

Temperature response of dominant disease lesion mutants — The disease lesion mimic mutants described previously (MNL 48:121) have been observed to be highly responsive to temperature. To establish the limits of the response kernels from back-cross ears that would segregate 1:1 for Les and Les2 (previously designated Spt) and from an ear of the cross Les/+, + + x + +, Les2/+ were planted. Sixty kernels from each ear (20 seeds per treatment) were given a 48-hour germination period at room temperature (78°F) and then transferred to one of three growth chambers. All three chambers were regulated for 16 hours of maximum light and 8 hours of darkness; the temperatures were held constant at 72°F, 80°F and 84°F, respectively. Each leaf on each seedling was noted for lesions as it became fully extended, and a leaf was recorded as positive only when it had at least one large lesion or three small lesions. Leaf counts from the first nine plants showing lesions in each treatment are summarized in Table 1; additional mutant plants were excluded

Table 1. Effect of temperature on the occurrence of lesions on successive leaves of seedlings carrying Les, Les2 or both.

Family	Temp (°F)	Leaf number									Mut/N
		1	2	3	4	5	6	7	8		
Large lesions (<u>Les</u> /+)											
37	72	9	9	9	8 //	1	0	0	0	9/20	
	80	7	7	5	3	2				*8/20	
	84	8	5	5	1	0				11/20	
Small lesions (<u>Les2</u> /+)											
38	72	7	7	5	6 //	5	8	9	8	9/18	
	80	1	9	8	7	4				9/19	
	84	9	9	9	9	5				10/19	
<u>Les</u> , <u>Les2</u> or <u>Les</u> and <u>Les2</u>											
39	72	Large	9	9	9	9 //	7	1	1	0	11/20
		Small	0	1	0	0 //	1	9	9	8	10/20
	80	Large	9	9	8	4	2				10/19
		Small	4	3	6	5	5				*6/19
	84	Large	9	9	7	8	5				12/20
		Small	9	9	9	9	8				9/20

//Temperature changed from 72°F to 84°F at the four-leaf stage.

* Less than nine mutant plants, due to either poor expression or chance deviation from a 1:1 ratio.

to make the data from all three treatments directly comparable. Nonmutant plants were recorded as zero and have no weight in the table except in the two cases when less than nine mutant plants were found. If the reduced number is due to poor expression, the data are valid; if due to chance variation from a 1:1 ratio, a

slight bias (not enough to change the significance of the observations) is introduced.

The plants from the cross Les/+ x Les2/+ (family 39) included Les, Les2, Les Les2 and normal individuals. Since both mutants can be observed in the double heterozygote, the data were recorded separately for each type; thus, the double mutant plants were scored twice. To test for reversal of the temperature effect the plants in the 72° chamber were changed to 84° conditions after the four-leaf stage when lesion expression at 72° had stabilized. Table 2 summarizes the large and small lesion expression of only the double mutant plants in family 39 from Table 1.

Table 2. Effect of temperature on the occurrence of lesions on successive leaves of Les Les2 plants only.

Family	Temp (°F)	Lesion type	Leaf number								Mut/N	
			1	2	3	4	5	6	7	8		
39	72	Large	4	4	4	2	//	2	0	0	0	4/20
		Small	0	0	0	0	//	0	3	4	2	
	80	Large	4	4	4	3		2				4/19
		Small	3	3	4	4		3				
	84	Large	6	6	6	6		5				6/20
		Small	6	6	6	6		6				

//Temperature changed from 72°F to 84°F at the four-leaf stage.

A number of significant facts are evident from the data presented here.

(1) Les is expressed strongly on nearly all of the leaves of plants grown at 72°. At higher temperatures the lesions appear on the first few leaves but become less frequent with each succeeding leaf. Not expressed in the tables is the fact that the frequency of lesions per leaf varies widely and is inversely proportional to the temperature.

(2) For Les a change in temperature from 72° to 84° causes an abrupt halt in lesion appearance on subsequent leaves. The occurrence of lesions on the first two or three leaves of plants grown at higher temperatures may result from evaporative cooling of the soil and the lower plant parts.

(3) Les2 is expressed most strongly at 84° (more lesions per leaf as well as on a higher proportion of leaves) and less strongly at 80° and 72°, in that order. The low numbers in family 38 for leaf 1 at 80° and for leaf 5 at all temperatures resulted from temporary careless culture practices. Drought and low soil fertility have been observed to suppress lesion formation.

(4) Changing the growth temperature from 72° to 84° improves the expression of Les2, both in the frequency of lesions and in the number of leaves affected.

(5) Observations not expressed in the table show that the double heterozygotes have an intermediate phenotype with fewer and less striking large lesions and fewer small lesions, although both are clearly expressed.

(6) A comparison of large lesion plants from family 37 with the same type in family 39 (Table 1) shows no appreciable difference at the lowest temperature (72°) but a marked increase in the expression in family 39 at 84°. Table 2 shows this to be mostly due to the expression in the double mutant plants.

(7) The same comparison for the small lesion mutant (Les2) shows a striking reduction in the lesions on plants from the Les x Les2 cross (family 39) as compared to the backcross (family 38) at low temperatures while at the highest temperature there is no appreciable difference.

(8) Changing the temperature from 72° to 84° for the Les x Les2 material produces a striking and opposite reversal of expression for both lesion types; the effect is more abrupt and complete than in either family carrying the single mutants.

From these observations it can be concluded that Les is expressed best at low temperatures (around 72°) and not at high temperatures, while Les2 is expressed best at temperatures around 84° and reduced at lower temperatures. Changes in temperature may abruptly alter the expression of either mutant according to its prescribed response. Double heterozygotes have an intermediate expression with both mutants expressed at lower levels. In the double heterozygote the expression of Les2 is restricted or reduced at lower temperatures, while the expression of Les is enhanced in the higher temperature range. The Les x Les2 material also shows differences suggesting parental influence on the interaction of Les and Les2.

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Comparative efficiency of seed and pollen treatments in mutation experiments —

An important aspect of the design of mutation experiments is economy in terms of the plants to be handled in each generation. A common practice with autogamous plant species such as barley or Arabidopsis is to treat seed with a mutagenic agent, grow an M_1 , self that to produce M_2 seed, grow the M_2 and look for mutant segregants. The size of the experiment is usually determined by a trade-off between the number of M_2 plants required to provide a statistically adequate sample and the number of plants that can be handled. With corn, which has separate male and female flowers (geitonogamy), the M_1 may be selfed or outcrossed to untreated material and the progeny in either case may be selfed to produce an M_3 , which is examined for segregating mutants. The economics of the numbers grown in each generation and the samples taken is often not properly understood, but