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Report on modelling the distribution, economic damage caused by the two spotted spider mite and southern green stink bug

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ABSTRACT (FOR DISSEMINATION)	Soybean production in organic farming can be quite challenging from a pest management perspective. The two-spotted spider mite (*Tetranychus urticae*) is one of the most important soybean pests. Its occurrence is in close connection with weather conditions whereby dry and hot weather promotes reproduction and expansion. There are several cultural practices, that can suppress mites. The southern green stink bug (SGSB), *Nezara viridula* is another important soybean pest occurring in Europe. The level of SGSB damage on soybean depends on the variety and environmental conditions. SGSB do not influence soybean plant architecture, number of lateral branches and plant height, but affects number of seed per plant and number of damaged seed, yield and thousand seed weight.
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1. Executive summary

Soybean production in organic farming can be quite challenging from a pest management perspective.

The two-spotted spider mite (*Tetranychus urticae*) is one of the most important soybean pests. Its occurrence is in close connection with weather conditions whereby dry and hot weather promotes reproduction and expansion. Weather conditions during 2018 and 2019 were not favourable for mites and their occurrence in that period was sporadic, while 2020 was favourable for mites' occurrence which resulted in an average level of appearance. The screening of pest presence with an unmanned aerial vehicle (UAV) was performed in 2021, with subsequent analysis of collected multispectral (MS) images. There are several cultural practices that can suppress mites.

The southern green stink bug (SGSB), *Nezara viridula* is another important soybean pest occurring on the territory of Europe. The potential for SGSB damage on soybean was tested in isolation cages, during 2018, 2020 and 2021. There were two treatments, a control without stink bugs, and one with SGSB specimens. The level of SGSB damage on soybean depends on the variety and environmental conditions. SGSB do not influence soybean plant architecture, number of lateral branches and plant height, but affect the number of seeds per plant and number of damaged seed, yield and thousand seed weight.

Soybean fields should be regularly and carefully monitored for pest's activity. The period of pest occurrence and the stage of crop growth have a significant impact on damage to soybean plants. In extreme cases, damage from two-spotted spider mite and the southern green stink bug can be as high as 100% in terms of crop loss. The prevalence and number of spider mites are significantly influenced by weather conditions. It is crucial to routinely inspect soybean fields since spider mite abundance is not always highest on the field border. Spider mite influence on lowering soybean yield can be reduced by irrigation.

By encroaching on new areas, the southern green stink bug is multiplying rapidly. Natural enemies have the potential to drastically lower SGSB populations but it will take time for them to spread over Europe. Spider mites and southern green stink bugs can have a detrimental impact on soybean production, although correct variety selection and cultivation practices can help to mitigate these impacts. This is the plan for a stable soybean yield in organic production. One of the upcoming goals will be to look at trap crops strategy implementation and apply this strategy to combat the stink bug issue in organic farming.

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2. Introduction

Organic farming production brings many challenges for many crops with some being more difficult to grow organically than others. Soybean production in organic farming can be quite challenging and it is dependent, among other things, on pest presence and their pressure. One of the most important soybean pests are spider mites. The knowledge obtained about the biology, ecology and behaviour should be used for their management and control. There are several cultural practices that suppress mite pests and encourage their natural enemies, which should be used. An experienced organic farmer should know how to manipulate the natural enemies of spider mites to protect his crop. Water management plays a vital role since colony of mites can be severely influenced by high moisture and precipitation. Further, elimination of other crop residues on time can prevent mites from damaging subsequent crops.

During the last decade another important soybean pest occurred in Europe, *Nezara viridula*, the southern green stink bug (SGSB). Since it is a relatively new pest, data on its biology, ecology and management are limited. More data on this topic should help in decreasing yield losses in organic production.

In the last decades, we are witnessing climate change which substantially influences organic agricultural production and makes it even harder. That is why every knowledge about pests and production, global or specific for a certain region, is of great importance.

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Tetranychus urticae

Tetranychus urticae, 200x magnification. LVG Heidelberg

Nezara viridula

Fig. 1: *Tetranychus urticae* and *Nezara viridula*

3. Two-spotted spider mite

Mites are economically the most important soybean pests in Europe. The risk of damage is higher during dry and hot summer conditions. Several species can damage soybean plants but the two-spotted spider mite (*Tetranychus urticae*) is considered to be the most common (Fig. 1).

Description

The two-spotted spider mite is a cosmopolitan species and polyphagous. Adults are the size of salt grains and are greenish-yellow to brown, with two black spots (Fig. 1). Mature females are 0.5 mm long and egg-shaped while males are smaller and yellowish, with a sharp-pointed abdomen. Eggs are oval and approximately 0.15 mm in diameter. Larvae have three pairs of legs while nymphs and adults have four pairs of legs.

