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Heavy metal soil contamination delays the appearance of virus-induced symptoms on potato but favours virus accumulation

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Abstract
Here we report on the investigation of virus infection development in plants undergoing simultaneous heavy metal stress. We carried out small-scale field experiments using model system Potato virus X (PVX) – Solanum tuberosum cv. Povin’ (potato) plants. Heavy metals zinc (Zn), copper (Cu) and lead (Pb) were added to the soil separately (monometal contamination) at a range of concentrations. Our results show that heavy metal stress significantly delays the appearance and potentiates severity of virus-specific symptoms on infected potato plants. We also demonstrate that PVX content in plants may increase tremendously in response to this abiotic stressor. Finally, we provide links to the possible consequences in the context of virus epidemiology.

Keywords: Potato virus X, symptoms, virus content, heavy metal, abiotic stress

Introduction
Virus infections still represent a major challenge for plant-growers as there are no reliable measures to combat most of them in the field. Owing to this, the only widespread common practice for controlling virus diseases is to avoid infection rather than fight it. It comes parallel with producing virus-resistant cultivars and varieties of plants, as much is now known about the mechanisms of plant resistance to viruses (Soosaar et al. 2005).

Recently it has been shown that due to the non-compliance with generally approved agricultural practices, significant spreading of plant virus infections occurred in the territory of Ukraine (Polischuk et al. 2001). There are many environmental factors, biotic and non-biotic, that affect virus infection development in plants. Some Ukrainian regions are characterised by serious heavy metal contamination of land used for agricultural purposes. The main reason for such chemical pollution of the soil is industrial activity (Shevchenko et al. 2003).
In our previous work we traced the development of Tobacco mosaic virus (TMV) infection in tomato plants in order to find any changes in the efficiency of virus replication induced by additional stress of abiotic nature – heavy metals (Shevchenko et al. 2004). As we have seen from that experiment, the metals did not have any effects on the type or severity of the virus-specific symptoms but a 7-day delay in onset was noted for lead-treated plants. Furthermore, we also observed significant (more than two-fold) increment of TMV content in tomato plants induced, in particular, by zinc and lead.

In this work we used the ‘PVX-potato’ model system to see whether the phenomenon of favoured virus accumulation was unique for the TMV infection in tomatoes or, rather, uniform for various ‘virus-plant’ model systems.

Materials and methods

In these small-scale field experiments we used PVX and Solanum tuberosum cv. Povin’ (potato) plants, which are systemically invaded. To simulate soil contamination we used heavy metals copper, zinc and lead in the form of water-soluble salts (CuSO₄ × 5H₂O, ZnSO₄ × 7H₂O, and Pb(NO₃)₂) added to soil separately (monometal contamination) at a range of concentrations: 5×, 10×, and 50× maximum permissible concentration (MPC). Values of 1× MPC for the metals under study were as follows: Cu – 100 mg/kg of soil, Zn – 300 mg/kg, and Pb – 100 mg/kg (Kabbata-Pendias & Pendias 1986). The heavy metals were added to soil five days prior to plant inoculation with PVX.

Potato plants were virus-inoculated mechanically using carborundum powder in two upper leaves at the stage of four true leaves, concentration of inoculum constituted 150 μg/ml.

Development of systemic viral infection was monitored visually by symptoms, and quantitatively by measuring virus content in plants using indirect ELISA (Crowther 1995). Fresh plant samples for determining virus amounts were taken every seven days during the experiment. We used rabbit polyclonal antiserum raised against PVX (Aschersleben, Germany) and anti-rabbit antibodies conjugated with alkaline phosphatase (‘Sigma’, USA); results were counted at the wavelength of 405 nm on ELISA reader ‘Dynatech’. Obtained data was then evaluated and averaged using Microsoft Excel XP.

