This study
Lacanobia oleracea (L.) [bright-line brown-eye]		Host	Morley (1906)
Mamestra brassicae (L.) [cabbage moth[d]]		Host	Tobias (1971, 1976, 1986), Balevski (1995), Balevski (1999)
Mamestra configurata Walker [Bertha armyworm[d]]	KX281210	Poor ($n = 30$)	This study
Sideridis rosea (Harvey) [rosewing moth]	KX281215	Non-host ($n = 35$)	This study
Trichordestra lilacina (Harvey) [aster cutworm]	KX281213	Non-host ($n = 58$)	This study
Subfamily Heliothinae			
Helicoverpa armigera (Hübner) [cotton bollworm[d]]		Host	Tobias (1971, 1976, 1986)
Helicoverpa zea (Boddie) [corn earworm[d]]	KX281206	Poor ($n = 75$)	This study
Heliothis virescens (Fabricius) [tobacco budworm[d]]	KX281229	Excellent ($n = 30$)	This study
Heliothis viriplaca (Hufnagel) [marbled clover]		Host	Zhumanov (1980)
Subfamily Noctuinae			
Tribe Caradrinini			
Caradrina morpheus (Hufnagel) [mottled rustic]	KX281218	Excellent ($n = 35$)	This study
Tribe Eriopygini			
Lacinipolia renigera (Stephens) [bristly cutworm[d]]	KX281208	Non-host ($n = 25$)	This study
Lacinipolia sareta (Smith) [N/A]	KX281209	Non-host ($n = 34$)	This study
Tribe Leucaniini			
Mythimna litoralis (Curtis) [shore wainscot]		Host	Nixon (1974), Tobias (1986)
Mythimna unipuncta (Haworth) [true armyworm[d]]	KX281211	Intermediate ($n = 30$)	This study
Tribe Noctuini			
Abagrotis sp. [N/A[d]]	KX281230	Excellent ($n = 30$)	This study
Abagrotis reedi Buckett [N/A[d]]	KX281188	Excellent ($n = 29$)	This study
Actebia balanitis (Grote) [N/A]	KX281187	Excellent ($n = 27$)	This study
Actebia fugax (Treitschke) [N/A]		Host	Papp (1988)
Actebia praecox (Linnaeus) [Portland moth]		Host	Nixon (1974), Tobias (1986)
Agrotis ipsilon (Hufnagel) [black cutworm[d]]	KX281226	Intermediate ($n = 35$)	This study
Agrotis vancouverensis Grote [Vancouver dart]	KX281190	Intermediate ($n = 30$)	This study
Anaplectoides prasina (Denis & Schiffermüller) [green arches moth]	KX281192	Excellent ($n = 35$)	This study
Cryptocala acadiensis (Bethune) [catocaline dart]	KX28119	Excellent ($n = 35$)	This study
Euxoa auxiliaris (Grote) [army cutworm[d]]	KX281199	Excellent ($n = 30$)	This study

(*Continued*)

Table 1. (*Continued.*)

Taxonomy [common name]	GenBank accession no.[a]	Host suitability (n[b])	References[c]
Euxoa messoria (Harris) [darksided cutworm[d]]	KX281224	Excellent ($n = 11$)	This study
Eurois occulta (Linnaeus) [great brocade[d]]	KX281198	Intermediate ($n = 30$)	This study
Euxoa ochrogaster (Guenée) [red-backed cutworm[d]]	KX281201	Excellent ($n = 30$)	This study
Euxoa satis (Harvey) [N/A]	KX281200	Excellent ($n = 31$)	This study
Euxoa tristicula (Morrison) [early cutworm[d]]	KX281202	Excellent ($n = 22$)	This study
Feltia herilis (Grote) [master's dart[d]]	KX281203	Poor ($n = 30$)	This study
Feltia jaculifera (Guenée) [dingy cutworm[d]]	KX281204	Excellent ($n = 27$)	This study
Noctua pronuba (Linnaeus) [winter cutworm[d]]	KX281212	Excellent ($n = 29$)	This study
Spaelotis clandestina (Harris) [w-marked cutworm[d]]	KX281231	Intermediate ($n = 3$)	This study
Xestia c-nigrum (Linnaeus) [spotted cutworm[d]]	KX281223	Non-host ($n = 33$)	This study
Tribe Prodeniini			
Spodoptera exigua (Hübner) [beet armyworm[d]]	KX281220	Non-host ($n = 29$); host	This study; Tobias (1971), Nixon (1974), Tobias (1976, 1986)
Spodoptera eridania Stoll [southern armyworm[d]]	KX281219	Non-host ($n = 22$)	This study
Spodoptera frugiperda (J. E. Smith) [fall armyworm[d]]	KX281221	Non-host ($n = 18$)	This study
Subfamily Plusiinae			
Tribe Argyrogrammatini			
Chrysodeixis chalcites (Esper) [tomato looper[d]]	KC846709.1	Excellent ($n = 1000$s)	Hervet *et al.* (2014)
Chrysodeixis includens (Walker) [soybean looper[d]]	KX550951	Excellent ($n = 35$)	This study
Trichoplusia ni (Hübner) [cabbage looper[d]]	KX281222	Excellent ($n = 30$)	This study, Hervet *et al.* (2014)
Tribe Plusiini			
Autographa gamma (L.) [silver Y[d]]		Host	Tobias (1971, 1976, 1986)
Anagrapha falcifera (Kirby) [celery looper[d]]	KX281191	Excellent ($n = 35$)	This study
Autographa californica (Speyer) [alfalfa looper[d]]	KX281196	Excellent ($n = 31$)	This study
Cornutiplusia circumflexa (L.) [Essex Y]		Host	Tobias (1971, 1976, 1986)
Macdunnoughia confusa (Stephens) [Dewick's Plusia]		Host	Tobias (1971)
Family Nolidae			
Subfamily Chloephorinae			
Nycteola revayana (Scopoli) [oak nycteoline]		Host	Tobias (1971)
Family Notodontidae			
Subfamily Notodontinae			
Cerura vinula (L.) [puss moth]		Host	Tobias (1954)

Subfamily Notodontinae			
Notodonta ziczac (L.) [pebble prominent]		Host	Aue (1938)
Family Nymphalidae			
Subfamily Heliconiinae			
Speyeria aglaja (L.) [dark green fritillary]		Host	Fahringer (1936), Telenga (1955)
Subfamily Limenitidinae			
Limenitis camilla (L.) [(Eurasian) white admiral]		Host	Morley (1906), Fahringer (1936), Telenga (1955)
Subfamily Nymphalinae			
Aglais io (L.) [European peacock]		Host	Tobias (1971, 1976, 1986)
Aglais milberti (Godart) [Milbert's tortoiseshell]	KX281189	Intermediate ($n = 49$)	This study
Aglais urticae (L.) [small tortoiseshell]		Host	Aubert (1966), Tobias (1971, 1976), Papp (1988), Schwarz and Shaw (2000), Shaw *et al.* (2009)
Nymphalis polychloros (L.) [large tortoiseshell]		Host	Telenga (1955)
Vanessa atalanta (L.) [red admiral]		Host	Nixon (1974), Ford (1976), Haeselbarth (1983), Tobias (1986), Shaw *et al.* (2009), Obregón *et al.* (2015)
Vanessa cardui (L.) [painted lady[d]]		Host	Johnson (1913), Tobias (1971), Nixon (1974), Ford (1976), Tobias (1976), Doğanlar (1982), Tobias (1986), Owen (1987, 1989), Shaw *et al.* (2009), Papp (2011–2012), Stefanescu *et al.* (2012), Obregón *et al.* (2015)
Family Pieridae			
Subfamily Pierinae			
Pieris rapae (L.) [small cabbage white[d]]		Non-host ($n = 39$)	This study
Family Pterophoridae			
Subfamily Pterophorinae			
Cnaemidophorus rhododactylus (Denis & Schiffermüller) [rose plume moth]		Host	Tobias (1971)
Family Sphingidae			
Subfamily Macroglossinae			
Hyles euphorbiae (Linnaeus) [spurge hawk-moth]		Non-host ($n = 17$)	This study
Family Thaumetopoeidae			
Subfamily Arctiinae			
Thaumetopoea processionea (L.) [oak processionary[d]]		Host	Tobias (1971)

Host suitability categories reflect the percentage of successful parasitism (parameter 1) in the current study: excellent ([parameter 1] ≥80%), intermediate (20% ≤ [parameter 1] < 80%), poor (0% < [parameter 1] < 20%), or non-host (egg-to-adult development not supported). A host suitability of 'Host' is based on published reports that otherwise provide no indication of suitability.

