

Research Institute for Plant Protection, Budapest, Hungary

Virus Content and Symptom Expression in Samsun Tobacco Treated with Kinetin and a Benzimidazole Derivative

By

E. BALÁZS and Z. KIRÁLY

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While in virus hosts that react hypersensitively to infection, cytokinin-like compounds, such as kinetin and Benlate (a benzimidazole derivative), suppress the hypersensitive necrosis (ALDWINCKLE 1975, ALDWINCKLE and SELMAN 1967, BAILISS, COCKER and CASSELS 1977, BALÁZS, BARNA and KIRÁLY 1976 a, BALÁZS, BARNA and KIRÁLY 1976 b, KIRÁLY and SZIRMAI 1964, KIRÁLY, EL HAMMADY and POZSÁR 1968), there are conflicting reports as regards the effects on symptoms and virus multiplication in the case of a systemic host (BAILISS, COCKER and CASSELS 1977, FRASER and WHENHAM 1978, TOMLINSON, FAITHFULL and WARD 1976). These contradictory results may be due to the different hosts and viruses used in those experiments as well as to treatments carried out in different ways, including the time of treatment (whether before or after inoculation with the virus) (ALDWINCKLE 1975). Our earlier experiences have shown that leaf age may also influence the response. Results of the British authors regarding the symptom suppressing action as well as the virus inhibiting action of the benzimidazole derivatives lend support to the view that this type of pesticide may be used in some cases at least in the chemical control of virus diseases of plants.

In the present work we applied two viruses, namely tobacco mosaic virus (TMV) and cucumber mosaic virus (CMV) to a single tobacco variety (Samsun) to show the effect of cytokinin-like compounds both on symptom development and on the virus content.

Materials and Methods

Turkish Samsun tobacco plants (*Nicotiana tabacum* L.) at the six—eight leaf stage were inoculated by brushing 0.5 mg/ml TMV U1 onto celite dusted-leaves. Half leaves were treated with kinetin (6-furfurylaminopurine, Sigma Co.), 20 ppm in tap water, or with Fundazol (methyl-N-[1-butylcarbonyl]-2-benzimidazole, 50 %, Chinoïn Co.) in a 100 ppm tap water solution, twice daily after inoculation for 6—10 days using a sprayer.

Equivalent samples (500 mg fresh weight) were taken from the young upper fully expanded two leaves, from the next two middle leaves, and from the lower two old or matured leaves. Samples were homogenized in precooled mortars in 10 ml 0.06 M phosphate buffer, pH 6.9, and then centrifuged at 5000 g for 20 min at 4 °C, and the supernatants were subjected to ultracentrifugation at 60 000 g for 1 h. The pellets were resuspended and assayed on Xanthi-nc leaves in Latin square arrangements. Experiments were repeated five times. From the 5000 g supernatants equal amounts of samples were subjected to quantitative rocket immunoelectrophoresis. This procedure was carried out using a 1.5 mm layer of 1 % agarose and barbital buffer, pH 8.6 at 0.02 ionic strength. The antibody content of the gel was 0.1 μ l/cm² for the quantification of TMV in the samples. Details of this method have been published elsewhere (TÓBIÁS, HORNOK and BALÁZS 1979).

To study the role of kinetin and Fundazol on virus distribution and on symptom expression, we inoculated the two lower leaves of Samsun plants at the six leaf stage either with TMV or CMV. After inoculation we immediately sprayed the whole plants with kinetin or Fundazol twice daily for 10 days. Ten to twelve days after inoculation, when symptoms began to appear on the young leaves, samples were taken from the top leaves to cheque the virus content and determine the extent of viral symptom expression. The infectivity test was carried out as described above. In the case of CMV *Chenopodium quinoa* L. was used as test plant. With TMV infected plants, several measurements of the virus content of the leaves were made using immunoelectrophoresis.

Results and Conclusions

As is seen in Table 1, kinetin and Fundazol exerted a leaf age-dependent effect on the virus content of leaf tissues. In the old leaves treatments induced an increase in the virus content but the infectivity of those preparations were decreased. There were no significant difference in the virus content and infectivity of the middle leaves after the treatments. However, in the young leaves we found a considerable increment in the infective particles, but the virus content was less than in the control samples. It is surprising that the changes in infectivity induced by kinetin and Fundazol in both young and old leaves were in the opposite direction to the changes in virus content: increased infectivity occurred in the young leaves although their virus content was lower after treatment, and vice versa in the old leaves.

