Recovery Plan

for

Xanthomonas oryzae

Causing Bacterial Blight and Bacterial Leaf Streak of Rice

June 4, 2013

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This recovery plan is one of several disease-specific documents produced as part of the National Plant Disease Recovery System (NPDRS) called for in Homeland Security Presidential Directive Number 9 (HSPD-9). The purpose of the NPDRS is to ensure that the tools, infrastructure, communication networks, and capacity required to mitigate the impact of high consequence plant disease outbreaks can maintain a reasonable level of crop production.

Each disease-specific plan is intended to provide a brief primer on the disease, assess the status of critical recovery components, and identify disease management research, extension and education needs. These documents are not intended to be stand-alone documents that can address all of the many and varied aspects of plant disease outbreak and all of the decisions that must be made and actions taken to achieve effective response and recovery. They are, however, documents that will help USDA guide further efforts directed toward plant disease recovery.

Executive Summary

Bacterial blight (BB) and bacterial leaf streak (BLS) are the two most important bacterial diseases of rice worldwide. The diseases are caused by two pathovars of *Xanthomonas oryzae: X. oryzae* pv. *oryzae* (*Xoo*) that causes BB, and *X. oryzae* pv. *oryzicola* (*Xoc*) that causes BLS. *Xoo* and *Xoc* are not found in the USA. A third group of *X. oryzae,* referred to herein as *Xo*-USA, is found in LA and TX in the USA, causes very weak disease symptoms that resemble BB, is genetically distinct from *Xoo* and *Xoc,* and currently has no pathovar designation.

Xoo and *Xoc* are widely distributed and endemic in many countries in Asia, Africa and Australia, but they have not been found in North America. There are sporadic and/or single reports of *Xoo* in several other rice producing countries, but these have not been systematically verified.

Alternate hosts for both pathogens include weed species commonly found in rice production systems, including *Leersia spp.* and wild rice species (*Oryza* spp.). In only a few cases have Koch's postulates been performed to demonstrate that other weed species are indeed symptomatic or asymptomatic hosts for *Xoo* and *Xoc.*

Xo-USA*, Xoo* and *Xoc* can be reliably distinguished from one another using PCR-based approaches, and improved protocols for detecting the pathogens in seed- and plant-tissue are being evaluated internationally.

Control of BB and BLS are typically through genetic resistance. BB is most effectively controlled through the use of qualitative resistance governed by single resistance genes. Changes in the race structure of *Xoo* populations can render *R* genes ineffective, so there are continual efforts to identify new sources of resistance, including sources of quantitative resistance. To date, the sources of qualitative resistance for BLS are very limited, and no race structure for *Xoc* has been reported. Thus, most sources of resistance for BLS are quantitative.

Rice is an important commodity for USA agriculture, valued at approximately $2.63 billion in 2011. To protect this important commodity, quarantine efforts are in place to prevent entry of BB and BLS into the USA. As BB and BLS have not occurred in the USA, there have been no concerted efforts to incorporate resistance to these diseases into widely used USA germplasm. Over 30 single gene resistance sources are available for controlling BB, and a few sources of QTL-based resistance for both BLS and BB. Judicious use of resistance, however, requires understanding of the local pathogen populations to be controlled. Thus, if *Xoo* or *Xoc* were detected in the USA, a first critical effort would be to identify effective sources of resistance. To date, this can only be accomplished by analysis of virulence spectrum through plant inoculation.

The best protection of the USA rice industry from BB and/or BLS will be achieved by exclusion through effective statutory quarantines, early detection, and eradication by host destruction. Identification and development of resistant germplasm, and improvement of detection and race monitoring tools are key components of this recovery plan.

Recommended Actions:

1. Develop improved field-level detection tools, seed-detection protocols, and certification approaches. These must reliably distinguish *Xoo* and *Xoc* from each other, and from other *Xo* and *Xanthomonas* species.
2. Improve tools for rapid and accurate characterization of the race structure of the *Xoo* pathogen population. Understanding the effector repertoire is important to knowing what *R* gene combination will be effective in controlling disease [65,31].
3. Educate and train extension personnel, growers and crop advisors in the symptomatology and detection of BB and BLS in field conditions.
4. Assess key germplasm used in the USA in countries where BB and BLS are indigenous, to screen for resistance. An important caveat is that the resistance sources detected and integrated may not prove effective against the specific race introduced.
5. Improve genetic resistance by incorporating widely effective R genes, and identifying and incorporating sources of broad-spectrum resistance (effective against both *Xoo* and *Xoc*, and effective against all races of *Xoo*). This could be through novel transgenic approaches or through the introgression of novel QTL-based resistance.
6. Adopt uniform detection/diagnosis protocols among quarantine agencies worldwide.
7. Develop the physical resources to test, conserve, store, maintain strains or DNA of *Xo* pathogens in the USA.

Xanthomonas oryzae

Causing Bacterial Blight and Bacterial Leaf Streak of Rice

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I. Introduction

Xanthomonas oryzae is currently classified by two pathovars based on symptoms on the same host (rice, *Oryza sativa*). *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) causes bacterial blight (BB, formerly called bacterial leaf blight, BLB) and *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) causes bacterial leaf streak (BLS). Several excellent reviews of the two diseases and the pathogens that cause them are available [40,49,42,53]. Of the two diseases, BB is currently the more economically important [41]. Under disease-conducive conditions and in rice hosts with ineffective resistance, BB can cause yield losses up to 70% [55,42], although more typical reports range from 20-50% [53]. Collectively, because of its economic impact and its role as a well-established model system, *Xoo* has been ranked in the top-10 list of bacterial plant pathogens [39].

Relative to BB, BLS disease caused by *Xoc* is less widespread, occurring in tropical and subtropical regions of Asia, Africa and Australia [16], and is less severe, with losses usually ranging between 10-20%. However, in recent years, the disease has been observed with increasing frequency and wider distribution in Asia and Africa, likely due to the planting of susceptible varieties, including new high-yielding hybrids, and possibly, as a result of a changing environment [76,63,16,75].

BB and BLS has not been found in rice in the USA. Rice is an important commodity for USA agriculture. In 2011, rice production in the USA was valued at approximately $2.63 billion, half of which was exported (USDA National Ag Statistics Service, 2012). Rice production in the USA occurs on more than 2 million acres in Arkansas, California, Louisiana, Mississippi, Missouri, and Texas. Thus, with the value of rice, the potential for introduction of BB or BLS into the USA is of great concern.

