Late-Season Decline: A New Bacterial Disease of Corn Identified in the Texas Panhandle

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Abstract

The genus Pantoea has historically been associated with two diseases of corn, Stewart’s wilt caused by P. stewartii and necrotic or white leaf spots or streaks and stalk rot caused by P. ananatis. In 2020 and 2021, a sudden and unusual decline of corn stands was observed in corn fields in two counties in the Texas High Plains region. Symptoms observed included initial light green, elongated, slightly translucent, and nonchlorotic streaked lesions with nonwavy margins that developed on leaf blades during corn vegetative growth stages, with lesions becoming necrotic at the onset of crop reproduction. Additionally, stunting of affected plants, poor ear development, and stalk rot were associated with affected stands. Diagnosis of symptomatic tissues consistently recovered bacteria. BLAST searches of the partial 16S rRNA sequences of the bacterial isolates identified them as belonging to the genus Pantoea. Investigations of the pathogenicity of two bacterial isolates, B566 and B623, under greenhouse conditions relying on Koch’s postulates resulted in the development of symptoms identical to those observed on symptomatic field corn plants. The two bacteria were also successfully recovered from symptomatic leaf and stem tissues, thus satisfying Koch’s postulates. Sequence analysis showed that these isolates are closely related to P. ananatis but also phylogenetically distinct. The findings from this study provide evidence for a new disease of corn caused by two Pantoea species that can result in stand decline of infected corn plants.

Keywords: bacteriology, corn, plant pathology

Corn (Zea mays L.) is the most important agricultural crop in the United States based on production volume, with a 2017 production value of $49.5 billion from a total of 36.5 million hectares (USDA National Agricultural Statistics Service, 2017 Census of Agriculture. Complete data available at www.nass.usda.gov/AgCensus). In Texas, fungi are the most common causes of corn diseases. However, during the 2021 cropping season, sudden, drought-stress-like symptoms developed on previously green and healthy corn stands in a fungicide trial study at the Texas A&M AgriLife Experiment Station at Bushland, Texas (Fig. 1). The study comprised a total of 100 plots, including the nontreated control plots, and measured 12.5 m² each. Initial symptoms consisted of light green, elongated, slightly translucent, and nonchlorotic streaked lesions with nonwavy margins that developed on the leaf blades during corn vegetative growth stages (Fig. 2A). Under wet or humid conditions, such as after rain or irrigation, water-soaked margins were occasionally observed around the margins of the lesions. Later in the season, at the onset of the reproductive stage of growth (VT), the incidence and severity of the symptoms increased significantly. During this time, the foliar lesions often enlarged, coalesced, and became necrotic, resulting in leaf blight symptoms that progressed from the leaf tips toward the base of affected leaves. Infected plants also often exhibited stunted growth (Fig. 2B). During this phase of symptom development, affected plants appeared drought stressed. Other symptoms observed on affected plants included arrested ear development (Fig. 2C), with incompletely developed ears occasionally decomposing (Fig. 2D), and small and poorly developed corn ears with few or no kernels (Fig. 2E). Stalk rot is also associated with affected plants (Fig. 2F and G). Lodging of affected plant stands was also correlated with incidences of stalk rot in the affected plants (Fig. 2H). Overall, yield loss from the affected field averaged about 93%. Disease development spanned all of the study plots, treated and nontreated plots alike. Diagnosis of symptomatic plant tissues from both the early and later stages of symptom development for a potential causal organism frequently resulted in the isolation of bacteria, and in several cases no fungi, on culture media. Preliminary investigations of the identity of the two isolated bacteria, following single-colony purification (details below), through BLASTN searches of their partial 16S rRNA sequences indicated that they were most closely related to Pantoea ananatis. The observed foliar symptoms, however, are distinct from those caused by P. ananatis in corn, which results in necrotic or white leaf spots or streaks (Bomfeti et al. 2008; Krawczyk et al. 2021; Paccola-Meirelles et al. 2001) and stalk rot (Gosczynska et al. 2007).

