Medical and Veterinary Entomology (2000) 14, 339–344

Density and dispersal of the loaiasis vector *Chrysops dimidiata* in southern Cameroon

J.-P. CHIPPAUX, B. BOUCHITÉ, M. DEMANOU, I. MORLAIS and G. LE GOFF*

Institut de Recherche pour le Développement (IRD, ex-ORSTOM), Centre Pasteur du Cameroun, Yaoundé, Cameroon and * IRD, OCEAC, Yaoundé, Cameroon

Abstract. By mark–release–recapture experiments, we assessed the density of loaisis vectors, *Chrysops dimidiata* Wulp plus some *Chrysops silacea* Austen (Diptera: Tabanidae) and estimated their range of flight in the secondary forest of southern Cameroon. In 1993, the release point was at the centre of the study area and recapture points were at 1100 m radius. In 1994, releases were on the periphery of the study area and recapture sites were 400–8000 m from the release points. Results were concordant and showed *Chrysops* female densities of 785–3682 flies/km^2. The theoretical flight range was < 6000 m, with a maximum distance of 4500 m observed. These results are considered promising for the use of vector control methods against loaiasis.

Key words. *Chrysops dimidiata*, *Chrysops silacea*, flight range, large scale, loaiasis, mass chemotherapy, vector control, vector density, Cameroon.

Introduction

Loaiasis is a human disease caused by infection with the filarial nematode *Loa loa* (Cobbold) transmitted by haematophagous flies of the genus *Chrysops* (Diptera: Tabanidae). Being restricted to humid zones of tropical Africa (Thomson *et al.*, 2000) and causing only mild symptoms, loaiasis remains a neglected zoonotic disease (Pinder, 1988). Among communities where loaiasis is endemic, however, the pathology of this disease may be the third most frequent condition for which they seek medical consultation (Noireau *et al.*, 1990a). Mass treatment appeared to be dangerous, because hypermicrofilaremic patients treated with diethylcarbamazine or ivermectin could develop severe adverse reactions such as coma and death (Chippaux *et al.*, 1996). Vector control would be a good alternative method if the density and dispersal of vectors are limited, as suggested by previous studies. For example, the biting density of *Chrysops silacea* averaged 13/man-day in western Cameroon (Crewe & O'Rourke, 1951) and only 4/man-hour in the Chaillu mountains of Congo (Noireau *et al.*, 1990b). Our observations on *Chrysops dimidiata*, as well as *C. silacea* in southern Cameroon, agree. Furthermore, Beesley & Crewe (1963) mentioned that the flight range of *C. silacea* was < 4600 m. To increase our understanding of these factors, we carried out two mark–release–recapture experiments with adult *Chrysops* in May 1993 and April 1994.

Correspondence: Dr J.-P. Chippaux, IRD, BP 1386, Dakar, Sénégal. E-mail: chippaux@ird.sn

Materials and methods

Study area (Fig. 1)

The village of Ngat (3°20′N, 11°40′E, population ~500) is surrounded by secondary rain forest, 50 km south of Yaoundé. Along 10 km of earthen road, plantations of cocoa and other crops are accessible via a system of cross-tracks, with houses at well-spaced intervals by the roadside.

Bait collections

Chrysops females were captured outdoors, taking care not to allow them to bite. Preliminary samples during April and May 1993, the peak season for *Chrysops*, allowed us to assess their spatial distribution and to obtain healthy specimens for mark–release–recapture. Thirty collectors using hand-nets were spaced ~500 m apart along the road and tracks among plantations over an area of 3.8 km^2 in 1993 and 45 km^2 in 1994. *Chrysops* were first put singly in glass tubes (plugged with cotton wool), identified and counted, then grouped 100 per plastic bottle (1500 ml) and held until the evening.

