horticulturae

MDPI

Article

Epidemiology and Management of Bean Common Mosaic Virus (BCMV) in Traditional *Phaseolus vulgaris* L. Landraces within Protected Geographical Indications

Sonia Expósito-Goás, Lautaro Gabriel Pinacho-Lieti, Fernando Lago-Pena and Cristina Cabaleiro *

Department of Plant Production and Engineering Projects, EPSE, Campus Terra, University of Santiago de Compostela, E-27002 Lugo, Spain
* Correspondence: cristina.cabaleiro@usc.es; Tel.: +34-600-940-167

Abstract: Protected geographical indications (PGIs) share health problems related to plant propagation material. The PGI "Faba de Lourenzá" encompasses a 1660 km^2 area in northern Galicia, Spain, renowned for its "Faba Galaica" (FG) and Faba do marisco" (FM) bean cultivars. The lack of certified virus-free seeds poses a challenge. From 2019 to 2023, seeds from 60 lots were tested for BCMV. Plants from several plots were tested periodically to develop disease progress curves (DPCs). Control methods (plots out PGI zone, virus-free seedlings, rogueing, corn borders, and intercropping) were tested. Yields in five plots were used to assess BCMV's economic impact. Seed lots were 22.3% FG-infected and <5% FM-infected. The transmission rate of BCMV from infected FG plants to their seeds was 25.5 ± 5%, while for FM it was 12 ± 3%. FG yield losses were on average 31.6 ± 4.5%. Combining virus-free seedlings and infected plant removal in plots outside the PGI area proved effective at reducing infection rates; combining with intercropping resulted in the lowest incidence in an FG plot. Farmer training and off-site plot selection to produce healthy sowing beans are key to improving results.

Keywords: *Phaseolus*; PGI; ELISA; virus-free seeds; disease progress curves (DPCs); intercropping

Citation: Expósito-Goás, S.; Pinacho-Lieti, L.G.; Lago-Pena, F.; Cabaleiro, C. Epidemiology and Management of Bean Common Mosaic Virus (BCMV) in Traditional *Phaseolus vulgaris* L. Landraces within Protected Geographical Indications. *Horticulturae* **2024**, *10*, 699. https://doi.org/10.3390/horticulturae10070699

Academic Editors: Graciela Dolores Ávila-Quezada and Irasema Vargas-Arispuro

Received: 10 May 2024
Revised: 19 June 2024
Accepted: 24 June 2024
Published: 2 July 2024

1. Introduction

The germplasm of the common beans, *Phaseolus vulgaris* L. (*Fabaceae* family), brought to Europe after the XV century from different areas of South America, has evolved and adapted to new environments. The occasional crosses and the strong selection for consumer preferences in terms of bean types played an important role in the evolution of new genetic variants of dry beans in Europe. New evolutions that were not present in the American centres of origin appeared in Europe: the new germplasm came from recombination between Mesoamerican ones and Andine genetic improvements, which were better adapted to the conditions of diverse new agroecosystems [1].

In Spain, the North and Northwest of the Iberian Peninsula is the area where greater genetic variability of legumes has been found [1] and where traditional farming systems such as smallholdings and self-consumption agriculture are still maintained. The characteristics of local cultivars grown in special locations are recognized by the EU as Protected Geographical Indications (PGIs). In the North of Galicia (Spain), the area of A Mariña (1660 km^2) (Figure 1) was recognized in 2008 as the PGI "Faba de Lourenzá" [2]. The products protected are dried beans, which are separated from the pod of the local variety known as "Faba Galaica" (FG) that belongs to the International commercial class (ICC) 'Favada' [3]; another traditional cultivar in the same area is "Faba do Marisco" (FM), a different cultivar (also known as "verdina") included in the ICC "flageolet" [4]. Researchers from the "Consejo Superior de Investigaciones Científicas" (CSIC) [3] developed a line of improvement PMB-0382 "Faba Galaica" from the local variety PHA-0917, now in the

Spanish register of commercial varieties. "Faba do Marisco" is also registered by CSIC (2020/0103) [4].

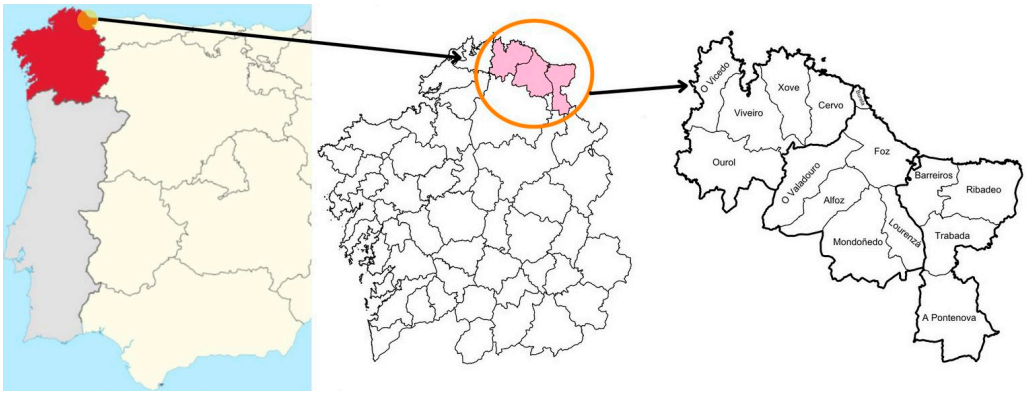

Figure 1. Geographical area included in the PGI "Faba de Lourenzá" in A Mariña, Galicia, Spain.

Cultivars within the PGIs have some qualitative characteristics that differentiate them from other beans and that make them highly demanded by consumers. FG is known for its exceptional culinary quality, due to its low proportion of skin (between 8 and 10%), its high-water absorption capacity (greater than 100%), and its behaviour when cooked, resulting, at the end of the process, in whole and complete grains in which the pastiness of the pulp stands out, free from lumps and barely differentiated from the skin. The plant has a climbing habit and indeterminate growth with long internodes [3]. FM seed is small (0.3–0.4 g), with a pale green colour thanks to the harvest conducted at a certain physiological stage; Its name in Galicia comes from the way it is cooked: a bean stew with shellfish ("marisco"). The plant is a low-stalk variety and has determined growth [4].

Beans can be infected with various pathogens transmitted through seeds, primarily viruses and bacteria. This complicates disease control in the field, especially for potyviruses, which can also be transmitted horizontally by aphids, pollen, or via mechanical means. Viruses transmission via seeds can occur through direct invasion of embryonic tissue, and/or indirectly by infecting pollen grains or ovules, which lead to an infected embryo after fertilization, resulting in an infected seed in a plant in which the virus is not detected [5]. Potyviruses are a major concern for *Phaseolus* spp. [6]. The most common are *bean yellow mosaic virus* (BYMV) and bean common mosaic, caused by two Potyvirus species: *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV). The difference between the two viruses is the phenotype generated in resistant cultivars. More than 200 aphid species, mainly in the Aphidinae subfamily (particularly the *Macrosiphum* and *Myzus* genera), can transmit potyviruses. In Spain, most potyviruses affecting beans have been identified [7–9]. Since potyviruses are transmitted non-persistently by aphids, epidemics can develop rapidly from even low seed-borne populations in areas where dry beans are the dominant crop. Utilizing aphicides to control non-persistently transmitted viruses like BCMV is generally ineffective [10].

The average seed transmission rate of BCMV and BCMNV from infected plants usually ranges from 15% to 35% [5,11,12] but can vary widely (3–95%) depending on the viral strain and bean variety [13]. This high seed transmission is a major cause of global BCMV epidemics, as it serves as the primary virus source in the field [14]. BCMV can survive for more than 30 years in seeds that keep their germination potential stored under suitable conditions [14].

In susceptible bean genotypes, BCMV infection induces a wide variety of symptoms, including mosaic, stunting, chlorosis, and leaf curling [15]. Mottling and malformation of the first true leaves indicate that the initial infection occurs through the seed [16]. The severity of these symptoms depends on various factors, including the viral strain, the bean cultivar, and the plant's age [17].

BCMV can seriously affect the common bean's yield or even cause total production loss [18]; however reported yield losses due to BCMV and BCMNV vary between 6 and 98% [6,16], depending on the cultivar, the time of infection [19], and the virus strain [20]. There are references to high losses due to potyvirus infections of aggressive breeds [19,21,22], but even mild or symptomless infections can decrease crop yield by 50% [22]. For many cultivars all over the world, progress has been made in combating BCMV through breeding bean varieties possessing the I gene, a dominant gene conferring resistance to most BCMV strains [6]. Potyvirus-resistant varieties have been the preferred control measure for extensive cultivation of green and dry beans because they are easier and more economical than trying to prevent transmission of potyviruses in the field [14,23,24]; however, for landraces in PGIs, this is not a viable option.

