Recovery Plan for

Rathayibacter Poisoning

caused by

Rathayibacter toxicus (syn. *Clavibacter toxicus*)

Updated March 2015 (Replaces February 2010 Version)

Contents		Page
Executive Summary		2
Contributors and Reviewers		5
I.	Introduction	5
II.	Disease Development and Symptoms	8
III.	Plant Infection, Spread of the Bacterium, and Animal Poisoning	11
IV.	Monitoring and Detection	12
V.	Response	13
VI.	USDA Pathogen Permits	14
VII.	Economic Impact and Compensation	15
VIII.	Mitigation and Disease Management	15
IX.	Infrastructure and Experts	16
X.	Research, Extension, and Education Needs	17
	References	20
	Web Resources	25
	Appendices	26

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 an 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.

Cite this document as:

Murray, T.D., I. Agarkova, S. Alderman, J. Allen, R. Bulluck, J. Chitambar, C. Divan, I. Riley, B. Schroeder, A. Sechler, and S. Subbotin. 2014. Recovery Plan for Rathayibacter Poisoning caused by *Rathayibacter toxicus* (syn. *Clavibacter toxicus*) National Plant Disease Recovery System, a cooperative project of The American Phytopathological Society and The United States Department of Agriculture, posted at http://www.ars.usda.gov/research/npdrs.

Executive Summary

Rathayibacter (*Clavibacter*) *toxicus* was originally added to the Select Agent List in 2008 and relisted in 2012, due primarily to the potential damage affecting domesticated forage-consuming animals in the U.S. *R. toxicus* is a cross-domain pathogen affecting plants and animals: a nematode-transmitted Gram-positive bacterium that causes plant disease (bacteriosis) 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 to a lesser extent in South Africa. In Australia, the plant disease has been found on over 10 million hectares of farmland in different parts of the country, with total losses attributed to *Rathayibacter* poisoning in Western Australia estimated at 40 million Australian dollars per year.

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 an accidental or deliberate release very difficult.

Several species of *Anguina* seed and leaf gall nematodes carry *R. toxicus* into the host plant, where it resides in the developing seed head and forms galls. Galls may be consumed by grazing animals or fall to the ground after the grass has senesced. Most infected plants do not show any visible symptoms. Thus, absence of visible yellow slime (gummosis) does not mean that a plant is free of *R. toxicus*. In many cases, bacterium 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, are members of the tunicamycin family of antibiotics and are produced as the plants become senescent and the bacteria reach their peak biomass. Corynetoxins produced by *R. toxicus* are heat stable, highly toxic (oral lethal dose for sheep is 3-6 mg/kg body weight) glycolipids with cumulative effects. Up to 16 different forms of corynetoxins may be produced, differing only in their side chains.

Animals that consume sufficient infected pasture grass suffer a toxicosis characterized by episodic neurological signs, 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 is not available commercially; its use is also 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 spring and summer grazing. However, it can happen at any time of the year in livestock fed with hay containing corynetoxins. *Rathayibacter* poisoning was diagnosed in Japan in cattle after they had eaten hay exported from Australia.

In the U.S., susceptible animals include the approximately 95 million cattle, 6 million sheep, 9 million horses, and an unknown number of bison.

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 U.S. are potentially susceptible to infection, as indicated by the occurrence of *Anguina* species, the nematode vectors, and other *Rathayibacter* species in the U.S. closely related to *R. toxicus.*

As seed and hay are moved about the country, *R. toxicus* and nematode vectors could 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 bacterium in close proximity to a nematode vector before there is evidence of the disease in grazing animals. This means the bacterium, nematode or infected seed could be introduced without detection or any suspicion. Currently, there is a cost-effective, rapid and sensitive identification test for *R. toxicus* but not for its nematode vectors.

Risk of *Rathayibacter* poisoning is generally mitigated by management practices such as crop rotation, stock rotation among grazed pastures, harvesting hay before corynetoxins are produced in seed-heads, 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. Establish strict quarantine measures at all ports of entry for hay products and forage grass seeds, especially those originating from Australia, New Zealand and South Africa. Develop reliable detection and identification tests for all *R. toxicus* hosts and vectors.
2. Establish an interdisciplinary working group of scientists familiar with these diseases, to coordinate research on the detection and identification of the pathogen and vectors, ecology and management of the diseases, and assess the applicability of Australian diagnostic practices and procedures to U.S. production systems. Because of the complexity of these diseases, this recommendation is critical to achieve an appropriate and early response to their appearance.
3. Educate veterinarians, plant pathologists, nematologists, extension personnel, crop consultants and grain and animal producers to be able to identify *Rathayibacter* poisoning and *Rathayibacter* bacteriosis and understand their ecology and management. Early response is critical for appropriate and effective control due to the complexity of the diseases.
4. Obtain additional specific information to improve disease management and animal protection.
    a. Bacteria: survey potentially susceptible grasses for gummosis, initially emphasizing areas of the U.S. with previous reports of *Rathayibacter* bacteriosis and/or staggers-like occurrences to determine the geographic distribution of *Rathayibacter* species with subsequent random surveys emphasizing plant hosts in important stock grazing areas; develop diagnostic tools for detection and differentiation of *R. toxicus* and related species; determine the role of bacteriophage and plasmids in toxin production; determine the role of the toxin in the ecology of the bacterium; sequence the genomes

of several *R. toxicus* and related *Rathayibacter* species to assess similarities and differences, and especially gene(s) related to corynetoxin production; develop methods to reproduce the disease under controlled environments to improve our understanding of the interactions between *R. toxicus*, its nematode vector, and the plant; and determine the potential for biological control of *R. toxicus* with non-toxigenic *Rathayibacter* species.

b. Nematodes: survey potentially susceptible grasses for presence of nematode galls emphasizing areas of the U.S. with previous reports of *Rathayibacter* bacteriosis and/or staggers-like occurrences to determine the geographic distribution of *Anguina* species as potential vectors of *R. toxicus* with subsequent random surveys emphasizing plant hosts in important stock grazing areas; establish pure cultures of the nematode vectors on greenhouse-grown plants for use in controlled environment studies; develop molecular detection and identification tools for *Anguina* species; assess the potential for *Anguina* species present in the U.S. to transmit *R. toxicus,* as well as the carrying capacity for *R. toxicus*; and, determine the potential for biological control of potential nematode vectors since chemical control is not feasible.

c. Plants: identify sources of resistance/tolerance to *R. toxicus* and/or the nematode vectors; determine viability and yield potential of plants bred for resistance to the bacterium and/or vector under U.S. conditions; determine applicability and feasibility of Australian pasture management practices to the U.S.

d. Animals: develop a better understanding of the mechanisms of toxin 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)*

This plan was updated in November 25, 2014 to incorporate new information that became available after the original plan was completed in February 2010.

