agriculture

MDPI

Article

Colletotrichum Species on Cultivated Solanaceae Crops in Russia

Maria Yarmeeva [1], Irina Kutuzova [1], Michael Kurchaev [2], Elena Chudinova [2], Ludmila Kokaeva [1,2], Arseniy Belosokhov [3], Grigory Belov [4], Alexander Elansky [2], Marina Pobedinskaya [1], Archil Tsindeliani [2], Yulia Tsvetkova [1,5] and Sergey Elansky [1,2,*]

1 Lomonosov Moscow State University, 119991 Moscow, Russia
2 Peoples' Friendship University of Russia (RUDN University), 117198 Moscow, Russia
3 Michurina St., 54, Michurinsk, 393760 Tambov, Russia
4 Russian Potato Research Centre, 140051 Moscow, Russia
5 All-Russian Plant Quarantine Center (VNIIKR), 140150 Moscow, Russia
* Correspondence: elanskiy_sn@pfur.ru

Abstract: *Colletotrichum* species are the causal agents of potato and tomato diseases, such as black dot and anthracnose. Several new species and species complexes were recently established. Thereby, a reassessment of the genus diversity is required. The study revealed two species, *Colletotrichum coccodes* and *Colletotrichum nigrum*, as Russia's main disease agents of cultivated Solanaceae plants. Black dot and anthracnose in potato were caused exclusively by *C. coccodes*, whereas the same diseases in tomato, eggplant, and pepper were predominately caused by *C. nigrum*. However, one isolate of *C. coccodes* was also identified as an agent of the tomato disease. Five potentially hybrid isolates were discovered. Morphological examination and pathogenicity assessment revealed no significant differences between the two *Colletotrichum* species. All isolates were sensitive to the fungicides azoxystrobin, difenoconazole, and thiabendazole, which are currently used in agriculture. This is the first report of the occurrence of *C. nigrum* in Russia.

Keywords: black dot; anthracnose; pathogen; potato; tomato; *Colletotrichum*; multi-gene phylogeny

Citation: Yarmeeva, M.; Kutuzova, I.; Kurchaev, M.; Chudinova, E.; Kokaeva, L.; Belosokhov, A.; Belov, G.; Elansky, A.; Pobedinskaya, M.; Tsindeliani, A.; et al. *Colletotrichum* Species on Cultivated Solanaceae Crops in Russia. *Agriculture* **2023**, *13*, 511. https://doi.org/10.3390/agriculture13030511

Academic Editor: Yuan Li

Received: 30 December 2022
Revised: 12 February 2023
Accepted: 16 February 2023
Published: 21 February 2023

1. Introduction

Colletotrichum is a well-known causal agent of potato and tomato diseases such as black dot and anthracnose, dramatically damaging both underground and aboveground plant parts. Although several revisions of the genus *Colletotrichum* were recently published [1–4], it still remains taxonomically puzzling. Currently, 16 species complexes and 15 singleton species (e.g., *C. coccodes* and *C. nigrum*) are established within *Colletotrichum* [5]. Given the complicated systematics of the genus, the species identification is based on morphological features, combined with molecular data.

C. coccodes predominantly infects Solanaceae plants, including chilli fruit [6], potato tubers [7,8], tomato [9], sweet pepper [10–12], black nightshade [13], and eggplant [14]. Nevertheless, its wide host range is not limited to Solanaceae, since the species was reported to infect strawberry [15], pumpkin [16], or onion [17].

There are very few reports concerning *C. nigrum*. The species was first described as an agent of pepper anthracnose from Gloucester County, New Jersey, USA [18], but it can be associated with tomato and eggplant diseases [19,20] as well. Liu and colleagues [17,21] reviewed the species' description, introduced a neotype to *C. coccodes*, selected an epitype to *C. nigrum*, and stated the ability of both species to induce anthracnose.

In Russia, the diversity within the genus is poorly described, due to the predominance of the morphological identification of causal agents. The review by Kotova and Kungurtseva [22] specified that *C. coccodes* is the only cause of potato and tomato black dot and anthracnose. Several studies in Russia investigated the diversity of *Colletotrichum* species on potato and tomato leaves using genetic markers [9,23,24] directly from the plant

material, without isolating the species in axenic cultures. Kazartsev and colleagues [25] recently scrutinized the diversity of *Colletotrichum* species on several wild and cultivated plants (no potato or tomato plants were included into the analysis), using molecular and morphological approaches to identify the species. *C. coccodes* strains were isolated from *Ambrosia artemisiifolia*, *Beta vulgaris*, *Brassica napus*, *Cannabis sativa*, *Galinsoga parviflora*, and *Portulaca oleracea*. Poluektova and colleagues [26] analysed the *glyceraldehyde-3-phosphate dehydrogenase* and *glutamine synthetase* genes of four *C. coccodes* strains from Russian potato. To the best of our knowledge, these are the only molecular investigations of the genus in Russia to date.

This study focuses on the disease agents of several cultivated Solanaceae crops in Russia. Our research combines both morphological and molecular approaches to reveal the diversity within the genus *Colletotrichum*. To this end, four genetic markers were used: ITS1-5.8S-ITS2 region (ITS) as a well-established barcode, the *glyceraldehyde-3-phosphate dehydrogenase* gene intron (*gaphd*), considered the most reliable genetic marker for *Colletotrichum* species, the *actin* intron (*act*), and the *glutamine synthetase* intron (*gs*). To estimate the agricultural risks of the disease spread, we assessed the sensitivity to some fungicides that are officially used for tuber treatment, and the pathogenicity range towards the tomato fruit and potato tuber slices.

2. Materials and Methods

2.1. Sampling and Isolation of Cultures

Samples were collected from the fruits of tomato, eggplant, and pepper (Table 1 and Figure 1) as well as from potato tubers, leaves, and stems. Seed potato tubers from the Netherlands, Germany, Australia, Cyprus, and Uganda were taken for comparison. All isolation sources were surface-sterilized with sodium hypochlorite (2% solution) to remove possible contamination, sliced, and put in wet chambers at 24 ± 1 °C. For isolation, small black sclerotia from the tuber peel or diseased tissue were taken using a preparation needle under a binocular microscope (MBS10, Russia), and transferred to culture media (potato-dextrose agar, PDA) amended with antibiotic (benzylpenicillin sodium salt, 100 mg/L).

Table 1. Details of isolates used in the study.

Strain Identifier	Origin * (Figure 1)	Host Plant	Isolation Source	Year of Isolation	GenBank Accession Numbers **			
					ITS	*gaphd*	*act*	*gs*
C13V(GH)PT1/1	1	Potato	Tuber	2013	OP718477	OP743730	OP743793	OP743860
C13K(S)PT11	2	Potato	Tuber	2013	OP718470	OP743723	OP743786	OP743853
C13K(S)PT14	2	Potato	Tuber	2013	OP718471	OP743724	OP743787	OP743854
C13K(S)PT15	2	Potato	Tuber	2013	OP718472	OP743725	OP743788	OP743855
C13K(S)PT17	2	Potato	Tuber	2013	OP718473	OP743726	OP743789	OP743856
C13K(S)PT21	2	Potato	Tuber	2013	OP718474	OP743727	OP743790	OP743857
C13K(S)PT34	2	Potato	Tuber	2013	OP718475	OP743728	OP743791	OP743858
C13K(S)PT58b	2	Potato	Tuber	2013	OP718476	OP743729	OP743792	OP743859
C13HPT29/2	3	Potato	Tuber	2013	OP718469	OP743722	OP743836	OP743890
C13G(B)PTde8/2	4	Potato	Tuber	2013	OP718463	OP743716	OP743830	OP743844
C13G(B)PTde9	4	Potato	Tuber	2013	OP718464	OP743717	OP743831	OP743845
C13G(B)PTde12	4	Potato	Tuber	2013	OP718461	OP743714	OP743828	OP743842
C13G(B)PTde23	4	Potato	Tuber	2013	OP718462	OP743715	OP743829	OP743843
C13G(B)PTes6	4	Potato	Tuber	2013	OP718466	OP743719	OP743833	OP743847
C13G(B)PTes19	4	Potato	Tuber	2013	OP718465	OP743718	OP743832	OP743846
C13G(B)PTal15	4	Potato	Tuber	2013	OP718456	OP743709	OP743823	OP743837
C13G(B)PTal19	4	Potato	Tuber	2013	OP718457	OP743710	OP743824	OP743838
C13G(B)PTal20	4	Potato	Tuber	2013	OP718458	OP743711	OP743825	OP743839
C13G(B)PTal23	4	Potato	Tuber	2013	OP718459	OP743712	OP743826	OP743840
C13G(B)PTal24	4	Potato	Tuber	2013	OP718460	OP743713	OP743827	OP743841
C13G(B-Sh)PTsa5	5	Potato	Tuber	2013	OP718468	OP743721	OP743835	OP743849
C13G(B-Sh)PTsa29	5	Potato	Tuber	2013	OP718467	OP743720	OP743834	OP743848
C14M(Ch)PT6	6	Potato	Tuber	2014	OP718479	OP743732	OP743795	OP743862