[a]The identity of species tested in the current study was verified using COI gene sequences that were deposited in GenBank under the specified accession number.

[b]n = number of caterpillars that experienced parasitism (with the exception of *S. virginica*, on which parasitism could not be observed).

[c]Nearly all the references cited for host records are from Yu *et al.* (2015). Where possible, we tried to verify the information in the original source.

[d]Occasional or common pest of economic significance.

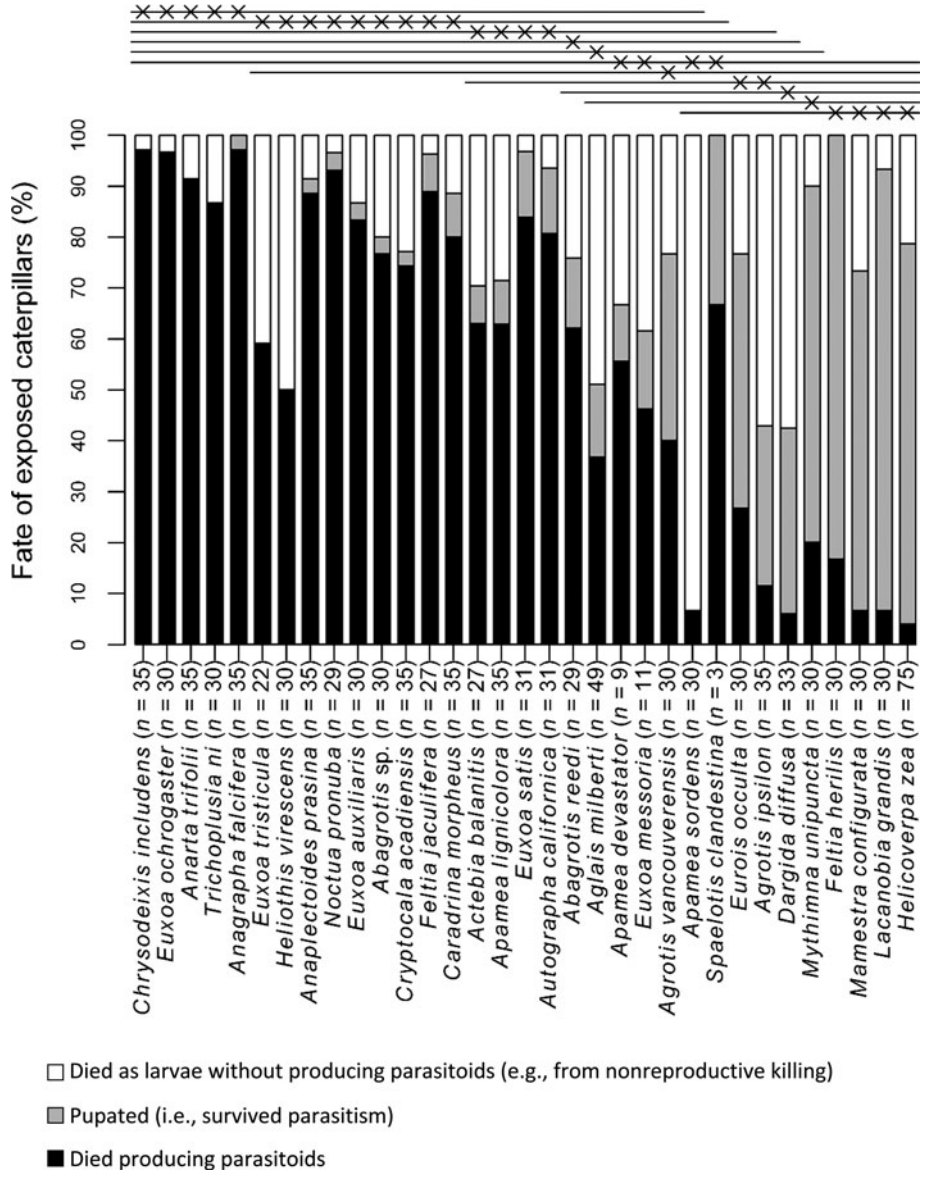

Figure 1. Fate of caterpillars of Lepidoptera species that produced *C. vanessae*. Rate of successful parasitism (i.e., per cent caterpillars that died producing parasitoids from all caterpillars of a particular species that experienced parasitism, not including these that died without producing parasitoids) of each host species was analysed with Fisher's exact tests comparing pairs of species ($\alpha = 0.05$, corrected according to Bonferroni). Significance is indicated by horizontal lines above the boxes. Each 'x' refers to a particular species, and the line it stands on indicates the species that did not have a significantly different successful parasitism rate from the species represented by the 'x'. Species order was chosen according to the most parsimonious display of the results of the statistical analysis. *A. tragopoginis* (Clerck) also produced *C. vanessae* but is not shown here because only one individual experienced parasitism.

Lasiocampidae (2), Nolidae (1), Pterophoridae (1), Crambidae (1), Thaumetopoeidae (1) (table 1). However, some of the earlier reports (i.e., those outside of family Noctuidae and subfamily Nymphalinae) should be interpreted with caution. *C. vanessae* previously has been reported to develop in *S. exigua* (Tobias, 1971), but neither it nor its congeners *Spodoptera eridania* and *S. frugiperda* supported parasitoid development in the current study (table 1). This does not necessarily negate the previous report, as exposure to parasitism may lead to different outcomes. During our tests of *A. trifolii*, subjection to parasitism of one cohort of caterpillars (n = 30) from adults collected in Lethbridge in 2013 resulted in all individuals dying of nonreproductive killing. In contrast, subjection to parasitism for a second cohort of caterpillars (n = 35) from adult *A. trifolii* collected in Lethbridge in 2014 (fig. 1) resulted in parasitoids emerging from almost all individuals. COI sequences of one specimen from both cohorts confirmed the identity as *A. trifolii* (GenBank accession numbers: KX281193 and KX281194). The larger concern is that previous reports of parasitoid–host associations rarely provide strong evidence of accurate identification of species, either for field-collected caterpillars or emerged parasitoids (Nixon, 1974). For this reason, we place greatest weight on reports of *C. vanessae* developing in species of Noctuidae and Nymphalinae, for which species determinations of both parasitoid and host either were verified with DNA barcoding (current study) or which derive from other reliable sources (Shaw *et al.*, 2009; Stefanescu *et al.*, 2012; Hervet *et al.*, 2014). Given its broad host range within these two taxonomic families, it is reasonable to suggest that *C. vanessae* has generalist tendencies, possibly allowing it to also develop in lepidopteran species of other families. However, we suggest that previous reports of hosts, particularly in Crambidae, Lasiocampidae, Nolidae, Notodontidae, Pterophoridae, and Thaumetopoeidae be validated further before acceptance. The broad fundamental host range of *C. vanessae* and the difficulties in collecting sufficient specimens of species in certain taxonomic groups means that more study is required to clearly delineate *C. vanessae*'s fundamental host range.