In the 1980s, there was concern that BB had entered the USA. Outbreaks of a disease with weak symptoms that were similar to BB occurred in Texas and Louisiana [25]. Yellow pigmented, Gram negative bacteria isolated from infested leaves were shown to be *Xanthomonas*, and these bacteria caused weak BB-like lesions on a few susceptible rice varieties [25]. Yield losses were less than 1 %. Although the organism was diagnosed as *X. campestris* pv. *oryzae* (note that *Xoo* was previously named

X. campestris pv. *oryzae*), the authors emphasized that the organism and the disease it caused were clearly distinct from BB caused by Asian strains of *Xoo* [25]. The USA strains were much less virulent than the Asian *Xoo* or *Xoc,* and the symptoms caused by the USA strains were similar among each other but different from those caused by Asian *Xoo*. The genomic fingerprint of the USA strains, as detected by restriction enzyme digestion of genomic DNA, or Restriction Fragment Length Polymorphism analysis using IS elements or an avirulence effector gene as probes, is clearly distinct from the ones reported for Asian and African *Xoo* and *Xoc* strains [25,33,16]. Later, using draft genome sequences in a comparative analysis, the bacterial pathogen from the USA rice was confirmed to be distinct from *Xoo* and *Xoc,* but declared to be within the species *Xanthomonas oryzae* (more below)[64].

Relationships of *X. oryzae:* Pathovars *Xoo* and *Xoc* are highly related, with over 85% DNA homology, and they are distinguished by only a few phenotypic features [68]. Presently, complete genome sequences for three strains of *Xoo* - Japanese strain MAFF311018 [51], Korean strain KACC10331 [34], and Philippine strain PXO99A [60] - and one strain of *Xoc,* Philippine strain BLS256 [9], are available. Draft genome sequences of two *Xo*-USA strains are published [64]. Phylogenetic analyses using these sequences defined three major genetic lineages among the species *X. oryzae*: Asian strains of *Xoo,* African strains of *Xoo*, and *Xoc* (from Asia and Africa) [20,16]. The *Xo*-USA are now grouped into a fourth genetic lineage of *X. oryzae* [64] but these strains are not yet designated a pathovar [64].

Pathogenic specialization: *Xoo,* but not *Xoc,* is characterized by a high degree of physiological race-cultivar specificity; races are classified by inoculation to a standard differential set of rice cultivars that contain single BB resistance genes (*Xa* genes) in the same rice genetic background [40,52]. For example, the differential set of near isogenic lines designated as IRBB contain a single *Xa* gene designated by the gene's number, e.g., IRBB10 contains the BB *R* gene *Xa10* [52]. Race designation is built from the complement of effector (avirulence) genes in the pathogen and the *R* genes in the host differential. This implies that as *Xoo* acquire or lose effector gene function, which happens frequently in the field, sources of resistance may no longer be effective [48,47,66,45,65,54,72].

Distribution: *Xoo* is widely distributed throughout rice growing countries in Africa (Benin, Burkina Faso, Cameroon, Egypt, Gabon, Gambia, Mali, Niger, Nigeria, Senegal, and Togo), Asia (Bangladesh, Cambodia, China, India, Indonesia, Iran, Japan, Korea, Laos, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand, and Vietnam), and Oceania (Australia) (CABI, 2011; EPPO, n.d.). Although a few old reports describe *Xoo* in Mexico and parts of Central and South America, consistent and validated reports from those areas, particularly within the past 30 years, are lacking, suggesting the disease is not endemic in those areas [38,80,53]. Rice with symptoms similar to BB were first reported in the United States (Texas and Louisiana) in the late 1980s [25]. However, molecular and genomic methods confirmed that these symptoms were caused by an undesignated pathovar of *Xo*, and not by *Xoo* or *Xoc* [30,57,64]. Currently, the *Xo*-USA strains are known to be present only in the United States.

The distribution range of *Xoc* includes Africa (Burkina Faso, Madagascar, Mali, Nigeria, and Senegal), Asia (Bangladesh, Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Thailand, and Vietnam), and Oceania (Australia) (CABI, 2012; EPPO, n.d.).

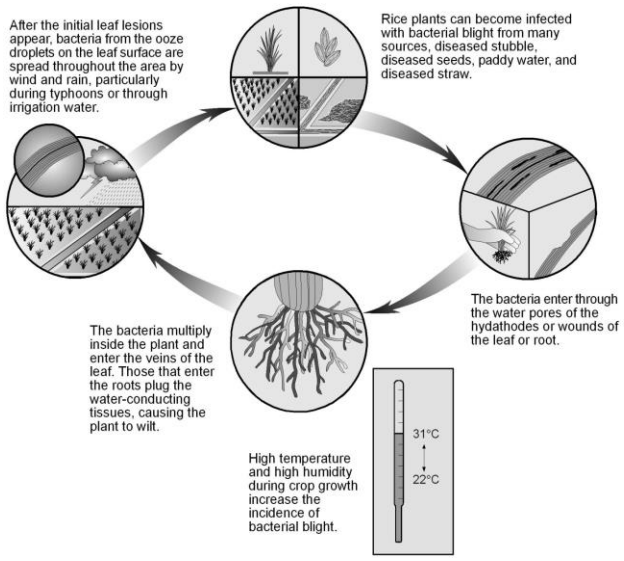
Alternate hosts: The primary host of the *Xanthomonas oryzae* pathovars is rice (*Oryza sativa*). In Asia, perennial weeds are considered a possible source of inoculum [53]. These minor hosts can be monocots such as wild rice (*Oryza* spp.) and wild grasses of the *Poaceae* family, Bermuda grass (*Cynodon dactylon*), sedges (*Cyperaceae*), small-flowered nutsedge (*Cyperus difformis*), purple nutsedge (*Cyperus rotundus*), barnyard grass (*Echinochloa crus-galli*), southern cut grass (*Leersia hexandra*), rice cutgrass (*Leersia*

oryzoides), Chinese sprangletop (*Leptochloa chinensis*), red sprangletop (*Leptochloa filiformis*), Guinea grass (*Panicum maximum*), ricegrass paspalum (*Paspalum scrobiculatum*), buffelgrass (*Pennisetum ciliare*), grasses (*Poaceae*), tall panicum (*Urochloa mutica*), annual wildrice (*Zizania aquatica*), northern wild rice (*Zizania palustris*), and zoysiagrass (*Zoysia japonica*) [53]. The *Xo*-USA pathovar has been isolated from *Leersia* spp., a weed that can serve as a host in the southern United States (Louisiana and Texas) [17].

Wild hosts of *Xoc* that have been reported include southern cut grass, grasses (*Poaceae*), annual wildrice, red sprangletop, ricegrass paspalum, northern wild rice, and zoysiagrass, although their significance in the life cycle of the pathogen is not known [53]; Wonni, Detemmerman et al. in preparation).

