

Incrimination of *Anopheles (Anopheles) intermedius* Peryassú, *An. (Nyssorhynchus) nuneztovari* Gabaldón, *An. (Nys.) oswaldoi* Peryassú as natural vectors of *Plasmodium falciparum* in French Guiana

Isabelle Dusfour⁺, Jean Issaly, Romuald Carinci, Pascal Gaborit, Romain Girod

Unité d'Entomologie Médicale, Institut Pasteur de la Guyane, 23 Av. Pasteur,
97306 Cayenne Cedex, Guyane Française

Anopheles darlingi Root is the major vector of human malaria in the Neotropics and has been considered to be the sole malaria vector in French Guiana. The presence of other potential vectors suggests that malaria may be transmitted by other species under certain conditions. From 2006-2011, all anopheline specimens collected from 11 localities were assayed to determine if the *Plasmodium circumsporozoite* protein was present. In addition to *An. darlingi*, we found *Anopheles oswaldoi*, *Anopheles intermedius* and *Anopheles nuneztovari* specimens that were infected with *Plasmodium* sp. Further investigations on the behaviour and ecology of *An. oswaldoi*, *An. intermedius* and *An. nuneztovari* are necessary to determine their role in malaria transmission in French Guiana.

Key words: *An. intermedius* - *An. nuneztovari* - *An. oswaldoi* - malaria vector - French Guiana

Anopheles darlingi Root is the major vector of human malaria in the Neotropics. In French Guiana, this anopheline species has been considered the sole vector of malaria for more than 50 years because of its high densities, high levels of anthropophilic behaviour and natural infectivity over a wide geographic range (Floch & Abonnenc 1943b, Mouchet et al. 1989, Claustre et al. 2001). Guianan populations of *Anopheles aquasalis* and *Anopheles triannulatus*, both known malaria vectors in South America (de Arruda et al. 1986, de Oliveira-Ferreira et al. 1990, Galardo et al. 2007, Sinka et al. 2010), were also studied by Floch and Abonnenc (1943a, 1944), and the capability of these mosquitoes to transmit *Plasmodium* parasites was demonstrated. However, no naturally infected specimen of these species was found in French Guiana. *Anopheles neivai*, another potential vector (Gutierrez et al. 2008), was suspected by Pajot et al. (1978) to transmit malaria in the Upper Oyapock valley based on observations of malaria cases, the high density of this species and the absence of *An. darlingi*. However, no infected specimens of this species have yet been identified. Therefore, all investigations appeared to confirm that *An. darlingi* was the only vector of *Plasmodium falciparum*, *Plasmodium vivax* and *Plasmodium malariae* (Mouchet et al. 1989, Claustre et al. 2001, Girod et al. 2008, 2011, Hiwat et al. 2009). However, certain malaria transmission patterns are still far from clear (Carme et al. 2009) and the presence of other anopheline species, such as *Anopheles braziliensis*, *Anopheles intermedius*, *Anopheles mediopunctatus* s.l., *Anopheles nuneztovari*

s.l., *Anopheles oswaldoi* s.l., *Anopheles strodei* and *An. triannulatus* s.l., known as primary, secondary or occasional malaria vectors across South America, suggests that other anopheline species may transmit malaria parasites in French Guiana (Panday 1977, de Arruda et al. 1986, Hayes et al. 1987, de Oliveira-Ferreira et al. 1990, Branquinho et al. 1993, Quinones et al. 2006, Galardo et al. 2007). Therefore, the collections performed by our team since 2006 have involved a systematic search for *Plasmodium* sporozoites in anopheline species. Preliminary results documenting *An. intermedius* and *An. nuneztovari* s.l. specimens that were naturally infected by *P. falciparum* have previously been cited in Carme et al. (2009). These findings are strengthened by the data and overall analysis presented here.

