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Microclimatic effect on vertical migration of *Haemonchus contortus* and *Haemonchus placei* third-stage larvae on irrigated Kikuyu pasture

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ABSTRACT

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The influence of microclimate on numbers of third-stage larvae of *Haemonchus contortus* and *Haemonchus placei* in four strata of irrigated Kikuyu pasture was assessed. On 36 different, interspersed days three replicates of pasture samples were collected on three occasions per day from 1990–1992 for larval recovery and the $\log_{10}$ mean counts of the larvae recovered were analysed by use of ANOVA models. Because the ground-surface area from which herbage was collected was standardized, estimated larval counts for the different strata could be compared; this was not possible in our previous studies.

For *H. contortus*, the estimated larval counts in the four strata were predicted by microclimatic air temperature, relative humidity and soil moisture, with the coefficient-of-determination (R^2) values ranging from 0,15–0,35. Of these, air temperature had the greatest effect. The same three predictors, together with illumination and wind speed, featured for *H. placei*, with R^2 values of 0,19–0,52.

With the exception of wind speed and illumination, which (for *H. placei*) had the opposite effect, all the microclimatic parameters listed, predicted an increase in numbers of larvae from a lower to an upper strata.

Keywords: Microclimatic effect, vertical migration, *Haemonchus contortus*, *Haemonchus placei*, third-stage larvae, irrigated Kikuyu pasture, climatic models

INTRODUCTION

The effect of environment on the ecology of free-living stages of *Haemonchus contortus* and *Haemonchus placei* is not well understood, particularly on irrigated pasture in Africa. Previous studies examined the effects of time of day, season and stratum (Krecek, Groeneveld & Van Wyk 1991) and of microclimate (Krecek, Murrell & Douglass 1990; Krecek, Groeneveld & Maritz 1992) on these nematodes. Barger, Benyon & Southcott (1972) compared actual field fluctuations in populations of *H. contortus* third-stage larvae (L_3) on clover pasture in Australia with computer-simulated larval counts on pasture. They measured more meteorological parameters than were measured in previous studies. However, recording was done once daily and data related to a standard height from the ground, presumably 2 m. In Australia, Southcott, Major & Barger (1976) examined mixed nematode infections which included *H. contortus* in

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sheep in a winter rainfall region. Though some climatic parameters were available, these were located 2 km from the study site.

The previous studies of Krecek *et al.* (1991) and Krecek *et al.* (1992) gave some indication of the environmental conditions under which L_3 of *H. contortus* were found on irrigated pasture and of the microclimatic factors which influenced their movements on the pasture during one year. The aims of the present study were twofold: to demonstrate the vertical migration of L_3 between strata derived by the influence of various microclimatic factors or predictors over a 2 year period and to use an improved sampling technique to allow a comparison of larval counts between the different strata; previously this was impossible.

MATERIALS AND METHODS

The nematode-larval-recovery techniques for processing herbage and soil samples, the description of the field study, donor animals, faecal material, collection of samples and climatic equipment, followed that of Krecek *et al.* (1991) and Krecek *et al.* (1992). Briefly, the nematode-larval-recovery technique for processing herbage samples included a modified Baermann apparatus which consisted of a coarse kitchen sieve placed inside a water-filled plastic bowl. The soil samples were processed by a centrifugal-flotation technique.

The 1000 m^2 site at the Onderstepoort Veterinary Institute on the Highveld of the Gauteng Province was planted with Kikuyu grass (*Pennisetum clandestinum*) only. Previously, Italian ryegrass was also used (Krecek *et al.* 1991; Krecek *et al.* 1992). Three lanes of pasture (50 m in length) were designated as replicates. The composition of the pasture soil indicated that while the clay component increased from 39,6 % in 1988 to 48,1 % in the present investigation, the sand component decreased from 37,2 % to 31,2 %.

Worm-free, 2-month-old Holstein calves and 5-month-old Dorper lambs were infected with *Haemonchus* L_3 which, when patent, provided donor animals. Subsequently, faeces containing *Haemonchus* eggs were placed on pasture in either faecal pats or pellets, depending on the host from which it originated. The faeces contained 533–1 333 eggs per gram of faeces (epg) for *H. placei* and 720–6 320 epg for *H. contortus*.

Samples of the herbage surrounding the pats were processed every 2–3 d after deposition until migration (translation) of L_3 to the herbage had occurred, usually from 7–23 d after deposition. After that, on 36 d, spread over 20 months from July 1990 to March 1992, herbage, mat and soil samples were processed. Collection on a given day included sampling of upper and lower herbage, mat and soil at three time intervals: 2 h after sunrise, at 12:00 and 2 h before sunset. In earlier studies, sampling was carried out five times daily (Krecek *et al.* 1991), but because no significant statistical differences were found for these intervals, they were reduced to three times daily in the present study.

