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Investigation in the storage of the predatory mite Amblyseius Swirskii under the condition of low temperature

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Goal. To determine the influence of storage of the mite *Amblyseius Swirskii* at the temperature range from 0 to 10°C on the biological and technological indicators of culture. **Methods.** Common in technical entomology and acarology methods of keeping and artificial reproduction of populations of mites. Methods of planning of experiment and experimental data processing: central composite rotatable plan, regression analysis. **Results.** The experiments showed a reduction of viability of predatory mites *Ambliseius Swirskii* with the increase of storage period in low temperatures. Minimization of the loss of predator can be achieved with storage temperature of about 6°C. The viability of the mite under these conditions remains at the optimum (close to the control) level for the storage conditions of not more than 8 days. The two-factor experiment gave the opportunity to obtain appropriate analytical expression — survival is dependent on storage time and temperature. Indicators of female fertility of *Ambliseius Swirsk*i after pre-storage at that temperature during 8 days remain at the optimal level. The effect is shown of low storage temperature on the migration activity of mites — it decreases after 8 days of storage. Keeping in the low-temperature conditions up to 8 days retain the migration activity of predators acceptable for practical use level. **Conclusions.** The optimal storage temperature, which secures the smallest loss of *Ambliseius Swirskii,* is 6°C. The maximum storage time of mite at this temperature — 8 days. Under these storage conditions the viability of the biological material approaching the control indices. Migration activity remains at the level acceptable to use predators in greenhouses. That ensures efficient use of *Ambliseius Swirskii* as an agent of the biological control method.

Key words: *bio method, female mite, Ambliseius Swirskii, survival, fertility, migration activity.*
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The predatory mite *Amblyseius swirskii* (Athias-Henriot, 1962) is used as an agent of the biological method in protecting vegetable and ornamental crops from pests in closed ground conditions (greenhouses, greenhouses, etc.). The specified predator is offered on the market by world famous manufacturers of biological control products for plant protection. According to the data published in scientific periodicals, *Amblyseius swirskii* belongs to those commercially available biocontrol agents for which there is the greatest demand in most countries of the world where the biological pest control is used [1]. Predatory mite *Amblyseius swirskii* beige in size, less than 1 mm. The female mite lays several eggs (from 2 to 4) daily on the leaf blade of plants. At a temperature of + 25 °C, the entire development cycle from an egg to an adult takes about 6 days. At all stages of development, the predator is very mobile and active. The main food source is eggs and whitefly larvae. Alternative food sources are young larvae of various types of thrips, some other small insects, as well as pollen. An individual *Amblyseius swirskii* per day is able to consume 15-20 eggs or 10-15 young whitefly larvae or up to 5 thrips larvae. Life expectancy of adults can reach 1,5 months.

It should be noted that over the past decade in economically developed countries there has been an increase in the attention of agricultural producers to an increase in the share of biological control products in the total volume of plant protection measures. This is due to both increased population demand for organic farming products and legislative initiatives aimed at reducing the chemical load, especially in greenhouses. At the same time, at different stages of the cycle of providing agricultural producers with plant protection products, producers and consumers of beneficial insects and mites face a number of problems. In particular, during the production and use of predatory mites during certain periods, the problem of inconsistency of the rate of delivery of finished products to the needs of the final consumer (farms) arises: sometimes the daily demand for products exceeds the average volumes of production that are determined by the design production capacities, sometimes vice versa - there is an excess of shipped biological control agents. That is, the problem of storing live mites arises. This fully applies to *Amblyseius swirskii*.

