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# The evaluation of potato virus Y transfer by some beneficial bugs (Hemiptera: Heteroptera)

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**Abstract.** *Pazyuk IM, Fominykh TS. 2019. The evaluation of potato virus Y transfer by some beneficial bugs (Hemiptera: Heteroptera). Biodiversitas 20: 1222-1227.* The predatory bugs *Podisus maculiventris*, *Orius majusculus*, *Nesidiocoris tenuis*, and *Macrolophus pygmaeus* were commonly used for control of pests in potato meristem culture in greenhouses. In this study, the risk of transmission of potato virus Y (PVY) by entomophagous bugs was estimated in cage experiments. It has been shown that none of the bugs tested was capable of transmitting the PVY. However, *N. tenuis* and *M. pygmaeus* have been shown to damage potato seedlings in the absence of animal food (pests or factitious food). The bug *N. tenuis* caused severer damage to potato plants than did the bug *M. pygmaeus*. Therefore, we can recommend only *P. maculiventris* and *O. majusculus* for pest control in potato meristem culture in greenhouses.

**Keywords:** *Macrolophus pygmaeus*, *Nesidiocoris tenuis*, *Orius majusculus*, *Podisus maculiventris*, potato virus Y

## INTRODUCTION

When growing meristem potatoes and producing minitubers in greenhouses, a number of measures are taken to prevent infection with potato viruses, such as utilization of high-quality meristem potato crops, cultivation of potatoes at geographically isolated sites, roguing, pesticides and mineral oils against virus vectors, e.g., aphids (Boiteau et al. 2009). However, year-round application of pesticides can be the reason why resistant pest populations emerge (Giordanengo et al. 2013), and can negatively affect the health of personnel. Biological protection of potatoes, i.e., the use of entomophages in greenhouses would make it possible to avoid such deleterious consequences. Currently, the main vectors of potato viruses among insects are aphids (Loebenstein et al. 2005). Heteroptera is very little known as vectors of viruses (Wheeler 2001; Mitchell 2004). A well-studied case of the virus transfer by a bug was capsid bug species *Engytatus nicotiana*e transferring velvet tobacco mottle virus (VTMoV), (Sobemovirus). The bug transferred this virus by feeding - excretion without involving salivary glands (no virus was detected in the salivary glands) (Randles et al. 1981, Wheeler 2001). Later, the transmission of phytoplasmas by Orius sp. and Nesidiocoris tenuis was noticed which caused the witch-brooms disease inProtea spp. and Paulownia spp. plants, respectively (Mitchell 2004).

Recently, the information has appeared that *Orius majusculus*, *Nesidiocoris tenuis* and *Macrolophus caliginosus* are capable of carrying Parietaria mottle virus (PMoV, family *Bromoviridae*), which causes mosaic and spots in tomato and pepper plants. Predacious bugs transmitted this virus in the event that infected plants were blooming and contained infected pollen, although the virus was not detected in the mouthparts and the head capsule of the bugs (Aramburu et al. 2010). *Macrolophus caliginosus* can transmit Pepino mosaic virus (PepMV) (Noël et al. 2016, Klapwijk and Stijger 2000). Belgian scientists noticed the interrelation among the density of the predacious bug *M. pygmaeus* on tomato plants, the PepMV infection rate and the severity of damage to tomato fruits (Moerkens et al. 2016).

In the Russian Federation, potatoes are mainly affected by 10 viruses. The five most deleterious are potato virus Y (PVY), potato virus X (PVX), potato virus S (PVS), potato virus M (PVM) and potato leaf roll virus (PLRV), and another five viruses have smaller effect in terms of spreading in Russia and harmfulness, e.g., potato virus A (PVA), potato aucuba mosaic virus (PAMV), potato mop top virus (PMTV), tobacco rattle virus (TRV) and tomato black ring virus (TBRV) (Anisimov 2010). As of today, PVY (species *Potato virus Y*, genus *Potyvirus*, family *Potyviridae*), which belongs to non-persistent viruses, is the most economically significant virus. It is spread everywhere and causes the most notable damage to potato cultivation. During the epiphytory, the yield decreases (56%) by PVY only, and the infection rate for high-value cultivars (cvs.) such as Red Scarlett and Impala reaches 85% and 95% respectively (Fominykh et al. 2017). Also, note that the literature specifies no transfer of any potato viruses by Heteroptera (Rogozina et al. 2016).