All herbage, mat and soil samples were collected from similar ground-surface areas. This allowed the numbers of larvae recovered to be compared between the four strata. Previously, strata samples were collected independently of one another. Relative larval counts were compared from strata; these are considered independent variables (Krecek *et al.* 1992). In the present study, because the sampling method used collected strata from similar ground-surface areas, these larval counts from the strata could be compared directly, and are considered dependent variables.

Half-circular wire hoops were used for each sampling. The hoop measured 90 mm in diameter and was placed over herbage near a faecal deposition place. The surface area for a half hoop was 31 800 mm^2. The upper herbage, lower herbage and mat

TABLE 1 P values for five microclimatic predictors and a goodness-of-fit statistic (R^2 or coefficient-of-determination) in the models which compare $\log_{10}$ mean third-stage larval counts between pairs of strata for *H. contortus*

Microclimatic parameter	Upper and lower herbage	Upper herbage and mat	Upper herbage and soil	Lower herbage and mat	Lower herbage and soil	Mat and soil
Air temperature (°C)	0,4180	**0,0022**	**0,0001**	**0,0147**	**0,0024**	0,3267
Relative humidity (%)	0,6326	**0,0543**	**0,0061**	**0,1240**	**0,0260**	0,3466
Illumination (%)	0,8047	0,4814	0,8203	0,6512	0,5815	0,2930
Soil moisture (%)	0,8834	0,6859	**0,1176**	0,5048	**0,0766**	0,1966
Wind speed (m/s)	0,9805	0,3830	0,2044	0,3052	0,1869	0,6712
R^2 value	0,19	**0,22**	**0,35**	**0,15**	**0,26**	0,09

TABLE 2 P values for five microclimatic predictors and a goodness-of-fit statistic (R^2 or coefficient-of-determination) in the models which compare $\log_{10}$ mean third-stage larval counts between pairs of strata for *H. placei*

Microclimatic parameter	Upper and lower herbage	Upper herbage and mat	Upper herbage and soil	Lower herbage and mat	Lower herbage and soil	Mat and soil
Air temperature (°C)	**0,0264**	**0,0017**	**0,0002**	0,6127	0,3815	0,5792
Relative humidity (%)	**0,0232**	**0,0283**	**0,0054**	0,5822	0,7842	0,6057
Illumination (%)	0,8792	0,9508	0,2691	0,9144	0,2881	**0,1242**
Soil moisture (%)	0,4332	0,2563	**0,0356**	0,8793	0,3827	0,2457
Wind speed (m/s)	0,6824	**0,0184**	**0,0085**	**0,0894**	**0,0789**	0,9956
R^2 value	**0,19**	**0,45**	**0,52**	**0,24**	**0,25**	**0,22**

were collected for three replicates each and then each of these strata was pooled. For a soil sample, a 30–40 mm core of soil was removed with a 475 mm Model HA probe T-soil sampler (Oakfield Apparatus, Inc., Wisconsin, USA). These core samples were pooled for the soil stratum.

Climatic equipment was mounted as near to the ground as possible since measuring the microclimate of the environment of the nematode was part of the aim. Equipment in the field comprised a datalogger, a data-capture apparatus and 11 microclimatic sensors mounted above cut-grass level when possible. Equipment in the laboratory included a personal computer, a reader card and a tape recorder. Sensors measuring relative humidity, air temperature, wind speed, illumination and radiation were mounted 4–6 cm above ground level. There were, however, three changes in the equipment used for the present study, as compared with that of Krecek *et al.* (1991). Firstly, the datalogger was changed to a Campbell Scientific Inc. CR21, a cassette-tape storage system. This was later upgraded to a Campbell Scientific Inc. CR10, which was able to accommodate a larger number of sensors. Secondly, a Rotronic Air-Probe YA-100 Hygromer relative-humidity-and-temperature sensor (Rotronic Ag, Zurich, Switzerland) was installed. Thirdly, the radiation was measured with a LI-COR LI-200SZ Pyranometer sensor (LICOR, NE, USA).

