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Description of a *Zoophthora radicans* (Zygomycetes: Entomophthoraceae) Epizootic in a Population of *Empoasca kraemeri* (Homoptera: Cicadellidae) on Beans in Central Brazil

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Development of a natural epizootic of *Zoophthora radicans* in an *Empoasca kraemeri* population on beans near Goiânia, Goiás, Brazil, was monitored over a 6-week period. At the initiation of monitoring on 23 April 1985, disease prevalence in the leafhopper population was 12.8%. Over the course of the epizootic, infection of second-, third-, fourth-, and fifth-instar nymphs was similar and reached a peak of approximately 55% on 13 May, while infection of adult leafhoppers never exceeded 19%. Fungal inoculum expressed as the number of host cadavers with active (sporulating) fungus attached to the bean foliage was the variable most closely correlated to disease prevalence. Variables quantifying moisture or moisture combined with temperature (degree hours under moist conditions) were the most important abiotic predictors. A moisture variable incorporating a quantitative measure of moisture (level of wetness of a leaf wetness sensor) and a variable based only on the presence or absence of free moisture (dew) were equivalent predictors of disease prevalence. Epizootic development appeared to be inhibited when foliage was wet for less than 9 hr during the night. Infection trends in relation to fungus inoculum levels indicated an inoculum density threshold for epizootic development of approximately 0.8 leafhopper cadavers per plant.

KEY WORDS: *Zoophthora radicans; Empoasca kraemeri;* Entomophthorales; leafhopper; fungal epizootic.

INTRODUCTION

Zoophthora radicans (=*Erynia radicans*, =*Entomophthora sphaerosperma*) is one of the most commonly encountered and widely studied species of the entomophthoralean fungi. Various strains of this fungus infect a broad range of insect pests from at least four orders (Glare et al., 1987) and frequently cause high levels of disease in host populations (Sawyer, 1929; Jaques and Patterson, 1962; Vandenberg and Soper, 1978; Milner and Lutton, 1983). Like many other species of entomophthoralean fungi, *Z. radicans* requires moist conditions for sporulation, spore germination, and host penetration (Wilding, 1981; Papierok, 1982; Soper, 1985). However, it is capable of reaching epizootic levels under drought conditions if free moisture is available in the form of dew (Milner et al., 1982; Carruthers and Soper, 1987).

Zoophthora radicans has been known as a pathogen of *Empoasca* leafhoppers for nearly 70 years. Beyer (1922) reported *Z. radicans* (=*E. sphaerosperma*) infecting *Empoasca fabae* (=*Empoasca mali*) in Florida, while Fenton and Hartzell (1923) in Iowa and Dudley (1926) in Wisconsin reported epizootics of this fungus in *E. fabae* populations on potatoes. More recently, it has been reported from *Empoasca flavescens* in China (Zang and Luo, 1976), *Empoasca* spp. in Israel (Ben-Ze'ev and

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Kenneth 1981), and *Empoasca kraemeri* in Brazil (Daoust et al., 1985).

Although there are numerous problems associated with the production, storage, and application of entomophthoralean propagules for pest control (Wilding, 1981), *Zoophthora radicans* was successfully introduced into *Therioaphis maculata* populations in Australia where it now provides significant long-term biological control of this pest (Milner et al., 1982; Milner and Lutton, 1983). Introductions into *Empoasca fabae* populations on potato in Illinois (McGuire et al., 1987a) and on alfalfa and beans in central New York (see Wraight and Roberts, 1987) produced localized epizootics and short-term reduction in pest numbers.

In light of these results, research into the use of *Z. radicans* as an introduced biological control agent is continuing through the development of introduction and establishment strategies, and by acquiring more extensive knowledge of host-pathogen dynamics. While investigations have been conducted on epizootics of *Z. radicans* in aphid and spruce budworm populations (Milner et al., 1982; Vandenberg and Soper, 1978; Perry and Whitfield, 1984), the only reported epizootiological studies of this fungus in *Empoasca* populations are those of McGuire et al. (1987a,b).

Empoasca kraemeri (a member of the *E. fabae* species complex) is a primary pest of beans (*Phaseolus vulgaris*) throughout Central and South America. Populations normally reach peak levels during periods of drought (Schoonhoven, 1978; King and Saunders, 1984). Extensive screening of *Phaseolus* lines has revealed no sources of high-level resistance to this pest (Kornegay and Temple, 1986), and alternative control strategies are needed.

In Central Brazil during the past several years, we have observed annual epizootics of *Z. radicans* in *E. kraemeri* populations in both irrigated and nonirrigated experimental plots of beans and cowpeas (Daoust et al., 1985). This investigation describes a natural epizootic of *Z. radicans* that occurred in an *E. kraemeri* population in beans in Central Brazil during 1985. Disease prevalence and incidence is examined as a function of temperature, moisture, inoculum level, and plant canopy and host population characteristics.

METHODS

Research Site and Monitoring Methods

The field site was located at the Empresa Brasileira de Pesquisa Agropecuaria/ Centro Nacional de Pesquisa de Arroz e Feijão (EMBRAPA/CNPAF) near Goiânia, Goiás, in Central Brazil. On 21 March 1985, a 70 × 70-m field was planted with black beans (*Phaseolus vulgaris*, var. Rio Tibagi) arranged in one hundred 6 × 6-m plots, each surrounded by 1 meter of fallow ground. Each plot consisted of 10 rows (0.67 m spacing) with approximately 70 plants per row. Fifteen of the plots were randomly selected and monitored throughout this study. Ditch irrigation was provided every 3 days beginning 23 April.

