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METEOROLOGICAL FACTORS AFFECTING THE SUCCESS OF THE GYPSY MOTH FUNGAL PATHOGEN *Entomophaga maimaiga* (ZYGOMYCETES: ENTOMOPHTHORALES) IN MICHIGAN

By

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ABSTRACT

METEOROLOGICAL FACTORS AFFECTING THE SUCCESS OF THE GYPSY MOTH FUNGAL PATHOGEN *Entomophaga maimaiga* (ZYGOMYCETES: ENTOMOPHTHORALES) IN MICHIGAN

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The fungal pathogen *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) has been responsible for significant declines in gypsy moth [(*Lymantria dispar* L.) (Lepidoptera: Lymantriidae)] population density in the northeastern U.S. since 1989. In Michigan, however, the pattern of *E. maimaiga* epizootics has been less consistent since its introduction in 1991. Although *E. maimaiga* is established throughout Michigan, high-density gypsy moth populations and severe defoliation have continued to occur. As the gypsy moth fungus is highly sensitive to variations in temperature and moisture, more information is needed concerning *E. maimaiga* infection rates in relation to climate in the North Central region of the United States. Meteorological factors affecting the success of *E. maimaiga* were examined using large-scale climate-matching analyses, and laboratory and field bioassays between 1999 and 2002 that compared *E. maimaiga* infection rates under optimal versus naturally-occurring conditions. Additionally, *E. maimaiga* and nuclear polyhedrosis virus infections of gypsy moth larvae during primary transmission were evaluated in an oak-dominated Michigan forest with low-density gypsy moth populations. Infection rates during 4-d intervals were related to microclimatic variables

occurring over a 6-wk period of gypsy moth larval development.

A relatively small area in the southern Great Lakes region was determined to be highly similar in long-term climatic patterns to the climatic conditions in regions of the U.S. where large-scale *E. maimaiga* epizootics have been documented. A high degree of climatic variability, however, occurs annually in portions of the North Central region. The number of years between 1971 and 2000, in which weather may have been favorable for the development of *E. maimaiga* epizootics in the North Central region, were estimated.

Bioassays using laboratory-reared 4th-instar gypsy moths were conducted to evaluate *E. maimaiga* infection rates in oak-dominated forests in Michigan. In field bioassays, infection rates of *E. maimaiga* were significantly lower under naturally-occurring conditions in Michigan than under laboratory conditions that were optimal for fungal germination. Increased levels of *E. maimaiga* infection in field bioassays were associated with June temperature and precipitation levels which were significantly greater than 30-year average conditions. Dynamics of the gypsy moth fungal pathogen *E. maimaiga* throughout much of the North Central region appear to be primarily limited by weather, specifically levels of June precipitation. The role of climatic variability in the success of *E. maimaiga* in the North Central region are discussed. Implications of this research for developing improved methods and recommendations to incorporate the biological control agent *E. maimaiga* into an integrated pest management system for the effective control of gypsy moth in forest ecosystems in the North Central region are presented.

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LIST OF SYMBOLS, ABBREVIATIONS AND NOMENCLATURE

°C	degrees Celsius
µm	micrometer
a	total precipitation constant (CLIMEX)
APHIS	Animal and Plant Health Inspection Service
ARSEF	Agricultural Research Service Collection of Entomopathogenic Fungal Cultures
BA	basal area
ca	circa
CI	confidence interval
Co.	county
CSIRO	Commonwealth Scientific & Industrial Research Organization (Australia)
d	day(s)
DBH	diameter at breast height
e_a	actual atmospheric water vapor pressure
e_s	saturation water vapor pressure
ESRI	Environmental Systems Research Institute
exp	inverse of the natural logarithm of a number
FBS	fecal bovine serum
g	gram(s)
h	hour(s)
ha	hectare(s)

I_{rpat}	precipitation pattern index (CLIMEX)
I_{rtot}	total precipitation index (CLIMEX)
I_t	average monthly temperature (CLIMEX)
I_{tmax}	maximum temperature index (CLIMEX)
I_{tmin}	minimum temperature index (CLIMEX)
IPM	integrated pest management
kPa	kilo-Pascal
k_p	precipitation pattern constant (CLIMEX)
k_R	total precipitation constant (CLIMEX)
k_T	temperature constant (CLIMEX)
LAI	leaf area index
MI	Match Index (CLIMEX)
MI DNR	Michigan Department of Natural Resources
MRCC	Midwestern Regional Climate Center
NAPIS	National Agricultural Pest Information Service
NOAA	National Oceanic and Atmospheric Administration
NPV	nucleopolyhedrosis (nuclear polyhedrosis) virus
PPQ	Plant Protection and Quarantine
r	correlation coefficient
r^2	coefficient of determination
R_D	average absolute difference between monthly precipitation of target and matching locations (CLIMEX)
R_M	annual precipitation at matching location (CLIMEX)

R_T	annual precipitation at target location (CLIMEX)
RH	relative humidity
SEM	standard error of the mean
SI	site index
T	air temperature
T_{dmax}	average absolute difference in maximum temperature between two locations (CLIMEX)
T_{dmin}	average absolute difference in minimum temperature between two locations (CLIMEX)
USDA	United States Department of Agriculture
wk	week(s)
yr	year(s)

INTRODUCTION

The distribution of the world's biota has historically been restricted by geographic and ecological barriers, such as oceans and mountain ranges. However, there has been a dramatic increase in the number of introductions of new species, as a result of increased international commerce, travel and ecosystem disturbance (Liebhold et al. 1995, Niemelä and Mattson 1996). Successful establishment of non-indigenous species in new geographic ranges may be facilitated by arriving without their native biotic constraints on growth, survival and reproduction (NRC 2002). Additionally, without their native complexes of predators, parasites or pathogens, many of these foreign species become important pests, causing substantial disturbance to forest ecosystems and often significant socioeconomic impacts (Liebhold et al. 1995).

Biological control of forest insects is an important technology for successfully managing economic pests. The principle strategy behind biological control is to use populations of other organisms (e.g. natural enemies) to limit the density and growth of an insect pest population (Van Driesche and Bellows 1996). Barbosa and Wagner (1989) identified 22 programs that utilized biological control in forest pest management globally, 18 of which were described as successful. Several non-indigenous forest pests, such as European pine shoot moth (*Rhyacionia buoliana* [Denis and Schiffermüller]) (Lepidoptera: Tortricidae), European spruce sawfly (*Gilipinia hercyniae* [Hartig]) (Hymenoptera: Diprionidae), larch casebearer (*Coleophora laricella* [Hübner])

(Lepidoptera: Coleophoridae) and winter moth (*Operophtera brumata* L.) (Lepidoptera: Geometridae) (Craighead 1950, Embrée 1966, Embrée and Otvos 1984, Dahlsten 1986, Long 1988), have been successfully managed using classical biological control strategies, which involve the importation and establishment of control agents from a pest's native natural enemy complex. Van Driesche et al. (1996) reviewed 28 exotic insect pest species in the United States and judged that 26 of them provided opportunities for their control via natural enemy introductions. The ecological and environmental threats posed by biological invasions necessitates continued efforts to examine classical biological control agents and evaluate their potential role in the effective control and management of exotic forest pests in North America.

Gypsy moth in North America

European gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae) is an exotic lepidopteran forest herbivore that was accidentally introduced to North America in the late 1860's by an entrepreneurial amateur entomologist in Massachusetts who was attempting to cross gypsy moth with native North American silkworms (Forbush and Fernald 1896, Liebhold et al. 1989). Despite great efforts to reduce the spread of this notorious forest defoliator, gypsy moth has continued to expand its geographic range. As of 2004, gypsy moth is currently known to be established in the southern portions of the Canadian provinces of Ontario and Quebec (CFIA 2004), throughout the northeastern states, and some southeastern states, Michigan and portions of the adjacent

North Central states of Illinois, Indiana, Ohio and Wisconsin (NAPIS 2004).

The life cycle of the gypsy moth was previously described by Forbush and Fernald (1896) and Leonard (1981). Gypsy moth spends the majority of its univoltine life cycle (i.e. one generation per year) as an egg, clustered in egg masses of 100 - 600 or more that are deposited on the undersides of tree branches, on tree trunks, buildings, fences or other suitable locations. In mid-spring, typically late April to early May, larvae hatch from eggs and begin to feed on tree foliage. Larvae are highly seteous (i.e. hairy) and first-instars typically disperse by spinning a strand of silk and "ballooning" via wind currents to new locations. Young larvae are dark-colored, while later instars develop prominent blue spots on their dorsal anterior body segments and red spots on their dorsal posterior body segments. Late-instar gypsy moths have voracious appetites and feed on hundreds of species of trees and shrubs, though oaks (*Quercus spp.*) are highly preferred. Late-instar larvae move down from the tree canopy at dawn and remain amongst the leaf litter or in cryptic locations on the boles of trees throughout the day (Forbush and Fernald 1896, Leonard 1981). At dusk, larvae ascend into the canopy again to feed. Feeding is completed in approximately seven weeks, at which point larvae find a sheltered location and pupate in a brownish-black pupal case. Adults eclose from pupal cases in approximately mid-July, with males typically eclosing several days earlier than females. Adult wingspans are about 50 mm and adult males are dark brown in color, while females are white with dark bands across their forewings. Female European gypsy moths do not fly. However, females of the closely-related

Asian gypsy moth, which is not known to currently be established in North America, do have the ability to fly. Female gypsy moths emit a sex pheromone that volatizes in the air and is highly attractive to male gypsy moths. Males follow the pheromone plume to females and mating occurs. Females cover egg masses with buff-colored setae from their bodies, which gives them the appearance of a sponge fungus—hence, its German name, the sponge fungus moth (Stanek 1969). Adults do not feed and soon die after mating and depositing eggs.

A large number of natural enemies of the gypsy moth, including parasitoids, predators and pathogens, have been studied and evaluated since its introduction to North America in an effort to successfully suppress this invasive pest. Reardon (1981), Griffiths and Quednau (1984), Van Driesche et al. (1996) and Nealis et al. (2002) provide thorough summaries of the history of the century-long effort to acquire a natural enemy complex for gypsy moth. Fuxa et al. (1998) provide a thorough summary of gypsy moth pathogens.

While several pathogens have been evaluated, a nuclear polyhedrosis virus (NPV) (Figure 1A) was detected in North America in the early 1900's (Glaser 1915) and has frequently been found to cause epizootics in high-density gypsy moth populations (Doane 1970, Leonard 1981, Woods and Elkinton 1987). Gypsy moth typically become infected with NPV by ingesting the virus (Murray and Elkinton 1989), though other modes of infection, such as transovum transmission (Doane 1969), are possible. Infected early-instar gypsy moth move to the ends of branches or the tops of trees (usually some elevated

position) where they die. As these dead larvae deteriorate, they contaminate the foliage and provide viral inoculum for infection of late-instar larvae (Woods and Elkinton 1987). Other biotic (e.g. other caterpillars and insects, parasitoids, birds, mammals) and abiotic factors (e.g. wind, rain) serve to further facilitate the spread and dispersal of the virus (Podgwaite et al. 1981). Until 1989, NPV remained the dominant gypsy moth pathogen in North America.

Entomophaga maimaiga in North America

Since 1989, the fungus *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) (Figure 1B, C) has become an important pathogen of gypsy moth in the northeastern U.S. (Andreadis and Weseloh 1990a, 1990b, Hajek et al. 1995b, Reardon and Hajek 1998, Hajek 1999). *Entomophaga maimaiga* is a desirable biological control agent because it is highly synchronized with gypsy moth larval development, has relatively few negative effects on non-target species (Soper et al. 1988, Vandenberg 1990, Hajek et al. 1995a, 1996a, 1996b, 2000), and is compatible with other natural enemies, including NPV (Andreadis and Weseloh 1990a, Hajek and Roberts 1992, Weseloh and Andreadis 1992b).

The source of *E. maimaiga* and the reason for it's appearance in North America in 1989 remain unknown (Hajek et al. 1995b, Hajek 1999). By the early 1900's, researchers in North America had learned of a fungal pathogen affecting gypsy moth in Japan and attempted to release it in the Boston area in 1910 and 1911 (Hajek 1999). There was no evidence, however, of successful

transmission of the fungus and local gypsy moth populations were substantially reduced by a viral epizootic in 1911, so the program was discontinued (Hajek 1999). Numerous surveys of gypsy moth populations for pathogens were conducted following the early release attempts (Campbell and Podgwaite 1971, Podgwaite 1981), however, the presence of entomophthoralean spores were not detected in larvae. Efforts to introduce *E. maimaiga* to North American gypsy moth populations were renewed in the mid-1980's, following damaging gypsy moth outbreaks (Hajek et al. 1995b). As had happened with the earlier release attempts, there was little to no evidence of successful transmission of *E. maimaiga* to the native gypsy moth populations at the experimental sites in New York and Virginia (Hajek et al. 1995b). *Entomophaga maimaiga* was absent in follow-up surveys at these sites in 1987 and 1989 to 1991, so the releases were considered to have failed (Hajek et al. 1995b). Unexpectedly, *E. maimaiga* was discovered causing epizootics in southwestern Connecticut in June 1989 and subsequent surveys during 1989 found that *E. maimaiga* was present in seven northeastern states (Andreadis and Weseloh 1990a, 1990b, Hajek et al. 1995b). Hajek et al. (1995b) and Weseloh (1998) discuss several hypotheses regarding the recent origin of *E. maimaiga* in North America.

Entomophaga maimaiga produces two types of spores, both of which may infect gypsy moth larvae (Reardon and Hajek 1998). Resting spores (i.e. azygospores) of *E. maimaiga* overwinter in the soil (Figure 1B), with the highest levels occurring in the organic layer of soil at the base of trees (Hajek et al. 1998a). Late-instar larval behavior of climbing down from the canopy to rest in

the leaf litter during the day increases the risk of fungal infection (Hajek 2001). A portion of these resting spores germinate in the spring when environmental conditions are suitable (i.e. primary transmission) (Hajek 1997b, Hajek and Humber 1997, Weseloh and Andreadis 1997). Gypsy moth larvae become infected when *E. maimaiga* spores adhere to the cuticle, and then gain entry to the host using a combination of mechanical pressure and enzymatic degradation (Hajek 1999). Early-instar larvae become infected and die. These infected cadavers externally produce *E. maimaiga* conidiophores that discharge conidia (Figure 1C) which may infect mid- to late-instar gypsy moth (i.e. secondary transmission). Late-instar larval cadavers principally produce resting spores and are usually found attached to lower tree trunks by their prolegs with their heads oriented downwards (Hajek and Soper 1991, Hajek et al. 1998b). Cadavers drop to the soil, decompose and resting spores remain dormant in the soil until the following spring.

Scope of the present study

Microclimatic conditions directly affect the transmissibility, germination and infection of many entomopathogenic fungal pathogens (Andreadis 1987). Temperature and forms of environmental moisture, such as humidity, dew, and free water tend to be particularly important factors (McCoy et al. 1988, Tanada and Kaya 1993, Burges 1998). Due to this dependency on climate, the success of fungal pathogens is invariably associated with climatic variability when viable host and pathogen populations are present.

Although *E. maimaiga* epizootics have effectively regulated gypsy moth populations in areas of the northeastern United States since its discovery in 1989 (Hajek et al. 1995b, 1996b, Hunter and Elkinton 1999), this fungal pathogen has been less consistent in other states, such as Michigan (Smitley et al. 1995). *Entomophaga maimaiga* was first introduced into Michigan in 1991 (Smitley et al. 1995), and additional introductions have been made subsequently (Buss 1997, L.J. Buss and D.G. McCullough unpubl. data, Michigan Department of Natural Resources [MDNR] and Michigan Department of Agriculture [MDA] unpubl. data). Despite these widespread introductions and establishment of *E. maimaiga* in Michigan, the development of epizootics has been inconsistent in suppressing gypsy moth populations. *Entomophaga maimaiga* appeared to contribute to a population collapse in 1993 and localized epizootics were frequently observed to effectively control gypsy moth populations in 1996 (Bauer and Smitley 1996). Both of these years had springs with above average precipitation (MRCC 2002). Although variable, substantial gypsy moth defoliation has continued to occur since 1996, with 242,361.2 ha of Michigan forests sustaining moderate to heavy defoliation between 1997 and 2003 (USDA-FS 2004).

Gypsy moth populations continue to expand into the North Central region, recently becoming established in Illinois, Indiana, Ohio and Wisconsin (NAPIS 2004). Much of the North Central region that contains extensive areas of highly susceptible forests (Liebhold et al. 1997a, 1997b). The costs of suppressing gypsy moth are high, ranging from $1 - 3.6 million per year in

Michigan alone between 1990 and 1998 (USDA-FS 2004), and resource managers in the North Central region are very interested in incorporating *E. maimaiga* into their gypsy moth management strategies. However, if the development of epizootics are regulated by specific climatic conditions, then *E. maimaiga* may not consistently suppress gypsy moth populations.

Many questions remain regarding the effectiveness of *E. maimaiga* as a successful biological control agent in the management of gypsy moth in the North Central region of the United States. More knowledge is still needed, including an understanding of climatic variability in the North Central region, its potential impact on the development of *E. maimaiga* epizootics and the role of weather in the infection dynamics of *E. maimaiga* in North American forests. The present study addresses associations between meteorological factors and the success of the gypsy moth fungal pathogen *E. maimaiga* in Michigan and the North Central region.

Dissertation organization

Results from extensive field bioassays and corresponding laboratory bioassays that were conducted from 1999 to 2001 to evaluate *E. maimaiga* infection of gypsy moth larvae are presented in Chapter 1. Pathogen infection rates under field conditions in Michigan versus controlled laboratory conditions, known to be optimal for fungal germination, were compared. Numerous climatic and site-related factors that may affect *E. maimaiga* infection rates, including *E. maimaiga* resting spore inoculum densities, were quantified. The proportion of

late-instar gypsy moth mortality in natural populations that was attributable to *E. maimaiga* versus other pathogens, such as NPV, was surveyed at the field sites and results are included in Chapter 1.

In Chapter 2, intensive 6-wk field bioassays that were conducted to evaluate *E. maimaiga* and NPV infections of 4th-instar gypsy moth larvae, in relation to hourly microclimatic conditions, are presented. Infection dynamics of both pathogens, during the initial phase in the development of epizootics (i.e. primary transmission), were evaluated at three field sites in Michigan over the course of gypsy moth larval development from late May to early July in 2001 and 2002.

In Chapter 3, the climatic conditions in regions of the United States where large-scale *E. maimaiga* epizootics have been documented, were compared to the climate of North America overall and, most rigorously, to the North Central region of the United States. I conjectured that regions with greater climatic similarity to epizootic-specific environmental conditions may be more likely to develop extensive *E. maimaiga* epizootics than regions that were less similar in climate. The climatological software CLIMEX (Sutherst and Maywald 1985, Sutherst et al. 1999) was used to compare epizootic-specific environmental conditions to average climatic conditions, based on 30-yr average maximum temperature, minimum temperature, total precipitation and precipitation pattern, at sites throughout North America. Annual departures from the average climatic condition, which may create ephemerally conducive conditions for *E. maimaiga* epizootics in an otherwise, generally non-conducive region, or vice versa, were

also examined for the North Central region.

A concise section on the possible implications of this research is presented in conclusion. Additionally, several appendices are included that are pertinent to this research. Appendix A contains a deposition record of voucher specimens and voucher specimen data. Michigan climate, including average daily temperature (°C), average daily maximum temperature (°C), average daily minimum temperature (°C), and average monthly precipitation (mm) for January through December, in relation to locations of *E. maimaiga* field bioassays is included in Appendix B. North American locations used in the CLIMEX climate-matching analyses are listed in Appendix C and summaries of the understory vegetation and ground flora at the *E. maimaiga* field bioassay sites are provided in Appendix D.

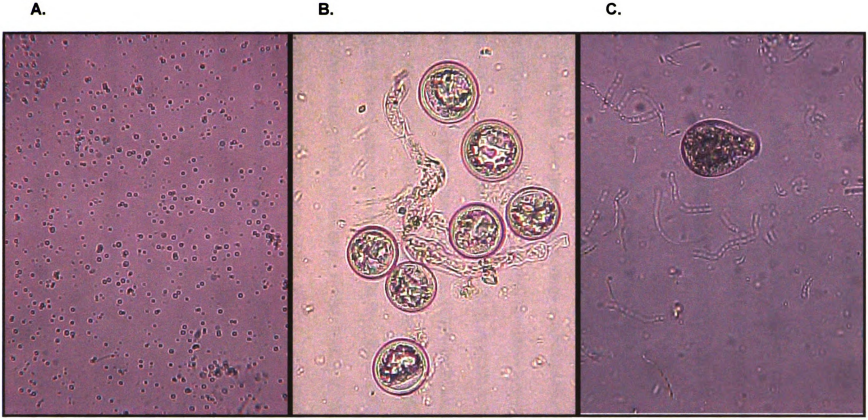

Figure 1. Polyhedral inclusion bodies of the nuclear polyhedrosis virus (NPV) (A), and resting spores (i.e. azygospores) (B) and a conidium (C) of the gypsy moth fungal pathogen *Entomophaga maimaiga*. Viral particles range in size from 1 - 10 µm. Pear-shaped conidia of *E. maimaiga* are approximately 20 × 25 µm in size and resting spores are approximately 30 µm in diameter. Images in this figure are presented in color.

CHAPTER 1

FIELD AND LABORATORY EVALUATION OF GYPSY MOTH INFECTION BY *Entomophaga maimaiga*: POTENTIAL INFLUENCE OF LARGE-SCALE METEOROLOGICAL EVENTS

INTRODUCTION

The fungal pathogen *Entomophaga maimaiga* Humber, Shimazu and Soper (Zygomycetes: Entomophthorales) has been responsible for significant declines in gypsy moth *Lymantria dispar* L. (Lepidoptera: Lymantriidae) defoliation in the northeastern U.S. since 1989 (Andreadis and Weseloh 1990a, 1990b, Hajek et al. 1990b, Hajek 1999). *Entomophaga maimaiga* is a desirable biological control agent because it affects few non-target species (Soper et al. 1988, Vandenberg 1990, Hajek et al. 1995a, 1996a, 1996b, 2000) and is compatible with other natural enemies, including the gypsy moth nucleopolyhederosis virus (NPV) (Andreadis and Weseloh 1990a, Hajek and Roberts 1992, Weseloh and Andreadis 1992b).

Gypsy moth larvae become infected when *E. maimaiga* spores adhere to the cuticle, then gain entry to the host using a combination of mechanical pressure and enzymatic degradation (Hajek 1999). *Entomophaga maimaiga* resting spores overwinter in the soil (Hajek et al. 1998a), with the highest levels of resting spores occur in the organic layer of soil at the base of trees (Hajek et

al. 1998a). Gypsy moth larval behavior, as they move up and down trees, increases the risk of fungal infection (Hajek 2001). Depending on ambient environmental conditions, a portion of these resting spores germinate in the spring (Hajek 1997b, Hajek and Humber 1997, Weseloh and Andreadis 1997) and infect early-instar gypsy moth larvae (i.e. primary transmission) (Hajek 2001). When these infected larvae die, *E. maimaiga* conidiophores that are produced externally on the cadavers discharge conidia and infect mid- to late-instar gypsy moth (i.e. secondary transmission). Late-instar cadavers principally produce resting spores and usually are found attached to lower tree trunks by their prolegs with their heads oriented downwards (Hajek and Soper 1991, Hajek et al. 1998b). These cadavers drop to the soil at the base of the tree, decompose and resting spores remain in the soil until the following spring.

The overall goal of this study was to evaluate the influence of climatic factors on *E. maimaiga* infection rates. Epizootics of *E. maimaiga* continue to occur frequently in much of the northeastern United States (Hajek et al. 1995b, 1996b, Hunter and Elkinton 1999). This fungal pathogen, however, has been less consistent in other states, such as Michigan (Smitley et al. 1995). *Entomophaga maimaiga* was first introduced into Michigan in 1991 and spread across much of the state by 1995 (Smitley et al. 1995). Substantial gypsy moth defoliation has continued to occur however since 1996, with 242,361 ha of Michigan forests sustaining moderate to heavy defoliation between 1997 and 2003 (USDA-FS 2004).

The primary objective of this project was to compare *E. maimaiga*

infection rates of gypsy moth larvae under field conditions with infection rates under laboratory conditions that were optimal for fungal germination. Field bioassays to assess infection of 4th-instar larvae were conducted from 1999 to 2001 in oak-dominated forests. Laboratory bioassays were conducted with larvae exposed to soil collected from field sites.

Germination of *E. maimaiga* resting spores is sensitive to moisture and temperature (Hajek et al. 1993, Weseloh et al. 1993), so it is likely that successful infection will vary annually depending on environmental conditions. My second objective was to compare regional monthly precipitation and temperature in northern Michigan in 1999 - 2001 with 30-yr averages to assess variability in precipitation and temperature and relate potential correlations to observed *E. maimaiga* infection rates.

MATERIALS AND METHODS

Study sites & field measurements

Thirty-two oak-dominated stands, each at least 10 ha in size, were selected in Michigan in 1999 (Figure 1.1). An additional stand that experienced an *E. maimaiga* epizootic in 1999 (N.W. Siegert, unpubl. data, site no. 33, Clare Co.) was included in the study in 2000 and 2001. All stands had at least one documented *E. maimaiga* epizootic between 1993 and 1998 (Buss 1997, L.J. Buss and D.G. McCullough unpubl. data, Michigan Department of Natural Resources [MDNR] and Michigan Department of Agriculture [MDA] unpubl. data). Stands were located on public land (Huron-Manistee National Forest, Michigan State University's W.K. Kellogg Experimental Forest, and several MDNR-managed state forests, including the Au Sable and Pere Marquette State Forests) and were separated by at least 5 km. Density of gypsy moth populations in each stand were quantified annually by averaging counts of egg masses in two to four 0.01 ha fixed-radius plots (Kolodny-Hirsch 1986).

Stands were characterized by a dominant mixed oak (*Quercus* spp.) overstory with ca 90% canopy closure (Table 1.1) and a sparse to moderately dense understory, which consisted of mixed oak, witch-hazel (*Hamamelis virginiana* L.), red maple (*Acer rubrum* L.), white pine (*Pinus strobus* L.), black cherry (*Prunus serotina* Ehrh.), sassafras (Sassafras albidum (Nutt.) Nees), and serviceberry (*Amelanchier arborea* (Michaux f.) Fern.) (Appendix D1). Ground flora tended to be moderately dense with bracken fern (*Pteridium aquilinum* (L.)

Kuhn), grasses and sedges (Gramineae and Cyperaceae), red maple and mixed oak regeneration, and low sweet blueberry (*Vaccinium angustifolium* Aiton) being the most common species (Appendix D2). Within each stand, I established a plot center and selected dominant oak trees at 10, 25 and 50 m along transects in each cardinal direction from the plot center (12 sample trees per stand). Sample trees averaged 38.4 ± 1.2 cm in diameter at breast height (DBH). Percentage canopy cover was measured in the four cardinal directions at each plot center with a concave spherical densiometer (Lemmon Forest Densiometers, Bartlesville, OK) and averaged (Table 1.1). Basal area was estimated with a 10-factor wedge prism at the plot center of each stand (Table 1.1). Soil in the selected stands was typically well-drained with a thin organic layer and a pH of ca 4.6 (Table 1.1). Soil pH was measured with a hand-held pH meter (WTW Measurement Systems, Inc., Ft. Myers, FL) from homogenized soil collected from the northern and southern aspects at the base of 12 sample trees in each stand.

To estimate the amount of fungal inoculum present at each stand, I quantified *E. maimaiga* resting spores in the soil. A soil sample, ca 85 cm^3, was collected at the base of each sample tree, where the highest levels of resting spores occur (Hajek et al. 1998a), using a modified bulb planter. All soil samples were collected at the beginning of the field bioassays, when cages of gypsy moth larvae were placed in the stands (see below). To avoid inadvertent transportation of *E. maimaiga* resting spores between field sites, disposable non-latex gloves (Medline Industries, Inc., Mundelein, IL) and boot covers

(McKesson General Medical Corporation, Richmond, VA) were used and disposed of following visits to each site. All equipment used in the stands was sterilized with 95% ethanol and thoroughly rinsed with distilled water. Equipment used to collect soil samples from each aspect of a sample tree was also sterilized and rinsed between each sample. In 1999, soil samples were composited by cardinal direction for each stand (e.g. four composite soil samples per stand) and securely stored in plastic resealable bags. In 2000 and 2001, soil samples were collected only from northern and southern aspects at the base of each sample tree and composited by aspect (e.g. two composite soil samples per stand). The homogenized soil samples were transported from the field to the laboratory in coolers with ice packs and stored at 5 °C to inhibit fungal germination prior to resting spore quantification.

Wet-sieving of the soil, followed by density-gradient centrifugation using Percoll (Sigma-Aldrich Company, St. Louis, MO), and microscopy was used to quantify *E. maimaiga* resting spores (number per gram of dry soil) for each composite sample in each stand in 1999 and 2000 (MacDonald and Spokes 1981, Li et al. 1988, Hajek and Wheeler 1994). Absolute counts of *E. maimaiga* resting spores in soil were not conducted in 2001 because of limited resources to complete the labor intensive sampling.

Field bioassays

To assess *E. maimaiga* infection rates under field conditions, I conducted 4-d field bioassays with freshly-molted 4th-instar gypsy moth larvae. Gypsy

moth egg masses were obtained from USDA APHIS, Otis Air National Guard Base, MA, and larvae were reared in early June on artificial diet (O'Dell et al. 1985) at the USDA APHIS PPQ Biological Control Laboratory, Niles, MI. Each morning, 4th-instar gypsy moth larvae that had molted in the previous 24 hr were collected for field bioassays. Field bioassays corresponded to the occurrence of 4th-instar larvae of the wild gypsy moth populations (typically early to mid-June in Michigan) to simulate the timing of naturally-occurring *E. maimaiga* infections. Larval development was staggered so that sufficient numbers of freshly molted 4th-instar larvae (approximately 4000 larvae per day in 1999 and approximately 2000 per day in 2000 and 2001) were available each morning for the duration of the field bioassays.

Field bioassays were conducted at each stand by placing 20 of the 4th-instar larvae in 15 × 20 cm cages made of 6 × 7 mesh/cm^2 aluminum screening (Hajek and Humber 1997). Cages used for field bioassays were sterilized annually with 95% ethanol. Two ca 15 g pieces of high wheat germ artificial diet (O'Dell et al. 1985), sufficient to last the duration of the field bioassay, were placed in each cage. One cage was placed on the soil surface at the base of each sample tree in each cardinal direction in each stand and collected four days later. After four days in the field, cages of larvae were collected, individually stored in plastic bags to prevent contamination during transport, and returned to the USDA APHIS PPQ Biological Control Laboratory. Larvae were reared individually in 50 mL cups on artificial diet following standard protocols for assessing fungal infections (Papierok and Hajek 1997). Larvae were reared

at 20 °C and 14:10 h (light:dark photoperiod) for 10 d, then placed in the dark for 3 d at 20 °C. After 3 d, the cadavers were checked for presence or absence of *E. maimaiga* conidia. If conidia were present, then cadavers were transferred to cold storage (4 °C and dark). If conidia were not present, then larvae were kept at 20 °C in the dark for an additional 7 d before being transferred to cold storage. Gypsy moth cadavers were dissected and examined with a microscope to determine whether *E. maimaiga* resting spores or NPV was present. Nearly 300 gypsy moth larvae were reared in the laboratory to check for possible laboratory contamination with *E. maimaiga* or NPV and 100% of the larvae survived to pupation.

Infection rates for *E. maimaiga* for the 4-day field and laboratory bioassays were calculated as the percentage of larvae infected by *E. maimaiga* out of the total number of larvae examined (i.e. total number of cadavers processed plus the number of larvae that survived to pupation). Because of the more rapid pathogenesis from *E. maimaiga* than NPV after simultaneous infection (Hajek 1997a, Malakar et al. 1999a, 1999b), larval cadavers found to be co-infected with *E. maimaiga* and NPV were assumed to have died from *E. maimaiga* infection.

Laboratory bioassays

Soil collected at the beginning of the field bioassays from the base of the sample trees (as described above) was used for laboratory bioassays, as well as resting spore analysis. The homogenized soil samples were shipped

overnight to Cornell University in coolers with ice packs to keep the *E. maimaiga* resting spores from germinating. At Cornell University, freshly-molted 4th-instar gypsy moth larvae (reared on artificial diet from egg masses obtained from USDA APHIS, Otis Air National Guard Base, MA) were placed on 35 g of soil from the field bioassay stands in polypropylene containers with lids (4.5 cm in height and 10.5 cm in diameter) at standardized moisture levels of 100% and reared at 15 °C and 14:10 h (light:dark photoperiod) for 4 d (Hajek et al. 2004). These conditions are optimal for *E. maimaiga* resting spore germination (Shimazu and Soper 1986, Shimazu 1987, Hajek et al. 1990b, Hajek 1997b). Thirty gypsy moth larvae per aspect were exposed to soil from each field bioassay stand in 1999 (120 larvae per stand; 3840 total larvae). In 2000 and 2001, 40 larvae per aspect were exposed to soil from each field bioassay stand (80 larvae per stand each year; 2640 total larvae each year). Larvae were then reared to detect fungal infections as described above for the field bioassays.

Infections in forest-collected gypsy moth larvae

Wild gypsy moth populations present at field sites were surveyed each year to examine prevalence of *E. maimaiga* and NPV mortality in late-instar larvae. Late-instar larval cadavers from wild gypsy moth populations present at field sites were collected as larvae start to pupate between late June and early July. Burlap bands (40 x 80 cm) were placed at breast height on the 25 m sample trees when cages were retrieved at the end of the field bioassays. Up to 40 gypsy moth cadavers per stand were collected from the burlap bands and

placed in individual containers. Cadavers were dissected and examined with a microscope to determine if *E. maimaiga* or NPV was responsible for mortality of the wild cadavers.

Precipitation & temperature departures from 30-yr averages

Since *E. maimaiga* resting spore germination is sensitive to moisture and temperature (Hajek et al. 1993, Weseloh et al. 1993), it is likely that successful infection will vary annually depending on environmental conditions. Weather data from the National Oceanic and Atmospheric Administration (NOAA) for climate divisions across northern lower Michigan, which encompassed the majority of the field sites, were used to approximate area-wide departures from the 30-yr averages of monthly air temperature and precipitation for 1999, 2000 and 2001 (MRCC 2002, NOAA 2002a, 2002b). A climate division is a region within a state that is as climatically homogeneous as possible (NOAA 2002a, 2002b). Climate divisions are often used for various research applications by climatologists to assess regional climatic trends over time (e.g. 30 year periods) (NOAA 2002a, 2002b). Departures were determined by calculating the differences between actual climatic conditions (MRCC 2002) and monthly 30-yr average conditions for the four climate divisions across northern lower Michigan for April, May and June between 1999 and 2001 (NOAA 2002a, 2002b).

