Histoplasma capsulatum Recovered from Bat Tissues

Abstract. *Histoplasma capsulatum* was recovered from the liver and spleen tissues of a species of predominantly insectivorous bats as well as from soil collected in a man-made harborage at Madden Air Field in the Republic of Panama.

Although the recovery of the fungus *Histoplasma capsulatum* from soils enriched with bat or bird guano has been documented (*1*), successful attempts to recover it from the tissues of bats have not been reported.

More than 100 bats were collected from a building adjacent to the air strip of the now-abandoned Madden Air Field, Republic of Panama. Within the building, the roosting habits of the bats were such that the guano collected could be related to the predominant bat using that particular roost. Three species of bats were recovered, *Chilonycterus rubiginosa fusca, Carollia perspiculata azteca,* and *Phyllostomus hastatus panamensis.* The latter species, an omnivore, was not examined for the presence of *Histoplasma capsulatum.* In addition to the bats and guano collected within the building, soil samples were taken at the entrance, under the eaves, and at the rear of the building.

Thirty *Chilonycterus rubiginosa fusca* were killed in groups of five; livers and spleens were pooled and made into a homogeneous suspension with the aid of sterile sand, mortar, and pestle. The supernatant from this suspension was then inoculated onto two blood-fortified mycosel (*2*) plates and into each of five mice (0.2 ml intraperitoneally). Forty-five *Carollia perspiculata azteca* were processed in similar fashion, after a holding period of 22 days at the Corozal Veterinary Quarantine Station. Thus a total of six pools of *Chilonycterus* and nine pools of *Carollia* liver-and-spleen suspensions were sampled for the presence of *Histoplasma capsulatum.*

None of the plates inoculated directly with the suspensions of liver and spleen revealed the presence of *H. capsulatum.* In addition, none of the mice inoculated with the tissues of *Carollia perspiculata azteca* yielded *Histoplasma capsulatum* when killed at varying times after inoculation. In contrast, two of the *Chilonycterus* pools (A445 and A447) yielded positive cultures. The details of these recoveries are of interest: of the five mice inoculated with pool A445, two were sacrificed on the 36th, two on the 37th, and one on the 38th day after inoculation. Liver and spleen suspensions were prepared and inoculated onto two blood-fortified mycosel plates which were then incubated at 26°C for approximately 15 days. Each of the two plates prepared on the 36th day after inoculation yielded *Histoplasma capsulatum.* One mouse of the five inoculated with pool A447 died on the second day after inoculation, and it was discarded. The remaining four mice were killed in two groups on the 36th and 37th days after inoculation. Each of the four plates prepared after their sacrifice yielded *H. capsulatum* after 2 weeks of incubation at 26°C.

The soil and guano specimens were placed at −20°C for 24 hours before processing to reduce the activity of minute fauna. Each 2-g sample of soil or guano was suspended in saline containing penicillin, streptomycin, and Achromycin (*3*) to a final concentration of 400 units, 400 and 40 μg/ml, respectively, and then shaken vigorously for 5 minutes. After 2 hours of settling, the supernatant was decanted and used to inoculate each of five mice intraperitoneally with 0.5 ml. All mice inoculated with supernatants from guano speciments died within 24 hours, despite prior treatment with antibiotics. Repeat isolation attempts from these materials, with more dilute inoculum, were unsuccessful, since the toxic principle in the guano persisted even after dilution.

Mice inoculated with the four soil samples were sacrificed at varying intervals of time after inoculation. Pools of liver and spleen from mice inoculated with soil samples collected at the entrance to the building were prepared on the 35th (two mice), the 37th (two mice), and the 41st day (one mouse) after inoculation. Each pool was inoculated onto duplicate blood-fortified mycosel plates and incubated at 26°C. When examined 15 days later, all six plates revealed the presence of *H. capsulatum.*

Thus, from one site in Panama, *Histoplasma capsulatum* was recovered from the tissues of two pools of bats and from the soil at the entrance to the artificial harborage. The multiple recoveries from bat pools, the recovery of the organism from mice inoculated with soil, and the numerous negative findings from similar materials processed at the same time substantiate the validity of these findings.