Several strategies can help ensure a healthy crop, including using seeds with low potyvirus levels and cultivating in highlands distant from major legume production areas. Other effective methods for preventing potyvirus transmission include removing infected or symptomatic plants (roughing), oil spraying [25], using straw or reflective mulches to reduce the attraction of vectors [26], using barrier crops, or intercropping [27]. These methods have been combined as an integrated strategy for effective protection against potyvirus in potato, cucurbit, maize, and bean crops [28–30].

Accurate and early diagnosis of BCMV and other potyviruses in *P. vulgaris* tissues is critical to be able to start with as low as possible levels of virus in the seed lots, to check when the epidemic reaches its exponential phase, and to predict the final level in the seeds. The potyvirus can spread easily, thus being able to detect it in asymptomatic plants is crucial. However, the most sensitive molecular methods are out of the question because they are too expensive and complex for routine testing of a high number of seedlings or plants in the field [24]. Some serological methods are very useful for quick and easy virus detection (Lateral flow tests, ImmunoStrips®) but again, their price makes their use impossible for high numbers of samples. Tissue printing assays have many advantages for epidemiological studies and for checking planting material [31]: samples are easy to obtain, deliver, and process; the use of commercial monoclonal and polyclonal antibodies at low concentrations is very efficient; nitrocellulose membranes or different kinds of paper are cheap support for assays; it uses common substances and standard buffers; the period of incubation and development of purple colour in positive samples is short; it is possible to check the tissue where a particular virus is detected; and it is possible to test groups of seedlings/petioles in a single print.

In a preliminary analysis of growers' FG seed lots in 2006 and 2014 in Lourenzá (A Mariña, Lugo, Spain), the prevalence of potyvirus was >95% [32]. Serological analysis indicated that BCMV was the most widespread potyvirus, and the presence of BCMNV was not detected. In addition to viruses, there could also be a high incidence of seeds infected with bacteria that cause rot during germination or plant damage when conditions are favourable, as was found in other local bean cultivars in the North of Spain [33].

Studying the epidemiology of potyvirus in these traditional cultivars, its seed transmission rate and the associated yield losses would undoubtedly help growers—in this and other similar PGIs—make informed decisions about the need for control measures and the advantages of coordination to do it properly. Obtaining seeds with the lowest possible levels of potyvirus could help delay the development of epidemics and reduce economic losses.

2. Materials and Methods

Phaseolus vulgaris seed lots: A total of 43 lots of Faba Galaica (FG) and 14 lots of Faba do Marisco (FM) bean seeds were obtained from various sources: the CSIC collections (Consejo Superior de Investigaciones Científicas), Cooperativa Terras da Mariña S.L., local growers within the PGI, and small shops selling bulk seeds from local farmers outside the PGI (Table 1).

Table 1. Identification of the lots of bean seeds from different groups that were analysed in this study.

Origin	Group	Year	#Lots	Identification [1]
CSIC	I	2014	1	C_G (1)
		2017	1	C_G (2)
		2018	1	C_G (3)
		2019	1	C_G (4)
		2021	1	C_G (5)
TERRAS DA MARIÑA S.COOP.GALEGA (LOURENZÁ)	II	2018	2	CTM_G (1–2)
		2019	6	CTM_G (3–8)
		2021	3	CTM_M (1–2.7)
		2022	4	CTM_M (3–6)
		2022	19	CTM_G (9–27)
ALFOZ & LOURENZÁ (out of cooperative)	III	2019	3	AL_G (1–3)
		2019	3	L_G (1–3)
		2019	1	LuC_G (1)
		2020	1	L_G (4)
		2021	1	L_G (5)
		2021	1	L_G (6)
		2021	1	LuC_G (2)
		<2021	2	LuC_M (1–2)
		2022	5	LuC_M (3–7)
Total # lots			57	

[1] C, CSIC (Consejo Superior de Investigacioes Científicas); G, faba Galaica; CTM, Cooperativa Terras da Mariña; M, faba do Marisco; AL, Alfóz; L, Lourenzá; LuC, Lugo, bulk sale in stores.

A total of 20–40 seeds were randomly selected from each seed lot and germinated in 14 cm diameter petri dishes at 25 °C in the dark. Seed development was monitored daily for signs of bacterial or fungal presence. Seed-borne viruses and bacteria were analysed using either root or leaf tissue from the same plants.

Fields: Tables 2 and 3 show details of the commercial and experimental plots sampled. While most plots were planted with cv. FG, several FM plots were included in the 2023 study. The plot code is as follows: first letter(s) for location (A, Alfoz; L, Lourenzá; M, Mondoñedo; SF, San Fiz; EI, plots in the USC (Campus Lugo), followed by the cultivar code (G, faba Galaica and M faba do Marisco). Next, letters indicate virus transmission control techniques (Mz, maize; B, border; I, Greenhouse). The last two numbers represent the year. Seeds were sown in trays, and all plants were tested before planting to ensure they were BCMV-free. In most cases, the experimental fields were planted with plantlets tested for BCMV originating from the same batch of seeds (CG_5).

Some plots were analysed only once, at the end of the growing season (LG_20, LG21, LM_23) while others were analysed on several dates to check the progress of the disease incidence and draw Disease Progress Curves (DPCs).

Harvest: in plots AG1_20, AG2_20, LG_22, LGBMz_23, and RGI_23, the plants that were sampled during the season were classified according to the date of virus detection. A total of 10–30 plants per class were harvested and the number of pods, the weight of pods before threshing, and the final dry weight of seeds were registered.

Virus transmission to seeds. Samples of seeds from the 7 fields where the incidence of BCMV had been estimated at harvest (Table 2) were germinated in seedbed trays and analysed (root, leaf, or both) to check the percentage of seeds with BCMV.

2020: From the AG1_20 and AG2_20 harvest, sixty seeds were analysed for BCMV and Ps·Ph.

2021: From the LG_21 harvest, the field where all plants tested positive for BCMV, two samples of 200 and 156 seeds were analysed.

2022: From the LG_22 harvest, four replicates of 10 seeds taken randomly from lots on each date of detection of infection were germinated in Petri dishes and analysed for BCMV,

Ps·Ph, and Xa·Ph in roots. Leaves of potted plantlets that were negative for BCMV in roots were tested again for BCMV.

2023: from the LGBMz_23 and RGI-23 harvest, more than 50 seeds taken randomly from the plants on each date of detection of the infection were germinated in pots and leaves analysed for BCMV. For FM, 10–40 seeds from infected plants from EIM_23 and SFM_23 were germinated separately and analysed for BCMV.

Table 2. Plots studied between 2020 and 2023 in several locations.

Plot	Year	cv	Location	Study			
				DPC [1]	Harvest	TTS [2]	One Test [3]
AG1_20	2020	FG	Alfoz	+	+	+	
AG2_20	2020	FG	Alfoz	+	+	+	
LG_20	2020	FG	Lourenzá	-	-	-	+
LG_21	2021	FG	Lourenzá	-	-	+	+
LG_22	2022	FG	Lourenzá	+	+	+	
LGBMz_23	2023	FG	Lourenzá	+	+	+	
MGI_22	2022	FG	Mondoñedo	-	-	-	
MGBMz_22	2022	FG	Mondoñedo	-	-	-	
RGI_23	2023	FG	Ribadeo	+	+	+	
EIG_22	2022	FG	Lugo	+	-	-	
EIM_23	2023	FM	Lugo	+	-	-	
SFM_23	2023	FM	Lugo	+	-	-	
SFGMz_23	2023	FG	Lugo	+	-	-	
LGMz1_23	2023	FG	Lourenzá	-	-	-	+
LGMz2_23	2023	FG	Lourenzá	-	-	-	+
LM_23	2023	FM	Lourenzá	-	-	-	+

[1] DPC: disease progress curve; [2] TTS, Transmission to seeds; [3] One test at the end of the season.

Table 3. Details on initial bean common mosaic virus incidence, sampling frequency, and number of plants analysed on each sampling date in the plots described in Table 2.

Plot	% BCMV Seedlings	Sampling	Frequency	Samples/Date
AG1_20	10%	Same plants [1]	monthly	107
AG2_20	10%	Same plants [1]	monthly	104
LG_20	nt [2] (>20%)	Random	at harvest	>500
LG_21	nt (>20%)	All plants in 2 lines	at harvest	200
LG_22	nt	Same plants [1]	10 days	200 (300)
LGBMz_23	0	Random & same	monthly	335
MGI_22	0	All plants	10 days	360
MGBMz_22	0	All plants	10 days	450
RGI_23	0	All plants	10 days	220
EIG_22	0	All plants	15 days	50
EIM_23	0	All plants	15–30 days	50
SFM_23	0	All plants	15–30 days	50
SFGMz_23	0	All plants	15–30 days	50
LGMz1_23	nt	Random	At harvest	75
LGMz2_23	nt	Random	At harvest	75
LM_23	nt	Random	At harvest	75

[1] after most plants were infected, a new batch of plants was tested; [2] nt: not tested (% based on average seed infection).

