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EFFECTS OF LOW DOSE CHRONIC RADIATION AND HEAVY METALS ON PLANTS AND THEIR FUNGAL AND VIRUS INFECTIONS

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ABSTRACT

The effects of low dose chronic radiation on plant disease resistance and fungal and virus infections have been studied. The results obtained in the 10-km Chernobyl zone demonstrated a decrease in plant disease resistance and appearance of a "new" population of stem rust agents of cereal with a high frequency of more virulent clones. Radionuclide contamination and heavy metals lead to wider virus spread and a higher diversity of virus species. The Chernobyl zone is a territory of enhanced risk and potential threats for the environment. A special type of monitoring of microevolution processes in plant pathogens should provide better understanding of how serious these potential threats are.

Keywords: Chernobyl catastrophe, Low dose chronic radiation, Heavy metals, Fungal and virus infections, Risk assessment

1 INTRODUCTION

Fungal and virus infections play a major role in determining plant performance in both agricultural and natural settings. Changes in plant disease resistance and in the virulence of plant pathogenic organisms under chronic radiation may pose a threat to agriculture, for at least two reasons. First, low dose chronic radiation can induce mutations and speed up the formation of new races. That can result in the appearance of more virulent clones in pathogen populations. Second, low doses may decrease phytoimmunity potential, i.e., innate plant disease resistance.

Since 1986, we have carried out studies of low dose radiation on plant-pathogen interactions in the 10-km Chernobyl exclusion (ChE) zone, the zone surrounding the Chernobyl nuclear power plant (NPP). The biochemical research was related to chronic radiation effects on the innate disease resistance of cultivated plants, especially cereals and corn. Test-systems, which could be useful for estimation of the low dose effects, were developed.

Early on, we had shown that some changes in plant pathogens virulence and aggressivity could take place in the ChE zone (Grodzinsky & Gudkov 2001; Dmitriev, Krizanovskaja, & Guscha, 2002). The aim of this study was to analyze low dose chronic radiation effects and other stresses on plant disease resistance and fungal and virus infections and also to investigate the population structure of plant pathogenic fungi in this zone.

Puccinia graminis Pers. (Uredinales, Basidiomycetes), the causal agent of stem rust in wheat, rye, and oats, is the most damaging disease of these crops. Rust diseases of small grain crops are difficult to control with resistant cultivars because there are many different pathogenic races of the rust fungi. Genes for resistance to wheat and rye rust diseases may be very effective against some races but fail completely against others (Dyakov, 1998).

Plants do not have an immune system of the kind known in animals but possess different inducible defenses against fungal pathogens. Among the defense responses are mechanical strengthening of cell walls, formation of pathogenesis-related (PR) proteins, synthesis of inducible antibiotic substances - phytoalexins, and the increase of enzyme activities, for example, proteinase inhibitors (Dmitriev, 2000). Some of these defenses can be used as quantitative parameters of plant resistance to biotic and abiotic stress factors on the environment.

2 DECREASE OF PLANT DISEASE RESISTANCE UNDER LOW DOSE CHRONIC IRRADIATION

Radionuclide contaminated wheat seeds (M_3) of three cultivars (Mironovskaya 808, Polesskaya 70, and Kiyanka) and rye seeds (cv. Saratovskaya) were collected in the 10-km ChE zone. The incidence and extent of wheat brown rust and powdery mildew were analyzed in the greenhouse. Field trials were carried out in the 10-km zone on three plots with matched soil parameters but differing in dose rates. Two lines of corn were also used in the experiments, original (W64A+/+) and high lysine opaque-mutation (W64A o2/o2), which demonstrate increased sensitivity to stress factors in the environment. The activity of plant proteinase inhibitors was measured in the albumine fraction of corn, wheat, rye leaves, and grains by inhibition of serine proteinase proteolytic activity, estimated by the casein method (Vinnichenko, Filonik, Bilchuk, & Mosolov, 1998). Areal and nutritional plant species for *P. graminis* were determined on grain crops in the 10-km ChE zone. In parallel, we analyzed wild cereals in natural biocenosis neighboring on common barberry (*Berberis vulgaris*), the host for the sexual stages of the stem rust fungus. The stage of urediniospores for fungal development was studied. Identification of pathogenic races of the *P. graminis f. sp. tritici* population was performed on classical Stackman's varieties-differentiators with known resistance genes to stem rust (Semenova, 1977). Three races of response reactions were taken into account: resistant (0-2 points), susceptible (3-4 points), and heterogeneous.

The results obtained suggest that low dose chronic radiation decreased plant immunity potential. Analysis of wheat powdery mildew incidence on three cultivars revealed that the extent of disease was 2-fold higher in plants grown from the seeds collected in the 10-km ChE zone than that in plants grown from control uncontaminated seeds. The data were confirmed in a set of experiments with artificial inoculation of wheat plants in a greenhouse. Seedlings of three wheat cultivars grown from contaminated seeds were infected at the second leaf stage by brown rust spores. It turned out that incidence and disease development in the seedlings was 2.6-fold higher than in seedlings grown from control seeds. Similar results were obtained for two other cultivar wheat seedlings grown from seeds affected by chronic radiation (data not shown).

To elucidate, alterations in cereals disease resistance field trials were carried out in the 10-km ChE zone on three plots with different dose rates. The absorbed doses during the vegetation period were 0.1, 0.8, and 2.7 Gy, respectively. Uncontaminated control wheat seeds were sown on the plots. Leaves were artificially inoculated at the beginning of the milk ripeness phase with brown rust spores. Phytopathological analysis made 5 and 10 days after inoculation revealed that the incidence and extent of brown rust was more severe on plants grown on heavily contaminated plots. The extent of brown rust disease 5 days after inoculation was 2-fold higher on plot 3 (with maximal gamma-background) than on plot 1 (Table 1). Ten days after inoculation, the extent of disease increased on all three plots but still remained highest (68 %) on plot 3.

Table 1. Incidence and extent of brown rust on wheat plants grown on plots with different levels of radionuclide contamination (cv. Kiyanka)

Plot No.	Gamma-background, mR/h	Date of analysis	Incidence of disease, %	Extent of disease, %
1	1-2	15.06 20.06	70 100	23 38
2	9-11	15.06 20.06	99 100	33 46
3	35-37	15.06 20.06	100 100	47 68

It appeared that the differences in wheat brown rust resistance were a result of differences in the absorbed dose of ionizing radiation. The external radiation dose for plants on plot 3 was 27-fold higher than that for plants on the low contaminated plot 1. However, based on data concerning the specific radioactivity of plants (data not shown), it was found that the internal absorbed dose for plants grown on plot 3 was 100-fold higher than that for plants on plot 1.

3 BIOCHEMICAL MECHANISM OF DECREASING IN PLANT DISEASE RESISTANCE

Three wheat cultivars, rye cv. Saratovskaya, and two lines of corn were grown on experimental plots in the 10-km ChE zone. The absorbed dose during the vegetation period was about 7-8 cGy for cereals and 3 cGy for corn. The data obtained show that the activity of proteinase inhibitors (trypsin, chemotrypsin, and subtilysine) in plants grown on radionuclide-contaminated plots decreased. In wheat and rye grains, the activity was decreased up to 15-60 % as compared to the control. The inhibitors could form stable complexes with proteolytic enzymes of pathogens and thus restrict disease development (Metlitskiy, Ozeretskovskaya, & Korableva, 1984). It is not clear, however, if other plant defense responses (phytoalexin synthesis and/or accumulation of PR-proteins) could also be affected by low dose chronic radiation. However, decreasing proteinase inhibitors activity appears to diminish plant disease resistance.

