Recovery Plan For

Rathayibacter Poisoning

Caused by
Rathayibacter toxicus (syn. *Clavibacter toxicus*)

February, 2010

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 minimize the impact of high consequence plant disease outbreaks are available so that a adequate level of crop production is maintained.

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 address all of the many and varied aspects of plant disease outbreaks 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 the USDA to further guide efforts toward plant disease recovery.

Executive Summary

Rathayibacter (*Clavibacter*) *toxicus* was added to the Select Agent List in 2008 due primarily to the potential damage affecting domesticated forage-consuming animals in the U.S. *R. toxicus* is a cross-domain pathogen: a nematode vectored Gram-positive bacterium causes plant disease (gummosis) and produces animal toxins in forage grasses. Consumption of infected grass often results in fatal poisoning of grazing animals. Rathayibacter poisoning has many names, the most common of which is annual ryegrass toxicity (ARGT). The disease is found mainly in Australia and possibly in South Africa. In Australia, the plant disease has been found in over 10 million hectares of farmland in different parts of the country and the total losses attributed to *Rathyibacter* poisoning are in the millions of dollars.

In Australia, the bacterium *R. toxicus* is most commonly found in *Lolium rigidum* (annual ryegrass) with the nematode *Anguina funesta*, but the bacterium is not vector/host specific and can potentially colonize and produce toxin in a wide range of cereals and fodder grasses, including species consumed by humans. The pathogen can go undetected for long periods, making visual detection of a deliberate release a serious challenge.

Several species of *Anguina* (seed and leaf gall nematodes) carry *R. toxicus* into the host plant where it resides in the inflorescence (developing seedhead) and galls are formed. Galls may fall to the ground at the end of the season. Most of the infected plants do not show any visible symptoms. Thus, absence of visible slime (gummosis) does not necessarily mean that a pasture is free of *R. toxicus*. In many cases, bacteria and nematode infections go undetected or the disease is misidentified. The nematode vector and bacterium can survive in the dry state for many years.

The toxins, termed corynetoxins, generally are produced as the plants become senescent and the bacterial biomass has peaked. Corynetoxins are heat stable, highly toxic (lethal dose for sheep is 3-6 mg/kg body weight) glycolipids with cumulative effects. As many as 16 toxins may be produced, differing only in their side chains.

Animals that consume infected pasture grass suffer a toxicosis characterized by episodic neurological symptoms, often leading to death. Animals exposed to the toxins do not develop immunity. Treatments for affected animals are limited. An antidote has been developed, but its use is constrained by the fact that outbreaks are unlikely to be detected before animals have died or have consumed too much toxin to be successfully treated. In Australia, the disease occurs during summer grazing. However, it can happen at any time of the year in livestock fed with toxic hay. Rathayibacter poisoning was diagnosed in Japan in cattle after they had eaten hay exported from Australia.

Susceptible animals include the approximately 95 million cattle (USDA-Economic Research Service, 2009), 6 million sheep (American Sheep Industry Association, 2009) and 9 million horses (American Horse Council, 2009).

The threat of introduction and establishment of *R. toxicus* in the U.S. is very high due to presence of susceptible grasses and potential nematode vectors. Pasture and rangeland throughout the United States are potentially susceptible to infection, as indicated by the occurrence in the U.S. of *Anguina* species, the nematode vectors and bacteria related to *R. toxicus*.

As seed and hay are moved about the country, *R. toxicus* and nematode vectors can be spread, most commonly through uncleaned or poorly cleaned grass seed, but also by wind dispersal and in hay, contaminated machinery, vehicles, animals or run-off water. It may take several years after the introduction of the nematode vector to see evidence of the disease in grazing animals. This means the bacterium, nematode or seed could be introduced without detection or any suspicion. Arguably, there is no cost-effective, rapid and sensitive identification test for *Rathayibacter*.

Risk of rathayibacter poisoning is generally mitigated by management practices such as crop rotation, rotation among grazed pastures, harvesting hay before seed-heads produce toxins, herbicide treatment of susceptible pasture grasses, inspection of fields for signs of infection and the use of certified seed free of the bacterium.

Recommendations

1. Strict quarantine measures in place at all ports of entry for hay products and forage grass seeds especially those originating from Australia, New Zealand and South Africa. Development of reliable identification tests for all *R. toxicus* hosts and vectors. Monitoring of grasses, particularly of pasture grasses, for gummosis.
2. Education of veterinarians, plant pathologists, nematologists, extension personnel, crop consultants and grain and animal producers to become familiar with the diseases, their ecology and management. It is critical for appropriate and early responses due to the complexity of the diseases.
3. Obtain additional information to improve disease management and animal protection.
    a. Bacteria: develop diagnostic tools for tracking and spread of *R. toxicus* and related species; determine the role of the toxin in the ecology of the bacterium; sequence the entire genome of at least three strains and one strain of related species to assess similarities and differences, including the assessment of gene(s) related to toxin production.
    b. Nematodes: assess the potential for biological control of prospective vectors; determine vector capability under optimal and adverse conditions, or carrying capacity for *R. toxicus.*
    c. Plants: determine viability and yield potential of plants bred for resistance to the toxin under U.S. conditions; determine feasibility of pasture management practices applicable to the U.S.
    d. Animals: assess applicability of Australian diagnostic practices and procedures to U.S. production systems; determine the toxicological mechanisms by which the corynetoxins damage animal tissues; understand the mechanisms of action to develop more effective diagnostics or treatment protocols; improve the protective efficacy of an Australian experimental vaccine or develop a new vaccine.

Rathayibacter poisoning

(caused by *Rathayibacter toxicus*; syn: *Clavibacter toxicus)*

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I. Introduction

Rathayibacter toxicus is a toxin producing Gram-positive bacterium that infests the foliage and floral structures of grasses. Ingestion of the toxin by grazing animals results in a serious poisoning that typically leads to their death. The bacterium is transferred from infested soils into plants by plant parasitic nematodes of the genus *Anguina*, which produce foliar or seed galls in several species of grasses. *R. toxicus* can proliferate within the nematode galls, killing the nematodes and completely filling the lumen of the gall (forming a bacterial gall). Often, bacterial growth in an inflorescence is sufficient to cause oozing from the floral structures as a toxic yellow bacterial slime (such symptoms caused by plant-colonizing bacteria are referred to as gummosis).

