



Varietal and environmental effects on the production of faba bean (*Vicia faba* L.) seeds for the food industry by confrontation of agricultural and nutritional traits with resistance against *Bruchus* spp. (Coleoptera: Chrysomelidae, Bruchinae)

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ABSTRACT

Faba bean is a globally produced agricultural crop due to the high protein content in seeds. However, yields strongly vary depending on biotic and abiotic factors. Here, we evaluated the combined effect of faba bean varieties and climate on crop productivity, seed quality and *Bruchus* spp. infestation to identify most promising faba bean varieties for use in the food industry as local protein source. Varietal and year related factors were studied during two cropping years to explain variation of field yield, seed protein/ash/lipid content, protein production, and infestation rates. Fourteen varieties including nine winter varieties and five spring varieties were compared, from which one variety presented stable and promising yield, seed composition and low infestation rates. Annual effects significantly impacted field yield and protein production in contrast with the varietal effect that significantly impacted seeds protein content and infestation rates. Principal components analysis showed that infestation rate and yield were not correlated; thus, these two parameters could be optimized independently. The spring variety Fanfare exhibited the best and most stable results over the two study periods. Winter varieties had higher yields, whereas spring varieties had higher seed protein content. The main parameters impacting bruchid infestations were variety, indicating the need to select certain varieties that reduce the impact of pests on seed quality. During 2020, a drought during growing season significantly impacted faba bean production, demonstrating the importance of developing drought-resistant varieties. Thus, fourteen faba bean varieties were characterized considering together key parameters for food uses, and were ranked to identify most interesting ones. We also highlighted most impacting parameters that should be taken into account for the future improvement of varietal resilience in European countries.

1. Introduction

Producing indigenous plant proteins is essential for the sustainability of the European cropping systems, with interest being expected to increase in the coming decades given their multiple benefits provided to cropping systems, environment and ecosystems (European Parliament,

2018). Leguminous plants constitute potential candidates to promote local plant protein productions (PPs). One such example is *Vicia faba* L., which is commonly named faba bean (as general denomination), but also broad bean, horse/field bean, and tic bean (depending on the traditional type of varietal groups). Faba bean crops provide many benefits to the environment by reducing the need for nitrogen fertilizers

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and, so reducing the greenhouse gases production associated with their production (Köpke and Nemecek, 2010). Moreover, the cultivation of faba bean benefits crop rotations by improving the yields of successive crops, preventing and limiting the spread of pests and diseases, and favoring beneficial organisms, such as pollinators or natural enemies of arthropod pests, due to their extensive floral resources (Jensen et al., 2010; Karkanis et al., 2018).

The main asset of cropping faba bean is the production of seeds that contain high amounts of proteins, about 30% weight/weight ($w w^{-1}$) of dry weight (DW) (Rempel et al., 2019). Faba bean seeds have high nutritional value and a comparable amino acid (AA) score to other legumes (FAO/WHO/UNU 2007; Vioque et al. 2012; Pastor-Cavada et al. 2014; Wongsiri et al. 2015). Besides proteins, faba bean seeds are composed of starch (around 40% DW), minerals ($\sim 3\text{--}4\%$ DW), and lipids ($\sim 2\%$) (Vioque et al., 2012; Landry et al., 2016). The relative percentages of each nutrient vary with variety (Micek et al., 2015).

Faba beans are used as food and feed. Food uses constitutes the most profitable outlet for growers, and was evaluated at 30–40 euros/t above the animal feed market (Biarnès et al., 2018; Bachmann et al., 2020). Food uses include seed consumption (whole or dehulled) in traditional meals (especially in Mediterranean countries). Transformation and formulation of new products in the food industry represents another food use opportunity for faba bean seeds, providing alternatives to traditional animal proteins. Their low lipids contents facilitate protein extraction, which leads to an improvement of the technological quality of proteins. Feed uses include pigs, poultry, and bovine feedings. The export of dehulled seeds for use in fish farms in Norway is currently rising (Biarnès et al., 2018). As feed, this crop provides a good alternative to imported soybean, and contributes to protein autonomy.

Despite these advantages, the promotion of faba bean is subject to sociological, technical, and logistical constraints (Simmen, 2020). The greatest constraint in using faba bean seeds as food is infestation by *Bruchus* spp. (Coleoptera: Chrysomelidae, Bruchinae). This group of pests ravaging faba bean is commonly called broad bean weevils or bruchids, and include five species occurring in Belgium; namely, *Bruchus affinis* Frölich 1799; *B. atomarius* (L. 1761), *B. brachialis* Fähræus 1839, *B. rufimanus* Boheman 1833 and *B. pisorum* (L. 1758). *Bruchus rufimanus* is the most abundant species in faba bean crops (Zampetti and Ricci, 2012; Baugnée et al., 2021). All five species are oligophagous univoltine species, the life cycle of which greatly depends on host plant phenology and climatic conditions (Segers et al., 2020). Adults overwinter in reproductive diapause in wooded sites, and colonize flowering crops during spring when temperatures exceed 15 °C (Hoffman et al., 1962). Individuals sexually mature while feeding on *V. faba* flowers, and reproduction occurs approximately two weeks after field colonization, with temperatures surrounding 20 °C (Tran and Huignard, 1992). Gravid females oviposit on newly formed pods and hatching larvae directly bore through the pods pericarp (Pölitiz and Reike, 2019). The whole post-embryonic development, including four larval instars (L1–L4) and a nymphal stage, takes place inside one forming seed, and spans approximately 100 days. Most adults emerge when dry seeds are harvested (Boughdad and Lauge, 1995). Emerging adults do not cause any further damage to stored seeds, as females cannot oviposit on dry seeds (Howe and Currie, 1964). Adults overwinter in reproductive diapause until the next spring when faba bean crop starts flowering (Huignard et al., 2011).

Bruchids cause both qualitative and quantitative damage to seeds including a reduction in seed weight due to the consumption of the endosperm by developing larvae. This reduction ranges from 5.0% to 9.4% of the dry seed weight (Boughdad and Lauge, 1995; Titouhi et al., 2015). The consumption of seeds by developing larvae also decrease the nutritional value and organoleptic properties caused by the accumulation of insects wastes such as urates (Huignard et al., 2011; Khelfane-Goucem and Medjdoub-Bensaad, 2016). Esthetic quality is also strongly altered due to the perforation of the seed coat by emerging adults. In addition, perforated seeds are more susceptible to fungal

infection in storage facilities or in crops after sowing (Kaniuczak, 2004; Ward, 2018). These damage prevent the seed valorization in food market that only accept seed batches with $< 2\text{--}3\%$ infestation rates (i.e., proportion of seeds presenting emergence hole of adults) (Bruce et al., 2011; Ward, 2018). This target is difficult to meet, as broad bean weevils typically generate 3–100% infestation rates, depending on country and cultivar (Boughdad and Lauge, 1995; Kaniuczak, 2004; Roubinet, 2016). As a consequence of bruchids infestations, the French export market of faba bean seeds to Egypt for traditional food uses (i.e., most important food market) collapsed during the last decade (Lacampagne, 2021). Solutions such as varietal selection must therefore be implemented to encourage this environment-friendly culture.

To date, few studies have assessed the susceptibility of different *V. faba* varieties to the development of bruchids, and underlined that no faba bean variety was yet able to support a seed production matching with food quality standards in Europe, as no resistance or tolerance could be observed (Roubinet, 2016). However, the selection of varieties exhibiting some level of resistance represents the most promising way of controlling such pests. Some accessions were recently supposed to exhibit antibiosis or antixenosis resistance mechanisms to the development of bruchids (Seidenglanz et al., 2011; Szafrowska, 2012; Carrillo-Perdomo et al., 2019). Further researches on the selection of candidate varieties for food industries (i.e., producing stable and optimal protein quantity and quality), should focus on resistances against bruchids that decrease seeds quality, but also on resistance to abiotic stresses that greatly impact seeds quantity, and that are expected to increase with current climate change. However, to date, studies have not investigated the traits of varieties considering all of these factors simultaneously.

This study aimed to identify the potential faba bean varieties to produce viable seeds for use in the food industry. To accomplish this, 14 varieties were tested in field trials, and seeds were characterized according to bruchids injury level and seed composition to detect those with the maximal amount of protein and ash content, and minimal amount of fatty matter and bruchids infestation rate. Our results are expected to provide baseline information on the best varieties for seed breeders to grow and towards selecting more resistant faba bean cultivars for the future.