Statistical analysis

Simple linear regression analyses were used to analyze relationships

between *E. maimaiga* resting spore counts and soil pH on *E. maimaiga* infection rates in field and laboratory bioassays (SYSTAT 2000). Overall differences in *E. maimaiga* resting spore counts and infection rates in field and laboratory bioassays among aspects were analyzed for each year using analysis of variance (ANOVA) (SYSTAT 2000). *Entomophaga maimaiga* infection rates in laboratory and field bioassays were not normally distributed among sites, so the nonparametric Wilcoxon signed-rank test (Sokal and Rohlf 1995) was used to test for differences in infection levels between laboratory and field bioassays each year (SYSTAT 2000).

Monthly precipitation and temperature values for climate divisions in northern lower Michigan were tested for differences from 30-yr averages using two-tailed t-tests, with critical values of $t_{(0.5,\,6)} = 2.447$ and $t_{(0.5,\,3)} = 3.182$ (Sokal and Rohlf 1995) for temperature and precipitation differences, respectively. The precipitation data were heteroscedastic, so the degrees of freedom for precipitation t-tests were reduced, as recommended by Sokal and Rohlf (1995), when the sizes of the two samples were equal.

RESULTS

Resting spore density in soil

The overall quantity of *E. maimaiga* resting spores in the soil at each site averaged 154 ± 12 and 232 ± 30 spores/g dry soil in 1999 and 2000, respectively (Table 1.2). Density of resting spores was highly variable in each year, ranging from 21 to 623 spores/g dry soil in 1999 and 33 to 932 spores/g dry soil in 2000. Differences in the number of *E. maimaiga* resting spores in the soil between aspects were not significant in 1999 ($P > 0.05$) or 2000 ($P > 0.05$) (Table 1.2).

Entomophaga maimaiga resting spore density in the soil was examined in relation to gypsy moth egg mass densities at the field sites. Gypsy moth egg mass densities in the field sites generally decreased from 1999 to 2001 (Table 1.1), with gypsy moth populations in many of the stands remaining at low levels through the duration of this project. The change in the number of resting spores was not significantly associated with the change in gypsy moth population densities between 1999 and 2000 ($r^2 = 0.01$, $P = 0.60$). Interestingly, three stands that had the largest decreases in gypsy moth egg mass densities from 1999 to 2000 (sites 5, 11, and 31) did not exhibit any change in *E. maimaiga* resting spore density.

Field bioassays

A total of 62,400 laboratory-reared gypsy moth larvae were used in the

field bioassays from 1999 to 2001, including 30,720 larvae in 1999, and 15,840 larvae in 2000 and in 2001. A portion of the larvae did not survive the 4-day exposure to field conditions each year, typically because of predation by ants or insectivorous rodents. In 1999, 21,769 gypsy moth larvae were returned intact from the 4-day field exposure, while 11,885 and 15,811 larvae were returned in 2000 and 2001, respectively.

In 1999, 3,953 gypsy moth larvae (18.2%) survived to pupation and a total of 10,436 larvae that died before pupating were processed. These larval cadavers were processed to determine whether *E. maimaiga* was the pathogen responsible for mortality. Infection by *E. maimaiga* killed 29.8 ± 2.3% of all larvae that were evaluated in the 1999 field bioassay (i.e. number of gypsy moth larvae that survived plus number of larval cadavers processed) (Table 1.2). The rest of the larval cadavers that were processed were determined to have been killed by NPV (43.4%).

In 2000, 2,204 gypsy moth larvae (18.5%) survived to pupation out of the 11,885 larvae that were returned from the 4-day field exposure. A total of 6,761 larval cadavers were processed. Percentage of infection by *E. maimaiga* dropped to less than half of that observed during the previous year's field bioassay. *Entomophaga maimaiga* infection was responsible for mortality of 10.8 ± 1.6% of the larvae that were evaluated (Table 1.2). The remaining larval cadavers that were processed were determined to have been killed by NPV (65.2%).

In 2001, nearly a third of the gypsy moth larvae (32.7%) that were

returned intact from the field exposure survived to pupation (5,173 out of 15,811 larvae). Infection by *E. maimaiga* was even lower in 2001. A total of 8,306 larval cadavers were processed and less than 4% of the larvae (3.4 ± 0.7%) were infected with *E. maimaiga* (Table 1.2). As in 1999 and 2000, the rest of the larval cadavers that were processed were determined to have been killed by NPV (55.2%).

Entomophaga maimaiga field infections were examined in relation to several site-related factors, including canopy cover, aspect, *E. maimaiga* resting spore density in the soil, and soil pH. Canopy cover was generally not associated with differences in *E. maimaiga* field infection rates (Figure 1.2). In 1999, however, the relationship between canopy cover and *E. maimaiga* field infection rates was marginally significant ($r^2 = 0.12$, $P = 0.051$) (Figure 1.2A). Differences in *E. maimaiga* field infection rates between aspects were not significant ($P > 0.05$) in 1999, 2000, or 2001 (Table 1.2). Infection of gypsy moth larvae under field conditions by *E. maimaiga* was not significantly correlated with the number of *E. maimaiga* resting spores in the soil in 1999 ($r^2 = 0.04$, $P = 0.27$) (Figure 1.3A). In 2000, however, increases in *E. maimaiga* field infection of gypsy moth was significantly correlated with increases in the quantity of fungal inoculum (Figure 1.3B). *Entomophaga maimaiga* infection of gypsy moth larvae in field bioassays was not significantly associated with soil pH between 1999 and 2001 ($P > 0.05$).

Laboratory bioassays

A total of 9,120 gypsy moth larvae were used in the laboratory bioassays from 1999 to 2001 (3,840 in 1999; 2,640 in 2000 and 2001). In 1999, infection of gypsy moth larvae by *E. maimaiga* was 20.9 ± 4.1% in the laboratory bioassays and was significantly lower than the level of *E. maimaiga* infection observed in the field bioassays in 1999 (Wilcoxon's $Z = -2.116$; $P = 0.034$) (Table 1.2). In 2000 and 2001, however, this trend was reversed and infection rates were significantly higher in laboratory bioassays versus field bioassays. *Entomophaga maimaiga* infected 43.7 ± 4.4% (Wilcoxon's $Z = 4.672$; $P < 0.0005$) and 59.7 ± 4.5% (Wilcoxon's $Z = 4.994$; $P < 0.0005$) of the gypsy moth larvae in laboratory bioassays in 2000 and 2001, respectively (Table 1.2). Infection of gypsy moth larvae by other pathogens, such as NPV, was extremely rare (< 0.5%) in laboratory bioassays.

Differences in *E. maimaiga* laboratory infection rates between aspects were not significant in 1999 ($P > 0.05$) or 2000 ($P > 0.05$). In 2001, however, *E. maimaiga* laboratory infection rates between aspects were significantly different ($P < 0.05$), with infection rates on northern aspects being greater than on southern aspects (Table 1.2). Laboratory infection of gypsy moth larvae by *E. maimaiga* increased linearly as the quantity of fungal inoculum (i.e. resting spore density) increased in 1999 and 2000 (Figure 1.4A, B). As few as 235 spores/g dry soil and 112 spores/g dry soil caused ≥60% infection of larvae reared under optimal laboratory conditions in 1999 and 2000, respectively (Figure 1.4A, B). *Entomophaga maimaiga* infection of gypsy moth larvae in

laboratory bioassays increased linearly in association with soil pH in 1999 (Table 1.1; Figure 1.5A). The linear relationship, however, was only marginally significant in 2000 ($r^2 = 0.12$, $P = 0.052$) (Figure 1.5B) and not significant in 2001 ($r^2 = 0.04$, $P = 0.25$) (Figure 1.5B). Infections of gypsy moth larvae by *E. maimaiga* in field and laboratory bioassays were not significantly associated with each other in 1999 ($r^2 = 0.001$, $P = 0.91$) and 2000 ($r^2 = 0.003$, $P = 0.76$), but were significantly associated with each other in 2001 ($r^2 = 0.14$, $P < 0.05$) (Figure 1.6).

Infections in forest-collected gypsy moth larvae

Up to 40 cadavers of late-instar gypsy moth larvae from wild populations present in field sites were collected from under burlap bands each year in early July from 1999 to 2000 (total of 917 larval cadavers). The number of field sites where gypsy moth cadavers were present ranged from 11 to 29 (Table 1.3). *Entomophaga maimaiga* was the dominant pathogen in the late-instar cadaver collections each year. Infections in forest-collected gypsy moth by *E. maimaiga* ranged from 76.6 to 90.8%, while NPV infections ranged from 9.2 to 29% between 1999 and 2001 (Table 1.3).

Precipitation & temperature departures from 30-yr averages

Long-term comparisons of climatic data are typically conducted with monthly 30-yr averages for variables such as precipitation and temperature (NOAA 2002a, 2002b). Precipitation and temperature for April, May and June

were evaluated during this study because that is the time period that germination of *E. maimaiga* resting spores and infection of gypsy moth larvae may occur (Hajek and Roberts 1991). Compared to 30-yr averages, June weather was significantly warmer and wetter in northern lower Michigan when field bioassays were conducted in 1999. June precipitation was 32.7 ± 12.5 mm greater than the 30-yr average ($t = 5.495$, $P < 0.05$). The average temperature was 1.9 ± 0.2 °C higher than the 30-yr average ($t = 5.822$, $P < 0.05$) for the region in 1999 (Table 1.4). April and May temperatures were also significantly higher than the 30-yr average ($t = 6.155$, $P < 0.05$; and $t = 5.547$, $P < 0.05$, respectively), but precipitation in those months did not significantly differ from the 30-yr average ($t = 0.244$, $P > 0.05$; and $t = 1.286$, $P > 0.05$, respectively) for the region in 1999 (Table 1.4).

The weather was much closer to average across the region in 2000 and June weather was not significantly different from normal conditions. June precipitation was only 4.5 ± 5.5 mm greater ($t = 1.499$, $P > 0.05$) and average temperature was only 0.2 ± 0.2 °C greater ($t = 0.623$, $P > 0.05$) than the respective 30-yr average for each variable (Table 1.4). Likewise, April precipitation and temperature was not significantly different from 30-yr averages ($t = 0.555$, $P > 0.05$; and $t = 0.000$, $P > 0.05$, respectively). May, however, was significantly warmer ($t = 3.579$, $P < 0.05$) and wetter than average ($t = 6.262$, $P < 0.05$) (Table 1.4).

In 2001, May was again significantly warmer ($t = 5.226$, $P < 0.05$) and wetter than average ($t = 8.284$, $P < 0.05$), but June weather was significantly

drier than normal compared to 30-yr averages ($t = 4.407$, $P < 0.05$) , with 10.6 ± 5.0 mm less precipitation than the 30-yr average (Table 1.4). Additionally, the average temperature in June of 2001 was only 0.5 ± 0.3 °C greater than the 30-yr average for northern lower Michigan and not significantly different ($t = 1.602$, $P > 0.05$). April temperature was significantly higher than the 30-yr average ($t = 5.905$, $P < 0.05$), however, April precipitation, though greater than the 30-yr average, was not significantly different from normal conditions ($t = 3.154$, $P > 0.05$) (Table 1.4).

DISCUSSION

Entomophaga maimaiga germination and infection rates have often been correlated with environmental moisture (Shimazu 1987, Hajek et al. 1990a, Hajek 1999). In a study examining the dynamics of resting spore development and germination, Hajek and Humber (1997) found that *E. maimaiga* infection increased with greater soil moisture. I hypothesized that *E. maimaiga* infection levels would be lower in field versus laboratory conditions because of *E. maimaiga*'s sensitivity to moisture and temperature (Hajek et al. 1993, Weseloh et al. 1993) and the considerable, inherent variability of climatic conditions in the field. Several manipulative experiments have demonstrated increased *E. maimaiga* infection with the application of water (Weseloh and Andreadis 1992a, 1992b, Hajek and Roberts 1991, Hajek et al. 1996b) and extensive *E. maimaiga* epizootics have also been associated with above average rainfall (Andreadis and Weseloh 1990a, 1990b, Hajek 1999, Webb et al. 1999). Weseloh and Andreadis (1992a) found that infection at ten locations in Connecticut was positively associated with June precipitation, but not May precipitation, despite abundant May rainfall. In contrast, Hajek et al. (1996b) found that precipitation in May was significantly correlated with infection levels, but June precipitation was not, at plots in Maryland, Pennsylvania, Virginia and West Virginia. Five of the seven experimental plots in 1992 (Hajek et al. 1996b), though, had greater than 60% *E. maimaiga* infection when more than 55 mm of precipitation fell in both May and June. At three locations in Michigan over the course of three

years, Smitley et al. (1995) found that infection was positively correlated with precipitation during the two week period in June prior to sampling. My results demonstrate that substantial annual variation in *E. maimaiga* infection levels may occur at individual sites and that increased levels of field infection were positively associated with abundant June precipitation. Similar to results of Weseloh and Andreadis (1992a), abundant May precipitation did not appear to positively influence *E. maimaiga* infections during this study in 2000 and 2001.

Pathogen-related mortality of gypsy moth larvae in the field bioassays was dominated by NPV, which was the major gypsy moth pathogen in North America (Doane 1970, Campbell and Podgwaite 1971) before *E. maimaiga* was discovered causing epizootics in the northeastern United States. Epizootics caused by NPV are generally considered to function as a density-dependent mortality factor (Doane 1970, Woods and Elkinton 1987) and sufficient NPV inoculum needs to accumulate before a viral epizootic may occur. Gypsy moth populations at the field sites were generally at high densities in 1999, but tended to decrease to low densities in 2000 and 2001. NPV was the dominant pathogen during this study from 1999 to 2001 and suggests that NPV inoculum at the sites was in large enough titers to potentially initiate a viral epizootic had high densities of gypsy moth been present. Viral infections in my laboratory bioassays, however, were extremely rare. The high levels of NPV infection could alternatively be explained by latent viral infections in larvae used in field bioassays that became activated during the 4-d field exposures. Stressful physical, chemical or physiological conditions have been suggested to induce

latent viral infections in some insects (Troitskaya and Chichigina 1980, Petre and Fuhrmann 1981, Hughes et al. 1993, Stoltz and Makkay 2003). In a closely related *Lymantria* spp., latent NPV infections were apparently activated by reductions in temperature (Bakhvalov et al. 1979). Whether or not latency may have been responsible for the high levels of viral infection observed in this study remains to be tested.

Results from this study demonstrate that the gypsy moth fungal pathogen, *E. maimaiga*, is capable of high levels of infection under favorable conditions, but may be limited by weather in some regions. Only a portion of the *E. maimaiga* resting spores in the soil may germinate annually depending on ambient conditions, which enables *E. maimaiga* to persist in the environment when gypsy moth populations are not present (Hajek and Humber 1997, Hajek 1999). In my field bioassays, *E. maimaiga* infection of gypsy moth larvae decreased from 1999 to 2001, while *E. maimaiga* infections in laboratory bioassays, using soil from the field bioassay sites, increased during that period. This pattern may reflect weather conditions in May and June. Interpretation, however, should be approached with caution because monthly-based meteorological factors are coarse descriptors of climate and may not accurately reflect site-specific environmental conditions during the phenology of gypsy moth larvae and the development of *E. maimaiga* epizootics. Despite these short comings, calendar months are the most available units for long-term comparisons of meteorological data and this approach may be insightful for initial exploration of *E. maimaiga*'s potential dependency on weather-related

factors. In 1999, sites experienced significantly wetter and warmer weather than normal in June, presumably more *E. maimaiga* resting spores germinated prior to the start of the bioassays, and infection levels were relatively high in the field bioassay. In 2000 and 2001, June weather was close to normal and drier than normal, respectively, and low levels of *E. maimaiga* infection were observed in the field. In laboratory bioassays, however, levels of *E. maimaiga* infection were much higher. This could be explained by fewer resting spores being available in soil samples for germination in the laboratory bioassays when the environmental conditions were favorable, such as 1999, compared to when the environmental conditions less than favorable, such as 2000 and 2001. If true, an inverse relationship should exist between field and laboratory infection rates. However, such a relationship is not supported by the current study.

An alternative explanation for the observed difference between field and laboratory infection levels could be that specific environmental cues that initiate germination of *E. maimaiga* resting spores vary in relation to the length of time that they have persisted in the soil. Variation in *E. maimaiga* germination in relation to resting spore age, however, remains to be tested. Another explanation could simply be that larval infection during 4-d field bioassays is strongly affected by variations in microclimate. *Entomophaga maimaiga* germination rates may be lower because favorable environmental conditions do not exist for long enough durations because of the diurnal periodicity of climatic conditions (e.g. air temperatures typically highest in the mid-afternoon and lowest at sunrise). Germination of *E. maimaiga* is known to vary in relation to

the duration of time under given conditions (Hajek and Humber 1997). Individual 4-d bioassays may be a good method for evaluating transmission of *E. maimaiga* during the four day time period, but should be used cautiously in assessing potential gypsy moth infections. Consecutive bioassays over longer periods of time during gypsy moth larval development, however, may be useful in evaluating *E. maimaiga* infections under varying conditions. Examination of forest-collected late-instar larvae may provide an estimate of *E. maimaiga* and NPV prevalence in wild gypsy moth populations.

Entomophaga maimaiga inoculum in the soil varied considerably among field sites. Several factors may account for this variation, such as the frequency of climatic conditions favorable for *E. maimaiga* germination, the history of gypsy moth population levels, and differential rates of germination in relation to length of persistence of resting spores in the environment. Previous field studies have shown that *E. maimaiga* infection levels of 80% or more may occur in gypsy moth larvae with as few as 255 resting spores per gram of dry soil present (Hajek and Roberts 1991). Similarly, results from this research confirm that relatively low levels of fungal inoculum (235 and 112 *E. maimaiga* spores per gram of dry soil in 1999 and 2000, respectively) in the soil were associated with high levels of *E. maimaiga* infection in the laboratory when environmental conditions were favorable for germination.

Soil pH may be an important factor in *E. maimaiga* resting spore germination and disease transmission. Valovage and Kosaraju (1992) found that the highest levels of *Entomophaga calopteni* resting spore germination

occurred in the pH range of 6 - 8. Results from this study suggest that soil pH may also affect the germination of *E. maimaiga* resting spores. More research is needed, however, to conclusively evaluate the effect of soil pH on resting spore germination and persistence. Future studies should address the role of soil pH in affecting *E. maimaiga* resting spore germination and persistence.

Entomophaga maimaiga is highly synchronized with gypsy moth phenology (Hajek et al. 1995a, Hajek and Humber 1997) and variations in environmental conditions, primarily moisture relations (i.e. precipitation) during the ca 2 months of larval development may play a critical role in the development, persistence and frequency of *E. maimaiga* epizootics. In regions where long-term, average environmental conditions are not favorable for *E. maimaiga* epizootics, highly variable areas are more likely to occasionally experience conditions necessary for epizootics than areas of low variability. Alternatively, in regions where long-term, average environmental conditions tend to be favorable for *E. maimaiga* epizootics, highly variable areas are more likely to experience adverse conditions and fewer epizootics than areas of low variability. Additional research involving landscape-level studies and long-term monitoring will be needed to fully assess the role of climatic variability in the success of *E. maimaiga* as an effective biological control agent of gypsy moth, as the range of gypsy moth continues to expand in North America.

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Table 1.1. Location and characteristics of oak stands in Michigan used for *Entomophaga maimaiga* field sites from 1999 to 2001.

Site No.	County	Canopy cover (%)	Basal area (m^2/ha)	Soil pH	Egg mass density (No./ha)		
					1999	2000	2001
1	Chippewa	96.4	30	4.6	0	0	0
2	Grand Traverse	91.2	28	5.1	0	0	100
3	Manistee	96.1	25	5.0	1050	50	0
4	Manistee	84.7	14	5.1	600	250	50
5	Mason	90.9	18	5.0	3600	150	550
6	Mason	95.8	25	5.2	1350	0	0
7	Mason	94.5	25	4.8	100	50	0
8	Oceana	95.6	25	4.3	0	0	0
9	Oceana	93.0	25	4.7	50	0	0
10	Oceana	90.9	30	4.5	300	50	0
11	Lake	86.7	16	4.2	7900	400	1550
12	Lake	90.6	28	4.4	700	850	2400
13	Lake	94.5	28	4.7	450	600	400
14	Lake	91.9	25	4.4	0	100	0
15	Lake	94.0	23	4.5	100	0	0

16	Newaygo	90.4	25	4.5	50	0	0
17	Newaygo	88.6	25	4.7	200	0	50
18	Newaygo	93.0	25	4.2	450	0	0
19	Newaygo	87.8	25	4.3	50	0	0
20	Newaygo	90.6	21	4.2	400	0	0
21	Newaygo	95.6	28	4.1	200	0	50
22	Newaygo	93.5	21	4.7	250	0	100
23	Newaygo	91.2	25	4.4	0	0	0
24	Newaygo	90.4	18	4.8	1150	0	0
25	Crawford	82.6	18	4.1	0	100	350
26	Kalkaska	93.5	25	5.4	0	50	150
27	Kalkaska	84.1	28	5.1	1300	250	200
28	Roscommon	89.6	23	4.3	200	0	0
29	Roscommon	89.6	23	4.3	1100	0	0
30	Clare	87.3	21	4.3	0	0	0
31	Arenac	80.0	23	4.1	14150	200	500
32	Kalamazoo	90.5	25	4.8	0	0	0
33	Clare	89.3	21	5.2	n/a	50	50

Table 1.2. Number of gypsy moth cadavers examined, mean (± SEM) percentage of gypsy moth larvae infected by *Entomophaga maimaiga* in field and laboratory bioassays in 1999, 2000 and 2001, and mean (± SEM) number of *E. maimaiga* resting spores (No./g dry soil) per aspect in 1999 and 2000*.

Year	No. gypsy moth cadavers processed	Aspect				Overall
		North	South	East	West	
Field bioassay						
1999	10,436	26.8 ± 1.4	32.2 ± 1.5	27.0 ± 1.4	28.9 ± 1.5	29.8 ± 2.3
2000	6,761	9.8 ± 0.9	10.4 ± 0.9	n/a	n/a	10.8 ± 1.6
2001	8,306	3.2 ± 0.4	3.8 ± 0.6	n/a	n/a	3.4 ± 0.7
Laboratory bioassay						
1999	3,840	26.6 ± 5.1	18.8 ± 4.3	21.0 ± 5.5	17.2 ± 4.4	20.9 ± 4.1
2000	2,640	49.2 ± 4.8	38.1 ± 5.0	n/a	n/a	43.7 ± 4.4
2001	2,640	69.5 ± 4.1	49.8 ± 5.5	n/a	n/a	59.7 ± 4.5
E. maimaiga resting spores (No./g dry soil)						
1999	n/a	138 ± 19	128 ± 18	167 ± 31	182 ± 34	154 ± 12
2000	n/a	261 ± 44	202 ± 30	n/a	n/a	232 ± 30

**Entomophaga maimaiga* resting spores were not quantified in 2001 because of limited resources.

Table 1.3. Percentage of gypsy moth cadavers from wild populations collected at field sites that were infected with *Entomophaga maimaiga* and NPV present at field sites. Up to 40 gypsy moth cadavers were collected from under burlap bands on four sample trees at each site.

Year	No. gypsy moth cadavers collected	No. field sites with gypsy moth cadavers present	*E. maimaiga*-killed cadavers (%)	NPV-killed cadavers (%)
1999	342	23	76.6	23.4
2000	436	29	90.8	9.2
2001	139	11	88.5	11.5

Table 1.4. Mean (± SEM) 30-yr averages of precipitation (mm) and temperature (°C) for April, May and June and departures from the 30-yr averages during 1999, 2000 and 2001 in northern lower Michigan where 30 of the 33 field sites were located. Climatic parameters that were significantly different from the 30-yr average ($P < 0.05$) are designated with a double asterisk (**) following the value.

Climatic parameter	Time period	30-yr average	Departures from the 30-yr average		
			1999	2000	2001
Precipitation (mm)	April	67.2 ± 4.4	2.0 ± 12.3	-4.1 ± 10.7	12.1 ± 3.9
	May	69.8 ± 1.7	7.7 ± 12.3	63.9 ± 19.5**	73.1 ± 17.8**
	June	77.2 ± 1.5	32.7 ± 12.5**	4.5 ± 5.5	-10.6 ± 5.0**
Temperature (°C)	April	6.0 ± 0.5	2.0 ± 0.1**	0.0 ± 0.1	2.2 ± 0.1**
	May	12.3 ± 0.5	2.0 ± 0.1**	1.3 ± 0.1**	1.8 ± 0.1**
	June	17.4 ± 0.5	1.9 ± 0.2**	0.2 ± 0.2	0.5 ± 0.3

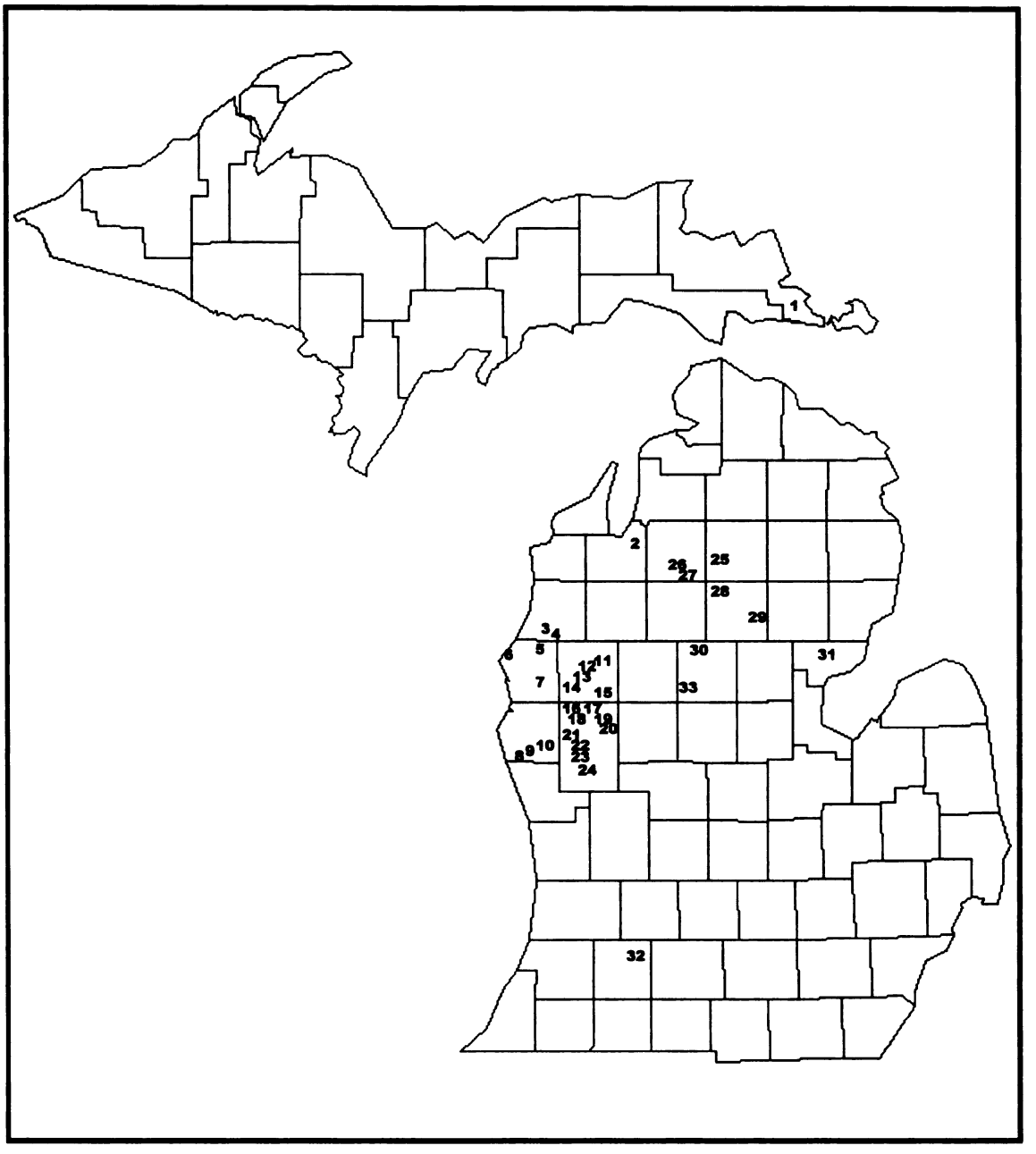

Figure 1.1. Numbers (1 - 32) mark the locations of *Entomophaga maimaiga* field sites in Michigan, 1999 to 2001. Site no. 33 was used as a field site in 2000 and 2001 after an *E. maimaiga* epizootic occurred there in 1999.

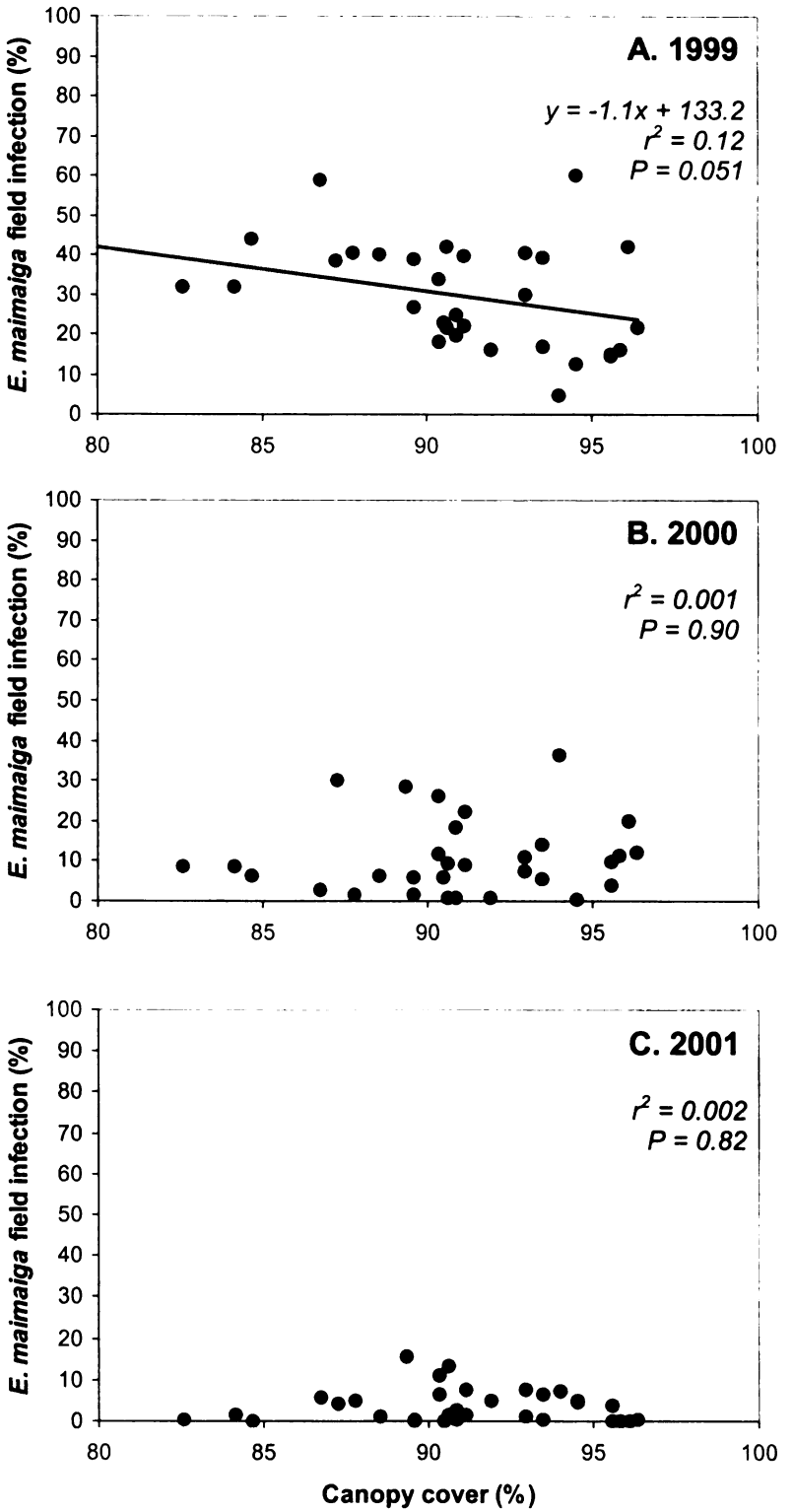

Figure 1.2. Field infection (%) of gypsy moth larvae by *Entomophaga maimaiga* in relation to canopy cover (%) in (A) 1999, (B) 2000, and (C) 2001. There was not a significant trend in *E. maimaiga* field infection by canopy cover in 2000 or 2001.

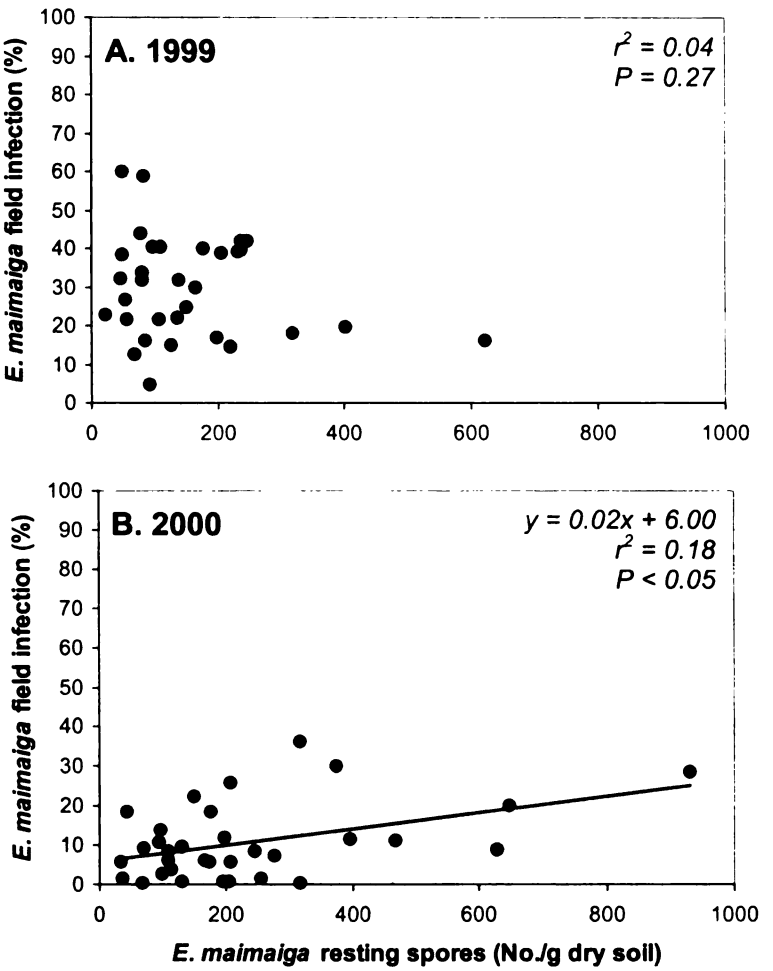

Figure 1.3. Field infection (%) of gypsy moth larvae by *Entomophaga maimaiga* in relation to the number of resting spores per gram of dry soil in (A) 1999 and (B) 2000. There was not a significant trend in *E. maimaiga* field infection by the number of resting spores in the soil in 1999.