Update Contributors:

Ian Riley, University of Adelaide, South Australia: Jeremy Allen, Department of Agriculture and Food Western Australia; John Chitambar and Sergei Subbotin, California Department of Food and Agriculture; Brenda Schroeder, University of Idaho; Irina Agarkova, University of Nebraska; Timothy Murray*, Washington State University; Russ Bulluck and Charles Divan, USDA-APHIS; Stephen Alderman and Aaron Sechler, USDA-ARS.

***** Chair and corresponding author: Tim Murray, email tim.murray@wsu.edu

Original Contributors:

Ian Riley, University of Adelaide, South Australia; Irina Agarkova, Michael Carlson, and Anne Vidaver***,** University of Nebraska; Russ Bulluck and Charles Divan, USDA-APHIS; Stephen Alderman, Norman Schaad and Wenbin Tuo, USDA-ARS.

*Chair and corresponding author: Anne Vidaver, email avidaver1@unl.edu

Authors are listed alphabetically by state and then federal agency.

Reviewers (proposed):

American Phytopathological Society (APS), American Veterinary Medical Association (AVMA), American Board of Veterinary Toxicology (ABVT), Society of Nematologists (SON)

I. Introduction

Rathayibacter toxicus is a toxin-producing Gram-positive bacterium that colonizes the vegetative stems and floral structures of grasses. Ingestion of sufficient amounts of the toxin by grazing animals results in a serious poisoning that frequently 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* proliferates within the nematode galls, killing the nematodes and completely filling the lumen resulting in formation of a bacterial gall. Bacterial growth in grass flowers is often sufficient to cause oozing from the floral structures of a yellow bacterial slime; such symptoms caused by plant-colonizing bacteria are referred to as gummosis. Damage resulting from livestock poisoning and associated effects is estimated at 40 million Australian dollars per year in Western Australia (Kessell, 2010). *R. toxicus* was originally added to the Select Agent List in 2008 and relisted in 2012, due primarily to the potential damage affecting domesticated forage-consuming animals in the U.S.

The common names given to the diseases caused by the association of *Rathayibacter* and *Anguina* are numerous. The common names for the plant and animal diseases are not used

consistently and therefore, we use *Rathayibacter* poisoning to emphasize the causal agent and primary concern for the consequences of ingestion of infected grasses by animals. *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 on a *R. toxicus*-infested pasture, hay or feed grain are susceptible to poisoning, as are other animals such as poultry fed *R. toxicus* contaminated feed. We use *Rathayibacter* bacteriosis to represent the plant disease.

Plant disease names

Yellow slime disease is a common name used to describe *R. toxicus* infection of grasses, but this name also has been used for other slime diseases, including *Rathayibacter tritici* in wheat. Most of the common names associated with the disease are related to toxic effects in animals (Table 1). A bacterial disease in orchard grass (*Dactylis glomerata*) was named Rathay's disease (Smith, (1914), after Rathay, who first described it in Austria in 1899. Rathay's disease, caused by *Rathayibacter rathayi*, is a common name used for the disease of orchard grass in Oregon, U.S. (see Appendix Table A1).

Table 1. Common names for *Rathayibacter toxicus* and/or *Anguina* associations.

Referring to	Common names of disease, galls, or pathogen
Toxicity of *Lolium rigidum* caused by *Rathayibacter toxicus*	annual ryegrass toxicity (ARGT) annual ryegrass toxicosis corynetoxin poisoning corynetoxicosis parasitized annual ryegrass toxicity toxic annual ryegrass tunicaminyluracil toxicosis Wimmera ryegrass toxicity Black Springs syndrome
Toxicity of *Lachnagrostis filiformis* (syn. *Agrostis avenacea*) or *Polypogon monspeliensis* caused by *Rathayibacter toxicus*	flood plain staggers blown grass/beard grass poisoning corynetoxin poisoning corynetoxicosis Stewarts Range syndrome tunicaminyluracil toxicosis
Anguina and/or *Rathayibacter toxicus*	bacterial galls gumming disease seed gall nematode

Bertozzi and Davies, 2009; Bertozzi and McKay, 1995; Edgar et al., 1994; Johnston et al., 1996; McKay et al., 1993; Riley and Barbetti, 2008; Riley, 1992a, 1995; Riley et al., 2001.

Animal disease names

Various names have been given to the disease in animals, based on the host, geographic area in which the disease occurred and the toxins involved (Table 1). For example, annual ryegrass toxicity (ARGT) is the most commonly used name to describe 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 ARGT).

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.

Hosts and vectors

Other plant hosts reported to be infected by *R. toxicus* are: *Austrodanthonia caespitosa, Avena sativa, Avena caespitosa, Danthonia caespitosa, Erharta longiflora, 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; Johnston et al., 1996; McKay & Ophel 1993; McKay et al., 1993; Riley, 1992a, 1995, 1996; Riley and Barbetti, 2008; Riley et al., 2001).

Known vectors for *R. toxicus* are four *Anguina* species (family Anguinidae): *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, 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 the agricultural areas of South and Western Australia, and a localized area of New South Wales thereafter (McKay and Ophel, 1993). A weather damaged grain-associated tunicaminyluracil toxicosis, not involving *R. toxicus*, occurred in pigs in New South Wales in 1985 (Bourke, 1987). Poisonings were reported in 1980 in South Africa (Schneider, 1981).