Biology

Mites have 10 to 14 generations per year. In the spring, females who over-winter lay their eggs on weeds which are intermediary hosts. Afterwards weed mites move to crops. Eggs, up to 190 per female, are laid on the underside of leaves. Mites quickly establish colonies protected by a delicate, silky web that shields them from predators and unfavourable weather conditions. Colonies usually consist of individuals of all growing stages.

Scouting and damage

The time for the beginning of the monitoring is in close relationship with weather conditions during June. Since warm and dry conditions are favourable for establishing mite colonies, in such cases, monitoring should be started in the second half of June. In other cases, the middle of July is optimal. High intensity and frequency of rainfall has negative effects on colonies. Since infestations occur usually at the field edges (Fig. 2) those parts should be the starting points for monitoring. Also, in time mites will move to middle parts of soybean fields and those parts should be monitored as well. Special attention should be paid when alfalfa is in the vicinity of soybean because after mowing mites relocate to soybean fields. The mites usually use wind for migration.

During monitoring, attention should be paid to the underside of the leaves since mites prefer there to populate. For easier visual identification of spider mites, the usage of a magnifying lens (at least 10x) is recommended. The mites are feeding by piercing leaves and sucking sap. The first symptoms are manifested as small yellow dots on leaves. Damaged plants are smaller and perform photosynthesis less efficiently. Additionally, they reach maturity earlier, yielding fewer pods whereby soybean yield can be reduced by 40-60%. Even total damage can occur in some circumstances.

Fig. 2: Presence of the spider mites on a soybean field.

Mites are economically the most important soybean pests.

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Control measures for spider mites

Spider mites' abundance can be controlled by various natural enemies. Predatory mites (such as Phytoseidae species), lesser mite destroyers (such as *Stethorus punctillum*), brown lacewings (Hemerobiidae), common green lacewings (*Chrysoperla carnea*), and predatory bugs (*Orius* sp.) make up the majority of these. These helpful arthropods need to be encouraged to prevent spider mites from reproducing in large numbers. All cultivation practices that lessen the plant's drought stress will also be beneficial.

The presence of rainfall during summer is of great importance. Heavy rains have very positive effects on decreasing spider mite colonies and at the same time positively influence the soybean plants. In that case, the fungus *Neozygites floridana* occurs and infests mites. The fungus requires temperatures not higher than 29°C and humidity not less than 90% to expand on the mites' population. Presence of this fungus is very beneficial for decreasing damage made by two-spotted spider mites.

Materials and methods

In order to assess the abundance of two-spotted mites on soybean fields in Vojvodina, the northern part of Serbia, monitoring was established during three years, from 2018 to 2020. For that purpose, three localities were chosen (fig. 3), Zmajevo (near Vrbas, N 45.430229°, E 19.732521°), Rimski šančevi (near Novi Sad, N 45.335093°, E 19.840764°) and Zlatica (near Zrenjanin, N 45.39247°, E 20.56669°). Counting of adults and nymphs was done on three soybean varieties, Galina (0), Sava (I) and Rubin (II), belonging to different maturity groups. Assessments were done on 20 leaves at the field edge and also at 20, 40 and 60 meters from field edge every 10 to 14 days during July and August.

Zmajevo; Rimski šančevi; Zlatica;

Fig. 3: Map with the localities for mite monitoring.

To determine the presence of the two-spotted mites in soybean, the screening of diverse germplasm was performed in 2021 at Rimski šančevi, Novi Sad, Serbia. The screening involved analysis of soybean multispectral (MS) images collected with an unmanned aerial vehicle (UAV). The UAV used for image acquisition was P4M (DJI, Shenzhen, China), equipped with five monochromatic sensors and one RGB camera. Each sensor covers a specific range of wavelengths providing information from the blue (B), green (G), red (R), red edge (RE), and near-infrared (NIR) part of the spectrum. The photos were taken on a cloud-free, sunny day three times during the soybean growing period. The first flight was performed on 21.6.2021 over pest-free soybean plants. The second was done two weeks later (5.7.2021), at the beginning of the mites' occurrence, when the presence was confirmed by visual inspection at which time there were no visible signs of damage on plants. The final photo acquisition was made on 26.7.2021 when infected soybean plots were heavily damaged. After each flight, the individual photos were stitched in orthomosaic following a clearly defined protocol. From the orthomosaic the reflectance data of five channels was extracted in the form of digital numbers (DNs) from infected and non-infected (control) soybean plots. The DNs of B, G, R, RE, and NIR channels were used to calculate triangular green index (TGI) and normalized difference vegetation index (NDVI). Based on the values of individual channels and two vegetation indices (VIs), the possibility of early detection of two-spotted mites in soybean was analysed.

