Article

Response of Two Local Common Bean Ecotypes of "Fagioli di Sarconi" PGI (*Phaseolus vulgaris* L.) to Seed-Borne Pathogens and Environmental Change

Antonella Vitti [1,2,*], **Vincenzo Bevilacqua** [1], **Giuseppina Logozzo** [1], **Stefania Marzario** [1], **Tania Gioia** [1,*] **and Maria Nuzzaci** [1]

1   School of Agricultural, Forestry and Environmental Sciences, University of Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy; vincenzogiovann1984@libero.it (V.B.); giuseppina.logozzo@unibas.it (G.L.); stefy.85s@libero.it (S.M.); maria.nuzzaci@unibas.it (M.N.)
2   Pharmacy Department, University of Salerno, Via Giovanni Paolo II n.132, 84084 Fisciano, Italy
*   Correspondence: avitti@unisa.it (A.V.); tania.gioia@unibas.it (T.G.)

Abstract: Among foods protected by the European Union with the PGI (Protected Geographical Indication) mark, several ecotypes of "Fagioli di Sarconi" common beans, typical legumes of Basilicata Region, are included. This work aimed to conduct a survey of seed-borne pathogens isolated from "Ciuoto" and "Cannellino rosso", ecotypes of "Fagioli di Sarconi" common beans, in two years and cultivation areas of the National Park of the Agri Valley, for identifying resistant and climatic changes well-adapted genotypes. Three validated methods were used for the seed-borne pathogens screening. Eighteen fungi were differently found for ecotype and year of observation by the washing test. Saprophyte contaminants pathogens isolated and detected by the blotter test were strongly reduced by 1% sodium hypochlorite treatment. Using the between paper test, specific for detecting *Colletotrichum lindemuthianum*, the presence of this pathogen for both ecotypes, years and cultivation areas, and also some bacteria were individuated. Therefore, area-, environment- and ecotype-dependent differences were revealed, probably also caused by a different polyphenolic content and thickness of integument of two ecotypes. This study represents a baseline information for further studies, development of forecasting models and management of seed-borne diseases associated with common beans.

Keywords: PGI (Protected Geographical Indication) marked common bean; "Ciuoto" and "Cannellino rosso" ecotypes; indigenous *Trichoderma* spp.; genotypes resistance

1. Introduction

Phaseolus vulgaris L., known as common bean, is the second most important legume in the world for food purposes thanks to its high nutritional value in terms of content of protein, vitamins, zinc, iron, and fiber [1–3]. It is widespread over a large area between 52° N and 32° S latitude, at low altitudes until sea level (USA and Europe) and high altitude (South America) [4–6]. In Italy, the cultivated area is 22,501 ha (17,161 and 5340 ha for green and dry beans, respectively); the Basilicata Region (South Italy) is invested with 125 ha of dry beans [7]. "Fagioli di Sarconi" common beans include about 21 different ecotypes protected by the European Union (Commission Regulation (EC) No 1263/96) with the mark PGI (Protected Geographical Indication). These legumes are used as a prevalent healthy food in Italian cuisine, and are cultivated in the National Park of the Agri Valley in Basilicata for dry seed production [8–10].

The "Consorzio di Tutela dei Fagioli di Sarconi" has preserved the germplasm on farm and its biochemical and nutraceutical traits have been characterized [5,8–16]; however, research on the response to diseases of common bean ecotypes is limited [17].

Seed-borne pathogenic fungi and bacteria can inhibit germination, infect the seedlings to death, or reduce plant growth by damaging the roots and vascular system, and affecting the transport of water and nutrients [17–20].

The main seed-borne pathogenic fungi that cause losses of yield and quality of common bean are *Alternaria* spp. *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cav, *Botrytis* spp., *Rhizopus* spp., *Cladosporium* spp., *Rhizoctonia solani* (Cooke) Wint [19–24]. All these fungi are seed-transmitted and can be preserved in the form of conidia in the coat or as mycelia on the seed surface [25], at extra- or intra-embryal level, or associated with the seeds as contaminants [26]. For this reason, all these fungi represent a limiting factor for common bean production in many countries.

According to the classic disease triangle, the host–pathogen–environment interactions are the cause of plant disease occurrence and severity [27]. Therefore, the environment has been always considered an important ring in plant pathology studies. Currently, climate change is strongly associated with seed-borne fungally induced disease incidence and severity. In fact, climate changes influence the growth, reproduction, survival, spread, pathogenicity, as well as the ability to modify a typical soil microflora, enabling the emergence of new species [28,29].

In this context, this study aimed to determine the influence of different cultivation areas on the presence of seed-borne pathogens isolated from seeds of two ecotypes of "Fagioli di Sarconi" common beans, "Ciuoto" and "Cannellino rosso", collected from two different cultivation areas of the Basilicata Region during the years 2018 and 2019, for the identification of genotypes resistant or little susceptible to seed-borne pathogens and well adapted to environmental changes.

2. Materials and Methods

2.1. Sample Collection

During the years 2018 and 2019, the phytosanitary status of seeds of two ecotypes of "Fagioli di Sarconi" PGI common bean, "Ciuoto" and "Cannellino rosso", characterized by a determinate growth habit (class 1) [30], was evaluated. The populations were multiplied in organic farms, in two different areas located in the National Park of the Agri Valley: Sarconi (latitude, 40°15′54″ N; longitude, 15°54′43″ E; altitude, 620 m a.s.l.) and Paterno (latitude, 40°20′25″ N; longitude, 15°46′03″ E; altitude, 595 m a.s.l.), Potenza, Italy. In both locations, soil is a fertile coarse lime soil, suitable for bean cultivation. Ecotype seeds were manually sown in rows (0.5 m between rows; 0.10 m between plants) during the first decade of June and harvested during the first decade of November, in each farm and in each location. Standard organic agronomic management and irrigation practices were applied to the experimental fields. After the manual harvesting and ginning of pods, all seeds collected were placed in plastic sterile bags and transported in cooler bags to the laboratories. The samples were immediately used for the following analyses.