The bacterium was isolated in the 1960s, but not described as a separate species (*Clavibacter toxicus*) 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 reduced the incidence of animal disease. Following the death of some cattle in Japan in 1996 from eating oaten hay exported from Australia (contaminated with ryegrass; Masters et al., 2006, 2011), routine inspection and testing for *R. toxicus* was introduced for all exported hay, and no deaths have occurred since. Since *R. toxicus* can be vectored by different nematode species to several grass species (Riley and Barbetti, 2008), the threat of introduction and establishment of *R. toxicus* in the U.S. includes other grass hosts and nematode vectors; therefore, a single inclusive name would be appropriate. Hence, we use *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. *R. toxicus* adheres to the cuticle of the seed-gall nematode, *Anguina*, and is carried to the developing seed heads of the plant (Fig. 2E). The infective juvenile stage of the nematode enters the growing plant and migrates to the seed head (Fig. 2C, D), where it infects individual developing seeds and transforms them into galls. Within the galls juvenile nematodes develop to adults, which produce several hundred eggs per gall. Eggs hatch within the galls releasing juvenile nematodes. Likewise, bacterial cells increase in number and may colonize the gall and grow outside of it to other parts of the seed head (Fig. 2A, B). During summer, galls fall to the ground and the nematodes within enter a dry (anyhydrobiotic) resting stage that allows them to survive through adverse, dry conditions for many years, if necessary. However, when exposed to favorable climatic conditions of sufficient periods of soil moisture or rainfall, galls rehydrate as water is absorbed. As a result, the nematodes rehydrate and once again become active. Soon the galls disintegrate releasing the nematodes onto the soil surface. As long as the soil surface is moist the nematodes will move towards the growing point (meristem) of nearby host plants, where they accumulate and wait until the immature seed head forms. Soon the seed head is invaded thereby, completing the life cycle (Riley and Barbetti, 2008; Subbotin and Riley, 2012).

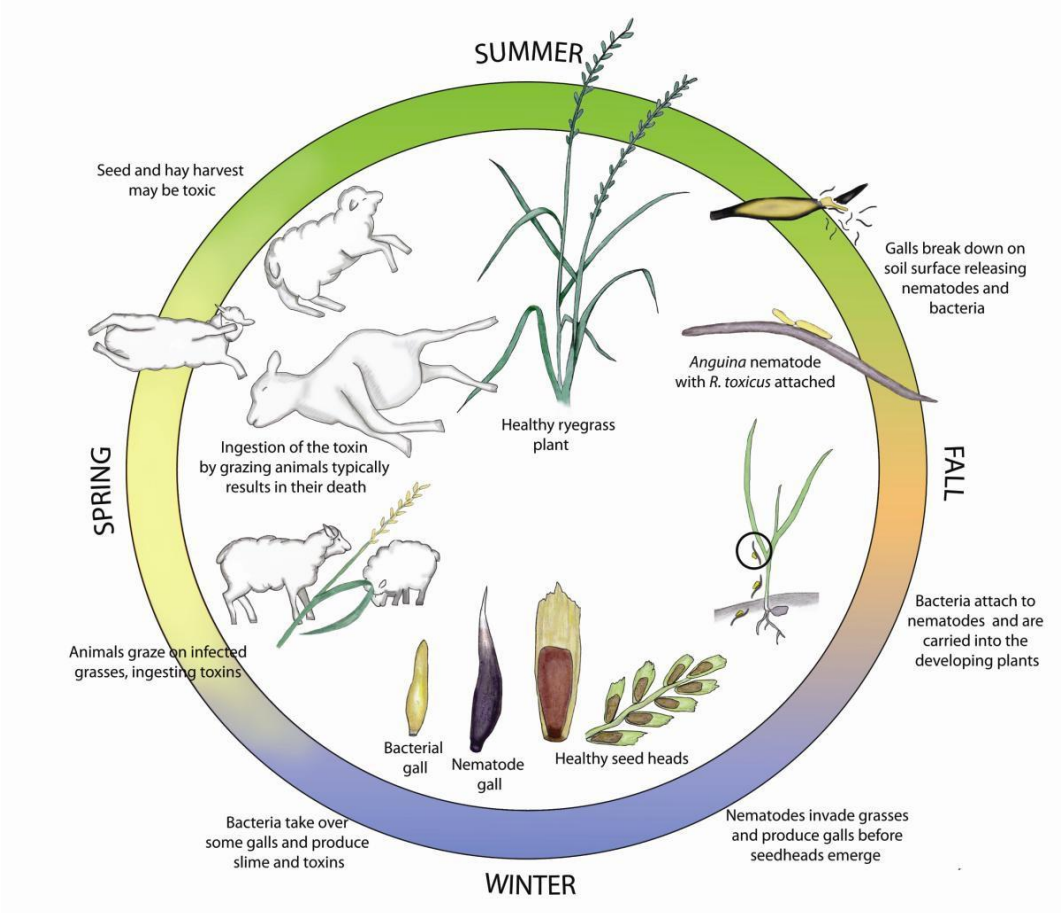


Figure 1. The annual life cycle of the bacterium causing Rathayibacter poisoning. This diagram is for illustrative purposes only and is not drawn to scale. Note that not all animals consuming infected grasses will die.

Host plants can occur in pastures or as weeds in crops. Nematode galls in annual ryegrass are difficult to detect in the field as they are small and covered by the outer seed coat (lemma and palea); once these coats are removed, the galls are spindle-shaped, shrunken in comparison with normal seed, and purplish-colored (Fig. 2F). Although most infected plants do not show any visible symptoms, some colonized grass seed heads become twisted and deformed, and may be covered with an orange-yellow exudate sometimes called yellow slime. Over time, the slime may harden and darken in color. Note that slime does not always form, or it may be washed-off by rain. Thus, absence of obvious slime does not mean that a pasture is free of *R. toxicus* (McKay and Riley, 1993).