Statistical analyses

Analyses similar to those of Krecek *et al.* (1992) were used and included SAS (SAS, NC, USA). The $\log_{10}$ mean larval counts per gram of dry matter (gdm) were analysed by step-wise regression, and microclimatic predictors identified for each stratum. The microclimatic predictors identified for these models after the regression step were relative humidity, soil moisture and air temperature for *H. contortus*. For *H. placei*, in addition to these three, air temperature and illumination were also identified and are discussed further below. The significance of the predictor was indicated by P (or exceedence probability). The goodness-of-fit of regression equations was measured by R^2 (coefficient-of-determination). Multi-colinearities for the microclimatic predictors were found. Because illumination and radiation were highly correlated, one of these had to be removed from the regression analyses. The same was true for temperatures, therefore radiation and all temperatures except that of air were removed from the analyses. Illumination was selected to remain since previous reports showed this to have an important effect on the vertical migration of rabbit trichostrongylid larvae (Crofton 1948). The criterium for the acceptance of a predictor in the models of the present study was a 90% level of confidence, but three in which $P < 0,15$ were accepted. Estimated larval counts for each pair of strata were calculated from the sample regression coefficients, for a value equally smaller and one larger than the mean value for the data set of each microclimatic predictor. The six pairs of strata compared, were: upper and lower herbage; upper herbage and mat; upper herbage and soil; lower herbage and mat; lower herbage and soil; and mat and soil. The mean values for microclimatic predictors are shown as two points, designated as the number of larvae per gram of dry matter. The mean values are: air temperature, 22°C; relative humidity, 52%; illumination, 46%; wind speed, 0,9 m/s; and soil moisture, 27%.

RESULTS

The microclimatic predictors for L_3 of *H. contortus* and *H. placei* for four strata of irrigated pasture, together with estimated larval counts between strata, coefficient-of-determination (R^2) values, and P values are shown in Fig. 1 and 2, respectively. With the exception of three with P values less than 0,15, no data are shown for those blocks for which the predictors

