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Control of Lettuce Mosaic with Virus

Fig. 1. Seedborne LMV-infected lettuce seedling with leaf mottling and uneven leaf margins.

Any disease or disease complex that threatens the lettuce industry in California is a cause for grave concern. California grows about 165,000 acres of lettuce with an annual value of approximately $265 million, a major portion of the total lettuce production in the United States during all months of the year.

The Early Challenge

After accepting a position with the University of California, Davis, I made my first field trip in early 1948 with M. W. Gardner, J. B. Kendrick, Sr., and W. C. Snyder. The trip was to the Salinas Valley, where 60,000 acres of lettuce are grown each year. I was shown a very damaging disease that was obviously creating a critical situation. In field after field, plants were yellowed and stunted, growth was uneven, and most plants had not formed heads or were too small for packing in standard market containers. Yield was low, quality and appearance were poor, and some fields were being disked before a single head had been harvested.

The industry was pleading for help to survive. Losses were twofold: 1) production costs were about $500/acre and 2) of equal and perhaps greater importance, uncertainty of yield resulted in periodic overproduction or underproduction, with widely fluctuating availability and prices.

Seeking the Etiology of June Yellows

In early 1948, the etiology of this lettuce disorder was uncertain and the subject of much controversy. Although lettuce mosaic (LM) was recognized in young, infected plants, the syndrome in older plants appeared different and was generally considered to result from an undefined disease complex called June yellows. In addition to lettuce mosaic virus (LMV) and other viruses, factors such as poor root development, excessive soil salinity, faulty nutrition, unadapted cultivars, and unfavorable climatic or soil factors were considered. Exploratory evaluation included fertilizer trials, incorporating applications of several minor elements, comparative tests for soil salinity in different fields, observation of cultivars for differences in tolerance, selection of individual tolerant plants, and tests of progeny for tolerance. Similar observations and tests were made in other lettuce-growing areas where incidence of June yellows was low or nil.

Results of these tests and observations were generally disappointing. Although some differences were noted, no single factor or group of factors was consistently associated with the incidence and severity of June yellows. Even though these essentially negative results did not elucidate the etiology of June yellows, they did reduce the list of possible causes and led to intensified efforts to determine the role of LMV in the etiology of the disease.

The conclusion that LMV was a major cause of June yellows led to a control program based primarily on the use of LMV-free seed. Before the program reached its current consistent effectiveness, frequent failures resulted from attempts to use cheap or easy shortcuts. With present information, similar mistakes can be avoided and comparable control programs can be developed more rapidly in the future.

When this work was initiated in 1948, much essential information on the epidemiology of LM already was available. Jagger (10) in 1921 had reported LM in Florida, South Carolina, and New York and had demonstrated aphid transmission. Newhall (14) in 1923 reported frequent seed transmission (3%) and, from observations of early seedling infection in the field, suggested that such transmission was the "most important source of primary inoculum in the spring." Kassanis (11) in 1947 confirmed that LMV is seed- and aphid-transmitted and studied natural spread in the field. He transplanted four infectors into a lettuce field in southern England and reported that 300 plants became infected within 12 wk. Further, he noted that spread was mostly to plants nearest to the infectors and thus surmised that "Spread of LMV is mainly a local phenomenon and the use of virus-free seed is the obvious measure, for infections coming from outside the field will be too few and too late to cause severe losses." Considerable additional information on LMV published since 1948 has been summarized by Tomlinson (16).

LMV is a typical member of the potato virus Y group with flexuous, filamentous particles approximately 750 × 13 nm. LMV is sap- or aphid-transmissible in a nonpersistent manner and has a wide host range, including 20 genera in 10 families. Because of its seed transmissibility, LMV occurs worldwide, wherever lettuce is grown.

Determining the Role of LMV

Mottled plants were evident in all fields in the Salinas Valley during the early stages of growth (Fig. 1), and tests of

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free Seed

numerous random samples for infection with sap-transmitted virus were positive. By conventional tests the virus was identified as LMV. Further, all of 50 commercial seed samples transmitted 1–3% LMV to seedlings. Because mottle symptoms in older plants were indistinct, determination of changes and variability of the LM syndrome in older plants was necessary. Young plants with mottle symptoms in several fields were staked, and detailed notes and color photographs were taken weekly. By this procedure, symptoms of LMV and their variability in all stages of growth were learned; LMV-infected plants could then be identified accurately by noting stunting, dull green to slightly yellow color, and downward rolling of the outer leaves (Fig. 2).