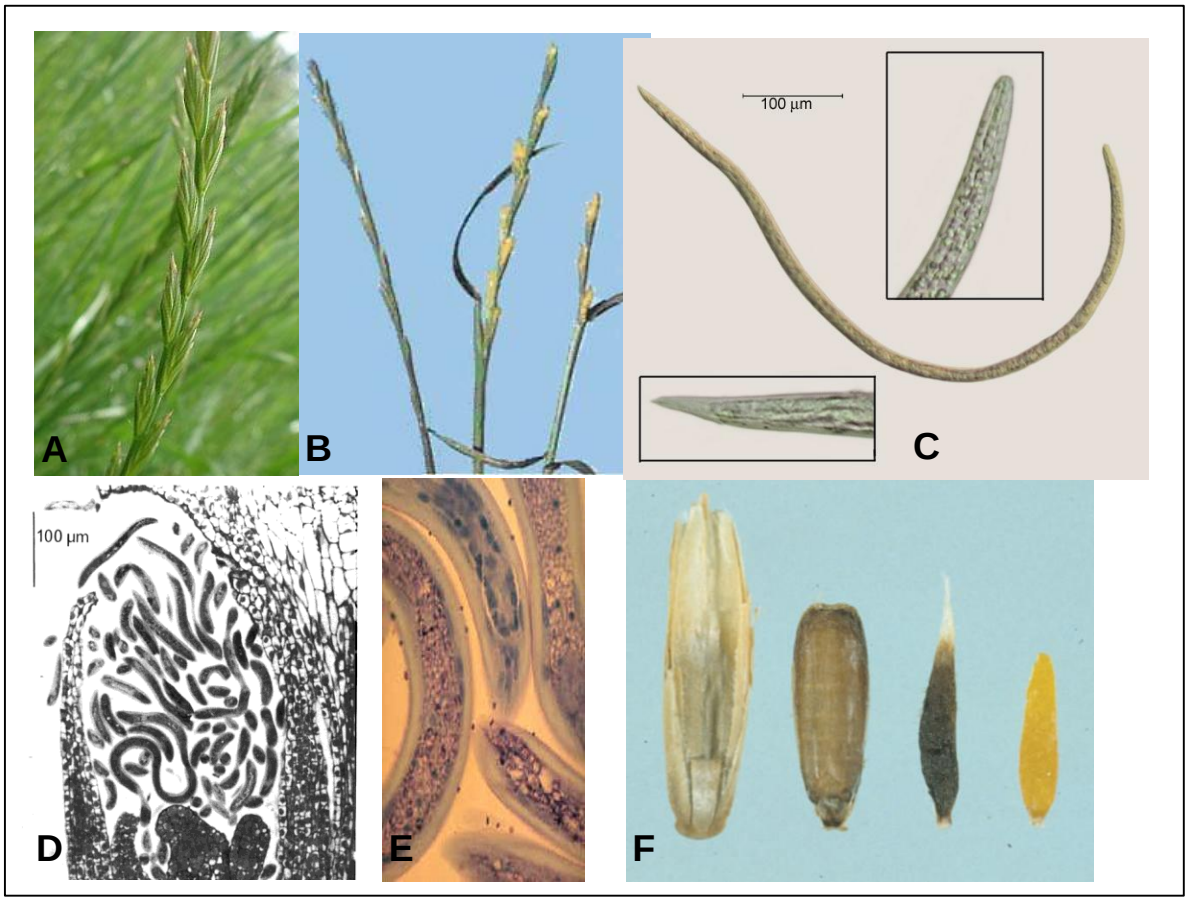


Figure 2. Healthy and diseased *Lolium rigidum*: (a) healthy ryegrass plants; (b) yellow slime of *Rathayibacter* on infected annual ryegrass heads; (c) microphotograph of *Anguina* juvenile (J2); (d) large numbers of *Anguina* juveniles (J2) clustered around a *L. rigidum* floret primordium (microphotograph 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. Photographs courtesy of: b- South Australian Animal Health Quarterly, December 2006; c- T.O. Powers, Univ. Nebraska; d- Stynes and Bird, 1982; e- J. Collier, Western Australian Department of Agriculture and Food; (f) I.T. Riley.

Galls form as the result of infection of the ovaries and replace the seed. Galls typically are either nematode galls (dark purple-colored, containing *Anguina* and no obvious bacteria) or bacterial galls (yellow-colored, containing bacteria and sometimes the remains of dead nematodes) (Fig. 2F). In many cases, bacteria and nematode infections go undetected or the disease is misidentified (Finnie, 1991). The nematode vector and bacterium can survive in the dry state for many years (Murray, 1986; Nickle, 1991). A related bacterium, *Rathayibacter iranicus*, was recently found in Turkey and isolated from asymptomatic wheat seeds on a semi-selective agar medium (Postnikova et al., 2009).

Production of corynetoxins varies within field populations of the bacterium, as toxin is not found in all mature galls colonized by bacteria. Corynetoxins are usually produced as infected plants become senescent and the bacteria reach their peak biomass. The toxins are heat stable glycolipids, known as corynetoxins or CTs, and are highly toxic (oral lethal dose for sheep is 3-6 mg/kg body-weight). Up to 16 toxins may be produced, differing only in their side-chains (Appendix Table A2; Figs. A1 and A2). The disease in animals may be confused with other diseases, such as ergot of *Paspalum*, caused by *Claviceps paspali*, which produces indole tremorgens, polioencephalomalacia enterotoxaemia, *Phalaris* toxicosis, perennial ryegrass staggers, grass tetany or botulism.