2. Material and methods

2.1. Plant material, field experiment and measurements

Fourteen faba bean varieties (including five spring varieties and nine winter varieties) were tested in Gembloux (Belgium) over two consecutive growing seasons (2018–2019 and 2019–2020). Geographical coordinates and altitude of the two field trials were 50°49'91"N, 4°74'34"E, 167.12 m (2018–2019), and 50°50'34"N, 4°73'19"E, 174.75 m (2019–2020). These locations had high bruchid population sizes based on previous faba bean infestation records. Thus, each variety was grown under hyper infestation conditions to discriminate sensitive and resistant varieties optimally. Spring varieties included commercial cultivars named “Bobas”, “Fanfare”, “Julia”, “LG Cartouche” and “Tiffany.” Winter varieties included commercial cultivars named “Augusta”, “Axel”, “Bering”, “Bumble”, “Diva”, “Honey”, “Irena”, “Nebraska” and “Tundra” (Appendix A in Supplementary material). Spring and winter varieties were tested in separate trials designed in four complete randomized blocks. The factor Type was used to discriminate winter and spring varieties. Faba beans were grown in plots of 14.0 m² (2.0 × 7.0 m) at a density of 35 seeds per m² (winter varieties) and 50 seeds per m² (spring varieties). These different sowing densities were used to obtain the same stem density in winter and spring crops. Winter varieties of faba bean branch many times at the base of the plant during the winter period, whereas spring varieties have one to two upright stems per seed (unpublished data). External plots were placed around the test areas to attenuate border side effects. Plots were

bordered by parcels of 27 m wide (called “generalized parcels”), which contained a single faba bean variety for winter and spring trials; namely “Nebraska” and “Fanfare,” respectively. A representative scheme of this field set-up is presented in [Appendix B in supplementary material](#). Under these trial conditions, we hypothesized that spring and winter varieties would grow in two distinct environments driven by respective weather conditions and pest pressure of the two years of study. No insecticides or fertilizers were applied in the experimental design. One fungicide treatment was applied at the bud-flowering stage of the crop to avoid any disease in the trial. Chemical weeding was performed after sowing. Seeds were mechanically harvested at maturity. The sowing and harvesting dates of spring and winter varieties are presented in [Table 3](#).

Bruchid populations were monitored in parallel with meteorological conditions and varietal phenology. Climatic parameters of temperature and rainfall, which are recognized as the key factors driving bruchid infestation ([Carrillo-Perdomo et al., 2019](#)), were recorded by a nearby weather station. The phenological traits measured for each variety included the flowering start date (*i.e.* 50.0% of flowering plants in plots) and the flowering duration, because both parameters strongly influence the oviposition periods of bruchids ([Seidenglanz and Hunady, 2016](#)). Populations of bruchids were monitored using manual catches in generalized parcels, to avoid disrupting adult behavior (*i.e.*, feeding and oviposition), which could be influenced by preferences for certain varieties. This manual method of monitoring was preferred over semi-chemical traps, because traps cannot efficiently detect the presence of adults during the flowering period, due to competition exerted by crop odor ([Ward, 2018; Segers et al., 2020](#)). Manual catches were performed weekly in generalized parcels following a standard method, in which a single operator followed a fixed pathway (100 m length \times 1 m width) at the same daily period (from 15:30 to 16:30), and caught active adults (*i.e.*, feeding or mating) in the flowers or apical leaves with a truncated cone reversed over a pill box. Insects were conserved in ethanol 70% ($v v^{-1}$) and brought to the laboratory for identification and counting. All these field measurements were computed in order to confront weekly bruchids field population with weekly averages of temperatures ($^{\circ}C$, T_{avg}), cumulated rainfall (mm, P_{cum}), and varietal phenological stage.

2.2. Analyses of harvested seeds

After harvesting the crop, agronomic traits including field yield (FY, $kg ha^{-1}$) and thousand seeds weight (TSW, g) were determined from a unique value based on the four plots pooled together for each variety. FY and TSW were determined at standardized 14% seed humidity. Seeds were then characterized based on damage and chemical composition analyses.

Damage analyses were performed on seeds stored at room temperature for one month to allow insects to complete their life cycle. Four randomly selected replicates of 50 seeds were sampled and seeds were ranked according to traces left by the post embryonic development of bruchids. The ranking approach implemented by [Carrillo-Perdomo et al. \(2019\)](#) was used: Class “HS” (*i.e.*, “healthy seed”) corresponded to non-infested seeds ([Fig. 1A](#)); class “SD” (*i.e.*, “surface damages”) corresponded to infested seeds, presenting traces of necrosis on their tegument, which was left by bruchid larvae ([Fig. 1B](#)); and class “EH” (*i.e.*

“emergence hole”) corresponded to infested seeds, presenting emergence holes of adults or “circular windows” on the seed coat, corresponding to the nymphal stage or adults remaining in the seed ([Fig. 1C–E](#)). Mean percentages of healthy seeds (HS), surface damage (SD), and emergence holes (EH) were computed from four replicates of 50 randomly selected seeds for each variety during the two year of study period.

Chemical composition analyses were performed (each in triplicates) on faba bean flour produced on a laboratory mill (M20 IKA Labor-technik, Staufen, Germany). Dry matter (DM, % $w.w^{-1}$) was determined according to the corresponding NREL method ([Sluiter et al., 2008a](#)). The nitrogen contents of the ground seeds were determined following the Dumas methodology using a Dumas Elementar Rapid N cube 161 15054 device (Donaustrasse, Germany). Crude protein contents (CP) were then calculated using the general factor 6.25 ([Ben Amira et al., 2017](#)), and were expressed as % (w/w) fresh matter (14% humidity). Protein productions (PP) were calculated as:

$$PP \text{ (kg proteins/ha)} = (FY * CP)/100$$

Ash contents (AC, % $w.w^{-1}$ fresh matter) were quantified after mineralization in a muffle furnace (Nabertherm controller b180) at $575^{\circ}C$ for 4 h ([Sluiter et al., 2008b](#)). Crude lipid contents (CL, % $w.w^{-1}$ of dry matter) were determined after the hydrolysis of milled seeds (60 min in boiling 4 M HCl, solid-liquid ratio of 1/5), in a Soxtherm equipment (Gerhard, Bonn, Germany), with the soxhlet method using petroleum ether (bp $40\text{--}60^{\circ}C$). AC and CL were expressed as % of dry matter.

2.3. Statistical analysis

Univariate and multivariate analyses were performed using Rstudio software® v.1.3.959 and Minitab® v 19.2, to identify the best varieties for food industry. Preliminary descriptions of all varietal characteristics were performed with analyzes of variance (one-way ANOVA, $\alpha = 0.05$) led on annual averages of CP, PP, AC, CL, HS, SD, EH. Varieties were then grouped with a Tukey *post-hoc* test for each growing year, independently. Then influences exerted by the factor years and variety (and their interaction) on seeds characteristics were assessed with a stepwise linear regression model ($\alpha = 0.1$) using *Year*, *Variety*, and their interaction as input factors, and using FY, CP, PP, HS, SD, and EH as response factors. Winter and spring varieties were analyzed in two distinct models because: (i) the development of winter and spring faba bean crops occurred under different climatic conditions, due to differences in sowing and harvesting dates ([Table 3](#)), and (ii) the factor *Variety* was naturally nested in the factor *Type*, which would mask the effect of variety in the regression model ([Nagakawa and Schielzeth, 2013](#)). The influence of the factor *Type* was assessed for each year separately with a one-way ANOVA test, and a Tukey *post hoc* test led on the annual average of each response factors (*i.e.*, average of FY, TSW, CP, PP, AC, HS, SD, and EH).

Multivariate analyses were performed to consider each quantitative variable combined (including agronomic, composition, and infestation characteristics of seeds) to rank varieties presenting the maximal PP/CP/HS and minimal CL/EH with biplot analyses. All scaled factors were

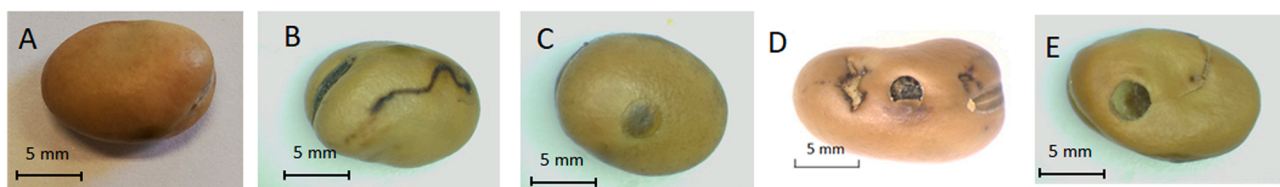


Fig. 1. Seed damage classes based on traces left by the post-embryonic development of *B. rufimanus*: A – non-infested seed; B – infested seed with traces of bruchid larvae; C – seed with a “circular window” on the coat corresponding to the nymphal stage or adult stage that stayed inside the seed; D – seed with adult that stayed in the seed; E – seed with emergence hole left by emerging adults (© A. Segers).

linearly combined, and Principal Component Analysis (PCA) were performed using the packages “FactoMineR” and “Factoextra” from Rstudio to summarize and visualize all the information of the 14 tested varieties (Kassambara and Mundt, 2020; Husson et al., 2020). Principal components (PC) were retained according to their eigenvalue (> 1), total variance explained in the dataset ($> 70\%$) (Kaiser, 1961), and correlation with variables of interest. Biplot representation of individuals (*i.e.*, *Variety*Year*) was performed, and groups were established according to the factors *Type* and *Year*. Each spring and winter variety was separately ranked according to the Euclidean distance to the point that optimally correspond to required characteristics for the food industry (*i.e.*, the point that maximize PP, CP, and/or HS according to result obtained within our dataset).