For consideration in future validation studies, we note that investigations of host range typically only use adult parasitoids recently emerged from cocoons (McCutcheon and Harrison,

https://doi.org/10.1017/S0007485322000025 Published online by Cambridge University Press

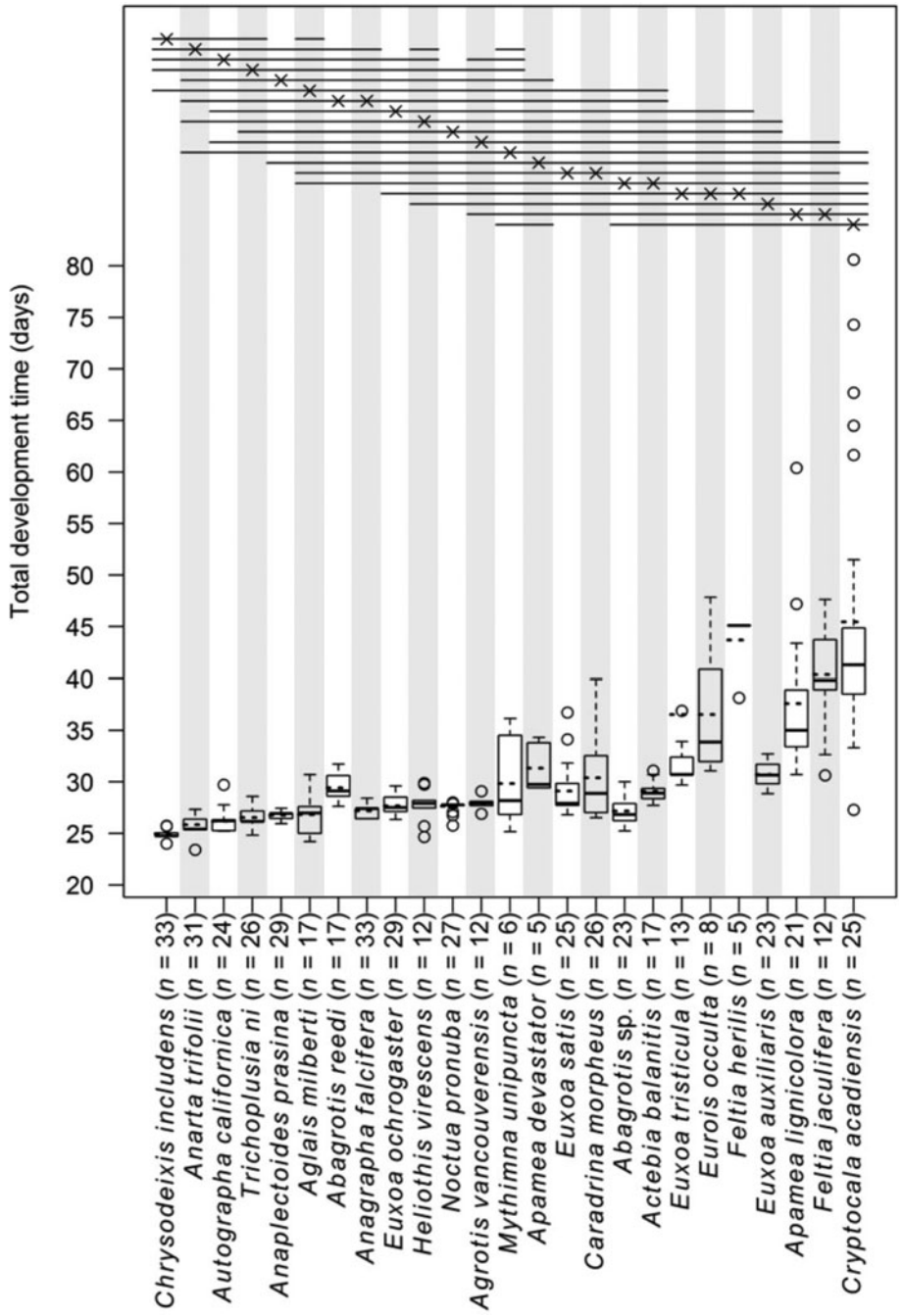

Figure 2. *C. vanessae* time for egg-to-adult development (total development time) in different host species. The boxes delimit the 25th and 75th percentiles (Q1 and Q3, respectively). The upper and lower whiskers indicate the highest observed value not greater than Q3 + 1.5 IQR and the lowest observed value not less than Q1 − 1.5 IQR, respectively (where IQR = interquartile range). Medians and means are represented by solid lines and dotted lines, respectively. Significance (Kruskal–Wallis test followed by post-hoc Dunn test, $\alpha = 0.05$ corrected according to Bonferroni) is indicated by horizontal lines above the boxes. Each species is represented by an 'x' standing on a line that indicates other species with similar successful parasitism rates. Species order was chosen according to the most parsimonious display of the results of the statistical analysis. For statistical rigour, analyses exclude species for which data derived from fewer than five parasitized individuals. Total development times (mean ± SE) in these latter species was: *A. ipsilon* (26.8 ± 0.2 days, $n = 4$), *A. sordens* (30.7 ± 0.8, $n = 2$), *D. diffusa* (31.3 ± 1.3, $n = 3$), *E. messoria* (Harris) (29.0 ± 2.5, $n = 3$), *H. zea* (27.6 ± 0.6, $n = 3$), *L. grandis* (28.2 ± 24.5, $n = 2$), *M. configurata* (27.8 ± 0.02, $n = 2$), and *S. clandestina* (28.5 ± 0.2, $n = 2$).

1987; Goldson *et al.*, 1992; Berndt *et al.*, 2007; Hoddle and Pandey, 2014) or do not indicate parasitoid age. However, host acceptance behaviour by female parasitoids is dynamic; i.e., low-life expectancy females are more likely than high-life expectancy females to accept low-quality hosts (Roitberg *et al.*, 1992; Roitberg *et al.*, 1993; Fletcher *et al.*, 1994; Sirot *et al.*, 1997). Observations with *C. vanessae* in this study showed that older adults accepted a broader diversity of caterpillar species than did younger adults. This has direct implications on the way host-range tests are conducted and suggests preferential use of low-life expectancy females.

Host species that supported more rapid parasitoid development tended to produce larger parasitoid broods and heavier individual parasitoids. This phenomenon has been reported by others (Doyon and Boivin, 2005; Gao *et al.*, 2016) and documents the effect of host species on a range of fitness parameters (Greenblatt and Barbosa, 1981). Conversely, host species characterized by prolonged larval development within the host also were associated with prolonged larval–pupal development outside of the host within cocoons. The two exceptions to this latter observation were for the host species *A. lignicolora* and *Cryptocala acadiensis*, wherein larval development in the host was prolonged, but larval–pupal development within cocoons was abbreviated, relative to other host species.

