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Development and Predation of *Picromerus bidens* (Hemiptera: Pentatomidae) on *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)

Развитие и хищничество *Picromerus bidens* (Heteroptera: Pentatomidae) на *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)

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КЛЮЧЕВЫЕ СЛОВА: колорадский картофельный жук, *Leptinotarsa decemlineata*, хищный клоп пикромерус двузубчатый, *Picromerus bidens*.

ABSTRACT: The Palearctic *Picromerus bidens* is a predacious spiny shield bug (Pentatomidae: Asopinae) that was studied as suitable as agents of biological control of Colorado beetle since 1997. This pentatomid is univoltine, overwinters in the egg stage, and preys mainly on coleopteran, hymenopteran, and lepidopteran larvae that feed on herbaceous and woody plants. In laboratory and field researches the ability of *P. bidens* to reduce the number of Colorado potato beetle larvae on potato plants was established. Depending on the predator age the biological efficiency of 80–90% is being achieved on 35th day (colonization in eggs stage), on 21–24th (colonization in stage of larvae I instar) or on 14–18th days (colonization in stage of larvae instar II).

РЕЗЮМЕ: Палеарктический хищный клоп пикромерус двузубчатый *Picromerus bidens* L (Pentatomidae: Asopinae) изучался в качестве агента биологической борьбы с колорадским жуком начиная с 1997 г. Клоп даёт одно поколение в год (унивольтинный), зимует в стадии яйца и охотится преимущественно на личинок из отрядов Coleoptera, Hymenoptera и Lepidoptera на травах и деревьях. В лабораторных и полевых исследованиях установлена способность *P. bidens* снижать численность личинок колорадского жука на картофеле. В зависимости от возраста пикромеруса 80–90-процентная эффективность колонизации достигается на 35-е (колонизация в фазе яйца), 21–24-е (колонизация в фазе личинок 1-го возраста) или 14–18-е сутки (колонизация в фазе личинок 2-го возраста).

Introduction

Colorado beetle *Leptinotarsa decemlineata* Say was registered on the territory of the European part of the Russian Federation after 1965 [Colorado potato beetle…, 1981]. Now it is spreading in Siberia and has reached the Far East of Russia.

Colorado beetle entomophages list counts 270 insects and 23 arachnid species [Gusev, 1991]. Most important for biological protection are two groups of predatory insects: beetles, 145 species among them - Carabidae and predatory heteropterans (Hemiptera) — 34 species.

In the beginning of 1990ies Oleg G. Volkov carried out the researches on exposure of local predatory pentatomids (Hemiptera: Pentatomidae: Asopinae) suitable as agents of biological control of Colorado beetle. Among all species studied the author chose shield bug *Picromerus bidens* (L.) fit with such criteria as: rather big sizes; hostility and voracity; easiness of cultivation in different conditions; the ability of use in rather wide growth conditions of different agricultural crops; the ability of mass production and rather long keeping of entomophage without losing the quality; the ability of transportation of entomophage for long distances; optimal correlation between searching abilities and migratory activity [Volkov & Tkacheva, 1997] (Figs 1–2).

P. bidens has a wide distribution in the western Palearctic region. It has also been introduced to North America [Puchkov, 1961; Thomas, 1994; Wheeler, 1999]. This is a big shield bug with expressed sexual dimorphism. Female can reach 13–15 mm length and 80–160 mg weight. Species has 2 spikes jutting out from its thorax. Imago color is brown with bronze shine. Mouthparts is piercing-sucking in the form of thickened proboscis.

In Central Russia shield bugs start to couple from the end of August and female lays eggs up to October on dry leaves, twigs and other vegetable remainders. This pentatomid unlike the other predacious bugs has a winter diapause on the egg stage.

It allows keeping entomophage, accumulating it in any amounts and transporting for long distances [Leston, 1955].

P. bidens is a generalised predator of a wide range of insects, including Hymenoptera, Coleoptera and Lepidoptera [Lariviere & Larochelle, 1989].

Group predatory behavior and joint feeding allows larvae and imago of *P .bidens* to cope with big prey. Piercing cover of a victim shield bugs inject saliva with lytic enzymes, after what the victim inevitably dies. Nymphs of shield bug are characterized of high searching activity. The elder shield bugs have low fly abilities, that is why they stay for a long time in the areas of distribution.

