

Studies on the Aphid Transmission of Some Cruciferous Viruses

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十字花科植物 바이러스의 진딧물 媒介에 關한 研究

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ABSTRACT

This is the first report on detailed aphid transmission studies of cruciferous virus in Korea, and experiments aimed to get basic informations for control of vectors.

Aphid transmission of turnip mosaic virus prevalent on radish in the field was studied.

Results obtained were as follows :

1. *Myzus persicae*, *Lipaphis erysimi*, *Aphis gossypii* and *Aphis craccivora* were found to transmit turnip mosaic virus.
2. The proper time for turnip mosaic virus transmission by *Myzus persicae* was 1 hour of fasting, 3 minutes for acquisition, and 1 minute for inoculation: *Lipaphis erysimi* was 2 hours for fasting, 5 minutes for acquisition, and 3 minutes for inoculation: while *Aphis gossypii* needed 1 hour for fasting, and 3 minutes for each of the acquisition and inoculation periods.
3. There was no great difference in probing patterns between nonfasted and fasted aphids for 2 hours. All the fasted aphids began feeding after 4 minutes.
4. When *Myzus persicae* were transferred artificially at 1-2 minute intervals, the number of probes with aphids fasted for 2 hours was much greater than that of nonfasted aphids. Aphids fasted for 2 hours mainly transmitted the virus before 4 minutes, with an acquisition feeding period of less than 3 minutes

INTRODUCTION

Several virus diseases of cruciferous crops have world-wide distribution, and virus disease of cruciferous crops such as radish and chinese cabbage are serious in Korea. Since Schultz¹⁾ reported virus diseases occurring on

chinese cabbage, mustard, and turnip in the U.S.A., many reports on cruciferous viruses had been published throughout the world.

In Korea, Paik⁸⁾ and Kwack⁷⁾ reviewed foreign literature concerning the reported cruciferous viruses. Park et al.¹⁰⁾ and Ko and Kim⁶⁾ classified turnip mosaic virus and cauliflower mosaic virus-like on diseased

cruciferous crops by means of symptoms and infection of indicator plants.

Aphids are vectors of turnip and cauliflower mosaic virus. Kennedy et al.⁵⁾ reported 40 species of aphids as vectors of turnip mosaic virus, and Park⁹⁾ reported 8 species of aphids were capable of transmitting turnip mosaic virus in Korea.

Sylvester¹³⁾ reported all instars of *Myzus persicae* could transmit turnip mosaic virus. Aphids fasted for 1-2 hours acquired the virus in less than 1 minute. Aphids could transmit turnip mosaic virus for 60 minutes after acquisition, and there was no latent transmissibility period remaining for up to 4 hours after acquisition.

Kasai⁴⁾ reported the acquisition and inoculation periods were less than 5 minutes, and the aphids could transmit the virus for 3 hours after acquisition.

In the Korean literature there have been scarce reports of the insect transmission of cruciferous viruses, with the exception of potato^{1,2)} and carnation viuses³⁾.

MATERIALS AND METHODS

The source of turnip mosaic virus were isolated from infected radish collected in the fields, and stock cultures of turnip mosaic virus obtained from Plant Pathology Department, Institute of Agricultural Sciences.

Indicator plants used were: *Chenopodium amaranticolor*, *Chrysanthemum coronarium*, *Nicotiana tabacum* var. Bright Yellow, *Raphanus sativus* and *Brassica rapa*. The physical properties of turnip mosaic virus were examined from the symptoms on *Chenopodium amaranticolor*.

Ten species of aphids were used: *Myzus persicae*, *Lipaphis erysimi*, *Aphis gossypii*, *Aphis craccivora*, *Aulacothum solani*, *Aphis rumicis*, *Macrosiphum euphorbiae*, *Macrosiphum avenae*, *Aphis spiraeicola*, and *Macrosiphoniella sanborni*.

Aphids collected in the fields were kept on healthy radish or other hosts, and tested as 3rd-4th instar apterae.

Aphids were kept in plastic or glass containers with lids and fasted for 2 hours in a cool place ($15 \pm 5^\circ\text{C}$) and then allowed to feed for less than 3 minutes on infected radish leaves. One aphid per plant was fed on

the Simudaigeun radish variety at 1-2 leaf stage for 1 day. They were killed with insecticides removing the screen cover. Aphids were transferred with No. 1 and 2 water-color paint brushes, and symptoms were checked after one week.