Comparison of genetic relatedness allows for tentative predictions regarding which lepidopteran species in North America are likely to be hosts for *C. vanessae*. Of particular interest from an economic perspective are results for tribe Noctuini and subfamily

https://doi.org/10.1017/S0007485322000025 Published online by Cambridge University Press

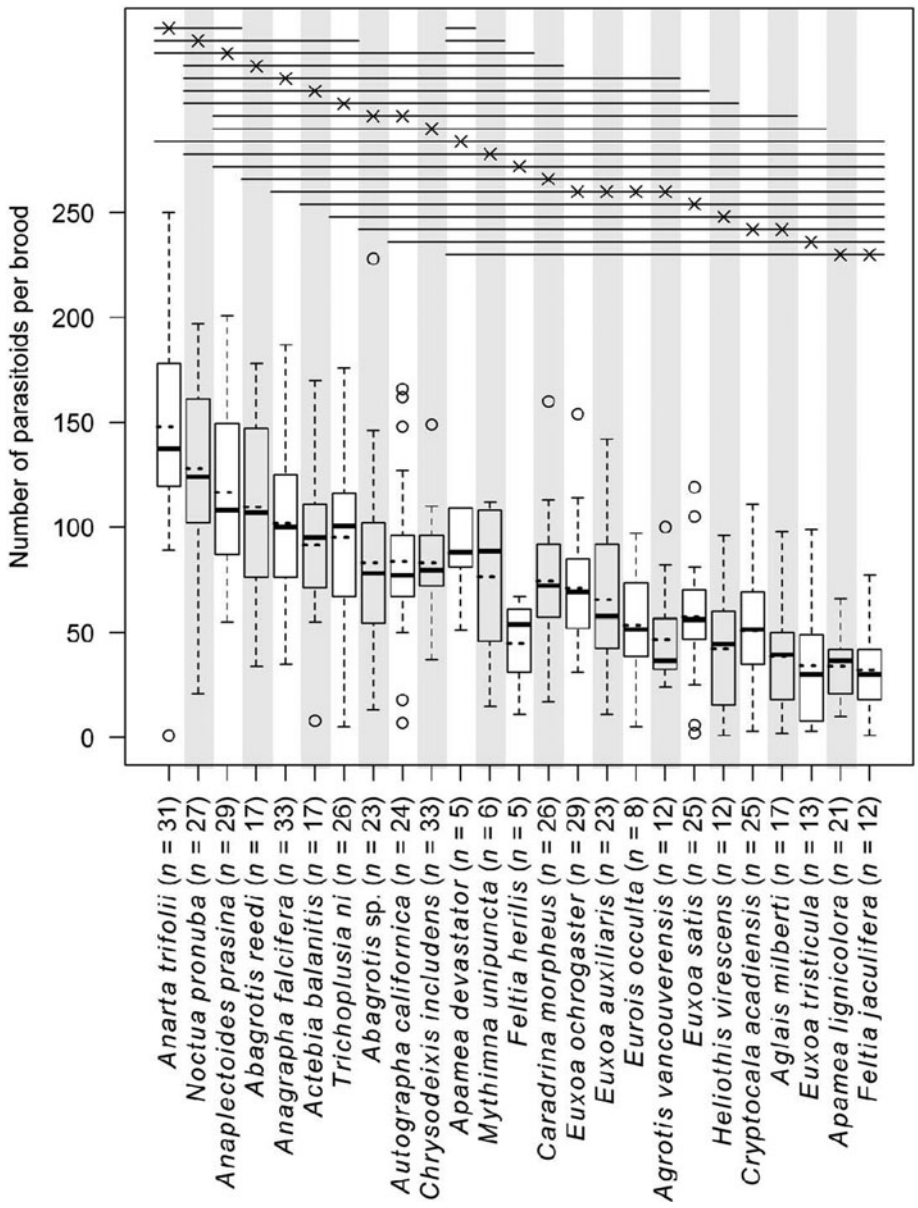

Figure 3. *C. vanessae* brood sizes on each host species. The boxes delimit the 25th and 75th percentiles (Q1 and Q3, respectively). The upper and lower whiskers indicate the highest observed value not greater than Q3 + 1.5 IQR and the lowest observed value not less than Q1 − 1.5 IQR, respectively. Medians and means are represented by solid lines and dotted lines, respectively. Significance (one-way ANOVA followed by post-hoc Tukey's HSD, $\alpha = 0.05$) is indicated by horizontal lines above the boxes. Each species is represented by an 'x' standing on a line that indicates other species with similar brood sizes. Species order was chosen according to the most parsimonious display of the results of the statistical analysis. For statistical rigour, analyses exclude species for which data derived from fewer than five parasitized individuals. Average brood size (mean ± SE) in these latter species was: *A. ipsilon* (71.5 ± 18.0, $n = 4$), *A. tragopoginis* (42, $n = 1$), *A. sordens* (1, $n = 1$), *D. diffusa* (28.0 ± 23.17, $n = 3$), *H. zea* (24.0 ± 16.3, $n = 3$), *L. grandis* (130.0 ± 24.5, $n = 2$), *M. configurata* (119.0 ± 16, $n = 2$), and *S. clandestina* (120.0 ± 20.5, $n = 2$).

Plusiinae. Within the tribe Noctuini, we identified the host suitability for species of *Abagrotis*, *Agrotis*, and *Euxoa* as 'intermediate' or 'excellent' (fig. 1, table 1, Supplementary fig. 1). These genera include many species that are economic pests of crops in North America and elsewhere (Floate, 2017). All four species tested in the subfamily Plusiinae were identified as excellent hosts (fig. 1, table 1, Supplementary fig. 1). Our findings suggest that further research is warranted on use of *C. vanessae* as a potential biocontrol agent against the pest species tested in the current study plus congeneric pest species that we did not test; e.g., pale western cutworm (*A. orthogonia*), granulate cutworm (*Feltia subterranea* (Fabricius)), and grey looper (*Rachiplusia ou* (Guenée)) (Lepidoptera: Noctuidae).

Nonreproductive killing and its implications for host suitability

Our observations of nonreproductive killing – wherein parasitism kills both the host and the parasitoid(s) within – have been reported in a number of studies but the underlying mechanisms are poorly understood (see Abram *et al.*, 2019 and references therein). Parasitoids can overcome the immune system of a host with variable outcomes, by injecting it with calyx fluid, alone or in combination with venom, polydnaviruses, and teratocytes. Larvae of *C. includens* injected with small amounts of calyx fluid from *Microplitis demolitor* Wilkinson (Hymenoptera: Braconidae) will form non-viable larval–pupal intermediates, whereas larvae of *H. virescens* injected with large amounts of this same fluid are little affected (Strand and Dover, 1991). A combination of venom and calyx fluid injected by *Cardiochiles nigriceps* (Viereck) (Hymenoptera: Braconidae) inhibits its host's ability to produce ecdysteroid and subsequently to pupate (Tanaka and Vincon, 1991). By injecting a polydnavirus (*Bracovirus*), parasitoids can inhibit feeding and gut contractions by the host to arrest its development at the prepupal stage (Soller and Lanzrein, 1996; Lanzrein *et al.*, 2012). Braconid teratocytes in the haemocoel of *H. virescens* can prolong the host's larval development, followed by either its death, normal pupation, or the formation of a non-viable larval–pupal intermediate (Vinson, 1970;

https://doi.org/10.1017/S0007485322000025 Published online by Cambridge University Press

