

## Association of Beet Western Yellows and Lettuce Mosaic Viruses with Internal Rib Necrosis of Lettuce

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### ABSTRACT

The internal rib necrosis (IRN) disease of *Lactuca sativa* L. 'Climax' that caused considerable damage in the Imperial Valley of California in 1969 was associated with two viruses prevalent in the area. Both beet western yellows virus (BWYV) and lettuce mosaic virus (LMV) were recovered from Imperial Valley lettuce cultivar Climax severely affected with IRN. The disorder was not reproduced in IRN-susceptible cultivars Climax and Vanguard by infection with BWYV. Infection with LMV produced IRN symptoms in a relatively low percentage of Climax plants, but none in Vanguard. The incidence of

IRN was higher in both Climax and Vanguard when infected with the combination BWYV + LMV. A synergistic effect on the growth of Climax and Vanguard was observed when both viruses were present. The IRN-resistant cultivars Great Lakes 118 and Calmar did not exhibit a synergistic response to the combination of the viruses. The genetic relationship of IRN-susceptible cultivars and the etiology of the disease on the basis of the combination of BWYV + LMV are discussed.

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In 1969, internal rib necrosis (IRN) of lettuce (*Lactuca sativa* L.) reached epidemic proportions in the Imperial Valley of California, in plantings that matured from mid-January to mid-February. The disorder appeared restricted to the crisp-headed cultivar Climax, for it was not found in a limited survey of other cultivars in commercial plantings (5). Both beet western yellows virus (BWYV) and lettuce mosaic virus (LMV) were recovered from plants with severe IRN. The destructiveness of the disease, and the importance of Climax as a winter cultivar, led to investigations of the relationship of BWYV and LMV to the occurrence of IRN.

**MATERIALS AND METHODS.**—The Imperial Valley isolates of BWYV and LMV were obtained from field grown lettuce cv. Climax plants with IRN. The Salinas Valley isolate of BWYV was obtained from sugar beet (*Beta vulgaris* L.), and the Salinas LMV from field grown lettuce cultivar Great Lakes. The BWYV isolates were maintained in radish (*Raphanus sativus* L.), and the LMV isolates in cultivar Great Lakes 118 lettuce.

Seed of Climax, Great Lakes 118, Calmar, and Vanguard were germinated in petri dishes. When 7 days old, the seedlings were transplanted to 6-inch pots, one plant/pot. All seedlings were free of seed-borne LMV. The plants were irrigated as necessary with 0.25-strength Hoaglands solution (4).

Nonviruliferous green peach aphids (*Myzus persicae* [Sulzer]) were reared on radish. After an acquisition feeding period of 24 hr on the virus source, 25 aphids were transferred to each healthy lettuce plant in an insectary compartment and allowed to feed for 48 hr. All plants were then sprayed with nicotine sulfate and placed in greenhouses which were fumigated at weekly intervals with nicotine sulfate.

The plants were inoculated at the sixth- or seventh-leaf stage of growth in Experiments 1, 2, and 3, and in the tenth- to twelfth-leaf stage in Experi-

ment 4. The plants were harvested 28 days after inoculation in Experiments 1, 2, and 3, and 42 days after inoculation in Experiment 4. Fresh weights of the aboveground portion of the plants were subjected to a factorial analysis of variance and to Duncan's multiple range test.

**RESULTS.**—*Symptoms of BWY.*—All cultivars tested showed typical BWY symptoms. There was irregular chlorotic blotching on the older leaves, and later the chlorotic areas tended to coalesce and produce interveinal yellowing near the leaf margins and base. No IRN was observed in any of the cultivars inoculated with BWYV.

*Symptoms of LM.*—All cultivars showed similar symptoms to the LMV isolates. There was vein-clearing, followed by development of a light green mosaic pattern in the leaf blades, and conspicuous stunting of the plants. Internal rib necrosis was noted in three of 24 Climax plants (12.5%) inoculated with LMV, but in none of eight Vanguard plants.

*Symptoms of BWY + LM.*—Striking differences in symptoms were apparent between cultivars. Climax and Vanguard developed mild-to-severe necrosis of the leaf blade, but Great Lakes 118 and Calmar did not develop necrotic symptoms. In any given experiment, stunting was more severe in Climax and Vanguard than in Great Lakes 118 or Calmar. Internal rib necrosis was present in four of 13 Vanguard plants (30.7%) and in 17 of 38 Climax plants (44.7%) (Tables 1, 2).