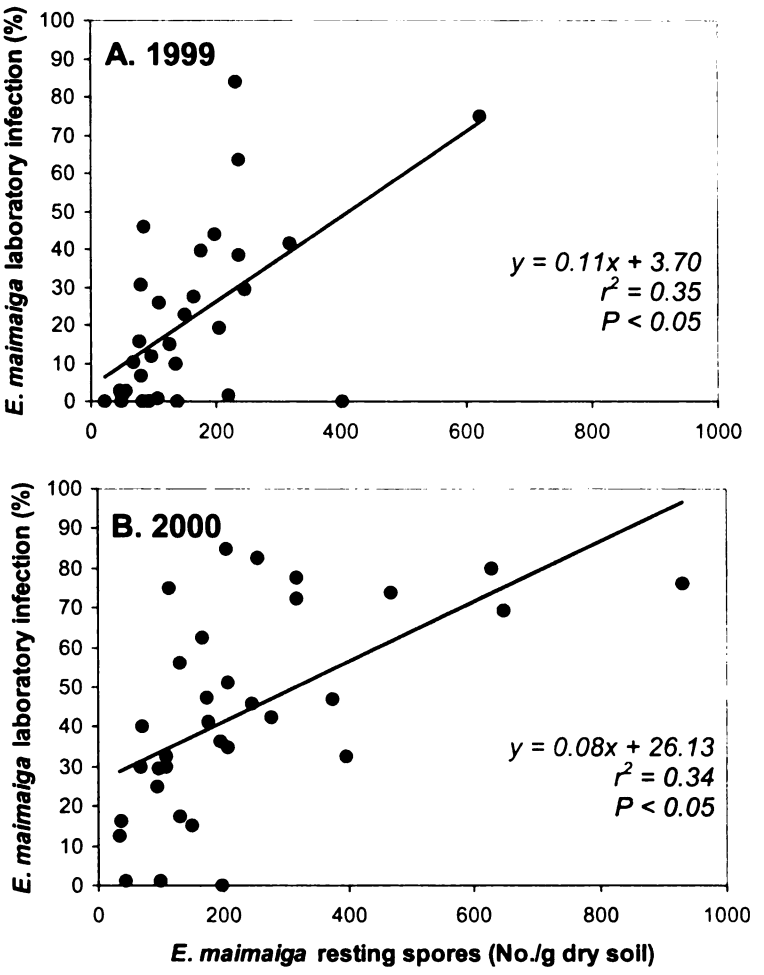

Figure 1.4. Laboratory infection (%) of gypsy moth larvae by *Entomophaga maimaiga* in relation to the number of resting spores per gram of dry soil in (A) 1999 and (B) 2000.

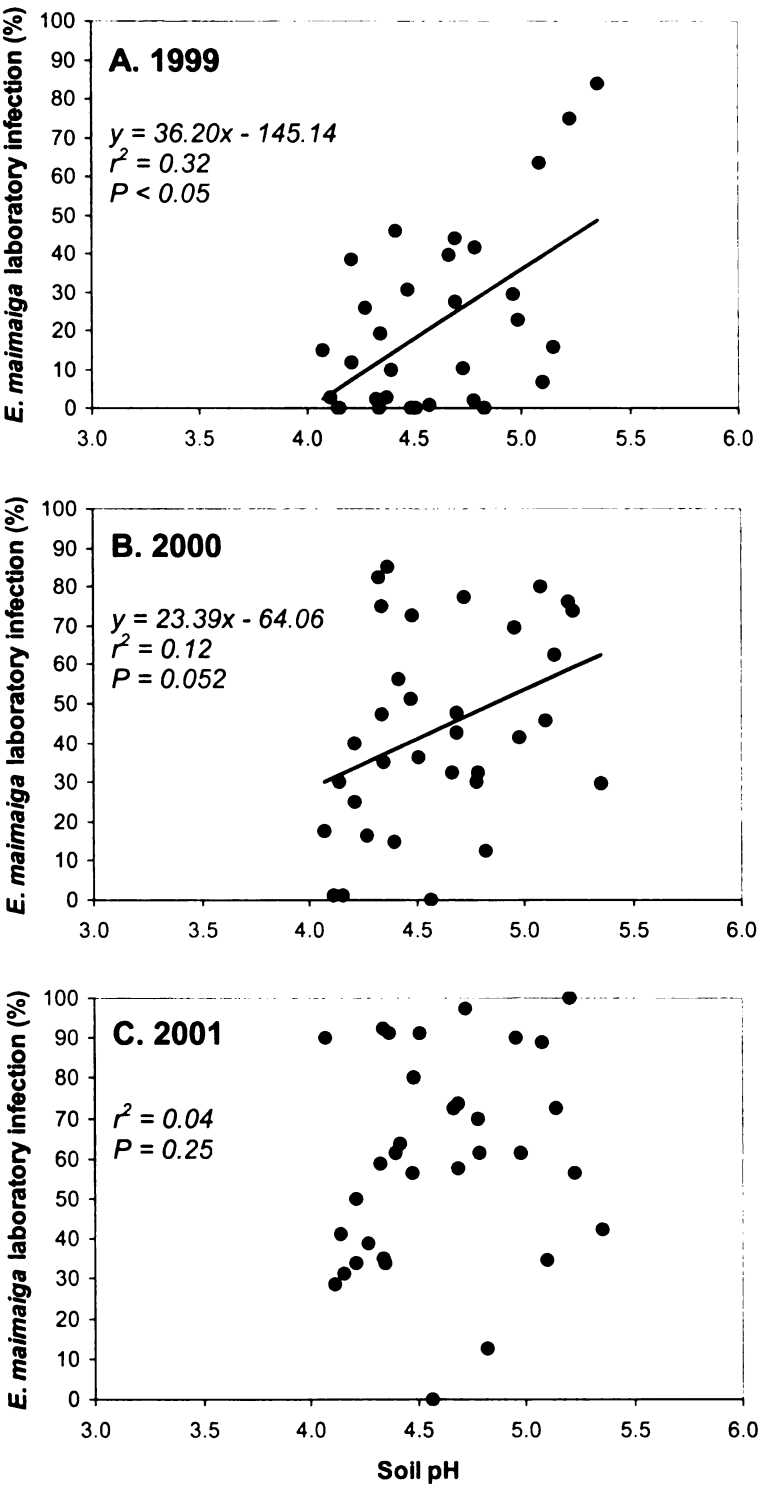

Figure 1.5. Laboratory infection (%) of gypsy moth larvae by *Entomophaga maimaiga* in relation to soil pH in (A) 1999, (B) 2000, and (C) 2001. There was not a significant trend in *E. maimaiga* laboratory infection by soil pH in 2001.

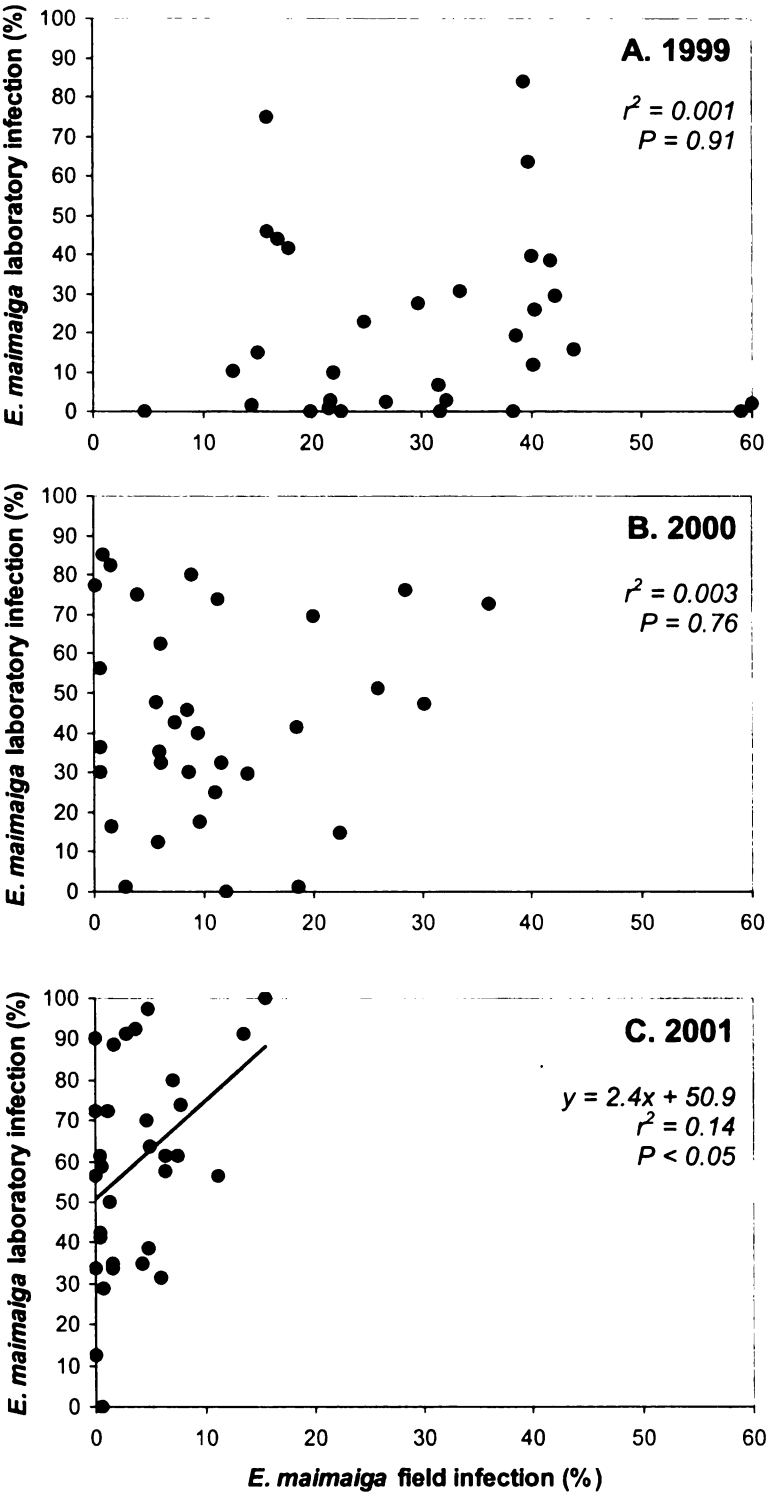

Figure 1.6. Laboratory infection (%) of gypsy moth larvae by *Entomophaga maimaiga* in relation to *E. maimaiga* field infections (%) in (A) 1999, (B) 2000, and (C) 2001. There was not a significant trend in *E. maimaiga* laboratory infection by *E. maimaiga* field infection in 1999 or 2000.

CHAPTER 2

FUNGAL & VIRAL INFECTIONS OF GYPSY MOTH (LEPIDOPTERA: LYMANTRIIDAE) LARVAE & EFFECTS OF MICROCLIMATIC CONDITIONS IN THE INITIAL DEVELOPMENT OF EPIZOOTICS

INTRODUCTION

Entomopathogens are capable of causing a rapid change in their prevalence over a short time period that results in a large-scale mortality event within a host population, known as an epizootic (Fuxa and Tanada 1987). Factors that initiate and affect the development of epizootics play a critical role in regulating insect pathogen dynamics, but are generally poorly understood. While host density is typically important for the amplification and intensity of epizootics (Watanabe 1987), environmental conditions strongly influence pathogen activity and are integral in the initial development of epizootics (Andreadis 1987, Benz 1987). Because of the inherent variability of environmental conditions in natural systems, the occurrence and intensity of entomopathogenic epizootics are usually difficult to accurately predict.

The gypsy moth nucleopolyhederosis virus (NPV) was first detected in North America in the early 1900's (Glaser 1915). It can cause dramatic epizootics in gypsy moth [(*Lymantria dispar* L.) (Lepidoptera: Lymantriidae)] populations, though it typically becomes abundant only when gypsy moth

population densities are high (Doane 1970, Leonard 1981, Woods and Elkinton 1987). Typically, larvae become infected with NPV by ingesting foliage contaminated with the virus (Murray and Elkinton 1989), though other modes of infection, such as transovum transmission (Doane 1969), are possible. Infected early-instar larvae move to an elevated position, such as the ends of branches or the tops of trees, where they die. As these dead larvae deteriorate, they recontaminate the foliage and bark and provide viral inoculum for infection of late-instar larvae (Woods and Elkinton 1987). Other biotic (e.g. other caterpillars and insects, parasitoids, birds, mammals) and abiotic factors (e.g. wind, rain) serve to further facilitate the spread and dispersal of the virus (Podgwaite et al. 1981). This viral pathogen has often been responsible for reducing outbreak gypsy moth populations to low densities and, until 1989, was the dominant gypsy moth pathogen in North America.

Since 1989, the fungus *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) has become an important pathogen of gypsy moth in the northeastern United States (Andreadis and Weseloh 1990a, 1990b, Hajek et al. 1995b, Hajek 1999). It is highly synchronized with gypsy moth larval development, has relatively few negative effects on non-target species (Soper et al. 1988, Vandenberg 1990, Hajek et al. 1995a, 1996a, 1996b, 2000), and is compatible with other natural enemies, including NPV (Andreadis and Weseloh 1990a, Hajek and Roberts 1992, Weseloh and Andreadis 1992a), making *E. maimaiga* a desirable biological control agent.

Entomophaga maimaiga produces two types of spores, both of which

may infect gypsy moth larvae (Hajek 1999). Resting spores of *E. maimaiga* overwinter in the soil, with the highest densities of spores occurring in the organic layer of soil at the base of trees (Hajek et al. 1998a). Behavior of late-instar gypsy moth larvae, such as diurnal movement up and down from the tree canopy (Forbush and Fernald 1896, Leonard 1981), increases the risk of fungal infection by putting larvae in contact with the spore-bearing soil (Hajek 2001). A portion of the *E. maimaiga* resting spores germinate in the spring depending on environmental conditions (Hajek 1997b, Hajek and Humber 1997, Weseloh and Andreadis 1997). These spores infect and kill early-instar gypsy moth larvae (i. e. primary transmission). Early-instar cadavers produce *E. maimaiga* conidiophores externally that discharge conidia to infect mid- to late-instar gypsy moth (i.e. secondary transmission). Late-instar larval cadavers principally produce resting spores and are usually found attached to lower tree trunks by their prolegs with their heads oriented downwards (Hajek and Soper 1991, Hajek et al. 1998b). Cadavers drop to the soil, decompose and resting spores remain dormant in the soil until the following spring.

Gypsy moth density and other host-associated factors do not appear to influence primary transmission of *E. maimaiga* (Hajek and Eastburn 2001), suggesting that environmental conditions are integral in the initial development of epizootics. Infection by resting spores, the primary transmission of *E. maimaiga*, was evaluated under field conditions using laboratory-reared 4th-instar gypsy moth in 2001 and 2002 in Michigan oak-dominated forests. The overall goal of this research was to acquire a better understanding of role of

microclimate in the initial development of *E. maimaiga* epizootics. The specific objective of this project was to evaluate the relative infection rates of gypsy moth larvae by the *E. maimaiga* and NPV pathogens in the field. Infection rates during 4-d intervals were related to site-specific microclimatic variables occurring over a 6-wk period of gypsy moth larval development.

MATERIALS AND METHODS

Study sites & field measurements

Three oak-dominated stands (Bitely, Jackson Corners, and Lilley) were selected in the Huron-Manistee National Forest in Newaygo County, Michigan, in 2001 (Table 2.1). Selected stands were at least 10 ha in size, known to have experienced at least one *E. maimaiga* epizootic in the past (Buss 1997, L.J. Buss and D.G. McCullough unpubl. data, Michigan Department of Natural Resources [MDNR] unpubl. data) and were within 9.5 km of one another. Additionally, stands had been utilized for related research the two years before the current study (N.W. Siegert chapter one). Density of gypsy moth populations in each stand were quantified annually by averaging counts of egg masses in two 0.01 ha fixed-radius plots (Kolodny-Hirsch 1986). Gypsy moth population densities were high in 1999 at these sites, but had decreased to low densities by 2000 and remained at low densities during this study in 2001 and 2002 (Table 2.1).

Stands were characterized by a dominant mixed oak (*Quercus* spp.) overstory with ca 90% canopy closure (Table 2.1) and a moderately dense understory, which consisted primarily of sassafras (*Sassafras albidum* (Nutt.) Nees) and witch-hazel (*Hamamelis virginiana* L.). Also lightly distributed in the understory was some mixed oak, red maple (*Acer rubrum* L.), white pine (*Pinus strobus* L.) and red pine (*Pinus resinosa* Aiton). Ground flora tended to be moderately dense with bracken fern (*Pteridium aquilinum* (L.) Kuhn), low sweet

blueberry (*Vaccinium angustifolium* Aiton), and red maple, mixed oak, sassafras and witch-hazel regeneration being the most common species.

A plot center was established in each stand. Dominant oak trees at 25 m along transects in each cardinal direction from the plot center were selected and tagged (4 trees per stand). Sample trees at the sites ranged from 36.6 to 41.0 cm and averaged 38.7 ± 1.3 cm in diameter at breast height (DBH). Soil at the sites was well-drained and consisted primarily of Coloma-Spinks-Metea sandy material (USDA-SCS 1995) with a thin organic layer and a pH of 4.4 ± 0.2 (N.W. Siegert chapter one). Percentage canopy cover was measured in the cardinal directions at each plot center with a concave spherical densiometer (Lemmon Forest Densiometers, Bartlesville, OK) and averaged 90.7 ± 1.3% (Table 2.1). Basal area was measured with a 10-factor wedge prism at the plot center of each stand and averaged 23.7 ± 1.3 m^2/ha (Table 2.1).

Disposable non-latex gloves (Medline Industries, Inc., Mundelein, IL) and boot covers (McKesson General Medical Corporation, Richmond, VA) were worn and disposed of following visits to each site to avoid inadvertent transportation of *E. maimaiga* between field sites. All equipment used in the stands was sterilized with 95% ethanol and thoroughly rinsed with distilled water between samples. Cages used for field bioassays were sterilized with 95% ethanol after each use.

Field bioassays

Gypsy moth egg masses were obtained from USDA APHIS, Otis Air

National Guard Base, Massachusetts. Larvae were reared on artificial diet (O'Dell et al. 1985) at the USDA APHIS PPQ Biological Control Laboratory, Niles, MI. Fourth-instar gypsy moth larvae that had molted within the previous 24 hr were selected daily for field bioassays. Larval development was staggered so that sufficient numbers of freshly-molted larvae (approximately 500 larvae per day in 2001 and 2002) were available each morning for the duration of the field bioassays.

Field bioassays were conducted at each stand by placing 20 of the 4th-instar larvae in 15 × 20 cm cages made of 6 × 7 mesh/cm^2 aluminum screening (Hajek and Humber 1997). Two ca 15 g pieces of high wheat germ artificial diet (O'Dell et al. 1985), which was sufficient enough to last the duration of the field bioassay, were placed in each cage. One cage was placed on the soil surface on the northern and southern aspects at the base of each sample tree in each stand and collected four days later (total of 8 cages per stand).

After four days in the field, cages of larvae were collected, individually stored in plastic bags to prevent contamination during transport, and returned to the USDA APHIS PPQ Biological Control Laboratory. Cages of larvae that had been in the field for 4-d were replaced with cages of fresh larvae. Field bioassays using 4th-instar larvae were continuously conducted for a 6-wk period, corresponding with gypsy moth larval development in wild populations, from 25 May to 4 July in 2001 and from 24 May to 3 July in 2002.

After their 4-d exposure period, larvae were reared individually in 50 mL cups on artificial diet following standard protocols for assessing fungal infections

(Papierok and Hajek 1997). Larvae were reared at 20 °C and 14:10 h (light: dark photoperiod) for 10 d, then placed in the dark for 3 d at 20 °C. After 3 d, the cadavers were checked for presence or absence of *E. maimaiga* conidia. If conidia were present, then cadavers were transferred to cold storage (4 °C and dark). If conidia were not present, then larvae were kept at 20 °C in the dark for an additional 7 d before being transferred to cold storage. Gypsy moth cadavers were dissected and examined with microscopy to determine whether *E. maimaiga* was present. To evaluate whether or not the laboratory was contaminated with *E. maimaiga* or NPV, 293 gypsy moth larvae were reared in the laboratory without undergoing field exposures and 100% of the larvae survived to pupation.

Infection rates for *E. maimaiga* for the 4-d field bioassays were calculated as the percentage of larvae in which *E. maimaiga* was found out of the total larvae examined (i.e. total number of cadavers processed plus the number of larvae that survived to pupation) for each 4-d period. Larval cadavers found to be co-infected with *E. maimaiga* and NPV were counted as mortality caused by *E. maimaiga* because of the more rapid pathogenesis from *E. maimaiga* than NPV (Hajek 1997a, Malakar et al. 1999a, 1999b). Co-infection with NPV occurred in 92.2 and 44.6% of *E. maimaiga*-killed cadavers in 2001 and 2002, respectively. Differences in overall infection levels between northern and southern aspects for all sites combined were analyzed using two-sample t-tests (SYSTAT 2000).

Microclimatic data

Several microclimatic variables at each site were collected every hour with on-site weather collection equipment, including air and soil temperatures, relative humidity, soil moisture and precipitation (Campbell Scientific, Inc., Logan, Utah). Weather collection equipment was positioned 40 - 50 m from the plot center at the base of a representative dominant oak tree. Air temperature and relative humidity data were collected with a temperature and relative humidity probe (Campbell Scientific, Inc., Logan, Utah) in a solar radiation shield positioned 1.5 m above the ground surface on a rebar pole. Soil temperatures and moisture levels were collected with temperature probes and water content reflectometers (Campbell Scientific, Inc., Logan, Utah), respectively. Temperature probes and water content reflectometers were positioned within the upper 3 to 4 cm of soil where the highest densities of *E. maimaiga* resting spores occur (Hajek et al. 1998a), to record relevant microclimatic conditions experienced by the fungus. Hourly precipitation measurements used in analyses were collected with a data-logging, tip-bucket rain gauge (Onset Computer Corporation, Bourne, Massachusetts) each year at the Jackson Corners site. Total precipitation during the 4-d bioassay periods, recorded using rain gauges (All-Weather Rain Gauge, Productive Alternatives, Inc., Fergus Falls, Minnesota) at each site, were similar over the study area.

Relative humidity was not used in analyses because of its nonlinear dependence on atmospheric temperature (Rosenberg et al. 1983). However, actual atmospheric water vapor pressure at each site was used in analyses and

was calculated using the respective on-site air temperature and relative humidity data, as:

$$e_a = RH \times e_s / 100,$$

where e_a = actual atmospheric water vapor pressure (kPa), RH = relative humidity (%), and e_s = saturation water vapor pressure (Rosenberg et al. 1983). Saturation water vapor pressure was calculated as:

$$e_s = 0.61078 \exp [(17.269 \times T) / (T + 237.30)],$$

where T = air temperature (°C) (Rosenberg et al. 1983).

Weather data were collected throughout the 6-wk period of gypsy moth field bioassays. In 2001, weather data were recorded for six weeks during gypsy moth larval development from 1200 hrs, 25 May to 1200 hrs, 4 July. In 2002, weather data were recorded from 1200 hrs, 24 May to 1200 hrs, 3 July, at each site. Microclimatic measurements were collected every hour for the duration of the study period.

In 2002, weather data was collected at only two of the sites due to an equipment malfunction at the Bitely site. Simple linear regression analyses were conducted using microclimatic variables in 2001 to develop equations to estimate microclimatic conditions for the Bitely site in 2002. Relationships between the three field sites and an independent weather station (Freemont station; MAWN 2003) were evaluated to determine which site most closely approximated microclimatic conditions at the Bitely field site. The equations used to approximate microclimatic conditions (followed the coefficient of determination) at Bitely in 2002 were: air temperature = 0.994 × (Freemont air

temperature) - 0.834 (r^2 = 0.96), relative humidity = 1.004 × (Freemont relative humidity) + 4.372 (r^2 = 0.87), northern aspect soil temperature = 1.083 × (Jackson Corner northern aspect soil temperature) - 0.616 (r^2 = 0.99), southern aspect soil temperature = 0.999 × (Jackson Corner southern aspect soil temperature) - 0.533 (r^2 = 0.96), northern aspect soil moisture = 1.078 × (Lilley northern aspect soil moisture) - 0.049 (r^2 = 0.78), and southern aspect soil moisture = 1.117 × (Lilley southern aspect soil moisture) - 0.030 (r^2 = 0.89). All linear regression relationships were significant at $P < 0.001$.

A backward-stepping multiple regression analysis was used to analyze effects of microclimatic variables on the levels of *E. maimaiga* and NPV infection for all sites combined each year (SYSTAT 2000). Because germination of *E. maimaiga* resting spores is greatest from 15 to 25 °C (Shimazu and Soper 1986, Hajek et al. 1990a, Hajek and Shimazu 1996), the sum of the hours that air and soil temperatures were between 15 and 25 °C were used in analyses. Other microclimatic variables used in analyses included the sum of the hours that volumetric soil moisture levels exceeded 10%, sum of the hours that precipitation occurred, total precipitation, and average atmospheric water vapor pressure over a given 4-d bioassay period. To reduce effects of multicollinearity, values for soil temperature and soil moisture were averaged between northern and southern aspects for each site over a given 4-d bioassay period.

RESULTS

Field bioassays

In 2001 and 2002, 4,800 laboratory-reared gypsy moth larvae were used in the field bioassays (total of 9,600 larvae). A portion of the larvae did not survive the 4-day exposure to field conditions each year (21.4% in 2001 and 12.1% in 2002), typically due to predation by ants or insectivorous rodents.

Overall, pathogen infection levels on northern and southern aspects were not significantly different in either year. In 2001, total *E. maimaiga* infection levels were 2.9 ± 0.5% on northern aspects and 2.6 ± 0.6% on southern aspects ($t = 0.273$, $df = 18$, $P = 0.79$), while total NPV infection levels were 41.7 ± 6.8% and 42.4 ± 6.3% on northern and southern aspects, respectively ($t = -0.082$, $df = 18$, $P = 0.94$). Similarly, in 2002, total *E. maimaiga* infection levels were 5.1 ± 1.9% on northern aspects and 3.9 ± 1.4% on southern aspects ($t = 0.501$, $df = 18$, $P = 0.62$), compared with total NPV infection levels of 14.3 ± 2.1% and 15.0 ± 3.0% on northern and southern aspects, respectively ($t = -0.195$, $df = 18$, $P = 0.85$).

Infection dynamics

In 2001, a total of 3,775 larvae were returned from the 4-d field exposures and reared in the laboratory until death or pupation. For all sites, the percentage of gypsy moth larvae infected with NPV was much greater than the percentage infected with *E. maimaiga*, regardless of the sample period (Figure

2.1).

Cumulatively, fungal and viral infections were responsible for mortality of 1,686 larvae (44.7%) in 2001. NPV was the dominant pathogen present in 1,584 cadavers (42.0% of the total larvae processed; 94.0% of the pathogen-killed cadavers). Infection by *E. maimaiga* was responsible for mortality of only 102 larvae (2.7% of the total larvae processed; 6.0% of the pathogen-killed cadavers). There were 195 larval cadavers (5.1%) in which neither NPV or *E. maimaiga* was present. Of the 3,775 larvae that were returned from the field, 1,894 gypsy moth larvae (52.2%) survived to pupation.

In 2002, 4,221 of the 4,800 larvae were returned from the 4-d field exposures and reared in the laboratory until death or pupation. In general, the percentage of gypsy moth larvae infected with NPV was again greater than the percentage infected with *E. maimaiga*, regardless of the sample period (Figure 2.2). Two exceptions occurred; at the Bitely site, during the 5 June and 13 June sample periods, the *E. maimaiga* infection rate was slightly greater than NPV infections.

Fungal and viral infection rates were much lower in 2002 than 2001, with 815 total larvae (19.3%) succumbing to either NPV or *E. maimaiga*. While NPV was again the dominant pathogen and was present in 622 cadavers (14.7% of the total larvae processed; 76.3% of the pathogen-killed cadavers), infection by *E. maimaiga* nearly doubled and was present in 193 cadavers (4.6% of the total larvae processed; 23.7% of the pathogen-killed cadavers). There were 88 larval cadavers (2.1%) in 2002 in which neither pathogen was present. Of the 4,221

larvae that were returned from the field, 3,318 gypsy moth larvae (78.6%) survived to pupation.

Microclimatic conditions

Several microclimatic variables suspected to influence primary transmission of *E. maimaiga*, including the sum of the hours that air and soil temperatures were between 15 and 25 °C, the sum of the hours that volumetric soil moisture levels were greater than 10%, sum of the hours that precipitation occurred, total precipitation, and average atmospheric water vapor pressure over a given 4-d bioassay period, were regressed on the percentage of gypsy moth larvae infected with *E. maimaiga* and the percentage of gypsy moth larvae infected with NPV in 2001 and 2002. While microclimatic conditions were relatively similar among sites each year, differences in environmental conditions between 4-d bioassay periods were considerable in 2001 (Figures 2.3 - 2.5) and 2002 (Figure 2.6 - 2.8).

In 2001, regression analysis of microclimatic variables on the percentage of gypsy moth larvae infected with *E. maimaiga* was not significant ($P = 0.091$) and the amount of variation explained was 22% (Table 2.2). However, regression analysis of microclimate on the percentage of gypsy moth larvae infected with NPV was significant ($P < 0.05$) and the amount of variation explained was 50%. Important predictors of NPV infection included the sum of the hours that air and soil temperatures were between 15 and 25 °C, total precipitation, and average atmospheric water vapor pressure over a given 4-d

bioassay period. Predictor coefficients for air temperature and atmospheric water vapor pressure were positive, indicating that NPV infection rates were higher during sample periods that had more hours with the air temperature between 15 and 25 °C and greater atmospheric water vapor pressure (Table 2.2).

In 2002, regression analysis of microclimatic variables on the percentage of gypsy moth larvae infected with *E. maimaiga* was significant ($P < 0.05$) and the amount of variation explained was 31% (Table 2.2). The sum of the hours that precipitation occurred over a given 4-d bioassay period was an important predictor of *E. maimaiga* infection. The predictor coefficient was positive, indicating that *E. maimaiga* infection rates were higher during sample periods that had precipitation occur over a longer period of time. Regression analysis of microclimate on the percentage of gypsy moth larvae infected with NPV was also significant ($P < 0.05$) and the amount of variation explained was 22%. The sum of the hours that precipitation occurred and average atmospheric water vapor pressure over a given 4-d bioassay period were important predictors of NPV infection. Predictor coefficients were again positive, indicating that NPV infections occurred more frequently when precipitation occurred for more hours and there was greater atmospheric water vapor pressure (Table 2.2).

DISCUSSION

This study provided a unique opportunity to examine the activity of two dominant gypsy moth pathogens, *E. maimaiga* and NPV, during primary transmission in the development of disease epizootics under field conditions. While the dynamics of these two pathogens are not identical, they do share some common characteristics. Specifically, primary transmission of the pathogen to early-instar hosts is the initial step in the development of an epizootic. The next step in the development of an epizootic involves secondary transmission of the pathogen from these infected early-instar hosts to later-instar hosts. It is during secondary transmission when amplification of disease takes place and the development of a large-scale epizootic may be realized (Hajek and Roberts 1991, Weseloh and Andreadis 1992a, 1992b, Hajek et al. 1993, Hajek 1997a). Gypsy moth populations at the three field sites were low since 1999 (N.W. Siegert chapter one) and wild gypsy moth larvae were rarely observed in these sites during this study in 2001 and 2002. This reduced the possibility of secondary transmission occurring, enabling me to focus on primary transmission of these pathogens over the course of gypsy moth larval development under field conditions.

Both *E. maimaiga* and NPV were common mortality agents in 2001 and 2002, though pathogen-related mortality of gypsy moth larvae was dominated by NPV. Until 1989, when *E. maimaiga* was discovered to be causing epizootics in the northeastern United States (Andreadis and Weseloh 1990a,

1990b, Hajek et al. 1990b), the major gypsy moth pathogen in North America was NPV (Doane 1970, Campbell and Podgwaite 1971). NPV remained the dominant gypsy moth pathogen in Michigan until *E. maimaiga* was introduced in the early to mid-1990's (Smitley et al. 1995, Buss 1997). Although NPV epizootics can be variable in nature, NPV is generally considered to function as a density-dependent mortality factor (Doane 1970, Woods and Elkinton 1987). Sufficient NPV inoculum generally must accumulate before a viral epizootic may occur. Since gypsy moth populations at the field sites were low in 2000 and 2001, I expected that infection rates by NPV during field bioassays would be low. However, NPV was the dominant pathogen during the 6-wk bioassays in both years, suggesting that NPV inoculum at the sites had persisted since 1999 in the soil and remained in large enough titers to potentially initiate a viral epizootic had high densities of gypsy moth been present. NPV of European pine sawfly (*Neodiprion sertifer* Geoffroy; Hymenoptera: Diprionidae) is known to persist in the soil at least 13 years (Olofsson 1988). Another possible explanation for the high levels of NPV infection, though, may be that the laboratory-reared larvae had latent viral infections that became activated during the 4-d field exposures. Previous research has suggested that stressful physical, chemical or physiological conditions may induce latent viral infections in some insects (Troitskaya and Chichigina 1980, Petre and Fuhrmann 1981, Hughes et al. 1993, Stoltz and Makkay 2003), but not others (Olofsson 1989). Reduction in temperature alone activated latent NPV infections in a *Lymantria* spp. closely related to gypsy moth (Bakhvalov et al. 1979). Further studies are

needed, however, to elucidate whether or not latency may have been responsible for the high levels of viral infection observed in this study.

Although several factors have been suggested to affect secondary transmission of NPV, and therefore the ultimate development of a viral epizootic (see D'Amico et al. 1996), precipitation or other forms of environmental moisture most likely drive viral infections, through contamination of food resources during primary transmission (Podgwaite et al. 1979, D'Amico and Elkinton 1995). In this study, infections of gypsy moth larvae with NPV were often associated with precipitation-related factors. Whether these factors are casual agents of viral infection or are merely correlated with precipitation-based contamination of food resources, however, remains to be tested.

Entomophaga maimaiga activity has often been associated with moisture (see Hajek 1999 and references therein), but few studies have evaluated the germination of resting spores during primary transmission. In a study of a related entomopathogen, Perry and Latgé (1982) found that free water was required for the germination of *Conidiobolus obscurus* (Petch) Hall & Dunn resting spores. Results from this study appear to further verify that infection by *E. maimaiga* is associated with environmental moisture. However, results were inconsistent between years and, in 2001, results of the multiple regression were not significant. Whether this was due to some unmeasured abiotic or biotic factor that affects *E. maimaiga* infection dynamics or is an artifact of stochasticity in resting spore germination between years, remains to be determined. In 2002, however, the sum of the hours that precipitation occurred

over a given 4-d bioassay period was an important predictor of *E. maimaiga* infection during primary transmission. This suggests that the primary transmission of *E. maimaiga*, and therefore the initial step in the development of epizootics, may be affected by the availability of free water.