Virus and bacteria detection. All the analyses were conducted using direct immunoprinting DIP-ELISA (Enzyme-linked Immunosorbent Assay), following the basic protocol in [34,35], with some modifications/simplifications in sample printing, antibody dilutions (Table 4), and buffers.

Table 4. Optimized dilutions of commercial antibodies used in tissue printing ELISA.

Reactive	Company	Procedure	Dilution	Buffers
BCMV + AP	Loewe® Biochemical GmbH, Sauerlach, Germany	Direct	1:800	BCB
BCMV − Ab		Indirect	1:200	Carbonate buffer
Anty-rabbit + AP	Bio-Rad Laboratories, S.A., Madrid, Spain	Indirect	1:3000	BCB
Ps·ph + AP	Agdia Inc. Elkhart, IN, USA	Direct	1:500	BCB
Xa·ph + AP		Direct	1:100	BCB
Ps·ph − Ab		Indirect	1:100	Carbonate buffer
Xa·ph − Ab		Indirect	1:100	Carbonate buffer

Xa·ph: *Xanthomonas axonopodis* pv phaseoli; Ps·ph *Pseudomonas syringae/savastanoi* pv phaseolicola.

Several plant tissues were used for analysis: roots (collected a few days after germination), leaf petioles, or leaf blades. Roots and petioles were printed, and leaf blades were gently squashed with skewers onto nitrocellulose membranes (0.45 µm pore size) from Sartorius (Göttingen, Germany). The target pathogens were BCMV and the bacteria *Xanthomonas axonopodis* pv. phaseoli (Xa·ph) and *Pseudomonas syringae/savastanoi* pv. phaseolicola (Ps·ph).

After blocking the membranes with a 1.5–2% skim milk solution in distilled water for one hour, two distinct detection procedures were employed:

Direct Procedure: Membranes were incubated with alkaline phosphatase (AP)-conjugated antibodies specific to each pathogen (dilutions in Table 4) in conjugate buffer (BCB, Bioreba AG, Reinach, Swizerland; ref. 110140/-42). Following washes with saline buffer (8.5% NaCl + 0.05% Tween 20) three times for 3–5 min each, the membranes were incubated with a ready-to-use BCIP-NBT liquid substrate (Sigma-Aldrich, St. Louis, MO, USA, ref. B-1911) for colour development.

Indirect Procedure: This method added an extra incubation step. Membranes were first incubated with coating antibodies specific to each pathogen in carbonate buffer for 1.5 h or overnight at 4 °C. After washing, they were incubated with an anti-rabbit antibody conjugated with AP (BIO-RAD, ref. 1706518) for 30 min. Washes and colour development were performed as in the direct procedure.

A blue colour indicating a positive reaction typically developed within 15–30 min, but sometimes later. The reaction was stopped under running tap water once positive controls showed clear distinction.

Disease progress curves for BCMV epidemics: DPC were drawn as "days after first analysis" (DAFA) and BCMV incidence for the 9 plots indicated in Table 2. Analysis of the DPCs was conducted using the EPIMODEL V3.4 software program, which fits temporal disease progress data to five temporal population growth models that are commonly used in the analysis of plant disease epidemics: monomolecular, exponential, logistic, Gompertz, and linear models. This process involves examining disease progress and rate curves and then, based on the shapes of these curves, choosing one or more models that are likely to provide the best fit for the raw data. Transformed datasets are then graphed and evaluated based on coefficients of determination (R^2), mean square errors (MSE), and standard deviations of parameter estimates [36,37].

Statistical analysis: Comparison of BCMV levels in the seed lots (dependent variable) from each zone/origin/plot/date of infection was conducted using analysis of variance (ANOVA) according to a general linear model (GLM); data in percentage (in the range 0–30%) were transformed [$\sqrt{}$(X + 0.5)]. Comparisons of the data for yield/plant in each plot were done using one-way ANOVA with the date of virus detection as the independent variable and yield/plant as the dependent variable. Tukey's b test with $p \leq 0.05$ significance level was used to separate the means of different treatments after ANOVA. To compare data (in percentage) from different seed lots and transmission to seeds, χ^2 tests were performed.

3. Results

3.1. BCMV and Bacteria in Seed Lots

The prevalence of BCMV was >95% in the sampled lots of FG, with only 2 out of 43 seed lots being negative. The percentage of positive seeds in each lot was quite variable (0–70%) and on average (±se), the incidence was 22.3 ± 2.6%, which was not significantly different for lots in the three groups (F: 0.155; p: 0.86 > 0.05) (Figure 2). The germination of lots of FG <5 years old was almost as good as fresh (more than 90%) and bacterial rot was a rare event; older seeds kept quite good germination levels if stored at 4–5 °C.

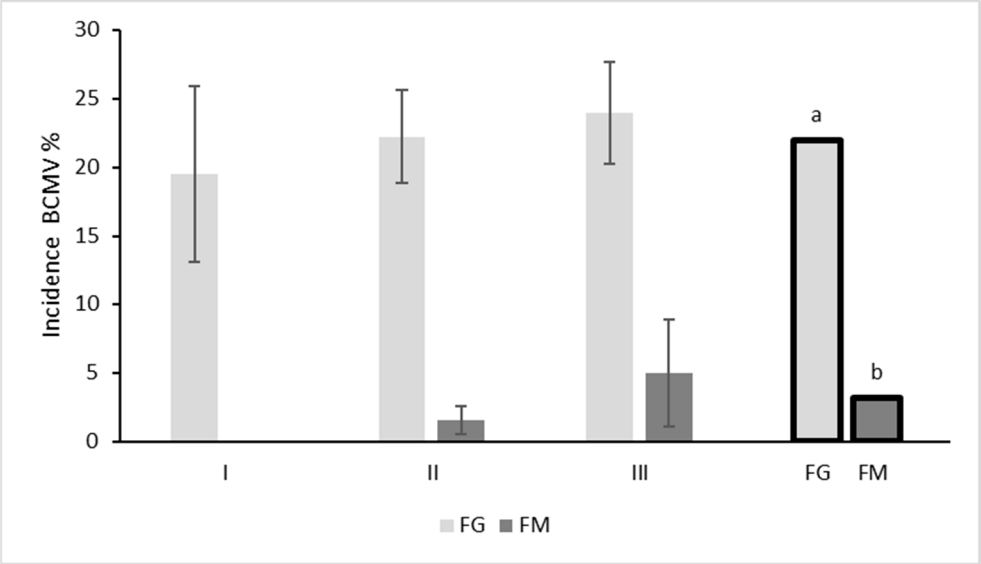

Figure 2. Average incidence of bean common mosaic virus (percentage ± se) in the lots with the three groups of Faba Galaica (FG) and two groups of Faba do Marisco (FM); (I, CSIC; II, Cooperative Terras da Mariña; III: PGI but out of Cooperative). Columns with black borders are the mean values of incidence for the two bean varieties, with different letters for significant differences after one-way ANOVA (F = 11.57; p = 0.01).

FM seed lots showed very poor or no germination when they were not from the last harvest. Most ungerminated seeds exhibited typical bacterial soft rot, with oozing observed. Additionally, both Ps·ph and Xa·ph were detected by tissue printing ELISA in these older seeds. BCMV was not detected in either the roots or leaves of six of the ten fresh FM seed lots. The average BCMV incidence in the remaining lots was less than 5% (Figure 2).

BCMV incidence in FG (22%) was significantly higher than in FM (3.2%) (Figure 2).

The prevalence of Ps·ph and Xa·ph in seed lots was 61.1% and 78.9%. However, the proportion of infected seeds within each lot with at least one positive seed was generally low. The average contamination rates were similar for both bacteria, with 9.2% for Xa·ph and 8.8% for Ps·ph. It is important to note that both these values had high standard deviations. Many seeds that tested positive for bacteria failed to complete germination, rotting shortly after radicle emergence. Interestingly, seeds infected with Ps·ph were frequently co-infected with Xa·ph.

3.2. Transmission of BCMV to Seeds

In plots AG1_20 and AG2_20, the seeds from harvest classified according to the date of detection of infection had BCMV levels of between 23 and 45%. In AG_1, there were significant differences between the only two dates of detection of BCMV in mother plants (χ^2 = 5.2 > 3.84, p < 0.05, df = 1). No significant differences (χ^2 = 4.3 < 7.81, p < 0.05, df = 3) or trends were observed in BCMV levels in AG_2 based on the date of detection of infection (Figure 3).

