

## **Influence of the time of inoculation of horse bean by the broad bean true mosaic virus (*Vicia virus varians* Quantz) on yield and seed transmission of the virus**

W. BŁASZCZAK

*Department of Phytopathology Agricultural University, Poznań, Poland*

### INTRODUCTION

A relatively large number of virus diseases has been found on leguminous plants on species belonging both to the large seed as well as small seed groups.

Harmfulness of those diseases varies and its extent is conditioned by interaction of three elements susceptibility of the host, virulence of the causal organism and environment. In case the mentioned factors are advantageous to the host losses are rather limited but when advantageous to the disease factor then the course of disease is heavy, plants frequently die, thus, losses prove to be very large. In many species of leguminous plants losses caused by defined viruses have not been determined as yet. Horse bean can among others be included into these species. The present plant protection very often requires a clear cut determination of the threshold value of harmfulness, i. e. of the intensity of the disease itself or disease factor at which measures of control are worth to be undertaken.

Presence of the broad bean true mosaic virus on horse bean in Poland has been stated in the year 1965 [2] and because of its transmittance through seeds investigation have been undertaken in order to determine its influence on the yield and degree of virus infection of seeds in dependence on the developmental stage at which the plant was inoculated.

### MATERIAL AND METHODS

The horse bean "Nadwiślański" and the true mosaic virus of horse bean (BBTMV) isolated from horse bean and identified by the author [2] constituted the experimental material. The virus was kept on horse bean in a greenhouse free of insects. In the year 1966 the experiment was conducted in the garden of the Plant Pathology Department, and in 1967 in the field of the Agricultural Experimental Farm Złotniki. Seeds of horse bean variety "Nadwiślański" treated with preparation "T" (150 gm/100 kg of seeds), were sown in rows at the depth of 5 cm, spaced 30-60-30-30-60-30 cm in 1966 (in the year 1967 spacing was increased from 60 to 70 cm). Fertilizers were applied before sowing at the rate of 38 kg of N, 60 kg K<sub>2</sub>O, and

39 kg P<sub>2</sub>O<sub>5</sub>/ha in form of ammonium nitrate, 40% of potash and 26% of silicophosphate. In 1966 horse bean was sown on one plot, and in 1967 on four plots applying space isolation of 10 m. In both years horse bean plants were inoculated at three stages of growth:

1st — plants with 2-3 pairs of leaves (17 V 66; 12 V 67),

2nd — plants in flowering stage (15 VI 66; 8 VI 67),

3rd — pod setting (1-3 cm in length) (5 VII 66; 27 VI 67).

The inoculum was prepared from horse bean plants and pea plants infected with the true mosaic virus. Test-plants were dusted with carborundum before inoculation and washed with water after it. Inoculations were always repeated after three days. Inoculated plants were labelled. In order to stop spreading of the disease from diseased plants onto healthy ones or plants inoculated at a later time the horse bean was sprayed three times with 0.1% Metasystox at 14 days intervals starting from the middle of May. In full summer only healthy plants without any disease symptoms, were labelled at random, theirs state of health being controlled periodically up to the harvest time and plants with symptoms of infection were eliminated. After maturing, plants were harvested by hand and dried. During the fall — winter period a biometrical elaboration of plants was carried out individually. Virus infection of seeds was determined, by means of sowing seeds of particular plants separately in the greenhouse, or in the greenhouse and garden, and by determining the number of virus infected plants on the basis of pathological symptoms. In dubious cases biological tests were made on horse bean and pea. In the experiment from 1967 germination ability and the health state of horse bean seeds were tested. Seeds were germinated on filter paper [4].