Table 1. *Cont.*

Strain Identifier	Origin * (Figure 1)	Host Plant	Isolation Source	Year of Isolation	GenBank Accession Numbers **			
					ITS	*gaphd*	*act*	*gs*
C14M(Ch)PT18/2	6	Potato	Tuber	2014	OP718478	OP743731	OP743794	OP743861
C15M(L)PT1	7	Potato	Tuber	2015	OP718480	OP743733	OP743796	OP743863
C15M(L)PT1/2	7	Potato	Tuber	2015	OP718481	OP743734	OP743797	OP743864
C15M(L)PT4	7	Potato	Tuber	2015	OP718482	OP743735	OP743798	OP743865
C15M(L)PT5	7	Potato	Tuber	2015	OP718483	OP743736	OP743799	OP743866
C15M(L)PT6	7	Potato	Tuber	2015	OP718484	OP743737	OP743800	OP743867
C15M(L)PT7	7	Potato	Tuber	2015	OP718485	OP743738	OP743801	OP743868
C16ME(Y-O)PL7	8	Potato	Leaf	2016	OP718490	OP743743	OP743806	OP743873
C16ME(Y-O)PL11	8	Potato	Leaf	2016	OP718489	OP743742	OP743802	OP743872
C16M(G)PS9	9	Potato	Stem	2016	OP718488	OP743741	OP743805	OP743871
C16M(G)PS15	9	Potato	Stem	2016	OP718486	OP743739	OP743803	OP743869
C16M(G)PS16b	9	Potato	Stem	2016	OP718487	OP743740	OP743804	OP743870
C17K(K)TF5-2	10	Tomato	Fruit	2017	OP718492	OP743745	OP743808	OP743875
C17K(K)TF5-14	10	Tomato	Fruit	2017	OP718491	OP743744	OP743807	OP743874
C17K(S)PTrs9	11	Potato	Tuber	2017	OP718494	OP743747	OP743810	OP743877
C17K(S)PTrs11/1	11	Potato	Tuber	2017	OP718493	OP743746	OP743809	OP743876
C18M(L)TF1/1	7	Tomato	Fruit	2018	OP718496	OP743749	OP743822	OP743889
C18K(S)TF1/2	12	Tomato	Fruit	2018	OP718495	OP743748	OP743811	OP743878
C18U(G)TF1/1	13	Tomato	Fruit	2018	OP716941	OP730520	OP743774	OP743898
C18U(G)PT4	13	Potato	Tuber	2018	OP718500	OP743753	OP743815	OP743882
C18U(G)PT6	13	Potato	Tuber	2018	OP718501	OP743754	OP743816	OP743883
C18U(G)PT7	13	Potato	Tuber	2018	OP718502	OP743755	OP743817	OP743884
C18U(G)PT11	13	Potato	Tuber	2018	OP718499	OP743752	OP743814	OP743881
C18TPS8	14	Potato	Stem	2018	OP718497	OP743750	OP743812	OP743879
C18TPS9	14	Potato	Stem	2018	OP718498	OP743751	OP743813	OP743880
C19CyPT1/2	15	Potato	Tuber	2019	OP718503	OP743756	OP743783	OP743850
C19CyPT2/1	15	Potato	Tuber	2019	OP718504	OP743757	OP743784	OP743851
C20AuPT5a	16	Potato	Tuber	2020	OP718505	OP743758	OP743785	OP743852
C20UgLaPT1/1	17	Potato	Tuber	2020	OL405711	OP743762	OP743821	OP743888
C20UgKgPT1	17	Potato	Tuber	2020	OP718506	OP743759	OP743818	OP743885
C20UgKgPT2	17	Potato	Tuber	2020	OP718508	OP743761	OP743820	OP743887
C20UgKgPT12	17	Potato	Tuber	2020	OP718507	OP743760	OP743819	OP743886
C21KST1F1	12	Tomato	Fruit	2021	OP716934	OP730512	OP743775	OP743891
C21KSTF9	12	Tomato	Fruit	2021	OP716939	OP730517	OP743780	OP743896
C21KST3F1	12	Tomato	Fruit	2021	OP716935	OP730513	OP743776	OP743892
C21KST3F2	12	Tomato	Fruit	2021	OP716936	OP730514	OP743777	OP743893
C21KSTF88	12	Tomato	Fruit	2021	OP716938	OP730516	OP743779	OP743895
C21KSTF77	12	Tomato	Fruit	2021	OP716937	OP730515	OP743778	OP743894
C21KSTF97	12	Tomato	Fruit	2021	OP716940	OP730518	OP743781	OP743897
C21KSTF98	12	Tomato	Fruit	2021	OP716941	OP730519	OP743782	OP743899
C21KSPeF3	12	Pepper	Fruit	2021	OP716931	OP743706	OP743771	OP743908
C21KSPeF4	12	Pepper	Fruit	2021	OP716932	OP743707	OP743772	OP743909
C21KSPeF6	12	Pepper	Fruit	2021	OP716933	OP743708	OP743773	OP743910
C21KSPeF20	12	Pepper	Fruit	2021	OP716930	OP743705	OP743770	OP743907
C21KSPeF19	12	Pepper	Fruit	2021	OP716929	OP743704	OP743769	OP743906
C21KSEgF1	12	Eggplant	Fruit	2021	OP716923	OP743698	OP743763	OP743900
C21KSEgF3	12	Eggplant	Fruit	2021	OP716924	OP743699	OP743764	OP743901
C21KSEgF4.1	12	Eggplant	Fruit	2021	OP716925	OP743700	OP743765	OP743902
C21KSEgF5	12	Eggplant	Fruit	2021	OP716926	OP743701	OP743766	OP743903
C21KSEgF6	12	Eggplant	Fruit	2021	OP716927	OP743702	OP743767	OP743904
C21KSEgF7	12	Eggplant	Fruit	2021	OP716928	OP743703	OP743768	OP743905

* Geographical origins of the isolates. Russia: 1—Vladimir Region; 2, 11—Kostroma Region; 6, 7, 9—Moscow Region; 8—the Mari El Republic; 10, 12—Krasnodar Krai; 13—Primorsky Krai; 14—the Republic of Tatarstan; 3—the Netherlands; 4, 5—Germany; 15—the Republic of Cyprus; 16—Australia; 17—Uganda. ** ITS—ITS1-5, 8S-ITS2 region, *gaphd*—glyceraldehyde-3-phosphate dehydrogenase gene intron, *act*—actin intron, *gs*—glutamine synthetase intron.

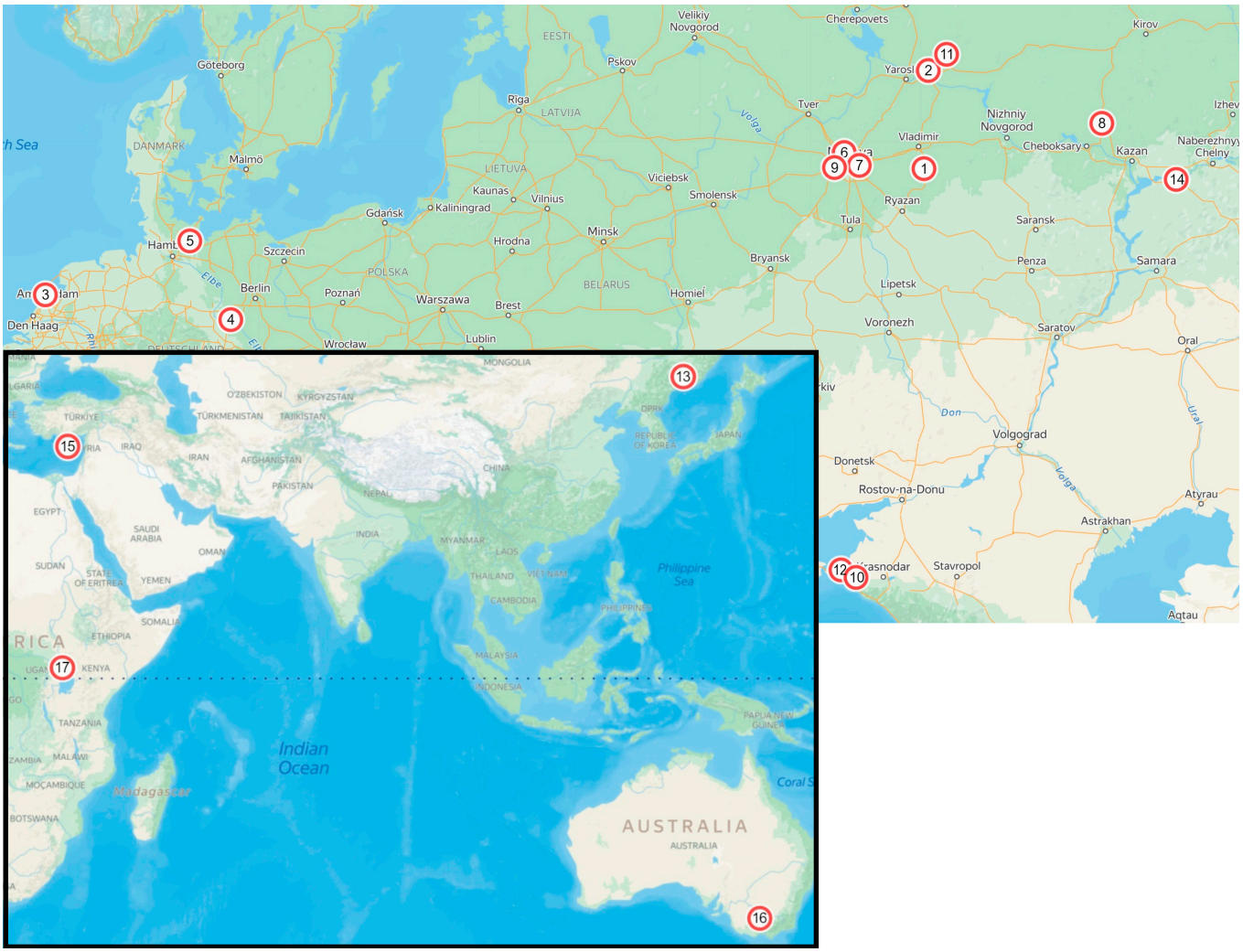

Figure 1. Location of collection sites (see also Table 1). Russia: 1—Vladimir Region; 2, 11—Kostroma Region; 6, 7, 9—Moscow Region; 8—the Mari El Republic; 10, 12—Krasnodar Krai; 13—Primorsky Krai; 14—the Republic of Tatarstan; 3—the Netherlands; 4, 5—Germany; 15—the Republic of Cyprus; 16—Australia; 17—Uganda.

2.2. DNA Isolation, PCR, Sequencing, and Phylogenetic Analysis

To extract DNA, the mycelium of fungi was grown on a liquid pea medium (180 g of green pea boiled for 10 min in 1 L of water, then filtered and autoclaved for 30 min at 1 atm). DNA was extracted according to the standard CTAB protocol [27,28]. ITS, *act*, and *gaphd* amplifications were performed in a SSI microtube strips in a 25 µL total volume reaction containing 1 µL of a DNA template (50 ng/µL), 2.5 µL of 10× PCR buffer (Applied Biosystems, Waltham, MA, USA), 0.5 µL of 10 mM each deoxyribonucleotide triphosphates (dNTP), 0.4 µL of 100µM each primer (Evrogen Co, Moscow, Russia), 1.5 U of Taq polymerase (5U/µL, Promega, Madison, WI, USA), and Milli-Q water (MQ). For the amplification of *gs* 2.8 µL of each dNTP was used; the concentrations of the other components remained the same. The following primers were used: ITS1 5′-TCCGTAGGTGAACCTGCGG-′3 and ITS4 5′-TCCTCCGCTTATTGATATGC-3′ for the ITS region [29], GSF1 5′-ATGGCCGAGTACATCTGG-′3 and GSR1 5′-GAACCGTCGAAGTTCCAC-′3 for the *gs* gene [30], GDF-1 5′-GCCGTCAACG ACCCCTTCATTGA-′3 and GDR-1 5′-GGGTGGAGTCGTACTTGAGCATGT-′3 for *gaphd* [31], and ACT-512F 5′-ATGTGCAAGGCCGGTTTCGC-′3 and ACT-783R 5′-TACGAGTCCTTCTGG CCCAT-′3 for *act* [32].

The PCR protocol included initial denaturation at 94 °C for 3 min, 35 amplification cycles, and an additional extending step at 72 °C for 3 min. For the primer pair ITS1/ITS4,

the amplification cycles were 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s. For the primer pairs GDF-1/GDR-1 and ACT-512F/ACT783R, the amplification cycles were 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s. For the primer pair GSF1/GSR1, the amplification cycles were 94 °C for 30 s, 61 °C for 30 s, and 72 °C for 120 s.

The amplification was performed on a T100 Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). PCR products were run in 0.7–1.2% agarose gel amended with ethidium bromide; the agarose concentration depended on the PCR fragment length. The gel extraction was performed with a Cleanup Mini Kit (Evrogen Co., Russia). The PCR fragments were sequenced using the BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and the Applied Biosystems 3730 × l automated sequencer (Applied Biosystems, USA). Each fragment was sequenced in both directions. Consensus sequences for each locus were assembled using Geneious version 7.1 (created using Biomatters) and MEGA X [33], and aligned with available type species sequences (Table 2). Species identification was based primarily on the *gaphd* sequence.

Table 2. Reference strains used in this study.

