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Distribution of *Amoebidium* and *Smittium* Species (Trichomycetes) in Mosquito Larvae on the Platte River Floodplain of Central Nebraska

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Trichomycetes (fungi) inhabit the digestive tract of insects and other arthropods. Two genera, *Smittium* and *Amoebidium*, were collected from mosquito larvae (Culicidae) from 36 sites in a six-county area of central Nebraska, U.S.A., in the Platte River floodplain during the summers of 1986 and 1987. When present, thirty mosquito larvae per site per month were identified at the fourth instar, checked for the epizooite *A. parasiticum*, and then dissected and the gut examined by phase-contrast microscopy for *S. culisetae* and *S. culicis*. In 1986, 17 species of mosquito larvae from six genera ($n = 665$) were dissected and 22.7% were infested with *Smittium* spp. and 14.7% with *Amoebidium* sp. In 1987, eight species from four genera ($n = 380$) were dissected and 26.6% were infested with *Smittium* spp. and 11.8% with *Amoebidium* sp. ($n+n = 1045$). The percentage of trichomycete infestation remained similar from year to year, although the yearly total of dissected potential hosts varied. Two sites had *Smittium* species only once each in two years, which suggests that host continuity is not necessary for trichomycete infestation.

‡ ‡ ‡

INTRODUCTION

Trichomycetes are considered a class (Alexopoulos, 1962; Scagel et al., 1982; Lichtwardt, 1986) and are now placed in Division Zygomycota (Lichtwardt, 1986) of the Kingdom Fungi. Members of the Class Trichomycetes are found inhabiting the interior chitinous gut lining of Arthropoda, except for the ectocommensal *Amoebidium parasiticum* Cienkowski, which is found attached to the external cuticle of various aquatic arthropods such as daphnids and insect larvae; it was the first trichomycete species to be axenically cultured (Whisler, 1960).

Smittium Poisson was the second trichomycete genus to be successfully cultured and two species, *S. culisetae* Lichtwardt and *S. culicis* Manier, occur with *Amoebidium* on mosquito larvae (Lichtwardt, 1964, 1986; Williams and Lichtwardt, 1972). Sweeney (1981) reported fatalities of 50–90% in laboratory-reared *Anopheles hilli* Woodhill & Less infested with *Smittium morbosum* Sweeney, but *S. culicis* and *S. culisetae* have not been reported as lethal. Williams and Lichtwardt (1972) reported *S. culisetae* as detrimental under suboptimal mosquito larval growth conditions.

Smittium culicis occurs most frequently in Europe, while *S. culisetae* occurs most often in the United States (Lichtwardt, 1986). *Smittium morbosum* has so far been reported only from Australia and to date is the only *Smittium* known to develop haustoria and to persist into the pupal and adult stages (Sweeney, 1981).

Few studies on distribution and infestation rates have been reported for Trichomycetes. Williams and Nagel (1980), Goettel (1987), and Wiener and Williams (1982) have reported on field investigations of the occurrence of Trichomycetes (*Smittium* spp., *Amoebidium* sp.) with mosquito larvae. Lichtwardt and Williams (1988) reported on the distribution and species diversity of trichomycete gut fungi in aquatic insect larvae other than mosquito larvae in two Rocky Mountain streams.

Our study area is in the Big Bend area of the Platte River in central Nebraska. It is primarily a floodplain on both sides of the river, with interspersed riparian forest, agricultural fields, and stream-flow dependent wetland prairie (Anonymous, 1981). Nagel (1975) reported collections of 22 mosquito species in a three-county area in the Big Bend. Mosquito larvae are the potential hosts of the three Trichomycetes of this study: *Smittium culicis*, *S. culisetae*, and *Amoebidium parasiticum*.

The purpose of this study is to determine the distribution of the three trichomycete species in mosquito larvae and the prevalence of host infestation. These data are compared to those from the study of Williams and Nagel (1980), which was limited to an area in Buffalo County near Kearney, Nebraska.

MATERIALS AND METHODS

Thirty-six sites (Fig. 1) were selected from within a 30x100 km section along the Platte River from south of Lexington to southwest of Grand Island, Nebraska. The area covered all or part of 36 townships in Buffalo, Dawson, Gosper, Hall, Kearney, and Phelps counties.

The field study was conducted from May through September in 1986 and 1987. Each site was sampled at least once a month. Mosquito larvae were collected with a drinking-water dipper with an extended handle. It was dipped ten times at each site to determine if larvae were present, usually near the pool edge and adjacent to vegetation. If no larvae were found in ten test dips, the site was considered devoid of larvae. When larvae were found, they were placed into plastic vials, labeled, and placed on ice for return to the laboratory. There, they were transferred to petri dishes that were one-third full of water and then refrigerated until they could be dissected. Sixty or more larvae

were usually collected to ensure that 30 would be available for dissection.

