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Identification and Pathogenicity of Causal Agents of Apple Canker Disease in Kazakhstan

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Abstract: Apples are widely consumed by people all over the world due to their taste and nutritional value. However, apple trees are prone to various environmental stresses, including fungal diseases. Among them, Cytospora canker (or Valsa canker) can cause dieback of branches and twigs. Although Kazakhstan is well known as an origin of apples, very little is known about canker diseases that spread across all commercial orchards. Therefore, an accurate identification of the causal agents of those diseases is needed for further application of informed disease management strategies. In this study, eleven isolates belonging to four *Cytospora* species were isolated from multiple cultivars, grown in six different orchards within the Almaty region, Kazakhstan. As a result of a multilocus phylogenetic analysis using ITS, LSU and *tef1-α* marker genes and morphological characterization, these isolates were described as *Cytospora parasitica*, *Cytospora sorbina*, *Cytospora pruinopsis* and *Cytospora chrysosperma*. Moreover, a pathogenicity test was conducted on detached twigs, and it demonstrated that two of these fungi were highly virulent. Overall, this paper is a first report of the causal agents of apple canker disease in Kazakhstan and could be a trigger for conducting future studies to better understand the disease epidemiology, as well as build management strategies.

Keywords: apple; Cytospora canker; phylogenetics; pathogenicity; *Malus domestica*

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1. Introduction

Apples (*Malus domestica* Borkh) are widely consumed all around the world due to their taste, juiciness, texture and nutritional contribution [1,2]. Moreover, apples have certain advantages, such as a good preservation capacity, and they are available at any time of the year in markets at affordable prices [3]. According to the existing literature, the accepted center of origin for the modern apple varieties, which dates to the end of the first millennium B.C., is the Tian Shan Mountains of Kazakhstan, in the village site of Tuzusai [4,5]. Apart from being consumed fresh, apples are constantly transformed into many different kinds of apple-based products depending on the processing technology used [6]. However, even though apples are proven to prevent the development of various

human diseases, they are themselves prone to many pathogenic infections, resulting in fruit quality and economic losses [7].

Among the fungal infections, *Cytospora* spp. are devastating ones that cause canker and dieback, often resulting in the death of apple trees worldwide [8]. The fungus infects trees through various wounds such as scars, frostbites and freshly pruned wounds [9]. Moreover, *Cytospora* spp. have the ability to remain dormant within the trees in orchards, occasionally infecting them whenever there is a relatively low resistance level [10]. To date, about 150 *Cytospora* spp. have been reported to cause branch canker and dieback on more than 130 woody plant species worldwide [11]. According to Feng et al. [12], canker disease is annually limiting apple production by more than 50% in China, the largest apple producer. This could be due to harsh weather conditions, as well as potassium deficiency, which leads to susceptibility of the trees to *Cytospora* spp. [13].

An accurate identification is needed to ensure informed disease management; however, it is challenging to distinguish between different causal agents of canker disease with the naked eye, as they all have similar visible symptoms. Moreover, the symptoms of a single *Cytospora* spp. can occur on different hosts, and multiple species of *Cytospora* may be simultaneously found on the same host [14]. Therefore, more informative morphological observations and phylogenetic analyses are required to study the relationship between different species of the fungus.

The present study was conducted to carry out molecular and morphological identification and pathogenicity testing of the causal agents of canker and dieback diseases of apple trees in Almaty region, Kazakhstan. Notably, this region is the main apple growing region within the country, with more than 15 commercial orchards, and is considered the an original home for *Malus sieversii*—a well-known wild apple specie and the primary progenitor of domesticated apples [15,16]. This article is a first formal report on four fungal species causing apple canker in Kazakhstan: *Cytospora parasitica*, *Cytospora sorbina*, *Cytospora pruinopsis* and *Cytospora chrysosperma*.

2. Materials and Methods

2.1. Sampling and Fungal Isolation

Infected branches and twigs (Figure 1) were collected from apple-cankered trees (cultivars: 'Gala', 'Granny Smith', 'Fuji', 'Sunrise', 'Star Crimson', 'Golden Rush', 'Enterprise', 'Red Delicious', 'Golden Delicious', 'Pink Lady', 'Red Jonaprince') in six different commercial orchards (Alma Issyk, Manshuk, Unifruit, Arnau Agro, Asyl Agro, Bakdala) within Almaty region, Kazakhstan (Table S1), and were sent to the laboratory in paper bags for fungal isolation during 2023 and the first half of 2024. For fungal isolation, the collected samples were initially disinfected using 70% ethanol, and then, tissue pieces (5 mm in diameter) were taken from the necrotic parts. The pieces were placed on Petri dishes containing potato dextrose agar (PDA, TM MEDIA, Delhi, India) and incubated at 27 °C for further single-spore isolations.

2.2. Morphological Characterization

The macromorphological characteristics of colonies were studied by recording measurements on days 3, 5, 7 and 14 of growth. Micromorphological features were examined by manually cutting pycnidia from tree branches using a scalpel and observing them under a ZEISS Axio Scope.A1 microscope (Carl Zeiss, Jena, Germany). Twenty to fifty measurements of conidiomata, conidia, conidiophores, locules and ostioles were recorded, and mean and standard deviation values were calculated.

