

## Effects of faba bean (*Vicia faba*) varieties on the development of *Bruchus rufimanus*

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### Abstract

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The development of *Bruchus rufimanus* was assessed in randomised field trials from 2005 to 2007 with eight faba bean genotypes (SU 5/13, SU-BT, Mistral, Merkur, Stabil Merlin, Melodie, Divine). Significant differences, reproducible between the years, were found between the faba bean lines. The varieties most attractive to *B. rufimanus* ovipositing females were SU-BT and Divine. Merkur showed a certain level of non-preference. The highest mortality rates of eggs and first instar larvae in pod valve tissues were recorded in Merkur (34–45%) and Divine (51–55%). The highest mortality rates of larvae and pupae during their development in seeds (effect of seed coat and cotyledon tissues) were recorded in Merkur (87–90%) and Melodie (87–99%). Parasitism by *Triaspis thoracicus* contributed to this mortality. The highest levels of *B. rufimanus* parasitism were found in SU-BT (32–35%), the lowest in Melodie (3–9%). The total reduction in *Bruchus rufimanus* individuals during their development was relatively high in all varieties, ranging from 72% to 99%.

**Keywords:** antibiosis; *Bruchus rufimanus* mortality in seeds; nonpreference; plant resistance to bruchids

The bean seed beetle, *Bruchus rufimanus* (Boheman), is an economically important pest of *Vicia faba* bean crops throughout Europe (CZERNIAKOWSKI *et al.* 1996; MEYER 1998; WARD & SMART 2011) and has been a serious insect pest in the Czech Republic (CZ). The adults are in reproductive diapause from September to April. They overwinter under the bark of trees, in leaf litter and also in seeds (MIDDLEKAUF 1961; TRAN *et al.* 1993) and return to the growing crop in the spring, once the beans begin to flower (usually at the end of May or in the first half of June in CZ). At the time of colonization, the males have active reproductive organs: their diapause has already terminated. The females remain in diapause and their sexual development begins in the fields (TRAN & HUIGNARD 1992; TRAN *et al.* 1993; KANIUCZAK 2006). Females lay eggs on the outside of developing pods, predominantly on the lowest fertile

nodes, where flowering starts (WARD & SMART 2011). Hatching larvae bore through the pod valves and develop within the seeds. This fact makes the larvae a difficult target for control with insecticide sprays and thus the adults are usually the main target of control attempts. The sprays have to coincide with the egg-laying period to be effective. Two applications are usually recommended (WARD & SMART 2011). However, egg monitoring on pods is complicated and very time-consuming for farmers, so the spray timing is often incorrect and the control is generally poor (WARD & SMART 2011). Moreover, even if the spray timing is correct, the effects of commonly available insecticides (pyrethroids, neonicotinoids) are often unsatisfactory (MATŁOSZ & KANIUCZAK 1997; BRUCE *et al.* 2011; SEIDENGLANZ *et al.* 2011). Problems with controlling this insect pest imply lost opportunities for growers in lucrative markets for

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seed stock production and human consumption (the latter is not the case for CZ growers) due to crop rejection on standards of quality. Because levels of seed infestation have ranged between 1% and 50% during the last decade (2000–2010) in Central and Western Europe, and as this can vary in individual seasons, an effective approach to a control system consisting of more than just one operation is essential for growers to expand their use of beans as a valuable break crop in both organic and conventional arable farming systems. BRUCE *et al.* (2011) aimed to develop a semiochemical-based monitoring system as part of an Integrated Pest Management (IPM) strategy. A monitoring system supported by better knowledge of the pest biology would allow more effective control with insecticides and would provide a reliable risk indicator for determining the need or optimum timing of sprays, reducing multiple applications and risk of resistance. In addition, the identification of genetic resources of plant resistance for breeding programmes would enhance a package of IPM approaches. It is known (MATŁOSZ 1998; KANIUCZAK 2004; unpublished data: LEGATO project KBBE.2013.1.2-02) that faba bean varieties used in individual European countries (UK, France, Germany, Spain, Poland, CZ) often react differently after contact with the pest, with their eggs or larvae. Some varieties are significantly more attractive to egg-laying females or constantly show significantly higher levels of seed infestation than others under

the same field conditions (MATŁOSZ 1998; KANIUCZAK 2004). Relatively resistant accessions should be present in genetic resource collections (unpublished data: LEGATO project KBBE.2013.1.2-02).

