Some Phenomena Observed in Systemic Infection of Alfalfa Mosaic Virus II. The Influences of Air Temperature and Shading on Symptpm Appearance

hoku Journal of Agricultural Research
Vol. 22, No. 4, December 1971
Printed in Japan

Some Phenomena Observed in Systemic Infection of Alfalfa Mosaic Virus

II. The Influences of Air Temperature and Shading on Symptom Appearance

Akira KUDO* and Tadao MISAWA

Laboratory of Phytopathology, Department of Agronomy, Faculty of Agriculture, Tohoku University, Sendai, Japan

(Received December 8, 1971)

Summary

Some factors concerning the appearance of the necrotic symptom which is caused by systemic infection were investigated by the combination of alfalfa mosaic virus (AMV) and broad bean.

The appearance of top leaf necrosis on apical leaves was influenced by the air temperature. Its ratio of appaerance was 25% at 17°C cand 75% at 24°C, respectively. Under low temperature, the virus multiplication decreased in the inoculated leaves than at 24°C.

When the inoculated leaf was shaded, the appearance ratio of top leaf necrosis decreased and its virus content was lower than in the control. Shading of the apical part influenced the appearance of top leaf necrosis, too.

When the entire seedling was placed in the dark after AMV multiplication in the inoculated leaf, the virus movement toward the apical part was inhibited and there top leaf necrosis never appeared. The necrotic symptom appeared only on the stem. The appearance ratio of top leaf necrosis increased with increasing illumination.

The symptoms which the plant shows when infected by virus are influenced by ·ironmental factors. In these factors, air temperature and light influence the nptoms most. It has been presumed that air temperature and light influence mulitplication and the movement of the virus, and thus indirectly the symp-ıs are influenced.

Cheo and Pound (1) reported changes of the symptom in spinach infected with ıumber virus 1 due to varying air temperatures. And also there are reports on influence of the strength of light and wave lengths upon the virus infection 4, 5). Yarwood (9), and Helms and McIntyre (4) indicated that when the ıt plant was placed in the dark, and illuminated just before inoculation, its ceptibility to the virus increased.

Present address: Horticultural Research Station, Hiratsuka, Japan

Broad bean infected with alfalfa mosaic virus (AMV) forms necrotic lesions in the inoculated leaf and top leaf necrosis in the systemically infected young top leaf.

Therefore it was observed that air temperature and light influenced these necrotic symptoms of broad bean by AMV. In this paper, these results will be presented.

Materials and Methods

The virus used was one isolate of alfalfa mosaic virus. For these experiments, broad bean (*Vicia faba* var. Wase soramame) was used in the 3–6 leaf stage. Inoculation was done by the rubbing method with carborundum. Seedling leaves showing necrosis were homogenized with an equal weight of 0.1 M phosphate buffer (pH 7.0) and this juice was used as the inoculum. The inoculation was performed on the compound leaves of a given leaf position. After the inoculation, the seedlings were placed in the phytotron (24°C). For the determination of AMV multiplication in the inoculated leaf, two leaf discs (7 mm in diameter) were cut out at random from the leaf blade, and homogenized with 0.1 ml of 0.1 M phosphate buffer (pH 7.0). This juice was inoculated on the five primary leaves of cowpea (*Vigna sinensis* var. Kurodane-Sanjaku) and the average number of appeared local lesions were estimated. The details of the method used will be described in each experiment.

Results

I) The influence of air temperature on top leaf necrosis development

Broad bean seedling in the 4–6 leaf stages were used and either the 3rd or the 4th leaf was inoculated. Then the inoculated seedlings were placed in rooms of 17°C or 24°C of the phytotron and the appearance of top leaf necrosis was investigated. Table 1 indicates the ratio of appearance of the top leaf necrosis in both temperatures. In the temperature of 24°C, top leaf necrosis began to appear from the 11th day after the inoculation and 12 seedlings in 16 seedling showed top leaf necrosis by the 18th day while another 4 seedlings showed none. On the contrary, in the temperature of 17°C, only one seedling showed top leaf necrosis by the 15th day. Two seedlings showed to leaf necrosis by the 18th day, but the number of the seedlings which showed top leaf necrosis were remarkably few. By three weeks after the inoculation, the number of the seedlings which showed top leaf necrosis had not increased.