While *E. maimaiga* infections were generally lower than NPV infections in this study, the infection levels I observed are not unreasonably low for primary transmission of this pathogen. Hajek et al. (1993) hypothesized that secondary transmission of *E. maimaiga* in gypsy moth populations was more critical in the ultimate development of epizootics. Indeed, several studies examining infection by *E. maimaiga* during gypsy moth larval development have documented low levels of infection during primary transmission of the pathogen comparable to levels observed in this study, followed by a rapid increase in *E. maimaiga* infection presumably due to secondary transmission (e.g. Weseloh and Andreadis 1992b, Hajek et al. 1996a, Hajek and Webb 1999, Webb et al. 1999). In a forest that had an *E. maimaiga* epizootic in the previous year in Connecticut, Weseloh and Andreadis (1992b) reported that 5% or less of forest-collected 1st to 3rd-instar gypsy moth larvae became infected in the first few weeks of larval development. Additionally, 2nd to 4th-instar laboratory-reared gypsy moth larvae that were caged and exposed 5 cm above the ground for 3-d periods had even lower infection rates until late 4th and 5th-instar larvae were present in the forest (Weseloh and Andreadis 1992b). In 5 out of 7 plots in Virginia, Hajek et al. (1996a) also found that 5% or less of forest-collected larvae became infected with *E. maimaiga* during the first six weeks of larval

development followed by a rapid increase in *E. maimaiga* infection levels. The remaining two plots exhibited a similar pattern of epizootic development, but *E. maimaiga* infections during the first several weeks of larval development were slightly greater (ca 5 to 15%) (Hajek et al. 1996a). Webb et al. (1999) documented similar results in forest-collected larvae from five "higher-population" woodlots in Virginia. It seems reasonable to suspect that the levels of *E. maimaiga* infection I observed during primary transmission would have been more than adequate for the development of a large-scale epizootic had higher-density gypsy moth populations been present and microclimatic conditions, such as environmental moisture (Hajek et al. 1999), been favorable for efficient secondary transmission.

Entomophaga maimaiga resting spores were quantified at these sites the two years before the current study in related research (N.W. Siegert chapter one). These sites averaged ca 169 ± 36 and 90 ± 18 resting spores per gram of dry soil in 1999 and 2000, respectively, but resting spore counts tended to be highly variable (N.W. Siegert chapter one). In 1999 and 2000, *E. maimaiga* resting spore counts at the Jackson Corners site were 235 ± 85 and 69 ± 15 resting spores per gram of dry soil, respectively. Resting spore counts at the Lilley site were 176 ± 58 and 108 ± 73 resting spores per gram of dry soil in 1999 and 2000, respectively. The Bitely site had the least variable counts with 97 ± 11 and 95 ± 15 resting spores per gram of dry soil in 1999 and 2000, respectively. Although only 112 to 235 *E. maimaiga* resting spores per gram of dry soil can cause more than 60% mortality in laboratory bioassays under

optimal conditions (N.W. Siegert chapter one), it is likely that greater fungal inoculum in the soil would increase *E. maimaiga* infection levels during primary transmission (Weseloh and Andreadis 1992a, Hajek and Webb 1999).

Prediction of naturally-occurring epizootics and their effects on gypsy moth populations has become more complicated because both *E. maimaiga* and NPV must be considered. Evaluation of hourly microclimatic conditions during larval development in stands with varying gypsy moth population densities would improve our understanding of the primary and secondary transmission dynamics of *E. maimaiga* and NPV. This information, in addition to a better understanding of the interaction of these two pathogens under varying climatic conditions, could significantly aid in the development of our ability to accurately predict epizootics in North American gypsy moth populations.

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Table 2.1. Location and characteristics of oak stands in Newaygo Co., Michigan, used for 6-wk bioassay field sites from 2001 to 2002.

Site	Latitude (N)	Longitude (W)	Canopy cover (%)	Basal area (m^2/ha)	Egg mass density (No./ha)			
					1999	2000	2001	2002
Bitely	43.76	85.91	93.0	25	450	0	0	0
Jackson Corners	43.75	85.80	90.6	21	400	0	0	0
Lilley	43.78	85.87	88.6	25	200	0	50	0

Table 2.2. Summary of regression equations for predicting *Entomophaga maimaiga* and NPV infections during primary transmission in the initial development of epizootics. Predictor variables (excluding intercept) are listed in order of contribution to r^2.

Year	Dependent variable	Predictor variable(s)	Coefficients	SE	F	df	r^2	P
2001	*E. maimaiga*	Intercept	6.872	2.709				
		Hrs soil temperature b/w 15 & 25°C	0.064	0.026				
		Atmospheric water vapor pressure	-5.516	2.640				
		Total precipitation	0.083	0.040	2.39	3, 26	0.22	0.091
	NPV	Intercept	-17.931	20.982				
		Hrs air temperature b/w 15 & 25°C	1.054	0.307				
		Hrs soil temperature b/w 15 & 25°C	-0.576	0.222				
		Atmospheric water vapor pressure	32.620	20.572				
		Total precipitation	-0.502	0.304	6.15	4, 25	0.50	0.001

2002	*E. maimaiga*	Intercept	1.339	1.239				
		Hrs with precipitation	0.621	0.177	12.26	1, 28	0.31	0.002
	NPV	Intercept	-7.550	9.907				
		Atmospheric water vapor pressure	10.345	5.217				
		Hrs with precipitation	1.117	0.417	3.84	2, 27	0.22	0.034

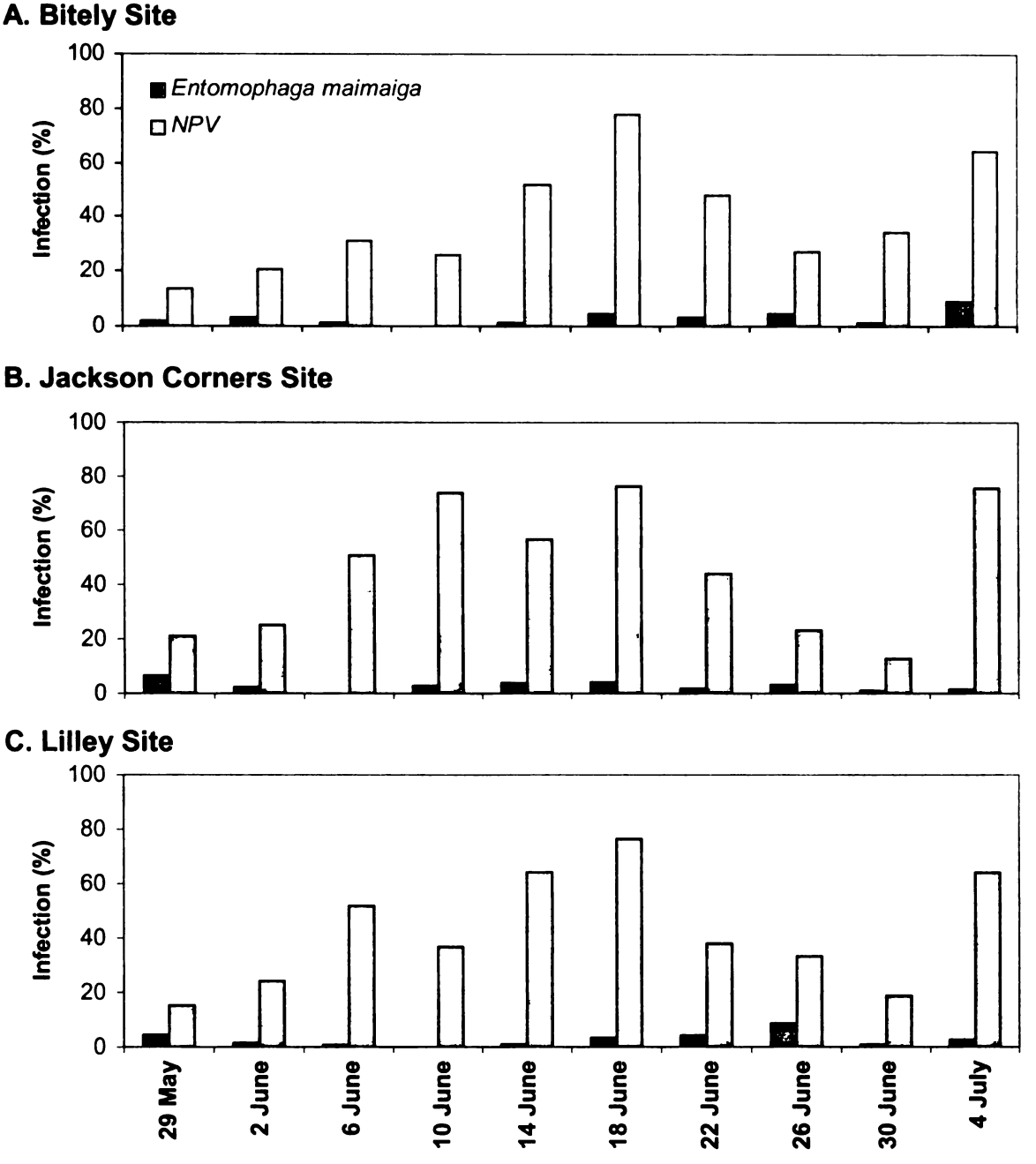

Figure 2.1. Cumulative percentage fungal and viral infections of 4th-instar gypsy moth larvae during 4-d bioassays conducted over a 6-wk period in 2001 at A) Bitely, B) Jackson Corners, and C) Lilley field sites.

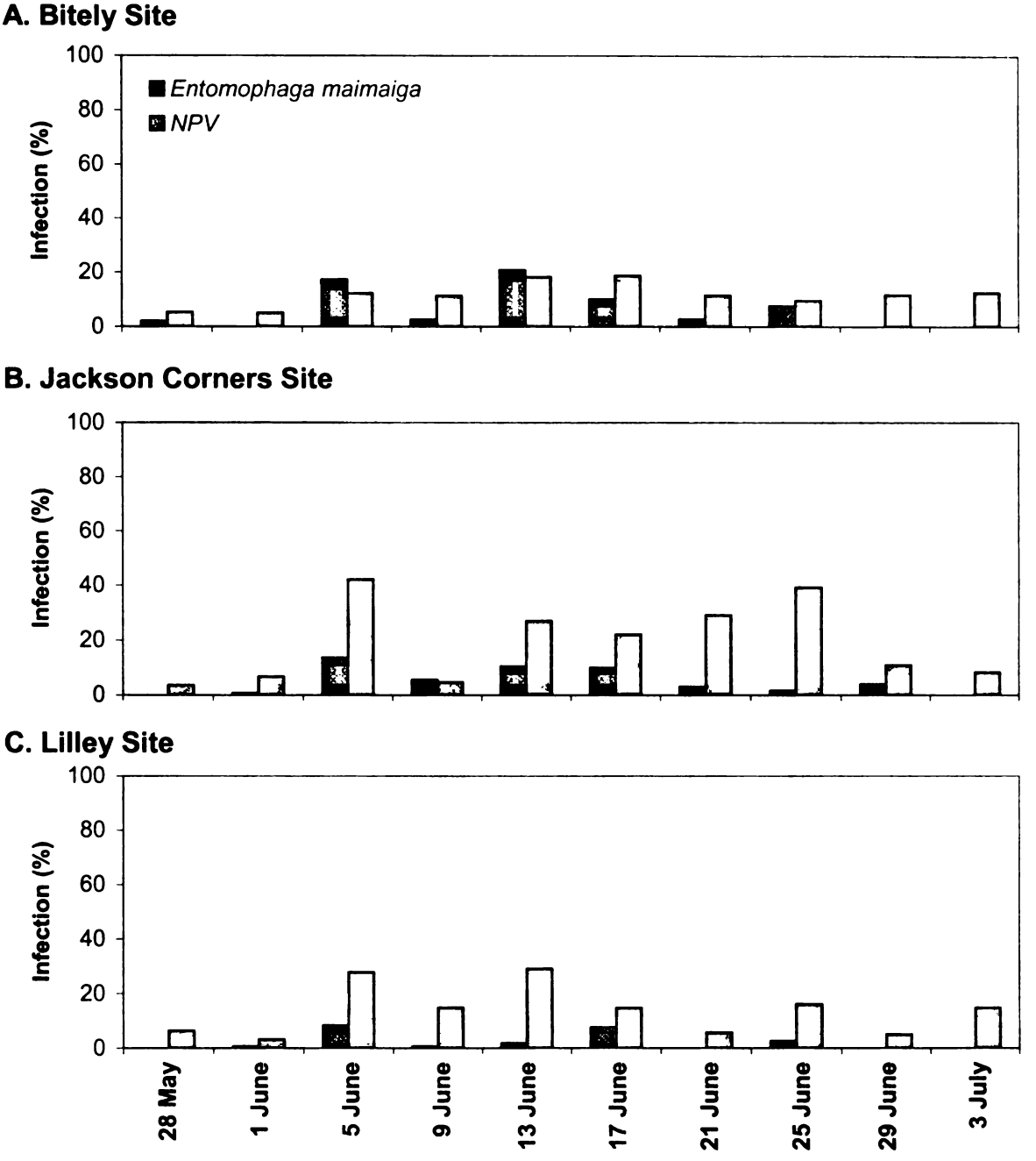

Figure 2.2. Cumulative percentage fungal and viral infections of 4th-instar gypsy moth larvae during 4-d bioassays conducted over a 6-wk period in 2002 at A) Bitely, B) Jackson Corners, and C) Lilley field sites.

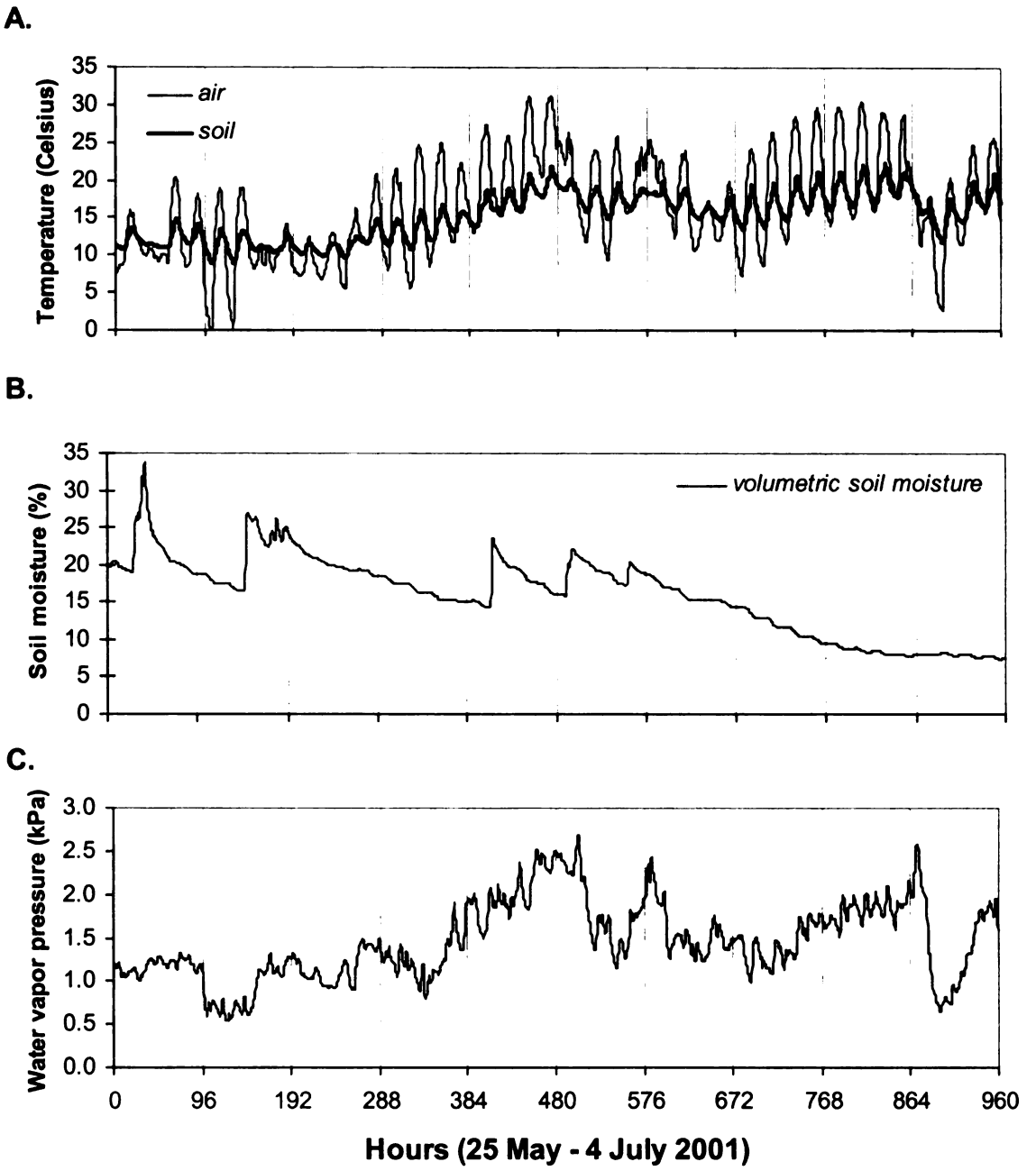

Figure 2.3. Microclimatic conditions that occurred at the Bitely site during the 6-wk field bioassays in 2001. Microclimatic variables included A) hourly air and soil temperatures (°C), B) soil moisture (%), and C) actual water vapor pressure (kPa). Soil temperatures and moistures shown were collected from southern aspects. Northern aspects were slightly lower in temperature (northern aspect soil temperature = 0.964 × (southern aspect soil temperature) + 0.299; r^2 = 0.99) and higher in moisture (northern aspect soil moisture = 1.069 × (southern aspect soil moisture) + 0.001; r^2 = 0.99).

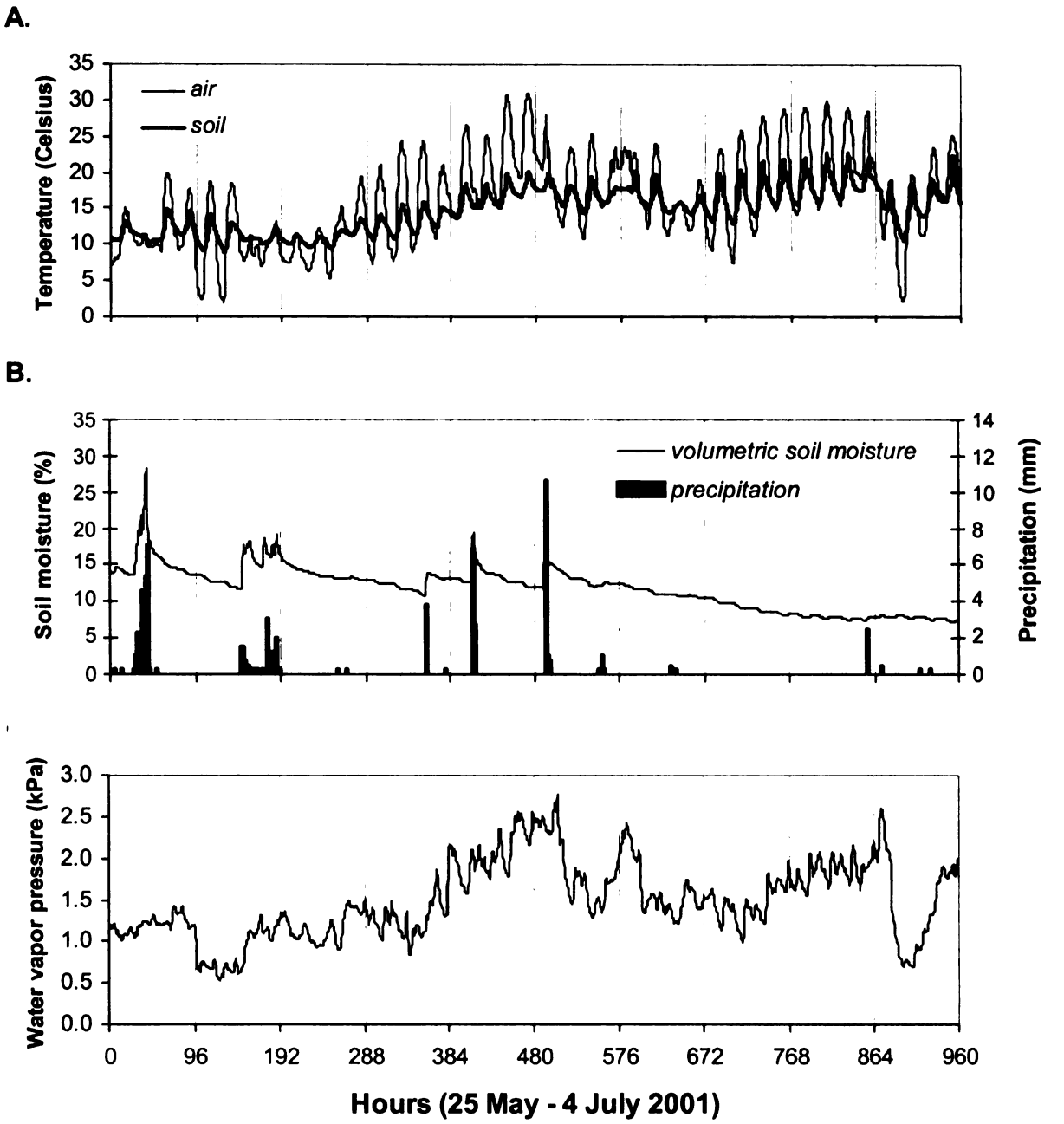

Figure 2.4. Microclimatic conditions that occurred at the Jackson Corners site during the 6-wk field bioassays in 2001. Microclimatic variables included A) hourly air and soil temperatures (°C), B) soil moisture (%) and precipitation (mm), and C) actual water vapor pressure (kPa). Soil temperatures and moistures shown were collected from southern aspects. Northern aspects were slightly lower in temperature (northern aspect soil temperature = 0.894 × (southern aspect soil temperature) + 1.264; $r^2 = 0.96$) and higher in moisture (northern aspect soil moisture = 1.116 × (southern aspect soil moisture) - 0.005; $r^2 = 0.97$).

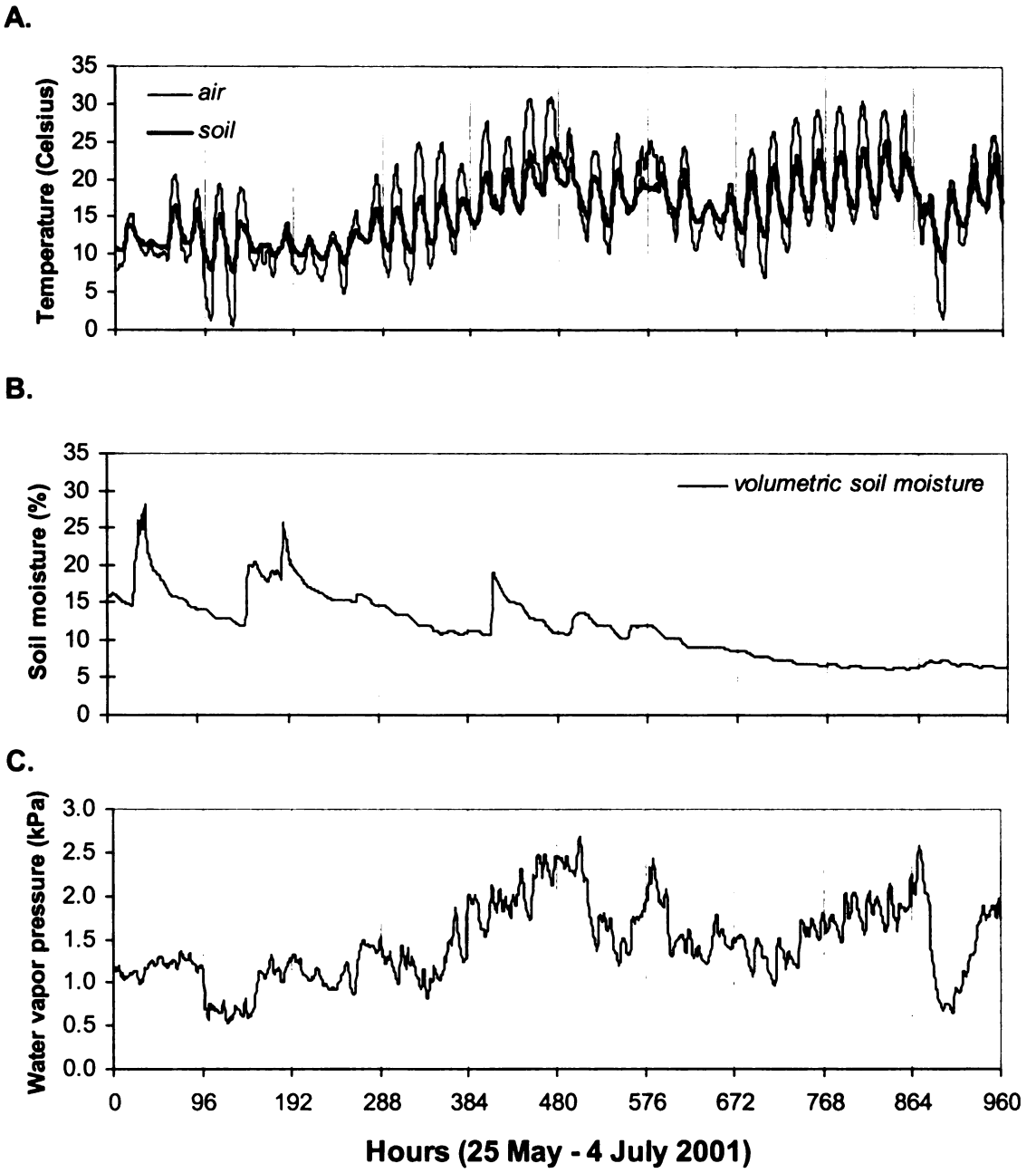

Figure 2.5. Microclimatic conditions that occurred at the Lilley site during the 6-wk field bioassays in 2001. Microclimatic variables included A) hourly air and soil temperatures (°C), B) soil moisture (%), and C) actual water vapor pressure (kPa). Soil temperatures and moistures shown were collected from southern aspects. Northern aspects were slightly lower in temperature (northern aspect soil temperature = 0.858 × (southern aspect soil temperature) + 1.553; r^2 = 0.98) and higher in moisture (northern aspect soil moisture = 1.016 × (southern aspect soil moisture) - 0.004; r^2 = 0.95).

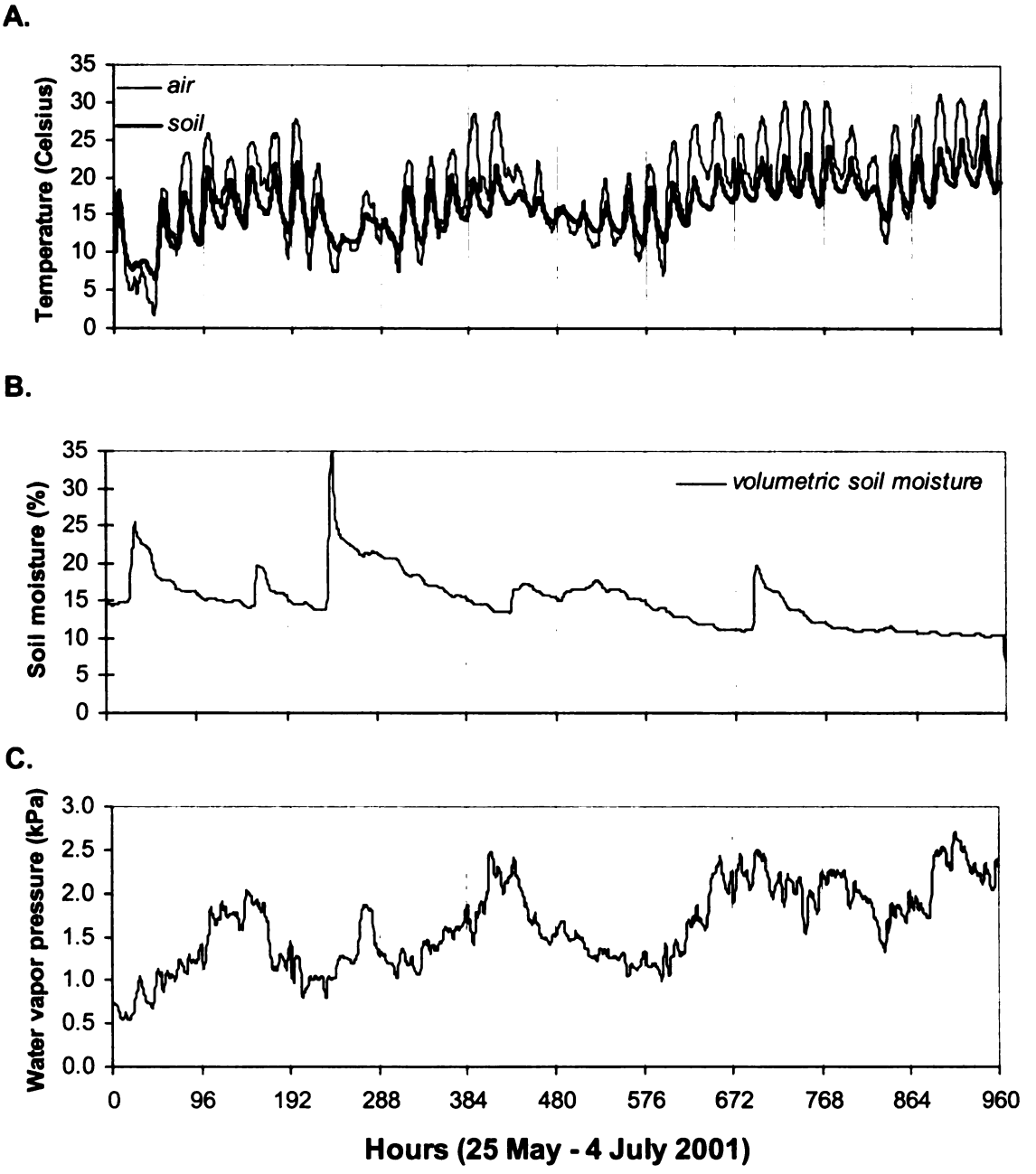

Figure 2.6. Microclimatic conditions that occurred at the Bitely site during the 6-wk field bioassays in 2002. Microclimatic variables included A) hourly air and soil temperatures (°C), B) soil moisture (%), and C) actual water vapor pressure (kPa). Soil temperatures and moistures shown were collected from southern aspects. Northern aspects were slightly lower in temperature (northern aspect soil temperature = 0.832 × (southern aspect soil temperature) + 2.583; r^2 = 0.85) and higher in moisture (northern aspect soil moisture = 1.134 × (southern aspect soil moisture) + 0.009; r^2 = 0.90).

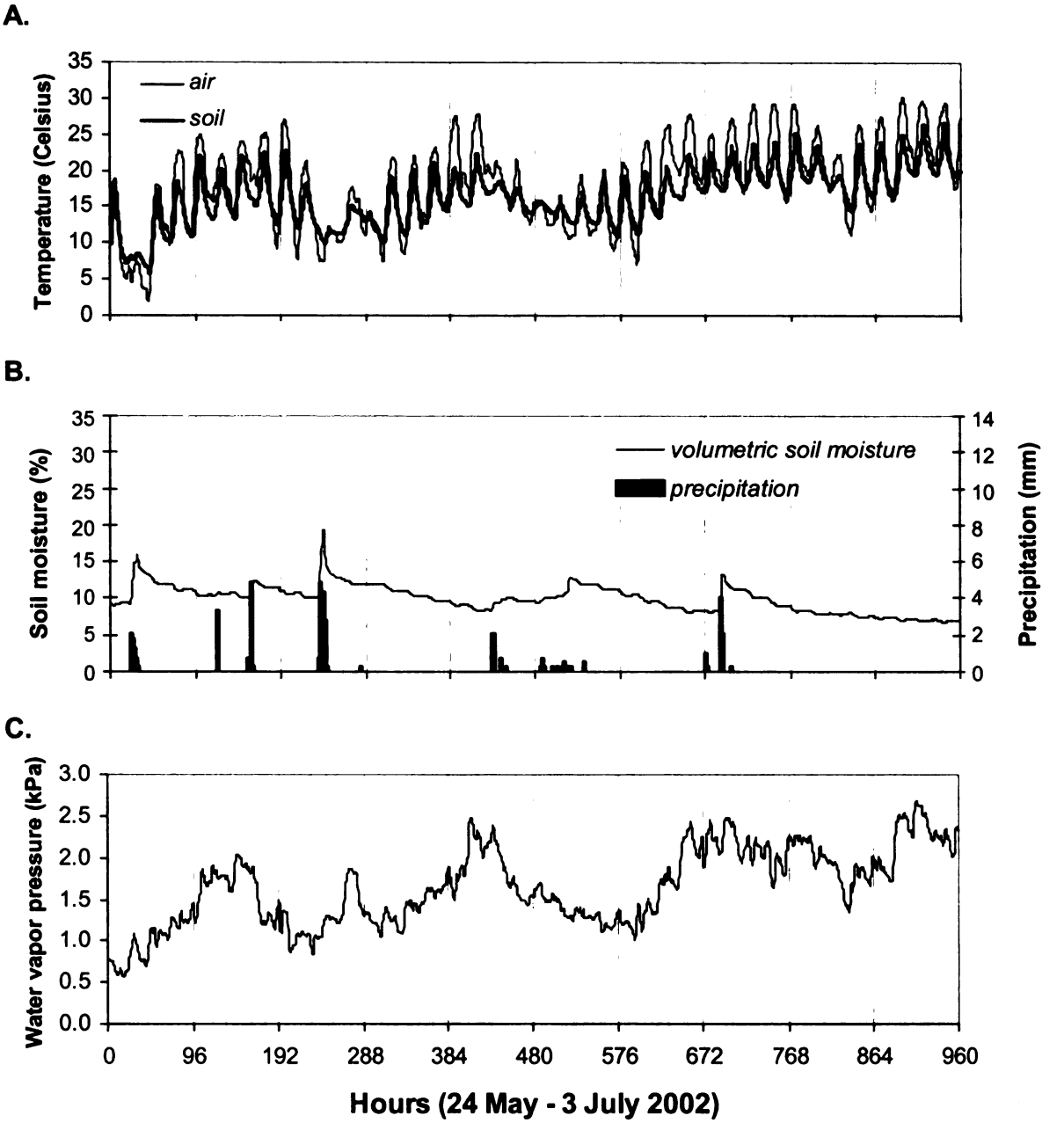

Figure 2.7. Microclimatic conditions that occurred at the Jackson Corners site during the 6-wk field bioassays in 2002. Microclimatic variables included A) hourly air and soil temperatures (°C), B) soil moisture (%) and precipitation (mm), and C) actual water vapor pressure (kPa). Soil temperatures and moistures shown were collected from southern aspects. Northern aspects were slightly lower in temperature (northern aspect soil temperature = 0.705 × (southern aspect soil temperature) + 3.871; r^2 = 0.85) and higher in moisture (northern aspect soil moisture = 1.558 × (southern aspect soil moisture) - 0.044; r^2 = 0.84).