Figure 1. Disease symptoms caused by *Cytospora* species on apple trees in Almaty region, Kazakhstan.

2.3. Molecular Identification

DNA was isolated using a modified version [17] of the classical phenol–chloroform method [18]. The mycelium of 11 fungal isolates was scraped from the surface of the culture, grown on a PDA medium, and placed in a sterile 2 mL microcentrifuge tube. To achieve better DNA purity, 100 µL of the aqueous phase was collected after centrifugation, and 600 µL of the phenol–chloroform mixture was re-added.

As a preliminary identification of the fungal isolate, the internal transcribed spacer (ITS) region was amplified using the primer pairs ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [19], and the large ribosomal subunit (LSU) was amplified using NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) [20]. Translation elongation factor 1-α (*tef1-α*) was amplified using forward EF1-688F (5′-CGGTCACTTGATCTACAAGTGC-3′) and a reverse primer (5′-CCTCGAACTCACCAGTACCG-3′) [21], while beta-tubulin (*tub2*) was amplified using Bt2a (5′-GGTAACCAAATCGGTGCTGCTTTC-3′) and Bt2b (5′-ACCCTCAGTGTAGTGACCCTTGGC-3′) [22]. The PCR was carried out in a total volume of 25 µL containing 1 µL of genomic DNA (~20 ng), 1 µL of forward and reverse primers (10 pM), 12.5 µL of HS-Taq (Biolabmix LLC, Russia) and 10.5 µL of nuclease-free water. The PCR reaction program included initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation (95 °C for 15 s), annealing (57.5 °C for 30 s), elongation (72 °C for

1 min) and final elongation at 72 °C for 10 min. PCR products were visualized on a 1.0% agarose gel.

2.4. Phylogenetic Analyses

The purification of PCR products was carried out using 2 µL of ExoSAP-IT (Applied Biosystems, Waltham, MA, USA) mixed with 5 µL of PCR product. The amplified DNA fragments were sequenced using the Sanger method using a BigDye Terminator v3.1 (NimaGen, Nijmegen, The Netherlands). The sequencing products were studied on an ABI 3130xl genetic analyzer (Applied Biosystems, Waltham, MA, USA). The sequences for four amplified genetic loci from eleven isolates, obtained in this study, were deposited in the GenBank database, and their accession numbers can be found in Table S2.

A bioinformatic analysis of the obtained nucleotide sequences was carried out using computer software for statistical analysis of the molecular evolution and construction of phylogenetic trees—MEGA 11. Multiple alignments were carried out using MUSCLE and the program included in the MEGA software package. The evolutionary history and divergence between sequences were inferred by using the maximum likelihood method and Tamura–Nei model [23]. The BioNJ algorithm was used to construct the phylogenetic tree for *Cytospora*. The clade support was estimated based on 1000 bootstrap replications.

2.5. Pathogenicity Trials

A pathogenicity test of four different causal agents of Cytospora canker (one isolate selected from each species) was performed on detached branches (20 cm in length and 1.5–2 cm in diameter) of 2-year-old apple trees (cv. 'Golden Delicious'). Firstly, the branches were surface-sterilized using 70% ethanol and washed three times using deionized water. The cortex was removed using a cork borer, and both ends of all branches were wrapped in Parafilm to prevent dehydration. Then, they were deeply wounded in the middle (6 mm in diameter), in direct contact with vascular tissue. Mycelium plugs (6 mm in diameter) from 5-day-old cultures were placed on the wounds and covered with wet sterile cotton and then sealed with Parafilm on top. A clean PDA plug was also inoculated as a control. To achieve the relevant humidity, all samples were placed in plastic containers containing wet paper towels at the bottom and maintained in the dark at room temperature. After 6 weeks, the lesion lengths were measured to assess the pathogenicity of the tested isolates. Mean values were calculated from the results of the experiment, with five replications.

3. Results

The species-level identifications for all 11 fungal isolates described in this study were achieved via morphological traits, molecular markers and phylogenetic analysis. Firstly, the morphological traits were examined in comparison with descriptions of *Cytospora* spp. from the existing literature. For instance, conidiophores of the isolate AC-97 were 31.7 µm in length, which is within the range of 27.2~34.0 µm that was previously reported by Ma et al. [24]. In contrast, the conidia of the isolate AC-77 measured 12 µm in length and 3.1 µm in diameter, consistent with the descriptions by Fan et al. [25].

Table 1 presents the results of the comparative analysis of the identification of Cytospora isolates using morphological and molecular methods. The obtained data confirmed the accurate matching of the isolates with known *Cytospora* spp. based on three genetic markers (ITS, LSU, *tef1-α*) and their phylogenetic clustering. All isolates formed clear clusters that were consistent with their putative species, confirming the reliability of the obtained molecular data and the validity of the phylogenetic approach.

Table 1. Comparison of the morphological traits, molecular matches and phylogenetic analysis of the *Cytospora* isolates described in this study.

