VARIABILITY OF PHENOTYPIC STRUCTURE OF THE COLORADO BEETLE (*Leptinotarsa decemlineata* SAY) (COLEOPTERA: CHRYSOMELIDAE) POPULATIONS UNDER THE ANTHROPOGENIC FACTOR INFLUENCE IN THE REPUBLIC OF BELARUS

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Abstract. The figure phenomorphic distribution of the Colorado beetle (*Leptinotarsa decemlineata* SAY) imago, front back central part, in Belarus is studied. It is shown the variability of the Colorado beetle population phenotypic structure under the influence of insecticides as anthropogenic factor. The pest population diversity according to pyrethroid chemical group preparations resistance in the northern, the central and the southern agroclimatic zones is specified by the morphotypic method. In the Republic of Belarus, there is still a presence of pyrethroid-resistant populations, as a high content of phenomorph №3 (resistance marker) shows. The tendencies in phenomorph №3 occurrence frequency change can be a basis for the further development of forecast or resistance reversion to insecticides. Colorado beetle imago populations susceptibility to preparations, based on imidacloprid and thiametoxam, is revealed after their application by the method of potato tubers treatment before planting. It is indicated by the computer Colorado beetle phenomorph imago database development with their frequency of occurrence in the regions of the Republic and its target use.

Keywords: Belarus, Colorado beetle, phenotypic structure, resistance.

Rezumat. Variabilitatea structurii fenotipice a populaţiilor gândacului de Colorado (*Leptinotarsa decemlineata* SAY) (Coleoptera: Chrysomelidae) sub influenţa factorului antropic în Republica Belarus. Este studiată distribuţia fenomorfică a gândacului de Colorado (*Leptinotarsa decemlineata* SAY), exemplare adulte, partea frontală centrală posterioară, din Belarus. Este ilustrată variabilitatea structurii fenotipice a populaţiilor gândacului de Colorado sub influenţa insecticidelor, ca factor antropic de acţiune. Diversitatea populaţiilor de dăunători conform rezistenţei la produsele chimice din grupul piretroide, în zonele agroclimatice din nord, centru şi sud, este specificată prin metoda morfotipică. În Republica Belarus, mai există încă populaţii rezistente la piretroide, aşa cum indică prezenţa mare a fenomorfului №3 (marker al rezistenţei). Tendinţele în schimbarea frecvenţei apariţiei fenomorfului №3 pot constitui un punct de plecare pentru dezvoltarea prognozării sau reversiei rezistenţei la insecticide. Susceptibilitatea populaţiilor de gândaci de Colorado adulţi la diferite preparate pe bază de imidacloprid şi tiametoxam este evidenţiată după aplicarea acestora prin tratarea tuberculilor de cartofi înainte de plantare. Este indicată dezvoltarea bazei de date privind fenomorfii adultului gândacului de Colorado, care redă frecvenţa apariţiei în diferite regiuni ale republicii.

Cuvinte cheie: Belarus, gândacul de Colorado, structură fenotipică, rezistenţă.

INTRODUCTION

On the territory of the Republic of Belarus, the Colorado beetle (*Leptinotarsa decemlineata*) has been present for more than 50 years and is a dominant pest, inhabiting 98–100% of the potato areas. In the potato agrocoenosis, the high number of harmful phytophagous species is constantly preserved, due to the high level of polymorphism, ecological flexibility and a large adaptive potential (USHATINSKAYA, 1981). In these conditions, the chemical method of potato protection against the pest is dominant. However, its systematic application in the Republic led to an increased resistance of the Colorado beetle at first to chlororganic, then to phosphororganic compounds and pyrethroids (BYHOVETS, 2000). As it is known, anthropogenic load enhancement in agrobiocoenosis, which is characterized by the extreme variety and changeability of ecological conditions, including the use of chemical means, increases the possibility of the appearance of a new adaptive harmful and often more aggressive species form (FASULATI & VILKOVA, 2000). Thus, the analysis of microevolutional processes of the phytophagous organisms according to the potato protection system improvement is important from any point of view, including pest resistance to insecticides formation problems. These processes are observed in relation to Colorado beetle, as they are accompanied by changes of its population phenotypic structure (according to shared ratio of standard morph imago), by the relationship of the main ecological adaptations of the species intrapopulation forms with the individual changeable outward signs, one of them is a type of figure beetle front back (FASULATI, 1985).