From 2006-2011, 11 localities were selected within the framework of various research programmes. The collection sites were distributed along the coastal area, where malaria transmission occurs sporadically and along the Maroni and the Oyapock valleys, where malaria is endemic (Figure). Different protocols were used to collect mosquitoes. Human landing collections (HLC) were performed from 6:00 am-8:00 am and 5:00 pm-7:00 pm (HLC-1), from 5:00 am-7:00 am, 9:00 am-11:00 am, 3:00 pm-5:00 pm and 6:00 pm-10:00 pm (HLC-2) or overnight (HLC-3). Animal bait (AB) was used overnight or during the day. Light traps (LT), LTs plus human bait, Mosquito Magnet (Woodstream Corporation, Lititz, PA, USA) traps and exposure-free bednet traps were used overnight (6:00 pm-6:00 am) (Supplementary data). Collections were made within villages or in the surrounding crop fields and forest. Female anopheline mosquitoes were individually labelled to reflect the site, mode and time of capture. Morphological identification was based on the keys of Shannon (1933), Floch and Abonnenc (1951), Forattini (1962), Faran (1980), Faran and Linthicum (1981), Linthicum (1988) and on unpublished observations from Dr Bruce Harrison to distinguish *An.*

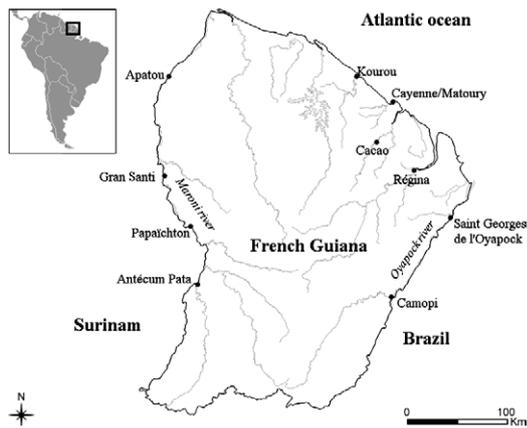
⁺ Corresponding author: idusfour@pasteur-cayenne.fr

Received 22 July 2011

Accepted 14 December 2011

intermedius from *Anopheles apicimacula*. The head and thorax of the anopheline females were tested with enzyme-linked-immunosorbent assays for *P. falciparum*, *P. vivax* (VK210 and VK247 variant epitopes) and *P. malariae* circumsporozoite proteins according to Burkot et al. (1984), as modified by Wirtz et al. (1987, 1992).

A total of 2,227 anopheline females were assayed for the presence of *Plasmodium* circumsporozoites. *An. darlingi* (n = 929) was the most abundant species collected (Table). The other potential malaria vectors found (Supplementary data) were *An. oswaldoi* s.l. (n = 483), *An. intermedius* (n = 246), *An. braziliensis* (n = 190), *An. nuneztovari* s.l. (n = 145) and *An. triannulatus* s.l. (n =



Location of collection sites in French Guiana.

68) (Table I). Two specimens of *An. darlingi* tested positive for *P. vivax* VK210, corresponding to an infection percentage of 0.22% (Supplementary data). These two infected females were both collected with the human-landing method between 6:00 pm-10:00 pm. The first female was captured in a forest camp in the Camopi area on 26 June 2007. The second infected female was caught in a crop field near Saint Georges de l'Oyapock on 5 May 2009. It is especially interesting that three additional highly abundant species tested positive for *P. falciparum* circumsporozoites: *An. oswaldoi* s.l. (n = 1), *An. intermedius* (n = 3) and *An. nuneztovari* s.l. (n = 1). Based on these results, 0.21% of *An. oswaldoi* s.l., 1.22% of *An. intermedius* and 0.69% of *An. nuneztovari* s.l. specimens collected were infected by *P. falciparum*. The infected *An. oswaldoi* s.l. specimen was collected with the Mosquito Magnet® trap in the forest near the village of Camopi. The infected *An. nuneztovari* s.l. specimen was caught using AB in the forest near the village of Saint Georges de l'Oyapock. The infected *An. intermedius* specimens were collected with the human-landing method between 6:00 pm-10:00 pm in the forest near the village of Saint Georges de l'Oyapock (n = 2) and in a crop field near the village of Cacao (n = 1). No other mosquito species naturally infected by *Plasmodium* species were found.