Natural levels of infection of *E. kraemeri* by *Z. radicans* were monitored approximately every 4 days over a 6-week period beginning 23 April. Two sample locations were randomly selected in each of the 15 plots. At each location, a small group of plants (ca. 1–4) was quickly covered by a clear, 60 × 90-cm plastic bag. The plants were shaken gently to dislodge adult leafhoppers from the plants, trapping them in the sample bag. A single plant within the bag nearest to the sample location point was then severed at ground level and enclosed within it. The number of plants covered by each bag was recorded to estimate adult densities. Collections were made during early morning. Processing of samples began immediately after collection and was completed within 24 hr, during which time the samples were stored at room temperature. All live leafhoppers were examined for the presence of infection and the number and condition of cadavers were re-

corded. Throughout the monitoring period, the total number of leafhoppers examined varied with leafhopper density (mean 6.9 to 17.0 leafhoppers per plant). Instar determinations were made using head capsule measurements and then the leafhoppers were squashed and examined using phase contrast microscopy for presence of *Z. radicans* hyphae.

Abiotic data were obtained by monitoring environmental conditions at 10-min intervals throughout the experimental period using electronic data loggers (OMNIDATA International, Inc., Logan, Utah). A temperature sensor (OMNIDATA International, Inc.) and a leaf wetness sensor (Wing Laboratories, Cincinnati, Ohio) were situated at canopy level shielded from direct sunlight.

Biotic and Abiotic Variables for Analysis

Thirty-three biotic and abiotic variables were generated from the collected data (Table 1). Stepwise multiple regression was used to relate these variables to disease prevalence and to identify those with the greatest influence on epizootic development. This was accomplished using the Maximum R^2 Improvement technique (SAS Institute, 1982).

Regressions were performed with two dependent variables, disease prevalence (percentage infection) and disease incidence estimated as the rate of change in prevalence between successive samples. Percentage infection was determined from the microscopic examinations of the living nymphs collected in the samples; fungus-infected cadavers were not included in prevalence determinations as their age was indeterminable. Since no distinction was made between neonate and mature first-instar nymphs, disease prevalence among first-instar leafhoppers was underestimated. Therefore, first-instar infection data were excluded from the regression analyses.

Our recent laboratory investigations indicated that the mean survival time of *E. kraemeri* fifth-instar nymphs inoculated with an LC50 dose of *Z. radicans* isolate ARSEF-1590 (originally isolated from a leafhopper from the epizootic) was approximately 80 hr at constant 20°C (Leite et al., 1989). On the basis of these data and considering the extended time required for processing of the samples, the temperature and moisture conditions over the 84-hr period prior to each sample were considered to have influenced the dependent variables. This included the 3 days and 4 nights preceding each sample period. However, since the method employed for determining infection (viz, squashing and microscopic examination) makes it difficult to detect very early infections, environmental conditions for the night immediately prior to the sample were not included in the analyses. The 72-hr time interval that includes the described 3 days and 3 nights prior to each sample is subsequently referred to as the presample period.

Leafhopper population density and fungal inoculum levels were the two principal biotic factors examined as predictor (or independent) variables. Leafhopper and cadaver densities existing during a given presample period were estimated by the means of the counts obtained from the two consecutive samples encompassing the presample period.

Fungal inoculum levels were estimated by taking counts of *Zoophthora*-killed leafhopper cadavers attached to the foliage of each sample plant. Only cadavers supporting active (sporulating) fungus were counted; since the plants were collected in early morning when the foliage was still dew-covered and then held under high humidity conditions in plastic bags, such cadavers were readily identifiable. The approximate instar of each cadaver was determined, and fungal inoculum expressed both as the number of large cadavers (fourth and fifth instars and adults) and as total cadavers per plant were used in the multiple regression analyses. Live leafhopper and cadaver densities used in the regressions

TABLE 1
DEPENDENT AND INDEPENDENT VARIABLES USED IN THE MULTIPLE REGRESSION ANALYSES OF A *Zoophthora radicans* EPIZOOTIC

Dependent variables	
PREV	Percentage infection of *Empoasca kraemeri* by *Z. radicans* (arcsine transformed).
INCID	Rate of change in percentage infection between sample dates.
Independent variables[a]	
	Biotic—Group 1
LGCPP	Inoculum expressed as number of large nymph (N_4–N_5) and adult leafhopper cadavers per plant ($\log_{10}$).
TOTCPP	Inoculum expressed as number of total leafhopper cadavers per plant ($\log_{10}$).
LHPP	Number of live leafhoppers per plant.
MEANAGE	Mean age (instar) of leafhopper population.
TRIFOL	Number of trifoliates per plant.
	Biotic—Group 2
RITOTCPP	Inoculum expressed as rate of increase (RI) of total leafhopper cadavers per plant.
RILGCPP	Inoculum expressed as rate of increase of large nymph and adult leafhopper cadavers per plant.
RILHPP	Rate of increase in number of live leafhoppers per plant.
MEANAGE	Mean age (instar) of leafhopper population.
TRIFOL	Number of trifoliates per plant.
	Abiotic—Temperature variables
DH	Total degree hours.
DDH	Total daytime degree hours.
NDH	Total night degree hours.
NDHMIN/MAX	Minimum/maximum night degree hours.
MEANMAXT	Mean of the maximum daytime temperatures.
MEANMINT	Mean of the minimum nighttime temperatures.
ABOVE30	Total hours temperature above 30°C threshold.
	Abiotic—Wetness hour (WH) variables
WH	Total wetness hours above 10°C threshold.
WHMIN/MAX	Minimum/maximum wetness hours above 10°C threshold.
	Abiotic—Moist condition (MC) variables
MC	Total hours of moist conditions above 10°C threshold.
MCMIN/MAX	Minimum/maximum hours of moist conditions above 10°C threshold.
DHMC	Total degree hours under moist conditions.
DHMCMIN/MAX	Minimum/maximum degree hours under moist conditions.
	Abiotic—Leaf wetness (LW) variables
LW	Total hours foliage wet above 10°C threshold.
LWMIN/MAX	Minimum/maximum hours foliage wet above 10°C threshold.
DHLW	Total degree hours while foliage wet.
DHLWMIN/MAX	Minimum/maximum degree hours while foliage wet.