In Belarus, the research on Colorado beetle population phenotypic structure were fragmentary and were carried out more than 30 years ago for the conditions of the south-west Brest region (KOKHMANJUK & KLIMETS, 1976; KLIMETS, 1988). But at present, the boundaries of the agroclimatic zones have changed; plant protection chemical means assortment is expanded. In this connection, the aim of the paper was the study of the Colorado beetle population phenotypic structure depending on habitat conditions and the determination of the distribution regularities of the phytophagous species resistance and of susceptible populations to widely used insecticides.

MATERIAL AND METHODS

Potato planting investigation of the overwintered collected Colorado beetle imagoes were done in 2007–2011. During the research, there were used imagoes collected from agricultural establishments and small holdings from all the agroclimatic zones (MELNIK, 2004). The beetle collecting was done during mass potato crops colonization, at full seedlings – blossoming stage. The number of studied populations was 60 from 24 districts of the Republic, in 2007, in 2008 – 61 from 37 districts, in 2009 – 30 (23 districts), in 2010 – 7 (5 districts), 2011 – 24 (21 districts).

During the years of research, 421 beetle samples were collected, of which more than 35,000 individuals were analysed; they fed on different potato varieties, both of domestic and foreign selection, widely cultivated in the Republic of Belarus. The comparative analysis of the pest populations phenotypic structure was done using 9 main morphs, selected according to the types of front back imago (pronotum) figure (Fig. 1) (FASULATI, 1985). According to these methods, the research studies were carried out in the Republic for the first time.

For Colorado beetle populations resistance diagnostics to the pyrethroid insecticides chemical group, a morphotypical method is used according to the following gradation: if №3 morph share from total morphs makes up to 15% - the population is sensitive, up to 20% - tolerant, up to 30% - resistant, up to 50% - highly-resistant (VASILIEVA *et al*., 2004). The sensitivity estimation of Colorado beetle zone populations to the insecticides from neonicotinoid chemical group was done after their application by tubers treatment method before planting on the basis of potato crops phytosanitary estimation.

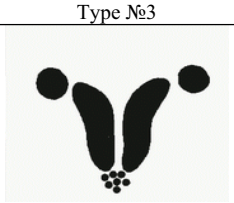
Features (phens)	**Stains A and B are merged: phen AB**	**Drawing is not symmetric: phen (AB)**	**Stains A and B are divided: phen B**
Stain P is brightly expressed: phen (P)	 Type №1	 Type №2	 Type №3
Stain P is poorly expressed: phen (p)	 Type №4	 Type №5	 Type №6
P stains are absent: phen (–)	 Type №7	 Type №8	 Type №9

Figure 1. The basic types of drawing of Colorado beetle imago front back central part, their signs constituents (phens) and arbitrary numbers (FASULATI, 1985). / Figura 1. Tipurile principale de pete prezente la gândacul de Colorado, partea frontală centrală posterioară, semnele constituente (phens) şi numerele arbitrare (FASULATI, 1985).

The insecticides application rate in 2006–2008 was indicated according to the data of SE "Main State Inspection on Seed Production, Quarantine and Plant Protection". Electronic bank of Colorado beetle phenomorphs is made with the help of the special database service programs Microsoft Access for Windows. The results of proper scientific researches were the primary information for the database filling on Colorado beetle imago phenomorphs with their occurrence frequency in the regions of the Republic.

RESULTS AND DISCUSSIONS

Nowadays, the insecticides assortment in the protection of potatoes against Colorado beetle includes 50 preparations, belonging to 8 chemical groups: phosphororganic, nereotoxins, pyrethroids, phenyl pyrazoles, neonicotinoids, anthranilamids, combined insecticides, semicarbozones. The allowed insecticides for application differ according to the mode of penetration into the pest body, the character and mechanism of action, and they are used both by the method of vegetative plants spraying and potato tubers treatment before planting. In connection with the zonal phytophagous incidence in the Republic of Belarus, the tendency of chemical means of plant protection use is traced. In the southern regions, where every year high threshold number is marked, the treatments number increases several times in comparison with the northern region.

In accordance with the estimation of plant protection products application amount, the phosphororganic preparations were replaced by pyrethroids: from 1995 to 2000, more than 98% of the total potato areas volume was treated with pyrethroid compounds. By 2006 pyrethroids and neonicotinoids ratio was equalled, potato areas subjected to treatment – 45.6 and 47.7%, accordingly, a proportion of the preparations from other chemical groups (phosphororganic, nereistoxins, phenyl pyrazoles) was about 6.7% (Fig. 2). In connection with this, in 2007, there was an increase of neonicotinoids application amount, rendering contact-intestinal and systemic action up to 74.4% potato areas. In 2008, their positive application dynamics was observed – 75.3%, pyrethroids are applied on 23.6% areas, the preparations from other chemical groups – 1.1%.

