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ON THE VECTOR PROPERTIES OF *HENOSEPILACHNA VIGINTIOCTOMACULATA* MOTSCHULSKY, 1858 (COLEOPTERA: COCCINELLIDAE) IN THE TRANSMISSION OF POTATO VIRUSES

O. A. Sobko

FSBSI "Federal Scientific Center of Agricultural Biotechnology of the Far East A.K. Chaiki", Timiryazevsky, Ussuriysk, 692539, Primorsky Krai, Russia. E-mail: o.eyvazova@gmail.com

Summary. The survival, transmission, and spread of most viruses depend on insect vectors. The potato ladybird *Henosepilachna vigintioctomaculata* transfers such potato viruses as PVY, PVM, PVX, PVS, PLRV, and PSTVd from diseased potato plants to healthy ones. The plant viruses were detected in all plants by the end of the experiment. The studied potato ladybird beetles had different orientation behavior while searching for food depending on whether they were infected with PVY, PVM, PVX, PVS, PLRV, and PSTVd or not. The infected *H. vigintioctomaculata* fed predominantly on healthy plants (72% of the beetles).

Key words: vector insects, potato ladybird**,** transmission, potato viruses, Primorsky Krai.

О. А. Собко. О векторных свойствах *Henosepilachna vigintioctomaculata* Motschulsky, 1858 (Coleoptera: Coccinellidae) в переносе фитовирусов картофеля // Дальневосточный энтомолог. 2024. N 501. С. 17-24.

Резюме. Выживание, передача и распространение большинства вирусов растений зависят от насекомых-переносчиков. Картофельная коровка *Henosepilachna vigintioctomaculata* переносит вирусы картофеля PVY, PVM, PVX, PVS, PLRV, PSTVd с больных растений картофеля на здоровые, во всех растениях к концу эксперимента обнаружены фитовирусы. Особи картофельной коровки, в зависимости от того, являются ли они носителями PVY, PVM, PVX, PVS, PLRV, PSTVd или были свободны от вирусов, проявляют разную ориентацию в поисках пищи. Инфицированные *H. vigintioctomaculata* в подавляющем большинстве (72% особей) питаются на здоровых растениях.

INTRODUCTION

The survival, transmission, and spread of most viruses depend on insect vectors. They transmit plant viruses in two main ways: circulatory (via the hemolymph) and non-circulatory one (via the cuticle of the mouthparts or the foregut). Circulating viruses enter the hemolymph of an insect through the intestine and travel to its salivary glands making the individual infectious to plants (Raccah & Fereres, 2015). Insect-transmitted plant viruses benefit from

different models of interaction between host plants and vectors. Specific dynamics between plant viruses and their vectors plays a key role in the successful transmission of viral infection. B Raccah and A Fereres have shown that viruses might affect the behavior of vectors in a way that enhances the transmission of infection (Raccah & Fereres, 2009). The spread of persistently transmitted viruses requires vectors to feed on an infected plant for a long time and subsequently transfer the virus to a new host plant. By contrast, non-persistent viruses can be transmitted successfully when vectors take in virions by feeding on an infected host plant for a relatively short time and then quickly spread the infection (Wendimu, 2023). Certain interactions between insects and plants are required for the effective transmission of plant viruses from an insect vector to a new host plant. First, vectors must approach an infected host (the stage of attraction) and then make contact with the host (usually during migration and feeding). Finally, the vector that acquired the virus must transfer it to a new non-infected host (the stage of infection spread) (Whitfield *et al*., 2015). Using insects as vectors, plant viruses are able to cover long distances from infected to healthy host plants. This relationship between an insect vector and a plant virus facilitates the rapid spread of infection and makes it difficult to control (Wendimu, 2023). Usually an insect vector transmits infection while chewing or sucking on the vegetative parts of host plants (Whitfield *et al*., 2015; Wendimu, 2023). Many viruses have a wide range of host plant species. This facilitates their introduction in new environments as well. Most insect vectors are species with either biting and chewing or piercing and sucking type of the mouthparts, such as aphids (Hemiptera, Aphididea), whiteflies (Hemiptera, Aleyrodidae), leafhoppers (Hemiptera, Cicadellidae), thrips (Thysanoptera, Thripidae), and beetles (Coleoptera). Among them, species from the order Hemipteran transmit more than 70% of all the discovered plant viruses spread by insect vectors (Whitfield *et al*., 2015). Our previous research established that *Henosepilachna vigintioctomaculata* Motschulsky, 1858 (Coleoptera: Coccinellidae) was a vector of potato leaf roll virus (PLRV), potato spindle tuber viroid (PSTVd), potato virus S (PVS), potato virus Y (PVY), potato virus M (PVM), and potato virus X (PVX) (Sobko & Matsishina, 2023). The present work is devoted to the study of the vector properties of *Henosepilachna vigintioctomaculata* against potato phytoviruses, as well as the distance orientation of the phytophage relative to healthy and phytovirus-infected potato leaves.

