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# SUSCEPTIBILITY OF FABA BEAN VARIETY 'MERKUR' TO BROADBEAN SEED BEETLE (*BRUCHUS RUFIMANUS*) IN LATVIA

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**Abstract**
The broadbean seed beetle (*Bruchus rufimanus*) is a significant pest of faba bean (*Vicia faba*) both in Latvia and in many other parts of the world. The objective of this study was to check the susceptibility of the faba bean variety 'Merkur' to the broadbean seed beetle in Latvia, comparing it with other popular varieties: 'Boxer' and 'Laura'. The trials were conducted in 2021 and 2022 at the Research and Study Farm 'Peterlauki' in Jelgava County and at a commercial farm in Cēsis County. The larval infestation rate of seeds of different varieties, the survival rate of individuals (larvae, pupae, imagines) in the seeds, as well as the percentage of seeds damaged by the pest in the yield were compared. It was observed that 'Merkur' seeds were significantly less infested than 'Boxer' and 'Laura' seeds. However, the survival rate of larvae in the seeds of 'Merkur' was similar to that in 'Laura' seeds and higher than in 'Boxer' seeds. The highest proportion of seeds damaged by the pest was found in the 'Merkur' and 'Boxer' yields, varying between 5–75% between years and trial locations. Therefore, it was concluded that the variety 'Merkur', evaluated from a practical point of view, does not differ from the other two varieties. Without taking additional plant protection measures, the percentage of seeds damaged by the broadbean seed beetle can significantly exceed the maximum limit (3%) specified in the buyers' quality criteria.
**Key words:** larvae, entrance holes, damaged seeds.

## Introduction

The broadbean seed beetle (*Bruchus rufimanus*) is a major pest of various legume crops, including faba bean (*Vicia faba*), in the largest part of its range. Without taking measures to control this pest, 70...80% yield losses should be expected (Segers *et al*., 2021; Gailis *et al.*, 2022). The seeds damaged by the seed beetle not only lose their nutritional value, but also become less valuable as a sowing material. The damaged seeds are significantly more susceptible to various pathogens, which, together with mechanical damage, reduce the germination rate. Plants grown from damaged seeds have lower biomass, shorter stems and roots (Almogdad, Jonaviciene, & Semaskiene, 2023; Huber *et al.*, 2023).

Recently, attention has been paid to the development of a successful integrated pest management strategy in the faba bean crops (Stoddard *et al*., 2010). One of the preventive measures in controlling harmful organisms is the use of low-susceptible or completely resistant varieties. Currently, several DNA markers are known that are responsible for the resistance of the faba beans to some harmful organisms, such as broomrapes (*Orobanche* spp.) and *Ascochyta*-caused blight, but not to arthropod pests (Rubiales & Khazaei, 2022). However, research continues to test the susceptibility of different varieties to the broadbean seed beetle, as well as to search for new genetic lines that would be as resistant as possible to this pest (Segers *et al.*, 2022; Dell'Aglio & Tayeh, 2023).

So far, in Europe, properties that reduce the susceptibility to the broadbean seed beetle have been studied in various faba bean varieties. For example, plants of the varieties 'Côte d'Or' (France) and 'Nova Gradiska' (Croatia) have increased mechanical strength, which reduces the ability of the seed beetle larvae to bore into them. The seeds of the variety 'Quasar' (United Kingdom) contain secondary metabolites that inhibit the development of pest larvae (Carillo-Perdomo *et al*., 2019). Therefore, the broadbean seed beetle infests the seeds of these varieties in relatively small quantities. All of these are winter varieties and are successfully grown in European regions with a distinctly Atlantic climate (mild and rainy winters) or an arid Mediterranean climate, where the cultivation of spring varieties is almost impossible due to long drought periods during the summers.

A study conducted in the Czech Republic analysed the susceptibility of several faba bean varieties (mostly spring varieties) to the broadbean seed beetle. It was found that the highest mortality of eggs and young larvae and, therefore, the least damaged yield can be observed in the sowings of two varieties – 'Merkur' and 'Melodie'. 'Merkur' is a medium-early spring variety developed in the Czech Republic with high yields and moderately good resistance to fungal diseases. However, 'Melodie' is a medium-early spring variety created in France. It is characterized as high yielding, low in vicine/convicine and high in seed protein. Broadbean seed beetle egg, larval and pupal mortality approaching 99% of the number of eggs laid on pods was observed in both varieties (Seidenglanz & Hunady, 2016). Both varieties have been tested in different European regions to check their growth and yield performance under different environmental conditions. Such testing has also been carried out in Estonia. As a result, it has been concluded that the variety 'Merkur' is suitable for growing in continental environmental conditions, which also prevail in Latvia. The performance indicators (e.g. amount of the yield, nutritional value) of this variety in such growing conditions do not significantly differ from the indicators of the relatively widely grown variety 'Fuego' in our country. However, 'Melodie' is not suitable for growing in the environmental conditions of the Baltic region, even though the amount of seed yield sometimes does not differ from the yield of 'Merkur' and 'Fuego' (Flores *et al*., 2013).