Species	Species Complex	Strain Identifier	Host	GenBank Accession Numbers *			
				ITS	*act*	*gaphd*	*gs*
C. nigrum	singleton	CBS 69.49	*Capsicum* sp.	NR163523	JX546646	JX546742	-
C. nigrum	singleton	CBS 132450	*Solanum lycopersicum*	JX546845	JX546653	JX546749	-
C. nigrum	singleton	CBS 127562	*Cichorium intybus*	JX546842	JX546650	JX546746	-
C. dianense	singleton	YMF 1.04943	*Alternanthera philoxeroides*	OL842189	OL981258	OL981284	-
C. coccodes	singleton	CBS 369.75	*Solanum tuberosum*	HM171679	HM171667	HM171673	HM171676
C. gigasporum	Gigasporum	CBS 101881	*Cyphomandra betacea*	KF687736	KF687797	KF687841	KF687745

* ITS—ITS1-5.8S-ITS2 region, *gaphd*—glyceraldehyde-3-phosphate dehydrogenase gene intron, *act*—actin intron, *gs*—glutamine synthetase intron.

2.3. Morphological Analysis

The cultures were grown on synthetic nutrient-poor agar medium (SNA) [34] amended with *Anthriscus sylvestris* double autoclaved stems [16] for 10 days. Microscopic preparations were made in clear lactic acid. The width and length of conidia were measured, with 30 measurements per structure, using a Leica DM 2500 (Leica Microsystems, Wetzlar, Germany).

2.4. Pathogenicity Tests

To compare the pathogenic activity, 10 isolates (two from potato, two from pepper, three from tomato, and three from eggplant) were chosen. Healthy cherry tomato fruits and potato tubers (cultivar "Gala") were washed and surface-sterilised in 0.5% sodium hypochlorite solution for 5 min, rinsed in distilled water, and air-dried. The potato tubers were sliced to imitate wounding. Two types of tomato fruit were used: wounded with sterile tips and unwounded. The experiment was conducted with three repeats for each strain of each kind of inoculation. The wounded fruits were internally inoculated with 100 µL of conidial suspension (concentration 10^5 spores/mL). The unwounded fruits and potato slices were surface-inoculated with mycelium and conidia, and placed in sterile wet chambers. The control fruits and tubers were surface or internally inoculated with distilled water. Each wet chamber was stored at 10 °C for 21 or 35 days, and the radius of the lesion was measured. To fulfil Koch's postulate, a small tissue sample was taken from the margin of the disease area with a sterile scalpel and placed in a Petri dish on PDA.

2.5. In Vitro Assessment of Fungicide Sensitivity

Three chemical fungicides: azoxystrobin (Quadris®, Syngenta, Basel, Switzerland), difenoconazole (Score®, Syngenta), and thiabendazole (Tecto®, Syngenta) were chosen to evaluate their efficiencies against *Colletotrichum* isolates. The fungicides were selected based on their current use in Russia for tuber or in-furrow treatment, and they were obtained from local suppliers. The sensitivity was evaluated in Petri plates with PDA. The fungicides were added at different concentrations to autoclaved PDA medium to produce a concentration series of 0, 0.1, 1, 10, and 100 mg/L for each fungicide (active ingredient). The mycelial plug (5 mm in diameter) of each isolate was punched from the margin of an actively growing colony of a 5-day-old culture and placed in the centre of a 90 mm PDA plate amended with fungicide, as well as on non-amended PDA plates (controls). Three replicates per treatment were produced, and the plates were incubated at 24 ± 1 °C for 4 days. The diameter of the fungal colony on each plate was measured at perpendicular angles. The average of the two measurements was used to calculate the fungicide concentration inhibiting linear colony growth of 50% over control (EC_{50}) [35,36].

3. Results

In total, 74 isolates were analysed: 50 from potato (*Solanum tuberosum* L.), 11 from tomato (*Solanum lycopersicum* L.), 7 from pepper (*Capsicum annuum* L.), and 6 from eggplant (*Solanum melongena* L.). All strains isolated from potato, regardless of the plant organ, were identified as *C. coccodes*, while those from eggplant and pepper proved to be related to *C. nigrum* (Figures 2–6). Almost all tomato isolates except one strain (C18M(L)TF1/1) were identified as *C. nigrum*. The aligned concatenated sequence dataset of the original isolates was 1690 bp long (440, 207, 233, and 810 bp for the ITS, *act*, *gaphd*, and *gs* sequences, respectively). It contained 65 variable sites: 2 in the ITS region, 2 in the *act* intron, 12 in the *gaphd* intron, and 49 the in *gs* second intron (including three deletions); among them, 2 in *act*, 8 in *gaphd*, and 17 in the *gs* intron seemed to be specific to either *C. coccodes* or *C. nigrum*, and they may be used to differentiate between these species.

Most *C. coccodes*, but also several *C. nigrum* (possible hybrids) isolates, contained a 25 bp insertion in the *gs* intron (Figure 7). All the nucleotide differences were found in the non-coding regions. All the phylogenetic trees, except one based on the ITS region (Figure 3), showed two well-delimited clades corresponding to two segregate species: *C. coccodes* and *C. nigrum* (Figures 2 and 4–6). The recently described *C. dianense* was in the same clade as *C. nigrum* (Figures 4 and 5).

Among the tomato and pepper *C. nigrum* strains, five intriguing isolates (C18U(G)TF1/1, C21KST1F1, C21KST3F1, C21KSTF77, and C21KSPeF20) were found (Figure 7). Presumably, they shared the features of both species—*C. coccodes* and *C. nigrum*. All of the isolates were identified as *C. nigrum*, based on the *gaphd* sequence (Table 3). The similarity to *C. dianense* is discussed further. In total, within 810 bp sequences of the *gs* gene, we revealed one 25 bp insertion (positions 225–249) and one 1 bp (position 470) insertion typical of *C. coccodes* CBS 369.75, and 17 single nucleotide polymorphisms (SNP) atypical of *C. coccodes* (positions 11, 51, 102, 122, 143, 144, 193, 320, 326, 357, 467, 471, 515, 588, 629, 675, and 724).

Morphological differences were not found between the sizes of the *C. coccodes* and *C. nigrum* conidia (for *C. coccodes*, the conidial length was 18.23 ± 6.13 µm and the width was 4.49 ± 1.04 µm; for *C. nigrum*, the conidial length was 21.17 ± 5.10 µm and the width was 4.34 ± 1.03 µm; Figure 8). The obtained conidial measurements overlapped with those of the type strains of *C. coccodes*, *C. nigrum*, and *C. dianense* [16,21,37]. All of the isolates produced aseptate, smooth-walled, hyaline, oval to cylindrical conidia with acute, subacute or obtuse apices, typical of *C. coccodes* or *C. nigrum* (Figure 9).

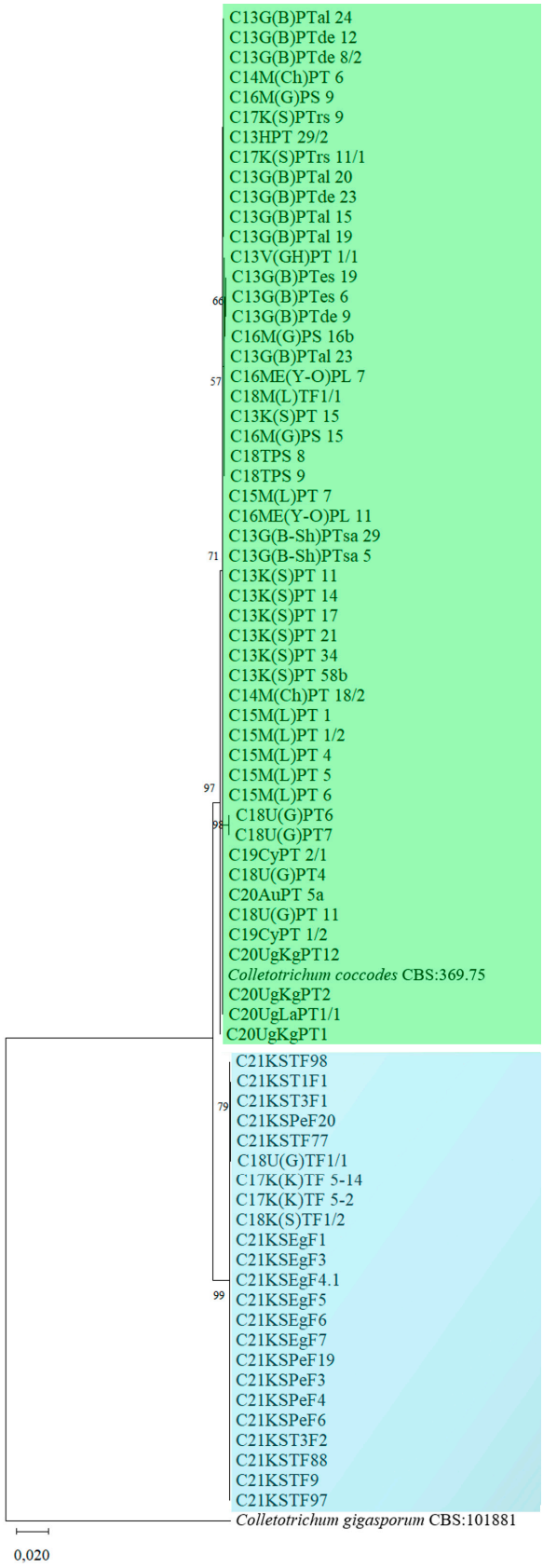

Figure 2. Phylogenetic tree inferred from maximum-likelihood analysis of the concatenated alignment, including the ITS region, and the partial *act*, *gaphd*, and *gs* gene regions. The confidence values are indicated at the branches. Green indicates *C. coccodes* clade, and blue indicates the *C. nigrum* clade.

