AN OVERVIEW REGARDING THE INCIDENCE OF SOME NECROTIC VIRUSES IN SEVERAL ROMANIAN AREAS

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Abstract
PMTV (Potato Mop-Top Virus) and PAMV (Potato Aucuba Mosaic Virus) are new damaging pathogens of potato crop in Romania. They can cause serious reduced yield and decrease of the tuber's quality. In case of these viruses, infected plants do not allways show signs of disease and the symptoms depend on potato cultivar, virus strain and the environment. PMTV and PAMV infection of material plants can produce spraing in tubers of sensitive varieties. PMTV causes heavy losses in northen Europe and it is very difficult to control. PAMV is a helping virus for the development of dangerous potyvirus like Potato Virus Y (PVY). Elimination of all these pathogens from the potato supply chain is essential for potato production and choosing resistant varieties to the infection with these viruses could be one of the measures recommended for farmers and producers. The results of this preliminary study shows that PMTV and PAMV were not found in the material collected from the following varieties: Armonia, Azaria, Christian and Millenium (samples collected from the counties Braşov, Covasna, Harghita, Suceava in 2019, 2020 and 2021).
Keywords: *Potato Mop-Top Virus, Potato Aucuba Mosaic Virus, necrotic viruses*

INTRODUCTION

The genetic and physiological traits specific to the potato, as well as the particularities of its crop technology, require compliance with strict requirements regarding the phytosanitary status of the material to be planted. During the growing season, plants can be easily infected with viruses that quickly lead to degeneration, by progressively decreasing the production capacity. The losses recorded due to viruses are much higher than with other plants, due to the fact that potato is propagated vegetatively. Damages and economic losses of potato virus diseases are due primarily to the reduction of plant growth leading to reduced production or even its destruction (Valkonen J.P.T., 2007). This is why protective measures of culture against viral infections, diagnosis and control of virus diseases play an important role in potato production technology and multiplication (Bădărău C.L. *et al.,* 2014 & 2016).

Potato Mop Top Virus (PMTV, family *Virgaviridae*, genus *Pomovirus*) and Potato Aucuba Mozaic Virus (PAMV, family *Alphaflexiviridae*, genus *Potexvirus*) are new viruses identified in the Romanian potato fields.

PMTV can be transmitted by planting infected tubers (Calvert E.L., 1968; Cojocaru N., 1987; Browning I. *et al*., 2001) and by zoospores of plasmodiophorid *Spongospora subterranea*, the powdery scab (Arif M. *et al*., 1995). PMTV tippically produces slightly raised lines and rings on the tubers surface and/or brown arcs and lines, commonly described as spraing, in the flesh of tubers of sensitive cultivars (Harrison B.D. and Kons R.A.C., 1971; Kurppa A.H.J. *et al.*, 1989; Loebenstein G., 2008). Plants produced from infected tubers may also produce misshapenor cracked tubers (Calvert E.L., 1966) often

with reticulated surface cracking, sometimes known as elephant hide blemishing, on the skin (Calvert E.L., 1968; Tenorio J. *et al.,* 2006). Symptomes may also develop on the foliar foliage of the plants, depending on the sensitivity of the cultivar. There are three type of foliar symptomes: yellow blotching or ‚V'-shaped chevrons on the leaves, disortion of leaflets in a dwarfed appearance, internode shortening wich is the classic „mop-top" pattern that gives the virus its name.

Potato Aucuba Mosaic Virus (PAMV) is transmitted mechanically and by *Myzus persicae* in a nonpersistent manner (Cojocaru N., 1987); aphid transmission requires a helping potyvirus such as PVA or PVY (Loebenstein G., 2008). Nucleotide sequence analysis of the coat protein gene of PAMV indicated that amino acids residues 14 and 16 from the N-terminus have the DAG sequence (Loebenstein G., 2008).This sequence, also found in the coat of potyviruses, is required for aphid transmissibility (Loebenstein G., 2008). Several virus isolates cause yellow leaf flecking, whereas other cause deformation and stunting. Some cultivars develope necrosis in the tubers or sunken patches on the tuber surface; such symptoms occur when tubers are stored at high (20-21^{o}C) temperature. The Potato Aucuba Mosaic virus symptoms resemble those of Potato Mop-Top virus; both viruses cause necrosis on the tuber surface, but those of PAMV are less clearly patterned than the necrotic rings caused by PMTV (Loebenstein G., 2008).

