Your article proof EN-13-172 from Environmental Entomology is available for download

_____

Dear Author,

This letter describes how to access and proof your article EN-13-172 slated for an upcoming issue of Environmental Entomology. If you have any questions, contact the Journal Production Manager noted below.

Please refer to this URL address:

http://rapidproof.cadmus.com/RapidProof/retrieval/index.jsp

Login: Your e-mail address
Password: 2kRU4B5cjPo2

This site contains one file. You will need to have Adobe Acrobat Reader software to read this file. This is free software that is available for user downloading at http://www.adobe.com/products/acrobat/readstep.html

This file contains the following:
1) Proofreading marks for your reference
2) ESA Publishing Agreement (copyright transfer form)--you must complete, sign, and return with proof
3) Reprint Order & Page Charges Form--you must complete and return with proof
4) Page proofs of your article and author queries, if applicable

After printing the PDF file, please read the page proofs carefully and limit any changes to those that alter scientific meaning, such as ambiguous sentences or errors in tables.
* Mark clearly legible corrections in red directly on the page proof, preferably in the margins;
* Answer all author queries, indicated as AQ:1, AQ:2, AQ:3, etc., in the margins of the proofs and listed on the last page of the proofs; and
* Verify that tables, figures, and figure captions are correct and in proper sequence.

Authors are entirely responsible for marking all errors.

Any changes you make that are not the result of editor or printer error or response to a query will be billed to you at a cost of $6.00 per affected line of text. Changes in figures will be charged at $50.00 per figure. Color proofs of color figures are available upon request.

Publishing Agreement
Your article will not be published unless this form is completed and returned with your page proof.

Ordering Reprints
If you wish to order reprints, please use the Reprint Order & Page Charges Form. (Please return the form even if you do not wish to order any reprints.) You can order paper reprints, an Open Access Reprint, or both. An Open Access Reprint is a PDF file of your article that is part of the online journal article and is freely accessible to anyone. You may immediately obtain the Open Access Reprint PDF file of your article from the ESA online journal website and post it on your home page and on other publicly accessible servers. You may also use it to send your article to colleagues as an e-mail attachment and to make as many "electronic reprints" of your article as you wish. Reprint orders for articles must be received before the issue goes to press and should be mailed to the address on the form at the same time you return your page proofs.

Within 48 hours, please e-mail (preferred), fax, or mail the page proofs and forms to the address below. Please do not send duplicates. Also include the completed Publishing Agreement and Reprint Order & Page Charges Form--otherwise publication of your article will be delayed.

Thank you for publishing with ESA. We hope that your experience has been positive. If you have any questions, contact the Journal Production Manager noted below.

Bert Shayte
Journal Production Manager
Environmental Entomology
Cadmus Communications, a Cenveo company
Publisher Services Group
5457 Twin Knolls Road, Suite 200
Columbia, MD 21045
Tel.: (410) 691-6290
Fax: 1-877-705-1381
E-mail: shayteb@cadmus.com
www.cadmus.com

Proofreader's Marks

MARK	EXPLANATION	EXAMPLE
ℰ	TAKE OUT CHARACTER INDICATED	ℰ Your prooƒf.
∧	LEFT OUT, INSERT	u Yor proof. ∧
#	INSERT SPACE	# Yourproof. ∧
9	TURN INVERTED LETTER	Your pɹoof. ∧
×	BROKEN LETTER	× Your prɤof.
eq#	EVEN SPACE	eq# A good proof.
◡	CLOSE UP: NO SPACE	Your pro⌒of.
Tr	TRANSPOSE	Tr A proof good
wf	WRONG FONT	wf Your proof.
lc	LOWER CASE	lc Your Proof.
≡ caps	CAPITALS	Your proof. caps Your proof.
‾ ital	ITALIC	Your proof. ital Your proof.
rom	ROMAN, NON ITALIC	rom Your proof.
bf	BOLD FACE	Your proof. bf Your proof.
...... stet	LET IT STAND	Your proof. stet Your proof.
out sc.	DELETE, SEE COPY	out sc. She Our proof. ∧
spell out	SPELL OUT	spell out Queen Eliz.
¶	START PARAGRAPH	¶ read. Your
no ¶	NO PARAGRAPH: RUN IN	no marked. ¶ Your proof.
⌞⌟	LOWER	⌞Your proof.⌟

MARK	EXPLANATION	EXAMPLE
⌐	RAISE	⌐ Your proof.
⊏	MOVE LEFT	⊏ Your proof.
⊐	MOVE RIGHT	⊐ Your proof.
‖	ALIGN TYPE	‖⊏ Three dogs. ‖ Two horses.
=	STRAIGHTEN LINE	= Your proof.
⊙	INSERT PERIOD	⊙ Your proof ∧
,/	INSERT COMMA	,/ Your proof ∧
:/	INSERT COLON	:/ Your proof ∧
;/	INSERT SEMICOLON	;/ Your proof ∧
∨	INSERT APOSTROPHE	∨ Your mans proof. ∧
∨ ∨	INSERT QUOTATION MARKS	∨∨ Marked it proof ∧ ∧
=/	INSERT HYPHEN	=/ A proofmark. ∧
/	INSERT EXCLAMATION MARK	/ Prove it ∧
?	INSERT QUESTION MARK	? Is it right ∧
⓺	QUERY FOR AUTHOR	⓺ was Your proof read by ∧
⊏/⊐	INSERT BRACKETS	⊏/⊐ The Smith girl ∧ ∧
(/)	INSERT PARENTHESES	(/) Your proof 1 ∧ ∧
⅟m	INSERT 1-EM DASH	⅟m Your proof. ∧
▯	INDENT 1 EM	▯Your proof
▯▯	INDENT 2 EMS	▯▯Your proof.
▯▯▯	INDENT 3 EMS	▯▯▯ Your proof.

Environmental Entomology

Publishing Agreement

You must submit this form with your proofs to the address provided with the page proofs. Complete and submit this form only when you have received your page proofs.

Manuscript Number: **EN-13-172**

Manuscript Title: _____

_____

_____

Author(s): _____

_____

1. I hereby transfer to the Entomological Society of America (ESA) full and complete ownership of any copyright I may have with respect to the work named above. If there is more than one author, I also certify that I am the duly authorized agent for any or all other authors of the work. (If the work is written as part of official duties as an employee of the United States or Canadian governments [federal, not state], check the box below. If the work is subject to the NIH Public Access Policy, check the box below.)

❑ This certifies that the work named above was prepared by an employee of the United States or Canadian governments as part of official duties and therefore cannot be copyrighted by ESA.

❑ The above work is subject to the NIH Public Access Policy because the research was funded by NIH. If this box is checked, the final published manuscript will be deposited by ESA on behalf of the author to PubMed Central (PMC). If the author purchased an Open Access Reprint (free PDF) of the article, it will be made accessible on PMC once the author approves the online version after being notified by PMC. If there is no Open Access Reprint available, then ESA will set a 12 month embargo period on access from the date of publication in the journal.

2. I understand that ESA assesses editorial review charges that are based upon the total number of pages published, reprint charges for any number of reprints I order, special charges for any color reproduction I require, and alteration charges for any changes deemed as author alterations made to page proofs of the article named above.

    Editorial review charges are billed to the senior or corresponding author within one month of publication at rates established by the ESA Governing Board. **Current rates are listed below and are applied based on at least one of the authors' membership status at the time of publication.** ESA members should ensure that their dues are up to date at the time of publi-

cation. Charges are made in full-page increments only, regardless of how much type appears on the last page of the article.

ESA Members: $32.00 per published page
Nonmembers: $81.00 per published page

3. I agree to be responsible for the payment of all appropriate and relevant publication charges associated with the production of the above named article. **All publication and reprint charges are due upon receipt of invoice.** Unpaid balances more than 30 days past due are subject to a finance charge of 1.5% per month (18% per annum). Authors with unpaid balances of more than 90 days will lose their right to publish with ESA until the outstanding balance is settled. There is a $25 fee for returned checks.

4. I have obtained written permission to use any quotations or excerpts from another work or from another's property not in the public domain or covered by fair use provisions of the U.S. Copyright law. Proper acknowledgment has been given in the article for the use of such material.

5. I understand that the page proofs of the above named article show how the figures will be reproduced.

6. I agree that I have reviewed the page proofs and marked on the page proofs any corrections that need to be made to the article. I understand that I am responsible for extra charges for any changes that are not a result of a printer or editor error or query.

7. I agree to refer to ESA all requests to republish or reprint the whole or any part of this article, including my own requests. Although ESA allows published articles to be posted on the author's web site two years after publication, ESA still holds the copyright on the original article and should be contacted for permission to republish or reprint it. ESA will respect the wishes of the author as to the use of the article or any material appearing in the article.

