Internal Rib Necrosis and Rusty Brown Discoloration of Climax Lettuce Induced by Lettuce Mosaic Virus

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ABSTRACT

Internal rib necrosis (IRN) and rusty brown discoloration (RBD) are pre- and postharvest disorders, respectively, that occur only in the crisp-head lettuce cultivar 'Climax'. IRN is characterized by necrosis of parenchyma cells in the midrib; whereas, RBD is characterized by necrosis of epidermal cells. Climax lettuce was inoculated with lettuce mosaic virus (LMV) at 30, 45, 60, and 75 days of age and usually incubated in growth chambers (at 7-18 C) until harvest. Twenty-one days after inoculation IRN symptoms began to develop; by harvest time, 50-60 days after inoculation, nearly every LMV-infected head had IRN. After harvest the heads were stored at 1 C for 2 weeks and RBD developed on most of the heads with LMV. No IRN or RBD developed on virus-free Climax or on healthy or LMV-infected heads of the lettuce cultivars 'Vanguard' and 'Calmar'. Beet western yellows, cucumber mosaic and alfalfa mosaic viruses did not induce IRN or RBD in Climax lettuce. LMV alone is, therefore, responsible for the development of IRN and RBD on Climax lettuce. 'Salad Bowl', a butter head type lettuce, develops a discoloration similar to IRN after infection by LMV.

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A serious internal defect of lettuce (*Lactuca sativa* L.) caused extensive crop loss during the 1968-69 growing season in the Imperial Valley of California (16). Similar disorders had been observed in previous years but had not caused extensive damage. Johnson et al. (11) selected the name "internal rib necrosis" (IRN) in preference to the other terms ("black heart", "gray rib", "rib blight", and "gray streak") used to describe this disorder. They considered cultivars, planting date, and irrigation management in relation to IRN development and concluded that near-freezing temperatures and rainfall were the major predisposing factors. Only the cultivar 'Climax' was susceptible to IRN; whereas, the cultivars 'Vanguard', 'Forty-niner', and 'Golden State' were not (11). Later the lettuce cultivars 'Merit', 'Calmar', 'Greenbay', 'Golden State C and D', 'Great Lakes 407', and 'Francisco' were also reported to be disease-free (22). Troutman et al. (21) reported that typical IRN-like symptoms were induced in Climax by side dressing nearly mature plants with NH_4OH. They attempted but failed to transmit any virus from the IRN-affected plants collected from the field. Previously, Grogan & Zink (8) described ammonium damage to lettuce. Zink & Duffus (23) suggested that IRN was a synergistic reaction from infection by both beet western yellows virus (BWYV) and lettuce mosaic virus (LMV). Their hypothesis, however, was based on only a few positive results obtained under greenhouse conditions.

Johnson et al. (11) suggested a possible connection between IRN and a postharvest defect of lettuce known as "rusty rib" that developed on Climax lettuce after storage for 7 days. Ceponis et al. (4) described and named this disorder "rusty brown discoloration" (RBD). They reported that RBD affected 90% of the lettuce arriving on the New York market in February, 1969. Ceponis & Kaufman (3) distinguished RBD from brown stain of lettuce caused by high carbon dioxide levels.

The above studies provide information on the occurrence of IRN and RBD, but have not satisfactorily explained the cause. An important factor may be LMV; until recently, no attempts have been made to control LMV in the Imperial Valley. LMV is a 750-nm-long flexuous rod, is seed-borne, and is aphid-transmitted in a nonpersistent manner (20). Grogan et al. (7) described the mosaic symptoms of LMV on lettuce, and a method of producing mosaic-free seed.

During the 1968-69 season, 28 heads of the IRN-affected Climax lettuce were assayed and found to be infected with LMV. All commercial fields had a very high percentage of plants with LMV that year. Indexed lettuce seed (no mosaic-infected plants from 30,000 seeds) was first planted in the Imperial Valley in the 1969-1970 season. Fields planted with these seeds were free from both IRN and LMV even though IRN was present in other fields. In the 1970-71 season, most of the lettuce seed planted was from indexed seedlots. Nevertheless, IRN appeared on Climax lettuce after midseason and continued for the remainder of the growing season. These observations suggested that the relationship of LMV to IRN and RBD should be tested, and that is the objective of this paper.

MATERIALS AND METHODS.—*Virus cultures and transmission.*—LMV was indexed and isolated by inoculation onto two or three plants of *Chenopodium quinoa* Willd. which develops systemic chlorotic spots with leaf distortion in 7-10 days (14). The presence of LMV may be confirmed by observing flexuous

rods in stained dips prepared by the method of Hitchborn & Hills (9). Isolates of LMV were maintained in *C. quinoa* by mechanical transfers every 21-28 days; one isolate was also propagated mechanically through successive transfers in Climax lettuce because mechanical transfer from *C. quinoa* to lettuce was difficult.