Analysis of recent studies and publications on the topic under study. As part of the search for methods for solving the above problem, in the world's specialized research centers, research is being carried out on new technological methods that will increase the shelf life of predatory mites with the least possible loss of production and deterioration of biological indicators that are important from the point of view of using plants. One of the main methods that allows you to store and transport predatory mites for more than 2 days at relatively low losses is the cooling of consumer packaging with substrate and biomaterial to temperatures in the range from 0 °C to + 15 °C. However, without special measures, product loss under such conditions is a significant percentage. Therefore, the researchers proposed a number of methods aimed at reducing the above losses. In particular, during the storage of mites at low temperatures, it was proposed to artificially create and maintain in the consumer packaging the maximum possible relative humidity – up to 100 %. Experiments have shown that such a local creation of hygrothermal conditions can significantly increase the survival of predatory mites [2-5]. Also, such a technique has proved itself as providing predatory mites as a feed for the usual spider mite *Tetranychus urticae* (C. L. Koch, 1836), which was previously introduced into diapause [2, 6, 7]. Such feeding is carried out for a certain time before storing predators at low temperatures. According to existing ideas, an increase in the ability of predators to withstand cold due to this measure is due to the synthesis of a spider mite in the body when it is introduced into the diapause of substances with cryoprotectant properties [7, 8]. The ability of an organism to increase its ability to withstand low temperatures with a sudden sharp drop in ambient temperature is known as «rapid cold hardening» (RCH). For predatory mites, RCH was studied relative to *Neoseiulus californicus* (McGregor, 1954) [9]. In the course of this study, the storage of this predator for 2 hours at a temperature of -10 °C led to the loss of 98 % of adult females. At the same time, if prior to the indicated cooling, preliminary acclimatization was carried out within 1 hour at a temperature of + 5 °C, the mite loss was only + 25 %. The greatest ability to RCH in this experiment was demonstrated by individuals of *Neoseiulus californicus* in the larval stage and especially in the egg stage. A similar induction of RCH in *Neoseiulus californicus* could be caused by acclimatizing it for 2 hours at a temperature of + 30 °C or by keeping the mites in a nitrogen atmosphere for 1-2 hours. Lowering the temperature is not the only and mandatory when storing predatory mites. For example, in the study [10], as a factor contributing to the better storage of Neoseiulus californicus, the maintenance of increased relative air humidity in the volume of mites was determined – 96%. However, unlike the works [2-5], in the publication [10] it was noted that mites remain at a temperature of + 25 °C. A very complete review of various aspects of the formation of certain physiological reactions of phytoseid mites to external influences, including the decrease in ambient temperature, presented in [11].

Investigations of the storage of exactly the mites *Amblyseius swirskii* were carried out by the authors of [12]. In this work, the results of a study of the storage of this predator at a temperature of + 15 °C for a week are presented. As indicated in [12], the density of the predatory mites in consumer packaging continued to decrease significantly over time. Other studies of the storage of *Amblyseius swirskii* at low temperatures, apart from [12], are not known to us.

Thus, the analysis of scientific publications indicates the absence of scientifically based recommendations on the regimes of prolonged tick maintenance, and determined the relevance of our research.

Research aim. Determination of the effect of shelf life of *Amblyseius swirskii* mites at temperatures from 0 °C to + 10 °C on biological and technological indicators of culture.

The determination of the optimal regime of ticks was carried out according to indicators of viability of the predator, fecundity of females, as well as migration activity.

Research methodology. At the first stage of the study, the number of mites that survived depending on the storage temperature and time of storage was determined, that is, a two-factor experiment took place. This involved constructing an appropriate response surface. For this situation, a central composite design was chosen with the condition that the variance of the predicted response value at a certain point depends only on the distance from this point to the center of the plan, and not on the direction to it. That is, the plan was rotatable. According to preliminary rough experiments, it was concluded that storing a predatory tick at temperatures below + 2 °C and above + 10 ° C leads to an unacceptably large loss of production. In addition, it was determined that if predators were stored at temperatures ranging from + 2 °C to + 10 °C for more than 10 days, the volume of losses also stipulated the economic inexpediency of using such a technique. Therefore, for the experiment, the following ranges were initially selected: temperature – from + 2 °C to + 10 °C, storage period – from 2 days to 10 days. Taking into account the use of the rotatable plan, these ranges were slightly expanded and ranged from + 0,3 °C to + 11,7 °C and from 0,3 days to 11,7 days, respectively. The number of complete uniformity trial was chosen equal to 3. For 70 mites were placed in plastic containers, tightly closed. Tanks were located in thermostats (Fig. 1 a)). Predators were kept for 0,3 days, 2 days, 6 days, 10 days and 11,7 days at a temperature of + 0,3 °C, + 2 °C, + 6 °C, + 10 °C, + 11,7 °C, in total – 11 storage options with three-fold repeatability (Table 1). At the end of the storage period of each container (a total of 33 containers with 70 mites in each were examined), the number of surviving mites was recorded.