[a] Abiotic variables describe conditions during the 72-hr (3 days and 3 nights) presample period described in the text. Values for temperature and moisture and combined temperature/moisture variables were calculated for each day and night separately, and the totals, maximums, and minimums from each presample period were obtained.

against incidence were expressed as the rate of change between the mean values from successive presample periods.

The temperature variables used in the analyses are described in Table 1. Temperatures of 30 and 10°C were used as the upper and lower developmental thresholds for fungal growth. These values were derived

from a radial growth experiment conducted with a strain of *Z. radicans* (ARSEF 789) isolated from *E. kraemeri* originally isolated at the experimental station in 1982 (S. P. Wraight and S. Galaini-Wraight, unpubl.). The isolate tested exhibited a temperature-dependent growth pattern similar to that reported by McGuire et al. (1987b) for a strain of *Z. radicans* isolated from *E. fabae* in Wisconsin.

Three different measures of moisture were calculated from the leaf wetness sensor readings of resistance in ohms transformed to a scale of 0–240 (Omnidata International, 1982). The maximum reading corresponding to a water-saturated probe (240) multiplied by a factor of 60 was defined as 1 wetness hour (WH). The number of wetness hours occurring during a specified period was thus calculated by multiplying the sensor reading by the length of time in minutes and dividing by 14,400 (240 × 60). For example, a reading of 120 for 30 min is equal to 0.25 wetness hr. The second measure of moisture, hours of moist conditions (MC), was defined as any time period when the sensor was absorbing moisture from the air or indicating a stable, moist condition. Thus, drying periods (when sensor readings dropped) were not considered as moist conditions, even if the readings fell from near saturation to a lower level of saturation. This procedure was adopted under the assumption that water loss from the sensor would correlate to a loss of water from the leaf surface or other microenvironment, which might adversely affect the rate of fungal development or sporulation on the host cadaver or the rate of penetration into the living host. The third moisture measurement, leaf wetness (LW), was calculated based on the minimum sensor reading corresponding to visible dew on the leaves. The number of hours that the sensor read above the threshold was recorded nightly.

The three measurements of moisture were ultimately used in two types of predictor variables for regression analysis. The first was defined as the number of wetness hours (WH), hours of moist conditions (MC), or hours of leaf wetness (LW) that occurred while temperature was above the lower developmental threshold for *Z. radicans*. The second type of variable incorporated both degree hours (DH) and the MC and LW moisture variables. The resulting variables (DHMC and DHLW) represent the degree hours accumulated under moist conditions as previously defined or while the foliage was wet. Relative humidity was recorded throughout the study; however, the data collected during the peak of the epizootic were lost and we were unable to evaluate this variable. The variables incorporating each of the different measurements of moisture were examined in combination with temperature and the biotic variables to determine the best representation of moisture as a predictor of epizootic development. In order to investigate the relative importance of abiotic variables in more detail, regressions were also conducted excluding biotic variables.

Individual multiple regression analyses (Table 2) were conducted with various combinations of the subsets of independent

TABLE 2
COMBINATIONS OF SUBSETS OF INDEPENDENT VARIABLES USED IN MULTIPLE REGRESSIONS AGAINST PREVALENCE AND INCIDENCE OF *Zoophthora radicans* INFECTION[a]

Biotic[b] + Temperature + Wetness Hour (WH) totals
Biotic + Temperature + WH variables including maximums and minimums
Biotic + Temperature + Moist Conditions (MC) totals
Biotic + Temperature + MC variables including maximums and minimums
Biotic + Temperature + Leaf Wetness (LW) totals
Biotic + Temperature + LW variables including maximums and minimums
Each of the above omitting biotic variables

[a] Independent variables within subsets shown in Table 1.

[b] Biotic variables in Group 1 regressed against prevalence and in Group 2 against incidence.

variables listed in Table 1. The SAS statistical package was also used in conducting standard analyses of variance (ANOVA).

In addition to the above procedures, direct observations of moribund leafhoppers and fungus-infected cadavers were made in the field to determine the state of the fungus as influenced by the widely differing environmental conditions that existed during the day and night and to provide a basic description of the natural, in situ development of the fungus following death of the host.

RESULTS AND DISCUSSION

Annual epizootics of *Z. radicans* in *E. kraemeri* populations in Central Brazil characteristically begin during the transition period between the rainy and the dry seasons (April and May) when the rains diminish and numbers of *Empoasca* increase. Infection levels usually peak during mid-May, after the rains have ceased, and then decline gradually as nighttime temperatures drop and the drought intensifies. Drought usually persists for 5 to 6 months. By July or August, *Z. radicans* is no longer detectable in the leafhopper population and does not reappear until near the end of the next rainy season (March or April).

General Observations

Plants in the experimental plots were ca. 1 month old at the initiation of monitoring. The plants were approximately 24 cm in height with a mean of 10 trifoliates per plant. *E. kraemeri* nymph and adult population densities were 6.7 and 5.3 per plant, respectively. The high adult population was a result of leafhopper migration from large fields of mature beans located near the study area. Examination of these fields revealed the presence of many *Z. radicans*-infected leafhopper cadavers. Infected adults from these fields were the likely source of the initial fungal inoculum.

Extremely heavy dews were recorded throughout the epizootic period. Foliage was wet most nights between 19:00 and 08:00 hr. Occasionally, overcast, breezy conditions prevented significant dew formation. Over the period of maximum fungal transmission (19 April–29 May), the mean minimum nightly temperature was 15.4°C (range 12.5–19.5°C). Daytime conditions were, with few exceptions, sunny, warm, and dry. The mean maximum daily temperature over the entire monitoring period was 30.4°C (range 27–33.5°C). Temperature rose above 30°C 33 days of the 50 days monitored, and remained above 30°C for an average of 2.1 hr on these days (maximum 4.5 hr). Rain was recorded only once during the entire monitoring period, and occurred during the evening. Thus free moisture was essentially available only during the nights.