Comparison of *B. rufimanus* development (from egg stage to hatching of new adults) in eight faba bean varieties / breeding materials is the aim of the paper.

## MATERIAL AND METHODS

The trials were conducted under field conditions in the Czech Republic (49°59.42497'N, 17°1.20018'E) over three years (2005–2007). The design of the trials was the same in each of the seasons. Trials were designed as four complete randomised blocks. Plots with each of the varieties (1–8) were in four repetitions. Size of plots: 1.2 × 1.2 m (1.44 m<sup>2</sup>). The individual faba bean plots were surrounded by a white mustard crop (width of mustard strips = 3 m). A description of the faba bean varieties used is given in Table 1.

Monitoring of bruchid egg counts on pods started at the time when the first eggs were observed on pods. This always occurred first on SU-BT plants. This variety started flowering earlier than the others in the test group. The monitoring was concentrated exclusively on the two bottom nodes which are more attractive to egg-laying females (WARD & SMART 2011). The assessments were done once or twice a week in the period of egg-laying (2005: 17 June–3 July;

Table 1. Brief descriptions of the varieties used in field trials (2005–2007)

Variety	Brief description
SU 5/13	Semi-early Czech spring faba bean line SU 5/13 with different coloured flowers is a source of increased resistance to <i>Ascochyta fabae</i> .
SU-BT	Very early spring faba bean white-flowered Czech breeding material with average seed yields. It shows higher susceptibility to pests and <i>Uromyces fabae</i> .
Mistral	Semi-early white-flowered Czech spring variety with low tannin content, average resistance to fungal diseases and high seed yields. Due to the shorter stem and weaker foliage it is not suitable for forage use.
Merlin	Semi-early white-flowered Czech spring variety with low tannin content, medium resistance to fungal diseases and high seed yields. Due to the shorter stem and weaker foliage it is not suitable for forage use.
Merkur	Semi-early Czech spring variety with different coloured flowers with high performance and medium resistance to fungal diseases.
Stabil	Semi-early Czech spring variety with different coloured flowers with lower resistance to stem brackling and medium resistance to fungal diseases.
Melodie	Semi-early French spring variety with different coloured flowers with low vicine/convicine content. This variety has very good performance and high protein content. TGW 550 g.
Divine	Early French spring variety with different coloured flowers with low vicine/convicine content. Resistant to lodging, high protein content. TGW 550 g.

2006: 25 June–11 July; 2007: 20 June–7 July; 20 pods from two bottom nodes per plot were assessed). The assessments with the highest mean counts per pod were used for further analysis.

Pods (exclusively from two bottom nodes) were taken by hand at harvest (August) and analysed for damage to seed by bruchids (20 pods per plot = 80 pods per treatment) in September/October. A pod was the basic unit for all assessments conducted in the laboratory. Each pod was assessed solely for the total number of seeds, for the number of larval entrances into seeds, number of *Bruchus* exit holes from seeds and for the number of *Triaspis thoracicus* (parasitoid of the insect pest) exit holes from seeds. From these primary data, the proportions of seeds left by *B. rufimanus* adults and *T. thoracicus* adults were calculated for each pod and, after that, the mean values for each variety ( $n = 80 = 4 \times 20$  pods per treatment) were determined. Proportions of *B. rufimanus* individuals that died in seeds and/or were parasitized by *T. thoracicus* in the compared varieties were inferred from the numbers of larval entrances into seeds and the numbers of *B. rufimanus* and/or *T. thoracicus* exit holes. Again, the proportions were first determined for each pod and, after that, for the individual varieties (expressed as variety means). The pods with seeds free from larval entrances were excluded from the two last assessments.