Due to the spread of biological plant protection method for meristem potatoes, the use of predaceous Hemiptera in the cultivation of minitubers raises the question of bugs' ability to carry potato viruses. The following bug species were taken for the experiments: *O. majusculus* Reuter (Anthocoridae), *P. maculiventris* Say (Pentatomidae), *M. pygmaeus* Rambur and *N. tenuis* Reuter (Miridae), i.e. species suitable for the biological protection of vegetable plants against a complex of sucking and gnawing pests

(Messelink et al. 2013; DeClercq et al. 2014; Pérez-Hedo and Urbaneja 2016; Sylla et al. 2016).

Predacious bugs *O. majusculus*, *P. maculiventris*, *M. pygmaeus* and *N. tenuis* are zoophytophages, i.e. they use plants as a source of moisture to a various extent (Ruberson et al. 1986; Cocuzza et al. 1997; Pérez-Hedo et al. 2015).

In spite of the fact that Hemiptera in general have been little studied for the transmission of viruses, the literature still refers to the cases of erroneous reckoning of some mirid species to virus vectors. In order to avoid this, the assessment of virus transmission by the bugs should be more grounded, and laboratory studies should be conducted using up-to-date techniques (Wheeler 2001). Therefore, the objective was to assess if the transmission of PVY is possible by *O. majusculus*, *P. maculiventris*, *M. pygmaeus* and *N. tenuis* in laboratory conditions during cultivation of minitubers from meristem potatoes.

## MATERIALS AND METHODS

### Crops, insects and virus cultures

Virus-free laboratory cultures of *O. majusculus*, *P. maculiventris*, *M. pygmaeus*, and *N. tenuis* predaceous bugs were kept in a thermostatically-controlled room at temperatures ranging from 22 to 24 °C, air humidity of 50-60%, photoperiod of 16L:8D. The feed for nymphs and adults of *O. majusculus* was eggs of *Sitotroga cerealella* Oliv. grain moth and *Schizaphis graminum* (Rondani) grain aphids; *M. pygmaeus* and *N. tenuis* were fed with grain moth eggs and pollen; *P. maculiventris* were fed with *Galleria mellonella* L. wax moth larvae. Virus-free laboratory culture of the test control species namely aphids — the green peach aphid, *Myzus persicae* (Sulzer) — was reared by the method of jars filled with water with lids in which holes are made. The bean roots inserted in holes.

PVY transmission by the test bugs species and the control aphids species was assessed using on tobacco cultivar of *Nicotiana tabacum* v. Samsun (line 959) and three potato cultivars namely: Udacha (RF selection), Red Scarlett, and Impala (Dutch selection). Meristem potato crop had been grown on Terra Vita soil for three weeks. NPK extra feeding was made once a week.

To infect the tobacco and potato plants, a PVY isolate collected from the potato cv. Red Scarlett (Harabalinskiy District, Astrakhan Region) was taken. This isolate caused veinal necrosis (VN) in tobacco (cv. Samsun), indicating that it belongs to the $PVY^N$ strain. The tobacco plants were maintained in greenhouse at temperatures ranging from 22 to 26 °C and the $PVY^N$ isolate was supported in tobacco plants by mechanical inoculation.

### Experimental set up

*Probability assessment for virus PVY transmission by bug adults*

Young male and female bugs were selected for the experiments. The healthy intact potato plants were placed along the perimeter of organza cages (40 × 60 × 40 cm): 10 plants per cage. The one tobacco plants infected with PVY were placed in the center of each cage. Further, a group of predaceous bugs *O. majusculus*, *P. maculiventris*, *M. pygmaeus*, or *N. tenuis* was placed onto infected tobacco plants on the basis of 12 adult individuals per intact potato plant. For 3 cultivars and 4 bug species, in total 12 cages were used. For the next 48 hours, the bugs moved freely inside the cage from the infected tobacco plants to the healthy intact potato ones (similar to the methodology described by Aramburu et al. (2010), after which the cages were opened, the bugs were caught, and the number of alive insect specimens was counted on infected tobacco plants, intact potato plants, and inside on the walls of the cage. In parallel, a portion of survived predators from initial number was determined. Potato plants were kept for about three weeks and then the ELISA test was performed. For the ELISA test, test sets for potato virus detection from Lorch Potato Research Institute were used.