Materials and Methods

The researched object

Moscow population of *Picromerus bidens* was used in the experiments, it was selected in the lab of Federal state establishment Russia Center for Plant Quarantine since 1992 for absence among shield bugs of imaginal diapause (aestivation). As is known in environmental conditions a summer reproductive diapause does not allow females of shield bug to lay diapausing eggs up to beginning of autumn [Musolin, 1996].

This peculiarity of lab population allows mass rearing of shield bug larvae and imago at equal light-regime and temperature which is much more convenient for them.

Terms of cultivation of Picromerus bidens

For cultivation of the stock colony of *P.bidens* was used popular methods of group rearing of pentatomidae [Mahdian et al., 2006b] as well as own developments. Wax moth *Galleria mellonella* L. (Lepidoptera: Galleriidae) and yellow mealworm beetle *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) were used as a feed for shield bug considering its polyphagia and the most cheapness and easiness of feed production in lab. Larvae of the wax moth should be kept in darkness under constant temperature of 33°C while its imago are kept under constant light-regime: 24 hours a day and temperature of 25°C.

A modified variant of feed was created for lab raising of *G. mellonella*, the main component of which is bran (550 g/kg), yeast, (100 g/kg), sucrose (100 g/kg), beeswax (50 g/kg) and water (200 g/kg). As antiseptics it is offered to use sorbic acid to the extent of 0.1% and potassium sorbate utilized in industrial canning. Larvae and imago of mealworm beetle were kept in darkness under constant temperature of 20°C. As a feed it was used a grain waste.

The developed methods of cultivation of shield bug are based on accumulation of entomophage in egg stage rather than larvae or imago as for all other types of predatory bugs.

For egg production mature shield bugs were kept at 23–25°C in chambers of variable construction made of mill gauze. The light intensity in the chambers of 180 to 250 lx was provided by fluorescent lamps 24 hours a day. Zigzag-folded paper was served as a substratum and shelters for egg-laying. On the bottom of a chambers there were placed Petri dishes with waterlogged wadding. The norm of settlement in chambers is 4 mature insects on 1 dm^3 extent. Shield bugs were feed 2–3 times a week by putting in chambers one caterpillar *G. mellonella*, larvae and a propupa of yellow mealworm for each two shield bugs.

Immediately after taking out from chambers diapausing eggs of shield bug on paper stripes were put to Petri dishes and placed in refrigerator in order to initiate embryonic development. In 0.1 m^3 refrigerator can be placed approximately 1 million of shield bug eggs. It is enough to keep layings in lab for 2 months at 2–4° that larvae become able to develop in 80% eggs.

For field and lab researches eggs were activated at permanent temperature at 20–25°C, at a higher temperature per cent of larvae coming out from eggs decreases.

Newly emerged larvae were transferred by soft brush in covered by gauze glass cages with capacity of 0.8–1 l (on the basis of 100 pieces per a cage) within 2–3 days. On the bottom of the cage a circle of filter paper was put for absorption of extra moisture. Besides, pleats of dark paper were placed in the cage, these are necessary for separation of larvae and creation of shelters for them, especially in a period of molting. A wet lump of wadding was placed on the lid as a source of water. The cages were placed on shelves (23–25°C, 70–80% relative humidity (RH); light conditions are the same as for imago *P. bidens.*

Caterpillars of *Galleria melonella* of IV–VI instar served as main food for shield bug larvae. Periodicity of feeding is 3 times a week. The norm of giving feed (at the ratio of predator/prey): for larvae of II instar (L2) = 10/1; L3 = 8/1; L4 = 5/1; L5 = 3/1. Shield bug larvae of IV–V instars were given at the same rate larvae and nymph of yellow mealworm.

Immediately after appearance of first adults shield bugs were transferred in chambers for eggs production as in the period of molting the highest per cent of cannibalism on imago is observed and shield bugs need maximum dispersal. Usually if the conditions are favorable, the death rate of the development period from the first instar to imago is not above 30%. A small dropout is indispensable for saving the aggressiveness between the species of *Picromerus*.