With this expertise, the rate of LMV spread could be determined. Many fields were found to be infected nearly 100% by harvest time. In fields with slower spread, infected plants were grouped in circular areas, and the source of primary inoculum could often be discerned because of greater stunting of plants with early or seedborne-infection (Fig. 3).

These observations led to the conclusions that the incidence of LM was very high, infected plants were severely stunted, and LMV appeared to be a major contributing factor to the frequent failure of lettuce fields. Further, seed-transmitted virus was being introduced into every field, and, as had been suggested by several reports (1,11,14), elimination of this source of inoculum might result in disease control. However, LMV-free seed for control of LM had never been tested.

Experimental LMV-free Seed

Seed-transmitted LMV-infected plants were eliminated by three careful inspections of seedlings grown in an insect-free greenhouse. The remaining healthy plants were transplanted to a field near Davis, well isolated from commercial lettuce-growing areas. No attempt was made to control aphids, but LMV-infected plants were not observed during the growing season and seedborne LMV was not detected in greenhouse tests. Thus, several pounds of LMV-free lettuce seed were obtained for experimentation.

The efficacy of LMV-free seed for control of LM was tested in commercial lettuce fields in the Salinas Valley. Plots about 180 ft^2 were planted with LMV-free seed near the centers of large commercial fields planted at the same time. LMV-infected and total plants were counted in nine beds of the commercial field on each side of the plots and in all beds within the plots. Although some LMV spread from commercial plantings into experimental plots, incidence in plot areas was significantly lowered (8). Moreover, onset of infection was delayed in LMV-free seed plots, and stunting was consequently reduced. Use of LMV-free seed thus appeared to control LM effectively.

The difference between growth of lettuce in LMV-free seed plots and in adjacent commercial fields was demonstrated at several field meetings with growers and seed company representatives. The striking difference generated a strong demand by growers for LMV-free seed. Unfortunately, the supply from commercial sources was limited.

Fig. 2. (Left) Healthy lettuce plant and (right) LMV-infected plant with severe stunting and downward rolling of leaves.

Attempts to Find "Acceptable" Level of LMV in Seed

For 10 yr after 1951, seed companies tried to meet the demand for LMV-free seed, but many attempts to produce such seed were unsuccessful. Reasons for these failures are not entirely known, but in some instances LMV-infected seedlings were not eliminated before transplanting into the field. In other instances, attempts to rogue infected plants from seed fields, with the intent of lowering LMV content of the seed to an acceptable level, were unsuccessful. The idea that some level of LMV seed transmission was permissible resulted in sale of seed with labels indicating seedborne virus content. For example, some labels indicated less than 0.1%, others less than 0.33%, and still others simply "low mosaic count." In most instances, disease control with such seed lots was unsatisfactory.

The question of how much LMV seed transmission could be tolerated was raised repeatedly. To answer this question, eight lettuce seed lots with LMV seed transmission of 0.0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6% were planted during 1955. This study (17) showed that the resulting percentage of infection depended on both the amount of seedborne infection and the number and motility of aphid vectors. Without exception, reduction in the amount of seedborne virus resulted in reduction in total percentage of LMV infection at harvest. Data from all trials indicated that seed transmission greater than 0.1% gave inadequate disease control; in one trial, even this relatively low percentage of seed transmission resulted in 23% LMV infection.

These results should have indicated that LM control was often inadequate with as little as 0.1% LMV seed transmission. However, the seed industry

Fig. 3. LMV-infection center in a commercial lettuce field. Plants near the middle of the center are extremely stunted and yellowed.

and growers generally accepted this level of seed transmission. Most commercial lettuce seed lots were so labeled because several lettuce-growing counties had passed ordinances against planting lettuce seed with more than 0.1% LMV seed transmission. Results with such seed were generally unsatisfactory, and LM continued to be a serious problem, especially late in the growing season when two crops were grown each year.

