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Research on the reproduction of the species *Orius laevigatus* depending on the nutritional substrate

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Abstract

Organic farming is currently one of the most important areas of development in the agricultural sector, responding to the need for healthy food produced in harmony with the environment. Defined by respect for natural cycles and the use of renewable resources, organic farming excludes the use of pesticides and synthetic chemical fertilizers, emphasizing natural soil fertility, biodiversity, and ecosystem balance. The species *Orius laevigatus* (natural predators of thrips, aphids, and other harmful insects) is widely used in biological control programs. Their reproduction in laboratories or biofactories is strongly influenced by the substrate on which they are raised, as this influences egg laying, larval development, and adult survival. Entomophagous predator species such as *Orius laevigatus, Orius niger, Macrolophus pygmaeus, and Amblyseius swirskii* have proven to be highly effective in controlling major pests of greenhouses and solariums, such as thrips, aphids, and phytophagous mites. In addition, these predators are highly adaptable to the specific microclimate conditions of protected areas and can use alternative food sources, which allows them to maintain their populations even during periods of low prey density.

Keywords: *Orius laevigatus*; Predators; Organic farming; Thrips; Nutrients

1. Introduction

The main goal of organic farming is to protect the biosphere and conserve natural resources by not using chemical fertilizers, synthetic pesticides, and herbicides. In this system, prevention methods play a key role in fighting pests, diseases, and weeds. In order to achieve production in harmony with nature, it is necessary to adapt biological techniques to local and socio-economic conditions, using agroecosystem resources in an efficient and responsible manner [2]. Although the principles of organic farming are universal, the technologies applied differ according to the specific pedoclimatic conditions, available resources, and traditions of each area [4]. This form of agriculture requires increased attention, observation, and constant reflection. It uses a high volume of labor, thus contributing to the creation of new jobs and the maintenance of the rural population on agricultural land—a crucial aspect in a period marked by unemployment and massive migration to urban areas [9]. Pests of agricultural plants act on plant tissues through several mechanisms: directly, by feeding on various parts of the plant, or indirectly, by producing galleries, excrement, or toxic substances. These interactions lead to damage, harm, and crop losses, which are fundamental concepts in understanding and applying integrated pest management [5].

The use of plant protection products such as fungicides, insecticides, and herbicides is crucial for controlling diseases and pests in agriculture, but their safety, cost, and availability are a growing concern [7], [1], [8], [3]. Potential adverse effects on human health and the environment have led to the development of risk exposure indicators [10], [12] and stricter legislative requirements [11], [6].

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2. Material and methods

2.1. Polyphagous material

All insects used in the experiments were kept at the University of Agronomic Sciences and Veterinary Medicine Bucharest and the Hortinvest Research Center. *Orius laevigatus* was obtained from Koppert BV (Berkel en Rodenrijs, Netherlands). All predator rearing systems were initiated in the spring of 2021-2023, and the species were reared continuously for at least 10 generations before being used in experiments. The predators were reared in plastic jars (Ø 11 cm × 13 cm) with lids covered with fine gauze (80 µm mesh size) for ventilation, provided with a green bean pod (*Phaseolus vulgaris L.)* as an oviposition substrate, fed ad libitum with a 50:50 mixture of *Ephestia kuehniella Zeller* (Lepidoptera: *Pyralidae*) eggs (Entofood, Koppert BV) and decapsulated cysts of *Artemia franciscana* Kellogg (Anostraca: *Artemiidae*) (BioArtFeed, BioBee Biological Systems, Sde Eliyahu, Israel). The beans and food sources were replenished every two weeks, and bean pods previously exposed to adult predators carrying eggs were placed in a new jar, starting a new synchronized unit. Nine growth units per predator species were maintained at 25 ± 1 °C, 70 ± 10% relative humidity.

2.2. Plant material

Frankliniella occidentalis and T. vaporariorum cultures were initiated in June 2021 from individuals collected in USAMVB greenhouses. These cultures were maintained in separate greenhouse compartments on pesticide-free pepper and eggplant plants. Pepper plants (cv. Bianca and eggplant cv. Mirval), at the six-leaf stage, were obtained through seedlings at a farm in Sălcioara, Dâmbovița, in greenhouses of 1000 m^2 each. Ninety plants were also transplanted into 10 L pots with mineral wool and grown individually in insect-proof cages (75 × 75 × 115 cm, mesh size 150 µm, insect growth tent in a greenhouse compartment (144 m^2). The plants were grown without pesticides and fed a standard vegetable nutrient solution until used in experiments. Liquid fertilizers were applied at a rate of 260 mL per day, adjusted as necessary depending on weather conditions.