Three months later (3 October 1961) a second collecting trip to this same site yielded 14 specimens of *Chilonycterus rubiginosa fusca* which were brought to the laboratory and sacrificed immediately for inoculation of liver and spleen suspensions onto blood-fortified mycosel media and into mice. From these direct inoculations 9 of the 14 bats examined yielded positive cultures for *Histoplasma capsulatum.*

The role of bats in the dissemination of *H. capsulatum* in nature remains to be determined. Whether they are able to disseminate the organism in their excreta or whether they experience overt disease cannot be stated at this time. However, the association of the organism with bat guano suggests this animal may eventually be implicated as one of the reservoirs in nature.

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References and Notes

1. C. W. Emmons, *Public Health Repts.* **73**, 590 (1958); ———, *ibid.* **76**, 591 (1961); L. Ajello, T. Briceno-Maaz, H. Campins, J. C. Moore, *Mycopathol. et Mycol. Appl.* **12**, 199 (1960).
2. Ten percent citrated human blood, obtained from Baltimore Biological Laboratories.
3. Achromycin is Lederle Laboratories' brand of tetracycline hydrocholoride.

20 October 1961

Irrigation and Nitrogen Effects on Sweet Corn Row Numbers at Various Growth Stages

Abstract. Kernel rows per ear of a hybrid of *Zea mays L.* were found to be affected by environmental conditions during the first 7 weeks after planting. Increased row numbers were evident on harvested ears if as little as 25 pounds per acre of nitrogen fertilizer was added at planting.

This investigation was conducted on Superstition loamy fine sand at Yuma, Arizona, in 1959 as part of a much larger 3-year study, 1957–1959 (*1*). Sweet corn, *Zea mays L.*, certified Golden Cross Bantam, was grown to determine the effect of quantity and timing of irrigation and nitrogen on the several basic components of yield: ears per plant, rows per ear, kernels per row, and weight of individual kernels. Three growth stages were defined and used

Table 1. Mean kernel rows per ear of sweet corn as affected by nitrogen and irrigation at the three growth stages.

Stage for application*	Rows	Stage for irrigation†	Rows
000	10.0	WWW	11.0
0X0	10.4	DDD	11.0
0XX	10.3	DDW	10.8
00X	10.5	DWW	10.7
XXX	11.3		
XX0	11.4		
X0X	11.4		
X00	11.7		

* Left letter = N application at planting; middle letter = N application at elongation; right letter = N application at tasseling. 0 = no N application; X = N application. † W = irrigated when tension reached 0.4 atm; D = irrigated when tension reached 8 to 10 atm; WWW = reirrigated when tension reached 0.4 atm throughout season; DDD = reirrigated when tension reached 8 to 10 atm throughout season; DDW = reirrigated when tension reached 8 to 10 atm before tasseling, 0.4 atm thereafter; DWW = reirrigated when tension reached 8 to 10 atm before coleoptile node elongation, 0.4 atm thereafter.

for studying the timing effect of N and irrigation. These three stages were: (i) establishment—from planting until the emergence of the coleoptile node at the soil surface, about 7 weeks after planting; (ii) higher internode elongation—the period of maximum culm elongation, 3 to 4 additional weeks; and (iii) reproduction—the period including pollination, fertilization, and caryopsis development, and terminating when the "roasting" ear was picked, about 3 additional weeks.

Plots were 115 by 63 feet, arranged in a split-plot design, with four moisture treatments as whole plots and 15 nitrogen applications randomly allocated on 23- by 21-ft subplots in each moisture treatment. There were three replications of the experiment, giving a total of 180 plots. In late February kernels were planted about 2 inches deep, six per foot, in rows 36 inches apart. Plants were thinned to two per foot when about 4 inches tall, 3 weeks after emergence.

Nitrogen applications involved a total of 75 or 300 lb of N per acre, applied at single or split times or both (for example: 25–25–25, 37.5–0–37.5, 100–100–100, 150–150–0) at the beginning of the defined growth stages as shown in Table 1, which gave 14 combinations plus a control. Various irrigation levels were maintained by using tensiometers with the porous sensing cups 8 inches deep. "Wet" plots were reirrigated when moisture tension reached 0.40 atm, whereas "dry" plots were reirrigated at or beyond 1.5 times the time to reach 0.7 atm. The latter treatment usually caused plants to wilt.