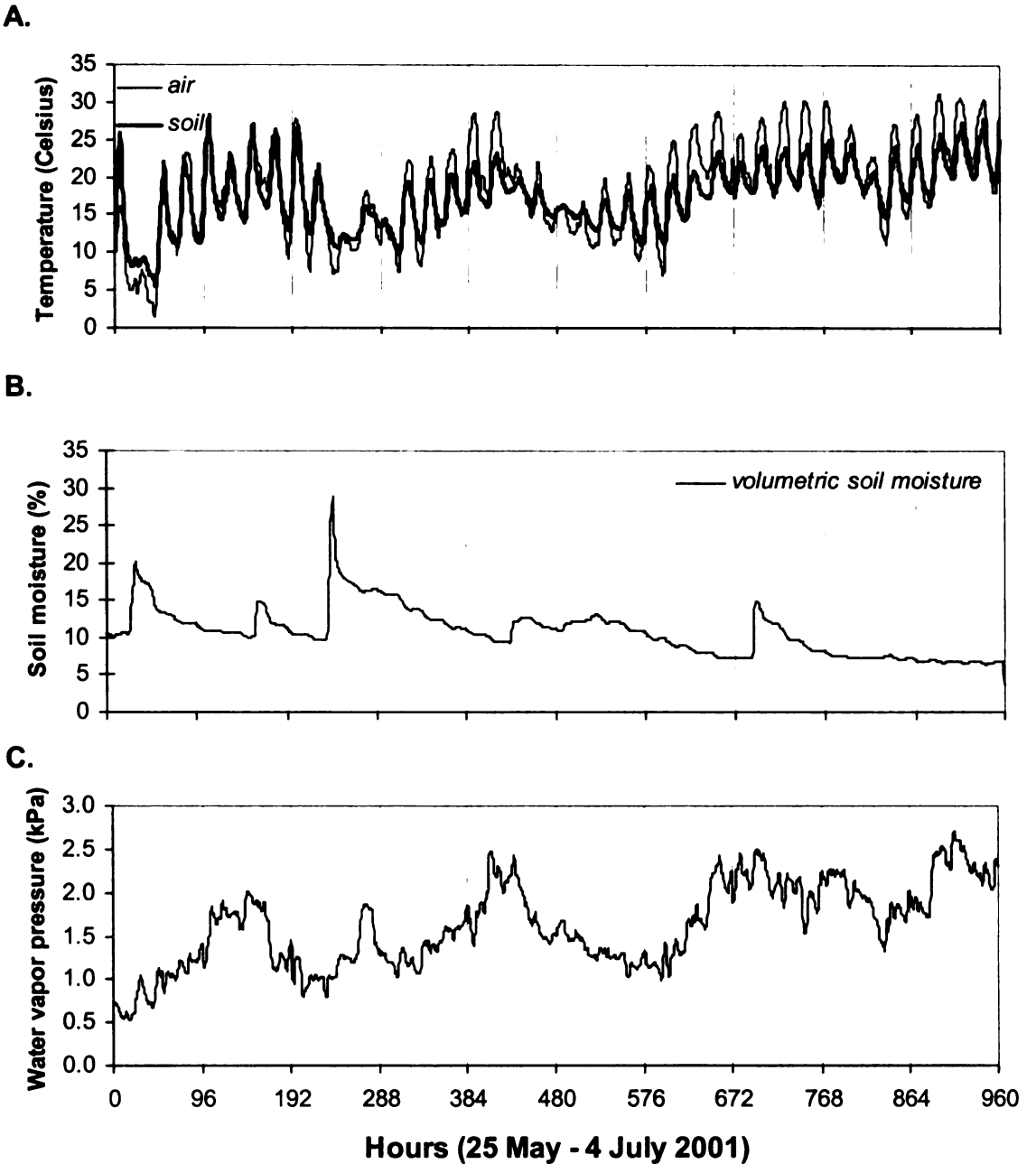

Figure 2.8. Microclimatic conditions that occurred at the Lilley site during the 6-wk field bioassays in 2002. Microclimatic variables included A) hourly air and soil temperatures (°C), B) soil moisture (%), and C) actual water vapor pressure (kPa). Soil temperatures and moistures shown were collected from southern aspects. Northern aspects were slightly lower in temperature (northern aspect soil temperature = 0.787 × (southern aspect soil temperature) + 2.521; r^2 = 0.91) and higher in moisture (northern aspect soil moisture = 1.175 × (southern aspect soil moisture) - 0.005; r^2 = 0.90).

CHAPTER 3

ASSESSING THE CLIMATIC POTENTIAL FOR EPIZOOTICS OF THE GYPSY MOTH FUNGAL PATHOGEN *Entomophaga maimaiga* IN THE NORTH CENTRAL UNITED STATES

INTRODUCTION

Substantial decreases in gypsy moth *Lymantria dispar* L. (Lepidoptera: Lymantriidae) defoliation in the northeastern United States in the last decade have been largely attributed to the occurrence of epizootics of the fungal pathogen *Entomophaga maimaiga* Humber, Shimazu et Soper (Zygomycetes: Entomophthorales). Originally from Japan, *E. maimaiga* epizootics in North America were first observed in 1989 (Andreadis and Weseloh 1990a, 1990b, Hajek et al. 1990b). *Entomophaga maimaiga* is a desirable biological control agent for gypsy moth. It has few impacts on non-target organisms (Hajek et al. 1995a, 1996a, 1996b, 2000) and is compatible with other natural enemies and pathogens, including a nuclear polyhedrosis virus (NPV) (Andreadis and Weseloh 1990a, Hajek and Roberts 1992, Weseloh and Andreadis 1992b). Unlike NPV (Doane 1970, Leonard 1981, Woods and Elkinton 1987), *E. maimaiga* functions in a density-independent manner (Hajek et al. 1990b, Hajek 1997a), so it is not necessary for gypsy moth populations to build to damaging levels before an epizootic may develop.

Entomophaga maimaiga produces two types of spores, both of which may infect gypsy moth larvae (Hajek 1999). Soil-borne *E. maimaiga* resting spores germinate in the spring when environmental conditions are suitable (Hajek 1997b, Hajek and Humber 1997, Weseloh and Andreadis 1997). Early-instar larvae become infected and die (Hajek et al. 1998b). These infected cadavers externally produce *E. maimaiga* conidiophores that discharge conidia which may infect mid- to late-instar gypsy moth. Late-instar cadavers drop to the soil, decompose and resting spores remain dormant in the soil until the following spring (Hajek et al. 1998a).

Entomophaga maimaiga has rapidly become a significant biological control agent for gypsy moth (Elkinton et al. 1991, Hajek et al. 1995b, Hajek 1999, Nealis et al. 1999) and has been widely introduced throughout the present North American range of gypsy moth (Smitley et al. 1995, Hajek et al. 1996b, Webb et al. 1999). Despite these widespread introductions and establishment of *E. maimaiga* in northeastern states, the occurrence of epizootics have been less consistent in other states, including Michigan (Smitley et al. 1995, Bauer and Smitley 1996, Buss 1997, Buss et al. 1999). Infrequency of epizootics in these areas has contributed to continued gypsy moth defoliation (USDA-FS 2004). This lack of consistency could be weather related and may be attributable to variation in spring climate.

Questions remain concerning how *E. maimaiga* will perform in the North Central states of Illinois, Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, Ohio and Wisconsin, where gypsy moth is more recently established

and continuing to expand into new areas. Forest health managers and pest specialists, especially in areas with high densities of suitable gypsy moth hosts near the leading edge of gypsy moth range expansion, such as Minnesota and Wisconsin, desire the ability to predict *E. maimaiga* success and the extent to which it should be incorporated into gypsy moth management strategies. Thorough evaluation of meteorological factors and their effect on *E. maimaiga* germination, however, is integral to predict how well this fungal pathogen will control gypsy moth in new areas.

Fungal entomopathogens can be highly efficacious (Carruthers and Soper 1987, McCoy et al. 1988, Hajek 1997b), but generally tend to be effective within a narrow range of environmental conditions (Benz 1987, Hajek and St. Leger 1994, Burges 1998). Previous studies have suggested that the *E. maimaiga* fungus is sensitive to abiotic conditions, particularly temperature and moisture (Shimazu and Soper 1986, Shimazu 1987, Hajek et al. 1990a, 1999, Weseloh et al. 1993, Hajek and Humber 1997) and that weather plays a critical role in the development of *E. maimaiga* epizootics (Elkinton et al. 1991, Weseloh and Andreadis 1992a, Hajek et al. 1993). Weather between April and June is likely to be critical because gypsy moth larvae are present during that time and certain unknown environmental conditions are needed for germination of *E. maimaiga* resting spores (Hajek and Roberts 1991). The variability of weather during the April to June period may be more important than average meteorological conditions in the long-term success of *E. maimaiga*.

The goal of this research was to assess the climatic conditions of the

North Central region and identify areas that are likely to frequently experience conditions suitable for the development of *E. maimaiga* epizootics. The objectives of the current study were to (1) compare year-specific weather from locations with documented *E. maimaiga* epizootics to the climate of the North Central region; (2) examine the temporal variability of precipitation and temperature in the North Central region to identify areas which should be climatically conducive for the development of *E. maimaiga* epizootics; and (3) estimate the number of years in the North Central region from 1971 to 2000, in which precipitation and temperature conditions may have been suitable for *E. maimaiga* epizootics. The response of *E. maimaiga* to general climate patterns remains unclear (Hajek 1999), so climate comparisons and climatic variability in the North Central region were examined using spring (April through June) and annual climate data.

METHODS AND MATERIALS

Documented epizootics

Environmental conditions specific to 11 documented *E. maimaiga* epizootics (Table 3.1) were used for climate comparisons and to estimate the number of years that may have been favorable for an epizootic to occur in the North Central region. Weather conditions during the specific years that the epizootics occurred were retrieved from the Midwestern, Southeastern and Northeastern Regional Climate Centers for weather stations nearest to the documented epizootics. These 11 epizootic locations with their respective year-specific climate data will be referred to herein as the "epizootic-specific sites." Locations were selected from the scientific literature if the level of *E. maimaiga* infection was greater than 60% or if it could otherwise be discerned that a large-scale epizootic had occurred. A few additional *E. maimaiga* epizootics have been documented in the literature (e.g. Hajek 1997a, Hajek and Humber 1997, Hajek et al. 1990b, 1999), but they were not included in the analyses because I was unable to obtain complete year-specific climate data for the respective locations.

Climate comparisons

Environmental conditions at the epizootic-specific sites (Table 3.1) were individually compared with 1132 locations in North America using the climatological software program CLIMEX for Windows Version 1.1

(Commonwealth Scientific and Industrial Research Organization [CSIRO] Publishing, Victoria, Australia) (Sutherst et al. 1999). Models in CLIMEX assume that temperature and moisture are primary determinants in a species' biogeography (Sutherst and Maywald 1985, Sutherst et al. 1995). The CLIMEX software contains a meteorological database of approximately 3000 locations worldwide and 300 locations in North America (Appendix C). Meteorological data from an additional 832 locations in nine North Central and two northeastern states were imported into the standard CLIMEX meteorological database to more thoroughly represent climatic variability within the region (Figure 3.1). Specifically, meteorological data from 77 locations in Illinois, 58 locations in Indiana, 113 locations in Iowa, 43 locations in Kentucky, 88 locations in Michigan, 100 locations in Minnesota, 79 locations in Missouri, 39 locations in New York, 84 locations in Ohio, 32 locations in Pennsylvania and 119 locations in Wisconsin were added to the database (Appendix C; MRCC 2002). The meteorological database is composed of 30-yr average monthly minimum and maximum air temperature, precipitation, and morning and afternoon relative humidity.

Climatic comparisons were based on minimum and maximum air temperature, total precipitation and precipitation pattern. Relative humidity data were not available for the additional locations imported into the CLIMEX database, so this parameter was excluded from the climate-matching analyses. The CLIMEX program expressed similarity between two locations as an index for each climatic parameter. Indices were scaled between 0 and 100, with

higher values reflecting greater similarity in a given climatic parameter between the two locations. The maximum similarity of a North American location to any one of the epizootic-specific sites was used in the climate-matching analyses.

The minimum and maximum temperature indices, I_{tmin} and I_{tmax}, were calculated by CLIMEX as:

$$I_{tmin} = exp(-k_T * T_{dmin}), \text{ and}$$

$$I_{tmax} = exp(-k_T * T_{dmax})$$

where, T_{dmin} and T_{dmax} are the average monthly absolute differences in minimum and maximum temperature, respectively, between two locations. By default, the constant, k_T, was set to 0.1.

CLIMEX calculated the total precipitation index, I_{rtot}, as:

$$I_{rtot} = exp(-k_R * R_d)$$

where, $R_d = (|R_T - R_M|)/[1 + a(R_T + R_M)]$, R_T was the annual precipitation at the target location, and R_M was the annual precipitation at the matching location. The CLIMEX software used default values of 0.001 and 0.004 for the constants a and k_R, respectively.

The precipitation pattern index, I_{rpat}, was calculated as:

$$I_{rpat} = exp(-k_P * R_D)$$

where, R_D was the average absolute difference between the monthly precipitation of the target and matching locations, after the monthly precipitation at the matching location was multiplied by R_z ($R_z = R_M/R_T$). CLIMEX used a value of 0.005 for the precipitation pattern constant, k_P.

An overall measure of climatic similarity was estimated by the CLIMEX

software as a "Match Index," which incorporated all of the equally-weighted indices, excluding the relative humidity index, and was scaled between 0 and 100, inclusively (Sutherst and Maywald 1985). The Match Index, *MI*, was calculated as:

$$MI = (I_t \times I_{rtot} \times I_{rpat})^{0.5} \times 100$$

where, I_t was the average monthly temperature.

The geographically-referenced overall climatic similarity index of a given North American location with maximum similarity to any one of the epizootic-specific sites was exported to the ArcView 3.2 geographic information system (Environmental Systems Research Institute [ESRI], Redlands, California). Isoclines were generated using the ArcView Spatial Analyst extension (ESRI).

Climatic deviations from 30-yr averages

Climatic variability in the North Central region was examined by evaluating the sum of the absolute departures from 30-yr averages (1971-2000) of precipitation and temperature for climate divisions in the North Central region (Figure 3.2; MRCC 2002). A climate division is a climatically homogeneous region within a state (NOAA 2002a, 2002b). Divisional climate data are used for numerous research applications, including assessment of large-scale climatic trends over long time periods (NOAA 2002a, 2002b).

Trends in variation of precipitation and temperature were examined for both spring (April through June) and annual climate data. Geographically-referenced absolute deviations for each climatic parameter were exported to

ArcView 3.2 and isoclines generated using the Spatial Analyst extension (ESRI).

Favorable years for epizootics in the North Central region, 1971-2000

Precipitation and temperature conditions during April, May and June at the epizootic-specific sites used for the climate comparisons (Table 3.1) were examined to determine the approximate range of environmental conditions that were associated with *E. maimaiga* epizootics. One outlier, June precipitation from Rockbridge County, Virginia in 1995 (Webb et al. 1999), was excluded from calculations because it was 328 mm greater than the 30-yr average for that site and skewed the distribution of the data.

Weather records for the North Central region from 1971 to 2000 (MRCC 2002) were assessed to estimate the number of years that may have been favorable for an *E. maimaiga* epizootic to occur. Meteorological records from the nearest available weather station to an epizootic were used to estimate an approximate value of a given climatic parameter. This exercise was hypothetical because *E. maimaiga* and gypsy moth were not established throughout the area investigated from 1971 to 2000. When estimating the number of years between 1971 and 2000 that may have been favorable for an *E. maimaiga* epizootic to occur, I assumed that *E. maimaiga* was initially present throughout the North Central region, that no varietal effects of fungal isolates occurred and that suitable gypsy moth hosts were sufficiently available for infection.

"Average scenarios" and "best-case scenarios" were estimated based on

the weather conditions at the documented epizootic locations for the years that epizootics occurred. A year was considered favorable in an average scenario if weather conditions met or exceeded the average for a given meteorological parameter estimated from the 11 documented epizootics. Average scenario estimates were 75.4, 127.5 and 116.9 mm of precipitation and temperatures of 10.6, 15.6 and 20.5 °C for April, May and June, respectively. Best-case scenarios were estimated in a similar manner using the average minus the standard deviation for a given meteorological parameter. Best-case scenario estimates were 43.0, 65.9 and 75.8 mm of precipitation and temperatures of 7.7, 13.8 and 18.7 °C for April, May and June, respectively. Scenarios were estimated based on precipitation only, temperature only, and both precipitation and temperature.

Departures from 30-yr average climatic conditions for the epizootic-specific sites were also examined (Table 3.1). Monthly precipitation and temperature values at locations where epizootics were documented to occur were tested for differences from 30-yr averages using two-tailed t-tests (Sokal and Rohlf 1995), with critical values of $t_{(0.5,\,20)} = 2.086$ and $t_{(0.5,\,9)} = 2.262$ for temperature and precipitation differences, respectively. The degrees of freedom for precipitation t-tests were reduced, as recommended by Sokal and Rohlf (1995) when sizes of the two samples are equal, because the precipitation data were heteroscedastic.

RESULTS

Documented epizootics

While precipitation was the meteorological parameter that most clearly varied from the 30-yr averages at the epizootic-specific sites (Table 3.1), precise upper and lower thresholds governing the development of epizootics remain unclear. Most of the sites had above average precipitation in May and June during years with epizootics, however, seven of the 11 epizootic-specific sites had below average precipitation in April. May precipitation was below average in three cases, while June precipitation was below average in only two cases (Table 3.1). Precipitation in April at the epizootic-specific sites ranged from 36 to 141 mm and April temperature ranged from 4.6 to 13.8 °C. May precipitation and temperature ranged from 52 to 242 mm and 12.8 to 17.9 °C, respectively. Precipitation and temperature in June at the epizootic-specific sites ranged from 42 to 432 mm and 16.8 to 22.8 °C, respectively. Overall, precipitation in April was not significantly different from 30-yr averages ($P > 0.05$), but precipitation in May and June were significantly different from 30-yr averages ($P < 0.05$). Temperatures at the epizootic-specific sites were not as variable as precipitation and, overall, departures in temperature did not differ significantly in April, May or June ($P > 0.05$).

Climate comparisons

Spring climatic conditions throughout most of the United States were

greater than 60% similar to any one of the epizootic-specific sites (Figure 3.3A). A relatively small area south of the Great Lakes region that extended from Kansas east to the Atlantic coast was greater than 80% similar in overall climate. Annual climatic conditions throughout much of the eastern half of the United States were 60 to 80% similar to any one of the epizootic-specific sites (Figure 3.3B). For spring climate comparisons (April to June), individual climatic similarity indices (i.e. similarity indices based solely on minimum air temperature, maximum air temperature, total precipitation or precipitation pattern) throughout the North Central region were typically greater than 80% similar to any one of the epizootic-specific sites.

Climatic deviations from 30-yr averages

Deviations in spring precipitation in the North Central region were greatest in the southwestern area of the region, extending from Iowa through Kentucky (Figure 3.4A). Northern Minnesota, northern Wisconsin, most of Michigan and northern Ohio were least variable in precipitation. Annual deviations in precipitation in the North Central region were greatest through southern Missouri, southern Illinois and western Kentucky (Figure 3.4B). The northern states, including Minnesota, northern Wisconsin, much of Michigan and the northern edge of Ohio, were least variable in precipitation over the 30 year time period, 1971 to 2000.

Deviations in spring temperature in the North Central region were greatest throughout most of Minnesota (except northeastern Minnesota),

Wisconsin (except for southern Wisconsin) and Michigan (except for the southeastern lower peninsula) (Figure 3.5A). Most of Ohio and southern areas of the North Central region were less variable in temperature. Overall, annual deviations in temperature in the North Central region were greatest in northern Iowa and Minnesota (Figure 3.5B). The southern and eastern edges of the North Central region, including most of the lower peninsula and the eastern half of the upper peninsula of Michigan, were least variable in annual temperature.

Favorable years for epizootics in the North Central region, 1971-2000

The spring climate experienced at the epizootic-specific sites was examined and compared to the 30-yr average of precipitation and temperature for each of those locations (Table 3.1). Mean departures from 30-yr averages of temperature were minimal, typically less than half a degree Celsius for April, May or June. Total precipitation in May and June, however, tended to be much greater than the 30-yr average (Table 3.1). Nearly 75% of the epizootic-specific sites had May or June precipitation that exceeded the 30-yr average precipitation by more than 40% and more than 90% of the epizootic-specific sites had May or June precipitation that exceeded the 30-yr average precipitation by more than 25%.

The average scenario estimates generally suggest that adequate precipitation for *E. maimaiga* epizootics occurred in less than a third of the years between 1971 and 2000 in the North Central region (Figure 3.6A-C). Large areas of Minnesota, Wisconsin and Michigan received adequate precipitation for

an *E. maimaiga* epizootic in 6 or fewer years. The best-case scenario estimates generally suggest that much of the North Central region received adequate precipitation for *E. maimaiga* epizootics to occur in at least 19 of the 30 years (Figure 3.6D-F). Portions of Minnesota, Wisconsin and Michigan, however, only received adequate precipitation in 13-18 of the years between 1971 and 2000.

Based on temperature alone, the number of years from 1971 to 2000 estimated to be favorable for the development of *E. maimaiga* epizootics in the North Central region exhibited a stronger geographic pattern than estimates solely based on precipitation (Figure 3.7A-F). The average scenario estimates generally suggest that only the southern portion of the North Central region received adequate temperature for *E. maimaiga* epizootics to occur in 13 or more years between 1971 and 2000 (Figure 3.7A-C). Temperatures in Minnesota, Wisconsin and Michigan generally met or exceeded average scenario estimates in 6 or fewer years from 1971 to 2000. The best-case scenario estimates followed a similar, though less robust, trend (Figure 3.6D-F), with northern portions of Minnesota, Wisconsin and Michigan reaching adequate temperatures in 6 or fewer years between 1971 and 2000.

Based on both precipitation and temperature, the number of years from 1971 to 2000 estimated to be favorable for the development of *E. maimaiga* epizootics in the North Central region also exhibited a strong geographic pattern. Northern areas generally had fewer favorable years than southern areas (Figure 3.8A-F). The average scenario estimates suggest that much of the North Central region, except for the most southern portions, met or

exceeded adequate levels of precipitation and temperature in 6 or fewer years over the 30-yr period (Figure 3.8A-C). The best-case scenario estimates were not as conservative and much of the area had adequate climate in 13 or more years. Only northern portions of Minnesota, Wisconsin and Michigan met or exceeded adequate levels of precipitation and temperature in 6 or fewer years between 1971 and 2000 (Figure 3.8D-F).

DISCUSSION

Climatic similarity to the current range of an organism is one of several factors that have been proposed to influence the likelihood of establishment of a nonindigenous species (Williamson 1996, NCR 2002). Previous researchers have used CLIMEX to estimate the potential geographic distributions of many exotic species (e.g. Samways et al. 1999, Scott and Yeoh 1999, Venette and Hutchison 1999, Holt and Boose 2000, Matsuki et al. 2001; see Sutherst et al. 1999 for list of other CLIMEX citations prior to 1999) and some biological control agents (e.g. Worner et al. 1989, Scott 1992, Julien et al. 1995, Palmer et al. 2000). Results from the CLIMEX climate-matching analyses in this study show that a relatively small area south of the Great Lakes region was fairly similar ($\geq$ 80%) in overall spring climate to one of the 11 epizootic-specific sites. I found that the area along the southern portion of the North Central region, extending from Kansas and Nebraska east to the Atlantic coast, experiences climatic conditions that are conducive for the development of *E. maimaiga* epizootics in an average year. However, while CLIMEX serves as a useful tool for initial estimation of a species potential distribution, it uses relatively coarse descriptors of climate. Phenology of gypsy moth larvae and the development of *E. maimaiga* epizootics are mediated by current-year meteorological factors and are not satisfactorily defined by calendar months. While monthly-based meteorological factors are coarse descriptors of climate and are not adequate for more sensitive analyses of *E. maimaiga* epizootic development, calendar

months are the most available units for long-term comparisons of meteorological data. Additionally, CLIMEX does not account for the temporal variability of precipitation and temperature which could strongly affect *E. maimaiga* and gypsy moth dynamics.

Organisms with broad ecological tolerances are more likely to become established in climatically variable environments, while those with narrow tolerances, such as the *E. maimaiga* fungal pathogen, are more likely to become established in climatically stable areas that are physiologically appropriate for that species (Leigh 1981, Crawley 1986). My assessment of climate in the North Central region shows that precipitation and temperature conditions are not uniformly consistent throughout the region and that some areas are more climatically variable on a year to year basis than others. Portions of the North Central region with high climatic variability may not experience the particular conditions necessary for the development of *E. maimaiga* epizootics as often as areas with low climatic variability. Alternatively, in regions where climate comparisons suggest that conditions may be not favorable for the development of *E. maimaiga* epizootics, highly variable areas are more likely than areas with low variability to periodically experience suitable climatic conditions for epizootics to occur, if adequate fungal inoculum and suitable hosts are present. This suggests that climate comparisons based on 30-yr averages only, may not provide an adequate description of an area's climatic conduciveness for *E. maimaiga*. Additionally, sites may vary more in one meteorological parameter than another at a given time of year which could

affect *E. maimaiga* germination and infection rates and, thus, the likelihood that an epizootic may develop. Areas, such as the southern portion of the North Central region, that are fairly similar in overall spring climate to one of the epizootic-specific sites are also the least variable in temperature but the most variable in precipitation.

In my assessment of climate in the North Central region, I conducted climate comparisons and examined deviations of meteorological factors from 30-yr averages for spring and annual climate data. It is currently unclear specifically how *E. maimaiga* is affected by spring versus annual climate (Hajek 1999). Spring climate is likely to be integral to the development of *E. maimaiga* epizootics, based on the organism's close phenological association with gypsy moth larval development (Hajek and Roberts 1991). Annual climate, on the other hand, may affect survival of *E. maimaiga* resting spores, their persistence in the environment, or perhaps the synchrony of resting spore germination with gypsy moth larvae. In addition, climatic deviations from 30-yr averages (i.e. normal conditions) are not uniform throughout the year and trends differ for precipitation and temperature. For instance, more than a quarter of the total departures in precipitation from normal conditions occur in the spring, with May and June precipitation being generally more variable than April precipitation. Alternatively, less than a quarter of the total departures in temperature from normal conditions occur in the spring, with April and May temperatures being generally more variable than June temperatures.

Based on the meteorological conditions associated with the epizootic-

specific sites, I estimated the number of years that conditions from 1971 to 2000 were met or exceeded in the North Central region. *Entomophaga maimaiga* epizootics used in this study occurred over a relatively broad range of meteorological conditions, but were not distributed evenly over the known range of gypsy moth and *E. maimaiga*. Whether this adequately represents the range of meteorological conditions necessary for the development of an *E. maimaiga* epizootic or is an artifact of the documented epizootics being relatively confined geographically, and therefore potentially limited in meteorological stochasticity, remains to be determined.

While moisture clearly influences *E. maimaiga* dynamics (Hajek 1999), few studies have associated levels of *E. maimaiga* infection with meteorological field conditions. Weseloh and Andreadis (1992b) found that a 1989 *E. maimaiga* epizootic in Fairfield County, Connecticut was positively associated with above average precipitation in May and June. In 1991, *E. maimaiga* infections were positively correlated with May precipitation across plots in four states (Hajek et al. 1996b). Following introductions in Michigan, Smitley et al. (1995) found *E. maimaiga* infection levels were positively associated with precipitation two weeks before gypsy moth larvae were sampled.

This study further substantiates that the majority of documented *E. maimaiga* epizootics that have occurred, with few possible exceptions, have been positively associated with abundant, above average precipitation in May and June. A few other *E. maimaiga* epizootics that have been documented in the literature (e.g. Hajek 1997a, Hajek and Humber 1997, Hajek et al. 1990b,

1999) were not included in these analyses because I was unable to obtain complete year-specific weather data for the locations. Hajek et al. (1990b) associated *E. maimaiga* epizootics in four research plots in central Massachusetts in 1989 with precipitation in May and June that was above the 30-yr average. However, epizootics in Tompkins County, New York, were recorded in 1992 (Hajek 1997a, Hajek and Humber 1997, Hajek et al. 1999), when only slightly above average precipitation occurred. Additionally, an *E. maimaiga* epizootic is reported to have occurred in Tompkins County, New York, in 1991 (Hajek 1997a, Hajek et al. 1999) which had below normal precipitation in May and June. Unfortunately, the level of *E. maimaiga* infection was not quantified and Hajek (1997a) reported that gypsy moth populations were not reduced substantially. Other locations with documented *E. maimaiga* infections were not included in analyses because I could not conclusively discern whether or not a large-scale epizootic (>60% mortality) or some lower level of infection had occurred.

The results of this research have implications for gypsy moth management in the North Central region, especially for areas along the leading edge where gypsy moth populations are expanding or have recently become established. States in the southern portion of the North Central region, including Indiana, Illinois, Kentucky, Missouri and Ohio, appear to be more climatically conducive for the development of frequent *E. maimaiga* epizootics than states in the northern portion of the region. The northern tier of states in the North Central region, specifically Minnesota, Wisconsin and Michigan, do not appear

to consistently receive adequate levels of precipitation or temperature necessary for frequent *E. maimaiga* epizootics. Additionally, these areas tend to be least variable in precipitation compared to the rest of the region, suggesting that the likelihood of a precipitation event necessary for an epizootic may be relatively uncommon. Unfortunately, Minnesota, Wisconsin and Michigan have some of the most susceptible forests to gypsy moth, as measured by the total basal area of preferred tree species, in the North Central region (Liebhold et al. 1997a, 1997b). This may result in larger, more damaging gypsy moth populations and potentially a greater rate of spread, though this remains to be determined. Lower, non-epizootic levels of *E. maimaiga* infection, however, may still be beneficial in managing gypsy moth populations in these regions. Complete assessment of the role of climatic variability and the occurrence of *E. maimaiga* epizootics in the North Central region will only be achieved through long-term monitoring and landscape-level studies. Thorough examination of *E. maimaiga* infection dynamics under varying meteorological conditions and corresponding interactions with other natural enemies will be needed to determine the extent to which this fungal pathogen will serve in the successful management of gypsy moth in North America.

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Table 3.1. Summary of *Entomophaga maimaiga* epizootic locations used in the CLIMEX climate-matching analyses. Following each location are the observed conditions, 30-yr averages and departures from 30-yr averages of precipitation (mm) and air temperature (°C) from the nearest available weather station for April, May and June. For all sites combined, climatic parameters that were significantly different from 30-yr averages ($P < 0.05$) are designated with a double asterisk (**) following the value.

Site of epizootic			Source	Month	Precipitation (mm)			Air temperature (°C)		
Year	State	County			Observed	30-yr average	Departure from 30-yr average	Observed	30-yr average	Departure from 30-yr average
1989	Connecticut	Fairfield	Andreadis and Weseloh 1990a, 1990b	April	80	101	-21	8.8	9.3	-0.5
			Weseloh and Andreadis 1992b	May	242	102	140	15.3	15.0	0.3
				June	142	90	52	20.3	20.1	0.2
1989	Massachusetts	Hampshire	Hajek and Roberts 1991	April	109	97	12	6.5	8.2	-1.7
				May	223	105	118	15.6	14.4	1.2
				June	146	97	49	20.2	19.2	1.0
1995	Maryland	Queen Anne's	Hajek et al. 1998a	April	88	72	16	10.6	10.8	-0.2
				May	140	96	44	13.8	15.8	-2.0
				June	97	91	6	18.2	20.2	-2.1
1996	Maryland	Worchester	Hajek et al. 1998b	April	141	74	67	4.6	6.4	-1.8
				May	72	75	-3	12.8	13.3	-0.5
				June	149	87	62	16.8	17.9	-1.1

1993	Michigan	Lake	Smitley et al. 1995	April	47	72	-25	12.8	10.7	2.1
				May	52	96	-44	13.4	15.7	-2.3
				June	42	91	-49	21.5	20.2	1.3
1999	Michigan	Clare	N.W. Siegert, unpublished data	April	44	80	-36	12.1	11.5	0.6
				May	151	100	51	16.3	16.3	0.0
				June	141	101	40	21.3	20.6	0.7
1992	Virginia	Augusta	Hajek et al. 1996a	April	55	88	-33	12.0	11.7	0.4
				May	137	108	29	16.9	16.9	0.0
				June	55	102	-47	22.4	21.7	0.7
1994	Virginia	Augusta	Hajek et al. 1996b	April	36	80	-44	12.9	12.9	0.1
				May	104	100	4	16.9	17.4	-0.5
				June	432	104	328	20.6	21.6	-1.0
1995	Virginia	Page	Hajek et al. 1998c	April	103	76	27	13.8	13.4	0.5
				May	96	93	3	17.9	18.1	-0.3
				June	123	87	36	22.8	22.3	0.5
1995	Virginia	Rockbridge	Webb et al. 1999	April	65	80	-15	13.3	12.8	0.5
				May	125	100	25	17.5	17.5	0.1
				June	110	103	7	22.3	21.6	0.7

1996	Virginia	Rockbridge	Webb et al. 1999	April	61	64	-3	8.1	6.7	1.4
				May	60	74	-14	14.5	13.4	1.2
				June	164	82	82	19.8	18.5	1.4
			All sites combined	April	75 ± 10	81 ± 3	-5 ± 10	10.5 ± 0.9	10.4 ± 0.7	0.1 ± 0.4
				May	128 ± 19	95 ± 3	32 ± 17**	15.5 ± 0.5	15.8 ± 0.5	-0.3 ± 0.3
				June	117 ± 13	94 ± 2	51 ± 30**	20.6 ± 0.5	20.3 ± 0.4	0.2 ± 0.3

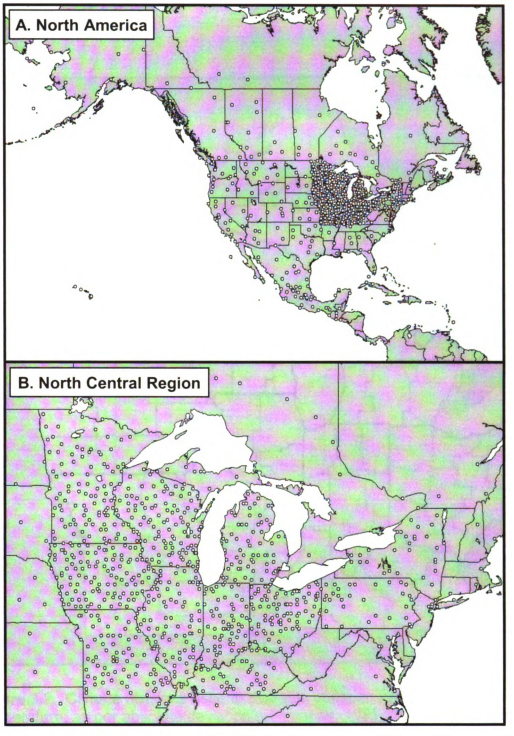

Figure 3.1. Symbols (○) mark the North American locations used in the CLIMEX climate-matching analyses (n = 1132 locations) in (A) North America. Additional locations were added to the CLIMEX database to better represent climatic variability in (B) the North Central region of the United States (n = 832 locations).