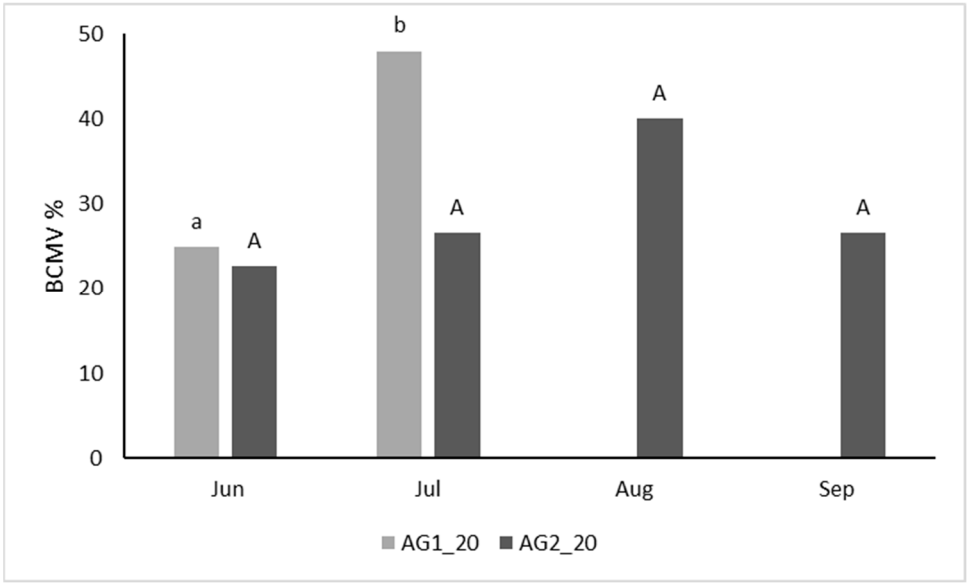

Figure 3. Percentage of transmission of bean common mosaic virus to bean seeds based on the date of detection of infection in mother bean plants in plots AG1_20 and AG2_20. Different letters indicate significant differences based on χ^2 tests for each plot.

Seeds from BCMV from the plot of FG tested at harvest in 2021 (LG_21) were 25.5 ± 5% infected. The transmission of BCMV to seeds in the harvest from LG_22 showed a slight trend towards a higher percentage of infection when transmission occurred at later dates (Figure 4), but the differences between dates were not significant based on χ^2 test (χ^2 = 12.54 < 14.06; p = 0.05; df: 7). The average transmission was 28.4 ± 3.2%.

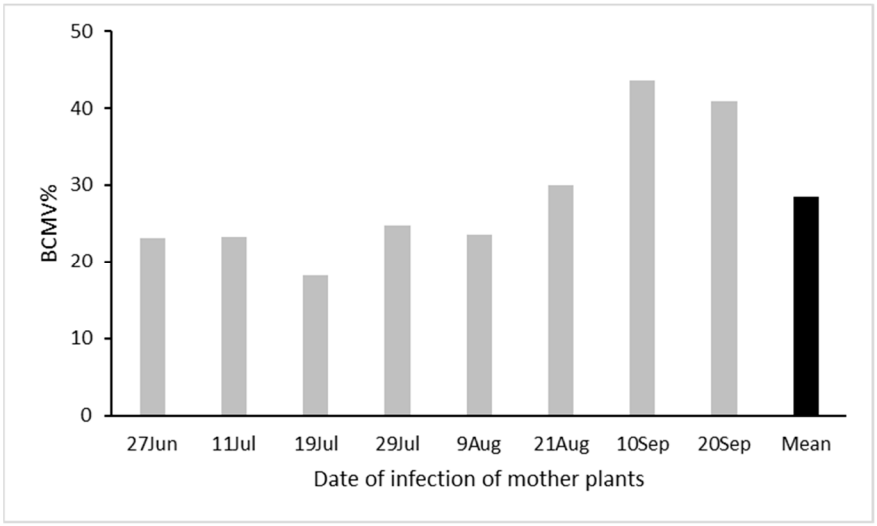

Figure 4. Percentage of transmission of bean common mosaic virus to seeds based on the date of detection of infection of bean plants in plot LG_22. There were no significant differences between dates of infection based on the χ^2 test.

In 2023, significant differences in seed transmission were observed between the two plots: LGBMz_23, surrounded by corn, and RGI_23, grown under protected cultivation (Figure 5) (F: 41.97, p = 0.003, df: 1). Seeds from LGBMz_23 had a higher proportion of infected seeds compared to those harvested from plants infected early in the season, but the chi-square test revealed no significant differences in infection rates among dates of detection of infection (χ^2 = 4.44 < 5.99, p = 0.05, df = 2). The average BCMV transmission rate in LGBMz_23 seeds was 26.1%. BCMV levels in seeds from greenhouse plants

were very low (2.5%), and no significant differences were detected across infection dates ($\chi^2 = 0.73 < 5.99$, $p = 0.05$, df = 2). Since all BCMV-positive plants were removed within the first month, there are no data from plants infected for longer periods in the greenhouse.

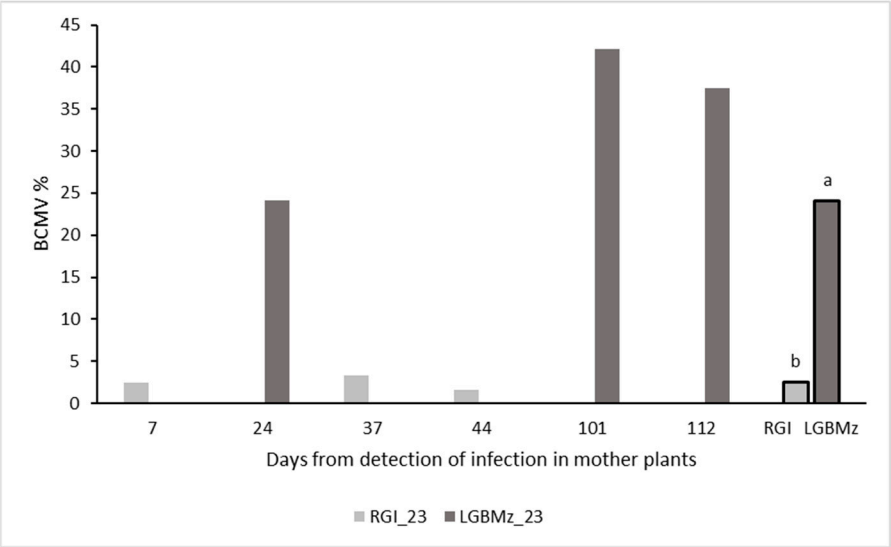

Figure 5. Percentage of transmission of bean common mosaic virus to bean seeds based on the elapsed time between the date of detection of infection of bean plants by BCMV and the harvest in LGBMz_23 and RGI_23. Columns with black borders are the mean incidence for the two 2023 plots; different letters indicate significant differences.

The prevalence of BCMV in seeds harvested from plants of FM in EIM_23 and SFM_23 at harvest was 76% (Figure 6). However, only seeds from two plants (one from each plot) had high levels (>40%) of BCMV detected in the radicle sample. The overall transmission of BCMV to seeds was 12.2 ± 3%. There were no significant differences between the two plots.

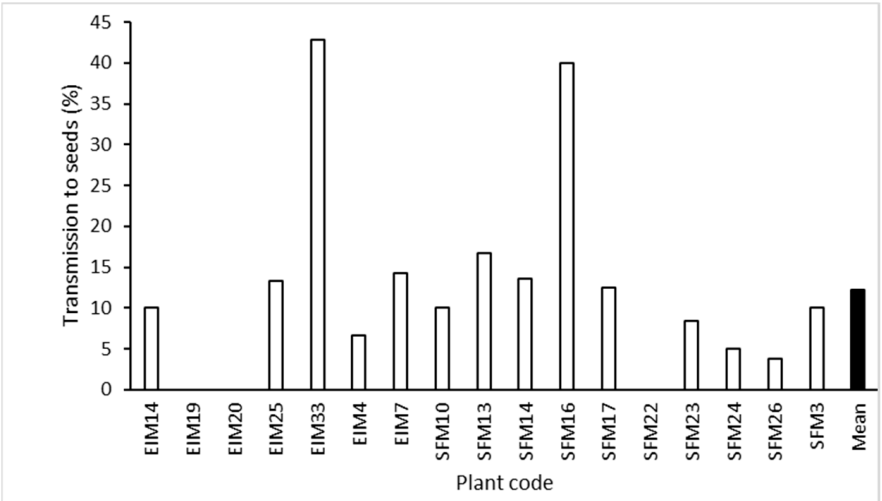

Figure 6. Percentage of transmission of bean common mosaic virus to bean seeds harvested from FM plants in EIM_23 and SFM_23; mean percentage of transmission in the two plots.

3.3. Harvest

Three out of five plots did not show significant differences in harvest weight based on the date of detection of infection. However, a trend towards higher yields was observed in plants infected at the end of the season and in plants testing negative for BCMV at harvest (Figure 7).