3. Results

The 14 commercial faba bean varieties tested over two growing seasons (2018–2019 and 2019–2020) exhibited different agronomic traits, seed composition, and bruchid infestation. Distinct differences were detected between varieties and when comparing certain results over the two years (Table 1). Comparison of each studied traits considering average per growing season (*i.e.*, *Year * Type*) is presented in Appendix C in supplementary material.

Agronomic characteristics (*i.e.*, FY and TSW) declined from 2019 to 2020 across varieties. Only one variety (*cf.*, Fanfare) was more productive in 2020. The winter varieties performed best in 2019, included Axel, Bumble, Diva, Honey and Nebraska, which produced more than 5000 kg ha⁻¹. However, FY strongly decreased in 2020. The highest relative decline in FY between 2020 and 2019 was 53.2% and 44.8% for LG Cartouche and Honey, respectively. The worst performing varieties were Bobas in 2019 (3740 kg ha⁻¹) and LG cartouche in 2020 (2017 kg ha⁻¹). The greatest decline was observed for the best performing varieties during 2019; for instance, relative TSW decreased by 28.8%, 31.5% and 35.9% for Bumble, Honey and Bering, respectively. Fanfare and Irena had the best TSW stability during 2019 and 2020, with a slight relative increase of 0.2% and 3.1%, respectively.

Seeds composition characteristics (*i.e.*, CP, CL, and AC) varied between 2019 and 2020. CP ranged from 23.4% w w⁻¹ (*cf.*, Diva 2020) to 28.4% w w⁻¹ (*cf.*, Irena 2019) fresh weight. CPs were generally lower in 2020 (p-value = 0.000, difference = 1.02%, IC_{95%} = [0.47; 1.56]). CL ranged from 0.88% to 1.76% w w⁻¹ (mean: 1.36 ± 0.17%). LG Cartouche, Julia, and Fanfare had the lowest CL. Ash ranged from 2.19% to 3.55% w.w⁻¹ (mean 2.96 ± 0.26%). On average, CL was statistically higher in 2020 (1.43%) compared to 2019 (1.30%) (p-value = 0.002). CL was higher in winter varieties (1.41%) compared to spring varieties (1.28%) (p-value = 0.007). However, this difference was very low when compared with the variability of other factors.

PP ranged from 538.2 kg ha⁻¹ (*cf.*, LG Cartouche 2020) to 1418 kg ha⁻¹ (*cf.*, Honey 2019). As a consequence of variation in FY and CP, PP also noticeably declined between 2019 and 2020. The most impacted varieties were LG Cartouche, Honey, and Bumble with relative decreases of 56.0%, 48.5% and 47.0%, respectively. These results were attributed to the major impact of FY and minor differences in CP across years.

Seed infestation characteristics (*i.e.*, HS, SD, and EH) strongly varied among and between varieties over the two years, with no clear correlations to seed composition. The mean proportion of healthy seeds (HS) ranged from 12% (*cf.*, Honey 2020) to 81% (*cf.*, Julia 2020). Surface damage (SD) ranged from 4.5% (*cf.*, Julia 2019) to 25.5% (*cf.*, Tundra 2020). For each variety, surface damage (SD) was inferior to emergence holes (EH), with major inter-annual variation being observed. SD increased in most varieties during 2020. Emergence holes (EH) ranged from 11.0% (*cf.*, Julia 2020) to 61.0% (*cf.*, Axel 2020). Overall, all varieties of faba bean had more healthy seeds (HS) in 2019 (61.3 ± 14.0%) compared to 2020 (53.8 ± 23.9%). Spring varieties had more healthy seeds (65.6 ± 8.7% in 2019 and 73.8 ± 7.3% in 2020) compared to

winter varieties in both years (58.9 ± 15.8% in 2019 and 42.7 ± 22.7% in 2020).

3.1. Environmental influence on seed characteristics

When comparing the two years of study, quantitative differences were detected, so environment was assessed with pest pressure and climatic conditions (Table 2). Bruchid populations were monitored for up to 15 weeks (from early April to mid-July) in both growing seasons. In 2019, infestation started four weeks after flowering, and lasted six weeks (from week 23 to week 28). Overall, 308 adults were caught in generalized winter parcels (GWP) and generalized spring parcels (GSP). Flowering periods in GWP and GSP occurred respectively from week 19 to 25 and week 22 to 26. In 2020, 288 adults were caught from week 19–30 in GWP and GSP, which bloomed from week 16 to 23 and week 22 to 26. Bruchids were present in crops for longer in 2020 than 2019, with the flowering period lasting 8 and 11 weeks, respectively.

Cumulative daily temperature and precipitation of all the cropping periods are presented in Table 3. Cumulated temperature was similar in both years throughout the entire cropping period of spring varieties while winter varieties were grown under more cumulated temperatures in 2019 rather than 2020. Cumulated precipitation noticeably differed between 2019 and 2020, especially for spring varieties. Precipitations was below the average for the area in 2019,² and was even lower in 2020, declining by 47.3% and 16.9% during the cropping period of spring and winter varieties, respectively.

Environmental observations (Bruchids population + climatic condition) indicated that pest pressure (*i.e.*, bruchid population size) was generally constant between the two years, with longer pest presence in 2020 compared to 2019. Additional extreme climatic conditions of drought occurred during the sensitive phases of crop production in 2020 (*i.e.*, transient phase between flower and seed production). Spring varieties were besides affected by the occurrence of *Uromyces fabae* (Pers.) de Bary, 1879 during pods forming period in 2020.

Multivariate linear modeling (stepwise regression analyses) was separately performed for spring and winter varieties to identify the respective influence of input factors *Year*, *Variety*, and their respective interactions on response factors FY, CP, PP, HS, SD and EH (Table 4). The interaction factor was not determined as significant in any studied model.

For winter varieties, *Year* (84.61%) contributed more compared to *Variety* (7.86%) in the FY model (R²adj = 0.90). The CP model was less explanatory (R²adj = 0.78), with *Variety* contributing strongly (47.24% compared to 36.35% for *Year*). This model had the highest error contribution (16.42%). *Year* variance (88.50%) contributed the most to the PP model, and had the lowest error contribution (5.77%). This last model was strongly impacted by FY data, which explained their high similarity.

Variety was the highest contributing factor to infestation factors (44.86% and 55.58% for HS and EH, respectively). Annual differences exhibited significant, but lower, contributions to models based on the evaluated parameters and related interactions. No model could be produced for SD, as the inadequacy of the model was significant (p-value = 0.013).

Models based on the spring varieties were of lower quality compared to the winter varieties. The highest R²adj was 0.73 (CP model), in which *Variety* contributed the most (64.57%), as obtained for the winter varieties. FY and PP models (R²adj of 0.32 and 0.34, respectively) differed to those of the winter, as *Year* was the sole contributing factor (< 37.7%), leading to a high error contribution (> 62.3%). The infestation-related models for spring varieties were of poorer quality. The highest contributions were related to *Variety* (31.2%), but this contribution was lower compared to that for winter varieties. The contributions of *Year* was

² <https://www.meteo.be/fr/belgique>.

Table 1

Field yield (kg ha⁻¹, FY), thousand seeds weight (g, TSW), and mean ± standard deviation of seed protein content (% w⁻¹, CP), protein production (kg ha⁻¹, PP), seed ash content (% w w⁻¹, AC), seed fatty matter content (% w w⁻¹, CL), mean healthy seeds (%), infested seeds presenting surface damage (%), and infested seeds with emergence holes (%). Per column, numbers with different letters are statistically different (p-value < 0.05).