#### WEATHER

The first three months of the vegetative period of 1966 were warm (Table 1). The mean monthly temperatures being higher by 0.7 to 1.7°C. During the remaining

Table 1  
Mean air temperatures and rainfalls in vegetation period of 1967 (according to Institute of Meteorology, College of Agriculture at Poznań)

Month	1966 mean temperatures in °C*	Deviations		Rainfalls total in mm*	Percentage of normal value	
		1966	1967		1966	1967
IV	7.8	+0.7	0.0	36	83.9	69.7
V	13.1	+1.0	+1.2	46	68.5	193.0
VI	17.1	+1.7	-0.7	57	169.8	222.1
VII	18.6	-0.2	+1.4	70	184.6	186.4
VIII	17.6	+0.1	-0.2	62	112.9	147.1
IX	13.7	-0.5	+2.2	43	36.3	120.7

\* Mean values for the years 1922-1946.

months the mean temperatures were similar to those of many-years. In 1967 higher month temperatures were recorded only in May and July. However, in both years during June, July and August, and in 1967 in May too, the amount of rainfalls largely exceeded the mean value of many-years. It seems, that the good growth and development of horse bean was due to these high rainfalls. Plants remained green for a long period of time which could be advantageous for spreading of virus diseases on plantations as well as for virus seed infection.

## RESULTS

Due to quantitative differences in the considered experimental material, in both these years, as well as due to different ecological conditions, the results obtained will be considered separately for each year but in a comparative way.

### INFLUENCE OF TIME OF INOCULATION ON THE GROWTH OF HORSE BEAN

In 1966, inoculation with BBTMV horse bean plants at three different developmental stages influenced the growth of plants in a relatively mild way (Table 2). Inoculation at an earlier stage inhibited the growth of plants and, thus, reduced their weight. Differences, however, in growth as well as weight of the horse bean plants affected by the disease at various stages were not very big. Plants infected with BBTMV tended to produce a greater number of shoots.

Table 2

Effect of the time of inoculation of horse bean "Nadwiślański" by the broad bean true mosaic virus on the growth of plants. Agr. Exp. St. Poznań—Sołacz 1966

Developmental phases of plants at the time of inoculation	Number of examined plants	Mean height of plants		Mean weight of plants		Mean number of shoots per plant	
		cm	%	g	%	absol.	%
I seedlings	105	106.7	86.2	34.8	77.8	1.88	135.2
II flowering	96	106.8	86.3	41.5	92.8	2.36	169.8
III pod-setting	107	116.1	93.8	47.0	105.1	1.87	134.5
Healthy plants	97	123.8	100.0	44.7	100.0	1.39	100.0

Bigger differences were noticed in the year 1967 (Table 3). Plants grew larger, the greatest growth inhibition and in consequence a drop of dry weight was observed in plants inoculated earliest. The noxious influence of the disease of plants inoculated at the two later stages was relatively small and very similar in both cases. Plants inoculated at the earliest date showed also a tendency to produce a larger number of stems non-bearing pods.

Table 3

Effect of the time of inoculation of horse bean "Nadwiślański" by the broad bean true mosaic virus on the growth of plants. Agr. Exp. St. Złotniki 1967

Developmental phases of plants at the time of inoculation	Number of examined plants	Mean height of plants		Mean weight of plants		Number of shoots non setting pods per plant	
		cm	%	g	%	absol.	%
I seedlings	492	103.6	75.7	28.2	54.7	0.23	766.6
II flowering	341	128.4	93.8	45.7	88.7	0.07	233.3
III pod-setting	209	134.0	97.9	45.2	87.8	0.07	233.3
Healthy plants	450	136.8	100.0	51.5	100.0	0.03	100.0

#### INFLUENCE OF THE TIME OF INOCULATION ON THE HORSE BEAN YIELD

The true mosaic virus of the horse bean which inhibited the plant growth caused in consequence, also a drop of the seed yield consisting of the total number of pods (one or *n*-seeded) on a plant. Plants infected with BBTMV at seedling stage and during flowering stage, gave in the year 1966 the lowest number of 4 seeded pods (Fig. 1). The number of these pods on plants inoculated at the third stage was much higher. Of course, the largest number of 4 seeded pods was produced by healthy plants. The number of 3 seeded pods increased as well with the delay of the time of inoculation.