Table 2. Specific activity of proteinase inhibitors in corn grains

N	Sample	Trypsin Inhibitor		Chemotrypsin inhibitor		Subtilisin inhibitor	
		mg/g protein	% of control	mg/g protein	% of control	mg/g protein	% of control
1	W64A+/+ Control	106	-	36	-	118	-
2	W64A+/+ Zone	66	62	23	64	90	76
3	W64Ao2/o2 Control	129	-	20	-	130	-
4	W64Ao2/o2 Zone	34	26	6	30	30	23

This assumption was confirmed by experiments with high lysine opaque-mutation of corn. The activity of proteinase inhibitors was decreased in both grains and leaves of the mutant (W64A o2/o2). In the mutant grains, the proteinase inhibitors activity was 3-4-fold less than in unirradiated plants and 2-fold less than in irradiated plants of the original corn line (Table 2). Clearly, therefore, corn mutation is highly susceptible to ionizing radiation. So far, opaque-mutation of corn as well as *waxy*-mutation of barley (Boubryak, Vilensky, Naumenko, & Grodzinsky, 1992) could be useful tools in understanding low dose effects. Thus, results obtained both in the greenhouse and in field trials demonstrate the decrease in plant disease resistance under low dose chronic radiation.

4 CHANGES IN POPULATION STRUCTURE OF P. *graminis* IN THE 10-KM ChE ZONE

Simultaneously, changes in virulence and aggressivity of plant pathogenic fungi could occur in the 10-km ChE zone. We analyzed such a possibility using *P. graminis*, the causal agent of stem rust in wheat and other cereals. The fungus regularly caused severe epidemics until the mid-1950s. Wheat leaf rust and oat crown rust caused countrywide losses of up to 10% in recent years (Lekomtseva, Volkova, & Tchayka, 2000). These epidemics occurred when new virulent races of rust suddenly increased to destructive levels before new cultivars could be developed with resistance to the new races.

It was an advantage of our research that since 1966 we have studied the sexual stage population of *P. graminis f. sp. tritici* in the "Manevoe" region near Kanev in Ukraine. That allowed us to have a distinctive "zero point" before the Chernobyl accident to analyze the changes in the stem rust population structure in the 10-km zone. To characterize the population structure, it was necessary to identify races with a variety of virulent phenotypes. An analysis of wheat, rye, barley, oats, and grasses in experimental plots in the 10-km ChE zone revealed stem rust development on 12 varieties of cereals. The incidence of disease was about 50-85 % in practically a 100 % damaged crop (Table 3).

Three main forms of the fungus were found: 1) wheat (*P. graminis* Pers. f. *tritici* Erikss. et Henn), which damage both wheat and barley; 2) rye (*P. graminis* Pers. f. *secale* Erikss. et Henn); and 3) oats (*P. graminis* Pers. f. *avenae* Erikss. et Henn). All forms were capable of developing on many cereal grass species, which serve as reservoirs of infection accumulation between vegetation periods. Six hundred forty-two monopustul clones of stem rust were isolated. Among them, 8 physiological races of the pathogen were revealed: 11, 21, 34, 40, 100, 189, 3k, including a race absent from the International register. We named it race "X". All races demonstrated high virulence based on their reactions on a set of 12 wheat lines with different genes for rust resistance (Table 4). Analysis of genotypes of *P. graminis* on monogenic lines showed that more virulent clones were present with higher frequency in the "Chernobyl" population.

Table 3. Stem rust lesions of cereal species on experimental plots

Species		Lesions intensity (%) / Type of lesions (points)			
		"Manevoe" region		10-km ChNPP zone	
1	*Triticum aestivum Will*	50	4	100	4
2	*Secale cereale L.*	80	4	100	4
3	*Hordeum vulgare L.*	65	3	80	4
4	*Avena sativa L.*	90	4	100	4
5	*Agrostis alba L.*	45	3	100	4
6	*Agrostis vulgaris With*	53	3	100	3
7	*Aspera spica-venti (L.) P.B.*	75	3	100	4
8	*Calamagrostis epigeios L.*	25	4	100	4
9	*Dactylis glomerata L.*	100	4	100	4
10	*Elytrigia repens (L.) P.B.*	100	4	100	4
11	*Lolium perenne L.*	40	3	100	3
12	*Poa pratensis L.*	70	3	100	3

Table 4. Reactions of physiological races of stem rust revealed in 10-km ChE zone

Race	Cultivars-differentiators											
	Little Club	Mar-quis	Relia-nce	Kota	Arna-utka	Min-dum	Spel-mar	Kub-anka	Acme	Ein-corn	Ver-nal	Khapli
11	4	4	3++	3	4	4	4	3	3	3	1	1
21	4	4	0	3	4	4	4	4	3	1+	0	1
34	4+	4	4	4	4	4	4	4	3	1	1	1
40	4+	4+	4	4+	4+	4+	4	4=	4	0	4=	1=
100	3	4	3	3	3	3	3	4	1	1	X	1
189	4	4	4	3++	4	4	4	4	4	4	4	4
3k	4	4	4	4	4	4	4	4	2	0	0	1

It was found in 1992 that race 3k (27 %) and 100 (23 %) were dominant in the "Chernobyl" population. Three years later, the majority of isolates contained races 34 (24 %). Races 11 (18 %), 21 (12 %), and 40 (6 %) were also present. Thus, only the widespread races 34 and 3k and the rare 189 remained during 1992-1995. Analysis of three wheat cultivars (Mironovskaya-808, Polesskaya 70, and Kiyanka) inoculated with different races of *P. graminis* revealed a high susceptibility (often "4" points, rarely "3"). Races 11, 21, and 34 are known worldwide. Race 21 was dominant during a few years in ex-USSR countries. Race 189 was of special interest. It induced very high susceptibility on all cultivars-differentiators with known resistance genes to stem rust. The data obtained suggest that active form- and race-producing processes occurred under chronic radiation due to an excess of infection materials in the ChE zone. As a result, a population structure of *P. graminis* has been changed by the appearance of a "new" population with a high frequency of more virulent clones.

Wheat stem rust can cause greater losses than any other wheat disease or pest in Ukraine, but controlling stem rust with resistant varieties is complicated by the diversity of stem rust races that occur and change from year to year. Rust fungi are highly variable, so race specific resistance rarely remains effective for more than a few years even if it seems completely effective at first. New rust races could arise from mutations caused by low dose chronic radiation and may spread into other parts of the country from the ChE zone and overcome the resistance of cultivars that was effective against the old races. Two approaches can improve the durability of rust resistance in cereals: 1) improve our understanding of how rust races compete within rust populations so that we can anticipate virulence shifts in rust populations or manipulate the populations to delay shifts in virulence and 2) identify new races of rust resistance that are either not race specific or that do not select for rapid increases in virulence in rust populations.