The degree of the multiplication of AMV in the inoculated 4th leaf was influenced by the air temperature as shown in Table 2. In the temperature of 17°C, the multiplication of AMV was distinctly lower than in that of 24°C and also the ratio of the appearance of top leaf necrosis was lower. Moreover severe brown necrosis was scarcely observed on the inoculated leaf at 17°C.

TABLE 1. *Ratio of Top Leaf Necrosis Appearance by Air Temperatures*

Temperature	Developing leaves	Position of inoculated leaf	Numbers of seedling showed top leaf necrosis / Numbers of inoculated seedling	Rate of appearance of top leaf necrosis
17°C	4	L-3	0/1	25%
	5	L-4	1/7	
	6	L-4	2/4	
24°C	4	L-3	1/1	75%
	5	L-4	8/8	
	6	L-4	3/7	

TABLE 2. *Multiplication of AMV in Inoculated Leaf*
(The 4th leaf)

Temperature	3 days*				5 days*			
	Replication			Average	Replication			Average
	I	II	III		I	II	III	
17°C	51	53	75	60	111	139	152	134
24°C	144	138	160	147	193	218	205	205

Each value is local lesion numbers per 16 mm leaf disc
* Period after inoculation

II) The influence of light on top leaf necrosis development

1) Shading of inoculated leaf

a) Influence upon appearance of top leaf necrosis

In this experiment, broad bean seedlings of the 4–5 leaf stage were used and every compound 3rd or the 4th leaf was inoculated. Immediately after the inoculation, the inoculated leaves were covered with alumi-foil to completely shut out the light. When the inoculated leaves were cut off from the light, the appearance of top leaf necrosis which is a systemic symptom was severely influenced. Table 3 indicates these results. At the 10th day after the inoculation, there were few seedlings which showed top leaf necrosis among the treated

TABLE 3. *Shadowing of Inoculated Leaf and Appearance of Top Leaf Necrosis*

Treatment	10 day*		19 days*	
	Exp. I	Exp. II	Exp. I	Exp. II
Shadoing	0*/6	1/11	0/6	3/11
Control	3/5	8/13	4/5	12/13

* Period after inoculation
** Numbers of seedling showing top leaf necrosis/Numbers of inoculated seedling

seedlings. This tendency was also observed at the 19th day after the inoculation. From the result that the virus activity of the stem of symptomless seedlings was examined, the transportation of AMV in the direction of the apical part from the inoculated leaves was not recognzied.

b) Virus concentration of the inoculated leaf

For observing the influence of shading on AMV multiplication in inoculated leaves, one leaflet of the compound leaf was covered with alumi-foil and another leaflet of the same compound leaf was not covered as a control. This treatment was done immediately after the inoculation. As another treatment, green cellophane paper for the purpose of lowering the photosynthesis was used instead of alumi-foil. In this case, clear cellophane was used as a control.

Table 4 indicates the concentration of multiplicated AMV as the number of local lesions on the primary leaf of cowpea. The multiplication of AMV in shaded leaves with alumi-foil or green cellophane paper, was always lower than that in the control leaves. Especially in the leaves shaded with alumi-foil, the multiplication of AMV was clearly inhibited. Necrotic lesions appeared on the untreated leaves at the 3rd day after the inoculation, but appeared on treated leaves at the 5th day. The concentration of AMV in shaded leaves with alumi-foil was approximately half of that in the untreated leaves by the 5th day after the inoculation. Moreover the necrotic lesions formed were brown in color on the untreated leaves, but black on

TABLE 4. *AMV Concentration in Treated Inoculated Leaf*

(1) *(The 4th leaf)*

Treatment	3 days*				4 days*			
	Replication			Average	Replication			Average
	I	II	III		I	II	III	
Alumifoil	103	91	94	96	118	125	128	124
Control	155	142	133	143	185	206	215	202