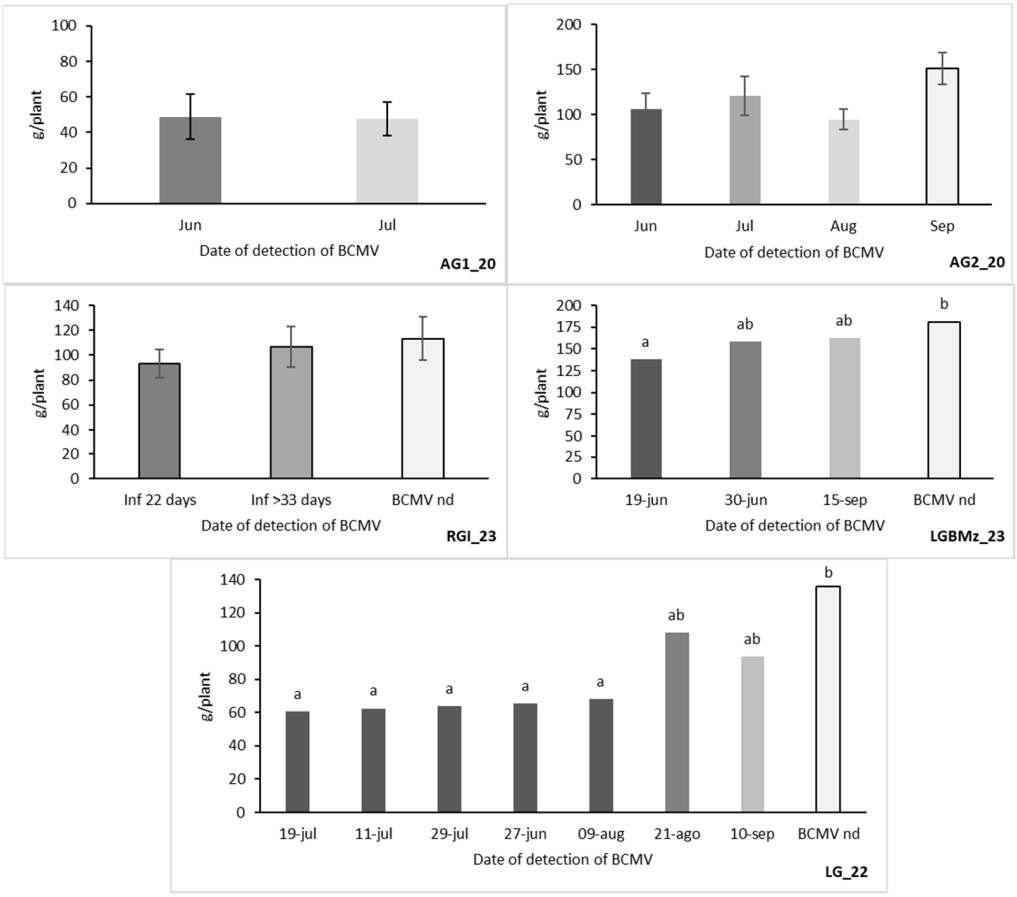

Figure 7. Harvest based on the date of detection of infection by bean common mosaic virus in bean plants in AG1_20, AG2_20, RGI_23, LGBmZ_23, and LG_22. The dry weights of seeds are shown in g/plant ±se when $p > 0.05$; the different letters represent significant differences based on the Tukey' b test, with $p < 0.05$.

In plots where significant differences were observed, they were typically between plants infected earliest and those infected later or not infected at all. The largest yield differences were observed in LG_22, reaching up to 50%, followed by LGBMz_23, with a maximum difference of 24%.

Similar trends were observed for fresh weight, number of pods per plant, and the percentage of seed weight relative to pod weight mirroring the pattern seen for dry seed weight.

3.4. Epidemiology and Control Strategies

As shown in Table 5 and Figure 8, the DPCs for BCMV incidence varied significantly across locations, years, and cultivars. FG consistently exhibited higher infection rates and final BCMV incidence compared to FM. In the absence of control measures, all FG plots (AG1_20, AG2_20, LG_21, LG_22) reached 100% BCMV incidence, often early in the season.

In 2020, two plots (AG1 and AG2) were planted with two lots of seeds with 10% BCMV. The initial infection rate was similar for both fields. However, by July, all sampled plants in AG1 were positive for BCMV, while those in AG2 reached 100% infection only at the final sampling (September). The rate of increase (slope of the linear regression line) was highest for AG1 throughout the study (Table 5). This rapid spread seems to be prevalent within the PGIs, as exemplified by LG_20; by harvest, nearly all plants in LG_20 were infected. To identify uninfected plants for harvest comparison, we had to analyse over 500 additional plants free of potyvirus symptoms, and most were positive for BCMV. Similarly, in 2021, LG_21 had 100% of plants infected at harvest and LG_22 displayed an intermediate spread

rate in 2022 (Table 4). However, similar to other plots, nearly all plants tested positive for BCMV at harvest.

Despite starting with virus-free seedlings, removing infected plants initially, and having no neighbouring bean crops, MGI_22 and MGBMz_22 experienced rapid disease spread (40% infection in MGI within a month and 75% infection in MGBMz within two months). This was likely due to poor weed management practices and late or no proper vine training. As a result, the grower abandoned these plots, and no further data collection was possible after July.

During the 2023 season, the DPCs showed promising results in managing BCMV infection using various approaches. In the two plots chosen randomly among those with traditional intercropping corn/bean as the only difference with respect to the standard monoculture, the final level of BCMV infection was under 60%. Despite initially removing infected plants and minimal aphid catches on the yellow sticky traps, the virus became established within the protected greenhouse (RGI_23) (Figure 8). However, by harvest time, only 45% of the plants tested positive for BCMV, indicating a reduction in infection rates over time. In the field plot LGBMz_23, the corn border strategy was initially ineffective in preventing transmission, likely due to delayed corn growth and poor weed management, but the rate of spread of the virus decreased later on and at harvest, it was 75%. As expected, all plots situated outside the PGI area yielded improved results. In SFGMz_23, the combination of techniques successfully delayed the epidemic, resulting in a low infection rate and 25.7% of infected plants at the end of a long season.

The three plots with FM had significantly lower rates of infection inside and outside the IGP area. Despite being sown with untested seeds and surrounded by FG fields that reached 100% infection by August, the 2023 Lourenzá plot (FM) showed a BCMV infection rate of 45% at that time. The two plots in Lugo planted with tested FM seeds had 25 and 35% infection at the end of the season, but considerably lower infection levels (9 and 20%) at the time this cultivar would be typically harvested (before maturity).

Table 5. Results of the epidemiological study. For bean common mosaic virus epidemics with enough data, values of R^2, slope, and SEEy are from the model that best fits the DPC.

Plot	LA [1]	Model	R^2	Slope	SEEy	% BCMV Expected	% BCMV Observed
AG1_20	bh	Linear	0.89	0.009	0.153	>100	100
AG2_20	bh	Linear	0.99	0.02	0.007	100	100
LG_20	bh					-	97
LG_21	bh					-	100
LG_22	bh	Linear	0.95	0.017	0.077	92	98
LGBMz_23	h	Monomolecular	0.82	0.012	0.28	80	75
MGI_22	35 d [2]			-		-	40
MGBMz_22	56 d [2]			-		-	75
RGI_23	h	Gompertz	0.74	0.02	0.750	40.5	45
EIG_22	h	Gompertz	0.80	0.04	0.62	55	60
EIM_23	h	Linear	0.92	0.003	0.037	24	25
SFGMz_23	bh	Gompertz	0.55	0.01	0.49	22	25.7
SFM_23	h	Gompertz	0.84	0.019	0.418	31	34.8
LGMz1_23	bh					-	52.6
LGMz2_23	bh					-	59.7
LM_23	h					-	45

[1] LA: last analysis; bh. before harvest; h, at harvest; ah, after harvest. [2] Monitoring stopped due to wrong management of the plot. SEE standard error of the estimate.