At the initiation of epizootiological monitoring, the prevalence of *Z. radicans* in the *E. kraemeri* population (second instar through adults) was 12.9% (Fig. 1). Infection levels then increased rapidly to 40.5% on 5 May and, following a brief decline in infections, peaked at approximately 50% on 13 May. Prevalence dropped markedly over the subsequent 12 days to less than 10%, increased to a final minor peak of 20% during the period 25–29 May, and then declined to less than 3% by 8 June when the beans were near maturity and leaf drop was excessive.

Visits to the field during the late afternoon (15:00–18:00 hr) throughout the monitoring period revealed many moribund and newly killed cadavers. Nearly all cadavers observed in the field were attached to the lower surfaces of the bean leaves. Although *Z. radicans* requires high moisture conditions for sporulation, such conditions are not required for rhizoid formation. We observed new cadavers strongly attached to the leaf surface several hours prior to dewset. After dewset *Z. radicans* produced a rapid outgrowth of hyphae onto the cadaver surface. A fully developed hymenium, frequently a bright green color (especially on adult leafhoppers) was generally visible within 2 to 3 hr following dewset. Spore deposits were detected four hours after

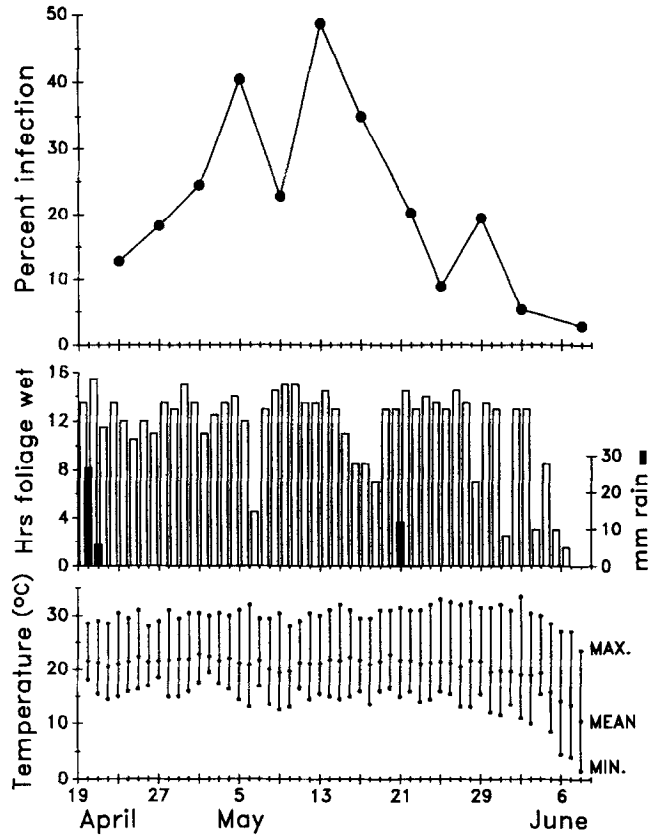

FIG. 1. Percentage infection of *Empoasca kraemeri* by *Zoophthora radicans* during an epizootic in Central Brazil with moisture and temperature data: moisture expressed as the number of hours foliage wet each night (□), daily rainfall in millimeters (■), and temperature as the daily maximum, minimum, and hourly mean values.

dewset. During the dry daytime periods, the cadavers were desiccated and the fungus was inactive. Observations of marked adult and fifth-instar cadavers showed that the fungus rehydrated and underwent sporulation on at least 5 consecutive nights. Infected cadavers were found at all levels on the plant, including the uppermost leaves.

No consistent differences were noted in rates of *Z. radicans* infection of the *E. kraemeri* second-, third-, fourth-, and fifth-instar nymph population (Fig. 2). In contrast, percentage infection of first-instar and adult leafhoppers was substantially lower over most of the epizootic period. The low infection rate observed for first instars may be explained by the difficulty in detecting very recent infections (see Methods). McGuire et al. (1987a), studying an epizootic of *Z. radicans* in an *E. fabae* population in potatoes in Illinois, also noted lower infection among adults than among

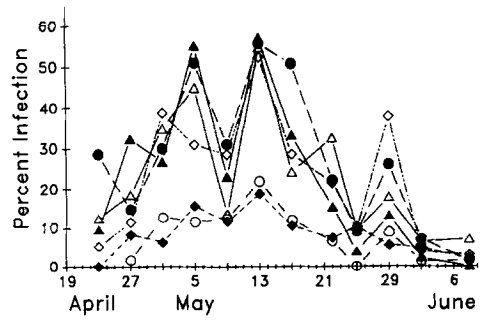

FIG. 2. Infection of *Empoasca kraemeri* first (○), second (●), third (△), fourth (▲), and fifth (◇) instars and adults (◆) during an epizootic in a Central Brazil bean culture.

nymphs. Since one of the primary sites of penetration of leafhopper nymphs is the intersegmental membranes of the abdomen (Wraight et al. 1990), the apparently greater resistance of adults to fungal infection may be due, in part, to the presence of the wings, which prevent spores from contacting the dorsal and lateral surfaces of the abdomen.

Analysis of variance indicated no significant differences ($P > 0.05$) in the numbers of *Z. radicans*-killed cadavers or living leafhoppers among plots during the early stages of the epizootic (23–27 April). Similar analyses of percentage infection levels obtained from the pooling of data from two plots in each of six areas of the field also revealed no differences throughout the 6-week monitoring period (data were pooled to reduce the variability in sample sizes for statistical analysis of arcsine transformed percentages). The observed homogeneity was likely influenced by the small size of the field relative to the large number of adults that migrated into the field. Since there were no significant between-plot differences in infection levels, prevalence and incidence values included in the regression analyses were calculated from data pooled over all plants sampled from all plots within each sample date.