The parsley strain of alfalfa mosaic virus (AMV), described by Campbell & Melugin (2), was propagated in *Nicotiana glutinosa* L.; *Phaseolus vulgaris* L. 'Bountiful' was used as a local lesion assay host.

Cucumber mosaic virus (CMV) was isolated from bell pepper (*Capsicum annuum* L.) and propagated in *Cucurbita pepo* L. 'Small Sugar'; cowpea (*Vigna sinensis* Savi ex Hassk. 'Blackeye') was used as a local lesion assay host (17).

LMV, AMV, and CMV were mechanically inoculated after triturating 1.5 - 2.0 gm of infected host tissue in ca. 3 ml of chilled buffer (0.05 M potassium phosphate + 0.1% mercaptoacetic acid, pH 7.6) in a sterilized mortar. Healthy plants were dusted with corundum and the tissue homogenate was finger-rubbed on the leaves. Control plants were rubbed with buffer in a similar manner.

Beet western yellows virus (BWYV) was isolated from lettuce grown in the Salinas Valley and maintained in radish (*Raphanus sativus* L. 'Icicle'). Shepherd's purse [*Capsella bursa-pastoris* (L.) Medic.] and radish were used as assay hosts (6).

Green peach aphids (*Myzus persicae* Sulzer) were used for all aphid transmissions of virus. Nonviruliferous aphids were maintained on healthy 'Tendergreen' mustard (*Brassica campestris* L., Perviridis group) in a room free from other plants or insects.

Since LMV is transmitted in a nonpersistent manner by *M. persicae* (20), aphids were starved for a minimum of 30 min before being transferred to *C. quinoa* leaves systemically infected with LMV. After the aphids began to probe they were allowed a 1-min acquisition period; five or ten aphids were then transferred to a small aphid cage clipped on each lettuce plant. After an inoculation access period of 2-3 hr, the cages were removed and the plants sprayed with nicotine sulfate.

BWYV is transmitted in a persistent manner by *M. persicae* (6). Nonviruliferous aphids were allowed an acquisition access period of 24 hr on the source plants. Ten aphids were transferred to each cage on healthy host plants. After an inoculation access period of 48 hr, the aphid cages were removed, and the plants sprayed with nicotine sulfate.

All test plants used in the greenhouse or the growth chambers were grown in pasteurized potting soil [U.C. mix No. C-2 (1)] and were kept either in screened greenhouses, which were routinely fumigated with Nicofume, or in insect-proof growth chambers. Lettuce was cultivar Climax unless otherwise stated.

Controlled-environment experiments.—Sherer controlled-environment chambers (Sherer-Gillett Co., Marshall, Michigan) were used for most experiments so that the day-length and temperature could be controlled. Lettuce seeds were sown and the plants raised in the greenhouse (temperature 24-29 C) for 30-45 days at which time they were moved to the chambers. In the initial experiments, the chambers were operated at 21 C during a 12-hr photoperiod and at 10 C for the 12-hr dark-period. To induce better heading in the later experiments, the temperature was lowered to 18 C and 7 C for the light and dark periods. In early trials, plants were in 12-cm diam plastic pots and were watered alternately with one-half strength Hoagland's solution (10) or distilled water. In later experiments, plants grown in 15-cm diam pots were fertilized with U.C. fertilizer-2C (1) every 30 days and watered with distilled water as needed.

Rating of IRN, RBD, and head maturity.—At harvest, lettuce heads were rated positive for IRN if a 45-degree cut across the midrib at the base of the outer leaves showed discolored parenchyma cells. The heads were then stored in separate plastic bags at 1 C and inspected 1, 2, and 3 weeks later for the development of RBD. "Exterior RBD" was visible without dissecting the head. "Interior RBD" was indicated by a superficial red discoloration on both surfaces of the leaves inside the head.

The lettuce was rated for head maturity when the heads were cut open at the end of storage. The 0-5 scale was based on head firmness: 0 = no head; 1 = very immature soft head; 2 = immature soft head; 3 = medium firmness with young leaves not compact (most desirable maturity for commercial use); 4 = mature head with compact young leaves; and 5 = very firm, overmature head with outer leaf midribs splitting.