The current changes in the structure of using chemical preparations, during the years, influenced the changes of the phytophagous morphological traits; one of them is a figure of the imago front back central part, represented as phens (A, B and P). Broken, distinguished from one another some sign variations are named phens. They reflect genetic constitution of the given individual, and their frequency – the populations genetic structure (TIMOFEEV-RESOVSKIJ & YABLOKOV, 1973; YABLOKOV, 1980).

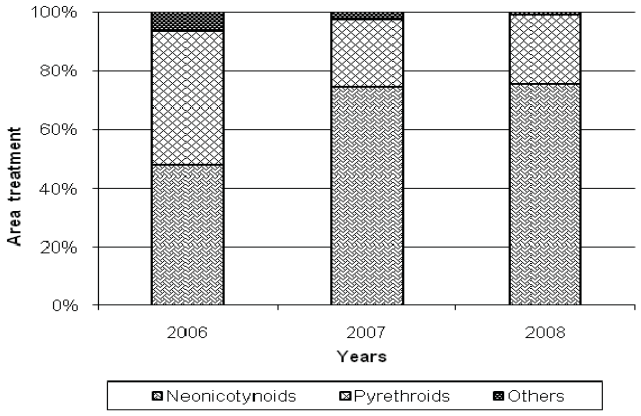


Figure 2. The change of different chemical class insecticides amount for potato protection against Colorado beetle (data SE "Main State Inspection on Seed Production, Quarantine and Plant Protection"). / Figura 2. Schimbarea cantităţii diferitelor clase de insecticide pentru protecţia cartofului împotriva gândacului de Colorado (date SE "Inspectoratul de Stat pentru Producerea de Seminţe, Carantina şi Protecţia Plantelor").

Note – Insecticides from other chemical groups – phosphororganic, nereistoxins, phenyl pyrazoles.

The Colorado beetle individuals phenotypic analysis done according to the figure of the imago front back central part has shown that the phenomorphs №1, №2, №3 (Table 1) are dominant. During the selective factors effect (temperature, insecticidal, etc.), the beetles are able to increase their share in phenotypes distribution in the population, what testifies the individuals increased viability. In our researches, the phenomorph №1 prevalence specifies this, as the frequency of occurrence fluctuated within 12.9–29.8%. Phenomorphs №7, №8, №9 are rare: their share was from 0.2 to 4.9%.

Table 1. Colorado beetle imago phenomorph occurrence frequency in the Republic of Belarus.
Tabel 1. Frecvenţa apariţiei fenomorfului la gândacul de Colorado în Republica Belarus.

Year	Phenomorph occurrence frequency, %								
	1	2	3	4	5	6	7	8	9
2007	29.8	18.0	22.5	6.5	5.1	9.4	2.5	1.9	4.3
2008	29.3	13.6	16.8	11.6	9.4	12.8	2.3	1.7	2.6
2009	22.4	13.6	16.0	10.0	9.3	15.0	4.9	2.9	6.2
2010	20.9	10.8	19.7	14.2	10.4	23.3	0.2	0.2	0.4
2011	12.9	9.8	19.1	9.9	12.1	25.9	1.5	2.5	6.4

In the literature, there is some information that the frequency of the beetle morphotypes occurrence is subjected to geographical, biotypical, seasonal variability. Besides, the Russian researchers, VASILIEVA *et al.*, 2004, determined that the Colorado beetle adaptation to seasonal temperatures is related to thermic-dependent phenomorphs №1, №2 and №6, and phenomorph №3 is a marker of the beetle population resistance to the preparations of pyrethroid chemical group (Fig. 3).