The primary mosquito collection activity since 2006 was conducted in areas in French Guiana where the malaria transmission patterns are variable: along the littoral zone, where malaria cases are sporadic, along the border with Suriname in the Maroni valley, where the number of cases has drastically decreased during the past few years, and along the border with Brazil in the Oyapock valley,

TABLE

List of anopheline females tested for *Plasmodium* infection, number of specimen tested and number of specimen found infected for *Plasmodium falciparum*, *Plasmodium vivax* variant 210, *P. vivax* variant 247 and *Plasmodium malariae*

<i>Anopheles</i> species	n	<i>P. falciparum</i> n (%)	<i>P. vivax</i> 210 n (%)	<i>P. vivax</i> 247 n (%)	<i>P. malariae</i> n (%)
<i>Anopheles darlingi</i>	929	-	2 (0.22)	-	-
<i>Anopheles oswaldoi</i> s.l.	483	1 (0.21)	-	-	-
<i>Anopheles intermedius</i>	246	3 (1.22)	-	-	-
<i>Anopheles braziliensis</i>	190	-	-	-	-
<i>Anopheles nuneztovari</i> s.l.	145	1 (0.69)	-	-	-
<i>Anopheles triannulatus</i> s.l.	68	-	-	-	-
<i>Anopheles</i> sp.	41	-	-	-	-
<i>Anopheles acanthotorynus/nimbus</i>	42	-	-	-	-
<i>Anopheles mediopunctatus</i> s.l.	29	-	-	-	-
<i>Anopheles ininii</i>	19	-	-	-	-
<i>Anopheles aquasalis</i>	17	-	-	-	-
<i>Anopheles peryassui</i>	11	-	-	-	-
<i>Anopheles minor</i>	3	-	-	-	-
<i>Anopheles neivai</i>	3	-	-	-	-
<i>Anopheles argyritarsis</i>	1	-	-	-	-
Total	2,227	5	2	-	-

where the malaria transmission pattern is still not clear (Carme et al. 2009). The present work confirms (i) the role of *An. darlingi* as a malaria vector and (ii) the suspected presence of other *Anopheles* species that are naturally infected by *Plasmodium* spp in French Guiana.

An. darlingi was the most abundant species of the study. Of the specimens collected, 0.22% were infected with and could transmit *P. vivax*. This percentage is lower than those obtained for *An. intermedius* (1.22%) and *An. nuneztovari* (0.69%), but does not challenge the status of *An. darlingi* as the principal malaria vector in French Guiana. Indeed, in many previous studies performed in this region, *An. darlingi* was collected in high densities, had a high sporozoite index and was the only species naturally infected by the four *Plasmodium* sp. and variants, especially along the border with Suriname in the Maroni valley (Girod et al. 2008, Hiwat et al. 2009, Fouque et al. 2010). In our study, specimens of this species infected by *P. vivax* were found along the Oyapock River, where this parasite causes most cases of malaria (Carme et al. 2009, Girod et al. 2011), whereas the other anophelines carried *P. falciparum*. Even if our dataset does not furnish conclusive evidence, the documented parasite infections define a pattern in which the status of *An. darlingi* as the major vector is certain and in which *An. intermedius*, *An. nuneztovari* and *An. oswaldoi* may play local roles in *P. falciparum* transmission.

It is rare to find and document a naturally infected *An. intermedius*. de Arruda (1986) found that 3.3% of the specimens tested contained oocysts in their midguts. Galardo et al. (2007) found the four *Plasmodium* species and variants in four distinct *An. intermedius* populations in the neighbouring state of Amapá, Brazil, but observed a low inoculation rate for this species. Even if little is known about the role of *An. intermedius* in malaria transmission, vigilance has always been required for this vector (dos Santos et al. 2005, Sinka et al. 2010). Our study found *An. intermedius* specimens that were naturally infected by *P. falciparum* in Saint Georges de l'Oyapock, at the Brazilian border. However, no study has yet demonstrated a role for this mosquito species in malaria transmission. In addition, this species is often described as exophilic or zoophilic and is found in sylvatic or peri-sylvatic environments (Forattini 1962, Zimmerman et al. 2006, Galardo et al. 2007). It is probable that contact between this species and humans is occasional and could occur in households or camps near the forest. *An. oswaldoi* s.l. has been identified as a secondary or local vector in many areas of South America (Hayes et al. 1987, Branquinho et al. 1993, Mouchet et al. 2004, Quinones et al. 2006, Sinka et al. 2010). The species complex includes at least four species (Marrelli et al. 1999) that can transmit *P. falciparum*, *P. vivax* variants and *P. malariae* (Hayes et al. 1987, Branquinho et al. 1993, Quinones et al. 2006). The biting behaviour of these mosquitoes is often described as exophilic and zoophilic. In this study, we found one specimen that was naturally infected with *P. falciparum* in a forested area near Camopi. However, we did not identify the species within the complex and the behavioural patterns of this species remain unknown. *An. nuneztovari* s.l. is described as a primary or

