

# Herbicide-Affected Plant Metabolism Reduces Virus Propagation

Gabriella Kazinczi<sup>a,\*</sup>, László Gáspár<sup>b</sup>, Péter Nyitrai<sup>b</sup>, Richard Gáborjányi<sup>c</sup>,  
Éva Sárvári<sup>b</sup>, András Takács<sup>a</sup>, and József Horváth<sup>c</sup>

<sup>a</sup> Office for Academy Research Groups Attached to Universities and Other Institutions, Virological Research Group, Pannon University, Georgikon Faculty of Agricultural Sciences, Institute for Plant Protection, H-8361 Keszthely, Deák F. str. 16, Hungary. Fax: 003683 5451 43. E-mail: kg@georgikon.hu

<sup>b</sup> Department of Plant Physiology and Molecular Plant Biology, Eötvös Loránd University, Budapest, Hungary

<sup>c</sup> Institute for Plant Protection, Pannon University, Georgikon Faculty of Agricultural Sciences, Keszthely, Hungary

\* Author for correspondence and reprint requests

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*Dedicated to the memory of Klaus Schmelzer PhD (1928–1976), a friend and fellow scientist of József Horváth*

It has been previously shown that certain herbicides or plant extracts inhibited the viral infection. The goal of this study was to investigate the effect of *Obuda pepper virus* (ObPV) infection and herbicide or plant extract treatments on the photosynthetic processes of the host plants to get informations about the interactions of these factors. In *Capsicum annuum*-ObPV host-virus relations the virus infection slightly increased the activity of photosystem II (PSII), as it was supposed from fluorescence induction parameters. Chlorophyll content of leaves was also elevated probably due to virus-induced growth inhibition. The herbicide Stomp (active ingredient: pendimethalin) incorporated into the soil one week before planting (preplant treatment) together with virus infection even strengthened these effects in agreement with previous observations that this herbicide always did not prevent virus infection or reduce virus concentration in hosts. In ObPV-infected *Nicotiana tabacum* the structural changes showed similar tendency like in ObPV-infected *C. annuum*, but PSII efficiency did not significantly differ from that of the control. However, non-photochemical quenching (NPQ) increased because of the strongly decreasing CO<sub>2</sub> fixation activity. Though simultaneous application of a water extract of *Cirsium arvense* shoot caused a little stronger inhibition of CO<sub>2</sub> fixation, little loss in production was obtained due to significant reduction in virus concentration. In *Solanum nigrum*-ObPV relation the slightly increasing tendency of the values of actual PSII quantum efficiency could be related to the probably elevated ratio of reaction centre components (increased chlorophyll *a/b* ratio) in the thylakoids. Application of the herbicide Fusilade S (active ingredient: fluazifop-P-butyl) at 4–6 leaf stage as a post-emergence treatment practically prevented systemic virus infection and the virus-induced changes of photosynthesis are probably due to inhibiting the virus infection/replication process.

**Key words:** Plant Viruses, Herbicides, Photosynthesis

## Introduction

Virus infection, as a biotic stress factor, has a complex effect on the host plant. Most frequently occurring symptoms, like chlorosis, mosaic, and dwarfing, suggest that viruses can influence photosynthetic processes of host plants. Virus infection was shown to change the structure of the photosynthetic apparatus, the activity of electron transport, photosynthetic enzymes, and other processes, which are in close relation to photosynthesis (Goodman *et al.*, 1986; Rahoutei *et al.*, 2000; Almási *et al.*, 2000, 2001; Funayama-Noguchi, 2001; Harsányi *et al.*, 2002; Pérez-Bueno *et al.*, 2004).

Photosynthesis is one of the most important plant physiological and biochemical processes affected by herbicides. The majority of herbicides have an indirect effect on photosynthesis (Tomlin, 1997). Some of them (*e.g.* triazines, carbamides) inhibit the photosynthetic electron transport in photosystem II (PSII), and others (*e.g.* diquat-dibromide, paraquat) have an effect on photosystem I (Cobb and Kirkwood, 2000; Szigei *et al.*, 2001). Inhibitory effects of *Cirsium arvense* plant extracts on the germination and growth of plant species have been intensively studied earlier (Bendall, 1975; Kazinczi *et al.*, 2001a). Nandal and Bhatti

(1983) observed that leaf extracts of *C. arvense* significantly reduced the mycelial dry weight of certain phytopathogen fungi (e.g. *Aspergillus*, *Alternaria*, *Rhizoctonia* spp.), and nematocide properties of *C. arvense* on some phytopathogen nematodes were also reported. The effect of *C. arvense* as reducing virus in some host-virus relations has become known recently (Kazinczi *et al.*, 2005a, b).