II. Signs and Symptoms

Fig 1. Disease cycle of bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae.* Reprinted from [67] with permission from APS Press.



The disease cycle of bacterial blight is shown in Figure 1. Despite the similarities of *Xoo* and *Xoc,* the two pathogens enter and reproduce in very different rice tissues. *Xoo* is a vascular pathogen [61,62]. It can enter the vessels directly through wounds generated during transplanting or by the wind-driven rains during typhoons. Alternatively, *Xoo* can gain access to vessels by moving with guttation fluids through natural openings called hydathode water pores located on the edges of rice leaves [19,43,62]. Once *Xoo* has entered the epithem, the chamber beneath the water pore, the bacteria multiply, move through the vascular pass and into the xylem vessels where they multiply and spread throughout the vascular system [62]. *Xoo* accumulates in high numbers in advance of lesions [6,70]. Younger plants are very susceptible to *Xoo*, particularly because of injuries caused during transplanting or by typhoons [41].

BB usually develops in the field at the tillering stage of rice plants. The first symptom of the disease is a water-soaked spot near the margins of fully expanded leaves [42]. BB lesions have a wavy margin and expand through the vascular tissue of the plant. The lesions rapidly enlarge in length and width along the veins, merging into wavy, elongated lesions. Older lesions appear as bleached white to straw colored necrotic areas that may cover most of the leaf (Figure 2A, C). The symptoms may be difficult to distinguish from physiological problems such as saline toxicity and drought sensitivity.

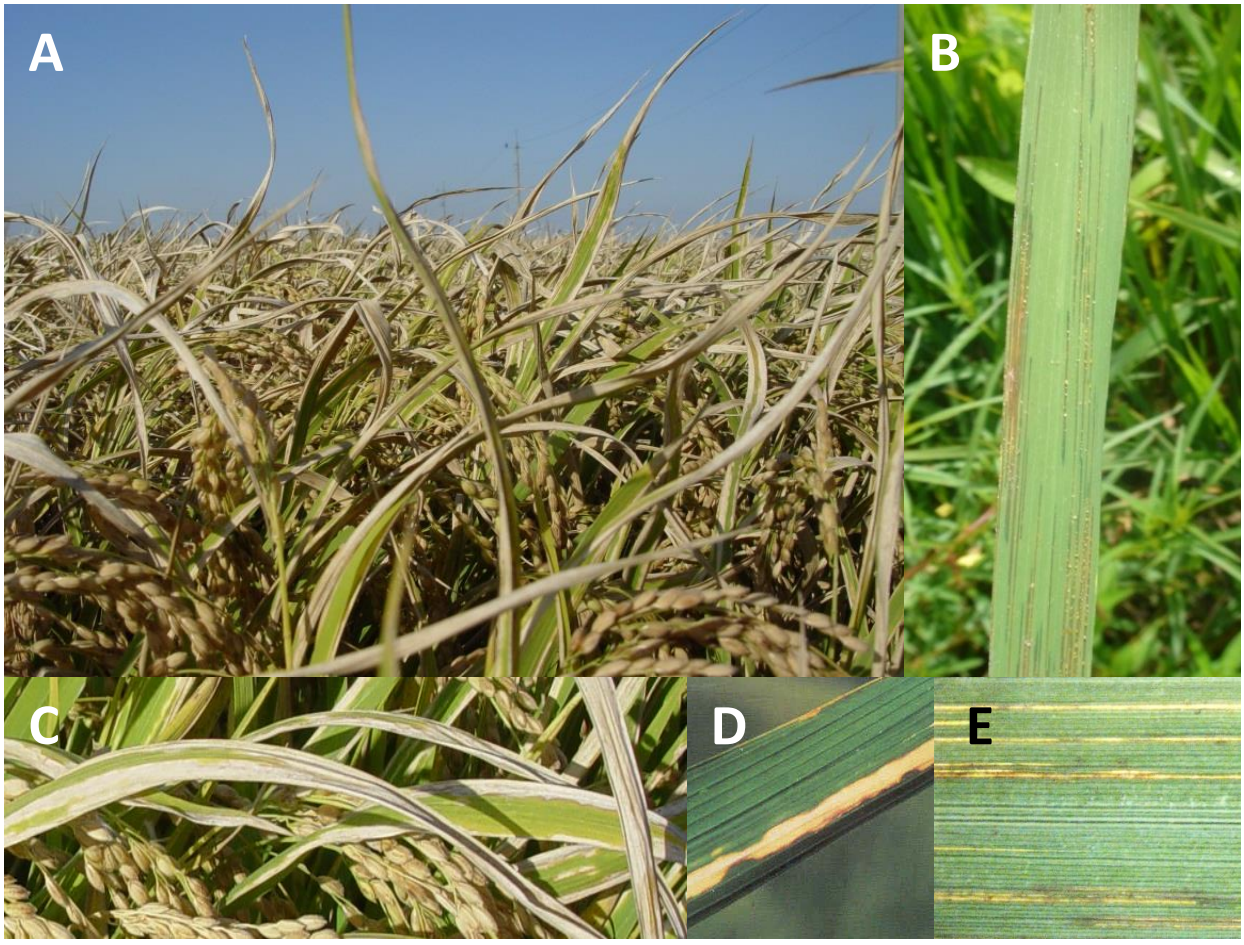
If infected at the seedling stage, a 'kresek symptom' may occur from 1 or 2 weeks after transplanting; in this case, the diseased leaves become greyish-green in color and fold then roll up along the midrib; the plants die within 2 to 3 weeks. Plants that survive kresek appear stunted in height and are overall yellowish-green in color [41,42]. A pale yellow leaf symptom may also occur under highly favorable

conditions, usually occurring on the youngest leaf of infected tiller, however no *Xoo* can be isolated from these leaves. This could be attributed to accumulation of the bacteria at the bottom of the stem or it could be due to toxin produced by *Xoo* [44,45]. Movement between leaves and plants occurs as bacterial exudates are blown by strong winds or splashing rains, or as leaves rub against one another. The *Xo* pathovars may also be present in asymptomatic tissue, passively multiplying for a quorum to initiate the synthesis of virulence factors and start a pathogenic function [70,23,85,6].

In case of severe BB infection, yellow bacterial exudates are visible in the guttation fluid, which oozes from the leaves' natural openings in the morning. These bacterial exudates form dried up clumps of bacteria on the underside of the leaf. Exudates may also become a secondary source of inoculum as they are moistened by high local humidity or precipitation. A yellowish stream emerging from the lower end of the lesion of a cut, infected leaf when placed in a tube with water is indicative of *Xoo* presence [53].