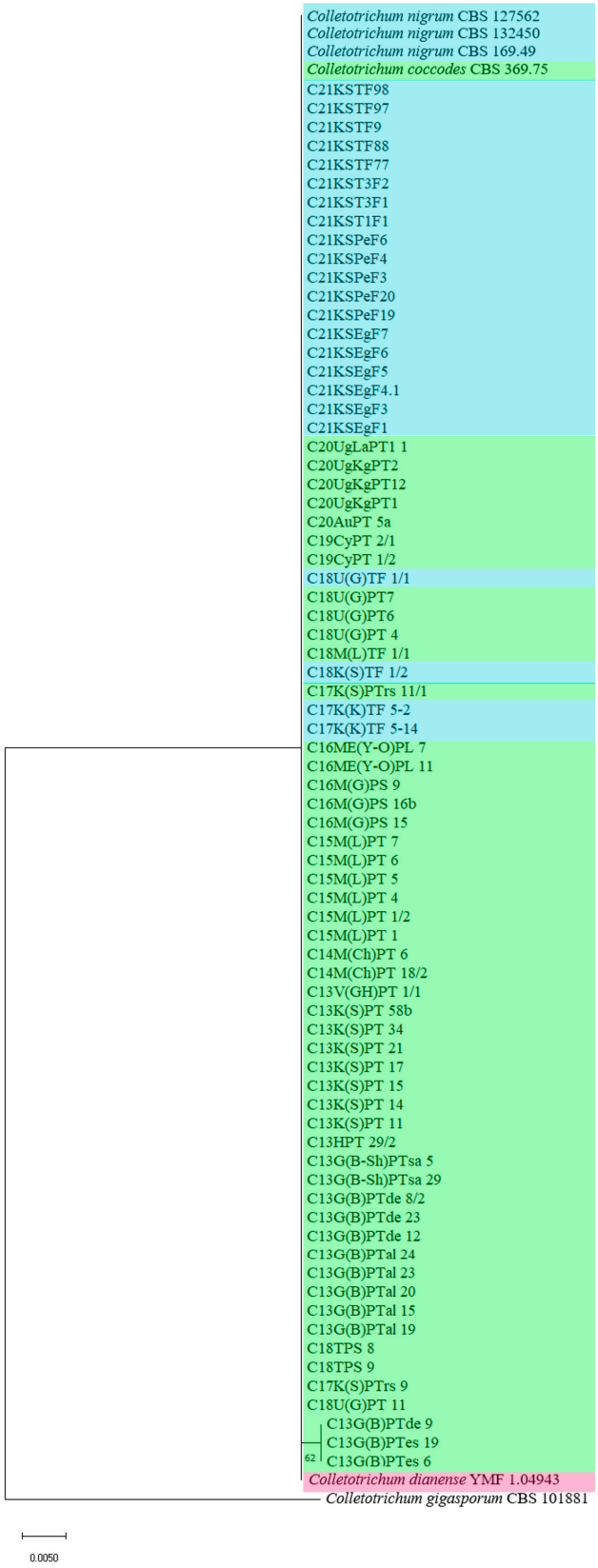

Figure 3. Phylogenetic tree inferred from maximum-likelihood analysis of the ITS1-5, 8S-ITS2 region alignment. Bootstrap 1000 replicates. The confidence values are indicated at the branches. Green indicates *C. coccodes* isolates; blue indicates *C. nigrum* isolates, and red marks *C. dianense* type species.

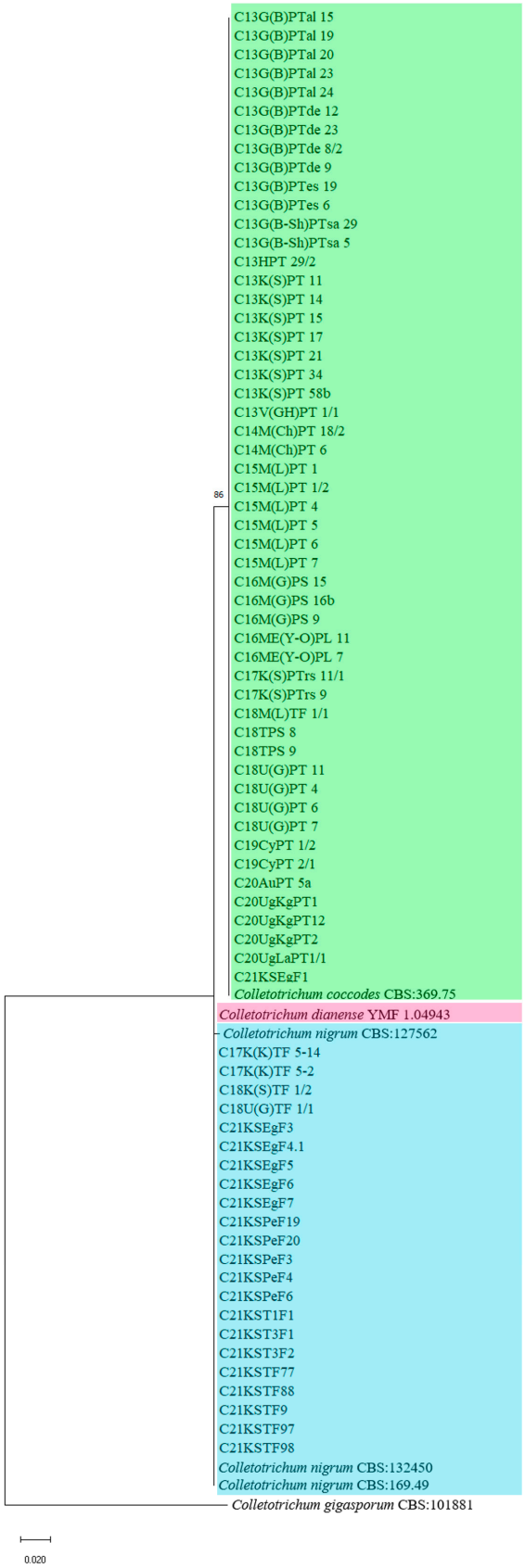

Figure 4. Phylogenetic tree inferred from maximum-likelihood analysis of the *actin* intron alignment. Bootstrap 1000 replicates. The confidence values are indicated at the branches. Green indicates *C. coccodes* clade, blue indicates the *C. nigrum* clade, and red marks *C. dianense* type species.

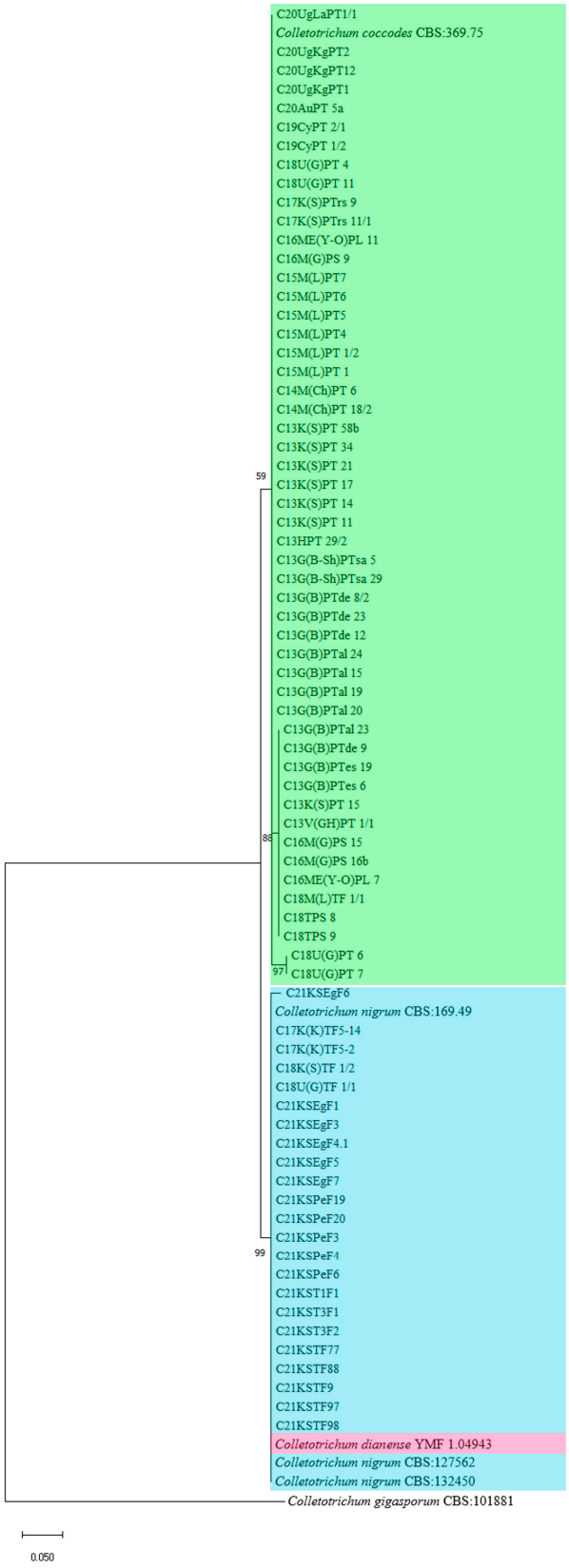

Figure 5. Phylogenetic tree inferred from maximum-likelihood analysis of the *glyceraldehyde-3-phosphate dehydrogenase* intron alignment. Bootstrap 1000 replicates. The confidence values are indicated at the branches. Green indicates *C. coccodes* clade, blue indicates the *C. nigrum* clade, and red marks *C. dianense* type species.

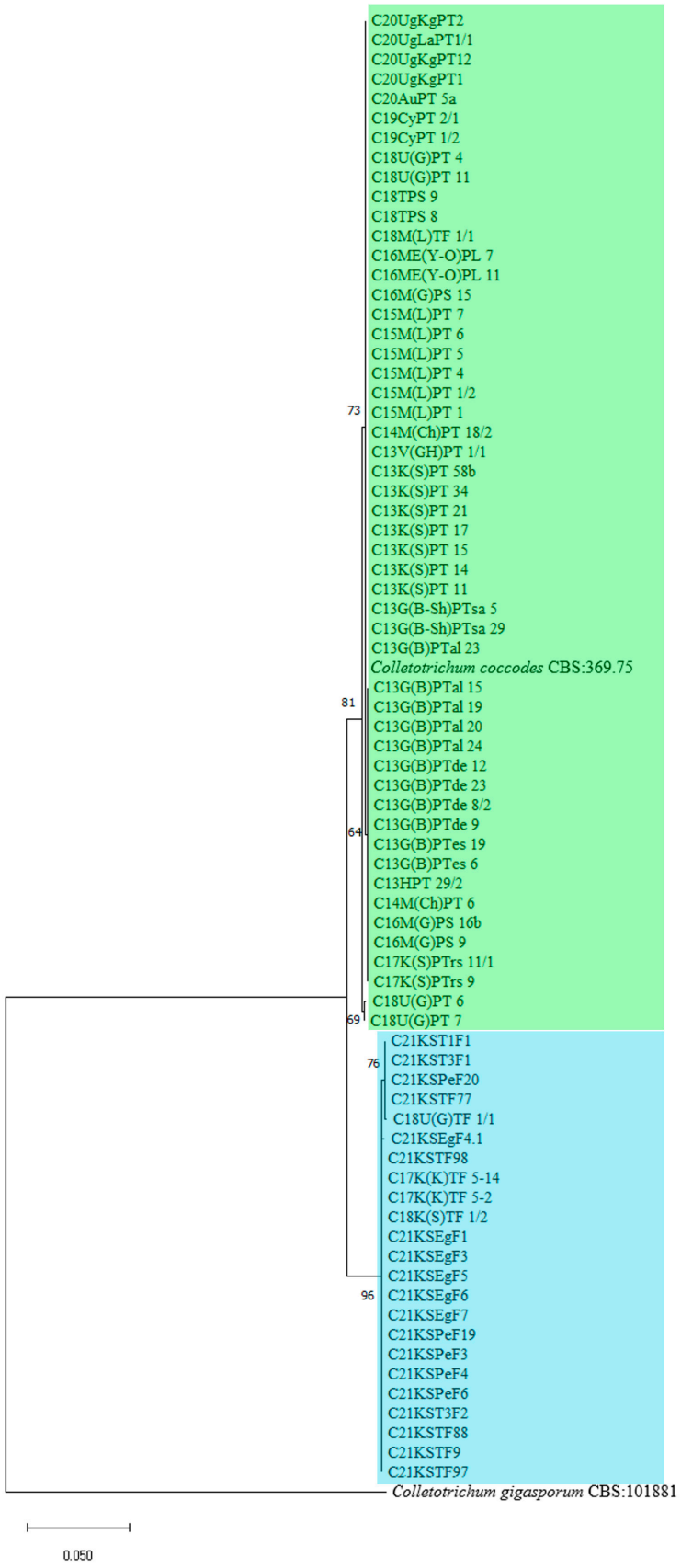

Figure 6. Phylogenetic tree inferred from maximum-likelihood analysis of the *glutamine synthetase* intron alignment. Bootstrap 1000 replicates. The confidence values are indicated at the branches. Green indicates *C. coccodes* clade; blue indicates *C. nigrum* clade.