Massive imports of potato in last years, the continuous "migration" of seed potatoes from one area to another, climate change, inadequate treatments for disease vector control (especially aphids), viral pressure, resistance of varieties are just some of the factors that may favor the spread of these new viruses that recently appeared in the culture.

This research work could help the spread limiting of these pathogens by identifying and implementing methodological control solutions of these viruses in the context of climate change . (Bădărău C.L*. et al.,* 2014 & 2016).

Potato Aucuba Mosaic Virus (PAMV) has several similar aphids vectors like another dangerous pathogen of potato Potato Virus Y Necrotic (PVYN) (Karasev A.V. *et al.,* 2011; Galvino-Costa S.B.F. *et al.*, 2012). These is the raison because in this research work, the samples collected for testing PAMV were examined in addition for PVYn.

The damage caused by these pathogens agent is both quantitative (reduction of production) and qualitative (commercial depreciation of tubers). In case of cultivation of sensitive varieties under favorable conditions, financial losses can be important both for potato consumption (it can become unmarketable) as for seed potatoes (it will be downgraded).

This study aimed to present some of the most important problems caused by the spread of PAMV (recently founded in the potato areas) and to present results regarding the level of infection with these viruses to several varieties cultivated in different Romanian counties in 2019, 2020 and 2021.

MATERIALS AND METHODS

The potato samples were taken from the following geographical regions of our country (different potato seed producers and farmers): Brasov, Covasna, Harghita and Suceava.

The varieties tested were:

- Christian, Sevastia, Brașovia, Castrum, Azaria, Cosiana, Gared, Armonia, Millenium, Temerar(Romanian varieties);
-Bellarosa, Riviera, Sante, Hermes and Carrera (foreign varieties).

The analysis was performed following the protocol Clark and Adams (1977) (Clark M.F. and Adams A.N., 1977) and for testing the tubers (collected in 2019 ans 2020) we used sap from tubers and from their sprouts (Bădărău C.L. *et a*l., 2014 & 2016). Rinsed microplates filled with substrate solution (p-nitro-phenyl-phosphate) were incubated one hour and the absorbance values were estimated at 405 nm (A_{405}) using a TecanSunRise reader (software

Magellan). The samples that have A_{405} values exceeding the cut-off (two times the healthy control samples average) were considered virus infected (Bădărău C.L. *et al.*, 2014 & 2016). The material was tested for 8 viruses (Potato Mop-Top Virus, Potato Aucuba Mosaic Virus, Potato virus Y, Potato Leaf roll Virus, Potato virus M, Potato virus X, Potato virus S and Potato virus A) and we keep only the infected material with the necrotic viruses. This biological material was retested using monoclonal antibodies (mAb) or polyclonal (PCA) (Bioreba, Switzerland).

RESULTS AND DISCUSSIONS

The results of the tests of samples collected from 4 geographical areas of Romania (know like potato seed producers) are presented in brief in the figures 1and 2.

As shown in figure 1, in case of samples collected in 2019, 2020 and 2021 the highest infection level with PAMV and PVY(N) was noticed to varieties Carrera, Bellarosa and Hermes for all viruses.