Results

Two-spotted spider mite occurrence is linked to weather conditions. Dry and hot weather promotes their reproduction and expansion. Weather conditions during 2018 and 2019 were not favourable for mites and that is why their occurrence in that period was sporadic. Weather conditions during 2020 were favourable for mite occurrence which resulted in their appearance that can be characterised as medium. Different responses of soybean varieties Galina (0), Sava (I) and Rubin (II), belonging to different maturity groups, are presented in Fig 4. It can be seen that the variety Rubin what the most affected by mites which can be explained by the phase of soybean growth when mites occurred in higher numbers. Sava and Galina were less attractive since they are earlier varieties than Rubin and their plants, especially of variety Galina, at the time of the second assessment, at the beginning of August, were almost mature. In the first decade of August, between the second and the third assessment, a steep drop in the number of mites occurred due to severe rainfall, which showed again an important influence of weather conditions on mite colony formation and growth. In Fig 4 are presented the average numbers of mites at the field edge, together with 20, 40 and 60 meters from field edge for all three localities and all three soybean varieties. As can be seen, the highest abundance was registered at 20 meters from the field edge. Abundance at the field edge was similar to at 60 meters while the least abundance was recorded at 40 meters. Setting up of the colonies is usually at the field edges but in cases of windy conditions, colonies can move in field where they will be more protected. Also, it should be noted that abundance in 2020 was characterised as medium and making conclusions based on data obtained only from that year should be done very cautiously. Unfortunately, abundance in 2018 and 2019 was very low so data obtained in these years could not contribute to the overall conclusion.

During 2018 and 2019 presence of spider mites in soybean was very low, without economic importance at all three localities.

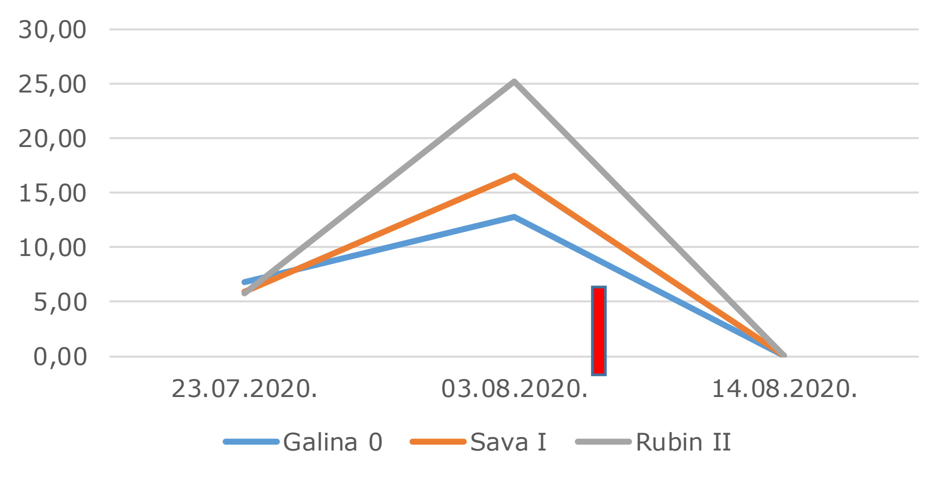

Fig. 4: Average mite abundance on different soybean varieties summarised for three locations.

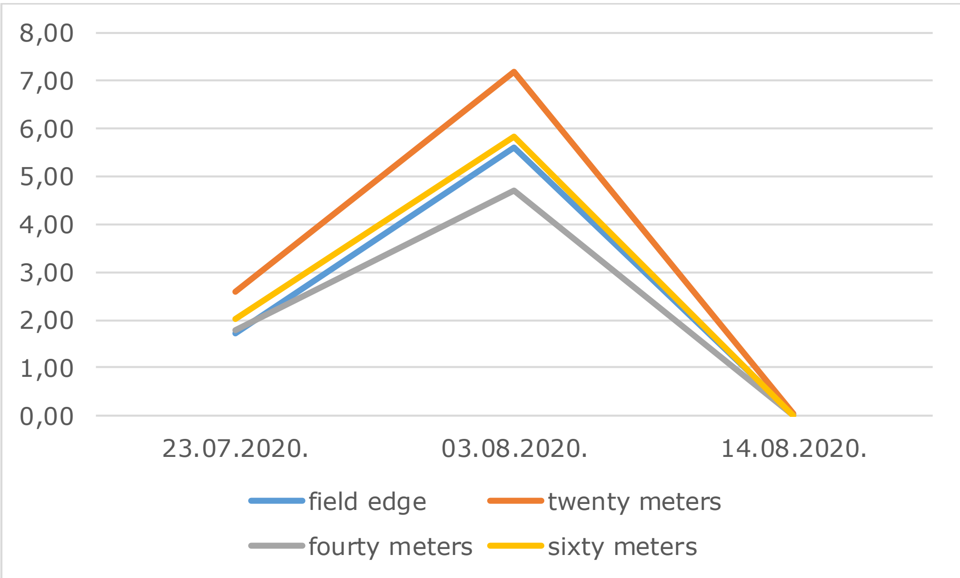

Fig. 5: Average mite abundance assessed at field edge, 20, 40 and 60 meters from the field edge summarised for three locations.