In contrast to *Xoo, Xoc* is an intercellular pathogen that enters plants either through wounds or by

Fig 2. Field symptoms of BB and BLS. A. BB epidemic in Korea (photo by J. E. Leach). B. BLS symptoms; note beads of yellow exudate (photo by V. Verdier). C and D are enlarged leaf symptoms of BB (Photos by J.E. Leach). E shows enlarged symptoms of BLS (Photo by V. Verdier).

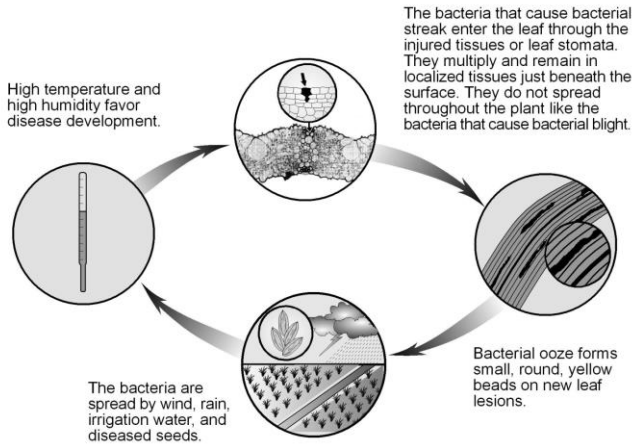


invading the open stomata [41,77,46] (Fig 3). Once inside the plants, *Xoc* multiplies between the mesophyll parenchyma cells, spreading up and down the leaf between the vascular bundles. *Xoc* can invade the host plant xylem tissue, but only at later stages of infection, when multiplication is limited [41]. BLS lesions may begin anywhere on the leaf between the veins as water soaked symptom and extend generally lengthwise throughout the leaf. Older lesions may extend over veins. The BLS lesion margin is characterized by fine water-soaked streaks.

The progression of BB or BLS lesions is determined by the susceptibility or resistance of the cultivar of rice. In areas where both *Xoo* and *Xoc* occur, BB and BLS symptoms may be present on the same leaf, which can complicate diagnosis [41].

Currently, it is not known if *Xo*-USA is a xylem-limited or an intercellular pathogen, although based on phenotype, it is predicted to be a xylem-limited pathogen [70]. Symptoms of susceptible rice infected with an *Xo*-USA strain begin as water-soaked lesions, typically associated with adult leaf margins [25]. Lesions turn chlorotic yellow, then necrotic (tan to white). Early in disease, the lesions are wavy, but mature lesions are vein delimited, and bounded by a necrotic red-brown stripe [25].

Fig 3. Disease cycle of bacterial leaf streak of rice caused by *Xanthomonas oryzae* pv. *oryzicola* [67] .



III. Spread and Risk Map

Leaves infected with both *Xoc* and *Xoo* exhibit exudates from lesions. Leaves with lesions and/or exudates may fall into the irrigation water of the flooded field, enabling pathogen spread. Irrigation water from one field can move the pathogen into another field, although free bacteria (outside of the leaf) do not survive long in the irrigation waters. Strong winds associated with rainstorms or typhoon also spread the bacteria to healthy leaves of host plants near-by the infected plant and may also wound the plant to allow an infection of the pathogen. Previously infected rice stubble may also serve as a source of inoculum [53].

Xoo is reported to overwinter on alternate hosts [53,40]. *Xoo* has been reported to survive in leaves in the soil from 1 to 3 months depending on humidity and acidic properties of the soil [53]. Infected leaf straw may also serve as inoculum for *Xoo* [53]. Humans walking through a field may also move exudates from an infected leaf to healthy leaf tissue.

While both *Xoo* and *Xoc* can be associated with the rice seed coat, only *Xoc* has been confirmed to be both seedborne and seed-transmitted [44,78]. The evidence that *Xoo* is seed-transmitted is controversial, and the epidemiological significance of *Xoo* for seedborne transmission has not been determined [44,59,13,67]. Pathogen-related symptoms of *Xo* are not observed on plants grown from infected seed may be due to a decline in bacterial populations during soaking of the seed in water prior

to sowing [13,28]. Although reported, dissemination following insect infestations or by birds has not been confirmed.

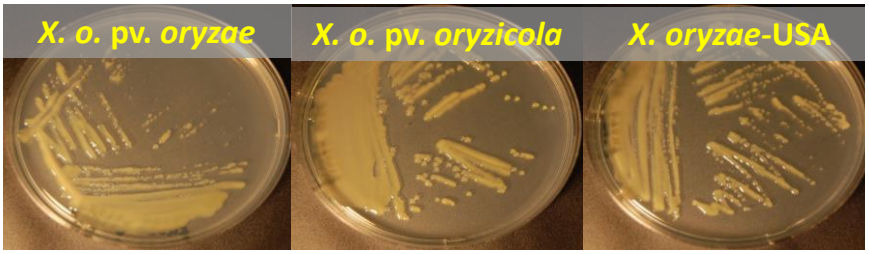
Risk maps for *Xoo* and *Xoc* in the USA that were developed by NAPPFAST are shown in Appendix A.

IV. Detection and Identification

Differentiating among the *X. oryzae* pathovars is impossible by colony appearance, as they all form bright yellow, mucoid colonies (Figure 4). Furthermore, there are non-pathogenic xanthomonads on rice leaves and seeds that are similar in appearance, and easily mistaken for *X. oryzae* [67]. The most definitive method for differentiation of the *Xo* pathovars is developed by microbiological and molecular tests [67].

The simplest method to distinguish which pathovar of *X. oryzae* is present in infected leaves is by studying the symptoms (Figure 2). However, if symptoms are observed on a late stage of infection, identifying the causal pathovar is difficult.

Fig. 4. Colony morphology of 72-hr old *Xanthomonas oryzae* pathovars on peptone sucrose agar.



Protocols from the European and Mediterranean Plant Protection Organization are currently recommended to isolate the bacterium from tissue or suspected seed (EPPO, 2007). However, a recently published set of protocols using rigorously tested methods for isolation and diagnosis, particularly from seed, should be considered for adoption [67].

After the bacterium is isolated, pathogenicity tests can be performed to distinguish the pathovars *Xoo* vs *Xoc* vs *Xo*-USA, which cause BLS, BB, or BB-like symptoms, respectively. To assess BLS causing *Xoc*, leaf infiltration (Figure 5) or mist inoculation are performed [56,79]. Leaf clip inoculation method is used to assess pathogenicity for the *Xoo* or *Xo*-USA strains [27] (Figure 5). It is important to use a susceptible cultivar of rice when evaluating pathogenicity. For example, many studies use Nipponbare, IR24 and Azucena as susceptible hosts to assess *Xoo* virulence, while Kitaake is used for *Xoc*.

