Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2021, 11(9), 115-121, doi: 10.15421/2021_304

ORIGINAL ARTICLE

Mass breeding of the predatory mite *Phytoseiulus* by the box method for plant protection

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Received: 01.11.2021. Accepted: 29.11.2021.

The predatory mite *Phytoseiulus persimilis* Athias-Henriot belongs to the Phytoseiidae family of the free-living gamasid mite subfamily Parasitiformes. Under the natural conditions, *Phytoseiulus* lives in warm coastal areas of Chile, Algeria, Lebanon, Italy, southern France, and Australia. In 1958, it was accidentally imported on orchid roots from Chile to Germany. From where it has already been specially delivered to many countries, including the former Soviet Union. In film shelters, greenhouses, winter gardens, and indoor conditions on windowsills, *Phytoseiulus* is successfully used with a very dangerous pest-spider mite. It is also used in the open ground on strawberries and berry bushes when night temperatures are not less than 10-12°C. The classic technology of *Phytoseiulus* mass breeding in industrial biological laboratories includes some technological processes: preparing a room for mites breeding; cultivation of fodder plants for spider mites; breeding of spider mites; breeding of *Phytoseiulus*; collecting and storage of *Phytoseiulus*. Mass breeding of *Phytoseiulus* is carried out year-round on spider mites, which are propagated on soybeans, beans, fodder beans, corn, and cucumber plants. In this article, the authors ground the box method of mass breeding of *Phytoseiulus*.

Keywords: *Phytoseiulus persimilis* Athias-Henriot, *Tetranychus urticae* Koch, Biological protection of plants, Biotechnology, Mass breeding technology, Box method.

Introduction

The predatory mite *Phytoseiulus persimilis* Athias-Henriot belongs to the Phytoseiidae family of the free-living gamasid mite of Parasitiformes order. Phytoseiids are microscopic mites (0.2-0.8 mm), in the Palearctic their size is only 0.6 mm. The dorsal scutellum of the idiosome is covered with 25-36 pairs of setae (a minimum number of 14 pairs was observed in representatives of the genus *Amblyseiulella*) in Palearctic forms. The body is oval, divided into gnathosoma and idiosome (the latter with one dorsal scutellum). The gnathosoma is a complex of oral parts, and the idiosome is the entire rest of the body, carrying 4 pairs of walking limbs.

Predators of other types of mites and some small insects can eat up to 20 phytophagous mites per day. They are connected to land plants and soil. In the fauna of Ukraine, most species are related to plants (about 100 species belonging to 18 of the 32 genera of three subfamilies known in the Patearctic), and geobionts are represented in only 4 genera of the Amblyseiini tribe (*Ambiyseius, Amblyseiulus, Xeoseiuhis, Chelaseius*). Geobiont species have the most ancestral features that bring them closer to their ancestral forms: a smoother surface and larger dorsal scutella covering most of the idiosome; in general, larger body size; dense sclerotization; reduced number of setae (in Palearctic species on the dorsal side of the idiosome, there are up to 23 pairs of them). These predatory mites are natural regulators of the number of different phytophages groups in natural and manufactured plant associations. The most highly effective types of phytoseiids are intensively used in the biological protection of plants in both open and closed ground conditions. More than 2 thousand species are described, grouped into 70 genera (https://ru.wikipedia.org/wiki/Phytoseiidae). The body size of acariphage is about 0.5 mm. The body color varies from orange-red to dark red, sometimes cherry (Fig. 1).

Fig. 1. Predatory mite *Phytoseiulus persimilis* Athias-Henriot and its feeding on spider mites. (http://nsau.edu.ru/images/people/lra/middle-middle-color-center-center-1-0-0-1446306449.673.jpg).

The eggs are oval, milky white with a yellowish-orange tint, measuring 0.18 × 0.21 mm. They differ well from smaller (0.14 mm) and spherical eggs of a spider mite. Eggs of acariphage are more demanding of hydrothermal conditions than larvae, nymphs, and adults. The six-legged larva is yellowish-orange, and the body size is 0.17-0.20 mm. The larvae are slow-moving and do not feed (http://www.biotech-systems.ru/ru/production/entomophages-and-acariphages/*Phytoseiulus*-persimilis/). Mating begins after the last molt of the nymph. Fertilized females lay their eggs among pest colonies, attaching them to webs or directly to the leaf surface. Predator eggs differ from spider mite eggs in their oval shape, pale pink color, and bigger size (Fig. 1). From the eggs, six-legged larvae are hatched, which live at the expense of the embryonic yolk. The larva is near the abandoned eggshell and goes into a dormant state, ending in molting. After the first molt, an eight-legged protonymph (nymph of the first age) appears, feeding mainly on pest eggs, consuming 5-7 pieces during the development period (one day). After finishing feeding, the protonymph molts and turns into a deutonymph (nymph of the second age). It differs from the previous phases in extreme mobility and voracity, destroying 9-16 eggs during the development period (one day). The development cycle ends with the appearance of an adult mite.