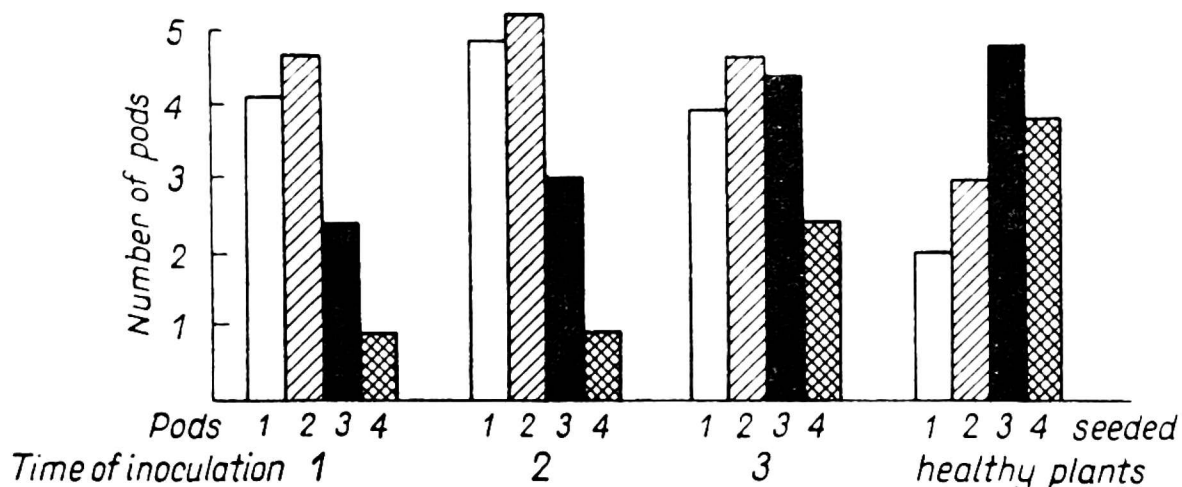


Fig. 1. The effect of the time of inoculation of horse bean "Nadwiślański" by the broad bean true mosaic virus on the number of 1-4 seeded pods per plant. Agr. Exp. st. Poznań—Sołacz 1966.

Similar relationships appeared also during the year 1967 (Table 4). Plants inoculated during the seedling stage gave the largest number of 1 and 2 seeded pods. Plants inoculated at the 2nd term doubled the number of pods with one two seeds, and the number of 3 seeded pods increased considerably as well. On plants inoculated at the stage of pod setting (3rd term) the number of pods with 3 and 4 seeds increased, whereas the number of pods with 1 or 2 seeds decreased. And, just as

in the previous year, the largest number of pods with 3 or 4-seeds was found on healthy plants.

Table 4

Influence of the time of inoculation of horse bean "Nadwiślański" by the broad bean true mosaic virus on the number of pods on main stem. Agr. Exp. St. Złotniki 1967

Developmental phases of plants at the time of inoculation	Mean number of pods per plant						Total
	0	1	2	3	4	5	
	seeded						
I seedlings	1.7	2.9	2.9	1.7	0.6	0.02	9.82
II flowering	3.8	5.9	6.1	3.3	0.8	0.02	19.92
III pod-setting	4.1	2.7	3.0	3.6	2.7	0.29	16.39
Healthy plants	3.2	1.8	2.9	4.4	3.1	0.27	15.67

Table 5

Influence of the time of inoculation of horse bean "Nadwiślański" by the broad bean true mosaic virus on seed yield. Agr. Exp. St. Poznań—Sołacz 1966

Developmental phases of plants at the time of inoculation	Mean number of seeds per plant		Mean weight of seeds per plant		Decrease of seed yield in %
	absol.	%	g	%	
I seedling	24.0	62.9	13.3	69.6	30.4
II flowering	28.0	73.5	13.7	71.7	28.3
III pod-setting	36.6	96.1	18.5	96.8	3.2
Healthy plants	38.1	100.0	19.1	100.0	—