Several diagnostic tools are currently available for identification of *Xoo* and *Xoc*, and for *Xo*-USA. In the late 1980s, a set of monoclonal antibodies was developed and widely used for diagnosis and distinction of *Xoo* and *Xoc* [7,4,3,5,15].

More recently, emphasis turned to DNA-based approaches to distinguish *Xoo* and *Xoc.* Early approaches involved amplification of the 16S rDNA followed by digestion with restriction enzymes [29,73]. However, as 16S rDNA sequences exhibit 98.6% similarity within the genus *Xanthomonas* [22], this approach cannot accurately distinguish these two *Xo* pathovars. The approach is only useful if supported by other sequence information such as the 16S-23S rRNA internal transcribed spacers. Primers based on the 16S-23S rDNA spacer region were designed for *Xoo*, but their design and testing were based on *Xoo* isolates

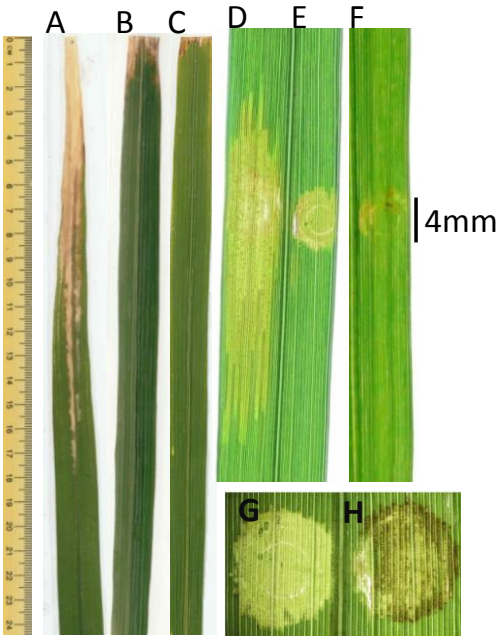
from only one country, and did not include *Xoc* isolates [1]. Hence, the reliability of these primers for accurate identification of geographically diverse collections is unknown. Repetitive DNA sequences, usually insertion sequence (IS) elements [32,58], can differentiate *Xo* pathovars from each other and from other *Xanthomonas* species by polymorphic hybridization [32] or PCR-amplification patterns [59,2,69]. However, the high degree of diversity of *Xanthomonas* isolates within and between countries, partially driven by movement of these mobile elements, complicates the analysis of patterns for diagnosis.

Early PCR-based assays developed for single gene targets, e.g., a membrane fusion protein [26], a putative siderophore receptor, and a *hrpF* gene in *X. campestris* species [8,84], while potentially reliable, were not validated on a diverse and wide array of strains.

More recently, a multiplex PCR with pathovar-specific primers, designed by *Xoo*/*Xoc* genome comparison, was developed [30] and is widely used in detection and diagnosis of *Xoo* and *Xoc* [74] Wonni, Detemmerman et al. in preparation). Additional primers were developed to distinguish the *Xo*-USA from Asian and African *Xoo* and *Xoc* [64]. Since the primers are based on comparative genome analyses, these primers are highly specific to the *X. oryzae* pathovars.

Currently used methods of detection and diagnosis for *Xoo* do not differentiate the races of the pathogen. Determining race structure for *Xoo* is important because it informs the specific resistance genes to be deployed to control the disease (see below). Determination of race is still best achieved by inoculation of rice differential hosts that contain single BB resistance genes (*Xa* genes).

Fig 5. Leaves inoculated by scissor clip inoculation [27] A, *Xoo;* B, *Xo*-USA*;* C, nonpathogenic *Xanthomonas* or leaf infiltration of D, *Xoc*; E, *Xo*-USA; F, nonpathogenic *Xanthomonas.* G, Susceptible and resistant responses to *Xoo,* respectively (Photos by R. Corral).



Near-isogenic lines (NILs) in the *indica* rice IR24 background (also known as IRBB lines for International Rice-Bacterial Blight) were developed at IRRI (International Rice Research Institute) and are commonly used to identify *Xoo* races [52]. Each NIL carries one specific resistance gene (*Xa* gene), which was incorporated into the recurrent backcross parent IR24 by conventional breeding techniques and/or using Marker-Assisted Selection (MAS). Similar NIL sets are available in *japonica* and *indica-japonica* genetic backgrounds [24,35,50]. New *Xoo* races are continuously being reported in countries where BB is endemic and are usually identified because they overcome deployed *R* genes. The development of a universal set of rice NILs and utilization of a set of *Xoo* reference strains for race typing on a global scale are needed to characterize and compare existing or emerging *Xoo* races.

Many seed-testing methods have been developed for diagnosis of *X. oryzae* pathovars, including growing-on tests [44], host inoculation with seed washings [78], semi-selective media [15] and serological assays [7]. These methods are time-consuming and often lack the needed sensitivity or specificity for routine seed testing. For example, the growing-on test, which involves detecting symptoms of infection from seeds sown on sterile soil, sand or water agar and allowing them to germinate, while uncomplicated, is relatively insensitive. Direct plating of seed extracts on semi-

selective medium is usually not sensitive enough for detecting the low pathogen levels because both bacteria grow slowly and are easily overgrown by the saprophytic flora (6). Nucleic acid-based methods that use PCR offer greater sensitivity and a shorter response time than conventional assays [30].

One method that has been recently adopted for plant pathogen diagnostics is loop-mediated isothermal amplification (LAMP). LAMP is isothermal and can be performed in a heat block or water bath thereby removing the need for specialized equipment, and allowing for implementation in the field. Adaptation of LAMP to *Xo*-USA*, Xoo,* and *Xoc* is in progress to facilitate rapid, accurate and sensitive detection and diagnosis as well as field surveys (Lang et al., unpublished results).

V. Response

While this plan is focused primarily on recovery, response to a new disease detection involves a continuum of activities from response to recovery. The response is under USDA, APHIS, Plant Protection and Quarantine's authority delegated from the Secretary under the Plant Protection Act of 2000.9

The ultimate authority for confirming a diagnosis of the disease rests with the Plant Protection and Quarantine (PPQ) division of APHIS: http://www.aphis.usda.gov/ppq. As such, this agency must use the most efficient and effective means to diagnose and differentiate *Xo* pathovars. At least two independent diagnostic methods are recommended to confirm the presence of *Xoc* or *Xoo.*

After a detection of *Xoo* or *Xoc* is confirmed by a USDA, APHIS, PPQ recognized authority, APHIS, in cooperation with the State Department of Agriculture, is responsible for the response. The response is immediate in the form of advance assessment teams of experts and survey personnel sent to the site of initial detection to place holds, conduct investigations, and initiate delimiting surveys. Actions that may be taken include regulatory measures to quarantine infected or potentially infected production areas, stop the movement of infected or potentially infected articles in commerce, and control measures which may include host removal and destruction, and/or insuring adherence to required sanitary practices. APHIS imposes quarantines and regulatory requirements to control and prevent the interstate movement of quarantine-significant diseases or regulated articles, and works in conjunction with states to impose these actions parallel to state regulatory actions which restrict intrastate movement.