Study of predatory activity

Shield bugs were kept in the Petri dishes (diameter 9 cm) by five individuals (larvae and nymphs) in a dish in

8 replications. Wet wadding tampon always was in the dish. Alive larvae of Colorado beetle of I–III instars were suggested as feed to shield bugs. Observations of the development and change of food were made every 2nd or 3rd day. The experiment was carried out in chambers with fluorescent lighting with photoperiod of 12 hours and temperature at 24–25°C.

An assessment of effective ratios in a system predator/prey was carried out in vegetation culture vessels on potato bushes. The experiment was made with artificial spreading of Colorado beetle larvae. Approximately 30 Colorado beetle larvae of II instar were preliminary placed on plants. The following variants at the numeral ratio in the predator/prey system were examined: 1:3; 1:4; 1:8; 1:15. Shield bug was put on the experimental plants relatively by 15, 8, 4 or 3 larvae of II instar.

The experiment on defining a functional reaction of predatory bug in connection with instar of a prey was made on potato plants artificially occupied as follows: 1) 20 eggs of Colorado beetle; 2) 20 Colorado beetle larvae of I–II instar 3) 10 Colorado beetle larvae of III instar 4) 5 Colorado beetle larvae of IV instar. There were placed by 5 shield bug larvae of II instar on each plant.

Study of the effectiveness of shield bug by method of seasonal colonization

Potato cultivar "Udacha" was plant manually in cut furrows on 18 May (experimental field of Russia Research Institute of Phytopathology, Moscow Area). Colorado beetles were introduced at experimental plots as egg clutches when plants have 5–6 leaves at the rate of one clutch per plant. Introduction of the shield bug was made (1) as eggs at plants previously infected with eggs of Colorado beetle and (2) as larvae of the first and second instars at plants previously infected with newly emerged larvae of Colorado beetle.

For each variant of the experiment 50 m^2 were allotted (10 beds by 10 plants each). Occupation was made on each plant. Follow-up of the dynamics of Colorado beetle larvae quantity (by instars) was made on 10–15 model plants for each variant of the experiment.

The following variants were tested:

1. Introduction at the rate: the 1 egg clutch of Colorado beetle and 1 egg clutch of the shield bug (22.06.09) — the predator/prey ratio is 1:1;

2. Introduction at the rate the larvae emerged from one egg clutch of Colorado beetle and 10 shield bug larvae of the I instar per plant (29.06.09);

3. Introduction at the rate: the larvae emerged from one egg clutch of Colorado beetle and 10 shield bug larvae of the II instar per plant (09.07.09).

4. Control variant — introduction of 1 egg clutch of Colorado beetle per plant without introduction of the shield bug (22.06.09).

As a whole it was introduced about 1000 eggs and by 100 shield bug larvae of I and II instars for the total area of 200 m^2. Biological effectiveness of shield bug introduction was defined by follow-up of pest quantity on experimental and control plots. The first calculation was made straight before output. The following observations on changes in quantity, stages of Colorado beetle development were carried out in 3–7 days within 8 weeks after colonization of predatory bug. The rate of potato bushes damage was also considered.

The biological efficiency (%) was calculated by the following formula:

$$BE = \left(1 - \frac{K_o \cdot N_n}{N_o \cdot K_n}\right) \cdot 100 \text{ , i.e.}$$

BE — biological efficiency; N_0, N_n — the number of Colorado beetle at experimental variants before and at *n*-day after shield bug colonization; K_0, K_n — the number of Colorado beetles at control variant.

In the end of the experiment the registration of the damage intensity of potato plants was made according to the 5-poit scale (0 — no damage, 1 — up to 25% of foliage surface is damaged, 2 — up to 50% is damaged, 3 — up to 75% is damaged, 4 — up to 100% of the leaves surface is damaged).

Results and Discussion

The research found that the development of *P. bidens* finishes successfully with feed by Colorado beetle larvae (Table 1). The duration of development of each stage of larvae and nymphs is about 5 days, the last instar is 1.6 times longer. Every individual of the predatory shield bug during 2nd and 3rd developmental instars is able to kill from 3 to 5 larvae of Colorado beetle.

The feeding rate of early instar nymph and especially final instar nymph is two or three times as large. The death rate is 20% at 24°C. Molting to the next instar

Table 1. Biological features of *P. bidens* at feeding by Colorado beetle larvae (t = 24°C; RH = 60–70%).
Таблица 1. Биологические особенности *P. bidens* при питании личинками колорадского жука (t = 24°C; RH = 60–70%).