The common names given to the diseases caused by the association of *Rathayibacter* and *Anguina* are numerous and differ depending primarily on the affected host. There is no widely-applicable common name for the plant disease or animal diseases. The name we have chosen, rathayibacter poisoning, illustrates the causative agent and the primary concern for the consequences of ingestion of infected grasses. Rathayibacter poisoning is a serious animal disease in Australia, but has also been reported in South Africa. Primarily sheep and cattle have been affected, but all grazing animals fed *R. toxicus* infested pasture, hay or feed grain are subject to poisoning. The term we have chosen to represent the plant disease is rathayibacter bacteriosis.

Plant disease names

Yellow slime disease has been used to describe *R. toxicus* infection of grasses, but not specifically or commonly because this name has also been used for other slime diseases, such as *R. tritici* in wheat. Most of the names associated with the disease are related to toxic effects in animals (Table 1). Rathay's disease has been used as a term for a bacterial disease in orchardgrass in Oregon, U.S. (see Appendix Table A1).

Animal disease names

Various names have been given to the disease in animals, depending upon the geographic area in which the disease occurred and plant host (Table 1). For example, annual ryegrass toxicity (ARGT) is the most common name used for describing the poisoning of animals consuming *Lolium rigidum* in which the nematode *Anguina funesta* is the vector of the bacterium, *R. toxicus* (ryegrass was previously separated into two words, viz. rye grass, which gave rise to the established acronym).

Names given to the disease by some veterinary scientists such as corynetoxicosis or tunicaminyluracil toxicosis have merit, but have not been widely adopted presumably because they do not relate to the plant disease (Table 1) and are not easily understood by non-technical audiences.

Table 1. Names for various *Rathyibacter* and/or *Anguina* associations.

Referring to toxicity of *Lolium rigidum*, caused by *Rathyibacter toxicus*
annual ryegrass toxicosis
annual ryegrass toxicity (ARGT)
annual ryegrass staggers
parasitized annual ryegrass
ryegrass toxicity
toxic annual ryegrass
tunicamycin poisoning
Wimmera ryegrass toxicity
Black Springs syndrome
Referring to toxicity of *Agrostis avenacea* (Syn. *Lachnagrostis filiformis*) or *Polypogon monospelensis*, caused by *Rathayibacter toxicus*
flood plain staggers (Johnson et al., 1996)
blown grass/beard grass poisoning
corynetoxin poisoning
corynetoxicosis
Stewarts range syndrome
tunicaminyluracil toxicosis
Referring to toxicity of *Erharta longiflora*, caused by *Rathayibacter toxicus*
veldtgrass staggers (experimental only, may not be accurate)
Referring to *Anguina* and/or *Rathayibacter toxicus*: disease, galls, or pathogen common names
bacterial galls
gumming disease
seed gall nematode

Hosts and vectors

Other plant hosts reported to be infected by *R. toxicus* are: *Austrodanthonia caespitosa, Avena sativa, Avena caespitosa, Danthonia caespitosa, Lolium multiflorum, L. perenne,L. persicum, L. strictum, L. temulentum, Phalaris* spp. and *Vulpia myuros* (Bertozzi and Davies, 2009; Bertozzi and McKay, 1995; Chatel et al., 1979; Edgar et al., 1994; McKay et al., 1993; Riley, 1992a ; Riley, 1995; Riley, 1996; Riley et al., 2001; Riley and Barbetti, 2008).

Known vectors for *R. toxicus* are *Anguina* species, including *A. funesta* (Riley, 1995), *A. tritici* (Riley, 1992a), *A. australis* (Riley et al., 2001), and *A. paludicola* (Bertozzi and Davies, 2009).

History

The first reports of plant disease or gummosis [pathological production of gummy or sticky exudates as a result of plant cell degeneration and bacterial cell proliferation and production of extracellular polysaccharides] of grasses associated with *R. toxicus* were in 1968 (Fisher, 1978). The first livestock poisonings reported occurred in 1956 in South Australia (Fisher et al., 1979). Poisonings spread throughout Australia thereafter (McKay and Ophel, 1993). Poisonings were reported in 1980 in South Africa (Schneider, 1981).

The bacterium was isolated in the 1960s, but not described as a separate species until 1992 (Riley and Ophel, 1992). In Australia the plant disease has been found in over 10 million hectares of farmland in different parts of the country (Carslake, 2006).

Experience in Australia with the plant and animal diseases has led to management practices that have minimized the incidence of plant disease and indirectly the incidence of animal disease. Hay for export (in a case of ryegrass contamination) is now routinely inspected and assayed for *R. toxicus*, and animal deaths, previously numbering in the thousands, are now rare.

It is clear that the same bacterium can be vectored by different nematode species to multiple grass species (Riley and McKay, 1990). The threat of introduction and establishment of *R. toxicus* in the U.S. includes other grass hosts and nematode vectors, so that a different specific name would be considered appropriate. Hence, we support use of rathayibacter poisoning for this document. However, in keeping with the literature, other names are used as published.

II. Disease Development and Symptoms

A disease cycle of *Rathayibacter toxicus* is shown in Fig. 1. In plants, *R. toxicus* is carried by the nematode to the inflorescence [seedhead] (Fig. 2). The nematodes enter the growing plant and migrate to the seedhead, where they infest individual seeds, transforming them into galls (Fig. 2). Bacterial cells in the soil adhere to the surface of nematodes as the nematodes emerge from galls. Galls typically fall to the ground at the end of the season, completing the life cycle. Host plants can include seedlings within a regenerating pasture, or weed grasses (Fig. 1). Most of the infected plants do not show any visible symptoms; however, a proportion of colonized grass seedheads become twisted and deformed, and may be covered with an orange-yellow exudate, called by some ‘yellow slime’. With time, the slime may harden and darken in color. Slime does not always form, and it may be washed off by rain. Thus, absence of visible slime does not necessarily mean that a pasture is free of *R. toxicus*.