© 2000 Blackwell Science Ltd

Fonds Documentaire IRD
010023358

Fonds Documentaire IRD
Cote : B* 23358 Ex : 1

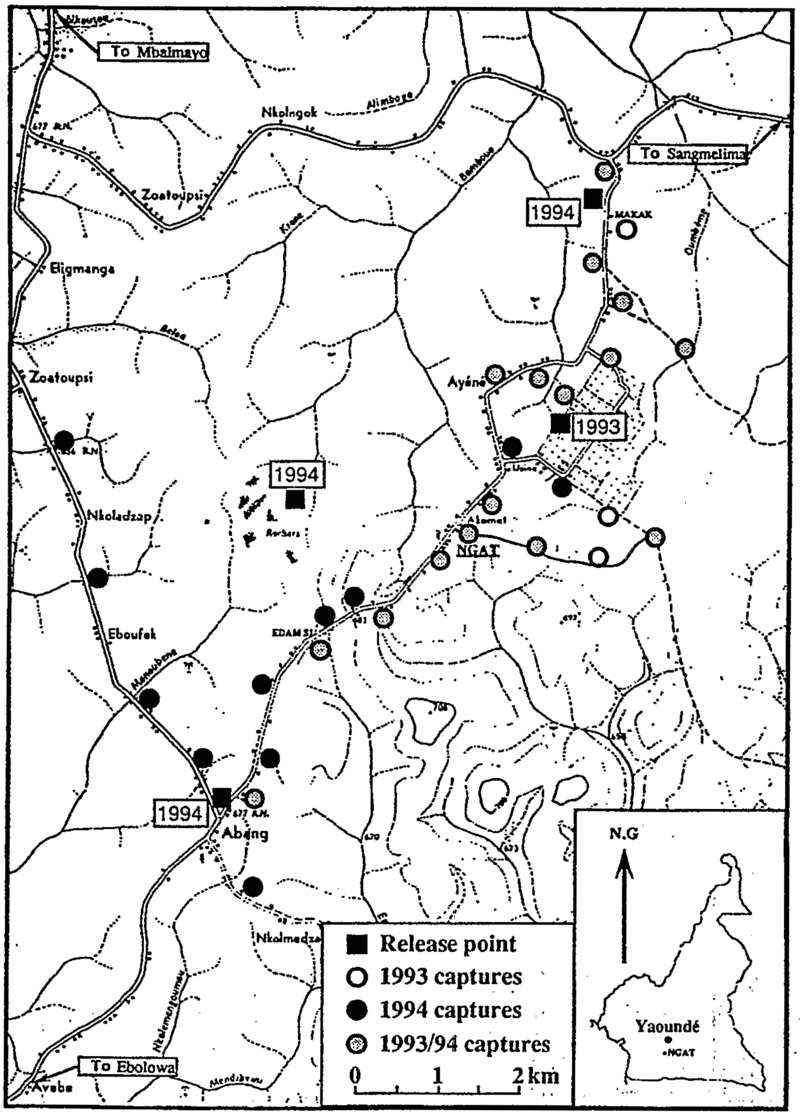


Fig. 1. Map of Ngat study area, 50 km south of Yaoundé, Cameroon, showing *Chrysops* release points and recapture positions for 1993 and 1994 experiments.

Mark and release

Flies were marked with dry fluorescent powders (Shannon Luminous Materials Inc., Bioquip Products, Gardena, CA, U.S.A.), using a different colour for each daily release: red for day 0 (d_0), yellow for day 2 (d_2) and green for day 3 (d_3) in 1993; red (d_0), yellow (d_1) and blue for day 3 (d_3) – more easily recognizable than the green – in 1994. The powder was dusted into each bottle of live *Chrysops* and spread by slowly rolling the bottle. Flies marked with these colours could be readily recognized with the naked eye, although an ultraviolet lamp illuminated the colours better.

In 1993, three groups of *Chrysops* were released from the same point in the middle of the study area: a clearing situated in a loop of the road, ~1 km from it. In 1994, three groups of *Chrysops* were released from different points on the west, north and south of the study area (Fig. 1). *Chrysops* collected during the day were released together during the evening. Any that were dead or unable to fly were identified (using the taxonomic key of Oldroyd, 1957), counted and their number deducted from those released.