secondary vector (Hayes et al. 1987, Tadei & Thatcher 2000, Mouchet et al. 2004, Quinones et al. 2006, Moreno et al. 2007, Sinka et al. 2010). Heterogeneous behaviours and variable vectorial capacities throughout the distribution of this mosquito taxon may be explained by the presence of a complex of species (Sinka et al. 2010), in which five genetic lineages are currently identified (Mirabello & Conn 2008). We did not identify species within the *An. oswaldoi* s.l. complex and the behavioural patterns of this group remain unknown. Based on the literature, we can hypothesise that the populations from French Guiana resemble those from Brazil and Suriname. Exophilic, exophagic and zoophilic behaviours have been observed in this region of South America (Panday 1977, Montoya-Lerma et al. 2011), whereas anthropophilic and endophagic behaviours are commonly found in Peru, Colombia and Venezuela (Montoya-Lerma et al. 2011). In addition, Mirabello and Conn (2008) identified lineage 1 in northeastern and central Amazonia, including the Guiana shield. In French Guiana, data on *An. intermedius*, *An. oswaldoi* s.l. and *An. nuneztovari* s.l. are still scarce. Further insights on the behaviour, ecology and genetics of these species are needed to confirm the roles that the species play in malaria transmission.

In addition to the present work, studies on other species known to be possible vectors in neighbouring countries (e.g., *An. triannulatus* s.l. or *An. aquasalis*) would complement the approach taken by our research (Charlwood & Wilkes 1981, Rubio-Palis 1994, Galardo et al. 2007, Sinka et al. 2010). Mosquito collections performed in the inland part of French Guiana would serve to complete the *Anopheles* species list in French Guiana. Indeed, it is surprising that species known as malaria vectors in the Brazilian Amazon and Suriname (e.g., the Albitarsis complex) have not yet been encountered in French Guiana.

ACKNOWLEDGEMENTS

To Dr RE Harbach, Dr B Harrison, Dr RC Wilkerson and Prof MAM Sallum, for providing help to clearly assess the identification of *An. intermedius*, and to Sarah C Chaney, for proofreading the paper.

REFERENCES

- Branquinho MS, Lagos CB, Rocha RM, Natal D, Barata JM, Cochrane AH, Nardin E, Nussenzweig RS, Kloetzel JK 1993. Anophelines in the state of Acre, Brazil, infected with *Plasmodium falciparum*, *P. vivax*, the variant *P. vivax* VK247 and *P. malariae*. *Trans R Soc Trop Med Hyg* 87: 391-394.
- Burkot TR, Williams JL, Schneider I 1984. Identification of *Plasmodium falciparum*-infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. *Am J Trop Med Hyg* 33: 783-788.
- Carme B, Ardillon V, Girod R, Grenier C, Joubert M, Djossou F, Ravachol F 2009. Update on the epidemiology of malaria in French Guiana. *Med Trop* 69: 19-25.
- Charlwood JD, Wilkes TJ 1981. Observations on the biting activity of *Anopheles triannulatus bachmanni* from Mato Grosso, Brazil. *Acta Amazonica* 11: 411-441.
- Claustre J, Venturin C, Nadire M, Fauran P 2001. Malarial vectors in French Guiana: study in an epidemic focus near Cayenne (1989-1998). *Bull Soc Pathol Exot* 94: 353-357.
- de Arruda M, Carvalho MB, Nussenzweig RS, Maracic M, Ferreira AW, Cochrane AH 1986. Potential vectors of malaria and their