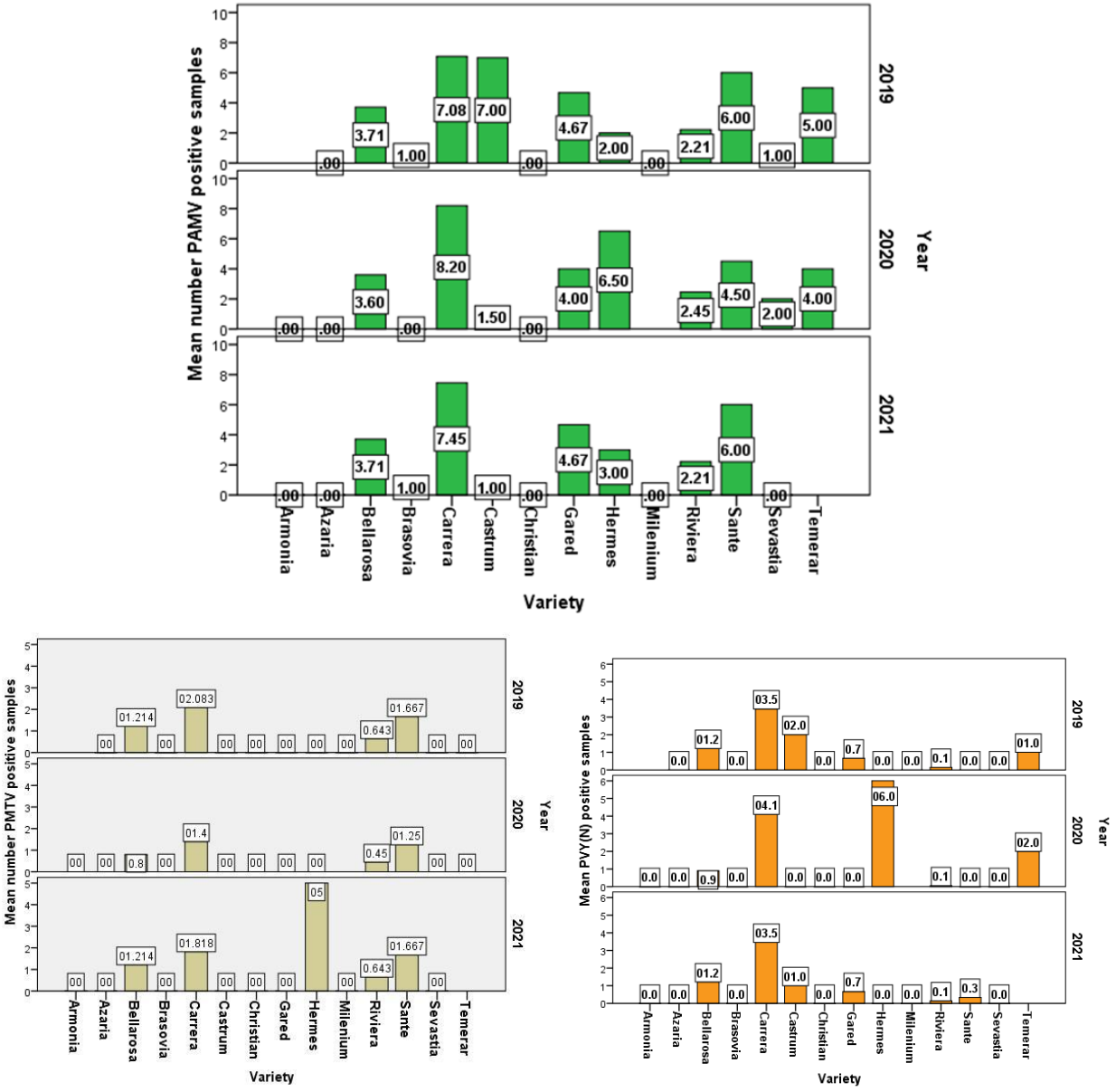

Figure 1. Results regarding the mean of the number of infected samples with PAMV, PMTV and PVY(N) identified in the material tested in 2019, 2020 and 2021 (several varieties more cultivated by seed potato producers). PAMV = Potato Aucuba Mosaic Virus; PVY(N) = Potato Virus Y Necrotic.

As it can be seen in figure 2A, PAMV, PMTV, PVY(N) viruses were identified in all areas, in a higher rate in 2019 and in the lowest rate in 2021. The PMTV virus was rarely encountered, the highest number of infected samples being reported also in 2019, with the exception of the Harghita areas (PMTV virus, year 2020).

The results regarding the distribution of viral infections and the percentage of infection of samples from the 4 areas (related to total tests) in all study years are presented in figure 2B.