Stage	Developmental duration (days)	Mortality of larvae and nymphs (%)	Predation rate (for the period of development)
Larvae first instar	4.1±0.1	0	0
Larvae second instar	5.2±0.7	5.0	1.9
Larvae third instar	5.9±0.5	5.8	2.5
Larvae fourth instar	5.7±0.4	6.3	3.1
Larvae fifth instar	9.1±0.9	7.1	4.5
Total larva–nymph period	25.9±0.6	24.2	12.0

takes 4–5 days. Thus, the developmental stage (larva to nymph) completes within 26 days.

During study of functional reaction of predator depending on the prey density it was found that the optimal predator/prey ratio is 1 to 8. In this variant effective killing of Colorado beetle larvae is reached within 7–10 days after introduction of 4 larvae of the shield bug per plant (Table 2).

Table 2. The decrease of the Colorado beetle larvae depending on the predator/prey ratio.
Таблица 2. Снижение численности личинок колорадского жука в зависимости от соотношения хищник/жертва.

Predator/prey ratio	The initial number of beetle larvae	Reducing of live Colorado potato beetle larvae day by day		
		5	8	12
1 to 3	30	7	2	0
1 to 4	30	12	3	2
1 to 8	30	15	8	4
1 to 15	30	24	20	10
Control	30	28	26	24

Table 3. The influence of Colorado beetle larvae age on shield bug larvae of instar II voracity.
Таблица 3. Прожорливость личинок II возраста *P. bidens* в зависимости от стадии развития колорадского жука.

Variant	The initial number of Colorado beetle eggs and larvae (June, 23)	The number of individuals during the experimemnt		
		July, 27	July, 30	August, 03
1	20 eggs	1 larvae of instar I, 11 larvae of instar II	0	0
2	20 larvae of instar I-II	10 larvae of instar II	0	0
3	10 larvae of instar III	1 larvae of instar III, 8 larvae of instar IV	4 larvae of instar IV	2 larvae of instar IV
4	5 larvae of instar IV	4 larvae of instar IV	0	0

Fig. 1. Nymphs of *P. bidens* feeding upon larvae of the Colorado potato beetle (Photo by Yuri I. Meshkov).
Рис. 1. Нимфы *P. bidens* поедают личинок колорадского жука (фото Ю. Мешкова).

During defining of functional response of *P. bidens* depending on prey age it was found that the predatory shield bug most effectively kills the Colorado beetle larvae of younger age at their numerical superiority (Table 3). During the transition of Colorado beetle larvae to instar IV their availability decreases a little. However after the transition of the shield bug larvae to instar III they become more active and able to suppress the pest density.

In 2009 indigenous spiecies of predatory shield bug *P. bidens* was tested in field conditions as an agent of biological struggle with Colorado beetle.

According to our observations, the emergence of Colorado beetle larvae started on June 29, 2009 (Table 4). The early July was cool and rainy. After July 16, the warm weather favored the development of the pest and its predator. At the control plots the maximum number of Colorado beetle was observed in early July (28.1 larvae per plant). Then the number of the pest gradually and smoothly decreased up to 2.5 larvae per plant in August, 16.

In the variant with introduction of I instar larvae shield bug started feeding in 3 days and in the variant with introduction of II instar larvae shield bug started feeding straight after colonization.

After July, 16 the weather was also favorable for development of predatory bug. Mass appearance of II instar larvae of shield bug was during the phase of potato flowering, after that they began destroying Colorado beetle.

The analysis of data revealed that the effect of the predatory shield bug release depends on his age and the date of colonization.

At earlier setting of shield bug in an egg phase (by 1 egg clutch per plant) the number of Colorado beetle larvae on plants was sharply decreasing and already on 42 day (in the beginning of August) it amounted to 0.3 species per plant, in the control variant in this time there were by 17 pest species per plant. Long terms are connected with the fact that at I instar shield bug larvae do not feed. Only after larvae moulting to II instar, predatory bug started destroying Colorado beetle larvae.

During introduction of predator larvae of I instar the biological efficiency of about 80% was reached on 21 day since setting of shield bug (Table 5). In this variant predator which molted on II instar started feeding Colorado beetle larvae earlier (Fig. 1).