- different susceptibility to *Plasmodium falciparum* and *Plasmodium vivax* in northern Brazil identified by immunoassay. *Am J Trop Med Hyg* 35: 873-881.
- de Oliveira-Ferreira J, Lourenço-de-Oliveira R, Teva A, Deane LM, Daniel-Ribeiro CT 1990. Natural malaria infections in anophelines in Rondônia state, Brazilian Amazon. *Am J Trop Med Hyg* 43: 6-10.
- dos Santos JM, Rodriguez GA, Maia J de F, Tadei WP 2005. Variability and genetic differentiation among *Anopheles (Ano.) intermedius* Chagas, 1908 and *Anopheles (Ano.) mattogrossensis* Lutz & Neiva, 1911 (Diptera: Culicidae) from the Brazilian Amazon. *Mem Inst Oswaldo Cruz* 100: 531-537.
- Faran ME 1980. Mosquito studies (Diptera: Culicidae): a revision of the Albimanus Section of the subgenus *Nyssorhynchus* of *Anopheles*. *Contrib Am Entomol Inst* 15: 1-215.
- Faran ME, Linthicum KJ 1981. A handbook of the Amazonian species of *Anopheles (Nyssorhynchus)* (Diptera: Culicidae). *Mosq Syst* 13: 1-81.
- Floch H, Abonnenc E 1943a. *A. aquasalis* Curry 1932 et paludisme en Guyane Française. Infection naturelle et infection expérimentale. In *Institut Pasteur de la Guyane et du Territoire de l'Inini*, Institut Pasteur de la Guyane et du Territoire de l'Inini, Cayenne, p. 8.
- Floch H, Abonnenc E 1943b. Sur le rôle de *A. darlingi* Root 1926 dans la transmission du paludisme en Guyane Française. In *Institut Pasteur de la Guyane et du Territoire de l'Inini*, Institut Pasteur de la Guyane et du Territoire de l'Inini, Cayenne, p. 10.
- Floch H, Abonnenc E 1944. *A. triannulatus* Neiva & Pinto en Guyane Française. Morphologie des oeufs et infection expérimentale par *P. falciparum* et *P. vivax*. In *Institut Pasteur de la Guyane et du Territoire de l'Inini*, Institut Pasteur de la Guyane et du Territoire de l'Inini, Cayenne, p. 7.
- Floch H, Abonnenc E 1951. Anophèles de la Guyane Française. *Arch Inst Pasteur Guyane Terr Inini* 236: 1-92.
- Forattini OP 1962. *Entomologia médica*, Vol. 1, Faculdade de Saúde Pública da Universidade de São Paulo, São Paulo, 662 pp.
- Fouque F, Gaborit P, Carinci R, Issaly J, Girod R 2010. Annual variations in the number of malaria cases related to two different patterns of *Anopheles darlingi* transmission potential in the Maroni area of French Guiana. *Malar J* 9: 80.
- Galardo AK, Arruda M, D'Almeida Couto AA, Wirtz R, Lounibos LP, Zimmerman RH 2007. Malaria vector incrimination in three rural riverine villages in the Brazilian Amazon. *Am J Trop Med Hyg* 76: 461-469.
- Girod R, Gaborit P, Carinci R, Issaly J, Fouque F 2008. *Anopheles darlingi* bionomics and transmission of *Plasmodium falciparum*, *Plasmodium vivax* and *Plasmodium malariae* in Amerindian villages of the Upper-Maroni Amazonian forest, French Guiana. *Mem Inst Oswaldo Cruz* 103: 702-710.
- Girod R, Roux E, Berger F, Stefani A, Gaborit P, Carinci R, Issaly J, Carme B, Dusfour I 2011. Unravelling the relationships between *Anopheles darlingi* (Diptera: Culicidae) densities, environmental factors and malaria incidence: understanding the variable patterns of malarial transmission in French Guiana (South America). *Ann Trop Med Parasitol* 105: 107-122.
- Gutierrez LA, Naranjo N, Jaramillo LM, Muskus C, Luckhart S, Conn JE, Correa MM 2008. Natural infectivity of *Anopheles* species from the Pacific and Atlantic regions of Colombia. *Acta Trop* 107: 99-105.
- Hayes J, Calderon G, Falcon R, Zambrano V 1987. Newly incriminated anopheline vectors of human malaria parasites in Junin department, Peru. *J Am Mosq Control Assoc* 3: 418-422.