Infected seeds are swollen and discolored. Galls are typically either nematode galls (containing *Anguina* and no *Rathayibacter*) or bacterial galls (containing *Rathayibacter* and no *Anguina*). In many cases, bacteria and nematode infections go undetected or the disease is misidentified. The nematode vector and bacterium can survive in the dry state for many years (Murray, 1986; Nickle, 1991). A related

bacterium was recently found in Turkey. *R. iranicus* was isolated from asymptomatic wheat seeds on a semiselective agar medium (Postnikova et al., 2009).

Toxin production may vary within field populations of the bacterium, as toxin is not found in all mature bacterially colonized galls. The toxins generally are produced as the plants become senescent and the bacterial biomass has peaked. The toxins are heat stable glycolipids, given the name corynetoxins or CTs and are highly toxic (lethal dose for sheep is 3-6 mg/kg body -weight). As many as 16 toxins may be produced, differing only in their sidechains (Appendix Table A2; Figs A1 and A2). The disease in animals may be confused with other diseases, such as ergot alkaloid toxicosis, perennial ryegrass staggers, grass tetany or botulism.

The involvement of a bacterial virus or bacteriophage was also considered highly likely in toxin production for some time, but has largely been discounted (Kowalski et al., 2007).

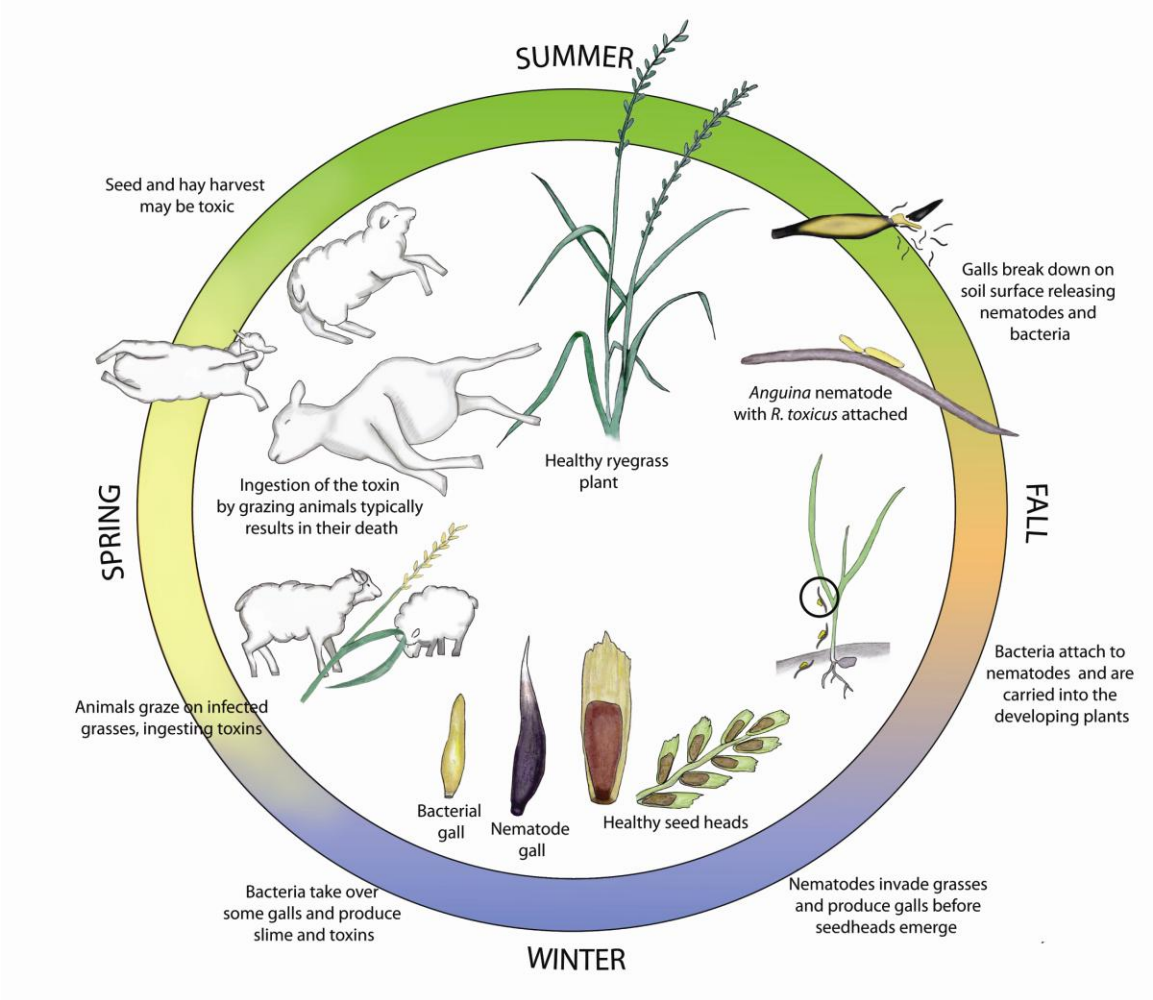

Figure 1. The annual life cycle of the bacterium causing rathayibacter poisoning. This diagram is for illustrative purposes only and is not drawn to scale.