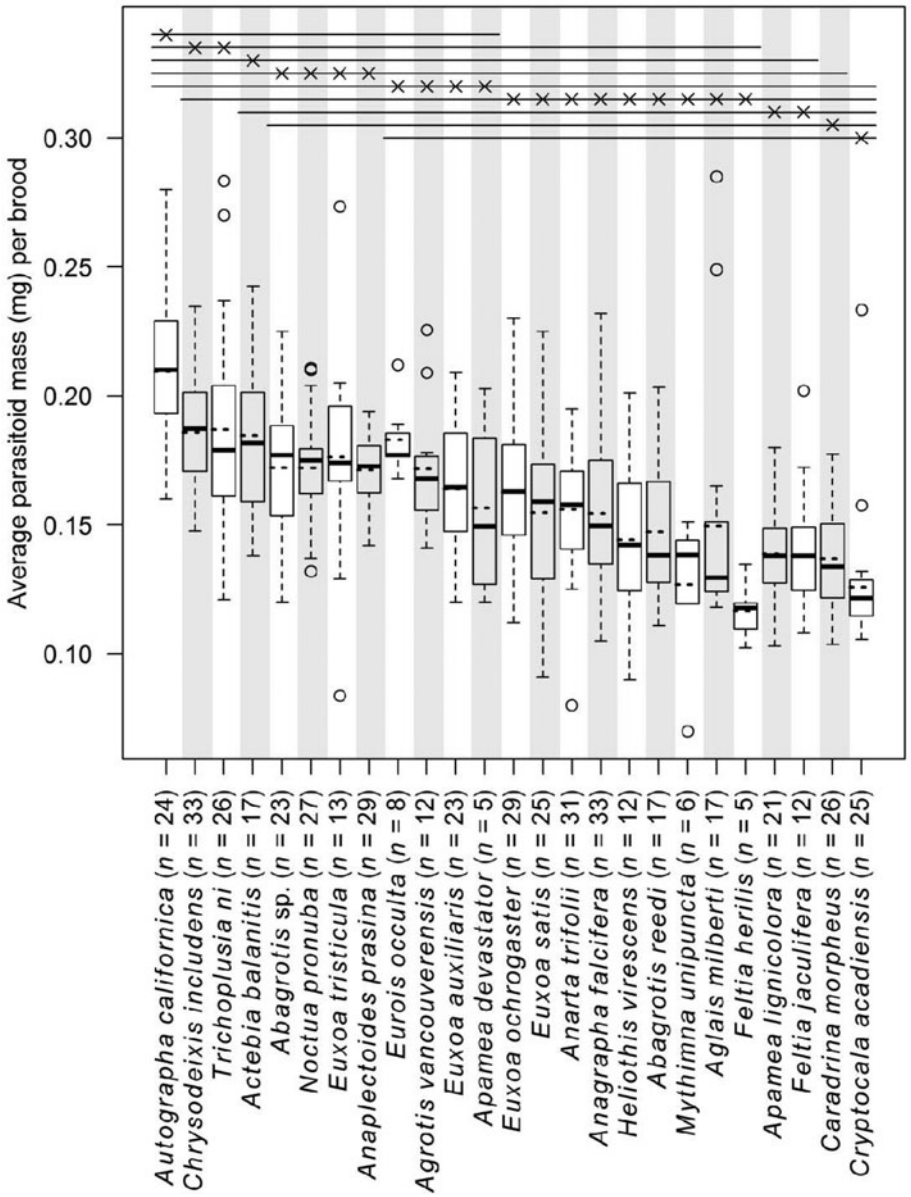

Figure 4. *C. vanessae* average adult individual dry mass per brood (average of 30 or fewer wasps per brood) on each host species. The boxes delimit the 25th and 75th percentiles (Q1 and Q3, respectively). The upper and lower whiskers indicate the highest observed value not greater than Q3 + 1.5 IQR and the lowest observed value not less than Q1 − 1.5 IQR, respectively. Medians and means are represented by solid lines and dotted lines, respectively. Significance (one-way ANOVA followed by post-hoc Tukey's HSD, $\alpha = 0.05$) is indicated by horizontal lines above the boxes. Each species is represented by an 'x' standing on a line that indicates other species with similar individual parasitoid mass. Species order was chosen according to the most parsimonious display of the results of the statistical analysis. For statistical rigour, analyses exclude species for which data derived from fewer than five parasitized individuals. The average mass of individual parasitoids per brood (mean ± SE) in these latter species was: *A. ipsilon* (0.15 ± 0.003 mg, $n = 4$), *A. sordens* (0.24 mg, $n = 1$), *D. diffusa* (0.14 ± 0.023 mg, $n = 3$), *H. zea* (0.17 ± 0.023 mg, $n = 3$), *L. grandis* (0.13 ± 0.004, $n = 2$), *M. configurata* (0.15 ± 0.010 mg, $n = 2$), and *S. clandestina* (0.16 ± 0.024 mg, $n = 2$).

Zhang and Dahlman, 1989). In those studies, the type of response was associated with the number of teratocytes injected, the age of these teratocytes, and the maturity of the host (Zhang and Dahlman, 1989). A higher number of teratocytes, and therefore a higher number of parasitoid larvae, would increase chances of overcoming a host's immune defences. This could explain why adult *C. vanessae* lay more eggs than can survive.

Fundamental vs. ecological niches

Laboratory studies can identify a parasitoid's fundamental niche (operationally defined here as the set of hosts in which it can survive, develop, and reproduce), but not necessarily its realized ecological niche (the smaller subset of hosts in which it actually does survive, develop, and reproduce, given ecologically realistic species interactions). Two factors that highlight this distinction in the current study are host diapause/overwintering dynamics and the role of the plant species on which the host feeds, both of which may affect whether potential hosts are actually utilized successfully under natural conditions.

Variation in use of host species provides insight into the effect of climate on *C. vanessae*'s ecological niche. Nymphalini appear to be the preferred hosts of *C. vanessae* in the south and western part of its Palaearctic range (Stefanescu *et al.*, 2012). However, at northern locations, species of Nymphalini commonly either overwinter as adults or migrate to southern latitudes for the winter (Scott, 1979; Dvořák *et al.*, 2002). *C. vanessae* appears to have solved this issue by overwintering in northern climes within noctuid caterpillars; e.g., Noctuidae parasitized by *C. vanessae* have been collected in October (Italy) and May (England) (Nixon, 1974).

In the current study, parasitoids were observed to develop more slowly on overwintering hosts, thus supporting the previous claim that they overwinter within overwintering hosts. Individuals of univoltine lepidopteran species that overwinter as larvae necessarily are at the larval stage during the fall. Such species were

https://doi.org/10.1017/S0007485322000025 Published online by Cambridge University Press