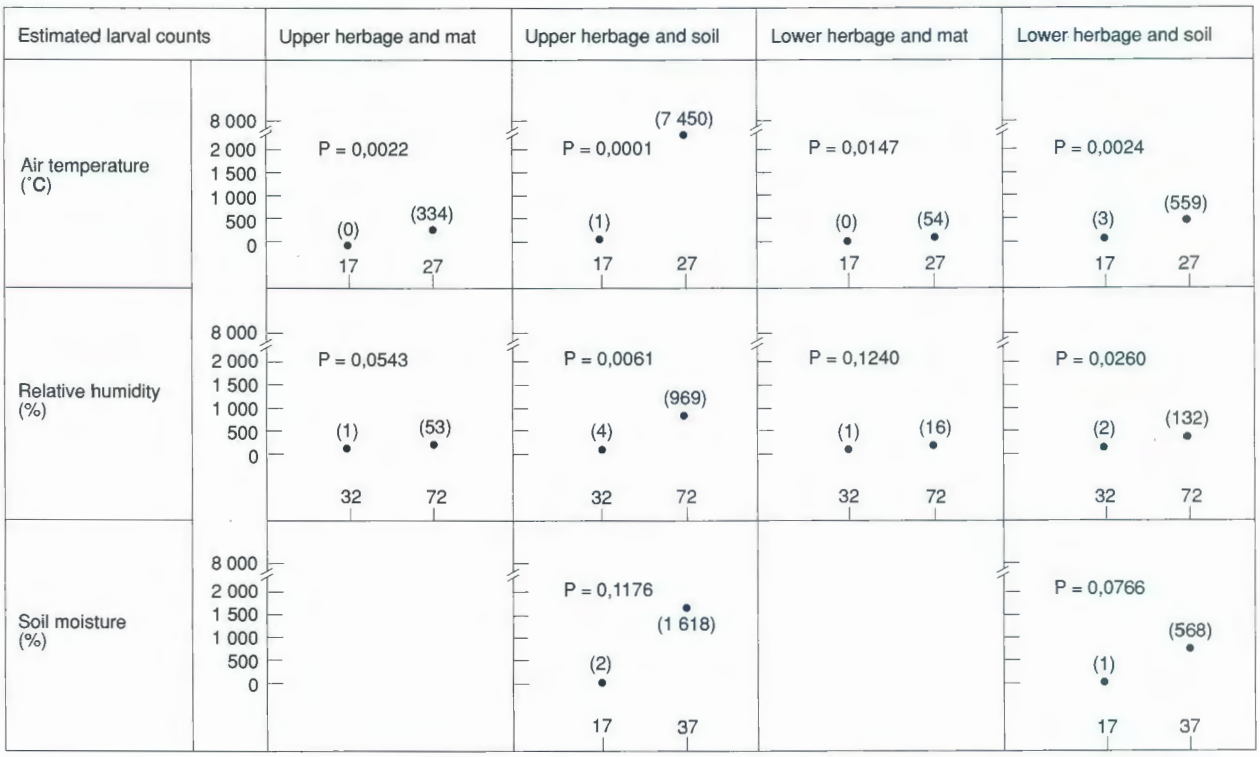

FIG. 1 Models explaining the effect of three microclimatic factors on the migration of estimated larval counts of third-stage larvae of *Haemonchus contortus* for pairs of four strata of irrigated pasture. P values are indicated for each model; the estimated numerical larval counts per gram of dry matter (gdm) of values equally smaller and larger than the mean for each variable are shown in parentheses

were not significant at a 90 % level of confidence. Tables 1 and 2 include the P values and goodness-of-fit statistics (R^2) in models which compare $\log_{10}$ mean larval counts for pairs of strata for *H. contortus* and *H. placei*, respectively.

The values in Fig. 1 indicate that air temperature and soil moisture had the greatest, and relative humidity a lesser effect on the numbers of L_3 of *H. contortus*. For example, when the effect of air temperature at 17 and 27 °C is examined on the estimated counts of L_3, the estimated larval counts increase with temperature. Generally, all of the predictors in the models of Fig. 1 showed a similar effect on larval numbers, with one exception. This was upper herbage and soil, where air temperature had a much greater effect; at 27 °C, estimated numbers of larvae had increased to 7450 from one at 17 °C.

For *H. placei* the trend was similar as regards the effect of air temperature, relative humidity and soil moisture on the estimated larval counts (Fig. 2). However, the opposite effect is evident for illumination and wind speed.

DISCUSSION

Information on how environment influences availability of *Haemonchus* larvae on pasture is essential for the development of novel control strategies for these nematode parasites. The present study, which includes 20 months' data, is more extensive than previous studies (Krecek *et al.* 1992). In addition, an improvement in the sampling method was possible in the present study, which allowed a comparison of larval counts between strata; a step which was not possible previously.

Krecek *et al.* (1992) reported that relative humidity (a moisture-related variable) together with air temperature were important predictors in the model for *H. contortus*. The results of the present study concur, except that, additionally, soil moisture was also a significant predictor. In contrast, for *H. placei*, moisture variables were not found to predict larval movements between strata (Krecek *et al.* 1992). In the present investigation both relative humidity and soil moisture were significant predictors.

This study and that of Krecek *et al.* (1992) are the first of their kind to attempt to correlate microclimate at ground level with parasitic nematode larval ecology. Levine (1963) cautioned researchers that standard weather stations at 2 m above the ground are a long distance from the actual habitat of the larval stages of these nematodes on the pasture and therefore may have little relevance to the biology of this organism. Other studies which examined the effect of climate, albeit macroclimate, on *H. contortus* include