Predator has no diapause in its life cycle and develops all year round. However, its development is greatly influenced by temperature and relative humidity. It takes 4.9 days to fully complete one predator generation at 30°C, 5.5 days at 27°C, 6.0 days at 25°C, and 49 days at 10°C. At the same temperatures, the *Phytoseiulus* develops on average 1.5-1.9 times faster than its victim-a spider mite. Predator is incredibly demanding of external conditions during embryonic development. For the development of acariphage, the most favorable temperature is 25-26°C. At a constant temperature of 35-37°C, its eggs do not develop. Larvae, nymphs, and adult mites are less demanding of temperature conditions. They develop satisfactorily at a temperature of 13-33°C, and for 3-4 hours, increasing even to 40-42°C does not affect their development, but when it decreases to 7°C, the development of acariphage stops.

Phytoseiulus is a hydrophilic species, so its development, voracity, and fertility are significantly affected by the level of relative humidity in the air. So, at a humidity of 50%, predator eggs dry out; at 60% egg development is possible only at high temperatures. Young and adult mites are more resistant to lower humidity and generally develop at 60%. At a relative humidity of 25-35%, regardless of the temperature, *Phytoseiulus* cannot develop. For the development of a predator, the optimal relative humidity is 70-80%. The high voracity of *Phytoseiulus* is fully manifested in optimal conditions and depends on the relative air humidity: when it increases, the amount of consumed food decreases. Thus, at a temperature of 25°C and humidity of 50-70%, one female destroys 21-23 spider mite individuals every day in different phases of its development (Fig. 1 and 3). At the same temperature, but with an air humidity of 98%, the predator destroys 11 individuals of the victim. The fertility of female predatory mites depends on several factors. As humidity decreases, the number of laid eggs decreases. At a temperature of 25°C and relative humidity of 30, 50.70-98%, the daily fertility of female *Phytoseiulus* is 0.8, 1.3, and 4.3 eggs, respectively.

The biological parameters of the *Phytoseiulus*, at other equal conditions (temperature, humidity), are mainly dependent on the fodder plant species on which the spider mite feeds. When breeding *Phytoseiulus* on a spider mite that developed on soybean, its fertility averages 69.1 eggs, a rose -59.6, a chrysanthemum-45.1 and a clove 32.5 eggs. A similar tendency is observed with the lifetime of females and the duration of the egg-laying period, which are maximal during the breeding of the victim on soybean and consistently decrease from rose to carnation. The sex ratio is usually 1:4 in favor of females (http://www.biotech-systems.ru/ru/production/entomophages-and-acariphages/*Phytoseiulus*-persimilis/). During the development of eggs, larvae, proto-, and deutonymphs, the natural mortality rate of the predator is observed only in the egg phase and is 2.5% for soybean, 5.4% for rose, 11.6% for chrysanthemum and 14.5% for carnation. The voracity of acariphage on all crops with different lifetime is relatively the same and in terms of the number of eaten eggs ranges from 270 to 340 pieces. At the same time, for each laid egg, the female *Phytoseiulus* eats 4.0 spider mite eggs on soybean, 4.6 on rose, 7.4 on chrysanthemum, and 9.1 on clove.

