

The study of *Perillus bioculatus* F. (Heteroptera, Pentatomidae) population as a potential key factor in the development of biological management strategies for *Leptinotarsa decemlineata* Say at *Solanum melongena* L.

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ABSTRACT

Perillus bioculatus F. (Heteroptera, Pentatomidae), or the two-spotted stink bug, is considered an important predator of Colorado potato beetle egg masses, larvae and adults. He is attracted to the volatile substances emitted by damaged plants. In Bacau County, the species was observed in 2022 in the eggplant culture at Vegetable Research and Development Station Bacau, during the survey of the Colorado potato beetle. The first apparition of the predatory insect was recorded at the beginning of August when we found 10 imago and 5 egg masses, that have been collected and transported into the laboratory. After a few days, 69 nymphs hatched from a total of 73 eggs. A total of 30 imago's and 25 first instar nymphs of the two-spotted stink bug were found in the second decade of August. The *P. bioculatus* dynamics proved to be affected by feeding conditions or conventional insecticide applications. In this study we report the presence of a new predator that is the natural enemy of the Colorado potato beetle in eggplants cultures, the focus of the present study being the density and dynamics of this insect as a potential key factor in the development of biological management strategies for *L. decemlineata* Say pest control at *Solanum melongena* L.

Keywords: eggplant crop, two-spotted stink bug, plant protection, predatory insects, biological control of Colorado potato beetles.

INTRODUCTION

Eggplant, *Solanum melongena* L. is one of the most popular vegetables in the world. It occupies a distinct place in the realm of vegetable crops. Is a perennial plant, but they are grown commercially as an annual crop. Rich in antioxidants, the plants play an essential role in the prevention of chronic and degenerative diseases, including heart disease and cancer (Daunay *et al.*, 2001; Frary *et al.*, 2007; Gürbüz *et al.*, 2018). In 2020, FAO statistics estimate that approximately 1,876,710 hectares (ha) were harvested worldwide, resulting in 56,618,843 tonnes (t) of production, 28,285 ha were harvested in Europe, yielding 960,227 t, and 4,710 ha were harvested in Romania, yielding 74,040 t (FAOSTATISTIC, 2022). Approximately 4.5 kg of eggplants are consumed per person annually in Romania (Kovács *et al.*, 2016). Among the most important eggplant, growing areas in Romania are Matca-Tecuci, Galati; Cosereni, Ialomita; Izbiceni-Cilieni, Olt; Vidra, Giurgiu; Seleusi-Curtici, Arad (Kovács *et al.*, 2016). In Romania the main pests of solanaceous plants in general and the eggplant in special are: aphids (*Aphis gossypii* Glov., *Myzus persicae* Sulzer), thrips (*Frankliniella*

occidentalis Pergande, *Thrips tabaci* Lind.) cotton bollworm (*Helicoverpa armigera* Hübner) and Colorado potato beetle (*Leptinotarsa decemlineata* Say) (Călin, 2005; Cindea, 1980; Costache *et al.*, 2003). At Vegetable Research and Development Station Bacau (VRDS Bacau) the main pest in the eggplant culture is Colorado potato beetle (*Leptinotarsa decemlineata* Say) (Coleoptera: Chrysomelidae).

L. decemlineata is the most important pest insect for potatoes, but it also attacks other solanaceous vegetables and causes significant damage to eggplants and can lead to a large yield loss. In the past, the insect has been successfully suppressed by pesticides, but in our days most conventional insecticides have had little effect on it because it has rapidly developed resistance to them (Kadoić Balaško *et al.*, 2020; Molnar and Rakosy-Tican, 2021). In 1950 this insect evolved resistance to DDT and more recently imidacloprid. Currently, many countries integrated pest management (IPM) programs on cultural and biological control methods, with a highly limited number of insecticide applications, in an attempt to minimize the Colorado potato beetle exhibits (Clements *et al.*, 2017; Schnaars-Uvino and Baker, 2021).