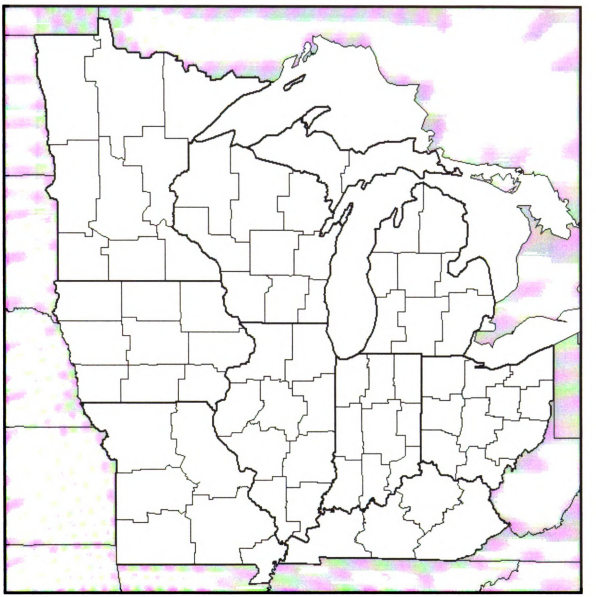

Figure 3.2. Climate divisions used to evaluate variation in precipitation and temperature, as per absolute deviation summaries, 1971-2000, in the North Central states of Illinois, Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, Ohio and Wisconsin (*adapted from* MRCC 2002).

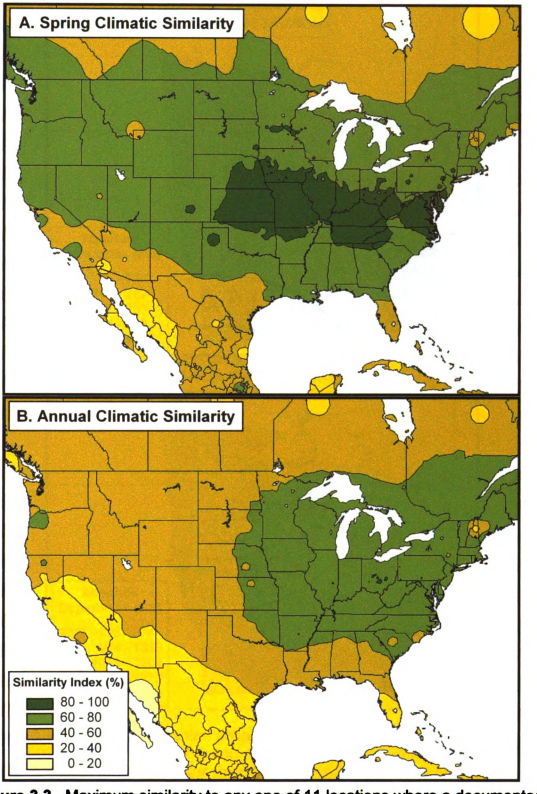

Figure 3.3. Maximum similarity to any one of 11 locations where a documented *Entomophaga maimaiga* epizootic occurred, based on overall climatic similarity (i.e. Match Index), using (A) spring and (B) annual climate data. Images in this figure are presented in color.

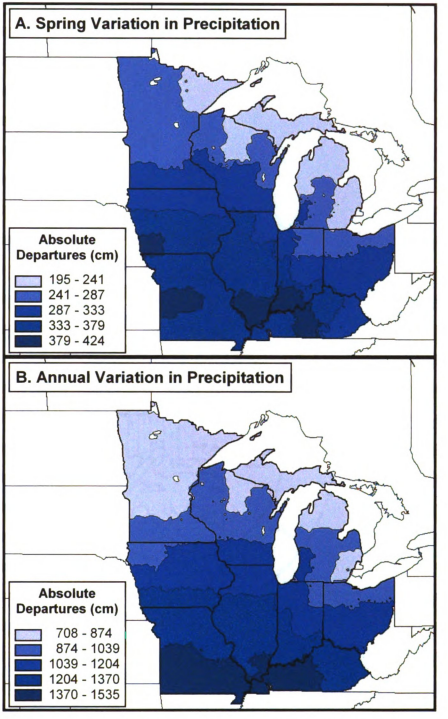

Figure 3.4. North Central region absolute departures from 30-yr averages, 1971-2000, of (A) spring and (B) annual precipitation (cm) (*adapted from* MRCC 2002). Areas covered by darker shades of blue indicate greater absolute departures from 30-yr averages (i.e. 30-yr sum of absolute departures) for precipitation than areas covered by lighter shades of blue for the North Central region. Images in this figure are presented in color.

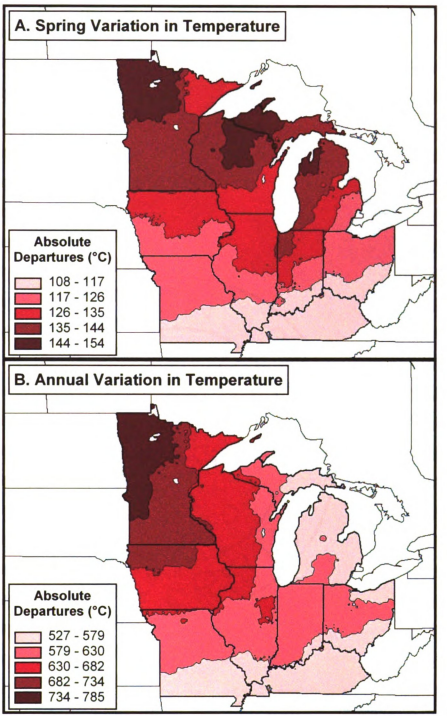

Figure 3.5. North Central region absolute departures from 30-yr averages, 1971-2000, of (A) spring and (B) annual temperature (°C) (*adapted from* MRCC 2002). Areas covered by darker shades of red indicate greater absolute departures from 30-yr averages (i.e. 30-yr sum of absolute departures) for temperature than areas covered by lighter shades of red for the North Central region. Images in this figure are presented in color.

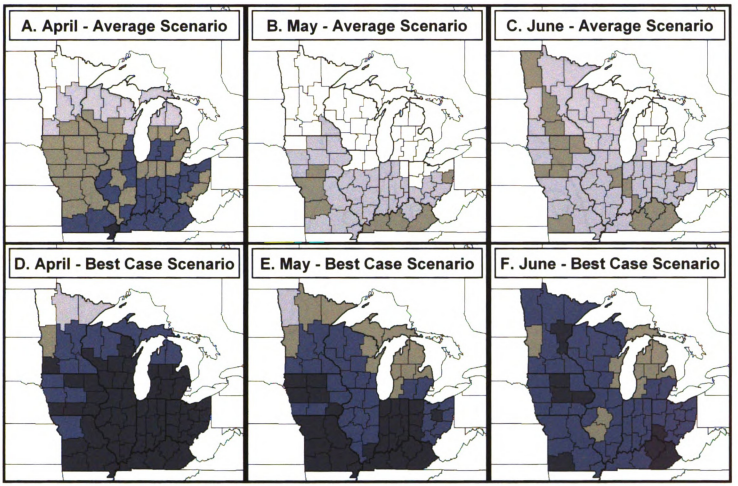

Figure 3.6. Number of years from 1971 to 2000 in the North Central region with precipitation similar to several documented *Entomophaga maimaiga* epizootics in April, May and June. A year was considered favorable for an epizootic if the precipitation during a given time period met or exceeded (A - C) the average precipitation or (D - F) the average precipitation minus the standard deviation (i.e. best case scenario) that occurred in areas with documented epizootics. Images in this figure are presented in color.

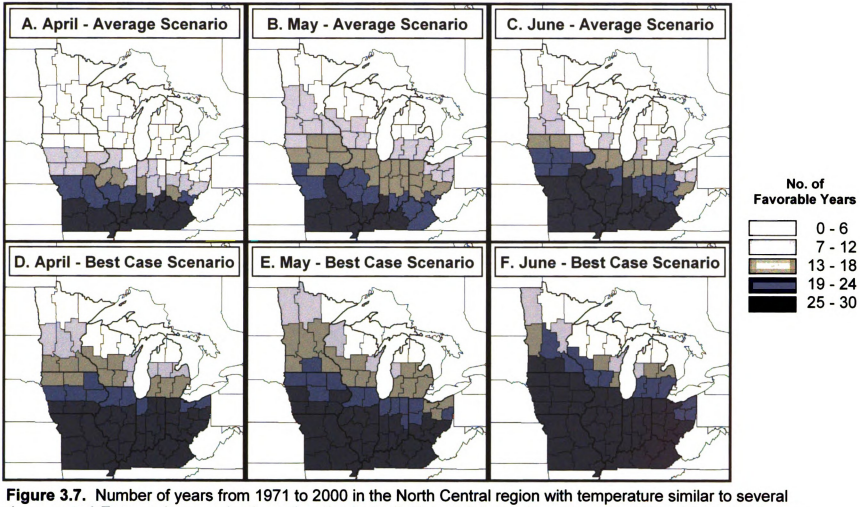

Figure 3.7. Number of years from 1971 to 2000 in the North Central region with temperature similar to several documented *Entomophaga maimaiga* epizootics in April, May and June. A year was considered favorable for an epizootic if the temperature during a given time period met or exceeded (A - C) the average temperature or (D - F) the average temperature minus the standard deviation (i.e. best case scenario) that occurred in areas with documented epizootics. Images in this figure are presented in color.

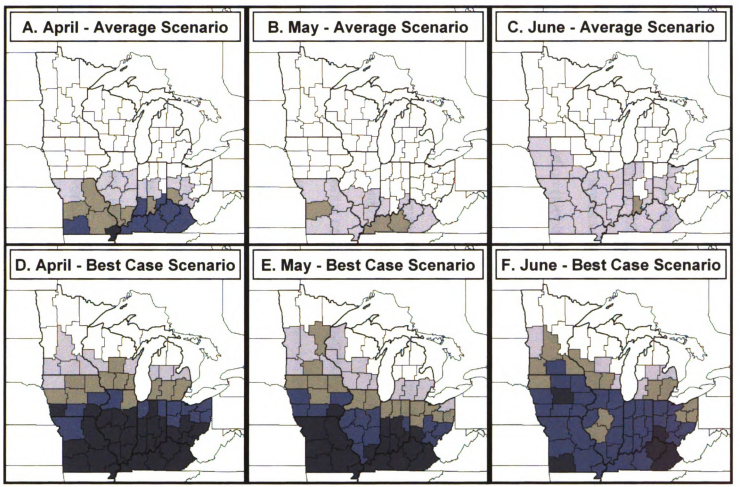

Figure 3.8. Number of years from 1971 to 2000 in the North Central region with precipitation and temperature similar to several documented *Entomophaga maimaiga* epizootics in April, May and June. A year was considered favorable for an epizootic if both the precipitation and temperature during a given time period met or exceeded (A - C) the average conditions or (D - F) the average conditions minus the respective standard deviations (i.e. best case scenario) that occurred in areas with documented epizootics. Images in this figure are presented in color.

MANAGEMENT IMPLICATIONS

The range of gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae), in North America continues to expand from its initial introduction in the northeastern United States. Gypsy moth populations are currently spreading into the North Central region and have recently become established in Illinois, Indiana, Ohio and Wisconsin (NAPIS 2004). Gypsy moth populations threaten to expand into areas with large extents of forest that are highly susceptible to defoliation (Liebhold et al. 1997a, 1997b). Resource managers in these areas are very interested in incorporating *E. maimaiga* into their gypsy moth management strategies. However, if the pattern of epizootics observed in Michigan is representative of what may be expected in other states in the North Central region, then *E. maimaiga* may not reliably suppress gypsy moth populations on a consistent basis. The success of *E. maimaiga* is directly dependent on environmental conditions and effective suppression of gypsy moth with this fungal pathogen in the North Central region will likely be mediated by local variability of microclimate.

Results from this research indicate that *E. maimaiga* infection is associated with environmental moisture and that above average precipitation may be necessary for the development of epizootics in the northern portions of the North Central region. Increased levels of *E. maimaiga* infection in field bioassays in Michigan were correlated with June precipitation levels which were significantly greater than 30-year average conditions. In a 6-wk field study,

infection of gypsy moth larvae through primary transmission by *E. maimaiga* occurred from late May to early July. Infection rates of *E. maimaiga during* primary transmission were somewhat variable but were generally associated with the number of hours that precipitation occurred.

The apparent association of increased levels of *E. maimaiga* infection with above average precipitation suggests that the development of epizootics may be strongly affected by climatic variability. The northern tier of states in the North Central region, specifically Minnesota, Wisconsin and Michigan, do not appear to consistently receive adequate levels of precipitation necessary for frequent *E. maimaiga* epizootics. Additionally, these areas tend to be least variable in precipitation compared to the rest of the region, suggesting that the likelihood of a precipitation event necessary to initiate an epizootic may be relatively uncommon. As these areas have some of the most susceptible forests to gypsy moth, this may result in larger, more damaging gypsy moth populations and potentially a greater rate of spread, though this remains to be determined.

Despite *E. maimaiga*'s apparent mediation by variability of microclimate in northern portions of the North Central region, continued efforts to introduce and establish this fungal pathogen in areas where gypsy moth has recently become established or is expanding into should still be attempted. Emphasis, however, should be placed on developing an integrated approach for managing gypsy moth in these areas and not assuming that *E. maimaiga* epizootics will consistently suppress gypsy moth populations. Lower levels of *E. maimaiga*

infection between large-scale epizootics may still be beneficial in managing gypsy moth populations by maintaining adequate levels of fungal inoculum in the soil. The frequency of epizootics and the interactions of *E. maimaiga* with other natural enemies between epizootics may ultimately determine the role that this fungal pathogen plays in managing gypsy moth in northern regions.

Much remains to be learned about *E. maimaiga* and its effect on gypsy moth populations in the North Central region. Future research should involve thorough examination of *E. maimaiga* infection dynamics and corresponding interactions with other natural enemies, especially NPV, under varying meteorological conditions. The infection dynamics of these pathogens and the role of microclimatic factors should be evaluated for transmission-specific (i.e. primary versus secondary transmission) stages in the development of epizootics. Evaluation of hourly microclimatic conditions during larval development in stands with a range of gypsy moth population densities would improve our understanding of the primary and secondary transmission dynamics of *E. maimaiga* and NPV. Additionally, this could improve our understanding of the requisite host and pathogen population sizes necessary for the development of large-scale epizootics. Future research involving landscape-level studies and long-term monitoring will be needed to fully assess the role of climatic variability and could significantly aid in the development of our ability to accurately predict epizootics in North American gypsy moth populations. This information would be useful in developing improved methods to successfully incorporate *E. maimaiga* into an integrated pest management system for the effective control

of gypsy moth, as it's range expands through in North America.

APPENDICES

APPENDIX A

Appendix A

Record of Deposition of Voucher Specimens

The specimens listed on the following sheets have been deposited in the named museum as samples of those species or other taxa which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: MSU 2003-04
ARSEF 6626 - 6630
ARSEF 6652 - 6657
ARSEF 6663 - 6668
ARSEF 6724 - 6729
ARSEF 7103, 7107 - 7111

Title of dissertation:

Meteorological factors affecting the success of the gypsy moth fungal pathogen *Entomophaga maimaiga* (Zygomycetes: Entomophthorales) in Michigan

Museums where deposited and abbreviations for table on following sheets:

1) A.J. Cook Arthropod Research Collection
Department of Entomology, Michigan State University (MSU)
243 Natural Sciences Building, East Lansing, Michigan 48824-1115

Gary L. Parsons, Curator

2) United States Department of Agriculture, Agricultural Research Service
Collection of Entomopathogenic Fungal Cultures (ARSEF)
United States Plant, Soil, and Nutrition Laboratory
Tower Road, Ithaca, New York 14853 - 2901

Richard A. Humber, Curator

Investigator's Name: Nathan Wade Siegert

Date: 13 March 2003

The Voucher No. are assigned by the respective curators at ARSEF & MSU.

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Lepidoptera: Lymantriidae *Lymantria dispar* L. Laboratory culture One *L. dispar* larval cadaver deposited per label.	*L. dispar* egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 2-6 June 1999 at T20N, R05E, S31, SW, Arenac Co. Michigan	MSU
	L. dispar egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 6-10 June 1999 at T42N, R02E, S15, NW, Chippewa Co. Michigan	MSU
	L. dispar egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 6-10 June 1999 at T20N, R05W, S17, SE, Clare Co. Michigan	MSU

Investigator's Name

Nathan Wade Siegert

Date 13-Mar-2003

Voucher No.: 2003-04
Received the above listed specimens for deposit in the A.J. Cook Arthropod Research Collection, Michigan State University.

Curator Date 14 MAY 2004

Appendix A

Voucher Specimen Data

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Lepidoptera: Lymantriidae *Lymantria dispar* L. Laboratory culture One *L. dispar* larval cadaver deposited per label.	*L. dispar* egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 6-10 June 1999 at T26N, R04W, S34, NE, Crawford Co. Michigan *L. dispar* egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 3-7 June 1999 at T27N, R09W, S28, NW, Grand Traverse Co. Michigan *L. dispar* egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 8-12 June 1999 at T01S, R09W, S22, SW, Kalamazoo Co. Michigan	MSU MSU MSU

Investigator's Name

Nathan Wade Siegert

Date 13-Mar-2003

Voucher No.: 2003-04
Received the above listed specimens for deposit in the A.J. Cook Arthropod Research Collection, Michigan State University.

Curator

14 May 2004
Date

Appendix A

Voucher Specimen Data

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Lepidoptera: Lymantriidae *Lymantria dispar* L. Laboratory culture One *L. dispar* larval cadaver deposited per label.	*L. dispar* egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 2-6 June 1999 at T25N, R06W, S22, NE, Kalkaska Co. Michigan *L. dispar* egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 2-6 June 1999 at T25N, R06W, S33, NE, Kalkaska Co. Michigan *L. dispar* egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 7-11 June 1999 at T18N, R14W, S04, SE, Lake Co. Michigan	MSU MSU MSU

Investigator's Name

Nathan Wade Siegert

Date 13-Mar-2003

Voucher No.: 2003-04

Received the above listed specimens for deposit in the A.J. Cook Arthropod Research Collection, Michigan State University.

Curator Date 14 May 2004

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Lepidoptera: Lymantriidae *Lymantria dispar* L. Laboratory culture	*L. dispar* egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 7-11 June 1999 at T19N, R14W, S13, NE, Lake Co. Michigan	MSU
One *L. dispar* larval cadaver deposited per label.	*L. dispar* egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 7-11 June 1999 at T19N, R14W, S22, SE, Lake Co. Michigan	MSU
	L. dispar egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 3-7 June 1999 at T20N, R13W, S35, NW, Lake Co. Michigan	MSU

Investigator's Name

Nathan Wade Siegert

Date 13-Mar-2003

Voucher No.: 2003-04
Received the above listed specimens for deposit in the A.J. Cook Arthropod Research Collection, Michigan State University.

Curator 14 MAY 2004 Date

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Lepidoptera: Lymantriidae *Lymantria dispar* L. Laboratory culture	*L. dispar* egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 5-9 June 1999 at T21N, R14W, S30, NW, Manistee Co. Michigan	MSU
One *L. dispar* larval cadaver deposited per label.	*L. dispar* egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 5-9 June 1999 at T18N, R15W, S31, NW, Mason Co. Michigan	MSU
	L. dispar egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 5-9 June 1999 at T20N, R15W, S18, SE, Mason Co. Michigan	MSU

Investigator's Name

Nathan Wade Siegert

Date 13-Mar-2003

Voucher No.: 2003-04
Received the above listed specimens for deposit in the A.J. Cook Arthropod Research Collection, Michigan State University.

14 MAY 2004

Curator Date

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Lepidoptera: Lymantriidae *Lymantria dispar* L. Laboratory culture	*L. dispar* egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 8-12 June 1999 at T13N, R13W, S12, SE, Newaygo Co. Michigan	MSU
One *L. dispar* larval cadaver deposited per label.	*L. dispar* egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 8-12 June 1999 at T14N, R13W, S26, SW, Newaygo Co. Michigan	MSU
	L. dispar egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 4-8 June 1999 at T15N, R13W, S07, NE, Newaygo Co. Michigan	MSU

Investigator's Name

Nathan Wade Siegert

Date 13-Mar-2003

Voucher No.: 2003-04
Received the above listed specimens for deposit in the A.J. Cook Arthropod Research Collection, Michigan State University.

14 MAY 2004

Curator Date

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Lepidoptera: Lymantriidae *Lymantria dispar* L. Laboratory culture One *L. dispar* larval cadaver deposited per label.	*L. dispar* egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 8-12 June 1999 at T15N, R13W, S34, SE, Newaygo Co. Michigan	MSU
	L. dispar egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 3-7 June 1999 at T16N, R12W, S30, NW, Newaygo Co. Michigan	MSU
	L. dispar egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 4-8 June 1999 at T16N, R13W, S16, SE, Newaygo Co. Michigan	MSU

Investigator's Name

Nathan Wade Siegert

Date 13-Mar-2003

Voucher No.: 2003-04
Received the above listed specimens for deposit in the A.J. Cook Arthropod Research Collection, Michigan State University.

14 May 2004
Curator Date

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Lepidoptera: Lymantriidae *Lymantria dispar* L. Laboratory culture One *L. dispar* larval cadaver deposited per label.	*L. dispar* egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 4-8 June 1999 at T16N, R13W, S19, SW, Newaygo Co. Michigan	MSU
	L. dispar egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 4-8 June 1999 at T16N, R14W, S11, NE, Newaygo Co. Michigan	MSU
	L. dispar egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 2-6 June 1999 at T22N, R01W, S23, NE, Roscommon Co. Michigan	MSU

Investigator's Name

Nathan Wade Siegert

Date 13-Mar-2003

Voucher No.: 2003-04
Received the above listed specimens for deposit in the A.J. Cook Arthropod Research Collection, Michigan State University.

14 MAY 2004
Curator Date

Appendix A

Voucher Specimen Data

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Lepidoptera: Lymantriidae *Lymantria dispar* L. Laboratory culture One *L. dispar* larval cadaver deposited per label.	*L. dispar* egg masses obtained from USDA APHIS, Otis Air National Guard Base, Massachusetts; reared on artificial diet at USDA APHIS PPQ Biological Control Laboratory, Niles, Michigan; 4th instars exposed to soil 6-10 June 1999 at T24N, R04W, S03, SE, Roscommon Co. Michigan	MSU

Investigator's Name

Nathan Wade Siegert

Date 13-Mar-2003

Voucher No.: 2003-04
Received the above listed specimens for deposit in the A.J. Cook Arthropod Research Collection, Michigan State University.

14 MAY 2004

Curator Date

Appendix A

Voucher Specimen Data

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Zygomycetes: Entomophthorales *Entomophaga maimaiga* Humber, Shimazu & Soper	ARSEF 6626 (00 MI 17-1-1): collected 16 June 2000 as soil from T16N, R13W, S16, SE, Newaygo Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 4-6 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF
	ARSEF 6628 (00 MI 19-1-1): collected 16 June 2000 as soil from T16N, R13W, S24, SE, Newaygo Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 4-6 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF
	ARSEF 6629 (00 MI 24-2-1): collected 13 June 2000 as soil from T13N, R13W, S12, SE, Newaygo Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 4-6 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF

Investigator's Name

Nathan Wade Siegert

Date

Appendix A

Voucher Specimen Data

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Zygomycetes: Entomophthorales *Entomophaga maimaiga* Humber, Shimazu & Soper	ARSEF 6630 (00 MI 27-1-1): collected 11 June 2000 as soil from T25N, R06W, S33, NE, Kalkaska Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 5-7 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF
	ARSEF 6652 (00 MI 2-1-1): collected 11 June 2000 as soil from T27N, R09W, S28, NW, Grand Traverse Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 2-5 October 2000; isolated into 95% Grace's 5% FBS 20 October 2000 by MM Wheeler.	ARSEF
	ARSEF 6653 (00 MI 4-2-1): collected 14 June 2000 as soil from T21N, R14W, S30, NW, Manistee Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 2-5 October 2000; isolated into 95% Grace's 5% FBS 20 October 2000 by MM Wheeler.	ARSEF

Investigator's Name

Nathan Wade Siegert

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Voucher Specimen Data

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Zygomycetes: Entomophthorales *Entomophaga maimaiga* Humber, Shimazu & Soper	ARSEF 6654 (00 MI 18-2-1): collected 16 June 2000 as soil from T16N, R13W, S19, SW, Newaygo Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 4-7 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF
	ARSEF 6655 (00 MI 28-2-1): collected 15 June 2000 as soil from T24N, R04W, S03, SE, Roscommon Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 5-8 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF
	ARSEF 6656 (00 MI 29-2-1): collected 15 June 2000 as soil from T22N, R01W, S23, NE, Roscommon Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 5-8 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF

Investigator's Name

Nathan Wade Siegert

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Voucher Specimen Data

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Zygomycetes: Entomophthorales *Entomophaga maimaiga* Humber, Shimazu & Soper	ARSEF 6657 (00 MI 33-1-1): collected 12 June 2000 as soil from T18N, R06W, S27, NE, Clare Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 5-8 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF
	ARSEF 6663 (00 MI 2-2-1): collected 11 June 2000 as soil from T27N, R09W, S28, NW, Grand Traverse Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 2-5 October 2000; isolated into 95% Grace's 5% FBS 20 October 2000 by MM Wheeler.	ARSEF
	ARSEF 6664 (00 MI 3-1-1): collected 14 June 2000 as soil from T21N, R15W, S23, NE, Manistee Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 2-5 October 2000; isolated into 95% Grace's 5% FBS 20 October 2000 by MM Wheeler.	ARSEF

Investigator's Name

Nathan Wade Siegert

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Appendix A

Voucher Specimen Data

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Zygomycetes: Entomophthorales *Entomophaga maimaiga* Humber, Shimazu & Soper	ARSEF 6665 (00 MI 3-1-2): collected 14 June 2000 as soil from T21N, R15W, S23, NE, Manistee Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 2-5 October 2000; isolated into 95% Grace's 5% FBS 20 October 2000 by MM Wheeler.	ARSEF
	ARSEF 6666 (00 MI 12-2-1): collected 10 June 2000 as soil from T19N, R14W, S13, NE, Lake Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 3-6 October 2000; isolated into 95% Grace's 5% FBS 20 November 2000 by MM Wheeler.	ARSEF
	ARSEF 6667 (00 MI 12-2-2): collected 10 June 2000 as soil from T19N, R14W, S13, NE, Lake Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 3-6 October 2000; isolated into 95% Grace's 5% FBS 20 November 2000 by MM Wheeler.	ARSEF

Investigator's Name

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Voucher Specimen Data

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Zygomycetes: Entomophthorales *Entomophaga maimaiga* Humber, Shimazu & Soper	ARSEF 6668 (00 MI 12-2-3): collected 10 June 2000 as soil from T19N, R14W, S13, NE, Lake Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 3-6 October 2000; isolated into 95% Grace's 5% FBS 20 November 2000 by MM Wheeler.	ARSEF
	ARSEF 6724 (00 MI 3-2-2): collected 14 June 2000 as soil from T21N, R15W, S23, NE, Manistee Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 2-5 October 2000; isolated into 95% Grace's 5% FBS 20 October 2000 by MM Wheeler.	ARSEF
	ARSEF 6725 (00 MI 19-2-1): collected 16 June 2000 as soil from T16N, R13W, S24, SE, Newaygo Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 4-7 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF

Investigator's Name

Nathan Wade Siegert

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Voucher Specimen Data

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Zygomycetes: Entomophthorales *Entomophaga maimaiga* Humber, Shimazu & Soper	ARSEF 6726 (00 MI 24-2-2): collected 13 June 2000 as soil from T13N, R13W, S12, SE, Newaygo Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 4-7 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF
	ARSEF 6727 (00 MI 27-1-2): collected 11 June 2000 as soil from T25N, R06W, S33, NE, Kalkaska Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 5-8 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF
	ARSEF 6728 (00 MI 17-1-2): collected 16 June 2000 as soil from T16N, R13W, S16, SE, Newaygo Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 4-7 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF

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Appendix A

Voucher Specimen Data

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Zygomycetes: Entomophthorales *Entomophaga maimaiga* Humber, Shimazu & Soper	ARSEF 6729 (00 MI 17-2-1): collected 16 June 2000 as soil from T16N, R13W, S16, SE, Newaygo Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 4-7 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF
	ARSEF 7103 (00 MI 29-1-2): collected 15 June 2000 as soil from stand 29, Roscommon Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 5-8 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF
	ARSEF 7107 (00 MI 28-1-1): collected 15 June 2000 as soil from T24N, R04W, S03, SE, Roscommon Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 5-8 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF

Investigator's Name

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Voucher Specimen Data

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Zygomycetes: Entomophthorales *Entomophaga maimaiga* Humber, Shimazu & Soper	ARSEF 7108 (00 MI 28-1-3): collected 15 June 2000 as soil from T24N, R04W, S03, SE, Roscommon Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 5-8 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF
	ARSEF 7109 (00 MI 29-1-1): collected 15 June 2000 as soil from T22N, R01W, S23, NE, Roscommon Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 5-8 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF
	ARSEF 7110 (00 MI 33-2-1): collected 12 June 2000 as soil from T18N, R06W, S27, NE, Clare Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 5-8 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF

Investigator's Name

Nathan Wade Siegert

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Appendix A

Voucher Specimen Data

Species or other taxon	Label data for specimens collected and deposited	Museum where deposited
Zygomycetes: Entomophthorales *Entomophaga maimaiga* Humber, Shimazu & Soper	ARSEF 7111 (00 MI 33-2-2): collected 12 June 2000 as soil from T18N, R06W, S27, NE, Clare Co. Michigan by NW Siegert. *L. dispar* exposed under laboratory conditions to soil 5-8 October 2000; isolated into 95% Grace's 5% FBS 12 October 2000 by MM Wheeler.	ARSEF

Investigator's Name

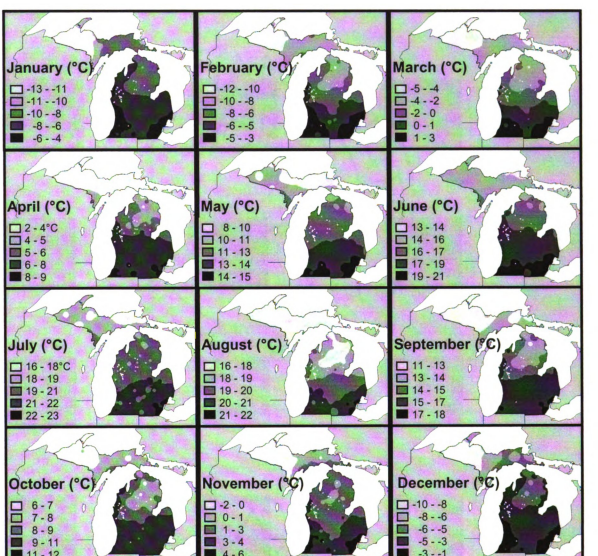
Nathan Wade Siegert

Date

APPENDIX B

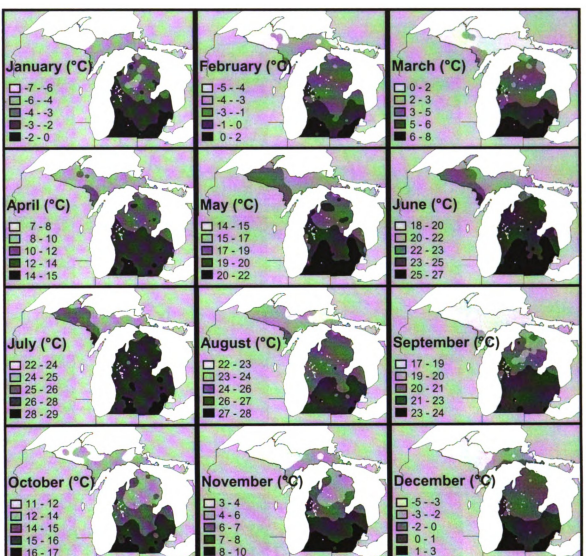
Appendix B

Figure B1. Michigan average daily temperature (°C) January through December, 1961-1990 (*adapted from* MRCC 2000). Temperature gradient varies by month, with areas covered by darker shades of gray indicating higher average daily temperatures than areas covered by lighter shades of gray. White dots indicate locations of *Entomophaga maimaiga* field bioassay sites in Michigan, 1999-2002.



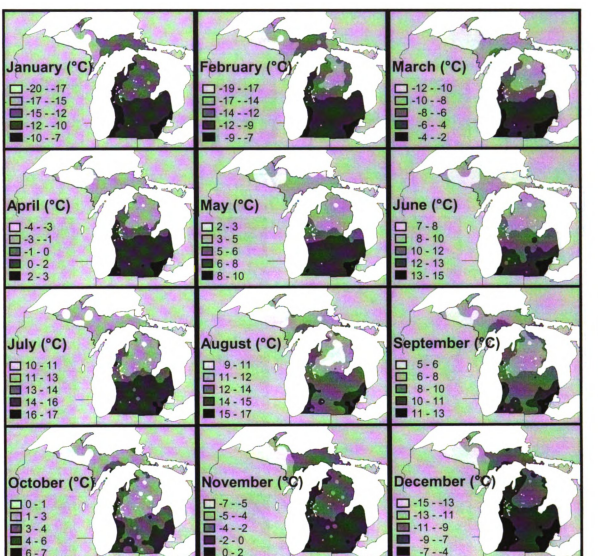
Appendix B

Figure B2. Michigan average daily maximum temperature (°C) January through December, 1961-1990 (*adapted from* MRCC 2000). Temperature gradient varies by month, with areas covered by darker shades of gray indicating higher average daily maximum temperatures than areas covered by lighter shades of gray. White dots indicate locations of *Entomophaga maimaiga* field bioassay sites in Michigan, 1999-2002.



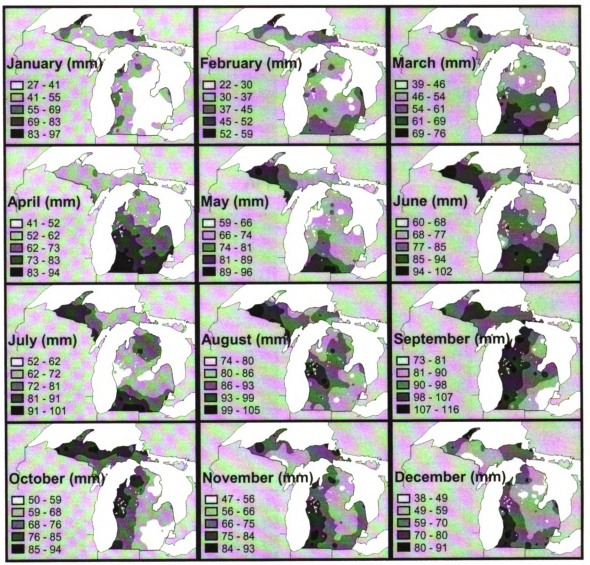
Appendix B

Figure B3. Michigan average daily minimum temperature (°C) January through December, 1961-1990 (*adapted from* MRCC 2000). Temperature gradient varies by month, with areas covered by darker shades of gray indicating higher average daily minimum temperatures than areas covered by lighter shades of gray. White dots indicate locations of *Entomophaga maimaiga* field bioassay sites in Michigan, 1999-2002.