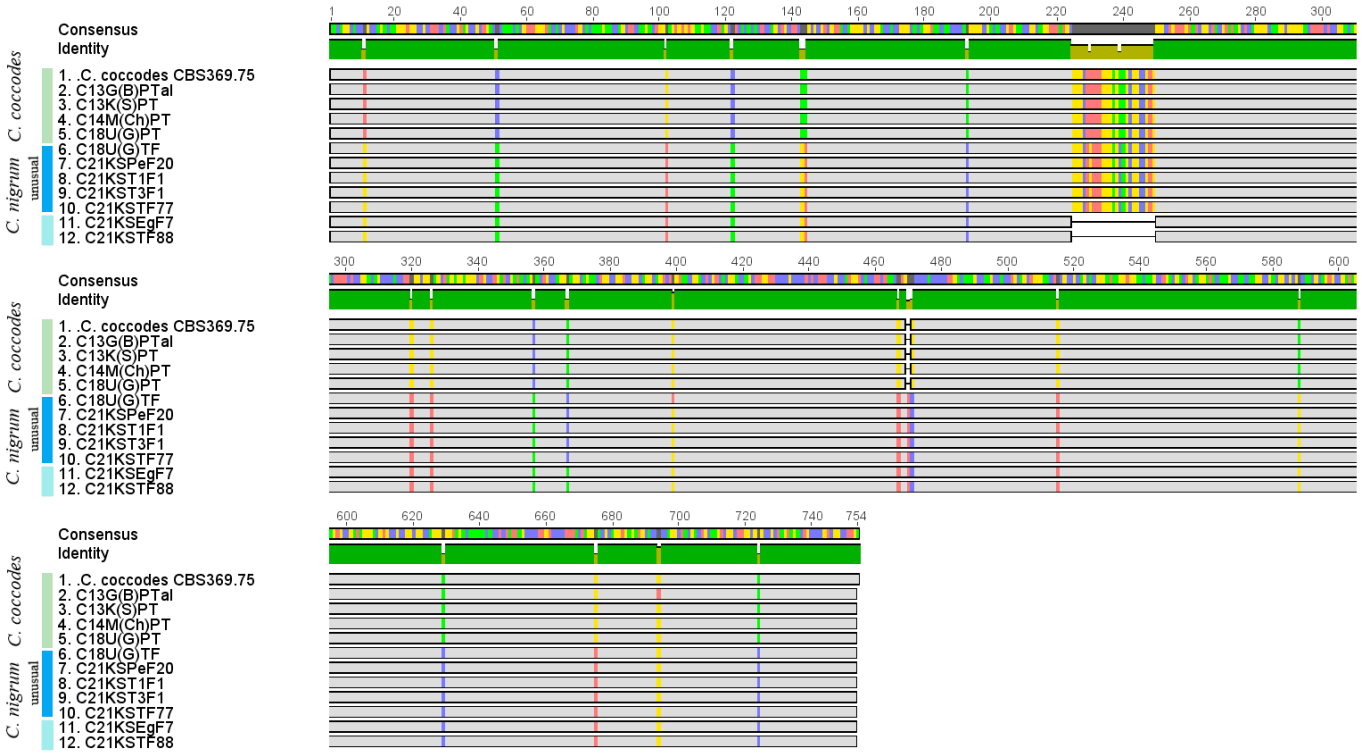

Figure 7. Comparison of *glutamine synthetase* second intron sequences of intriguing isolates (C18U(G)TF1/1, C21KST1F1, C21KST3F1, C21KSTF77, and C21KSPeF20) to type strain *C. coccodes* CBS 369.75. SNPs, including A, T, C, and G, are marked with red, green, blue, and yellow, respectively.

Table 3. Comparison of intriguing isolates to type strains *.

Isolate	Percentage of Similarity to Type Strains			
	ITS Sequence	***act* Sequence**	***gaphd* Sequence**	***gs* Sequence ****
C18U(G)TF1/1	100% *C. coccodes* 100% *C. dianense* 100% *C. nigrum*	100% *C. nigrum* 100% *C. dianense* 97% *C. coccodes*	100% *C. nigrum* 100% *C. dianense* 97% *C. coccodes*	97% *C. coccodes*
C21KST1F1	100% *C. coccodes* 100% *C. dianense* 100% *C. nigrum*	100% *C. nigrum* 100% *C. dianense* 97% *C. coccodes*	100% *C. nigrum* 100% *C. dianense* 97% *C. coccodes*	97% *C. coccodes*
C21KST3F1	100% *C. coccodes* 100% *C. dianense* 100% *C. nigrum*	100% *C. nigrum* 100% *C. dianense* 97% *C. coccodes*	100% *C. nigrum* 100% *C. dianense* 97% *C. coccodes*	97% *C. coccodes*
C21KSTF77	100% *C. coccodes* 100% *C. dianense* 100% *C. nigrum*	100% *C. nigrum* 100% *C. dianense* 97% *C. coccodes*	100% *C. nigrum* 100% *C. dianense* 97% *C. coccodes*	97% *C. coccodes*
C21KSPeF20	100% *C. coccodes* 100% *C. dianense* 100% *C. nigrum*	100% *C. nigrum* 100% *C. dianense* 97% *C. coccodes*	100% *C. nigrum* 100% *C. dianense* 97% *C. coccodes*	97% *C. coccodes*

* *C. coccodes* CBS:369.75, *C. nigrum* CBS:169.49, and *C. dianense* YMF 1.04943. ITS—ITS1-5.8S-ITS2 region, *gaphd*—glyceraldehyde-3-phosphate dehydrogenase gene intron, *act*—actin intron, *gs*—glutamine synthetase intron. ** No *gs* sequences of type *C. nigrum* or *C. dianense* isolates were found.

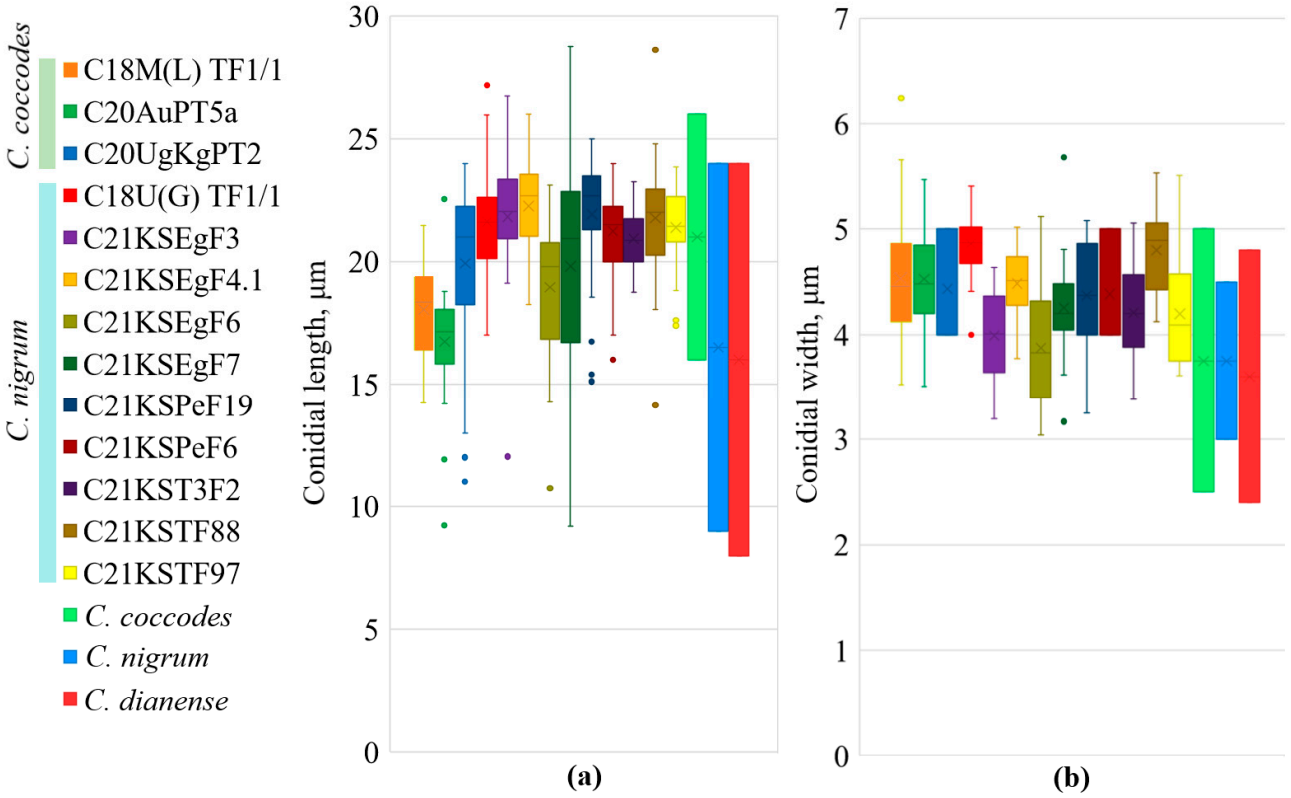

Figure 8. Comparison of the conidial lengths (**a**) and widths (**b**) of *Colletotrichum* isolates and type strains. Boxes indicate quartiles (first and third), whiskers indicate the minimum and the maximum values, and points outside the boundary are outliers.

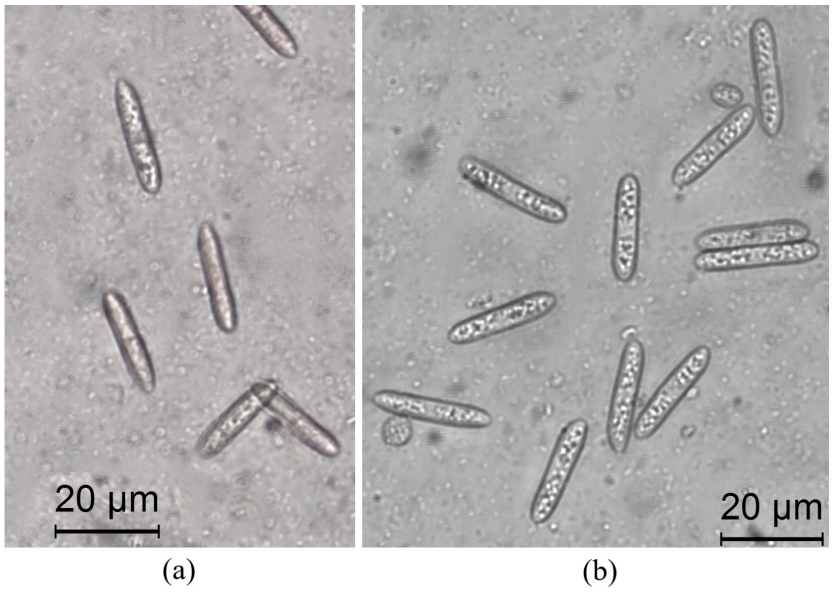

Figure 9. Conidia of *C. coccodes* isolate C20UgKgPT2 (**a**) and *C. nigrum* isolate C21KSPeF6 (**b**).