The European entomologists began studying the Colorado potato beetle's natural enemies not long after it acclimated in Europe and became a pest species. An insect predator called two-spotted stink bug (*Perillus bioculatus* Fabricius), whose biology had been studied in North America, was introduced into Europe in order to restrain the Colorado potato beetle (Elisovețcaia *et al.*, 2016). The first attempts to acclimatize the two-spotted stink bug species were conducted by French researchers in the 30's of the 20th century. Due to the outbreak of the Second World War, the investigation stopped and was not resumed until 50-60 years later, but this time, in several European countries: France, Germany, Belgium, Yugoslavia, Czechoslovakia, Hungary, Bulgaria, Poland and the USSR (Derjanschi and Elisovetskaya, 2014).

This attractive species is easy to recognise. The common color form is red and black but the species show color polymorphism. They have a distinct black "Yshaped" marking on the pronotum and two black spots on the thorax. This species has five instars. The body length of first instar have approximately 1-1.5 mm; 2nd instar 2-3 mm; 3rd instar 4-5 mm; 4th instar 6-7 mm; 5th instar are about 8 to 9 mm long and the imago has a length between 8.5-11.5 mm (Elisovețcaia *et al.*, 2016; Paiero *et al.*, 2013). A study conducted during 1960-90 examined the reproduction, application, and acclimatization of *P. bioculatus* species in ex-USSR. It was concluded that two-spotted stink bugs develop in the north and mountainous areas in two generations, but in the south, three or four generations (Derjanschi and Elisovetskaya, 2014). As a result of its climatic conditions, it is estimated that in the Republic of Moldova, *P. bioculatus* develops 3 generations during the season, one in June-July, one in July-August, and one in August-September (Elisovețcaia *et al.*, 2016). It is possible that in Romania this insect also develops up to three generations during the season, but more studies are needed on the bioecological peculiarities of our Country. The nymphs and adults of the two-spotted stink bug prey with egg mass, larvae, and adults of *L. decemlineata* (Tarla and Tarla, 2018) but they prefer eggs and larvae of the Colorado potato beetle, and only adult of predator feed on the imago of *L. decemlineata* (Lacey *et al.*, 2001). A minimum of 1.5 individuals (nymphs or adults) of *P. bioculatus* per plant reduces the number of Colorado potato beetles to zero in seven days (Ferro, 1994 quoted by Elisovețcaia *et al.*, 2016). Despite all the efforts made by European researchers, the species was not able to adapt to the climatic conditions of the new continent (Jermy, 1980). In 2004, the species *P. bioculatus* was discovered on the potato plants in the European part of Turkey and Greece for the first time (Kivan, 2004; Péricart and Heiss, 2010). Russian scientists discovered several populations of two-spotted stink bugs in 2008 in Krasnodar region. There were between 10-20 larvae and adults per 1 m² on *Ambrosia artemisiifolia* L. plants (Ismailov and Agasieva, 2010). Based on a few samples of *P. bioculatus*, new faunistic reports were published in

Bulgaria (Simov et al., 2012) and Serbia (Protić and Živić, 2012) in 2012 and in Romania (Rădac and Teodorescu, 2021) in 2021. As far as we know from the literature, in Romania, are no mention of *P. bioculatus* until 2021 and there were no recent releases done in agriculture (Kazimir, 2018; Rădac and Teodorescu, 2021). In the north-eastern area of Romania, Bacau County, (46.34566°N, 26.57144°E), the species *P. bioculatus* was identified in an agricultural crop in field conditions, namely on eggplants. So far, the studied specimens were found in three sites in southern Romania in 2017, 2018 and 2020 (2017-2018 - 45.07366°N, 28.80058°E/ 45.13205°N, 28.58267°E; 2020 - 44.40906°N, 26.18689°E) and they are not associated with agricultural lands (Rădac and Teodorescu, 2021). *P. bioculatus* probably arrived in Romania naturally, either from the Republic of Moldova or Bulgaria (Rădac and Teodorescu, 2021).

The species can be a potential key factor in the development of biological management strategies for *Leptinotarsa decemlineata*. This study aimed to report that the species *P. bioculatus* was found for the first time in a crop of economic importance for Romania such as eggplant in the northeast region, and to record the population density of this predatory bug under the conditions of an eggplant crop in conventional agriculture.