8. I have read the ethics in publishing statement that appears on the ESA web site (http://www.entsoc.org/pubs/publish/Policies) and affirm that this article is in compliance.

9. I understand that ESA will invoice me for the article after the issue of the journal in which the article appears is mailed and that I will make payment in full upon receipt of the invoice.

10. I hereby provide a billing address on the next page for the article mentioned above

Signature: _____

Printed name: _____

Author acting as agent for all other authors

10/18/2013

Environmental Entomology

Page Charges & Reprint Order–2014

You must submit this form with your proofs to the address provided with the page proofs even if you do not order reprints. The order form should accompany the return of your page proofs to ensure that your reprint order is produced on time. Be sure to fill out the order form completely and sign your name where indicated under Billing Information. Reprint prices are subject to change without notice. Paper reprints are shipped within 2 weeks of the journal issue's mailing date.

1 Article Information (Please type or print clearly)

Manuscript number **EN-13-172**   Number of pages _____

Reprint number **3861193**

2 Billing Information

ESA members will be invoiced. Please provide an invoice address.

Name _____

Address _____

_____

City _____ State/Province _____

Postal Code _____ Country _____

Email _____ Phone: _____

For all non-member international orders, a valid credit card number is requested or payment in advance of publication will be required.

❏ Visa ❏ Mastercard

Account No. _____ CSC No. ____ Exp. Date ____

Cardholder Signature _____

Cardholder Name _____

❏ Check/Money Order (payable to Entomological Society of America)
❏ Purchase Order
Purchase Order must:
- accompany this form
- list Entomological Society of America as vendor
- include manuscript number
- include journal title, article title, and author's name

3 Reprint Prices ($US) (Domestic shipping included)

Black & White Paper Reprints					Open Access Rep.
Quantity					**Quantity**
Pages	25	50	100	200	Unlimited
1-2	$ 48	68	88	111	$ 88
3-4	81	106	133	183	133
5-8	148	171	217	275	217
9-12	220	240	310	366	310
13-16	283	309	403	482	403
Add'l 1-4 pages	55	77	96	132	96
Covers*	127	134	141	188	N/A
Color Paper Reprints					**Open Access Rep.**
1-2	$ 78	136	204	317	Use B&W prices
3-4	101	183	244	353	Use B&W prices
5-8	206	299	431	656	Use B&W prices
9-12	313	418	617	961	Use B&W prices
13-16	418	534	805	1,266	Use B&W prices
Add'l 1-4 pages	106	118	187	305	Use B&W prices

* Covers stating the title, authors, and journal issue are printed in black ink on heavy weight gray paper.

4 Reprint Information

Two kinds of reprints are offered. **Paper Reprints**, whether black and white or color, are printed on high quality, gloss coated paper, trimmed, and stapled at the fold. An **Open Access Reprint** is a PDF file of your article that is posted with the online journal issue and is freely available in unlimited quantities.

Open Access Reprint (online PDF) $ _____

Paper Reprints (quantity) _____ $ _____

Covers (quantity) _____ $ _____

Total reprint cost $ _____

5 Shipping (International orders only, Paper) $ _____

All paper reprint orders in the United States are shipped UPS, unless otherwise specified, and shipping is included in the price of the reprints. **Paper reprint orders shipped outside the United States must have Expedited Shipping. Add 20% of the paper reprint cost for Expedited Shipping for each address.** Expedited Shipping provides delivery in 5–9 days and includes package tracing and proof of delivery.

6 Total Reprint Amount (add 4 and 5) $ _____

7 Page Charges
Number of pages _____ x Rate ($US) _____ $ _____
($32/page, ESA member; $81/page nonmember)

8 Color Charges (if applicable, $US) $ _____

9 Total Reprint, Page, and Color Charges ($US) (add 6, 7, and 8) $ _____

10 Shipping Information (No PO Boxes allowed)

Ship _____ copies to:
Name _____
Address _____

_____

City _____ State/Province _____
Postal Code _____ Country _____
Telephone _____ Fax _____

Ship _____ copies to:
Name _____
Address _____

_____

City _____ State/Province _____
Postal Code _____ Country _____
Telephone _____ Fax _____

11 Complete and send at once with your page proofs to:

Journal Production Manager, Environmental Entomology
Cenveo Publisher Services
5457 Twin Knolls Road, Suite 200
Columbia, MD 21045
Fax: 1-877-705-1381; Phone: 410-691-6290
e-mail: Bert.Shayte@cenveo.com

10/18/2013

ENTOMOLOGICAL SOCIETY OF AMERICA
Color Printing Agreement

If you submitted a manuscript to ESA that contained color figures, ESA permits figures to be printed in color providing that authors agree to pay the extra expense associated with color printing.

The price for printing each page of color in the journal is $200 per page.

The journal production editor will put more than one color figure on a page when feasible. If you wish to have any of your figures printed in color, please sign and complete this form and return it to the address below as soon as possible. Your manuscript cannot be processed any further until we receive this form.

There is no addtional charge to have figures in color online.

Journal name: **Environmental Entomology**

Manuscript number: **EN-13-172**

Figures to be printed in color: _____

I agree to pay full costs for the color printing and hereby authorize the Entomological Society of America to invoice me for these costs.

Signature: _____

Name: _____

Address: _____

City: _____

State/Province:_____ Zip/Postal Code: _____

Country:_____

Telephone:_____ Fax: _____

E-mail: _____

Mail, e-mail, or fax this form to:
ESA Journal Production Manager
Cadmus Professional Communications
8621 Robert Fulton Drive
Columbia, MD 21046
Fax: 1-877-705-1381
Phone: 410-691-6998
E-mail: m-ibrahiml@cadmus.com

4/5/13

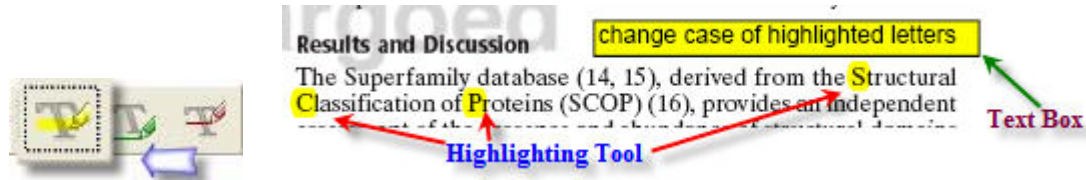# Instructions for Annotating
Your .PDF Proof

- Use Text Boxes and the Callout Tool to indicate changes to the text.

- Use the Strike-Out tool to indicate deletions to the text.
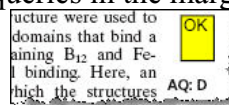
- Use the Highlighting Tool to indicate font problems, bad breaks, and other textual inconsistencies.
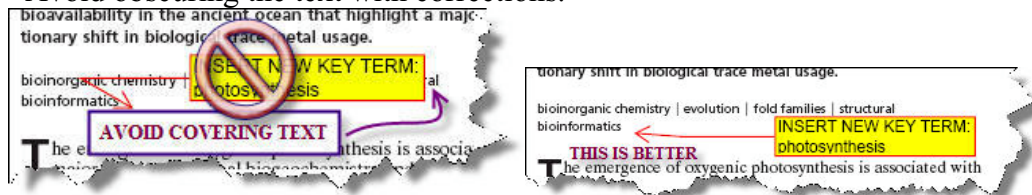
- Clearly indicate where changes need to be made using arrow, lines, and the Call-Out Tool.

- Mark changes and answer queries in the margins and other areas of white space.

- Avoid obscuring the text with corrections.

Optimal Conditions to Rear Phorid Parasitoids (Diptera: Phoridae) of *Atta vollenweideri* and *Acromyrmex lundii* (Hymenoptera: Formicidae)

ANDREA C. GUILLADE[1] AND PATRICIA J. FOLGARAIT

Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Sáenz Peña 352, Bernal BXD1876, Buenos Aires, Argentina

ABSTRACT Phorid flies have been considered viable options for biological control of leaf-cutter ants because they are highly specific to these hosts, producing direct mortality and also affecting the normal functioning of colonies. Designing protocols for mass rearing of these insects requires knowing the temperature and humidity conditions that optimize their development in terms of duration, survivorship and longevity of adults. We reared *Apocephalus setitarsus* Brown, *Eibesfeldtphora trilobata* Disney, and *Myrmosicarius brandaoi* Disney, which are specific leaf-cutter ant parasitoids of *Atta vollenweideri* Forel, and *Apocephalus neivai* Borgmeier and *Myrmosicarius catharinensis* Borgmeier, which are parasitoids of *Acromyrmex lundii* Guérin-Méneville. Phorids were maintained under one of five different rearing conditions, 20°C high humidity (20HH), 24°C low and high humidity (24LH and 24HH), and 28°C low- and high humidity (28LH and 28HH). Flies from all species could complete their development under all the conditions tested. As expected, the 20HH treatment significantly lengthened all developmental periods in all species, whereas the shortest duration was achieved under 28HH. Although pupal survivorship was highest at 24HH, the greatest longevity of adults was achieved at 20HH. Percentage of pupae obtained and pupal survivorship also exhibited the highest values at 24HH. The rearing efficiency index was highest for all species at 24HH. Therefore, we suggest this temperature and humidity combination as the most suitable for rearing these species, whereas the 20HH treatment is ideal for maintaining adults for longer periods, which would be advantageous for synchronizing matings and mass releases of adults in the field.