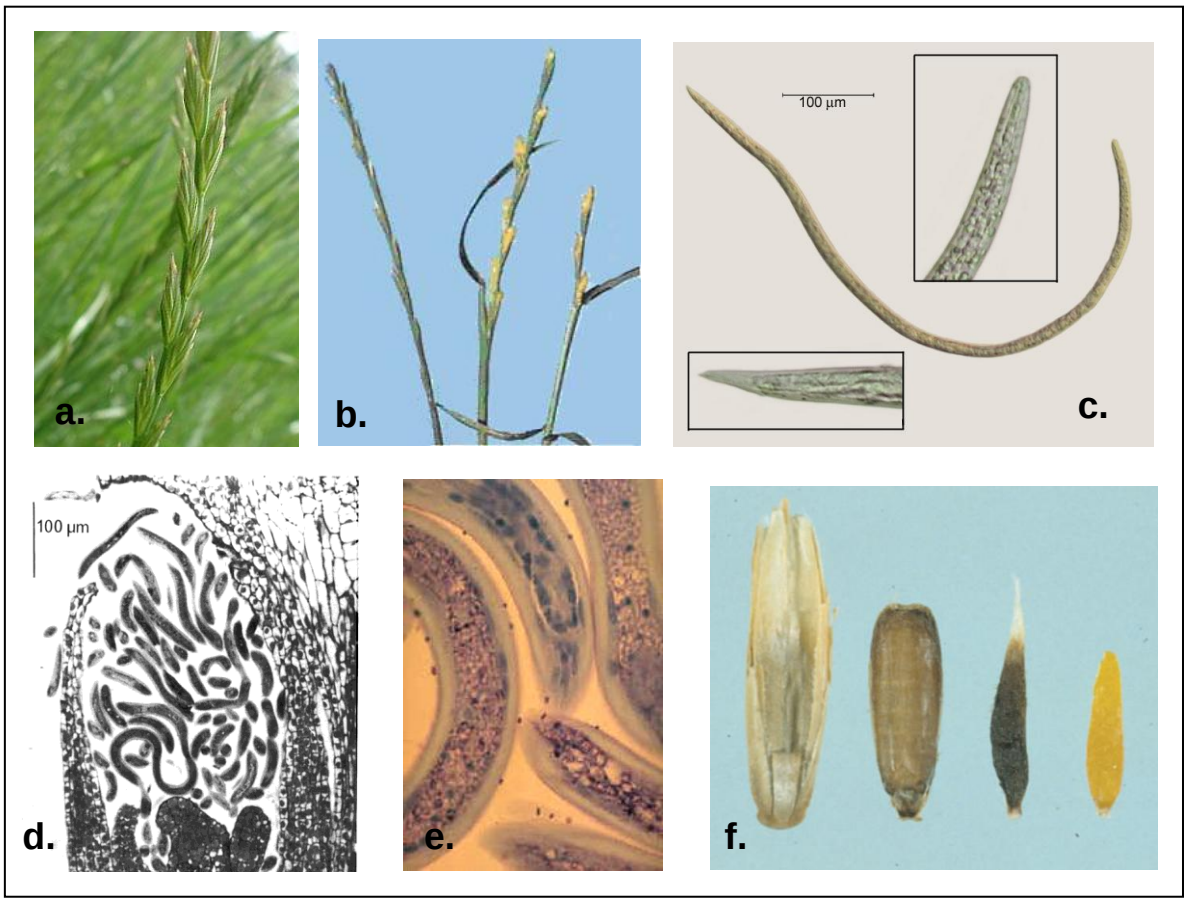

Figure 2. Healthy and diseased *Lolium rigidum*: (a) healthy ryegrass plants; (b) yellow slime of *Rathayibacter* on infected annual ryegrass heads; (c) microphotograph of *Anguina* sp. nematode juvenile (J2); (d) large numbers of *A. agrostis* juveniles (J2) clustered around a *L. rigidum* floret primordium (microphotograph of sections through developing inflorescences); (e) nematode *Anguina funesta* juveniles with *R. toxicus* (seen as dark dots on the surface of nematodes) adhered to the cuticle; (f) from left to right: *L. rigidum* dispersal unit (diaspore), *L. rigidum* healthy seed, *A. funesta* gall, bacterial gall. (photo (b) is extracted from South Australian Animal Health Quarterly, December 2006; (c) courtesy of Dr. T.O. Powers; (d) extracted from Stynes and Bird, Phytopathology, 1982, **72**:336-46; (e) J. Collier; (f) courtesy of Dr. I.T. Riley.

Animals consuming *R. toxicus*-infected plants can develop a fatal neurological disease characterized by convulsions. All grazing animals, regardless of age or sex are susceptible to the toxin. Early clinical signs are loss of coordination in the legs, followed by high-held heads and arched backs. Later, muscle tremors, intermittent body convulsions, head nodding, tooth grinding and involuntary eye movement can occur. In the final stages, the animals lie on their sides and make walking motions. Poisoning signs can occur over several weeks, and removal of animals from a toxic paddock varies

in being successful in their recovery and survival (animals can die within 24 hours of the first signs of poisoning). Pregnant animals can abort after exposure to contaminated forage, and it is believed that the incidence of this problem is underestimated. Post mortem findings may include small hemorrhages commonly seen in the gallbladder and also in other organs including the rumen, small intestine, kidney and lymph nodes throughout the body, and altered color and appearance of the liver. Elevated liver enzymes in blood indicate hepatic damage caused by the toxins. Only if toxin is detected can such symptoms be directly attributed to this bacterium. Despite neurological symptoms, brain tissue can appear normal histologically.

Animals exposed to the toxins do not develop immunity because the corynetoxin molecules are too small to be antigenic (McWilliam and Vogel, 1988). Additionally, a second exposure to the toxins can produce even more severe symptoms, as a consequence of the toxin accumulating in some tissues (Jago and Culvenor, 1987).

III. Plant Infection, Spread of the Bacterium and Animal Poisoning

Pasture and rangeland throughout the United States is potentially susceptible to infection, as indicated by the occurrence in the U.S. of *Anguina* species, the nematode vectors and bacteria related to *R. toxicus* (Alderman et al., 2003), (Postnikova et al., 2004; Riley et al., 2004).

