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LADY BEETLES FOR BIOCONTROL OF APHIDS, THE VECTORS OF VIRUSES, ON SEED POTATO PLANTS IN GREENHOUSES

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Abstract

Summer greenhouses are used for the production of seed-potato mini-tubers in many Russian regions, so developing technologies for the effective and environmentally friendly protection of seed potatoes against insects that are vectors of viruses in greenhouses is relevant for domestic potato growing. Predatory coccinellids (Coleoptera, Coccinellidae) are successfully used for aphids' control in greenhouses. However, these natural enemies have not yet been applied to protect seed-potato plants. This research is aimed at developing new approaches for applying Harmonia axyridis Pall. and Cheilomenes sexmaculatus Fabr. in the preventive biological control of aphids on seed-potato plants in greenhouses. Coccinellid larvae tolerance to fasting was evaluated in laboratory conditions at 24 °C and 60-70 % humidity. The optimal weight of the larvae to be preventively released into the greenhouses was determined. The IV instar larvae were kept with food in abundance for 1-2 days after molting. The larvae selected daily were grouped depending on their weight. The average time of starvation and the percentage of the individuals pupated were calculated for each size class of the larvae. To select indigenous coccinellids that can be used for aphid control on potatoes in greenhouses we monitored insects in natural habitats nearby the seed potato farm (Volosovskii Region, Leningrad Province). Monitoring of local entomophages and release of coccinellids were carried out in the seed potato farm. H. axyridis larvae were released in a film greenhouse (600 m^2) on the potato plants of cultivar Red Scarlett. The experiment continued from June 12 to July 10, 2017. The number of coccinellids (larvae, pupae, and adults) was estimated weekly. It was shown that even a 2-day starvation of the H. axyridis II-III instar larvae caused an 8-12 % decrease in the survival rate. Therefore, their use unfits for preventive colonization of potato plants. It is optimal to use the IV instar larvae if they weigh enough for pupation with the likelihood about 50 %. We recommend releasing H. axyridis larvae weighted 20-29 mg and Ch. sexmaculatus larvae weighted less than 9 mg. H. axyridis IV instar larvae found on the plants at the seed farm 7 days after releasing averaged 22 % of the total released number. A decrease in the number of H. axyridis larvae and mass pupation occurred 14 days after when pupated individuals were 9.3 % of the initial larvae number. The emergence of the H. axyridis imago was observed 21 days after the release. The obtained results prove there are some good reasons for application of the H. axyridis IV instar larva in greenhouses on seed potatoes. Monitoring of aphidophages in their natural habitats near the greenhouses allowed us to select local species of coccinellids which are promising for potato plant protection in greenhouses. In June-July 2017, potato aphids (Macrosiphum euphorbiae Thomas) appeared on nettle plants (Urtica dioica L.). Adalia bipunctata L. (imago and larvae) and Coccinella septempunctata L. (larvae) were found in the pest hotspots. A. bipunctata imagoes may be used in greenhouses because this species needs lower prey abundance for egg laying and consumes most aphids colonizing potato plants.

Keywords: biological control, *Coccinellidae*, *Harmonia axyridis*, *Cheilomenes sexmaculatus*, preventive colonization, resistance to food stress, cannibalism

The development of new environmentally friendly methods of protecting the seed potatoes is a new area in potato growing. Production of mini-tubers from test tube plants is one of stages of the original seed production. Summer greenhouses are used for their production in summertime in many regions of the Russian Federation, for example, Krasnodar Territory [1], North Ossetia [2], Tatarstan [3], the Sverdlovsk Province (4), the Sakhalin region (5), and in the Leningrad Province. Growing mini-tubers in summer greatly increases the risk of attack of planting by insects, the carriers of viruses. The development of an efficient and environmentally friendly protection of seed potatoes against such insects in greenhouses of such type is challenging for the domestic potato growing industry.

The main strategy of protecting seed potatoes is preventive use of pesticides [3, 6]. It is necessary, because the hazard is not the damage caused by virus vectors (mainly, aphids), but the virus infection carried by them. Even test pricks of individual specimens of non-specialized aphid species can cause infection of potato plants by viruses [7]. When growing mini-tubers no infection of plants by viruses is allowed [8]. Consequently, the system of biological protection of seed potatoes shall also be based on preventive colonization of planting with entomophages.