Seed color types were detected by visual inspection, according to bean seed imaging from Basic Protocol 2 [31], for future quality control and postprocessing. As reported in Figure 1 and Table 1, "Ciuoto" and "Cannellino rosso" have different seed qualitative and quantitative morphological traits.
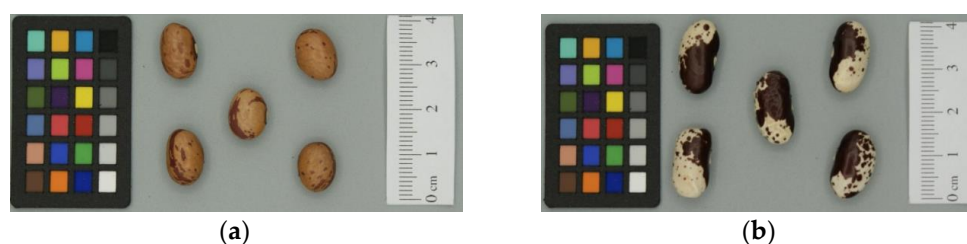

(**a**) (**b**)

Figure 1. Common bean seed ecotypes of "Fagioli di Sarconi" PGI (Protected Geographical Indication): "Ciuoto" (**a**) and "Cannellino rosso" (**b**).

Table 1. Common bean seed traits in "Fagioli di Sarconi" PGI (Protected Geographical Indication) ecotypes used in the present study.

Fagioli di Sarconi PGI Ecotype	Seed Qualitative Trait						
	Coat Darker Color	Coat Lighter Color	Coat Patter	Longitudinal Shape	Transverse Shape	Veining	Brilliance
Ciuoto	Cream/Brown	Purple/red	Stripe	Oval	Large elliptic	Weak	Medium
Cannellino rosso	Whitish	Purple	Spotted bicolor	Kidney shaped	Elliptic	Weak	Medium

Fagioli di Sarconi PGI ecotype	Seed Quantitative Trait			
	Length (mm)	Height (mm)	Width (mm)	Weight (g)
Ciuoto	11.13 ± 1.02	7.54 ± 0.62	6.53 ± 0.55	0.38 ± 0.08
Cannellino rosso	15.91 ± 0.81	8.09 ± 0.49	6.54 ± 0.48	0.63 ± 0.09

Data are expressed as the mean of 25 seeds for each lot ± SDs.

The two locations are in an internal intensive horticultural area, characterized by a temperate Mediterranean climate with cool winters and warm dry summers. The data of maximum, minimum and average temperature and rainfall for the growing season (June to November 2018 and 2019) were obtained from the nearest weather station, the agrometeorological station of the Agenzia Lucana per lo Sviluppo e l'Innovazione in Agricoltura (ALSIA) of the Basilicata Region located about 8 and 14 km from Paterno's and Sarconi's farm, respectively.

2.2. Isolation of Seed Microflora

The phytopathological analysis of seeds was carried out with three different methods [32,33].

2.2.1. Washing Test

For each test, 400 seeds (4 replicates × 100) were considered, according to [32], with some modifications. The separation of spores and mycelium from the integument surface was obtained by stirring the seeds in presence of sterile, distilled water. After filtration, the suspension was centrifuged at 5000 × *g* for 11 min. The precipitate was resuspended in 200 μL sterile, distilled water, and samples were set up for observation under the optical microscope. In addition to this procedure, seeds were sown on Petri dish containing Potato Dextrose Agar (PDA) and the antibiotics ampicillin and stremptomycin (Sigma-Aldrich, Italy), and incubated at 22 °C in the dark, for 5–8 days. Then afterwards, slides were prepared for observation under the optical microscope.

2.2.2. Blotter Test

According to [32], with some modifications, filter paper discs were placed in 140 mm diameter Petri dishes and moistened with 2 mL of sterile, distilled water. Four hundred seeds (4 replicates × 100) were treated with a 1% sodium hypochlorite solution for 10 min and distributed on the moistened paper. The dishes were incubated at 20 °C for 10 days, alternating 12 h of 380 nm NUV-light (near-UV-light) tubes and 12 h of dark. After the incubation, the seeds were examined under the optical microscope. Untreated seeds represented the control.

2.2.3. Between Paper Test

The identification and subsequent characterization of *Colletotrichum* spp. was carried out on 200 seeds (4 replicates × 50) according to [33], with small modifications. After seeds disinfection and air-drying, 4 sub-samples of 50 seeds were placed between two double sheets of toweling paper and soaked in sterile distilled water. The paper toweling was folded lengthwise and covered with a polyethylene sheet to keep a high moisture during incubation (7 days at 20 °C). Then, the seeds and cotyledons were observed, and

black depressed areas with well-defined contours were searched. The typical signs of the anthracnose presence were visually and under optical microscope checked (acervules with or without bristles).

2.3. Identification of Pathogens

After the incubations and the preparation of slides, the macroscopic (appearance of the colony and mycelium) and microscopic (appearance of the mycelium, presence or absence of septa in hyphae, color, shape and size of conidia, reproductive structures) characteristics were revealed under the optical microscope (Axioskop, Zeiss, Jena, Germany) at $20\times$, $40\times$, $60\times$ and $100\times$ resolution. For the microscopic and morphological identification of the fungal isolates, the scientific literature and mycological atlases with related taxonomic keys were used [34–42].