The results obtained through analysis of soybean MS images for early detection of the two-spotted mite attack showed that there were differences in reflection between three UAV flights and the presence of the pest.

The reflectance-based data extracted from the orthomosaic created after the first flight showed uniformity across the trial. The values of individual channels were almost the same with no deviations recorded between different plots. After two weeks the soybean plot infected with the two-spotted mites started to reflect sunlight differently. These plants increased reflectance of the light from the visible part of the spectrum and RE channel while NIR remained the same compared to the control. As the season progressed, the attack of the pest became more and more intense which caused yellowing of the leaves and eventually necrosis. The visual changes were supported by DNs of the channels extracted from the final, third flight. Further damage of the leaf tissue caused an additional increase in reflection of all channels except the NIR whose values dropped compared to the control.

The difference in TGI and NDVI values between infected and control soybean plots was shown in Fig. 6.

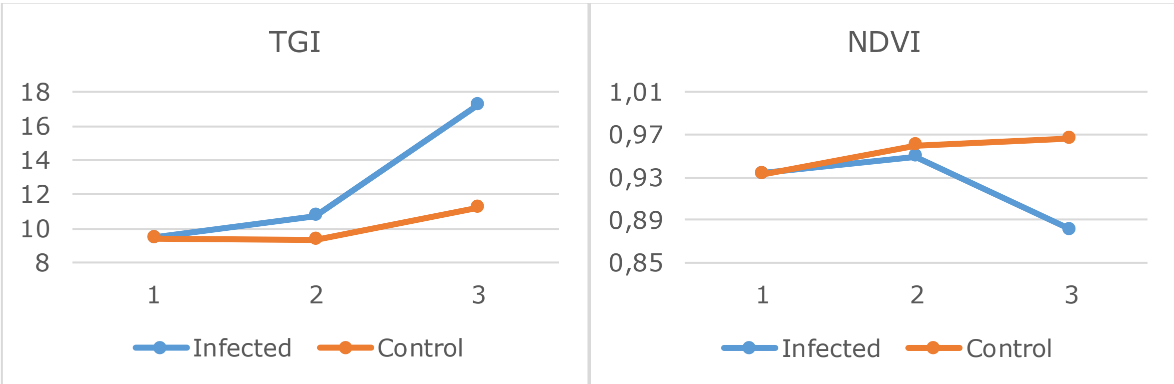

Fig 6: Temporal change of triangular green index (TGI) and normalised difference vegetation index (NDVI) of soybean plot infected with two-spotted mite and the control on the following dates in 2021: 1) 21.6; 2) 5.7 and 3) 26.7.

The results obtained indicated a rapid increase of TGI on the plot affected by the mites while at the same time, NDVI dropped compared to the control. The opposite trend of these two VIs was likely a consequence of their different origin. Namely, the TGI was based on the B, G, and R channels whose values increased as the attack of the pest progressed, leading to higher values of TGI. On the other side, a smaller difference between NIR and the G channel caused lower values of NDVI on the second and especially on the third photo acquisition.

The breaking point for early detection of two-spotted mites was obtained from the second flight. Fig 6 shows the visual progress of the mites' attack on soybean in the span of a month.

Fig 7: Visual changes of soybean plots during the season. Red circle marks plot infected with two-spotted mite, green circle represents plot in absence of the pest in 2021 on a) 21 June; b) 5 July and c) 26 July.

On the 5th of July presence of the mites was recorded by visual inspection in soybean. At this moment, the pest didn't cause any damage visible to the naked eye. Still, the values of TGI and NDVI calculated from UAV photos showed that infected plants were already under stress and that measures needed to be taken to prevent further progression. The obtained results suggest that remote sensing tools and techniques could be used for the early detection of the two-spotted mite in soybean.

Rainfall during summer months plays significant role in decreasing damage made by mites.

Recommendations for organic farmers:

1. Following the weather conditions at each region, monitoring usually starts during July in South-eastern Europe. In cases of higher temperatures and low precipitation, monitoring should be started during June.
2. Spider mites first occupy field edges and that is why additional attention should be given to those parts of fields.
3. Early spider mite detection in an irrigation system (where applicable) leads to drastically reduced chances for yield loss.
4. Irrigation has multi-fold positive effects on soybean production. It helps soybean plants to grow optimally, flushes spider mites from the leaves and gives ambient conditions for spider mite pathogenic fungi to develop.
5. Variety selection and proper cultivation practices play a very important role in overcoming the negative effects of spider mite on soybean.