Isolate	Morphological Match	BLAST Match (ITS)	BLAST Match (LSU)	BLAST Match (*tef1-α*)	Phylogenetic Clade	Final Species Identification
AC-118	*C. chrysosperma*	*C. chrysosperma* (98.3%)	*C. chrysosperma* (100%)	*C. chrysosperma* (98.8%)	Clusters with *C. chrysosperma*	*Cytospora chrysosperma*
AC-6	*C. parasitica*	*C. parasitica* (99.4%)	*C. parasitica* (99.1%)	*C. parasitica* (98.4%)	Clusters with *C. parasitica*	*Cytospora parasitica*
AC-97		*C. parasitica* (98.3%)	*C. parasitica* (98.4%)	*C. parasitica* (97.6%)	Clusters with *C. parasitica*	*Cytospora parasitica*
AC-106		*C. parasitica* (99.1%)	*C. parasitica* (99%)	*C. parasitica* (100%)	Clusters with *C. parasitica*	*Cytospora parasitica*
AC-108		*C. parasitica* (99.6%)	*C. parasitica* (98.2%)	*C. parasitica* (100%)	Clusters with *C. parasitica*	*Cytospora parasitica*
AC-77	*C. pruinopsis*	*C. pruinopsis* (99.6%)	*C. pruinopsis* (98.9%)	*C. pruinopsis* (99.8%)	Clusters with *C. pruinopsis*	*Cytospora pruinopsis*
AC-21	*C. sorbina*	*C. sorbina* (99%)	*C. sorbina* (98.2%)	*C. sorbina* (98%)	Clusters with *C. sorbina*	*Cytospora sorbina*
AC-23		*C. sorbina* (99%)	*C. sorbina* (98%)	*C. sorbina* (97.8%)	Clusters with *C. sorbina*	*Cytospora sorbina*
AC-27		*C. sorbina* (98.7%)	*C. sorbina* (98.9%)	*C. sorbina* (97.3%)	Clusters with *C. sorbina*	*Cytospora sorbina*
AC-34		*C. sorbina* (99.6%)	*C. sorbina* (99.3%)	*C. sorbina* (99.1%)	Clusters with *C. sorbina*	*Cytospora sorbina*
AC-46		*C. sorbina* (98.9%)	*C. sorbina* (98.2%)	*C. sorbina* (97.1%)	Clusters with *C. sorbina*	*Cytospora sorbina*

3.1. Morphological Descriptions

3.1.1. *Cytospora parasitica*

Description: Conidiomata were dark brown, scattered, immersed in the host tissues, rounded, multilocular and slightly protruding through the surface of the bark (Figure 2A,B). The average diameter of the conidiomata was 635 µm (n = 20; SD = 82.67). A single circular ostiole with a diameter of 202 µm (n = 20; SD = 33.87) was located in the center of each dark-gray-to-black conidial disc and surrounded by multiple irregularly arranged locules with common walls (Figure 2C). The mean diameter of the locules was 46.7 µm (n = 20; SD = 8.4). The conidiophores were hyaline, colorless and 33.2 µm in length (n = 30; SD = 7.8) (Figure 2D). The conidia were transparent, curved and allantoid (Figure 2E). The length and diameter of the conidia were 10.7 µm (SD = 1.7) and 2.9 µm (SD = 0.4), respectively (n = 50). Culture characteristics: Fast-growing colonies, initially light gray with fluffy aerial mycelium (3–5 days); later, the middle of the colony began to darken, acquiring a dark gray color. Colonies were aerial, with torn uneven edges, of uniform consistency (day 5). After 2 weeks, the colonies acquired a dense structure of a light-yellow hue (Figure 2F).

Host and location: Overall, four isolates were collected from cankered twigs and branches of apple trees in Almaty region, Kazakhstan. AC-6: collected on 24 August 2023 from 'Gala' in Alma Issyk orchard; AC-97: collected on 25 August 2023 from 'Gala' in Unifruit orchard; AC-106 and AC-108: collected on 25 August 2023 from 'Fuji' in Unifruit orchard.