A moderate influence of the disease on the yield of horse bean seeds was observed in the year of 1966 (Table 5). The harmful effect of the BBTMV was the greater the earlier plants were inoculated. The decrease in yield of seeds amounted to 30.4% when plants were inoculated at the stage of 2-3 pairs of leaves. The harmful influence of the disease during the year 1967 was much greater as far as the number of seeds and seed yield is concerned (Table 6). When horse bean plants were ino

Table 6

Influence of time of inoculation of horse bean "Nadwiślański" by the broad bean true mosaic virus on seed yield. Agr. Exp. St. Złotniki 1967

Developmental phases of plants at the time of inoculation	Mean number of seeds per plant		Mean weight of seeds per plant		Decrease of seed yield in %
	absol.	%	g	%	
I seedlings	19.3	47.3	8.1	40.9	59.1
II flowering	34.6	84.8	15.4	77.9	22.1
III pod-setting	36.0	88.2	13.8	69.7	30.3
Healthy plants	40.8	100.0	19.8	100.0	—

culated at the earliest stage the number of seeds decreased by about 53% and the weight of seeds almost by 60%. Infection of plants at later developmental stages appeared to be detrimental to a much lower extent.

INFLUENCE OF TIME OF INOCULATION OF HORSE BEAN BY BBTMV  
ON VIRUS SEED TRANSMISSION

The stage of plants at which inoculation took place strongly influenced the infection of seeds by the virus. Seeds originating from horse bean plants inoculated at the 1st date in 1966, were infected with the virus in 2.51% (Table 7); at the 2nd date of inoculation the percentage of virus infected seeds was equal to 3.13 and at the 3rd to 1.04%. Virus infected seeds were also found in the group of seeds harvested from symptomless plants at the final stage of their vegetative period. Many plants infected with the BBTMV, with clearly symptoms of the disease, yielded healthy seeds, and number of virus diseased plants giving virus infected seeds decreased with the delay of inoculation. The number of virus infected seeds on a plant ranged from 1.29 to 17.24%.

Table 7

Transmission of the broad bean true mosaic virus by seeds of horse bean "Nadwiślański" depending on the time of plant inoculation. Agr. Exp. St. Poznań—Sołacz 1966

Developmental phases of plants at the time of inoculation	Number of seeds sown	Percentage of virus infected seedlings	Number of examined plants		Range of virus infected seeds found per plant (%)
			with virus infected seeds	total	
I seedlings	1524	2.51	26	58	1.76-12.28
II flowering	1563	3.13	21	52	1.47-17.24
III pod-setting	1592	1.04	10	43	2.66-10.00
Healthy plants	1039	0.39	4	28	1.29-5.26

Table 8

Transmission of the broad bean true mosaic virus by seeds of horse bean "Nadwiślański" depending on the time of plant inoculation. Agr. Exp. St. Złotniki 1967

Developmental phases of plants at the time of inoculation	Place of testing	Number of seeds sown	Percentage of virus infected seedlings	Number of examined plants		Percentage of infected plants giving virus infected seeds
				total	with virus infected seeds	
I seedlings	greenhouse	432	2.08	26	6	30.2
	garden	1226	2.36	60	20	
II flowering	greenhouse	468	3.20	12	7	44.8
	garden	1265	3.32	46	19	
III pod-setting	greenhouse	476	0.63	10	1	9.8
	garden	1168	0.43	41	4	
Healthy plants	greenhouse	490	0.00	16	0	5.2
	garden	1311	0.30	42	3	

Virus infection of horse bean seeds harvested in the year 1967 did not show much difference, the results of greenhouse evaluation being very similar to those obtained with seeds sown out in the garden (Table 8). A greater virus infection was recorded only in the group of seeds harvested from plants inoculated at the 3rd date. About 90% of diseased plants inoculated at the 3rd stage gave healthy seeds. It should also be stressed that in that year infection of seeds with a virus, originating from plants inoculated at the 2nd developmental stage, was greater than of seeds collected from plants inoculated at the earliest stage.