KEY WORDS Phoridae, leaf-cutter ant, temperature, humidity, rearing efficiency

Leaf-cutting ants in the tribe Attini harvest fresh plant material from a wide variety of plants (Cherret 1986), and are the most abundant herbivores in the Neotropics (Weber 1972, Hölldobler and Wilson 1990). Both genera in this tribe, *Atta* F. and *Acromyrmex* Mayr, have been regarded as some of the most harmful pests to a wide variety of crops, as well as forestry and rangelands (Cherret 1986).

Chemical control of these social insects, although the most popular pest control method used (Della Lucia 1993, Boaretto and Forti 1997), has proven to be ineffective in the long-term. Increasing evidence of resistance (Ouyang et al. 2010, Rodríguez et al. 2010) and undesired effects against nontarget organisms (Al-Deeb et al. 2001, Chauzat et al. 2006, Medina et al. 2007, Bagnères et al. 2009, Adán et al. 2011, Guillade and Folgarait 2013) have led environmental protection organizations to ban several agrochemicals, whereas many others are being restricted for different uses, most notably forestry (Forest Stewardship Council 2007). As the options for chemical control became increasingly limited, researchers began focusing on other potential control agents.

After intensively studying several key aspects of their biology and ecology, mass rearing of several *Pseudacteon* species, which attack fire ants, became possible (Cónsoli et al. 2001; Folgarait et al. 2002a,b, 2005, 2006; Vogt et al. 2003). It was established that most species needed high humidity to successfully develop in the laboratory (80% or higher), and that below this limit rearing was difficult, if not impossible (P.J.F., unpublished data). The rearing temperature for this genus also needs to be high, with most species developing well on temperatures between 27 (Vogt et al. 2003) and 28°C (Folgarait et al. 2002a,b, 2005, 2006). Once the best rearing conditions had been determined, these parasitoids were successfully introduced and established in the United States for the control of the invading *Solenopsis invicta* Buren and *Solenopsis richteri* Buren fire ants (Vázquez et al. 2006, Porter 2010, Plowes et al. 2012). The favorable results obtained (Henne et al. 2007, Porter 2010) encouraged researchers to study phorid flies attacking leaf-cutter ant species. Among these, the parasitoid flies in the Phoridae family have been considered for leaf-cutter

[1] Corresponding author, e-mail: andreaguillade@gmail.com.

ant biological control programs (Elizalde and Folgarait 2011, Guillade and Folgarait 2011) because of many interesting features (Folgarait 2013).

Several phorid species in the genera *Apocephalus, Eibesfeldtphora, Myrmosicarius,* and *Neodohrniphora* are highly specific to leaf-cutter ants, with evidence pointing to parasitoids of *Atta* spp. not attacking *Acromyrmex* spp. and vice versa (Elizalde and Folgarait 2010). The flies develop within the body of their hosts, consuming living tissue and eventually killing them at the time of pupation; therefore, causing direct mortality to their targets (Erthal and Tonhasca 2000, Tonhasca et al. 2001, Guillade and Folgarait 2011). The host body part preferred for oviposition and pupation varies with each fly species (head, thorax, and abdomen), as well as the size of the host (Feener and Brown 1993, Tonhasca 1996, Bragança et al. 2009, Guillade and Folgarait 2011) and the site at which ants are attacked (nest entrances, foraging trails, and cutting sites; Erthal and Tonhasca 2000, Bragança et al. 2003, Elizalde and Folgarait 2011), which could allow different species of flies to have complementary effects, increasing the total parasitism rates under a biological control program (Folgarait 2013). Finally, phorids interfere with the normal functioning of the ant nests by eliciting both individual and colony-level responses in their hosts (Elizalde and Folgarait 2012), with ants abandoning their loads to retreat into the nest, and shifts in the size of ants allocated to foraging, to avoid the sizes preferred by the phorids (Orr 1992, Feener and Brown 1993, Tonhasca et al. 2001, Guillade and Folgarait 2013). The features discussed, plus several others, make them ideal candidates for biological control programs (Folgarait 2013).

Many key aspects of the biology, ecology, and behavior of several species of Phoridae attacking leaf-cutter ants have been revealed (Tonhasca 1996; Tonhasca et al. 2001; Bragança et al. 2003; Guillade and Folgarait 2011; Elizalde and Folgarait 2011, 2012), and the taxonomy of several genera has been revised (Disney et al. 2006, 2009; Brown et al. 2010). However, to date, no study has assessed the comparative efficacy of different combinations of temperature and humidity for rearing these parasitoids. In particular, humidity has always been set at very high values for rearing purposes, but many of these species are found in geographic regions where annual relative humidity is considerably lower than that artificially maintained in the laboratory. Several instances in which we needed to rear leaf-cutter phorids in the field, without the possibility of providing constant temperature and humidity conditions, have revealed that these parasitoids could complete their development under less than ideal circumstances, and were rather tolerant to low humidity and fluctuating temperatures (A.C.G., unpublished data). This speaks of a certain degree of physiological plasticity, and thus bears the question of whether the conditions generally accepted as optimal for rearing phorid parasitoids are indeed the best for phorids of leaf-cutter ants. To mass-rear these flies for biological control releases, it is indispensable to have the knowledge for optimal rearing efficiency. This implies knowing the best temperature and humidity rearing conditions to obtain the optimal ones regarding several aspects of their life cycle, such as the length of the different developmental stages and their survivorship, as well as manipulating and synchronizing adult emergence for mass release programs (Thead and Streett 2006). Our study provides such information for *Apocephalus setitarsus* Brown, *Eibesfeldtphora trilobata* Disney, and *Myrmosicarius brandaoi* Disney, which are parasitoids of *Atta vollenweideri* Forel, as well as *Apocephalus neivai* Borgmeier and *Myrmosicarius catharinensis* Borgmeier, which are parasitoids of *Acromyrmex lundii* Guérin-Méneville.

Materials and Methods

Flies were obtained through passive sampling (Elizalde and Folgarait 2011), which consisted of collecting worker ants from foraging trails from 18 *A. vollenweideri* nests in San Cristobal, Santa Fe Province, Argentina (Chaco phytogeographic province; Cabrera 1994), as well as from 18 *A. lundii* nests in Villa Elisa, Parque Pereyra Iraola, Manuel B. Gonnet, and La Plata, Buenos Aires Province, Argentina (Pampa phytogeographic province; Cabrera 1994), during the springs of 2010 and 2011. From each nest (sampling unit) we collected five 300-ant batches, one per rearing regimen (see below).

Samples were taken to the laboratory, where ants were placed in plastic containers with water and sugar water ad libitum. These containers were kept under different temperature and humidity regimens, and were periodically inspected to remove dead ants. Dead ants were transferred to plastic containers with a plaster base and searched for phorid pupae, which were then removed and placed individually in smaller containers covered with a fine-mesh cloth until the emergence of the adult. Immediately after emergence, adults were moved to specially modified test tubes, with ventilation on one end, covered with fine-mesh cloth, and a cap adapted to provide sugar water ad libitum to the fly. We recorded date of collection of the ants, date of death of ants, date of pupation, date of emergence of adult, and death of adult. Dead adults were then inspected under a Nikon SMZ800 (Nikon, Tokyo, Japan) stereoscopic microscope to determine species, gender, and size (width of mesonotum). We also measured the size of the host head each fly emerged from (width below the eyes of the ant head) for *M. brandaoi, M. catharinensis,* and *E. trilobata;* we could not measure host size for *A. setitarsus* and *A. neivai* because both species have free-living pupae.

Taxonomic identification of phorids was carried out according to the keys by Disney (1996), Brown (1997), Disney et al. (2006, 2009), and Brown et al. (2010). Ants were identified according to Bonetto (1959), Gonçalves (1961), and Kusnezov (1978). Given that during the samplings the ants were randomly assigned to one of the five batches that would then be kept under one of the five rearing regimens, the percentage of pupae obtained might be viewed in this assay as larval survivorship, if the assumption is

made that every batch carried the same proportion of parasitized ants. Nonetheless, we chose the term percentage of pupae obtained because such assumption could only have been confirmed by dissecting every ant collected in search of phorid larvae. We calculated percentage of pupae obtained for each species and treatment from each 300-ant batch, whereas pupal survivorship was determined for each species under each rearing regimen as the ratio between the number of adults emerged and the number of pupae obtained per batch. We then multiplied percentage of pupae obtained by pupal survivorship, for each species under each rearing regimen, to calculate a rearing efficiency index (REI), to determine the best rearing regimen for each species studied (a greater REI indicating greater rearing efficiency).