Year	Variety	Type	FY	TSW	CP	PP	AC	CL	HS	SD	EH
2019	Augusta	W	4652	527	26.5 ± 0.5 ^c	1234.5 ± 22.3 ^{de}	2.8 ± 0.0 ^{bc}	1.3 ± 0.1 ^{bc}	68.0 ± 14.79 ^{ab}	5.5 ± 3.8 ^a	26 ± 13.0 ^{abc}
	Axel	W	5533	500	25.6 ± 0.4 ^c	1414.8 ± 20.7^a	3.0 ± 0.1 ^{ab}	1.4 ± 0 ^{ab}	42 ± 13.95 ^b	15.5 ± 11.4 ^a	42 ± 10.2 ^{ab}
	Bering	W	4571	593	26.8 ± 0.2 ^{bc}	1223.2 ± 8.1 ^{de}	3.1 ± 0.0^a	1.3 ± 0.0 ^{bc}	52.5 ± 15.44 ^{ab}	7.5 ± 5.5 ^a	39 ± 13.5 ^{abc}
	Bumble	W	5264	626	25.8 ± 0.6 ^c	1356.4 ± 29.6 ^{ab}	2.7 ± 0.0 ^c	1.2 ± 0.1 ^{bc}	56.5 ± 18.79 ^{ab}	14 ± 4.3 ^a	28 ± 19.3 ^{abc}
	Diva	W	5250	478	25.7 ± 0.3 ^c	1348.7 ± 17.4 ^{ab}	3.0 ± 0.1 ^{ab}	1.3 ± 0.0 ^b	70 ± 2.83^a	13.5 ± 6.6 ^a	16.5 ± 4.4 ^c
	Honey	W	5535	613	25.6 ± 0.2 ^c	1418.3 ± 13.1^a	2.3 ± 0.1 ^d	1.0 ± 0.2 ^c	60 ± 9.93 ^{ab}	5.5 ± 3.0 ^a	33.5 ± 9 ^{abc}
	Irena	W	4731	454	28.4 ± 0.7^a	1341.9 ± 35.4 ^{bc}	3.1 ± 0.0^a	1.4 ± 0.0 ^{ab}	43 ± 5.77 ^b	9.5 ± 6.4 ^a	46.5 ± 8.2 ^a
	Nebraska	W	5103	447	23.5 ± 0.7 ^d	1200.1 ± 37.8 ^e	3.0 ± 0.1 ^{ab}	1.6 ± 0.1^a	60.5 ± 7 ^{ab}	14.5 ± 8.0 ^a	25 ± 11.0 ^{abc}
	Tundra	W	4816	520	26.5 ± 0.6 ^c	1277.5 ± 29.4 ^{cd}	3.0 ± 0.1 ^{ab}	1.2 ± 0.1 ^{bc}	78 ± 13.06^a	5 ± 1.2 ^a	16.5 ± 11.5 ^c
	Bobas	S	3740	452	25.8 ± 0.3 ^c	964.4 ± 9.8 ^g	2.9 ± 0.0 ^{abc}	1.3 ± 0.1 ^b	71.5 ± 9.57^a	6 ± 7.7 ^a	22 ± 3.7 ^{abc}
	Fanfare	S	4120	402	25.4 ± 0.6 ^c	1045.2 ± 26.6 ^f	2.9 ± 0.0 ^{bc}	1.2 ± 0.2 ^{bc}	61.5 ± 5.26 ^{ab}	6 ± 7.1 ^a	30 ± 2.8 ^{abc}
	Julia	S	3892	382	26.1 ± 0.4 ^c	1014.4 ± 14.1 ^{fg}	2.9 ± 0.1 ^{bc}	1.2 ± 0.0 ^{bc}	74 ± 2.31^a	4.5 ± 3.0 ^a	21 ± 3.8 ^{bc}
	LG cartouche	S	4314	469	28.3 ± 0.6 ^{ab}	1218.7 ± 25 ^{de}	3.0 ± 0.1 ^{ab}	1.0 ± 0.0 ^c	58 ± 3.27 ^{ab}	6.5 ± 6.6 ^a	34.5 ± 8.2 ^{abc}
	Tiffany	S	4101	401	25.3 ± 0.4 ^c	1037.1 ± 17.6 ^f	3.0 ± 0.1 ^{ab}	1.3 ± 0.0 ^{bc}	63 ± 10 ^{ab}	8.5 ± 6.4 ^a	28 ± 5.9 ^{abc}
	2020	Augusta	W	3455	384	25.5 ± 0.8 ^{bcd}	881.3 ± 26.1 ^d	2.7 ± 0.1 ^{fg}	1.2 ± 0.0^a	60.0 ± 8.49 ^{abc}	10.5 ± 6.61 ^{abc}
Axel		W	3703	428	25.5 ± 0.3 ^{bcd}	942.4 ± 12.3 ^{bc}	2.9 ± 0.1 ^{efg}	1.5 ± 0.1^a	19 ± 8.87 ^{de}	19.5 ± 7.19 ^{abc}	61 ± 3.46 ^a
Bering		W	3511	380	25.7 ± 0.6 ^{bcd}	897.5 ± 20.8 ^{cd}	2.9 ± 0.1 ^{efg}	1.5 ± 0.0^a	41.5 ± 13.4 ^{bcd}	20 ± 4.32 ^{abc}	38 ± 12 ^{bc}
Bumble		W	3049	446	23.6 ± 0.6 ^g	718.8 ± 17.1 ^g	2.8 ± 0.0 ^{fg}	1.5 ± 0.1^a	36 ± 7.12 ^{cde}	24 ± 1.633 ^a	40 ± 7.66 ^{bc}
Diva		W	3576	334	23.4 ± 0.6 ^g	835.5 ± 22.4 ^e	2.9 ± 0.0 ^{efg}	1.5 ± 0.1^a	70 ± 8.16^a	5.5 ± 7.19 ^c	24.5 ± 5 ^{cd}
Honey		W	3058	420	23.9 ± 0.6 ^{fg}	729.8 ± 18.5 ^g	2.8 ± 0.0 ^{fg}	1.5 ± 0.1^a	12 ± 9.93 ^e	23.5 ± 9.15 ^{ab}	65 ± 4.76 ^a
Irena		W	3552	468	27.0 ± 0.2^a	960.1 ± 7.7 ^b	3.1 ± 0.1 ^{cd}	1.1 ± 0.0^a	37 ± 18.8 ^{cde}	11 ± 7.75 ^{abc}	52 ± 12.96 ^{ab}
Nebraska		W	3776	377	24.2 ± 0.3 ^{defg}	916.2 ± 10.2 ^{bcd}	2.9 ± 0.0 ^{defg}	1.5 ± 0.1^a	71.5 ± 5^a	5.5 ± 3.79 ^c	22.5 ± 4.12 ^{cd}
Tundra		W	3724	402	23.9 ± 0.2 ^{fg}	889.5 ± 6.6 ^d	3.1 ± 0.0 ^{cde}	1.5 ± 0.1^a	37 ± 20.8 ^{cde}	25.5 ± 11.82 ^a	37.5 ± 14.08 ^{bc}
Bobas		S	3517	410	25.2 ± 0.3 ^{cdef}	886.3 ± 11.6 ^d	3.3 ± 0.0 ^{bc}	1.5 ± 0.0 ^{bc}	72.5 ± 3.79 ^a	6 ± 2.83 ^c	21.5 ± 4.12 ^{cd}
Fanfare		S	4420	403	25.8 ± 0.2 ^{abc}	1141.4 ± 9.02^a	2.7 ± 0.1 ^g	1.3 ± 0.1^a	75.5 ± 1^a	7 ± 3.83 ^c	17.5 ± 4.43 ^d
Julia		S	2679	338	26.6 ± 0.5 ^{ab}	713.3 ± 12.8 ^g	3.5 ± 0.0^a	1.3 ± 0.0^a	81 ± 5.29^a	8 ± 6.93 ^{bc}	11 ± 9.59 ^d
LG cartouche		S	2017	415	26.7 ± 0.3 ^{ab}	538.2 ± 11.18 ^h	3.4 ± 0.0 ^{ab}	1.3 ± 0.1 ^{ab}	64.5 ± 6.81 ^{ab}	6.5 ± 3 ^c	29 ± 4.76 ^{cd}
Tiffany		S	3267	338	24.1 ± 0.3 ^{efg}	786.4 ± 8.8 ^f	3.5 ± 0.1^a	1.5 ± 0.0^a	75.5 ± 7.55^a	5.5 ± 3 ^d	17.5 ± 6.61 ^d

Table 2

Climatic condition and faba bean phenology associated with bruchid population dynamics. Climatic conditions were quantified from the average temperature (Tavg, °C) and cumulated precipitation (Pcum, mm) per week. Bruchid populations were quantified from the number of individuals caught in generalized winter parcels (GWP) and generalized spring parcels (GSP) in parallel to faba bean phenology (F = flowering, P = Pods setting).

Week no.	2019				2020					
	Tavg (°C)	Pcum (mm)	Phenology GWP	Phenology GSP	Bruchus spp. (GWP + GSP)	Tavg (°C)	Pcum (mm)	Phenology GWP	Phenology GSP	Bruchus spp. (GWP + GSP)
16	13.2 ± 3.16	0.1				10.39 ± 3.06	4.8	F		
17	12.43 ± 3.53	7.7				12.6 ± 1.94	0	F		
18	8.24 ± 2.35	19.6				11.14 ± 1.69	11.2	F		
19	9.37 ± 1.91	17.2	F			13.2 ± 2.76	2.3	F + P		1 (1 + 0)
20	11.34 ± 1.49	19.6	F			8.93 ± 1.84	0	F + P		3 (3 + 0)
21	13.57 ± 1.81	26.8	F + P			16.06 ± 2.29	1.8	F + P		2 (2 + 0)
22	16.17 ± 3.96	8.7	F + P	F		16.1 ± 0.59	0	F + P	F	78 (78 + 0)
23	14.96 ± 2.25	26.1	F + P	F	15 (15 + 0)	14.63 ± 3.75	21.3	F + P	F	5 (5 + 0)
24	15.29 ± 1.40	33.7	F + P	F + P	113 (87 + 26)	15.14 ± 2.73	9.7	P	F + P	6 (6 + 0)
25	18.26 ± 2.45	10.3	F + P	F + P	97 (57 + 40)	17.14 ± 1.05	30	P	F + P	29 (24 + 5)
26	21.8 ± 2.75	0	P	F + P	53 (21 + 32)	20.24 ± 2.62	7	P	F + P	28 (20 + 8)
27	16.87 ± 1.53	0.4	P	P	28 (2 + 26)	16.69 ± 0.92	15.5	P	P	58 (26 + 32)
28	15.99 ± 2.13	3.7	P	P	2 (0 + 2)	15.73 ± 1.76	10	P	P	61 (5 + 56)
29	17.59 ± 2.36	0.4		P		16.89 ± 1.70	10		P	12 (0 + 12)
30	23.61 ± 4.80	22.2		P		17.29 ± 1.69	12.9		P	5 (0 + 5)
tot	228.69	196.5			308 (182 + 126)	222.17	136.5			288 (176 + 112)

Table 3

Climatic conditions from sowing to harvesting of spring and winter faba bean varieties during the two years of the study (cumulated daily average temperature, Tcum; cumulated daily precipitation, Pcum).