- Hiwat H, Issaly J, Gaborit P, Somai A, Samjawan A, Sardjoe P, Soekhoe T, Girod R 2009. Behavioral heterogeneity of *Anopheles darlingi* (Diptera: Culicidae) and malaria transmission dynamics along the Maroni River, Suriname, French Guiana. *Trans R Soc Trop Med Hyg* 104: 207-213.
- Linthicum KJ 1988. A revision of the Argyritarsis Section of the subgenus *Nyssorhynchus* of *Anopheles* (Diptera: Culicidae). *Mosq Syst* 20: 98-271.
- Marrelli MT, Malafronte RS, Flores-Mendoza C, Lourenço-de-Oliveira R, Kloetzel JK, Marinotti O 1999. Sequence analysis of the second internal transcribed spacer of ribosomal DNA in *Anopheles oswaldoi* (Diptera: Culicidae). *J Med Entomol* 36: 679-684.
- Mirabello L, Conn JE 2008. Population analysis using the nuclear white gene detects Pliocene/Pleistocene lineage divergence within *Anopheles nuneztovari* in South America. *Med Vet Entomol* 22: 109-119.
- Montoya-Lerma J, Solarte YA, Giraldo-Calderón GI, Quiñones ML, Ruiz-López F, Wilkerson RC, González R 2011. Malaria vector species in Colombia - A Review. *Mem Inst Oswaldo Cruz* 106 (Suppl. 1): 223-238.
- Moreno JE, Rubio-Palis Y, Paez E, Perez E, Sanchez V 2007. Abundance, biting behaviour and parous rate of anopheline mosquito species in relation to malaria incidence in gold-mining areas of southern Venezuela. *Med Vet Entomol* 21: 339-349.
- Mouchet J, Carnevale P, Coosemans M, Julvez J, Manguin S, Richard-Lenoble D, Sircoulon J 2004. *Biodiversité du paludisme dans le monde*, John Libbey, Eurotext, Paris, 428 pp.
- Mouchet J, Nadire-Galliot M, Gay F, Poman JP, Lepelletier L, Clautre J, Bellony S 1989. Malaria in Guiana. II. The characteristics of different foci and antimalarial control. *Bull Soc Pathol Exot Filiales* 82: 393-405.
- Pajot FX, Molez JF, Le Pont F 1978. Anophèles et paludisme sur le Haut-Oyapock (Guyane Française). *Cahiers de l'ORSTOM series entomologie médicale et Parasitologie XVI*: 105-111.
- Panday SR 1977. *Anopheles nuneztovari* and malaria transmission in Surinam. *Mosq Syst* 37: 728-737.
- Quinones ML, Ruiz F, Calle DA, Harbach RE, Erazo HF, Linton YM 2006. Incrimination of *Anopheles (Nyssorhynchus) rangeli* and *An. (Nys.) oswaldoi* as natural vectors of *Plasmodium vivax* in Southern Colombia. *Mem Inst Oswaldo Cruz* 101: 617-623.
- Rubio-Palis Y 1994. Variation of the vectorial capacity of some anophelines in western Venezuela. *Am J Trop Med Hyg* 50: 420-424.
- Shannon RC 1933. Anophelines of the Amazon Valley. *Proceedings of the Entomological Society of Washington* 35: 117-143.
- Sinka ME, Rubio-Palis Y, Manguin S, Patil AP, Temperley WH, Gething PW, Van Boeckel T, Kabaria CW, Harbach RE, Hay SI 2010. The dominant *Anopheles* vectors of human malaria in the Americas: occurrence data, distribution maps and bionomic precursors. *Parasit Vectors* 3: 72.
- Tadei WP, Thatcher BD 2000. Malaria vectors in the Brazilian Amazon: *Anopheles* of the subgenus *Nyssorhynchus*. *Rev Inst Med Trop Sao Paulo* 42: 87-94.
- Wirtz RA, Burkot TR, Graves PM, Andre RG 1987. Field evaluation of enzyme-linked immunosorbent assays for *Plasmodium falciparum* and *Plasmodium vivax* sporozoites in mosquitoes (Diptera: Culicidae) from Papua New Guinea. *J Med Entomol* 24: 433-437.
- Wirtz RA, Sattabongkot J, Hall T, Burkot TR, Rosenberg R 1992. Development and evaluation of an enzyme-linked immunosorbent assay for *Plasmodium vivax*-VK247 sporozoites. *J Med Entomol* 29: 854-857.
- Zimmerman RH, Galardo AK, Lounibos LP, Arruda M, Wirtz R 2006. Bloodmeal hosts of *Anopheles* species (Diptera: Culicidae) in a malaria-endemic area of the Brazilian Amazon. *J Med Entomol* 43: 947-956.