4. The southern green stink bug (SGSB)

The southern green stink bug *(Nezara viridula*) is a relatively new soybean pest in Europe. Every year it becomes more abundant and it is expected to become a serious pest in the future. Although we found that yield loss can be up to 100% uder experimental conditions these levels are rare in commercial production. Still, damage can be significant and should not be neglected. Scouting for the presence of the pests should start in May or June and continue during July and August.

SGSB is polyphagous which makes its control even more demanding.

Description

Stink bugs have glands that excrete a strong, characteristic odour which is how they got their name. Adults of the green southern stink bug are 12 to 15 mm long and 7 to 8 mm wide. The body has a shield-like shape similar to most species from the Pentatomidae family. Three white dots and two smaller ones, all of them in line, can be seen on the scutellum which serves for species identification. The green shield bug, *Palomena prasina*, can be easily confused with *Nezara viridula*, but the green shield bug does not have white dots on the scutellum. The larvae (nymphs) of *Nezara viridula* are with white, red and black parts of the body.

Biology

This species can have up to 5 generations per year, depending on climatic region. It is considered that in most parts of Europe, it has two generations per year. The origin of the species is Ethiopia. Due to the mild winters during previous decades this species has expanded its habitat. The females lay up to 300 eggs in clusters of 30 to 130 on the underside of leaves of many plants. After hatching, the nymphs remain in a group near the egg cluster until the second instar (Fig. 7). Adults enter houses, buildings, barns, greenhouses and other structures in the late autumn for over-wintering.

SGSB can have up to five generations per year but in the most parts of Europe it has two.