*Effect of the viruses on fresh weight.*—Infection with BWYV alone resulted in no significant reduction in mean fresh weight per plant when compared with the control for a given cultivar (Tables 1, 3). Also, there were no significant differences in fresh weight among cultivars infected with BWYV in a given experiment.

Infection with LMV resulted in a significant decrease in mean fresh weight per plant in any given

TABLE 1. Effect of two isolates of beet western yellows virus (BWYV) and two isolates of lettuce mosaic virus (LMV), and a combination of the viruses, on incidence of internal rib necrosis (IRN), and on fresh weight of cultivar climax, Experiment 1

Virus used as inoculum	No. plants with IRN/ no. plants in treatment	Mean fresh weight/plant(g)
None (control)	0/5	75.6 a <sup>a</sup>
BWYV Salinas	0/5	74.2 a
BWYV Imperial	0/5	72.0 a
LMV Imperial	1/5	42.3 b
LMV Salinas	0/5	38.1 b
BWYV Salinas + LMV Imperial	1/5	25.1 c
BWYV Imperial + LMV Imperial	2/5	20.3 c
BWYV Salinas + LMV Salinas	2/5	20.2 c
BWYV Imperial + LMV Salinas	3/5	9.1 d

<sup>a</sup>Two means having any letters in common are not significantly different from each other at the 1% level.

TABLE 2. Effect of beet western yellows virus (BWYV), lettuce mosaic virus (LMV), and the combination of the two viruses on the incidence of internal rib necrosis (IRN) in cultivars of lettuce, Experiments 2, 3, and 4

Experiment no.	Cultivar	No. plants with IRN/no. of plants in treatment when indicated virus was used as inoculum			
		None	BWYV	LMV	BWYV + LMV
2	Great Lakes 118	0/11	0/11	0/11	0/11
	Climax	0/11	0/11	1/11	5/11
3	Great Lakes 118	0/5	0/5		0/5
	Calmar	0/5	0/5		0/5
	Vanguard	0/5	0/5		2/5
	Climax	0/5	0/5		3/5
4	Great Lakes 118	0/8	0/8	0/8	0/8
	Calmar	0/8	0/8	0/8	0/8
	Vanguard	0/8	0/8	0/8	2/8
	Climax	1/8	0/8	1/8	1/8

TABLE 3. Effect of beet western yellows virus, lettuce mosaic virus, and a combination of the two viruses, on fresh weight of several cultivars, Experiments 2, 3, and 4

Experiment no.	Cultivar	Mean fresh weight (g)/plant when indicated virus was used as inoculum <sup>a</sup>			
		None	BWYV	LMV	BWYV + LMV
2	Great Lakes 118	56.9 b	51.6 b,c	45.5 c,d	40.6 d
	Climax	67.4 a	58.6 b	45.8 c,d	23.4 e
3	Great Lakes 118	66.3 a	59.3 a,b		48.0 b,c
	Calmar	56.4 a,b	52.6 b		36.8 c
	Vanguard	54.9 a,b	53.9 a,b		24.6 d
	Climax	60.9 a,b	56.7 a,b		15.7 d
4	Great Lakes 118	99.2 a,b	99.8 a,b	69.6 c	68.9 c
	Calmar	98.2 a,b	94.4 b	68.5 c	63.9 c
	Vanguard	108.3 a	101.4 a,b	73.4 c	51.8 d
	Climax	105.8 a,b	97.9 a,b	68.5 c	36.5 e

<sup>a</sup>Two means in a given experiment having any letters in common are not significantly different from each other at the 1% level.

cultivar, as compared to that of the control. However, in no experiment was there a significant difference in fresh weight between cultivars infected with LMV.

The combination of BWYV and LMV infections in Great Lakes 118 and Calmar induced no significant decrease in fresh weight as compared with that resulting from LMV infection alone. In contrast, the combination of BWYV + LMV in Climax and Vanguard appears to be synergistic, resulting in a significant decrease in fresh weight as compared with LMV infection alone, with the reduction being greater in Climax. In Experiment 1, in which the two isolates of BWYV and the two isolates of LMV were inoculated into Climax in the four possible combinations, the combination of Imperial BWYV + Salinas LMV induced a significantly lower mean fresh weight than did the other combinations.