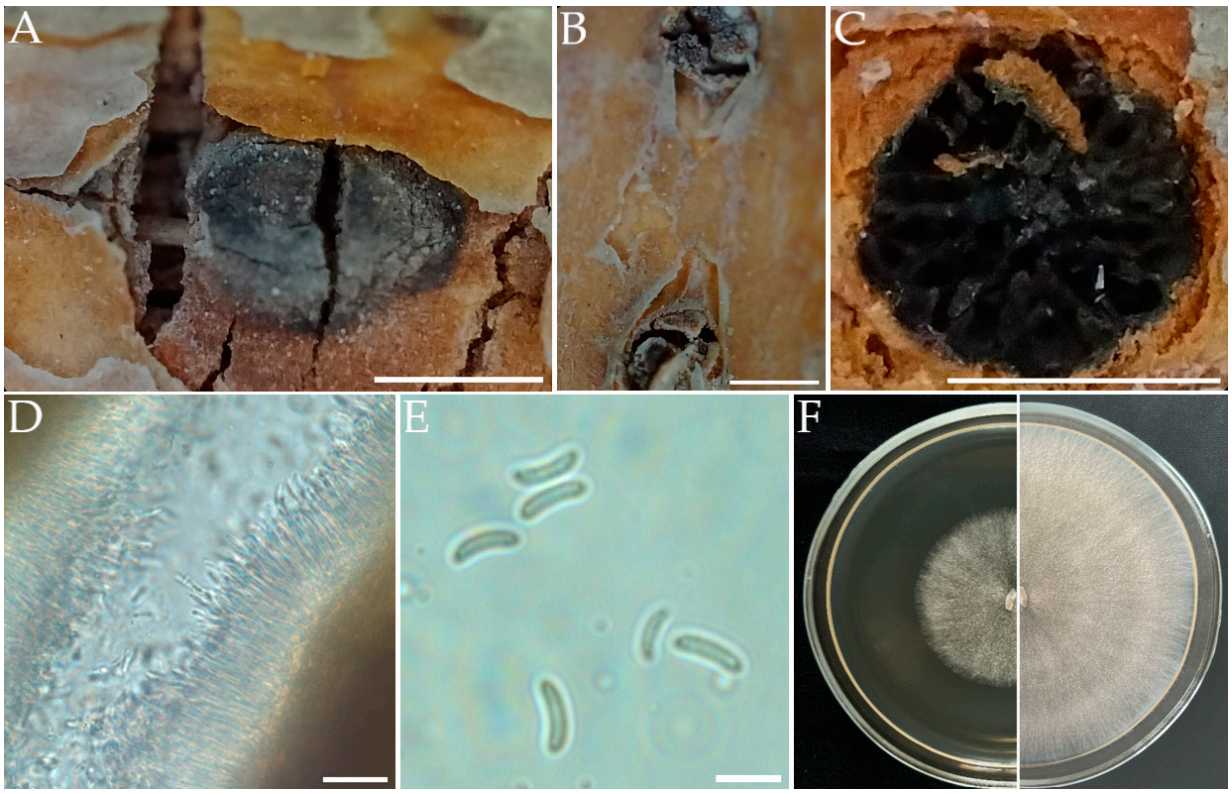

Figure 2. Morphology of *Cytospora parasitica*. (**A**,**B**) Habit of conidiomata on twigs. (**C**) Transverse section of conidioma. (**D**) Conidiophores and conidiogenous cells. (**E**) Conidia. (**F**) Three-day-old colony (left) and 14-day-old colony (right) on potato dextrose agar medium. Scale bars: (**A**–**C**) = 500 µm; (**D**) = 20 µm; (**E**) = 10 µm.

3.1.2. *Cytospora sorbina*

Description: Conidiomata were dark brown, immersed in the bark and 614 µm in diameter (n = 20; SD = 73.19) (Figure 3A,B). There was a single irregular circular ostiole, 199 µm in diameter (n = 20; SD = 27.36) in the center of a dark brown multilocular disc (Figure 3C). The locules were irregularly arranged and with an average diameter of 35.4 µm (n = 20; SD = 7.2). The conidiophores were colorless with a length of 33.7 µm (n = 30; SD = 5.8) (Figure 3D). Conidia were hyaline, smooth-walled, allantoid and aseptate (Figure 3E). The length of the conidia was 12.2 µm (SD = 2.6), and the diameter was 3.4 µm (SD = 0.6) (n = 50). Culture characteristics: Slow-growing colonies, with white mycelium (day 5), later darkening, acquiring a light brown and/or dark yellow color. The colony structure was radiant, of a uniform consistency, and the edges were uneven (day 14) (Figure 3F).

Host and location: Five isolates were collected from cankered twigs and branches of apple trees in Almaty region, Kazakhstan. AC-21, AC-23 and AC-27: collected on 24 August 2023 from 'Golden Rush' in Alma Issyk orchard; AC-34: collected on 24 August 2023 from 'Star Crimson' in Alma Issyk orchard; AC-46: collected on 24 August 2023 from 'Star Crimson' in Manshuk orchard.

3.1.3. *Cytospora pruinopsis*

Description: Conidiomata were light brown, rounded and immersed in the bark but slightly protruding through the surface of the bark (Figure 4A,B). The mean diameter of the conidiomata was 622 µm (n = 20; SD = 64.89). The disc was black, with a single irregular ostiole in the center (Figure 4C). The diameter of the ostiole and locule were 198 µm (SD = 40.53) and 37.3 µm (SD = 7.1), respectively (n = 20) (Figure 4D). The conidia were hyaline, smooth-walled, allantoid, aseptate and curved (Figure 4E). The length of the

conidia was 12 µm (SD = 2.5), and the diameter was 3.1 µm (SD = 0.7) (n = 50). Culture characteristics: Fast-growing colonies, initially white (day 3) with a light-yellow radial ring; later (day 5), the radial ring acquired an olive-yellow tint. Colonies were felt-like, with uneven edges (day 14) (Figure 4F).