MATERIALS AND METHODS

Seedlings of the eggplant, 'Alexandra' cultivar, were transplanted in the field on 13 May 2022, at a distance of 70 cm between rows and 40 cm between plants per row, resulting in a plant density of 35 714 plants per hectare. Observations on the presence of *P. bioculatus* in eggplant crops were carried out on an area of 1500 m² in the experimental field for conventional agriculture at VRDS Bacau in 2022. To determine the dynamics of *L. decemlineata* in eggplant, the attack estimation was determined using the following indicators: frequency of attack (F%) and intensity of attack (I%), every 10 days, for 100 plants, against a background of natural infestation. With the data obtained, the degree of attack (DA%) was calculated. The dynamic of the insect was graphically represented.

During the observations of Colorado potato beetle dynamics in eggplant, specimens of imago *Pentatomidae* were observed feeding on larvae of *L. decemlineata* in the eggplant experimental field. The predator was observed in culture starting with the first decade of August. Five adult of stink bug specimens were collected by hand for laboratory determination. The determination of specie was performed using the literature available in international databases but also using diagnostic characters keys from *Perillus* species provided by Paiero *et al.* (2013) in "Stink bugs (*Pentatomidae*) and parent bugs (*Acanthosomatidae*) of Ontario and adjacent areas: A key to species and a review of the fauna". The collected specimens were preserved in 70% alcohol for preservation in the entomological collection of VRDS Bacau. Also, five egg masse were collected and transported to the laboratory for counting. The eggs were numbered at a stereomicroscope and then each egg mass was placed in individual test tubes to see how many nymphs will hatch.

In situ images were taken with the Xiaomi Redmi Note 9 PRO phone camera and stereomicroscope images were taken using the Toupcam L3CMOS digital camera and ToupView software version x64, 4.11.18081.20201205 compatible with Windows 7/8/10. The climatic condition were registered with the FielClimate weather station.

RESULTS AND DISCUSSIONS

In the climatic conditions of 2022, with average temperatures not exceeding 25°C in the summer months and low rainfall (figure1 A and B), this is the first time when a two-spotted stink bug, a predatory pentatomid bug, has been found in Bacau County. In our Country, the

species was recorded for the first time by Rădac and Teodorescu in 2021 in southern Romania.

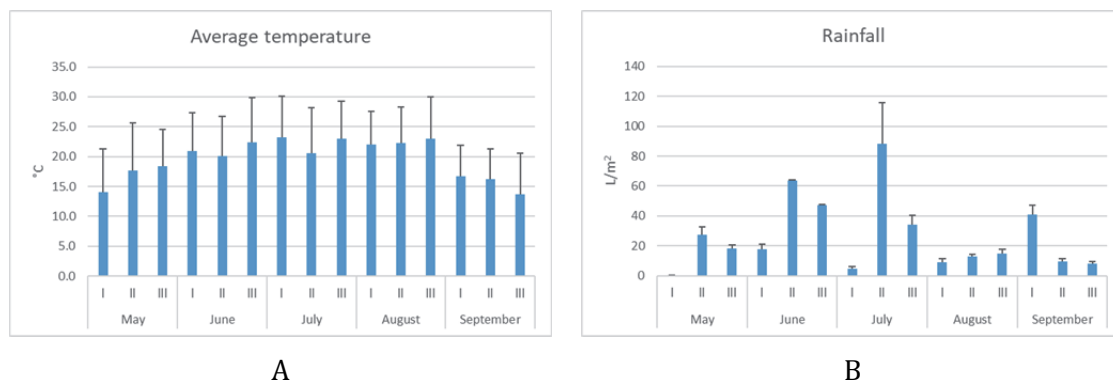


Figure 1. (A) The average air temperature recorded by the Field Climate weather station at VRDS on decades in 2022; (B) The sum of precipitation recorded by the Field Climate weather station at VRDS on decades in 2022