All the tested strains of both species were able to cause tomato fruit and potato tuber infection (Table 4). Regardless of the species used to infect the tomatoes, the infected fruit had typical dark lesions of anthracnose with sclerotia, milky-white swellings with conidia occasionally developed. In the case of tomato wound inoculation, the *C. coccodes*- and *C. nigrum*-caused disease radii were 5.2–6.2 mm and 3.2–8.3 mm, respectively, after 21 days of infection. On the intact fruit, the disease radius did not exceed 1.2 mm after 21 days

for all the isolates of both species, but after 35 days, one anthracnose-causing strain of *C. nigrum* reached a disease radius of 9.8 mm in width. All the tested strains of *C. coccodes* and *C. nigrum* were able to spread on potato slices. The disease radius was 2.5–5.2 mm for *C. coccodes* and 1.2–8.3 mm for *C. nigrum* after 21 days. No correlation between the disease severity and the original host was found: the isolates from tomatoes and potatoes could infect plants of both species. Nevertheless, the tomato fruit disease caused by both species was more rapid and extensive under the same temperature conditions compared with the potato tuber disease.

Table 4. Pathogenicity tests.

Strain Identifier	Species	Host	Average Disease Radius (mm) on Tomato after			Average Disease Radius (mm) on Potato after
			21 days		35 days	21 days
			Wound Inoculation	Surface Inoculation	Surface Inoculation	Wound Inoculation
C21KSEgF7	*C. nigrum*	Eggplant	4.8	0.0	0.9	2.3
C21KSEgF3	*C. nigrum*	Eggplant	3.2	0.1	2.0	2.5
C21KSEgF4.1	*C. nigrum*	Eggplant	3.2	0.1	2.3	4.3
C21KSPeF6	*C. nigrum*	Pepper	3.7	1.0	9.8	2.0
C21KSPeF19	*C. nigrum*	Pepper	5.2	0.3	2.5	8.3
C20AuPT5a	*C. coccodes*	Potato	6.2	0.2	2.0	5.2
C20UgKgPT2	*C. coccodes*	Potato	5.2	1.2	2.2	2.5
C21KSTF88	*C. nigrum*	Tomato	8.3	0.5	3.3	1.8
C21KSTF97	*C. nigrum*	Tomato	7.5	0.5	1.2	1.2
C21KST3F2	*C. nigrum*	Tomato	6.3	0.2	3.2	4.0

No isolate resistant to any examined fungicide was found (Table 5). Thiabendazole EC_{50} for *C. coccodes* was 0.65–58.38 mg/L, and that for *C. nigrum* was 0.58–20.29 mg/L. Six isolates (five *C. coccodes* from potato tubers and stem, and one *C. nigrum* from tomato fruit) were less sensitive to the chemical (EC_{50} > 10 mg/L). No resistance was found for azoxystrobin, EC_{50} for *C. coccodes* was 0.05 and 9.07 mg/L, EC_{50} for *C. nigrum* was 0.08–8.50 mg/L. Difenoconazole was the most effective chemical; EC_{50} for all the tested isolates was less than 0.12 mg/L.

Table 5. Sensitivity to fungicides.

Strain Identifier	Species	Isolation Source *	EC_{50}, mg/L **		
			Thiabendazole	Azoxystrobin	Difenoconazole
C13V(GH)PT1/1	*C. coccodes*	PT	4.24	0.08	0.06
C13K(S)PT11	*C. coccodes*	PT	5.07	7.75	0.07
C13K(S)PT14	*C. coccodes*	PT	7.75	0.08	0.06
C13K(S)PT15	*C. coccodes*	PT	4.47	0.28	0.07
C13K(S)PT17	*C. coccodes*	PT	10.20	5.82	0.12
C13K(S)PT21	*C. coccodes*	PT	7.90	3.68	-
C13K(S)PT34	*C. coccodes*	PT	0.78	0.07	0.06
C13K(S)PT58b	*C. coccodes*	PT	3.47	-	0.07
C13HPT29/2	*C. coccodes*	PT	0.91	0.05	0.05
C13G(B)PTde9	*C. coccodes*	PT	50.30	0.07	0.06
C13G(B)PTde12	*C. coccodes*	PT	0.85	0.06	0.05
C13G(B)PTde23	*C. coccodes*	PT	0.93	0.09	0.06
C13G(B)PTes6	*C. coccodes*	PT	33.38	0.10	0.06
C13G(B)PTes19	*C. coccodes*	PT	8.78	0.10	0.06
C13G(B)PTal15	*C. coccodes*	PT	0.96	0.09	0.09

Table 5. *Cont.*

Strain Identifier	Species	Isolation Source *	EC_{50}, mg/L **		
			Thiabendazole	Azoxystrobin	Difenoconazole
C13G(B)PTal19	*C. coccodes*	PT	0.96	0.09	0.06
C13G(B)PTal20	*C. coccodes*	PT	0.99	0.08	0.06
C13G(B)PTal23	*C. coccodes*	PT	6.13	0.07	0.07
C13G(B)PTal24	*C. coccodes*	PT	0.85	0.06	0.05
C13G(B-Sh)PTsa29	*C. coccodes*	PT	1.00	0.08	0.06
C14M(Ch)PT6	*C. coccodes*	PT	-	0.09	0.06
C14M(Ch)PT18/2	*C. coccodes*	PT	-	0.08	0.06
C15M(L)PT1	*C. coccodes*	PT	0.82	0.06	0.08
C15M(L)PT1/2	*C. coccodes*	PT	0.94	-	0.09
C15M(L)PT4	*C. coccodes*	PT	0.89	0.08	0.09
C15M(L)PT5	*C. coccodes*	PT	0.85	0.08	0.09
C15M(L)PT6	*C. coccodes*	PT	-	0.08	0.06
C15M(L)PT7	*C. coccodes*	PT	0.95	-	0.09
C16ME(Y-O)PL7	*C. coccodes*	PL	-	-	0.09
C16ME(Y-O)PL11	*C. coccodes*	PL	-	-	0.08
C16M(G)PS9	*C. coccodes*	PS	0.85	0.08	0.09
C16M(G)PS15	*C. coccodes*	PS	0.84	4.09	0.06
C16M(G)PS16b	*C. coccodes*	PS	58.38	0.07	0.08
C17K(K)TF5-2	*C. nigrum*	TF	0.91	0.09	0.09
C17K(K)TF5-14	*C. nigrum*	TF	-	0.08	0.09
C17K(S)PTrs9	*C. coccodes*	PT	-	0.08	0.06
C17K(S)PTrs11/1	*C. coccodes*	PT	0.87	0.08	0.07
C18M(L)TF1/1	*C. coccodes*	TF	0.74	6.32	0.12
C18K(S)TF1/2	*C. nigrum*	TF	20.29	8.50	-
C18U(G)TF1/1	*C. nigrum*	TF	-	-	0.07
C18U(G)PT4	*C. coccodes*	PT	6.07	-	-
C18U(G)PT7	*C. coccodes*	PT	0.65	7.75	0.10
C18U(G)PT11	*C. coccodes*	PT	25.43	9.07	0.08
C18TPS8	*C. coccodes*	PS	-	7.75	0.09
C18TPS9	*C. coccodes*	PS	-	3.31	0.09
C19CyPT1/2	*C. coccodes*	PT	0.95	0.07	0.09
C19CyPT2/1	*C. coccodes*	PT	0.85	0.07	0.09
C20AuPT5a	*C. coccodes*	PT	0.75	0.08	0.07
C20UgLaPT1/1	*C. coccodes*	PT	0.71	0.07	0.07
C20UgKgPT1	*C. coccodes*	PT	0.82	0.08	0.07
C20UgKgPT2	*C. coccodes*	PT	0.73	0.08	0.07
C20UgKgPT12	*C. coccodes*	PT	0.83	0.07	0.07
C21KST3F2	*C. nigrum*	TF	0.66	0.08	0.07
C21KSTF88	*C. nigrum*	TF	0.67	0.08	0.07
C21KSTF97	*C. nigrum*	TF	0.65	0.08	0.07
C21KSPeF6	*C. nigrum*	PeF	0.68	0.08	0.07
C21KSPeF19	*C. nigrum*	PeF	0.68	0.08	0.07
C21KSEgF3	*C. nigrum*	EF	0.58	0.08	0.07
C21KSEgF4.1	*C. nigrum*	EF	0.64	0.08	0.07
C21KSEgF6	*C. nigrum*	EF	0.71	0.08	0.07
C21KSEgF7	*C. nigrum*	EF	0.66	0.09	0.07

* PT—potato tuber, PS—potato stem, PL—potato leaf, TF—tomato fruit, PeF—pepper fruit, EF—eggplant fruit.
** EC_{50}—effective concentration.

4. Discussion

The efficiencies of the known genetic markers in differentiating *Colletotrichum* species vary among different species complexes [4]. The ITS region is widely used in routine studies, although the result may be doubtful. For instance, in northern Italy, *C. coccodes* was reported as an agent of pepper root disease [10]. Undoubtedly, the species can cause root

disease; still, ITS-based identification remains insufficient. In Turkey, unusual symptoms of *Colletotrichum* disease leading to extremely high crop losses were discovered, and the pathogen was identified as *C. coccodes* [38]. However, the only molecular marker used in the study was the ITS region, so the identification seems uncertain.

Dos Santos Vieira and colleagues [39] propose using *gaphd* and several other regions to distinguish between *Colletotrichum* species, while ITS and *act* are less effective. According to our study, both the *act* and *gaphd* genes are suitable, at least for *C. coccodes* and *C. nigrum* division (Figures 5 and 6).

The *Gs* intron also proved useful for delineating *Colletotrichum* species. This gene sequence is mainly used to distinguish the species within *C. gigasporum*, *C. orbiculare*, and *C. gloeosporoides* species complexes. Thus, up to date, GenBank lacks the *gs* region sequences of the type material for many species. Several GenBank accession numbers marked as the *C. coccodes gs* gene (GU935816 and GU935817) presumably belong to *C. nigrum*, as they differ by approximately 2–3% from *C. coccodes* CBS164.49 or CBS369.75 (GenBank accession numbers HM171675 and HM171676, respectively) but they are similar to our strains that are identified as *C. nigrum*, based on the *act* or *gaphd* genes. We propose that at least 17 single nucleotide changes underlie the differences between the *gs* second intron of the two species. The *C. nigrum* currently presumed occurrence and host range seem to be lower than the real ranges. We assume that several reports of *C. coccodes*, for example [17], may display *C. nigrum* disease instead.