Multiple Regression Analyses

The regression analyses identified only a few biotic and abiotic variables that were highly correlated to both prevalence and incidence. Variables expressing moisture and temperature, inoculum level, and host population density were retained at the $\alpha = 0.1$ level by the Stepwise MAXR^2 procedure. Regressions with the dependent variables prevalence and incidence produced markedly different results.

Multiple Regression of Prevalence

Moisture and temperature. Night temperatures fell below the 10°C threshold for *Z. radicans* development only during the 3 nights of the final assessment period. The variables WH, MC, and LW were therefore influenced only minimally by temperature and thus describe moisture essentially independent of temperature. These variables are subsequently referred to as moisture variables. The variables incorporating both degree hours and moisture (DHMC and DHLW) will be referred to as degree-hour moisture variables.

Moisture and degree-hour moisture variables were indicated by the MAXR^2 procedure to be the most significant abiotic variables in predicting disease prevalence. Model r^2 values were similar, regardless of whether moisture was expressed as WH, MC, or LW (Table 3). High r^2 values ranging from 0.867 to 0.875 were obtained from models incorporating WH or DHMC in combination with biotic variables quantifying host density and/or inoculum, and DHLW in combination with inoculum alone.

Degree-hour moisture variables were the most highly correlated to prevalence when regressions were conducted with the exclusion of all biotic variables. The fact that the temperature variable DH replaced the moisture variable WH as the best abiotic predictor when biotic variables were excluded is not necessarily an indication that use of WH to express moisture is inferior to MC or LW. This apparent weakness in WH as a predictor actually derives from the fact that while combining DH with MC and LW enhances the correlations obtained with these variables, resulting in their selection by the MAXR procedure (Table 3), it is not possible to create a comparable variable combining DH and WH. In fact, the similar predictive power of the three measures of moisture is revealed by examination of the moisture variables. The simple correlation coefficients (*r*) obtained from the regressions of WH, MC, and LW against prevalence (0.342, 0.407, and 0.364, respectively) are not significantly different ($\chi^2 = 0.027$, 2 *df*).

The observation that the more quantita-

TABLE 3
MULTIPLE REGRESSION STATISTICS FOR PREDICTION OF *Zoophthora radicans* PREVALENCE

	Regression conducted with WH moisture variables		Regression conducted with MC moisture variables		Regression conducted with LW moisture variables	
	β ± SE[a]	*F* (Prob. > *F*)	β ± SE	*F* (Prob. > *F*)	β ± SE	*F* (Prob. > *F*)
Variables						
WH	0.46 ± 0.202	5.3 (0.0550)				
LHPP	−1.44 ± 0.743	3.8 (0.0935)				
TOTCPP	22.04 ± 6.689	10.9 (0.0132)	25.22 ± 5.860	18.5 (0.0026)	25.98 ± 5.663	21.0 (0.0018)
DHMC			0.07 ± 0.017	16.8 (0.0034)		
DHLW					0.07 ± 0.015	18.0 (0.0028)
Intercept	26.41		9.65		9.97	
Model *F*	15.2 (0.0019)		26.5 (0.0003)		27.9 (0.0002)	
Model r^2	0.867		0.869		0.875	
Regressions conducted as above but excluding biotic variables						
DH	0.06 ± 0.022	6.8 (0.0265)				
DHMC			0.09 ± 0.028	9.6 (0.0113)		
DHLW					0.07 ± 0.027	7.5 (0.0210)
Intercept	−12.78		6.49		8.44	
Model *F*	6.8 (0.0265)		9.6 (0.0113)		7.5 (0.0210)	
Model r^2	0.403		0.490		0.428	

[a] Model coefficient β ± standard error.

tive moisture variables (WH and MC) did not prove to be better predictors than the simple threshold variable LW supports views that moisture in entomophthoralean epizootics functions as an on–off switch (Carruthers and Soper, 1987). In this system, very high levels of moisture (at or near saturation) were recorded over long periods on most nights. This excess moisture apparently contributed a disproportionately high number of wetness hours relative to its epizootiological importance, reducing the sensitivity of the WH variable. It is possible, however, that in drier systems, or systems subject to greater fluctuation in moisture level, this variable might be more effective than LW as a measure of moisture. Millstein et al. (1983) determined that spore production by an unidentified *Zoophthora* species (probably *Z. phytonomi*) was directly related to moisture over the range of 90 to 100% RH, and Nordin et al. (1983) concluded that cumulative moisture (humidity hours) was an important factor in the epizootic development of this fungus in alfalfa weevil populations.

The inclusion of moisture or degree-hour moisture variables as the only abiotic variables in five of the six models in Table 3 is an indication of the primary importance of moisture in *Z. radicans* epizootic development. For example, if the variables DHMC and DHLW are removed from the regression analyses, they are replaced by MC and LW (at $P = 0.0184$ and $P = 0.0138$) and the resulting model r^2s are 0.805 and 0.818, respectively.