RESULTS.—*Description of internal rib necrosis and rusty brown discoloration.*—In February and March, 1971, lettuce heads were received from the Imperial Valley. A majority of the heads had exterior symptoms of IRN; RBD developed after storage for 7 days or more. Externally IRN is a grayish-black discoloration visible on the lower side of the leaf along the midrib, and the discoloration is the most intense at the basal end of the leaf midrib, as shown in color photographs published elsewhere (11, 13). In paradermal sections the discoloration was observed between the vascular bundles. In cross sections of the midrib observed with a microscope, the necrotic cells occur in groups or singly in the parenchyma tissue between the vascular bundles; they are reddish brown, and appear granular from the dead and coagulated protoplasm (Fig. 1-B). The discolored parenchyma extends up the midrib in severe cases but often is limited to a few cm; in all cases, the intensity of discoloration decreases with distance from the basal end of the head.

RBD also occurs predominantly on the midribs, but in severely affected heads extends to the entire leaf blade; symptoms are shown in color photographs (11, 13). RBD begins on the outer leaves at the basal end of the head and extends upward on these leaves as well as affecting the younger leaves. Microscopically, the epidermal cells collapse and have a red pigmentation. In mild cases, irregular groups of

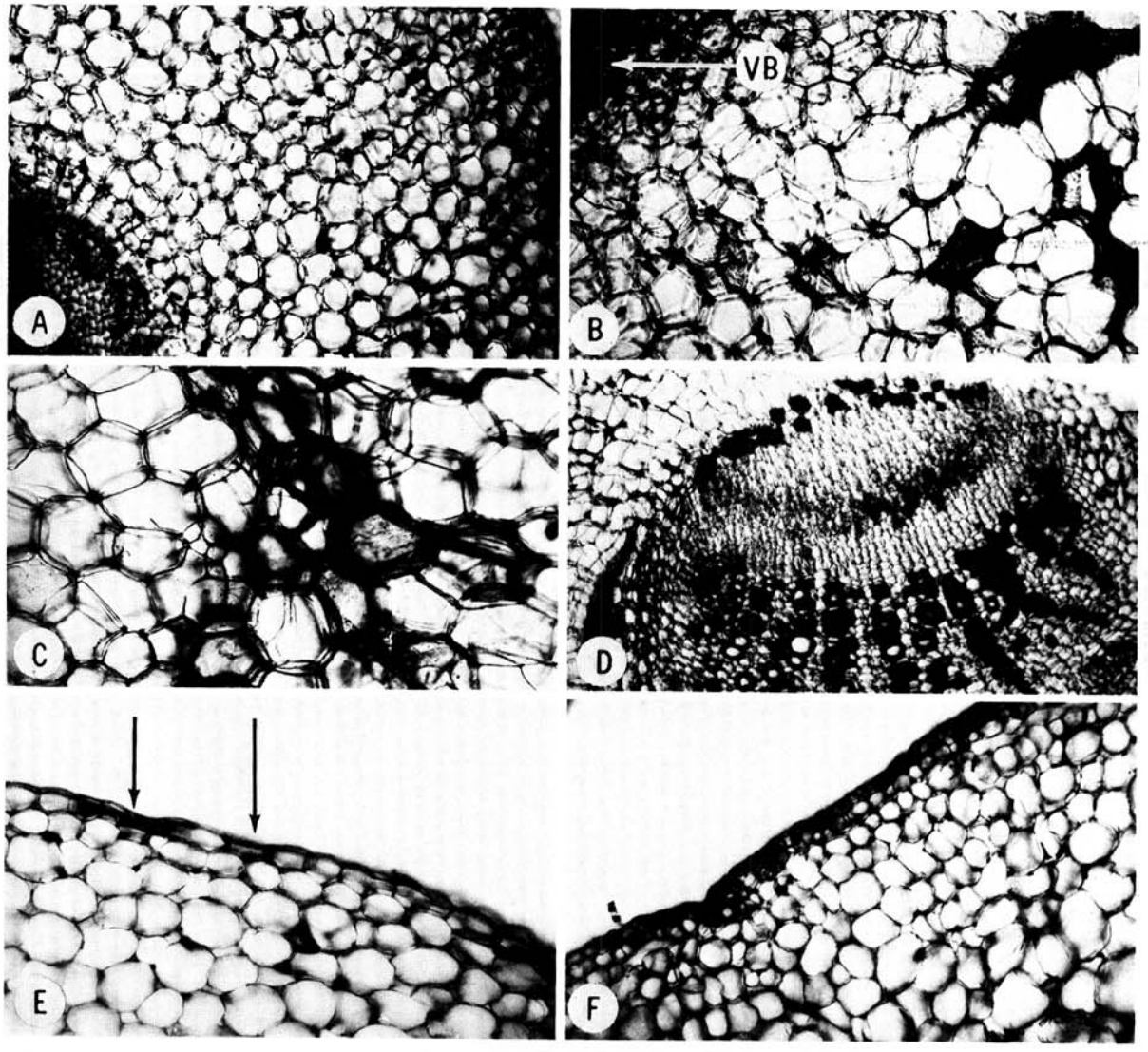

Fig. 1. Cross sections of midribs from 'Climax' lettuce plants illustrating three disorders. **A)** Virus-free control plant (×59). **B)** Plant with internal rib necrosis (IRN) from a commercial field, necrotic parenchyma cells (arrow) are a symptom of IRN, vb = vascular bundle (×75). **C)** Experimental, lettuce mosaic virus-infected plant with necrotic parenchyma cells typical of IRN (×91). **D)** Plant with ammonia damage, note xylem is necrotic and occluded (×75). **E)** Plant with mild rusty-brown discoloration (RBD), only a few epidermal cells are necrotic (arrow) (×75). **F)** Plant with severe RBD, most of the epidermis has collapsed (×48).

cells are affected (Fig. 1-E) but as the severity increases, the entire epidermal layer collapses, the red discoloration becomes more intense and underlying parenchyma cells are involved (Fig. 1-F).

Isolation and identification of LMV.—Lettuce with and without IRN was collected during January and February, 1971, and shipped to Davis. Assays showed that all 17 of the plants with IRN had LMV as well as 6 of the 11 heads that had no IRN. Flexuous rods were found in negatively stained dips of tissue from diseased lettuce plants, and in *C. quinoa* plants inoculated with samples from this lettuce. Eight of the lettuce heads collected in late February were also assayed on a host range which included *Chenopodium amaranticolor* Coste & Reyn., *Nicotiana glutinosa* L., *N. tabacum* L. 'Havana 425', *Datura stramonium* L., *Cucumis sativus* L. 'National Pickling', Bountiful bean and Small Sugar pumpkin. No symptoms of any mechanically transmitted virus except LMV developed on the host range. Furthermore, assays for BWYV from each of the 28 lettuce plants were negative. LMV isolates from three areas of the Imperial Valley and one isolate from the Salinas Valley were selected for use in further experiments.