The presence of the *Xo*-USA strains, which are indigenous to Louisiana and Texas, and which produce symptoms resembling BB argues that considerable care must be made to avoid raising unnecessary concern. The *Xo*-USA has not been a threat to rice grain yield. Once a sample displaying BLS or BB symptoms sample is confirmed as positive for *Xoo* or *Xoc* by an APHIS recognized authority, an advanced technical team may be sent to the site as the first step in a response. A larger team would then be deployed, consisting of state and federal regulatory personnel operating under a unified command within the Incident Command System. Survey teams will conduct delimiting surveys in the area using trace back and trace forward information and with various appropriate stratified delimiting sampling schemes for surveys in the area of detection. It is important as part of the response to control the movement of diseased host tissues of infested counties since this may serve as an avenue to infect other rice production areas.

After the results of delimiting survey are known, if the disease is considered generally distributed

through commercial rice in an area, options for control are very limited. If the disease is isolated to a small area, eradication may be effective if all infected rice plants and grain are detected.

While rice germplasm with bacterial blight and bacterial leaf streak resistance is available, most US germplasm is susceptible to both diseases. The USDA World Collection has approximately 18,000 entries of which some are known sources of resistance. However, no recent screening has been done on the collection. Resistance sources are available in the International Rice Research Institute's T.T. Chang Genetic Resources Center which houses the world rice collection of over 120,000 accessions. Plant quarantine restrictions and the strict quality standards of US rice will delay the incorporation of resistance into the US germplasm. No breeding program is actively incorporating resistance to these diseases into the US lines.

There are no active surveys to detect BB or BLS in the US. Current monitoring programs are limited to training cooperative extension service personnel on the existence of these diseases, proper sample collection, and identification techniques.

VI. USDA Pathogen Permits and Regulations

USDA/APHIS/PPQ permit and registration requirements for plant diseases and laboratories fall under two authorities, the Plant Protection Act (7 CFR Part 330) and the Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331). Laboratories receiving suspect infected plant material or cultures are required to have PPQ permits. Laboratories possessing, using, or transferring select agents such as *Xanthomonas oryzae*, the causal agents of BLS and BB and the weak BB-like disease found in the USA, are required to be registered. Diagnostic laboratories that identify select agents are exempt from this requirement as long as they complete an APHIS/CDC Form 4 and destroy the culture within 7 days.

The Plant Protection Act permit requirements apply to all plant pests and infected material, including diagnostic samples, regardless of their quarantine status, which when shipped interstate require the receiving laboratory to have a permit. For further guidance on permitting of plant pest material, consult the PPQ permit website at: http://www.aphis.usda.gov/ppq/permits/ or contact PPQ Permit Services at (301) 734-8758.

The Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331) specifies requirements for possession, use, and transfer of organisms listed as select agents such as the *Xanthomonas oryzae* pathovars. Once an unregistered diagnostic laboratory identifies a select agent, they must immediately notify the APHIS Select Agent Program, complete an APHIS/CDC Form 4 within 7 days, and either destroy or transfer the agent to a registered entity within 7 days. In compliance with this Act, if a diagnostic laboratory holds back part of a screened sample or culture for voucher purposes, and that sample when forwarded to the USDA Beltsville Laboratory comes back as positive for a select agent, the diagnostic laboratory is required to notify the APHIS Select Agent Program immediately. This must take place within seven (7) days of results notification and a PPQ Officer must be given the opportunity to witness the destruction of the sample or culture within that time period. Clarification of this and other information related to adherence to the select agent regulations is available on the following APHIS website: http://www.aphis.usda.gov/programs/ag_selectagent/index.html, or call (301) 734-5960.

VII. Economic Impact and Compensation

Crop insurance covers production losses due to BB and BLS if the losses are unavoidable and result from naturally occurring events during the insurance period. Producers must follow good farming practices, and should work with agricultural experts and document all actions to control and manage the diseases.

The Risk Management Agency (RMA) defines what constitute good farming practices. To determine if producers followed good farming practices, agricultural experts answer, at least, the following questions: Will the control measure:

1) allow the insured crop to make normal progress toward maturity?
2) produce at least the yield used to determine the production guarantee?
3) not reduce or adversely affect the yield?

The answers to these questions must be "Yes." If the answer to any of the above questions is "No," RMA may not consider the control measure as a good farming practice. RMA does not consider the cost or economics of the control measure in determining good farming practices.

RMA recommends that producers document their actions and the data they used in making their decisions, including data from:

- Local weather stations;
- Farm Service Agency (FSA) reports;
- Published articles in newspapers, newsletters, magazines, and Web information from:
    - Land grant universities;
    - Extension Service;
    - Crop consultants; or
    - Other agricultural experts
- Journals and logs that list the date of control measures, application method(s), product(s) (include labels), and conditions, etc.

RMA does not prevent producers from mitigating their losses and taking care of their crop as they see fit. Insurance covers losses due to unavoidable circumstances during the insurance period, assuming the producer followed good farming practices. Production losses due to bacterial leaf streak are covered by the insurance.

VIII. Mitigation and Disease Management

Any disease mitigation strategy that is employed should be coordinated with federal, state and local regulatory officials.

Chemical control measures are available [10,12], but their use and effectiveness are limited by cost and high variability in response or susceptibility among strains [14,12,18,81]. Recommended cultural controls include field sanitation, drainage, plant spacing, and fertilizer management [36].

The rice germplasm system in the United States is one of the most secure in the world. No rice is allowed for direct planting in USA fields. Small amounts may be allowed for research purposes only after going through strict quarantine procedures that may include inspection of the seed, hot water treatment, de-hulling, surface sterilization, and growing out of contaminated-free seedlings in a quarantine greenhouse. Rough rice (with hulls), brown rice, and white rice are allowed in the United States for consumption. Most of the germplasm in the United States is not evaluated for BB and BLS because these diseases are not known to occur in the USA.