Susceptible animals include the approximately 95 million cattle (USDA-Economic Research Service, 2009), 6 million sheep (American Sheep Industry Association, 2009) and 9 million horses (American Horse Council, 2009). No attempt has been made to isolate *R. toxicus* from toxigenic fescue grass in the Southeastern U.S. Pigs, llamas and alpaca animals may also be at risk, as well as any animals consuming toxins from hay or feed grains. The most valuable animals on an individual basis that are at risk are race horses.

In addition, it is important to note that *R. toxicus* can potentially colonize and produce toxins in a wide range of cereals consumed by humans, such as those put in dry organic breakfast cereals.

As seed and hay are moved about the country, *R. toxicus* can be spread, most commonly through uncleaned or poorly cleaned grass seed, but also by wind dispersal and in hay. Galls typically fall to the ground at the end of the season where they overwinter and nematodes infest grasses the following spring. The bacterium and nematode vector can also be spread by contaminated machinery, vehicles, animals or run-off water. Given the relatively low rate of disease development, it may take several years after the introduction of the nematode vector to see evidence of the disease in grazing animals. This means the bacterium, nematode or seed could be introduced without detection or any suspicion.

Susceptible grasses and potential nematode vectors reside in the U.S. It is possible that poisoning of cattle and sheep associated with consumption of Chewing's fescue screenings (infested with nematodes), recorded in the 1940s on the west coast (Shaw and Muth, 1949; Galloway, 1961), may have been due to *R. toxicus* or a relative. Herbarium specimens from that time enabled detection of a glycolipid toxin, but it differed from those of *R. toxicus* (Riley et al., 2003; Riley et al. 2004).

IV. Monitoring and Detection

Monitoring of grasses, particularly of pasture grasses, for gummosis should be considered. Surveys for *R. toxicus*, even in Oregon where cattle poisonings have occurred, have not been conducted since the 1960s. Thus, it is unknown whether a low level of bacterial infestation persists within fields or among native or weed grasses.

A number of published methods for detecting the bacterium in infected plants are available. The bacterium is not easily isolated and grows slowly on agar media. Seed scarification, a process of breaking, scratching or mechanically altering seed coats to break seed dormancy and enable germination, can be used for detection of the nematode and the related bacterium, *R. rathayi*, in seed lots (Alderman et al., 2003). The newer methods such as PCR detection of *R. toxicus* (Kowalski et al., 2007), (real-time PCR, Schaad, unpublished); and enzyme-linked immunosorbent assays [ELISA] (Masters et al., 2006), appear more precise, more sensitive, quicker and perhaps cheaper, but their validation data have not been published. Like *R. iranicus*, *R. toxicus* can be presumptively identified by 16S rDNA sequencing and confirmed by AFLP analysis (Postnikova et al., 2009). Rumen fluid and feces may also be tested for the presence of the bacterium, although that does not confirm an ill animal is suffering from nor that a dead animal died from the toxicosis. At least for ARGT, a large proportion of livestock without any symptoms will give a positive rumen test because the bacterium is widely distributed (Bourke, 2007). Necropsy of dead animals, especially brain and liver, are used to provide definitive diagnosis (see II) (Finnie, 2006).

ELISA can be performed on ryegrass seedheads pre-anthesis to detect the bacterium as a means of predicting the likelihood of toxicity later in the season (Riley and McKay, 1991; Riley, 1992b) and allowing stock-owners to undertake measures to reduce the development of toxin (e.g. early grazing, herbicide application or mowing). ELISA is more commonly applied to mature ryegrass samples indicating the likelihood that the material is already toxic (e.g. when applied to hay or standing pasture) or could potentially contribute to the spread of the causal organisms (McKay and Riley, 1993).

A number of methods have been published (e.g. McKay and Riley, 1993) for detecting potential vectors, but their detection is not necessarily associated with the diagnosis of disease in the plant or toxicosis in the animal.

<u>*Rathayibacter toxicus* diagnostic laboratories (all are in Australia):</u>

South Australian Research and Development Institute (SARDI)
SARDI Plant and Soil Health

Locked Bag 100
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Animal Health Laboratories, Department of Agriculture Western Australia
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V. Response

The response to all plant health emergencies is under USDA-APHIS-Plant Protection under The Plant Protection Act of 2000 (7 CFR Part 330) and the Agricultural Bioterrorism Protection Act of 2002 (7CFR Part 331).

The planned immediate response to suspect instances of either rathayibacter bacteriosis of pasture grasses or rathayibacter poisoning of animals would be to determine the causal agent. It is necessary to rule out other potential causes that present similar symptoms in the plants or animals. Thus, diagnosis and detection of the bacterium are essential first steps. Nematode detection in galls would be an indirect indicator of high concern. A survey of asymptomatic seed for the bacterium such as conducted in Turkey (Postnikova et al., 2009) would be a high priority.

After a confirmed detection of *R. toxicus* by the USDA-APHIS-PPQ recognized authority, APHIS, in cooperation with the affected state's Department of Agriculture, is in control of the response. The response is an immediate assessment of the disease by a Rapid Assessment Team (RAT) that would include regulatory personnel and recognized *R. toxicus* experts. The assessment will consist of investigation and delimitation of the site of initial detection to prevent pathogen spread and to establish extent of the affected area. The RAT team will also assess if the introduction was intentional or accidental. As a plant pathogen on the select agent list, *R. toxicus* is covered under the Agricultural Bioterrorism Protection Act of 2002; federal and local law enforcement may be involved to determine if a bioterrorism event has occurred.

APHIS imposes quarantines and regulatory requirements to control and prevent the interstate movement of quarantine-significant pathogens or regulated articles and works in conjunction with states to impose these actions parallel to state regulatory actions to restrict intrastate movement.

The USDA-APHIS-PPQ response will also depend on where *R. toxicus* is found and how widespread, based on the initial survey by the RAT. If eradication of the

pathogen is impossible as in the event of widespread establishment, a decision can be made to continue, expand, or modify regulatory actions. Since the disease in plants could be easily overlooked, it may spread considerable distances before being detected. In that case, alternate management and mitigation techniques would be needed, as outlined below under Mitigation and Disease Management.