Knowledge about chronic radiation-induced pathogen population changes has been growing for 20 years since the Chernobyl catastrophe. Other groups have also confirmed certain changes in harmful organism populations, which have occurred in the ChE zone. For example, 18 physiological races of the causal agent of powdery mildew of wheat were discovered on cv. Kiyanka grown at an exposure dose of 180 mR/h. Among those 18 races, five new races were revealed in the radionuclide contaminated area. Two of the new races possess a high virulence, but also a new virulent race X_4 unknown in Europe was found (Garnaga, 2001). Besides, 15 physiological races of powdery mildew were identified on wheat cv. Polesskaya, three of which were not yet described in the European Cadastre of Species. It is also of interest that the "Chernobyl" population of the Colorado beetle contains a larger number of individuals with less weight (about 120-160 mg compared to 180-200 mg in control) but with enhanced feeding speed, ca. 17 mg/h.

Many of the newly recognized effects of chronic radiation are similar to systemic stress or immune responses, in that there is no simple relationship between exposure and effect and the outcome is not obviously dependent on the dose (Mazurik, 2005). Plant viruses are less numerous than fungal pathogens, but they also could be harmful if the transformed environment facilitated virus infections.

5 POLLUTANTS AND VIRUS DISTRIBUTION IN CENOSES

As viruses are obligatory intracellular parasites of animals, plants, and bacteria, they fully depend on the host organism in their life cycle. This statement is logically followed by the conclusion that all stresses exerted on the host may, in the end, somehow affect virus replication and interaction with its host (unless the host has adequate and fully competent defense mechanisms to diminish or resist the stresses completely). In addition, it is known that various chemical and physical factors do have noteworthy mutagenic potential, and hence, virtually any organism possessing DNA or RNA may experience genetic changes if subjected to, for instance, radioactivity influence.

Moreover, the majority of plant viruses are RNA-containing viruses, and hence, they lack proof-reading mechanisms to repair 'mistakes' in RNA made by polymerases. In consequence, they are even more prone to mutations than cell genomes and other plant pathogens. Despite the lethal character of many mutations in the virus genome, some do not have this characteristic. Derived mutants hypothetically may attain novel properties in terms, for the most part, of the way the virus interacts with its host's (i.e., visual appearance of the disease, replication rate, quantity of virus particles produced per infected cell) ability to utilize new vectors or invade new hosts, etc. Therefore, we were also interested to learn if low dose chronic radiation and heavy metals could increase the spread of viruses. It is not known whether this combined stress affects the development of plant virus infections, as a possible increase of virus content due to an abiotic stress factor might potentiate further easier/faster spreading of the virus, thus raising questions of biosafety and epidemiology.

As we demonstrated earlier, ionizing radiation and heavy metals contamination of soil may lead to significant changes in symptoms induced by virus infection, elevation of virus content in plants, and possible mutations in the plant virus genome (Tyvonchuk, Polischuk, & Boyko, 1998; Budzanivska, Polischuk, & Boyko, 2001; Shevchenko, Budzanivska, Patyka, Boyko, & Polischuk, 2003; Shevchenko, Budzanivska, Shevchenko, Polischuk, & Spaar, 2004). However, our greatest concern lay at the population level of virus infections, as we suspected that there might be an increase in the prevalence and diversity of viruses – raising questions about biosafety. These questions and possible consequences for the environment and plant production are discussed in the following paragraphs.

We are attempting to find links between heavy metal or radioactive contamination and the relative abundance of plant viruses in the region. From one point of view, abiotic stresses might have caused an inhibition of natural plant defense responses, leading to their higher susceptibility to viruses. From another, being applied constantly, these environmental factors could have induced the narrowing of living conditions for plants.

Similar outcomes were demonstrated by Schwartz, Gerard, Perronnet, & Morel (2001). They studied representation of plant species on a Zn-polluted territory near a zinc smelter site. Interestingly, only Zn-hyperaccumulators were capable of growing on the severely contaminated area: *Arabidopsis halleri*, *Armeria maritime*, and *Arrhenatherum elatius*. On the assumption of total biomass, *A. maritima* was the most abundant plant species growing in immediate proximity to the plant (and hence considered to be most tolerant to zinc). With a gradual decrease of Zn content in soil, *A. maritima* was then replaced by *A. halleri* which, in turn, was superseded by *A. elatius* on soils with comparatively lower metal concentration (Schwartz et al., 2001). This data clearly proves that abiotic stresses may limit the number of plant species able to tolerate and survive in an adverse environment. The narrowing of living conditions for plants (and, therefore, for their respective pathogens) could possibly provoke a situation where not all plants would be able to survive in unfavorable conditions, and hence, perhaps not every pathogen would still be able to exert its 'power' on the hosts.

Indeed, that was demonstrated for the Ni-hyperaccumulator plant *Streptanthus polygaloides*. When grown in Ni-rich soil, this species accumulated approximately twice as much *Turnip mosaic virus* (TuMV) as the same plants growing in non-contaminated soil. However, this was not the case for its relative, *Streptanthus insignis*, which is not a Ni-hyperaccumulator. The virus content in systemically infected *Streptanthus insignis* did not depend on the Ni concentration in soil (Davis, Murphy, & Boyd, 2001). In addition, Boyd and Martens (1999) demonstrated that high Ni content in tissues of Ni-hyperaccumulating *Streptanthus polygaloides* did not affect the behavior of aphids which normally feed on these plants in California (i.e., the insects kept on feeding on host plants with elevated content of nickel). This, in turn, means that given higher virus content in plants and availability of vectors, virus transmission may be favoured in heavy metal-contaminated areas.

Keeping this in mind, we selected three regions in Ukraine with different ecological backgrounds: the ChE zone with a durable high rate of radioactivity, the Kharkiv region (Zmiyiv Power Plant area) with confirmed serious heavy metal contamination of soil, and the Volyn region (Shatsk National Park), a clean resort area. In some experiments, we used samples from the Kyiv region as 'clean' controls – even though somewhat polluted (Figure 1). For radioactive contamination, gamma-radiation dose rates for compared regions were 300-520 μR/h for the ChE zone and 5-10 μR/h for the territory of Shatsk National Park.

Figure 1. Map of Ukraine with sampling points indicated. Red dots, from left to right, represent Shatsk National Park (Volyn region), Kyiv region, and Zmiyiv Power Plant area (Kharkiv region), respectively. The black dot represents the sampling area in the Chernobyl region

For radioactive contamination assay, we sampled several different areas within the Chernobyl region: Kopachy, Novo- and Staro-Shepelychi, Chernobyl surroundings, Opatchychi, arid areas in Shepelychi, and Buryakivka villages. For the purpose of chemical pollution analysis, we collected plant and soil samples from the Kyiv region and the area of the Zmiyiv Power Plant in the Kharkiv region for heavy metal content investigation using atomic absorbance spectroscopy (AAS). As reference points, we also collected samples of plants and soil from the area of Shatsk National Park (evidently an ecologically safe region) as referent samples. Further on, all plant samples were tested in an enzyme-linked immunosorbent assay (ELISA) for the presence of *Tobacco mosaic virus* (TMV), *Potato virus X* (PVX), *Potato virus Y* (PVY), *Beet mosaic virus* (BMV), *Barley yellow dwarf virus* (BYDV), and *Cucumber mosaic virus* (CMV), which are widespread in Ukraine (Polischuk, Budzanivska, Ryzhuk, Patyka, & Boyko, 2001). The investigations have been focused on the assessment of plant viruses' distribution and their frequency of occurrence in wild and cultural flora of the contaminated region.