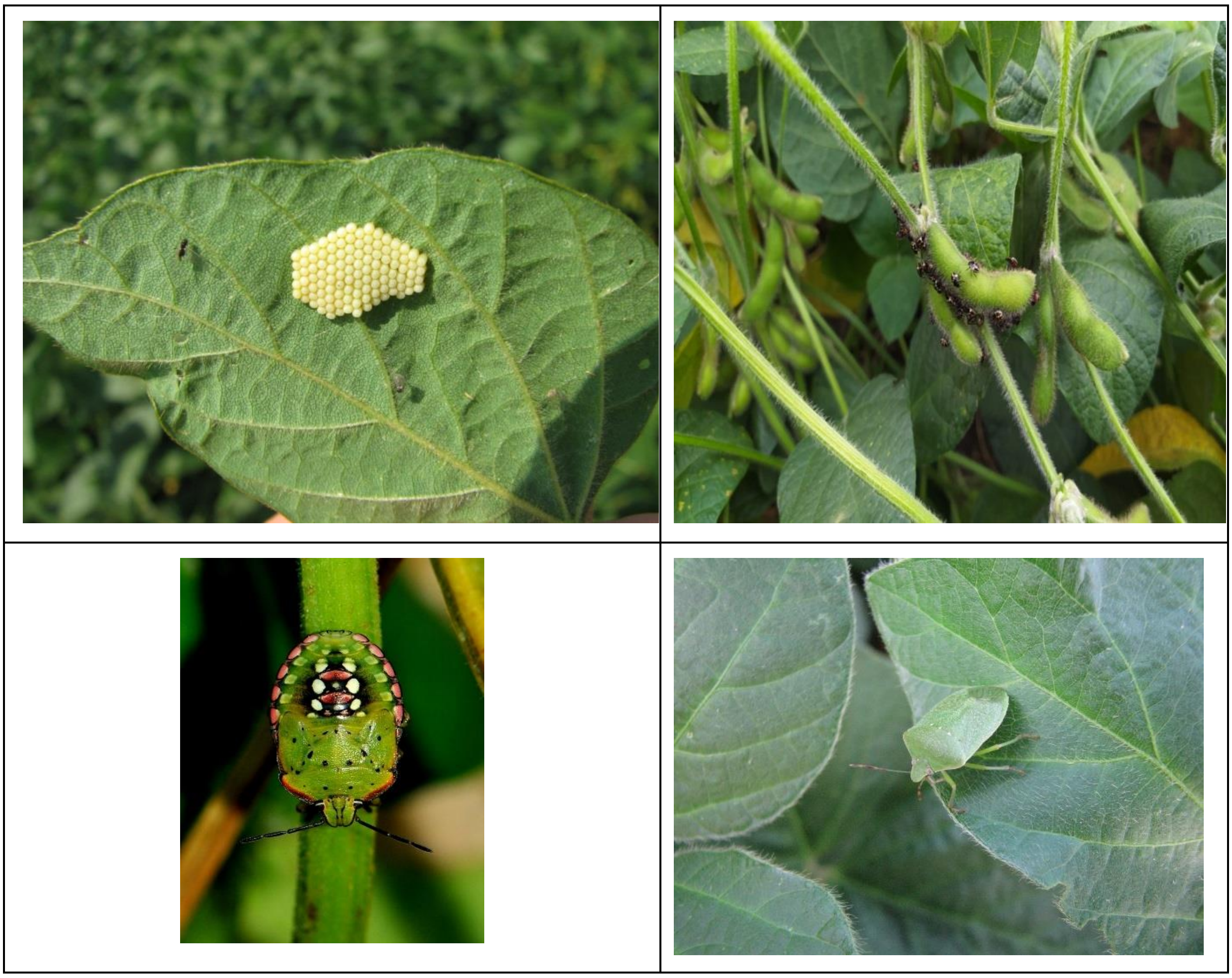

Fig. 8: Different life stages of SGSB.

Damage

The SGSB is a polyphagous pest which feeds by piercing plant tissue with needle-like stylets and sucking plant sap. Adults and nearly all nymphal stages (2nd to 5th nymphal stage) feed on different plant tissues causing damage. Soft parts of plants, mostly leaves and fruits, are preferred. Feeding injury can occasionally be seen as yellow or dark blotches, or it may even lead to necrosis. Premature abscission can happen if flower buds are damaged by feeding.

Damage during the initial phases of formation is the main danger to the soybean seeds. Feeding on pods results in seed damage and affects the development of the pods (Fig. 9). For now, in central Europe, the SGSB are migrating to soybean fields in larger numbers during pod ripening and therefore damage is limited. Since the pest is becoming more and more abundant, time of occurrence will probably change to earlier phases. That is why it is expected for the green southern stink bug to become a more serious problem in the coming years.

SGSB feeding injury can occasionally be seen as yellow or dark blotches, or it may even lead to necrosis.

Control measures

Biocontrol of the SGSB is a challenge since predators and parasitoids are not yet sufficiently established in Europe. Egg parasitoids from the genera *Trissolcus*, *Telenomus* and *Cryon* and some tachinid flies are considered to have the greatest potential for biocontrol. Furthermore, trap crops, such as forage pea, bean, brassicaceous crops, and their utilisation should be analysed in detail. The purpose of trap crops is to attract stink bugs to lay eggs on them and not on the protected crop, in this case soybean.

Fig. 9: Isolation cages with *Nezara viridula* in a soybean field.

Material and methods

To assess the potential SGSB damage on soybean a set of trials was conducted in semi-controlled, field conditions at location Rimski šančevi (near Novi Sad, N 45,3345°, E 19.8361°), Serbia. Isolation cages, 1 m in diameter and 1.8 m in height, were mounted during 2018, 2020 and 2021 (Fig. 9). Testing was done on five different soybean varieties that belong from the 00 to III maturity groups (Fortuna, Romansa, Princeza, Sava and

Senka). Two treatments were used, a control without stink bugs, and a treatment with SGSB specimens. In each isolation cage with stink bugs, five specimens per plant were introduced at the end of July. Soybean plants were harvested at maturity and analysed in detail for plant height, number of lateral branches, number of seeds per plant, number of completely damaged seeds and thousand seed weight.

Fig. 10: Soybean seed damage caused by *Nezara viridula*.

Results

One of the goals of this set of trials was to evaluate reactions of different soybean varieties to biotic stress caused by SGSB feeding since it has a different influence on several aspects of the soybean plant. Overall, the level of SGSB damage on soybean depends on the variety and environmental conditions. SGSB does not influence soybean plant architecture, which is seen through a consistent number of lateral branches and plant height in the control and SGSB cages (Table 1).

Table 1: Plant height and number of lateral branches on five soybean varieties during 2018, 2020 and 2021.

Year	Variety	Fortuna (00)		Princeza (0)		Romansa (I)		Sava (I)		Senka (III)	
	Trait	Con.	Treat.	Con.	Treat.	Con.	Treat.	Con.	Treat.	Con.	Treat.
2018	Plant height (cm)	61.2	59	97.3	85.3	74.3	66.8	72.7	79	100.7	96.57
	Number of lateral branches	2.9	1.6	2.4	5	2.1	2.22	0.5	0.44	0.71	1
2020	Plant height (cm)	87.1	82.5	65.2	72.4	75.5	73.9	85.5	89.78	108.1	98.5
	Number of lateral branches	0.4	0.7	4.6	2.4	2.8	4.5	0.6	1.33	3.7	2.5
2021	Plant height (cm)	66.32	64.9	64.8	71.5	57.2	64.38	68.2	72.89	95.56	90.44
	Number of lateral branches	1	1.1	1.5	2.5	2	2.12	0.8	0.56	2.33	1.67