Method of preventive application of entomophages in greenhouses has been developed for vegetable crops [9-11]. However, they require significant adjustment for use on seed potatoes. Growing virusless potatoes either precludes or restricts significantly the implementation of some standard techniques, which are usually applied for preventive colonization with aphidophages (for example, bringing accumulating plants with aphids into greenhouses). The aphid species for accumulating plants shall be selected depending on the nutritional adaptation of insects. The main requirement is that aphidophage shall not develop on the crop being grown. Greenbugs (*Metopolophium dirhodum* Walker, *Rhopalosiphum padi* L., *Schizaphis graminum* Rond., *Sitobiont avenae*Fabr.) or cabbage aphids (*Brevicoryn ebrassicae* L.) are often used [9, 11]. However, the said species often carry potato virus Y [12-14], due to what they are prohibited in case of seed planting.

Taking into account the limitations caused by peculiarities of cultivation of seed potatoes, it is necessary to compensate any losses and increase the strength margin of the protection system being developed, for example, by means of including new species of entomophages. In addition, it is necessary to adjust the methods of application of those entomophages, which are already used against aphids in the protected soil, but have not been approbated on potato crop yet.

A promising group of polyphagous entomophages is Coccinellidae (*Coleoptera*, *Coccinellidae*). Coccinellidae are widely used for protection of vegetable and decorative crops against aphids in greenhouses. Releases of younger larvae (or introduction of eggs), and releases of imago [9, 15-17] are used. The carrying of viruses by predatory Coccinellidae is almost impossible. They do not drink sap and oviposit onto the surface of leaves and culms without any damage of tissue. In addition, as distinct from specialist aphidophages (*Aphidiidae* and *Cecidomyiidae*), Coccinellidae exhibit an accelerated protective effect. Once released into greenhouses, Coccinellidae are capable of killing the victim (including virus carrying aphids) immediately, which precludes any possibility of the infection dissemination.

We have assessed the possibility of preventive colonization of seed potato planting with larvae of Coccinellidae *Harmonia axyridis* Pall. and *Cheilomenes sexmaculatus* Fabr for the first time. The basic criterion of screening of the species was stress resistance, which was defined as the time for which larvae preserved vitality when starving or absence of optimal feed. The approach we offered allowed selecting physiologically labile species capable of decreasing the intensity of metabolism in absence of feed faster than others. Species from various size classes at different development stages were tested, which allowed obtaining original data on the influence of the body weight and instar of larvae on their ability to survive on potato crop in the absence of the pest.

The goal of the research was the development of new methods of applying aphidophage Coccinellidae in the system of preventive protection of potatoes against aphids in greenhouses.

Techniques. The targets of the research were two species of predatory Coccinellidae — *Harmonia axyridis* Pall. and *Cheilomenes sexmaculatus* Fabr. The resistance of Coccinellidae larvae to starving was assessed in laboratory conditions at 24 °C and 60-70% humidity. Larvae of instars II and III were kept individually in Petri dishes and in groups of 5 bionts in 300 ml plastic containers without feed till pupation or death. In the control group, larvae were fed with greenbugs on cut wheat plants. The number of survived bionts, which molted into the next instar and pupated, was assessed daily.

For preventive releases of larvae into greenhouses, the optimal weight of bionts was determined. Larvae of instar IV were kept with the excess of feed (greenbugs or *Myzus persicae*) for 1 or 2 days after molting, and then bionts were selected for the experiment daily. The selected larvae were weighed on scales HTR-80CE (Shinko Denshi Co., Ltd, Japan), distributed over groups, depending on weight. The bionts in the experiment were kept individually without any feed until emergence of imago or death. In each of the separated larvae size classes, the average starving duration and the share of the pupated bionts were calculated.

Monitoring of local entomophages and release of Coccinellidae was performed in seed farm of Oktyabrskoye CJSC (Volosovskii Region, Leningrad). Larvae of Coccinellidae *H. axyridis* were released in a 600 m² film greenhouse onto Red Scarlett fluke potatoes. The test tube plants were planted on May 17, 2017 into 5 l vegetative pots (in total 8000 pcs). No pre-plant treatment of the root system with insecticides was carried out. The same-age larvae *H. axyridis* were used for releasing. They were grown from eggs collected within one day. The date of mass molting to instar IV was noted, and in 1 day larvae were selected for release into the greenhouse. The release was carried out from June 12 until July 10, 2017.