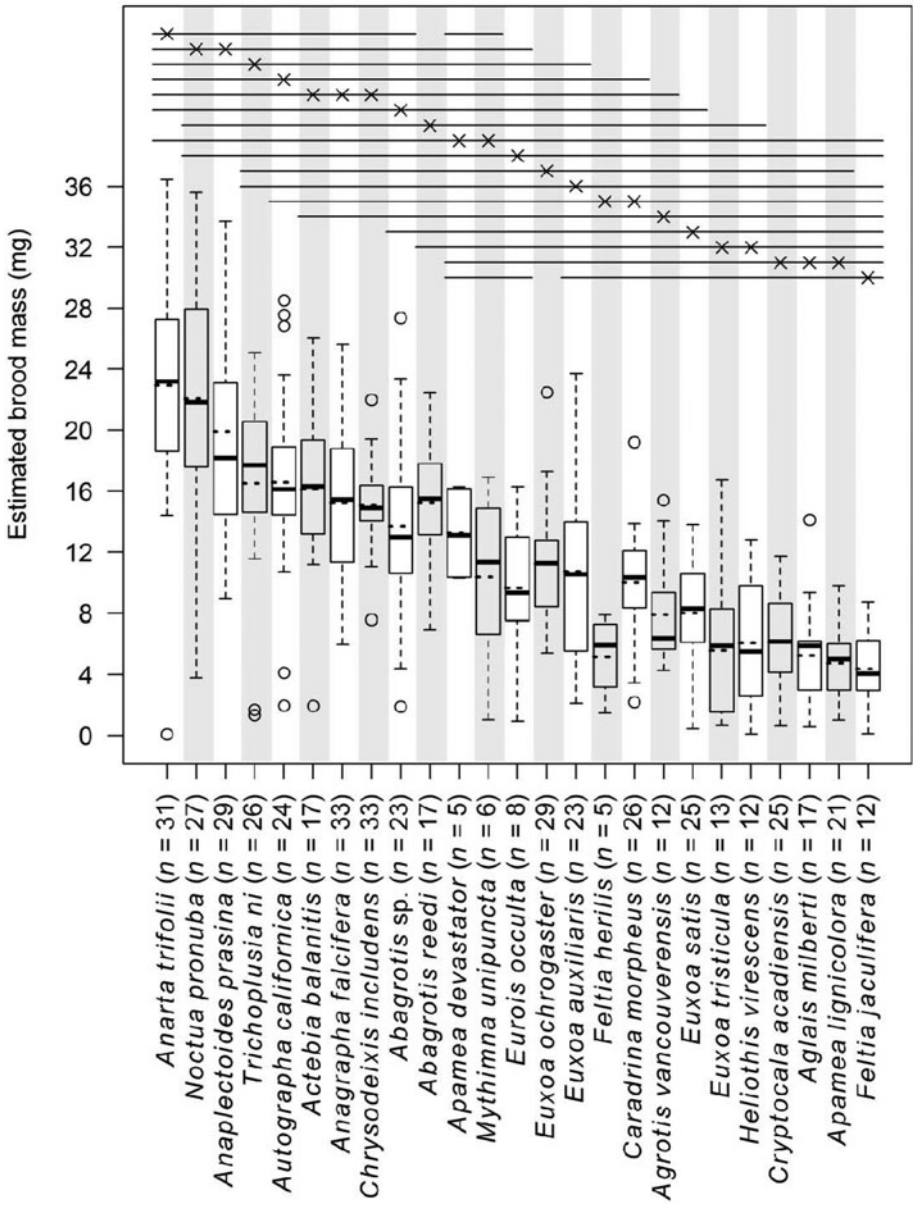

Figure 5. *C. vanessae* estimated brood mass per host (average mass of 30 or fewer wasps per brood × brood size) on each host species. The boxes delimit the 25th and 75th percentiles (Q1 and Q3, respectively). The upper and lower whiskers indicate the highest observed value not greater than Q3 + 1.5 IQR and the lowest observed value not less than Q1 − 1.5 IQR, respectively. Medians and means are represented by solid lines and dotted lines, respectively. Significance (Kruskal–Wallis test followed by post-hoc Dunn tests, $\alpha = 0.05$ corrected according to Bonferroni) is indicated by horizontal lines above the boxes. Each species is represented by an 'x' standing on a line that indicates other species with similar individual parasitoid mass. Species order was chosen according to the most parsimonious display of the results of the statistical analysis. For statistical rigour, analyses exclude species for which data derived from fewer than five parasitized individuals. The average brood mass (mean ± SE) in these latter species was: *A. ipsilon* (10.6 ± 2.4 mg, $n = 4$), *A. sordens* (0.2 mg, $n = 1$), *D. diffusa* (3.9 ± 3.1 mg, $n = 3$), *H. zea* (4.4 ± 3.1 mg, $n = 3$), *L. grandis* (17.2 ± 3.7 mg, $n = 2$), *M. configurata* (18.4 ± 3.6 mg, $n = 2$), and *S. clandestina* (18.7 ± 0.5 mg, $n = 2$).

subjected to parasitism by *C. vanessae* in the fall while they were diapausing or soon to do so, although they remained at 20 °C under experimental conditions throughout their entire development. In fig. 2, the species between (and including) *A. devastator* and *C. acadiensis* (Bethune) (Lepidoptera: Noctuidae) all overwinter as larvae and are univoltine throughout their range, except for *Euxoa tristicula* (Morrison), a species that can be multivoltine. However, personal observations and previous studies (Jacobson, 1969) show that *E. tristicula* must necessarily go through a diapause at the larval stage, which lasts at least 2 months at 20 °C. Parasitoids took the longest time to develop within these diapausing hosts. This indicates that development within overwintering hosts likely triggered parasitoids to initiate overwintering, but after a short diapause (from a few days, up to 2 months in *C. acadiensis*; fig. 2) parasitoids eventually resumed their development, likely because of conditions non-optimal for overwintering (including too high temperature, too long light cycle, and too strong light intensity). Regarding the other species in fig. 2, only *Abagrotis* spp., *Agrotis vancouverensis*, and *A. prasina* also overwinter as larvae and are likely to be obligately univoltine throughout their range. The reason why parasitoids did not attempt to diapause within these hosts is unknown.

The plant species on which hosts feed are a crucial aspect of parasitoid–host specificity. While this topic is not our central focus here, we include some additional comments about it in Supplementary Text 1. The current study focuses on host suitability. Therefore, results reflect the fundamental host range of *C. vanessae*. Future studies should attempt to bridge this gap by studying host range under more natural conditions.

Speculation on the fate of C. vanessae *in North America*

We expect *C. vanessae* to become broadly established across North America beyond its current known distribution; i.e., southern regions of Ontario, Manitoba, and Alberta (this study, Hervet *et al.*, 2014). *C. vanessae* can develop in numerous host species.

https://doi.org/10.1017/S0007485322000025 Published online by Cambridge University Press

Into each host, they lay a supernumerary egg load to optimize offspring production for hosts of varying size and possibly also to increase their chances of overcoming the host immune system. The larvae display an antagonistic behaviour that potentially confers inter-specific competitiveness and increase their ability to develop in a broader range of hosts (Hervet *et al*., 2018). Finally, larvae have a facultative diapause that allows for multiple generations until cooler temperatures induce a larval overwintering period.

We do not know the extent to which *C. vanessae* will suppress pest populations, although recent work identifies it as a potential biocontrol agent of the pests *T. ni* and *C. chalcites* (Pacheco *et al*., 2018; Pacheco *et al*., 2021). Future studies will be required to characterize the effects of *C. vanessae* on populations of both pest and non-pest species of Lepidoptera and to provide greater insight into its expansion and establishment as a new member of the North American parasitoid community.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0007485322000025.

Acknowledgements. For technical support and enthusiasm, we thank Kayla Johnson, Jayden Dyck, Briana Smith, Braidon Schaufert, and Marko Mićović. Bob Byers and Paul Coghlin were instrumental in helping to collect and identify species and provided additional support. We thank Patrick M. Barks for the montage of the video 'Parasitoid larvae (*Cotesia vanessae*) emerging from their caterpillar host (*Trichoplusia ni*)'. This research was supported by the Canola Council of Canada (Canola Agronomic Research Program Project 2012-1, to K.D.F.), Alberta Conservation Association (Grants in Biodiversity, to V.A.D.H.), and the Natural Science and Engineering Research Council of Canada (NSERC Discovery Grant RGPIN-2015-05486 to R.A.L.).

Conflict of interest. The authors declare none.

References

Abram PK, Brodeur J, Urbaneja A and Tena A (2019) Nonreproductive effects of insect parasitoids on their hosts. *Annual Review of Entomology* **64**, 259–276.

Aubert JF (1966) Liste d'identification no. 7 (Présentée par le Service d'Identification des Entomophages). *Entomophaga* **11**, 135–151.

Aue AUE (1938) Entomologisches Allerlei XVI. *Entomologische Rundschau* **55**, 602–604.

Balevski NA (1995) New phytophagous hosts of hymenopterous braconid parasitoids (Hymenoptera, Braconidae) in Bulgaria. *Acta Entomologica Bulgarica* **2**, 69–73.

Balevski NA (1999) *Catalogue of the Braconid Parasitoids (Hymenoptera: Braconidae) Isolated from Various Phytophagous Insect Hosts in Bulgaria*. Sofia & Moscow: Pensoft Publishers.

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J and Wheeler DJ (2005) GenBank. *Nucleic Acids Research* **33**, D34–D38.

Berndt LA, Mansfield S and Withers TM (2007) A method for host range testing of a biological control agent for *Uraba lugens*. *New Zealand Plant Protection* **60**, 286–290.

Dinno A (2016) dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums. R package version 1.3.2. https://CRAN.R-project.org/package=dunn.test.

Doğanlar M (1982) Hymenopter parasites of some lepidopterous pests in eastern Anatolia. *Turkiye Bitki Koruma Dergisi* **6**, 197–205.

Doyon J and Boivin G (2005) The effect of development time on the fitness of female *Trichogramma evanescens*. *Journal of Insect Science* **5**:4.

Dvořák L, Belicek J and Fric Z (2002) Observations of overwintering nymphalid butterflies in underground shelters in SW and W Bohemia (Czech Republic) (Lepidoptera: Nymphalidae: Nymphalini). *Journal of Research on the Lepidoptera* **41**, 45–52.