2.4. Disease Assessment

Seed disease incidence caused by the seed-borne fungi (*R. solani*, *F. oxysporum*, *C. lindemuthianum*) and bacteria (*Pseudomonas syringae* pv. *phaseolicola* and *Xanthomonas campestris* pv. *phaseoli*) was examined 14 days post-germination using the scores 0–heathy and 1–infected. Disease incidence was obtained by dividing the number of infected seeds by total number of seeds and then multiplying by 100.

2.5. Statistical Analysis

Meteorological parameters (precipitation, minimum, maximum and average temperature, minimum, maximum and average relative humidity and potential evapotranspiration) for the two years of observation 2018 and 2019 were analyzed with SAS OnDemand for Academics (SAS Institute, Inc., Cary, NC, USA) [43] by proc ANOVA (one-way analysis of variance). The statistical data on disease were performed using R Stats Package (Version 4.0.2 (2020-06-22); R Core Team, 2020) using the RStudio environment (Version 1.3.1093; RStudio Team, 2020) [44] by Pearson's chi-squared test.

3. Results

3.1. Meteorological Parameters

The rain, temperature and relative humidity data for the Agri Valley territory during the cultivation period, from June to November for both years 2018 and 2019, are summarized in Table 2 and in Figure 2.

Table 2. Meteorological parameters provided by the ALSIA agro-meteorological station in the Agri Valley for the two years of observation, 2018 and 2019.

Meteorological Parameter	2018				2019				*pr* (>F)
	Min	**Max**	**Range**	**Mean**	**Min**	**Max**	**Range**	**Mean**	
Rain (mm day^{-1})	0.00	38.60	38.60	2.09	0.00	56.40	56.40	1.69	0.530
Temperature air (°C day^{-1})									
Minimum	−4.30	19.80	24.10	10.03	−0.50	16.50	17.00	9.58	0.301
Maximum	8.40	36.40	28.00	26.13	11.00	39.40	28.40	27.78	0.016 *
Average	2.83	25.78	22.94	17.20	6.28	25.93	19.64	18.08	0.094
Relative humidity (% day^{-1})									
Minimum	19.00	90.00	71.00	42.39	13.00	91.00	78.00	37.58	0.001 ***
Maximum	67.80	100.00	32.20	96.96	82.00	99.80	17.80	96.96	0.998
Average	46.25	96.42	50.17	75.09	49.67	96.79	47.13	71.25	0.000 ***
Evapotranspiration (mm day^{-1})	0.76	8.13	7.37	4.44	0.90	8.08	7.18	4.87	0.049 *

Asterisks indicate significance at *** $pr < 0.001$; * $pr < 0.05$, Data from [45].
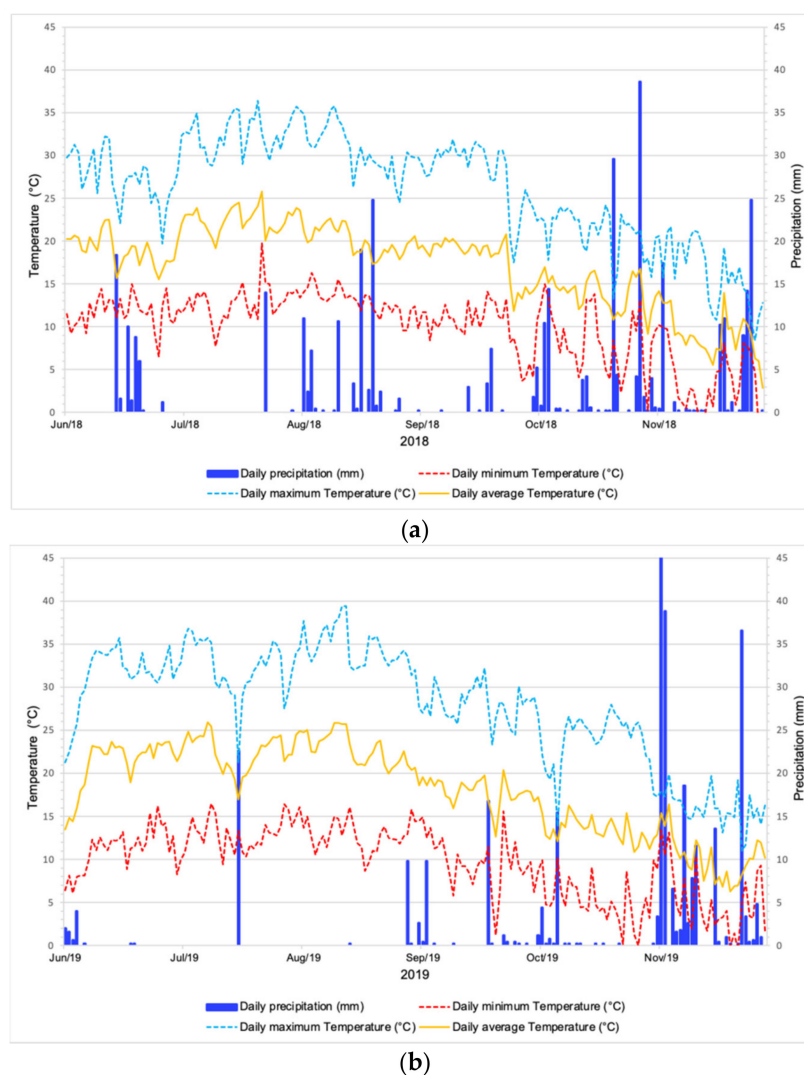
(**a**)

(**b**)

Figure 2. Total precipitation and minimum, maximum and average temperatures during the bean growing seasons of June–November 2018 (**a**) and 2019 (**b**).