The response of lettuce to LMV infection.—Nine

experiments were done to determine whether LMV caused IRN and RBD in Climax lettuce; the cultivars Calmar and Vanguard were also used because they were resistant to IRN and RBD (11, 22). LMV was either mechanically or aphid-inoculated to plants ca. 30, 45, 60, or 75 days old, and the plants were placed in growth chambers or the greenhouse. Controls consisted of noninoculated plants and plants inoculated with buffer or fed upon by nonviruliferous aphids and were evenly divided among the age groups in each experiment. The plants were harvested when they were 90-120 days old (i.e., 25-80 days after inoculation, average 50-60 days), rated for exterior IRN, and stored at 1 C for possible development of RBD. Each plant was indexed for LMV infection. In the first six experiments, each plant was indexed on *C. quinoa*. Since plants with mosaic symptoms always indexed positive, the indexing of such plants seemed redundant and was discontinued in the last three trials. Development of IRN will be discussed first.

Since IRN severity and frequency of occurrence seemed to be equivalent whether the plants were aphid- or mechanically-inoculated with any of the four isolates of LMV, the results of nine experiments done in growth chambers have been combined (Table 1). IRN developed only on the cultivar Climax. The response of lettuce to LMV infection depended on the age of the plant at the time of inoculation. Plants infected at 30 days of age were the most severely affected with the classical mosaic symptoms; the leaves showed a severe light-dark green mottle, and were twisted and reflexed. There was a general stunting of the plants, they formed only very loose heads, and often bolted prematurely. Plants of this age were therefore not used in later trials. IRN developed on ca. 50% of these plants. Plants infected at 45 and 60 days of age formed small to medium heads with a mild mottle or reflexing of leaf margins and IRN developed on almost all of them. Virus-free plants were considerably larger and fleshier with turgid, white midribs in contrast to virus-infected plants which had flattened green midribs. IRN did not develop on noninoculated Climax plants, on inoculated but noninfected Climax, or on any plants of Calmar or Vanguard, regardless of LMV infection. In two trials, an equal number of additional plants were included in each treatment but these plants were incubated in the greenhouse (Table 1). Again only Climax plants infected with LMV developed IRN.