Results and discussion

We have analysed the impact of soil chemical contamination with heavy metals on the development of plant viral infection. As we have seen from natural and agricultural ecosystems, heavy metal pollution of soil may result in the extensive relative abundance and diversity of plant viruses at the population level (Polischuk et al. 2001). This gave us the idea to simulate such conditions in small-scale field experiments in order to trace the development of virus-specific visual symptoms of the infection, and accumulation of virus antigens in the plants.

In this work, we applied zinc, lead and copper at a range of concentrations (5×, 10× and 50× MPC) to attain low, medium and high level of soil contamination. PVX-infected potato plants grown in soil not amended with heavy metals developed visual symptoms of virus infection by 14 days post infection (dpi); the symptoms were typical – mild leaf mosaic (Figure 1A). Input of heavy metal to soil at any concentration tested substantially delayed the appearance of the symptoms up to 21 dpi. Moreover, Zn and Cu at 10× MPC induced more severe symptoms on the later stage of virus infection (28 dpi), namely strong leaf mosaic followed by deformation of the upper leaves (Figure 1B).

From these results it is evident that plants undergoing additional stress during systemic virus infection may and do respond differently upon inoculation with the pathogen. Soil
contamination with heavy metals added at $10^6$ MPC invoked the appearance of more severe visual symptoms of PVX infection of potato – deformation of leaves, which was not the case for PVX-infected potato plants grown in non-polluted soil. It should be said that from the three metals tested only Zn and Cu inflicted the appearance of atypical virus-specific symptoms. Soil contamination with lead ions did not have any effects on the visual development of the infection. However, a 7-day delay in onset of the symptoms was noted for all heavy metals under study – Cu, Zn and Pb.

It is worth noting that in our previous work (Shevchenko et al. 2004) we have not observed any changes in virus-specific symptoms induced by TMV on tomato plants. From the same metals used in the ‘TMV-tomato’ model system at $10^6$ MPC, the delay in the appearance of

Figure 1. Symptoms induced by PVX on potato plants. (A) Plants grown in non-contaminated soil – mild mosaic of leaves by 14 dpi; (B) plants grown in soil amended with Zn in $10^6$ MPC – delayed development of severe leaf mosaic by 21 dpi, followed by deformation of younger leaves by 28 dpi.
symptoms was characteristic only for Pb-treated plants, but not for Zn or Cu. Despite the existing differences in the reaction of tomato and potato plants on the inoculation with respective viruses, we deem that there might be general commonalities in the pattern of virus infection development in plants stressed with heavy metals, such as the delay in the onset of virus-specific symptoms on metal-treated plants.

The next part of our investigations was directed at establishing temporal dynamics of virus content in potato plants, whether treated with heavy metals or not. We sought variations in the accumulation of PVX antigens in potatoes treated with contaminants, in comparison with ‘normal’ development of the disease in non-treated plants.

Our study of PVX in potatoes showed that untreated virus-infected plants accumulated up to 25 μg/g of fresh leaf tissue by 14–21 dpi (Figure 2A). The temporal dynamics of PVX accumulation was common for a systemic virus invasion of a susceptible host plant – increase in virus amount (0–14 dpi) followed by a steady-state stage (‘plateau’, 14–21 dpi) with a gradual decrease of virus concentration to basal levels (21–42 dpi). This type of dynamics of virus amount in systemically invaded leaf tissues is typical for many plants (Matthews 1992).

All the heavy metals tested at 5× MPC induced statistically significant elevation in PVX concentration. Zn caused the greatest changes in virus content (1.6-fold increase on 14 dpi;

![Figure 2. Temporal dynamics of PVX content in fresh leaf tissue of potato plants as affected by heavy metals lead (Pb), zinc (Zn), and copper (Cu) applied at a range of concentrations: (A) 5× MPC of heavy metals; (B) 10× MPC of heavy metals; (C) 50× MPC of heavy metals; V, virus infection.](image)
42 µg/g of tissue) and also in temporal dynamics (Figure 2A). From our point of view, it is of importance that residual levels of virus content (post 21 dpi) remained substantially higher in metal-treated plants. By 42 dpi, PVX concentration in Pb-treated potatoes has constituted approximately 18–20 µg/g, in Cu-treated plants – 22–25 µg/g, and in Zn-treated plants – almost as much as 38–40 µg/g. Conversely, virus amounts in non-treated infected potatoes have decreased to approximately 10 µg/g by this time (Figure 2A).