During the 2019 cultivation cycle the average daily rainfall, equal to 1.69 mm day^{-1}, was lower than the previous year (2.09 mm day^{-1}), but was not significantly different. Regarding the temperatures, only the maximum showed a significantly different value between the two years. In the same way, the minimum and average relative humidity were significantly different between the two years, with values in 2019 being lower than in the previous year. As a consequence, the average evapotranspiration during 2019 was significantly higher with respect to 2018.

3.2. Isolation and Identification of Seed Mycoflora by Washing Test

Eighteen seed-borne fungal species were found contaminating or infecting the two ecotypes of "Fagioli di Sarconi" PGI common bean seeds in the two different areas and during the two years 2018 and 2019 (Table 3).

Table 3. Seed-borne fungal species isolated and detected (+) or not (−) by washing test on the two ecotypes of "Fagioli di Sarconi" PGI common bean seeds.

	2018				2019			
Ecotype	Ciuoto		Cannellino Rosso		Ciuoto		Cannellino Rosso	
Locality	Sarconi	Paterno	Sarconi	Paterno	Sarconi	Paterno	Sarconi	Paterno
Fungal microflora								
Alternaria spp.	+	-	-	-	-	+	+	-
Alternaria alternata	-	−	−	−	−	+	−	+
Aspergillus spp.	−	−	+	+	−	−	−	−
Aspergillus flavus	−	−	+	+	+	−	−	−
Aspergillus niger	+	−	−	−	−	−	+	−
Cladosporium cladosporioides	−	−	−	+	+	+	+	−
Botritys spp.	−	−	+	−	−	+	−	+
Colletotrichum lindemuthianum	−	+	−	−	−	+	−	−
Fusarium oxysporum	−	−	−	−	−	+	−	+
Fusarium solani	−	+	−	−	−	+	−	−
Mucor hiemalis	+	−	−	−	−	−	+	−
Penicillium spp.	−	−	+	+	−	+	+	+
Penicillium expansum	−	+	−	−	−	+	−	−
Rhizoctonia solani	−	+	−	+	+	+	+	+
Rhizophus nigricans	+	−	−	−	−	−	−	−
Trichoderma harzianum	−	−	−	+	−	−	−	−
Tricoderma viridae	+	−	−	−	−	−	−	−
Uromyces appendiculatus	−	−	−	−	−	+	−	+

Data from [45].

Microscopic morphological structures identifying the fungi *Rhizoctonia solani* isolated from "Cannellino rosso" ecotype in Paterno (2018), *Colletotrichum lindemuthianum* and *Fusarium oxysporum* isolated from "Ciuoto" in 2019 in Paterno and Sarconi, respectively, are shown in Figure 3. In particular, Figure 3a shows the typical durable moniloid cells of *R. solani*; the conidia of *C. lindemuthianum* showed in Figure 3b are typically cylindrical, fusiform or sickle−shaped, hyaline with smooth wall and with two guttules; the macroconidia of *F. oxysporum* were individuated by their typical three to five septa, the apical and basal cells being moderately curved and foot shaped, respectively, while the microconidia for their drop shape were without septa (Figure 3c).
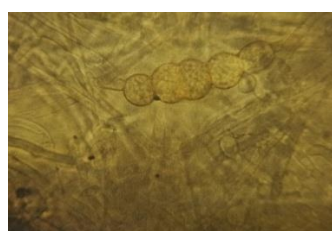
(a)　　　　　　　　　　　(b)　　　　　　　　　　　(c)

Figure 3. (**a**) Moniloid cells of *R. solani* (resolution 60×); (**b**) conidia of *C. lindemuthianum* (resolution 20×); (**c**) macroconidia and microconidia of *F. oxysporum* (resolution 40×).

3.3. Identification and Incidence of R. solani on Treated and Untreated Seeds with Blotter Test, and of C. lindemuthianum, F. oxysporum and Bacterial Diseases Causal Agents with between Paper Test

The blotter test was able to identify, in the 1% sodium hypochlorite treated seeds, the following seed−borne fungi: *R. solani* (both years, ecotypes and localities); *Aspergillus niger* (2018, Paterno, both ecotypes; 2019, Sarconi, "Cannellino rosso"); *F. solani* (2019, Paterno, both ecotypes); *Penicillium expansum* (2018, Paterno, "Ciuoto").

On the other hand, the following fungi were detected in untreated seeds: *Aspergillus flavus* (2019, Sarconi, "Cannellino rosso"); *A. niger* (2018, Paterno, both ecotypes; 2019, Sarconi, both ecotypes); *Cladosporium cladosporioides* (2019, Sarconi, "Cannellino rosso"); *F. solani* (2018, Paterno, "Ciuoto"; 2019, Paterno, "Cannellino rosso"); *Mucor hiemalis* (2018, Sarconi, "Ciuoto" and Paterno, both ecotypes; 2019, Paterno, both ecotypes); *Penicillium* spp. (2018, Sarconi, "Cannellino rosso"); *P. expansum* (2018, Paterno, "Ciuoto"); *Rhizophus nigricans* (2018, Sarconi, "Ciuoto").

With the between paper test, although specific for *C. lindemuthianum*, the fungus *F. oxysporum* and two bacterial diseases causal agents were isolated and detected, as reported in Table 4.

Table 4. Seed-borne fungal and bacterial species isolated detected (+) or not (−) by blotter and between paper tests on the two ecotypes of "Fagioli di Sarconi" PGI common bean seeds. The percentage of disease incidence indicates rhizoctoniosis from *R. solani* and the presence of typical fungal or bacterial symptoms for the other pathogens.