The close correlation between disease prevalence and moisture expressed as hours of leafwetness is evident in Figure 1. The epizootic peak on 13 May occurred following a period of 6 nights during which foliage was wet for an average of 14.1 hr per night. The sharp decline in infection levels following 13 May correspond to a dry period between 15 and 19 May when foliage was wet less than 9 hr each night. This response is in accordance with the findings of Milner et al. (1982) who concluded that 9 hr of moisture (relative humidity greater than 90%) was required for *Z. radicans* transmission in aphid populations and that longer periods induced higher levels of infection. As we noted previously, fungus which killed the leafhopper host during late afternoon was capable of penetrating to the cadaver surface, forming a dense layer of hymenium, and initiating sporulation within

4 hr after the onset of moisture-saturated conditions. In light of this, and including the 4–6 hr required for host penetration (Wraight et al., 1990), it is possible for *Z. radicans* to kill and secure its host to the leaf substrate and then exit the host, undergo sporulation, and come into contact with and infect a new host all within 8–10 hr after the onset of moist conditions. The timing of this sporulation/host infection process is the probable explanation of the observed transmission threshold of approximately 9 hr of moisture. It is notable, in this respect, that 1989 was an exceptionally dry year in Goiânia and marked the first occasion since we initiated research at CNPAF in 1982 that *Z. radicans* failed to appear in our experimental fields. Between 24 April and 21 May, the normal epizootic period, weather conditions were unstable and dewfall was frequently inhibited by warm, breezy conditions; foliage was wet for an average of only 6.8 hr per night. No infected *E. kraemeri* were found, even in populations that exceeded two leafhoppers per trifoliate.

Figure 1 shows that a marked decline in infection occurred between 22 and 25 May, even though high levels of nightly moisture and moderate temperatures prevailed during this period. We attribute this to another factor (inoculum level) discussed below.

The relative lack of importance of temperature alone as a predictor of prevalence in this study is not unexpected considering that moist conditions prevailed only during the night, and, during the peak period of the epizootic, minimum nightly temperatures remained well above the 10°C threshold. Furthermore, maximum temperature during the day only exceeded the 30°C threshold for a few hours on any given day (and then usually by only one or two degrees), and while this might have delayed *Z. radicans* development slightly, it likely did not seriously interfere with disease development. McGuire et al. (1987b) showed significant inhibition of *Z. radicans* expression in *E. fabae* only when infected leafhoppers were exposed to a temperature of 32°C for more than 8 hr. Nevertheless, the result that degree-hour moisture variables were the most significant single-variable predictors of prevalence (Table 3) and that DHLW combined with inoculum alone to produce an r^2 of 0.875 indicates that fluctuations in nighttime temperature above the 10°C threshold do, in fact, have an important effect on epizootic development. Numerous studies have shown strong relationships between temperature and *Z. radicans* in vivo incubation time and the rate of spore production and germination (Milner and Lutton, 1983; Van Roermund et al., 1984; Soper, 1985; McGuire et al., 1987b).

Examination of the graphic representation of prevalence in relation to moisture and temperature (Fig. 1) revealed an apparent close correlation between prevalence and moisture conditions recorded during the driest night of each presample period. This was especially evident in the case of the 9 May sample when dry conditions on the night of 7 May preceded a substantial decline in prevalence. In view of this, variables relating maximum (MAX) and minimum (MIN) daily and nightly temperature and moisture conditions within each presample period were added to the regressions. The initial observation was confirmed in that the moisture variables or moisture components of the degree-hour moisture variables originally selected (Table 3) were replaced by MIN or MAX moisture variables and model r^2 values actually increased slightly (Table 4). Increases in r^2 were largest in the WH and MC models, both of which incorporated a host population density variable (LHPP).

The explanation for a strong correlation among environmental conditions occurring on a single night and *Z. radicans* prevalence likely relates to the brevity of the assessment period. If, as the mean incubation times (Leite et al., 1989) suggest, prevalence at any given time is governed by environmental conditions existing over the preceding 3 to 4 days and nights, then in-

TABLE 4

MULTIPLE REGRESSION STATISTICS FOR PREDICTION OF *Zoophthora radicans* PREVALENCE: ANALYSES INCLUDING MAXIMUM AND MINIMUM VALUES[a] FOR ABIOTIC VARIABLES

	Regression conducted with WH moisture variables		Regression conducted with MC moisture variables		Regression conducted with LW moisture variables	
	β ± SE[b]	*F* (Prob. > *F*)	β ± SE	*F* (Prob. > *F*)	β ± SE	*F* (Prob. > *F*)
Variables						
WHMIN	1.00 ± 0.348	8.2 (0.0243)				
LHPP	−1.39 ± 0.664	4.4 (0.0742)	−1.14 ± 0.503	5.1 (0.0584)		
TOTCPP	25.13 ± 5.925	18.0 (0.0038)	24.74 ± 4.378	31.9 (0.0008)	30.44 ± 5.150	34.9 (0.0004)
MCMIN			1.17 ± 0.258	20.8 (0.0026)		
LWMIN					1.30 ± 0.284	21.0 (0.0018)
Intercept	31.35		24.35		13.07	
Model F	19.3 (0.0009)		37.3 (0.0001)		31.6 (0.0002)	
Model r^2	0.892		0.941		0.888	
	Regressions conducted as above but excluding biotic variables					
DH	0.06 ± 0.022	6.8 (0.0265)				
DHMCMAX			0.29 ± 0.082	9.6 (0.0053)		
DHLWMIN					0.21 ± 0.072	8.6 (0.0152)
Intercept	−12.78		−0.48		13.04	
Model *F*	6.8 (0.0265)		12.5 (0.0053)		8.6 (0.0152)	
Model r^2	0.403		0.556		0.461	

[a] Maximum and minimum nightly values recorded during each presample period as described in text.
[b] Model coefficient β ± standard error.

fections initiated (or not initiated) on a single night could represent a significant proportion of the infections actually or potentially existing at the time of sampling. The high correlations obtained support the hypothesis that the observed infections were initiated over the few nights constituting each presample period.

Inoculum. Inoculum expressed as the log of the total number of cadavers per plant (TOTCPP) was the variable most highly correlated to disease prevalence (partial *F* significant at $P < 0.015$) (Table 3). The r^2 value obtained from regression of prevalence on the log number of total cadavers existing during each immediate presample period was 0.593, and this high level of association is evident in Figure 3. This close relationship between inoculum levels and levels of prevalence has been observed in other epizootics of entomophthoralean fungi (Soper and McLeod, 1981; Carruthers et al., 1985). Correlations of these variables, however, are difficult to interpret, because inoculum is both a cause of and a direct result of infection, and therefore inherently closely correlated to fluctuations in prevalence over time.