Addition of Zn and Cu at 10× MPC led to exceptional differences in time dynamics of PVX content. Virus concentration in these plants climbed to 63 µg/g on 42 dpi (2.5-fold elevation). Moreover, even by 42 dpi, we had not detected any decrease in PVX content (which normally follows a maximum of concentration by that time) in plants grown in Cu-/Zn-contaminated soil. This situation is totally different from the ‘normal’ pattern of PVX behaviour on systemically infected potato. Conversely, Pb at 10× MPC did not induce any differences compared to 5× MPC values (Figure 2B).

Contrary to previous data, no heavy metals at 50× MPC induced any statistically significant changes in PVX concentration when compared to infected plants grown in non-amended soil (Figure 2C). We believe that the reason for diminished virus replication might be a substantial inhibition of major physiological processes of the host grown in soil treated with very high amounts of heavy metals. Potato plants cultivated in soil with Cu or Zn at 50× MPC were generally smaller and demonstrated thinner leaves, sometimes with yellowing along the veins and brown edges (data not shown).

In summary, we showed that heavy metals lead, copper and zinc induced an elevation of PVX content in systemically invaded potato plants. According to our results (Figure 2A and 2B), this process may be dependent on the nature of the metal applied, as Cu and Zn invoked more substantial virus accumulation compared to Pb ions. Analysing the ability of these metals to induce an increase in virus amounts in plants, we may order them as Pb → Cu → Zn. Furthermore, we may speculate on the peculiar effects of each metal tested in the process of virus replication. However it is of significant interest that such an order (Pb → Cu → Zn) also depicts the ability of plants for long-distance trafficking of these ions (Kabbata-Pendias & Pendias 1986). Taking this into account, it is possible that variations in virus content intrinsic for a given metal (Pb, Cu, and Zn) may be explained purely on the basis of the physical accessibility of the metal at a definite period of time.

Our data also showed that heavy metal influence on PVX concentration seemed to be dose-dependent, as metals applied at 10× MPC induced statistically higher virus amounts in plants compared to metals at 5× MPC, with exclusion of Pb (Figure 2B and 2A, respectively). The reasons for this remain unclear. In our opinion, it might be related to the importance of Cu and Zn ions for many biochemical processes of a plant (Shevchenko et al. 2003). As a virus uses plant synthetic machinery for its own replication, redirecting plant transcriptional activity (Técsi et al. 1996; Havelda & Maule 2000), we suggest that input of heavy metals in soil may favour virus replication by providing necessary biochemical elements directed to the synthesis of virus components.

Added at 50× MPC, the metals possessed an inhibitory effect on the development of potatoes, inducing minor stunting of the plants and yellowing of the leaves (data not shown). We believe that these conditions are rather adverse for systemic virus infection as the host may not be able to maintain the progressive multiplication of the pathogen in addition to its normal physiological functions.

We also noted that more severe symptoms on PVX-infected potato grown in soil contaminated with Cu or Zn at 10× MPC, namely strong mosaic and deformation of younger leaves (Figure 1B), correlated with remarkably high virus content in these plants (Figure 2B) on the late stage of the infection process. It remains unknown whether it was coincidence or not,
however, this seems to be coherent with our understanding of the systemic virus infection on susceptible host.

It is worth mentioning that in our previous work we detected significant elevation of TMV content in tomatoes in response to treatment of the soil with lead and zinc at $10^6$ MPC (Shevchenko et al. 2004). Virus accumulation was delayed in stressed plants but reached values approximately 2.3–2.5 times higher comparing to non-treated plants. Thus, despite the existing differences between these model systems, there are noticeable similarities in the meaning of virus amounts present in plant tissues as influenced by heavy metals added to soil.