Effective number of aphids

Myzus persicae, *Lipaphis erysimi*, *Aphis gossypii* and *Aphis craccivora* were used, with ten replications for each of 1, 3, 5, and 7 individuals fed on a test plant. The other methods were the same as described before.

Effect of fasting

Myzus persicae, *Lipaphis erysimi*, and *Aphis gossypii* were used. Aphids were fasted for 0, 0.5, 1, 2, 4, and 8 hours, and inoculated on radish with up to 12 replications. The other methods were the same as described before.

Acquisition and inoculation feeding period

Myzus persicae, *Lipaphis erysimi*, and *Aphis gossypii* were employed and tested for periods of 1, 3, 5, 10, 30, and 60 minutes using up to 12 replications. Acquisition and inoculation feeding periods were recorded by stop-watch. To test acquisition feeding periods, aphids were fasted for 2 hours and fed on the host plants for 1 day. To test inoculation feeding periods, they had been fasted for 2 hours and fed on the infected plant for the periods of less than 3 minutes to acquire the virus.

Probing, feeding and infectivity of aphids

Myzus persicae was used in these tests. Nonfasted aphids were reared on the turnip mosaic virus infected radish, and fasted for 2 hours were treated according to the methods described before. Nonfasted aphids for 2 hours were released on the healthy plants and examined the number of probes, feeding patterns and transmissibility. The aphids were transferred one at two minute intervals. The other methods were the same as described before.

RESULTS

Symptoms of turnip mosaic virus on several indicator plants are as follows: local lesion on *Nicotiana tabacum* var. Bright Yellow and *Chenopodium amaranticolor*. Mosaic symptom on *Chrysanthemum coronarium* and *Raphanus sativus*.

The physical properties of turnip mosaic virus v

as follows: thermal inactivation point was 55°C, dilution end point was $1 \cdot 10^4$ and longevity in vitro was 3 days.

The symptom of turnip mosaic virus inoculated mechanically to eleven radish varieties was generally a yellowish-green mosaic, ranging from insignificant to distinct and clear symptoms. Simudaigeun showed clearer symptoms than others with a higher susceptible reaction

Confirmation as vectors

The transmissibility of turnip mosaic virus is shown in Table 1. Four species of aphids: *Myzus persicae*, *Lipaphis erysimi*, *Aphis gossypii*, and *Aphis craccivora* were found as vectors of turnip mosaic virus among the ten species tested. *Myzus persicae* showed 80% infectivity, *Lipaphis erysimi* showed 50%, *Aphis gossypii* showed 20% and *Aphis craccivora* showed 10%. As a result, different infectivity levels were found depending on aphid species.

Table 1. Aphid transmission of turnip mosaic virus on radish.

Aphid species	Infection rate	Number of inoculated plant
<i>Myzus persicae</i>	82%	28
<i>Lipaphis erysimi</i>	50	42
<i>Aphis craccivora</i>	33	36
<i>Aphis gossypii</i>	20	30
<i>Uroleiscus solani</i>	0	23
<i>Aphis rumicis</i>	0	20

<i>Macrosiphum euphorbiae</i>	0	16
<i>Macrosiphum avenae</i>	0	22
<i>Aphis spiraeicola</i>	0	21
<i>Macrosiphoniella sanborni</i>	0	19

Effective number of aphids

The effects of the different number of aphids on transmission of turnip mosaic virus are shown in Table 2.

In polts with single *Myzus persicae*, *Lipaphis erysimi*, *Aphis gossypii*, and *Aphis craccivora* showed as high a rate of transmission as where 5 and 7 aphids were introduced. Although the higher infectivity was generally related to number of individuals, there was no significant difference in virus transmission in relation to actual number of aphids.

