LETTUCE MOSAIC VIRUS:

STELLA M. COAKLEY · R. N. CAMPBELL · K. A. KIMBLE

Lettuce has been the most important winter vegetable crop in the Imperial Valley for the past four years, averaging about 42,000 acres grown, and an average gross value of about $35 million. Lettuce grown for harvest in midwinter (December 15 to March 15) is planted from about September 10 to November 5. Plantings during October are largely of the cultivar Climax. During the winter harvest season of 1968–69, a disorder, later known an internal rib necrosis (IRN), occurred throughout the Imperial Valley in epidemic proportions just prior to harvest and caused serious losses in the field and in transit. Persons associated with the lettuce industry recalled the disease from past years but it had never been ranked as a major disease until 1969. No valid estimate was made of the losses from internal rib necrosis during the 1968–69 season but it was common for growers to abandon entire fields. Since then IRN has appeared sporadically in lettuce planted for midwinter harvest but not in epidemic proportions. In addition, a postharvest disorder (then called rusty rib) now called rusty brown discoloration (RBD), occurred on lettuce during storage and transit at 35°F. In February 1969, 90% of the lettuce shipments arriving in New York had RBD. The cause (or causes) of IRN and RBD has not been identified but environmental conditions (freezing temperature and rainfall), aqua ammonia damage and a synergistic reaction between beet western yellows virus and lettuce mosaic virus have been speculated as causes. Two papers presented here report the results of cooperative research by personnel of the California Agricultural Experiment Station, the California Agricultural Extension Service, and the US Department of Agricultural to determine the causes of IRN and RBD.

THE STUDIES reported here (in two articles) were conducted to determine the role of lettuce mosaic virus (LMV) in the development of internal rib necrosis (IRN) and rusty brown discoloration (RBD). This virus has been consistently isolated from IRN-affected lettuce heads since 1969. Three cultures of lettuce mosaic virus were inoculated to lettuce plants grown in the greenhouse or growth chambers so they were known to be free of other viruses. Lettuce plants were of the susceptible cultivar Climax, as well as nonsusceptible cultivars, Vanguard and Calmar. After inoculation the plants were kept in growth chambers equipped to provide a 12-hour "day." Temperatures were set at 70° F day–50° F night in some trials or at 65° F day–45° F night in other trials. As the plants grew, the lower leaves were examined for IRN. When the plants formed heads and matured, they were harvested, examined again for IRN, assayed for LMV infection, and stored at 33–35° F for up to two weeks to allow RBD to develop.

The results of nine trials were similar and have been combined (see table). Plants of various ages were inoculated. Thirty-day-old plants developed severe mosaic symptoms with mottled leaves and severe twisting of the leaves. These plants developed some IRN but usually failed to head. Plants of this age were therefore not used in most trials. Plants that were 45 or 60 days old at inoculation generally did not show pronounced symptoms of infection by LMV but a high proportion of these plants developed IRN and RBD. Careful inoculation and dissection records showed that IRN generally started to form on leaves that were about one-half to one inch long inside the young head at the time of inoculation, but it did not start to form until about 21 days after inoculation

Microscopic examination of these plants showed that the pattern of dead cells was the same in the experimental plants as in plants collected from the Imperial Valley. In the case of IRN there were groups of dead cells between the veins at the midrib, whereas RBD caused death of epidermal cells that became red-dish-brown. No IRN or RBD developed on Climax plants that were inoculated but failed to become infected with LMV or on noninoculated Climax plants. Additionally, none of the Calmar or Vanguard plants developed IRN or RBD whether they were infected by LMV (20 plants) or not (40 plants).

These results show that LMV alone causes IRN in growing plants and that it is an important factor in the induction of severe RBD in stored heads. They also confirm that IRN and RBD occur only in the cultivar Climax. The lettuce heads produced in these growth chamber experiments did not reach the same degree of maturity or head firmness that is encountered in field-grown lettuce. Thus, the mild discoloration similar to RBD but occurring on field-grown, mature to overmature heads without LMV infection was not observed (see next article).