Rearing Conditions. Ants collected from each nest of each species were separated into five batches of 300 individuals. Each batch was kept from the beginning to the end of the experiment in a climatically controlled rearing chamber with a photoperiod of 12:12 (L:D) h, and one of the following temperature and humidity regimens: 20HH: $20 \pm 1°C$ and $80 \pm 10\%$ relative humidity (RH); 24HH: $24 \pm 1°C$ and $80 \pm 10\%$ RH; 24LH: $24 \pm 1°C$ and $30 \pm 10\%$ RH; 28HH: $28 \pm 1°C$ and $80 \pm 10\%$ RH; and 28LH, $28 \pm 1°C$ and $30 \pm 10\%$ RH. Although it would have been desirable to test rearing at low temperature and RH, our low-temperature rearing chamber could not sustain constant relative humidity when set at 30%, resulting in an unacceptably wide fluctuation in this variable.

Statistical Analyses. After transformation the data met the assumptions required for parametric analyses. Developmental times and sizes of host ants and adult flies were LogN transformed, whereas for percentage of pupae obtained and pupal survivorship we applied square-root transformation; t-tests were conducted for comparisons between males and females for each life history trait studied. Because no significant differences were found between males and females for any of the species and parameters studied ($P > 0.05$ for all contrasts), we pooled data from both genders of each species. We then performed analyses of variance for all multiple comparisons, using Statistix 2.0 for Windows (Analytical Software 1998). We subsequently carried out Bonferroni's a posteriori contrasts, maintaining the significance level at 0.05. Pearson's correlations were also performed between size of adults and total developmental time, as well as size of adults and adult longevity, for every species under each rearing regimen, using Statistix 2.0 for Windows.

Results

Parasitoids of *A. vollenweideri*. Developmental times for *A. setitarsus*, *E. trilobata*, and *M. brandaoi* under the five rearing regimens tested are shown in Fig. 1A–C. We found significant differences in larval time between treatments for all the species tested (*A. setitarsus*: $F = 160.76$; df = 4,124; $P = 0.0000$; *E. trilobata*: $F = 450.94$; df = 4, 332; $P = 0.0000$; *M. brandaoi*: $F = 123.46$; df = 4, 131; $P = 0.0000$). A posteriori

contrasts show that larval time was lengthened by the 20HH treatment for all three species, whereas the shortest time was always recorded under the 28HH treatment ($P < 0.05$ each). The only contrasts that did not exhibit significant differences were between the 24HH and 28LH treatments for *E. trilobata*, and between 24HH and 24LH for *A. setitarsus* and *M. brandaoi* ($P > 0.05$ each).

Pupal time varied for all species under different treatments (*A. setitarsus*: $F = 4.24$; df = 4, 67; $P = 0.0042$; *E. trilobata*: $F = 10.18$; df = 4, 238; $P = 0.0000$; *M. brandaoi*: $F = 16.68$; df = 4, 88; $P = 0.0000$). According to a posteriori contrasts, the longest time was achieved at 20HH for all species but *A. setitarsus*, for which pupal time was shortest at 24HH ($P < 0.05$ each). The shortest time was recorded at 28HH, although we found no significant differences between this treatment and 24HH except for *M. brandaoi* ($P < 0.05$).

Total developmental time varied between treatments for all species (*A. setitarsus*: $F = 11.39$; df = 4, 67; $P = 0.0000$; *E. trilobata*: $F = 156.42$; df = 4, 238; $P = 0.0000$; *M. brandaoi*: $F = 74.46$; df = 4, 88; $P = 0.0000$). A posteriori contrasts show that, for all species, the shortest time was obtained under the 28HH treatment ($P < 0.05$). The times at 20, 24, and 24LH were shortest for *A. setitarsus*, whereas the 28LH treatment resulted in an intermediate value. For *E. trilobata* the shortest time was recorded at 28HH, the longest at 20 and 24LH, with 24HH and 28LH yielding intermediate values ($P < 0.05$). For *M. brandaoi*, the shortest time was obtained at 28HH, followed by 28LH, then 24°C (with no significant differences between HH and LH), and finally 20HH ($P < 0.05$).

Adult longevity also varied across treatments (*A. setitarsus*: $F = 11.46$; df = 4, 66; $P = 0.0000$; *E. trilobata*: $F = 82.79$; df = 4, 237; $P = 0.0000$; *M. brandaoi*: $F = 31.44$; df = 4, 95; $P = 0.0000$), with a posteriori contrasts showing that it was always longest at 20HH ($P < 0.05$). Both higher temperature and lower humidity significantly decreased longevity. We found significant differences ($P < 0.05$) within the same temperature between high and low humidity for *A. setitarsus* (24°C), *E. trilobata* (24°C), and *M. brandaoi* (24 and 28°C).

Within each species, the size of adults was not significantly different between treatments (Table 1). However, we found significant differences across treatments for host ants of *E. trilobata* ($F = 13.72$; df = 4, 332; $P = 0.0000$), which were larger under the 24°C treatment (both 24HH and 24LH). Size of hosts did not vary significantly under our treatments for *M. brandaoi*.

The percentage of pupae obtained varied across treatments for all species (*A. setitarsus*: $F = 10.28$; df = 4, 85; $P = 0.0000$; *E. trilobata*: $F = 4.81$; df = 4, 85; $P = 0.0016$; *M. brandaoi*: $F = 9.05$; df = 4, 85; $P = 0.0000$), being in all cases significantly higher at 24HH (Fig. 2A). We also found differences across treatments for pupal survivorship (*A. setitarsus*: $F = 8.9$; df = 4, 40; $P = 0.0000$; *E. trilobata*: $F = 10.12$; df = 4, 44; $P = 0.0000$; *M. brandaoi*: $F = 3.45$; df = 4, 39; $P = 0.0165$), with a
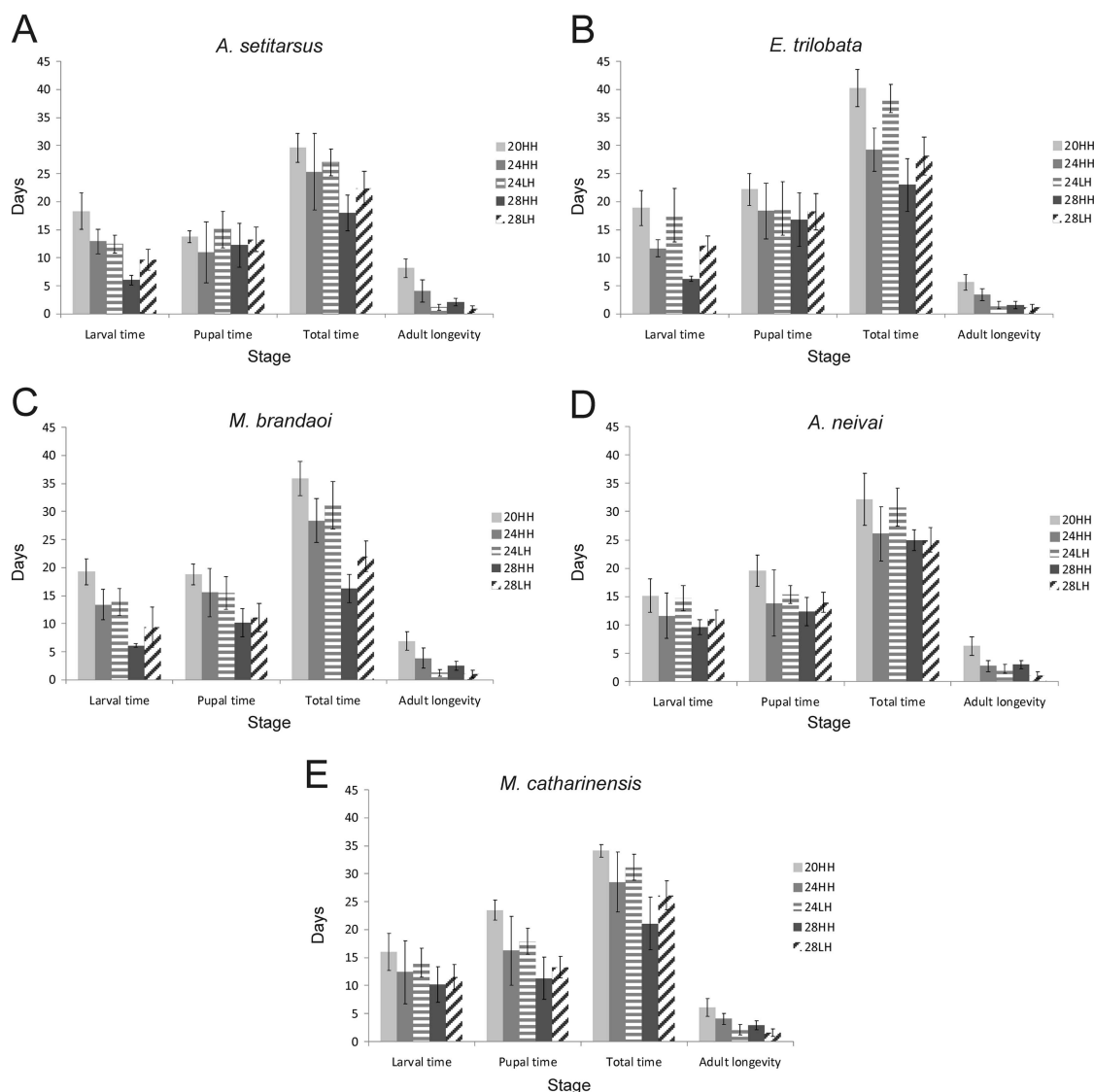
Fig. 1. Developmental times and adult longevity (mean and SD) of parasitoids of *A. vollenweideri* (A–C) and *A. lundii* (D–E) under five different rearing regimens.

