

RESEARCH COMMUNICATION

EFFECTS OF MICROCLIMATIC VARIABLES ON THE AVAILABILITY AND MOVEMENT OF THIRD-STAGE LARVAE OF *OSTERTAGIA OSTERTAGI* ON HERBAGE

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ABSTRACT

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Assessments were made of the influence of several microclimatic variables on the availability of third-stage larvae of *Ostertagia ostertagi*, on pasture herbage. Variables most closely related to recovery of larvae from the lower herbage samples were: maximum air, mat and dung temperatures. Recovery of larvae from the upper portion of the herbage was closely correlated with dung temperature, sampling-time air temperature and maximum air temperature. Bearing in mind that the moisture threshold was maintained throughout the trial the results of this study suggest that under field conditions, larval movement of third-stage *O. ostertagi* larvae on herbage is regulated primarily by temperature.

Climate, especially as regards temperature and humidity, profoundly influences the movement of nematode larvae on herbage, particularly vertical migration (Crofton, 1948; Williams & Bilkovich, 1973; Al Saqur, Bairden, Armour & Gettinby, 1982; Callinan & Westcott, 1986). The findings of earlier studies have suggested that, because of microclimate differences, nematode larvae of ruminants and equids tend to remain near the base of the herbage (Crofton, 1948; Silangwa & Todd, 1964; English, 1979). Many of these studies, however, were conducted under controlled laboratory conditions rather than in the field or were designed to monitor larval movement in relation to air temperature and relative humidity each measured on a single occasion during any 24-hour period. Insufficient effort has been made to study the dynamics of larval movement on herbage in response to short-interval microclimatic changes. Thomas (1974) has pointed out that bioclimatographs from laboratory determinations of the parasite's temperature-humidity constraints may be unreliable because the microclimate experienced by larvae in the soil-herbage zone is difficult to measure. Levine & Todd (1975) pointed out that standard weather shelter measurements are usually made 1.6 m above the ground and are not satisfactory for obtaining information on the influence of microclimate on nematodes at ground level.

The purpose of the study was to investigate the relationship of microclimatic variables to migration of third-stage larvae ( $L_3$ ) of *Ostertagia ostertagi* on herbage under natural field conditions. Such information would help to provide both a better understanding of the ecology of the free-living stages and to improve control strategies.

The study period of 28 weeks began in April 1986 and terminated in October 1986. Two pastures were utilized for observations from April–June: Pasture A, predominantly orchard grass, *Dactylis glomerata*, and Pasture B, predominantly red clover, *Trifolium pratense*. Thereafter a 3rd pasture, C, chiefly composed of Kentucky bluegrass, *Poa pratensis*, was used. Soil analyses revealed that Pasture A was sandy loam with a pH of 6.7; Pasture B was sandy

clay loam with a pH of 6.9; and Pasture C sandy loam, with pH 5.3.

Every 6 weeks, two 4-month-old calves were infected orally with 200 000 *O. ostertagi*  $L_3$ . Faeces containing ca. 200 *O. ostertagi* eggs per g were collected from these experimentally infected calves on Days 21–26 of infection, shaped into 4–6 1 000 g pats and placed on pasture. Every 2–3 days thereafter, herbage surrounding each pat was collected and processed as described in the following paragraph to indicate when  $L_3$  had migrated (translated) from the dung pat to the herbage. Throughout the 3 seasons studied (spring, summer and autumn), the average period of translation ranged from 14–21 days. Sixty-four fresh faecal pats were placed on pasture every 6 weeks and sampled for 6 weeks after larval translation.

Once  $L_3$  had translated, samples of upper and lower herbage were collected weekly at 6 time intervals during daylight hours, at sunrise, 9:00, 12:00, 15:00, 18:00 and sunset. Throughout the study, the height of the herbage was maintained at 8–10 cm by regular clipping. For sampling purposes, the top 4–5 cm was snipped with scissors and designated the upper herbage while the bottom 4–5 cm comprised the lower herbage. At each sampling, herbage was randomly collected from a 15 cm radius around 3–4 of each group of 4–6 dung pats, upper and lower herbage samples being pooled separately. The herbage was refrigerated at 4 °C until processed, usually within 24 h of collection. The wet mass of the samples was generally 25–50 g. Larvae were recovered from these herbage samples with a modified Baermann apparatus, which consisted of 12.5 × 9.5 × 2.5 cm rectangular stainless steel baskets that were constructed from type SS304 4 × 4 wire mesh (diameter 1.2 cm) and were lined with paper facial cloths (Scotties<sup>R</sup>) before being placed in 17.5 cm plastic plant pot liners and flooded with water for 24 h. The washings were sieved over a standard testing sieve with 25 µm apertures, and sedimented before the *O. ostertagi* larvae were counted with the aid of a compound microscope. *O. ostertagi*  $L_3$  were identified according to Douvres (1957) and non-parasitic nematodes were differentiated from the parasitic  $L_3$  by greater development in their internal morphology then lack of a sheath (Caveness, 1964). After they had been processed for larval recovery, herbage samples were dried for 72 h individually in an oven at 40 °C and were mass-measured for computing larval densities as larvae per kg of dry herbage (KDH).