The involvement of a bacterial virus or bacteriophage has been considered important for toxin production by *R. toxicus* for some time, but there is uncertainty over the significance of bacteriophage in disease outbreaks. Bird et al. (1980) and Stynes and Bird (1983) demonstrated the presence of a bacteriophage in toxic galls from *Lolium rigidum*. McKay et al. (1993) isolated the same bacteriophage from toxic galls found in *Lachnagrostis filiformis* and *Polypogon monspeliensis*, and Ophel et al. (1993) showed that only *R. toxicus* infected with bacteriophage and producing bacteriophage structural proteins, produced corynetoxins in laboratory experiments. Furthermore, culturing toxin-producing *R. toxicus* in the presence of an antiserum to the bacteriophage resulted in the bacterium losing the ability to produce corynetoxins. All of these studies were considered reasonable evidence that a bacteriophage was involved in the production of toxins by the bacterium. However, Kowalski et al. (2007) conducted a study of toxic galls obtained from the screenings of harvested grain, and seed heads collected from ryegrass pasture samples, and found a poor correlation between the presence of corynetoxins and the bacteriophage. In toxic galls from grain screenings, 100% contained *R. toxicus* and corynetoxins, but only 68% contained the bacteriophage. In ryegrass seed heads, 86% contained *R. toxicus*, 47% of these bacterium-positive galls contained corynetoxins, and only 5% of all the seed heads were positive for the bacteriophage. The seed heads with bacteriophage also contained *R. toxicus* and corynetoxins.

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 poisoning by the corynetoxins. Early clinical signs are loss of coordination in the legs, followed by high-held heads and arched backs. Later, muscle tremors, head nodding, tooth grinding, involuntary eye movement, and body convulsions can occur. Initially, these clinical signs are intermittent and episodic, with periods of relative normality in between. In the final stages, the animals lie on their sides and make walking motions. Poisoning signs can occur over several weeks, and animals can die within 24 hours of the first signs of poisoning. When an outbreak of the disease occurs, animals may continue to develop clinical signs and die for up to 10 days

after removal from the toxic pasture. 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. There is no test for corynetoxins in animal tissues and consequently, the disease is diagnosed on the basis of known exposure to the toxin, level of toxin exposure (determined by quantity of bacterial galls in the feed), clinical signs, gross and microscopic pathology (lesions suggestive of corynetoxicosis) and the exclusion of other causes. Despite neurological signs, brain tissue can appear normal histologically.

Animals exposed to corynetoxins do not develop immunity because the toxin molecules are too small to be antigenic (McWilliam and Vogel, 1988). Additionally, a second exposure to the toxins can result in more severe signs due to an accumulation of the toxin in certain tissues (Jago and Culvenor, 1987).

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

Pasture and rangeland throughout the U.S. are potentially susceptible to infection, as indicated by the occurrence of *Anguina* species in the U.S., the nematode vectors, and bacteria related to *R. toxicus* (Alderman et al., 2003; Postnikova et al., 2004; Riley et al., 2004). No attempt has been made to isolate *R. toxicus* from toxigenic fescue grass in the southeastern U.S.

Susceptible animals at risk in the U.S. include the approximately 95 million cattle (USDA-Economic Research Service, 2009), 6 million sheep (American Sheep Industry Association, 2009), 9 million horses (American Horse Council, 2009) and an unknown number of Bison. Pigs, llamas and alpacas may also be at risk, as well as animals consuming toxins from hay or feed grains (e.g. poultry). The most valuable animals at risk are race horses.

It is important to note that *R. toxicus* can potentially colonize and produce corynetoxins in a wide range of cereals consumed by humans, such as those included in dry 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, hay, and by wind. Galls fall to the ground after the grass has senesced where the bacteria and nematodes survive, and nematodes infest grasses the following autumn or spring. The bacterium and its 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 bacterium and nematode vector to see evidence of the disease in grazing animals. This means the bacterium or nematode could be introduced without detection or any suspicion.

Regular grazing helps keep nematode populations low enough to prevent development of *Rathayibacter* bacteriosis to an economic level. It is an important but subtle point that in all recorded cases of ARGT in Western and South Australia, the plant host was protected from grazing through production of seed heads for one or more seasons, which allowed the nematode populations to increase to damaging amounts. For example, *A. funesta* in ryegrass within another

crop as a dominant and hard to control weed, wheat as a host of *A. tritici*, *Anguina spp.* in orchard grass and Chewings fescue grown for seed production, *A. paludicola* in a pasture that was not grazed because the field was flooded, and *A. paludicola* in blowngrass where rare flood events resulted in a host population well above that which could be consumed by low livestock densities.

Susceptible grasses and potential nematode vectors are present in the U.S. It is possible that poisoning of cattle and sheep associated with consumption of Chewings 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 related species. Herbarium specimens from that time enabled detection of a glycolipid toxin, but it differed from those produced by *R. toxicus* (Riley et al., 2003, 2004).

IV. Monitoring and Detection

Surveys for *R. toxicus*, even in Oregon where cattle poisonings have occurred (Riley et al., 2003; Shaw and Muth, 1949), 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. Surveys of grasses, particularly pasture grasses, for *R. toxicus* should be considered.

Several published methods for detecting *R. toxicus* in infected plants are available. The bacterium is not difficult to isolate, but grows slowly on agar media and may be missed. 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 orchard grass seed lots (Alderman et al., 2003). Newer methods including PCR (Kowalski et al., 2007), real-time PCR (Schaad et al., unpublished), and enzyme-linked immunosorbent assays (ELISA; Masters et al., 2006, 2011, 2014) for detection of *R. toxicus* are more precise, more sensitive, faster and perhaps less expensive, 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 *R. toxicus*, although its presence does not confirm that an ill animal is suffering from, nor that a dead animal died from corynetoxins poisoning (Allen and Gregory, 2011). At least for ARGT, a large proportion of livestock without any clinical signs will give a positive rumen test because *R. toxicus* is widely distributed (Bourke, 2007). Necropsy of animals, with microscopic examination of the brain and liver especially, is used to provide support for diagnosis (see Section II) (Finnie, 2006).

ELISA can be performed on ryegrass seed heads pre-anthesis to detect *R. toxicus* 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 to determine if the bacterium is present, and if so, the risk of disease that the material presents if consumed by livestock (e.g. when applied to hay or standing pasture) or could potentially contribute to the spread of the causal organisms (McKay and Riley, 1993; Masters et al., 2014).