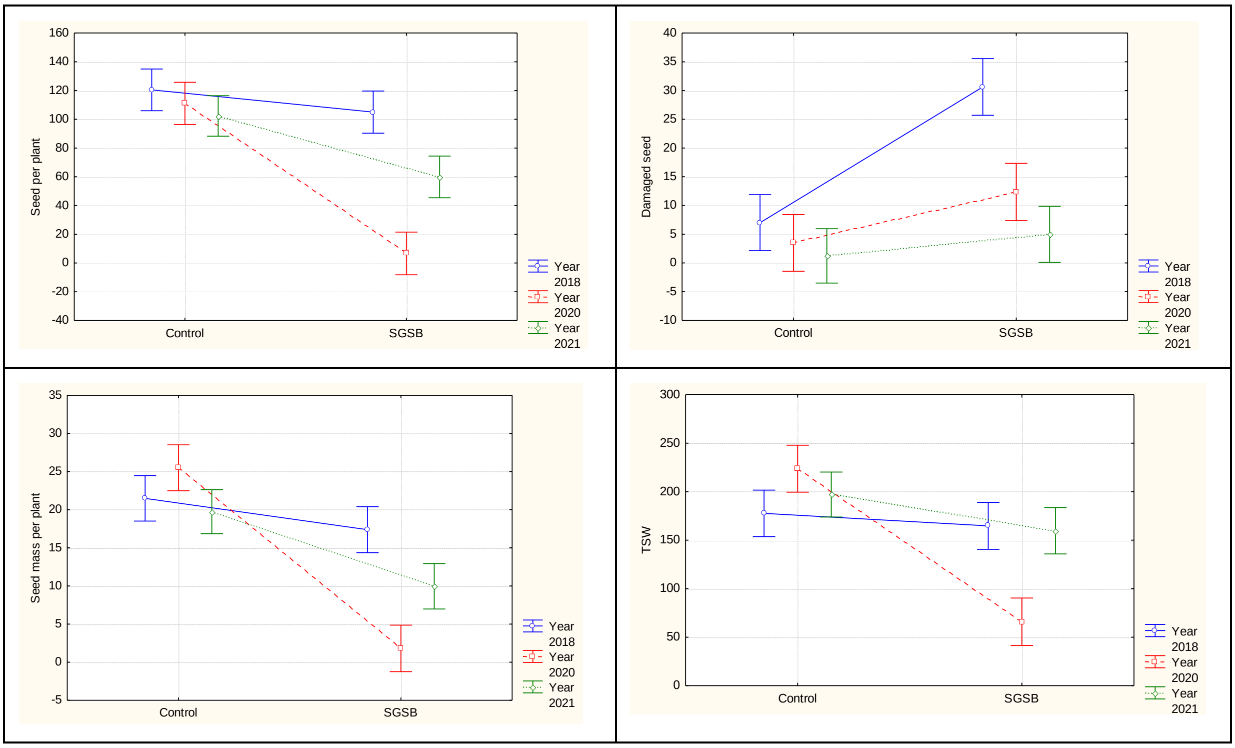

Fig. 11: Differences between control and SGSSB treatment expressed through number of seeds per plant, number of damaged seeds, seed weight per plant and the thousand seed weight.

Even though plant architecture is not influenced by SGSB, yield-related traits are more prone to insect feeding. The average number of seeds per plant in every experimental year was lower in the SGSB treatment. The number of seeds per plant on the variety Fortuna did not show a difference between the control and SGSB treatments which can be partly explained by maturity group (00) since it is a very early variety. In contrast, all other varieties showed significant differences between control and SGSB treatments, especially in 2020 while the level of damage was influenced by environmental conditions.

Feeding of SGSB on soybean plants result in damaged seed, which is not suitable for planting or processing. Year 2018 was with the most evident differences and with the highest number of damaged seeds which is not consistent with data presented previously since in 2020 damage was so severe that seeds were not formed in most cases. Again, the variety Fortuna (00) did not show significant differences between the control and SGSB treatments.

Seed weight was also influenced by SGSB feeding. Even SGSB cause total soybean seed damage, feeding of this insect causes reduction in seed size on non-damaged seed. In year 2020, the most severe damage was observed. Moreover, SGSB caused direct damage on soybean seed, also insect feeding resulted in indirect effects through physiological response of soybean plant by the reduction of thousand seed weight (TSW).