TABLE 1. Development of internal rib necrosis (IRN) and rusty brown discoloration (RBD) on lettuce inoculated with lettuce mosaic virus (LMV) at 30, 45, 60, and 75 days of age

Cultivars	Age at inoculation (days)	No. plants	No. with IRN / No. with LMV	No. with RBD / No. stored[a]
A) Plants incubated in growth chambers at 10-21 or 7-18 C				
'Climax'	30	18	7/13	4/17
	45	15	12/13	12/15
	60	38	28/28	23/31
	75	2	0/2	0/2
	Controls	42	0/0	0/42
'Calmar'	30	7	0/2	0/7
	45	10	0/7	0/9
	60	10	0/3	0/6
	75	3	0/2	0/3
	Controls	18	0/0	0/18
'Vanguard'	60	9	0/6	0/6
	Controls	3	0/0	0/3
B) Plants incubated in greenhouse at 24-29 C				
Climax	30	3	0/1	1/3
	45	6	2/4	1/2
	60	6	4/4	1/2
	75	3	0/0	0/3
	Controls	12	0/0	0/4
Calmar	30	3	0/2	0/1
	45	6	0/5	0/6
	60	6	0/5	0/1
	75	3	0/0	0/3
	Controls	12	0/0	0/4

[a] Total number of heads stored. Although not all were infected with LMV, RBD developed only on heads infected with LMV.

At the time of inoculation, the leaves on each plant were numbered and the inoculated leaves recorded. Lower leaves were removed when the oldest ones began to senesce, but usually at 21 and 34 days after inoculation and at harvest. After storage the heads were dissected. IRN appeared in a minimum of 21 days and was indistinguishable from that found in field-grown lettuce (Fig. 1-C). The IRN discoloration extended from 1 to 15 cm up the leaf and first appeared on the leaf which was 1- to 2.5-cm long at the time of inoculation, except that in one trial symptoms occurred on the inoculated leaves. Severe exterior symptoms of IRN developed 34 days after inoculation on the plants grown in the larger containers. The plants headed well in these containers and the older leaves did not senesce rapidly which allowed the development of the exterior symptoms.

From these experiments, it is evident that IRN occurs at ordinary temperatures and that near-freezing temperatures are not necessary for disease development. In the first experiment done in the growth chambers, five plants were subjected to two 14-hour nights in a 3- to 4-C coldroom before harvest. There was no effect on the IRN symptoms. Temperature affects the growth of the plants; constant warm temperatures such as in the greenhouse (24-29 C) are unfavorable for good growth, and consequently, for IRN development.

The cultivars Calmar and Climax were sown in experimental plots at Davis in late August 1971 to determine whether IRN could develop on lettuce grown in fields other than in the Imperial Valley. The heads were harvested 65-100 days after planting and assayed for LMV. Of 56 Climax plants 32 were infected with LMV as a result of aphid dissemination of the virus from local sources. Sixteen of 32 infected heads had IRN but none of the 24 virus-free heads had IRN. None of the Calmar plants developed IRN although 34 of 54 were infected with LMV.

A field trial was done at the USDA Imperial Valley Field Station in the 1971-72 lettuce season to

confirm the results obtained in growth chambers. The results, which are similar to the present results, will be presented elsewhere.

Postharvest response of Climax lettuce to LMV infection.—Rusty brown discoloration (RBD) is a postharvest disease; discoloration begins ca. 7 days after storage, and reaches a maximum intensity in 14 days. When the lettuce plants raised in the greenhouse or growth chamber were stored for 2 weeks, most of the Climax heads infected with LMV developed RBD (Table 1). Microscopically, this RBD was indistinguishable from that observed on plants from the field. RBD developed poorly, if at all, on heads which were soft (a maturity rating <3) at the time of harvest, as they often were when produced in growth chambers. The heads raised in the large containers were more mature (average maturity = 3) than those in earlier experiments, and all such LMV-infected Climax plants developed RBD in 2 weeks. None of the virus-free Climax lettuce developed RBD. The Vanguard and Calmar lettuce with or without LMV did not develop RBD.

The heads of lettuce raised in the fields at Davis were stored at 1 C after harvest and in 14 days typical RBD developed on 16 of 32 heads of Climax with LMV. The 24 virus-free Climax, 20 virus-free Calmar, and 34 virus-infected Calmar heads did not develop RBD.

The response of the lettuce variety Climax to infection by other viruses.—Beet western yellows virus: Plants infected with BWYV may be found in many lettuce fields. The symptoms associated with this virus are a thickening of lower leaves and an interveinal chlorosis. Because BWYV infections are common in some lettuce plantings, it was necessary to consider what effect this virus might have on IRN. Two trials were done in growth chambers.

Groups of plants (with 10-13 leaves) received one of the following treatments: noninoculated controls (fed on by virus-free aphids); aphid-inoculated with BWYV; mechanically inoculated with LMV; or inoculated with both BWYV and LMV. The plants were harvested 55-75 days after inoculation, and the heads stored at 1 C. The combined results from two trials showed that IRN was no more severe on the heads with both viruses than on the heads with LMV alone and that the heads with BWYV alone did not develop IRN or RBD (Table 2).