posteriori contrasts showing the highest survivorship recorded at 24HH and the lowest at 28LH, whereas the differences between all other treatments were not significant (Fig. 2B). Interestingly, for *M. brandaoi*, the highest survivorship was recorded at 24HH and the lowest at 20HH, whereas differences between all other treatments were not significant.

REIs for all three species under the five rearing regimens are given in Table 1. Although the highest REIs were obtained at 24HH for all species, *E. trilobata* also showed good rearing efficiency at 28HH.

We found a positive correlation between size of adults and total developmental time only for *M. brandaoi* at 24HH ($r = 0.49$; $P = 0.003$). However, size of adults and adult longevity were positively correlated only for *A. setitarsus* at 24LH ($r = 0.51$; $P = 0.03$). All

other correlations were not statistically significant ($P > 0.05$).

Parasitoids of *A. lundii*. Developmental times for *A. neivai* and *M. catharinensis* under the five rearing regimens tested are shown in Fig. 1D and E. Larval time varied significantly for *A. neivai*, ($F = 18.06$; df $= 4, 123$; $P = 0.0000$), being longer at 20HH and 24LH and shorter under the rest of the treatments ($P < 0.05$). *M. catharinensis* also showed significant variations ($F = 9.47$; df $= 4, 157$; $P = 0.0000$), with a posteriori contrasts revealing that the longest time was recorded at 20HH and the shortest at 28HH ($P < 0.05$), whereas the other contrasts showed no significant differences.

Pupal time varied across treatments for *A. neivai* ($F = 8.57$; df $= 4, 95$; $P = 0.0000$), being shortest at 24 and 28HH and longest at 20HH according to a poste-

Table 1. Size (mm) of host ants and adult phorids (mean ± SD) and REI, product of multiplying natural percent parasitism by pupal survivorship, for phorids of *A. vollenweideri* and *A. lundii* under all treatments

Treatment	A. vollenweideri									A. lundii				
	A. setitarsus		E. trilobata			M. brandaoi				A. neivai		M. catharinensis		
	Size of adults	REI	Size of hosts	Size of adults	REI	Size of hosts	Size of adults	REI		Size of adults	REI	Size of hosts	Size of adults	REI
20HH	0.54 ± 0.02 (*9*)	0.25	1.95 ± 0.29b (*81*)	0.53 ± 0.04 (*65*)	0.36	1.62 ± 0.25 (*37*)	0.46 ± 0.04 (*17*)	0.17		0.52 ± 0.01 (*15*)	0.53	1.58 ± 0.15 (*33*)	0.41 ± 0.02 (*15*)	0.27
24HH	0.53 ± 0.04 (*29*)	1.59	2.08 ± 0.26a (*94*)	0.54 ± 0.04 (*94*)	1.40	1.78 ± 0.30 (*41*)	0.43 ± 0.04 (*39*)	1.17		0.49 ± 0.05 (*29*)	1.11	1.49 ± 0.21 (*33*)	0.42 ± 0.04 (*44*)	0.95
24LH	0.54 ± 0.04 (*18*)	0.14	2.10 ± 0.25a (*63*)	0.55 ± 0.03 (*36*)	0.48	1.81 ± 0.32 (*27*)	0.42 ± 0.05 (*23*)	0.32		0.52 ± 0.02 (*10*)	0.09	1.56 ± 0.15 (*38*)	0.41 ± 0.02 (*20*)	0.21
28HH	0.51 ± 0.03 (*13*)	0.33	1.86 ± 0.22b (*73*)	0.53 ± 0.04 (*49*)	0.87	1.58 ± 0.15 (*20*)	0.42 ± 0.04 (*15*)	0.40		0.50 ± 0.06 (*15*)	0.27	1.58 ± 0.15 (*33*)	0.41 ± 0.02 (*11*)	0.28
28LH	0.51 ± 0.03 (*8*)	0.06	1.79 ± 0.19b (*31*)	0.54 ± 0.03 (*15*)	0.12	1.56 ± 0.16 (*16*)	0.42 ± 0.05 (*11*)	0.18		0.52 ± 0.02 (*9*)	0.05	1.59 ± 0.16 (*22*)	0.42 ± 0.02 (*9*)	0.11

Size of hosts for *A. setitarsus* and *A. neivai* are not given (see Materials and Methods section). Number of individuals measured per species and treatment is shown in italics. Equal lowercase letters in a column indicate significant differences across treatments.

riori contrasts, with the low humidity treatments exhibiting intermediate values ($P < 0.05$ each). Significant differences were also found across treatments for *M. catharinensis* ($F = 8.6$; df = 4, 97; $P = 0.0000$), which according to a posteriori contrasts exhibited the longest pupal time at 20HH and the shortest at 28HH, with the other treatments showing intermediate values ($P < 0.05$ each).

Accordingly, total developmental time also varied significantly depending on the rearing regimen for both species (*A. neivai*: $F = 10.67$; df = 4, 95; $P = 0.0000$; *M. catharinensis*: $F = 15.9$; df = 4, 97; $P = 0.0000$). A posteriori contrasts showed that for *A. neivai* it was longest at 20HH and shortest at 28HH and 28LH, whereas *M. catharinensis* exhibited the longest time at 20HH and the shortest at 28HH ($P < 0.05$ each).

Adult longevity was also significantly affected by the rearing regimen for both *A. neivai* ($F = 36.18$; df = 4, 85; $P = 0.0000$) and *M. catharinensis* ($F = 44.62$; df = 4, 97; $P = 0.0000$). Adults of both species lived the longest at 20HH and shortest at 28LH, the treatments in between exhibiting intermediate values, according to a posteriori contrasts ($P < 0.05$ each).

We found no significant differences between sizes of adult flies under any treatment (Table 1) for both *A. neivai* ($F = 2.12$; df = 4, 68; $P = 0.08$) and *M. catharinensis* ($F = 0.04$; df = 4, 89; $P = 0.99$). Furthermore, we did not record significant differences for the size of host ants parasitized by *M. catharinensis* ($F = 12.32$; df = 4, 176; $P = 0.086$).

The rearing regimens tested significantly affected the percentage of pupae obtained for both *A. neivai* ($F = 6.12$; df = 4, 62; $P = 0.0004$) and *M. catharinensis* ($F = 4.49$; df = 4, 77; $P = 0.0027$). A posteriori contrasts showed that, for *A. neivai*, this percentage was greatest at 24HH and lowest at 24 and 28LH ($P < 0.05$ each), whereas for *M. catharinensis* the highest value was recorded at 20HH and the lowest at 24LH, 28HH, and 28LH (Fig. 2A), with the treatment 24HH resulting in intermediate levels of parasitism.

Pupal survivorship was significantly affected by the treatments for both species (*A. neivai*: $F = 3.98$; df = 4, 56; $P = 0.0066$; *M. catharinensis*: $F = 5.25$; df = 4, 77; $P = 0.0009$; Fig. 2b). For *A. neivai* survivorship was greatest at 24HH and lowest at 24LH, according to a posteriori contrasts ($P < 0.05$ each), whereas the differences among the other treatments for either species were not significant. However, a posteriori contrasts showed that survivorship was greatest at 24HH and lowest at 28LH for *M. catharinensis* ($P < 0.05$ each), with the other treatments showing intermediate values.

REIs for both species under the five rearing regimens are given in Table 1. The 24HH regimen was found to be the most adequate for both *A. neivai* and *M. catharinensis*.