MATERIAL AND METHODS

The experiments were conducted on imagines of the 28-spotted potato ladybird beetle *H. vigintioctomaculata*. The laboratory culture was maintained according to the standard methodology (Matsishina *et al*., 2021). Twenty-five beetles were selected for each of three repetitions. The insects fed on the potato leaves infected with PVY, PVM, PVX, PVS, PLRV, and PSTVd and then were transferred to virus-free potato plants. Before the onset of the experiment, the insects were examined for viral infection. The potato ladybird beetles were transferred to rearing cages with virus-free plants. The potato plants were grown in an aeroponic system VeFarm-Aero (Sharapov *et al*., 2023). Samples were obtained from the plants in 5, 10, 30, 60, 120, 180, and 240 minutes, in one day, and on the fourth, eighth, and tenth day.

The insect orientation behavior was studied according to the standard methodology. The studied sample included twenty-five beetles in each of three repetitions; the exposure time was 240 minutes. We assessed the number of beetles on healthy potato leaves and leaves with mixed viral infection in 5, 10, 30, 60, 120, 180, and 240 minutes (Kapustkin, 2008).

All the insect and plant specimens were examined for viral infection by RT-PCR. The presence/ absence of plant viruses in the samples was analyzed by one-step RT-PCR with fluorescent detection in real time using an amplifier QuantStudio 5 (Applied Biosystems) and

commercial kits “Potato Virus X. Y. M. L. S. A – PB” (Syntol Llc) from the series Phytoscreen designed for the identification of PVX, PVY, PVM, PLRV, PVS, PVA, and PSTVd. (Ryabushkina *et al*., 2012). The software Past v.4.03 was employed for the statistical processing of the research data (Hammer *et al*., 2001; Murtagh & Legendre, 2014). The results obtained were visualized using MS Exel.

RESULTS AND DISCUSSION

Our research established that potato ladybird beetles transferred the following viruses to the healthy potato plants that they fed on: potato virus Y (PVY), potato virus M (PVM), potato virus X (PVX), potato virus S (PVS), potato leaf roll virus (PLRV), and potato spindle tuber viroid (PSTVd). PVM, PVY, and PVX were observed to rapidly spread in the plants. PVM was found in potato leaves in five minutes after infected insects fed on the plant, PVY and PVX – in ten minutes, and PSTVd – in 180 minutes. PVS and PLRV were found in tubers and stolons in eight days. PVY, PVM, and PVX were detected in tubers in two days.