This leaf-age-dependent effect may be a possible explanation for the discrepancies found in the literature in relation to the effects of kinetin and benzimidazole derivatives on systemic hosts (BAILISS, COCKER and CASSELS 1977, FRASER and WHENHAM 1978, TOMLINSON, FAITHFULL and WARD 1976). In earlier investigations we demonstrated the existence of a "cytokinin gradient" in tobacco plants (BALÁZS, BARNA and KIRÁLY 1976 b) and showed that exogenously applied cytokinins had different effects on leaves of different ages. ATKINSON and MATTHEWS (1967) have shown that the green parts of virus infected leaves contain less virus than the yellow parts of leaves having

Table 1
Effect of kinetin and Fundazol on TMV content of Samsun tobacco*)

Samples	Treatments continued for eight days after inoculation			
	Kinetin		Fundazol	
	changes in infectivity expressed as % of untreated half**)	TMV content expressed as % of untreated half***)	changes in infectivity expressed as % of untreated half**)	TMV content expressed as % of untreated half***)
Young leaves	+23.5*	-13.1*	+17.2*	-15.0*
Middle leaves	+ 1.1	+ 1.1	+ 3.0	+ 1.2
Old leaves	-16.7*	+24.6*	-14.2*	+18.6*

*) Experimental plants were in the six—eight leaf stage.

***) Infectivity assay was done on Xanthi-nc plants in Latin square arrangement in five replications.

****) TMV content was measured by quantitative immunoelectrophoresis 100 % = 16 µg/mg.

* Values significantly different from assay control $P = 0.05$.

the mosaic symptom. One can hypothesize that virus replication is inhibited in the green parts of the virus infected leaves with the mosaic symptom because of the relatively high cytokinin level in those parts (SZIRÁKI and BALÁZS 1977).

Regarding TMV and CMV symptoms in Samsun tobacco after kinetin and Fundazol treatments see Table 2. Symptoms caused by TMV were suppressed by both compounds, but the virus content did not change as compared to the control (data not shown). This result is similar to the finding of TOMLINSON, FAITHFULL and WARD (1976). However, in the case of CMV, symptom development was promoted as a consequence of kinetin treatment, while the virus content remained unchanged. This may be due to the fact that the white parts in the mosaic seem more conspicuous when the rest of the leaf tissue and the whole plant are more green after treatment with kinetin. The virus content in the kinetin and Fundazol treated plants (in the fully expanded leaves) were -3.1 % and -1.5 %, respectively, as compared to the control, ten days after inoculation. After fifteen days the figures were +4.1 % and +1.2 %, respectively.

Thus, treatments with Fundazol effectively suppressed symptom expression without any significant change in the virus content.

These results are similar to those published by TOMLINSON, FAITHFULL and WARD (1976), and also are in agreement, partly at least, with the findings of FRASER and WHENHAM (1978).

If we consider the different actions of hormones and hormone like substances on leaves that are of different ages, the rather complicated picture received in our experiments is perhaps better understood. Further investigations are needed to understand how cytokinins and similar substances control viral replication in tissues and symptom expression in plants.

Table 2
Effect of kinetin and Fundazol treatments on symptom expression induced by TMV and CMV in Samsun tobacco*)

Days after inoculation	Control		Kinetin treated		Fundazol treated	
	TMV	CMV	TMV	CMV	TMV	CMV
8	++*)	+	—	+	—	—
9	++	+	+	++	—	—
10	+++	+	+	++	+	—
11	++++	++	+	+++	+	—
12	++++	++	+	++++	++	+
13	++++	+++	++	++++	++	++
14	++++	+++	++	++++	++	++
15	++++	++++	++	++++	+++	++

*) Experimental plants were in the six leaf stage. Symptoms recorded on the top expanding leaves.

**) Severity of symptoms: — no symptoms, + mild mosaic, ++ medium mosaic, +++ distinct mosaic, ++++ severe mosaic on the leaves.

Summary

A leaf age-dependent effect of kinetin and the benzimidazole derivative Fundazol treatments was found on the virus content of Samsun tobacco systemically infected with tobacco mosaic virus. Increased infectivity occurred in the young leaves although their virus content was lower after treatment, and vice versa in the old leaves.