The quantity of Coccinellidae (larvae, chrysalides and imagoes) in the greenhouse was assessed daily. The accounts were performed by means of visual inspection of randomly chosen 1000 potato plants. Aphids and other pests were accounted simultaneously.

The weighted average (*M*) and the weighted average error (\pm SEM) were calculated using a package of statistical software Statistica10.0 (StatSoft Inc., USA). The difference confidence estimation was carried out using Student *t*-test. The differences were considered statistically significant at p < 0.05.

Results. Previously *H. axyridis* and *Ch. sexmaculatus* were selected as promising entomophages for protection in greenhouses based on high reproductive potential and trophic connections with aphids, which are common in potato agrocenosis [18]. In addition, they are found on potatoes on the field [19, 20]. For protection of vegetable and flower crops against aphids in greenhouses, larvae *Harmonia dimidiata* Fabr., *Coleomegilla maculata* Degeer, *Coccinella septempunctata* L., *Cycloneda limbifer* Casey, *Adalia bipunctata* L., *Propylea quatuor-decimpunctata* L., *P. japonica* Thunb. are also used. It is recommended to release mainly larvae of ages I-II to the pest concentration centers. If the food is available, larvae complete their development and pupate in greenhouses. Duration of the protective effect of the release is 7 to 14 days, depending on temperature and species of Coccinellidae [9, 16, 21-23]. The protective activities on potato plants shall ensure the total absence of aphids in the greenhouses. Therefore, the strate-

gy of releasing younger larvae in expectation of further development of Coccinellidae due to eating phytophages cannot be implemented. In the absence of the pest, larvae *H. axyridis* of ages II-III perish without food within 2 days. Even if a larva finds a victim after 2 day starving, it cannot compensate its loss of weight and vitality. The compensatory growth of larvae after short-term starving is only possible at instar IV [24].

According to our data obtained as a result of mass and individual growing of *H. axyridis*, 2-day deficiency of feed for larvae results in irreversible adverse effects, which was expressed in decrease of larvae which molted to instar IV down to 8-12% at individual growing. In case of mass growing this index used to rise to 20-23%, evidently due to cannibalism (Table).

Survival capacity of larvae Harmonia axyridis Pall. after2-day starving (laboratory test)

Age	Content	Variant	Quantity,	Molted to instar IV		Pupated	
			pcs	total, pcs	M±SEM, %	total, pcs	M±SEM, %
II	Individual	E	50	4	8±1.0*	1	2±0.3*
		С	50	35	70 ± 3.0	32	64±3.3
II	Mass	E	100	20	20±1.6**	6	6±0.6*
		С	100	65	65±2.3	58	58±2.4
III	Individual	E	60	7	12±1.3**	0	0*
		С	50	39	78 ± 2.4	27	54 ± 3.5
III	Mass	E	100	23	23±1.8**	11	11±1.0**
		С	120	84	70 ± 1.9	58	48 ± 2.3
Note.	E and C stand for	or experiment	and control g	roups respective	ely (the feed in	the control g	group is green-

bugs). *, ** Differences with control group are statistically significant respectively at $p \le 0.001$ and $p \le 0.01$.

Consequently, it is not expedient to release larvae of Coccinellidae aged II-III on potatoes for preventive colonization. It is necessary to seek other approaches in their use. One of possible solutions is releasing instar IV larvae, which are capable of compensating losses caused by temporary limitation of food resource. It is necessary to note that at instar IV larvae of Coccinellidae are highly gluttonous. They eat about 70% of the total amount of aphid consumed during larval development. Therefore, their potential as entomophages at instar IV is much higher than at all preceding ages combined [15, 21-23]. At the preventive colonization, the efficiency of releasing the larvae ages IV will depend on their resistance to nutritional stress (absence of feed) which is determined by the duration of starving before pupation or death.

Based on the results of laboratory testing, the optimum is release of larvae *H. axyridis* weighing 20-29 mg and larvae *Ch. sexmaculatus* weighing < 9 mg, because in these variants of the experiment the larvae starving duration was maximum (p < 0.05). In particular, on day 4 in these size classes 30 to 40 % of larvae showed delay of metamorphosis and preservation of motor activity. In other variants of the experiment on the 4th day of starving the majority of larvae used to pupate (Fig. 1). In the selected size classes 42% of larvae *C. sexmaculatus* and 60 % of *H. axyridis* pupated. Consequently, potentially about a half of the released bionts were able to develop to imago stage and with breed. In the environment of age-synchronised crop, the larvae achieved the optimal weight 1 or 2 days after molting to instar IV, depending on the species.