Several methods have been published for detection and identification of potential nematode

vectors, but their use is not necessarily associated with the diagnosis of disease in the plant or toxicosis in the animal (e.g. McKay and Riley, 1993; Powers et al., 2001).

Rathayibacter toxicus diagnostic laboratories (all are in Australia):

Crop Diagnostics, Plant and Soil Health
South Australian Research and Development Institute
Locked Bag 100
Glen Osmond, SA 5064
Australia
Tel + 61 8 8303 9375; Fax: + 61 8 8303 9393

Animal Health Laboratories, Department of Agriculture and Food Western Australia
3 Baron-Hay Court
South Perth WA 6151
Australia
Tel: + 61 8 9368 3351; Fax: + 61 8 9474 1881

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 (7 CFR Part 331).

The planned immediate response to suspect instances of *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 signs in the 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 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 an act of bioterrorism 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 Services (AgSAS), Biotechnology Regulatory Services (BRS), Plant Protection and Quarantine (PPQ), and Veterinary Services. Additional information about APHIS permits can be found at http://www.aphis.usda.gov/wps/portal/aphis/resources/permits or contact PPQ permit services at (301) 734-8758.

Access to e-Permits requires USDA level-2 e-authentication, including Select Agents. Applications and permits for Select agents can only be viewed and processed by the Agriculture Select Agent Services staff.

Select Agent Permits require the following process:

1. E-submission available and preferred over paper applications of PPQ Form 526.
2. The applicant does not need to have an e-authentication (for paper applications), but must be Security Risk Assessment (SRA)-approved and registered with the Agriculture Select Agent Services (AgSAS) or the Division of Select Agents and Toxins (DSAT) at the Centers for Disease Control and Prevention.
3. Only the AgSAS staff may receive and input the paper applications into e-Permits.
4. Only the AgSAS staff can "see" Select Agent permits in e-Permits.
5. The Unit Director for Operational Services (AgSAS) reviews applications and writes permit conditions.
6. Permit conditions are not sent to the state.
7. Final Permit review and approval is by the Unit Director for Operational Services (AgSAS), not the PPQ Permits Branch Chief.
8. The Unit Director for Operational Services (AgSAS) signs the final permit and sends it to applicant.
9. The Select Agent pathogen permit looks like any other PPQ Form 526 permit generated through e-Permits by PPQ.
10. 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 AgSAS and/or DSAT inspection staff.
    c. Select Agent site-inspections can satisfy a "general" plant pathogen inspection, but not the reverse.

Additional information on APHIS Select Agent PPQ 526 permits can be found at: http://www.selectagents.gov or AGSAS@aphis.usda.gov or at 301-851-3300 option 3.

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 *Rathayibacter* poisoning in Australia (Davis et al., 1995). In Australia, annual losses of production and cost of control from *Rathayibacter* poisoning have been estimated in the millions of dollars (Kessell, 2010; Stirling et al., 1992).

If the bacterium 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 several effective methods. Less effective and experimental methods are also mentioned for comparison. Some methods may be transferable to the U.S.; however, cultivars, biological control agents, and vaccines might need to be developed specifically for the U.S., and possibly for different regions within the U.S.

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' was developed (Erickson, 2014) for use in South Australia. For Western Australia, an early flowering, nematode-resistant cultivar 'Safeguard' was developed (Erickson, 2014).

Herbicides. Pre- 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, the development of herbicide resistance has been reported to correspond with an increase in the use of herbicides for the control of annual ryegrass in order to control ARGT (Riley and Gill, 1994). Furthermore, resistance in ryegrass and other weedy grasses is widespread in parts of the U.S. (Jasieniuk et al, 2008).

Pasture management. Hay can be cut before the seed heads become toxic. Topping, or spraying the tops of plants with an herbicide before flowering is another possibility, although not always practical. Alternatively, heavy grazing can be used to reduce seed head production, but this is only possible if there is a sufficient number of animals to remove all seed heads and any re-emergent seed heads before they can become toxic. Because of the number of animals required, this can only be applied to one or a few fields in a given year. In Australia, selective burning of dry pasture after harvest is another management tool used to reduce infected seeds, remains of toxic hay, and nematode galls. Sowing of clean and certified seed is recommended. Avoiding seed produced in areas where yellow slime disease is known to occur is a prudent practice. Close observation is needed to determine that seed heads are free from galls. Seed heads should be examined to watch for the emergence of yellow bacterial slime on the heads. Grass and seed heads should be tested for the presence of the nematode and/or bacteria. Crop rotation and clean 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.

Biological control. Research on biological control of vectors has shown promise (e.g. Barbetti and Riley, 2006), but has not been widely used on a practical basis. The twist fungus, *Dilophospora alopecuri*, has been used by the Western Australia Department of Agriculture and Food to reduce the population of nematodes (Riley, 1994; Riley 1996; Yan and Riley, 2003), but is not commercially available at this time. Applying non-toxigenic *Rathayibacter* species to soil that could compete with *R. toxicus* for binding sites on the nematode cuticle has potential, but needs additional research (Riley and Barbetti, 2008).

Animal treatments. Treatments for affected animals are limited. Care must be taken not to graze animals on heavily infested pastures during dangerous periods, which extends from the seed-setting stage until the infested grass has weathered away, been burnt or ploughed under. If alternate pastures are available, animals may be removed from contaminated areas. Stock 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 corynetoxins 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 (Than et al., 1998) 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, stock rotation among grazed pastures, harvesting hay before bacteria produce toxins in seed heads, 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 must be submitted to USDA-APHIS-PPZ-CPHST in Beltsville, MD for identification. Diagnosticians in those labs will need educational materials for such identification.