Year	2018				2019			
Ecotype	Ciuoto		Cannellino Rosso		Ciuoto		Cannellino Rosso	
Locality	Sarconi	Paterno	Sarconi	Paterno	Sarconi	Paterno	Sarconi	Paterno
Blotter Method								
Rhizoctonia solani								
treated seed	+	+	+	+	+	+	+	+
Disease incidence (%) [1]	27	23	8	15	12	15	4	8
untreated seed	+	+	+	+	+	+	+	+
Disease incidence (%) [1]	19	35	24	27	11	23	12	24
Between Paper Test								
Fusarium oxysporum	+	+	−	+	−	+	−	+
Disease incidence (%) [2]	1	4	0	2	0	10	0	6
C. lindemuthianum	+	+	+	+	+	+	+	+
Disease incidence (%) [2]	4	40	8	20	4	65	4	30
Bacterial disease causal agents								
P. syringae pv. *phaseolicola*	+	+	+	+	+	+	+	−
X. campestris pv. *phaseoli*	+	+	+	−	−	+	−	+
Disease incidence (%) [2]	28	30	18	24	12	12	6	16

[1] The differences of rhizoctoniosis from *R. solani* between untreated and treated seeds are significant for χ^2 = 87.04, at $p < 0.0001$; [2] the differences of symptoms between ecotype, year and locality are significant for χ^2 = 9.71, at $p \leq 0.046$ for *C. lindemuthianum*, for χ^2 = 8.50, at $p < 0.037$ for *F. oxysporum*, and for χ^2 = 10.84, at $p \leq 0.028$ for the bacterial disease. Data from [45].

The seed-borne pathogenic fungi and bacteria considered the most dangerous in determining losses of yield and quality in the common bean were deeply considered in terms of their disease. The percentage of rhizoctoniosis from *R. solani*, as well as the presence of typical symptoms on integument and cotyledons induced by *C. lindemuthianum*, *F. oxysporum* and bacterial diseases causal agents are depicted (Table 4).

R. solani was detected in both ecotypes and years. The incidence of rhizoctoniosis in common bean seeds treated with hypochlorite for both years was higher for "Ciuoto" with respect to the "Cannellino rosso" ecotype in both Paterno and Sarconi. On the other hand, during the year 2019, the rhizoctoniosis incidence for all treated seeds decreased.

Conversely, *R. solani* induced a higher disease incidence in the untreated seeds, in both ecotypes and years of cultivation, in the Paterno area.

As showed in Table 4, χ^2 test indicated differences between ecotype, year and cultivation area for the incidence of fusarium disease as highly significant. The highest incidence of phytopathy was recorded in 2019 for "Ciuoto" in the Paterno area. In Sarconi, in the same year, the presence of fusariosis on seed was not found.

The incidence of anthracnose symptoms from *C. lindemuthianum* was evaluated on integument and cotyledons by between paper test. The differences between ecotype, year

and area were highly significant, as shown by χ^2 test. In particular, for this pathogen, the Paterno area was the most infected one, with the year 2019 being more serious than the previous year and "Cannellino rosso" showing less susceptibility to anthracnose than the "Ciuoto" ecotype.

In summary, the treatment of the seeds with 1% Na−hypochlorite allowed a reduction of most fungal microflora adhering to the seed, such as *Aspergillus flavus*, but did not eliminate the systemic pathogens infecting the internal structures of the seed, such as *C. lindemuthianum*, *F. solani*, and *R. solani*.

Furthermore, the different environmental factors in the two years and cultivation areas determined a different incidence of the considered diseases

3.4. Macroscopic and Microscopic Characterization of R. solani, C. lindemuthianum and F. oxysporum

The typical damping−off symptoms of *R. solani* on the stem of the "Cannellino rosso" common bean ecotype, located in Sarconi (2019) and "Ciuoto", and located in Paterno (2018), as revealed by blotter test, are shown in Figure 4a,b.
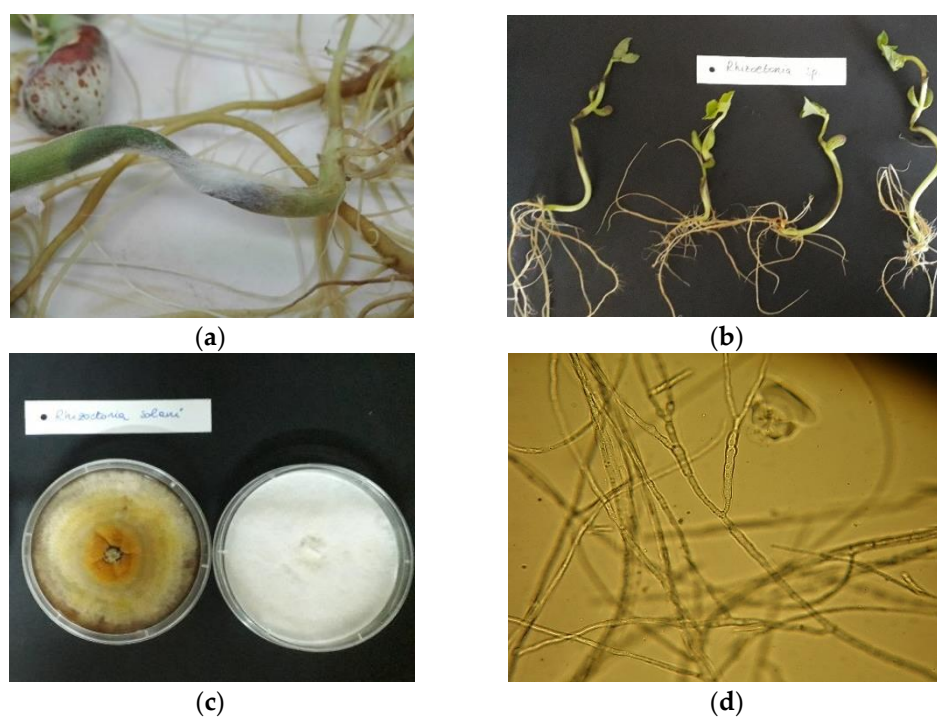