To test significant differences in mean values determined for the studied variables in the compared varieties in the three years (2005, 2006, 2007) and totally (2005–2007) analysis of variance (ANOVA) and an appropriate post-test (Tukey's test) were performed. The statistical analysis was performed by the Statistica software v.10 (StatSoft, Inc. 1984–2013).

## RESULTS

The Divine variety was significantly more attractive to egg-laying *B. rufimanus* females than the majority of the other varieties compared in the group in all three years. The SU-BT variety also showed significantly (in many instances) higher numbers of bruchid eggs on pods, especially in the first two years. In contrast, the lowest numbers of eggs were recorded on the Merkur variety in all three years. So the variety showed a certain level of nonpreference (Table 2).

The highest numbers of bruchid larvae entering the seeds were recorded with the SU-BT variety in all three years. Interestingly, the mean numbers of larvae entering the Divine seeds, the most attractive

variety to ovipositing females, were markedly lower in all three years (Table 2). The reason was the markedly higher mortality of young bruchid individuals (eggs and 1<sup>st</sup> instar larvae) on pod valves and within the pod in this variety compared to SU-BT (Table 3). The lowest numbers of larval entrances to seeds were recorded with the Merkur variety (Table 2). This coincides with the relatively low numbers of eggs on pods and it is also a result of the high mortality of eggs and young larvae on pod valves and in within the pod (Table 3).

Most insect adults (*B. rufimanus* + its parasitoid *T. thoracicus*) successfully finished their development, hatched and left seeds on the SU-BT variety. There were significantly higher mean numbers of exit holes from seeds in all three years in this variety. In contrast, substantially fewer insects left seeds of the Merkur and Melodie varieties during these years (Table 2). The recorded levels of bruchid larvae mortality in seeds correspond with such development (Table 3). Seeds of the Melodie variety proved to be the most unfavourable place for development of bruchid larvae (the variety showed the highest level of antibiosis) in the tested group, even if this variety was relatively attractive to egg-laying females and the mortality of eggs/larvae on pod valves and within the pod was rather lower at the same time (with the exception of the 2005 season; Table 3).

The highest numbers of *B. rufimanus* adults were found leaving SU-BT pods (mean number of bruchid exit holes from seeds per pod) every year. In 2005 a high number of *B. rufimanus* adults also hatched in SU 5/13. In 2006 and 2007 relatively high numbers of *B. rufimanus* adults completed their development in the Merlin variety. The fewest adults left pods of the Merkur and Melodie varieties (Table 2). This coincides with the levels of mortality in their seeds (high level of antibiosis in Merkur and Melodie, lower in SU-BT) and on pod valves and within the pod (high level of antibiosis in Merkur, average for Melodie, low – average in SU-BT). However, this does not correspond with the levels of parasitisation (high in SU-BT, average in Merkur, low in Melodie). Even if the *B. rufimanus* parasitisation (induced by *T. thoracicus*) reached the highest levels in SU-BT (32.08–34.96%), the variety remained the most intensively damaged by the pest (17.58–27.06% of seeds left by *Bruchus* adults). The least damaged varieties were Merkur (1.98–4.90% of seeds left by *Bruchus* adults) and Melodie (1.81–9.90% of seeds left by *Bruchus* adults) (Table 4).

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Table 2. Effects of faba bean (*Vicia faba*) varieties and parasitoid (*Triaspis thoracicus*) on the development of broad bean weevil (*Bruchus rufimanus*) under field conditions (2005–2007)