The most reliable means of controlling BB is through the use of resistant germplasm. In rice producing areas where the disease occurs, several sources of single gene resistance that can control BB are available. The currently available *R* genes (Table 1) were recently reviewed [71]. Deployment of appropriate genes requires an understanding of the race structure of the invading *Xoo* population; without such knowledge, it would be impossible to predict which *R* genes would be effective. Breeding programs in the US have not focused on introduction of resistance to BB into germplasm because the risk of disease is not considered high. However, introduction of available genes into USA rice varieties through breeding can produce resistant varieties in 4-8 years. Efficient genetic transformation techniques are available for rice, but very few BB *R* genes have been cloned, and their effectiveness against the *Xoo* population (races) needs to be evaluated. New genome editing technologies using engineered nucleases are allowing for novel approaches to developing disease resistance that is not considered transgenic [37].

Table 1. *Xa R* genes currently available for BB resistance and their characteristics.

R gene	Subpopulation	Accession name
Xa2, Xa4, Xa11, Xa16, Xa18, Xa25(a) and (b), Xa26, xa28, xa34(t)*	*indica*	Tetep, TKM6, IR8, IR944, IR24, Tetep, HX-3, Minghui63, LotaSail, BG1222
Xa3, Xa14, Xa17, Xa18, Xa31(t)	*japonica*	Wase Aikoku, TN1, Asominori, Toyonishiki, Zhachanglong
Xa1,Xa12	temperate *japonica*	Kogyoku
xa5, Xa7*, xa24*	aus	DZ192, DV85, DV86-DV85-Aus295
Xa21, Xa23, Xa27, Xa29, Xa30(t), Xa32(t), Xa35*	wild species	*O. longistaminata, rufipogon, minuta, officinalis, nivara, australiensis, minuta*
Xa6/Xa3, xa8, xa9, Xa10, xa13, Xa15, Xa22(t), xa33(t), Xa36(t)*	-	Zenith, PI231129, Khao Lay Nhay, CAS209, BJ1, XM41, Zhachanglong, Ba7, C4059
xa19, xa20	mutant	XM5, XM6

- : No data, ND: not determined

**: Xa gene released in Asia*

Note: Xa30(t) from *O. nivara* is now designated as *Xa38*; *Xa9* is dominant gene and is allelic to *Xa3* and *Xa6;* BJ1 carrying *xa13* is an aus cultivar; *Xa15* from XM41 also a mutant line?

Currently, no single rice resistance gene source is available that can control BLS; the only resistance sources from rice are multi-genic or quantitative resistance [11,21,63]. An *R* gene, *Rxo1,* which is

effective against Asian and some African *Xoc* populations, was identified from maize [82,83,16]. This gene functions in rice, and, if introduced into local varieties by biotechnology approaches, can be useful in controlling BLS, given that the pathogen population carries the corresponding *avrRxo1* effector [82].

IX. Research, Education and Extension Priorities

The following lines of research are needed to enhance detection and management of BB and BLS. They would improve our ability to block the entrance, detect the presence, and help manage the impact of BB and/or BLS. The research priorities are listed according to their relative importance.

1. Develop improved field-level detection tools, seed-detection protocols, and certification approaches. These must reliably distinguish *Xoo* and *Xoc* from each other, and from other *X. oryzae* and other *Xanthomonas* species.
2. Improve tools for rapid and accurate characterization of the race structure of the *Xoo* pathogen population. Understanding the repertoire of avirulence effectors is important to knowing what *R* gene combination will be effective in controlling disease [65,31].
3. Educate and train extension personnel, growers and crop advisors in the symptomatology and detection of BB and BLS in field conditions.
4. Assess key germplasm used in the USA in countries where BB and BLS are indigenous, to screen for resistance. An important caveat is that the resistance sources detected and integrated may not prove effective against the specific race introduced.
5. Improve genetic resistance by incorporating widely effective R genes, and identifying and incorporating sources of broad-spectrum resistance (effective against both *Xoo* and *Xoc*, and effective against all races of *Xoo*). This could be through novel transgenic approaches or through the introgression of novel QTL-based resistance.
6. Adopt uniform detection/diagnosis protocols among quarantine agencies worldwide.
7. Develop the physical resources to test, conserve, store, maintain strains or DNA of *X. oryzae* strains in the USA.
8. Determine the feasibility of bacteriophage for biocontrol. Phage can be easily selected, propagated, and mutated to overcome any developed resistance.

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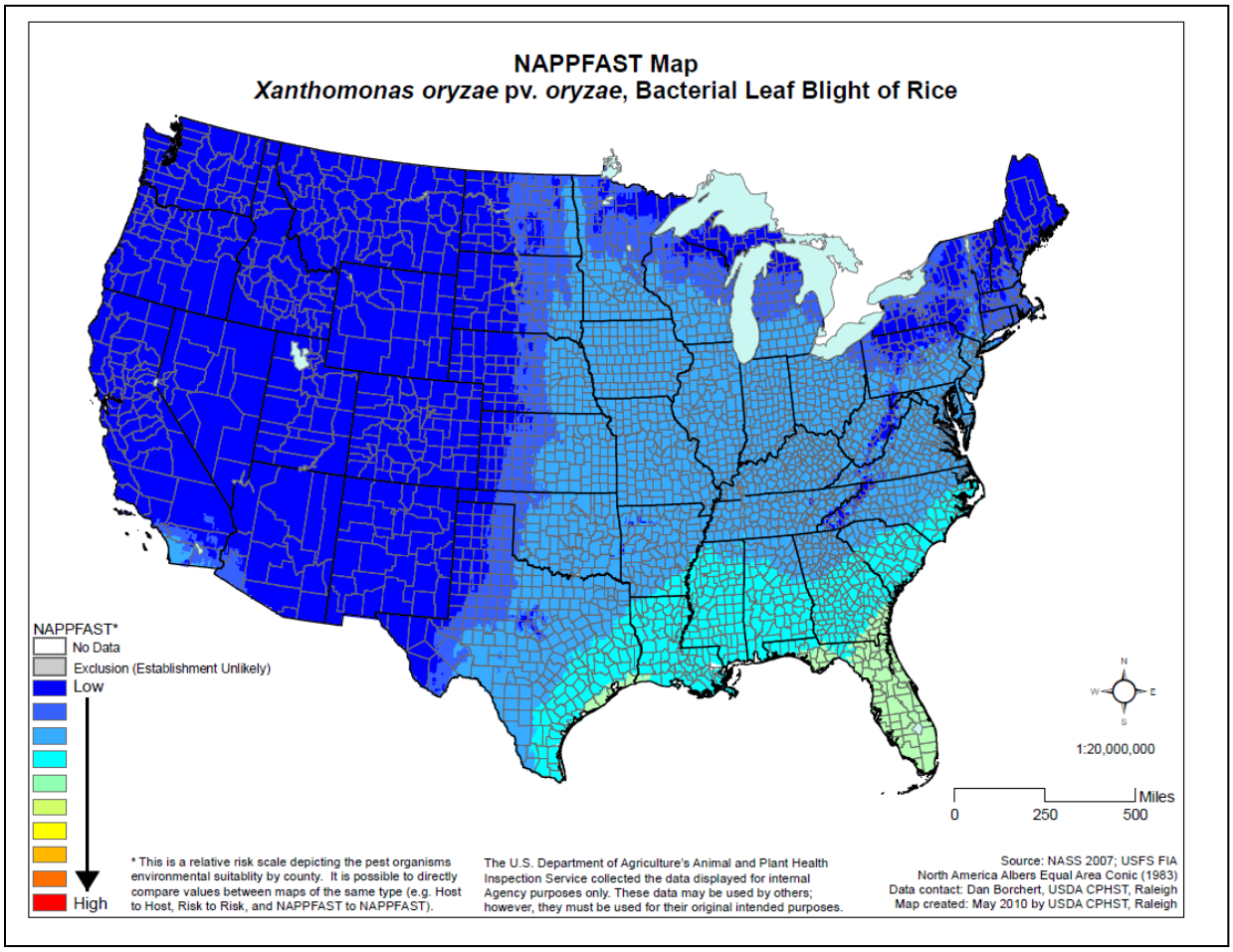
Appendix A.