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Pantoea ananatis was first identified and reported as a plant pathogen causing fruitlet rot of pineapple in the Philippines (Serrano 1928). It has since been designated as an emerging pathogen because of its increasing numbers of previously unreported hosts, including as a pathogen of corn; Palea browning (or dark brown leaf lesions) and stalk rot disease of rice (Cother et al. 2004); sudangrass leaf blotches and streaks (Azad et al. 2000); onion leaf blight, seed stalk rot, and bulb decay (Gitaitis and Gay 1997); wheat brownish leaf lesion (Krawczyk et al. 2020); and tomato gray wall disease (Stall et al. 1969). Symptoms of P. ananatis infection of corn are distinct from those of Stewart’s wilt (Roper 2011), caused by Pantoea stewartii. Symptoms of Stewart’s wilt include water-soaked light green lesions with necrotic centers and wavy and chlorotic margins that run parallel to the leaf veins (Roper 2011).

Subsequent to these initial observations of symptoms of a potentially new corn disease, identical foliar symptoms were observed on corn plant samples from Potter County submitted to the Texas High Plains Plant Disease Diagnostic laboratory, located in Amarillo, Texas, for disease diagnosis. Bacteria closely related to P. ananatis, on the basis of their respective 16S rRNA partial sequences, were recovered from all diseased samples. In 2021, widespread lodging attributed to stalk rot was observed in a grower’s irrigated corn field that resulted in a loss of 4.4 to 5.7 thousand kg/ha. Pantoea sp. closely related to P. ananatis on the basis of 16S rRNA was similarly recovered from plant stands. Then, in 2022, identical symptoms were observed in growers’ corn fields in seven additional counties in the Texas Panhandle, specifically in Dallam, Hartley, Sherman, Moore, Hutchinson, Hansford, and Oldham counties. Diagnosis of diseased plants identified during surveys of and visits to suspect fields also recovered bacteria belonging to the genus Pantoea. BLASTN searches of the partial 16S rRNA resulting sequences indicated that the new isolates were closely related to P. ananatis. Sequence alignments of the 16S rRNA sequences of isolates from 2021 and 2022 shared nucleotide identities ranging between 98.7 and 100% (data not shown). Disease severity, however, varied across the affected fields in the different counties. Because neither P. stewartii nor any other previously reported corn bacterial pathogens were recovered from symptomatic tissues, the objective of this study was to investigate the pathogenicity of the newly recovered bacterial isolates.

**FIGURE 1**
Field-grown corn in 2021 during the A, vegetative growth stage (V5-V7) showing no disease symptoms, B, a section of the field, and C, partial field view during the dough/dent growth stage (R4/R5) showing severe foliar blight and drought-like symptoms of the late-season decline disease on corn plants.

**FIGURE 2**
Symptoms of the late-season decline disease on field corn plants including A, pale-green foliar lesions and blight, B, stunting, C, poorly developed and D, unfertilized corn ears, E, hypertrophied kernels, F, tassel senescence, G, silk rot and abscission, and H, stalk rot-induced lodging.
Materials and Methods

Bacterial isolation, purification, and identification
Symptomatic corn leaf and stem tissues from naturally infected field corn plants or inoculated greenhouse plants were rinsed in sterile-distilled water and surface sterilized by soaking in 10% hypochlorite solution for 1 min, then rinsed in five changes of sterile double-distilled water. The surface-sterilized leaf and stem tissues were blotted dry on Kimwipes (catalog #34155; Kimberly-Clark, Roswell, GA) and cut into 0.5 to 1 cm-long sections with sterile scalpel blades. The cut tissue sections were placed in Petri dishes containing water agar using sterile forceps, and the plates were incubated in the dark at 28°C. Representational colonies from the resulting single colonies were selected and further purified through a second round of streaking on LB agar plates and incubated overnight in the dark at 28°C. Represenative colonies from the resulting single colonies were selected and used in downstream analysis. The 16S rRNA polymerase chain reaction (PCR) amplification was carried out using the primer pair 27F (AGAGTTTGTATCMTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) (Galkiewicz and Kellogg 2008) at a final concentration of 0.5 µM and 1 × final concentration of Bio-Rad 2× HF Master Mix (BIO-RAD, Hercules, CA) in a 25 µl total reaction volume. The PCR amplification cycle consisted of an initial denaturing step at 98°C for 3 min; 35 cycles of three steps consisting of 98°C for 10 s, 57°C for 30 s, and 72°C for 30 s; and a final step of 72°C for 10 min. The PCR products, ~1,500-bp amplicons, were sequenced, and the resulting sequences were used to identify the respective bacteria through BLAST searches.