Table 2. Virus transmission in relation to number of aphid

Aphid species	Number of aphid			
	1	3	5	7
<i>Myzus persicae</i>	80(10)	75 (8)	100(6)	75(4)
<i>Lipaphis erysimi</i>	47(15)	50(12)	42(12)	60(5)
<i>Aphis gossypii</i>	17(12)	22 (9)	17(6)	33(3)
<i>Aphis craccivora</i>	25(12)	33 (9)	44(9)	33(6)

the figure; percentage of infectivity figure in the parenthesis; inoculated plants

Effects of fasting

Figure 1 shows the data on the effects of the fasting

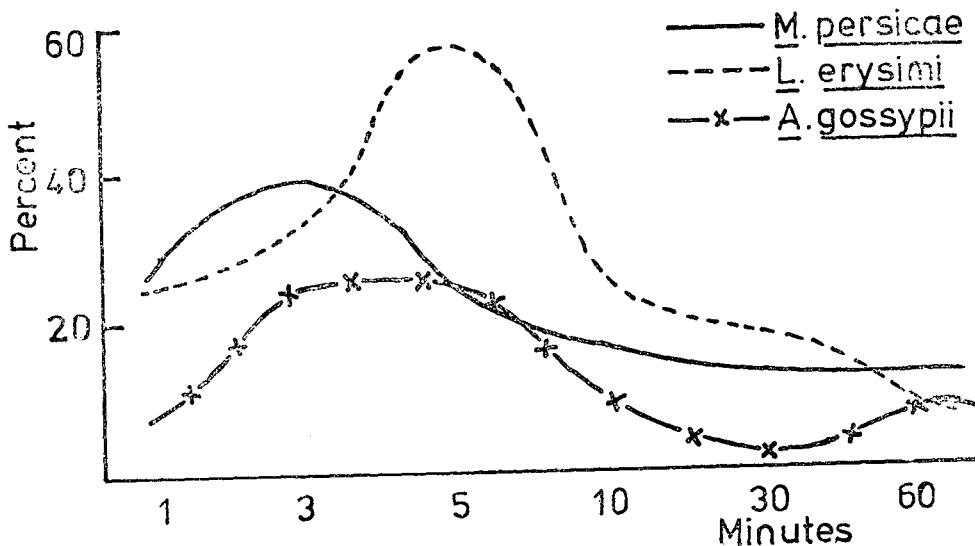


Figure 1 Effect of fasting on the transmission of the turnip mosaic virus

period on the aphids on transmitting turnip mosaic virus.

With *Myzus persicae* there were maximum effects at 1 hour of fasting to 2 hours. *Lipaphis erysimi* showed maximum effects of fasting at 2 hours, and *Aphis gossypii* showed the maximum effects of fasting at 1 hour, which decreased gradually and was highest at 4 hours.

Acquisition feeding period

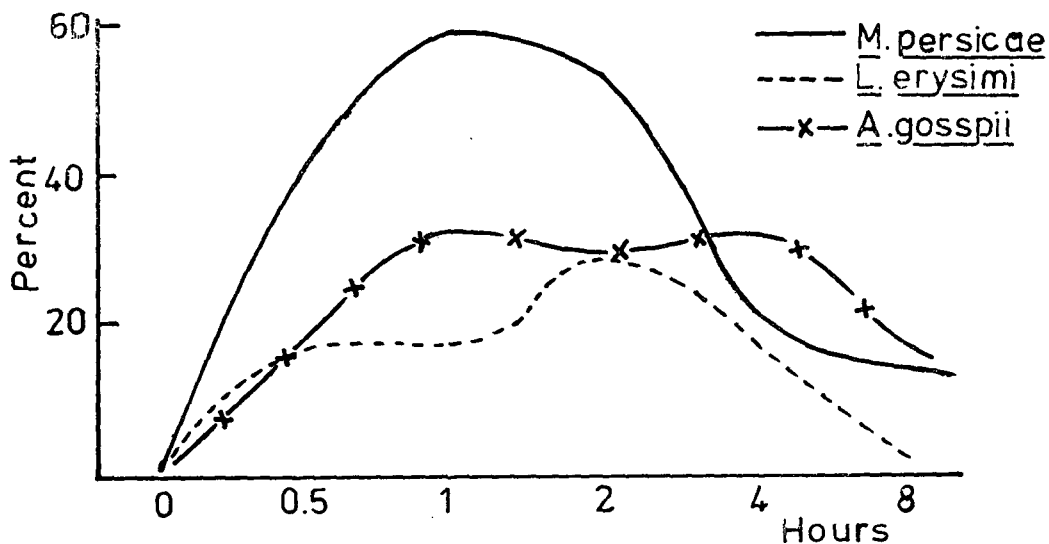


Figure 2 Effective acquisition feeding period for turnip mosaic virus

Inoculation feeding period

Figure 3 shows the most effective inoculation feeding period of *Myzus persicae*, *Lipaphis erysimi*, and *Aphis gossypii*.