Recaptures

After their release, attempts to recapture marked *Chrysops* followed the same collecting procedures described above. The exact position of recapture sites and their distances from the release point were determined by Global Positioning System (GPS). To minimize any effects of personal bias, each collector changed position sequentially each day.

In 1993, because of bad weather, recaptures began on the second day (d_2) instead of the first day (d_1) after the first release. At first (on d_2), all collectors were within a radius of ~1100 m. Thereafter, recapture points were moved out to 8000 m from the release site. In 1994, recapture sites ranged from 400 to 10 000 m from the release sites, and recaptures began on the first day (d_1) following release.

Daily captures continued for 2 weeks after the third release, i.e. until 17 days after those marked red were released, 15 or 16 days after those marked yellow were released, and 14 days after those marked green were released.

Density was estimated from d_2 data because, according to Beesley & Crewe (1963), recaptures were best on the second day.

Chrysops distribution

Spatial distribution of *Chrysops* spp. (pooled *C. dimidiata* and *C. silacea*) was estimated from the frequency distribution of the number captured daily at each site. For those recaptured (marked) we determined whether dispersal was random, clumped or uniform (Ludwig & Reynolds, 1988). We fitted the frequency distribution of the number of *Chrysops* per sample to classic models of population spatial patterns: random dispersal (Poisson model), clumped dispersal (negative binomial model) or uniform dispersal (binomial model) using the software developed by Ludwig & Reynolds (1988). The significance of fit was tested by χ^2.

Chrysops density

We used the Lincoln–Petersen method, designed for single mark–release–recapture sampling, whereby the population density (D) can be estimated from the following equation:

$$D = \frac{M.\,n}{m}$$

where M is the number of released individuals, n is the total number of individuals caught during the recapture operation and m is the number of marked individuals recaptured (Caughley, 1977). The standard deviation of D is given by the equation:

$$\sigma = \frac{M^2(n+1)(n-m)}{(m+1)^2(m+2)}$$

We applied the Lincoln–Petersen equation independently for each of the three daily mark–release–recapture experiments, and for both sets of experiments in 1993 and 1994.

To obtain the average *Chrysops* density from multiple mark–released operations, we used the Schumacher & Eschmeyer equation:

$$D = \frac{\Sigma(M_i^2.\,n_i)}{\Sigma(M_i.\,m_i)}$$

where M_i represents the total number of individuals marked with each of the three colours, n_i the total number of captured individuals during the recapture period and m_i is the number of marked individuals observed in each sample (Caughley, 1977). The standard deviation of D cannot be calculated, but it is possible to express the standard deviation of $1/D$ by the following equation:

$$\sigma = \frac{\dfrac{\Sigma(m_i^2/n_i) - (\Sigma M_i m_i)^2/\Sigma(M_i^2 n_i)}{j-1}}{\Sigma(M_i^2 n_i)}$$

which allowed us to compare results of 1993 with those of 1994.

Chrysops flight range

We assessed the flight range of *Chrysops* by two methods. First we measured the relative proportions (cumulated frequency) of marked *Chrysops* in relation to distance from their site of release (Beesley & Crewe, 1963). We also correlated the density of marked *Chrysops* with distance between points of release and recapture. The density of recapture (Dr) integrates the number of marked *Chrysops* and the efficiency of the capture at the same place, according to the equation:

$$Dr = \frac{(m/n)}{M} \times 100$$

where m is the number of marked *Chrysops* at a given point, n the total number of *Chrysops* caught at the same place, and M, is the number of *Chrysops* released. Each day, the number of marked *Chrysops* caught was deduced from M, the number of released *Chrysops*.