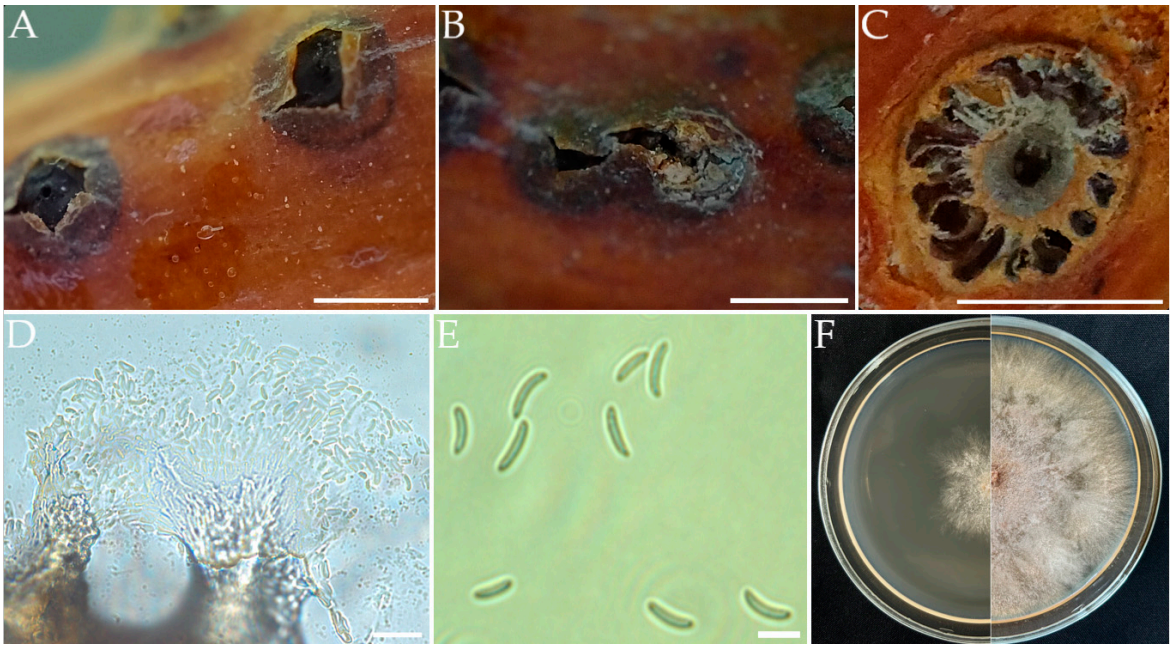

Figure 3. Morphology of *Cytospora sorbina*. (**A**,**B**) Habit of conidiomata on twigs. (**C**) Transverse section of conidioma. (**D**) Conidiophores and conidiogenous cells. (**E**) Conidia. (**F**) Five-day-old colony (left) and 14-day-old colony (right) on potato dextrose agar medium. Scale bars: (**A**–**C**) = 500 µm; (**D**) = 20 µm; (**E**) = 10 µm.