There are examples that one type of stress treatment may give some protection against other stresses (Goodman *et al.*, 1986; Nilsen and Orcutt, 1996). The effects of other stress factors on virus infection have been also investigated (Ghoshroy *et al.*, 1998). Favourable side effects of some herbicides and plant extracts, as virus inhibitors, have been discovered (Schuster, 1972, 1982; Horváth and Hunyadi, 1973; Rao *et al.*, 1994; Kazinczi *et al.*, 2002, 2003, 2005a, b). Some of them decreased the virus concentration or practically prevented virus infection of host plants. However, their mechanism of interaction and influence on plant metabolism are not known.

In the present paper our goal was to study the effects of virus infection and some herbicide or plant extract treatments on the photosynthesis of the host plants in three systemic, compatible host-virus relations to get some informations about the site of interaction of these factors.

## Materials and Methods

### *Model host-virus relations and treatments*

Three models of host-virus relations with three different treatments depending on the severity of virus inhibition were chosen for photosynthetic studies: (i) Fusilade S herbicide, the active ingredient of which was 12.5% (w/v) fluazifop-P-butyl {(R)-2-[4-(5-trifluoromethyl-2-pyridyloxy)phenoxy]propionic acid}, was mixed at 1.5% content to the inoculum, when *S. nigrum* plants were mechanically inoculated at 4–6 leaf stage with *Obuda pepper virus* (ObPV). This herbicide as post-emergence treatment practically prevented virus infection of the host. (ii) In *Nicotiana tabacum* ‘Samsun’-ObPV relation, water extract of *C. arvense* shoot significantly reduced the virus concentration in host plants. Fresh shoots of *C. arvense* were cut into small pieces in a grinder. After grinding, 25 g fresh biomass were stirred in 100 ml distilled water and left for a day. Then the mixture was filtered through filter paper (MN 640w), and the water extract was used to spray daily *N. taba-*

*cum* plants from their 2–4 leaf stage. (iii) Stomp 330 {33% (w/v) pendimethalin [N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine]} as a preplant treatment (incorporated into the soil one week before planting) at a dosage of 1320 g active ingredient ha<sup>-1</sup> did not influence the virus concentration in *Capsicum annuum*-ObPV relation. All plants at 4–6 leaf stage were mechanically inoculated with ObPV. The DAS ELISA serological method after Clark and Adams (1977) was used five weeks after inoculations, where from the extinction values one could conclude to the virus concentration. Test samples were considered resistant to virus infection if their extinction values did not exceed two times those of the negative (uninfected) control ones. Analysis of variance (ANOVA) has been done with susceptible, virus-infected plant samples, in order to determine the effect of the treatments on the virus concentration in hosts as compared to the positive control samples. Virus-infected plants without herbicide or plant extract treatments served as positive control. Details of the methodology of virus infections, different treatments and evaluation of their effects on virus infections are given in our previous papers (Kazinczi *et al.*, 2002, 2005a).

### *Chlorophyll content*

Chlorophyll content of leaves was determined in 80% acetone extracts using the extinction coefficients and equations of Porra *et al.* (1989).

### *Fluorescence induction measurements*

Fluorescence induction measurements of leaf samples were performed using a PAM Chlorophyll Fluorometer (Walz, Effeltrich, Germany).  $F_o$  level of fluorescence was determined after 20 min of dark-adaptation. The maximum fluorescence yield in the dark ( $F_m$ ) and the light-adapted state ( $F_m'$ ) was measured by applying a 0.7 s pulse of white light (3500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; light source: Schott, KL 1500 electronic). For the quenching analysis actinic light (120  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) was provided.  $F_o'$  was measured with turning off the actinic light and applying 3 s of weak far-red light (emission peak at 735 nm). Fluorescence induction parameters were calculated according to van Kooten and Snel (1990) and Genty *et al.* (1989).