Treatment of other plants with aqueous ammonia induced a discoloration of the midrib that superficially resembled IRN. Microscopic examination showed, however, that this discoloration was in the veins. It is clear that ammonia damage is distinct from IRN.

Other trials were conducted to determine if other viruses might cause IRN and/or RBD on Climax lettuce. Inoculations with alfalfa mosaic virus, cucumber mosaic virus and beet western yellows virus separately did not cause IRN or RBD. Similarly, inoculations of beet western yellows virus together with LMV did not cause more severe IRN than that produced by LMV alone.

Since LMV causes IRN and RBD, the most direct method of preventing these diseases is to control LMV. Thus, these results strongly support the continuation and improvement of the seed indexing program curently being used in Imperial County. Breeding a variety to replace Climax can be accomplished but this will require several years.

DEVELOPMENT OF INTERNAL RIB NECROSIS (IRN) AND RUSTY BROWN DISCOLORATION (RBD) ON CLIMAX LETTUCE INFECTED WITH LETTUCE MOSAIC VIRUS (LMV)

		Results		
Age at Inoculation	No. of heads	No. with LMV	No. with LMV & IRN	No. with LMV & RBD
30	18	13	7	4
45	15	13	12	12
60	38	28	28	23
Noninoculated	42	0	0	0

. . . *causes* INTERNAL RIB NECROSIS,
. . . *stimulates* RUSTY BROWN DISCOLORATION
. . . *in* CULTIVAR CLIMAX

DEVELOPMENT OF INTERNAL RIB NECROSIS AND RUSTY BROWN DISCOLORATION IN CLIMAX LETTUCE INOCULATED WITH LETTUCE MOSIAC VIRUS, BRAWLEY, CALIFORNIA 1972

Treatment	Internal rib necrosis*		Location of Internal rib necrosis†			Rusty brown discoloration‡	
	LMV-infected plants	Plants free of LMV	Wrapper	Cap	Internal	LMV-infected plants	Plants free of LMV
Inoculated:							
50 days	40/40	0/0	4	27	40	39/39	0/0
70 days	38/38	0/3	29	33	38	37/37	0/3
90 days	40/40	0/0	34	35	40	38/38	0/0
Noninoculated:							
Control shelter	8/14	0/33	0	0	8	10/14	0/33
Other shelters	15/18	0/23	2	3	15	14/18	0/23

* Number of heads with internal rib necrosis/number of plants in category.
† Number of heads with internal rib necrosis showing in wrapper leaves, cap leaves or within the head.
‡ Number of heads with rusty brown discoloration/number of plants in category as determined after storage for 14 days at 34°F.

Field studies of
INTERNAL RIB NECROSIS AND RUSTY BROWN DISCOLORATION OF THE LETTUCE CULTIVAR CLIMAX

STELLA M. COAKLEY · R. N. CAMPBELL · K. A. KIMBLE
H. JOHNSON, JR. · K. MAYBERRY · T. W. WHITAKER

By the end of the 1970 harvest season Climax was known to be the lettuce cultivar most severely affected by internal rib necrosis. Investigations in 1970–71 were planned to test additional commercial varieties and all possible seed sources of Climax, including seed from the original release of Climax for their IRN reaction to determine if some change had occurred since the cultivar was released. Seed was planted on four dates: October 7, October 14, October 24 and October 28. The results of these tests were surprisingly uniform and clearcut:

(a) Only Climax showed the typical internal rib necrosis (IRN) symptoms. The other cultivars tested—Merit, Calmar, Greenland, Green Bay, Golden State D, GL 407 and Francisco—were uniformly negative for IRN.