Figure 2 present data on the acquisition feeding period of *Myzus persicae*, *Lipaphis erysimi*, and *Aphis gossypii*.

The maximum acquisition of the virus was obtained with 3 minute feeding periods for *Myzus persicae*, and 5 minutes for *Lipaphis erysimi*. with *Aphis gossypii*, the peak was produced in acquisition feeding period in from 3 to 5 minutes.

with *Aphis gossypii* and *Lipaphis erysimi* the rate of transmission reached a maximum at 3 minutes. It gradually decreased with time and continued, while *Myzus persicae* showed a peak at 1 minute, with 1

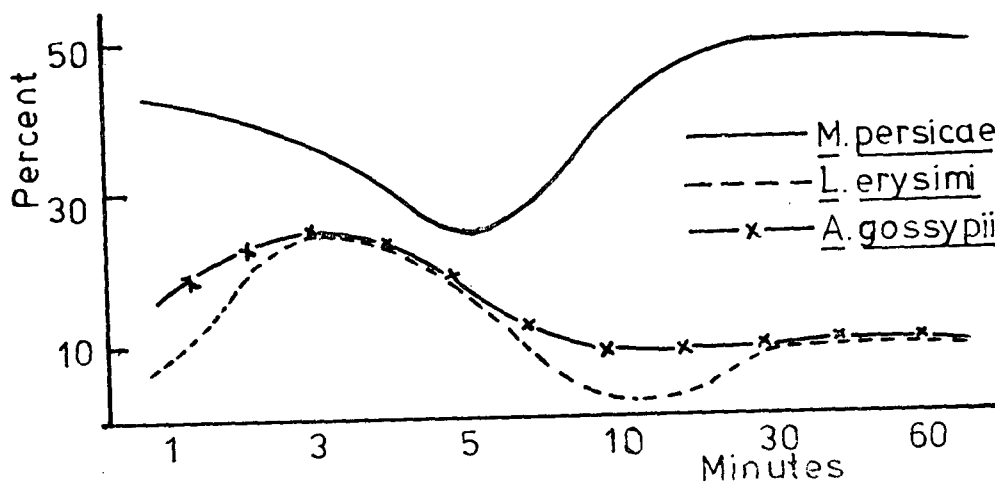


Figure 3 Effective inoculation feeding period for turnip mosaic virus

same tendency of arriving to a maximum at an early stage. This decreased gradually with time and showed a peak again at 30 and 60 minutes.

Probing and feeding patterns

The probing and feeding patterns of *Myzus persicae* are shown in Table 3.

There were no differences in probing times between

nonfasted aphids and those fasted for 2 hours. However, the nonfasted aphids showed various types of feeding: one fed many times, the other a few times, and others fed not at all. The aphids fasted for 2 hours showed a certain pattern of feeding: all fed a long time, and there was probing before feeding.

Table 3. Probing and feeding patterns of the nonfasted and fasted for 2 hours aphids

Time(min)	1	2	3	4	5	6	7	8	9	10
aphid nonfasted		v		v	v.....				v.....	
	vv	vv.....			v.....			v.....		
	vvv	vv.....								
					v					
			v.....			v		v.....		
			v.....							
aphid fasted for 2 hours	v	v.....								
	vv	v.....								
	v	vv	v	v.....						
	vvv	v.....								

v; one time probing

Different phases of aphid transmission

Table 4 presents the differences in aphid transmission of turnip mosaic virus between nonfasted and 2 hours fasted *Myzus persicae*.

When *Myzus persicae* were transferred artificially at 2 minute intervals, some differences were observed.