Appendix B

Figure B4. Michigan average monthly precipitation (mm) January through December, 1961-1990 (*adapted from* MRCC 2000). Precipitation gradient varies by month, with areas covered by darker shades of gray indicating higher average monthly precipitation averages than areas covered by lighter shades of gray. White dots indicate locations of *Entomophaga maimaiga* field bioassay sites in Michigan, 1999-2002.



APPENDIX C

Appendix C

Table C1. North American locations used in the CLIMEX climate-matching analyses (n = 1132 locations). Additional locations added to the CLIMEX meteorological database to better represent climatic variability in the North Central region (n = 832 locations) are listed in upper-case characters.

Country	Province/State	Location	Latitude	Longitude
Barbados	*no states*	Bridgetown	13.2 N	59.7 W
Canada	**Alberta**	Banff	51.2 N	115.6 W
		Beaverlodge	55.2 N	119.4 W
		Calgary	51.1 N	114.0 W
		Edmonton	53.6 N	113.5 W
		Embarras	58.2 N	111.4 W
		Fort McMurray	56.7 N	111.2 W
		Grande Prairie	55.2 N	118.9 W
		Jasper	52.9 N	118.1 W
		Keg River	57.8 N	117.9 W
		Lethbridge	49.6 N	112.8 W
		Medicine Hat	50.0 N	110.7 W
	British Columbia	Bull Harbour	50.9 N	127.9 W
		Cranbrook	49.5 N	115.8 W
		Estevan Point	49.4 N	126.5 W
		Fort Nelson	58.8 N	122.6 W
		Hope	49.4 N	121.4 W
		Penticton	49.5 N	119.6 W
		Prince George	53.9 N	122.7 W
		Prince Rupert	54.3 N	130.4 W
		Vancouver	49.2 N	123.2 W
		Victoria	48.4 N	123.3 W
	Manitoba	Churchill	58.8 N	94.1 W
		Dauphin	51.1 N	100.1 W
		Gillam	56.3 N	94.7 W
		Rivers	50.0 N	100.3 W
		The Pas	53.9 N	101.2 W
		Winnipeg	49.9 N	97.2 W
	New Brunswick	Chatham	47.0 N	65.4 W
		Moncton	46.1 N	64.7 W
	Newfoundland	Belle Isle	51.9 N	55.4 W
		Cape Race	46.7 N	53.1 W
		Cartwright	53.7 N	57.0 W
		Fogo	49.7 N	54.3 W
		Goose Bay	53.3 N	60.4 W

Table C1 (cont'd.)

		Grand Bank	47.1 N	55.8 W
		Hopedale	55.5 N	60.2 W
		St Johns	47.6 N	52.7 W
	Northwest Terr.	Fort Resolution	61.2 N	113.7 W
		Fort Simpson	61.9 N	121.3 W
		Nottingham Island	63.1 N	77.9 W
		Resolution Island	61.4 N	64.9 W
	Nova Scotia	Halifax	44.7 N	63.6 W
		Yarmouth	43.8 N	66.1 W
	Ontario	Armstrong	50.3 N	89.0 W
		Earlton	47.7 N	79.8 W
		Kapuskasing	49.4 N	82.5 W
		Lansdowne House	52.2 N	87.9 W
		London	43.0 N	81.2 W
		Moosonee	51.3 N	80.7 W
		Nakina	50.2 N	86.7 W
		North Bay	46.4 N	79.4 W
		Ottawa	45.4 N	75.7 W
		Pagwa	50.0 N	85.3 W
		Pickle Lake	51.5 N	90.3 W
		Porquis Junction	48.7 N	80.8 W
		Sioux Lookout	50.1 N	91.9 W
		Toronto	43.7 N	79.4 W
		Trout Lake	53.8 N	89.9 W
	Prince Edward Is.	Summerside	46.4 N	63.8 W
	Quebec	Fort Chimo	58.1 N	68.4 W
		Great Whale River	55.3 N	77.8 W
		Grindstone Island	47.4 N	61.9 W
		Harrington Harbour	50.5 N	59.5 W
		Inoucdjouac	58.4 N	78.3 W
		Megantic	45.6 N	70.8 W
		Montreal	45.5 N	73.6 W
		Nitchequon	53.2 N	70.9 W
		Quebec	46.8 N	71.3 W
	Saskatchewan	Regina	50.5 N	104.6 W
		Saskatoon	52.1 N	106.6 W
		Swift Current	50.3 N	107.7 W
	Yukon Territory	Watson Lake	60.1 N	128.8 W
		Whitehorse	60.7 N	135.1 W
Cuba	*no states*	Colon	22.7 N	80.8 W
		Habana	23.2 N	82.5 W

Table C1 (cont'd.)

El Salvador	*no states*	San Salvador	13.7 N	89.2 W
Guatemala	*no states*	Coban	15.5 N	90.3 W
		Guatemala City	14.7 N	90.3 W
		Puerto San Jose	13.9 N	90.5 W
		Santa Elena	16.6 N	89.6 W
Mexico	*no states*	Acapulco de Juarez	16.8 N	99.9 W
		Aguascalientes	21.9 N	102.3 W
		Campeche	19.9 N	90.6 W
		Chihuahua	28.6 N	106.1 W
		Chilpancingo	17.5 N	99.5 W
		Ciudad Lerdo	25.5 N	103.5 W
		Colima	19.2 N	103.7 W
		Comitan	16.3 N	92.1 W
		Cozumel	20.5 N	86.9 W
		Culiacan	24.8 N	107.4 W
		Durango	24.0 N	104.7 W
		Ensenada	31.9 N	116.6 W
		Guadalajara	20.7 N	103.4 W
		Guanajuato	21.0 N	101.3 W
		Guaymas	28.0 N	111.0 W
		Hermosillo	29.1 N	111.0 W
		Huejucar	22.4 N	103.2 W
		Isla Guadalupe	29.2 N	118.3 W
		Jalapa Enriquez	19.5 N	96.9 W
		La Paz	24.2 N	110.2 W
		Lagos de Moreno	21.4 N	101.9 W
		Leon	21.1 N	101.7 W
		Manzanillo	19.0 N	104.3 W
		Mazatlan	23.2 N	106.4 W
		Merida	21.0 N	89.6 W
		Mexico	19.4 N	99.2 W
		Monclova	26.9 N	101.4 W
		Monterrey	25.7 N	100.3 W
		Morelia	19.7 N	101.2 W
		Oaxaca de Juarez	17.1 N	96.7 W
		Orizaba	18.9 N	97.1 W
		Pachuca	20.1 N	98.7 W
		Piedras Negras	28.7 N	100.5 W
		Progreso	21.3 N	89.7 W
		Puebla	19.0 N	98.2 W
		Puerto Cortes	24.4 N	111.9 W
		Queretaro	20.6 N	100.4 W
		Rio Verde	21.9 N	100.0 W
		S.Cristobel de Cas	16.7 N	92.6 W
		Salina Cruz	16.2 N	95.2 W
		Saltillo	25.5 N	101.0 W
		San Luis Potosi	22.1 N	101.0 W
		Soto la Marina	23.8 N	98.2 W

Table C1 (cont'd.)

		Tampico	22.2 N	97.8 W
		Tapachula	14.9 N	92.3 W
		Tepic	21.5 N	104.9 W
		Tlaxcala de Xico	19.3 N	98.2 W
		Toluca	19.3 N	99.7 W
		Torreon	25.5 N	103.4 W
		Tulancingo	20.1 N	98.4 W
		Tuxtla Gutierrez	16.8 N	93.1 W
		Veracruz	19.2 N	96.1 W
U.S.A.	**Alabama**	Birmingham	33.6 N	86.8 W
		Mobile	30.7 N	88.0 W
		Montgomery	32.3 N	86.4 W
	Alaska	Anchorage	61.2 N	149.8 W
		Bethel	60.8 N	161.8 W
		Eagle	64.8 N	141.2 W
		Fairbanks	64.3 N	147.9 W
		Gambell	63.8 N	171.8 W
		Juneau	58.3 N	134.3 W
		Ketchikan	55.4 N	131.7 W
		Nome	64.5 N	165.5 W
		St Paul Island	57.2 N	170.2 W
	Arizona	Flagstaff	35.1 N	111.7 W
		Phoenix	33.4 N	112.0 W
		Tucson	32.3 N	110.9 W
		Winslow	35.0 N	110.7 W
		Yuma	32.7 N	114.6 W
	Arkansas	Fort Smith	35.3 N	94.4 W
		Little Rock	34.7 N	92.2 W
	California	Bakersfield	35.4 N	119.0 W
		Bishop	37.3 N	118.4 W
		Eureka	40.8 N	124.2 W
		Fresno	36.8 N	119.7 W
		Los Angeles	33.9 N	118.4 W
		Mount Wilson	34.2 N	118.1 W
		Mt. Shasta	41.3 N	122.3 W
		Red Bluff	40.2 N	122.3 W
		Sacramento	38.5 N	121.5 W
		San Diego	32.7 N	117.2 W
		San Francisco	37.6 N	122.4 W
		San Jose	37.3 N	121.9 W
		Santa Maria	34.9 N	120.4 W
		Stockton	38.0 N	121.3 W
	Colorado	Colorado Springs	38.8 N	104.7 W
		Denver	39.8 N	104.9 W

Table C1 (cont'd.)

	Grand Junction	39.1 N	108.5 W
	Pueblo	38.3 N	104.5 W
Connecticut	New Haven	41.3 N	72.9 W
District of Columbia	Washington	38.9 N	77.1 W
Florida	Jacksonville	30.4 N	81.7 W
	Key West	24.5 N	81.8 W
	Miami	25.8 N	80.3 W
	Pensacola	30.4 N	87.2 W
	Tampa	28.0 N	82.5 W
Georgia	Atlanta	33.7 N	84.4 W
	Thomasville	30.8 N	84.0 W
Hawaii	Hilo	19.7 N	155.1 W
	Honolulu	21.3 N	157.8 W
	Lihue	22.0 N	159.4 W
Idaho	Boise	43.6 N	116.2 W
	Pocatello	42.9 N	112.6 W
Illinois	ALBION	38.4 N	88.1 W
	ALEDO	41.2 N	90.7 W
	ALTON_DAM_26	38.9 N	90.2 W
	ANNA_1_E	37.5 N	89.2 W
	ANTIOCH	42.5 N	88.1 W
	AURORA	41.8 N	88.3 W
	BELLEVILLE_SIU_RES	38.5 N	89.8 W
	BROOKPORT_DAM_52	37.1 N	88.7 W
	CAIRO_3_N	37.0 N	89.2 W
	CARBONDALE_SEWAGE_	37.7 N	89.2 W
	CARLINVILLE	39.3 N	89.9 W
	CHARLESTON	39.5 N	88.2 W
	CHENOA	40.7 N	88.7 W
	CHICAGO_MIDWAY_AP_	41.7 N	87.8 W
	CHICAGO_O'HARE_WSO	42.0 N	87.9 W
	CHICAGO_UNIVERSITY	41.8 N	87.6 W
	DANVILLE	40.1 N	87.7 W
	DECATUR	39.8 N	89.0 W
	DIXON_1_NW	41.8 N	89.5 W
	DU_QUOIN_2_S	38.0 N	89.2 W
	EFFINGHAM	39.1 N	88.5 W
	FAIRFIELD_RADIO_WF	38.4 N	88.3 W
	FLORA_5_NW	38.7 N	88.6 W
	FULTON_LOCK_&_DAM_	41.9 N	90.2 W
	GALESBURG	41.0 N	90.4 W
	GALVA	41.2 N	90.1 W
	GENESEO	41.5 N	90.2 W

Table C1 (cont'd.)

GOLDEN	40.1 N	91.0 W
GRIGGSVILLE	39.7 N	90.7 W
HARRISBURG	37.7 N	88.5 W
HILLSBORO_2_SSW	39.2 N	89.5 W
HOOPESTON	40.5 N	87.7 W
JACKSONVILLE_2_E	39.7 N	90.2 W
JERSEYVILLE_2_SW	39.1 N	90.3 W
LACON_1_N	41.0 N	89.4 W
LA_HARPE_1_SW	40.6 N	91.0 W
LINCOLN	40.2 N	89.4 W
MARENGO	42.3 N	88.6 W
MASON_CITY_1_W	40.2 N	89.7 W
MATTOON	39.5 N	88.3 W
MC_LEANSBORO_2_E	38.1 N	88.5 W
MINONK	40.9 N	89.1 W
MOLINE_WSO_AP	41.5 N	90.5 W
MONMOUTH	40.9 N	90.7 W
MORRISON	41.8 N	90.0 W
MOUNT_CARROLL	42.1 N	90.0 W
MT_VERNON_3_NE	38.3 N	88.9 W
NASHVILLE_4_NE	38.4 N	89.3 W
NEWTON_6_SSE	38.9 N	88.1 W
OLNEY	38.7 N	88.1 W
OTTAWA_4_SW	41.3 N	88.9 W
PALESTINE	39.0 N	87.6 W
PANA	39.4 N	89.1 W
PARIS_WATERWORKS	39.6 N	87.7 W
PARK_FOREST	41.5 N	87.7 W
PAW_PAW_1_E	41.7 N	89.0 W
PEORIA_WSO_AIRPORT	40.7 N	89.7 W
PONTIAC	40.9 N	88.6 W
PRINCEVILLE	40.9 N	89.8 W
QUINCY_FAA_AIRPORT	39.9 N	91.2 W
RANTOUL	40.3 N	88.2 W
ROCKFORD_WSO_AP	42.2 N	89.1 W
RUSHVILLE	40.1 N	90.6 W
SALEM	38.6 N	88.9 W
SPARTA_3_N	38.2 N	89.7 W
SPRINGFIELD_WSO_AP	39.8 N	89.7 W
STOCKTON_1_N	42.3 N	90.0 W
TUSCOLA	39.8 N	88.3 W
URBANA	40.1 N	88.2 W
VIRDEN_1_N	39.5 N	89.8 W
WALNUT	41.5 N	89.6 W
WATERLOO	38.3 N	90.2 W
WATSEKA_2_NW	40.8 N	87.8 W
WAUKEGAN_2_WNW	42.3 N	87.9 W
WHEATON_3_SE	41.8 N	88.1 W
WHITE_HALL_1_E	39.4 N	90.4 W
WINDSOR	39.4 N	88.6 W

Table C1 (cont'd.)

Indiana	ANDERSON_QUARTZ_PL	40.1 N	85.7 W
	BERNE	40.7 N	84.9 W
	BLOOMINGTON	39.2 N	86.5 W
	BROOKVILLE	39.4 N	85.0 W
	CAMBRIDGE_CITY	39.8 N	85.2 W
	COLUMBIA_CITY	41.2 N	85.5 W
	COLUMBUS	39.2 N	85.9 W
	CRANE_NAVAL_DEPOT	38.9 N	86.8 W
	DELPHI	40.6 N	86.7 W
	DUBOIS_S_IND_FORAG	38.5 N	86.7 W
	ELWOOD	40.3 N	85.8 W
	EVANSVILLE	38.0 N	87.6 W
	Evansville	38.0 N	87.5 W
	EVANSVILLE_WSO_AP	38.0 N	87.5 W
	FARMLAND_5_NNW	40.3 N	85.2 W
	Fort Wayne	41.0 N	85.2 W
	FORT_WAYNE_WSO_AP	41.0 N	85.2 W
	FRANKFORT_DISPOSAL	40.3 N	86.5 W
	GOSHEN_COLLEGE	41.6 N	85.8 W
	GREENCASTLE_1_E	39.7 N	86.8 W
	GREENFIELD	39.8 N	85.8 W
	GREENSBURG	39.3 N	85.5 W
	HOBART_2_WNW	41.5 N	87.3 W
	Indianapolis	39.7 N	86.2 W
	INDIANAPOLIS_SE_SI	39.7 N	86.0 W
	INDIANAPOLIS_WSFO_	39.7 N	86.3 W
	KENTLAND	40.8 N	87.4 W
	LAFAYETTE_5_S	40.3 N	86.9 W
	LAGRANGE_SEWAGE_PL	41.7 N	85.4 W
	LA_PORTE	41.6 N	86.7 W
	LOWELL	41.3 N	87.4 W
	MADISON	38.7 N	85.4 W
	MARION_2_N	40.6 N	85.7 W
	MARTINSVILLE_2_SW	39.4 N	86.4 W
	MOUNT_VERNON_WATER	37.9 N	87.9 W
	NEW_CASTLE	39.9 N	85.4 W
	NORTH_VERNON_2_SW	39.0 N	85.7 W
	OAKLANDON_GEIST_RE	39.9 N	86.0 W
	OOLITIC_EXP_FARM	38.9 N	86.5 W
	PAOLI	38.6 N	86.5 W
	PRINCETON_1_W	38.3 N	87.6 W
	ROCHESTER	41.1 N	86.2 W
	ROCKVILLE	39.8 N	87.2 W
	RUSHVILLE_SEWAGE_P	39.6 N	85.4 W
	SAINT_MEINRAD	38.2 N	86.8 W
	SCOTTSBURG	38.7 N	85.8 W
	SEYMOUR_1_N	39.0 N	85.9 W
	SHELBYVILLE	39.5 N	85.8 W
	SHOALS_HIWAY_50_BR	38.7 N	86.8 W
	South Bend	41.7 N	86.3 W

Table C1 (cont'd.)

	SOUTH_BEND_WSO_AIR	41.8 N	86.2 W
	SPENCER	39.3 N	86.8 W
	TELL_CITY	38.0 N	86.8 W
	VALPARAISO_WATER_W	41.5 N	87.0 W
	VEVAY	38.8 N	85.1 W
	WABASH	40.8 N	85.8 W
	WANATAH_2_WNW	41.4 N	86.9 W
	WASHINGTON	38.7 N	87.2 W
	WEST_LAFAYETTE_6_N	40.5 N	87.0 W
	WHITESTOWN	40.0 N	86.3 W
	WINAMAC	41.0 N	86.6 W
	WINCHESTER_AIRPORT	40.2 N	84.9 W
Iowa	ALBIA_3_NNE	41.1 N	92.8 W
	ALGONA_3_W	43.1 N	94.3 W
	ALLISON	42.8 N	92.8 W
	ANAMOSA_1_NW	42.1 N	91.3 W
	ANKENY	41.7 N	93.6 W
	ATLANTIC_1_NE	41.4 N	95.0 W
	AUDUBON_1_SSE	41.7 N	94.9 W
	BEACONSFIELD_2_N	40.8 N	94.1 W
	BEDFORD	40.7 N	94.7 W
	BELLEVUE_LOCK_&_DA	42.3 N	90.4 W
	BELLE_PLAINE	41.9 N	92.3 W
	BLOOMFIELD_1_WNW	40.8 N	92.4 W
	BOONE	42.0 N	93.9 W
	BRITT	43.1 N	93.8 W
	CARROLL	42.1 N	94.8 W
	CASCADE	42.3 N	91.0 W
	CASTANA_EXPERIMENT	42.1 N	95.8 W
	CEDAR_RAPIDS_AP	41.9 N	91.7 W
	CEDAR_RAPIDS_NO_1	42.0 N	91.6 W
	CENTERVILLE	40.7 N	92.9 W
	CHARITON_1_E	41.0 N	93.3 W
	CHARLES_CITY	43.0 N	92.7 W
	CHEROKEE_2_S	42.8 N	95.5 W
	CLARINDA	40.7 N	95.0 W
	CLARION	42.7 N	93.7 W
	CLINTON_1	41.8 N	90.3 W
	COLUMBUS_JUNCT_2_S	41.3 N	91.4 W
	CORNING	41.0 N	94.8 W
	CRESCO_1_NE	43.4 N	92.1 W
	CRESTON_2_SW	41.0 N	94.4 W
	DECORAH	43.3 N	91.8 W
	DENISON	42.0 N	95.3 W
	Des Moines	41.5 N	93.7 W
	DES_MOINES_WSFO_AR	41.5 N	93.7 W
	DUBUQUE_LOCK_&_DAM	42.5 N	90.7 W
	DUBUQUE_WSO_AP	42.4 N	90.7 W
	ELDORA	42.3 N	93.1 W

Table C1 (cont'd.)

ELKADER_5_SSW	42.8 N	91.4 W
EMMETSBURG	43.1 N	94.7 W
ESTHERVILLE	43.4 N	94.8 W
FAIRFIELD	41.0 N	91.9 W
FAYETTE	42.8 N	91.8 W
FOREST_CITY_2_NNE	43.3 N	93.6 W
FORT_DODGE	42.5 N	94.2 W
FORT_MADISON	40.6 N	91.3 W
GLENWOOD_3_SW	41.0 N	95.8 W
GREENFIELD_1_WNW	41.3 N	94.5 W
GRINNELL_3_SW	41.7 N	92.7 W
GRUNDY_CENTER	42.4 N	92.8 W
GUTTENBERG_L_&_D_1	42.8 N	91.1 W
HAMPTON	42.8 N	93.2 W
HARLAN	41.7 N	95.3 W
HAWARDEN	43.0 N	96.5 W
HUMBOLDT_3_W	42.7 N	94.3 W
IDA_GROVE_5_NW	42.4 N	95.5 W
INDIANOLA	41.4 N	93.6 W
IOWA_CITY_1_S	41.7 N	91.5 W
IOWA_FALLS	42.5 N	93.3 W
JEFFERSON_1_S	42.0 N	94.4 W
KEOKUK	40.4 N	91.4 W
KEOSAUQUA_STATE_PA	40.7 N	92.0 W
KNOXVILLE	41.3 N	93.1 W
LAKE_PARK	43.5 N	95.3 W
LEON_6_ESE	40.7 N	93.6 W
LE_CLAIRE_L_&_D_14	41.6 N	90.4 W
LE_MARS	42.8 N	96.2 W
LOGAN	41.6 N	95.8 W
MAPLETON_NO_2	42.2 N	95.8 W
MAQUOKETA_3_S	42.0 N	90.7 W
MARSHALLTOWN_2	42.1 N	92.9 W
MASON_CITY	43.2 N	93.2 W
MASON_CITY_AP	43.2 N	93.3 W
MILFORD_4_NW	43.4 N	95.2 W
MOUNT_AYR_4_SW	40.7 N	94.3 W
MOUNT_PLEASANT_1_S	41.0 N	91.6 W
MUSCATINE	41.4 N	91.1 W
NEWTON_2_E	41.7 N	93.1 W
NEW_HAMPTON_1_E	43.0 N	92.3 W
NORTHWOOD	43.5 N	93.2 W
OAKLAND_4_WSW	41.3 N	95.5 W
OELWEIN	42.7 N	91.9 W
ONAWA	42.0 N	96.1 W
OSAGE	43.3 N	92.8 W
OSKALOOSA	41.3 N	92.7 W
OTTUMWA_AIRPORT	41.1 N	92.4 W
PERRY	41.8 N	94.1 W
POCAHONTAS_2_SE	42.7 N	94.7 W

Table C1 (cont'd.)

	PRIMGHAR	43.1 N	95.6 W
	RED_OAK	41.0 N	95.2 W
	ROCKWELL_CITY	42.4 N	94.6 W
	ROCK_RAPIDS	43.4 N	96.2 W
	SAC_CITY	42.4 N	95.0 W
	SANBORN	43.2 N	95.7 W
	SHELDON	43.2 N	95.8 W
	SHENANDOAH	40.8 N	95.4 W
	SIBLEY_5_NNE	43.5 N	95.7 W
	SIDNEY_1_NNW	40.8 N	95.7 W
	SIGOURNEY	41.3 N	92.2 W
	Sioux City	42.4 N	96.4 W
	SIOUX_CENTER_2_SE	43.0 N	96.2 W
	SIOUX_CITY_WSO_AP	42.4 N	96.4 W
	SIOUX_RAPIDS_4_E	42.9 N	95.1 W
	SPENCER_1_N	43.0 N	95.2 W
	STORM_LAKE_2_E	42.6 N	95.2 W
	SWEA_CITY	43.4 N	94.3 W
	TIPTON_4_NE	41.8 N	91.1 W
	TOLEDO	42.0 N	92.6 W
	TRIPOLI	42.8 N	92.3 W
	VINTON	42.2 N	92.0 W
	WASHINGTON	41.3 N	91.7 W
	WATERLOO_WSO_AP	42.5 N	92.4 W
	WAUKON	43.3 N	91.5 W
	WEBSTER_CITY	42.5 N	93.8 W
	WILLIAMSBURG	41.7 N	92.0 W
	WINTERSET_2_NNW	41.4 N	94.0 W
Kansas	Concordia	39.5 N	97.7 W
	Dodge City	37.8 N	100.0 W
	Goodland	39.4 N	101.7 W
	Topeka	39.1 N	95.6 W
	Wichita	37.7 N	97.4 W
Kentucky	ASHLAND_DAM_29	38.5 N	82.6 W
	BARBOURVILLE_WATER	36.9 N	83.9 W
	BARREN_RIVER_RESER	36.9 N	86.1 W
	BAXTER	36.8 N	83.3 W
	BEAVER_DAM	37.4 N	86.9 W
	BEREA_COLLEGE	37.6 N	84.3 W
	BOWLING_GREEN_FAA_	37.0 N	86.4 W
	CARROLLTON_LOCK_1	38.7 N	85.2 W
	COVINGTON_WSO_AIRP	39.0 N	84.7 W
	DANVILLE	37.7 N	84.8 W
	DIX_DAM	37.8 N	84.7 W
	FALMOUTH	38.7 N	84.3 W
	FARMERS_2_S	38.1 N	83.6 W
	FRANKFORT_LOCK_4	38.2 N	84.9 W
	GLASGOW_WKAY	37.0 N	85.9 W

Table C1 (cont'd.)

	GOLDEN_POND_8_N	36.9 N	88.0 W
	GREENSBURG	37.3 N	85.5 W
	HEIDELBERG	37.5 N	83.8 W
	HENDERSON_7_SSW	37.8 N	87.6 W
	HOPKINSVILLE	36.8 N	87.5 W
	LEITCHFIELD_2_N	37.5 N	86.3 W
	Lexington	38.0 N	84.6 W
	LEXINGTON_WSO_AIRP	38.0 N	84.6 W
	LONDON_FAA_AIRPORT	37.1 N	84.1 W
	Louisville	38.2 N	85.7 W
	LOUISVILLE_WSO_AIR	38.2 N	85.7 W
	LOVELACEVILLE	37.0 N	88.8 W
	MADISONVILLE	37.3 N	87.5 W
	MAMMOTH_CAVE_PARK	37.2 N	86.1 W
	MANCHESTER_4_W	37.2 N	83.8 W
	MAYSVILLE_SEWAGE_P	38.7 N	83.8 W
	MONTICELLO_3_NE	36.9 N	84.8 W
	MOUNT_VERNON	37.3 N	84.3 W
	MURRAY	36.6 N	88.3 W
	OWENSBORO_3_W	37.8 N	87.2 W
	PADUCAH_WSO	37.1 N	88.8 W
	ROUGH_RIVER_DAM	37.6 N	86.5 W
	SCOTTSVILLE	36.7 N	86.2 W
	SHELBYVILLE_1_E	38.2 N	85.2 W
	SOMERSET_2_N	37.1 N	84.6 W
	SUMMER_SHADE	36.9 N	85.7 W
	WARSAW_MARKLAND_DA	38.8 N	85.0 W
	WEST_LIBERTY	37.9 N	83.3 W
	WILLIAMSBURG	36.7 N	84.2 W
	WILLIAMSTOWN_5_WSW	38.7 N	84.6 W
Louisiana	New Orleans	30.0 N	90.2 W
Maine	Eastport	44.9 N	67.0 W
	Portland	43.7 N	70.3 W
Maryland	Baltimore	39.3 N	76.6 W
Massachusetts	Boston	42.4 N	71.1 W
Michigan	ADRIAN_2_NNE	41.9 N	84.0 W
	ALBERTA_FORD_FORST	46.7 N	88.5 W
	ALLEGAN_5_NE	42.6 N	85.8 W
	ALMA	43.4 N	84.7 W
	ALPENA_SEWAGE_PLAN	45.1 N	83.4 W
	ALPENA_WSO_AIRPORT	45.1 N	83.6 W
	ANN_ARBOR_UNIV_OF_	42.3 N	83.7 W
	BAD_AXE	43.8 N	83.0 W
	BALDWIN_STATE_FORE	43.9 N	85.8 W
	BATTLE_CREEK_5_NW	42.4 N	85.3 W

Table C1 (cont'd.)

BENTON_HARBOR_ARPT	42.1 N	86.4 W
BERGLAND_HYDRO_PLA	46.6 N	89.6 W
BIG_RAPIDS_WATERWO	43.7 N	85.5 W
BLOOMINGDALE	42.4 N	86.0 W
BOYNE_FALLS	45.2 N	84.9 W
CADILLAC	44.3 N	85.4 W
CARO_REGIONAL_CENT	43.5 N	83.4 W
CHAMPION_VAN_RIPER	46.5 N	88.0 W
CHARLOTTE	42.5 N	84.8 W
CHEBOYGAN	45.7 N	84.5 W
COLDWATER_ST_SCHOO	42.0 N	85.0 W
DEARBORN	42.3 N	83.2 W
DETOUR_VILLAGE	46.0 N	83.9 W
Detroit	42.4 N	83.0 W
DETROIT_METRO_WSO_	42.2 N	83.3 W
DOWAGIAC_1_W	42.0 N	86.1 W
EAST_JORDAN	45.2 N	85.1 W
EAST_LANSING_4_S	42.7 N	84.5 W
EAST_TAWAS	44.3 N	83.5 W
EAU_CLAIRE_4_NE	42.0 N	86.3 W
Escanaba	45.8 N	87.1 W
FAYETTE_4_SW	45.7 N	86.7 W
Flint	43.0 N	83.7 W
FLINT_WSO_AP	43.0 N	83.8 W
GAYLORD	45.0 N	84.7 W
GLADWIN	44.0 N	84.5 W
Grand Rapids	42.9 N	85.5 W
GRAND_HAVEN_FIRE_D	43.1 N	86.2 W
GRAND_MARAIS_2_E	46.7 N	85.9 W
GRAND_RAPIDS_WSO_A	42.9 N	85.5 W
GRAYLING	44.7 N	84.7 W
GREENVILLE	43.2 N	85.3 W
GROSSE_POINTE_FARM	42.4 N	82.9 W
GULL_LAKE_EXPERIME	42.4 N	85.4 W
HALE_LOUD_DAM	44.5 N	83.7 W
HARBOR_BEACH_1_SSE	43.8 N	82.6 W
HART	43.7 N	86.3 W
HASTINGS	42.7 N	85.3 W
HESPERIA_4_WNW	43.6 N	86.1 W
HILLSDALE	41.9 N	84.6 W
HOLLAND_HOPE_COLLE	42.8 N	86.1 W
HOUGHTON_FAA_AIRPO	47.2 N	88.5 W
HOUGHTON_LAKE_6_WS	44.3 N	84.9 W
IONIA_1_WNW	43.0 N	85.1 W
IRONWOOD_DAILY_GLO	46.5 N	90.2 W
IRON_MTN-KINGSFORD	45.8 N	88.1 W
JACKSON_FAA_ARPT	42.3 N	84.5 W
LAKE_CITY_EXP_FARM	44.3 N	85.2 W
Lansing	42.8 N	84.6 W
LANSING_WSO_AIRPOR	42.8 N	84.6 W

Table C1 (cont'd.)

	LAPEER	43.0 N	83.3 W
	LUDINGTON_5_SE	43.9 N	86.4 W
	LUPTON_1_SW	44.4 N	84.0 W
	MANISTEE_3_SE	44.2 N	86.3 W
	MAPLE_CITY	44.8 N	85.8 W
	MARQUETTE	46.5 N	87.4 W
	MARQUETTE_WSO	46.5 N	87.6 W
	MILFORD_GM_PROVING	42.6 N	83.7 W
	MIO_HYDRO_PLANT	44.7 N	84.1 W
	MONROE_WATERWORKS	41.9 N	83.4 W
	MONTAGUE_4_NW	43.5 N	86.4 W
	MOUNT_PLEASANT_COL	43.6 N	84.8 W
	MUSKEGON_WSO_AIRPO	43.2 N	86.2 W
	NEWBERRY_STATE_HOS	46.3 N	85.5 W
	ONAWAY_BLACK_L_FOR	45.4 N	84.2 W
	OWOSSO_3_NNW	43.0 N	84.2 W
	PELLSTON_FAA_AIRPO	45.6 N	84.8 W
	PETOSKEY	45.4 N	85.0 W
	PONTIAC_STATE_HOSP	42.7 N	83.3 W
	PORT_HURON_SEWAGE_	43.0 N	82.4 W
	SAGINAW_FAA_AIRPOR	43.5 N	84.1 W
	SAULT_STE_MARIE_WS	46.5 N	84.3 W
	SOUTH_HAVEN	42.4 N	86.3 W
	STAMBAUGH_2_SSE	46.0 N	88.6 W
	STANDISH_5_SW	44.0 N	84.0 W
	STEPHENSON_8_WNW	45.5 N	87.8 W
	ST_JAMES_2_S_BEAVE	45.7 N	85.5 W
	ST_JOHNS	43.0 N	84.5 W
	THREE_RIVERS	41.9 N	85.6 W
	TRAVERSE_CITY_FAA_	44.7 N	85.6 W
	VANDERBILT_STATE_F	45.2 N	84.4 W
	WEST_BRANCH_3_SE	44.3 N	84.2 W
	WHITEFISH_POINT	46.8 N	85.0 W
Minnesota	ADA	47.3 N	96.5 W
	AGASSIZ_REFUGE	48.3 N	96.0 W
	ALBERT_LEA_3_SE	43.7 N	93.3 W
	ALEXANDRIA_FAA_AIR	45.9 N	95.4 W
	ARGYLE_4_E	48.3 N	96.7 W
	ARTICHOKE_LAKE	45.4 N	96.1 W
	AUSTIN_3_S	43.6 N	93.0 W
	BAUDETTE	48.7 N	94.6 W
	BEMIDJI	47.5 N	94.9 W
	BENSON	45.3 N	95.6 W
	BIG_FALLS	48.2 N	93.8 W
	BUFFALO	45.2 N	93.9 W
	CALEDONIA	43.6 N	91.4 W
	CAMBRIDGE_STATE_HO	45.6 N	93.2 W
	CANBY	44.7 N	96.3 W
	CASS_LAKE	47.4 N	94.6 W

Table C1 (cont'd.)