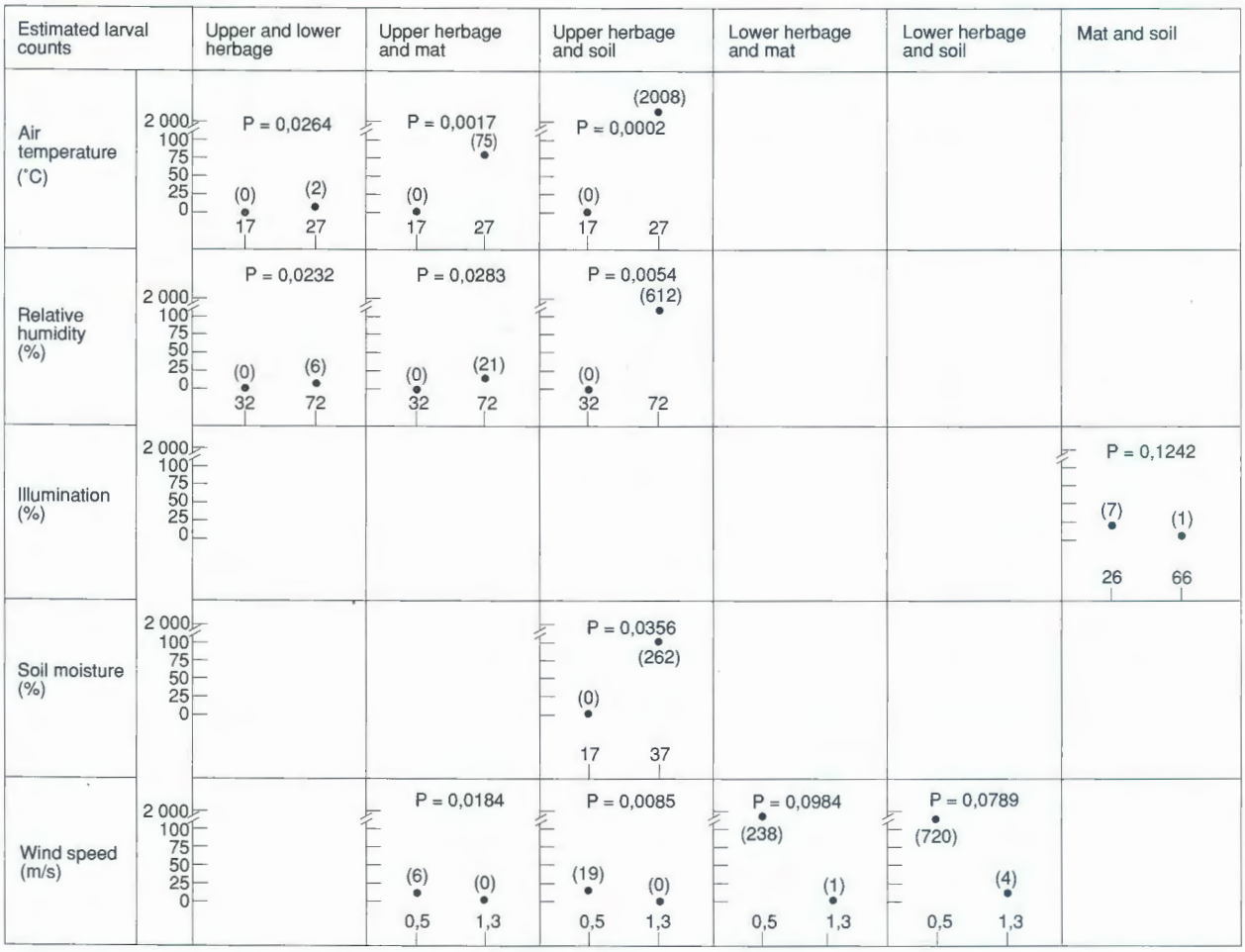

FIG. 2 Models explaining the effect of five microclimatic factors on the migration of estimated larval counts of third-stage larvae of *Haemonchus placei* for pairs of four strata of irrigated pasture. P values are indicated for each model; the estimated numerical larval counts per gram of dry matter (gdm) of values equally smaller and larger than the mean for each microclimatic variable are shown in parentheses

Barger *et al.* (1972), Barger, Lewis & Brown (1984), Gibson & Everett (1976a; 1976b) and Southcott *et al.* (1976). The meteorological data for these studies were often measured some distance from the study pasture and included daily readings of maximum and minimum air temperatures, soil temperature, relative humidity, evaporation and rainfall. A major advance concerning methodology is evident in the present study. A wider spectrum of measurements, including soil temperatures, radiation and soil moisture, were included, as well as more frequent (i.e. hourly) recordings of these parameters in the microclimate of the immediate habitat of the larvae.

Other improvements are that the present study included three replicates for all parasitic samples and two years' data, as well as more frequent and comprehensive measurements of the climatic conditions. The soil type was often not mentioned in earlier studies and yet this is a critical factor in the ecology of free-living stages of ruminant nematodes. The role which soil plays is underlined in studies by Krecek & Murrell (1988) who recovered *O. ostertagi* L_3 from pasture after a migration 15 cm down and 15 cm back to the surface in a soil which consisted largely of 72 % sand. Fincher & Stewart (1979) recovered *O. ostertagi* L_3 at depths of 12,5 cm in studies where eggs had been placed below the surface of a pasture. In the present study substantial estimated larval counts of both nematodes were observed for the models which included soil. This suggests that this stratum of pasture served here as a reservoir for larvae.

The R^2 models in the present study range from 0,15–0,35 for *H. contortus* and 0,19–0,52 for *H. placei*. These goodness-of-fit statistics (R^2 values) of the models are improvements on earlier studies which reported considerably smaller R^2 values, the maximum being 0,21 for *H. contortus* and 0,12 for *H. placei* (Krecek *et al.* 1992). Improved values in the present study may be explained by larger numbers of samples which were collected over a more extensive period. Certainly, the larger R^2 values further

expand the explanation as to the extent to which larval migration is affected by climate predictors in the model. We remain of the opinion that these models could be tested under controlled conditions (i.e. environmental chambers) to better determine the separate effect of each climatic measurement on the migration of parasitic nematode larvae.

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