We found a statistically significant correlation between size of adults and total developmental time for *A. neivai* at 24HH ($r = 0.61$; $P = 0.004$), 24LH ($r = 0.78$; $P = 0.01$), and 28HH ($r = 0.72$; $P = 0.003$), whereas for *M. catharinensis* these two variables were not significantly correlated under any of the rearing regimens. Neither *A. neivai* nor *M. catharinensis* showed signif-
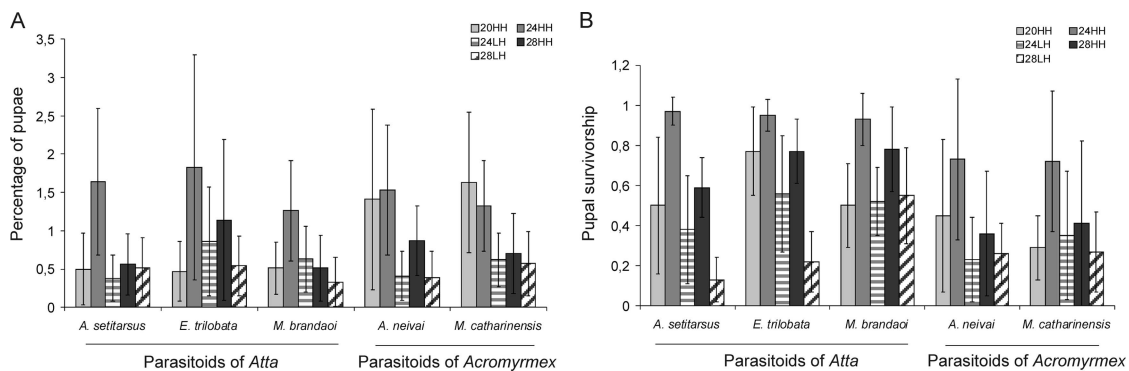
Fig. 2. Percentage of pupae obtained (A) and pupal survivorship (B) for *A. setitarsus*, *E. trilobata*, and *M. brandaoi*—parasitoids of *A. vollenweideri*—and *A. neivai* and *M. brandaoi*—parasitoids of *A. lundii*—under five different rearing regimens.

icant correlations between size of adults and adult longevity, under any of the treatments.

Discussion

Our results provide information on key aspects of the life history of the most important genera of leaf-cutting ant phorids, which may prove to be of great value for designing mass-rearing techniques for these parasitoids. Although flies from all five species studied here could develop under all the rearing regimens tested, immature performance in terms of developmental times differed significantly among treatments, and the same could be observed for adult longevity. Both percentage of pupae obtained and pupal survivorship were also affected, resulting in varying rearing efficiencies.

To our knowledge, few other works have been published on life cycles of leaf-cutter ant phorids under temperatures lower than 26.5°C and none under humidities lower than 65%, with the exception of our previous work, in which the rearing temperature was 25 ± 1°C (Guillade and Folgarait 2011), and only one study has been published in which leaf-cutting ant parasitoids were reared at 22°C (Elizalde and Folgarait 2011). Our findings differ from those reported by the latter authors, particularly regarding larval times, which were consistently longer in our study for all the species reared. These differences seem to suggest a great sensitivity of phorid species to even small changes in temperature or humidity. However, the fact that all five species studied here could successfully complete their development and survive at least 2 d as adults show the great plasticity and tolerance they have to varying environmental conditions, which is a highly desirable trait for organisms that will be released in the field under variable situations, given their wide geographic distribution (Elizalde and Folgarait 2010) and the variety of habitats occupied by their hosts (Farji Brener and Ruggiero 1994).

Both phorids of *A. vollenweideri* from Santa Fe Province and those of *A. lundii* from Buenos Aires province exhibited their best performance in terms of rearing efficiency at the 24HH treatment (Table 1). Interest-

ingly, the three *A. vollenweideri* phorid species exhibited very low coefficients of variation of pupal survivorship under this treatment, ranging from 0.07 to 0.14, whereas the coefficients of variation for *A. lundii* phorids at 24HH were circa 0.5. One factor that might explain the great variation observed in pupal survivorship across treatments between phorids of *A. vollenweideri* and *A. lundii* is the fact that *A. vollenweideri* ants deposit their litter in refuse chambers within the nest (Jonkman 1976). Ants are well-known for regulating the temperature within their nests to maintain optimal conditions for culturing their fungus gardens (Bollazzi and Roces 2010); therefore, if phorids develop within internal refuse dumps, then they are likely to do so, comparatively, under largely constant temperature and humidity conditions. However, *A. lundii* deposits litter in either external or internal refuse dumps, and thus phorids developing in them should be adapted to greater variations in climatic conditions. Nonetheless, the litter deposited in external refuse dumps might act as mulch, buffering the phorid pupae to an extent from the lower humidity in the surrounding air. For phorids that must complete their development under such varying circumstances it would likely not be advantageous, from an evolutionary point of view, to be adapted to a particular temperature and humidity regimen, but rather to achieve a reasonably high survivorship under a wider range of environmental conditions. A great genotypic variability regarding environmental tolerance during development would be an advantage to their populations, ensuring that enough individuals reach adulthood, adverse climatic conditions notwithstanding. This hypothesis seems to be consistent with the lesser variation across treatments observed for *A. lundii* phorids compared with the variation observed for phorids reared from *A. vollenweideri.* The mean coefficients of variation in pupal survivorship for each species across the five treatments was 0.55 for *A. setitarsus*, 0.39 for *E. trilobata*, and 0.43 for *M. brandaoi*, whereas for *A. lundii* phorids it was much greater with 0.81 for *A. neivai* and 0.78 for *M. catharinensis.* It would be interesting to test this hypothesis by contrasting the REIs obtained under different rearing regimens in this

study for phorids parasitizing *A. vollenweideri*, which deposits refuse in internal chambers, with those of *Atta colombica* Guérin-Méneville, a species with external refuse dumps.

Henne and Johnson (2007) reported, in an experiment conducted under laboratory conditions in an artificial setup, that *S. invicta* workers parasitized by *Pseudacteon tricuspis* Borgmeier remained within lateral tunnels in the nest, avoiding foraging, and that they only left the nest through lateral, secondary trails shortly before dying, a behavior they hypothesized to be dictated by the phorid parasitoid to keep it protected from unfavorable environmental conditions. These ants were incapable of biting because of the damage the parasitoids had inflicted to their mandibular muscles. If this was the case for leaf-cutting ants, very few parasitized workers would be collected during a passive sampling, and they would die shortly after capture because only those workers close to death by a few hours would leave the nest. We found that larval time from field-collected ants in our experiments fluctuated from ≈5 to 18 d, depending on the rearing regimen. Elizalde and Folgarait (2011) also found prolonged larval times for several phorid species reared from ants collected in the field. Moreover, although the percentage of pupae recovered in our experiment was rather low, this seems to be a result of the environmental conditions, as discussed below, because Elizalde and Folgarait (2011) reported parasitism rates as high as ≈30% for *E. trilobata* in the same study site but from different years. Because both in our experiment and the latter authors' work, the ants were collected from foraging trails, the hypothesis that ants remain hidden in the nest until a few hours before their death seems to be contradicted, at least in leaf-cutter ants.

Because rearing conditions clearly affected the larval development for the species studied, we can compare our results to other studies assessing parasitism rates only when rearing conditions were similar to one of those tested here. Elizalde and Folgarait (2011) reported parasitism rates for *A. lundii* during 2005 that were similar to those we found. However, they reported much higher levels of parasitism for 2006. The opposite can be observed for *A. vollenweideri* parasitoids, which in their study were more abundant in 2005. Phorid abundance is markedly seasonal (Elizalde and Folgarait 2011, Guillade and Folgarait 2011), and climatic factors have been shown to also affect their richness to some extent (Elizalde and Folgarait 2010). Our samplings took place a year after one of the worst droughts to have ever affected Argentina, and phorid fly populations were only beginning to recover after their drop in abundance during 2008 and 2009. This caused the REIs to be particularly low; however, samplings from more favorable years, such as that of Elizalde and Folgarait (2011) would yield much higher values of this index. Considering the maximum parasitism rates these authors reported, the REI would be 5.82 at 24HH for *A. setitarsus*, 28.5 for *E. trilobata*, and 13.95 for *M. brandoai.* As for parasitoids of *A. lundii*, the REI would be 4.38 for *A. neivai* and 4.32 for

M. catharinensis . Furthermore, we have reported a very high pupal survivorship for *A. setitarsus*, *E. trilobata*, and *M. brandoai* at the 24HH treatment, much higher than what Tonhasca et al. (2001) reported for *Myrmosicarius grandicornis* Borgmeier or Bragança et al. (2009) reported for *Neodohrniphora* ~~elongate~~ Brown, both at 26.5°C. Such high pupal survivorship is a greatly desirable trait for any mass rearing program because it would ensure that ≈95% of all the parasitoids brought into the laboratory would successfully complete their development.