Currently, plant and animal experts with the greatest knowledge of *R. toxicus* include:

I. Agarkova, Department of Plant Pathology, University of Nebraska, Lincoln, NE, irina@unl.edu
S. Alderman, USDA-ARS National Forage Seed Production Research Center, Corvallis, OR, stephen.alderman@ars.usda.gov
J. Allen, Animal Health Laboratories, Department of Agriculture and Food Western Australia, jeremy.allen@agric.wa.gov.au
J. Chitambar, California Department of Food and Agriculture, Sacramento, CA, john.chitambar@cdfa.ca.gov
S. Colegate, USDA-ARS, Poisonous Plant Research Laboratory, Logan, UT, steven.colegate@ars.usda.gov
D. Luster, USDA-ARS, Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD, doug.luster@ars.usda.gov
A. McKay, South Australian Research and Development Institute, alan.mckay@sa.gov.au
T. Murray, Department of Plant Pathology, Washington State University, Pullman, WA, tim.murray@wsu.edu
I. Riley, School of Agriculture, Food and Wine, University of Adelaide Plant Research Centre, Waite Campus, Australia, ian@riley.asia
B. Rodoni, Biosciences Research Division, Victorian Department of Primary Industries, brendan.rodoni@dpi.vic.gov.au
B. Schroeder, Department of Plant, Soil, and Entomological Sciences, University of Idaho, Moscow, ID, bschroeder@uidaho.edu
A. Sechler, USDA-ARS, Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD, aaron.sechler@ars.usda.gov
W. Schneider, USDA-ARS, Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD, william.schneider@ars.usda.gov
J. Stack, Department of Plant Pathology, Kansas State University, KS, jstack@ksu.edu
S. Subbotin, California Department of Food and Agriculture, Sacramento, CA, sergei.subbotin@ucr.edu
A. Vidaver, Department of Plant Pathology, University of Nebraska, NE, avidaver1@unl.edu

X. Research, Extension, and Education Needs

Needs for research, extension and education vary depending on whether the bacterium, nematode, plant or animal component is addressed. Three over-arching recommendations and several specific recommendations are listed below.

1. Establish strict quarantine measures at all ports of entry for hay products and forage grass seeds, especially those originating from Australia, New Zealand and South Africa. Develop reliable detection and identification tests for *R. toxicus*, its hosts and vectors.
2. Establish an interdisciplinary working group of scientists familiar with these diseases, to coordinate research on the detection and identification of the pathogen and vectors, ecology and management of the diseases, and assess the applicability of Australian diagnostic practices and procedures to U.S. production systems. Because of the complexity of these diseases, this recommendation is critical to achieve an appropriate and early response to their appearance.

3. Develop educational 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 and/or workshops. Materials should first be provided to APHIS and the National Plant Diagnostic Network.

Specific Needs

For bacteria:

1. Determine the occurrence and geographical distribution of *Rathayibacter* species in the U.S. by conducting surveys that initially target plant species and areas with previous reports of *Rathayibacter* bacteriosis and/or staggers-like occurrences (e.g. California, Idaho, Oregon, Montana, Utah, and the northern Appalachians). Subsequent surveys may be more random emphasizing plant hosts in important stock grazing areas. Analysis should include microbial populations in soil (community analyses) from collection sites.
2. Improve our understanding of genetic diversity among *Rathayibacter* species by such methods as Multilocus Sequence Typing (MLST) and Amplified Fragment Length Polymorphism (AFLP).
3. Develop and/or improve rapid diagnostic and detection tools for *R. toxicus* and related species.
4. Determine the presence and role of bacteriophage and plasmids in corynetoxin production. A bacteriophage has been implicated in toxin production by *R. toxicus*, but further study is needed to determine its role in ARGT outbreaks. In some bacteria, plasmids carry genetic determinants for toxic products. *R. toxicus* is known to lose the ability to produce corynetoxins in culture; loss of plasmids may be one of the reasons.
5. Determine the potential for biological control using non-toxigenic *Rathayibacter* species.
6. Determine the role of corynetoxins in the ecology of the bacterium.
7. Sequence the entire genome of at least six *R. toxicus* strains (one from each geographical group) and two strains of related species to assess similarities and differences, including the assessment of gene(s) related to toxin production.

For nematodes:

1. Determine the occurrence and geographical distribution of *Anguina* species in the U.S. by conducting surveys that initially target plant species and areas with previous reports of *Rathayibacter* bacteriosis and/or staggers-like occurrences (e.g. California, Idaho, Oregon, Montana, Utah, and the northern Appalachians). Subsequent surveys may be more random emphasizing plant hosts in important stock grazing areas. Analysis should include microbial populations in soil (community analyses) from collection sites.
2. Establish pure cultures of the nematode vectors on greenhouse-grown plants and develop methods for reproducing the disease under controlled environment conditions to improve our understanding of the interactions between *R. toxicus*, its nematode vector, and the plant. Being able to reproduce the disease under containment (greenhouse) conditions is essential to accomplishing this goal.

3. Develop tools for molecular detection and identification of *Anguina* species that may serve as vectors for *R. toxicus*.

4. Determine vector capability under optimal and adverse conditions, or carrying capacity for *R. toxicus.*

5. Assess the potential for biological control of prospective vectors. Agent(s), ease of use, cost, distribution network and shelf life are factors to be examined. Chemical control in the U.S. is not likely to be approved for the nematodes, although herbicides might be used for control of host plants.

For plants:

1. Determine feasibility of developing plant resistance to nematode and/or bacterial colonization for the U.S.

2. Determine viability and yield potential of plants bred to be resistant to the nematode, bacterium or toxin production under U.S. conditions.

3. Determine applicability and feasibility of Australian pasture management practices to the U.S.

For animals:

1. Determine the toxicological mechanisms by which corynetoxins produced by *R. toxicus* damage animal tissues. Methods using global genomic and proteomic analyses to determine specific biomarkers have provided some information (Retallick et al., 2006; Penno et al., 2009, 2012). A greater understanding of the mechanism of action needed to develop more effective diagnostics or treatment protocols.