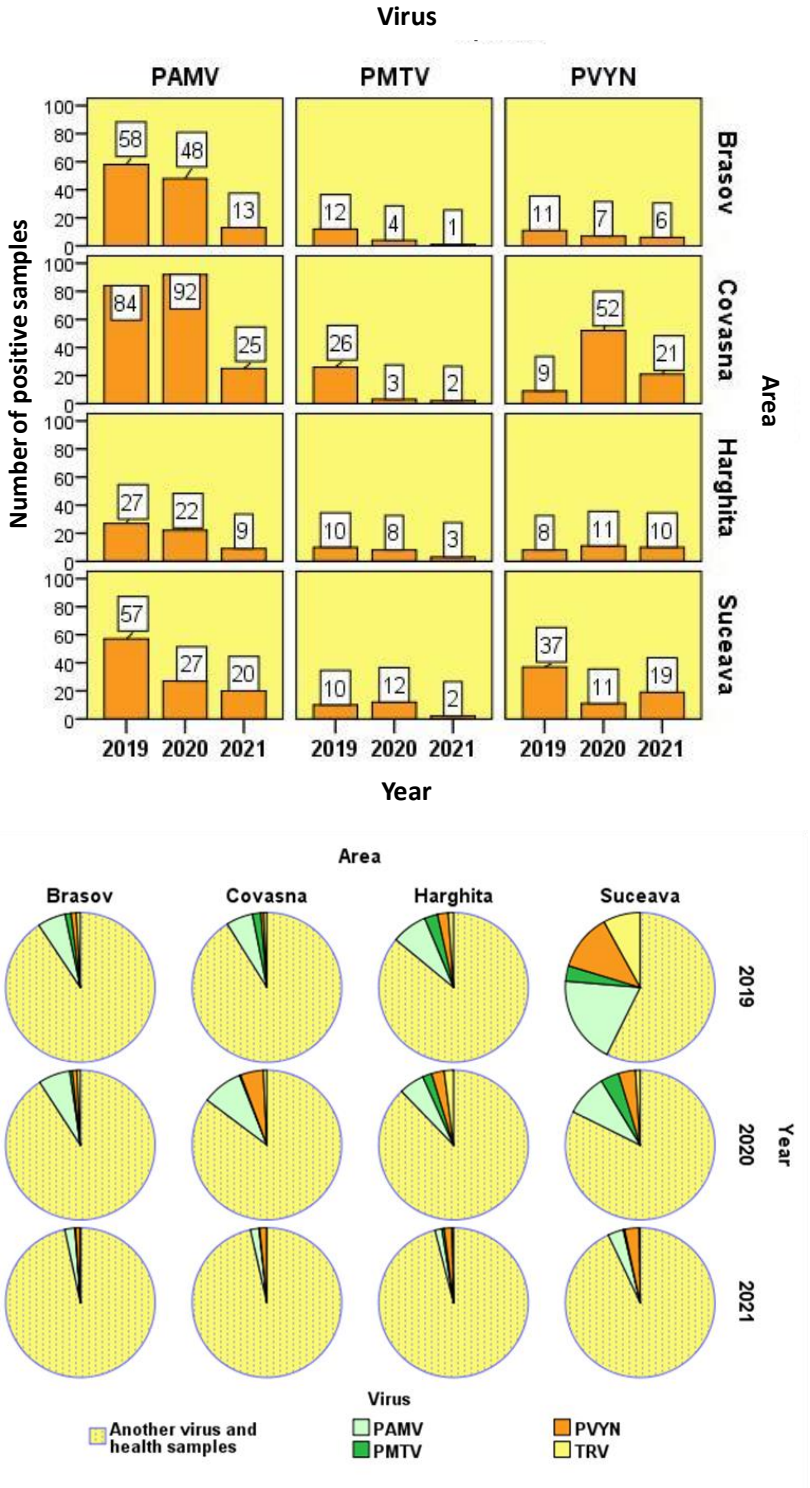

Figure 2. Results regarding the number of infected samples with PAMV, PMTV and PVY (necrotic) identified in the material tested in 2019, 2020 and 2021 (A) and their distribution în the areas (B). PMTV= PotatoMop Top Virus; PAMV = PotatoAucubaMosaic Virus; PVYN = Potato Virus Y Necrotic.