For the purpose of control, the possibility PVY transfer by peach aphids *M. persicae* was assessed. Flightless species were used, which prior to the experiment had been carried with thin brushes onto moistened filter paper in Petri dishes to leave them with no feed for an hour. After starving, the aphids were moved onto the infected tobacco plants (5 insect specimens per leaf), where they were kept for an hour and provided with feed. Then, the aphids were carried with a brush onto the healthy potato plants in cages (40 × 60 × 40 cm), on the basis of 20 specimens per plant. The aphids fed on healthy plants for the next 48 hours, moved freely, after which the cages were opened and the number of alive aphids was counted on the plants and inside the cage on the walls. In parallel, a portion of survived specimens from initial number was determined.

*Assessment of the damage caused by the bugs to potato plants*

The rate of damage to potato plants was identified 48 hours after the bugs had been kept in the cages with the infected tobacco and the intact potato plants. Immediately after the bugs had been caught, the portion of the plants with lesions in the form of punctures along the edge of the leaf was identified. The portion of the plants damaged by the bugs was considered as a percentage of the total number of the plants. The plants were cared for three weeks. After that, the density of the next generation bugs (adults and nymphs) F1 on the plants was estimated. All plants of the three potato cultivars (Udacha, Red Scarlett, and Impala) were taken into account, all leaves and stems were inspected. The percentage of damaged leaves and the mean number of lesions per leaf was evaluated for all plants.

*Probability assessment for PVY Transfer by bug nymphs*

The experiment was carried out on *O.majusculus* larvae of 3 to 4 ages and *P. maculiventris* larvae of 1 to 2 ages. The intact potato plants of the three potato cultivars (Udacha, Red Scarlett and Impala) were placed along the perimeter of organza cages (40 × 60 × 40 cm) on the basis of 10 plants per cage. Using a brush, the bug nymphs were placed on the tobacco plants infected with PVY for 1 hour. Then, they were carefully moved onto the intact potato plants on the basis of 12 larvae per plant. The nymphs were given no animal feed, motivating them to search and feed

on the plants. The bug nymphs were kept on the plants for 24 hours, after which the nymphs were removed, and the potato plants were cared for (watering, extra nutrition) during three weeks followed by the ELISA test for the presence of PVY.

**Statistical analysis**

To assess the difference between the two bug species (*M. pygmaeus* and *N. tenuis*), ANOVA, single-factor analysis of variance, was applied based on the mean percentage of plants damaged by the bugs after 48 hours of stay, and the means were compared using the Tukey's test. To analyze the effect of the bugs activity on the damaged leaves percentage, the mean number of lesions per leaf, the mean number of F1 larvae per leaf, and the mean number of F1 adult per leaf, ANOVA, one-way analysis of variance, was applied, where the single factor was the "potato cultivar", and then the single factor was the "bug species". The mean values were compared using Tukey's test. The analysis was performed using SYSTAT 12.0 software.

## RESULTS AND DISCUSSION

**Probability assessment for potato PVY transfer by bug adults**

The outcome of the probability assessment for PVY transfer by bug adults based on ELISA tests shows that the bug adults, which stayed 48 hours in an organza cage with the PVY-infected tobacco plants and the healthy intact potato plants, did not transfer PVY to the healthy potato plants (Table 1). However, the transfer of PVY by *M. persicae* on Impala (10%) and Udacha (10%) cultivars took place. In case of mechanical damage, 100% infection was observed.

**Assessment of damages caused by the bug adults and F1 generation to potato plants**

In the course of the experiment, lesions in the form of punctures along the edge of the leaf made by *M. pygmaeus* and *N. tenuis* bug adults were observed on all potato cultivars after 48 hours (Table 2). The mean percentage of the potato plants damaged during *M. pygmaeus* and *N. tenuis* feeding had no significant differences (Tukey HSD, p=0.385). *O. majusculus* and *P. maculiventris* bug adults did not damage any of the tested potato plants.