Living fungal material has fewer artifacts and less size distortion than preserved specimens, so living specimens were used for this study. No staining was used except in preservation of selected specimens after initial observations were done. All specimens were examined using phase-contrast microscopy to observe the hyaline features of the fungi, especially the fungal trichospores by which the species of *Smittium* were identified. *Smittium culicis* and *S. culisetae* are differentiated by the size and shape of their trichospores. The spore of *S. culicis* has its greatest width at its median, while that of *S. culisetae* has its greatest width submedially. The spore length of *S. culicis* as well as the collar length are each approximately 4 um longer than those of *S. culisetae*.

Mosquito larvae were identified using Stojanovich (1961), Ross and Horsfall (1965), or both. All voucher specimens were confirmed using Darsie and Ward (1981). Thirty larvae were dissected from each monthly collection having larvae, examined for Trichomycetes, and also checked for haustoria or other signs of fungal intrusion such as the exterior dark spot described by Sweeney (1981) for *S. morbosum*.

Larvae were dissected in a petri dish under a stereoscopic dissecting microscope, using two pairs of very finely-honed jeweler's forceps. The larval hindgut was removed by grasping the anal saddle with one pair of forceps and the thorax with the other and gently pulling out the hindgut. The severed head, midgut, and hindgut (still attached to the saddle) were transferred into a drop of sterile distilled water on a microscope slide. The hindgut was opened and all three segments were covered by a coverslip. The antennae and saddle brushes were checked for *Amoebidium parasiticum* and the hindgut was checked for *Smittium* spp.

RESULTS AND DISCUSSION

Of 97 collections taken in 1986, 26 (26.8%) had mosquito larvae; 17 of 54 (31.5%) collections had larvae in 1987, and the two-year average was 43 of 151 collections (28.5%). The Platte River flooded in 1986 but not in 1987, which probably accounts for the higher number of larvae in 1986. There were 665 larvae dissected in 1986 and 380 in 1987. Nagel's (1975) study of mosquito populations in this area reported 1556 larvae in 1973 and 345 in 1974. Nagel (1975) reported that the majority of favorable mosquito breeding habitats were produced by flooding and rainfall in 1973 (a wet year) and by irrigation in 1974 (a dry year).

Aedes vexans (Meigen) were the predominant larvae in 1986, with 204 (30.7%) specimens, *Culex tarsalis* Coquillet larvae were second with 198 (29.8%) specimens, and *Aedes spencerii* (Theobald) larvae were third with 105 (15.8%) specimens. These three species accounted for 507 (76.2%) of the 665 larvae dissected in 1986. *Aedes vexans* were also the predominant larvae in 1987, with 184 (48.4%) specimens, and *Culex tarsalis* were second with 109 (28.7%) specimens; they accounted for 293 (77.1%) of the 380 larvae dissected in 1987. For both years, the three species accounted for 800 (76.6%) specimens of the 1045 dissected. There were 17 species collected in 1986 but only eight in 1987. The mosquito larval species numbers and infestation rates are tabulated in Table I for 1986 and Table II for 1987.