A comparison of the Chernobyl region with that of the Shatsk National Park revealed much less abundant plant species' diversity in the radioactively contaminated area, probably reflecting pressure exerted on plants by stress. Furthermore, an analysis of some specimens of *Elytrigia repens*, *Taraxacum officinale*, *Plantago major,* and *Cirsium arvense* demonstrated substantial differences in virus amounts and representation between the Chernobyl samples and those of other areas. Investigations in two fields near the Opatchychi settlement showed that plant virus frequency distribution was much greater in the Chernobyl samples than in samples from Shatsk National Park. PVX, TMV, and PVY were the most abundant (Figure 2). Similar to the Opatchychi area, plant virus frequency distribution was greater in the samples from the ChE area compared to control plants. This could be a reflection of impoverished species diversity. It should be stressed that this research supports the tendency towards polluted ecosystems being hotbeds of viruses capable of fuelling epidemics (Polischuk et al., 2001).

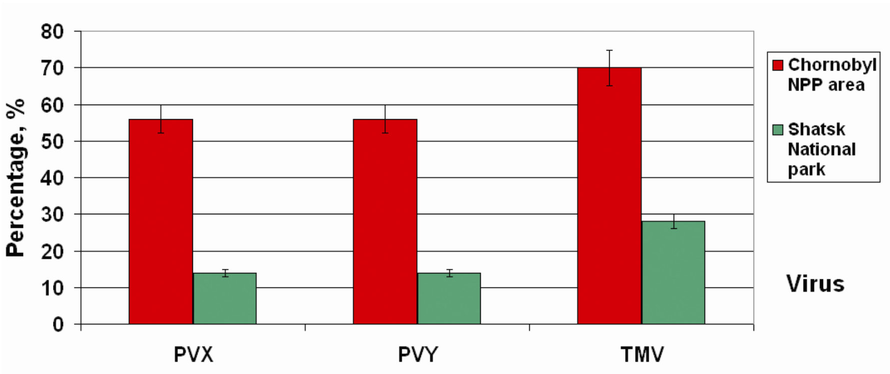


Figure 2. Comparison of encountering frequency for PVX, PVY, and TMV in plant samples from regions differing in the level of radioactivity pollution: Chernobyl NPP area and Shatsk National Park

Analyzing the possible effects of chemical contamination of soil on the spread of plant virus infections, we used atomic absorbance spectroscopy to measure the concentration of several heavy metals in soil samples from the area of the Zmiyiv Power Plant in the Kharkiv region. We have used soil samples from the Kyiv region ('medium' pollution level) and Shatsk National Park as well. As we expected, remarkably high values of metals' content in soil were characteristic for the Kharkiv region. Surprisingly, a comparison of metals' concentrations in the Kyiv region and the area of Shatsk National Park did not show any statistically significant differences for the majority of metal ions tested (Figure 3). It is worth mentioning that the soil samples from these geographical regions analyzed in the study had similar absorbing capacity (Peterson, 1983). Obviously, increased heavy metal concentration in the area of the Zmiyiv Power Plant was due mainly to the activity of the power plant.

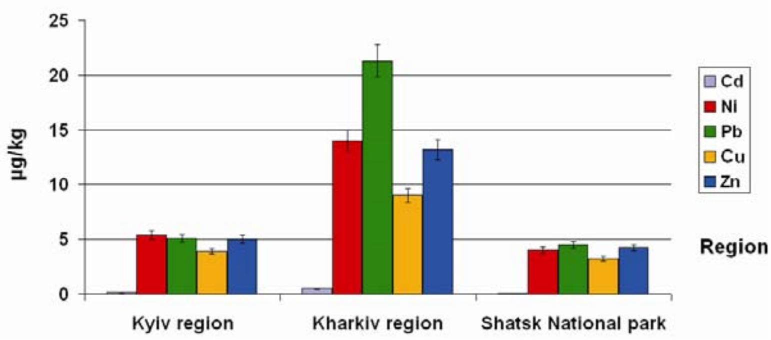


Figure 3. Content of heavy metal ions in soil sampled in Kyiv region, Kharkiv region (Zmiyiv Power Plant area) and Shatsk National Park

Afterwards, we studied plant samples from these sites for the presence of virus antigens in ELISA. Our results showed extremely frequent occurrences of virtually all viruses tested – TMV, BMV, CMV, BYDV, and PVY –

in plant samples from the chemically polluted area (Zmiyiv PP), as opposed to plants from wild flora from the Shatsk National Park. Samples from the Kyiv region demonstrated an intermediate position (Figure 4). Indeed, the Kyiv region was quite similar to the Shatsk area from the point of view of heavy metal content in soil (Figure 3). We suggest that the increased abundance of plant viruses was due to the agricultural practices conducted there. Thus, another reason for low frequency of virus occurrence in the samples from Shatsk National Park might be the absence of any agricultural production. This is not, however, the case for the Kharkiv region (Zmiyiv Power Plant area), as plant samples from this region were collected from uncultivated lands (Polischuk, Senchugova, Budzanivska, Holovenko, & Boyko, 1998).

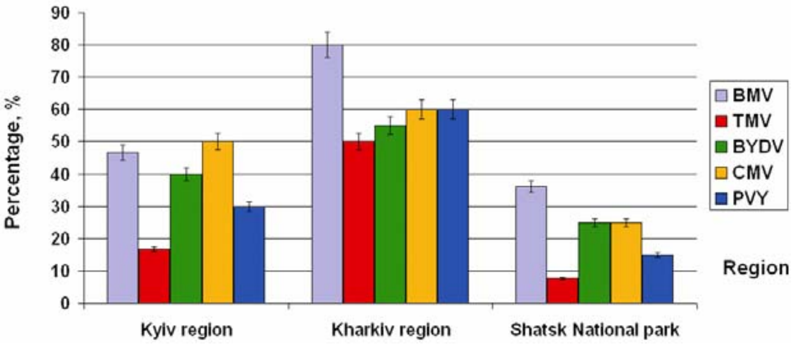


Figure 4. Comparison of virus frequency distribution in plant samples from regions differing in the level of heavy metal soil contamination: Kyiv region, Kharkiv region (Zmiyiv Power Plant area), and Shatsk National Park

The results obtained suggest that abiotic environmental stresses, namely radioactive pollution of the cenoses and heavy metal soil contamination, have significant impact on virus distribution. A primary reason for this may be the narrowing of the diversity of plant species growing on contaminated soil. A second reason is the possible decrease in plant disease resistance as shown for corn and cereals in the ChE zone (Tables 1-2). Whatever the reasons, the consequence might be, again, more efficient spreading of plant viruses, as the plants growing in environmentally polluted areas represent potent foci of infection.