VI. USDA Pathogen Permits

The Animal and Plant Health Inspection Service (APHIS) uses an electronic database, designated as e-Permits. Current users are: the Agriculture Select Agent Program (ASAP), Biotechnology Regulatory Services (BRS), Plant Protection and Quarantine (PPQ), and Veterinary Services. Access to e-Permits requires USDA level-2 e-authentication, except for Select Agents. Additional information about APHIS permits can be found at **http://www.aphis.usda.gov/ppq/permits/ or contact PPQ permit services at (301) 734-8758.**

Select Agent Permits require the following process:

1. No e-submission available, applications must be on a paper PPQ Form 526.
2. The applicant does not need to have an e-authentication, but must be Security Risk Assessment (SRA)-approved and registered with the Agriculture Select Agent Program (ASAP) or the Division of Select Agents and Toxins at the Centers for Disease Control and Prevention.
3. The applicant has no e-Permits access to this type of application and does not receive electronic updates.
4. Only the ASAP staff may receive and input the application into e-Permits.
5. Only the ASAP staff can "see" Select Agent permits in e-Permits.
6. The Senior Agriculture Microbiologist in ASAP reviews applications and writes permit conditions.
7. Permit conditions are not sent to the state.
8. Final Permit review and approval is by the ASAP Director, not the PPQ Permits Branch Chief.
9. The Senior Agriculture Microbiologist signs the final permit and sends it to applicant.
10. The Select Agent pathogen permit looks like any other PPQ Form 526 permit generated through e-Permits by PPQ.
11. Site-Inspections:
    a. "General" or non-Select Agent plant pathogen and pest inspections are conducted by PPQ-Containment Staff, PPQ field inspectors, or State Ag inspectors.
    b. Select Agent site-inspections are conducted only by ASAP staff.

c. Select Agent site-inspections can satisfy a "general" plant pathogen inspection, but not the reverse.

VII. Economic Impact and Compensation

As late as the 1990s, thousands of sheep and cattle, as well as some horses died from ailments (Table 1) attributed to *Rathyibacter* poisoning in Australia (Davis et al., 1995). In Australia, loss of production and cost of control from rathayibacter poisoning has been in the millions of dollars (Stirling et al., 1992).

If the disease enters the U.S., compensation by the USDA Risk Management Agency (RMA) may be available for losses caused by *Rathayibacter toxicus* either to plants or animals.

VIII. Mitigation and Disease Management

Control and management of rathayibacter bacteriosis and poisoning in Australia relies on a number of effective methods. Less effective and experimental methods are also mentioned for comparison. Some methods may be transferable to the U.S. Cultivars, biocontrol agents, and vaccines are likely to be specific for the U.S.A, and possibly even for different regions.

Resistant cultivars. There are few commercially known resistant varieties of grasses, although research in this area is continuing in Australia. A single nematode resistant ryegrass cultivar, 'Guard' of *Lolium rigidum* has been reported (Anonymous, 1994) for use in South Australia. For Western Australia, an early flowering, nematode-resistant cultivar 'Safeguard' has been bred (Department of Agriculture and Food Western Australia, 2009).

Herbicides. Pre-emergent herbicides and post-emergent herbicides are suggested to kill grasses and decrease disease presence. This process makes crop rotation easier for management of infested grasses. However, herbicide resistance has been reported to correspond with an increase in ARGT (Riley and Gill, 1994).

Pasture management. Hay can be cut before the seed heads reach a dangerous stage. Topping, or spraying the tops of plants with a herbicide before flowering is another possibility, although not always practical. In Australia, selective burning of dry pasture after harvest is also a management tool to reduce infected seeds, remains of toxic hay, and nematode galls. Sowing of clean and certified seed is recommended. Avoiding seed produced in areas with the disease is a prudent practice. Close observation is needed to determine that seed heads are free from galls or not. Seed heads should be examined to watch for the emergence of yellow bacterial slime on the heads. The seed heads can also be tested for the presence of the bacteria. Grass and seed head samples could be tested for the presence of the nematode and/or bacteria. Crop rotation and fallow, eradication of host plants and alternative pasture forages have been recommended.

Nematicides. Efforts to remove the vectors by chemical treatments have not proven effective or are considered too costly or impractical.

Biocontrol. Research on biocontrol of vectors shows promise (e.g. Barbetti and Riley, 2008), but has not been widely used on a practical basis. A 'twist fungus', *Dilophospora alopecuri*, developed by the Australian Department of Agriculture has been used to reduce the level of nematodes (Riley, 1994; Riley 1996; Yan and Riley, 2003).

Animal treatments. Treatments for affected animals are limited. Care must be taken not to graze animals on affected pastures during the dangerous periods which extend from the seed-setting stage until the time when the affected grass has weathered away or been burnt or ploughed under. If alternate pastures are available, animals may be removed from contaminated areas. Stocks suspected of being affected by the disease should be moved as expeditiously as possible to a clean site. Veterinary advice should be sought.

Currently there are no government approved or commercial antidotes or vaccines available. An antidote to the toxin has been reported (May and Stewart, 1998; CSIRO, unpublished), but its use is constrained by the fact that outbreaks are unlikely to be detected before animals have died or have consumed too much toxin to be successfully treated. Also, the stress of administering the treatment can induce an episode of convulsions leading to death. An experimental vaccine has been developed (CSIRO, unpublished) but it has limitations. Multiple immunizations are needed to give acceptable protection. Even if such protections were available, they may not be sufficient to permit animals to graze toxic pastures without close monitoring (McKay and Riley, 1993).

Integration of Mitigation and Disease Management Strategies. Risk of rathayibacter poisoning is generally mitigated by management practices such as crop rotation, which includes changes in grasses grown in pastures, rotation among grazed pastures, harvesting hay before seed-heads produce toxins, herbicide treatment of susceptible pasture grasses before flowering to minimize vector and bacterial colonization, inspection of fields for signs of infection and the use of certified seed free of the bacterium. The latter is determined through serological or molecular assays and is currently done by both private and government laboratories in Australia.