The number of probes for aphids fasted for 2 hours was slightly greater than that of nonfasted aphids, and the virus infectivity of fasted aphids was much higher. Aphid transmission of turnip mosaic virus was mainly performed before 4 minutes.

Table 4. Number of probes and infected plants

Time(min)	1	2	3	4	5	6	7	8	9	10	12	14	16	18	20
aphid nonfasted	-	+	⊕	-	+	-	+	-	-	+					
	-	-	+	⊕	-	-	-	+	+	-					
	-	+	+	-	-	+	-	+	+	-					
	-	+	⊕	-	+	-	+	+	-	-					
	0	3	5(2)	1(1)	3	1	3	4	2	1	23(3)				
aphid fasted	⊕	+	-	+	+	+	+	-	+	+					
2 hours	-	+	⊕	-	+	-	-	+	+	⊕					
trans	⊕	-	+	⊕	+	-	-	+	⊕	+					
	-	⊕	+	-	-	+	⊕	+	-	+					
	⊕	⊕	-	+	+	⊕	-	-	-	+					
	3(3)	4(2)	3(1)	3(1)	4	3(1)	2(1)	3	3(1)	5(1)	33(11)				

aphid	+	+	-	+	+-	-	⊕	-	+	+
nonfast	-	-	+	+	-	+	+	+	-	-
	--	+	⊕	-	-	-	+	-	+	+
	+	-	-	-	+	+	+	-	+	-
	-	-	+	+	-	+	+	-	-	+
	2	2	3(1)	3	3	3	5(1)	1	3	3 28(2)
aphid	-	⊕	-	+	+	+	⊕	-	+	+
fasted	⊕	⊕	+	+	-	+	-	+	+	+
for 2	-	+	+	+	-	+	-	+	+	+
hours	⊕	+	-	+	+	+	⊕	-	+	-
	⊕	⊕	+	-	+	+	-	+	-	+
	3(3)	5(3)	3	4	3	5	2(2)	3	4	4 38(8)

--; no probing
 +; one time probing
 ++; twice probing
 ⊕; infected plant

figure; number of probes
 figure in parenthesis; infected plants

DISCUSSION

Symptoms of turnip mosaic virus on indicator plant coincided well with the results of Tomlinson¹⁴. The varieties showing highest infectivity were Simudaigeun, Cheongsugungjung, Seouldanchu, and Junggugcheongpi. Simudaigeun was used as a test plant throughout this experiment, because it was found to be the most highly infected variety and showed the clearest symptoms.

Four species of aphids: *Myzus persicae*, *Lipaphis erysimi*, *Aphis gossypii* and *Aphis craccivora*, were found to transmit turnip mosaic virus among the ten species of aphids tested for transmission; and infectivity rates varied. The differences in infectivity rate may be attributed to the fine structure of the outer surface of the stylets, as suggested by van der Want¹⁶. Van Hoof¹⁵ supported Want's suggestion by examining the differential structure of aphid stylets with the electron microscope. In addition, there are possible differences in probing and feeding sites of aphid species, according to host plants. *Aulacorthum solani* and *Macrosiphoniella sanborni*, both of which have been reported as vectors of turnip mosaic virus by Kennedy et al.⁵ and Paik⁹ could not transmit the virus in this experiment.

In relation to fasting time, *Aphis gossypii* showed maximum infectivity at 1 and 4 hours of fasting. With *Myzus persicae* and *Lipaphis erysimi* this was at 1 and 2 hours of fasting. The bimodal or double peak in effect of fasting time on *Aphis gossypii* could be interpreted as a continuous level of infection capability

ranging from one to four hours. The reason for decreased infectivity at 2 hours of fasting was in part due to differences in small replications. Severin and Tompkins¹² and Kasai⁴ also reported maximum infectivity with aphids fasted for 2-3 hours.

The maximum infectivity for the acquisition feeding time of *Lipaphis erysimi* was 5 minutes, 3 minutes for *Myzus persicae*, and 3 and 5 minutes for *Aphis gossypii*. Kasai⁴ used a 5 minute period in his test and Sylvester¹³ and others¹⁴ found 1 minute was sufficient. The time in this test was not actual acquisition feeding period, but involved the lapse time in the period on infected plants. Considering the duration of probing and feeding period in these experiments, results were the same as those obtained by other investigators.