Variety	<i>n</i>	Mean No. of eggs per pod (SD)			Mean No. of larval entrances into the seeds per pod (SD)			Mean No. of exit holes from seeds per pod (SD)			Mean No. of <i>Bruchus</i> exit holes per pod (SD)			Mean No. of <i>Triaspis</i> exit holes per pod (SD)		
		2005	2006	2007	2005	2006	2007	2005	2006	2007	2005	2006	2007	2005	2006	2007
SU 5/13	80	2.53 <sup>b</sup> (1.90)	2.43 <sup>b</sup> (2.19)	2.23 <sup>d</sup> (2.12)	2.21 <sup>c</sup> (1.51)	1.83 <sup>b</sup> (1.05)	1.76 <sup>cd</sup> (1.87)	1.28 <sup>b</sup> (1.06)	0.69 <sup>bc</sup> (0.61)	0.64 <sup>bcd</sup> (0.97)	0.71 <sup>ab</sup> (0.58)	0.35 <sup>bc</sup> (0.48)	0.35 <sup>bc</sup> (0.64)	0.56 <sup>b</sup> (0.73)	0.34 <sup>bcd</sup> (0.57)	0.29 <sup>bc</sup> (0.66)
SU-BT	80	4.40 <sup>a</sup> (3.09)	3.96 <sup>a</sup> (3.33)	3.74 <sup>b</sup> (3.81)	3.93 <sup>a</sup> (2.26)	3.66 <sup>a</sup> (2.56)	3.68 <sup>a</sup> (3.20)	2.21 <sup>a</sup> (1.19)	1.91 <sup>a</sup> (1.09)	1.83 <sup>a</sup> (1.59)	0.93 <sup>a</sup> (0.44)	0.70 <sup>a</sup> (0.58)	0.61 <sup>a</sup> (0.79)	1.29 <sup>a</sup> (0.96)	1.21 <sup>a</sup> (0.91)	1.21 <sup>a</sup> (1.28)
Mistral	80	2.25 <sup>bc</sup> (1.89)	2.51 <sup>b</sup> (2.05)	2.61 <sup>bcd</sup> (2.13)	1.93 <sup>c</sup> (1.40)	2.09 <sup>b</sup> (1.55)	2.36 <sup>bc</sup> (2.21)	0.86 <sup>bc</sup> (0.78)	0.83 <sup>b</sup> (0.79)	0.89 <sup>bc</sup> (0.99)	0.48 <sup>cd</sup> (0.50)	0.23 <sup>c</sup> (0.42)	0.35 <sup>abc</sup> (0.53)	0.39 <sup>bc</sup> (0.63)	0.60 <sup>b</sup> (0.74)	0.54 <sup>b</sup> (0.86)
Merlin	80	2.13 <sup>bc</sup> (1.50)	2.53 <sup>b</sup> (2.33)	2.46 <sup>cd</sup> (2.13)	1.78 <sup>c</sup> (1.57)	1.90 <sup>b</sup> (1.67)	1.69 <sup>cd</sup> (1.85)	0.81 <sup>c</sup> (0.87)	0.95 <sup>b</sup> (0.97)	0.80 <sup>bc</sup> (1.10)	0.41 <sup>d</sup> (0.50)	0.53 <sup>ab</sup> (0.64)	0.46 <sup>ab</sup> (0.69)	0.40 <sup>bc</sup> (0.59)	0.43 <sup>bc</sup> (0.59)	0.34 <sup>bc</sup> (0.73)