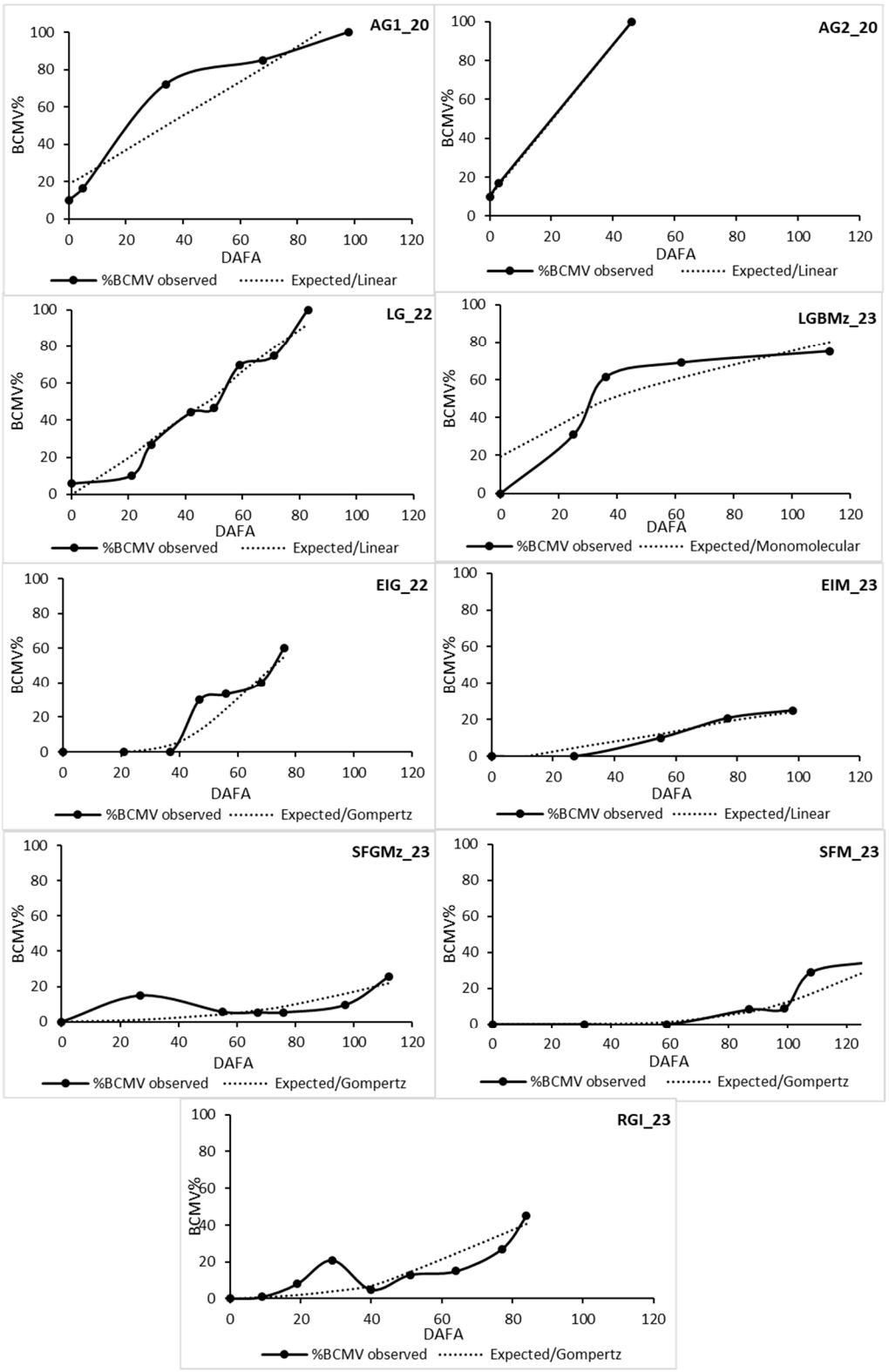

Figure 8. Disease progress curves for 9 of the bean common mosaic virus epidemics studied between 2020 and 2023. DAFA: days after first analysis of bean plants. The regression lines correspond to the expected values based on the epidemiological model that best fits the DPC values (Table 5).

4. Discussion

Dry beans are one of the most important crops in the world and BCMV is considered economically important throughout Africa, Europe, North America, and Latin America,

with infection levels that often reach 100% and estimated yield losses of 35–98% [16]. Our findings with Faba Galaica (FG) align with these general trends in BCMV prevalence and damage. For large-scale cultivation, various control strategies exist to minimize losses from seed-borne pathogens, ranging from certified seeds to resistant cultivars (CABI, 2023). In Protected Geographical Indications (PGIs), managing plant and seed health is critical for the long-term viability of traditional cultivars and farming practices. These elements define the unique characteristics (typicity) required for PGI certification. That makes PGIs interesting crop systems to study plant disease epidemics: they are limited to a certain area, use one or few cultivars in small fields with traditional growing systems, and usually get high prices in specific markets. For small PGIs like 'Faba de Lourenzá', the total seed requirement is low, and like in many other PGIs in the EU and bean crops in developing countries [38], farmers often save or exchange seeds from the previous year. In this context, we observed a very high prevalence of seed-borne pathogens, particularly viruses. Despite these findings and potential yield losses (Figure 7), growers often express minimal concern, likely because, similar to potato varieties infected with Potato Virus Y (PVY), plants infected during the season may not exhibit clear disease symptoms.

This study analysed thousands of samples between 2020 and 2024, a throughput impossible with other virus detection methods due to cost constraints. Tissue printing proved to be the most efficient technique for field screening and evaluating propagation material in our previous studies with grapevine and potatoes [29]. Tissue printing has been used since the 1990s for the detection of viruses, bacteria, and MLO in cereals, citrus, plums, and potatoes, and for polyphagous viruses such as cucumber mosaic virus (CMV) or tomato spotted wilt virus (TSWV) among many others [39]. Legume viruses were among the first viruses detected using this technique [34,39]. ELISA tests for bacteria, including tissue printing ELISA, have been approved as one of the European Plant Protection Organization (EPPO) Standards (2010–09) [40] but to our knowledge, it has not been reported for Xa·ph or Ps·ph in bean germinated seeds or leaves.

Analysis of FG seed lots from PGI fields and seeds from known BCMV-infected mother plants yielded similar average infection rates in the range of 20–30%. That seed infection rate translates to near-complete field infection by harvest, as observed in our samplings from Lourenzá (2020–2022) where no control measures were implemented. Cifuentes et al. [23] reported that sowing seeds with only 4% infection resulted in 100% plant infection at harvest. In our study, with a much higher seed infection rate (>20%), this level of virus prevalence will likely be reached well before the end of the season, as exemplified by plot AG2_20. The only exception to the high seed transmission rate observed in FG is RGI_23, which showed a very low value of 2.5%. This could be due to greenhouse conditions, but further confirmation is needed. There are a number of factors that influence seed transmission rates, including environmental conditions [41]. Greenhouse environments differ significantly from open fields in the region, potentially explaining the lower transmission rate observed in RGI_23. FM lower incidence in seed lots (<5%) corresponds to lower levels of transmission to seed (2.5%).

The BCMV levels observed in FG within the PGI Faba de Lourenzá are comparable to those reported for other Spanish PGIs [23,42]. However, Asturias reported a significantly lower incidence of potyviruses [43]. FM, cultivated alongside FG, exhibits a contrasting pattern. FM seed lots have lower infection rates, field plants typically test negative until later in the season, and seed transmission from infected plants is also lower compared to FG. This lower level of BCMV infection in FM might be attributed to two factors: its determinate growth habit leading to a shorter growing season, and the harvesting of green pods before full maturity and drying. The lower BCMV seed transmission in FM compared to FG translates to a reduced initial inoculum for aphids within FM plots. Additionally, late-season transmission from surrounding infected FG fields, via aphids, would likely occur after FM seed harvest. The two FM individual plants with over 40% infected seeds (Figure 6) were likely exceptions, possibly due to harvesting at a later stage when seeds were more mature.

Our findings revealed significant harvest weight differences, primarily between plants infected early in the season and those testing negative at harvest. This highlights the importance of planting seeds with minimal BCMV infection. Yield reductions in fields sown with 20–40% infected seeds might be more substantial than growers acknowledge. Studies in other Spanish PGIs report bean yield losses of 40–50% due to BCMV [42,43]. Though traditional varieties cultivated for decades may seem unaffected, the true impact of potyvirus infections is likely underestimated due to the absence of data on potential yields achievable with disease-free seeds. Local minor cultivars grown in small, often non-irrigated fields, exhibit high yield variability due to various factors. This makes it challenging for farmers to attribute yield losses specifically to viral diseases, especially when symptoms are mild or absent in plants infected later in the season [42]. Tolerance is assumed when symptoms are very mild or not shown at all in plants infected after the first month [42]. Current seed certification programs, effective in reducing seed-borne diseases, are often impractical for small regions cultivating local varieties due to limited economic viability. In these cases where seeds are reused and exchanged and vector control in the field is absent, the risk of reaching 100% infection at harvest and progressive plant degeneration is very high [20]. Moreover, although these traditional beans fetch a higher price in gourmet shops than common varieties (15–25 €/kg in 2023), their production costs are also higher due to being less intensively cultivated and requiring more manual labour compared to fully mechanized improved varieties. Therefore, minimizing virus-related losses becomes crucial for maintaining their profitability.

Our efforts to reduce virus transmission were sometimes hampered by grower decisions related to weed control, planting density, or vine training. In some cases (MGI_22, MGBMz_22, and early stages of LGBMz_23), this was due to inadequate weed and vine management just after planting. This is because weeds, including leguminous species (*Trifolium* spp.) that can harbour BCMV, attract aphids at the beginning of summer. Carazo and Romero [44] identified 15 additional reservoir species for BCMV beyond legumes. In Nigeria, virus incidence in weeds around the legume plots was 2.5% [45]. Studies have shown that mulching reduces early-season infection in seed potatoes [26].