The results obtained during laboratory testing of *H. axyridis*, witness the prospectivity of using instar IV larvae in greenhouses for the purpose of protecting the seed potatoes against aphids. For estimate of *Ch. sexmaculatus* further studies of trophic connections are required. So far, there is no data if the predator feeds on *Macrosiphum euphorbiae* Thomas, the potato virus Y carrier [18]. Direct evidence is required that this entomophage can liquidate *M. euphorbiae*, especially taking into account relatively small size of larvae from the size class

which is optimal for release (less than 9 mg).

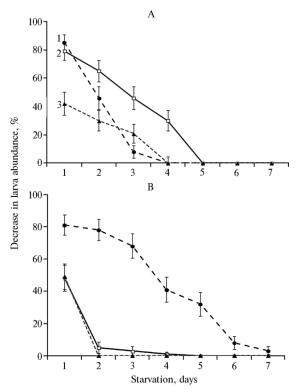


Fig. 1. Dynamics of decreasing quantity of instar IV larvae *Harmonia axyridis* (A) \blacksquare *Cheilomenes sexmaculatus* (B) (as a result of pupation or death) in relation to the initial quantity during starvation: 1 — weight of larvae < 19 mg (A: n = 39; B: n = 73), 2 — 20-29 mg (A: n = 105; B: n = 328), 3 — 30-39 mg (A: n = 121; B: n = 97) (laboratory test).

Laboratory experiments for assessment of resistance of Coccinellidae larvae to nutritional stress were carried out at 24 °C which was optimal for larvae development. As temperature decreases, the larvae vitality preservation time shall increase. This supposition was confirmed in production tests. Instar IV larvae of H. axyridis were released in the greenhouse at mean daily temperature 18-21 °C. From June 12 until July 10, 2017 680 larvae were released onto 3400 plants.

Individual *Macrosiphum euphorbiae* were found on potatoes, mostly winged disseminating females. The share of plants colonized by aphids did not exceed 0.1 % (7-8 plants per greenhouse). Despite the almost total absence of the pest, 30.4% of the released *H. axyridis* were detected on the plants 7 days after the release. The share of larvae amounted 21.8% of the initial value (Fig. 2). In 14 days a confident (p < 0.05) decrease

of number of larvae, *H. axyridis* and mass pupation was observed. A total of 9.3% of the initial number of larvae were found as pupated bionts on plants. Emergence of imago *H. axyridis* was noticed 21 days after the release of larvae.

The share of larvae, which remained on the plants in our experiments, was on average comparable or more than in the traditional method of colonization (release of younger larvae to the pest centers). For example, after instar I larvae *H. axyridis* release to greenhouses onto hautbois plants, 22.0% of the initial number of Coccinellidae were found 7 days later [25]; 7.5-12.5% of bionts were found on herbaceous crops 4 days after the release of instar I-II larvae *H. axyridis* [26]. Similar results have been obtained when other Coccinellidae species larvae were released. For example, 8 days after the release of instar I larvae *A. bipunctata* and *C. septempunctata* 19.0% and 1.3% of the initial number of Coccinellidae were found respectively [27]. It means that as early as 4 to 8 days after release of younger larvae their number decreases significantly (by 80-90%), despite there are aphids in agrocenosis. It is evident that in the absence of victim the decrease of the number of released larvae will be even faster.

A possible environmental and genetic mechanism ensuring the efficiency of our suggested variant of preventive colonization of H. axyridis was heterogeneity of physiological response of this species to nutritional stress (absence of aphids). The population contained some share of instar IV larvae which delayed metamorphosis, despite the quantity of the nutrients accumulated by them enabled their pupation. It can be supposed that such larvae remained on the plant and contributed into the protective effect of the preventive colonization.

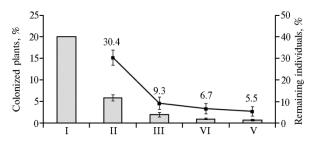


Fig. 2. The share of colonized plants (diagram) and *Harmonia axyridis* bionts remained on them (plot) after release of instar IV larvae: I — release (larvae), II — day 7 (71% of larvae, 29 % of chrysalides), III — day 14 (chrysalides), VI — day 21 (imago), VII — day 28 (imago) (Oktyabrskoye CJSC, Leningrad Province, June-July, 2017).