Merkur	80	1.25 <sup>c</sup> (1.13)	1.80 <sup>b</sup> (1.84)	2.05 <sup>d</sup> (1.53)	0.83 <sup>d</sup> (0.74)	0.95 <sup>c</sup> (0.95)	1.13 <sup>d</sup> (1.47)	0.26 <sup>d</sup> (0.44)	0.38 <sup>c</sup> (0.54)	0.45 <sup>cd</sup> (0.84)	0.08 <sup>e</sup> (0.27)	0.13 <sup>c</sup> (0.33)	0.16 <sup>c</sup> (0.40)	0.19 <sup>c</sup> (0.39)	0.25 <sup>cd</sup> (0.44)	0.29 <sup>bc</sup> (0.62)
Stabil	80	2.43 <sup>b</sup> (1.83)	2.21 <sup>b</sup> (1.86)	3.55 <sup>bc</sup> (3.01)	1.96 <sup>c</sup> (1.58)	1.66 <sup>bc</sup> (1.55)	3.34 <sup>ab</sup> (2.83)	0.89 <sup>bc</sup> (0.97)	0.60 <sup>bc</sup> (0.72)	1.03 <sup>b</sup> (1.26)	0.65 <sup>bc</sup> (0.62)	0.28 <sup>c</sup> (0.45)	0.39 <sup>abc</sup> (0.52)	0.24 <sup>c</sup> (0.56)	0.33 <sup>bcd</sup> (0.52)	0.64 <sup>b</sup> (0.97)
Melodie	80	4.83 <sup>a</sup> (2.40)	2.63 <sup>b</sup> (2.34)	2.39 <sup>cd</sup> (1.82)	3.11 <sup>b</sup> (2.17)	2.46 <sup>b</sup> (2.01)	2.18 <sup>cd</sup> (2.43)	0.38 <sup>d</sup> (0.56)	0.35 <sup>c</sup> (0.48)	0.25 <sup>d</sup> (0.68)	0.06 <sup>e</sup> (0.24)	0.28 <sup>c</sup> (0.45)	0.14 <sup>c</sup> (0.38)	0.31 <sup>bc</sup> (0.54)	0.06 <sup>d</sup> (0.24)	0.11 <sup>c</sup> (0.42)
Divine	80	4.51 <sup>a</sup> (2.73)	4.53 <sup>a</sup> (4.04)	5.04 <sup>a</sup> (3.53)	2.11 <sup>c</sup> (1.48)	2.21 <sup>b</sup> (1.98)	2.26 <sup>bc</sup> (2.27)	0.81 <sup>c</sup> (0.92)	0.90 <sup>b</sup> (0.85)	0.81 <sup>bc</sup> (1.11)	0.55 <sup>bcd</sup> (0.55)	0.33 <sup>b</sup> (0.50)	0.28 <sup>b</sup> (0.50)	0.26 <sup>bc</sup> (0.55)	0.58 <sup>b</sup> (0.71)	0.54 <sup>b</sup> (0.98)
<i>F</i>		31.048	10.137	12.096	25.232	15.990	10.643	37.801	31.817	14.660	31.209	11.054	5.8883	25.104	24.815	12.544