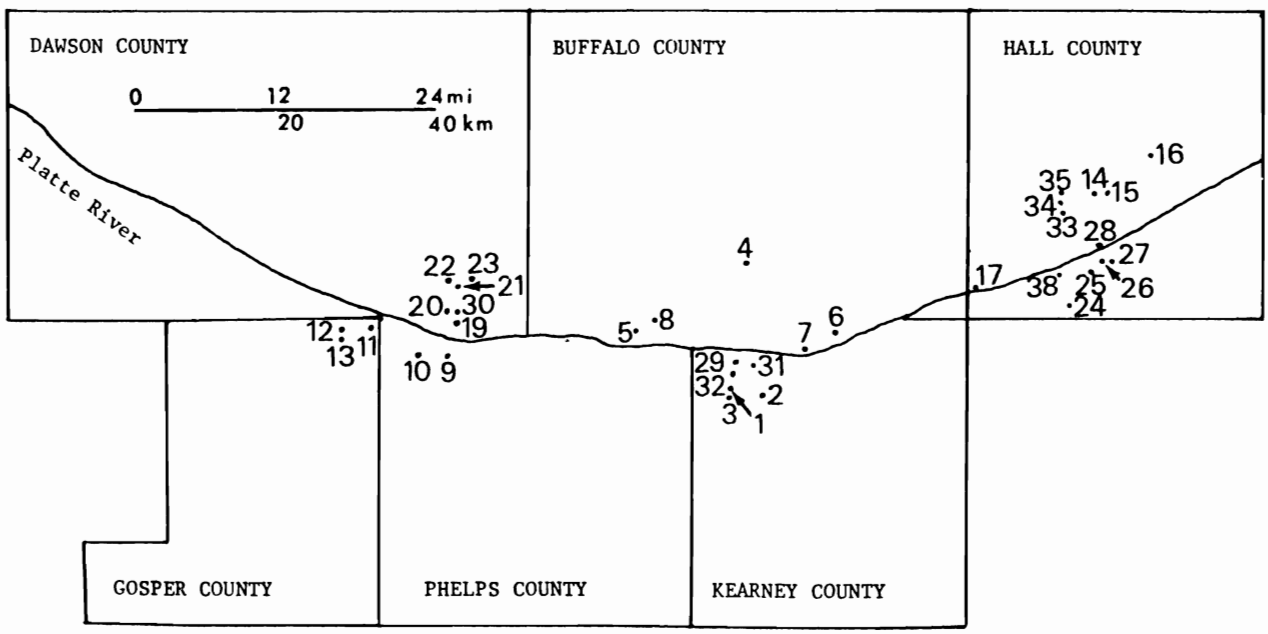

FIGURE 1. Study sites on the Platte River floodplain in central Nebraska.

TABLE I. Prevalence of Trichomyecte (*Smittium* and *Amoebidium* spp.) infestation by species of larval mosquito (1986).

	Specimen	***S. culisetae***		***S. culicis***		***Smittium spp.***		***A.parasiticum***	
	No.	No.	%	No.	%	No.	%	No.	%
Aedes campestris	2	0	0	0	0	0	0	0	0
Ae. cinereus	2	0	0	0	0	0	0	0	0
Ae. dorsalis	1	0	0	0	0	0	0	0	0
Ae. flavescens	2	0	0	0	0	0	0	1	50
Ae. nigromaculis	10	0	0	0	0	0	0	0	0
Ae. spencerii	105	32	30	0	0	10	9.5	0	0
Ae. trivittatus	20	0	0	0	0	0	0	0	0
Ae. vexans	204	21	10	0	0	25	12	7	3.4
Ae. sp.	1	0	0	0	0	1	100	0	0
Anopheles punctipennis	49	0	0	0	0	0	0	0	0
An. quadrimaculatus	2	0	0	0	0	0	0	0	0
Culex restuans	15	0	0	0	0	1	6.6	2	13.3
Cx. tarsalis	198	6	3	3	1.5	2	1	49	24.7
Culiseta impatiens	1	1	100	0	0	0	0	1	100
Cs. incidens	2	1	50	1	50	0	0	2	100
Cs. inornata	48	32	66.6	8	16.6	6	12.5	36	75
Psorophora ciliata	2	1	50	0	0	0	0	0	0
Uranotaenia sapphirina	1	0	0	0	0	0	0	0	0
Total	665	94	14.1	12	1.8	45	6.8	98	14.7

6 genera

18 species

In 1986, the ectocommensal *Amoebidium parasiticum* was observed on 98 (14.7%) of the 665 larvae dissected, and in 1987 it was found on 45 (11.8%) of the 380 specimens. The collection site repeatability was 83% (access to five sites was precluded by bridge construction in 1987, and one site was added; however, none of these produced *Amoebidium*). The two-year average infestation was 143 (13.7%) of 1045 specimens. The number of infested larvae varied by site and by county, but the overall percentage of infestation remained similar for the two years when measured for the entire study area.

In 1986, *Smittium culisetae* was found in 94 (14.1%) of 665 specimens, and in 63 of 380 (16.6%) specimens in 1987, the two-year average being 15% (157 of 1045 specimens). In 1986, *S. culicis* was found in 12 (1.8%) of 665 specimens, and in 1987 it was in 12 (3.2%) of 380 specimens. The unidentified *Smittium* species (immature thalli) for 1986 were 45 (6.8%) of 665, and in 1987 they were 26 (6.8%) of 380 specimens. There were eleven larvae with both *S. culisetae* and *S. culicis*, and this occurred at only one site in 1986. The combined *Smittium* species for 1986 was 151 (22.7%) of 665 and for 1987 it was 101 (26.6%) of 380 larvae. The infestation rate of the representative trichomycete groups — *S. culisetae* (14.1% and 16.6%), combined *Smittium* spp. (22.7% and 26.6%), *Amoebidium parasiticum* (14.7% and 11.8%) — were similar in 1986 and 1987, respectively. This similarity of trichomycete infestation rates from one year to the next was quite different from that reported by Williams and Nagel (1980). In 1977, they reported 3.8% and in 1978, 59.8% infestation for *A. parasiticum*, and for combined *Smittium* spp. they reported 1.7% and 40% respectively. The size of the present study area (3,000 sq km) is approximately 15 times the size (200 sq km) of Williams and Nagel's (1980) area. The larger size of our study area could be a factor in averaging fluctuations.