Albeit not as high as that of *A. vollenweideri* phorids, mean pupal survivorship for *A. neivai* and *M. catharinensis* was also highest at the 24HH treatment; nonetheless, survivorship for these two species was highly variable across treatments, as indicated by the great coefficients of variation obtained, which ranged from 0.48 to 1, and were greater in comparison to those from *Atta* phorids (0.07–0.92). One possible hypothesis that may account for the lower pupal survivorship in *Acromyrmex* in comparison to *Atta* could be related to the presence of actinomycetes, in particular the alleged symbiont *Pseudonocardia* (Cafaro et al. 2011). The presence of the bacteria seems to protect these ants against pathogenic fungi (Currie et al. 2003). However, the existence of these symbionts in *Atta* ants has become the subject of much controversy, whereas its presence in *Acromyrmex* is undisputed (Fernández-Marín et al. 2009). If this is truly the case, it can be argued that antibiotics secreted by the aforementioned actinomycetes could have been coopted to negatively affect the coevolution with specific parasitoids, which would then lead to a lower parasitism success in *Acromyrmex* ants that have *Pseudonocardia* or other actinomycetes with similar roles. Obviously, as in any arms race, the parasitoids would have counter-evolved defenses against actinomycetes antibiotics, but in any case, they could still be less efficient in comparison to *Atta* parasitoids that do not have to deal with them. Future research should aim to study the effect of several coevolving factors that can interact in complex manners if the goal is to improve survivorship for these parasitoids under laboratory conditions, to maximize their rearing efficiency.

The high sensitivity that all species tested here exhibited to variations in both temperature and humidity could help explain the marked seasonality of these parasitoids in the field (Guillade and Folgarait 2011, Elizalde and Folgarait 2012). This should be taken into account when designing schedules for mass release programs of these flies, to ensure the survival and establishment of the parasitoids released. Our results should prove highly valuable for mass rearing programs, because such information is necessary, for example, for timing the emergence of adults or conserving them until the dates set for release (Thead and Streett 2006). We then conclude that the 24HH treatment is the most suitable for rearing the species of parasitoids studied here, and the 20HH treatment would be highly recommended for keeping emerged adults until their release in the field.

Acknowledgments

References Cited

Adán, A., E. Viñuela, P. Bengochea, F. Budia, P. Del Estal, P. Aguado, and P. Medina. 2011. Lethal and sublethal toxicity of fipronil and imidacloprid on *Psyttalia concolor* (Hymenoptera: Braconidae). J. Econ. Entomol. 104: 1541–1549.

Al-Deeb, M. A., G. E. Wilde, and K. Y. Zhu. 2001. Effect of insecticides used in corn, sorghum, and alfalfa on the predator *Orius insidiosus* (Hemiptera: Anthocoridae). J. Econ. Entomol. 94: 1353–1360.

Analytical Software. 1998. Statistix for Windows User's manual. Analytical Software, Tallahassee, FL.

Bagnères, A. G., A. Pichon, J. Hope, R. W. Davis, and J. L. Clèment. 2009. Contact versus feeding intoxication by fipronil in *Reticulitermes* termites (Isoptera: Rhinotermitidae): laboratory evaluation of toxicity, uptake, clearance, and transfer among individuals. J. Econ. Entomol. 102: 347–356.

Boaretto, M.A.C., and L. C. Forti. 1997. Perspectivas no controle de formigas cortadeiras. Serie Técnica IPEF 11: 31–46.

Bollazzi, M., and F. Roces. 2010. Leaf-cutting ant workers (*Acromyrmex heyeri*) trade off nest thermoregulation for humidity control. J. Ethol. 28: 399–403.

Bonetto, A. 1959. Las hormigas cortadoras de la provincia de Santa Fe (géneros *Atta* y *Acromyrmex*). Dirección General de Recursos Naturales. Ministerio de Agricultura y Ganadería. Provincia de Santa Fe, Argentina.

Bragança, M.A.L., T.M.C. Della Lucia, and A. Tonhasca Jr. 2003. First record of phorid parasitoids (Diptera: Phoridae) of the leaf-cutting ant *Atta bisphaerica* Forel (Hymenoptera: Formicidae). Neotrop. Entomol. 32: 169–171.

Bragança, M.A.L., A. Tonhasca Jr. and T.M.C. Della Lucia. 2009. Características biológicas e comportamentais de *Neodohrniphora elongata* Brown (Diptera: Phoridae), um parasitóide da saúva *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae). Revista Brasileira de Entomologia 53: 600–606.

Brown, B. V. 1997. Revision of the *Apocephalus* attophilus-group of ant-decapitaiting flies (Diptera: Phoridae). Contrib. Sci. 468: 1–60.

Brown, B. V., R.H.L. Disney, L. Elizalde, and P. J. Folgarait. 2010. New species and new records of *Apocephalus* Coquillet (Diptera: Phoridae) that parasitize ants (Hymenoptera: Formicidae) in South America. Sociobiology 55: 165–190.

Cabrera, A. L. 1994. Regiones fitogeograficas argentinas. Editorial ACME. Buenos Aires, Argentina.

Cafaro, M. J., M. Poulsen, A. E. Little, S. L. Price, N. M. Gerardo, B. Wong, A. E. Stuart, B. Larget, P. Abbot, and C. R. Currie. 2011. Specificity in the symbiotic association between fungus-growing ants and protective *Pseudonocardia* bacteria. Proc. Biol. Sci. 278: 1814–1822.

Chauzat, M. -P., J. P Faucon, A. C Martel, J. Lachaize, N. Cougoule, and M. Aubert. 2006. A survey of pesticide residues in pollen loads collected by honey bees in France. J. Econ. Entomol. 99: 253–262.

Cherret, J. M. 1986. The economic importance and control of leaf-cutting ants, pp. 165–192. *In* S. Bradleigh Vinson (ed.), Economic Impact and Control of Social Insects. Praeger Publishers, New York, NY.

Cónsoli, F. L., C. T. Wuellner, S. Bradleigh Vinson, and L. E. Gilbert. 2001. Immature development of *Pseudacteon tricuspis* (Diptera: Phoridae), an endoparasitoid of the red imported fire ant (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 94: 97–109.

Currie, C. R., A.N.M. Bot, and J. J. Boomsma. 2003. Experimental evidence of a tripartite mutualism: bacteria protect ant fungus gardens from specialized parasites. Oikos 101: 91–102.

Della Lucia, T.M.C. 1993. As formigas cortadeiras. Bib. Cent. Univ. Fed. Viçosa, Brasil.

Disney, R.H.L. 1996. A key to *Neodohrniphora* (Diptera: Phoridae), parasites of leaf-cutting ants (Hymenoptera: Formicidae). J. Nat. Hist. 30: 1377–1389.

Disney, R.L.H., L. Elizalde, and P. J. Folgarait. 2006. New species and revision of *Myrmosicarius* (Diptera: Phoridae) that parasitize leaf-cutter ants (Hymenoptera: Formicidae). Sociobiology 47: 771–809.

Disney, R.H.L., L. Elizalde, and P. J. Folgarait. 2009. New species and new records of scuttle flies (Diptera: Phoridae) that parasitize leaf-cutter and army ants (Hymenoptera: Formicidae). Sociobiology 54: 601–631.

Elizalde, L., and P. J. Folgarait. 2010. Host diversity and environmental variables as determinants of leafcutter phorid parasitoid's species richness. J. Biogeogr. 37: 2305–2316.

Elizalde, L., and P. J. Folgarait. 2011. Biological attributes of Argentinian phorid parasitoids (Insecta: Diptera: Phoridae) of leaf-cutting ants, *Acromyrmex* and *Atta.* J. Nat. Hist. 45: 2701–2723.

Elizalde, E., and P. J. Folgarait. 2012. Behavioral strategies of phorid parasitoids and responses of their hosts, the leafcutter ants. J. Insect Sci. 12: 135.

Erthal Jr., M. and A. Tonhasca Jr. 2000. Biology and oviposition behavior of the phorid *Apocephalus attophilus* and the response of its host, the leaf-cutting ant *Atta laevigata.* Entomol. Exp. Appl. 95: 71–75.

Farji Brener, A. G., and A. Ruggiero. 1994. Leaf-cutting ants (*Atta* and *Acromyrmex*) inhabiting Argentina: patterns in species richness and geographical range sizes. J. Biogeogr. 21: 391–399.