Whole-genome sequencing and alignments
DNA library preparation and sequencing. Bacterial DNA library preparation and sequencing for two representative bacterial isolates, B566 and B623, were carried out as described by Obasa and Haynes (2022). For Illumina short-read sequencing, the genomic DNA of each sample was randomly sheared into short fragments of about 350 bp, which were used for library construction using the NEBNext DNA Library Prep Kit (New England Biolabs, Ipswich, MA) according to the manufacturer’s instructions. This was followed by end reparing, de-tailing, and ligation with the NEBNext adapter. The required fragments (300 to 500 bp size) were PCR enriched by P5 and indexed P7 oligos. The prepared DNA library was then purified and verified for concentration and quality using a Qubit 2.0 fluorometer and subsequently assessed for insert size using the Agilent 2100 bioanalyzer (Agilent Technologies, CA). Finally, the constructed libraries were pooled and paired-end sequenced on the Illumina HiSeq (Illumina, San Diego, CA) sequencing platform. For long-read sequencing with Oxford Nanopore MinION (Oxford Nanopore Technologies, Cambridge,

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MA), library preparation was carried out with the Rapid Barcoding Sequencing kit (SQK-RBK004) according to the manufacturer’s instructions. Sequencing was done using the R9 flow cells according to the manufacturer’s instructions.

Assembly and annotation of sequence reads. The generated raw reads from all sequencing outputs were processed to remove adapters and primers using the post-run processing software for the respective sequencing technologies. The processed reads were assembled using the default settings of the Geneious SPAdes DNA de novo assembler (Biomatters, San Diego, CA; Bankevich et al. 2012; Gurevich et al. 2013). The assembly was analyzed to identify prokaryotic genes using GLIMMER (Delcher et al. 1999); the identified prokaryotic genes were subjected to a BLAST search, and the gene ontology (GO) terms associated with hits obtained from the BLAST search were retrieved by mapping (Gotz et al. 2008). Next, Blast2Go annotation (Gotz et al. 2008) and InterProScan annotation (Jones et al. 2014) were independently run on the mapped assembly, and the resulting annotation results merged. A final round of annotation using EggNOG mapper version 1.0.3 with EggNOG 5.0.0 (Huerta-Cepas et al. 2019) for the functional annotation of prokaryotic gene products.

FIGURE 3
novel sequences using precomputed EggNOG-based orthology assignments was carried out, and the resulting annotations similarly merged with those from the earlier annotations.

**Phylogenetic analysis.** Multilocus sequence analysis using six genes (\textit{dnaK, fumC, gyrB, murG, trpB, and tuf}) obtained from the respective draft genome sequences of two selected isolates, B566 and B62, was used for phylogenetic analysis. The sequences were concatenated and aligned to closely related species’ similarly concatenated sequences (Table 1) identified using BLASTN (Altschul et al. 1990). A phylogenetic tree was constructed using the Tamura-Nei genetic distance model (Tamura and Nei 1993), using global alignment with free end gaps, a gap open penalty of 12, a gap extension penalty of 3, and a 65% similarity cost matrix for the pairwise alignment options for building a distance matrix available in the default settings of the Geneious Tree Builder application in Geneious Prime (Kearse et al. 2012). Furthermore, alignments of the respective assembled genomes of the corn bacterial isolates with those of the type strains of their respective closest relative identified by BLASTN were carried out using the JSpeciesWS online taxonomic threshold service (Richter et al. 2015; jspecies.ribohost.com/jspeciesws/#home) to determine the average nucleotide identity (ANI) between each pair.

