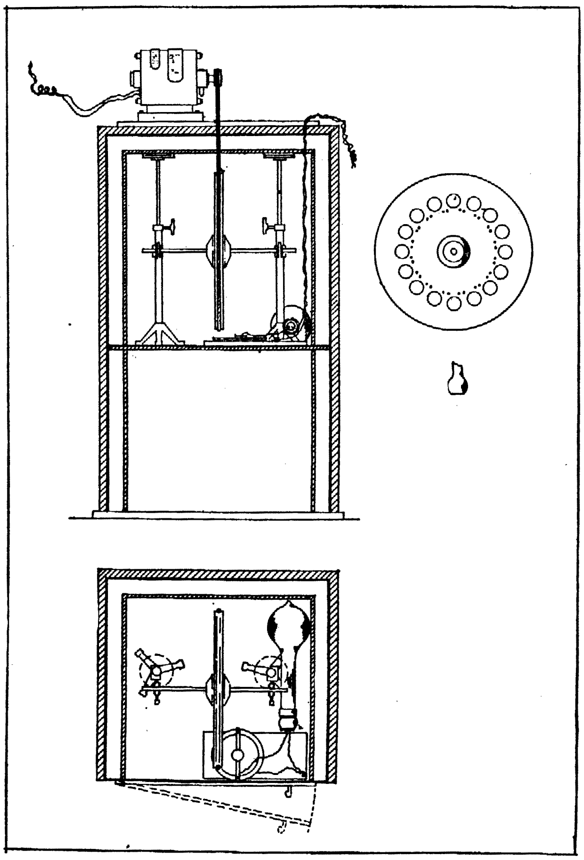

FIG. 1.

the possibility of absorption, the corks of the small bottles were covered with paraffin. This apparatus is to be particularly recommended for studies of this nature, in view of the fact that it can be duplicated readily from materials commonly found in the ordinary chemical laboratory and at a very low cost. If an incubator is not available any similar apparatus properly insulated can be used instead.

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SPECIAL ARTICLES

FURTHER EVIDENCE OF INSECT DISSEMINATION OF BACTERIAL WILT OF CORN

IN two previous papers[1] the writers have discussed some of the results of their experiments and field observations relative to bacterial wilt or Stewart's disease (*Aplanobacter stewarti* EFS emend McCul.) of corn. The cage experiments of 1922 with the brassy flea beetle (*Chaetocnema pulicaria* Melsh.)[2] demonstrated its agency as a carrier in the secondary spread of wilt during mid-season—a hitherto puzzling factor. Similar experiments in 1923 have abundantly confirmed these results and have added the toothed flea beetle (*C. denticulata* Ill.)[2] as a direct disseminator. Briefly, in several field tests aggregating sixteen cloth covered hill-cages, into each of which had been introduced twelve to fifty wilt-fed flea beetles, the final result gave a total of eight healthy plants and forty plants with bacterial wilt. That is, 83 per cent. of the plants in the cage tests contracted the disease.

In 149 control cages into which no insects were experimentally introduced there were 436 healthy plants and five plants with wilt. Of the latter, three cases with abundant yellow ooze characteristic of the disease, and accompanied by small larval channels in the base of the stem, occurred in two hills not caged until the plants were two to three inches tall. A fourth case with yellow ooze and larval channel occurred in a cage together with four healthy plants bearing external evidence of insect injuries at the base of the stem. The last case showed no ooze or bacteria in the stem, but on a leaf blade there were several wilt streaks starting from injuries due to flea beetles. Thus, with exception of five cases of external origin there was no wilt throughout the season in the 441 caged control plants.

The direct dissemination from diseased to healthy plants through the agency of flea beetles easily explains a large part of the mid-season spread of corn wilt, which often makes up the bulk of its seasonal incidence. They leave largely unexplained the sporadic cases of primary infection which appear early in the season without any apparent relation to external dissemination and which seem to originate from within the plant itself, that is, from the stem upward. These cases were at first supposed to come largely from infected seed and possibly also in some instances from infected soil. However, our field and greenhouse experiments during several seasons have clearly pointed away from soil infection as a factor and have apparently brought seed transmission within much narrower limits. From data previously discussed (*loc. cit.*) it may be repeated that under controlled conditions 2 per cent. of wilt is the highest amount obtained from seed collected from badly diseased plants. This evidence, however, does not minimize the importance of seed transmission in introducing wilt into

[1] Rand, Frederick V., and Lillian C. Cash, "Stewart's disease of corn," *Jour. Agric. Research,* 21: 263–264, 1921.