The data presented synthetic in figures 2 and 3 will be used in the future for identify favorable and risk areas and improving potato microzoning - based on spatial and temporary assessment of necrotic viruses PAMV, PMTV and PVY(N), the degree of infection with these viruses correlated with climate change in Romania. So, the source of the samples tested in this study was several geographical areas, different counties (5 producers from Brasov, 15 farmers from Covasna, 7 from Harghita and 7 from Suceava). Regarding the total number of samples tested, 2550 samples were taken from Brasov, 4050 from Covasna, 1500 from Harghita and 1200 from Suceava.

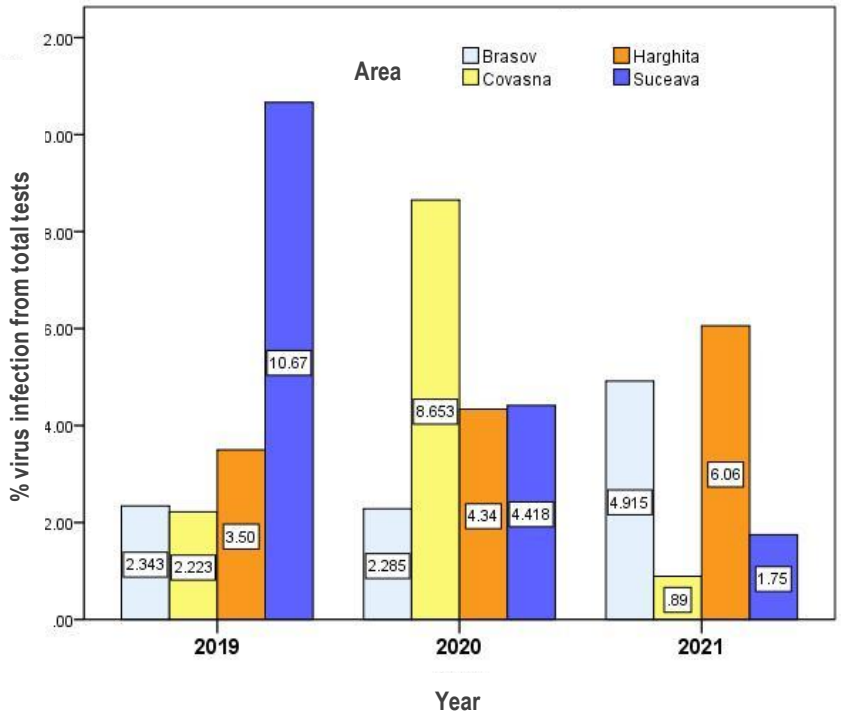

Figure 3. Percentages of samples infected with necrotic viruses (% of total number of samples collected in each region), in function of the county.

Also, the main objective was to identify some varieties non infected with PMTV, PAMV and PVY (necrotic strains), material collected from several Romanian counties. These varieties found total noninfected in our study (in the conditions of the studied counties in 2019, 2020 and 2021) were the following: Armonia, Azaria, Christian and Millenium.

It is important to identify the more PMTV and PAMVsensitive varieties because symptomless infection by these pathogens is known to occur in potato tubers (Sokmen M.A. *et al.*, 1998) but its occurence in foliage is less documented (Browning *et al*., 2002; Montero- Astua *et al.*, 2008). The foliar symptoms of PMTV is influenced especially by environmental temperature (Carnegie S.F. *et al*., 2009 & 2010). Some varieties never or rarely develop foliar symptoms because they are insensitive to PMTV infection on foliage (Davey *et al*., 2006).