After determining the optimal storage temperature (see Section "Research Results") in the next, second stage, the fecundity of females that were stored under precisely these conditions was investigated. To this end, adult fertilized females were placed in Petri dishes on cut out round plates of pepper. As a feed, pepper pollen was introduced into Petri dishes. Petri dishes were tightly closed with cling film. Every day mites were transplanted into new Petri dishes and the number of eggs laid was counted. For comparison, the number of eggs that were laid by the females was counted; it was not previously stored at a low temperature (control).

At the last, third stage, the dispersal activity of mites was studied; they were previously stored at a low temperature. For this, in 200 ml polyethylene containers, 100 individuals of the *Amblyseius swirskii* predator were placed. The containers were located in the thermostat at the previously determined optimal storage temperature (see Section "Research Results"). The storage time of mites ranged from 1 day to 8 days. After storage, for each separate period, the mites were placed in a graduated glass of measuring glassware (Fig. 1 b)) with a bottom diameter of 120 mm. The height of the glass is 150 mm. The temperature of the room during this experiment was maintained in the range from + 23 °C to + 25 °C – the temperature range recommended in greenhouses for growing tomatoes and cucumbers. The time the predators reached the edge was recorded in a laboratory journal. Observations were carried out for 2 hours. The repetition of the experiment – three times. The following indicators were determined: the percentage of predators that within 2 hours were not able to reach the edge of the glass, and the time during which 50% of the mites reached the edge. Regression analysis was applied to process the results.

a *b*

Fig. 1. Laboratory equipment where the study was provided: a – thermostat; b – graduated glass of measuring glassware

Research Results. The data obtained during a series of experiments to determine the optimal storage temperature are shown in the table (Table 1).

1. Dependence of the survival of Amblyseius swirskii from temperature and storage time

Storage time, day	Storage temperature, °C	The mean value of number of ticks that survived, individuals ± SE
0,3	+ 6,0	69,7 ± 0,6
2,0	+ 2,0	38,7 ± 0,6
2,0	+ 10,0	39,3 ± 0,6
6,0	+ 0,3	7,0 ± 1,0
6,0	+ 11,7	7,0 ± 1,0
6,0	+ 6,0	68,3 ± 0,6
6,0	+ 6,0	67,7 ± 0,6
6,0	+ 6,0	68,3 ± 0,6
10,0	+ 2,0	33,0 ± 1,0
10,0	+ 10,0	33,3 ± 0,6
11,7	+ 6,0	61,0 ± 1,0

Processing of experimental data provided such a report surface:

$$s = 0,17 + 0,34 \times \tau - 0,09 \times \tau^2 + 23,02 \times t - 1,91 \times t^2 \quad , \qquad (1)$$

where *s* – objective function – the number of surviving individuals, individuals;

τ – storage time, days;

t – storage temperature, °C.

The response surface is shown in the figure (Fig. 2).

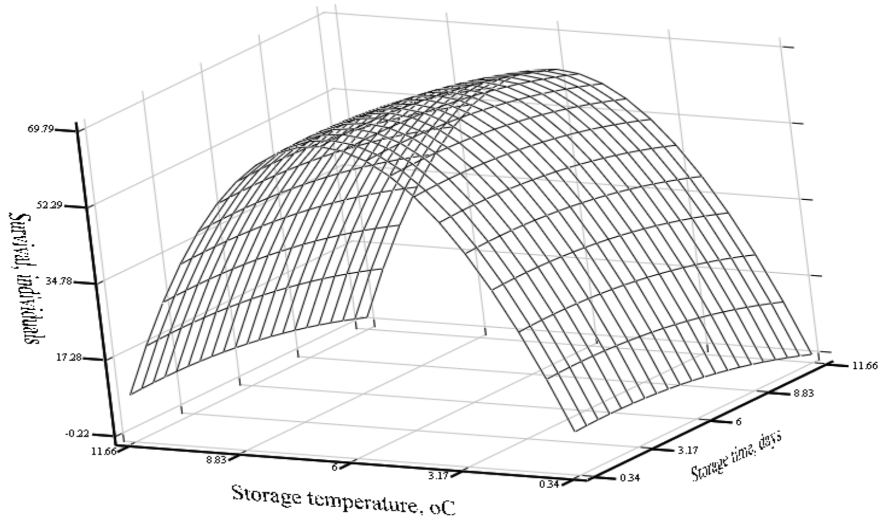

Fig. 2. The dependence of the survival of individuals Amblyseius swirskii on temperature and storage time