	Winter varieties				Spring varieties			
	Sowing date	Harvesting date	Tcum (°C)	Pcum (mm)	Sowing date	Harvesting date	Tcum (°C)	Pcum (mm)
2018–2019	08/11/2018	12/08/2019	2703.9	551.4	28/03/2019	22/08/2019	2179.7	289.9
2019–2020	21/11/2019	30/07/2020	2566.0	458.3	09/04/2020	20/08/2020	2183.5	152.6

higher for spring varieties, accounting for 20.7% of variance. As with the winter varieties, no SD model could be produced. The lower quality of the spring varieties models may be explained, at least in part, by the

lower number of studied varieties in this group (5 compared to 9 winter varieties).

Overall, these models indicated that CP and seed infestation

Table 4
Regression analysis modeling the contributions of the factors Year, Variety (and their interactions) on FY, CP, PP, HS, SD, and EH. NS = not selected by the model.

	FY		CP		PP		HS		SD		EH	
	p-value	Contribution (%)	p-value	Contribution (%)	p-value	Contribution (%)	p-value	Contribution (%)	p-value	Contribution (%)	p-value	Contribution (%)
Winter												
Year	< 0.001	84.61	< 0.001	36.35	< 0.001	88.50	< 0.001	14.92	NS	NS	0.002	8.54
Variety	0.005	7.86	< 0.001	47.24	0.006	5.77	< 0.001	44.86	NS	NS	< 0.001	55.58
n	18		54		54		72		72		72	
Error (%)	7.54		16.42		5.73		40.22		NS		35.89	
Adjusted R ²	0.9004		0.7831		0.9243		0.5073		NS		0.5604	
Spring												
Year	0.005	35.00	< 0.001	15.17	0.003	37.68	0.012	20.65	NS	NS	0.013	20.68
Variety	NS	NS	0.009	64.57	NS	NS	0.022	31.20	NS	NS	0.022	31.21
n	10		30		30		40		40		40	
Error (%)	65.00		20.26		62.32		48.14		NS		48.20	
Adjusted R ²	0.3158		0.7299		0.3440		0.4091		NS		0.4085	

parameters were more impacted by *Variety*, whereas FY and PP were more related to environmental conditions. Differences were also observed between spring and winter types in terms of factors implicated in the models, their individual contributions to the models, and so model quality.

3.2. Grouping and ranking of varieties for the food industry

Three first principal components (PC) computed from the linear combination of scaled factors explained more than 80% of total variance combined (Fig. 2 - a) with respective PC1, PC2 and PC3 explained variances of 40.5%, 27.9%, and 15.1%. The contribution of each factor to all computed PCs is presented in the [Supplementary material \(Appendix E in Supplementary material\)](#). The two first PCs (i.e., Dim1 and Dim2) presented a biplot representation of each pair (i.e., *Variety * Year*), with PC1 being strongly correlated with PP, FY, and TSW, and PC2 being strongly correlated with HS and EH (Fig. 2 - b). These two axes clearly separated the varieties with respect to protein production and bruchid infestation rate (i.e., lower right region of Fig. 2 - c containing optimal varieties). Other biplots for PC1–PC3 and PC2–PC3 did not present any additional explanatory information.

Groupings of each cultural cycle (cf., *Type × Year*) are presented in Fig. 3. The influence of *Year* and *Type* on seed characteristics was visualized, extending stepwise regression modeling. The differentiation winter and spring varieties according to the year of study are more pronounced along PC1 illustrating the general decrease of the productivity-related factors (i.e., FY and PP). In comparison, differentiation of years of study according the type of faba beans was more pronounced along PC2, showing that winter varieties were more impacted by bruchids compared to spring varieties. This result supported that winter varieties could achieve higher FY compared to spring varieties.

Based on these groups, separated means comparisons (on the factor *Type*) were performed (Table 5). Winter varieties had higher FY in 2019 (difference = 1017 kg ha⁻¹, IC95% = 814; 1220) compared to spring varieties, but differences were minimal in 2020. The major difference in FY between spring and winter varieties led to a major difference in PP in 2020 (256.8 kg ha⁻¹, IC95% = 201.6; 312.0). In the same year, spring varieties had higher CP (difference = 0.958%, IC95% = 1.730; 0.186), highlighting the strong influence of annual variation in FY on PP.

The optimal varieties for the food industry (i.e., producing high and stable amounts of protein, and low bruchid infestation) were located in the lower right region of the biplot (Fig. 2 - c). The point that maximized these factors (named “optimal point”) corresponded to biplot coordinates (5; -3). Each spring and winter variety was ranked according to mean Euclidean distance computed from this point in the two years (Fig. 4). Varietal Euclidean distances ranged from 4.93 ± 0.65 (cf., Fanfare) to 6.99 ± 2.16 (cf., Axel). While no statistical differences were found, varieties were ranked according to the characteristics of interest for food industries. The most stable varieties over the two years had the lowest standard deviations, as observed for Fanfare.

Individually, some varieties demonstrated high performance. For example, in 2019, the varieties Honey had the highest FY/PP, while this cultivar was the worst performing one during 2020. Mean Euclidean distances may hide such extreme results, which are more expressed by important standard deviations. Equally, Tundra 2019 seems to be the closer sample to the hypothetical ideal point (5; -3). However, its overall quality was diminished when taken into account the performance of this variety in 2020. These highlights once more the level of annual differences demonstrated in previous analysis and underlines the need to consider overall performances (including bruchids infestation and PP) on several years of test.

4. Discussion

It is important to promote indigenous leguminous crops with high PPs to support European cropping systems; however, biotic and abiotic

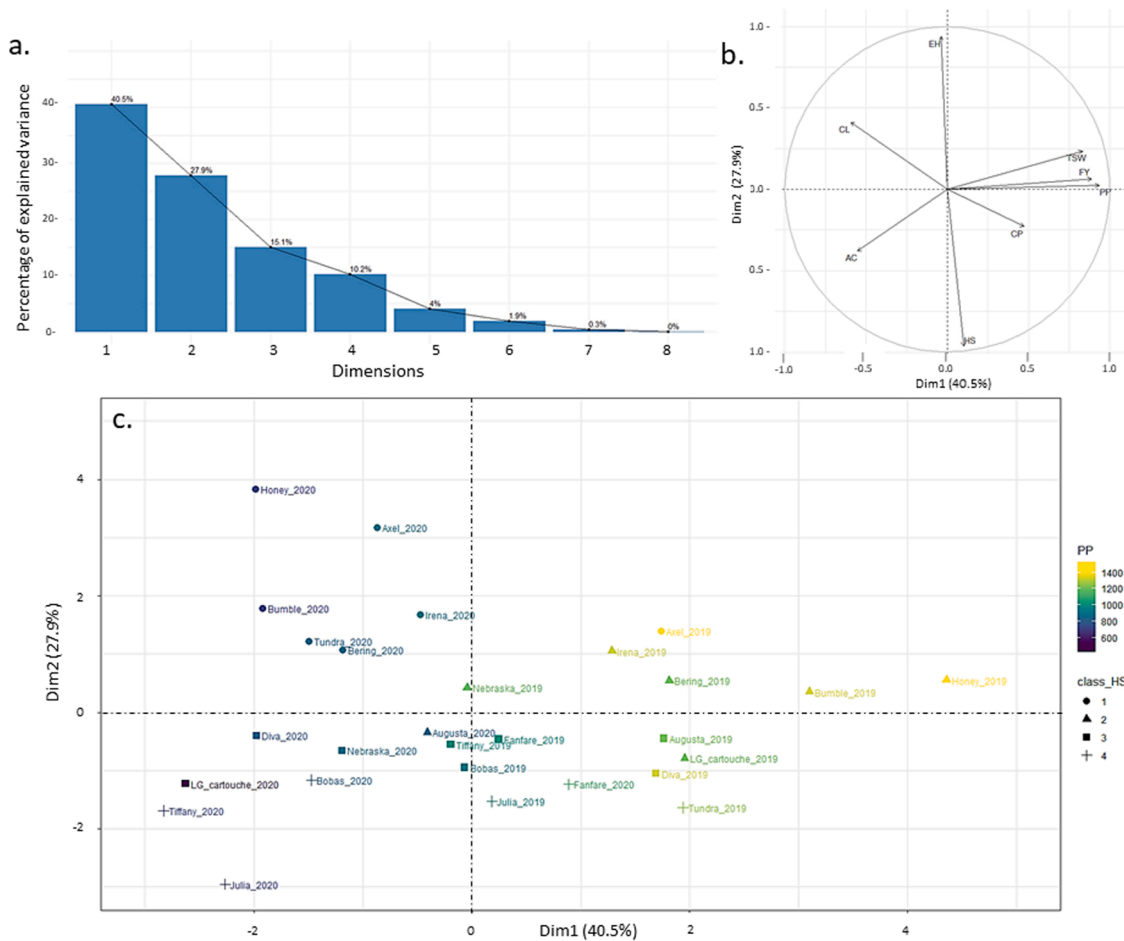


Fig. 2. Principal component analysis (PCA) results: a. Scree plot indicating the percentage of explained variance with each PCs; b. Correlation circle indicating the contribution of each factor with PC1 and PC2; c. Biplot representation of individuals according to Dim 1. (PC1) and Dim. 2 (PC2). Groupings were made according to quartiles of HS (1 to fourth quartile) and protein production (kg ha^{-1}).