From a practical point of view, the topics of predator population growth on various fodder plants are of the most significant interest. It was found that during spider mite feeding on soybean, the population of *Phytoseiulus* increases by 28 times on the 10th day, on roses by 20, on chrysanthemums by 16, and on carnations only 11 times. Therefore, under the same conditions for reproduction and equal pest numbers, the acariphage population on a rose will increase almost 2 times faster than on a carnation. This fact should be taken into account when using *Phytoseiulus* on various crops and when choosing a fodder crop during mass breeding of *Phytoseiulus*. Being a specialized predator of spider mites, *Phytoseiulus* cannot remain for a long time on plants that are free of its victim, and soon (in three to four days) dies (Bilyk, 2012). Under the natural conditions, *Phytoseiulus* lives in warm coastal areas of Chile, Algeria, Lebanon, Italy, southern France, and Australia (Bilyk, 2012). In 1958, it was accidentally imported on orchid roots from Chile to Germany. It has already been specially delivered to many countries, including the former Soviet Union (http://fontgarden.ru/sad/179-fitosejulyus). In film shelters, greenhouses, winter gardens, and indoor conditions on windowsills, *Phytoseiulus* is successfully used with a very dangerous pest-spider mite. It is also used in the open ground on strawberries, berry bushes during the period when night temperatures are not less than 10-12˚C (the lowest threshold for the development is 7˚C) (http://fontgarden.ru/sad/179-fitosejulyus).

Materials and Methods

We used an improved technology for mass breeding of the predatory mite *Phytoseiulus* by box method, a technological map of mass predator reproduction has been developed, and technology for its use in the biological protection of plants from spider mites have been proposed.

Results and Discussion

By food specialization, *Phytoseiulus* is a typical oligophagus. It feeds on the spider mite family (two-spotted spider mite, garden spider mite, hawthorn spider mite). There is information about the predator's ability to destroy the species *Bryobia lagodechiana* (early) and *Panonychus citri*. The victim is completely sucked out. *Phytoseiulus* can feed on mites both in the active phases of their development and on their eggs. Females of *Phytoseiulus* destroy up to 30 eggs daily or up to 25 individuals of the pest of later phases of its development. It is better to eat new-laid eggs of the victim. When feeding on diapause females of the spider mite, the predator destroys 4-5 female pests per day, but the acariphage's fertility decreases. Without food, female predators die in 4 days. Adult predators with an excess of victim destroy mainly adult pests and larger nymphs, leaving some nymphs, larvae and eggs to feed the larvae of their own offspring. After destroying the main part of the pest colony, adult mites migrate to other leaves inhabited by spider mites, where they lay eggs again and continue their activities. Nymphs of acariphage almost completely abolish the remains of the pest colony and also move to other leaves in search of food. *Phytoseiulus* is an aggressive predator that is largely adapted to living colonies of spider mites which are highly spun with spider's web. Thanks to morphological adaptations, long dorsal setae and specially arranged praetarsus of limbs, mites can slide between the threads of the web without getting tangled in it. Cannibalism in *Phytoseiulus* is poorly expressed (http://planeta2012.com.ua/produktsiya/biologicheckie-agenti/101-pomoshniki-klechi?start=2/).

Because of the constantly growing demand for environmentally friendly products, *Phytoseiulus* is becoming increasingly popular, which stimulates the necessity of predator mass breeding. The classic technology of mass breeding of *Phytoseiulus* in industrial biological laboratories includes several technological processes: 1) preparation of the room for mites breeding; 2) growing fodder plants for spider mites; 3) spider mites breeding; 4) breeding of *Phytoseiulus*; 5) collecting and storage of *Phytoseiulus* (Bilyk, 2012). We can create a biological laboratory for mass breeding of a predator in any heated room where the air temperature can be maintained within 20-25°C, and the relative humidity is not lower than 70%. Practice has shown that it is most advisable to use specially built winter greenhouses, which are placed at a certain distance from the main production greenhouses, in order to prevent unwanted entering of spider mites the production plantings of vegetable crops. Depending on the time of year, we can also use spring greenhouses under film or glass for this purpose. In summer, for example, when the air temperature does not fall below 10-15°C, it is more expedient to propagate acariphages in spring greenhouses under a film, because they are not inferior in productivity to winter ones and the cost of *Phytoseiulus* is significantly reduced. We often organize mass breeding of predatory mites on a section which is isolated from the production of greenhouse, but the entrance to it is separated.

Continuous, stable getting a predator in such a biological laboratory is possible only with the correct organization and clear sequence of performing the main technological elements of acariphage breeding. The size of the greenhouse of the biological laboratory is determined for every specific case, based on the volume of greenhouse areas planned for biological protection against spider mites. Currently, the following approximate standards have been adopted when planning the size of the greenhouse of a biological laboratory (breeding), depending on the volume of areas intended for biological plants protection from spider mites. So, when protecting cucumbers in winter greenhouses, the area of such a greenhouse should be about 1%, and in spring greenhouses-0.5% of the area planned for biosecurity. Approximately 10% of the area of the breeding greenhouse is allocated for breeding of the mother crop of spider mites. This part of the greenhouse is carefully isolated from its main part in order to prevent unauthorized penetration of *Phytoseiulus* into it, since this can lead to an early termination of the biological laboratory as a result of the destruction of the mother culture of spider mite by a predator.