**DISCUSSION.**—Internal rib necrosis has been reported to be a physiological disorder, but without sufficient evidence to support this conclusion (5). Several growers have suggested that low temperatures and a prolonged rainy period may have been the major predisposing factors responsible for the prevalence of the disorder in 1969. However, a single year's data of Johnson et al. (5) indicate that wet soil near market maturity and cold weather do not cause IRN.

In contrast to the report by Johnson et al. (5) that Vanguard is resistant to IRN, in our laboratory study we found Vanguard to be susceptible; and during the 1970 winter lettuce season, we observed IRN in commercial plantings of Vanguard. However, field observations on the incidence of IRN, and the synergistic reaction of Vanguard and Climax plants to the combination of BWYV + LMV, indicate that Vanguard is less susceptible than Climax to IRN.

Troutman et al. (7) reported that symptoms like those described for IRN were induced by sidedress applications of  $\text{NH}_4\text{OH}$  to nearly mature, field-grown Climax plants, and that the severity of IRN was directly correlated with concentrations of  $\text{NH}_4\text{OH}$  applied. Grogan & Zink (3) described a discoloration of the midribs and the xylem core of the roots of IRN-resistant Great Lakes cultivars. These symptoms were reproduced by application of inorganic commercial fertilizer materials or animal manure. A comparison of the relative toxicity of the several fertilizers revealed that those containing  $\text{NH}_4\text{OH}$  and free ammonia were the most toxic. In contrast to IRN, the midrib discoloration caused by fertilizers was in the vascular bundles, and always associated with damage of the root systems.

The symptoms of IRN in this study were similar to those reported by Johnson et al. (5), but less severe than those observed in the Imperial Valley in 1969. Cold weather has been observed to be associated with the occurrence of IRN, and may be a contributing factor to the development of symptoms. This could account for the relatively low incidence of IRN in greenhouse Climax and Vanguard plants infected with LMV or with BWYV + LMV. Although IRN was observed in Climax infected with LMV alone, the

incidence was considerably lower than in the BWYV + LMV infection. These data suggest that IRN symptoms may be the result of LMV infection alone; however, the shock effect of BWYV + LMV infection is more important. The synergistic reaction of the combination of the two viruses in IRN-susceptible cultivars would support this hypothesis.

Sources of BWYV in the Imperial Valley are abundant and widely distributed. Several crop plants grown in the area, as well as weeds, are known to be susceptible to infection (2). It is interesting that in 1969, the IRN epidemic year, BWYV was also epidemic in the sugar beet crop. The primary source of LMV in the Imperial Valley appears to be seed-borne infection in lettuce. The recovery of BWYV and LMV from Climax plants with IRN, the development of IRN symptoms in Climax and Vanguard plants inoculated with BWYV + LMV, and the synergistic effect of the virus combination in IRN susceptible cultivars are all evidence of a causal relationship.

We favor the theory that the symptoms of IRN are caused primarily by a combination of BWYV + LMV in association with cool weather. Zink & Kimble (10) have reported that infection by LMV late in the growth of the crop does not have a severe stunting effect on head lettuce. Infection of lettuce with BWYV does not cause a significant stunting. Consequently, infection with BWYV during growth of the crop, followed by late infection with LMV, could account for the production of marketable sized heads with IRN symptoms.

Several facts of this investigation merit further discussion, as they are of general genetic and pathological significance. IRN-susceptible Climax and Vanguard have two common parents, 4157 and 5550, in their pedigrees (6). Cultivars Golden State C and D, which have been reported to be resistant to IRN (K. S. Mayberry & J. K. House, Farm Advisors, Imperial County, Calif., *personal communication*) have one common parent, 4157, with Climax and Vanguard (6). This suggests that parent 5550, which was later named Climax, carried the genetic factors for susceptibility. The IRN-resistant Merit, Calmar, Great Lakes, and selections from Great Lakes (Forty-Niner, Greenland, and Green Bay) do not have a common parent with Climax and Vanguard (1, 6, 8, 9). The synergistic reaction of BWYV + LMV may be a useful technique in the screening of cultivars and breeding lines for IRN resistance. Further testing will be necessary to determine the effectiveness of this procedure.

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