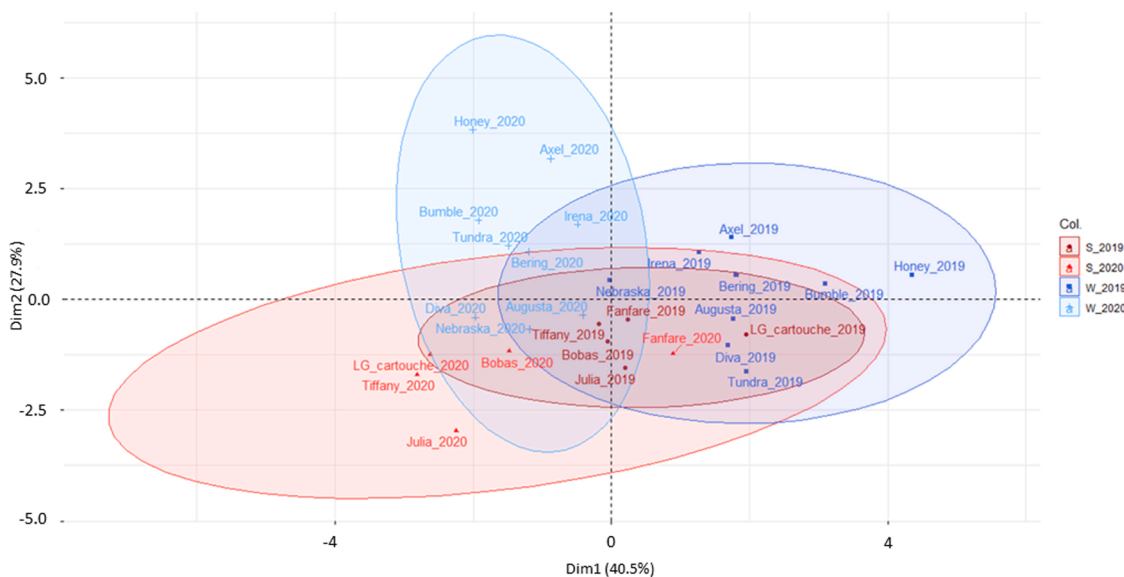


Fig. 3. Individual groups according to the year of study and type of faba bean (W = winter varieties; S = spring varieties).

factors cause variable crop quality and quantity in food industry production. Here, we screened the quality (*i.e.*, bruchids resistance) and quantity (*i.e.*, protein production) of 14 commercial faba bean cultivars

(including five spring varieties and nine winter varieties) over two years with distinct climatic conditions. Multivariate analyses showed that meteorological conditions (*Year*) strongly influenced FY and PP,

Table 5Annual one-way ANOVA p-value comparing the significance of *Type* on CP, FY, and PP. ° = p-value < 0.1, * = p-value < 0.5, *** = p-value < 0.001.

	PC (% w/w)	FY (kg ha ⁻¹)	PP (kg ha ⁻¹)	HS (%)	SD (%)	EH (%)
2018–2019	0.785	< 0.001***	< 0.001***	0.088	0.044*	0.355
2019–2020	0.016*	0.081°	0.271	< 0.001***	< 0.001***	< 0.001***

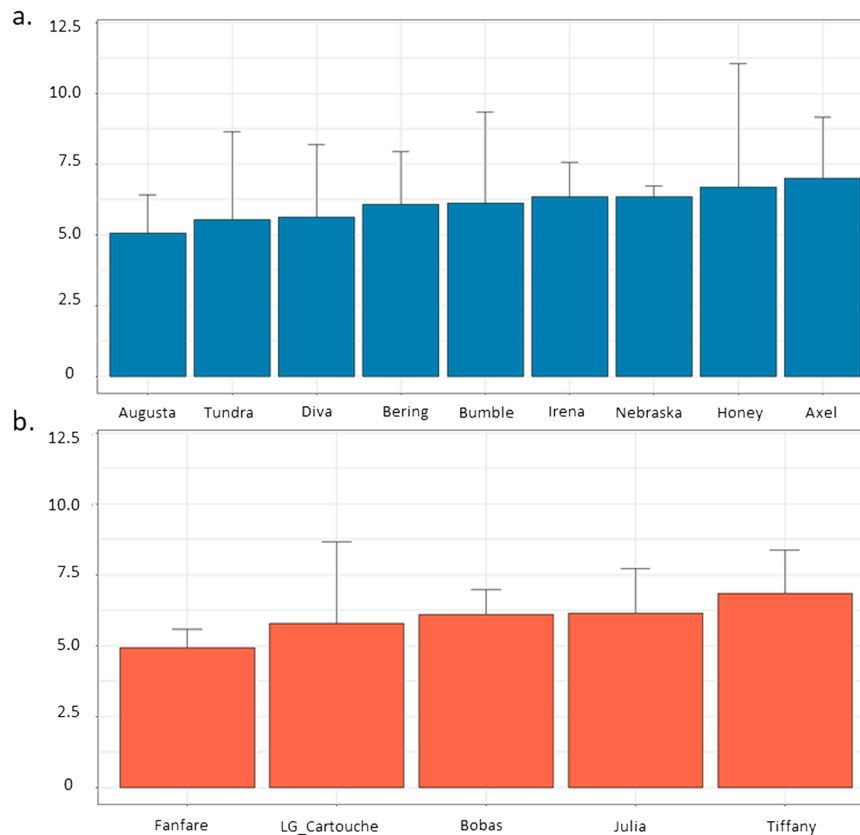


Fig. 4. Varietal ranking according to mean Euclidean distances computed between each individual (*i.e.*, *Variety*Year*) and the optimal (*i.e.*, coordinates [5; - 3]) in the biplot space. (a) Winter varieties. (b) Spring varieties.

whereas *Variety* strongly influenced CP and infestation rates. Biplot analysis discriminated (though not significantly) the most promising cultivars with respect to seeds quality and quantity. Our results provide preliminary information on field infestations by *Bruchus spp.* in the Walloon region (Belgium), and a methodological baseline is proposed for the characterization of varieties based on productivity, bruchid resistance and seeds composition.

4.1. *Bruchus spp.* infestation in relation to faba bean variety

Bruchid infestation of faba bean crops has been described for several countries, along with assessments of resistance mechanisms in different varieties (Boughdad, 1994; Szafirowska, 2012; Medjdoub-Bensaad et al., 2015; Seidenglanz and Huñady, 2016; Carrillo-Perdomo et al., 2019; Pölitiz and Reike, 2019). Bruchid populations occur in regions where faba beans are harvested as dry seeds, allowing the completion of the life cycle and rise in pest populations during the following growing season (Ward, 2018). Adults overwinter in wooded sites, and start colonizing fields when temperatures reach 15 °C, before or during the crop flowering period. This pattern was observed in Belgium, in an area presenting high infestation rates of *Bruchus spp.* (Table 2). Field infestation started when crops were flowering and mean temperatures were about 15 °C. The bruchid population peaked at week 24 (*cf.*, year 2019) and week 22 (*cf.*, year 2020), supporting observations made in neighboring

countries, like Germany (2018) where infestation peaked in week 25 (Pölitiz and Reike, 2019).

Pest pressure (*i.e.*, total amount of individuals recorded during monitoring sessions) was stronger in 2019 (308 individuals) compared to 2020 (288 individuals), with bruchids remaining in crops longer during 2020 compared to 2019 (12 weeks vs. 7 weeks respectively), leading to greater subsequent damage to harvested seeds in 2020. This phenomenon could be explained by the longer flowering period observed in 2020, which provided food supplies and extended the egg laying period, as suggested by Seidenglanz and Huñady (2016). Pest pressure was stronger on winter varieties during both years, resulting in greater subsequent damage to seeds. This phenomenon was also reported by Seidenglanz and Huñady (2016), who suggested that earlier flowering is a stronger driver when early and late flowering varieties are grown together. Early flowering varieties attract females earlier and oviposition lasts longer, as food supply is extended by late flowering varieties, leading to greater subsequent damage. This hypothesis can now be confirmed by the monitoring of pest populations proving that bruchids populations remained longer and in greater numbers on winter varieties. However, such differences of infestation could not be observed among varieties from winter or spring type, *i.e.*, no clear relation could be established concerning the precocity-duration of flowering (Appendix D in supplementary material) and the subsequent damages observed.