**Bacterial inoculum preparation**

Bacterial cultures were grown overnight in LB broth at 28°C and 240 rpm. From the overnight cultures, a subculture was transferred into fresh LB broth and similarly incubated at 28°C and 240 rpm to an absorbance value (OD\textsubscript{600}) of 0.5 as measured with the Denovix DS-11 FX + spectrophotometer (DeNovix, Wilmington, DE). To estimate the cell density in colony-forming units (CFU) for each bacterial culture, a subsample of the final culture was serially diluted in sterilized nuclease-free water (Ambion - Life Technologies, Austin, TX), spread on LB agar plates in triplicate, and incubated overnight in the dark at 28°C.

**Pathogenicity assay of bacterial isolates on corn**

Seeds of Pioneer corn hybrid “P1151” were planted in sterile potting media (Berger BM1 Nutrient Retention General Purpose Media; Hummert International, Topeka, KS) contained in 25-gallon plastic pots at the rate of four seeds per pot. The pots were lightly watered daily and maintained at 28°C, and a 14-h photoperiod was achieved with supplemental lighting under greenhouse conditions. Seventy-two days after germination, two randomly selected upper canopy leaves or stems were inoculated with the suspension of one of two randomly selected bacterial isolates, B566 or B623, at a final concentration of $7.5 \times 10^6$ CFU. Stem inoculation was done with 100 µl of bacterial suspension using a pipette. For leaf inoculation, either the leaf mid-rib was inoculated with 20 µl of bacterial suspension or the leaf lamina was inoculated with bacterial cells collected from single colony-purified cultures using the tip of a sterile toothpick to create up to 15 randomly distributed inoculation foci per inoculated leaf. All four plants in a pot were similarly inoculated, and two replicate pots per bacterial treatment were set up. Pots were arranged in a complete randomized design and observed for symp-

![FIGURE 4](image-url)  
Koch’s postulate evaluation of two bacterial isolates, B566 and B623, recovered from field corn plants affected by the late-season decline disease, under greenhouse conditions. Postinoculation pictures: A, the development of pale green foliar lesions (black arrow) during the vegetative growth stage, B, foliar blight (black arrow) after the onset of the reproductive growth stage, C, lesion with water-soaked appearance (black arrow) in inoculated leaf mid-rib during the vegetative growth stage and D, necrotized leaf mid-rib lesion (black arrow) after the onset of the reproductive growth stage, E, development of slightly sunken spherical stem lesion with water-soaked appearance (black arrow) during the vegetative growth stage that similarly became necrotic F, (black arrow) after the onset of the reproductive growth stage on inoculated plants.
tom development. Pots with plants similarly inoculated, but with sterile double-distilled water, in the stem, mid-rib, or leaf lamina with the tip of a sterile toothpick, served as the respective noninoculated controls. Following symptom development, representative tissues were collected and diagnosed to identify the causal pathogen. The collected symptomatic tissues were surface sterilized, placed on LB agar in Petri dishes, incubated, and subsequently diagnosed as described above for the presence of the bacteria used to inoculate the particular plant. Recovered bacteria were single-colony purified by streaking. The 16S rRNA sequence fragments from recovered bacterial isolates were aligned and compared with those of the bacterial isolates originally used to inoculate the respective plants. The study was conducted twice.