2.3. Work mode

Twenty-eight plants of each species (4 months after transplanting) were standardized by removing all leaves except nine (three young, incompletely expanded leaves; three mature, fully expanded leaves; and three old leaves from the base of the rosette) and two flowers (one mature, fully expanded, with a stem of ~60 cm, and one immature, developing flower with a stem of ~10 cm). Each plant was then placed individually in an insect-proof cage (60 × 60 × 90 cm, mesh size 250 µm), and eight adult female *Orius* predators, 1 week old, mated, were released into each cage. To support predator oviposition during the experiment, at the start of the experiment, a single *Ephestia kuehniella* egg card was provided in each cage as a food source, placed either on the cage wall with adhesive tape or on a single leaf. These cards, prepared on green cardboard with double-sided adhesive tape on an area of 11.5 × 11.6 mm, contain approximately 550 eggs, sufficient for the consumption needs of the predators during the period and under the conditions of this experiment.

3. Results and discussion

The species *Orius laevigatus* (natural predators of thrips, aphids, and other harmful insects) is widely used in biological control programs. Their reproduction in laboratories or biofactories is strongly influenced by the substrate on which they are raised, as this affects egg laying, larval development, and adult survival. (Figure 1)

(source: original)

Figure 1 Results regarding the reproduction of the species *Orius laevigatus.*

Table 1 Results regarding the type of plant substrate/plant for oviposition and development in *Orius laevigatus.*

N0	Substrate type	Average number of individuals/10 plants in 2021	Average number of individuals / 10 plants in 2022	Average number of individuals / 10 plants in 2023	Average ind./plant substrate
1.	pepper	180	240	199	206,3
2.	eggplants	120	184	172	158,6
3.	beans	80	75	62	72,3
	Overall	126,6	166,3	144,3	145,7
	Overall average/substrate type	145,7			

(source:original)

Table 1 shows the results regarding the type of plant substrate/plant for oviposition and development in *Orius laevigatus*. It can be seen that when using three species of vegetable plants (peppers, eggplants, and beans, 10 plants of each species) as a breeding substrate for the species *Orius laevigatus*, the most favorable substrate was peppers, with an average of 206.3 individuals/plant plant, followed by the eggplant substrate with 158.6 individuals/plant, while beans recorded lower values of 72.7 individuals/plant. The average on this substrate (crop plant) was 145.7 individuals/plant, a good average that recommends the substrate for practical application on farms.

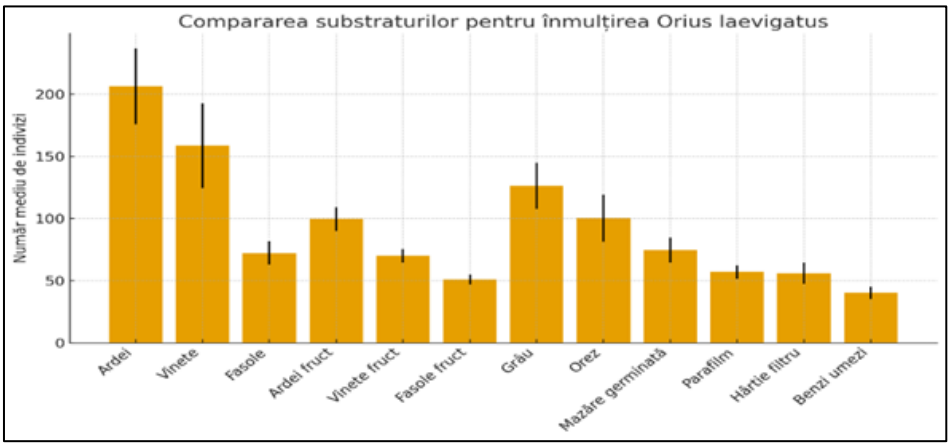

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Figure 2 The average differences between the types of substrates used for the reproduction of *Orius laevigatus*

Figure 2, represented as bars with standard errors, highlights the average differences between the types of substrates used for the reproduction of *Orius laevigatus*. It is clear that natural plant substrates (especially peppers) lead to the

highest values, with a significantly higher average number of individuals compared to cut fruit/vegetables, sprouts, and artificial substrates. The error bars also indicate greater variability in the case of plant substrates, reflecting differences between plant species (peppers being clearly superior to beans).