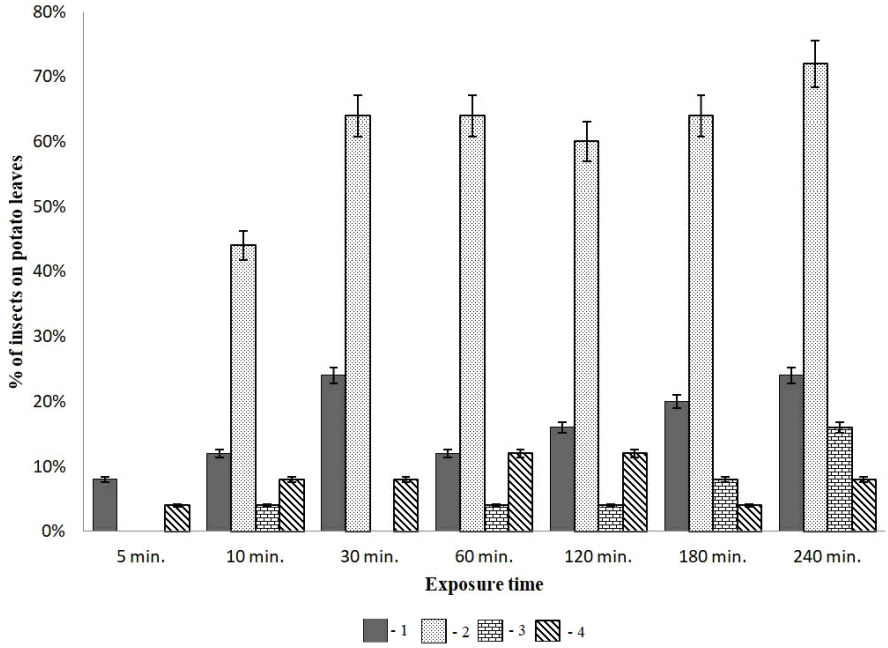

Fig. 1. Chemo-orientation of *Henosepilachna vigintioctomaculata*. Reduction: 1 – % of healthy insects on leaves of healthy plants; 2 – % of infected insects on leaves of healthy plants; 3 – % of healthy insects on potato leaves infected with plant viruses (PVY, PVM, PVX, PVS, PLRV, PSTVd); 4 – % of infected insects on potato leaves infected with plant virus (PVY, PVM, PVX, PVS, PLRV, PSTVd); healthy insects – *H. vigintioctomaculata* with no potato viruses detected in their bodies; infected insects – *H. vigintioctomaculata* with certain potato viruses detected in their bodies (PVY, PVM, PVX, PVS, PLRV, and PSTVd); healthy plants – the potato leaves that were not infected with any plant virus; infected plants – the potato leaves that were infected with one or several plant viruses (PVY, PVM, PVX, PVS, PLRV, and PSTVd).

The following viruses were found in the egg mass laid by infected potato ladybird beetles: PVY, PVM, PVX, PVS, PLRV, and PSTVd. While feeding on plants, *Henosepilachna vigintioctomaculata* damages the tissues of their leaves and thus allow viruses to multiply in them. The viruses travel through plasmodesma to neighbor cells and eventually spread in the tissues. A number of authors suppose that the multiplication rate and biological activity of a given virus depend largely on the physiology of its host plant. This dependence stems from the ability of a virus to exist as an obligate intracellular parasite (Sukhov, 1965; Whitfield *et al*., 2015).