Although spring varieties were less impacted by bruchids (lower

infestation) in our study, these varieties did not necessarily produce seeds of better quality and quantity for the food industry. In our field trials, winter and spring varieties were grown side by side, with the delayed phenology of spring varieties impacting earlier flowering winter varieties. However, when spring and winter varieties are grown in distinct geographical locations, winter varieties flower earlier during the cooler and rainier periods. Thus reducing the risk of bruchid infestation, which is favored by high temperatures and sunny climate (Szafrrowska, 2012; Simmen, 2020). Winter varieties tending to have a early and short flowering period, are more recommendable compared to spring varieties.

Damage clearly varied across the 14 varieties. During both years, the most resistant commercial cultivars (with > 70% of non-infested seeds) were Julia, Bobas, and Diva. In contrast, Axel, Honey, and Irena had < 40% of non-infested seeds. Regression models showed that the most important factor influencing variation was *Variety* (44.86% for winter varieties and 31.20% for spring varieties), followed by *Year* (14.92% for winter varieties and 20.65% for spring varieties). This result supports recent studies showing that the infestation rates of cultivars are driven by different potential resistance mechanisms, such as antibiosis and antixenosis, which develop at different stages of the pest life cycle. The attractiveness of plants to adult bruchids may vary depending on odor profiles for feeding and oviposition (Ferry et al., 2004; Carrillo-Perdomo et al., 2019). The composition of epicuticular wax on faba plant pods might also influence the acceptance of oviposition substrate by gravid females. Zhao et al. (2019) showed that the profiles of epicuticular wax on *V. faba* pods vary with cultivars. Furthermore, the fatty acid profile of epicuticular wax impacted the oviposition preference of other species of bruchids (i.e., *Callosobruchus maculatus* (Fabricius 1775)). Higher relative quantities of oleic acid tend to reduce oviposition (Parr et al., 1998). Plants might also have resistance mechanisms to penetration by hatching larvae, such as physical barriers (e.g., trichome of pods) and the production of biochemicals, like lectins, polyphenols, proteinase inhibitors, and alpha amylases inhibitors (Mitchell et al., 2016; Mishra et al., 2018). Doss et al. (2000) provided evidence of plant induced defenses against *B. pisorum* by the neoplasmic growth of pod tissues caused by lipid compounds (named “bruchins”), which prevented larvae from penetrating pods. The present study explored post-embryonic resistance mechanisms by analyzing seed damage, rather elucidating the mechanisms that influence the oviposition of adults. Our results did not provide insights on resistance mechanisms of faba to the development of larvae. This is because larval mortality (SD) was considered less important than the emergence of adults (EH). SD also strongly varied, preventing us from drawing any statistical conclusions. These results support the observations of Seidenglanz and Huñady (2016), who found that polyphenol content (i.e., tannins, vicine, and convicine content) did not affect the mortality of *B. rufimanus*. However, Lattanzio et al. (2005) showed that *C. maculatus* mortality was linked to the polyphenol content, while Carrillo-Perdomo et al. (2019) identified an accession of *V. faba* (Quasar) that had superior SD to EH. Thus, future studies should focus on identifying the presence of biochemicals other than tannins, vicine, and convicine to elucidate resistance mechanisms in *V. faba* cultivars. Differences to HS among the 14 tested cultivars in the current study should be based on oviposition preferences rather than detrimental biochemicals for larval development.

4.2. Field yield and protein production

The FY (mass of grain harvested per hectare) of faba beans varies considerably in the world, ranging from 2.3 to 6.8 t ha⁻¹. Maximal theoretical yield was estimated as high as 6.56 t ha⁻¹ or 7.7 t ha⁻¹, respectively under Mediterranean conditions with irrigation or temperate conditions (Mínguez and Rubiales, 2021). In Belgium, mean yields of 4 t ha⁻¹ were obtained for *V. faba* between 2014 and 2019. These yields increased after 2017, with maximal yields of 4.9 t ha⁻¹ in 2019. Faba yields in Belgium are part of the most performing ones

compared to other countries in Europe and globally, which have yields of 3 t ha⁻¹ and 2 t ha⁻¹, respectively (FAOSTAT, 2021). The current study obtained highly variable FY (3.8–5.5 t ha⁻¹), but remained consistent with mean yields in Belgium, despite both years having lower precipitation than usual (798 mm and 731 mm in each year, respectively (IRM, 2021). Axel, Bumble, Diva, Honey, and Nebraska had the highest FY. However, slight changes to climatic conditions in the two years resulted in lower FY. Fanfare had the highest PP stability under water limited environments, and should be promoted for use due to its tolerance to low precipitation.

Stepwise regression analyses (Table 5) showed that *Year* (summarizing meteorological changes) strongly affected FY and PP, particularly for winter varieties. This phenomenon might be attributed to the flowering and grain filling physiology of faba beans being strongly influenced by environmental conditions. The flowering and grain filling stages (i.e., key growing stages influencing yield) relies on several environmental and intra-plant factors (Patrick and Stoddard, 2010). On average, just 24.0% of produced ovules will develop mature seeds (Rowlands, 1960). This lack of flowers retention is caused by inefficient fertilization and pollination, which occur when flowers are 6-days old only (Stoddard, 1986; Stoddard and Bond, 1987). Flower retention is highly sensitive to transient stresses, such as drought or high temperatures, which make flowers abort (Khan et al., 2010). Intra-plant competition between reproductive structures and vegetative/r-reproductive structures for assimilate availability also hinders the retention (Jaquiere and Keller, 1980). Consequently, climatic conditions and transient stresses during the fertilization of flowers and subsequent seed formation strongly affect yield. In the current study, pollinator activity was consistent in both years (unpublished field observations), whereas climatic conditions (particularly rainfall) distinctly differed at the onset of flowering, with dryer conditions occurring in 2020 compared to 2019. During 2019, winter and spring faba beans received 36.8 mm and 34.8 mm rainfall, respectively, during the first two weeks of flowering before pod formation. In 2020, rainfall did not exceed 4.8 mm during the flowering period of winter faba bean crops, which probably contributed to the loss of the first flowers that were produced (i.e., pod formation was delayed by three weeks after flowering). In comparison, the spring varieties received 21.3 mm rainfall during the second week of flowering. This suggests that drought was more impacting on FY than temperatures as temperatures were quite similar. Early flowering varieties would therefore be preferable as it would delay the sensitive phase of flower fertilization and seed formations with risk of drought in Belgium and should contribute to the field yield (Duc, 1997; Link et al., 2010; Korsvold, 2020). Besides, cool temperatures of the early growing season in Belgium (and other North-Western European countries) will slightly affect FYs of faba beans as it is considered as part of the “cool-season species” (Mínguez and Rubiales, 2021) on one hand, and it should delay the pod formation period with the period of bruchids presence on the other hand.

The favorable precipitation conditions (1078 mm in 2002, a typical year) found in Belgium during the flowering and grain filling stages promotes the cultivation of faba with high PPs (FAOSTAT, 2021). The frequency and intensity of extreme events (such as heat waves) are predicted to increase during spring-summer in future years in Belgium. Winter precipitation is also projected to rise over the 21st century, whereas summer precipitation is projected to decline (Hoyaux et al., 2010). Thus, it would be necessary to adapt existing faba bean varieties or develop new ones that correspond to changing climatic conditions in Belgium and Europe, in general, to maintain optimal production. The genes involved in the early initiation of flowering (genes *E*), response to vernalization (gene *HR*), and late flowering (gene *LF*) have already been identified for the pea, but not yet faba beans. Thus, these genes need to be identified to allow breeders to develop cultivars that are better adapted to stressful environments (i.e., with early and short duration flowering periods) (Patrick and Stoddard, 2010).

The wide variety of faba bean cultivars available on the market in

many countries makes difficult the comparison of results across studies, as no single cultivar has been defined as a general control over the years. Only a few varieties that were evaluated here have also been assessed in previous papers, such as “Fanfare.” Compared to the current study, this variety had higher yields (5 t ha^{-1}) with lower variability in Denmark and Finland (Skovbjerg et al., 2020). The PP recorded in our study during 2019 was higher compared to previous studies, whereas the PP recorded in 2020 was comparable, and even higher (Stoltz et al., 2013; Micek et al., 2015; Reckling et al., 2018; Barlóg et al., 2019). Even though the varieties that performed well in 2020 did not reach the level of production obtained in 2019, they still matched or exceeded the PPs listed in the literature over past decades. Higher PP was only obtained in a study conducted in Serbia, demonstrating that this parameter can be enhanced in specific locations (Mihailović et al., 2010; Mínguez and Rubiales, 2021).