Figure 8 showcases the diverse effects of location, cultivar, and control strategies on Disease Progress Curves (DPCs). While some models provide superior fits to the epidemic data, the slopes of these best-fit DPCs are also demonstrably influenced. Interestingly, natural epidemics were best modelled by linear functions with high R-squared values and low errors (Table 4). Conversely, controlled epidemics were primarily described by Gompertz models, albeit with a consistently lower goodness-of-fit. In most cases, the predicted final infection level aligned well with observed values (Table 5). This information could be valuable for pre-emptively determining crop destination based on anticipated infection severity. Early removal of infected plants most significantly impacts DPC trajectory and model fit.

The parallels between BCMV and Potato Virus Y (PVY) in seed potato production are evident. Consequently, established management strategies for potatoes can be effectively applied to control seed-borne viruses in beans and other crops [26,28,29,46,47]. This strategy combines using low-infection seeds, removing symptomatic young plants, and implementing intercropping. Maize as an intercrop offers distinct advantages over border crops, particularly in small fields [26].

For indeterminate growth cultivars like FG, intercropping with maize is a simple practice. It provides support for climbing vines and leads to significant cost reductions. Interestingly, the traditional cultivation system for FG has been associated with corn. However, this practice is declining in favour of monoculture. While there is no such tradition for FM, intercropping with sweetcorn has been proposed for bush bean cultivars in other regions of Spain as well [48].

Similar to potato intercropping, interplanted crops surrounding the bean plants act as a mechanical barrier for aphids and a virus sink. These companion crops attract aphids away from the main bean crop [26], which likely explains the positive results observed in

SFGMz_23 and the lower level of BCMV at the end of the season in both LGMz_23 plots. An integrated approach that combines multiple techniques like these is most effective. Additionally, utilizing virus detection methods like tissue printing ELISA for pre-planting seed lot evaluation is crucial.

5. Conclusions

The high prevalence of Bean common mosaic virus (BCMV) in FG fields is primarily caused by infected seeds. This is due to the virus's high seed transmissibility and the occurrence of early horizontal transmission by aphids.

The significant differences in BCMV incidence and seed transmission between the two evaluated bean cultivars seem to be related to their different growing seasons and durations in the field. Harvesting before full maturity in FM results in poor germination of seeds older than the previous season ones but also in lower seed transmission.

The best way to reduce the virus incidence and minimize yield losses is through the commitment of farmers in the PGIs to:

(a) locate plots for seed production far from the growing area where a combination of techniques for preventing virus transmission will be applied by trained growers using an IPM approach,
(b) test representative samples of the seeds produced in those plots and use exclusively lots with the lowest possible virus level, and
(c) train growers to pay special attention to soil and plant management from the beginning of crop planting to delay virus transmission.

Author Contributions: Conceptualization, S.E.-G. and C.C.; Methodology, S.E.-G. and C.C.; Software, C.C.; Validation, S.E.-G. and C.C.; Formal Analysis, C.C. and F.L.-P.; Investigation, S.E.-G., C.C. and L.G.P.-L.; Resources, C.C.; Data Curation, S.E.-G., C.C. and L.G.P.-L.; Writing—Original Draft Preparation, C.C.; Writing—Review and Editing, C.C., S.E.-G., F.L.-P. and L.P; Visualization, F.L.-P.; Supervision, C.C.; Project Administration, C.C.; Funding Acquisition, C.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially funded by a contract with Unión de Cooperativas AGACA through the "Axencia Galega da Calidade Alimentaria", Proxectos Piloto CALL (DOG 33 18/022021Exp: FEADER2021/066A).

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors upon request.

Acknowledgments: Thanks to Antonio de Ron (CSIC), the Cooperative "Terras da Mariña" and other growers in and outside the PGI "Faba de Lourenzá" for technical support, seed lots, and field access.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Santalla, M.; Rodiño, A.P.; De Ron, A.M. Allozyme evidence supporting southwestern Europe as a secondary center of genetic diversity for common bean. *Theor. Appl. Genet.* **2002**, *104*, 934–944. [CrossRef] [PubMed]
2. De Ron, A.M.; Rodiño, A.P.; Santalla, M.; Martínez-Sierra, V. Denominación de Origen Protegida Faba de Lourenzá. In *Nuevos Retos y Oportunidades de las Leguminosas en el Sector Agroalimentario Español*; De los Mozos, M., Giménez, M.J., Rodríguez Conde, M.F., Sánchez, R., Eds.; Consejería de Agricultura de Castilla—La Mancha: Toledo, Spain, 2006; pp. 387–392.
3. Boletín Oficial del estado-A-2018-6283. Orden APM/477/2018, de 26 de abril, por la que se dispone la inclusión de diversas variedades de distintas especies en el Registro de Variedades Comerciales: 20170135 faba galaica: 49361-49364. Available online: https://www.boe.es/diario_boe/txt.php?id=BOE-A-2018-6283 (accessed on 31 May 2024).
4. Ministerio de Ciencia e Innovación/Consejo Superior de Investigaciones Científicas. Ficha "Faba do Marisco". Available online: https://www.mbg.csic.es/wp-content/uploads/2021/03/Ficha-Faba-do-Marisco.pdf (accessed on 31 May 2024).
5. Pagán, I. Transmission through seeds: The unknown life of plant viruses. *PLoS Pathog.* **2022**, *18*, e1010707. [CrossRef] [PubMed]
6. Dell Olmo, E.; Tiberini, A.; Sigillo, L. Leguminous seedborne pathogens: Seed health and sustainable crop management. *Plants* **2023**, *19*, 2040. [CrossRef] [PubMed]
7. Saiz, M.; de Blas, C.; Carazo, G.; Fresno, J.; Romero, J.; Castro, S. Incidence and characterization of bean common mosaic virus isolates in Spanish bean fields. *Plant Dis.* **1993**, *79*, 79–81. [CrossRef]