Whereas *Chrysops* density was based on all recapture sites, in order to estimate the flight range we used data from selected recapture sites on the basis of the following criteria: average density > 15/day and data available from > 8 days of recapturing (well distributed during the sampling period). Correlations were calculated after logarithmic transformation of both the recapture density and the distance from the release point.

Results

Species and their spatial distribution (Table 1)

Chrysops dimidiata comprised ~90% and *C. silacea* ~10% of the females captured. The frequency distribution of the number per day of *Chrysops* spp. at each site fitted the Poisson model, meaning that they were randomly distributed in the study area ($\chi^2 = 1.55$; d.f. = 4; $P > 0.5$). Furthermore, we found that the Poisson model fits all recaptures made during 1993 and

© 2000 Blackwell Science Ltd,

1994 ($P>0.05$). The spatial pattern also fitted the negative binomial model for slightly aggregated distribution, but less significantly than to the Poisson model (Table 1).

Density of Chrysops dimidiata *(Table 2)*

In the 1993, we released a total of 2347 marked *C. dimidiata* females. During the following 2 weeks, 1089 *C. dimidiata* were collected among which 284 were marked. Using the Schumacher & Eschmeyer equation, we estimated the density of *C. dimidiata* at 3405 individuals ($\sigma=0.11.10^{-3}$) in the study area of 3.8 km^2, i.e. 896/km^2. Taking the releases into account, according to the Lincoln–Petersen equation, the density of *C. dimidiata* varied between 785 and 1429/km^2.

In 1994, we released a total of 3931 marked *C. dimidiata* females and recaptures were attempted over an area of ~45 km^2. During the 2 weeks of collections, we obtained 3429 *Chrysops*, among which 76 were marked. From the Lincoln–Petersen equation the densities were estimated at 880–3682/km^2 (not significantly different between years). Using the Schumacher & Eschmeyer equation, the density of *C. dimidiata* was estimated at 54 657 in the area of 45 km^2 ($\sigma=0.88.10^{-5}$), i.e. 1215/km^2. The difference of density between 1994 and 1993 was slightly significant ($t=2.31$; $P=0.02$).

Table 1. Numbers (frequency distribution) of *Chrysops* captured before marking.

Class	Number	1993 captures	1994 captures	Both years
0	10–19	3	1	4
1	20–29	1	2	3
2	30–39	5	3	8
3	40–49	7	8	15
4	50–59	3	4	7
5	60–69	4	6	10
6	70–79	1	2	3
7	80–89	0	3	3
8	90–99	1	4	5
9	100–109	0	3	3

χ^2 (fit to Poisson model): 0,47 (d.f. = 2); 2,13 (d.f. = 3); 7,52 (d.f. = 5)
χ^2 (fit to negative binomial model): 2,35 (d.f. = 1); 1,89 (d.f. = 2); 5,11 (d.f. = 4)

Flight range of Chrysops

In 1993, on the second day post-release, we recaptured marked individuals at every site, all < 1100 m from where they were released. Evidently the flight range exceeded 1 km in 2 days. The correlation coefficient between the distance of recapture and density of *Chrysops* spp. was significant ($r=-0.80$; d.f. = 28; $P<0.01$). Considering only *C. dimidiata*, the correlation coefficient was highly significant ($r=-0.91$; d.f. = 17; $P<0.01$). Too few *C. silacea* were recaptured for us to evaluate its flight range. We observed no significant differences of recapture rates according to the colours used for marking *Chrysops*, implying that they were equally tolerated.

In 1994, the greatest observed dispersal of *Chrysops* by d_2 was 4000 m. The correlation coefficient between density and distance from the release point was significant ($r=-0.58$; d.f. = 66; $P<10^{-2}$). The maximum observed dispersal was 4500 m for *C. dimidiata* and 2200 m for *C. silacea* during the 2 weeks of sampling.