Results

Bacteria associated with symptomatic corn tissues

BLASTN searches of the 16S rRNA partial sequence of the bacterial isolates B566 (GenBank accession: OP268626) and B623 (GenBank accession: OP268628), as well as those of other recovered isolates from the field, determined that they all belong to the genus *Pantoea*, with *P. ananatis* (GenBank accession: MG681216.1, MK156144.1, and MK414947.1) identified as their most closely related species. Multilocus sequence analysis using six genes (*dnaK*, *fumC*, *gyrB*, *murG*, *trpB*, and *tuf*) obtained from the respective draft genome sequences of two selected isolates, B566 and B623, and phylogenetic analysis, however, indicated that bacterial isolate B566 is the most closely related to *P. ananatis* (Fig. 3). The analysis determined that B623 was most closely related to *Pantoea* sp. isolate B270 (GenBank accession: JAHCSX000000000), a recently discovered bacterial pathogen of peanut that causes bacterial early decline disease of peanut in Texas (Obasa and Haynes 2022). Nevertheless, the branching of the two bacteria indicated that they are in distinct genotype groups and likely represent unique species that are distinct from both *Pantoea* sp. isolate B270 and *P. ananatis* (Fig. 3). To further evaluate the relatedness of the two bacterial isolates, B566 and B623, with their closest relatives identified by the phylogenetic analysis, separate alignments of the whole-genome sequence of each bacterium with *Pantoea* sp. isolate B270 (GenBank accession: JAHCSX000000000), and a type strain of *P. ananatis* LMG 2665 (GenBank accession: GCA_000661975.1; Table 1) was performed. Additionally, the relatedness of B566 to B623 was assessed by conducting a whole-genome sequence alignment with a type strain of *P. stewartii* subsp. *stewartii* CCUG 26359 (GenBank accession: GCF_011044475.1; Table 1).

For the alignments of the genome of isolate B566 with those of *Pantoea* sp. B270, *P. ananatis* LMG 2665, and *P. stewartii* subsp. *stewartii* CCUG 26359, the calculated ANIs (and aligned nucleotide percentages) were 77.1 (61.9), 98.9 (89.4), and 83.6% (71.8%), respectively. For the alignments of the genome of isolate B623 with those of *Pantoea* sp. B270, *P. ananatis* LMG 2665, and *P. stewartii* subsp. *stewartii* CCUG 26359, the calculated ANIs (and aligned nucleotide percentages) were 77.1 (61.8), 98.8 (90.7), and 83.4% (71.9%), respectively. Additionally, the calculated ANI (and aligned nucleotide percentages) from the alignment of the genomes of isolates B566 and B623 was 98.8% (89.6%), indicating that isolates B566 and B623 are genetically distinct from each other, as well as *Pantoea* sp. B270, *P. ananatis*, and *P. stewartia*, respectively. Therefore, the two bacteria were designated *Pantoea* sp. isolate B566 (GenBank accession: JANUQL000000000) and *Pantoea* sp. isolate B623 (GenBank accession: JANUXM000000000).

Bacterial isolates pathogenic on inoculated corn plants

Inoculation of corn leaf margins with isolate B566 or B623 resulted in identical symptoms as those observed on affected field corn plants, including the development of light green, elongated, slightly translucent, and nonchlorotic streak lesions with nonwavy margins (Fig. 4A). Symptoms were initially quiescent, during the vegetative growth stage, but became active and necrotic from around the onset of tasseling (VT) (Fig. 4B). Inoculated mid-rib tissues de-

![FIGURE 5](https://example.com/Figure5.jpg)

Koch’s postulate evaluation of two bacterial isolates, B566 and B623, recovered from field corn plants affected by the late-season decline disease, under greenhouse conditions. Postinoculation pictures: A, normal growth of noninoculated control plants, and stunting and delayed onset of the VT growth stage in B, B566- and C, B623-inoculated plants.
veloped expanding water-soaked lesions around inoculation foci (Fig. 4C) that similarly turned necrotic around the VT growth stage (Fig. 4D). Plants inoculated through the stems also developed spherical, slightly sunken, and water-soaked lesions around inoculation foci (Fig. 4E), which also turned necrotic around the VT growth stage (Fig. 4F). Relative to the noninoculated control plants, all the stem-inoculated plants were also visibly stunted by the VT stage of growth, the onset of which was also delayed on the inoculated plants (Fig. 5). Stem pigmentation on inoculated plants (Fig. 6B and D) was visibly less compared with that on the noninoculated control plants around the dough to dent stage of growth (R4-R5) through maturity (Fig. 6A to D). Furthermore, at maturity, necrosis of vascular tissues extending from the inoculation foci was visible in stems of inoculated plants but not the noninoculated control plants (Fig. 6E and F). Finally, ears that developed on inoculated plants, relative to those on the noninoculated control plants, showed varying levels of malformation, ranging from poorly developed cobs to absence of kernels, as well as the presence of few and hypertrophied kernels on cobs (Fig. 7). Bacterial isolates recovered from leaf and stem lesions had 16S rRNA sequence identities that were 100% identical (data not shown) to those of the original bacterial isolates used to inoculate the respective plant tissues, thus satisfying Koch’s postulates. Neither bacterium was recovered from the noninoculated plants.