However, the leading role in the preventive release of *H. axyridis* was evidently played by instar IV larvae, which did not reach the threshold weight necessary for metamorphosis. In the laboratory experiments in individual maintenance without feed, the larvae with weight deficit preserved motor activity 3 to 4 times as long as the bionts which have already reached the pupation threshold. Among

the larvae released by us to the greenhouse (20-29 mg) the share of bionts with subthreshold weight was significant (about 50%). To survive in the greenhouse, they were to find food. Such food could be aphids or bionts of the same species (larva, chrysalide, young imago).

Cannibalism is characteristic of Harmonia larvae. Eating bionts of its species is an integral part of the behavior pattern of *H. axyridis* [28]. Possibly, it was cannibalism that enabled surviving in the greenhouse for larvae with sub-threshold weight. When using the larvae from our recommended size class (20-29 mg) the supposed losses due to cannibalism will be partially compensated with decrease of mortality among the subthreshold weight larvae.

Releasing instar IV larvae *H. axyridis*, we noticed the accumulation of entomophage in agrocenosis at pupal stage. If younger larvae are used, then their pupation without feed in the greenhouse is unlikely. For example, in case of paucity of aphids on chrysanthemums in the greenhouse 0.6% of the total released instar I larvae *A. bipunctata* pupated, and when larvae *C. septempunctata* were released, no chrysalides were found [27]. Concerning the quantity of *H. axyridis* chrysalides in the greenhouse, one should allow for the fact that the accounts were on the plants only. Possibly, some part of released larvae pupated outside the plants: on the walls of vegetative pots, on the film covering the greenhouse floor, etc. It is highly probable that some chrysalides were eaten by larvae of the same species.

In addition to well spread cannibalism, *H. axyridis* has one more species feature, which should theoretically prevent from accumulation of entomophages in the greenhouse. In the lifecycle of *H. axyridis* there is a mandatory period of migration state, which follows right after the emergence from chrysalide and ensures dispersal of the new generation bugs from hatchings [15]. During colonization in the greenhouse, one can forecast migration of a part of young imago to territories near the greenhouse. However, as applied to conditions of Oktyabrskaya CJSC, we assess the migration of *H. axyridis* as a positive phenomenon, because the Coccinellidae, which flew away, could control the pest on potato plantings on the field just near the greenhouses. In addition, Coccinellidae imagoes, which migrated to territories near the greenhouse, will strengthen the natural populations of entomophages, which restrict the development of aphids in natural stations around the greenhouses.

As a whole, it can be concluded that the greenhouse planting of virusless potatoes is an agrocenosis the conditions in which facilitate seasonal coloniza-

tion of Coccinellidae. Thanks to long-term crop vegetation time (3 to 4 months), availability of safe pupation locations and absence of active manipulations with plants, it becomes possible to accumulate the released ladybirds.

For reference, on lettuce lines the seasonal Coccinellidae colonization failed despite high release rates [9]. Lettuce crop has short vegetation time (30 days on average). Entomophages with finished products are removed from the greenhouses. The emptied hydroponic bed installations are disinfected as plants are removed, destroying the predators pupated on them. Due to permanent movements of boxes with plants, the ladybird larvae may fall under the beds. Since the potato planting in soil greenhouses are free from the above listed drawbacks, we consider the application of Coccinellidae larvae in these agrocenoses to be very promising.

Taking stock of our production tests, we can issue the preliminary recommendations concerning the frequency of releases of *H. axvridis* for protection of seed potatoes in the greenhouses. Since 1 week after the release only 22% of H. axyridis remained at the larva stage, the releases should be carried out at least once per 7 days, so that the larvae ensuring the protective effect were permanently present on the plants. Further research is necessary to determine the optimal release rates. Herewith, it should be taken into account that the excessive increase of the density of ladybirds on the plant is not expedient due to high risk of larvae cannibalism in the absence of aphids, as it was noted in the experiments with *H. dimidiata* on cucumber crop in the greenhouse [22]. However, there is a risk of the insufficient Coccinellidae larvae release rates resulting in the protection system weakening, because not all plants will be inspected by the larvae. To enhance the protective effect reliability, it is expedient to consider a variant of the combined application of ladybird larvae with other aphidophages. The data has been obtained that Aphidiidae avoid the plants with traces of Coccinellidae larvae and imago for 24 hours [29]. Consequently, the repellent effect caused on Aphidiidae by ladybirds makes the parasites to inspect mostly those plants, which have not been visited by predators. It can be supposed that the combined use of the predator and parasite will facilitate the continuous protection of the maximum amount of plants. The ladybird imagoes exhibit high search activity and are characterized by significant life duration (no less than 20 to 30 days for small-size species and 2 to 3 months for big-size ones). Due to such features, the ladybird imagoes are promising for protection of potatoes in the greenhouses and on the territory near the greenhouse.