Virus transmission of *Aphis gossypii* and *Lipaphis erysimi* in relation to inoculation feeding period reached a maximum at 3 minutes and gradually decreased with time and continued. *Myzus persicae* showed a peak at 1 minute, with the same tendency for gradual decrease with time, and a resurgence of the peak at 30 and 60 minutes. This result indicated 3 minutes is sufficient for inoculation, although the reason which *Myzus persicae* showed maximum peaks at 30 and 60 minutes might be due to unknown factors or experimental error. The consequence after 1 hour in infectivity did not occur in this experiment, but Sylvester¹³ and others^{4,18} reported infectivity could persist up to 4 hours. The reason 3 minute is a maximum inoculation time and infectivity decreases gradually afterwards, may

e to aphid stylet penetration through epidermal cells which the stylet-borne virus particles concentrated in a plant. As for the acquisition feeding periods, Kasai¹³⁾ reported a time of less than 5 minutes and Sylvester¹³⁾ and Tomlinson¹⁴⁾ reported 1 minute was sufficient for inoculation.

No differences in the number of the probes was found between fasted and nonfasted aphids. But aphids forced fast for 2 hours started to feed within 4 minutes, while nonfasted aphids tended to feed intermittently. The activity of turnip mosaic virus was the highest for fasted aphids. It was also found that the number of probes of fasted aphids could be increased by transferring artificially at 1 and 2 minute intervals. The fasted aphids supposedly transfer the virus to other plants during the early period of probing because of the high activity of plants on which fasted aphids probed freely. The reason for aphid transmission of the virus during early stages is supposed to be due to the form, which is more stable of introduced virus. Van Hoof¹⁵⁾ and the aphid transmissible stylet-borne viruses were of viral nucleic acid type, rather than protein-coated virus particles.

In summary, the effect of fasting was recognized in aphid transmission of stylet-borne viruses by Tomlinson¹⁷⁾. He attributed this to "preliminary fasting" which caused physiological changes within aphids. Research on this subject has been conducted by many workers, and Watson's idea is still considered valid. For the case of acquisition and inoculation feeding period, those of less than a few minutes are more effective than longer periods. These results coincided with the evidence to date. The reason is believed to be due to aphid stylet penetration through the epidermal cells in which stylet-borne viruses are concentrated. This also perhaps explains, in part, why allowing aphids to feed for prolonged periods does not increase virus infection. There are still questions regarding the transmission of stylet-borne viruses are related to the complexity of aphid behaviour and other factors. Additional research is required for a more full understanding of the mechanism on virus transmission by aphids.

摘 要

외의 무우에서 많이 발생하고 있는 turnip mosaic 바이러스에 대한 성질과 이의 虫媒傳染에 대하여 몇 가지

실험 결과의 다음과 같은 결론을 얻었다.

1) 採集된 TuMV의 物理的性質은 假令의 1시간 단속을 하였을 때 2시간 단속의 採集량이 가장 높았다.

2) TuMV의 媒介昆蟲은 *Myzus persicae*, *Lipaphis erysimi*, *Aphis gossypii*, *Aphis craccivora*의 4種이었다.

3) TuMV의 진딧물에 의한 傳染에서 가장 適當한 處理時間은 *Myzus persicae*는 1時間 絶食, 3分 吸汁, 1分 接種이었고, *Lipaphis erysimi*는 2時間 絶食, 5分 吸汁, 3分 接種이었고, *Aphis gossypii*는 1時間 絶食 3分 吸汁, 3分 接種이었다.

4) 진딧물의 Probing 횟수는 2時間 絶食시킨 진딧물과 絶食시키지 않는 것에 큰 差異가 없었고, 絶食시킨 진딧물의 全媒體가 4分 經過時부터 攝食하기 始作하였다.

5) 복충아 혹은 진딧물을 1~2分 間隔으로 人爲的으로 옮겨주었을 때, 2時間 絶食시킨 진딧물의 Probing 횟수가 絶食시키지 않은 것의 Probing 횟수보다 훨씬 많았고, 2時間 絶食시킨 진딧물이 주로 3分以内의 獲得吸汁時間後 4分以内에 Virus를 媒介하였다.

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