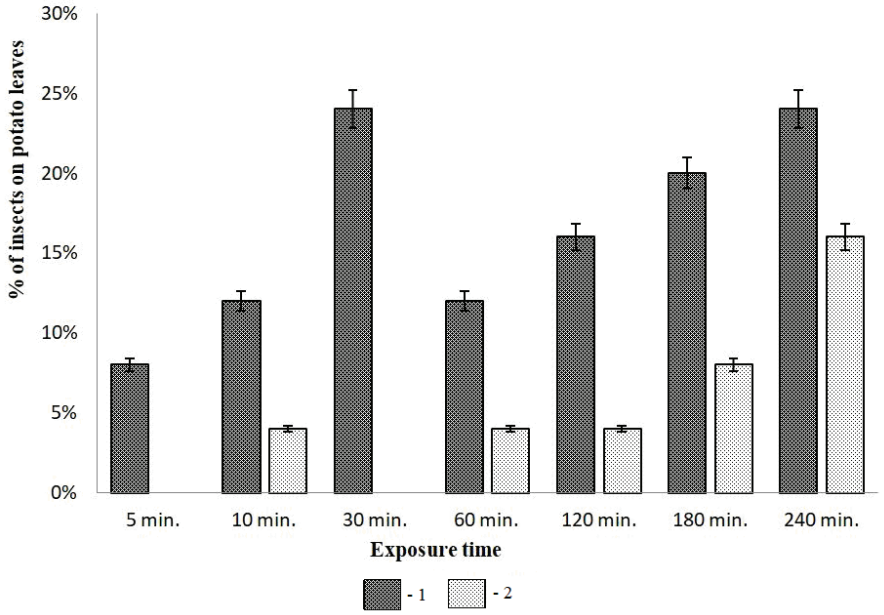

Fig. 2. Chemo-orientation of healthy *Henosepilachna vigintioctomaculata*. 1 – % of healthy insects on leaves of healthy plants; 2 – % of healthy insects on potato leaves infected with plant viruses (PVY, PVM, PVX, PVS, PLRV, PSTVd); healthy insects – *H. vigintioctomaculata* with no potato viruses detected in their bodies; healthy plants – the potato leaves that were not infected with any plant virus; infected plants – the potato leaves that were infected with one or several plant viruses (PVY, PVM, PVX, PVS, PLRV, and PSTVd).

As is commonly known, trophic relationships are among the most important contacts that an organism makes with its environment (Pavlov, 2016). In the course of evolution, leaf-eating insects developed a specific feeding strategy that guaranteed them the regular intake of nutrients and the restoration of energy balance (Pavlov, 2016). In their search for food, insects adopt chemo-orientation – a step-by-step odor inspection, which allows them to find the most suitable spot for feeding (Pavlov, 2016). The behavior strategy of the phytophagous insects towards virus-free and infected potato leaves differed drastically in our research on the chemo-orientation of the potato ladybird beetle. There were more beetles on healthy leaves, they feed actively and laid eggs (Fig. 1).

The feeding activity varied among the insects as well. The imagines that did not transmit viral infection (presumably "healthy") were less active than the insect-vectors. Over the course of the experiment, they pursued the strategy of dispersal trying to distance from each other as far as possible – up to 60% of the total number of beetles in the sample. "Healthy" insects were not observed neither on diseased nor on infected potato plants for the first thirty minutes of the experiment. From 12% to 24% of the total number of beetles were found on virus-free plants within 60-180 minutes after the onset of the experiment. The number of the phytophagous insects started to increase on diseased plants as well in 240 minutes (16%) (Figs 1, 2).

The *H. vigintioctomaculata* individuals with detected plant viruses fed predominantly on healthy potato leaves. Up to 44% of the beetles were found on healthy leaves in 10 minutes. By the end of the experiment (in 240 minutes), 72% of the beetles fed on healthy potato leaves, 8% – on diseased leaves, and 20% crawled inside the rearing cages (Figs 1, 3).