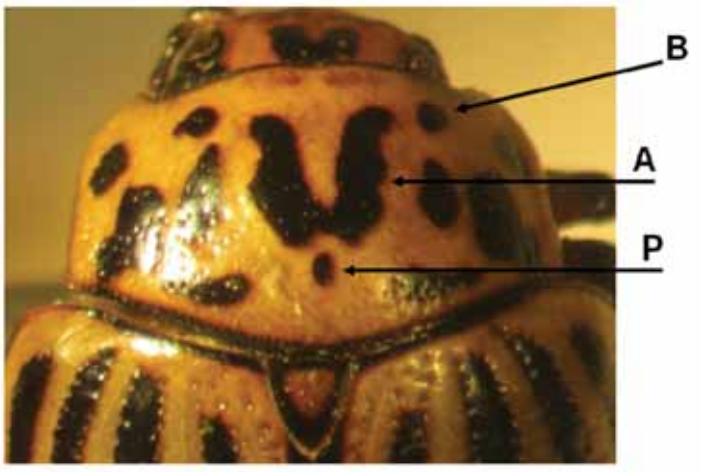


Figure 3. Phenomorph №3 of a figure of the imago front back central part (pronotum). / Figura 3. Fenomorful №3 al unei cifre, cazul unui adult, partea frontală centrală posterioară (pronot) (original).

Note – Spots B and strips A are separated: phen B, dot P is clearly expressed (according to FASULATI, 1985)

While Colorado beetle's microevolutional processes studying, it is revealed that under the pyrethroid intensive use influence in our Republic, the beetles average morph №3 share in 2007 during pest mass reproduction in the Belarusian population has reached 22.5% of the total amount of individuals (Table 1). Resistant populations were not only in the southern agroclimatic zones (23.5%), but in the central (21.3%) and northern (22.7%) ones.

In 2008–2009 (populations low density), a decrease of phenomorph №3 occurrence frequency to 16.8–16.0%, was noticed, accordingly, diagnosing the population as tolerant. In our opinion, the direction of these processes is caused both by the changes in applied insecticides assortment, i.e. the increasing of neonicotinoids use share, and by the meteorological conditions and biological peculiarities of the Colorado beetle. In 2010–2011, the phenomorph №3 share characterized the population in relation to pyrethroids as tolerant (19.7 and 19.1%, accordingly). We can assume that the neonicotinoids application promotes the gradual reversion (sensitivity return) to pyrethroids.

In connection with the different degree of potato plantings colonization by the Colorado beetle and the intensity of pyrethroids application in the zonal aspect phenomorph №3 incidence variation according to the agroclimatic zones is revealed. Thus, resistant populations are met in the southern zone in 53.4% cases, in the central one – in 26.7% cases, whereas, in the northern one they were not revealed. High-resistant populations were revealed in 20.0% cases in the southern

agroclimatic zone. Therefore, in spite of the fact that the last years in applied insecticides structure neonicotinoids prevail, there is a presence of the populations resistant to pyrethroids, what testifies a high content of phenomorph №3.

Since 2008 we annually do the estimation of the Colorado beetle population sensitivity to insecticides from neonicotinoids group by the method of phytosanitary monitoring of potato plantings after the preparations application by the method of tubers treatment before planting. During the researches carried out, sensitivity to imidacloprid and thiamethoxam was revealed in 45 regional Colorado beetle in imago stage populations (at 100% biological efficiency of the preparations).

The results of phenomorph research of the Colorado beetle imago front back central part were the basis for the creation of the database with open access, with the help of which the pest population diagnosis is possible according to insecticides resistance, which belong to pyrethroids chemical class. In connection with different level sensitivity of the pest population to pyrethroids presented in database on phenomorph №3 incidence, it is possible, based on over-wintered or young beetles, to make a decision in advance according to scientific-proved choice of insecticides for phytophagous control in the proper agrobiocoenosis, justify their application tactics and form the assortment during chemical means purchase.

CONCLUSIONS

Our results testify the microevolutional processes intensity in Colorado beetle populations on the territory of the Republic of Belarus. The Phenomorph №3 figure of the pest imago front back central part is a sensitive marker, which helps to determine the processes direction and allocate such anthropogenic factor as insecticidal load. Its incidence changes depending on the intensity of insecticides application from pyrethroids chemical class. During the zonal characteristics determination it is revealed that the resistant populations are met in the southern zone in 53.4% cases, in the central one – in 26.7% cases, whereas, in the northern one, they were not revealed. High-resistant populations were revealed in 20.0% cases in the southern agroclimatic zone. The populations resistant to insecticides from neonicotinoids chemical class were not revealed. The computer database on Colorado beetle imago phenomorphs is composed with the structure of information by years, regions, farms, varieties, incidence in percentage of phenomorphs №1–9, which let justify the tactics of application of insecticides. In connection with the Colorado beetle phenomorph incidence changing, depending on the research year, population geographical belonging, chemical means, there is a need of the phytophagous phen shape annual monitoring, that further let forecast the pest sensitivity changes to applied insecticides.

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