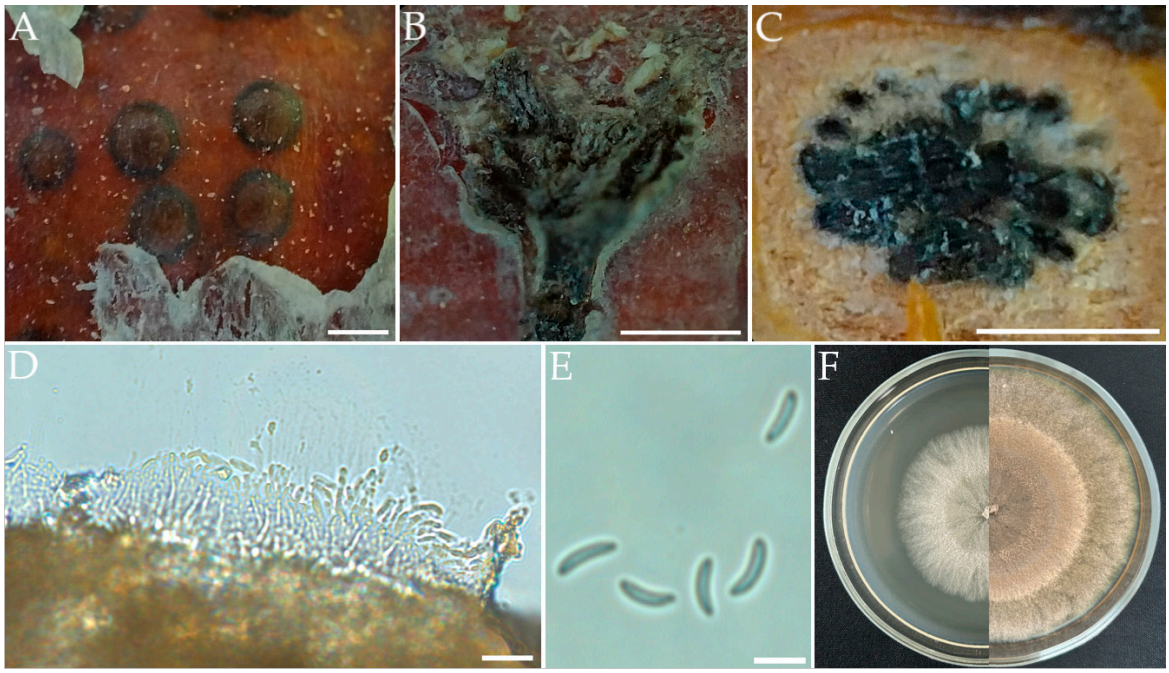

Figure 4. Morphology of *Cytospora pruinopsis*. (**A**,**B**) Habit of conidiomata on twigs. (**C**) Transverse section of conidioma. (**D**) Conidiophores and conidiogenous cells. (**E**) Conidia. (**F**) Three-day-old colony (left) and 14-day-old colony (right) on potato dextrose agar medium. Scale bars: (**A**–**C**) = 500 µm; (**D**) = 20 µm; (**E**) = 10 µm.

Host and location: A single isolate, AC-77, was collected from cankered twigs and branches of 'Golden Delicious' in Manshuk orchard within Almaty region, Kazakhstan, on 24 August 2023.

3.1.4. *Cytospora chrysosperma*

Description: Conidiomata were dark brown and/or black, flat, multilocular, immersed in the bark and 665 µm in diameter (n = 20; SD = 83.31) (Figure 5A,B). The disc was black with a single big rounded ostiole in the center (Figure 5C). The average diameter of the ostiole and locule were 223 µm (SD = 38.27) and 50.5 µm (SD = 4.4), respectively (n = 20) (Figure 5D). Conidia were hyaline, smooth-walled, allantoid and aseptate (Figure 5E). The length and diameter of the conidia were 10.8 µm (SD = 1.9) and 2.6 µm (SD = 0.5), respectively (n = 50). Culture characteristics: Fast-growing colonies with white fluffy aerial mycelium (5–7 days), later acquiring a light-yellow hue; the edges of the colonies were uneven (Figure 5F).

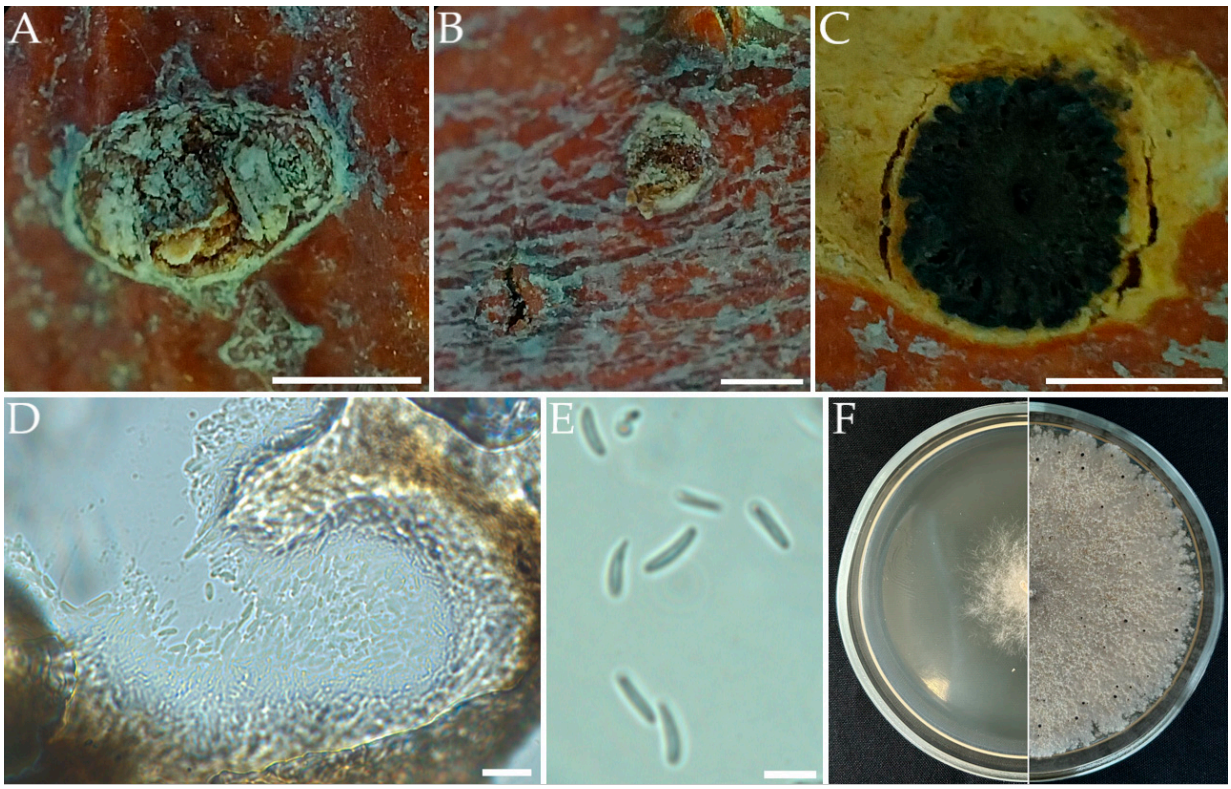

Figure 5. Morphology of *Cytospora chrysosperma*. (**A**,**B**) Habit of conidiomata on twigs. (**C**) Transverse section of conidioma. (**D**) Conidiophores and conidiogenous cells. (**E**) Conidia. (**F**) Three-day-old colony (left) and 14-day-old colony (right) on potato dextrose agar medium. Scale bars: (**A**–**C**) = 500 µm; (**D**) = 20 µm; (**E**) = 10 µm.