6 CHANGES IN THE DEVELOPMENT OF VIRUS INFECTION

Following the monitoring of the spread of viruses in various regions in Ukraine, we isolated the *Tobacco mosaic virus* (TMV) in association with a range of different symptoms in the same host; *Plantago major*. Two TMV isolates were compared with a reference isolate (TMV strain U1): one from the ChE zone (designated TMV_{ch}) and one from the Shatsk National Park in the Volyn region (TMV_{sh}). Gamma-radiation dose rates in these regions were in the range of 300-520 μR/h for the ChE zone and 5-10 μR/h for the ecologically ‘clean’ territory of Shatsk National Park. We expected the TMV isolates to differ because of the constant radioactive pressure driving mutation and possibly evolution in the virus genome. Indeed, the TMV_{ch} differed from U1 and TMV_{sh} in bioassay tests (Tyvonchuk, Polischuk, & Boyko, 1998).

We have also analyzed 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. This gave us the idea to simulate such conditions in the lab and 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.

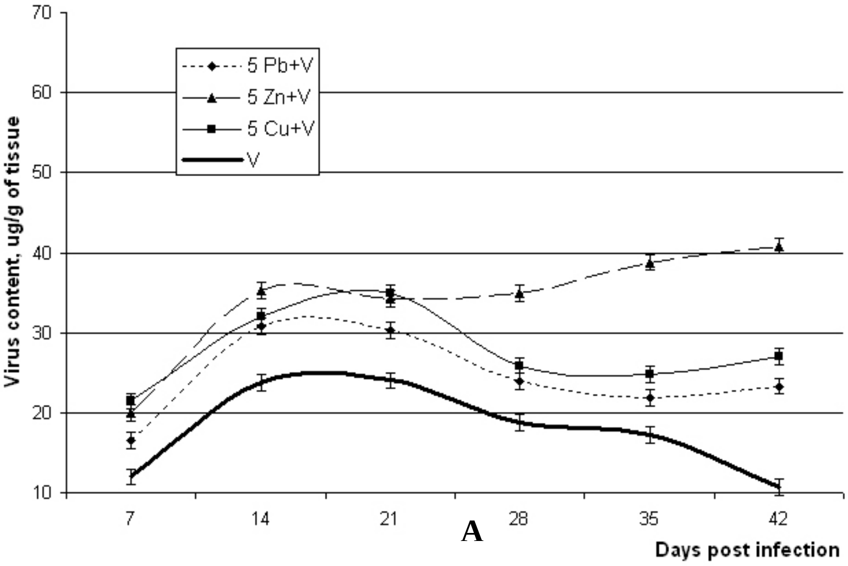
In the first set of experiments, we used TMV and *Lycopersicon esculentum* (tomato), which is systemically invaded. To simulate contamination, we added the heavy metals copper, zinc, and lead in the form of water-soluble salts added to the soil separately (monometal contamination) at 10 times the maximum permissible concentration (MPC) (Kabbata-Pendias & Pendias, 1986). We observed no difference in the type of symptoms induced by TMV on tomato plants, whether heavy metals were applied or not. All infected plants developed mild leaf mosaic followed by deformation of upper leaves. However, it is worth mentioning that among the metals tested, lead caused a delay in the time of appearance of virus-induced symptoms on the plants. Whereas, on all the remaining TMV-infected tomatoes (either grown in sterile or Cu-/Zn-contaminated soil), the symptoms developed by 19 day post infection (dpi), and virus-infected *L. esculentum* plants grown in Pb-treated soil developed similar visual signs of infection only by 26 dpi.

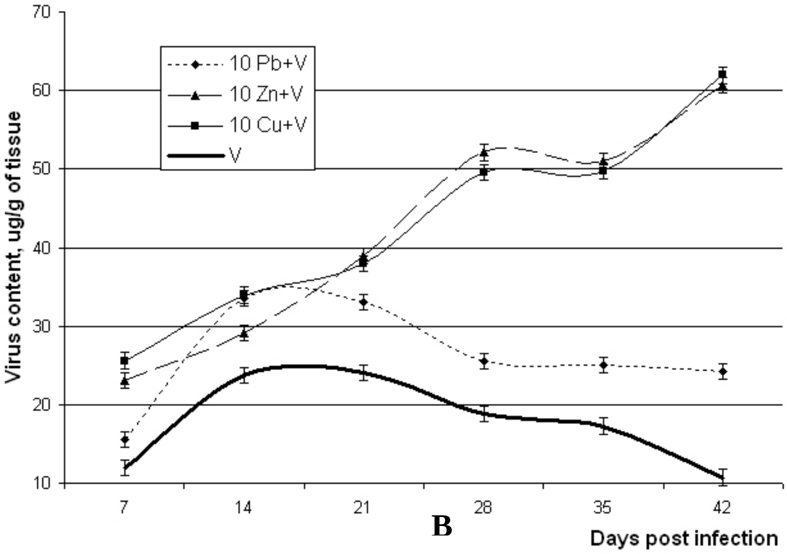
In these experiments, we have also investigated whether there were any changes in the virus content in tomatoes undergoing additional heavy metal stress. It was evident that TMV-infected plants grown in non-amended soil have been accumulating virus for up to 14-16 dpi followed by gradual decrease up to 28 dpi – as is common in many plants (Matthews, 1992). The maximum TMV concentration in infected tomatoes not being treated with heavy metals constituted approximately 70 μg/g of fresh leaf tissue. Conversely, the virus content in Cu-treated plants reached its maximum (21 μg/g of fresh tissue) with 22 dpi, in Pb-treated (192 μg/g of fresh tissue) and in Zn-treated (160 μg/g of fresh tissue) plants – with 22-24 dpi (Shevchenko, Budzanivska, Shevchenko, Polischuk, & Spaar, 2004). From this result, it is clear that zinc and lead actually did induce a significant (more that 2-fold) increment of TMV content in tomato plants. 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.

We used another model system: *Potato virus X* (PVX) – *Solanum tuberosum* cv. Povin' (potato) plants. In this case, we applied zinc, lead, and copper at a range of concentrations (5X, 10X, and 50X MPC) to attain low, medium, and high levels of soil contamination. PVX-infected potato plants grown in soil not amended with heavy metals developed visual symptoms of virus infection by 14 dpi; the symptoms were typical – mild leaf mosaic (Figure 5, *A*). Input of heavy metal to the soil at any concentration tested substantially delayed the appearance of the symptoms up to 21 dpi. Moreover, Zn and Cu at 10X 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 5, *B*) (Shevchenko, Kamzel, Budzanivska, Shevchenko, Spaar, & Polischuk, 2007).

Figure 5. 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 10X MPC; delayed development of severe leaf mosaic by 21 dpi, followed by deformation of younger leaves by 28 dpi

Our study of PVX content in potatoes showed that untreated virus-infected plants accumulated up to 25 µg/g of fresh leaf tissue by 14-21 dpi. All the heavy metals tested in 5X MPC induced a statistically significant elevation in PVX concentration. Zn caused the greatest changes in virus content (1.6-fold increase with 42 dpi; 42 µg/g of tissue) and also in temporal dynamics (Figure 6, *A*). Amendment of Zn and Cu in 10X MPC led to exceptional differences in time dynamics of PVX content. Virus concentration in these plants climbed to 63 µg/g for 42 dpi (2.5-fold elevation). Moreover, even by 42 dpi, we did not detect any decrease in PVX content (which normally follows a maximum of concentration) in plants grown in Cu-/Zn-contaminated soil. Pb in 10X MPC did not induce any differences when compared to 5X MPC values (Figure 6, *B*). Contrary to these data, all heavy metals in 50X MPC did not induce any statistically significant changes in PVX concentration when comparing to infected plants grown in non-amended soil (Figure 6, *C*) (Shevchenko et al., 2007).