This year, during the survey of Colorado potato beetle (figure. 2.A), in the experimental field for conventional agriculture at VRDS Bacau, it has been seen that imago *Pentatomidae* were feeding on the larvae of *L. decemlineata* (figure 2.B), which are pests for the eggplant. From 10 imago observed per 100 plants examined 5 specimens were collected by hand and transported to the laboratory for determination. A literature search of international databases was conducted to determine the species, as well as diagnostic character keys from *Perillus* species provided by Paiero *et al.* (2013) in "Stink bugs (*Pentatomidae*) and parent bugs (*Acanthosomatidae*) of Ontario and adjacent areas: A key to species and a review of the fauna". The literature suggests that *P. bioculatus* is a monophagous species that feed exclusively on Colorado potato beetles, but in the laboratory studies, some alternative host foods have been used (Tarla and Tarla, 2018). Dina Elisovetcaia *et al.* (2020) studied the reproduction of *P. bioculatus* species and observed that when nymphs were fed exclusively on *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae, the percentage of malformed individuals of two-spotted stink bugs during molting increased in comparison with other nymphs that fed additionally with Colorado potato beetle. Also, the study revealed that in the first group, the death rate reached 20 to 75% compared with the individuals fed and with Colorado potato beetle larvae (Elisovetcaia *et al.*, 2020). At VRDS Bacau the species were observed feeding in the eggplant crop only on eggs and larvae of *L. decemlineata*. The species show color polymorphism (Ismailov *et al.*, 2019) in eggplant culture being identified with two color variations (red/black and ivory/brown) (figure 2, C and D) but in the literature, three forms of color are shown for this species (red, yellow, and black & white form) (Elisovetcaia *et al.*, 2016). Some authors claim that the color form depended on the temperature conditions (Elisovetcaia *et al.*, 2016) and other authors have reported that the insect's food is responsible for its color (Kaur *et al.*, 2017). However, there is a possibility that the amount of carotin deposited in the hypodermis of insects can be permanently modified by the environment (Palmer and Knight, 1924)

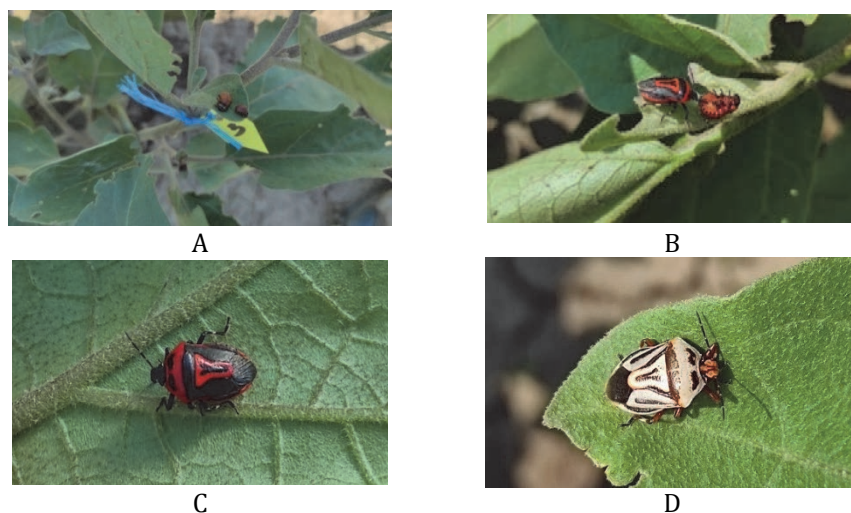


Figure. 2. (A) *Leptinotarsa decemlineata* Say larvae on a *Solanum melongena* L. plant; (B) *Perillus bioculatus* F. imago feeding with a *Leptinotarsa decemlineata* Say larvae on a *Solanum melongena* L. leaf; (C) *Perillus bioculatus* F. red/black color variation; (D) *Perillus bioculatus* F. ivory/brown color variation

The eggs found are laid by the female on the upper part of the eggplant leaves (figure 3, A). The egg mass is arranged in compact rows containing a total of 10 to 18 barrel-shaped eggs. At first, the egg has a yellow color after which it turns black and are cemented to the substrate. The chorion has reticulated markings (figure 3, B). After hatching, nymphs are red/black, round to oval in shape, and stay in groups until the exoskeleton hardens (figure 3, B and C). Prasad and Pal (2015) studied the incubation period and duration of other stages of the life cycle of *P. bioculatus* and they observe that the first instar nymph consumed 1-2 grubs of *Zygogramma bicolorata* Pallister (Coleoptera, Chrysomelidae), 2nd and 3rd instar nymphs consumed 3 grubs, 4th instar nymph consumed 10-11 grubs and the adult bug consumed 6-7 grubs. During their life period, a single two-spotted stink bug consumed a ranged of 20-22 *Z. bicolorata* grubs (Prasad and Pal, 2015).