The remaining area of the greenhouse is divided into seven equable sections on which fodder plants are grown. Plants can be grown both directly in the ground and in boxes, the height of the side walls of which is 25-30 cm, on racks. Of great importance is the rational placement of racks in the greenhouse. Racks are made of boards in the form of decking on props with a height of 60 cm. The width of the side racks of the greenhouse should not exceed 60-70 cm, the middle ones-120 cm. The width of the aisles

between the racks is about 120 cm, and at the ends-50 cm. The distance from the side wall of the outermost racks to the side cover of the greenhouse should be about 40 cm. The length of the racks is determined by the size of the greenhouse. When using racks in winter, it is easier to maintain optimal conditions for plants and mites, since no underground heating is required. To organize a kind of conveyor for obtaining a predator during the entire growing season of cucumber plants, seeds of fodder crop are sown at each of the seven sections at intervals of five to seven days. Accounting for the quantity of *Phytoseiulus* is carried out by calculating its absolute number on test plants with further interpolation of the average value for the entire area of the surveyed rack or section. To obtain a satisfactory level of probability of conducted accounting of acariphage, at least 20 plants must be taken for counting from each square meter of the accounting area. Due to the fact that when calculating *Phytoseiulus*, on average, about 3 hours are spent on an area of 10 m^2, it is not difficult to calculate the total cost of working time associated with performing this operation.

There is a method of accounting for a predator using a special separator, the use of which significantly reduces the unproductive cost of working time associated with estimating the number of acariphages. The technological map of *Phytoseiulus* mass breeding includes the following main stages:

Stage 1: Preparation of the room we divided the area of the greenhouse for spider mites breeding into four to five sections to create a green conveyor. The timing of sowing seeds and the number of sections on the conveyor is determined by the seasonal need for the number of *Phytoseiulus*. Sowing seeds on separate sections is carried out in six to seven days. It should be kept in mind that with this technology, there are no sections for *Phytoseiulus* breeding in the greenhouse, and plants are cut during the period of the greatest number of spider mites on them, so the total area of the breeding greenhouse is reduced by a third of the usual one.

Stage 2: Growing a fodder plant (soybean) for spider mites with this method of predator breeding, soybean plants accumulate not only many spider mites but also thanks to the fibrous stems of the crop, good conditions are created for aeration of the cage, and the plants keep their fodder suitability when placed in the cage during the seven days. Sowing soybean seeds and caring for plants is the same as described above.

Stage 3: Breeding of spider mites 15-20 days after sowing, fodder plants (the phase of three or four real leaves) are populated with spider mites at 40-50 individuals per plant. The accumulation of spider mites on soybean plants by the time they are cut continues until clearly visible marbling appears on the leaves (10-15 days after the mites' colonization). We can use again sections allocated for spider mites breeding for growing soybeans in 30-35 days.

Stage 4: Breeding of *Phytoseiulus* is carried out in a rectangular insect vivarium (box) made of transparent material (organic glass), with a bottom and a cone-shaped or flat roof (Fig. 2).

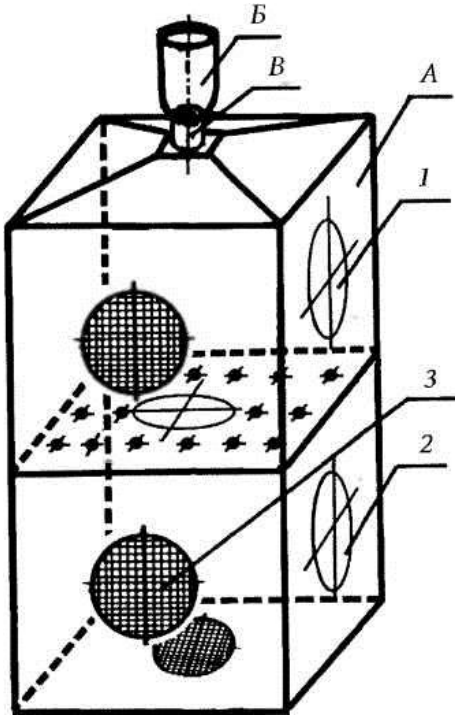

Fig. 2. Vivarium box for *Phytoseiulus* breeding: *A*-vivarium case: 1) loading hole; 2) unloading hole; 3) ventilation hole; *B*-vivarium-receiver; *C*-adapter (according to Bilyk, 2012).

