being less selective, nontarget organisms were more susceptible, especially at higher rates. Both larvicides were extremely effective against *Anopheles*. Dursban appeared to have a residual effect lasting from a week to 10 days in some cases (0.01 and 0.021 lb./ac.), whereas no residual effect was observed for Abate.

Although application rates reported herein are based on expected effective swaths of 100 feet, wider swaths were usually attained. This might be a desirable condition since it would allow a margin of error even at the extremely low dosages used. However, it appears feasible to continue evaluation toward a narrower swath or alternate right, left, or two-nozzle spray equipment to concentrate spray when required by field conditions.

STEMPELLIA MAGNA (KUDO) (NOSEMATIDAE: MICROSPORIDIA) IN *CULEX RESTUANS* THEOBALD FROM VIRGINIA

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A new geographic record of the microsporidian *Stempellia magna* is reported. Late instar *Culex restuans* larvae collected on June 23, 1966 at Falls Church, Virginia had a 35 percent incidence of infection. Later collections of mixed species from the same site showed infections only in *C. restuans*. *Anopheles stephensi* larvae reared in water from the site produced a frank infection, with mature spores, in a single larva and several larvae with early developmental stages of the parasite indicating *per os* transmission.

INTRODUCTION. In 1920, Kudo described a new microsporidian parasite, *Thelohania magna,* from larvae of *Culex pipiens* collected near Urbana, Illinois. The next year heavy infections of this parasite were reported from *Culex territans* collected at Warren, Pennsylvania (Kudo, 1921). In 1925 Kudo placed this species in the genus *Stempellia*. *Stempellia magna* was not reisolated until 1962 when Kudo collected it from *Culex restuans* and *C. pipiens* on the campus of Southern Illinois University at Carbondale, Illinois. *Stempellia magna* was also reported from *Culex restuans* at the Gettysburg Battlefield, Gettysburg, Pennsylvania, by Wills and Beaudoin (1965). Thus the distribution and host records of *Stempellia magna* are few, with the parasite recorded from two states and three mosquito species. This species is the only member of the genus *Stempellia* that has been described from mosquitoes. The other species, *S. mutabilis* Léger and Hesse, was described from an ephemerid in France.

METHODS AND MATERIALS. On June 23, 1966 a larval collection of *Culex restuans* was made at Falls Church, Virginia. The larvae were brought into the laboratory and placed in a white enamel pan for observation. All dead and moribund larvae were removed from the pan daily, smeared on microscope slides, air-dried, fixed in absolute methanol for one minute, and stained for 30 minutes with a 1:20 ratio of Giemsa stock solution and tap water. On July 5, four live larvae and five live

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pupae remained in the pan, at which time they were smeared and stained. These larvae and pupae were very active with no visible evidence of morbidity. Since all adults emerging from this collection were used in an attempt to colonize the species, none were examined for infection.

The collection site was an eddy pool along a small stream supplied with water by the effluent from a storm sewer and by underground seepage. Bacteriological analysis of the water showed high contamination by a species of *Klebsiella.* Heavy rains on June 25 flushed all larvae from the pool, but the larval population built up again in about 2 weeks.

On July 14 large numbers of eggs and larvae were collected at the Falls Church site. This collection was a mixed population containing larvae of *Anopheles walkeri, Culex territans, C. quinquefasciatus,* and *C. restuans* and eggs of *C. quinquefasciatus* and *C. restuans.* The *Anopheles* larvae were removed and placed in a separate pan. Due to the possibility of injury to the larvae and the time involved in identification and sorting a total of possibly 10,000 larvae, all *Culex* species were kept together in several white enamel pans. Dead larvae and pupae were smeared and stained daily and examined for infection. On July 27 all but 104 larvae had died or pupated. These larvae, all of which appeared to be healthy, were identified, smeared and stained for examination. On July 28, 36 *Culex quinquefasciatus* and *C. restuans* adults were prepared and examined for parasites. In addition, about 100 dead adults of these two species were triturated in a Ten Broeck grinder, smeared and stained for examination. All other adults were used in a continuing effort to establish laboratory colonies of the mosquito species.