Williams and Nagel (1980) suggested that a continued host population is necessary to maintain trichomycete infestations. At two sites, one in Phelps and one in Buffalo County, larvae were found only once during the two years of the study, although the sites were routinely checked. The Phelps County site produced *S. culisetae* and immature thalli (probably *S. culisetae*) at 90% prevalence, and the Buffalo County sites produced *S. culicis* and immature thalli (probably *S. culicis*) at 76.7% prevalence. This does not support the suggestion of Williams and Nagel (1980) that a continued host population is necessary to maintain a trichomycete population at a site.

Goettel's (1987) report does not support Williams and Nagel's (1980) suggestion because he observed *Smittium* species at sites that had been dry for extended periods. This suggests that *Smittium* (at least *S. culisetae*) spores can survive extended periods when no hosts are available, as suggested by Williams (1983a). On the other hand, *Smittium* may have some as yet unknown method of rapidly colonizing isolated sites.

Of the six sites that produced larvae both years, only Site 1 had *Smittium* spp. present both years, and only Sites 1 and 19 had *Amoebidium parasiticum* present both years (Table III).

The only county record previously reported of trichomycete distribution in Nebraska was *Smittium culisetae* from Kearney County (Williams, 1983b). We collected *S. culisetae* in Buffalo, Dawson, Hall, and Phelps counties; *Smittium culicis* in Buffalo and Kearney counties; and *Amoebidium parasiticum* in Buffalo, Dawson, Gosper, Hall, and Kearney counties.

We collected six genera of host mosquitoes: *Aedes*, *Anopheles*, *Culex*, *Culiseta*, *Psorophora*, and *Uranotaenia*. Two, *Anopheles* and *Uranotaenia*, did not have *Smittium* thalli, and they and *Psorophora* were not infested with *Amoebidium parasiticum*.

Mosquito species we collected but not previously reported in the literature (Nagel, 1975; Lunt and Rapp, 1981) were *Culex territans* Walker in Kearney County; *Psorophora columbiae* (Dyar and Knab) in Hall County; *Culiseta incidens* (Thomson) in Hitchcock County (a county not in our survey area but we sampled it for trichomycete larval hosts); *Culex restuans* Theobald in Hitchcock County; and *Aedes epactius* Dyar and Knab (as confirmed in Darsie and Ward, 1981) in Hitchcock County. *Aedes epactius* had not been reported in Nebraska, and numerous unsuccessful attempts to collect more specimens were made in the area just below Trenton Dam. The mosquitoes were collected and identified as larvae, and only *Culex territans* and *Culiseta incidens* produced adult specimens.

New host records for *Smittium culisetae* include *Aedes spencerii* and *Psorophora ciliata* (Fabricius), with *Culiseta impatiens* (Walker) and *Cs. incidens* being new host records for Nebraska. *Culiseta incidens* is a new host record for *Smittium culicis*; new host records for *Amoebidium parasiticum* include *Aedes flavescens* (Muller), *Culiseta impatiens*, and *Culiseta incidens*.

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TABLE II. Prevalence of Trichomycete (*Smittium* and *Amoebidium* spp.) infestation by species of larval mosquito (1987).

	Specimen	*S. culisetae*		*S. culicis*		*Smittium spp.*		*A. parasiticum*	
	No.	No.	%	No.	%	No.	%	No.	%
Aedes dorsalis	4	0	0	0	0	0	0	0	0
Ae. nigromaculis	20	0	0	0	0	0	0	0	0
Ae. triseriatus	1	0	0	0	0	0	0	0	0
Ae. vexans	184	19	10.3	12	6.5	19	10.3	13	7.1
Anopheles quadrimaculatus	2	0	0	0	0	0	0	0	0
Culex territans	30	0	0	0	0	0	0	0	0
Cx. tarsalis	109	28	25.7	0	0	4	3.7	5	4.6
Culiseta inornata	30	16	53.3	0	0	3	10	27	90
Total	380	63	16.6	12	3.2	26	6.9	45	11.8

TABLE III. Trichomycete comparison of sites with larvae present in both years.

Site	1986		1987	
	Smittium spp.	*Amoebidium parasiticum*	*Smittium spp.*	*Amoebidium parasiticum*
1	+	+	+	+
12	-	+	-	-
13	-	+	-	-
19	+	+	-	+
20	+	-	-	-
30	-	-	-	-

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