(source:original)

Figures 3. and 4. Aspects regarding the evolution of Orius laevigatus predators in eggplant crops

Table 2 Results regarding the type of substrate fruits and vegetables, for oviposition and development in Orius Laevigatus

No.	Substrate type	Average number of individuals /10 pieces of fruit in 2021	Average number of individuals / 10 pieces of fruit in 2022	Average number of individuals / 10 pieces of fruit in 2023	Average ind./substrate
1.	Peppers - pieces of fruit	110	98	91	99,6
2.	Eggplants - pieces of fruit	75	64	71	70,0
3.	Beans- pieces of fruit	51	47	55	51,0
	Overall	78,6	69,6	72,3	73,53
	Overall average/substrate type	73,53			

(source:original)

Table 2 shows the results regarding the type of substrate (cut fruit and vegetables) for oviposition and development in *Orius laevigatus*, (Figure 3 and 4).The results generated from the table show that the highest average values were recorded for the pepper substrate, with 99.6 individuals/10 pieces of substrate, followed by the eggplant substrate with 70 individuals/10 pieces of substrate, and finally the bean substrate with values of 51 individuals/10 pieces of substrate. The average for this type of substrate is 73.53 individuals/10 pieces of cut fruit, which is less than the plant substrate with 145.7 individuals.

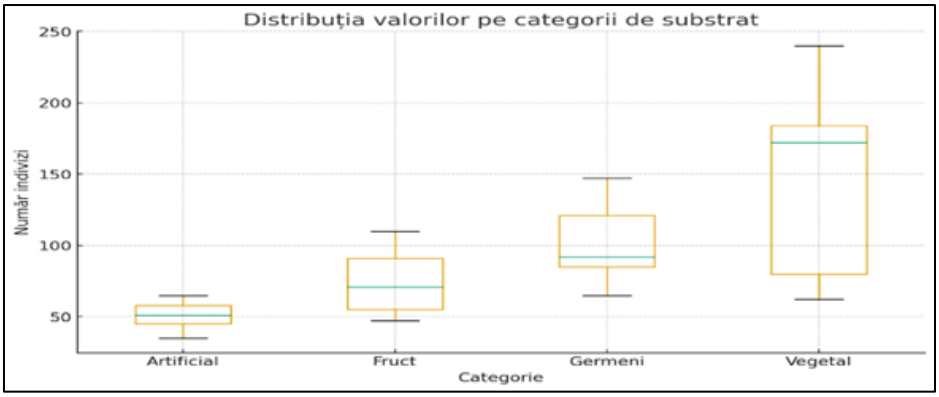

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Figure 5 Results regarding the distribution of variability in the predator/substrate relationship

Figure 5. A boxplot by substrate category provides an overview of the distribution and variability of the data. Plant substrates have a high median but also a high dispersion, confirming their overall efficiency but also notable differences between plant types. Sprouts have a more compact distribution with intermediate values, while cut fruits/vegetables have a low median and low variability. Artificial substrates are distinguished by very constant values, but at a minimum level, thus confirming their low efficiency.

Table 3 Results regarding the type of germ and shoot substrate for oviposition and development in *Orius laevigatus*

NO.	Substrate type	Average number of individuals /10 plants germinated in 2021	Average number of individuals / 10 plants germinated in 2022	Average number of individuals / 10 plants germinated in 2023	Average ind./substrate
1.	GRAIN	147	111	121	126,3
2.	RICE	122	87	92	100,3
3.	Sprouted peas	65	74	85	74,6
	Overall	111,3	90,6	99,3	100,4
	Overall average/substrate type	100,4			

(source:original)

Table 3 shows the results regarding the type of substrate of germs and shoots for oviposition and development in *Orius laevigatus*. The average results show that the highest value was recorded for the wheat sprout substrate with 126.3 individuals, followed by the rice sprout substrate with values of 100.3 and finally the sprouted pea substrate with values of 73.6. The average value for the sprouted plant material substrate was 100.4 individuals, a value immediately below that of the plant substrate, but the disadvantage is that it is highly perishable. This type of substrate may be a temporary or transitional alternative for the reproduction and development of the species *Orius laevigatus*.

Table 4 Results regarding the type of artificial substrate for oviposition and development in *Orius laevigatus*

N0.	Substrate type	Average number of individuals in 2021	Average number of individuals in 2022	Average number of individuals in 2023	Average ind./substrate
1.	parafilm	62	58	51	57,0
2.	Filter paper	55	65	48	56,0
3.	Wet strips	35	41	45	40,3
	Overall	50,6	54,6	48	51,1

	Overall average/substrate type	51,1			

(source:original)

Table 4 shows the results for the type of artificial substrate (type, parafilm, filter paper, wet strips) for oviposition and development in *Orius laevigatus.* The highest average number of individuals was recorded on the parafilm substrate with 57 individuals, followed by the filter paper substrate with 56 individuals, and finally the wet strips substrate with 40.3 individuals. The average value for this type of artificial substrate is 51.1 individuals, which is low compared to the values for the other types of substrate.