To select Coccinellidae suitable for protection of potatoes in the greenhouses, a monitoring was carried out. It covered the local entomophage species, which live in natural stations near the greenhouse system of Oktyabrskoye CJSC. In June and July, 2017 *Macrosiphum euphorbiae* was discovered on nettle (*Urticadioica* L.) plants. In the pest centers Aphidiidae (aphid mummies), *A. bipunctata* (imago and larvae) and *septempunctata* (larvae) were noticed. It should be noticed that *Macrosiphum euphorbiae* form rarefied colonies. It should be taken into account during screening of entomophages suitable for pest control. It is expedient to select the ladybird species for females of which individual aphid bionts are sufficient for oviposition. One of such species is Adalia [30].

The ladybirds' ability to lay eggs on the background of low number of aphids is in negative correlation with the imago weight [31]. *A. bipunctata* is included into the small-size class, imago weight is 10-18 mg). Herewith, the following aphid species commonly found in potato argocenoses were noticed among victims of *A. bipunctata: Acyrthosiphon pisum* Harris, *Aphis fabae* Scopoli, *Aphis gossypii* Glover, *Aphis nasturtii* Kalten., *Aulacorthum solani* Kalten, *Brachycaudus heli-*

chrysi Kalten, M. euphorbiae, Myzus persicae Sulzer, Rh. padi [18].

In addition to the variety of victims and low oviposition induction threshold, there is one more argument in favor of using Adalia or similar-sized species on potatoes. During preventive colonization, not high gluttony (characteristic of big ladybirds) is required from the predator, but the ability to find and destroy the primary pest center. Herewith, the cost of growing one biont of small-sized Coccinellidae species is 2 to 3 times as cheap as that of big-sized ladybirds. Therefore, it is economically more expedient to release imagoes of small-sized Coccinellidae species. Besides, in case of migration from the greenhouse it is more probable that such ladybirds will stay on the territories near the greenhouses, eating the pests, which are regularly brought by the wind from natural stations and personal subsidiary plots.

Concerning the prospects of applying C. septempunctata, the big size of the ladybird should be taken into account (imago weight is 25-50 mg), due to which is needs high aphid density for development of the breed. The C. sep*tempunctata* oviposition is induced by aphid colonies consisting of dozens bionts, and for the development of the next generation over 100 bionts are necessary [32]. The emergence of such unfavorable phytosanitary situation is unlikely on the territories near the greenhouse close to seed potatoes planting. Evidently, insecticides will be applied against aphids in case of a risk of pest outbreak. Therefore, we think that it is inexpedient to include C. septempunctata imago into the complex of entomophages for preventive releases. However, it should be taken into account that the said species is one of dominating predators in potato agrocenoses on the field [19, 33, 34]. Herewith, C. septempunctata is known as a wide polyphage, and in the absence of aphids, it can feed on arthopods, and plant pollen and fungal spores [35]. The dominating position of C. septempunctata among natural predators on potatoes and the wide nutritional adaptation allow recommending the activities for preservation of its wintering areas near greenhouse seed potatoes production complexes. High population numbers of this entomophage will facilitate enhancement of phytosanitary situation as a whole.

Thus, based on the results of the conducted screening of Coccinellidae aphidophages for protection of seed potatoes, we selected *Harmonia axyridis* Pall species, which we recommend to release at larval stage, 1 or 2 days at instar IV. For the guaranteed protective effect, the *H. axyridis* larvae releases should be carried out at least once per 7 days. The natural populations of *C. septempunctata* and *A. bipunctata* for protection of seed potatoes is possible if a technological solution is found which would allow to purposefully attract these entomophages to the territories near greenhouses, for example, by planting nectariferous plants. As a whole, the technology of cultivation of virusless potatoes allows accumulating the released ladybirds, so the application of Coccinellidae larvae in such agrocenoses is highly promising.

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