CEDAR	45.3 N	93.3 W
CHASKA	44.8 N	93.6 W
CLOQUET	46.7 N	92.5 W
COLLEGEVILLE_ST_JO	45.6 N	94.4 W
COOK_18_W	47.9 N	93.1 W
CROOKSTON_NW_EXP_S	47.8 N	96.6 W
DETROIT_LAKES_1_NN	46.8 N	95.8 W
Duluth	46.8 N	92.2 W
DULUTH_WSO_AP	46.8 N	92.2 W
FAIRMONT	43.6 N	94.5 W
FARIBAULT	44.3 N	93.3 W
FARMINGTON_3_NW	44.7 N	93.2 W
FERGUS_FALLS	46.3 N	96.1 W
FOREST_LAKE_5_NE	45.3 N	92.9 W
FOSSTON_1_E	47.6 N	95.8 W
GAYLORD	44.5 N	94.2 W
GLENWOOD_2_WNW	45.7 N	95.4 W
GRAND_MARAIS	47.7 N	90.3 W
GRAND_MEADOW	43.7 N	92.6 W
GRAND_RAPIDS_FORES	47.2 N	93.5 W
GULL_LAKE_DAM	46.4 N	94.3 W
HALLOCK	48.8 N	96.9 W
HIBBING_FAA_AIRPOR	47.4 N	92.9 W
HINCKLEY	46.0 N	92.9 W
HUTCHINSON_1_N	44.9 N	94.4 W
INTERNL_FALLS_WSO_	48.6 N	93.4 W
ITASCA_UNIV_OF_MIN	47.2 N	95.2 W
JORDAN_1_S	44.7 N	93.6 W
LAMBERTON_SW_EXP_S	44.3 N	95.3 W
LEECH_LAKE_FEDERAL	47.3 N	94.2 W
LITCHFIELD	45.1 N	94.5 W
LITTLE_FALLS_1_N	46.0 N	94.3 W
LONG_PRAIRIE	46.0 N	94.8 W
LUVERNE	43.7 N	96.2 W
MADISON_SEWAGE_PLA	45.0 N	96.2 W
MAHNOMEN_1_W	47.3 N	96.0 W
MARSHALL	44.5 N	95.8 W
MELROSE	45.7 N	94.8 W
MILACA_1_ENE	45.8 N	93.7 W
MILAN_1_NW	45.1 N	95.9 W
Minneapolis	44.8 N	93.3 W
MINNEAPOLIS_WSFO_A	44.9 N	93.2 W
MONTEVIDEO_1_SW	44.9 N	95.8 W
MOOSE_LAKE_1_SSE	46.5 N	92.8 W
MORA	45.9 N	93.3 W
MORRIS_WC_EXP_STN	45.6 N	95.9 W
NEW_ULM	44.3 N	94.4 W
OTTERTAIL	46.4 N	95.6 W
OWATONNA	44.1 N	93.2 W
PARK_RAPIDS_2_S	46.9 N	95.1 W

Table C1 (cont'd.)

	PINE_RIVER_DAM	46.7 N	94.1 W
	PIPESTONE	44.0 N	96.3 W
	POKEGAMA_DAM	47.3 N	93.6 W
	PRESTON	43.7 N	92.1 W
	REDWOOD_FALLS_FAA_	44.5 N	95.1 W
	RED_LAKE_FALLS	47.9 N	96.3 W
	RED_LAKE_INDIAN_AG	47.9 N	95.0 W
	ROCHESTER_WSO_AP	43.9 N	92.5 W
	ROSEMOUNT_AGRI_EXP	44.7 N	93.1 W
	ROTHSAY	46.5 N	96.3 W
	SANDY_LAKE_DAM_LIB	46.8 N	93.3 W
	SANTIAGO_3_E	45.5 N	93.8 W
	SPRINGFIELD_1_NW	44.3 N	95.0 W
	STEWART	44.7 N	94.5 W
	STILLWATER_1_SE	45.0 N	92.8 W
	ST_CLOUD_WSO_AP	45.5 N	94.1 W
	ST_JAMES_FILT_PLAN	44.0 N	94.6 W
	ST_PAUL	45.0 N	93.1 W
	ST_PETER_2_SW	44.3 N	94.0 W
	THEILMAN	44.3 N	92.2 W
	TOWER_3_S	47.8 N	92.3 W
	TRACY	44.2 N	95.6 W
	TWO_HARBORS	47.0 N	91.7 W
	WADENA_3_S	46.4 N	95.2 W
	WALKER_AH_GWAH_CHI	47.1 N	94.6 W
	WARROAD	48.9 N	95.3 W
	WASECA_EXP_STATION	44.1 N	93.5 W
	WHEATON	45.8 N	96.5 W
	WILLMAR_CNTY_HWY_G	45.1 N	95.0 W
	WINDOM	43.9 N	95.1 W
	WINNEBAGO	43.8 N	94.2 W
	WINNIBIGOSHISH_DAM	47.4 N	94.1 W
	WINONA	44.0 N	91.6 W
	WINTON_POWER_PLANT	47.9 N	91.8 W
	WRIGHT_4_NW	46.7 N	93.1 W
	ZUMBROTA	44.3 N	92.7 W
Mississippi	Vicksburg	32.3 N	90.9 W
Missouri	ADVANCE_1_S	37.1 N	89.9 W
	ANDERSON	36.7 N	94.4 W
	APPLETON_CITY	38.2 N	94.0 W
	ARCADIA	37.6 N	90.6 W
	BETHANY	40.3 N	94.1 W
	BOLIVAR_1_NE	37.6 N	93.4 W
	BOONVILLE	39.0 N	92.8 W
	BROOKFIELD	39.8 N	93.1 W
	BRUNSWICK	39.4 N	93.1 W
	BUFFALO_3_S	37.6 N	93.1 W
	BUTLER	38.3 N	94.3 W

Table C1 (cont'd.)

CALIFORNIA	38.6 N	92.6 W
CAMDENTON_2_NW	38.2 N	92.8 W
CANTON_L_AND_D_20	40.2 N	91.5 W
CAPE_GIRARDEAU_FAA	37.2 N	89.6 W
CARROLLTON	39.4 N	93.5 W
CARUTHERSVILLE	36.2 N	89.7 W
CLEARWATER_DAM	37.1 N	90.8 W
CLINTON	38.4 N	93.8 W
CONCEPTION	40.3 N	94.7 W
DONIPHAN	36.6 N	90.8 W
ELDON	38.3 N	92.6 W
ELSBERRY_1_S	39.2 N	90.8 W
FARMINGTON	37.8 N	90.4 W
FREDERICKTOWN	37.6 N	90.3 W
FREEDOM	38.5 N	91.7 W
FULTON	38.8 N	91.9 W
GRANT_CITY	40.5 N	94.4 W
GREENVILLE_6_N	37.2 N	90.4 W
HAMILTON_2_W	39.8 N	94.0 W
HANNIBAL_WATER_WOR	39.7 N	91.4 W
JACKSON	37.4 N	89.7 W
JEFFERSON_CITY_WAT	38.6 N	92.2 W
JOPLIN_FAA_AIRPORT	37.2 N	94.5 W
Kansas City	39.2 N	94.7 W
KENNETT_RADIO_KBOA	36.2 N	90.1 W
KIRKSVILLE	40.2 N	92.6 W
LAKESIDE	38.2 N	92.6 W
LAMAR	37.5 N	94.3 W
LEBANON_2_W	37.7 N	92.7 W
LEES_SUMMIT_REED_W	38.9 N	94.3 W
LEXINGTON_3_NE	39.2 N	93.9 W
LICKING_4_N	37.5 N	91.9 W
LOCKWOOD	37.4 N	93.9 W
MARBLE_HILL	37.3 N	90.0 W
MARSHFIELD	37.3 N	92.9 W
MARYVILLE_2_E	40.3 N	94.8 W
MEXICO	39.2 N	91.9 W
MOBERLY	39.4 N	92.4 W
MOUNTAIN_GROVE_2_N	37.2 N	92.3 W
MT_VERNON_M_U_SW_C	37.1 N	93.9 W
NEOSHO	36.9 N	94.4 W
NEVADA_SEWAGE_PLAN	37.8 N	94.4 W
NEW_FRANKLIN_1_W	39.0 N	92.8 W
OSCEOLA	38.0 N	93.7 W
OZARK_BEACH	36.7 N	93.1 W
POMME_DE_TERRE_DAM	37.9 N	93.3 W
POPLAR_BLUFF	36.8 N	90.4 W
PRINCETON_6_SW	40.3 N	93.7 W
SALEM	37.6 N	91.5 W
SALISBURY	39.4 N	92.8 W

Table C1 (cont'd.)

	SAVERTON_L_&_D_22	39.6 N	91.3 W
	SEDALIA_WATER_PLAN	38.7 N	93.2 W
	SHELBINA	39.7 N	92.1 W
	SPICKARD_7_W	40.3 N	93.7 W
	Springfield	37.2 N	93.4 W
	SPRINGFIELD_WSO_AP	37.2 N	93.4 W
	St Louis	38.8 N	90.4 W
	STEELVILLE_2_N	38.0 N	91.4 W
	STEFFENVILLE	40.0 N	91.9 W
	ST_CHARLES	38.8 N	90.5 W
	ST_LOUIS_WSCMO_AIR	38.8 N	90.4 W
	SWEET_SPRINGS	39.0 N	93.4 W
	TRENTON	40.1 N	93.6 W
	UNION	38.5 N	91.0 W
	VANDALIA	39.3 N	91.5 W
	VERSAILLES	38.4 N	92.8 W
	VIENNA_2_WNW	38.2 N	92.0 W
	WAPPAPELLO_DAM	36.9 N	90.3 W
	WAYNESVILLE_2_W	37.8 N	92.2 W
	WEST_PLAINS	36.7 N	91.8 W
	WILLOW_SPRG_RADIO_	37.0 N	92.0 W
Montana	Billings	45.8 N	108.5 W
	Glasgow	48.2 N	106.6 W
	Great Falls	47.5 N	111.4 W
	Havre	48.6 N	109.7 W
	Helena	46.6 N	112.0 W
	Kalispell	48.3 N	114.3 W
	Lewiston	46.4 N	117.0 W
	Miles City	46.4 N	105.9 W
	Missoula	46.9 N	114.0 W
Nebraska	Grand Island	41.0 N	98.3 W
	Lincoln	40.8 N	96.8 W
	Norfolk	42.0 N	97.4 W
	North Platte	41.1 N	100.7 W
	Omaha	41.3 N	95.9 W
Nevada	Ely	39.3 N	114.9 W
	Las Vegas	36.1 N	115.2 W
	Reno	39.5 N	119.8 W
	Winnemucca	41.0 N	117.8 W
New Hampshire	Mount Washington	44.3 N	71.3 W
New Jersey	New York	40.7 N	74.0 W
New Mexico	Roswell	33.4 N	104.5 W
	Santa Fe	35.7 N	105.9 W

Table C1 (cont'd.)

New York	Albany	42.7 N	73.8 W
	ALBANY_WSFO_AP	42.8 N	73.8 W
	AURORA_RESEARCH_FA	42.7 N	76.7 W
	BATAVIA	43.0 N	78.2 W
	BINGHAMPTOM_WSO_AP	42.2 N	76.0 W
	BOONVILLE_2_SSW	43.5 N	75.3 W
	BUFFALO_WSCMO_AP	43.0 N	78.7 W
	CANTON_3_SE	44.5 N	75.2 W
	COLDEN_1_N	42.7 N	78.7 W
	DANNEMORA	44.7 N	73.7 W
	DOBBS_FERRY	41.0 N	73.8 W
	ELMIRA	42.2 N	76.8 W
	FREDONIA	42.5 N	79.2 W
	GLENS_FALLS_AP	43.3 N	73.7 W
	HUDSON_CORRECTIONL	42.3 N	73.8 W
	INDIAN_LAKE_2_SW	43.8 N	74.3 W
	ITHACA_CORNELL_UNI	42.5 N	76.5 W
	LAKE_PLACID_2_S	44.3 N	74.0 W
	LOWVILLE	43.8 N	75.5 W
	MASSENA_AP	45.0 N	74.8 W
	MINEOLA	40.7 N	73.7 W
	MOUNT_MORRIS_2_W	42.7 N	77.8 W
	NEW_YORK_LAGUARDIA	40.8 N	74.0 W
	NY_WESTERLEIGH_STA	40.7 N	74.2 W
	OLD_FORGE	43.7 N	75.0 W
	OSWEGO_EAST	43.5 N	76.5 W
	POUGHKEEPSIE_FAA_A	41.7 N	74.0 W
	RIVERHEAD_RESEARCH	41.0 N	72.7 W
	ROCHESTER_WSO_AP	43.2 N	77.7 W
	SCARDALE	41.0 N	73.8 W
	SLIDE_MOUNTAIN	42.0 N	74.5 W
	SPENCER_2_N	42.3 N	76.5 W
	STILLWATER_RESERVI	43.8 N	75.0 W
	SYRACUSE_WSO_AIRPO	43.2 N	76.2 W
	UTICA_FAA_AP	43.2 N	75.3 W
	WANAKENA_RANGER_SC	44.2 N	74.8 W
	WARSAW_6_SW	42.7 N	78.2 W
	WATERTOWN	44.0 N	75.8 W
	WATERTOWN_AP	44.0 N	76.0 W
	WHITEHALL	43.5 N	73.3 W
North Carolina	Asheville	35.6 N	82.5 W
	Wilmington	34.3 N	77.9 W
North Dakota	Bismark	46.8 N	100.8 W
	Ellendale	46.0 N	98.5 W
	Fargo	46.9 N	96.8 W
	Williston	48.2 N	103.6 W

Table C1 (cont'd.)

Ohio	Akron	41.1 N	81.5 W
	AKRON_CANTON_WSO_A	40.9 N	81.4 W
	ASHLAND_2_SW	40.8 N	82.3 W
	ASHTABULA	41.8 N	80.8 W
	BARNESVILLE	40.0 N	81.2 W
	BELLEFONTAINE	40.3 N	83.8 W
	BOWLING_GREEN_WWTP	41.4 N	83.6 W
	BUCYRUS_SEWAGE_PLA	40.8 N	83.0 W
	CADIZ	40.3 N	81.0 W
	CANFIELD_1_S	41.0 N	80.8 W
	CELINA_3_NE	40.6 N	84.5 W
	CENTERBURG_2_SE	40.3 N	82.7 W
	CHARDON	41.6 N	81.2 W
	Chicago	41.8 N	87.8 W
	CHILO_MELDAHL_L&D	38.8 N	84.2 W
	CHIPPEWA_LAKE	41.0 N	81.9 W
	CINCINNATI_LUNKEN_	39.1 N	84.4 W
	CIRCLEVILLE	39.6 N	82.9 W
	Cleveland	41.4 N	81.8 W
	CLEVELAND_WSFO_AP	41.4 N	81.9 W
	Columbus	40.0 N	83.0 W
	COLUMBUS_VLY_CROSS	39.9 N	82.9 W
	COLUMBUS_WSO_AIRPO	40.0 N	82.9 W
	COSHOCTON_AGR_RES_	40.4 N	81.8 W
	COSHOCTON_WPC_PLAN	40.3 N	81.9 W
	DANVILLE_2_W	40.4 N	82.3 W
	Dayton	39.9 N	84.2 W
	DAYTON_MCD	39.8 N	84.2 W
	DAYTON_WSO_AP	39.9 N	84.2 W
	DEFIANCE	41.3 N	84.4 W
	DELAWARE	40.3 N	83.1 W
	DORSET_2_E	41.7 N	80.7 W
	EATON	39.7 N	84.6 W
	ELYRIA_3_E	41.4 N	82.1 W
	FINDLAY_FAA_AIRPOR	41.0 N	83.7 W
	FINDLAY_WPCC	41.0 N	83.7 W
	FRANKLIN_2_W	39.5 N	84.3 W
	FREDERICKTOWN_4_S	40.4 N	82.5 W
	FREMONT_WATER_WORK	41.3 N	83.1 W
	GALLIPOLIS	38.8 N	82.2 W
	GREENVILLE_SEWAGE_	40.1 N	84.7 W
	HILLSBORO	39.2 N	83.6 W
	HIRAM	41.3 N	81.2 W
	HOYTVILLE_2_NE	41.2 N	83.8 W
	IRWIN	40.1 N	83.5 W
	KENTON	40.7 N	83.6 W
	LANCASTER_2_NW	39.7 N	82.6 W
	LIMA_WWTP	40.7 N	84.1 W
	LONDON	39.9 N	83.4 W
	MANSFIELD_6_W	40.8 N	82.6 W

Table C1 (cont'd.)

	MANSFIELD_WSO_AP	40.8 N	82.5 W
	MARIETTA_WWTP	39.4 N	81.4 W
	MARION_2_N	40.6 N	83.1 W
	MARYSVILLE	40.2 N	83.4 W
	MC_CONNELSVILLE_LO	39.7 N	81.8 W
	MILLPORT_2_NW	40.7 N	80.9 W
	MINERAL_RIDGE_WTR_	41.2 N	80.8 W
	MONTPELIER	41.6 N	84.6 W
	NAPOLEON	41.4 N	84.2 W
	NEWARK_WATER_WORKS	40.1 N	82.4 W
	NEW_LEXINGTON_2_NW	39.7 N	82.2 W
	NEW_PHILADELPHIA	40.5 N	81.4 W
	NORWALK_WWTP	41.3 N	82.6 W
	OBERLIN	41.3 N	82.2 W
	PAINESVILLE_2_N	41.8 N	81.3 W
	PANDORA	41.0 N	84.0 W
	PAULDING_1_S	41.1 N	84.6 W
	Peoria	40.7 N	89.7 W
	PHILO_3_SW	39.8 N	81.9 W
	PORTSMOUTH_SCIOTOV	38.8 N	82.9 W
	PUT-IN-BAY	41.7 N	82.8 W
	RIPLEY_EXP_FARM	38.8 N	83.8 W
	SANDUSKY	41.5 N	82.7 W
	STEUBENVILLE	40.4 N	80.6 W
	TIFFIN	41.1 N	83.2 W
	Toledo	41.6 N	83.6 W
	TOLEDO_BLADE	41.7 N	83.5 W
	TOLEDO_EXPRESS_WSO	41.6 N	83.8 W
	UPPER_SANDUSKY	40.8 N	83.3 W
	URBANA_WWTP	40.1 N	83.8 W
	VAN_WERT	40.8 N	84.6 W
	WARREN_3_S	41.2 N	80.8 W
	WASHINGTON_COURT_H	39.5 N	83.4 W
	WAUSEON_WATER_PLAN	41.5 N	84.2 W
	WAVERLY	39.1 N	83.0 W
	WESTERVILLE	40.1 N	82.9 W
	WILMINGTON_3_N	39.5 N	83.8 W
	WOOSTER_EXP_STN	40.8 N	81.9 W
	XENIA_6_SSE	39.6 N	83.9 W
	Youngstown	41.2 N	80.7 W
	YOUNGSTOWN_WSO_AP	41.3 N	80.7 W
	ZANESVILLE_FAA_AIR	40.0 N	81.9 W
Oklahoma	Oklahoma City	35.4 N	97.6 W
	Tulsa	36.2 N	95.9 W
Oregon	Astoria	46.2 N	123.9 W
	Baker	44.8 N	117.8 W
	Burns	43.6 N	119.1 W
	Eugene	44.1 N	123.1 W

Table C1 (cont'd.)

	Pendleton	45.7 N	118.8 W
	Portland	45.5 N	122.7 W
	Roseburg	43.2 N	123.3 W
	Salem	44.9 N	123.0 W
Pennsylvania	ALLENTOWN_BETHLEHE	40.7 N	75.5 W
	BLOSERVILLE_1_N	40.3 N	77.3 W
	CHAMBERSBURG	40.0 N	77.7 W
	CLARION_3_SW	41.2 N	79.5 W
	CLERMONT_8_SW	41.7 N	78.5 W
	CONFLUENCE_1_SW_DA	39.8 N	79.3 W
	DUBOIS_FAA_AP	41.2 N	78.8 W
	ERIE_WSO_ARPT	42.2 N	80.2 W
	FRANKLIN	41.3 N	79.8 W
	GRATERFORD_1_E	40.2 N	75.5 W
	INDIANA_3_SE	40.7 N	79.2 W
	JAMESTOWN_2_NW	41.5 N	80.5 W
	KANE_1_NNE	41.7 N	78.8 W
	LANDISVILLE	40.2 N	76.5 W
	MEADVILLE_1_S	41.7 N	80.2 W
	MERCER	41.2 N	80.2 W
	MONTROSE_1_E	41.8 N	75.8 W
	NEW_CASTLE_1_N	41.0 N	80.3 W
	Philadelphia	39.9 N	75.3 W
	PHILADELPHIA_WSO_A	39.8 N	75.2 W
	PHILIPSBURG_8_E	41.0 N	78.0 W
	PHOENIXVILLE_1_E	40.2 N	75.5 W
	Pittsburgh	40.5 N	80.2 W
	PITTSBURGH_WSCOM_2	40.5 N	80.2 W
	PLEASANT_MOUNT_1_W	41.7 N	75.5 W
	PUTNEYVILLE_2_SE_D	41.0 N	79.3 W
	Scranton	41.3 N	75.7 W
	SLIPPERY_ROCK	41.0 N	80.0 W
	STROUDSBURG_2_E	41.0 N	75.2 W
	TIONESTA_2_SE_LAKE	41.5 N	79.5 W
	TITUSVILLE_WATER_W	41.7 N	79.7 W
	TOBYHANNA	41.2 N	75.3 W
	TOWANDA_1_ESE	41.8 N	76.5 W
	WAYNESBURG_1_E	39.8 N	80.2 W
	WELLSBORO_3_S	41.7 N	77.3 W
Rhode Island	Providence	41.7 N	71.4 W
South Carolina	Charleston	32.9 N	80.1 W
	Columbia	34.0 N	81.1 W
South Dakota	Huron	44.4 N	98.2 W
	Pierre	44.4 N	100.3 W
	Rapid City	44.0 N	103.1 W
	Sioux Falls	43.6 N	96.7 W

Table C1 (cont'd.)

Tennessee	Nashville	36.1 N	86.7 W
Texas	Abilene	32.4 N	99.7 W
	Amarillo	35.2 N	101.7 W
	Austin	30.3 N	97.7 W
	Brownsville	25.9 N	97.4 W
	Dallas	32.8 N	96.8 W
	El Paso	31.8 N	106.4 W
	Houston	29.8 N	95.4 W
	Lubbock	33.7 N	101.8 W
	Waco	31.6 N	97.2 W
	Wichita Falls	33.9 N	98.5 W
Utah	Milford	38.4 N	113.0 W
	Modena	37.8 N	113.9 W
	Salt Lake City	40.8 N	111.9 W
Virginia	Norfolk	36.8 N	76.2 W
	Richmond	37.5 N	77.3 W
	Wytheville	36.9 N	81.1 W
Washington	North Head	46.3 N	124.1 W
	Seattle	47.5 N	122.3 W
	Spokane	47.6 N	117.5 W
	Tacoma	47.3 N	122.4 W
	Tatoosh Island	48.4 N	124.7 W
	Walla Walla	46.0 N	118.3 W
	Yakima	46.6 N	120.5 W
Wisconsin	ALMA_DAM_4	44.3 N	91.9 W
	AMERY_2_N	45.3 N	92.4 W
	ANTIGO_1_SSW	45.1 N	89.2 W
	APPLETON	44.3 N	88.4 W
	ARLINGTON_EXP_FARM	43.3 N	89.3 W
	ASHLAND_EXP_FARM	46.6 N	91.0 W
	BARABOO_WATER_WORK	43.5 N	89.7 W
	BAYFIELD_6_N	46.9 N	90.8 W
	BEAVER_DAM	43.5 N	88.8 W
	BELOIT_COLLEGE	42.5 N	89.0 W
	BLAIR	44.3 N	91.2 W
	BLOOMER_CITY_HALL	45.1 N	91.5 W
	BOWLER_RANGER_STN	44.9 N	89.0 W
	BREED_6_SSE	45.0 N	88.4 W
	BRODHEAD_1_SW	42.6 N	89.4 W
	BURLINGTON	42.7 N	88.3 W
	CHARMANY_FARM	43.0 N	89.5 W
	CHILTON_SEWAGE_PLA	44.0 N	88.2 W
	CLINTONVILLE_SEWAG	44.6 N	88.8 W
	CRIVITZ_HIGH_FALLS	45.3 N	88.2 W
	CUMBERLAND	45.5 N	92.0 W

Table C1 (cont'd.)

DALTON	43.7 N	89.2 W
DANBURY	46.0 N	92.4 W
DARLINGTON	42.7 N	90.1 W
DODGE	44.1 N	91.6 W
DODGEVILLE	43.0 N	90.1 W
EAU_CLAIRE_FAA_AIR	44.9 N	91.5 W
ELLSWORTH_1_E	44.7 N	92.5 W
FAIRCHILD_RANGER_S	44.6 N	91.0 W
FOND_DU_LAC	43.8 N	88.4 W
FORT_ATKINSON_2_SS	42.9 N	88.8 W
FOXBORO	46.5 N	92.3 W
GENOA_DAM_8	43.6 N	91.2 W
GERMANTOWN_2_W	43.2 N	88.1 W
GOODMAN	45.6 N	88.3 W
GORDON	46.3 N	91.8 W
GRANTSBURG	45.8 N	92.7 W
GREEN_BAY_WSO_AIRP	44.5 N	88.1 W
GURNEY	46.5 N	90.5 W
HANCOCK_EXP_FARM	44.1 N	89.5 W
HARTFORD_SEWAGE_PL	43.3 N	88.4 W
HATFIELD_DAM	44.4 N	90.7 W
HILLSBORO_SEWAGE_P	43.7 N	90.3 W
HOLCOMBE_1_W	45.2 N	91.1 W
JUMP_RIVER_1_ESE	45.4 N	90.8 W
KENOSHA	42.5 N	87.8 W
KEWAUNEE	44.4 N	87.5 W
LAKE_GENEVA	42.6 N	88.4 W
LAKE_MILLS	43.1 N	88.9 W
LANCASTER_4_WSW	42.8 N	90.8 W
LAONA_6_SW	45.5 N	88.8 W
LA_CROSSE_WSO_AIRP	43.9 N	91.3 W
LONG_LAKE_DAM	45.9 N	89.1 W
LYNXVILLE_DAM_9	43.2 N	91.1 W
MADELINE_ISLAND	46.8 N	90.7 W
Madison	43.1 N	89.3 W
MADISON_WSO_AIRPOR	43.1 N	89.3 W
MANITOWOC	44.1 N	87.7 W
MARINETTE	45.1 N	87.6 W
MARSHFIELD_EXP_FAR	44.7 N	90.1 W
MATHER_3_NW	44.2 N	90.4 W
MAUSTON	43.8 N	90.1 W
MEDFORD_1_SW	45.1 N	90.3 W
MELLEN_4_NE	46.4 N	90.6 W
MENOMONIE_SEWAGE_P	44.9 N	91.9 W
MERRILL	45.2 N	89.7 W
MILWAUKEE_MT_MARY_	43.1 N	88.0 W
MILWAUKEE_WSO	43.1 N	87.9 W
MINOCQUA_DAM	45.9 N	89.7 W
MONDOVI	44.6 N	91.7 W
MONTELLO	43.8 N	89.3 W

Table C1 (cont'd.)

NECEDAH	44.0 N	90.1 W
NEILLSVILLE_3_SW	44.5 N	90.6 W
NEWALD_4_N	45.8 N	88.7 W
NEW_LONDON	44.4 N	88.7 W
NORTH_PELICAN	45.6 N	89.3 W
OCONOMOWOC_1_SW	43.1 N	88.5 W
OCONTO_4_W	44.9 N	87.9 W
OSHKOSH	44.0 N	88.6 W
OWEN	45.0 N	90.5 W
PARK_FALLS	45.9 N	90.4 W
PLATTEVILLE	42.8 N	90.5 W
PLYMOUTH	43.8 N	88.0 W
PORTAGE	43.5 N	89.4 W
PORT_WASHINGTON	43.4 N	87.9 W
PRAIRIE_DU_CHIEN	43.0 N	91.2 W
PRAIRIE_DU_SAC_2_N	43.3 N	89.7 W
PRENTICE_NO._2	45.5 N	90.3 W
RACINE	42.7 N	87.8 W
RAINBOW_RESERVOIR	45.8 N	89.6 W
REST_LAKE	46.1 N	89.9 W
RHINELANDER_WATER_	45.6 N	89.4 W
RICE_LAKE	45.5 N	91.7 W
RICHLAND_CENTER	43.3 N	90.4 W
RIDGELAND	45.2 N	91.9 W
RIVER_FALLS	44.9 N	92.6 W
ROSHOLT_9_NNE	44.8 N	89.3 W
SHAWANO_2_SSW	44.8 N	88.6 W
SHEBOYGAN	43.8 N	87.7 W
SOLON_SPRINGS	46.3 N	91.8 W
SPOONER_EXPERMNT_F	45.8 N	91.9 W
STANLEY	45.0 N	90.9 W
STEVENS_POINT	44.5 N	89.6 W
STURGEON_BAY_EXP_F	44.9 N	87.3 W
ST_CROIX_FALLS	45.4 N	92.7 W
SUPERIOR	46.7 N	92.0 W
TREMPEALEAU_DAM_6	44.0 N	91.4 W
TWO_RIVERS	44.2 N	87.6 W
VIROQUA_2_NW	43.6 N	90.9 W
WASHINGTON_ISLAND_	45.4 N	86.9 W
WATERTOWN	43.2 N	88.7 W
WAUPACA	44.3 N	89.1 W
WAUSAU_AIRPORT	44.9 N	89.6 W
WEST_BEND	43.4 N	88.2 W
WEYERHAUSER	45.4 N	91.4 W
WHITEWATER	42.8 N	88.7 W
WILLOW_RESERVOIR	45.7 N	89.8 W
WINTER_5_NW	45.9 N	91.1 W
WISCONSIN_DELLS	43.6 N	89.8 W
WISCONSIN_RAPIDS	44.4 N	89.8 W

Table C1 (cont'd.)

Wyoming	Casper	42.9 N	106.5 W
	Cheyenne	41.2 N	104.8 W
	Lander	42.8 N	108.7 W
	Sheridan	44.8 N	107.0 W
	Yellowstone Park	45.0 N	110.7 W

APPENDIX D

Appendix D

Table D1. Summary of understory vegetation present along a single, randomly-selected 100 m × 1 m transect in 32 of the 33* Michigan field sites used for the *Entomophaga maimaiga* bioassays. Included in the summary is a list of species present, the percentage of field sites that species were present along transects, the overall mean (±SEM) number of specimens (per m^2) across field sites, and the range in the number of specimens (per m^2) across field sites.

Common name	Scientific name	Sites in which species were present (%)	Mean (±SEM) no. of specimens (per m^2)	Range in no. of specimens (per m^2)
Oak	*Quercus* spp.	59.4	3.56 ± 1.03	0-21
Witch-hazel	*Hamamelis virginiana* L.	53.1	3.41 ± 0.92	0-18
Red maple	*Acer rubrum* L.	50.0	2.56 ± 0.65	0-13
White pine	*Pinus strobus* L.	46.9	1.47 ± 0.58	0-14
Black cherry	*Prunus serotina* Ehrh.	43.8	2.34 ± 0.88	0-22
Sassafras	*Sassafras albidum* (Nutt.) Nees	31.3	1.66 ± 0.57	0-14
Serviceberry	*Amelanchier arborea* (Michaux f.) F	28.1	1.16 ± 0.38	0-8
American beech	*Fagus grandifolia* Ehrh.	9.4	0.34 ± 0.20	0-5
Largetooth aspen	*Populus grandidentata* Michaux	9.4	0.31 ± 0.20	0-5
Red pine	*Pinus resinosa* Aiton	6.3	0.25 ± 0.22	0-7

Table D1 (cont'd.)

Ironwood	*Ostrya virginiana* L.	3.1	0.13 ± 0.13	0-4
American hornbeam	*Carpinus caroliniana* Walter	3.1	0.09 ± 0.09	0-3
Honeysuckle	*Lonicera canadensis* Marshall	3.1	0.06 ± 0.06	0-2

*One site (No. 32; Kalamazoo Co.) was not quantitatively-surveyed because a partial harvest occurred before the survey could be conducted. However, earlier observations indicated that the site had a sparse understory with some brambles (*Rubus* spp.) and barberry (*Berberis* spp.) present.

Appendix D

Table D2. Summary of ground flora (<1 m in height) present in four 1 m^2 quadrants along a single, randomly-selected 100 m × 1 m transect in 32 of the 33* Michigan field sites used for the *Entomophaga maimaiga* bioassays. Included in the summary is a list of species present, the percentage of field sites that species were present in quadrants, the overall mean (±SEM) number of specimens (per m^2) across field sites, and the range in the number of specimens (per m^2) across field sites.

Common name	Scientific name	Sites in which species were present (%)	Mean (±SEM) no. of specimens (per m^2)	Range in no. of specimens (per m^2)
Bracken fern	*Pteridium aquilinum* (L.) Kuhn	96.9	3.63 ± 0.55	0-16
Grasses/sedges**	Gramineae/Cyperaceae	90.6	11.59 ± 2.59**	0-61
Red maple	*Acer rubrum* L.	87.5	2.24 ± 0.37	0-8
Oak	*Quercus* spp.	84.4	3.82 ± 0.65	0-16
Low sweet blueberry	*Vaccinium angustifolium* Aiton	81.3	4.88 ± 0.78	0-19
Witch-hazel	*Hamamelis virginiana* L.	56.3	0.70 ± 0.18	0-4
Serviceberry	*Amelanchier arborea* (Michaux f.) F	56.3	0.67 ± 0.14	0-3
Black cherry	*Prunus serotina* Ehrh.	50.0	0.71 ± 0.18	0-4
Spotted wintergreen	*Chimaphila maculata* (L.) Pursh	46.9	4.32 ± 1.73	0-50
Sassafras	*Sassafras albidum* (Nutt.) Nees	40.6	0.88 ± 0.26	0-5
Violet	*Viola* spp.	18.8	0.89 ± 0.48	0-14

Table D2 (cont'd.)

Sweetfern	*Comptonia peregrina* (L.) Coulter	15.6	0.15 ± 0.09	0-3
Star-flower	*Trientalis borealis* Raf.	12.5	0.14 ± 0.08	0-2
Solomon-seal	*Polygonatum biflorum* (Walter) Ell.	6.3	0.04 ± 0.03	0-1
Bunchberry	*Cornus canadensis* L.	3.1	0.19 ± .019	0-6
Ironwood	*Ostrya virginiana* L.	3.1	0.14 ± 0.14	0-5
Barren-strawberry	*Waldsteinia fragarioides* (Michaux) Tra	3.1	0.14 ± 0.14	0-5
American ginseng	*Panax quinquefolius* L.	3.1	0.10 ± 0.10	0-3
Wild strawberry	*Fragaria virginiana* Miller	3.1	0.09 ± 0.09	0-3
Indian-pipe	*Monotropa uniflora* L.	3.1	0.05 ± 0.05	0-2
Ash	*Fraxinus* spp.	3.1	0.03 ± 0.03	0-1
American hornbeam	*Carpinus caroliniana* Walter	3.1	0.03 ± 0.03	0-1
White pine	*Pinus strobus* L.	3.1	0.02 ± 0.02	0-1
Largetooth aspen	*Populus grandidentata* Michaux	3.1	0.02 ± 0.02	0-1
Honeysuckle	*Lonicera canadensis* Marshall	3.1	0.01 ± 0.01	0-1

*One site (No. 32; Kalamazoo Co.) was not quantitatively-surveyed because a partial harvest occurred before the survey could be conducted. However, earlier observations indicated that ground flora at the site was predominantly poison ivy (*Toxicodendron radicans* (L.) Kuntze) and Virginia creeper (*Parthenocissus* spp.).

**Grasses/sedges were quantified as percentage of cover in 1 m^2 quadrants.

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