NAPPFAST Map
Xanthomonas oryzae pv. oryzae, Bacterial Leaf Blight of Rice
NAPPFAST*
No Data
Exclusion (Establishment Unlikely)
Low
High
* This is a relative risk scale depicting the pest organisms environmental suitablity by county. It is possible to directly compare values between maps of the same type (e.g. Host to Host, Risk to Risk, and NAPPFAST to NAPPFAST).
The U.S. Department of Agriculture's Animal and Plant Health Inspection Service collected the data displayed for internal Agency purposes only. These data may be used by others; however, they must be used for their original intended purposes.
Source: NASS 2007; USFS FIA
North America Albers Equal Area Conic (1983)
Data contact: Dan Borchert, USDA CPHST, Raleigh
Map created: May 2010 by USDA CPHST, Raleigh
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S
1:20,000,000
Miles
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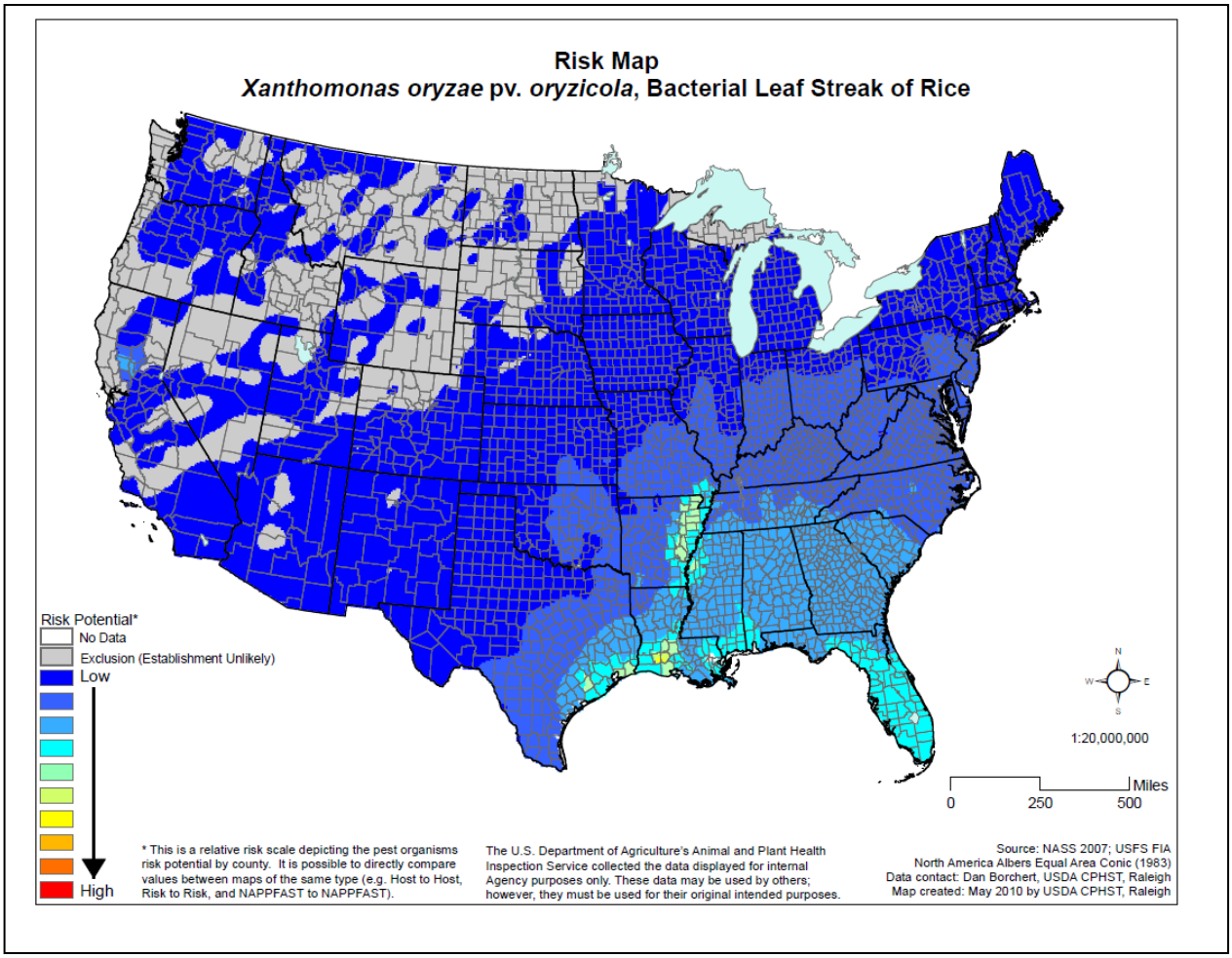
250
500


Risk Map
Xanthomonas oryzae pv. oryzicola, Bacterial Leaf Streak of Rice
Risk Potential*
No Data
Exclusion (Establishment Unlikely)
Low
High
* This is a relative risk scale depicting the pest organisms risk potential by county. It is possible to directly compare values between maps of the same type (e.g. Host to Host, Risk to Risk, and NAPPFAST to NAPPFAST).
The U.S. Department of Agriculture's Animal and Plant Health Inspection Service collected the data displayed for internal Agency purposes only. These data may be used by others; however, they must be used for their original intended purposes.
Source: NASS 2007; USFS FIA
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