Rand, Frederick V., "Bacterial wilt or Stewart's disease of corn," *The Canner,* 56: 164–166, 1 fig., 1923 (No. 10, Pt. II).

[2] Identified by the Bureau of Entomology.

new sections nor the possibility in some instances of serious direct injury therefrom.

It became increasingly apparent that the facts would be explained if it could be shown that the early seasonal incidence of the disease is due largely to the introduction of the bacterial parasites by insects working at the roots or base of the stem. The following observations and experiments are pertinent to this working hypothesis.

(1) The known geographical distribution of bacterial wilt of maize corresponds closely with the prevalence of the southern corn root worm or 12-spotted cucumber beetle (*Diabrotica duodecempunctata* Oliv.). (2) This insect is reported to be most injurious in wet seasons and on low land. Our experience with wilt has been similar, and, furthermore, during two dry seasons there has been a higher percentage in irrigated than in contiguous unirrigated plots. (3) In a large number of wire-covered cages, which kept out Diabroticas but not flea beetles, there were no early or primary cases. From mid-season onward, however, wilt began to appear from flea beetle injuries on the leaves. (4) Seed of susceptible varieties obtained from wilt-free sections (Maine) has tended to develop an even higher percentage of wilt when planted in five lowland and mountainous sections of Maryland and West Virginia where the disease is prevalent than has seed of the same varieties obtained from and planted in these wilt-prevalent localities. Also under usual field conditions in Maryland and West Virginia seed from Maryland-grown healthy plants is as likely to give a high percentage of wilt as is that collected from badly diseased plants from the same original seed and plot. Furthermore, in both instances just discussed the percentage of *early cases* is as great from "wilt-free" seed as from open market or from "wilt-infected" seed of the same variety. (5) The sporadic appearance and spread of wilt in rows from thoroughly mixed lots of seed and in blocks planted with the same or different seed lots of the same variety suggest insect dissemination in primary as well as secondary cases. (6) Large seed-disinfection tests in the field have averaged as high a percentage of both primary and secondary wilt from treated as from untreated seed of the same lots. (7) In experimental fields in five localities during 1923 a high percentage (62–100 per cent.) of primary stem wilt cases showed small larval channels at the base of the stem. Plants without wilt pulled at random in the thinning out process in these same fields gave a uniformly low percentage (0–17 per cent.) of these larval channels. (8) The amount of wilt has always dwindled nearly or quite to zero in the late summer plantings, even when weather conditions have been apparently as favorable as in case of early plantings developing abundant wilt. This absence of wilt has coincided with striking decreases in number of flea beetles and with the well-known postponement of the egg-laying by the last or hibernating brood of Diabroticas until the following spring.

To the field evidence outlined above have been added a few results obtained under controlled conditions. During the period from mid-June to late August wilt-fed Diabroticas—usually four to six to a cage—were introduced into a total of 129 cloth-covered hill cages in the field. No wilt resulted from the August experiments aggregating fifty cages, but from those of June and July, six clean-cut cases of bacterial wilt developed in five cages, and five of these six plants showed definite small larval channels at the base of the stem. The time involved was a long period of drought. From what we know of other cases it is improbable that more than a small proportion of the beetles would become carriers, and then only under specially favorable conditions would they bring about infection through contamination of egg and larva and finally of the host. Therefore, six cases of wilt out of a total of several hundred caged plants, while giving a very small percentage, is of some significance when taken in connection with the negative results in the check cages above discussed.

Direct inoculations with intestinal contents of beetles of the southern corn rootworm have given a much higher proportion of infections. During June and August, 1923, three such tests were carried out in the field, in cloth cages, in which a total of about 85 plants were inoculated from wilt-fed beetles. Several of these inoculated plants developed within the first week a soft rot at the base and were thrown out of the test. Out of a total of 74 plants left, 15 developed typical wilt with yellow ooze, *i.e.*, 21 per cent. Three similar tests with inoculation at one, two and three days after wilt feeding were carried out in September in the greenhouse with like results, except that a somewhat smaller percentage developed typical signs, due, probably, to the larger proportion of infected plants rejected on account of soft-rot.