### Photosynthetic activity

Photosynthetic activity of leaves was determined by  $^{14}\text{CO}_2$  fixation according to Láng *et al.* (1985).  $^{14}\text{CO}_2$  fixation was carried out in leaves placed into a mercury-locked chamber.  $\text{CO}_2$  content was 1% (released from one part active – 10.387 MBq/mg activity – and nine parts inactive  $\text{BaCO}_3$  with  $\text{HClO}_4$ ) and photon flux density was  $1000 \mu\text{E m}^{-2} \text{s}^{-1}$ . After 2 min fixation, leaf discs (each with a 23.4 mm<sup>2</sup> area) were dried at high temperature by ironing the discs. The radioactivity of discs was measured by a Beckman LS 5000 TD liquid-scintillation equipment. The scintillation mixture contained 0.5% 2,5-diphenyl-oxazol and 0.005% 1,4-di[2-(5-phenyl)-oxazolyl]-benzene dissolved in toluene.

## Results

### Chlorophyll content

Chlorophyll content of ObPV-infected pepper plants (*C. annuum*) was significantly enhanced compared to that of the uninfected plants, and a further significant increase was observed due to the combined virus-herbicide treatment (Fig. 1A). Changes of similar tendency were found in the case of *N. tabacum*, but they were not significant. Neither ObPV nor the combined virus-Fusilade S treatment influenced significantly the chlorophyll content of *S. nigrum* leaves compared to the control, though it was slightly reduced by virus infection.

Chlorophyll *a/b* ratio of ObPV-infected *S. nigrum* significantly increased, while it hardly rose in the other two host-virus interactions (Fig. 1B).

However, it was slightly elevated in the other two virus-host relations after Stomp-330 or *C. arvense* shoot extract treatments.

### Fluorescence induction measurements

In *C. annuum*-ObPV actual ( $\Delta F/F_m'$ ) and maximum ( $F_v/F_m$ ) quantum efficiency of PSII, and efficiency of energy utilization in the open PSII reaction centers ( $F_v'/F_m'$ ) were slightly elevated by either virus infection or the combined treatment (Figs. 2A–C). Neither virus infection nor virus infection and herbicide treatment together changed the proportion of the opened reaction centres (qP) (Fig. 2D). The extent of non-photochemical quenching (NPQ) was somewhat reduced after virus infection, but it was reversed to the level of control due to herbicide treatment (Fig. 2E).

In *N. tabacum*-ObPV relation PSII efficiency hardly changed after virus infection and the combined treatment, only NPQ was enhanced in both cases (Fig. 2).

In *S. nigrum*-ObPV relation almost all parameters ( $F_v/F_m$ ,  $F_v'/F_m'$ , qP,  $F/F_m'$ ) increased slightly due to ObPV infection, while non-photochemical quenching processes (NPQ) were somewhat decreased. However, all parameters remained at control level after the combined virus-herbicide treatment (Fig. 2).

### Carbon assimilation

ObPV infection did not influence the photosynthetic activity of pepper (*C. annuum*), nevertheless

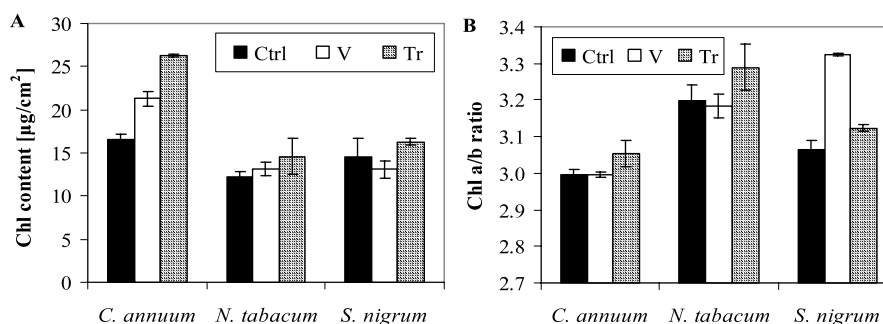


Fig. 1. (A) Chlorophyll (Chl) content and (B) chlorophyll *a/b* ratio in leaves of host plants as affected by virus infection and different treatments. Ctrl, control; V, virus-infected; Tr, combined virus infection and different treatments depending on the host plants (Stomp, *C. arvense* shoot water extract and Fusilade S treatments in *C. annuum*, *N. tabacum* and *S. nigrum* hosts, respectively).