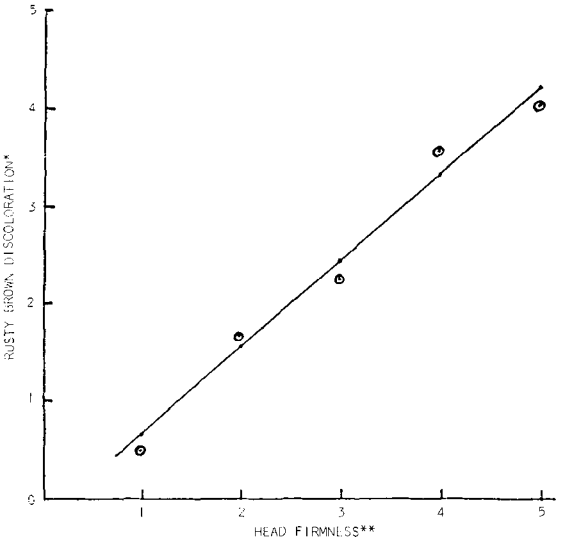
(b) Nine different lots of Climax seeds provided by various seed companies, as well as the original release, produced plants that had equally severe IRN. A few plants were free of symptoms of IRN. These plants were saved, transported to the greenhouse and seed was obtained from them. The seed from these apparently disease-free plants was tested for IRN susceptibility during the 1971–72 growing season. All were susceptible to IRN, indicating that these particular plants were escapes rather than resistant or tolerant to IRN.

Experimental plots were designed in the 1971–72 growing season to field test whether lettuce mosaic virus could cause IRN and RBD, as had been demonstrated under controlled greenhouse conditions (as reported in the companion article in this issue). Four shelters were constructed and covered with tobacco cloth to exclude aphids. Each 12-ft-long shelter was installed over two beds planted to Climax lettuce and irrigation was not done until the shelters were completed. One shelter served as a control and was entered once to thin the plants. Each of the remaining three shelters were entered once to thin the plants and two more times to inoculate selected plants with lettuce mosaic virus (LMV) at 50, 70 and 90 days before harvest. At harvest time the plants were evaluated for internal rib necrosis; they were then bagged, shipped to Davis, individually assayed on *Chenopodium quinoa* for lettuce mosaic virus infection, stored for two weeks at 35 F, and evaluated for head firmness and rusty brown discoloration. The cages were not insect proof. At harvest on February 28 and 29, 1972, green peach aphids that are effective vectors of LMV were found in two shelters.

The results (see table) are of interest despite the failure to exclude aphids. All but three of the plants inoculated at 50, 70 or 90 days were infected with LMV; all the LMV-infected plants developed IRN and RBD. Because of the aphid infestation some of the noninoculated plants became infected with LMV and many of these developed IRN and RBD. It is likely that not all of these plants developed IRN and RBD because they were inoculated by aphids within one or two weeks of harvest. This is supported by the fact that most of the IRN observed in these plants was on younger leaves within the head, whereas most inoculated plants had IRN in the older wrapper and cap leaves as well. None of the plants that were free of LMV developed either IRN or RBD.

Since aphids had infested the plants in two chambers and might have introduced other viruses, especially beet western yellows virus (BWYV), additional assays for BWYV were done from some plants. Eight plants with severe IRN were selected from each of three shelters. These plus eight plants from the control shelter were assayed but only one IRN-affected, aphid-infested plant yielded BWYV. Thus, the trial gives strong confirmation that LMV alone causes IRN and RBD in Climax.

REGRESSION CORRELATION OF HEAD FIRMNESS AND RUSTY BROWN DISCOLORATION IN CLIMAX PLANTS INOCULATED WITH LETTUCE MOSAIC VIRUS (37 plants from each of 3 inoculation dates were analyzed) r = 0.989



* 0 = no leaf discoloration
5 = severe discoloration
† 1 = immature, soft head
3 = optimum market maturity
5 = hard, overmature head

When the heads were stored for two weeks for RBD to develop, additional tests were done. In one test half of the LMV-inoculated heads were placed in pairs in a large plastic bag and an additional virus-free Climax head collected from a commercial field was also placed in the bag to determine if a volatile compound produced in the LMV-infected heads could cause RBD on the virus-free head. Although RBD developed on all 60 LMV-infected heads, none of the 30 healthy heads developed RBD. In addition, there was a direct correlation between head firmness (i.e. maturity) and RBD severity with the more mature heads developing more severe RBD (see graph).