4.3. Seed composition

CP ranged from 23.4% to 28.4% (w w^{-1}) of fresh seed weight (*cf.*, 14.0% HR), supporting the findings of Sharan et al. (2021). Regression analysis confirmed that year had a low impact on CP ($1\% \text{ w w}^{-1}$ difference in seeds between 2019 and 2020; Table 4), contributing to 36.35% and 15.17% variability in winter and spring varieties, respectively. In contrast, Variety strongly impacted seed composition (61.79% of variability in CP), reaffirming the need for research to optimize varieties. As protein function is minimally impacted by changes of varieties (Singhal et al., 2016), new varieties should be developed that enhance CP, in parallel to improving yield and resistance to infestation. The CP of winter and spring varieties differed. The CP of winter varieties was 24.3% higher compared to that of spring varieties (Table 5). Thus, winter varieties should be developed in Belgium and similar temperate countries, due to their lower sensitivity to drought and higher or equivalent field yields compared to spring-sown varieties (Neu-gschwandtner et al., 2015, 2019).

To improve existing varieties, it is necessary to develop new genetic resources by more sequencing (Khazaei et al., 2020). This is highlighted by the interesting results of the 14 varieties investigated in the present study; yet, only some have been referenced in the literature, and no genetic information is available. Various studies have sought to enhance the CP of faba bean genetically. Proteins found in the cotyledons are mainly non-enzymatically active, and serve as nutrients for the future embryo. Storage proteins belong to multiple families, including globulins (major family) and albumins. Globulins are separated in two groups with different functions, depending on their sedimentation coefficients: legumins-types (11S) and viciline-types (7S) (Warsame et al., 2018). The legumin/vicilin ratio affects certain properties of faba bean when developing end-products for consumers (Singhal et al., 2016). Higher legumin content is preferred to improve protein function; thus, their accumulation was investigated. Legumins are stored at the end of protein storage in seeds, after vicilin. Therefore, the legumin/vicilin ratio is initially impacted by pedoclimatic conditions. However, different genes are involved in producing these two types of proteins. Improved genotypes can be used to enhance legumin storage in the protein bodies of the cotyledon. The nutritional value of faba bean can be enhanced by modifying genes and increasing the repetition of coding for different protein types and amino acid profiles. Increasing sulfur-containing amino acids and tryptophan content could increase the nutritional value of the faba beans seeds (Singh et al., 2012; Warsame et al., 2018). Studies have begun to map the faba bean genome, and to link genes to phenotypes; however, further research is needed to obtain a complete understanding (Cooper et al., 2017). Databases must be constructed to gather genetic information on different faba bean varieties, which could then be used to guide genetic research to enhance FY, CP, and *Bruchus* resistance.

In our study, most varieties had equivalent ash content, except for Bumble, Augusta, Fanfare, and Honey, which had lower ash content.

The overall obtained ash content was in the lower range of that presented in published literature (Gasim et al., 2015), indicating that mineral intake during ingestion by humans would be low. Legumes usually have high mineral content, even though their bioavailability is not always favorable. The presence of anti-nutritional factors (such as phytic acid) in faba bean lowers the availability of minerals for assimilation in the digestive tract (Zhang et al., 2020). CLs were very low and did not vary enough to be used as a discriminating factor between varieties or types. The very low lipid content meant that it would not present any issues when faba bean seeds are transformed to processed food or feed.

4.4. Biplot analysis

The strong influence of Year on FY in the regression analysis was the consequence of extreme climatic conditions encountered during the second year of study. No correlation was observed between infestation factors and FY (data not shown). This phenomenon might be attributed to (i) the climatic requirements of pests for development and reproduction being fulfilled in both years of study; (ii) pest pressure being similar in 2019 and 2020, and (iii) quantitatively low of seed weight caused by bruchids (from 5.0% to 10.0% of dry seed weight). This weak quantitative loss was due to the development of endophytic pests occurring in forming seeds, and so did not impact the physiology and yield of host plants (Boughdad and Lauge, 1995; Shearman et al., 2005; Titouhi et al., 2015; Roubinet, 2016; Chapelin-Viscardi et al., 2019). Such losses became insignificant when considering variation in FY caused by extreme climatic conditions. Unlike previous studies, we obtained no correlation between FY and CP (Skovbjerg et al., 2020). Separate analysis for spring and winter varieties did not reveal any partial correlation, with an FY-CP correlation of 0.17 and 0.37, respectively.

PCA (Fig. 2) and individual biplots (*i.e.*, Variety*Year) on PC1 and PC2 provided information on the contributing characteristics (Figs. 2 and 3). For instance, infestation characteristics (HS and EH) were independent of productivity characteristics, and were strongly correlated with PC1 and PC2 (Fig. 2. B). Moreover, these first two PCs present eigenvalues > 1 (Appendix E in Supplementary material), meaning they account for more variance than accounted by each individual variable from standardized data. The two first components were retained because the third computed PC (which was also correlated with productivity characteristics) could not provide a more efficient and precise separation of individuals. Despite the quality of this representation, a limitation of this study relies in the gathering of all observation by their annual means for each variety which may induce a loss of information. However, this still indicates global trends considering all seeds characteristics, and this allow a better visualization of the influence exerted by the years of study (*cf.*, the climate that occurred during growing seasons) and the type of varieties. Future studies should consider the same number of replicates in order to represent all individual observations in the biplot space, which would allow a better characterization of the varietal stabilities in different environment.

These biplots were particularly useful while grouping varieties according to year of study and the type of faba bean, visually demonstrating the greater productivity and infestation of winter faba beans and influence of climate on all tested varieties. This approach allowed us to visualize stable varieties in relation to environmental influences, and to classify varieties based on the productivity and quality of seeds. The ranking of varieties based on Euclidean distances led to more nuanced results than by considering seed infestation and protein production separately. Best performing varieties seemed to be Fanfare, Augusta and Tundra, instead of Julia, Bobas and Diva according to bruchid resistances, or Axel, Irena and Fanfare according to protein productivities. The variability of Euclidean distances is essential in the characterization of varietal stabilities which are not expressed by the averages. This was typically the case of variety Honey that presented best performing PP in

2019 and worst performing PP in 2020. In this study, we highlight yield stability in water limited environment (*cf.*, climatic condition of year 2020) by computing distances from an optimal point based on the variation of our dataset which does not suggest quantitative prerequisites. Each experiment performed under these conditions will have its own point of interest depending on the data set itself.

Fanfare was slightly better ranked than Nebraska (difference of 0.14 in mean Euclidean distances) but Fanfare was the most stable variety with a lower standard deviation. As water limitation is considered to be the most important environmental constraint to crop productivity, and is expected to increase in both frequency and intensity (Khan et al., 2010), Fanfare was the best adapted cultivar for the quality, quantity, and stability of seed production in Belgium (out of the 14 tested cultivars). However, infestation rates still exceeded export quality standards for human consumption. Despite this, valorization opportunities may arise for fractioning processes for food industries where visual quality standards are expected to be less constraining (Traore and Simmen 2021). Following clarification on the safety concerns of seed infestation before seed fractioning, the method of ranking varieties represents a promising direction for future characterizations.

5. Conclusions

This study investigated the ability of 14 faba bean varieties to meet the standards required for use in the food industry. Deciding factors included bruchid infestation rate, seed composition, field yield, and protein production. To study year-dependent variations, the 14 varieties were studied over 2 years and were mainly impacted by climatic conditions variation. Variation in infestation rate was strongly associated with faba bean cultivar. The best performing cultivars were Julia, Bobas, and Diva. Damage analyses excluded post embryonic mechanisms, and suggest probable oviposition preferences of bruchids on the 14 tested cultivars. Because damage differed between winter and spring varieties, future cultivars should have early and short flowering periods. Our results on field yield and protein production confirmed *V. faba* as a promising crop for local protein production. However, environmental conditions exerted a much stronger influence compared to the effect of variety. Winter varieties were generally more productive, and should be preferentially selected over spring varieties. However, only five spring varieties were compared to nine winter ones. More varieties need to be compared to confirm our findings. Different levels of stability in yield were observed under water limited environments; thus, stable varieties, such as Fanfare, should be preferentially selected over high yield performing but less stable varieties, such as Honey. This study proposed a new tool for ranking varieties by considering multiple characteristics of interest for food industries. PCA showed little to no correlation between infestation rate and productivity parameters; thus, these characteristics could be improved separately. This knowledge is of great importance for breeders, as they could implement complementary improvements on both bruchid resistance and seed productivity, with no anticipated co-effects. This tool could be improved by adding complementary factors related to other pests and disease resistance, seed processability, protein quality (*e.g.*, amino acid profile, anti-nutritional factors content, digestibility), and protein functionality (*e.g.*, ability to form foam, emulsion, dough). This study also highlighted the importance of developing varieties that fit current and future European climatic conditions. Based on the presented results, future studies should focus on yield stability over years and the resistance of varieties to bruchids, with Fanfare being promising. However, the capacity for genetic improvement should be investigated for winter varieties, in terms of early flowering and seed filling stages, which enhance field yield (*cf.*, quantity), as well as bruchid resistances and protein composition (*cf.*, quality). Such advances could potentially supply cropping systems in Belgium and other countries in Europe with sustainable plant protein production.

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CRedit authorship contribution statement

AS, LD, CC, RCM and FF: Conceptualization; Methodology; Formal analysis. AS, LD: Writing – original draft. RCM, NJ, CC, CC, PM, JP, AR, CB and FF: Writing – review & editing; Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2021.107831](https://doi.org/10.1016/j.agee.2021.107831).

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