Three heads of Climax were harvested from a field plot at Davis in May 1972. All had LMV, IRN, and foliar symptoms of BWYV infection. BWYV was recovered from each of the samples. Again, BWYV did not appear to make IRN more severe than in experimental plants infected with only LMV.

Cucumber mosaic and alfalfa mosaic viruses: In each of two trials groups of five plants were inoculated with either LMV, CMV, or AMV or kept as noninoculated controls. The plants were kept in growth chambers and harvested ca. 50 days after inoculation. All ten plants infected with LMV showed a severe mottling and leaf reflexing; external symptoms of IRN were visible on all these plants 34 days after inoculation. Nine of the 11 plants inoculated with CMV developed yellow spotting; then, ca. 14 days after inoculation, the inoculated leaves collapsed, symptoms became less visible, and at harvest only two plants showed a very mild systemic blotching at leaf edges. In assays of the nine plants at harvest time, only three had CMV and extracts from these caused only a few local lesions on cowpea. The virus titer was evidently very low in these plants. Six of the 10 plants inoculated with AMV developed necrotic etching on inoculated leaves in ca. 14 days, and AMV was successfully transmitted from one plant to a host range 3 weeks after inoculation. At harvest, however, assays from all the plants were negative. No IRN-type of parenchyma discoloration occurred in the 16 healthy plants or in those inoculated with CMV or AMV. After storage for 2 weeks, all the heads with LMV developed severe RBD, but none of the heads inoculated with AMV or CMV showed any exterior discoloration.

Response of the lettuce cultivar Salad Bowl to LMV infection.—Plants of the leaf lettuce cultivar Salad Bowl growing in a field plot at Davis in the spring of 1972 developed discoloration along the midribs. Assays indicated all plants were infected with LMV. The discoloration was in the parenchyma cells, sometimes in those adjacent to the phloem, and was usually confined to the older leaves. When the heads were stored at 1 C for 2 weeks, no RBD-like surface discoloration occurred.

To determine if LMV was responsible for this parenchyma discoloration, inoculations were done on plants grown in the greenhouse. Eight plants (28 days old) were inoculated with LMV and five plants were not inoculated. Twenty-four days later, IRN-type of discoloration had developed on the older leaves of all the plants infected with LMV but the virus-free plants remained free of discoloration. Cross sections of the discolored midribs showed the discoloration to be in the parenchyma cells and sometimes in those adjacent to the phloem. The plants were stored at 1 C for 2 weeks, but no RBD-type of discoloration developed.

The effect of ammonium hydroxide on lettuce.—NH_4OH was applied at the rates of 5.14 -

TABLE 2. The effect of beet western yellows virus and lettuce mosaic virus alone and in combination on Climax lettuce

Treatment	No. plants	Symptoms: Internal rib necrosis[a]	Symptoms: Rusty brown discoloration[b]
BWYV	14	0/8	0/14
LMV	11	11/11	3/4
BWYV + LMV	6	4/4	1/3
Controls	10	0/0	0/10

[a] Results as number of heads with internal rib necrosis/number of heads infected with virus(es). BWYV infections based on symptoms. Lettuce mosaic virus infection based on symptoms or assay to *Chenopodium quinoa*.

[b] Number of heads with RBD/number of heads stored at 1 C for 2 weeks; decay prevented storage of the other heads.

102.8 kg (28 -560 lb) of nitrogen per hectare (acre) to greenhouse-grown lettuce (ca. 75 days old) in 12-cm pots by diluting 1.6 N NH_4OH appropriately to apply 50 ml solution/pot; control pots received 50 ml of distilled water. Exterior symptoms began to appear after ca. 24 hr as dark streaks along the vascular elements of the midrib. Cross sections of the root and midrib showed the discoloration began in the xylem elements of the root and continued upward through the xylem (Fig. 1-D). The severity of discoloration increased with increasing concentration of the ammonium solution.

The effect of ethylene on lettuce.—Ethylene is associated with russet spotting, a postharvest disorder of lettuce (12, 18). To determine whether ethylene played a role in the development of RBD, an experiment was done in cooperation with Jim Klaustermeyer (Dept. of Vegetable Crops, U.C. Davis). Closed containers each with four healthy heads or four IRN-affected heads were kept at 1 C and treatments of 0, 0.1, or 1 ppm ethylene in air were applied for 7 days using a flow-through system. Each treatment was replicated three times. Ethylene damage occurred at 0.1 and 1.0 ppm on both healthy and IRN-affected heads. The plants with IRN developed RBD in this length of time. RBD and russet spotting were distinctly different on Climax lettuce; ethylene-induced russet spots were deep necrotic pits on the midribs in contrast to the shallow necrosis of RBD.