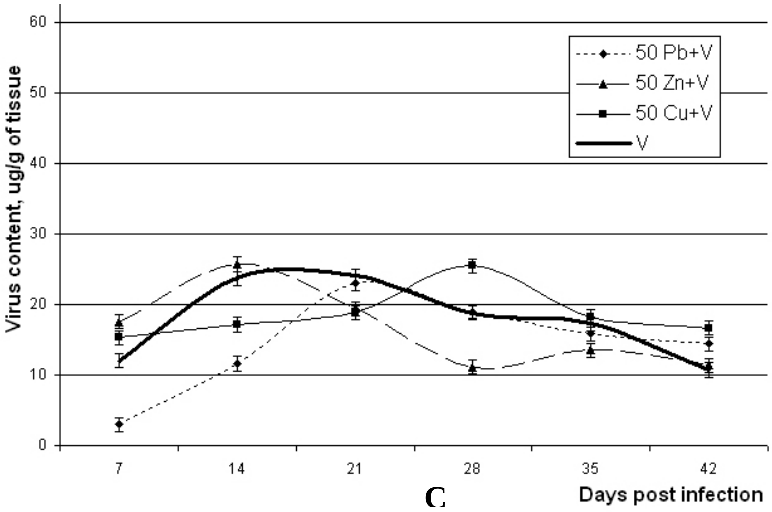


Figure 6. Temporal dynamics of PVX content in fresh leaf tissue of potato plants as affected by heavy metals lead (Pb), zinc (Zn), and copper (Cu) applied in a range of concentrations. *A* – 5X MPC of heavy metals. *B* – 10X MPC of heavy metals. *C* – 50X MPC of heavy metals. *V – virus infection*

Then we tried to establish a correlation between higher virus content and heavy metal concentration in plants. In one of a few such works (Miteva, Maneva, Hristova, & Bojinova 2001), it was shown that tomatoes undergoing virus disease (invoked by *Cucumber mosaic virus* or *Tomato mosaic virus*) tended to have significantly increased concentration of Cd, As, Pb, and Zn, i.e., a biotic stress factor (virus infection) favoured the development of an abiotic one (heavy metals). On the contrary, when grown in Ni-rich soil, the Ni-hyperaccumulator plant *St. polygaloides* accumulated approximately twice as much TuMV as the same plants growing in non-contaminated soil. In this case, conversely, the abiotic stress factor stimulated the advance of the biotic cause – virus infection. However, this was not proven to be the same for its non-hyperaccumulating relative, *St. insignis*. The virus content in systemically infected *St. insignis* did not depend on the Ni concentration in the soil (Davis et al, 2001). As most agricultural plants are not metal hyperaccumulators, data presented by Davis et al. (2001), although of great scientific importance, is insufficient in its practical implications.

Hence, we used a typical model system including TMV and systemically infected tobacco plants (*Nicotiana tabacum* cv. Samsun) for evaluation of relationships between a high content of heavy metal (Zn) in the soil (and thus in plant tissues) and the establishment of TMV infection. The effect of Zn was considered primary as tobaccos were grown in Zn-amended soil prior to inoculation with the pathogen. AAS measurements of Zn content in plant tissues are shown in Figure 7.

Apparently, tobaccos growing in Zn-contaminated soil did accumulate more metal. Analysis of the same plants in ELISA for determination of virus content revealed that plants cultivated in metal-amended soil had increased levels of virus antigens by 7 dpi, in comparison to inoculated tobaccos grown in sterile substrate (Figure 8) (Kamzel, Petrenko, Budzanivska, & Shevchenko, 2007).

In our opinion, together with results of Miteva et al. (2001) and Davis et al. (2001), the presented outcomes demonstrate an interesting phenomenon of interaction between stresses of different origins when exerted on the plant organism. Independent of the initial nature of the stress (whether heavy metal or virus infection), it favors the establishment/progress of another stress.

Results obtained regarding the capability of heavy metals to induce elevation of virus content in systemically invaded plants gave us the idea to investigate the formation of virus-specific inclusion bodies in infected plant cells. Leaf epidermal cells of both intact and TMV-infected tobacco plants (either grown in non-contaminated or Zn-amended soil) were analyzed via dyeing with acridine orange and subsequent luminescent microscopy (Konstantinova-Shlesinger, 1961). Preliminary outcomes of these experiments showed that zinc can possibly influence the generation of virus-induced inclusions in cytoplasm of infected cells in tobacco. No RNA-containing bodies could be identified in the cell cytoplasm of intact tobaccos independent of Zn content in soil. Conversely, as shown in Figure 9, *A*, TMV infection induced the formation of typical cytoplasmic inclusions (irregularly shaped bodies, often distorted rectangles), normally near or around the nuclei. We should stress that most of the TMV inclusions analyzed in absence of zinc contamination were characterized by compact and dense structure without visible splits. On the contrary, TMV-specific inclusions found in epidermal cells of tobaccos grown in Zn-polluted soil were shown to be rather amorphous, greatly varying in form and having numerous splits (Figure 9, *B*).

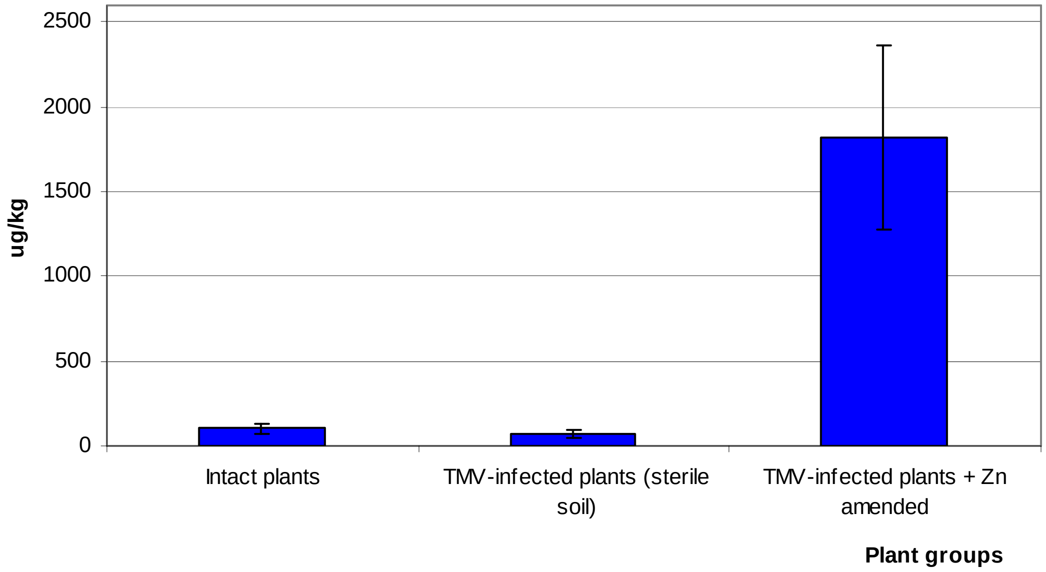


Figure 7. Content of Zn ions in dried plant tissues of tobacco (as sampled by 10 days post amendment of water-soluble Zn-containing compound into soil)