Each value is local lesion numbers per 16 mm leaf disc
* Period after inoculation

(2)

Treatment	2 days*				3 days*			
	Replication			Average	Replication			Average
	I	II	III		I	II	III	
Green cellophane	40	51	54	48	114	121	133	122
Clear cellophane	73	84	84	80	181	171	156	169

Each value is local lesion numbers per 16 mm leaf disc
* Period after inoculation

the treated leaves. There was no difference in the size of the lesion between the treated and untreated leaves. Shading with green cellophane paper did not influence them so much as the alumi-foil. The multiplication of AMV and the appearance of necrotic lesions was delayed and appeared by the 4th day after the inoculation.

c) Period of shading and appearance of top leaf necrosis

By shading the inoculated leaves with alumi-foil, the appearance of top leaf necrosis was remarkably inhibited. Therefore, the relation between the period of shading and the appearance of top leaf necrosis was investigated. The periods of shading were set as follows. Two days after the inoculation and full period from the inoculation to the inspection. Table 5 indicates the rate of appearance

TABLE 5. *Ratio of Appearance of Top Leaf Necrosis on Shading-treated Seedling*

Treatment	Appearance percentage of top leaf necrosis
All days*-shading	10%
2 days**-shading	57%
Non-treatment	71%

20 seedlings were used at each treatment
* The day from inoculation to inspection
** Period after inoculation

of top leaf necrosis at the 11th day after the inoculation. The shading of both periods influenced the appearance of top leaf necrosis and the appearance of top necrosis was remarkably inhibited by shading of full period but shading of two days did not inhibited so much. Thus, the inhibition increased with the increase of the shading-period.

2) Influence of shading of the top-leaf

In this experiment, broad bean seedling of the 3 and 4 leaf stage were used. The 3rd or the 4th leaf was inoculated. The top part of the stem having unexpanded leaves was covered with green cellophane paper on the 2nd day after the inoculation: As a control, the top part of the stem of other seedlings was covered with clear cellophane paper. In addition, about twenty pin holes were made in the cellophane cover for aeration. Six seedlings were used for each treatment and the experiment was replicated two times. As similar results were obtained, a mean result is indicated in Table 6. In the case of untreated seedlings, top leaf necrosis appeared often in the 5th leaf. On the contrary, in seedlings treated with green cellophane paper, the necrotic symptom was observed only on the leaf petiole and the stem, and top leaf necrosis was not found even on the 10th day after the inoculation.

TABLE 6. *Ratio of Appearance of Top Leaf Necrosis on Treated Top Leaves (at the 10th day asfter inoculation)*

Treatment with green cellophane		Treatment with clear cellophane	
Inoculated leaf	Leaf position showing necrosis	Inoculated leaf	Leaf position showing necrosis
L-3	-*	L-3	-
L-3	-	L-3	-
L-3	-	L-3	L-5
L-3	-	L-3	L-5
L-3	-	L-3	L-5
L-4	L-6	L-4	L-5,6

* non symptom

3) Influence of shading of the entire seedling

Broad bean seedlings of the 5 and 6 leaf stage were used. The 2nd and the 3rd leaf in seedlings of the 5 leaf stage, and the 3rd and the 4th leaf in seedlings of the 6 leaf stage, were inoculated, respectively. The inoculated seedlings were placed under natural light conditions for 4 days in order to form necrotic lesion in the inoculated leaves. At the 4th day, the entire seedling was placed in the box covered with black vinyl film. In the treatment of lighting, the inside of the covered box was illuminated continuously by an electrib bulb of 100 or 200 watts. The investigation for the appearance of top leaf necrosis was done on the 13th day after the inoculation.