Nevertheless, data collected during the early and late stages of the epizootic provide a basis for estimating the level of inoculum required to inititate an epizootic. Prevalence declined sharply (from 20 to 9%) between 22 and 25 May while moisture and temperature conditions were near optimum for disease transmission (Fig. 1). The most apparent explanation for this is the very low level of inoculum (approximately 0.5 cadavers per plant) that existed in the field at that time (Fig. 3). This low inoculum level was a direct result of the dry conditions that caused the rapid decline in prevalence following the epizootic peak on 13 May. Moist conditions returned after 19 May, but a violent thunderstorm with high winds occurred at 17:30 hr on 21 May, likely further contributing to inoculum reduction by removing many moribund leafhoppers, cadavers, and spore deposits from the bean foliage. This series of events and its large negative impact on cadaver density probably account for the substantially lagged increase in prevalence recorded on 29 June, and the data indicate that an inoculum density of 0.4 to 0.5 cadavers per plant will support only a minimal level of *Z.*

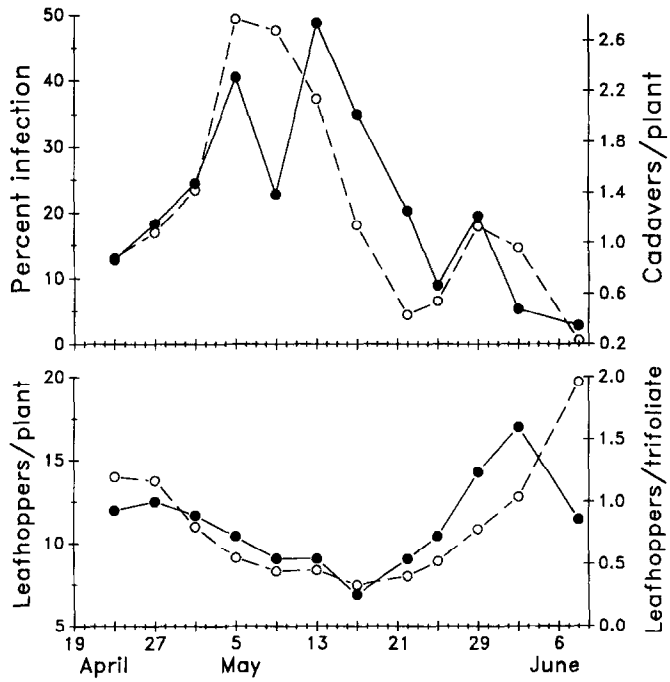

FIG. 3. Percentage infection (●) of *Empoasca kraemeri* by *Zoophthora radicans* with associated levels of inoculum expressed as the number of leafhopper cadavers supporting active (sporulating) fungus (○) and host population levels expressed on a per plant (●) and per trifoliate (○) level.

radicans transmission in low-density host populations (less than 10 leafhoppers per mature bean plant). The level of inoculum increased slowly from 0.43 cadavers per plant on 23 May to 0.53 on 25 May, and then rapidly to 1.13 cadavers per plant on 29 May when a minor secondary epizootic peak was recorded as infection increased from 8.9 to 19.5% (Fig. 3). The inoculum level of 0.84 cadavers per plant (mean of the 25 and 29 May inoculum estimates) that corresponds to this increase in infection may represent the approximate inoculum density threshold for epizootic development. This conclusion is supported by the observation that a similar level of inoculum (mean of 0.98 cadavers per plant) was present during the interval between the first two samples of this study (23 and 27 April) when infection increased from 12.9 to 18.2%.

Host density. Host density expressed as the number of leafhoppers per plant (LHPP) was the least significant of the variables selected by the stepwise regression. It was included in only one of the models in Table 3 (with WH) and at a low level of significance ($P = 0.0935$). In the regressions including the maximum and minimum variables (Table 4), LHPP was included in both the WH and MC models, but in both cases at $P > 0.05$. The fact that β in these models is negative indicates that rather than functioning as a biotic predictor variable, population density actually correlates to prevalence as a dependent variable. This is simply a reflection of leafhopper population response to pressure from a highly virulent pathogen. The leafhopper population was suppressed throughout the epizootic period and began to recover when conditions became cooler and drier and the fungus was no longer a controlling factor (Fig. 3). These observations, however, relate only to host population and *Z. radicans* infection trends occurring within the limited time frame of this study. Our monitoring was initiated too late in the epizootic cycle

to have detected a strongly lagged host density-dependent increase in infection level.

The complex relationships such as normally exist between host density and pathogen prevalence are not definable from this type of brief descriptive study and regression analysis. In addition, interpretation of the described *E. kraemeri-Z. radicans* host pathogen system is limited by the lack of information regarding leafhopper migration patterns. *Empoasca kraemeri* is a year-round resident of the state of Goiás, especially in areas where crops are maintained over the long dry season through irrigation. However, it is not known if large numbers of leafhoppers also migrate into the region from the South where the dry season is less severe and *Z. radicans* epizootics are also widespread. Nevertheless, the results of this investigation in conjunction with the 1989 observations and available data on leafhopper population dynamics indicate that host density alone is not a controlling factor in the development of *Z. radicans* epizootics in *E. kraemeri* populations in Central Brazil bean cultures. Magalhães et al. (1988) conducted a 2-year investigation of *E. kraemeri* populations in seven consecutive plantings of beans at CNPAF. The data reveal that population densities of *E. kraemeri* in excess of that observed at the beginning of the present study exist throughout much of the year. Yet, we have observed *Z. radicans* epizootics only during April and May. We have observed resting spores in cadavers attached to foliage during epizootics, and while we have never determined resting spore production rates, it is reasonable to assume that the fungus survives interepizootic periods in this stage. It may be that the moderate to cool nighttime temperatures and the heavy dews that occur during early April act in combination with an increase in host population to some unknown threshold density to initiate disease transmission. After the pathogen becomes active in the host population (perhaps on weed species in noncultivated areas) and dispersal via infected adult leafhoppers is initiated, epizootics may then develop essentially independent of host density as was observed in this study.