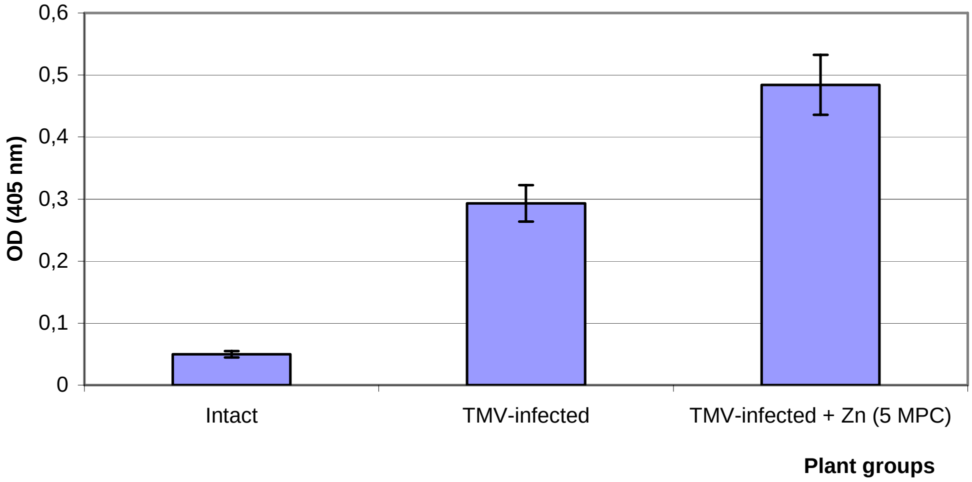


Figure 8. TMV content in the fresh leaf tissue of tobacco plants as affected by the heavy metal Zn (as sampled by 7 days post virus inoculation). Plants grown in Zn-amended soil accumulate more viruses.

In relation to zinc amendment into soil, no differences in localization of virus-specific inclusion bodies have been found. However, these outcomes are preliminary and require careful double-checking. There is a very small amount of data (especially recently published) on the interplay between metal ions and virus particles. It is generally known that virus particles do possess superficial sites for divalent metal cations. In particular, Loring, Fujimoto, and Tu (1962) revealed that TMV virions bind Ca^{2+}, Mg^{2+}, and Pb^{2+}. Authors argued on the need of these sites (and presumably attached cations) in maintaining the stability of virus particles outside the host cell. Further, Durham and Hendry (1977) and Wilson (1984) demonstrated that Ca^{2+} dissociation from these surface sites is required when a virion enters the cell for destabilization of the 3D structure of the virus particle and removal of the subunits of coat protein to release the 5'-end of the virus RNA and start the round of replication. Similar data were achieved for different viruses including *Tobacco rattle virus* (Durham & Haidar, 1977), *Brome mosaic virus*, *Turnip yellow mosaic virus* (Durham, Hendry, & Von Wechmar, 1977), etc. However, there is virtually no available information on the effects metals can play inside the virus-infected cell. Possibly, given the fact that viruses have specific superficial metal-binding sites, metal ions may be capable of specific arrangements of virus particles and, hence, play their role in the formation of virus-induced inclusions in the cell. Indeed, investigations conducted *in vitro* showed that ions of Cd^{2+}, Pb^{2+}, Cu^{2+}, Zn^{2+}, and Ni^{2+} may potentiate side-to-side linking of TMV virions and even precipitation of coupled particles, in contrast to divalent cations of calcium and magnesium (Nedoluzhko & Douglas, 2001). Presented literature data do not allow making any early conclusions about our results on TMV inclusion formation but demonstrate the principal importance of metal ions in the life cycle of plant viruses and their assembly. Taking into account the very high measured content of Zn in tissues of tobacco (Figure 7) and the respectively increased concentration of TMV in these plants, we assume that metals do play some role in observed cellular changes, in yet unknown ways, however.

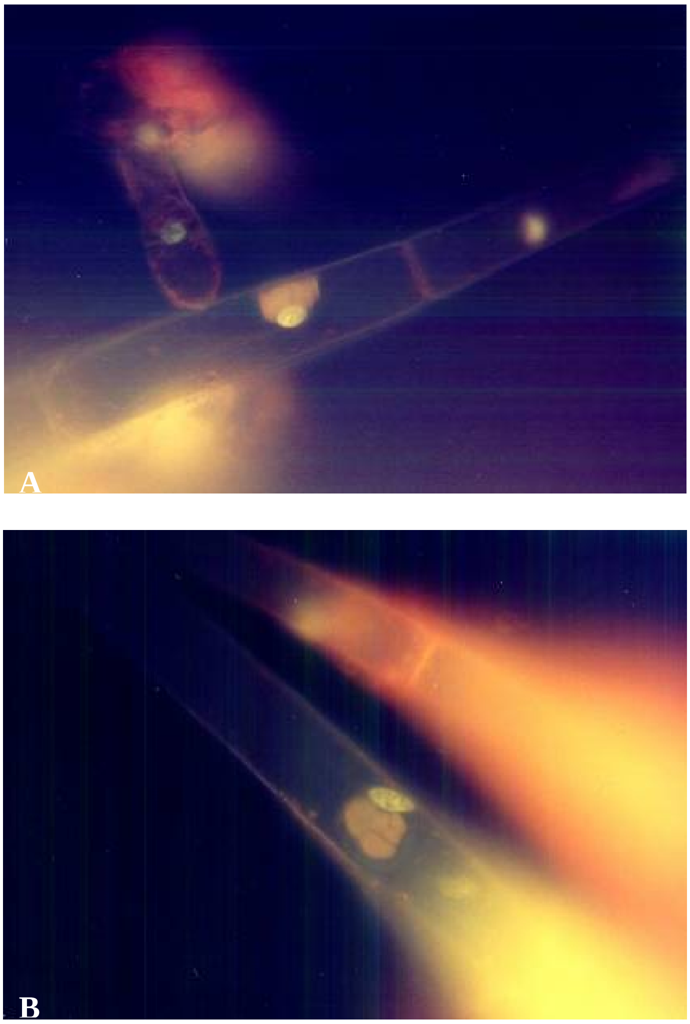


Figure 9. Observation of TMV-induced inclusion bodies in epidermal hairs of tobacco leaves via luminescent microscopy (UV light). ***A*** – TMV-infected tobacco plants cultivated in sterile substrate. TVM-specific orange-colored RNA-containing inclusions with compact structure were detected near/around nucleus. ***B*** – TMV-infected tobacco plants grown in Zn-amended soil. TVM-specific inclusions with non-compact structure (numerous splits) were identified. Dye – acridine orange; Light filter – green; Magnification – x630

Taking this altogether, we believe that abiotic stresses, either heavy metals or radioactivity, may alter and facilitate the development of plant virus infections. These alterations can affect type, severity, and time of appearance of the symptoms invoked by a virus in a systemically invaded host. More significantly, we explicitly show that heavy metals may provoke an enormous increment in virus content in the host tissues. Sometimes this effect seems to be dependent on the nature of the metal, as in the 'TMV-tomato' model system. Conversely, 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 any decrease in virus concentration at the late stage of infection process. At the cellular level, as preliminary results show, formation of virus-induced inclusion bodies may undergo alterations due to chronic effects of heavy metals. Finally, interference seems to exist in mutual action of both stress factors. Constant high concentration of the virus in the host may further increase the prospects of virus transmission to other plants in the ecosystem. Furthermore, the duration of infectivity when plants were under abiotic stress seemed longer comparing to the virus-infected untreated plants.