In some instances, even the first crop of the season was badly damaged because labels often were erroneous. Each seed company was responsible for indexing its seed, and the only legal sanction for correctness of labeling was spot checks of seed samples by the California State Department of Agriculture. Many seed stocks were not sampled for indexing, and even those that were could have as high as 0.36% LMV seed transmission without being cited for label violation. This apparent discrepancy resulted from the application of a tolerance for sampling and indexing error so that only airtight cases of violation would be prosecuted. Thus, during the 10-yr period after demonstration of the efficacy of LMV-free seed for control of LM, the disease still was causing severe losses in most of the lettuce acreage.

A "Try for Perfection" Program

A meeting was held in 1961 with lettuce growers and seedsmen to discuss better control. An LM-control committee of growers agreed to develop and test a pilot control program in a 10,000-acre portion of the Salinas Valley where LM had previously caused very severe losses. The program was described as "a try for perfection" (2) and was intended to eliminate or decrease spread of LMV from four potential sources by: 1) planting only indexed lettuce seed with zero seedborne LMV in 30,000 seedlings, 2) destroying potential weed hosts of LMV in and adjacent to lettuce fields, 3) disking lettuce fields immediately after harvest, and 4) avoiding the planting of new lettuce fields adjacent to old fields.

The 0 in 30,000 standard was adopted with the realization that a seed lot passing the index would not necessarily be virus-free. Assuming that the index had been done correctly, T. M. Little, biometrician for the Agricultural Extension Service, calculated a 99.9% confidence level in the actual percentage of LMV seed transmission being between 0 and 0.022%.

To identify 0 in 30,000 seed lots for planting, growers agreed to an assessment of about $1/acre to pay for renting glasshouse space and other costs of indexing seed lots. The index program was supervised by A. S. Greathead, farm advisor of the Cooperative Extension Service in Salinas Valley.

Each seed company participating in the program was asked for a list of seed lot numbers that they had tested and found to index 0 in 30,000. One-pound samples of the designated seed lots were drawn in a random manner by a representative of the County Department of Agriculture, and each sample was given a code number so that personnel doing the indexing would not know the source and identity of the seed lot.

The indexing procedure used was the seedling grow-out test in an insectproof glasshouse. About 155 boxes of soil with approximately 200 seedlings in each were required for indexing each seed lot. About 20 days after seeding, each plant was pulled and inspected. Any that appeared abnormal (uneven leaf margins, stunting, mottle) were transplanted into small pots and later tested for virus infection by attempting to transmit the virus from the suspect plants to healthy plants. Of 20 seed lots submitted for indexing by seed companies, only 10 indexed 0 in 30,000; these were used to plant about 5,000 acres in the pilot-program area. All growers in the area used only these seed lots for planting.

The other three parts of the program were not accomplished completely. Weed control was improved by using a roving spray rig to kill weeds along roadsides, fence rows, and edges of fields, but this method was far from perfect. Also, planting new fields adjacent to old ones could not always be avoided, although in most instances old fields were disked under quickly after harvest.

The Spectacular Results

Even without strict adherence to all parts of the program, the results were spectacular. Extension agent Greathead, working closely with the control committee, recorded the incidence of LMV infection within and outside the control district at regular intervals. His midseason observations (2,7) were as follows:

1. No LM was observed within the control district until several weeks after its appearance in fields outside the district. At midseason, all fields within the district had a lower incidence of LM than comparable fields throughout the Salinas Valley.
2. LM was most prevalent in fields at the perimeter of the control area.
3. LMV was not widely disseminated from large weedy areas, such as those alongside the Salinas River, where control of potential weed hosts was not possible. (Some 27 weed and crop species that commonly grow in the Salinas Valley are known to be susceptible to LMV.) However, a few instances of localized spread from weed sources were noted.
4. Despite heavy aphid infestation during 1962, no fields in the pilot control district were destroyed by LMV, and the cost to participating growers, less than $1/acre, was returned manyfold by increased yields of better quality lettuce.