**FIGURE 6**
Comparison of the effect of stem inoculation on stem pigmentation shows
A and C, conspicuous reddish pigmentation on the stem and leaf mid-ribs of noninoculated control plants and
B and D, less prominent pigmentation of stems and leaf mid-ribs of inoculated plants. Longitudinal stem sections of E, noninoculated and F, inoculated plants showing the presence of stalk rot only in the stems of inoculated plants.

**Discussion**

The discovery of symptoms of a new disease on corn plants, initially in Potter County, Texas, in 2020, and again in 2021, that affected leaves, stem vascular tissues, and ears and caused stunting led to the identification of bacteria belonging to the genus *Pantoea* as the causative agents of the disease. The initial identity of the bacterial pathogens was determined to be most closely related to *P. ananatis* based on the alignments of their respective 16S rRNA partial sequence fragments against those of closely related species identified through BLASTN searches of the National Center for Biotechnology Information database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Two isolates, B566 and B623, whose 787- and 624-bp 16S rRNA sequences respectively returned a 99.62 and 100% identity match with *P. ananatis* in BLASTN searches, were selected for further characterization, pathogenicity, and disease epidemiology studies. Whereas BLASTN analysis of the 16S rRNA sequences of bacterial isolates of the new corn disease identified *P. ananatis* as their closest relative, results of the MLSA and whole-genome sequence analysis suggest that the isolates of the new corn disease are genetically distinct from *P. ananatis* and could represent new species within the genus *Pantoea*. This distinction is also supported by the fact that the foliar symptoms of the new disease are phenotypically distinct from the necrotic spots and streak symptoms...
of foliar symptoms in different corn hybrids, suggesting possible
transmission, and epidemiology of this new and rapidly spread-
ing bacterial pathogen that has shown potential to cause sig-
ificant economic losses. This is particularly important for Texas
given that a substantial acreage of corn production is irrigated
and thus can create a favorable microclimate for bacterial disease
establishment.

(Bomfeti et al. 2008; Krawczyk et al. 2021; Paccola-Meirelles et al.
2001) induced by *P. ananatis* in corn. This new disease is there-
fore designated bacterial late-season decline disease of corn (LSD).
Overall, symptoms from inoculation of corn plants with the two
bacterial isolates, B566 and B623, were identical to those observed
on symptomatic corn plants in the field and included the light green,
elongated, slightly translucent, and nonchlorotic streak lesions with
nonwavy margins, stunting, and vascular necrosis, as well as small
and poorly developed corn ears with few or no kernels that often
were also hypertrophied. Foliar and stem lesions also became exac-
cerbated and turned necrotic from around the onset of the reproduc-
tive growth stage. The onset of pigment development on stem in the
hybrid used is associated with the period of increased stem lignifi-
cation and ear development. The observed reduced stem pigmentation
is therefore hypothesized to be related to the stalk rot induced by the
bacteria in the stems of the inoculated plants. Additionally, although
no lodging was observed during the two independent greenhouse
studies, the stems of the inoculated plants at maturity were notice-
ably less rigid and prone to breaking when slightly leaned. The
stem breakages were also observed to occur around the regions of
the stems that had extensive stalk rot. A similar corn stand with
stalk rot-induced weakened stems would be susceptible to lodging
under the field conditions of often strong winds common in the
Texas Panhandle, similar to observations of naturally infected field
corn. The successful recovery of the two bacteria, B566 and B623,
from the respective inoculated plants, but not from the noninoc-
ulated control plants, satisfied Koch’s postulate and confirms that
the bacteria induced the observed symptoms and, consequently, are
the causative agents of the new disease. The fact that inoculations
with isolates B566 and B623 resulted in identical symptoms, al-
though their original source locations are about 116 miles apart in
two separate counties (Potter and Hansford, respectively), further
supports a causal role of these new *Pantoea* species in the disease
development.