The distribution of both tobacco mosaic virus and cucumber mosaic virus in tobacco was influenced by both of the treatments. In the young leaves we found a suppression in symptom development caused by tobacco mosaic virus after treatment of the leaves with either kinetin or Fundazol. However, a promotion of symptoms caused by cucumber mosaic virus was experienced as a consequence of treatment with kinetin.

Zusammenfassung

Virusgehalt und Symptomausprägung in Samsun-Tabak behandelt mit Kinetin und einem Benzimidazolderivat

Ein vom Blattalter abhängiger Effekt von Behandlungen mit Kinetin und dem Benzimidazolderivat Fundazol wurde beim Virusgehalt von Samsun-Tabak, systemisch infiziert mit Tabakmosaikvirus, gefunden. Erhöhte Infektiosität trat in jungen Blättern auf, obwohl deren Virusgehalt nach der Behandlung geringer war; das umgekehrte war in alten Blättern der Fall.

Sowohl die Verteilung von Tabakmosaikvirus als auch von Gurkenmosaikvirus im Tabak wurde durch beide Behandlungen beeinflusst. In jungen Blättern fanden wir eine Unterdrückung der durch Tabakmosaikvirus ver-

ursachten Symptome nach der Behandlung der Blätter mit Kinetin oder Fun-dazol. Als Folge der Kinetinbehandlung wurde jedoch eine Verstärkung der Gurkenmosaikvirus-Symptome beobachtet.

Literature

- ALDWINCKLE, H. S., 1975: Stimulation and inhibition of plant virus infectivity in vivo by 6-benzylaminopurine. *Virology* **66**, 341—343.
- , and I. W. SELMAN, 1967: Some effects of supplying benzyladenine to leaves and plants inoculated with viruses. *Ann. Appl. Biol.* **60**, 49—58.
- ATKINSON, P. H., and R. E. F. MATTHEWS, 1967: Distribution of tobacco mosaic virus in systemically infected tobacco leaves. *Virology* **32**, 171—173.
- BAILISS, K. W., F. M. COCKER, and A. C. CASSELS, 1977: The effect of Benlate and cytokinins on the content of tobacco mosaic virus in tomato leaf disks and cucumber mosaic virus in cucurbit cotyledon disks and seedlings. *Ann. Appl. Biol.* **87**, 383—392.
- BALÁSZ, E., B. BARNÁ, and Z. KIRÁLY, 1976a: Effect of kinetin on lesion development and infection sites in Xanthi-nc tobacco infected by TMV: Single-cell local lesion. *Acta Phytopath. Hung.* **11**, 1—9.
- , —, and —, 1976b: A comparison of the number of infection sites with the number of local lesions in TMV-infected Xanthi-nc tobacco treated with kinetin. *Abstr. 8th Congr. Czechoslovak Plant Virologists*, Bratislava.
- FRASER, R. S. S., and R. S. WHENHAM, 1978: Inhibition of the multiplication of tobacco mosaic virus by methyl-benzimidazol-2-yl-carbamate. *J. Gen. Virol.* **39**, 191—194.
- KIRÁLY, Z., and J. SZIRMAI, 1964: The influence of kinetin on tobacco mosaic virus production in *Nicotiana glutinosa* leaf disks. *Virology* **23**, 286—288.
- , M. EL HAMMADY, and B. I. POZSÁR, 1968: Susceptibility to tobacco mosaic virus in relation to RNA and protein synthesis in tobacco and bean plants. *Phytopath. Z.* **63**, 47—63.
- SZIRÁKI, I., and E. BALÁSZ, 1977: The effect of infection by TMV on cytokinin level of tobacco plants, and cytokinin in TMV-RNA. In: Z. KIRÁLY (Ed.), *Current Topics in Plant Pathology*, 345—352. Akad. Kiadó, Budapest.
- TÓBIÁS, I., L. HORNOK, and E. BALÁSZ, 1979: The use of rocket immunoelectrophoresis to detect tobacco mosaic virus in infected pepper tissues. *Acta Phytopath. Hung.* **14**, 233—241.
- TOMLINSON, J. A., E. M. FAITHFULL, and C. M. WARD, 1976: Chemical suppression of the symptoms of two virus diseases. *Ann. Appl. Biol.* **84**, 31—41.

Authors' address: Research Institute for Plant Protection, Department of Pathophysiology, P. O. Box 102, H-1525 Budapest (Hungary).