It is interesting that at later introduction of II instar larvae the biological efficiency reached 80% level on 14 day after setting. Mass development of shield bug larvae of II instar on plants took place in a phase of potato development — “start of flowering”.

The efficiency of 90% was reached within 35 day at introduction of shield bug by spreading of eggs clutches, 24 days at setting I instar larvae and 18 days at setting II instar larvae.

During the flowering phase of potato plants, the most vulnerable for damage by Colorado beetle, the registration of the damage intensity was made. The

Fig. 2. Nymph and an adult of *P. bidens* feeding upon adults of the Colorado potato beetle (Photo by Yuri I. Meshkov).
Рис. 2. Личинка и имаго *P. bidens* поедают имаго колорадских жуков (фото Ю. Мешкова).

Table 4. Effects of shield bug colonization on the larvae Colorado beetle abundance (Potato cultivar "Udacha", Moscow Area, 2009).
Таблица 4. Влияние колонизации P. bidens на обилие личинок колорадского жука (картофель сорта "Удача", Московская область, 2009).

Stages of Colorado beetle	Average quantity of Colorado beetle species per 1 plant *										
Date	22.06	29.06	06.07	13.07	20.07	23.07	27.07	03.08	06.08	10.08	16.08
Variant 1	*22.06* (the rate of shield bug colonization — 1 egg clutch per plant)										
Days after colonization	0	7	14	21	28	31	35	42	45	49	55
Eggs *	33.4	30.6	2.8								
Larvae I+II		1.8	24.0	12.2	3.4	0.3					
Larvae III				6.6	2.0	2.4	1.3				
Larvae IV				0.8	2.7	2.7	0.8	0.3			
All larvae (M)	0	1.8	24.0	19.6	8.1	5.4	2.1	0.3	0	0	0
SE		0.6	11.8	10.0	6.0	3.6	2.8	0.4			
Variant 2	*29.06* (the rate of shield bug colonization — 10 larvae of the instar I per plant)										
Days after colonization	0	0	7	14	21	24	28	35	38	42	48
Eggs *	40.6	35.6									
Larvae I+II		1.9	36.0	5.9	0.5	0.1					
Larvae III				14.3	1.8	0.6	0.1				
Larvae IV				1.2	2.6	1.6	0.6				
All larvae (M)	0	1.9	36.0	21.4	4.9	2.3	0.7	0	0	0	0
SE		0.9	15.1	8.0	2.9	3.0	1.3				
Variant 3	*09.07* (the rate of shield bug colonization — 10 larvae of the instar II per plant)										
Days after colonization	0	0	0	4	11	14	18	25	28	32	38
Eggs *	39.1	35.5	7.2								
Larvae I+II		3.6	30.6	20.3	1.3	1.0	0.7				
Larvae III				5.2	3.8	0.9	0.3	0.3			
Larvae IV					6.3	3.6	1.3	0.4	0.5	0.1	
All larvae (M)	0	3.6	30.6	25.5	11.4	5.5	2.3	0.7	0.5	0.1	0
SE		2.7	11.6	14.7	7.9	4.1	1.6	0.3	0.2	0.1	
Control	(without colonisation of the shield bug)										
Days after colonization	0	0	0	0	0	0	0	0	0	0	0
Eggs *	33.2	27.7									
Larvae I+II		4.5	27.3	13.7	5.5	3.3	1.2	0.1			
Larvae III			0.8	10.8	9.6	6.2	3.8	2.1	0.1	0.1	
Larvae IV				1.6	10.0	14.3	15.5	14.7	14.3	11.6	2.5
All larvae (M)	0	4.5	28.1	26.2	25.1	23.8	20.5	16.9	14.4	11.7	2.5
SE		1.4	10.2	9.1	7.0	5.4	1.5	2.5	3.2	2.2	1.8

* 22.06 settlement of Colorado beetle on potato plants — 1 egg clutch per 1 plant.
* 22.06 колонизация колорадским жуком растений картофеля — 1 кладка яиц на 1 растение.

Table 5. Biological efficiency of the *P. bidens* colonization (Potato cultivar “Udacha”, Moscow Area, 2009).
Таблица 5. Биологическая эффективность колонизации *P. bidens* (картофель сорта “Удача”, Московская область, 2009).