Egg rafts were kept in individual containers in the laboratory to identify the species and determine if any were infected with *Stempellia magna* by transovarian transmission.

Photomicrographs were made of various stages of the parasite, using a compound light microscope equipped with a 100 x oil immersion objective and a 35mm camera with a 5 x lens. Measurements were made of 25 mature spores and the standard error of the mean calculated.

RESULTS AND DISCUSSION. From the collection made on June 23, 19 living larvae, 60 dead larvae, and 5 living pupae were examined. Of these, 15 living larvae, 8 dead larvae and 5 living pupae were infected with *Stempellia magna,* making a total of 28 specimens infected of 84 examined.

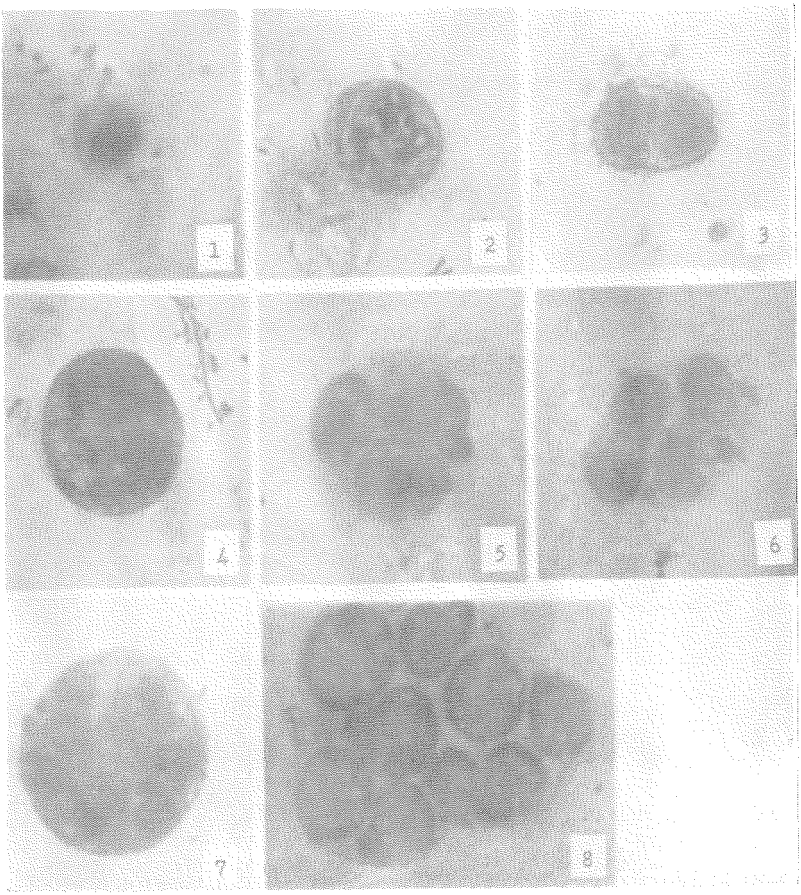
During the study of the collection made on July 14, 68 larvae and 71 pupae died. Of these 52 larvae and 35 pupae were infected with *Stempellia magna.* In addition, 13 of 20 live larvae of *Culex restuans* examined were infected with the parasite. No infections were found among 79 live larvae of *Culex quinquefasciatus* and 5 of *C. territans.* No infection was observed in the 36 smeared adults, but a few spores were seen on the slides prepared from triturated adults. Of 363 specimens examined from both collections, 128 were infected with *Stempellia magna.*

The external appearance and activity of some larvae and pupae suggested they were healthy individuals, but microscopic examination revealed that some had only early parasitic stages while others had early stages and mature spores. This suggests that even more specimens may have been parasitized, but the infection was not fatal and persisted into the pupal and adult stages. Kudo (1962) and Wills and Beaudoin (1965) collected egg rafts in the field which produced infected larvae in the laboratory, indicating that transovarian transmission may exist due to latent infections carried over into the adult mosquitoes. We believe that such reports are inconclusive unless egg rafts are surface-sterilized and shown to be free of parasitism by microscopic examination. A preliminary study to determine infectivity of water-borne stages of *Stempellia* to *Anopheles stephensi* larvae resulted in one frank infection in which spores were present and several cases where early