Feener Jr., D. H., and B. V. Brown. 1993. Oviposition behavior of an ant-parasitizing fly, *Neodohrniphora curvinervis* (Diptera: Phoridae), and defense behavior by its leaf-cutting ant host *Atta cephalotes* (Hymenoptera: Formicidae). J. Insect Behav. 6: 675–688.

Fernández-Marín, H., J. K. Zimmerman, D. R. Nash, J. J. Boomsma, and W. T. Wcislo. 2009. Reduced biological control and enhanced chemical pest management in the evolution of fungus farming in ants. Proc. R. Soc. B 276: 2263–2269.

Folgarait, P. J. 2013. Leaf-cutter ants' parasitoids: current knowledge. Psyche. doi: http://dx.doi.org/10.1155/2013/539780.

Folgarait, P. J. and A. C. Guillade. 2013. Leaf-cutter ant foraging affected by parasitoid density: implications for biological control, p. 166. *In* Anais do XXI Simpósio de Mirmecologia, 1–5 December 2013. Fortaleza (CE), Brazil. Universidade Estadual do Ceará, Fortaleza, Ceará.

Folgarait, P. J., O. A. Bruzzone, R.J.W. Patrock, and L. E. Gilbert. 2002a. Developmental rates and host specificity for *Pseudacteon* parasitoids (Diptera: Phoridae) of fire ants (Hymenoptera: Formicidae) in Argentina. J. Econ. Entomol. 95: 1151–1158.

Folgarait, P. J., O. A. Bruzzone, and L. E. Gilbert. 2002b. Development of *Pseudacteon culltellatus* (Diptera: Phoridae) on *Solenopsis invicta* and *Solenopsis richteri* fire ants (Hymenoptera: Formicidae). Environ. Entomol. 31: 403–410.

Folgarait, P. J., M. G. Chirino, R.J.W. Patrock, and L. E. Gilbert. 2005. Development of *Pseudacteon obtusus* (Diptera: Phoridae) on *Solenopsis invicta* and *Solenopsis richteri* fire ants (Hymenoptera: Formicidae). Environ. Entomol. 34: 308–316.

Folgarait, P. J., R.J.W. Patrock, and L. E. Gilbert. 2006. Development of *Pseudacteon nocens* (Diptera: Phoridae) on *Solenopsis invicta* and *Solenopsis richteri* fire ants (Hymenoptera: Formicidae). J. Econ. Entomol. 99: 295–307.

Forest Stewardship Council. 2007. FSC guidance document. FSC Pesticides policy: guidance on implementation. (http://ebookbrowse.com/fsc-pol-30–001-en-fsc-pesticides-policy-2005-pdf-d342630382).

Gonçalves, C. R. 1961. O género *Acromyrmex* no Brasil (Hymenoptera: Formicidae). Studia Entomology 4: 113–180.

Guillade, A. C., and P. J. Folgarait. 2011. Life history traits and parasitism rates of four phorid species (Diptera: Phoridae), parasitoids of *Atta vollenweideri* (Hymenoptera: Formicidae) in Argentina. J. Econ. Entomol. 104: 32–40.

Guillade, A. C. and P. J. Folgarait. 2013. Natural enemies of *Atta vollenweideri* (Hymenoptera: Formicidae) leaf-cutter ants negatively affected by synthetic pesticides, chlorpyrifos and fipronil. J. Econ. Entomol. (in press).

Henne, D. C., and S. J. Johnson. 2007. Zombie fire ant workers: behavior controlled by decapitating fly parasitoids. Insect Soc. 54: 150–153.

Henne, D. C., S. J. Johnson, and S. D. Porter. 2007. Status of the fire ant decapitating fly *Pseudacteon tricuspis* (Diptera: Phoridae) in Louisiana. Fla. Entomol. 90: 565–569.

Hölldobler, B. and E. O. Wilson. 1990. The ants. Cambridge University Press, New York, NY.

Jonkman, J.C.M. 1976. Biology and ecology of the leaf cutting ant *Atta vollenweideri* Forel, 1893. Z. Angew. Entomol. 81: 140–148.

Kusnezov, N. 1978. Zoogeografía de las hormigas en Sudamérica. Acta Zoológica Lilloana 19: 25–186.

Medina, P., J. J. Morales, F. Budia, A. Adán, P. Del Estal, and E. Viñuela. 2007. Compatibility of endoparasitoid *Hyposoter didymator* (Hymenoptera: Ichneumonidae) protected stages with five selected insecticides. J. Econ. Entomol. 100: 1789–1796.

Orr, M. R. 1992. Parasitic flies (Diptera: Phoridae) influence foraging rhythms and caste division of labor in the leaf-cutter ant, *Atta cephalotes* (Hymenoptera: Formicidae). Behav. Ecol. Sociobiol. 30: 395–402.

Ouyang, Y., P. Chueca, S. J. Scott, G. H. Montez, and E. E. Grafton-Cardwell. 2010. Chlorpyrifos bioassay and resistance monitoring of San Joaquin Valley California citricola scale populations. J. Econ. Entomol. 103: 1400–1404.

Plowes, R. M., P. J. Folgarait, and L. E. Gilbert. 2012. The introduction of the fire ant parasitoid *Pseudacteon nocens* in North America: challenges when establishing small populations. Biocontrol 57: 503–514.

Porter, S. D. 2010. Distribution of the Formosa strain of the fire ant decapitating fly *Pseudacteon curvatus* (Diptera: Phoridae) three and a half years after releases in North Florida. Fla. Entomol. 93: 107–112.

Rodríguez, M. A., D. Bosch, B. Sauphanor, and J. Avilla. 2010. Susceptibility to organophosphate insecticides and activity of detoxifying enzymes in Spanish populations of *Cydia pomonella* (Lepidoptera, Tortricidae). J. Econ. Entomol. 103: 482–491.

Thead, L. G., and D. A. Streett. 2006. Duration of *Pseudacteon curvatus* Borgmeier (Diptera: Phoridae) pupal stages at reduced temperatures. J. Entomol. Sci. 41: 120–125.

Tonhasca Jr., A. 1996. Interactions between a parasitic fly, *Neodhorniphora declinata* (Diptera: Phoridae) and its host, the leaf-cutting ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae). Ecotropica 2: 157–164.

Tonhasca Jr., A., M. A. L. Bragança and M. Erthal Jr. 2001. Parasitism and biology of *Myrmosicarius grandicornis* (Diptera: Phoridae) in relationship to its host, the leaf-cutting ant *Atta sexdens* (Hymenoptera: Formicidae). Insect. Soc. 48: 154–158.

Vázquez, R. J., S. D. Porter, and J. A. Briano. 2006. Field release and establishment of the decapitating fly *Pseudacteon curvatus* on red imported fire ants in Florida. BioControl 57: 207–216.

Vogt J. T., S. D. Porter, D. A. Nordlund and R. Smith. 2003. A modified rearing system for production of *Pseudacteon curvatus* (Diptera: Phoridae), a parasitoid of imported fire ants. Biol. Control 28: 346–353.

Weber, N. A. 1972. Gardening ants, the Attines. The American Philosophical Society, Philadelphia, PA.

AQ: 12

The article is in press, therefore I do not know yet the volume and page range. I have cited the article following the instructions provided in the E.S.A. style guide for such an instance.

AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES 1

1—AQ1: Per ESA, either Genus species or ESA-accepted common name can be there in the title but not both. Please confirm the changes in the article title and amend if necessary.

2—AQ2: This article has been lightly edited for grammar, style, and usage throughout the main text as well as the figure legends and table caption, table bodies, and footnotes, as appropriate. Please limit your corrections to substantive changes that affect meaning. If no change is required in response to a query, please write "OK as set" in the margin. Thank you.

3—AQ3: Please confirm whether letter of permission has been granted for the unpublished data "P. J. F., unpublished data."

4—AQ4: Please confirm whether letter of permission has been granted for the unpublished data "A. C. G., unpublished data."

5—AQ5: ESA style prefers to be more specific when mentioning locations within the article (see Materials and Methods vs. see below). Please be more specific.

6—AQ6: Please check if the expression "LogN transformed" in the sentence construction "Development times and sizes. . . " is correct as given. Please amend if necessary.

7—AQ7: Please check if the changes made to the sentence construction "Considering the maximum parasitism rates. . . " are correct. Please amend if necessary.

8—AQ8: Please check if the change from "coevolving actors" to "coevolving factors" is correct. Please amend if necessary.

9—AQ9: Per ESA, acronyms or abbreviations are to be spelled out at the first instance. Accordingly, please spell out "CONICET" in the Acknowledgments section.

10—AQ10: Per ESA style, journal titles in foreign references must be spelled in full. Please spell out the journal title.

11—AQ11: Please note that the reference "Folgarait and Guillade (2013)" is not cited in the text. Please cite it in text or delete from the reference list.

12—AQ12: Please provide volume and page range for reference "Guillade and Folgarait (2013)."