The dimensions of the structure are 30 × 30 × 60 cm. In the center of the roof, there is a neck with an adapter. The adapter corresponds to the size of the neck of the vivarium-receiver, which is a glass jar with a capacity of 0.5-3.0 liters. In the vivarium walls, there are two holes with a diameter of up to 20 cm, through which plants are loaded and removed. These holes are tightly closed with lids. Five holes with a diameter of 15 cm, tightened with nylon net (No. 55), provide aeration of the vivarium. The area for ventilation should be 15-20% of the total area of the vivarium walls. Inside, a horizontal partition, which has a hole with a diameter of 15 cm in the center and many holes with a diameter of 1 cm over the entire surface divided into two identical parts the vivarium box. The bottom of the box has four legs with a height of 5 cm. When breeding a predator, the box is placed in a tray with

water; the holes are covered with lids, the neck is covered with a thick cloth. In a room with a vivarium, the temperature should be maintained at the level of 26-28°C and relative humidity at 40-60%.

To feed the *Phytoseiulus*, plants on which a spider mite's damage to the leaves is 70-80% of the surface are cut. We carried soybean plants cut under the root with a spider mite to the predator breeding room, and we fill the upper part of the vivarium through the loading hole of 1/3 of the volume. Plants are laid out in an equable friable layer, *Phytoseiulus* is settled at 1,000 adults per vivarium. We carried new plants with the same amount of spider mites in every two days to feed the predator. Mobile phases of the *Phytoseiulus* (nymphs and adults) migrate to fresh plants, and predator and victim's eggs remain in the lower layer, serving as food for acariphage larvae hatching. If *Phytoseiulus* accumulates near the neck of the vivarium, the amount of feed must be increased. Five days after the start of *Phytoseiulus* breeding, we redistributed plant remnants; we opened the unloading hole and transferred the first laid layer of plants to the lower part of the box.

In the same way, they act before each subsequent "feeding" of the acariphage. We removed plant remnants from the lower cage of the box every two-three days when the larvae of the *Phytoseiulus* hatched from the eggs move to the plants with the victim in the upper part of the box. The breeding cycle of *Phytoseiulus* in the box lasts 14 days, and during this period, its number increases 30-40 times (Table 1).

Table 1. Technological map of mass breeding of *Phytoseiulus* on spider mites (according to M. O. Bilyk, 2012).

S.No	Types of Work	Terms of Conducting		Purpose and Character of the Work	Optimal conditions		Devices and Equipment	Notes
		Beginning	Duration		Temperature	Humidity		
1	Preparation of the greenhouse room for spider mites			Preparation of the greenhouse room for spider mites breeding	-	-	Greenhouse, racks	Dividing the greenhouse into 4-5 sections to create a
2	Growing fodder plants for spider mites			Growing fodder plants for spider mites	20-25ºC	70%	Greenhouse, racks	400-450 seeds per 1 m^2
3	Settling of fodder plants by spider mites	13-15 days after sowing seeds		Settling of fodder plants by spider mites	20-25ºC	70%	Greenhouse, racks	40-60 individuals per plant
4	Release of the predatory mite *Phytoseiulus*	14-15 days after the release of spider mite		Release of the predatory mite *Phytoseiulus*	20-25ºC	70%	Greenhouse, vivarium 30 × 30 × 60 cm	10-15 individuals per plant
5	Collecting a predator	15-20 days after the release of the predator		Collecting a predator	20-25ºC	70%	Separator	Collecting of 1.5 thousand predator individuals from 1 m^2
6	Short-term predator storage			Short-term predator storage	3-5ºC	80-90%	Refrigerating plant	Storage up to 7 days