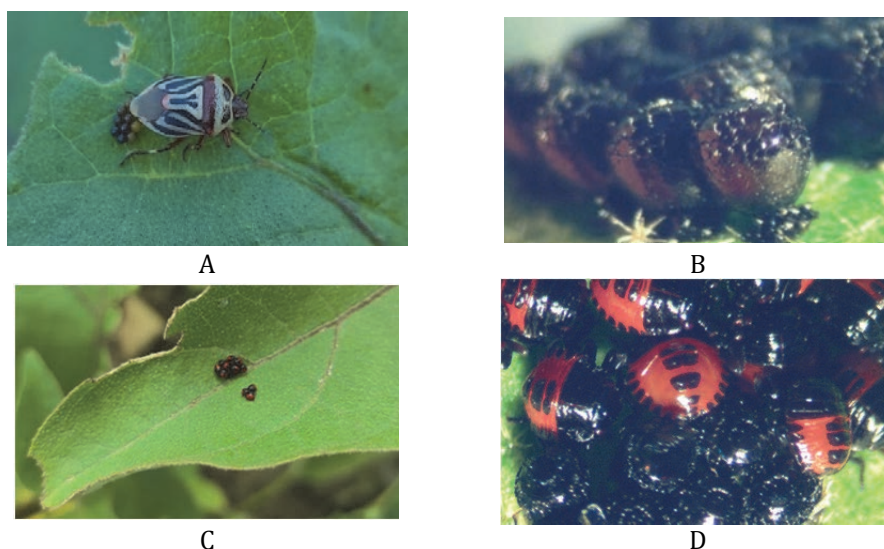


Figure 3 (A) *Perillus bioculatus* F. female laying eggs on a *Solanum melongena* L. leaf; (B) *Perillus bioculatus* F. eggs seen at stereomicroscope, detail of the chorion with reticulated markings; (C) *Perillus bioculatus* F. nymphs hatching on a *Solanum melongena* L. leaf; (D) *Perillus bioculatus* F. nymphs seen at stereomicroscope

The experimental field for conventional agriculture at VRDS Bacau was used to observe the dynamics of the *L. decemlineata* species under 2022 climatic conditions. The Colorado potato beetle began attacking eggplant crops in the first decade of July (figure 4.A) and the degree of attack increased until the second decade of August. During the first decade of August, 10 individuals (figure 4, A) and 5 egg masses of the predatory insect *P. bioculatus* were found per 100 eggplants examined (figure 4, B). Five adults and the egg masses were collected by hand for laboratory determination. The collected eggs were numbered and then each egg masse was placed in individual test tubes. Out of a total of 73 eggs collected, after a few days, 69 nymphs hatch from the eggs. The next day after counting all nymphs were released into the same eggplant crop. In the second decade of August, 30 imago of two-spotted stink bug were found per 100 eggplants examined and a number of 25 first-instar nymphs was a count (figure 4,B). Weissbecker B. *et al.* (2000) studied the volatile substances emitted by damaged potato plants to observe whether the species *P. bioculatus* is attracted to these substances. It has been observed that the two-spotted stink bug loses its attractiveness after one hour if the plant is mechanically damaged, however, the attraction was long-lasting if the plants were damaged by the Colorado potato beetle (Weissbecker *et al.*, 2000). This may explain why the density of *P. bioculatus* increased in the eggplant culture in the second decade of August at VRDS Bacau with the increase of the degree of attack of the species *L. decemlineata*.