INFLUENCE OF THE TIME OF INOCULATION OF HORSE BEAN BY BBTMV ON THE SEEDS QUALITY

In the experiment of 1967 the weight of 1000 seeds of horse bean, the energy and germination ability were also determined according to the method applied in seed evaluation [4]. A quantitative determination of the percentage of seeds affected by fungi (Table 9) was also carried out.

Table 9

Influence of the time of inoculation of horse bean "Nadwiślański" by the broad bean true mosaic virus on germination capacity, fungal infection and on the weight of 1000 seeds. Agr. Exp. St. Złotniki 1967

Seeds from plants inoculated in phase	Weight of 1000 seeds (g)	Germination energy (%)	Germination capacity (%)	Percentage of seeds infected by fungi
I seedlings	398.5	33.0	79.0	21.0
II flowering	406.5	43.0	83.5	14.5
III pod-setting	367.2	49.5	87.0	13.5
Healthy plants	491.8	58.5	91.5	2.5

In general virus diseased plants — especially plants inoculated at the time of pod setting gave seeds of smaller size. Great differences were also found as far as energy and germinative ability were concerned. The noxious influence of the disease diminished with the delay of inoculation. A considerable percentage of seeds harvested from virus diseased plants proved to be infected by fungi. Among seeds collected from healthy plants only 2.5% of seeds were infected by fungi, but seeds collected from plants inoculated at the seedling stage were infected in 21%.

DISCUSSION

It results from the carried out investigations that BBTMV inhibits the growth of infected horse bean plants to a large extent; its harmfulness being the greater the earlier the plant was infected. The noxious effect of BBTMV was more intensive in the year 1967. when the plant growth decreased by 25% (inoculation at 1st stage),

and the weight from 12 to 45%. In another comparative experiment carried out in the same year, when horse bean plants were inoculated at the stage of 2 pairs of leaves, the BBTMV caused a decrease of growth and weight by 27% and 44% respectively [3]. As it is seen the results were very much alike.

A decrease of fresh weight of the plant results in a lower yield. It has been shown in the present paper infected horse bean plants produce less 4 and 3-seeded pods. As shown in Fig. 1.

It has been also stated in the above mentioned comparative experiment that due to plant infection by the BBTMV the number of 4 and 3-seeded pods decreased by 86 and 67%, respectively [3]. In England a decrease of pod number and a drop of the seed yield by 20%, in case of an early infection of plants, was also observed [6]. In our experiments the decrease of seed yield from a plant inoculated at the 1st stage amounted, in the year 1966, to 30%, and in the year 1967 to 59%. Plants inoculated at the 2nd stage reacted with a similar yield drop in both years; the reaction being, however, different when plants were inoculated at the 3rd stage (pod setting). A drop of seed yield by 59.4% [3] was also observed in the already mentioned experiment carried out in the same year. Quantz [5] gives on, that plants grown out of virus infected seeds developed weakly, set pods poorly, and frequently gave no seeds at all. A lower yield of seeds was mainly due to a lower number of seeds.

The virus infection of seeds was similar in both years. It should be stressed that plants inoculated at the flowering stage gave more virus infected seeds than plants inoculated at the stage of 2-3 pairs of leaves. This difference was exceptionally great and outstanding in the year 1967. This, however, disagrees with the generally appreciated regularity that the number of virus infected seeds is lower when infection takes place at a later developmental stage of the plant [1]. This may be related with a considerable drop of seed number produced in general by earlier infected plants. Plants affected during the time of pod setting gave less virus infected seeds, although from the epidemiological point of view this value was not low at all. In his investigations on seeds from normal plantations, Quantz [5] found that virus infection of horse bean seeds ranged from 0 to 1%, sometimes even a bit more. The infection of seeds of broad bean was greater and reached 2.8% [5]. The virus infection of seeds of a single plant ranged in Quantz's investigations from 2.5 to 15%, the part of plants infected at an early stage of development gave healthy seeds. This was confirmed by our investigations.