Following the first observed cases of the new disease in Texas
corn in 2020 and again in 2021 in Potter and Hansford counties, in
the Texas High Plains, the disease has since been confirmed in seven
additional counties in 2022 in the Texas High Plains. Ongoing ob-
servations from investigations of suspected cases from corn fields in
2022 have revealed the occurrence of variations in the appearance
of foliar symptoms in different corn hybrids, suggesting possible
differential outcomes from host-pathogen interactions. Such inter-
actions may or may not influence disease severity. However, it is
noteworthy that the observed disease severity in 2022 varied across
corn fields with different hybrids. In some fields, normal ear de-
velopment did not appear to have been noticeably affected, along
with minimal incidences of minor stalk rot, whereas in others, late-
season foliar blight, evidence of abnormal ear development, and
extensive stalk rots were observed. Factors such as the corn hybrid
genotypes grown, ecological and local environmental conditions in-
cluding weather, crop production, and pest management practices in
the different fields could have been contributing factors. The rapid
multicounty spread of the bacterial pathogen in the 12 months since
the first confirmation of the disease in 2021 suggests the possibility
of the existence of more than one mechanism of transmission of the
causative bacteria. In the case of the disease outbreak that occurred
in 2021 in the fungicide study plots at Bushland, Texas, the Pioneer
P1151 corn hybrid seeds planted to the study plots were from the
same seed batch that was used in previous field and greenhouse stud-
ies in 2019 and 2020, the two years immediately preceding 2021. In
those studies, no instance of the disease was noticed, thus eliminat-
ing a seedborne origin in the particular Pioneer P1151 seed batch.
The absence of the bacterial pathogens in the seed batch was also
confirmed through seed testing (data not shown). Consequently, the
2021 disease outbreak likely resulted from pathogen introduction
through a mechanism other than seed. Preliminary testing of a lim-
ited set of seeds from ears collected from two corn fields affected
by the disease in 2021, however, successfully recovered bacteria
whose 16S rRNA sequences showed high similarity to those of
bacteria recovered from symptomatic leaf and stem tissues of the
respective plants. BLASTN searches also identified *P. ananatis*
as their closest relative (data not shown). Observations on this aspect
have been inconsistent, and additional work is needed to elucidate
any potential seed pathway in the spread of the disease bacterial
pathogens.

Additional work is needed to determine sources of inoculum,
transmission, and epidemiology of this new and rapidly spread-
ing bacterial pathogen that has shown potential to cause sig-
ificant economic losses. This is particularly important for Texas
given that a substantial acreage of corn production is irrigated
and thus can create a favorable microclimate for bacterial disease
establishment.

**FIGURE 7**
Effect of inoculation on corn ear development shows A, normal ears from noninoculated control plants, whereas B, B566- and C, B623-inoculated plants pro-
duced poorly developed and deformed ears with few or no kernels, some of which were also hypertrophied.
If seed transmission is confirmed based on extensive seed testing in the future, the use of certified seed and preplanting seed treatment methods would be important for farmers to consider. It may also offer an opportunity for detection of potential seedborne inoculum through seed testing. This can help to identify infected seed lots for subsequent exclusion from trade and planting, as a way to limit the spread of the disease. Implementation of such a management strategy can help to mitigate the risk to farmers of the potential economic losses associated with the disease.

**Conclusion**

The findings from this work provide evidence for a new bacterial disease of corn that is distinct from previously reported bacterial diseases of corn. Although infection can become established early during the vegetative growth stage, disease severity increases during the reproductive growth stages of affected corn crops. The resulting foliar blight associated with symptom development during the reproductive growth stages, coupled with the potential to cause stalk rot, can adversely impact yield. Stalk rot-induced stand lodge, for productive growth stages, coupled with the potential to cause stalk diseases of corn. Although infection can become established early through seed testing. This can help to identify infected seed lots and its application to single-cell sequencing. J. Comput. Biol. 19:455-477.