According to the pertinent literature, the sexual process is unknown for *C. coccodes* or *C. nigrum*. The only way for strains of these species to exchange genetic material is via a parasexual process or through a vegetative compatibility reaction [40]. Based on the *gs* sequence of five isolates (C18U(G)TF1/1, C21KST1F1, C21KST3F1, C21KSTF77, and C21KSPeF20), we suppose that they might represent hybrids between *C. coccodes* and *C. nigrum*. Whereas we detected SNPs in all the isolates identified as *C. nigrum* based on *gaphd*, we assume these SNPs to be specific to *C. nigrum* (Figure 2). At least one of the isolates (C18U(G)TF1/1) was collected from tomato fruit grown near potato plants; therefore, it might have had a possibility of interfering with *C. coccodes* strains. Notwithstanding these putative hybrid isolates, we assume that the second intron of the *gs* gene is useful for distinguishing between *C. coccodes* and *C. nigrum*, and we propose a more active use of the GSF1—GSR1 primers for identifying the *Colletotrichum* species.

Both *C. coccodes* and *C. nigrum* are currently considered as singleton species. According to Liu et al. [16], all potato-associated isolates belong to *C. coccodes*. At the same time, both *C. nigrum* and *C. coccodes* were able to infect tomato and pepper. The statement is supported by other studies [3–5] and by our data. Until now, we found no information regarding *C. nigrum* in Russia.

Based on ITS region sequencing, only one *Colletotrichum* species—*C. coccodes*—was previously reported from potato and tomato leaves in Russia [9,23,24,26]. Belov and colleagues [9] used a specific primer pair (Cc1F1 and Cc2R1) [41] to detect *C. coccodes* [41]. Both test systems [23,42] were developed based on the ITS region, considered the universal fungi barcode [43]. However, they were of limited use in distinguishing *C. coccodes* and *C. nigrum*.

C. dianense, which is very similar to *C. nigrum*, was recently described [37]. The authors of the study stated that it could be distinguished from *C. nigrum* by its conidial shape and apex. The ITS region and the *act* gene of the *C. dianense* type isolate YMF 1.04943 is 100% identical, and the *gaphd* gene is 99.66% similar to the *C. nigrum* type strain CBS 169.49 (one nucleotide difference, position 185). We compared our isolates to both species. Although, in our opinion, the two species are slightly different, we named our isolates from tomato, pepper, and eggplant *C. nigrum*, as *C. nigrum* is a well-known and earlier described species.

The cross-virulence of *Colletotrichum* species was reported a while ago [44]. Yet, there are no literature reports of *C. nigrum* potato infections, as the species was only found on tomato and pepper [3,16], while the GenBank database contains several *C. nigrum* isolates (e.g., KU821311) reported from potatoes. *Colletotrichum* disease is known as post-harvest,

and is particularly harmful to climacteric fruits (e.g., tomato) [45]. Even though our study demonstrated the possibility of *C. nigrum* potato infection, no *C. nigrum* strains were isolated from potato.

Another *Colletotrichum* species, *C. acutatum s. str.,* was reported as a tomato and pepper infectious agent [5,46]. The presence of *C. acutatum s.l.* in potato leaves and mini tubers was revealed in our previous studies (unpublished data) based on the reaction with species-specific primers CaInt2/ITS4 [47]. Although *C. acutatum s.l.* has not been proven to be a potato disease agent, the possibility of its presence on potato tubers and leaves should be kept in mind.

Liu and colleagues [16,21] mentioned that *C. coccodes* strains from tomato or other hosts produce larger conidia than *C. coccodes* from potato, and *C. nigrum* forms longer conidia than *C. coccodes*. Our results do not support these statements and show no significant difference between the conidial length or width among the three studied species (Figure 8). Contrary to Zheng and colleagues [37], we suppose that the morphological differentiation within the *C. coccodes*—*C. nigrum*—*C. dianense* clade may not be significant.

All the tested chemicals—azoxystrobin, thiabendazole, and difenoconazole—proved to be effective against *Colletotrichum* spread. The results were in line with previous studies [48–50]: normally, EC_{50} is less than 1 mg/L for all of the tested chemicals. In addition, azoxystrobin reduced black dot on tubers in field conditions [50]. Resistance to azoxystrobin or thiabendazole was reported in other *Colletotrichum* species complexes [51,52]. We discovered five strains (C13G(B)PTde9, C13G(B)PTes6, C16M(G)PS16b, C18K(S)TF1/2, and C18U(G)PT11), with thiabendazole EC_{50} ranging over 20–50 mg/L. Sanders and Korsten [52] classified strains with 66–70% growth on 0.5–2.5 mg/L thiabendazole as resistant, but we named them as less sensitive after Leite [53]. In our previous study, we examined the β-tubulin gene, but no specific mutations in any of the *Colletotrichum* isolates was found [54], contrary to *Colletotrichum musae* [53], less sensitive strains, *Colletotrichum siamense* [55], or *Helminthosporium solani* highly resistant strains [56]. Because even the highest EC_{50} values for *Colletotrichum* spp. are much lower than the concentrations in the working liquid for treatment (e.g., 170–250 mg/L for azoxystrobin, 4800–5600 mg/L for thiabendazole, and 187–625 mg/L for difenoconazole), we conclude that in general, all of the studied chemicals could still be considered as an effective strategy for anthracnose control on Solanaceae in Russia.

5. Conclusions

Here, we present the results of the first extensive molecular and morphological analysis of *Colletotrichum* species affecting Solanaceae plants in Russia. Two morphologically indistinguishable species, *C. coccodes* and *C. nigrum,* were revealed. The *act* and *gaphd* gene introns are suggested as the most suitable molecular markers to differentiate between these species. The *Gs* intron sequences give rise to the hypothesis of a parasexual process between these two species; therefore, further research is required. Eggplant and pepper plants were found to be infected exclusively by *C. nigrum*; tomato plants were infected by both species. Potato infection was caused only by *C. coccodes*. However, in vitro, both species showed an ability to infect tomato fruit and potato tubers. Three studied chemicals, azoxystrobin, difenoconazole, and thiabendazole, were effective against the isolates of both species, although several isolates were less sensitive to thiabendazole.

Author Contributions: Conceptualization, S.E.; methodology, E.C., S.E. and M.Y.; software, E.C., S.E. and L.K.; validation, I.K., M.Y., M.K., A.B., E.C. and L.K.; formal analysis, E.C., S.E., M.Y. and M.K.; investigation, I.K., M.K., M.Y., A.B., G.B., A.E., M.P., A.T. and Y.T.; resources, S.E., E.C., A.B., M.Y. and I.K.; data curation, E.C., M.Y. and S.E.; writing—original draft preparation, M.Y.; writing—review and editing, S.E., A.B. and L.K.; visualization, M.Y.; supervision, S.E.; project administration, S.E.; funding acquisition, S.E., L.K. and E.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the RUDN University Scientific Grant System (project № 202193-2-000), and by the Russian Foundation for Basic Research (grant № 20-016-00139).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Newly generated ITS, *act*, *gaphd*, and *gs* sequences are deposited in GenBank under the accession numbers specified in Table 1.

Acknowledgments: The authors are grateful to Anastasia Sharapkova and Tatiana Gavrilova (Rosetta Stone MSU) for the English improvement of the article.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Marin-Felix, Y.; Hernández-Restrepo, M.; Iturrieta-González, I.; García, D.; Gené, J.; Groenewald, J.Z.; Cai, L.; Chen, Q.; Quaedvlieg, W.; Schumacher, R.K.; et al. Genera of phytopathogenic fungi: GOPHY 1. *Stud. Mycol.* **2017**, *86*, 99–216. [CrossRef] [PubMed]
2. Jayawardena, R.S.; Bhunjun, C.S.; Hyde, K.D.; Gentekaki, E.; Itthayakorn, P. Colletotrichum: Lifestyles, biology, morpho-species, species complexes and accepted species. *Mycosphere* **2021**, *12*, 519–669. [CrossRef]
3. Talhinhas, P.; Baroncelli, R. Colletotrichum species and complexes: Geographic distribution, host range and conservation status. *Fungal Divers.* **2021**, *110*, 109–198. [CrossRef]
4. Bhunjun, C.S.; Phukhamsakda, C.; Jayawardena, R.S.; Jeewon, R.; Promputtha, I.; Hyde, K.D. Investigating species boundaries in Colletotrichum. *Fungal Divers.* **2021**, *107*, 107–127. [CrossRef]
5. Liu, F.; Ma, Z.Y.; Hou, L.W.; Diao, Y.Z.; Wu, W.P.; Damm, U.; Cai, L. Updating species diversity of Colletotrichum, with a phylogenomic overview. *Stud. Mycol.* **2022**, *101*, 1–86. [CrossRef]
6. Banya, M.; Garg, S.; Meena, N.L. A review: Chilli anthracnose, its spread and management. *J. Pharmacogn. Phytochem.* **2020**, *9*, 1432–1438.
7. Fiers, M.; Edel-Hermann, V.; Chatot, C.; Le Hingrat, Y.; Alabouvette, C.; Steinberg, C. Potato soil-borne diseases. A review. *Agron. Sustain. Dev.* **2012**, *32*, 93–132. [CrossRef]
8. Johnson, D.A.; Geary, B.; (Lahkim) Tsror, L. Potato Black Dot—The Elusive Pathogen, Disease Development and Management. *Am. J. Potato Res.* **2018**, *95*, 340–350. [CrossRef]
9. Belov, G.L.; Belosokhov, A.F.; Kutuzova, I.A.; Statsyuk, N.V.; Chudinova, E.M.; Alexandrova, A.V.; Kokaeva, L.Y. Colletotrichum coccodes in potato and tomato leaves in Russia. *J. Plant Dis. Prot.* **2018**, *125*, 311–317. [CrossRef]
10. Garibaldi, A.; Gilardi, G.; Baudino, M.; Ortu, G.; Gullino, M.L. Colletotrichum coccodes: A soil-borne pathogen dangerous to a pepper rootstock in Italy. *J. Plant Pathol.* **2012**, *94*, 91.
11. Ali, A.; Bordoh, P.K.; Singh, A.; Siddiqui, Y.; Droby, S. Post-harvest development of anthracnose in pepper (Capsicum spp): Etiology and management strategies. *Crop Prot.* **2016**, *90*, 132–141. [CrossRef]
12. Tyvaert, L.; Everaert, E.; Lippens, L.; Cuijpers, W.J.M.; França, S.C.; Höfte, M. Interaction of Colletotrichum coccodes and Verticillium dahliae in pepper plants. *European J. Plant Pathol.* **2019**, *155*, 1303–1317. [CrossRef]
13. Boyette, C.D.; Hoagland, R.E.; Stetina, K.C. Bioherbicidal enhancement and host range expansion of a mycoherbicidal fungus via formulation approaches. *Biocontrol Sci. Technol.* **2018**, *28*, 307–315. [CrossRef]
14. Manova, V.; Stoyanova, Z.; Rodeva, R.; Boycheva, I.; Korpelainen, H.; Vesterinen, E.; Wirta, H.; Bonchev, G. Morphological, Pathological and Genetic Diversity of the Colletotrichum Species, Pathogenic on Solanaceous Vegetable Crops in Bulgaria. *J. Fungi* **2022**, *8*, 1123. [CrossRef] [PubMed]
15. Shivas, R.G.; Tan, Y.P.; Edwards, J.; Dinh, Q.; Maxwell, A.; Andjic, V.; Liberato, J.R.; Anderson, C.; Beasley, D.R.; Bransgrove, K.; et al. Colletotrichum species in Australia. *Australas. Plant Pathol.* **2016**, *45*, 447–464. [CrossRef]
16. Liu, F.; Cai, L.; Crous, P.W.; Damm, U. Circumscription of the anthracnose pathogens Colletotrichum lindemuthianum and C. nigrum. *Mycologia* **2013**, *105*, 844–860. [CrossRef]
17. Rodriguez-Salamanca, L.M.; Enzenbacher, T.B.; Derie, M.L.; du Toit, L.J.; Feng, C.; Correll, J.C.; Hausbeck, M.K. First report of Colletotrichum coccodes causing leaf and neck anthracnose on onions (Allium cepa) in Michigan and the United States. *Plant Dis.* **2012**, *96*, 769. [CrossRef]
18. Halsted, B.D. A new anthracnose of peppers. *Bull. Torrey Bot. Club* **1891**, *18*, 14–15. [CrossRef]
19. Rivera, Y.; Stommel, J.; Dumm, J.; Ismaiel, A.; Wyenandt, C.A.; Crouch, J.A. First report of Colletotrichum nigrum causing anthracnose disease on tomato fruit in New Jersey. *Plant Dis.* **2016**, *100*, 2162. [CrossRef]
20. Fungal Databases. Available online: http://nt.ars-grin.gov/fungaldatabases (accessed on 10 June 2022).
21. Liu, F.; Hyde, K.D.; Cai, L. Neotypification of Colletotrichum coccodes, the causal agent of potato black dot disease and tomato anthracnose. *Mycologia* **2011**, *2*, 248–254.