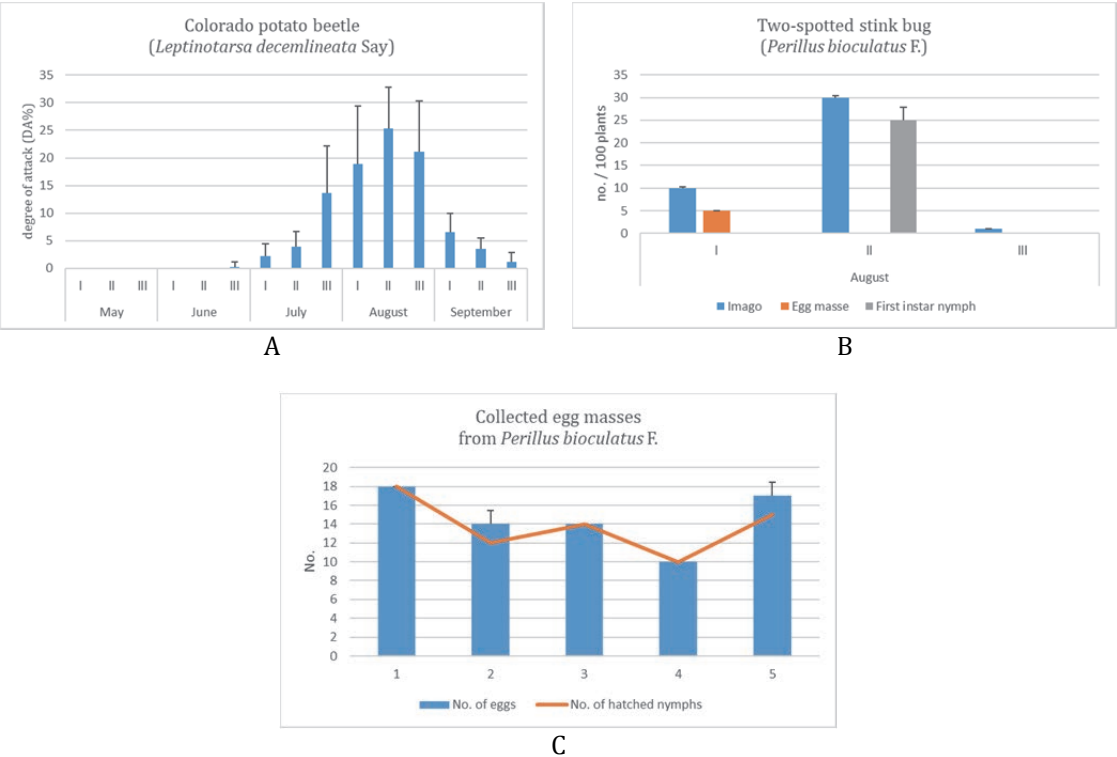


Figure 4. (A) The dynamic of *Leptinotarsa decemlineata* Say in *Solanum melongena* L. crop under 2022 climatic conditions; (B) The density of *Perillus bioculatus* F. imago, egg masses and first instar nymphs per 100 eggplants examined; (C) Number of *Perillus bioculatus* F. eggs collected and number of hatched nymphs

A decline of the species in the third decade of August may be the result of the insecticide Mospilan being used against the Colorado potato beetle in the eggplant crop (figure 4.B). The abundance of *P. bioculatus* may be influenced by feeding conditions or by the

application of conventional insecticides. In small-scale field cage experiments has been reported 100% mortality of *P. bioculatus* imago exposed to various chemical pesticides (Hough, 1991). Also, the temperature can play an important role in the embryologic and post-embryologic development for two-spotted stink bug (Coudron *et al.*, 2009; Jermy, 1980). Therefore, more studies on the impact of this species in crops affected by *L. decemlineata* should be carried out in Romania, because the two-spotted stink bug can be used in biological control which offers a more sustainable and less expensive alternative to chemicals.

CONCLUSIONS

The present study reports the presence of an important predator of the Colorado potato beetle in eggplant crops from northeast Romania. The detection of *P. bioculatus* in natural conditions on the territory of our Country shows that this species, which originates from the North American region, has acclimatized spontaneously because there are no official reports of releases in agro-ecosystems from Romania. Also, the data presented will contribute to a better understanding of the species range in Europe and Romania. Chemical control of *L. decemlineata* is still used by farmers. However, alternatives are being explored since the species is increasingly resistant to chemicals. Chemicals can also be the cause of major human health and environmental problems. Biological control approaches seem very promising but require a number of conservation measures to protect natural enemies for a long period. The integration of the predatory heteropteran species *P. bioculatus* in solanaceous crops (especially in potato and eggplant crops) will allow the natural reduction of the Colorado beetle population. Therefore, in the coming period, new research will be needed in Romania to help us understand more about the distribution, ecological relationships, and introduction of two-spotted stink bugs in natural and agricultural environments.

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