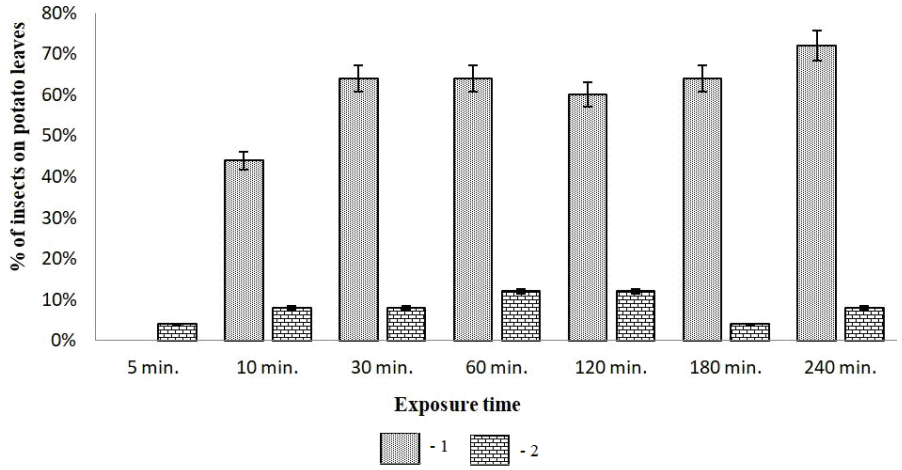

Fig. 3. Chemo-orientation of infected *Henosepilachna vigintioctomaculata.* Reduction: 1 – % of infected insects on leaves of healthy plants; 2 – % of infected insects on potato leaves infected with plant virus (PVY, PVM, PVX, PVS, PLRV, PSTVd); infected insects – *H. vigintioctomaculata* with certain potato viruses detected in their bodies (PVY, PVM, PVX, PVS, PLRV, and PSTVd); healthy plants – the potato leaves that were not infected with any plant virus; infected plants – the potato leaves that were infected with one or several plant viruses (PVY, PVM, PVX, PVS, PLRV, and PSTVd).

Plant viruses use different mechanisms to spread more effectively including the manipulation of the activity of their vector and the efficiency of their transmission either directly or through mutual host plants (Boquel *et al*., 2011). It has been shown that the volatile compounds change quantitatively and qualitatively in plants after infection (Lacroix *et al*., 2014; Rajabaskar *et al*., 2014). Diseased plants become more attractive for insects, especially those not infected with a plant virus (Eigenbrode *et al*., 2002). The infection caused by PLRV leads to a change in the total amount of volatile organic compounds in potato plants affecting the pre-contact behavior of *Myzus persicae* (Sulzer) (Rajabaskar *et al*., 2013). The virus-free *M. persicae* were attracted to the potato plants infected with PLRV at first but after the acquisition

of a plant virus their preferences changed – newly infected aphids preferred healthy plants (Rajabaskar *et al*., 2014; Fingu-Mabola & Francis, 2021). The circulating plant viruses directly manipulate the preferences of their vectors to orient them to healthy plants, which is consistent with our research results.

The potato ladybird beetle is a vector of potato viruses because this species fits all the criteria that determine the specificity of the relationships between vectors and viral agents (Alekseev *et al*., 1988; Chunikhin, 1989). Potato viruses are relatively harmless for the potato ladybird beetle. PVY multiplies in *H. vigintioctomaculata* because it remains in the bodies of insects during their whole life cycle and can be transmitted from one generation to the next (Sobko & Matsishina, 2023). The phytophagous insect displays highly efficient mechanisms of infection transmission, i.e. it is capable of long-distance flights, feeds and lays eggs predominantly on potato plants, and appears on plants already at the germination stage (Ermak *et al*., 2022). Moreover, the potato ladybird beetle feeds on weeds, which are the reservoirs of viral infections, before migrating to potato fields from forests and after harvest (Sobko, 2023). After feeding on a PVS-infected *Sonchus arvensis* L., the potato ladybird beetle migrates to potato plants and then back to weeds. The feeding of phytophagous insects on plant reservoirs as well as the circulation of plant viruses in the hemolymph of vectors facilitate the survival of the pathogen during the period between growing seasons.

CONCLUSION

Henosepilachna vigintioctomaculata transfers such potato viruses as PVY, PVM, PVX, PVS, PLRV, and PSTVd from diseased potato plants to healthy ones. The plant viruses were detected in all plants by the end of the experiment. The studied potato ladybird beetles had different orientation behavior while searching for food depending on whether they were infected with PVY, PVM, PVX, PVS, PLRV, and PSTVd or not. The infected *H. vigintioctomaculata* fed predominantly on healthy plants (72% of the beetles). The potato ladybird beetles that were free of any viral infection tried to spread farther from each other at first and only later started to feed on the potato plants infected with the plant viruses.

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Address: Federal Scientific Center of the East Asia Terrestrial Biodiversity (former Institute of Biology and Soil Science), Far East Branch of the Russian Academy of Sciences, 690022, Vladivostok-22, Russia.
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