Table 2. Analysis of variance of data in Table 1.

Source of variation	D.F.	M.S.
Replication	2	2.97
Irrigation (I)	3	.83
Error *a*	6	.59
Nitrogen (N)	14	5.78*
I × N	42	.34
Error *b*	112	.26

* Significant at the 1-percent level.

To estimate the number of kernel rows per ear for a treatment, all harvestable ears in the three middle rows of each plot were placed randomly side by side and a systematic sample, with a random start, was taken to give ten ears from each plot.

Originally the number of kernel rows per ear was considered to be solely a complex genetic character (*2*). Emerson and Smith (*3*) found that "Since inbreds proven to be essentially homozygous for row-number genes vary in this character over three, four, or five classes, it is evident that there is a nongenetic component of variability of considerable magnitude. The genes concerned do not rigidly control the number of kernel rows that are formed during the morphological development of the ear." They state further, "Row number is less affected by environmental diversity than most characters of this type as observed over many years by the senior author." Also, "The relatively small differences in fertility encountered in a uniform well-chosen small field . . . have no appreciable influence on row number." They failed to obtain a significant row increase from N applications (or other cultural treatments) in any single case, although the trend was significant when ten such N tests were combined. Alexander (*4*) did not obtain significant increases with 320 lb of N per acre, possibly because of adequate indigenous N in the soil. Superstition sand as used in this study usually contains less than 20 lb of available N per acre-foot.

Both genetic constitution and environment play a role in the expression of the attribute under study. Although probably affected predominantly by genetic factors, results presented in Tables 1 and 2 and in Fig. 1 provide strong evidence that row numbers also have been increased by cultural practices, specifically that rows per ear were determined within our stage of establishment, before the plants were 8 inches tall. When the nitrogen application effects were subdivided into individual degrees of freedom, highly significant effects were noted for rate of N over all applications and for application at planting for either rate, with no subsequent effect of N on kernel row numbers for subsequent times of application. In fact 80 percent of the variation for nitrogen was directly attributable to the effect of N application in the planting stage. Means in Table 1 may indicate an odd number of rows, though of the 1800 individual ears examined, all had an even number, varying from 8 to 16. Row numbers appeared to be unaffected by the irrigation levels used, and to be increased by N only in the first stage of growth. Supplemental N at rates to 100 lb per acre during plant establishment resulted in further increases.

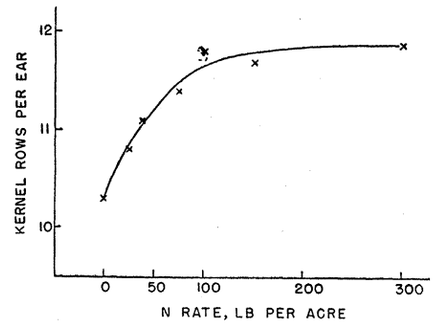

Fig. 1. Effect of nitrogen applied at planting on mean kernel row numbers of harvested sweet corn ears.

Although row number is considered a component of yield, and was increased by supplemental N, yields were not increased. Apparently this was because fewer rows per ear led to compensating increases in individual kernel number and size (*5*).

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References and Notes

1. C. O. Stanberry, H. A. Schreiber, M. Lowrey, C. L. Jenson, in preparation.
2. W. A. Huelsen, *Sweet Corn* (Interscience, New York, 1954), pp. 86–94.
3. R. A. Emerson and H. H. Smith, *Cornell Univ. Agr. Expt. Sta., Mem. No. 296* (1950).
4. D. E. Alexander, *Agron. J.* **44**, 430 (1952).
5. This report is a joint contribution of the Soil and Water Conservation Research Division, U.S. Agricultural Research Service, and the Arizona Agricultural Experiment Station; it is published as Arizona Technical Paper 648. We thank Ruth Cypert for measuring ears and analyzing the data.

30 November 1961