TABLE 7. *Shading of Whole Seedling and Appearance of Top Leaf of Stem Necrosis*

Exp.	Treatment	Developing leaves*	Position of inoculated leaf	Numbers of seedling showing stem necrosis	Numbers of seedling showing top leaf necrisis
I	Lighting (100 W)	5	L-2,3	17/20**	9/20**
	Shading	5	L-2,3	11/18	0/18
II	Lighting (200 W)	6	L-3,4	18/20	17/20
	Shading	6	L-3,4	5/18	0/18

* At the inoculated time

** Numbers of inoculated seedling

The results are shown in Table 7. Every treated seedling grew abnormally for the deficiency of light and the length of internode became longer than the control. In the case of shading, top leaf necrosis was not observed, but necrotic symptom of the stem occurred only in few seedlings. However the necrotic symptom did not occur evenly in all parts of the stem, but the occurence of the symptom decreased

radually in the direction of the top part and further the virus activity could not ıe observed in the top part. On the contrary, under the illumination of 100 watt lectrib bulb, many seedlings showed clear necrotic symptom in the stem, but top eaf necrosis was not observed so often in the top leaves (L–7, 8) which were ındeveloped at the inoculation. But the virus activity was investigated distinct-y in the top leaf which did not showed top leaf necrosis. Further symptomless nfected seedlings were found (three in twenty seedlings). When seedlings were lluminated with a 200 watt bulb, the appearance of stem necrosis and top leaf ıecrosis was remarkable.

Discussion

Air temperature remarkably influenced the appearance of the symptom of ɔroad bean by alfalfa mosaic virus. These results consist with known papers. Under temperatures as low as 17°C, the rate of appearance of top leaf necrosis was less than that of high temperatures as 24°C. Also a longer period was necessary to display top leaf necrosis. Cheo and Pound (1) reported that the symptom of spinach by cucumber virus 1 was influenced by air temperature, and the appearance of the symptom of susceptible varieties delayed and resistant varieties did not show the symptom. They concluded that the reason was the multiplication of the virus being lowered by the low air temperature.

As well as our previous report (6), also in this experiment the multiplication of AMV was inhibited by an air temperature of 17 C. Also the necrotic symptom of inoculated leaves was scarce. It is assumed that the formation of top leaf necrosis is related with the virus content of the inoculated leaves.

Pound and Bancroft (7), and Takahashi (8) reported that the virusm ultiplicated more vigorously in light conditions rather than in dark conditions. When broad bean leaves inoculated with AMV were covered with alumi-foil or green cellophane paper for the purpose of shading, the multiplication of AMV was inhibited in the inoculated leaf. Zaitlin and Jagendorf (10), and Goffeau and Bové (3) explained that the decreased multiplication of the virus by shading was caused by lowering of ATP production by photosynthesis. But whether or not their explanation is reasonable, is unknown.

In order to display top leaf necrosis, AMV must be transported from the inoculated leaf to apical leaves as a matter of course. However when the inoculated leaves were shaded or the entire inoculated broad bean was placed under dark conditions, top leaf necrosis never occurred. Further, when the entire plant is shaded, necrosis appeared only on the part of the stem near the inoculated leaf, but was not found at the upper part of stem and also the virus activity was not detected there. These results indicate that the transportation of AMV is prevented and the multiplication of AMV in the new infected tissue is inhibited, by shading or dark conditions.

Under environmental conditions which are unbeneficial for plant growth as weak light or low air temperature, the multiplication of AMV is inhibited indirectly and the transportation of metabolic substances changes. Thus the movement of AMV is inhibited and top leaf necrosis seems to be prevented.

References

1) Cheo, P.C. and Pound, G. Phytopath. **42**, 306. (1952)
2) Coast, E.M. and Chant, S.R. Ann. appl. Biol. **65**, 403. (1970)
3) Goffeau, A. and Bové, J.M. Virology **27**, 243. (1965)
4) Helms, K. and McIntyre, G.A. Virology **31**, 191 (1967)
5) Matthews, R.E.F. Ann. appl. Biol. **40**, 556. (1953)
6) Misawa, T. and Kudo, A. Virus (in press). (1971)
7) Pound, G.S. and Bancroft, J. Virology **2**, 44. (1956)
8) Takahashi, W. Am. J. Botany **34**, 496. (1947)
9) Yarwood, C.E. Phytopath. **46**, 32. (1956)
10) Zaitlin, M. and Jagendorf, A.T. Virology **12**, 477. (1960)