7 CONCLUSIONS

Results obtained both in greenhouse and in field trials in the ChE zone demonstrate the decrease in disease resistance of different wheat, rye, and corn cultivars under low dose chronic radiation. Analysis of biochemical mechanisms underlying the decrease in plant disease resistance revealed that activity of proteinase inhibitors in grains of wheat and rye under low dose radiation was decreased to 30-55 % as compared to a control. The opaque-mutation of corn was highly susceptible to ionizing radiation. This mutation could be a useful tool in understanding low dose radiation effects. The data obtained suggest that active form- and race-producing processes occurred under chronic radiation due to an excess of infection material in the 10-km ChE zone. As a result, a population structure of fungal pathogen *P. graminis* has been changed by the appearance of a "new" population with a high frequency of more virulent clones. We believe that a special type of monitoring of the evolutionary processes in plant pathogens under chronic radiation should provide better understanding of how serious these threats to agriculture are.

Low dose chronic radiation exerts various effects on both host plants and their pathogens. The resulting interaction complicates the understanding of the mode of action of radionuclide contamination on the host-pathogen system. A better understanding of this complex system might be achieved using near-isogenic lines of both hosts and pathogens. If such lines can be produced for a host-pathogen system, it will enable scientists to distinguish between the relative contributions of the host and the pathogen to plant pathogenesis.

Thus, taken together, these data clearly demonstrate that the ChE zone is a territory of enhanced risk and potential threat for the environment due to mutations of plant pathogens under low dose chronic radiation.

It is too early to make definitive conclusions about the decrease of plant disease resistance and the increase of plant pathogen virulence under low dose chronic radiation. It is well known that plants have evolved on the Earth under a high radiation background. Formerly, however, the radiation stimulation of plant defense responses compensated for the increased virulence of new-forming pathogen races. At this time, however, plant defense mechanisms are weakened for a number of reasons. It is sufficient to mention the orientation of selection research during the last decades toward higher plant productivity, which has resulted in the real decrease of plant disease resistance. Furthermore, pesticide and infection pressure on cultivated plants often exceed their abilities for adaptation.

We have also analyzed possible 'crosstalk' between the negative effects of abiotic environmental stress factors, radioactivity and heavy metals, on plants and the development of virus infections in these hosts. Two main aspects of this analysis were: i) virus infection progress in a single plant (possibly at cellular/organism level) and ii) distribution of virus infections in plants at the population level. Through this analysis, we discovered that abiotic stressors can induce changes in the appearance of virus-specific symptoms on the plants. From the results of a set of laboratory and small-scale field experiments with two model systems, it is clear that chemical contamination of soil may and does favor an enhanced accumulation of viruses in host plants. Sometimes, heavy metals invoked more than a 2.5-fold increment in virus content, compared to virus-infected plants grown in non-polluted soil. The major point is that this elevation of virus content was not temporary; it remained high for a long time. Moreover, it is probable that there is interdependency between heavy metal action and virus infection influence on plants. This effect is probably preceded by alterations in virus infection at the cellular level as shown in our experiments analyzing TMV inclusions in epidermal cells.

Another side of the story was revealed at the population level. We showed that both the abiotic stressors we studied potentially inflicted broader harm from viruses on a given territory. Viruses have been detected in their respective hosts more frequently. As this has been shown for five different viruses isolated from different plant species and for two different stress factors separately, we suggest it is indeed the case. The outcomes and suggestions are summarized in Figure 10. However, what exactly is happening to the plants undergoing stresses of abiotic nature at molecular level, which makes possible easier/faster/more efficient development and further spread of virus infection, still remains elusive.

Furthermore, more work is needed to determine if there is any influence of radioactivity/chemical pollution on virus vectors which would, in turn, affect plant virus epidemics. For instance, there are quite a few ecological and toxicological papers describing effects of heavy metal contamination on insects and related taxa that present somewhat contradictory data. Laskowski (2001) observed the inhibitory effect of Cd on *Acyrthosiphon pisum* population numbers for a long time. In addition, Culliney and Pimentel (1986) argued that plant cultivation in soil treated with sewage waters (characterized by a high concentration of various heavy metals and some organic compounds) severely reduced the intensity of reproduction of *Myzus persicae*, a typical aphid vector for many plant viruses. However, the unequivocal role of heavy metals could not be established in that case. The negative influence of Cd, Hg, and Pb ions (although not chronic) was also shown on *Aiolopus thalassinus* (Schmidt & Ibrahim, 1994). As a contradiction to previous data, Boyd and Martens (1999) demonstrated that Ni-hyperaccumulating *St. polygaloides* plants still remain a feed source for aphids *Acyrthosiphon pisum*, as well as for *Melanotrichus boydi*; the latter are able to accumulate as much as 800 ug of Ni/g of dry weight (Schwartz & Wall, 2001). Also, increased levels of Cd and Cu were demonstrated not to affect populations of another widespread aphid, *Aphis fabae* – an important virus vector (Crawford, Hodkinson, & Lepp, 1995). As aphids represent the most important class of plant virus vectors at both the intra- and interpopulational level, there is a probability of appearance of new reservoirs of aphids and viruses they transmit at phytoremediation and contamination sites (Davis et al., 2001).

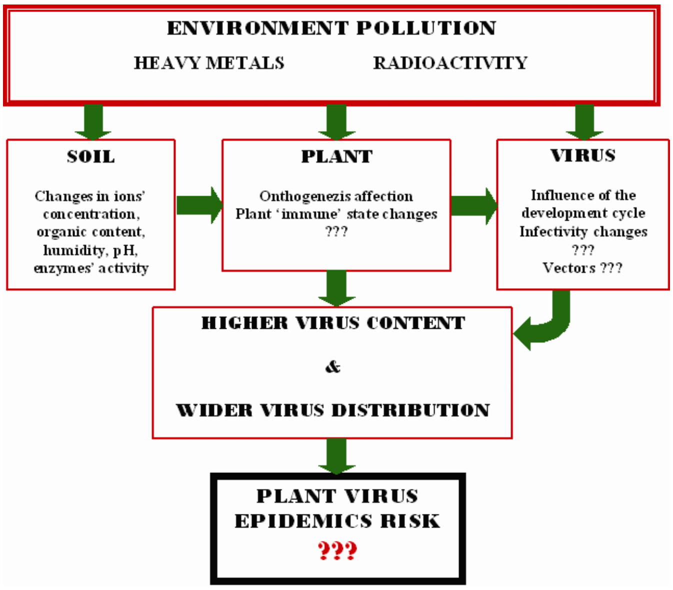


Figure 10. Schematic representation of possible connections and consequences in the development and distribution of virus infections in plants undergoing additional abiotic stress

Finally, we propose that virus infections behave quite differently when their hosts undergo additional stresses of abiotic nature. Viruses tend to accumulate to higher levels in plant tissues, and virus infections tend to spread more successfully. 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 (radioactively/chemically) contaminated environments to avoid their spreading to the neighboring agrocenoses.

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