The objective of our study was to compare the susceptibility of the faba bean variety 'Merkur' and other faba bean varieties to the broadbean seed beetle in Latvia. The following tasks were implemented:
1) The comparison of the infestation rate of seeds of different varieties with the broadbean seed beetle's larvae;
2) The comparison of the survival rate of the seed beetle individuals (larvae, pupae, imagines) within the seeds of different varieties during the vegetation season;
3) The comparison of the proportion of the pest-damaged seeds in the yield among different varieties.

**Materials and Methods**

The study was conducted in the vegetation seasons of 2021 and 2022. In the first season, one trial was set up at the Latvia University of Life Sciences and Technologies Research and Study Farm 'Peterlauki', Jelgava County, Platone Parish (coordinates of the trial site: 56°32'41.6"N 23°43'50.5"E). That year, three varieties – 'Merkur', 'Laura' and 'Boxer' – were compared for their susceptibility to the broadbean seed beetle. In 2022, two trials were set up: at the Research and Study Farm 'Peterlauki' (coordinates of the trial site: 56°32'47.3"N 23°43'25.8"E) and at a commercial farm in Ligatne Parish, Cesis County (coordinates of the trial site: 57°12'56.8"N 25°01'26.8"E). In that growing season, two field bean varieties were compared: 'Boxer' and 'Merkur'.
In the fields where the trials were set up, the pre-crop was spring barley (*Hordeum vulgare*). The main soil treatment was ploughing to a depth of 18–20 cm. The beans were sown at a depth of 5 cm on 14 April 2021. In 2022, the sowing was performed on 12 and 20 April in the Research and Study Farm and the commercial farm, respectively. The applied sowing rate was 50 germinate able seeds $m^{-2}$. Each variety was sown in four replicates; each experimental plot was 30 $m^2$ (3×10 m) in size. The grids of experimental plots were located inside the commercial faba bean fields. The narrowest place between the plots and the edge of the field was 30 m wide. During the growing seasons, the experimental plots were treated with plant protection products simultaneously with the surrounding bean fields, according to traditional agricultural practices. The exception was insecticides, which were not applied in the experimental plots.
In 2021, when the first seed beetle eggs were observed on pods at the lowest level of plants (29 June), pods were sampled once a week. At each sampling, nine pods from each plant level were randomly collected from each plot. Later in the laboratory, entrance holes of the broadbean seed beetle larvae, as well as individuals (larvae, pupae, imagines) and exit holes of imagines in each seed, were counted. The pods were last collected and the seeds were analysed on 17 August. In total, 135 pods were collected and analysed in each experimental plot during the growing season.
The infestation rate of the pest in seeds was assessed by counting larval entry holes; each entrance hole was assumed to be created by a single larva that successfully penetrated the seed. Each seed was considered as one replicate for the mathematical analysis of the data to compare the susceptibility of the varieties.
The survival rate of the pest's individuals in seeds was calculated as the percentage ratio between the sum of individuals found in all seeds and the sum of larval entrance holes. Initially, in the first sampling times, the sum of surviving individuals in the seeds was only the number of larvae. In subsequent samplings, the number of pupae, imagines and exit holes was also added to the number of larvae.
The beans were harvested on 5 September. From each plot, 500 seeds were randomly taken, which were used to determine the proportion of seeds damaged by the seed beetle in the yield. The seeds were considered damaged if at least one broadbean seed beetle imago was noticed inside of them or they had at least one exit hole.
In 2022, only the proportion of seeds damaged by the broadbean seed beetle in the yield was analysed, comparing both varieties. This was done similarly to 2021, with 500 seeds randomly taken from each plot after the harvest.
Observations obtained at local meteorological stations showed that in 2021, there was a drought in the second half of April and the first ten-day period of May - the amount of precipitation was a few millimetres in 30 days. The average air temperature was 14.4 °C, significantly higher than the long-term average. Suitable growing conditions for faba beans prevailed only in the second and third ten-day period of May, when the total amount of precipitation was 50.2 mm, and the average air temperature was 13.1 °C. Drought also prevailed in June and July, with 15 mm and 5 mm precipitation, respectively, while the average air temperature was 5.2 °C higher than the long-term average. As a result, faba beans did not develop the upper third pods.
In 2022, meteorological conditions were more suitable for the faba bean development. In April, during the sowing, the average air temperature was 0.5 °C higher than the long-term average. The amount of precipitation in the Farm 'Peterlauki' corresponded, but in the commercial farm, it was five times less than the long-term average. In the subsequent months of the vegetation period, the amount of precipitation in both research locations was similar or slightly higher than the long-term average, but the average air temperature exceeded the long-term observations by 3...4 °C. Therefore, in the second year of the study, faba beans could fully develop.
The resulting data were processed using MS Excel 2016 with Daniel's XL Toolbox NG plug-in (version 7.3.4). Descriptive statistics (arithmetic means, standard errors) were obtained with it, as well as