The fact of facilitated virus accumulation in plants undergoing heavy metal stress is intriguing, as it was previously demonstrated that heavy metal ions – Pb, Cd and Zn in particular – were shown to inhibit the mitotic activity of the cell affecting microtubules and intracellular trafficking (Guralchuk 1994; Melnichuk 1990). In turn, this would mean a decreased capability of the cell to sustain virus replication, and following cellular and long-distance transport of the virus throughout the host, which is contradictory to our results on PVX infection of potato plants.

In an elegant set of experiments, Citovsky et al. (1998) showed that plants treated with non-toxic concentrations of cadmium did not fully support systemic transport of Tobacco vein clearing virus (TVCV) in tobacco plants. The authors revealed that TVCV trafficking was affected at the stage of phloem unloading into systemic leaves, but not at the stage of virus loading into the phloem from the primarily infected leaves. On the contrary, the same concentrations of Cd ions did not influence long-distance transport of another unrelated virus, Tobacco etch virus, in tobacco (Citovsky et al. 1998). This might mean that mechanisms of phloem loading and unloading differ for a given virus. Furthermore, another related study demonstrated that not all metal ions were able to induce such an inhibition of systemic transport of TVCV. Thus, from a range of metals tested, zinc was not able to affect plant virus movement (Ueki & Citovsky 2001). These outcomes, again, are in conflict with the data we obtained for the ‘TMV-tomato’ and ‘PVX-potato’ model systems. Contradictions may be explained by means of possible differences in systemic trafficking of unrelated viruses, TVCV and PVX, however remaining elusive.

**Conclusions**

Here we have demonstrated that abiotic stressors can induce changes in the development of plant virus infection. Following the results of a set of small-scale field experiments, we believe that heavy metals altered the expression of virus-induced symptoms on plants. These alterations can affect the type and severity of symptoms invoked by a virus. However, a 7-day delay in onset of the symptoms was noted for all heavy metals under study.

More significantly, we explicitly showed that heavy metals may provoke an enormous increment in virus content in the host tissues. In the ‘PVX-potato’ system, practically all metals tested (zinc, copper and lead) induced an elevation in virus concentration. However, for zinc and copper, the most peculiar feature of virus content temporal dynamics has been the absence of the ‘plateau’ stage and the absence of a decrease in virus amounts at the late stage of the infection process. Interestingly, the appearance of severe virus-specific symptoms on potato plants treated with Cu and Zn at $10^6$ MPC coincided with the maximum virus content in these plants.

Constantly high concentrations of the virus in the host may further increase the prospects of virus transmission to other plants. Furthermore, the duration of infectiousness when plants were under abiotic stress seemed longer compared to the virus-infected untreated plants. Our understanding of virus infection development in plants undergoing simultaneous heavy metal stress is summarised in Figure 3.
However, precise reasons for such an ability of heavy metal ions to potentiate virus accumulation in systemic host tissues remain unclear. We are also uncertain about why the metals behaved differently influencing amounts of the virus. Connections between more severe visual symptoms of the disease and high virus content in plant tissues, if any, are ambiguous as well. It remains obscure whether there are any variations in biological properties (i.e. infectiousness, host range, etc.) of the virus when it is replicated in a heavy metal-treated host plant. It would be useful to conduct field experiments focusing on the investigation of the potential of a virus to be transmitted further to other susceptible species (especially for vector-transmitted viruses), in order to reveal an epidemiological capacity of the virus replicated in heavy metal-treated plants. It is also worth investigating the advance of virus infection in a considerably different model plant species, for example in a monocot system, to investigate whether the disease would also be facilitated in the means of virus content if influenced by heavy metals.

Generally, we propose that virus infections behave quite differently when their hosts are undergoing additional abiotic stresses of an abiotic nature. Speculating, we believe this may pose a significant risk in the context of uncontrollable distribution of these pathogens, proving a need for careful monitoring of virus circulation in chemically contaminated environments to avoid their spread to the neighbouring agrocenoses.

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