In addition, the number of F1 generation bugs was counted, which was the result of egg laying by the bugs within 48 hours (Table 3). The one-way analysis of variance showed that the mean number of nymphs per potato plant did not differ among the cultivars for both *M. pygmaeus* (df=2, F=0, p=1.0) and *N. tenuis* (df=2, F=0.156, p=0.856). Similarly, the mean number of nymphs did not differ among *M. pygmaeus* and *N. tenuis* for cv. Udacha (df=1, F=1.327, p=0.264), cv. Red Scarlett (df=1, F=1.2, p=0.287), and cv. Impala (df=1, F=0.9, p=0.355). The mean number of adult per potato plant did not differ among the cultivars for both *M. pygmaeus* (df=2, F=1, p=0.381) and *N. tenuis* (df=2, F=2.25, p=0.124). The mean number of adult on the potato plants did not differ among *M. pygmaeus* and *N. tenuis* for cv. Udacha, cv. Red Scarlett (df=1, F=2.25, p=0.150), and cv. Impala (df=1, F=1, p=0.330). Furthermore, the mean percentage of damaged leaves per plant did not differ among the cultivars for *M. pygmaeus* (Tukey SHD, p=0.154, p=0.184, p=0.994), while for *N. tenuis* it was significantly higher on cv. Impala (Tukey SHD, p=0.002) and cv. Red Scarlett (Tukey SHD, p=0.0001), than that on cv. Udacha. *N. tenuis* damaged the plants much more than *M. pygmaeus* on cv. Red Scarlet (Tukey SHD, p=0.00001) and cv. Impala (Tukey SHD, p=0.00007). The mean number of lesions per potato leaf was similar among the cultivars for both *M. pygmaeus* (df=2, F=0.967, p=0.386) and *N. tenuis* (df=2, F=0.206, p=0.813). However, *N. tenuis* damaged the leaf areas significantly more, than *M. pygmaeus* did on cv. Udacha (Tukey SHD, p=0.00031), on cv. Red Scarlett (Tukey SHD, p=0.00005), and on cv. Impala (Tukey SHD, p=0.00999).

**Probability assessment for PVY transfer by bug nymphs**

Due to the fact that negative effects of releases on potato plants of two species — *N. tenuis* and *M. pygmaeus* — were observed, only *O. majuscules* and *P. maculiventris* were selected for the experiment with nymphs.

Based on ELISA tests, no transfer of PVY by nymphs of *O. majuscules* and *P. maculiventris* was observed during their staying on infected tobacco plants for one hour and on the healthy intact potato plants for 24 hours (Table 4).

**Table 1.** ELISA test results of probability assessment for PVY transfer by adults of *O. majusculus*, *P. maculiventris*, *N. tenuis*, *M. pygmaeus* bugs and flightless *M. persicae* on three cultivars of meristem potatoes

| Insect species | Potato cultivars | | |
|---|---|---|---|
| | Udacha | Red Scarlett | Impala |
| *O. majusculus* | 0/10* | 0/10 | 0/10 |
| *P. maculiventris* | 0/10 | 0/10 | 0/10 |
| *M. pygmaeus* | 0/10 | 0/10 | 0/10 |
| *N. tenuis* | 0/10 | 0/10 | 0/10 |
| *M. persicae* | 1/10 | 0/10 | 1/10 |
| Mechanical Damage | 3/3 | 3/3 | 3/3 |

Note: * Number of the infected plants/ number of the plants tested

**Table 2.** Percentage of plants damaged by the bugs after 48 hours of stay

| Bug species | Potato cultivars | | | Mean percentage of the damaged plants (Mean±SE) |
|---|---|---|---|---|
| | Udacha | Red Scarlett | Impala | |
| *O. majusculus* | 0% | 0% | 0% | 0% |
| *P. maculiventris* | 0% | 0% | 0% | 0% |
| *M. pygmaeus* | 70% | 80% | 100% | 63±19% a |
| *N. tenuis* | 40% | 50% | 100% | 83±9% a |

Note: The same letters indicate no significant statistical based on Tukey HSD test, p>0.05