Eichlin TD (1975) Guide to the adult and larval Plusiinae of California (Lepidoptera: Noctuidae). *California Department of Food and Agriculture Occasional Papers in Entomology* No. 21, 1–73.

Eichlin TD and Cunningham HB (1978) The Plusiinae (Lepidoptera: Noctuidae) of America north of Mexico, emphasizing genitalic and larval morphology. *United States Department of Agriculture Technical Bulletin* No. 1567, 1–122.

Fahringer J (1936) Opuscula braconologica. Band 4. Palaearktische Region. Lieferung 1–3. Wien, Austria, Wagner, F.

Fernández-Triana J, Shaw MR, Boudreault C, Beaudin M and Broad GR (2020) Annotated and illustrated world checklist of Microgastrinae parasitoid wasps (Hymenoptera, Braconidae). *Zookeys* **920**, 1–1089.

Fletcher JP, Hughes JP and Harvey IF (1994) Life expectancy and egg load affect oviposition decisions of a solitary parasitoid. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **258**, 163–167.

Floate KD (2017) Cutworm pests of crops on the Canadian Prairies: identification and management field guide. Agriculture and Agri-Food Canada, Lethbridge, Alberta, Catalogue No. A59-42/2017E-PDF, AAFC publication No. 12614E.

Floate KD and Hervet VA (2017) Noctuid (Lepidoptera: Noctuidae) pests of canola in North America. In Reddy GVP (ed.), *Integrated Management of Insect Pests on Canola and Other Brassica Oilseed Crops*. Wallingford, Oxfordshire, UK and Boston, MA, USA: The Centre for Agriculture and Bioscience International (CABI), pp. 96–113.

Ford RLE (1976) Influence of the Microgasterini on the populations of British Rhopalocera (Hymenoptera: Braconidae). *Entomologist's Gazette* **27**, 205–210.

Fox J and Weisberg S (2011) *An R Companion to Applied Regression*, 2nd Edn. Thousand Oaks, CA: Sage. http://socserv.socsci.mcmaster.ca/jfox/Books/Companion.

Gao S, Tang Y, Wei K, Wang X, Yang Z and Zhang Y (2016) Relationships between body size and parasitic fitness and offspring performance of *Sclerodermus pupariae* Yang *et* Yao (Hymenoptera: Bethylidae). *PLoS ONE* **11**, e0156831. doi: 10.1371/journal.pone.0156831

Godfray HCJ (1994) *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton, NJ, USA: Princeton University Press.

Goldson S, McNeill M, Phillips C and Proffitt J (1992) Host specificity testing and suitability of the parasitoid *Microctonus hyperodae* (Hym.: Braconidae, Euphorinae) as a biological control agent of *Listronotus bonariensis* (Col.: Curculionidae) in New Zealand. *Entomophaga* **37**, 483–498.

Greenblatt JA and Barbosa P (1981) Effects of host's diet on two pupal parasitoids of the gypsy moth: *Brachymeria intermedia* (Nees) and *Coccygomimus turionellae* (L.). *Journal of Applied Ecology* **18**, 1–10.

Grisdale D (1973) Large volume preparation and processing of a synthetic diet for insect rearing. *The Canadian Entomologist* **105**, 1553–1557.

Haeselbarth E (1983) 9th Determination list of entomophagous insects. West Palearctic Regional Section, International Organization for Biological Control, Bulletin 6(1), pp. 1–49.

Hajibabaei M, Janzen DH, Burns JM, Hallwachs W and Hebert PD (2006) DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 968–971.

Harvey JA (2005) Factors affecting the evolution of development strategies in parasitoid wasps: the importance of functional constraints and incorporating complexity. *Entomologia Experimentalis et Applicata* **117**, 1–13.

Hebert PDN, Cywinska A, Ball SL and deWaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **270**, 313–321.

Hervet VAD (2017) *Host range and multitrophic interactions between the parasitoid Cotesia vanessae (Hymenoptera: Braconidae) and Noctuidae (Lepidoptera) hosts in North America* (Doctoral dissertation). Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada.

Hervet VAD, Murillo H, Fernández-Triana JL, Shaw MR, Laird RA and Floate KD (2014) First report of *Cotesia vanessae* (Hymenoptera: Braconidae) in North America. *The Canadian Entomologist* **146**, 560–566.

Hervet VAD, Laird RA and Floate KD (2016) A review of the McMorran diet for rearing Lepidoptera species with addition of a further 39 species. *Journal of Insect Science* **19**, 1–7.

Hervet VAD, Laird RA and Floate KD (2018) Siblicidal behaviour by larvae of the gregarious parasitoid *Cotesia vanessae*. *Journal of Hymenoptera Research* **67**, 55–62.

Hoddle MS and Pandey R (2014) Host range testing of *Tamarixia radiata* (Hymenoptera: Eulophidae) sourced from the Punjab of Pakistan for classical biological control of *Diaphorina citri* (Hemiptera: Liviidae: Euphyllurinae: Diaphorinini) in California. *Journal of Economic Entomology* **107**, 125–136.

Jacobson LA (1969) Biology of *Euxoa tristicula* in the field and laboratory. *Journal of Economic Entomology* **62**, 1053–1054.

Johnson W (1913) Some Irish Ichneumonidae and Braconidae. *The Irish Naturalist* **22**, 138–141.

Karimpour Y, Fathipour Y, Talebi A and Moharramipour S (2001) Report of two endoparasitoid wasps, *Cotesia ofella* (Nixon) and *C. vanessae* (Reinhard) (Hym.: Braconidae) on larvae of *Simyra dentinosa* Freyer (Lep.: Noctuidae) from Iran. *Journal of Entomological Society of Iran* **21**, 105–106.

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**, 111–120.

Lanzrein B, Pfister-Wilhelm R, Kaeslin M, Wespi G and Roth T (2012) The orchestrated manipulation of the host by *Chelonus inanitus* and its polydnavirus. In Beckage N and Drezen J-M (eds), *Parasitoid Viruses: Symbionts and Pathogens*. Amsterdam, Boston, Heidelberg, London, New York, Oxford, Paris, San Diego, San Francisco, Singapore, Sidney, Tokyo: Elsevier, Academic Press, pp. 169–178.

Lavine MD and Strand MR (2002) Insect hemocytes and their role in immunity. *Insect Biochemistry and Molecular Biology* **32**, 1295–1309.

Lindgren P and Green G (1984) Suppression and management of cabbage looper populations. United States Department of Agriculture, Technical Bulletin No. 1684.

McCutcheon G and Harrison W (1987) Host range and development of *Microplitis rufiventris* (Hymenoptera: Braconidae), an imported parasitoid of several lepidopterous pests. *Environmental Entomology* **16**, 855–858.

McMillan E (1935) A survey of cutworm damage in a specimen locality in Saskatchewan. *Journal of Economic Entomology* **28**, 428–431.

McMorran A (1965) A synthetic diet for the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). *The Canadian Entomologist* **97**, 58–62.

Meyer NF (1927) Parasites (Ichneumonidae and Braconidae) bred in Russia from injurious insects during 1881–1926. *Izvestiya Otdela Prikladnoi Entomologii* **3**, 75–91.

Morley C (1906) Notes on the hymenopterous family Microgasteridae. *The Entomologist* **39**, 99–105.

Murillo H, Hunt DWA and VanLaerhoven SL (2013) First records of *Chrysodeixis chalcites* (Lepidoptera: Noctuidae: Plusiinae) for east-central Canada. *The Canadian Entomologist* **145**, 338–342.

Nixon GEJ (1974) A revision of the north-western European species of the *glomeratus*-group of *Apanteles* Förster (Hymenoptera: Braconidae). *Bulletin of Entomological Research* **64**, 453–524.