Varieties Fortuna and Sava seemed to be less influenced by SGSB for the most yield-related traits. Variety Fortuna avoided higher damage due to its maturity group (00). Maturity group of the soybean variety can be of key importance for avoiding significant damage of SGSB in the field. Considering that in organic production pesticide use is forbidden, careful selection of soybean variety and timely application of cultivation practices are two strategies for overcoming the problem of crop failure due to SGSB damage. The third pillar is the use of traps and trap crops that need further evaluation in a range of environments.

All tested soybean varieties were negatively influenced by SGSB presence but still there were some differences between varieties.

Recommendations for organic farmers:

1. Soybean is the most susceptible to damage if SGSB feeding occurs at the end of flowering and at the beginning of pod formation.
2. SGSB first occupy field edges and then move further within the field.
3. Presence of SGSB natural enemies is not yet sufficient in Europe but still, it should be considered by working on conservation of habitats for natural enemies.
4. More attention should be given to the sowing of trap crops and their wider utilisation in practice. SGSB is polyphagous which makes this topic even more complex.
5. Variety selection and proper cultivation practices play a very important role in overcoming the negative effects of SGSB in organic soybean production.
6. Small fields under soybean, especially in semi-urban conditions,

5. Conclusions

After three years of trials and pest monitoring it can be concluded that:

- Soybean fields should be monitored regularly and systematically for the presence of pests.
- Damage on soybean plants is greatly influenced by the time of pest occurrence and the phase of crop development.
- Damage, expressed through yield loss can be up to 100% in an extreme situation caused by spider mite and the southern green stink bug.
- Weather conditions play a significant role in the occurrence and abundance of spider mites.
- Abundance of spider mites is not always highest at the field edge and it is important to systematically monitor soybean fields.
- Irrigation can reduce spider mite influence on reducing soybean yield.
- Southern green stink bug is becoming more and more abundant by invading new territories.
- Natural enemies can significantly reduce the population of southern green stink bug but time is needed to introduce and establish these in Europe. When alien species are introduced to a new territory there is a concern about their influence on biodiversity.
- Variety selection and proper cultivation practices can play a very important role in overcoming the negative effects of spider mites and southern green stink bugs on soybean. This is the strategy for soybean yield stability.
- One of the future tasks would be to examine trap crops and use this approach for overcoming the problem of stink bugs in organic production.

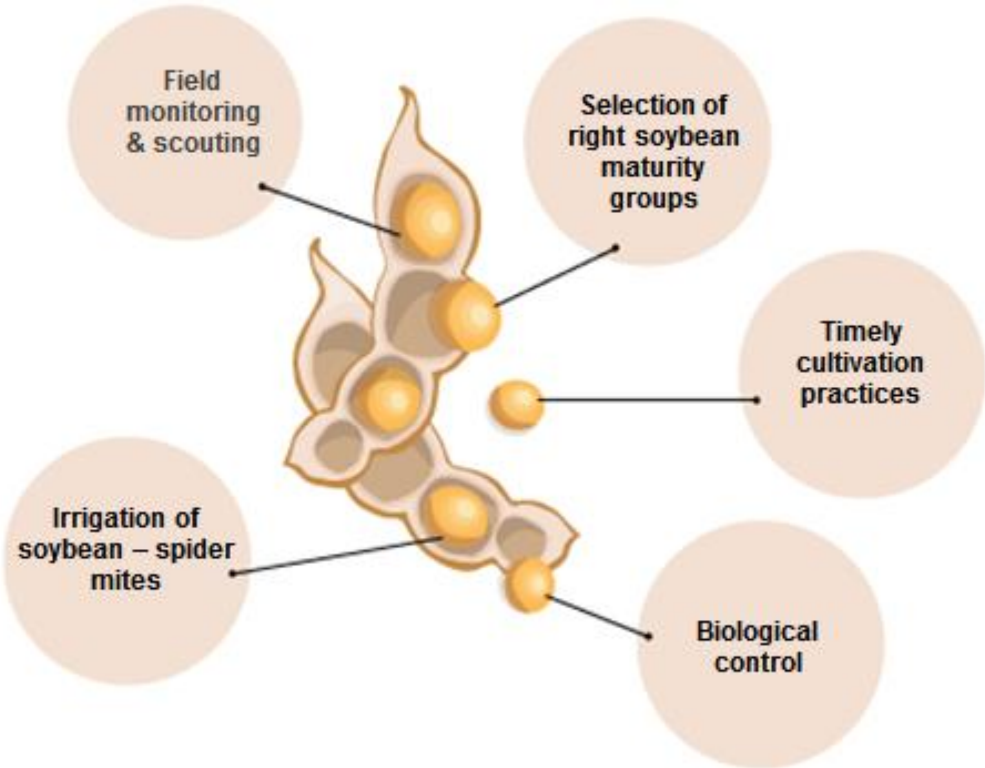

Fig 12: Five steps for organic farmers to overcome pest damage in soybean production.

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