IX. Infrastructure and Experts

Diagnosis and identification of *R. toxicus* to species is likely to be relatively easy if the plant host species is known and symptoms are clearly distinguishable from other pathogens of grasses. Samples from sick animals would be examined by local veterinary services, and may be confused with other agents or maladies. It would be important to have cross communication of plant and animal diagnosticians. If the bacterium is identified, then regional centers of the National Plant Diagnostic Network (NPDN) need to know, and samples submitted to USDA-APHIS-PPZ-CPHST in Beltsville, MD for

identification. Diagnosticians in those labs will need educational materials for such identification.

Currently, the experts in the U.S. with the most knowledge of *R. toxicus* are authors of this report and Dr. S.M. Colegate (USDA- ARS-Poisonous Plant Research Lab). This includes plant and animal experts. All other experts we are aware of reside in Australia (Western and Southern), including consultant Dr. I.T. Riley.

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X. Research, Extension, and Education

Needs for research, extension and education vary depending on whether the bacterium, nematode, plant or animal component is addressed.

The primary recommendation is to establish an interdisciplinary group of scientists to become familiar with the diseases, their ecology and management. Because of the complexity of these diseases, this recommendation is critical to achieve an appropriate and early response to their appearance.

A second high priority recommendation is to develop information and training materials for veterinarians, plant pathologists, nematologists, extension personnel, crop consultants and producers of grains and animals for food, recreation, and wool. These materials could be produced in print, web-based materials, symposia or workshops. Materials should first be provided to APHIS and the National Plant Diagnostic Network.

For bacteria, the following needs are paramount:

1. Determining the current geographical occurrence of *Rathayibacter* species in the U.S. Surveys need to be conducted on suspect plants in likely areas (e.g. Oregon, Appalachian area) and analyses of microbial populations in soil (community analyses) from such grasslands.
2. Improve analysis of genetic diversity among and between *Rathayibacter* species, by such methods as Multilocus Sequence Typing (MLST) and Amplified Fragment Length Polymorphism (AFLP).
3. Develop and/or improve diagnostic tools for tracking and spread of *R. toxicus* and related species.
4. Determine the presence and role of plasmids in toxin production. In some bacteria, plasmids carry genetic determinants for toxic products. *R. toxicus* is known to lose toxin ability in culture; loss of plasmids may be one of the reasons.
5. Determine the role of the toxin in the ecology of the bacterium.

6. Sequence the entire genome of least three *R. toxicus* strains (one from each geographical group) and one strain of related species to assess similarities and differences, including the assessment of gene(s) related to toxin production.
7. Improve reproduction of the disease under containment (greenhouse) conditions. Current methods with hypodermic inoculation of the bacterium or with added nematodes 'mostly fails' (I. Riley, personal communication).

For nematodes, the following needs are paramount:

1. Assess the potential for biological control of prospective vectors. (Chemical control in the U.S. is not likely to be approved for the nematodes, though herbicides might be used for control of host plants). Agent(s), ease of use, cost, distribution network and shelf life are factors to be examined.
2. Determine vector capability under optimal and adverse conditions, or carrying capacity for *R. toxicus.*

In plants, the following needs are significant:

1. Determine feasibility of plant resistance to nematodes or bacterium colonization for the U.S.
2. Determine viability and yield potential of plants bred for resistance to the toxin under U.S. conditions.
3. Determine feasibility of pasture management practices applicable to the U.S.

In animals, the following needs remain:

1. Determine the toxicological mechanisms by which the corynetoxins damage animal tissues. Methods using global genomic and proteomic analyses determining specific biomarkers are providing some information (Retallick et al., 2006). Much more information about the mechanism of action is needed to develop more effective diagnostics or treatment protocols.

2. Determine whether animals or even humans suffering from the toxicosis have suppressed immune function and are more susceptible to pathogenic infections. Various authors have suggested animals or even humans exposed to natural toxins similar to corynetoxins may be at greater risk to infection by opportunistic pathogens, including viruses.

3. Determine potential model systems to study the effects of corynetoxin exposure on susceptibility to subsequent pathogenic infection. *Neospora caninum* and *Toxoplasma gondii* are intracellular protozoan parasites affecting most mammals and can cause abortions in cattle and sheep (though *N. caninum* has not been shown to infect humans). Studies show that the parasites exist in their latent forms for a long time or even life time in immunocompetent individuals, but they can be re-activated to replicating form and cause clinical diseases when the animals are immunologically compromised. Thus, corynetoxins may serve as a

co-factor for opportunistic pathogens, rendering the animals more susceptible to infections or re-activation of latent forms of the parasites leading to chronic diseases.

4. Improve the protective efficacy of the experimental vaccine (CSIRO, unpublished) or develop a new generation of the vaccine using novel conjugation and highly potent adjuvant technologies. Efficacy and cross-neutralization for all corynetoxins would need to be determined, as well as cost effectiveness and practicality.

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Web Resources

Currently, there are no appropriate web resources for this recovery plan.

Some information can be obtained from searches using “ARGT” as a key word on websites of:
Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO) http://www.csiro.au/;
Department of Agriculture and Food Western Australia (DAFWA) http://www.agric.wa.gov.au;
Department of Primary Industries and Resources South Australia (PIRSA), http://www.pir.sa.gov.au/;
South Australian Research and Development Institute (SARDI) http://www.sardi.sa.gov.au/.

Appendix

Table A1. Names associated with *Anguina* spp. and *Rathayibacter* species other than *R. toxicus*.

Referring to *Anguina tritici* and/or *Rathayibacter tritici* in wheat: common names for galls, disease, or pathogen
anguinosis
cereal nematode
earcockle
earcockle nematode
earcockle eelworm
seed gall nematode
spike blight
tundu
wheat eelworm
wheat nematode
wheat gall nematode
yellow ear-rot
yellow slime disease
žitna nematode
Referring to *Anguina agrostis* (or *Anguina* sp.) and/or *Rathayibacter rathayi* in *Dactylis glomerata*
bacterial gummosis
bacteriosis
gumming disease
Rathay's disease
seed gall nematode
yellow slime disease
Referring to *Anguina agrostis* in bentgrass (*Agrostis* spp.)
bentgrass nematode

Structure of corynetoxins (CTs).