Host and location: A single isolate, AC-118, was collected from cankered twigs and branches of 'Fuji' in Arnau Agro orchard within Almaty region, Kazakhstan, on 14 September 2023.

3.2. Phylogeny

Of the four amplified loci (ITS, LSU, tef1-α, tub2), three loci (ITS (598 bp), LSU (507 bp) and *tef1-α* (488 bp)) were used to construct the phylogenetic tree. The *tub2* locus was not involved in constructing the phylogenetic tree due to the fact that the *tub2* locus did not yield amplicons for some of our isolates. Several reference isolates belonging to three species of *Cytospora* (*Cytospora pruni-mume, Cytospora paulowniae* and *Cytospora viticola*),

which have previously been identified by the same three loci that were used for analysis in this study, were chosen for the tree construction.

The phylogenetic analysis involved 75 concatenate partial ITS, LSU and *tef1-α* genes' nucleotide sequences for *Cytospora* species (Figure 6). There was a total of 5358 positions in the final dataset. The tree was rooted with an outgroup of *Phaeoacremonium hispanicum*. The choice of this group was due to its phylogenetic remoteness from representatives of the genus *Cytospora*, allowing for efficient tree rooting and ensuring the reliability of the analysis. As a representative of a genus that is not related to *Cytospora*, *P. hispanicum* acted as a control point, demonstrating the degree of genetic diversity within and between the analyzed species. We also included reference sequences from each species to show how closely related they are (Table S2). The tree was scaled, with branch lengths being measured by the number of substitutions per site.

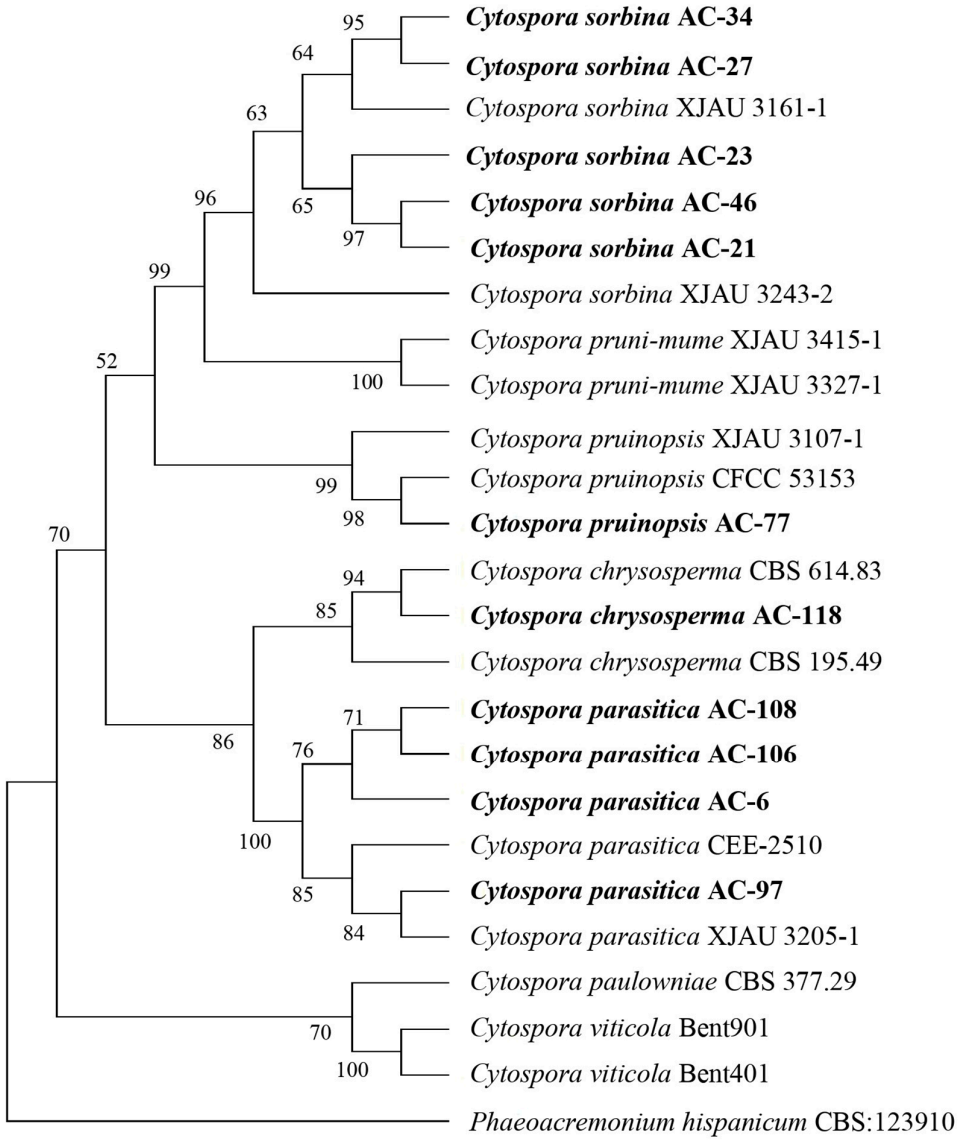

Figure 6. Phylogram of *Cytospora* species was constructed using maximum likelihood and maximum parsimony analyses based on concatenated partial ITS, LSU and *tef1-α*. For analysis, tree was constructed using BioNJ algorithm with bootstrap test (1000 replicates). Isolates obtained in this study are shown in bold.