22. Kotova, V.V.; Kungurtseva, O.V. Anthracnose of agricultural plants. *Vestn. Zashchity Rastenii* **2014**, *11*, 3–132.
23. Nikitin, M.; Deych, K.; Grevtseva, I.; Girsova, N.; Kuznetsova, M.; Pridannikov, M.; Golikov, A. Preserved microarrays for simultaneous detection and identification of six fungal potato pathogens with the use of real-time PCR in matrix format. *Biosensors* **2018**, *8*, 129. [CrossRef] [PubMed]
24. Kokaeva, L.; Chudinova, E.; Berezov, A.; Yarmeeva, M.; Balabko, P.; Belosokhov, A.; Elansky, S. Fungal diversity in tomato (Solanum lycopersicum) leaves and fruits in Russia. *J. Central Eur. Agric.* **2020**, *21*, 809–816. [CrossRef]
25. Kazartsev, I.A.; Gomzhinaa, M.M.; Gasicha, E.L.; Khlopunova, L.B.; Gannibal, P.B. Biodiversity of Colletotrichum spp. on Several Wild and Cultivated Plants. *Mikol. Fitopatol.* **2021**, *56*, 127–139.
26. Poluektova, E.V.; Berestetskiy, A.O.; Kutuzova, I.A.; Kokaeva, L.Y.; Yarmeeva, M.M.; Khusnetdinova, T.I.; Balabko, P.N.; Kurchaev, M.L.; Elansky, S.N. Biological properties and susceptibility to fungicides of Colletotrichum coccodes strains of different geographic origin. *Probl. Agrochem. Ecol.* **2021**, *3–4*, 45–54.
27. Belosokhov, A.F.; Yarmeeva, M.M.; Kokaeva, L.Y.; Chudinova, E.M.; Mislavsky, S.M.; Elansky, S.N. Trichocladium solani sp. nov.—A new pathogen on potato tubers causing yellow rot. *J. Fungi* **2022**, *8*, 1160. [CrossRef]
28. Elansky, S.N.; Chudinova, E.M.; Elansky, A.S.; Kah, M.O.; Sandzhieva, D.A.; Mukabenova, B.A.; Dedov, A.G. Microorganisms in spent water-miscible metalworking fluids as a resource of strains for their disposal. *J. Cleaner Prod.* **2022**, *350*, 1–9. [CrossRef]
29. White, T.J.; Bruns, T.; Lee, S.J.W.T.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Academic Press: Cambridge, MA, USA, 1990; Volume 18, pp. 315–322.
30. Stephenson, S.A.; Green, J.R.; Manners, J.M.; Maclean, D.J. Cloning and characterisation of glutamine synthetase from Colletotrichum gloeosporioides and demonstration of elevated expression during pathogenesis on Stylosanthes guianensis. *Curr. Genet.* **1997**, *31*, 447–454. [CrossRef]
31. Templeton, M.D.; Rikkerink, E.H.; Solon, S.L.; Crowhurst, R.N. Cloning and molecular characterization of the glyceraldehyde-3-phosphate dehydrogenase-encoding gene and cDNA from the plant pathogenic fungus Glomerella cingulata. *Gene* **1992**, *122*, 225–230. [CrossRef]
32. Carbone, I.; Kohn, L.M. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* **1999**, *91*, 553–556. [CrossRef]
33. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [CrossRef] [PubMed]
34. Nirenberg, H. *Untersuchungen über die Morphologische und Biologische Differenzierung in der Fusarium-Sektion Liseola*; Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem; Kommissionsverlag Paul Parey: Berlin, Germany, 1976; pp. 1–93.
35. Horsfall, J.G. *Chapter II—Fungicidal Action and Its Measurement. Principles of Fungicidal Action*; Chronica Botanica: Waltham, MA, USA, 1956; pp. 15–29.
36. Yarmeeva, M.M.; Kokaeva, L.Y.; Chudinova, E.M.; Kah, M.O.; Kurchaev, M.L.; Zeyruk, V.N.; Elansky, S.N. Anastomosis groups and sensitivity to fungicides of *Rhizoctonia solani* strains isolated from potato in Russia. *J. Plant Dis. Prot.* **2021**, *128*, 1253–1261. [CrossRef]
37. Zheng, H.; Yu, Z.; Jiang, X.; Fang, L.; Qiao, M. Endophytic Colletotrichum species from aquatic plants in southwest China. *J. Fungi* **2022**, *8*, 87. [CrossRef] [PubMed]
38. Çakır, E.; Karahan, A.; Kurbetli, İ. Involvement of Colletotrichum coccodes causing atypical symptoms of potato tubers in Turkey. *J. Plant Dis. Prot.* **2019**, *126*, 173–176. [CrossRef]
39. Dos Santos Vieira, W.A.; Bezerra, P.A.; da Silva, A.C.; Veloso, J.S.; Câmara, M.P.S.; Doyle, V.P. Optimal markers for the identification of Colletotrichum species. *Mol. Phylogenetics Evol.* **2020**, *143*, 1–19.
40. Aqeel, A.M.; Pasche, J.S.; Gudmestad, N.C. Variability in morphology and aggressiveness among North American vegetative compatibility groups of Colletotrichum coccodes. *Phytopathology* **2008**, *98*, 901–909. [CrossRef]
41. Tozze Júnior, H.J.; Firmino, A.C.; Fischer, I.H.; Furtado, E.L.; Massola Júnior, N.S. Characterization of Colletotrichum spp. isolates associated with fruit trees in the state of São Paulo. *Summa Phytopathol.* **2015**, *41*, 270–280. [CrossRef]
42. Cullen, D.W.; Lees, A.K.; Toth, I.K.; Duncan, J.M. Detection of Colletotrichum coccodes from soil and potato tubers by conventional and quantitative real-time PCR. *Plant Pathol.* **2002**, *51*, 281–292. [CrossRef]
43. Schoch, C.L.; Seifert, K.A.; Huhndorf, S.; Robert, V.; Spouge, J.L.; Levesque, C.A.; Chen, W.; Bolchacova, E.; Voigt, K.; Crous, P.W.; et al. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 6241–6246. [CrossRef]
44. Hadden, J.F.; Black, L.L. *Anthracnose of Pepper Caused by Colletotrichum spp.*; AVRDC: Tainan, Taiwan, 1989; pp. 189–199.
45. Ciofini, A.; Negrini, F.; Baroncelli, R.; Baraldi, E. Management of Post-Harvest Anthracnose: Current Approaches and Future Perspectives. *Plants* **2022**, *11*, 1856. [CrossRef]
46. Damm, U.; Cannon, P.F.; Woudenberg, J.H.C.; Crous, P.W. The Colletotrichum acutatum species complex. *Stud. Mycol.* **2012**, *73*, 37–113. [CrossRef] [PubMed]
47. Sreenivasaprasad, S.; Sharada, K.; Brown, A.E.; Mills, P.R. PCR-based detection of Colletotrichum acutatum on strawberry. *Plant Pathol.* **1996**, *45*, 650–655. [CrossRef]
48. Aqeel, A.M. Response of Colletotrichum coccodes to QoI fungicide in vitro. *Plant Pathol. J.* **2007**, *4*, 283–290. [CrossRef]

49. Chapin, L.J.G.; Wang, Y.; Lutton, E.; Gardener, B.B.M. Distribution and fungicide sensitivity of fungal pathogens causing anthracnose-like lesions on tomatoes grown in Ohio. *Plant Dis.* **2006**, *90*, 397–403. [CrossRef] [PubMed]
50. Nitzan, N.; Cummings, T.F.; Johnson, D.A. Effect of seed-tuber generation, soilborne inoculum, and azoxystrobin application on development of potato black dot caused by Colletotrichum coccodes. *Plant Dis.* **2005**, *89*, 1181–1185. [CrossRef]
51. Torres-Calzada, C.; Tapia-Tussell, R.; Higuera-Ciapara, I.; Martin-Mex, R.; Nexticapan-Garcez, A.; Perez-Brito, D. Sensitivity of Colletotrichum truncatum to four fungicides and characterization of thiabendazole-resistant isolates. *Plant Dis.* **2015**, *99*, 1590–1595. [CrossRef]
52. Sanders, G.M.; Korsten, L. Survey of fungicide resistance of Colletotrichum gloeosporioides from different avocado production areas. *Afr. Avocado Grow. Assoc.* **1999**, *22*, 35–38.
53. Tsindeliani, A.A.; Skokov, D.A.; Yarmeeva, M.M.; Elansky, S.N.; Chudinova, E.M. Thiabendazole sensitiveness of strains isolated from Solanaceae plants. In *Current Mycology in Russia. Materials of the Fifth Mycological Congress in Russia*; Academy of Mycology: Moscow, Russia, 2022; pp. 258–259. (In Russian)
54. Leite, I.C.; Silva, R.A.; Santos, J.E.; Freitas-Lopes, R.L.; Câmara, M.P.; Michereff, S.J.; Lopes, U.P. Analysis of Colletotrichum musae populations from Brazil reveals the presence of isolates with high competitive ability and reduced sensitivity to postharvest fungicides. *Plant Pathol.* **2020**, *69*, 1529–1539. [CrossRef]
55. Hu, M.J.; Grabke, A.; Dowling, M.E.; Holstein, H.J.; Schnabel, G. Resistance in Colletotrichum siamense from peach and blueberry to thiophanate-methyl and azoxystrobin. *Plant Dis.* **2015**, *99*, 806–814. [CrossRef]
56. Chudinova, E.M.; Kokaeva, L.Y.; Elansky, S.N.; Kutuzova, I.A.; Pertsev, A.S.; Pobendinskaya, M.A. The occurrence of thiabendazole-resistant isolates of Helminthosporium solani on potato seed tubers in Russia. *J. Plant Dis. Prot.* **2020**, *127*, 421–423. [CrossRef]