Results for 1993 and 1994 were similar. By combining all the data, the theoretical maximum dispersal of *C. dimidiata* would be 5441 m by d_2 ($r=-0.83$; d.f. = 96; $P<0.01$; Fig. 2) and 4633 m during the 2 weeks ($r=-0.77$; d.f. = 45; $P<0.01$; Fig. 3). The cumulative frequency of recapture at different distances from the release points (Fig. 4) showed that 50% of *C. dimidiata* were recaptured within 800 m and < 1% were recaptured > 3500 m from the release point within 2 weeks of their release.

Discussion

We chose the village of Ngat for these studies because it is has abundant *Chrysops* and endemic loaiasis among accessible plantations and secondary forest, only 50 km from Yaoundé. Of the two main vectors of human loaiasis, *C. dimidiata* was found to predominate in this study area, whereas *C. silacea* predominates in other parts of Cameroon (Oldroyd, 1957). The relative isolation of Ngat village was an advantage for the investigation of loaiasis epidemiology and potential control in this highly endemic area (Boussinesq & Gardon, 1997).

Knowledge of *Chrysops* density and range of flight is essential to develop a vector control strategy. Short flight range reduces the risk of reinvasion by infected vectors from outside a zone under control, whereas greater range would necessitate more widespread control measures. Many factors influence the

Table 2. Density of *Chrysops dimidiata* estimates 2 days post-release.

Powder	No. released (M)	No. sample (n)	No. marked (m)	Density in study area	σ	Density/km^2
Red 1993	990	766	226	3355	186	883
Yellow 1993	611	159	17	5431	1174	1,429
Green 1993	746	164	41	2984	386	785
Red 1994	1732	1464	64	39 620	4697	880
Yellow 1994	1018	984	6	143 247	50 465	3,183
Blue 1994	1181	981	6	165 677	58 367	3,682

flight behaviour and dispersal of *Chrysops* adults (Duke, 1972). By capturing *Chrysops* females on human bait at ground level we ignored their vertical distribution, although adult *Chrysops* spend most of their life in the canopy (Duke, 1955a, 1957). Also, *Chrysops* biting densities vary considerably between ecological zones (Duke, 1955b; Noireau *et al.*, 1990b). Therefore, we chose sampling sites of in a limited area of relatively homogeneous vegetation, where *Chrysops* dispersal was expected to be random (negative binomial model). If *Chrysops* dispersal in forest is rather clumped, this could explain our data also fitting the Poisson model.

Estimation of population density by mark–release–recapture supposes that there is no change in demographic condition during the experiment. We assessed data from d_2 (samples collected two days post-release) because it involved minimal delay and gave the best recapture results (Beesley & Crewe, 1963). Among the factors affecting *Chrysops* demography and survival of released flies, assuming that powders are not toxic, the coloration may have increased risks of natural predation by making flies more conspicuous. For fear of obtaining insufficient *Chrysops* in 1993 we used many collectors close to the release point. This could have depleted the density of *Chrysops* in the catching area. The number of unmarked *Chrysops* caught after successive release might have been suppressed (especially in 1993) by intensive captures during preceding days, reducing the numerator of the Lincoln–Petersen equation. If so, we would have underestimated the density of *Chrysops*. In 1994, therefore we changed the location of releases and made recaptures further from the release points. Despite these experimental differences, the density estimates of *Chrysops* populations were of similar magnitude in both years: 785–1429/km^2 in 1993; 880–3682/km^2 in 1994 (not significantly different).

Almost certainly the overall flight range of *Chrysops* was underestimated, partly due to the limited sampling times and because the probability to capture a marked fly decreased geometrically, whereas the distance is a linear function. According to Crewe & O'Rourke (1951), *Chrysops* seem capable to seek their target by sight from afar, and we assume that our collectors missed none. The cumulative frequency of marked *Chrysops* according to distance from the release point was affected by the density (sampling favourability for *Chrysops*) at each site of collection. Even so, correlation between the logarithm of the density of marked *Chrysops* and the logarithm of the distance from the point of release permitted us to evaluate (by regression analysis) the maximal theoretical range of flight as more than 5 km, although < 1% of marked flies recaptured were > 3.5 km from their release point.