CTs are structurally and functionally similar to tunicamycins (TMs) that are produced by the bacterium *Streptomyces iysosuperficus* (Takatsuki et al., 1971; Takatsuki and Tamura, 1971a; Takatsuki and Tamura, 1971b). Both molecules share a common N-acetylglucosamine tunicaminyluracil core structure and vary in length, terminal branching formation and hydrosylation states of the fatty acid chains (Eckardt, 1983) (Figs. A1 and A2 and Table A2). The structural and functional similarity between the CTs and TMs allows the use of TMs as a substitute in evaluating CT functions *in vitro* and *in vivo*. Indeed, many effects of CTs on cells and animals were obtained using TMs in cell and animal models, and numerous reports confirmed that the effects of CTs and TMs on cells and animals are indistinguishable (Allen et al., 2006; Finnie, 2006). The half life for CTs should be short, based on studies on TMs, which is about 4 hr in sheep (Stuart et al., 1992). In general, glycolipids in their native forms are poor immunogens and not surprisingly, natural immunity against CTs as glycolipids has never been reported in affected animal species. CTs may be extracted from the bacterial gall-rich concentrate

and followed by purification with HPLC (Than et al., 2002; Vogel et al., 1981).

Table A2. The fatty-acid residues (R in Fig. 1) on tunicamycins (TMs) and corynetoxins (CTs)

TM	Fatty acid residues of TM (R)	CT	Fatty acid residues of CT (R)
TM-I	$(CH_3)_2$-CH-$(CH_2)_7$-CH=CH-	CT-S15a	CH_3-$(CH_{2/3})_2$-CH-$(CH_2)_{10}$-
TM-II	$(CH_3)_2$-CH-$(CH_2)_8$-CH=CH-	CT-H16i	$(CH_3)_2$-CH-$(CH_2)_{10}$-CHOH-CH_2-
TM-III	CH_3-$(CH2)_{10}$-CH=CH-	CT-U16i	$(CH_3)_2$-CH-$(CH_2)_{10}$-CH=CH-
TM-IV	$C_{12}H_{25}$CH=CH-	CT-H17a	CH_3-$(CH_{2/3})_2$-CH-$(CH_2)_{10}$-CHOH-CH_2-
TM-V	$(CH_3)_2$-CH-$(CH_2)_9$-CH=CH-	CT-S16i	$(CH_3)_2$-CH-$(CH_2)_{12}$-
TM-VI	(CH_3)-CH-$(CH_2)_{11}$-	CT-U17a	CH_3-$(CH_{2/3})_2$-CH-$(CH_2)_{10}$-CH=CH-
TM-VII	$(CH_3)_2$-CH-$(CH_2)_{10}$-CH=CH-	CT-U17i	$(CH_3)_2$-CH-$(CH_2)_{11}$-CH=CH-
TM-VIII	CH_3-$(CH_2)_{12}$-CH=CH-	CT-S17a	CH_3-$(CH_{2/3})_2$-CH-$(CH_2)_{12}$-
TM-IX	$C_{14}H_{29}$-CH=CH-	CT-H18i	$(CH_3)_2$-CH-$(CH_2)_{12}$-CHOH-CH_2-
TM-X	$(CH_3)_2$-CH-$(CH_2)_{11}$-CH-CH-	CT-U18i	$(CH_3)_2$-CH-$(CH_2)_{12}$-CH=CH-
		CT-H19a	CH_3-$(CH_{2/3})_2$-CH-$(CH_2)_{12}$-CHOH-CH_2-
		CT-S18i	$(CH_3)_2$-CH-$(CH_2)_{14}$-
		CT-U19a	CH_3-$(CH_{2/3})_2$-CH-$(CH_2)_{12}$-CH=CH-
		CT-S19a	CH_3-$(CH_{2/3})_2$-CH-$(CH_2)_{14}$-
		CT-U16i	Same as TM-VII
		CT-U17i	Same as TM-X

S, saturated fatty acid; U, unsaturated fatty acid; H, β-hydroxy fatty acid; i, iso; a, anteiso (Eckardt, 1983).

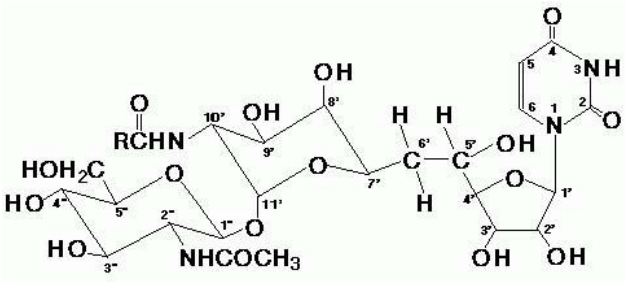

Figure A1. The identical tunicaminyluracil core structure of corynetoxins and tunicamycins (Eckardt, 1983). R represents the fatty acid residues in various lengths, terminal branching and hydrosylation states (see Appendix Table A2).

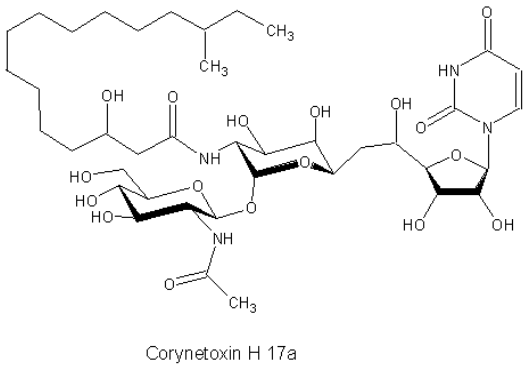

Figure A2. Corynetoxin H 17a, one of the major components of the CTs (Eckardt, 1983).