The numbers marked with different letters in the columns differ significantly (Tukey's test;  $P < 0.05$ )

Table 3. The course of *B. rufimanus* mortality during its development in the compared faba bean varieties (2005–2007)

Treatment No.	Variety	Approximate mean levels of mortality of eggs on pods + I. instar larvae unable to enter successfully the seeds in pods (%) <sup>1</sup>					Mean level of larval mortality in seeds (%) <sup>#</sup> (SD)					Mean level of parasitisation (%) <sup>#</sup> (SD)					<i>n</i> <sup>*</sup>
		2005	2006	2007	2005	2006	2007	2005	2006	2007	2005	2006	2007	2005	2006	2007	
1	SU 5/13	12.65	24.69	21.08	65.59 <sup>b</sup> (23.75)	78.93 <sup>ab</sup> (29.99)	75.99 <sup>ab</sup> (35.36)	22.69 <sup>ab</sup> (23.10)	17.50 <sup>b</sup> (28.84)	14.26 <sup>bc</sup> (26.98)	65	70	52	65	70	52	
2	SU-BT	10.68	7.58	1.60	72.55 <sup>b</sup> (16.50)	73.16 <sup>b</sup> (30.00)	80.91 <sup>ab</sup> (24.48)	32.08 <sup>a</sup> (18.46)	34.96 <sup>a</sup> (23.75)	34.25 <sup>a</sup> (29.19)	71	68	64	71	68	64	
3	Mistral	14.22	16.73	9.58	72.30 <sup>b</sup> (29.94)	87.96 <sup>a</sup> (21.47)	78.88 <sup>ab</sup> (33.73)	17.57 <sup>bcd</sup> (26.89)	28.68 <sup>ab</sup> (30.53)	22.10 <sup>ab</sup> (30.33)	68	63	64	68	63	64	
4	Merlin	16.43	24.90	31.30	72.27 <sup>b</sup> (34.93)	72.95 <sup>b</sup> (27.05)	73.91 <sup>b</sup> (30.74)	18.50 <sup>bcd</sup> (23.49)	24.02 <sup>ab</sup> (30.15)	17.81 <sup>bc</sup> (29.93)	61	59	52	61	59	52	
5	Merkur	33.60	47.22	44.88	89.82 <sup>a</sup> (29.75)	87.32 <sup>ab</sup> (26.34)	86.71 <sup>ab</sup> (25.97)	22.22 <sup>bc</sup> (39.25)	24.64 <sup>ab</sup> (32.15)	23.89 <sup>ab</sup> (34.55)	54	46	42	54	46	42	
6	Stabil	19.34	24.89	5.92	64.05 <sup>b</sup> (31.86)	80.94 <sup>ab</sup> (29.34)	84.11 <sup>ab</sup> (26.12)	6.67 <sup>d</sup> (13.48)	16.14 <sup>bc</sup> (19.81)	19.96 <sup>abc</sup> (27.73)	66	56	70	66	56	70	
7	Melodie	35.61	6.46	8.79	98.45 <sup>a</sup> (5.91)	88.71 <sup>a</sup> (16.22)	91.32 <sup>a</sup> (23.86)	9.43 <sup>d</sup> (15.17)	3.21 <sup>c</sup> (11.54)	5.50 <sup>c</sup> (19.34)	69	59	61	69	59	61	
8	Divine	53.22	51.21	55.16	68.90 <sup>b</sup> (32.47)	84.14 <sup>ab</sup> (21.59)	82.66 <sup>ab</sup> (32.57)	10.52 <sup>cd</sup> (19.47)	27.27 <sup>ab</sup> (26.59)	20.68 <sup>abc</sup> (31.12)	68	57	61	68	57	61	
		$F_{(7,514)} = 13.208$					$F_{(7,458)} = 3.6820$	$F_{(7,514)} = 9.0185$	$F_{(7,470)} = 8.4764$	$F_{(7,458)} = 4.9867$							

The numbers marked with different letters in the columns differ significantly (Tukey's test;  $P < 0.05$ ); <sup>1</sup>the rates of mortality are only approximate, because the monitoring of eggs was done on different pods than all the other assessments; <sup>#</sup>as a basis for calculation the number of larval entrances to seeds per pod was used (= 100%) and the numbers of *Bruchus* or *Triaspis* exit holes from seeds per pod were expressed in %; *n* – No. of pods analysed per variety; \*the pods with seeds without larval entrances were excluded from the assessments marked with #, so the *n* values are lower than 80



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Table 4. Final levels of seed damage caused by *B. rufimanus* and total proportions of seeds left by the pest and/or its parasitoid *T. thoracicus* (2005–2007)