DISCUSSION.—As a result of infection by LMV, Climax lettuce develops IRN while growing in the field and RBD during storage and transit. The age of the lettuce at the time of LMV infection affects the type of symptom expressed. Plants infected at an early age show typical mosaic symptoms, head poorly, are unmarketable, and develop little IRN and RBD. Plants infected later usually do not develop mosaic symptoms but show extensive symptoms of IRN and RBD. Plants apparently must be infected with LMV for at least 3 weeks before symptoms of IRN or RBD develop. The suggestion that cold temperatures (<0 C) in combination with rainfall might cause IRN (11) is probably incorrect. Those plants were subjected to LMV infection as well as an adverse physical environment. The present results show that LMV infection alone can induce IRN without the adverse physical factors and therefore, LMV is regarded as the critical component of the environment that results in IRN. It has not been possible to test the adverse physical factors by themselves. Likewise, ammonium injury (21) is rejected as the cause of IRN because these conditions are microscopically distinct. BWYV, AMV, and CMV also failed to cause IRN and RBD in Climax lettuce. Zink & Duffus (23) suggested that IRN was caused by a synergistic reaction between BWYV and LMV. They failed, however, to present data to show a constant association of IRN with both BWYV and LMV in field-grown plants. In 1971, we could not isolate BWYV from any of 28 plants, including 17 with IRN, from the Imperial Valley. Our isolation technique seems to be adequate because BWYV was consistently isolated from plants grown at Davis and in the Salinas Valley and showing typical BWYV symptoms (S. M. Coakley, *unpublished*). Young plants were inoculated and incubated only 28 days in two of the three greenhouse trials done by Zink & Duffus (23) to compare LMV alone to LMV plus BWYV. These plants correspond to the 30-day-old plants inoculated in the present trials and their response differs from that of older plants. Furthermore, the 28-day incubation period is scarcely more than the 21-day minimum time for symptom expression in our trials. In the last trial of Zink & Duffus, older plants were inoculated and incubated 42 days, but the control, LMV alone, and BWYV plus LMV treatments had equal IRN incidences; i.e., 1 of 8. Finally, the temperature regime used by Zink & Duffus (23) was not given and there apparently was no confirmation that all inoculated plants were infected. Thus, we do not believe that Zink & Duffus (23) have presented adequate evidence to substantiate their hypothesis. The present work shows that infection by LMV alone can cause virtually 100% incidence of IRN in large plants that do not otherwise show obvious LMV symptoms and this is the pattern commonly observed in the Imperial Valley. The addition of BWYV to such plants might increase the severity of IRN but we could find no evidence for this.

LMV in Imperial Valley lettuce fields has usually been introduced annually with LMV-infected lettuce seeds and aphids have provided secondary movement of the virus. The secondary spread in any year depends upon vector activity which varies with environmental conditions. The interrelation between seed-borne inoculum and aphid movement accounts for the great variation in the amount of LMV present each year, and therefore, the occurrence of IRN and RBD.

Climax is the only head lettuce cultivar known to develop IRN and RBD when infected with LMV. Vanguard, Forty-niner, Merit, Calmar, Greenbay, Golden State C and D, Great Lakes 407, and Francisco were disease-free under the same conditions that Climax lettuce developed IRN and RBD (11, 22). The resistance of Vanguard and Calmar was confirmed in our research although Zink & Duffus (23) reported IRN-type symptoms in Vanguard infected with BWYV and LMV. Climax and Golden State strains have a common parent, breeding line No. 4157, and Climax is one parent of the nonsusceptible Vanguard (19). The leaf lettuce cultivar Salad Bowl, however, develops a similar discoloration along the midribs of the older leaves. This suggests that new cultivars should be screened for susceptibility to these disorders.

The physiological processes responsible for these disorders are not known. An increase in respiration rates of virus-infected plants is common (5, 15). Respiration rates were 1.5 times greater in virus-infected than in virus-free heads (J. Klaustermeyer & S. M. Coakley, *unpublished*). RBD was more severe on the more mature, virus-infected heads; furthermore, a "normal" discoloration that resembled RBD occurred when virus-free Climax was

stored for extended periods of time. This "normal" discoloration developed more rapidly on firmer heads but on soft heads it failed to develop even after 120 days of storage (S. M. Coakley, *unpublished*). Thus, RBD may be the result of accelerated senescence brought on by LMV infection and the consequent increase in respiration rate.