Obregón R, Shaw MR, Fernández-Haeger J and Jordano D (2015) Parasitoid and ant interactions of some Iberian butterflies (Insecta: Lepidoptera). *SHILAP Revista de Lepidopterología* **43**, 439–454.

Owen D (1987) Winter breeding by *Cynthia cardui* (L.) (Lepidoptera: Nymphalidae) in Crete and Madeira, and the possible significance of parasitoids in initiating migration. *Entomologist's Gazette* **38**, 11–12.

Owen D (1989) Possible origin of the migratory *Cynthia cardui* L. (Lep.: Nymphalidae). *Entomologist's Record and Journal of Variation* **101**, 51–52.

Özbek H and Çoruh S (2012) Larval parasitoids and larval diseases of *Malacosoma neustria* L. (Lepidoptera: Lasiocampidae) detected in Erzurum Province, Turkey. *Turkish Journal of Zoology* **36**, 447–459.

Pacheco HM, Vanlaerhoven SL, García MÁM and Hunt DW (2018) Food web associations and effect of trophic resources and environmental factors on parasitoids expanding their host range into non-native hosts. *Entomologia Experimentalis et Applicata* **166**, 277–288.

Pacheco HM, Vanlaerhoven S and Garcia MAM (2021) Host suitability of *Trichoplusia ni* and *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) for native and nonnative parasitoids expanding their host range. *The Canadian Entomologist* **153**, 137–149.

Papp J (1988) Contribution to the braconid fauna of Hungary, VIII. Microgasterinae (Hymenoptera: Braconidae). *Folia Entomologica Hungarica* **44**, 167–184.

Papp J (2011–2012) A contribution to the braconid fauna of Israel (Hymenoptera: Braconidae). *Israel Journal of Entomology* **41–42**, 165–219.

Ramirez-Cabral NYZ, Kumar L and Shabani F (2017) Future climate scenarios project a decrease in the risk of fall armyworm outbreaks. *Journal of Agricultural Science* **155**, 1219–1238.

Ratnasingham S and Hebert PDN (2007) BOLD the barcode of life data system (http://www.barcodinglife.org). *Molecular Ecology Notes* **7**, 355–364.

R Core Team (2016) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Roitberg BD, Mangel M, Lalonde RG, Roitberg CA, van Alphen JJ and Vet L (1992) Seasonal dynamic shifts in patch exploitation by parasitic wasps. *Behavioral Ecology* **3**, 156–165.

Roitberg BD, Sircom J, Roitberg CA, van Alphen JJ and Mangel M (1993) Life expectancy and reproduction. *Nature* **364**, 108.

Roitberg BD, Boivin G and Vet LEM (2001) Fitness, parasitoids, and biological control: an opinion. *The Canadian Entomologist* **133**, 429–438.

Rose DJW, Dewhurst CF and Page WW (1995) The bionomics of the African armyworm *Spodoptera exempta* in relation to its status as a migrant pest. *Integrated Pest Management Reviews* **1**, 49–64.

Schmidt BC (2015) Revision of the *Lacinipolia vicina* (Grote) complex (Noctuidae, Noctuinae, Eriopygini). *Zookeys* **527**, 103–126.

Schwarz M and Shaw MR (2000) Western Palaearctic Cryptinae (Hymenoptera: Ichneumonidae) in the National Museums of Scotland, with nomenclatural changes, taxonomic notes, rearing records and special reference to the British check list. Part 3. Tribe Phygadeuontini, subtribea Chiroticina, Acrolytina, Hemitelina and Gelina (excluding *Gelis*), with descriptions of new species. *Entomologist's Gazette* **51**, 147–186.

Scott JA (1979) Hibernal diapause of North American Papilionoidea and Hesperioidea. *Journal of Research on the Lepidoptera* **18**, 171–200.

Sharma HC, Gaur PM, Srinivasan S and Gowda CLL (2014) Exploiting host plant resistance for pest management in chickpea. *Legume Perspectives* **3**, 25–28.

Shaw MR, Stefanescu C and van Nouhuys S (2009) Parasitoids of European butterflies. In Settele J, Shereeve TG, Konvicka M and van Dyck H (eds), *Ecology of Butterflies in Europe*. Cambridge: Cambridge University Press, pp. 130–156.

Sirot E, Ploye H and Bernstein C (1997) State dependent superparasitism in a solitary parasitoid: egg load and survival. *Behavioral Ecology* **8**, 226–232.

Soller M and Lanzrein B (1996) Polydnavirus and venom of the egg-larval parasitoid *Chelonus inanitus* (Braconidae) induce developmental arrest in the prepupa of its host *Spodoptera littoralis* (Noctuidae). *Journal of Insect Physiology* **42**, 471–481.

Stefanescu C, Askew RR, Corbera J and Shaw MR (2012) Parasitism and migration in southern Palaearctic populations of the painted lady butterfly, *Vanessa cardui* (Lepidoptera: Nymphalidae). *European Journal of Entomology* **109**, 85–94.

Strand MR and Dover BA (1991) Developmental disruption of *Pseudoplusia includens* and *Heliothis virescens* larvae by calyx fluid and venom of *Microplitis demolitor*. *Archives of Insect Biochemistry and Physiology* **18**, 131–145.

Tanaka T and Vincon SB (1991) Depression of prothoracic gland activity of *Heliothis virescens* by venom and calyx fluids from the parasitoid, *Cardiochiles nigriceps*. *Journal of Insect Physiology* **37**, 139–144.

Telenga NA (1955) Braconidae, subfamily Microgasterinae, subfamily Agathinae. *Fauna USSR, Hymenoptera* **5**, 311 pp.

Tobias VI (1954) Materials for the fauna and biology of the Ichneumons of the family Braconidae of western Kazakhstan. *Trudy Zoologicheskogo Instituta* **16**, 417–426.

Tobias VI (1971) Review of the Braconidae (Hymenoptera) of the U.S.S.R. *Trudy Vsesoyuznogo Entomologicheskogo Obshchestva* **54**, 156–268.

Tobias VI (1976) *Braconids of the Caucasus (Hymenoptera, Braconidae). Opred. Faune SSSR No. 110*. Leningrad: Nauka Press.

Tobias VI (1986) Acaeliinae, Cardiochilinae, Microgastrinae, Miracinae. Supplement. In Medvedev GS (ed). *Opredelitel Nasekomych Evrospeiskoi Tsasti SSSR 3, Peredpontdatokrylye 4. Opred*. Faune SSSR, pp. 336–501.

https://doi.org/10.1017/S0007485322000025 Published online by Cambridge University Press

USDA (1977) The biologic and economic assessment of Endrin. United States Department of Agriculture, Technical Bulletin No. 1623.

Vinson SB (1970) Development and possible functions of teratocytes in the host–parasite association. *Journal of Invertebrate Pathology* **16**, 93–101.

Wapshere A (1974) A strategy for evaluating the safety of organisms for biological weed control. *Annals of Applied Biology* **77**, 201–211.

Yu DSK, Van Achterberg C and Horstmann K (2015) *Taxapad 2016, Ichneumonoidea 2015*. Database on flash-drive. Version 20160623. Nepean, Ontario, Canada.

Zahiri R (2012) *Molecular systematics of Noctuoidea (Insecta, Lepidoptera)* (Doctoral dissertation). Department of Biology, Turun Yliopisto, Turku, Finland.

Zhang BC (1994) *Index of Economically Important Lepidoptera*. Wallingford: CABI.

Zhang D and Dahlman DL (1989) *Microplitis croceipes* teratocytes cause developmental arrest of *Heliothis virescens* larvae. *Archives of Insect Biochemistry and Physiology* **12**, 51–61.

Zhumanov BZ (1980) *Chloridea viriplaca* and its parasites. *Zashchita Rastenii* **8**, 42.

https://doi.org/10.1017/S0007485322000025 Published online by Cambridge University Press