ANOVA with Bonferroni-Holm post hoc test was performed.

**Results and Discussion**

*Level of seed infestation*

The first pods were sampled and analysed on 29 June 2021. They had developed in the lower third of the stems, and broadbean seed beetle eggs were found on them. Seven days later (6 July) pods of the middle third had also developed. The pods in the upper third of the stems did not develop for plants of any variety in the vegetation season of 2021; this is explained by the insufficient amount of precipitation in the spring and the first two months of summer.

The highest number of larval entrance holes in the seeds was observed on the third sampling (13 July), when it was significantly higher than that observed in the first two weeks. After that, it did not increase significantly and slightly fluctuated in the plots of any variety (Table 1), which indicates that at that moment, intensive broadbean seed beetle egg laying had already ended. The egg-laying period of this species is believed to last usually six weeks (Segers *et al*., 2021). However, studies conducted in Latvia show that the egg-laying period usually lasts 4–5 weeks, reaching its peak in the third week (Gailis *et al*., 2022). Fluctuations in the number of larval entrance holes after reaching the peak could be explained by several factors. This could be the uneven distribution of the broadbean seed beetle population in the experimental plots, resulting in the collection of pods with slightly different levels of infestation at different samplings. Also, this result could be determined by different survival success of eggs and newly hatched larvae on different plants.

Comparing the studied varieties, it should be concluded that the seeds of 'Merkur' were significantly less infested than the seeds of the other two varieties. This trend appeared especially well in the first three weeks of observation, when the number of larval entrance holes in 'Merkur' seeds was approximately two to three times less compared to 'Boxer' and 'Laura' seeds (Table 1). It has already been studied that 'Merkur' is a faba bean variety, on the pods of which broadbean seed beetle females lay eggs in a smaller amount than on the pods of other varieties ('Boxer' and 'Laura' were not examined in that study). Similarly, 34–45% of laid eggs and first instar larvae die on 'Merkur' pods and in pod valve tissues, which has been significantly more compared to various other faba bean varieties (Seidenglanz & Hunady, 2016).

Table 1

**The average number of *Bruchus rufimanus* larval entrance holes in the seeds of three faba bean varieties in the vegetation season of 2021**

| The date (the phenological developmental stage of plants according to BBCH-scale) | Merkur | Boxer | Laura |
|---|---|---|---|
| 29 June (68–71) | $0.28^{a}_{A}$ | $1.02^{a}_{B}$ | $0.80^{a}_{B}$ |
| 6 July (71–74) | $0.53^{a}_{A}$ | $1.23^{a}_{B}$ | $1.20^{a,d}_{B}$ |
| 13 July (71–75) | $1.39^{b}_{A}$ | $2.16^{b}_{B}$ | $2.40^{b}_{B}$ |
| 20 July (75–80) | $1.36^{b}_{A}$ | $1.88^{b,c}_{B}$ | $1.66^{c,d}_{A,B}$ |
| 27 July (80–85) | $1.36^{b}_{A}$ | $1.78^{b,c}_{B}$ | $1.93^{c}_{B}$ |
| 3 August (85–87) | $1.05^{b}_{A}$ | $1.41^{c}_{B}$ | $1.48^{d}_{B}$ |
| 10 August (87–88) | $1.26^{b}_{A}$ | $1.49^{c}_{A}$ | $1.37^{d}_{A}$ |
| 17 August (89) | $1.16^{b}_{A}$ | $1.50^{c}_{B}$ | $1.39^{d}_{A,B}$ |

Note: lowercase letters (a, b, c, d) indicate significantly different ($p<0.05$) values among various dates within each variety; uppercases letters (A, B) indicate significantly different values among the varieties in each date of observations.