Since it would take several years to breed a cultivar having the horticultural characteristics of Climax, the current clean seed program offers a more rapid control. In this program the *C. quinoa* test (14) has been adapted to a mosaic-indexing program which has been used to certify seedlots which have zero mosaic in 30,000 seeds. Planting indexed seeds eliminates major sources of primary inoculum and thereby should eliminate LMV epidemics and the development of IRN and RBD. Such a control program (enforced by a county ordinance) was used in the Imperial Valley in the 1971-72 season and LMV, IRN, and RBD were successfully controlled.

LITERATURE CITED

1. BAKER, K. F. 1957. The U. C. system for producing healthy container-grown plants. Univ. Calif. Agric. Exp. Stn. Manual 23. 332 p.
2. CAMPBELL, R. N., & STELLA A. MELUGIN. 1971. Alfalfa mosaic virus strains from carrot and parsley. Plant Dis. Reptr. 55:322-323.
3. CEPONIS, M. J., & J. KAUFMAN. 1970. Brown stain of Western head lettuce on the New York market. Plant Dis. Reptr. 54:856-857.
4. CEPONIS, M. J., F. M. PORTER, & J. KAUFMAN. 1970. Rusty-brown discoloration, a serious market disorder of Western winter head lettuce. Hort. Sci. 5:219-221.
5. DIENER, T. O. 1963. Physiology of virus-infected plants. Annu. Rev. Phytopathol. 1:197-218.
6. DUFFUS, J. E. 1960. Radish yellows, a disease of radish, sugar beet and other crops. Phytopathology 50:389-394.
7. GROGAN, R. G., J. E. WELCH, & R. BARDIN. 1952. Common lettuce mosaic and its control by the use of mosaic-free seed. Phytopathology 42:573-578.
8. GROGAN, R. G., & F. W. ZINK. 1956. Fertilizer injury and its relationship to several previously described diseases of lettuce. Phytopathology 46:416-422.
9. HITCHBORN, J. H., & G. J. HILLS. 1965. The use of negative staining in the electron microscopic examination of plant viruses in crude extracts. Virology 27:528-540.
10. HOAGLAND, D. R., & D. I. ARNON. 1950. The water-culture method for growing plants without soil. U. C. Agric. Exp. Stn. Circ. 347. 32 p.
11. JOHNSON, H., JR., D. R. WOODRUFF, & T. W. WHITAKER. 1970. Internal rib necrosis of head lettuce in Imperial Valley. Calif. Agric. 24(9):8-10.
12. LIPTON, W. J. 1961. Anatomical observations on russet spotting and pink rib of lettuce. Amer. Soc. Hort. Sci. Proc. 78:367-374.
13. LIPTON, W. J., J. K. STEWART, & T. W. WHITAKER. 1972. An illustrated guide to the identification of some market disorders of head lettuce. U.S. Dept. Agric., Marketing Res. Rept. No. 950. 7 p., 19 plates.
14. MARROU, J., & C. M. MESSIAEN. 1967. The Chenopodium quinoa test: a critical method for detecting seed transmission of lettuce mosaic virus. Proc. Int. Seed Test. Assoc. 32(1):49-57.
15. MATTHEWS, R. E. F. 1970. Plant Virology. Academic Press, New York. 778 p.
16. MC COY, O. D. 1969. Serious internal defect of lettuce in Imperial County during the 1968-1969 season. Veg. Res. Mimeo. No. 27. Univ. Calif. Agric. Exp. Stn. Meloland. 5 p.
17. MILNE, K. S., R. G. GROGAN, & K. A. KIMBLE. 1969. Identification of viruses infecting cucurbits in California. Phytopathology 59:819-823.
18. ROOD, P. 1956. Relation of ethylene and post-harvest temperatures to brown spot of lettuce. Amer. Soc. Hort. Sci. Proc. 68:296-303.
19. THOMPSON, R. C., & E. J. RYDER. 1961. Descriptions and pedigrees of nine varieties of lettuce. USDA Tech. Bull. No. 1244. 19 p.
20. TOMLINSON, J. A. 1970. Lettuce mosaic virus. Commonwealth Mycological Institute, Descriptions of Plant Viruses No. 9.
21. TROUTMAN, J. L., B. R. GARDNER, & W. D. PEW. 1971. Internal rib necrosis of lettuce associated with ammonium concentration. Phytopathology 61:1324 (Abstr.).
22. WHITAKER, T. W., H. JOHNSON, & K. MAYBERRY. 1971. Internal rib necrosis. Western Grower and Shipper 42(5):28.
23. ZINK, F. W., & J. E. DUFFUS. 1972. Association of beet western yellows and lettuce mosaic viruses with internal rib necrosis of lettuce. Phytopathology 62:1141-1142.