The spectacular results of the 1962 pilot control program were observed by many growers other than those participating. Therefore, since 1963, the lettuce acreage of the whole Salinas Valley has operated under the four-point control program that proved so successful in the 1962 pilot program. Costs of the program, which now amount to about 50¢/acre, are paid by assessing seed companies for each seed lot submitted for LMV index and by supplementary grants, as needed, by a local lettuce-growers association. Although grower compliance with rules of the program is generally voluntary, a county ordinance requires the participation of all growers. As a result, LMV is no longer a threat to production. In fact, on several occasions when I have taken advanced plant pathology classes on field trips into the area, LM could not be found, even for demonstration!

When the work on LMV was started in the Salinas Valley, the disease occurred occasionally in the Imperial Valley in southern California but was not nearly so prevalent in lettuce grown for winter harvest. However, during the 1968–1969 season, the occurrence of internal rib necrosis and rusty brown necrosis in the cultivar Climax, grown extensively in the Imperial Valley, was shown to be a cultivar-specific reaction to LMV infection (3). These disorders were initially thought to be due to a complex of factors such as ammonia toxicity, near-freezing temperatures, improper irrigation, and so on. However, since 1971, a seed-indexing program for LMV with a 0 in 30,000 standard has been used, and internal rib necrosis in Climax has not recurred.

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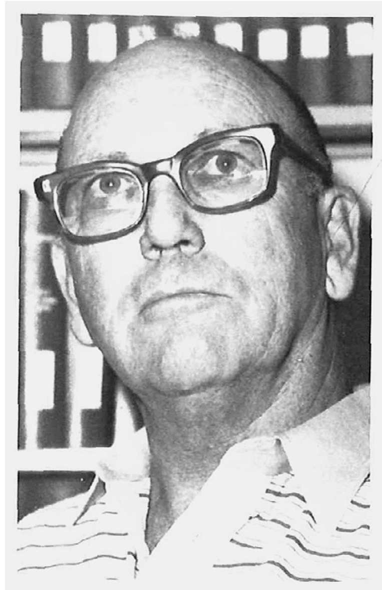

Control Now and in the Future

The principle used for control of LMV (elimination or decrease in amount or effectiveness of inoculum) is not unique and, in fact, is the basis for nearly all methods used for plant disease control. For example, inoculum can be reduced or eliminated by heat or chemical treatment, hostfree periods, rotation to nonsusceptible crops, and growing seed crops in areas where climate restricts multiplication of pathogens.

The difficulties in developing an effective LMV-control program were due in large part, if not entirely, to the use of less expensive shortcuts that failed to eliminate the major source of inoculum. With a seedborne virus disease such as LM that spreads rapidly when aphid vectors are abundant, even a small percentage of virus seed transmission can result in high incidence of disease. If seed producers are not doing everything possible to eliminate the inoculum instead of reducing it to some "acceptable" level, mistakes will be made and periodic failures will be inevitable.

The effective control of LM by eliminating LMV from seed despite its numerous hosts appears to be unique. This does illustrate that seedborne inoculum, distributed randomly throughout a crop, is considerably more effective than inoculum originating from outside. Further, as shown by Costa and Duffus for LMV (4), the natural host range of a virus may not coincide with that determined experimentally.

In the future, LMV probably will be controlled by using resistant cultivars (15). However, because nearly all cultivars currently in use are susceptible, control still largely depends on the use of LMV-free seed stocks that can be identified by several indexing methods.

The expensive and time-consuming grow-out method of indexing has been largely replaced by inoculation of *Chenopodium quinoa* to detect virus in comminuted samples of seed (12,13). In the future, testing for LMV in seed will probably be done with serologic techniques that are more sensitive and less expensive than either the grow-out or *Chenopodium* method (6,9).

Until now I have ignored other aphid-borne viruses that can infect lettuce. Two were reported to cause yellowing typical of June yellows (5). Doubtless, June yellows was not caused by LMV alone. However, the immediate and dramatic improvement in lettuce production resulting from the LMV-control program and the failure of June yellows to recur in epidemic proportions during the past 17 yr strongly support my contention that factors other than LMV are sporadic and of relatively minor importance. However, improved weed control apparently has lowered the incidence of insect-borne virus and mycoplasma diseases of lettuce. For example, spotted wilt (thrips-borne virus) and aster yellows (leafhopper-borne mycoplasma) occurred sporadically in most lettuce fields during the 1950s but are now difficult to find.

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