**Table 3.** Damages to the plants of three potato cultivars caused by F1 generation bugs

| Bug species | Mean percentage of damaged leaves per plant (Mean±SE) % | | | Mean number of lesions per leaf (Mean±SE) | | | Mean number of F1 larvae per leaf (Mean±SE) | | | Mean number of F1 adult per leaf (Mean±SE) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Udacha | Red scarlet | Impala | Udacha | Red scarlet | Impala | Udacha | Red scarlet | Impala | Udacha | Red scarlet | Impala |
| *M. pygmaeus* | 21.7±4.6aA | 12.5±1.6aA | 22.2±3.9aA | 4.25±0.71aA | 5.7±0.79aA | 4.65±0.77aA | 0.2±0.13aA | 0.2±0.13aA | 0.2±0.13aA | 0aA | 0aA | 0.1±0.10aA |
| *N. tenuis* | 29.8±4.0 aA | 56.4±3.7 bB | 50.8±3.9 bB | 12.9±2.05aB | 13.0±1.36aB | 11.4±2.36aB | 0.5±0.22aA | 0.6±0.34aA | 0.4±0.16aA | 0aA | 0.2±0.13aA | 0aA |

Note: The count was performed 3 weeks after the bugs had been released. a, b: the different small letters designate differences between the cultivars; A, B: the different capital letters designate differences between bug species according to Tukey HSD test, $p < 0.05$

**Table 4.** ELISA test results of probability assessment for PVY transfer by *O. majusculus* and *P. maculiventris* nymphs on the meristem cultures of three potato cultivars

| Bug species | Potato cultivars | | |
|---|---|---|---|
| | Udacha | Red Scarlett | Impala |
| *O. majusculus* | 0/10* | 0/10 | 0/10 |
| *P. maculiventris* | 0/10 | 0/10 | 0/10 |

Note: * Number of the infected plants/ Total number of the plants tested

**Table 5.** Evaluation of the survival rate for the adult and nymph stages of bugs released onto potato plants for PVY transfer assessment

| Insect species | Potato cultivars | | |
|---|---|---|---|
| | Udacha | Red scarlet | Impala |
| | ***Adults*** | | |
| *O. majusculus* | 45% | 57% | 73% |
| *P. maculiventris* | 78% | 88% | 79% |
| *M. pygmaeus* | 73% | 78% | 79% |
| *N. tenuis* | 46% | 74% | 58% |
| *M. persicae* | 39% | 20% | 30% |
| | ***Nymphs*** | | |
| *O. majusculus* | 53% | 72% | 53% |
| *P. maculiventris* | 92% | 90% | 97% |

Percentage of specimens survived after the exposure on potato plants in 48 hours after the release for adults and in 24 hours for nymphs.

## Discussion

As a result of the research, it was established that adult bugs *O. majusculus, P. maculiventris, M. pygmaeus*, and *N. tenuis* mostly remained alive, except for *O. majusculus* bugs on cv. Udacha, where 45% of adults survived, and *N. tenuis* bugs on cv. Udacha, where 46% survived. Similar results were obtained for nymphs of *O. majusculus* and *P. maculiventris* (Table 5). Hence, most specimens had the possibility to transfer PVY. It was noticed that the percentage of aphids surviving the experiment was less than that of the bugs, nonetheless, the peach aphid transferred PVY on cv. Udacha and cv. Impala.

Moreover, damages caused by mirid bugs when feeding on potato plants did not result in their infection with viruses contrary to what was expected. A great number of potato plant lesions in case of *M. pygmaeus* and *N.tenuis* is primarily caused by a high rate of the bugs released — 12 individuals per potato plant of 9-11 leaves. The release standard for these bug species in greenhouses is small (by the example of *M. pygmaeus* — 0.25-5 insects per $m^2$ (https://www.koppert.com/ pests/whiteflies/product-against/mirical/). It is believed that when feeding on plants, mirid bugs, unlike aphids, destroy plant cells, which greatly impairs the ability of the viruses to breed as obligate parasites (Wheeler 2001).

As a result of the statistical analysis of potato plant damages by two bug species, it was found that *N. tenuis* damaged the Udacha cultivar to a lesser extent, than Red Scarlett and Impala. At the same time, the *M. pygmaeus* damaged Red Scarlett and Impala to a lesser extent, than the *N. tenuis* did. The experiment showed the dependence of damages on the plant cultivar and the bug species. This fact should be taken into account when releasing bugs in greenhouses onto potatoes.

Mirid bugs, when feeding on plants, destroy plant cells. They tend to induce reactions in host plant tissues and are capable of provoking responses when wounding the plants which inhibit certain viruses (Wheeler 2001). Potato plants were not blooming during the experiment. This might be the reason why the chances to transfer the virus by the bugs decrease.

In conclusion, we recommend using only *O. majusculus* and *P. maculiventris* species for biological protection meristem potatoes from pests in greenhouses.

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