Variety	n	Mean No. of seeds per pod (SD)			Mean portion of seeds leaved by <i>Bruchus</i> adults (%) (SD)			Mean portion of seeds leaved by <i>Bruchus</i> or (and) <i>Triaspis</i> adults (%) (SD)		
		2005	2006	2007	2005	2006	2007	2005	2006	2007
SU 5/13	80	3.28 <sup>bc</sup> (1.12)	3.43 <sup>cd</sup> (0.85)	3.23 <sup>ab</sup> (0.67)	26.40 <sup>a</sup> (27.48)	11.17 <sup>abcd</sup> (15.85)	10.83 <sup>abc</sup> (19.77)	41.69 <sup>b</sup> (36.58)	21.50 <sup>bcd</sup> (20.37)	19.48 <sup>bcd</sup> (28.90)
SU-BT	80	3.81 <sup>a</sup> (1.07)	3.91 <sup>ab</sup> (0.96)	3.51 <sup>a</sup> (0.66)	27.06 <sup>a</sup> (17.13)	18.04 <sup>a</sup> (16.13)	17.58 <sup>a</sup> (23.13)	59.46 <sup>a</sup> (34.32)	49.13 <sup>a</sup> (28.79)	47.92 <sup>a</sup> (38.74)
Mistral	80	3.33 <sup>abc</sup> (1.19)	3.50 <sup>bcd</sup> (0.97)	3.35 <sup>a</sup> (0.75)	15.81 <sup>b</sup> (19.87)	7.17 <sup>cd</sup> (14.29)	10.77 <sup>abc</sup> (16.85)	30.08 <sup>bc</sup> (30.69)	24.81 <sup>bc</sup> (24.61)	26.13 <sup>bc</sup> (27.32)
Merlin	80	3.63 <sup>ab</sup> (1.19)	3.79 <sup>abc</sup> (0.91)	3.59 <sup>a</sup> (0.79)	13.38 <sup>b</sup> (18.95)	15.06 <sup>ab</sup> (18.86)	13.23 <sup>ab</sup> (21.82)	23.98 <sup>cd</sup> (27.21)	26.88 <sup>bc</sup> (28.99)	22.10 <sup>bc</sup> (29.93)
Merkur	80	3.45 <sup>abc</sup> (0.93)	3.81 <sup>abc</sup> (1.08)	3.39 <sup>a</sup> (0.74)	1.98 <sup>c</sup> (7.17)	3.73 <sup>d</sup> (10.38)	4.90 <sup>bc</sup> (12.42)	8.94 <sup>e</sup> (17.70)	10.96 <sup>d</sup> (17.16)	12.85 <sup>cd</sup> (24.46)
Stabil	80	3.71 <sup>ab</sup> (1.10)	3.98 <sup>a</sup> (0.76)	3.54 <sup>a</sup> (0.84)	19.27 <sup>ab</sup> (18.93)	7.19 <sup>cd</sup> (12.31)	11.96 <sup>abc</sup> (16.74)	26.02 <sup>cd</sup> (29.17)	15.56 <sup>cd</sup> (18.48)	27.31 <sup>b</sup> (29.51)
Melodie	80	2.95 <sup>c</sup> (1.02)	3.16 <sup>d</sup> (0.86)	2.88 <sup>b</sup> (0.92)	1.81 <sup>c</sup> (7.20)	9.90 <sup>bcd</sup> (16.72)	4.58 <sup>c</sup> (13.12)	14.35 <sup>de</sup> (23.56)	12.33 <sup>d</sup> (17.65)	7.50 <sup>d</sup> (17.67)
Divine	80	3.56 <sup>ab</sup> (0.81)	3.53 <sup>abcd</sup> (1.32)	3.31 <sup>a</sup> (0.77)	15.90 <sup>b</sup> (16.27)	11.56 <sup>abc</sup> (21.17)	8.85 <sup>bc</sup> (16.69)	23.71 <sup>cd</sup> (27.84)	29.23 <sup>b</sup> (30.61)	23.75 <sup>bc</sup> (31.46)
F		5.4595	6.5141	6.9479	23.169	6.5549	4.5819	24.142	21.003	13.615

The numbers marked with different letters in the columns differ significantly (Tukey's test;  $P < 0.05$ )

## DISCUSSION

The reproduction of *B. rufimanus* is synchronised with the flowering and fructification cycles of its host plant (MEDJDOUB-BENSAAD *et al.* 2007). When temperatures rise above 15°C, the insects leave their hibernation sites and migrate towards the broad bean cultures (HOFFMANN *et al.* 1962). In Europe oviposition is usually triggered and the *B. rufimanus* activity in crops starts increasing when temperatures of 20°C or more are reached (MUEL personal communication 2006; WARD & SMART 2011). The colonisation of faba bean cultures by *B. rufimanus* adults depends on plant flower availability; the pollen consumption provides the insect with nutrition and induces female reproduction. When important trophic supplies are available on stems, females are attracted by these resources, forage on such stems for a long period and have higher chances of encountering suitable pods for oviposition there (MEDJDOUB-BENSAAD *et al.* 2007). In a field where more than one faba bean variety is grown together, early flowering varieties attract *B. rufimanus* females earlier and the oviposition starts earlier and lasts longer; at least under CZ field conditions, where the oviposition period lasts about two to three weeks maximally. This probably explains the differences in mean numbers of eggs recorded in the study. The SU-BT and Divine varieties with the highest mean numbers of eggs on pods entered the flowering period earlier (by about one week) than the others in the group every year. The relationship between the time of flowering and attractiveness of faba bean to *B. rufimanus* females was also confirmed in screening trials (300 faba bean accessions compared) conducted under CZ field conditions in 2014 (unpublished data: LEGATO project KBBE.2013.1.2-02) and also for some closely related species (for example in *B. pisorum*: BUROV 1980; ANNIS 1983; CLEMENT 1992; SMITH & HEPWORTH 1992). Among the varieties with somewhat delayed start of flowering (compared to SU-BT and Divine) Merkur showed the highest level of nonpreference (the lowest attractiveness to ovipositing females).