Our findings with *C. dimidiata* agree generally with those obtained for *C. silacea* by different procedures: Beesley & Crewe (1951) marked and released *Chrysops* on the day of collection, day after day throughout the year (total ~10 000 individuals). However, their lack of sampling standardization obviates statistical analysis comparable to ours. From an epidemiological point of view, our findings on the dispersal of loiasis vectors must be considered with caution. They were obtained in a limited and homogeneous area of secondary forest and plantations, not representative of the primary forest natural *Chrysops* habitat. Although this was taken to be more associated with human exposure, the study area was also chosen for the likely random spatial pattern of *Chrysops*

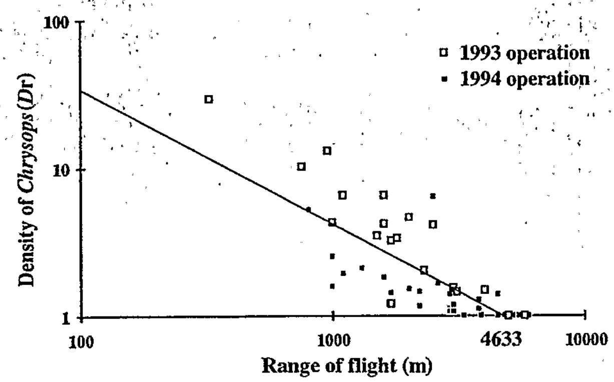


Fig. 3. Correlation between density of marked *Chrysops* and distance from the release point during the entire experiment (1993 and 1994).

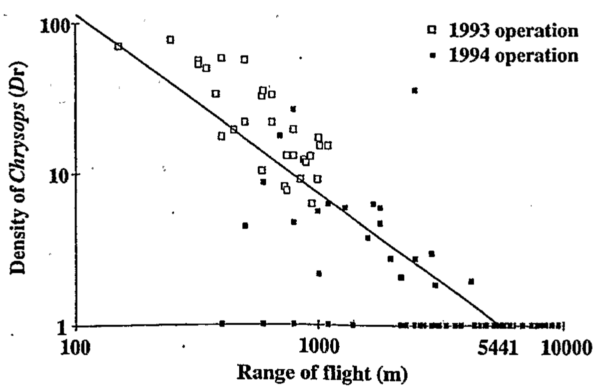


Fig. 2. Correlation between density of marked *Chrysops* and distance from the release point on d_2 (1993 and 1994 experiments).

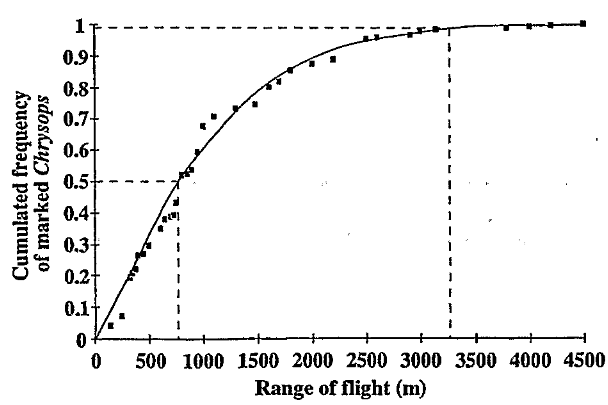


Fig. 4. Cumulative frequency of marked *Chrysops* according to distance from release point during entire experiment (1993 + 1994).

dispersal. On a larger scale the vector dispersal could be clumped, with focally increased density of vector populations and greater risks of transmission.

This study confirms the fairly low population density of adult *Chrysops* (~1000/km^2) and that their flight range is usually not great in secondary forest. These results are very promising for use to limit loaiasis if an effective method of vector control is developed. *Chrysops* control, if economically feasible, would be a good alternative to mass human chemotherapy (e.g. with ivermectin) in the loaiasis endemic zone of tropical Africa (Thomson *et al.*, 2000).