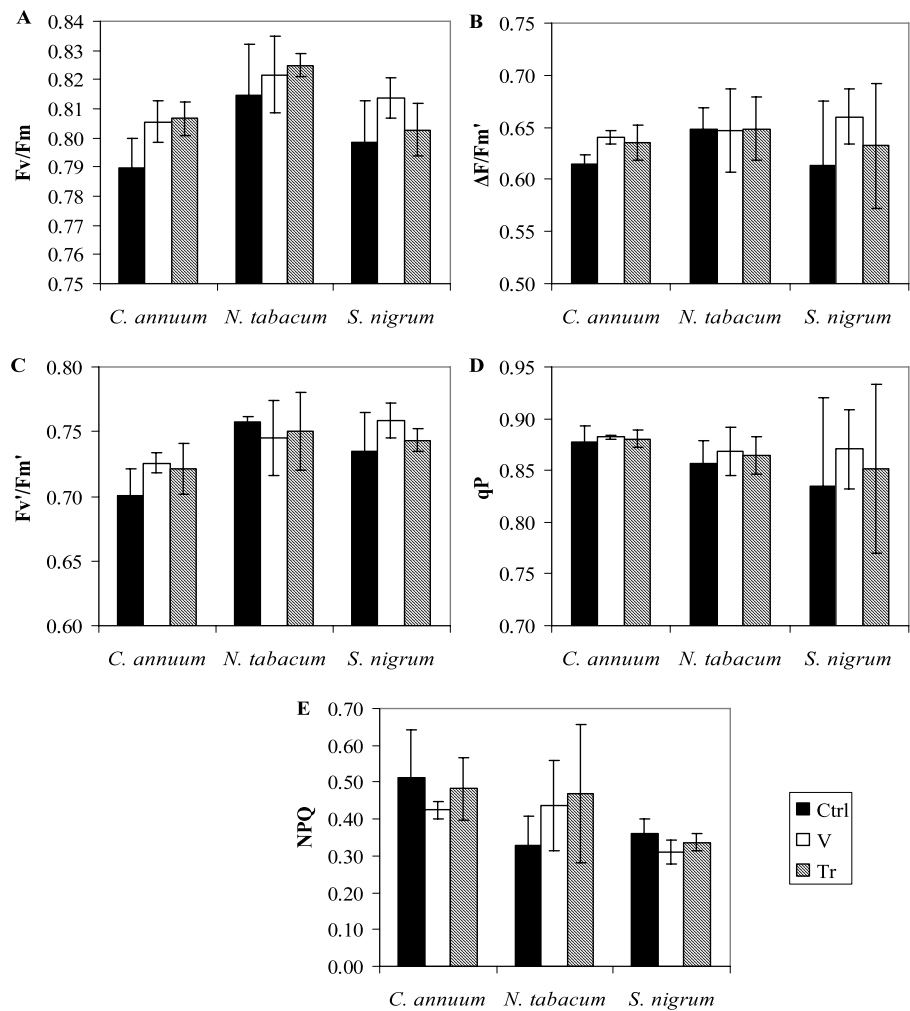


Fig. 2. Fluorescence induction parameters of differently treated plants: (A) Maximal quantum efficiency of PSII in dark-adapted samples ( $F_v/F_m$ ); (B) actual quantum efficiency ( $\Delta F/F_m'$ ); (C) intrinsic energy utilization efficiency ( $F_v'/F_m'$ ) of PSII; (D) ratio of open reaction centres ( $qP$ ); and (E) non-photochemical quenching (NPQ) at steady state photosynthesis. Treatments were the same as in Fig. 1.

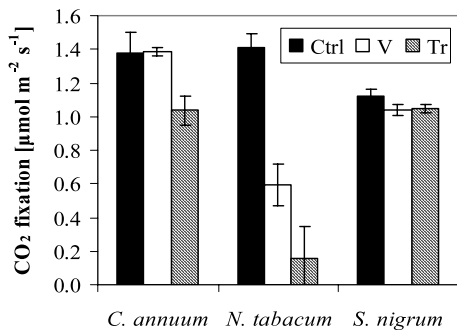


Fig. 3.  $CO_2$  fixation of differently treated plants. Treatments were the same as in Fig. 1.

a slight (not significant) reduction in the  $^{14}\text{CO}_2$  fixation was observed due to virus infection together with herbicide treatment. In contrast, considerable decrease in  $^{14}\text{CO}_2$  fixation was observed in *N. tabacum* after virus infection and a further decrease was observed due to the combined (virus infection plus *C. arvense* water extract) treatment. Neither ObPV infection nor combined treatment (virus infection plus herbicide treatment with Fusilade S) influenced significantly  $^{14}\text{CO}_2$  fixation in *S. nigrum* hosts (Fig. 3).