In the context of intensify the measures to prevent and control necrotic viruses, the contribution of this paper to the current state of research will result in estimation of PMTV, PAMV and PVY(N) spread to some genotypes grown in our country in order to assess the degree of infection with these viruses to several national and foreign varieties more cultivated in different geographical areas of the country and to identify some potato varieties with high resistance or tolerance to infection with viruses PMTV, PAMV. Also, we

tested the sampes for another necrotic virus PVY(N) because this pathogen facilitates the presence of PAMV.
Transmission of PAMV occurs in the presence of a potyvirus helping virus, (viruses A and Y are the most effective); bothviruses can be acquired simoultaneously by several same aphids (*Myzuspersicae,Aulacorthumcircumflexus, A. Solanişi Aphis nasturtii)*.The viruses that are the subject of this study (PMTV, PAMV, PVYN) are not diagnosed in the seed material certification process.
No detailed studies have been done on the presence of these pathogens in potato crops in our country. So, it will be useful to continue the research work regarding these necrotic viruses especially for the farmers worried about the emergence of new symptoms

CONCLUSIONS.

In our country, although it is known that financial damages brought by necrotic viruses are major in case of growing susceptible varieties under favorable conditions both for consumption potatoes (it can become unmarketable) and for seed potato (it will be downgraded or rejected from certification), to date there has not been conducted a comprehensive study on a spatial expansion of the spectrum of these viruses in our country, study that will contribute to the development of the control of emerging necrotic potato virus like PAMV, PMTV and PVY(N).
In this study, between the varieties tested in 2019, 2020 and 2021 (samples collected from the following counties: Suceava, Brasov, Harghita and Covasna) the lowest level of infection with PMTV, PAMV and PVY (necrotic strains) had the following varieties: Armonia, Azaria, Christian and Millenium.

ACKNOWLEDGEMENTS

This work was supported by the project ADER 511, "*Research regarding the seed potato viruses control and the study of the behavior of Romanian potato breeding lines and varieties in the presence of new viruses with high damaging potential in the context of climate change*", project number 511/27.09.2019 and ADER 522, „*Research on the development of an innovative system for monitoring aphid fauna and systems for monitoring, controlling and testing the biological quality of the seed potato*".

REFERENCES

Arif M., Torrance L., Reavy B., (1995) - *Acquisition and transmission of potato mop-top furovirus by a culture of Spongospora subbterranea f. sp. subbterranea derived from a single cystosorus.* Annals of Applied Biology, 126: 493-503.

Bădărău C.L, Damşa F., Chiru N., (2014) - *Effects of some electrotherapy treatments of PVX infected potato plantlets cv. Roclas, on several biological development indicators*. Journal of Horticulture, Forestry and Biotechnology, 18(3): 25- 29.

Bădărău C.L., Chiru S.C., Rakosy E., Aurori A., OlteanuGh., Ghinea A., Nistor A., Cioloca M., (2016) *–Preliminary srudies regarding the incidence of potato virus Y in seed potato in Romania (for several cultivars)*. Lucrări ştiinţifice.Seria Agronomie, 56(1): 43-48.

Browning I., Craigid J., Darling M., Darling D., Holmes R., (2001)- *Studies on the detection, transmission to progeny and symptom expression of potato mop top virus in potato*. Abstracts of Virology section Meeting of EAPR, 7-13 october 2001, Havlicckuv Brod Trest (Czech Republic). Potato Research 45(2):106.

Calvert E.L., Harrison B.D., (1966) - *Potato mop-top, a soil borne virus.* Plant pathology, 15:134-139.

Calvert E.L. (1968) - *The reaction of potato varieties to potato mop-top virus*. Report of Agriculture Research Minister Agriculture North Ireland, 17: 31-40.

Carnegie S.F., Davey T., Saddler G.S., (2009) - *Effects of the temperature on the transmission of Potato Mop Top Virus from seed tuber and by its vector, Spongospora subterranea.* Plant Pathology, 59:22-30.

Carnegie S.F., Cameron A.M.., McCreath M., (2010) - *Symptoms caused by Potato mop-top virus on potato plants during vegetative propagation in scotland and their association with tuber yield, speriang and tuber infection*. Potato Research, 53 (2), p.83-92.

