Occurrence of the Alfalfa Mosaic Virus in Sugar Beet in California

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Received for publication June 26, 1964

A number of different aphid-borne viruses have been found in naturally infected sugar beet. Several of these, including the malva yellows, western yellows and yellow net viruses are retained by the aphid vector for an indefinite period, whereas others are stylet-borne with shorter periods of retention by the insect. In the latter group are the viruses of beet yellows, beet mosaic, cucumber mosaic and the recently described beet ring mottle (2)[2]. Here the occurrence of a fourth stylet-borne virus, alfalfa mosaic, is reported in naturally infected beets.

Infected plants were found in 3 different fields in the Sacramento Valley in the spring of 1962. All fields were located near alfalfa plantings in the Colusa County area. In the first field in which the disease was observed, only 2 infected plants were found. In this case an alfalfa field was located immediately across a county road from the sugar beet planting. In the second instance in which the disease was encountered a relatively small localized area in a field was found with about 25 diseased plants as if secondary spread had occurred within the field following introduction of the disease. An alfalfa field was located less than one-fourth mile from the infection center in beets. In the third case, many infected plants were found more-or-less randomly scattered throughout one edge of a beet field bordering an alfalfa seed field. Approximately 10% of the beet plants were showing symptoms in that area of the field near alfalfa. The percentage of diseased plants, however, decreased markedly at distances away from the alfalfa with only an occasional plant showing symptoms at the opposite edge of the field. The proximity of the diseased plants to alfalfa and the nature of the symptoms on beets suggested that the disease might be caused by the alfalfa mosaic virus.

The most characteristic symptoms of the disease consisted of prominent yellow blotches and ringspots (Figure 1). The most conspicuous symptoms were exhibited on the older leaves of affected plants with few or no symptoms being manifest on the upper leaves, thus suggesting that plants probably recover rapidly

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[2] Numbers in parentheses refer to literature cited.

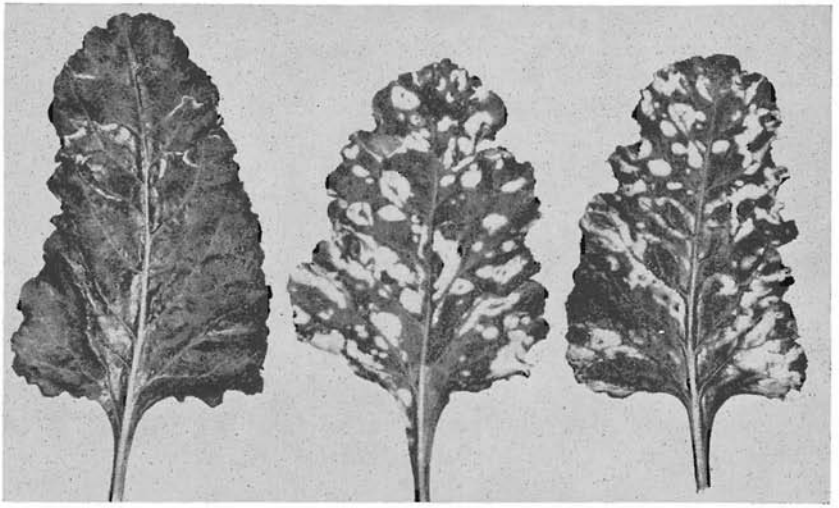

Figure 1.—Symptoms of the alfalfa mosaic virus on naturally infected sugar beet.

from the disease. The irregular blotches and ringspots were usually sharply delimited from the surrounding green-colored leaf tissue and were usually not associated with the veins or other portions of the leaves in any particular way. They were usually accompanied by chlorotic lines and oak-leaf patterns on affected laminae.

On younger plants in a 6 to 8 leaf stage, the symptoms were not generally as conspicuous and consisted chiefly of irregular chlorotic spots on the older leaves. Little, if any, distortion or blistering was present on any of the diseased plants.

Diseased plants were removed and transplanted in the greenhouse. The symptoms persisted on these plants for a period but the new foliage developed no further signs of the disease. Leaf tissue showing symptoms was removed and homogenized in 0.05 M phosphate, pH 7.5, and used to inoculate seedlings of other species known to be susceptible to the alfalfa mosaic virus. Inoculated primary leaves of beans, *Phaseolus vulgaris* L. var. Bountiful, and cowpea, *Vigna sinensis,* var. Early Wilt Resistant Ramshorn, developed a few necrotic local lesions following inoculation. These developed as well-defined circular lesions of a reddish-brown color unlike the more-or-less irregular necrotic flecks associated with bacteria frequently present in beet tissue (3). Several of the lesions on bean and cowpea were removed with a small cork-borer and homogenized in phosphate for further transfer to other plants.

Tobacco, *Nicotiana tabacum* L. var. Wisconsin Havana 425, and *Nicotiana glutinosa* L., showed a transient chlorotic etching followed by complete recovery, and cucumber, *Cucumis sativus* L. var. National Pickling, developed chlorotic local lesions following inoculation with material from bean and cowpea. The virus was readily recovered from beans, cowpeas, tobacco, *Nicotiana glutinosa* and cucumber upon subsequent inoculation of cowpea thus demonstrating that a mechanically transmissible virus was present in diseased beets.

The identity of the virus was confirmed by serological tests using antiserum to a strain of the alfalfa mosaic virus isolated from alfalfa.

Agar gel diffusion tests were used as these have proved very convenient for the routine identification of the spherical or shorter rod-shaped plant viruses which diffuse well in agar-gels. This technique consists of pouring a shallow layer of melted agar into a plastic Petri dish and allowing it to solidify. Cylindrical wells to accommodate the test reagents are then cut in the gel with a suitably sized cork-borer, followed by removal of the agar pieces. The antiserum is usually added to a larger center well and an extract of the virus infected material under study to smaller wells radially placed at an appropriate distance from the antiserum depot. The plates are then incubated for a day or two at room temperature before their evaluation. During this period the virus and antiserum diffuse outward toward one another, meet and combine in optimum proportions to form lines of precipitation.

For the tests used here, 11 ml of a solution of purified agar at a concentration of 0.8%, containing 0.85% sodium chloride and 0.04% sodium azide was poured into plastic Petri plates 9 cm in diameter and allowed to solidify. This gave a layer of semi-solid gel about 3 mm in depth. A center antiserum depot 8 mm in diameter and 8 evenly spaced peripheral wells each 4 mm in diameter at a distance of 3 mm from the edge of the center well were then removed. Sap expressed from naturally infected sugar beet leaves using a juice extractor was pipetted into some of the peripheral wells. Sap from healthy beets was placed in other wells to serve as controls. Following addition of the antiserum the plates were incubated for 24 hr at room temperature.

Samples from plants collected at each of three different fields showed positive reactions for the alfalfa mosaic virus. Clearly apparent lines of precipitation were present near the edge of

antigen wells filled with the extracted sap from diseased but not from healthy plants indicating that the plants were infected with the alfalfa mosaic virus. These results in addition to the host reactions mentioned previously appear adequate to show the alfalfa mosaic virus was the cause of the prominent mottling on affected plants.

The disease described here is probably distinct from the yellow blotching of leaves reported by Bennett (1) in beets in California and elsewhere. The irregular yellow spots associated with virus are superficially similar to those described by Bennett but are more sharply delimited from the surrounding green leaf tissue and are generally accompanied by ringspots and line patterns. In two cases a disease similar to that described by Bennett has been observed in upper San Joaquin Valley fields. In neither case could virus be recovered by inoculation of beans, cowpeas or tobacco, nor were positive serological tests obtained.

LITERATURE CITED

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