Most of the plants infected by BBTMV gave virus free seeds, and the number of diseased plants transferring the virus through seeds decreased with the delay of time of inoculation. It is also possible, that plants showing no macroscopical pathological symptoms may give virus diseased seeds.

Apart from causing a lower yield of seeds the BBTMV also considerably decreased their quality. This in turn caused a drop of weight of 1000 seeds, relatively highest when plants were inoculated at the stage of pod setting. Thus, the virus influenced less the pod setting as well as seed setting but inhibited the growth and development of seeds. A similar phenomenon was also observed in yellow lupine



infected, with narrow leaf virus [1]. The harmful influence of the virus disease was reflected also in a lower energy and activity of seed germination; moreover a large percentage of those seeds being infected by fungi.

### CONCLUSIONS

On the basis of the carried out experiments following conclusions can be drawn:

1. The BBTMV inhibits the growth horse bean plants and lowers their yield.
2. The harmful influence of BBTMV is the larger, the earlier the infection takes place. The most dangerous infection is at the seedling stage.
3. The BBTMV is transferred through the seeds of horse bean collected from diseased plants. However, only some diseased plants give infected seeds. The percentage of virus infected seeds from plants transmitting the BBTMV through seeds ranged from 1.29 to 17.24%.
4. The largest number of virus infected seeds was obtained from plants inoculated at the flowering stage — 3.13 to 3.32%. The virus infection of seeds from plants inoculated at the stage of pod setting was much lower.
5. Horse bean plants showing no symptoms of the disease at the final phase of their vegetative period may also give virus infected seeds, although in a much lower number.
6. Horse bean plants infected with the BBTMV produced undergrown seeds of lower energy and germinative activity, infected to a high degree with fungi.

### SUMMARY

Experiments were carried out in garden (1966) and in the field (1967). Plants of the horse bean "Nadwiślański" were inoculated with the broad bean true mosaic virus (BBTMV) at three developmental stages: seedlings, flowering and pod setting. Over 400 plants were elaborated individually in 1966 and about 1500 plants in 1967. The rate of virus infection of seeds was determined on the ground of seedlings which grew out of them in a glasshouse or in a glasshouse and garden.

A considerable inhibition of plant growth by (BBTMV) and in consequence a smaller size and lower weight of the diseased plants has been stated. The detrimental effect of the BBTMV was the greater the earlier in its developmental stage the plant was infected. Infected plants set a smaller number of pods specially of multi-seeded ones (Fig. 1, Table 4). The decrease of seed yield amounted to 30% and 59% in 1966 and 1967 respectively. Infection at an earlier developmental stage caused a higher decrease of seed yield.

The average rate of virus infection varied in the range from a fraction to over 3% in both years in dependence on the stage of development at which the plants were inoculated. A considerable percentage of horse bean plants infected by the BBTMV produced healthy, virus free seeds. The BBTMV caused a decrease of the weight of 1000 horse bean seeds and a drop of their energy and germination activity. Broad bean seeds from plants infected by BBTMV were infected by fungi (Table 9) at a considerably higher rate.

## REFERENCES

1. Błaszczak W., 1963. *Roczniki Wyższej Szkoły Rolniczej Poznań*, XV: 1-78.
2. Błaszczak W., 1967. Communication Delivered on Virus Conference in Warszawa, 27-28 October 1967. (In press).
3. Błaszczak W., Jamróg K., 1968. (A manuscript).
4. Dorywalski J., Wojciechowicz M., Bartz J., 1964. *Metodyka Oceny Nasion*, PWRiL, Warszawa.
5. Quantz W., 1963. *Phytopath. Z.* 20: 421-448.
6. Report of the Rothamsted Experimental station for 1960. RAM 40 (1961).