Clark M.F. and Adams, A.N., (1977) - *Characterization of the microplate method of the enzyme-linked immunosorbent assay for the detection of plant virus*. Journal of General Virology, 34:475-483

Cojocaru N., (1987) – *Viroze.* In: "*Protecţia cartofului: boli, dăunători, buruieni*", Coordonator Plămădeală B., ed. Ceres: 60-84.

Cooper J.I., Harrison B.D., (1976) - *Field and glasshouse experiments on the control of Potato mop top*. Annals of Applied Biology, 83: 215-230.

Davey T., Browning I., Carnegie S.F., Saddler G.S., (2006) - *The importance of potato mop top virus (PMTV) in Scotish seed potatoes*. Proceedings Crop Protection in Northen Britain 2006:375-380

Davey T., Browning I., Carnegie S.F., Mitchell W.J., Saddler G.S., (2008)- *Soil: the principal source of Potato Mop Top virus (PMTV) infection*. Proceedings Crop Protection in Northen Britain 2008:205-210.

Galvino-Costa S.B.F., Figueira A., Camargos V.V., Geraldino P.W., Hu X., Nikolaeva O.V., Kerlan C., Karasev A.V., (2012) - *A novel type of Potato virus Y recombinant genome, determined for the genetic strain PVY^{E}.* Plant Pathology, 61:388-398.

Harrison B.D., Jones R.A.C., (1970) - *Host range and some properties of potato mop-top virus*. Annals of Applied Biology, 65:393-402

Harrison B.D., Jones R.A.C., (1971) - *Factors affecting the development of spraing in potato tubers infected with potato mop-top virus*. Annals of Applied Biology, 68:281-289.

Jones R.A.C.,(1988) - *Epidemiology and control of Potato mop top virus*. In: Cooper J.I., Asher M.J.C., (eds), *Developments in Applied Biology 2: viruses with fungal vectors*. Association of Applied Biologists, Wellwsbourne, UK, p.255-270.

Karasev A.V., Hu X., Brown C.J., Kerlan C.,. Nikolaeva O.V, Crosslin J.M., Gray S.M., (2011) - *Genetic diversity of the ordinary strain of Potato virus Y (PVY) and origin of recombinant PVY strains*. Phytopathology, 101:778-785.

Kirk H.G., (2008) - *Mop-top virus, relationship to its vector*. American Journal for Potato Research, 85:261-265.

Kurppa A.H.J., (1989) - *Reaction of potato cultivars to primary and secondary infection by potato mop-top furovirus and strategies for virus detection*. *EPPO Bulletin,* 19:593-598.

Loebenstein G., (2001) – *Potato Mop-Top Virus (PMTV; Genus Pomovirus).* In: Loebestein G., Berger P.H., Brunt A.A., Lawson R.H. (eds), *Virus and Virus-like Diseases od Potatoes and Production of Seed-Potatoes.* Springer, Dordrecht, p. 95-100.

Loebenstein G., (2001) – *Potato Aucuba Mosaic Virus (PAMV; Genus Potexvirus).* In: Loebestein G., Berger P.H., Brunt A.A., Lawson R.H. (eds), *Virus and Virus-like Diseases od Potatoes and Production of Seed-Potatoes.* Springer, Dordrecht, p. 117-119.

Montero-Astua. M., Vasquez V., Turechek WW., Merz U., Rivera C., (2008)- *Incidence, distribution and association of Spongospora subterranea and Potato Mop Top virus in Costa Rica.* Plant deseases, 92:1171-1176.

Sokmen M.A., Barker H., Torrance L., (1988)- *Factors affecting the detection on potato mop top virus in potato tubers and improvement of tests procedures for more reliable assays.* Annals of Applied Biology, 133:55-63.

Tenorio J., Franco Y., Chuquillanqui C., Owens R.A., Salazar L.F., (2006) - *Reaction of potato varieties to Potato mop top virus infection in the Andes.* American Journal for Potato research, 83: 423-431.

Valkonen J.P.T., (2007) – *Viruses economical losses and biotechnological potential.* In: Valkonen J.P.T. (ed), *Potato Biology and Biotechnology. Advances and perspectives*, Chapter 28, p. 619-641.