8. Melgarejo, P.; García-Jiménez, J.; Jordá, M.C.; López, M.M.; Andrés, M.F.; Duran-Vila, N. *Patógenos de plantas descritos en España*; Ministerio de Medio Ambiente y Medio Rural y Marino: Madrid, Spain, 2010; p. 49.
9. Romero, J. Mosaicos de la judía. In *Enfermedades de las Plantas Causadas por Virus y Viroides*; Ayllón, M.A., Cambra, M., Llave, C., Moriones, E., Eds.; Bubok Publishing S.L. y Sociedad Española de Fitopatología: Madrid, Spain, 2016; pp. 455–466.
10. Walkey, D.G.A. Ecology and Epidemiology of Plant Viruses 2nd ed. In *Applied Plant Virology*; Springer: Dordrecht, The Netherlands, 2012; Chapter 8, pp. 210–225.
11. Morales, F.J.; Castaño, M. Seed transmission characteristics of selected bean common mosaic virus strains in differential bean cultivars. *Plant Dis.* **1987**, *71*, 51–53. [CrossRef]
12. Shukla, D.D.; Ward, C.W.; Brunt, A.A. *The Potyviridae*; CAB International: Wallingford, UK, 1994; 516p.
13. Aishwarya, P.; Rangaswamy, K.T.; Basavaraju, S.; Achari, R.; Prameela, H.A. Evaluation of the seed-borne nature of bean common mosaic virus (BCMV) in cowpea. *Int. J. Curr. Microbiol. Appl. Sci.* **2020**, *9*, 239–245. [CrossRef]
14. Tang, M.; Feng, X. Bean Common Mosaic Disease: Etiology, Resistance Resource, and Future Prospects. *Agronomy* **2023**, *13*, 58. [CrossRef]
15. Gonzalez-Cruces, A.; Arista-Carmona, E.; Díaz-Arias, K.V.; Ramírez-Razo, K.; Hernández-Livera, A.; Acevedo-Sánchez, G.; Mendoza-Ramos, C.; Mora-Aguilera, G. Epidemiology of bean common mosaic virus and *Alternaria alternata* in 12 *Phaseolus vulgaris* genotypes. *Mex. J. Phytopathol.* **2022**, *40*, 188–220. [CrossRef]
16. Galvez, E. Aphid-transmitted viruses. In *Bean Production Problems: Disease, Insect, Soil and Climatic Constraints of Phaseolus vulgaris*; Schwartz, H.F., Gálvez, E., Guillermo, E., Eds.; Centro Internacional de Agricultura Tropical (CIAT): Cali, Colombia, 1980; pp. 211–238.
17. Meziadi, C.; Blanchet, S.; Geffroy, V.; Pflieger, S. Genetic resistance against viruses in *Phaseolus vulgaris* L.: State of the art and future prospects. *Plant Sci.* **2017**, *265*, 39–50. [CrossRef]
18. Mangeni, B.H.; Were, K.; Ndong'a, M.; Mukoye, B. Incidence and severity of bean common mosaic disease and resistance of popular bean cultivars to the disease in western Kenya. *J. Phytopathol.* **2020**, *168*, 501–515. [CrossRef]
19. Worrall, E.A.; Wamonje, F.O.; Mukeshimana, G.; Harvey, J.J.W.; Carr, J.P.; Mitter, N. Bean common mosaic virus and bean common mosaic necrosis virus: Relationships, biology, and prospects for control. *Adv. Virus Res.* **2015**, *93*, 1–46.
20. Sastry, K.S. *Seed-Borne Plant Virus Diseases*; Springer: New Delhi, India, 2013; 353p.
21. Hampton, R.O. The nature of bean yield reduction by bean yellow and bean common mosaic virus. *Phytopathology* **1975**, *65*, 1342–1346. [CrossRef]
22. Morales, F.J. Common Bean. In *Virus and Virus-like Diseases of Major Crops in Developing Countries*; Loebenstein, G., Thottappilly, G., Eds.; Springer: Dordrecht, The Netherlands, 2003; pp. 425–445.
23. Cifuentes, G.; Castro, S.; Romero, J. Los Potyvirus de judía transmitidos por semilla: Incidencia de la enfermedad y su efecto en los rendimientos. In Proceedings of the II Seminario de la Judía de la Península Ibérica, Villaviciosa, Spain, 5–7 September 2000.
24. Çelik, A.; Emiralioğlu, O.; Yeken, M.Z.; Çiftçi, V.; Özer, G.; Kim, Y.; Baloch, F.S.; Chung, Y.S. A novel study on bean common mosaic virus accumulation shows disease resistance at the initial stage of infection in Phaseolus vulgaris. *Front. Genet.* **2023**, *14*, 1136794. [CrossRef] [PubMed]
25. Walkley, A.D.G.; Dance, C.M. The effect of oil sprays on aphid transmission of Turnip Mosaic, Beet Yellows, Bean Common and Bean Yellow Mosaic Viruses. *Plant Dis.* **1979**, *63*, 877–881.
26. Dupuis, B.; Cadby, J.; Goy, G.; Tallant, M.; Derron, J.; Schwaerzel, R.; Steinger, T. Control of potato virus Y (PVY) in seed potatoes by oil spraying, straw mulching and intercropping. *Plant Pathol.* **2017**, *66*, 960–969. [CrossRef]
27. Dupuis, B.; Schwaerzel, R.; Goy, G.; Tallant, M.; Derron, J. Stepwise development of an efficient method to control Potato virus Y spread in seed potato fields. In Proceedings of the European Association for Potato Research, Virology Section, Hamar, Norway, 4–9 July 2010; Spetz, C., Blystad, D.R., Eds.; Bioforsk Fokus: Hamar, Norway, 2010; Volume 5, p. 22.
28. Hooks, C.R.R.; Fereres, A. Protecting crops from non-persistently aphid-transmitted viruses: A review on the use of barrier plants as a management tool. *Virus Res.* **2006**, *120*, 1–16. [CrossRef]
29. Martin-Lopez, B.; Varela, I.; Marnotes, S.; Cabaleiro, C. Use of oils combined with low doses of insecticide for the control of *Myzus persicae* and PVY epidemics. *Pest. Manag. Sci.* **2006**, *62*, 372–378. [CrossRef]
30. Gadhave, K.R.; Gautam, S.; Rasmussen, D.A.; Srinivasan, R. Aphid transmission of Potyvirus: The largest plant-Infecting RNA virus genus. *Viruses* **2020**, *12*, 773. [CrossRef] [PubMed]
31. Abd El-Aziz, M.H. Three modern serological methods to detect plant viruses. *J. Plant Sci. Phytopathol.* **2019**, *3*, 101–106. [CrossRef]
32. Cabaleiro, C.; Marnotes, S.; Couceiro, C.; Martínez, M.A.; Lastra, B.; Martín, B.; García, L.; Alvarez, S. Uso de cultivos borde o asociados para el control de virus no persistentes en patata (PVY) y alubias (BCMV). In Proceedings of the XIV Congreso de la SEF, Lugo, Spain, 15–19 September 2008.
33. Legorburu, F.J.; Ruiz de Galarreta, J.I.; Abad, J.M.; Pérez de San Román, C. Bean Common (BCMV) and Bean common mosaic necrosis (BCMNV) potyvirus in relation to bean landraces in the Basque Country. *Investig. Agr. Prod. Prot. Veg.* **1998**, *13*, 153–158.
34. Lin, N.S.; Hsu, Y.H.; Hsu, H.T. Immunological detection of plant viruses and a mycoplasmalike organism by direct tissue blotting on nitrocellulose membranes. *Phytopathol.* **1990**, *80*, 824–828. [CrossRef]

35. Cambra, M.; Gorris, M.T.; Roman, M.P.; Terrada, E.; Garnsey, S.M.; Camarasa, E.; Olmos, A.; Colomer, M. Routine detection of Citrus tristeza virus by direct Immunoprinting-ELISA method using specific monoclonal and recombinant antibodies. In Proceedings of the 14th Conference IOCV, Campinas, Brazil, 13–18 September 1998; da Graça, J.V., Lee, R.F., Yokomi, R.K., Eds.; International Organization of Citrus Virologists c/o Department of Plant Pathology: Riverside, CA, USA, 2000.
36. Madden, L.V.; Hughes, G.; van den Bosch, F. *The Study of Plant Disease Epidemics*; American Phytopathological Society: St. Paul, MN, USA, 2007; 421p.
37. Nutter, F.W., Jr.; Eggenberger, S.K.; Littlejohn, K.J. Visualizing, describing, and modeling Disease Progress Curves using EPIMODEL. In *Exercises in Plant Disease Epidemiology*, 2nd ed.; Stevenson, K.L., Jeger, M.J., Eds.; American Phytopathological Society: St. Paul, MN, USA, 2015; pp. 21–30.
38. Mwaipopo, B.; Nchimbi-Msolla, S.; Njau, P.; Tairo, F.; William, M.; Binagwa, P.; Kweka, E.; Kilango, M.; Mbanzibwa, D. Viruses infecting common bean (*Phaseolus vulgaris* L.) in Tanzania: A review on molecular characterization, detection and disease management options. *Afr. J. Agric. Res.* **2017**, *12*, 1486–1500.
39. Makkouk, K.M.; Kumari, S.G. Detection of ten viruses by the tissue-blot immunoassay (TBIA). *Arab. J. Plant Protection* **1996**, *14*, 3–9.
40. European Plant Protection Organization. ELISA tests for plant pathogenic bacteria. *Bull. OEPP/EPPO* **2010**, *40*, 369–372. [CrossRef]
41. Simmons, H.; Munkvold, G. Seed Transmission in the Potyviridae. In *Global Perspectives on the Health of Seeds and Plant Propagation Material*; Gullino, M.L., Munkvold, G., Eds.; Plant Pathology in the 21st Century 6; Springer Science+Business Media: Dordrecht, The Netherlands, 2014; pp. 3–15.
42. Campelo, M.P.; Reinoso, B.; González, A.J. Incidencia y transmisión de Potyvirus en semillas de judía de la I.G.P. "Alubia de la Bañeza-León". In Proceedings of the XIII Congreso Nacional de la Sociedad Española de Fitopatología, Murcia, Spain, 18–22 September 2006.
43. González, A.J. Virus fitopatógenos transmisibles por semilla en judía tipo "granja asturiana". *Bol. San. Veg. Plagas* **2004**, *30*, 595–603.
44. Carazo, G.; Romero, J. Malas hierbas reservorios de los Potyvirus que infectan judías. In Proceedings of the IV Seminario de Judías de la Península Ibérica, Valladolid, Spain, 2–4 September 2008.
45. Odedara, O.O.; Kumar, P.L. Incidence and diversity of viruses in cowpeas and weeds in the unmanaged farming systems of savanna zones in Nigeria. *Arch. Phytopathol. Plant Prot.* **2017**, *50*, 1–12. [CrossRef]
46. Boiteau, G.; Singh, M.; Lavoie, J. Crop border and mineral oil sprays used in combination as physical control methods of the aphid-transmitted potato virus Y in potato. *Pest. Manag. Sci.* **2009**, *65*, 255–259. [CrossRef]
47. Lacomme, C.; Pickup, J.; Fox, A.; Glais, L.; Dupuis, B.; Steinger, T.; Rolot, J.L.; Valkonen, J.P.T.; Kruger, K.; Nie, X.; et al. Transmission and Epidemiology of Potato virus Y. In *Potato virus Y: Biodiversity, Pathogenicity, Epidemiology and Management*; Lacomme, C., Glais, L., Bellstedt, D., Dupuis, B., Karasev, A., Jacquot, E., Eds.; Springer: Cham, Germany, 2017; pp. 141–176.
48. Santalla, M.; Rodiño, A.P.; Casquero, P.; de Ron, A.M. Interactions of bush bean intercropped with field and sweet maize. *Eur. J. Agron.* **2001**, *15*, 185–196. [CrossRef]