Other entomopathogenic fungi, including entomophthoralean species, have been reported to cycle in a density-independent manner (see Benz, 1987). Generally, these involve situations where the pathogen has become widely distributed throughout the host habitat. The rapid and efficient dispersal of *Z. radicans* by adult leafhoppers and the fact that *Empoasca* leafhopper populations tend to be aggregated and stratified on the host plant (Helm et al., 1980; Ramalho and Ramos, 1979) are undoubtedly dominant factors in the ability of *Z. radicans* to attain high levels of prevalence in low-density host populations.

Damage from *E. kraemeri* is in large part a function of plant vigor, especially as affected by drought stress. The reported threshold levels for initiation of control efforts are therefore variable, being dependent upon test location and time. Nymph populations are frequently used as indicators because of the relative ease of sampling this lifestage, and economic damage thresholds from most studies range from one to a few nymphs per trifoliate (Valderrama et al., 1977; Pedrosa, 1977; King and Saunders, 1984). Our observation of high levels of *Z. radicans* infection in leafhopper populations substantially lower than one nymph per trifoliate indicates the high microbial control potential of this entomopathogen.

Multiple Regression of Incidence

Regressions with incidence as the dependent variable produced substantially lower r^2 and significance levels than the regressions with prevalence (Table 5). This was anticipated because the sampling protocol was designed to measure prevalence and provides only an approximation of incidence (see Fuxa and Geaghan, 1983). The results are of interest, however, especially with respect to the identification of important predictor variables.

TABLE 5
MULTIPLE REGRESSION STATISTICS FOR PREDICTION OF *Zoophthora radicans* INCIDENCE

	Regression conducted with WH moisture variables		Regression conducted with MC moisture variables		Regression conducted with LW moisture variables	
	β ± SE[a]	*F* (Prob. > *F*)	β ± SE	*F* (Prob. > *F*)	β ± SE	*F* (Prob. > *F*)
Variables						
RILGCPP	0.73 ± 0.238	9.5 (0.0132)			0.73 ± 0.238	9.5 (0.0132)
MC			0.06 ± 0.021	9.5 (0.0150)		
ABOVE30			−0.10 ± 0.050	3.7 (0.0914)		
Intercept	0.26		−0.63		0.26	
Model F	9.5 (0.0132)		5.2 (0.0358)		9.5 (0.0132)	
Model r^2	0.513		0.565		0.513	
	Regressions conducted as above but excluding biotic variables					
WH	0.05 ± 0.021	6.5 (0.0316)				
MC			0.06 ± 0.021	9.5 (0.0150)		
ABOVE30			−0.10 ± 0.050	3.7 (0.0914)		
LW					0.04 ± 0.022	4.0 (0.0770)
Intercept	−0.28		−0.63		−0.47	
Model *F*	6.5 (0.0316)		5.2 (0.0358)		4.0 (0.0770)	
Model r^2	0.418		0.565		0.307	

[a] Model coefficient β ± standard error.

Moisture was clearly the dominant abiotic variable in regressions against incidence. A temperature variable (ABOVE30) was included in only one model (in combination with the moisture variable MC) and at a low level of significance ($P = 0.0914$). MC appeared to be a better predictor of incidence than WH or LW, since in the regressions including all biotic and abiotic variables, it was the only moisture variable included in the models.

As was observed with the prevalence data, addition of the MAX and MIN variables to the regressions of incidence resulted in the replacement of each moisture variable (WH, MC, or LW) with the corresponding MIN moisture variable with a consequent increase in model r^2. For example, in the two-variable model including ABOVE30 and MC ($r^2 = 0.565$), MC was replaced by MCMIN and then combined with RILGCPP to produce an r^2 of 0.677, and while the single-variable model incorporating LW had a low r^2 of 0.307, replacement with LWMIN produced an r^2 of 0.466.

Only one biotic variable, the rate of increase of large cadavers, was selected. Because spore production is directly related to cadaver instar (size), we initially hypothesized that a variable expressing inoculum as large cadavers would be nearly as good a predictor as total cadavers. In this case, the simple correlation coefficients (r) from the regressions of incidence against the rate of increase of large and total cadavers (viz., 0.716 and 0.618, respectively) were not significantly different ($\chi^2 = 0.141$, 1 *df*).

Microbial Control Potential

The observed high epizootic potential of *Z. radicans* in *Empoasca* leafhopper populations can be attributed to many factors which, when considered in total, describe a pathogen well adapted to parasitism of a highly mobile, rapidly developing host which attains high population densities during drought periods. Despite its high microbial control potential, however, this study in conjunction with the work of McGuire et al. (1987b) indicates that a major obstacle to the utilization of this fungus for leafhopper control will be its requirement for high moisture conditions and moderate temperatures for a substantial part of each day. This problem is compounded by the fact that in many crop systems in various regions, damage from *Empoasca* leafhoppers is associated with hot, dry weather. Nevertheless, through additional studies designed to gain a more thorough understanding of

Z. radicans dynamics, it may be possible to design application/introduction strategies integrated with cultural management practices to maximize expression of the inherent epizootic potential of this pathogen. Crop management recommendations to enhance natural pathogen prevalence is becoming an increasingly important component of applied insect pathology (see Carruthers and Soper, 1987) and will likely continue to become more important in the future, especially with respect to utilization of entomopathogenic fungi.

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