*The survival rate of B. rufimanus individuals inside the seeds*

Larvae were detected in the seeds of all varieties already on 29 June, while the first pupae – on 27 July. At the same time, the first imagines and their exit holes were also observed in the seeds of the variety 'Merkur', but in the seeds of other varieties, the first imagines were detected a week later – on 3 August. On the same date, the first imago exit holes were also observed in 'Boxer' seeds, while in 'Laura' seeds they were first detected on 10 August. No phenological regularities were observed when assessing the survival success of broadbean seed beetle individuals infesting seeds. The percentage ratio of the number of individuals in the seeds found on 29 June to the number of larval entrance holes did not change significantly until the end of the observation period on 17 August (Table 2). Therefore, data on the seed beetle survival rates obtained from all observation dates were pooled into common sample sets, and the growing season average was calculated and compared between the varieties. Of the number of larvae that initially infested the seeds, 58.9% of broadbean seed beetle individuals survived in 'Merkur' seeds, 48.4% in 'Boxer' seeds and 54.3% in 'Laura' seeds. A statistically significant difference ($p=0.014$) existed between 'Merkur' and 'Boxer'. No statistically significant differences in the survival rate of individuals were found between the other pairs ('Merkur' – 'Laura' ($p=0.305$) and 'Boxer' – 'Laura' ($p=0.117$)) of varieties.

Factors that could cause the mortality of broadbean seed beetle larvae and pupae in legume seeds are still not fully understood. Therefore, it is difficult to judge what effect varieties might have on mortality rates. Several authors have found that the infestation of various legume seeds by *Bruchus* seed beetles is negatively correlated with the content and concentration of iron, manganese, phenols, tannins and other chemical compounds (Mohamed & Abd-El Hameed, 2014; Tsialtas *et al.*, 2020; Boulata, Irakli, & Tsialtas, 2022). However, these studies mostly show that the chemical composition of pods and seeds prevents seed beetle larvae from entering the seeds, causing their death at an early stage of development. On the other hand, it is not yet possible to make such a convincing conclusion about the mortality of larvae in later stages of development, which have managed to enter the seeds. Larval mortality can be caused by cannibalism. This has been observed in pea seed beetle (*Bruchus pisorum*) populations; when one larva accidentally encounters another larva while boring its tunnel, it kills it by partly eating it (Larson, Brindlay, & Hinman, 1938). This species is a close relative of the broadbean seed beetle, so similar behaviour could be present. Larvae are more likely to kill each other in more infested seeds. This could explain the lowest individual survival rate in 'Boxer' seeds, as they had the highest number of larval entrance holes. However, for now, this should only be considered a hypothesis, as neither our study nor any other study can confirm it. The broadbean seed beetle larvae and pupae are regularly parasitized by the parasitoid braconid wasps *Triaspis thoracicus* and *T. luteipes* (Medjdoub-Bensaad *et al*., 2015; Seidenglanz & Huňady, 2016; Tsialtas, Irakli, & Lazaridou, 2018; Tsialtas *et al.*, 2020; Boulata, Irakli, & Tsialtas, 2022). Little data is available on the distribution of these species in Europe, but they could also be found in Latvia. Observations show that traces of parasitoid activity – exit holes – are less frequently observed in legume seeds whose chemical composition repels seed beetles (Boulata, Irakli, & Tsialtas, 2022). However, likely, this is primarily related to a smaller food base of the parasitoid rather than to the characteristics of the legume variety, which, by attracting or repelling the natural enemy, promotes or reduces the survival rate of the seed beetle larvae and pupae.

Table 2

**The survival rate (%) of *Bruchus rufimanus* individuals in the seeds of three faba bean varieties during the 2021 growing season**

| The date (the phenological developmental stage of plants according to BBCH-scale) | Merkur | Boxer | Laura |
|---|---|---|---|
| 29 June (68–71) | 66.7 | 43.1 | 73.2 |
| 6 July (71–74) | 67.3 | 51.0 | 54.2 |
| 13 July (71–75) | 52.3 | 53.4 | 44.4 |
| 20 July (75–80) | 56.3 | 50.8 | 54.6[b] |
| 27 July (80–85) | 42.3 | 30.9 | 30.2[a] |
| 3 August (85–87) | 55.0 | 40.3 | 53.6 |
| 10 August (87–88) | 64.1 | 59.5 | 57.2 |
| 17 August (89) | 67.3 | 58.34 | 66.9[a,b] |

Note: lowercase letters (a, b) indicate two pairs of dates (pair a and pair b) for variety 'Laura' between which there were significant ($p<0.05$) differences; no significant differences were observed among other pairs of dates for all varieties.