References

Beesley, W.N. & Crewe, W. (1963) The bionomics of *Chrysops silacea* Austen, 1907 – II. The biting rhythm and dispersal in rain-forest. *Annals of Tropical Medicine and Parasitology*, **57**, 191–203.

Boussinesq, M. & Gardon, J. (1997) Prevalences of *Loa loa* microfilaraemia throughout the area endemic for the infection. *Annals of Tropical Medicine and Parasitology*, **91**, 573–589.

Caughley, G. (1977) *Analysis of Vertebrate Populations*. John Wiley & Sons, New York.

Chippaux, J.-P., Boussinesq, M., Gardon, J., Gardon-Wendel, N. & Ernould, J.-C. (1996) Severe adverse reaction risks during mass treatment with ivermectin in loiasis-endemic areas. *Parasitology Today*, **12**, 448–450.

Crewe, W. & O'Rourke, F.J. (1951) The biting habits of *Chrysops silacea* in the forest at Kumba, British Cameroon. *Annals of Tropical Medicine and Parasitology*, **45**, 38–50.

Duke, B.O.L. (1955a) Studies on the biting habits of *Chrysops*. I – The biting-cycles of *Chrysops silacea*, at various heights above the ground in the rain-forest at Kumba, British Cameroons. *Annals of Tropical Medicine and Parasitology*, **49**, 193–202.

Duke, B.O.L. (1955b) Studies on the biting habits of *Chrysops*. IV – The dispersal of *Chrysops silacea* over cleared areas from the rain-forest at Kumba, British Cameroons. *Annals of Tropical Medicine and Parasitology*, **49**, 368–375.

Duke, B.O.L. (1957) Studies on the biting habits of *Chrysops*. V – The biting-cycles and infection rates of *Chrysops silacea*, *C. dimidiata*, *C. langi*, and *C. centurionis* at canopy level in the rain-forest at Bombe, British Cameroons. *Annals of Tropical Medicine and Parasitology*, **51**, 24–35.

Duke, B.O.L. (1972) Behavioural aspects of the life cycle of *Loa*. *Behavioural Aspects of Parasite Transmission* (ed. by E. U. Canning and C. A. Wright), pp. 97–107. *Zoological Journal of the Linnean Society* (Suppl. 1). Academic Press. London.

Ludwig, J.A. & Reynolds, J.F. (1988) *Statistical Ecology*. John Wiley & Sons, New York.

Noireau, F., Apembet, J.D., Nzoulani, A. & Carme, B. (1990a) Clinical manifestations of loiasis in an endemic area in the Congo. *Tropical Medicine and Parasitology*, **41**, 37–39.

Noireau, F., Nzoulani, A., Sinda, D. & Itoua, A. (1990b) *Chrysops silacea* and *C. dimidiata*: fly densities and infection rates with *Loa loa* in the Chaillu mountains, Congo Republic. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **84**, 153–155.

Oldroyd, H. (1957) *The horse-flies (Diptera: Tabanidae) of the Ethiopian Region, III. Subfamilies Chrysopinae, Secepsinae and Pangoniinae and a Revised Classification*. British Museum (Natural History), London.

Pinder, M. (1988) *Loa loa* – a neglected filaria. *Parasitology Today*, **4**, 279–284.

Thomson, M.C., Obsomer, V., Dunne, M., Connor, S.J. & Molyneux, D.H. (2000) Predicting trouble: satellite mapping of *Loa loa* prevalence in relation to ivermectin use in west/central Africa. *Lancet*, in press.