(a)

(b)

(c)

(d)

Figure 4. Symptoms, macroscopic and microscopic characters of *R. solani*: (**a**) hydropic halo and felted mycelium, wrapped in a ring on the stem of a "Cannellino rosso" ecotype seedling (data from [45]); (**b**) collar and root rot symptoms on "Ciuoto" ecotype; (**c**) different macroscopic mycelium characteristics of the fungus isolated on "Cannellino rosso" ecotype; (**d**) fungal branching arranged at acute−right angle and restricted in their insertion point (resolution 40×).

Figure 4c clearly shows binucleated *R. solani* isolates with considerable morphological variability from white to creamy−yellow, with a felty appearance for the white mycelium, while a daily growth rings with variable tonality in the other. The conidiophores branches of the *R. solani* isolates resulted fold in the direction of growth and slightly narrowed at the point of insertion on main hypha; moreover, the secondary conidiophore branches reached right−acute angles on the main hypha at maturity (Figure 4d).

Figure 5a reports typical anthracnose symptoms on seeds detected by between paper test as spots, more or less wide, and surrounded by a purple halo, as well as some macroscopic and microscopic morphological structures identified as the fungus *C. lindemuthianum* isolated from "Ciuoto" ecotype, located in Paterno (2018).
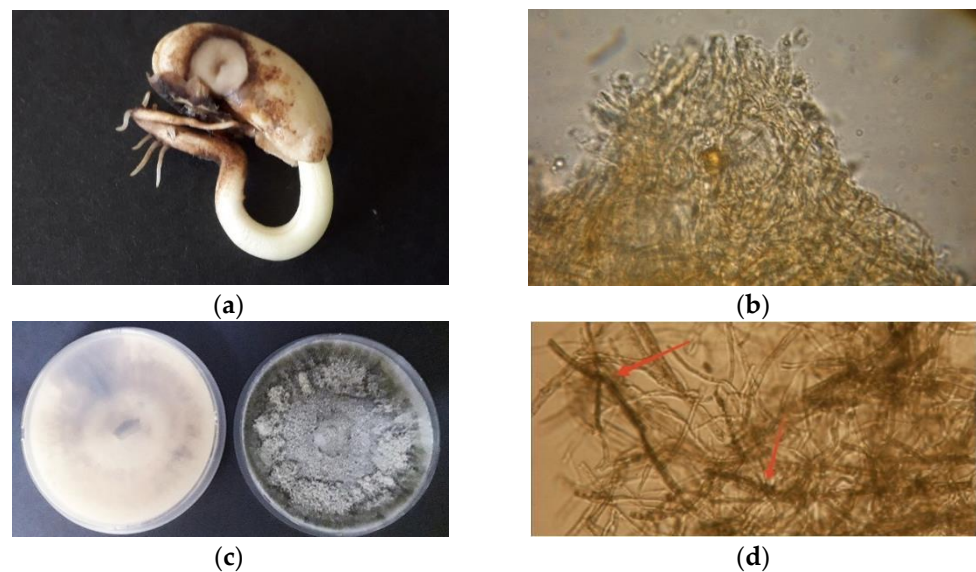
Figure 5. Symptoms, macroscopic and microscopic characters of *C. lindemuthianum*: (**a**) symptoms of anthracnose on integuments and cotyledons of "Ciuoto" ecotype located in Paterno (2018); (**b**) acervulus similar to sporodochium from seeds, with bristles in evidence (resolution 20×); (**c**) macroscopic mouse−grey colored aerial mycelium characteristic of the fungus; (**d**) detail of acervulus similar to sporodochium from plates, with subulate, terminal and septate bristles in evidence, where the red arrow indicates their basal portion (resolution 100×).

C. lindemuthianum is a mitosporic fungus with four physiological breeds known to have different degrees of pathogenicity compared to common bean cultivars. Colonies are from salmon−gray to mouse−gray in color, as shown in our isolates (Figure 5c), and produce acervules consisting of a compact layer of cylindrical, hyaline, curved, pigmented phialides (Figure 5b). The typical acervules of *C. lindemuthianum* with dark, scattered, 5–9 septate and pigmented bristles are depicted in Figure 5d.

The morphological characteristics of *F. oxysporum* are reported in Figure 6. Typical rot seeds were revealed, characterized by the presence of abundant aerial, white, felty, flaky with a purplish central mass mycelium, as also confirmed by the macroscopic appearance of the colonies on the plate (Figure 6a,b). The macroconidia were slightly curved, with three to five septa, and hooked basal and short apical cells, as is typical of this fungus; even the microconidia appeared to be typically formed at the end of the phialidia, and were abundant, fusiform, reniform, and produced by branched polyphialides (Figure 6c).
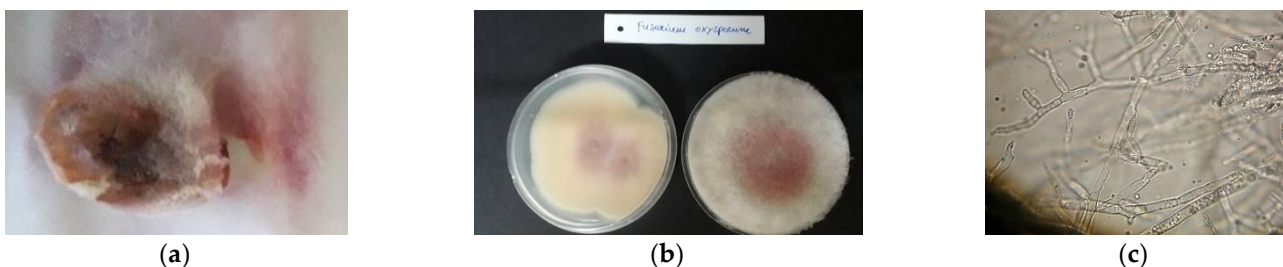