*The rate of the seed yield damage*

The percentage of faba bean seed yield damaged by the broadbean seed beetle was significantly different in the two years of the study. In the 2021 season, it was in the range of 57–75%, while in 2022, the proportion of damaged seeds in the yield fluctuated in the range of 4.5–9.5%. No statistically significant differences were found between the volume of damaged seeds of 'Merkur' and 'Boxer' in both years. However, in 2021, the percentage of damaged seeds in the yield of 'Laura' was significantly lower than that of the other two varieties (Table 3).

The percentage of damaged seeds found in our study was similar to that observed previously both in Latvia and in other places where the broadbean seed beetle is a significant pest of faba beans (Segers *et al.*, 2021; Gailis *et al.*, 2022). We cannot explain with absolute certainty the large variation in the percentage of damaged seeds between the years. But one reason could cause differences in faba bean plant development. In 2021, during the first half of the growing season, plants suffered from drought, as a result of which the plants did not develop the pods in the upper third of the stems. In contrast, in 2022, the amount of precipitation was sufficient, and the plants successfully developed pods in all thirds of the stem. Pods in the upper third are significantly less infested than pods in the lower third, where the number of infested seeds is always the highest (Gailis *et al.*, 2022). Therefore, if plants develop pods in all thirds of the stem, the yield may have a higher proportion of intact seeds. This is unlikely to have been the only factor responsible for the differences. Fluctuations in the density of broadbean seed beetle populations between different years are also possible, but no studies are yet available on these.

The proportion of damaged seeds in the harvest is the most important indicator from a practical point of view. In Latvia, the requirements of different buyers tend to vary, however, usually, no more than 3% of damaged seeds are allowed in the material of the first food group faba beans. In the material of the second food group, the proportion of seeds damaged by pests should not exceed 4–6%. The results of our research show that it is impossible to achieve such indicators in the seed yield of the 'Merkur' variety in Latvia without applying pest control measures. 'Merkur' was less susceptible to the broadbean seed beetle (fewer larval entrance holes) compared to the other two varieties. However, the number of larvae entering the seeds and their survival success were sufficient for the percentage of damaged seeds in the yield to be as high as or higher than for 'Boxer' and 'Laura'.

Table 3

**Percentage of seeds damaged by *Bruchus rufimanus* in the faba bean yield obtained in three field trials in 2021 and 2022**

| | 2021 (Farm 'Peterlauki') | | | 2022 (Farm 'Peterlauki') | | 2022 (Commercial Farm) | |
|---|---|---|---|---|---|---|---|
| | Merkur | Boxer | Laura | Merkur | Boxer | Merkur | Boxer |
| Mean | 74.7[a] | 70.8[a] | 57.4[b] | 4.5[a] | 9.4[a] | 7.9[a] | 6.3[a] |
| Standard Error | 5.1 | 3.6 | 2.1 | 1.8 | 2.2 | 1.9 | 2.1 |

Note: lowercase letters (a, b) indicate significantly different ($p<0.05$) values in each farm within each year.

## Conclusions

1. The seeds of the faba bean variety 'Merkur' were infested by significantly fewer broadbean seed beetle larvae compared to 'Boxer' and 'Laura'. Judging by this factor, 'Merkur' was less susceptible to the broadbean seed beetle compared to the other two varieties.
2. The survival success of the broadbean seed beetle individuals (larvae, pupae) that entered the seeds differed significantly between the varieties 'Merkur' and 'Boxer'. In the seeds of 'Merkur', the largest part of all the larvae that entered them developed to the imago stage. Between the other two variety pairs ('Merkur' – 'Laura' and 'Boxer' – 'Laura') no significant differences in the survival rate of the seed beetle individuals were observed.
3. The highest proportion of damaged seeds was found in the yield of 'Merkur' and 'Boxer'; it was significantly lower in the yield of 'Laura'. In the agroecological conditions of Latvia, without pest control, the yield of 'Merkur' may contain 5–75% of seeds damaged by the broadbean seed beetle. This amount of damaged harvest is greater than the maximum allowable proportion of damaged seeds specified in the buyers' quality criteria.
4. In Latvian conditions, the faba bean variety 'Merkur' showed less susceptibility to the broadbean seed beetle during the vegetation period, when the pest lays eggs and first instar larvae penetrate the seeds. However, this is of no practical significance as the percentage of damaged seeds in the yield was similar or higher than other varieties.

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