## Discussion

In *C. annuum*-ObPV relation the virus infection changed the structure and activity of the photosynthetic apparatus. Compared to reports publishing structural and functional defects on PSII under viral infection (Almási *et al.*, 2000; Rahoutei *et al.*, 2000; Funayama-Noguchi, 2001; Pérez-Bueno *et al.*, 2004), however, these changes were the opposite. PSII activity somewhat increased as it was evidenced from the tendency of changes in fluorescence induction parameters (increasing  $\Delta F/F_m'$ ,  $F_v/F_m$ ,  $F_v'/F_m'$ , decreasing NPQ).  $\text{CO}_2$  fixation was not influenced. The viral effect also appeared as an elevated chlorophyll content in leaves. It could be also connected, however, with growth inhibition caused by the virus infection. Tobamoviruses, such as ObPV, *Tobacco mosaic virus* (TMV) are considered to have a strong growth inhibitory effect on systemic host plants (Kazinczi *et al.*, 2001b; Takács *et al.*, 2004). A preplant treatment with Stomp (active ingredient: pendimethalin) together with virus infection even strengthened these effects in agreement with previous observations that this herbicide did not behave as a virus inhibitor in this relation (Kazinczi *et al.*, 2002). The stronger effects of the combined treatment can be well explained by the known characteristics of pendimethalin, which is a selective herbicide absorbed by roots and leaves and similarly as the above mentioned virus treatment inhibits cell division and elongation. It is used for the control of most annual grasses and many dicot weeds in crop fields as a preplant incorporated, pre-emergence, pre-transplanting and early post-emergence treatment. The not significant decline of  $\text{CO}_2$  fixation, which was observed tendentially, may refer to some adverse effect of this herbicide on the dark phase of photosynthesis.

In *N. tabacum*-ObPV relation the effects of virus infection on the photosynthetic apparatus of the host were different from those observed in the case of *C. annuum*. Structural changes were of similar tendency, but PSII efficiency did not significantly differ from that of the control. However, NPQ increased probably because of the strongly decreasing  $\text{CO}_2$  fixation activity. This may be either due to the inhibition of some Calvin cycle enzymes or to stomatal closure. The decreased activity of ribulose-biphosphate carboxylase was shown in some virus-plant relation (Mohamed, 1973). Simultaneous application of a water extract of *C. arvense* shoot, which significantly reduced the virus concentration in host plants, caused even stronger inhibition of  $\text{CO}_2$  fixation. This fact let us think that the extract itself may inhibit the dark reactions or decrease the opening of stomata, which may also somehow connected with the inhibition of systemic virus infection. ObPV infection is considered to cause great reduction of biomass production of host plants, as compared to healthy control ones, but only a slight, not significant (6%) reduction in fresh weight of ObPV-infected *N. tabacum* was observed due to the treatment with *C. arvense* extract (Kazinczi *et al.*, 2005b, 2006). In our experiments main components of *C. arvense* shoot extracts were not characterized, but phenolic compounds responsible for allelopathic activity were identified in previous investigations (Wilson, 1981).

In *S. nigrum*-ObPV relation virus infection may have caused a significant alteration in thylakoid composition as it is evidenced from the increased chlorophyll *a/b* ratio of leaves. There were indications that virus infection reduced the amount of light harvesting complexes (Funayama-Noguchi, 2001; Szigeti *et al.*, 2002). The slightly increasing tendency of the values of fluorescence induction parameters  $\Delta F/F_m'$ ,  $F_v/F_m$ ,  $F_v'/F_m'$  could be related to the elevated ratio of reaction centre components in the thylakoids, which contain chlorophyll *a*. Post-emergence co-application of Fusilade S (active ingredient: fluazifop-P-butyl) herbicide practically prevented systemic virus infection of the host and the virus-induced changes of photosynthesis. This fact also pointed out that this herbicide did not influence the photosynthetic activity of the host plant. For this reason, it is a more promising virus inhibitor. It must be further tested, however, in different virus-host relationships. Flu-

azifop-P-butyl is a selective systemic herbicide absorbed by the leaves and used for post-emergence control of annual and perennial grass weeds in broad-leaved crops. It inhibits fatty acid synthesis. The mechanism of inhibition of virus infection by this herbicide is not known, but it was different

from that of the *C. arvense* shoot extract. It may be connected either with inhibition of virus infection or the virus replication process (Gáborjányi and Tóbiás, 1986). Concerning virus protection of plants, both protective agents have great interest and agricultural importance.

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