Figure 6. Symptoms, macroscopic and microscopic characteristics of *F. oxysporum*: (**a**) mycelium on the integument, with a characteristic vinous aura, on "Ciuoto" ecotype seed, located in Paterno (2019); (**b**) macroscopic mycelium of the fungus with its characteristic dark purple color; (**c**) fungal conidiophora with branched polyphialids (resolution 100×).

4. Discussion

Seed health is a critical factor in achieving a good product and high yields. The presence of pathogens in or on the seed influences all the plant vegetative and productive

phases. Seeds intended for food may not only have little nutritional value, they may also be contaminated with mycotoxins. Therefore, the health of the seed is an indispensable requirement for the productivity and quality of the crop. For the isolation and identification of the seed-borne pathogens of common beans, diagnostic methods with different sensitivities were used. The washing test method allows to identify the spores of the fungi contaminating the seed surface [32,33]. Therefore, it proved to be the most effective and sensitive compared to the other ones for this purpose. In fact, the washing test made it possible to detect most of the fungi contaminating the integument and infecting the cotyledons and the embryo. There were 18 seed-borne fungal agents. Most of these (*R. solani*, *C. lindemuthianum*, *F. oxysporum*, *F. solani*, *U. appendiculatus*) are of great importance due to the damages they cause to the seed in pre− and post−emergence, thus causing economic losses [46]; the other fungal pathogens detected are no less dangerous (*Alternaria alternata*, *Penicillium* spp., *A. flavus*, *A. niger*, *M. hiemalis*, *Fusarium* spp.), and determine substantial problems linked to mycotoxin production during post−harvest storage [16]. This method also made it possible to identify beneficial fungi (*T. harzianum* and *T. viridae*) known to be used as antagonists of harmful pathogens [47–49].

The blotter method, unlike the washing test, is more selective towards some pathogens with saprophytic behavior (*Aspergillus* spp., *Cladosporium* spp., *Botritys* spp., *Mucor* spp., *Rhizophus* spp.). The treatment of the seeds with 1% Na−hypochlorite allowed a reduction of most of fungal microflora adhering to the seed, but did not make it possible to eliminate the systemic pathogens infecting the internal structures of the seed, such as *C. lindemuthianum*, *F. solani*, and *R. solani*. Nevertheless, the treatment was able to decrease the presence of some conidia and/or mycelia in the coat and on the seed surface, thus decreasing the disease incidence of *R. solani*. The between paper method, applied as international protocol specific for detecting *C. lindemuthianum*, revealed its presence in both ecotypes, years and areas.

Regarding thermo−hygrometric data, collected during the entire crop cycle, the average air temperature was 17.20 °C in 2018 and 18.08 °C in 2019, without significant difference between the two years, while the maximum temperatures, equal to 26.13 °C and 27.78 °C during 2018 and 2019, respectively, were significatively different. This finding could explain the prevalent presence of the mesophilic fungal species detected during the year 2018, when their spores were able to germinate at humidity values of 55–75%, such as *Alternaria* spp., *A. alternata*, *C. cladosporioides*, *Penicillium* spp., *Fusarium* spp., *Colletotrichum* spp., and *R. solani* [38,42].

Temperature plays an important role in sporulation, germination of conidia and in the development of fungal pathogens. Table 5, built taking into account scientific studies investigating the development of the same isolated and identified phytopathogenic fungi in the current study, clearly indicates that they can develop between −5 and 50 °C, with optimal growth around 25 °C.

In particular, fungal species found on the two "Fagioli di Sarconi" PGI common bean ecotypes seeds, belonging to thermophilic fungi (development at maximum temperature of 45–50 °C, and minimum growth temperature not lower than 20 °C), were *A. niger* and *M. hiemalis.* The detected mesophilic fungi (development between 10 and 40 °C, with optimum at 25 °C) were *Alternaria* spp., *A. alternata*, *Aspergillus* spp., *A. flavus*, *C. lindemuthianum*, *F. oxysporum*, *F. solani*, *R. solani*, *U. appendiculatus* and *Trichoderma* spp. Finally, *C. cladosporioides*, *Botrytis* spp., *R. nigricans*, *Penicillium* spp., *P. expansum* were the detected cryophilic fungi (optimum development between 5 and 10 °C).

Table 5. Referenced temperature ranges (°C) for all isolated seed-borne fungal species.

Mycoflora	−5	0	5	10	15	20	25	30	35	40	45	50	References
Alternaria spp.						x	x	x	x				[50]
Alternaria alternata (Fr.) Keissler						x	x	x	x				[51]
Aspergillus spp.			x	x	x	x	x	x	x				[38]
Aspergillus flavus Link				x	x	x	x	x	x	x			[38]
Aspergillus niger van Tieghem							x	x	x	x	x	x	[39]
Cladosporium cladosporioides G.A. de Vries	x	x	x	x	x	x	x	x	x	x			[38]
Botrytis spp.	x	x	x	x	x	x	x	x	x				[38]
Colletothricum lindemuthianum Briosi e Cavara					x	x	x	x					[42]
Fusarium oxysporum von Schlechtendal					x	x	x	x					[50]
Fusarium solani (Mart.) Sacc.					x	x	x	x					[50]
Mucor hiemalis Wehmer						x	x	x	x	x	x	x	[42]
Penicillium expansum Link	x	x	x	x	x	x	x	x					[42]
Penicillium spp.	x	x	x	x	x	x	x						[42]
Rhizoctonia solani (Cooke) Wint.				x	x	x	x						[52]
Rhizophus nigricans Vuillemin		x	x	x	x	x	x	x	x				[50]
Trichoderma spp.						x	x						[39]
Uromyces appendiculatus (Pers.) Link						x	x	x					[51]

The temperatures are indicated by the symbol x.