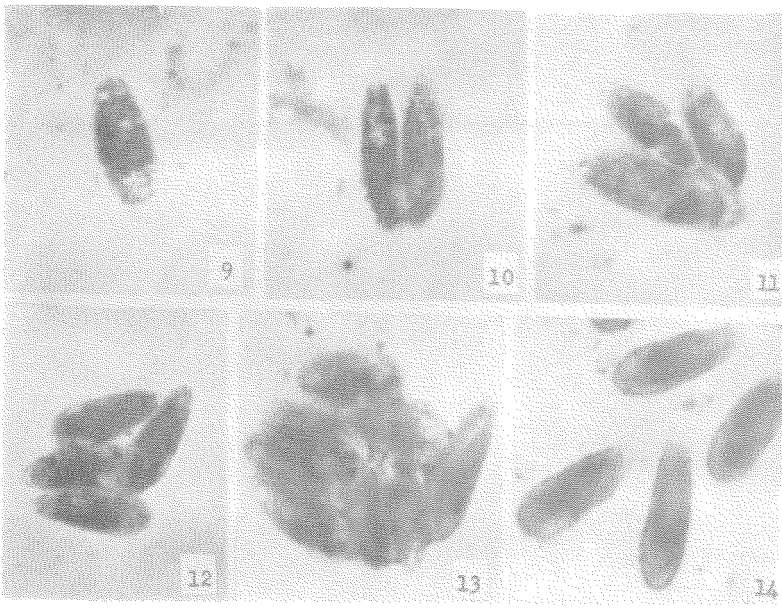
stages were suspected. Thus, *per os* infection cannot be discounted as a mode of transmission of *Stempellia* in nature.

Of the 29 egg rafts collected from the pool, 5 rafts had hatched in the field and the remaining 24 hatched in pans. The resulting larvae from 2 rafts were identified as *Culex restuans* while the remaining 22 rafts produced *C. quinquefasciatus*. None of the resulting larvae were infected with *Stempellia magna*.

Figures 1–14 show some of the stages of *Stempellia magna* that we observed in stained smears. Figure 1 shows a young schizont. In Figure 2 nuclear division is taking place that will result in binary fission, as seen in Figure 3. Figures 4–8 show several sporonts with various numbers of nuclei. Although Kudo (1921) states that sporonts produce a single spore, or divide into two, four, or eight sporoblasts, several sporonts were observed in the present study with an odd number of nuclei. Figures 5 and 8



FIGS. 1–8.—Photomicrographs of some early stages of *Stempellia magna* from stained smears of *Culex restuans* larvae. 1. Young schizont. 2. Nuclear division. 3. Binary fission. 4. Binucleate sporont. 5. Trinucleate sporont. 6. Quadrinucleate sporont. 7. Octonucleate sporont. 8. Nononucleate sporont.



FIGS. 9–14—Photomicrographs of *Stempellia magna* spores from stained smears of *Culex restuans* larvae. 9. Young spore. 10. Group of two spores. 11. Group of three spores (one a macrospore). 12. Group of four spores. 13. Group of eight spores. 14. Individual mature spores.

show two such sporonts with three and nine nuclei, respectively. Young and mature spores are shown in Figures 9–14. Figure 11 shows a three-spore group, one a large macrospore. This may have resulted in the failure of one nucleus in a binucleate sporont to divide, thus producing a trinucleate rather than the normal quadrinucleate sporoblast. Figures 9, 10, 12, and 13 show spores that have been produced in normal fashion in one-, two-, four- or eight-spore-groups.

The mature spores of *Stempellia magna* (Figure 14) measured $12.44 \pm 0.19\mu \times 3.84 \pm 0.11\mu$. This compares with "12 to 13.5μ long and 4μ broad" as stated by Kudo (1921), and "$12.1 \pm (?)$[4] microns by $3.34 \pm .06$ microns" given by Wills and Beaudoin (1965).

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[4] An apparent misprint in the paper by Wills and Beaudoin (1965) omitted the standard error of the mean on their measurements of spore length.