Variant of shield bug colonization	Biological efficiency (%)										Rate of damage
	29.06	06.07	13.07	20.07	23.07	27.07	03.08	06.08	10.08	16.08	
Days after	22.06 (the rate of shield bug colonization — 1 egg clutch per plant)										
colonization	*7*	*14*	*21*	*28*	*31*	*35*	*42*	*45*			
Variant 1	3.0	15.1	25.6	67.9	77.4	89.8	98.2	100			2.3
Days after	29.06 (the rate of shield bug colonization — 10 larvae of the instar I per plant)										
colonization	*0*	*7*	*14*	*21*	*24*	*28*	*35*				
Variant 2	–	0	27.7	82.7	91.4	97.0	100				2.5
Days after	09.07 (the rate of shield bug colonization — 10 larvae of the instar II per plant)										
colonization	*0*	*0*	*4*	*11*	*14*	*18*	*25*	*28*	*32*	*38*	
Variant 3	–	–	27.6	66.2	82.8	92.8	97.5	97.8	99.5	100	1.6
Control	without colonization of the shield bug										3.8

lowest damage of potato plants was observed in variant 3 after colonization of the shield bug larvae of the instar II in the rate of 10 larvae per plant. This effective norm of the predator colonization in places of the pest concentration (in its foci) permits to decrease the number of the pest by 80–90%.

P. bidens is long known as an active predator for caterpillars and other insects with soft cover [Mayne & Breny, 1948]. During lab cultivation of *P. bidens* was fed larvae of the Mediterranean flour moth, *Anagasta kuehniella* (Zeller), greater wax moth, *Galleria mellonella*, and *Spodoptera littoralis* (Boisduval) [Javahery, 1986; Mahdian et al., 2006a]. It also develops successfully on pupa and larvae of darking beetles *Tenebrio molitor*. Shield bug is also a predator for Colorado beetle [Volkov & Tkacheva, 1997; Mayne & Breny, 1948].

In our lab researches it was established that shield bug is able to attack Colorado beetle larvae and feed them in lab as well as in field conditions. The developmental rate of juvenile stages (2^{nd}–5^{th} instars) *P. bidens* on larvae of *L. decemlineata* was similar to the development rates on lepidopteran larvae [Mahdian et al., 2006b, 2008]. In connection with the fact that shield bug is able to finish it individual development on Colorado beetle the larvae of leaf-cutting beetle can be considered as optimal food for development of the predator.

In environmental conditions predatory shield bug is an inhabitant of grassy and bush plants. Its predatory activity is enough high also on some Solanaceae (tomato, sweet pepper and eggplant) [Mahdian et al., 2007]. In our researches it was established that shield bug has a high searching and predatory activity at all development rates on potato plants. In field conditions firstly a number of shield bug larvae of I–II instars goes down, further a number of shield bug larvae of III instar also goes down. At a delay with setting and high quantity if shield bug larvae many Colorado beetle larvae manage to pass into next instar. However then predator clears plants almost from all pests and leaves the plants in a search of other prey on other plants (Fig. 2).

Conclusion

In connection with the analysis of the received results under change of Colorado beetle larvae number a conclusion can be drawn that the efficiency of *P. bidens* setting depends, firstly, on time of colonization and then, on predator physiological age.

It is optimal to spread shield bug in the beginning of mass eggs laying by Colorado beetle. In this case during setting of shield bug a complementarity between Colorado beetle and shield bug development rates is maintained. The norm of setting is defined from daily voracity of shield bug and with consideration of its migration features. The abovementioned norms of setting of shield bug are approximate and can be taken as a base during protection measures. In each specific case the norms of settings should be connected with average density of Colorado beetle on plants and phenophases of protected plants. Usually entomophage sucks Colorado beetle larvae not completely, especially of elder instars, preferring to utilize muscular tissue. Such influence mechanism increases efficiency of shield bug as an agent of biological control.

The following regulations must be followed during the shield bug use:

1. The first introductions of the predator must be made in the pest foci. It decreases the necessary number of the predator and restrains the pest spreading over the area.

2. To consider critical developmental stages of the protected plants (the period of flowering in potato). During this period even low density of Colorado beetle (up to 0.5 egg clutch per plant) may result in notable loss of the yield. In this connection the rate of introduction must be not less than 50 thousand of larvae or 100 thousand of eggs per hectare.

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