During September, 36 adult beetles were collected at random in the vicinity of several of the experimental fields and fed only upon healthy young maize plants for three weeks. Similar inoculations were then made with the intestinal contents of the 26 beetles still alive at the end of this period. At the present writing three typical cases of wilt are noted, although they have not yet been tested by isolation and subsequent reinoculation of the parasite, as was done for a portion of the plants from all the other tests. A similar positive result has just been obtained with beetles fed for five days on wilted plants, then kept for a month out-of-doors on healthy plants before using the intestinal contents for inoculation.

The origin of a large part of the sporadic early cases of bacterial wilt is, then, still *subjudice* with probabilities strongly in favor of its being due to Diabrotica 12-punctata; as an important means of mid-season spread of the disease flea beetles have been definitely indicated.

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PHYSIOLOGICAL STABILITY IN MAIZE*

THE significant results of Davidson and LeClerc[1] and of Gericke,[2] who obtained increased protein content of wheat grain by delaying the application of nitrates to the soil, raise pertinent questions regarding the possibility of similarly modifying other species and genera of plants. Particular interest attaches to maize in this respect, because of the leading position which it occupies among the crops treated with commercial fertilizers.

Woods[3] has shown that the percentage of protein in maize grain remains constant, despite large increases in yield of crop, with the addition of nitrate of soda to a carrier of potassium and phosphorus. With sand cultures of this plant, Duley and Miller[4] found a decreased percentage of nitrogen in the ears when the concentration of nutrient solution supplied was deficient during the development of this organ, but it should be noted that the supply of nitrogen was not varied independently in this case.

The writer has completed two tests in the greenhouse and one in the field with maize, following the procedure applied to wheat by LeClerc and by Gericke. In the field test the Golden Glow dent variety was grown on Miami silt loam lightly top dressed with farm manure. For the greenhouse test Leaming's Yellow Dent variety was grown on silt loam impoverished by dilution with an equal weight of sand. To this were added liberal supplies of all the essential elements of fertility. In applying the nitrate of soda either one third was added when the plants were about one foot high and the remainder well after the onset of ear filling or the reverse order was followed. In these tests the variations of nitrate supply did not modify the nitrogenous content of the cured seed, excepting in one of the greenhouse tests which was conducted during deficient illumination of the winter months. In this case the application of the greater part of the nitrate at the later stage of growth increased the protein content by 1.5 per cent.

Not only does maize withstand modification of composition through variation of nutrient treatment, but it is also peculiarly free from such modification through variation of climatic factors. The latter characteristic was early observed by Richardson,[5] who found inappreciable differences in the nitrogen content of maize seed reared in widely separated sections of the United States. In their comprehensive treatises on this plant species both Hunt[6] and Burt-Davy[7] quote data showing little influence of climate upon the protein content of the grain. Cooperatively with Professor E. J. Delwiche of the Department of Agronomy at this institution, the writer has found in three out of four years the same protein content of maize grain reared from common seed stock and under closely similar cultural conditions at Ashland and Madison. These Wisconsin stations represent a difference of 250 miles in latitude.

The behavior here emphasized shows marked contrast in comparison with the susceptibility of the sugar beet root and wheat grain to compositional modification through the influence of environmental factors, as shown by Wiley[8] and by LeClerc.[9]

TABLE I

COMPARATIVE NITROGENOUS COMPOSITION OF LEAVES

Date of Sampling	Species of Plant	Dry Matter in Fresh Leaf	Total Nitrogen in Dry Matter	Soluble Nitrogen in Total Nitrogen	Distribution in the Soluble Nitrogen: Protein	Mono Amino	Ammonia	Basic (a)
		%	%	%	%	%	%	%
8/14	maize	27.8	2.0	19	28	29	7	36
8/14	mangold	11.3	5.1	77	80	2	trace	18
8/26	maize	30.6	2.4	21	33	23	3	41
8/26	mangold	13.2	4.4	82	81	5	1	13

(a) Mixed forms, determined by difference.

* Presented in abstract at the annual meeting of the Wisconsin Academy of Sciences, Arts and Letters, Beloit, April 6, 1923. Published with permission of the Director of the Wis. Agric. Expt. Station.

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2 SCIENCE 52: 446, 1920.

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