Comparison of the calculated Cochren coefficient G_p with the critical value of the Cochren coefficient G_m from the table, as well as the calculated Fisher coefficient F_p with the critical value of the Fisher coefficient F_m from the table (p=0,05), showed that the obtained model, a second-order polynomial, is consistent with the results of experimental studies.

The surface clearly demonstrates that at a certain temperature (+ 6 °C) there is a pronounced decrease in production losses. However, it should be noted that it is possible to speak of such a reduction in losses only in the context of the circumstances of the storage of ticks at low temperatures. Indeed – if the ticks are at a temperature optimal for their life, then the survival rate will be higher than at a certain optimal storage temperature. As for the dependence of production loss on storage over time, as the response surface shows, although the temperature is low, the survival rate decreases with increasing shelf life, but the influence of this factor is very weak. In this case, no fluctuations and extremes are observed

After preliminary storage at the temperature indicated above for 8 days, the fecundity indices of the female *Amblyseius swirskii* remains at the level that is acceptable for the practical use of predators.

The results of the experiment to determine the maintain of predatory mites dispersal activity after storage at the above optimal temperature are shown in the table (Table 2).

2. Results obtained during the experiment to determine the ability of Amblyseius swirskii to keep dispersal activity after cold storage

Storage time, days	The number of individuals that did not reach the edge during 2 hours, %	Time for which 50% of individuals reached the edge, min..
1	6	11
2	9	12
3	13	12
4	16	13
5	20	15
6	23	17
7	27	19
8	30	22

In the course of the regression analysis, the dependence of the percentage of predators that did not reach the edge of the laboratory capacity within 2 hours was obtained — a linear equation with the corresponding coefficients. The percentage of ticks Y_1 (%), that did not reach the edge is determined by the formula:

$$Y_1 = 2,286 + 3,500 \cdot X, \quad (2)$$

where X – duration of preliminary storage, days;

The dependence of the time for which 50 % of predators reached the edge of the tank on the number of days the mites were stored at low temperatures is provided by the quadratic parabola equation with coefficients obtained by performing regression analysis. Time Y_2 (хв.), during which 50 % of the mites reached the edge, is determined by the formula:

$$Y_2 = 11,540 - 0,420 \cdot X + 0,210 \cdot X^2, \quad (3)$$

where X – duration of preliminary storage, days;

The results of the study were presented in a report at the profile scientific and practical conference [13].

Conclusions

The experiments showed an increase in the losses of the predatory mite of Amblyseius swirskii with an increase in the storage time at low temperatures. Minimization of predator losses can be achieved at a storage temperature of about + 6 °C. The mite survival under such conditions remains at the level of 95 %, if the storage time does not exceed 8 days. That is, under such conditions, survival is at a level approaching the control. After preliminary storage at the temperature indicated above for 8 days, the fecundity indices of the female Amblyseius swirskii remains at the level that is acceptable for the practical use of predators. The study of the dispersal activity of predators showed that in a significant way this indicator worsens starting from the 8th day of storage. Until the indicated period of use, Amblyseius swirskii is possible according to this criterion. Thus, the optimum storage temperature at which there is the least loss of production is + 6 °C, the maximum storage time of the tick at the indicated temperature is 8 days. Under these storage conditions, acceptable predator survival is observed, they retain the necessary biological indicators and the further use of Amblyseius swirskii as a biological control agent is possible and appropriate. According to our estimates, the storage of mites at low temperatures for a period of more than 8 days at mass rearing for use on plants is not justified. As we noted during the statement of the problem, at different stages of the supply cycle of biological plant protection agents, producers and consumers of beneficial insects and ticks face inconsistencies in the rate of delivery of finished products to the needs of the final consumer. In our opinion, the approach we proposed allows us to solve this problem to a large extent by introducing the storage of Amblyseius swirskii at low temperatures.

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