Climax lettuce heads collected from a commercial field were assayed and found to be free of LMV. When these heads were stored for three weeks or more, they often developed a mild discoloration that was similar to RBD. This mild discoloration also was more severe on the firmer heads but it never became as severe as RBD caused by LMV; it would rate less than 1 on a 0–5 scale of increasing RBD severity. The similarity of this discoloration and RBD suggests that LMV infection accelerates physiological processes that normally do not produce effects on Climax lettuce until the lettuce is overmature. This work was done only with Climax so the reaction of other varieties is unknown, but judging from their resistance to RBD in other tests it seems likely that susceptibility to the mild discoloration represents an undesirable characteristic of the variety Climax, which is accentuated by LMV infection.

A double-wall plastic shelter covering two lettuce beds, 12 ft long, was constructed to evaluate the effect of minimum temperature on the incidence of IRN. Large vents on each end served to keep the inside temperature near ambient during daytime and also allowed free access to all insects. A thermostatically controlled electric heater was installed to keep the minimum temperature above 40° F, during the night when the vents were closed. The shelter was installed over young plants on November 30, 1971, and at the same time several plants were inoculated with lettuce mosaic virus. At maturity, on January 25, 1972, the plants from the shelter were evaluated for IRN. Of the 41 plants within the shelter all but two exhibited typical symptoms of IRN. This experiment confirms that temperatures below 40° F are not required for the development of IRN symptom in Climax.

This work demonstrates that IRN and RBD are limited to the variety Climax among the varieties which are commonly grown in the Imperial Valley, California. It also confirms the results of carefully controlled experiments which have shown that lettuce mosaic virus is the cause of IRN and RBD, and it emphasizes the necessity for the control of lettuce mosaic virus through a clean-seed program. A clean seed program in which the only lettuce seed lots certified for planting are those with zero mosaic in 30,000 seeds was instituted in Imperial County in 1971. This has given excellent control of LMV in subsequent seasons. A similar program has effectively controlled LMV for 10 years in the summer lettuce crops of the Salinas Valley.

Stella M. Coakley is Research Assistant; R. N. Campbell is Professor; and K. A. Kimble is Staff Research Associate, Department of Plant Pathology, University of California, Davis. Hunter Johnson, Jr. is Extension Vegetable Specialist, University of California, Riverside; K. Mayberry is Farm Advisor, Imperial County, and T. W. Whitaker is Research Geneticist, USDA, U. S. Horticultural Field Station, La Jolla. *This research was supported in part by a National Science Foundation Scholarship and by a grant from the Imperial Valley Vegetable Growers Association.*

NEW

VICTOR VOTH · R. S. BRINGHURST
HOWARD J. BOWEN · TOM MOCK

Water placement is very important to strawberry production. Under the conditions of this experiment, plants on a conventional 40-inch bed with standard furrow irrigation required the greatest amount of water and yielded the least fruit, whether figured on a per-plant or per-acre basis. In contrast, the experimental 60-inch midbed furrow-irrigated plants yielded significantly more fruit on a per-plant or per-acre basis, even though there was a 35% increase in plant population. Apparently surface application, with the water constantly moving downward, improves plant performance. Of the new application systems investigated here, only the use of porous pipe placed under the soil surface was of questionable value from the point of view of plant performance.

THE MILD CLIMATE of coastal California contributes to high strawberry yields, although urbanization is reducing the available land in the best areas. These experiments were designed to study the production efficiency of novel irrigation methods, but also involved bed shape and plant placement.

Drip system

Promising results were obtained in 1967–68 at San Diego in preliminary experiments with the "drip" irrigation system utilizing plastic pipe positioned between the plant rows on top of the conventional 40-inch, double-row bed with orifices every two feet. Water con-