In total, the (approximate) rates of bruchid egg/first instar larvae mortalities on pod valves and within the pod for all compared varieties in the study ranged from 1.60% to 55.16% during the three years (Table 3). There were significant differences in the decreases between the mean numbers of *B. rufimanus* eggs per pod and the mean numbers of *B. rufimanus* larval entrances into seeds per pod in the varieties Divine,

Merkur, Merlin and Melodie (Table 2). In the other varieties in the group the differences were less clear (SU 5/13, Stabil, Mistral) or almost negligible (SU-BT) (Table 3). The results show there is no clear (and simple) relationship between the tannin content (or vicine/convicine content) in the faba bean variety or the colour of its flowers and the mortality of *B. rufimanus* eggs/larvae on pod valves and within the pod. This finding agrees with those of some other studies (MATŁOSZ & KANIUCZAK 1997; MATŁOSZ 1998; KANIUCZAK 2006) and indicates different levels of antibiosis in pod valve tissues of the varieties. The pod valves of the Divine and Merkur varieties seem to be a markedly more difficult obstacle for young bruchid individuals than the pod valves of the other varieties in the group. In the two varieties we also recorded more neoplastic structures on pod valves. Whether the growth of the tumour-like structures was stimulated by the previous presence of *B. rufimanus* eggs laid there we were unable to determine. However, the presence of some form of induced resistance in the two varieties should not be excluded (Doss *et al.* 2000). The approximate mean mortalities of *B. rufimanus* in the pod valves of the two varieties ranged from about 40% (Merkur) to 50% (Divine) during the three years (Table 3).

In general the rates of *B. rufimanus* larvae mortalities in seeds were relatively high in all varieties and in all years (they ranged from 64.05% to 98.45%) and were markedly higher than those observed for pod valves. Seed tissues (seed coat and cotyledon tissues) showed higher levels of antibiosis compared to pod valve tissues for *B. rufimanus*. Mortality in seeds alone was the most important reduction factor of bruchid individuals in all varieties compared, even if there were significant differences between them (Table 3). In comparison with the recorded rates of mortality of *B. rufimanus* larvae presented in this study, the rates of mortality determined for *B. pisorum* were almost negligible in seeds of common CZ field pea varieties tested under comparable field conditions (unpublished data: LEGATO project KBBE.2013.1.2-02). The initial levels of infestation (mean number of eggs per pod; mean number of larval entrances into seeds per pod) are usually markedly higher for *B. rufimanus* than for *B. pisorum* under common field conditions in CZ.

The successful development of *B. rufimanus* in different faba bean varieties can differ substantially. In general, mortality rates of *B. rufimanus* individuals (eggs, larvae, pupas) in the course of their develop-

ment on faba beans, even where common European varieties are compared, are relatively high. Total (egg-imago) reduction of *Bruchus rufimanus* individuals ranged from 72% to 99% in the present study. The highest rates of mortality were recorded among larvae in seeds (64–99%). In some varieties pod valves seem to be a dangerous obstacle for young bruchid individuals. Parasitisation by *Triaspis thoracicus* can also play an important role in decreasing the numbers of new emerged *B. rufimanus* adults (reduction of new overwintering population).

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