Microhabitat measurements included mat temperature, soil temperature at a depth of 2 cm and

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TABLE 1 Relationship between meteorological variables and the recovery of  $\log_{10}$  *O. ostertagi* L<sub>3</sub> on pasture herbage from all study plots

Variable	Lower herbage n = 130		Upper herbage n = 125	
	r	b	r	b
Dung temperature (°C)	-0,31***	-0,21 (±0,12)*	-0,11	-0,13(±0,05)**
Mat temperature (°C)	-0,27***	+0,20(±0,11)*	-0,12	
Soil temperature (°C)	-0,30***		-0,12	
Sampling-time air temperature (°C)	-0,27***		-0,23**	-0,10 (±0,03)**
Maximum (24 h) air temperature (°C)	-0,37***	-0,10(±0,04)**	-0,22**	-0,09(±0,04)**
Minimum (24 h) air temperature (°C)	-0,32**		-0,16	
Relative humidity (%)	+0,04		+0,09	
Rainfall (cm)	-0,03		-0,02	
Antecedent precipitation index	+0,08		-0,04	
Vapour pressure deficit	-0,17**		-0,23**	
r-square value <sup>a</sup>	0,16		0,12	

r: simple correlation coefficient (± SE of b); b: partial regression coefficient for the best 3 variable model

\* P < 0,10; \*\* P < 0,05; \*\*\* P < 0,01

<sup>a</sup> r-square is the proportion of  $\log_{10}$  larval count variation explained by the regression model

temperature under the dung pat. These measurements, as well as air temperature and relative humidity, were made at the time of each herbage collection. Maximum and minimum air temperatures and rainfall during the various 24-hour intervals during which samples were collected were taken with instruments placed about 50 cm above ground level. Rainfall was measured in a Tru-Check-Rain Gauge<sup>1</sup>, and maximum and minimum air temperatures with a Taylor No. 5458 Maximum Minimum Self-Registering Thermometer<sup>2</sup>. Mat, soil and dung pat temperatures were measured with a Cole-Parmer Digi-Sense Type K thermocouple thermometer with penetration probe<sup>3</sup>. Soil moisture was measured by a gravimetric method (Baver, 1956), and data for relative humidity were obtained from a station of the National Weather Service, situated approximately 30 km from the site of sampling.

Integrated measures of temperature and moisture were derived. Firstly, the antecedent precipitation index (API), an estimate of moisture deficiency derived from soil moisture and rainfall, was calculated according to Linsley, Kohler & Paulhus (1975). Furthermore, vapour pressure deficit (VPD) was calculated to integrate both relative humidity and interval air temperature (Oke, 1978).

Correlation and regression analyses were employed and, on upper and lower herbage data were analyzed separately to examine the effects of microclimatic factors on  $\log_{10}$  larval counts of data pooled from all 3 study pastures (Steel & Torrie, 1960).

The relationship of larval recoveries to the meteorological variables is shown in Table 1. Lower herbage larval counts appear to be most closely related to maximum air temperature, with some added explanation coming from mat and dung temperatures. When dung and maximum air

temperatures increased, larval recoveries diminished whereas mat temperature increases were accompanied by larger larval numbers. The upper herbage larval counts show a relationship with dung temperature, sampling-time air temperature and maximum air temperature in which an increase in any one of these temperatures was accompanied by a decrease in larval recoveries. Generally, there was little correlation between moisture variables and larval recoveries.

Our data indicate that the movement of L<sub>3</sub> on herbage is associated more closely with temperature than with humidity (Table 1). However, it must be recalled that relative humidity was measured several metres above ground and that two of the four moisture variables reported would be affected since these include humidity itself and vapour pressure deficit. Other factors undoubtedly playing a role in the results of this trial include the brevity of the 8 month study, lack of replicates and the pasture moisture level. Irrigation was employed when drought conditions occurred (i.e. summer), therefore, the moisture threshold was never lacking but maintained. This alone could have deemphasized the correlation with moisture and therefore diminished the role which it contributed.

Others have shown that the availability of nematode larvae is also very dependent upon moisture (Lancaster & Hong, 1987; Bryan & Kerr, 1989). The explanation for this apparent difference is probably related to the need for moisture to facilitate larval development and translation to herbage, while larval movement and survival with herbage biotope being more dependent upon daily temperature variations, providing the moisture level within the herbage canopy is relatively stable.

The results presented here point to the inherent weakness associated with both semi-field and laboratory-derived data for development of epidemiological models (Callinan, Morley, Arundel & White, 1982; Callinan & Westcott, 1986). Although studies conducted under semi-field conditions can provide important information on the relationship between climate and the behaviour of nematode larvae, models utilizing such data may not allow for the complexity that exists under real pasture conditions

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