For the years 2018 and 2019 the mean minimum relative humidity was 42.39% and 37.58%, respectively, while the average relative humidity values were 75.09% and 71.25%. These values indicate a small, but significant difference between the two years. The humidity, as well as air temperature, is important for the development of phytopathogenic fungi. In particular, xerophilic fungi prefer dry environment, where spores germinate with low relative humidity values (<55%), as indicated in Table 6, built in the same way of that of temperature. *Aspergillus* spp., *A. niger* and *A. flavus* were the xerophilic fungi detected in the current study.

Table 6. Referenced relative humidity ranges (%) for all isolated seed-borne fungal species.

Mycoflora	<55	55 < 75	>75	References
Alternaria spp.		x		[50]
Alternaria alternata (Fr.) Keissler		x		[51]
Aspergillus spp.	x			[38]
Aspergillus flavus Link	x			[38]
Aspergillus niger van Tieghem	x			[39]
Cladosporium cladosporioides G.A. de Vries		x		[38]
Botrytis spp.			x	[38]
Colletothricum lindemuthianum Briosi e Cavara		x		[42]
Fusarium oxysporum von Schlechtendal		x		[50]
Fusarium solani (Mart.) Sacc.		x		[50]
Mucor hiemalis Wehmer			x	[42]
Penicillium expansum Link		x		[42]
Penicillium spp.		x		[42]
Rhizoctonia solani (Cooke) Wint.			x	[52]
Rhizophus nigricans Vuillemin			x	[50]
Trichoderma spp.		x		[39]
Uromyces appendiculatus (Pers.) Link		x		[51]

The relative humidities are indicated by the symbol x.

We found that most seed-borne isolated fungi were mesophilic with optimal growth relative humidity values between 55 and 75%, as mentioned above, including *Alternaria* spp., *A. alternata*, *C. cladosporioides*, *Penicillium* spp., *Fusarium* spp., *Colletotrichum* spp. Among

the hygrophilous fungi, having spores that germinate with humidity above 75%, we found *R. solani*, *Botrytis* spp., and *Mucor* spp.

R. solani is a polyphagous pathogen that attacks different plant portions from the soil surface. The early symptoms are detected on the cotyledon leaves, at the base of the stem with typical dropsy areas followed by bottlenecks and brown spots on the young roots. This pathogen multiplies by repeatedly spreading its mycelium. In fact, the infections occur due to the mycelium, which is able to spread into the young tissues of the plant. The preservation of the fungus occurs in the soil as mycelium and as pseudosclerotia able to survive 6–7 years. *R. solani* has a wide distribution in temperate regions, where the optimal development temperature ranges from 15 to 22 °C, with frequent rainfall [53].

The different temperature and relative humidity in the two reference years explains the different disease incidence of some seed-borne fungi. In fact, during 2018, characterized by lower maximum temperature and higher minimum and average humidity than to 2019, we recorded a greater rhizoctoniosis incidence induced by *R. solani* for both ecotypes and areas.

Anthracnose is the most severe fungal adversity of common bean. The etiological agent *C. lindemuthianum* is kept in the seed and in the soil as mycelium for up to 5 years [19]. *F. oxysporum* is a pathogen perpetuated in the soil, where it is kept for several years thanks to the chlamydospores [24]. The development of these two pathogens, mesophilic species, is affected by high rainfall and humidity. For this reason, it is possible also for anthracnose and fusariosis to explain the higher incidence of the disease in the year 2019 compared to the previous one as being due to the lower humidity.

In addition, concerning the incidence of anthracnose by *C. lindemuthianum*, of fusariosis by *F. oxysporum* and of bacteriosis, the results highlighted that the "Cannellino rosso" ecotype was less susceptible to plant diseases compared to the "Ciuoto" one. The reasons are to be found not only in the higher integumental thickness of "Cannellino rosso" with respect to others local ecotypes [12,54], but probably also in the higher content of polyphenolic compounds (hydrolysable tannins and condensed tannins) present in the integument, which are considered protective factors for the seed against seed-borne pathogens [55–57] and also considered potentially able to constitute a defense mechanism against fungal attack and mycotoxin production in beans [58]. Conversely, only the Na−hypochlorite treatment induced less susceptibility to rhizoctoniosis by *R. solani* in the "Cannellino rosso" ecotype.

5. Conclusions

The phytosanitary screening of seeds of "Fagioli di Sarconi" PGI common bean ecotypes "Ciuoto" and "Cannellino rosso", cultivated in the areas of Paterno and Sarconi in the Agri Valley, made it possible to detect the fungal and bacterial microflora harmful to the quality of the seeds. It was possible to individuate the "Cannellino rosso" as being less susceptible to the majority of diseases caused by the fungi and bacteria identified. Moreover, the incidence of the pathogens analyzed in the current study was area−, environment− and ecotype−dependent. Data on seed health with respect to different common bean cultivars, meteorological conditions or cultivation location are missing. Therefore, the present study represents baseline information for further disease resistance breeding studies, development of forecasting models and management of seed−borne diseases associated with common beans.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interests.

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