The data presented in Tables 1–4 allow a comparative analysis of the effectiveness of the different types of substrate used for oviposition and development of the species *Orius laevigatus.* In the case of natural plant substrates (Table 1), the overall average number of individuals per plant was 145.7, with significant variations between the plant species tested. The pepper substrate obtained the highest average (206.3 individuals/plant), followed by eggplant (158.6) and beans (72.3), indicating a clear preference of insects for the soft and succulent tissues of peppers. Analysis of variance over the years (2021–2023) revealed some interannual variability, with annual averages of 126.6, 166.3, and 144.3, respectively, suggesting the influence of environmental conditions or uncontrolled factors on reproduction. For substrates based on chopped fruit and vegetables (Table 2), the overall average was significantly lower (73.53 individuals/10 pieces) compared to intact plant substrates. Peppers again achieved the best results (99.6), followed by eggplants (70.0) and beans (51.0). The decrease in performance compared to the intact plant can be attributed to the rapid degradation of the cut plant material and the lack of a sustainable microhabitat.

In the case of sprouts and shoots (Table 3), the overall average was 100.4 individuals/10 sprouted plants, placing them between intact and cut natural substrates. Wheat sprouts provided the best conditions (126.3), followed by rice (100.3) and peas (74.6). These substrates, although easy to obtain, have disadvantages related to perishability and the need for frequent refreshment.

Artificial substrates (Table 4) performed the worst, with an overall average of only 51.1 individuals. Parafilm (57.0) and filter paper (56.0) provided modestly acceptable conditions, while wet strips (40.3) proved unsuitable. These results highlight the limitations of artificial substrates in the absence of nutritional supplements or a natural plant environment. From a descriptive statistics perspective, there is a consistent trend in the ranking of substrates among the categories tested: peppers > eggplants > beans in the case of natural vegetables and similarly in the case of cut fruits. Interannual variability was moderate, except for artificial substrates, where values remained constant and low. These findings support the conclusion that natural vegetable substrates, especially soft and succulent ones, are significantly superior for the efficient multiplication of *Orius laevigatus*, both in terms of oviposition and post-embryonic development survival.

4. Conclusions

Conclusions regarding the reproduction of *Orius laevigatus* depending on the substrate:

- Regarding the egg-laying process, the following observations can be made:
    - Orius laevigatus females lay their eggs in plant tissue by inserting their ovipositor.
    - The substrate must be soft and succulent (e.g., beans, peppers, eggplant, cotton leaves, or even wheat sprouts).
    - Plants with thick, succulent leaves and well-developed veins favor a higher egg-laying rate.
- Regarding the development of nymphs, the following observations were made:
    - Hatchling nymphs need both food (thrips, aphids, mites, or moth/fly eggs) and a suitable microclimate (moderate humidity).
    - The plant substrate has a dual role: it provides a microhabitat for food and a place for hiding and protection.
    - Artificial substrates (filter paper, wet sponges) can be used in intensive production systems, but their effectiveness depends on supplementation with artificial or live food.
- With regard to adults, it has been observed that:
    - They thrive better in containers with branches or leaves from host plants.
    - The presence of a plant substrate increases longevity and fertility.

- In the absence of substrate, even if food is available, egg laying is greatly reduced because the female cannot find a suitable place for oviposition.
- However, the following substrates have been highlighted as frequently used in propagation:
  - Natural: beans, peppers, eggplant, chrysanthemums.
  - Alternative (in biofactories): pieces of fruit/vegetables (e.g., chopped bell peppers), specially grown bean leaves, wheat or barley sprouts.
  - Artificial: parafilm strips or impregnated paper, but with reduced efficiency for egg laying.

The most effective substrate for the multiplication of *Orius laevigatus* is natural plant substrate (beans, peppers, eggplant). These provide the necessary conditions for oviposition, nymph development, and adult maintenance. Artificial substrates are useful only for controlled experiments, but not for intensive production. The reproduction of *Orius laevigatus* depends largely on the type of substrate used. Soft and succulent plant substrates (beans, peppers, cotton) stimulate oviposition and complete life cycle development, while artificial substrates are more difficult to use and require special supplements to achieve good results.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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