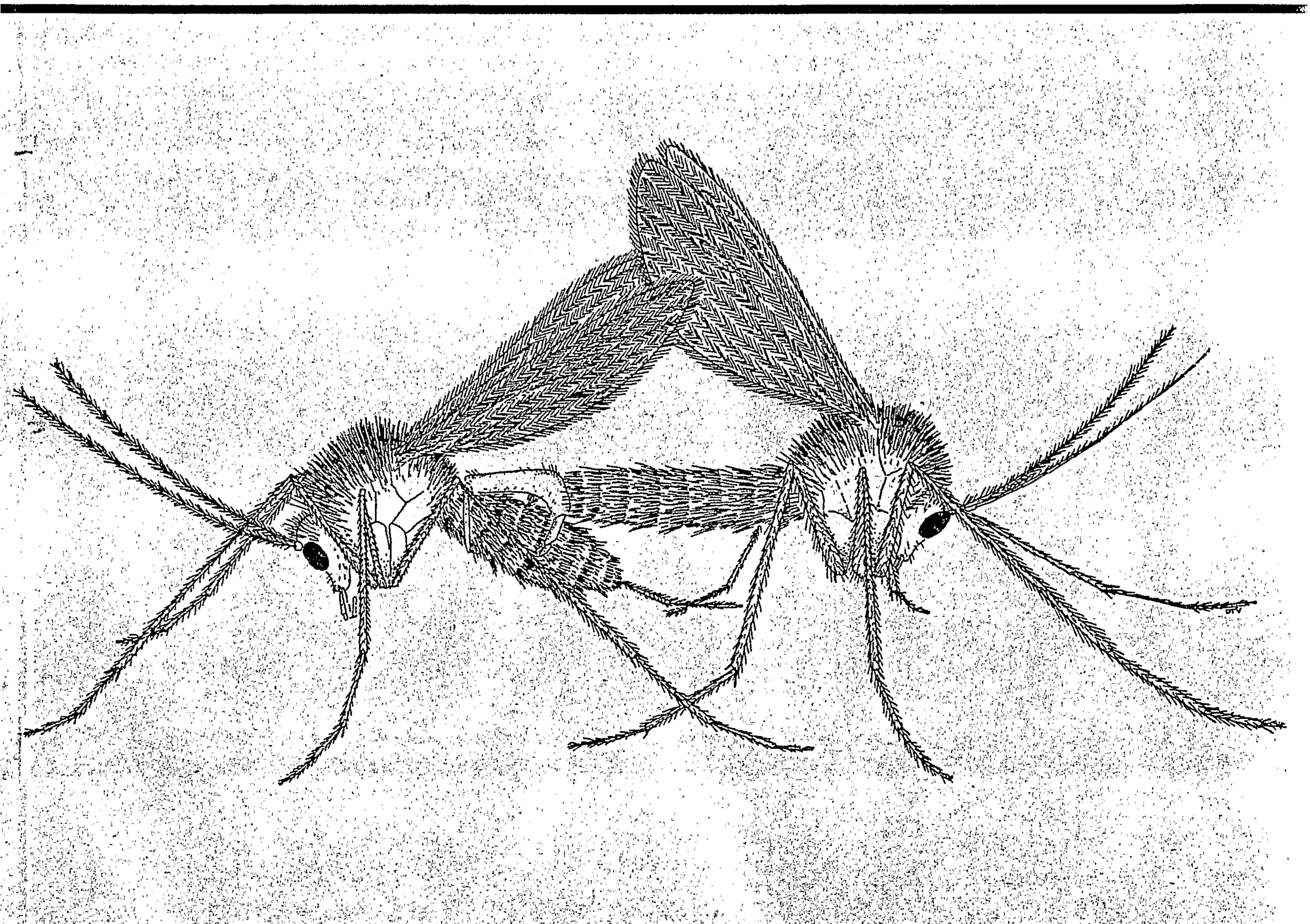
Accepted 25 May 2000

Volume 14 • Number 3 • September 2000

ISSN 0269-283X

Medical and Veterinary Entomology

Editors: G. B. White (Medical) and R. Wall (Veterinary)



Published for the Royal Entomological Society

Blackwell Science

PM 278

Saute,