Stage 5: Collecting *Phytoseiulus*, two or three days before the acariphage is collected, we do not give the predator food. If the ratio of its and its victim approaches 1:1, it leaves the plants and migrates up. On the opening with an adapter in the roof of the vivarium box, install a cage receiver (a glass jar with a capacity of 1.0 liters) with the neck down. To prevent the acariphage from spreading, we seal the gap between the jar and the receiver wall with adhesive plaster. When *Phytoseiulus* accumulates in the cage-receiver (the walls of the jar are entirely covered with mites), it is replaced with another one (four to five times) until the complete collection of mites. We then filled each jar with 50-100 cm^3 of the crumbly substrate (bran). Mix the substrate in a circular motion and collect the mites from the walls of the jar. The contents of all jars are strewed into one container, and the volume is adjusted to 1 liter. Three or four samples of 1 cm^3 are taken from the received mixture, and we counted the amount of *Phytoseiulus* in each. To do this, pour out each sample on white paper, count the mites coming out of the substrate, and determine the total number of predators in 1 liter of filler. For one cycle of *Phytoseiulus* breeding in the vivarium box, a spider mite collected from plants grown per 1 m^2 is consumed. This ensures getting of 30-40 thousand predator adults. Short-term storage of *Phytoseiulus*-up to seven days is possible at a temperature of 3-5 °C and 80-90% humidity. We apply *Phytoseiulus* on peppers, tomatoes, potatoes, beans, corn, cucumbers, melons, strawberries, eggplants, gerberas, roses and other ornamental plants.

Local method-release near the pest focus, which includes examining greenhouses once a week. Depending on how the leaf blade is damaged, 10-60 individuals are released per plant on average. *Phytoseiulus* is applied on soybean leaves, each of which contains up to 10 individuals. In foci with a high density, spider mites are released in the predator-victim ratio 1:50 and 20-30 individuals per neighboring plants that are not infected with the pest.

Fig. 3. Application of *Phytoseiulus* in a greenhouse on tomatoes (http://www.saninskoe.ru/images/saninskoe06/saninskoe06-210.png).

Mass method

soybean leaves with a predator are laid out evenly throughout the greenhouse, and in the detected foci, they are laid out locally. It is better to use the mass method as a preventive one. 3-4 weeks after plants planting, 30-40 individuals are released per 1 m^2. As the leaf surface increases, the number of releases should be increased. With preventive colonization, it is better to carry out three-fourfold releases of acariphage after 10-12 days, increasing the norm-30 individuals per 1 m^2 for the first time, 40-for the second, 60-for the third, and 80-for the fourth. This contributes to the complete protection of greenhouses from the pest.

Mass colonization

Involves a well-established and reliable breeding system for *Phytoseiulus*. It is necessary to have a greenhouse for breeding, which is 0.5 the biological method will protect-1.0% of the total area of greenhouses. We should note that it significantly reduced the effectiveness of the predator on strongly pubescent crops (http://ggiskzr.promblogus.com/goods/2053-fitoseyulyus_*Phytoseiulus*_persimilis_Ath_Henr.html/). An indicator of the death or destruction of the number of harmful organisms or damage to plants by them expressed biological efficiency.

Conclusion

The predatory mite *Phytoseiulus persimilis* Athias-Henriot belongs to the Phytoseiidae family of the free-living gamasid mite subfamily Parasitiformes. Under the natural conditions, *Phytoseiulus* lives in warm coastal areas of Chile, Algeria, Lebanon, Italy, southern France, and Australia. In 1958, they accidentally imported it on orchid roots from Chile to Germany, from where it has already been specially delivered to many countries, including the former Soviet Union.

In film shelters, greenhouses, winter gardens, and indoor conditions on windowsills, we successfully used *Phytoseiulus* with a very dangerous pest-spider mite. It is also used in the open ground on strawberries and berry bushes when night temperatures are not less than 10-12°C.

The classic technology of *Phytoseiulus* mass breeding in industrial biological laboratories includes some technological processes: preparation of a room for mites breeding; cultivation of fodder plants for spider mites; breeding of spider mites; breeding of *Phytoseiulus*; collecting and storage of *Phytoseiulus*.

We carried mass breeding of *Phytoseiulus* whole year on spider mites, which are propagated on plants of soybeans, beans, fodder beans, corn, cucumber. We suggested the box method of mass breeding of *Phytoseiulus*.

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Citation:
Stankevych, S., Zabrodina, I., Melenti, V., Vasylieva, Yu.V., Kava, L., Golovan, L., Nepran, I., Gavryliuk, A., Kosylovych, H., Holiachuk, Yu., Maslikova, K., Romanov, O., Romanova, T., Bragin, O., Hordiienko, I., Hudym, O., Derevyanko, I. (2021). Mass breeding of the predatory mite *Phytoseiulus* by the box method for plant protection. *Ukrainian Journal of Ecology* 11 (9), 115-121.