Within the species *C. sorbina*, subgroups can be observed (e.g., isolates AC-21, AC-46 and AC-23) with bootstrap support of 65, which may indicate the presence of genetic

variations within a single species. The bootstrap support at the nodes of the *C. parasitica* group is quite high (76–85), indicating a good reliability of the clustering of these isolates. In contrast, the *C. pruinopsis* clade is separated from other species such as *C. parasitica* and forms a separate branch. *C. chrysosperma* formed a separate clade, with bootstrap 94 replicates, indicating the strong reliability of clustering of these isolates.

3.3. Pathogenicity Assay

According to the results of the pathogenicity test, all four species of Cytospora were pathogenic but with different virulence levels (Figure 7). Particularly, the *C. parasitica* and *C. chrysosperma* isolates demonstrated very high virulence, whereas *C. sorbina* caused relatively fewer lesion symptoms. Thus, the *C. pruinopsis* isolate was the least aggressive based on the mean lesion length in this experiment. However, multiple isolates per species need to be tested to draw robust conclusions about variations in aggressiveness between species. No apparent symptoms were observed on the control samples.

Figure 7. Pathogenicity tests of four causal agents of Cytospora canker disease on apple saplings. Visual necrotic symptoms caused by (**A**) *Cytospora parasitica*, (**B**) *Cytospora sorbina*, (**C**) *Cytospora pruinopsis* and (**D**) *Cytospora chrysosperma*. Control samples were inoculated with clean potato dextrose agar plugs.

4. Discussion

This study was conducted to identify and characterize causal agents of canker and dieback diseases of apple trees in Almaty region, Kazakhstan, for the first time. Although 43 species of *Cytospora* were previously reported in the south-eastern regions of Kazakhstan by Rakhimova et al. [26], all their isolates were collected from different plants, excluding apple trees. Furthermore, all the 43 species were identified based on only morphological observations and were not confirmed via phylogenetical analysis. In recent years, the number of commercial orchards in Almaty region has increased due to demand in the market. However, severe pre-harvest disease symptoms have been observed increasingly often in almost all orchards. This could be because of the import of more apple cultivars into the country, as well as various environmental factors due to climatic changes.

To date, more than 30 species of *Cytospora* have been reported to cause canker on apple trees worldwide [27–34]. Among these, *C. parasitica* was reported for the first time in this study in Kazakhstan, which was previously reported to cause canker disease in apple in China [24], Iran [29] and Turkey [32]. The other three fungal species, namely *C. sorbina*, *C.*

pruinopsis and *C. chrysosperma,* were also isolated from cankered apple trees for the first time in Kazakhstan. Both the molecular and morphological identification confirmed the presence of the four species. Moreover, the isolates of *C. parasitica* and *C. chrysosperma* from this study showed a high level of virulence on detached twigs of 2-year-old apple trees.

To conclude, the present study reported, for the first time, the characterization and pathogenicity of four causal agents of Cytospora canker disease in six major commercial orchards within the Almaty region, Kazakhstan. However, further pathogenicity tests on live tree samples are needed for a better understanding of the level of aggressiveness of different fungal isolates. Pan et al. [14] stated that the *Cytospora* species occurrence is more related to geographical and environmental factors than it being host-specific. Therefore, similar studies are required to discover new species of *Cytospora* in south-east Kazakhstan, as this region has the most suitable environment for apple growth in the country and borders with China, which is considered a hot spot for Cytospora canker occurrence. In addition, six *Cytospora* strains were reported by Liu et al. [35] on wild apple trees in Tianshan Forest, China. Accordingly, wild apple forests within the Almaty region also require studying for the presence of any potential canker diseases. Nevertheless, we believe that this paper will provide useful information and serve as a foundation for future epidemiological studies and the development of appropriate management strategies against apple canker disease within this region.

5. Conclusions

In this study, both morphological and phylogenetic analyses confirmed a total of 11 fungal isolates belonging to *Cytospora* species. In addition, a pathogenicity test performed on detached twigs demonstrated that *Cytospora parasitica* and *Cytospora chrysosperma* were highly pathogenic. The outcomes are significant, as this is a first formal report of causal agents of apple canker in Kazakhstan. However, further ecological and epidemiological studies are needed to develop informed management strategies against canker diseases in this region.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae11010045/s1: Table S1: Information on sample collection: orchards and cultivars; Table S2: Details of the isolates used in the phylogenetic analysis in this study.

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

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