2. Improve the protective efficacy of experimental vaccines (Than et al., 1998) or develop a new generation of vaccines using novel conjugation and highly potent adjuvant technologies. Additional data on efficacy, cross-neutralization cost effectiveness and practicality are needed for all corynetoxins to be successful.

References

Alderman, S.C., D.M. Bilsland, J.A. Griesback, G.M. Milbrath, N.W. Schaad and E. Postnikova. 2003. Use of a novel seed scarifier for detection and enumeration of galls of *Anguina* and *Rathayibacter* species in orchard grass seed. Plant Dis. 87:320-323.

Allen, J. 2004. Annual ryegrass. In: Clinical Veterinary Toxicology. K.H. Plumlee (ed.). Mosby, St. Louis, USA: 422-424.

Allen, J. 2012. Annual ryegrass toxicity–an animal disease caused by toxins produced by a bacterial plant pathogen. Microbiology Australia, March 2012 edition.

Allen, J.G. and A.R. Gregory. 2011. The diagnostic significance of detecting *Rathayibacter toxicus* in the rumen contents and feces of sheep that may be affected by annual ryegrass toxicity. In, Poisoning by Plants, Mycotoxins and Related Toxins, editors F. Riet-Correa, J. Pfister, A.L. Schild and T. Wierenga. CAB International, Wallingford: 325-330.

Allen, J.G., M. Retallick, M. Kowalski, S.M. Colegate, E.J. Speijers, T. Doran and W. Michalski. 2006. A study of the bioequivalence of corynetoxins and tunicamycins: the effect of these toxins on the viability and morphology of rat hepatocytes in tissue culture. Poisonous Plants: Global Research Solutions. K.E. Panter, T.L. Wierenga and J.A. Pfister (eds). Cambridge, MA, CABI: 504-510.

American Horse Council. 2009. National Economic Impact of the U.S. Horse Industry. http://www.horsecouncil.org/nationaleconomics.php.

American Sheep Industry Association. 2009. U.S. Sheep Industry Production Information. http://www.sheepusa.org/Fast_Facts.

Anonymous. 1994. Annual Ryegrass, *Lolium rigidum*, GUARD. Plant Var. J. Aust. **7**:16.

Barbetti, M.J., and Riley, I.T. 2006. Field application of *Dilophospora alopecuri* to manage annual ryegrass toxicity caused by *Rathayibacter toxicus*. Plant Disease 90:229-232.

Bertozzi, T. and K.A. Davies. 2009. *Anguina paludicola* sp. n. (Tylenchida: Anguinidae): The nematode associated with *Rathayibacter toxicus* infection in *Polypogon monspeliensis* and *Lachnagrostis filiformis* in Australia. Zootaxa 2060:33-46.

Bertozzi, T. and A.C. McKay. 1995. Incidence on *Polypogon monspeliensis* of *Clavibacter toxicus* and *Anguina* sp., the organisms associated with 'flood plain staggers' in South Australia. Aust. J. Exp. Agr. 35:567-569.

Bird, A.F., B.A. Stynes and W.W. Thomson. 1980. A comparison of nematode and bacteria-colonized galls induced by *Anguina agrostis* in *Lolium rigidum*. Phytopathology 70:1104-1109.

Bourke, C.A. 1987. A naturally occurring tunicamycin-like intoxication in pigs eating water damaged wheat. Aust. Vet. J. 64:127-128.

Bourke, C. 2007. Annual ryegrass toxicity and blown grass/beard grass poisoning. Primefact 429. New South Wales (NSW) - Department of Primary Industries (DPN). February 2007.

Carslake, T. 2006. ARGT attack fought on several fronts. Farming Ahead. 11: 62-63. (Australia).

Chatel, D.L., J.L. Wise and A.G. Marfleet. 1979. Ryegrass toxicity organism found on other

grasses. J Agr. West. Aust. (4th Series) 20:89.

Davis, E.O., G.E. Curran, W.T. Hetherington, D.A. Norris, G.A. Wise, I.J. Roth, A.A. Seawright and W.L. Bryden. 1995. Clinical, pathological and epidemiological aspects of flood plain staggers, a corynetoxicosis of livestock grazing *Agrostis avenacea*. Aust Vet J. **72**:187-190.

Eckardt, K. 1983. Tunicamycins, streptovirudins, and corynetoxins, a special subclass of nucleoside antibiotics. J. Nat. Prod. 46:544-550.

Edgar, J.A., P.A. Cockrum, P.L. Stewart, N. Anderton and A.L. Payne. 1994. Identification of corynetoxins as the cause of poisoning associated with annual beardgrass (*Polypogon monspeliensis* (L) Desf.) and blown grass (*Agrostis avenacea* C. Gemelin). Plant Associated Toxins: Agricultural, Phytochemical and Ecological Aspects. S.M. Colegate and P.R. Dorling eds., CAB International, Wallingford, UK, pages 393-398.

Erickson, A. 2014. Controlling annual ryegrass toxicity (ARGT) through management of ryegrass pasture. https://www.agric.wa.gov.au/livestock-biosecurity/controlling-annual-ryegrass-toxicity-argt-through-management-ryegrass-pasture.

Finnie, J.W. 1991. Corynetoxin poisoning in sheep in the south-east of South Australia associated with annual beard grass (*Polypogon monspeliensis*). Aust. Vet. J. 68:370-370.

Finnie, J.W. 2006. Review of corynetoxins poisoning of livestock, a neurological disorder produced by a nematode-bacterium complex. Aust. Vet. J. 84:271–277.

Fisher, J.M. 1978. Annual ryegrass toxicity. Bien. Rep. Waite Agric. Res. Inst. Glen Osmond, South Australia: 7-11.

Fisher, J.M., A.J. Dube and C.M. Watson. 1979. Distribution in South Australia of *Anguina funesta*, the nematode associated with annual ryegrass toxicity. Aust. J. Exp. Agric. Anim. Husb. 19:48-52.

Galloway, J.H. 1961. Grass seed nematode poisoning in livestock. J. Am. Vet. Med. Assoc. 139:1212-1214.

Jago, M.V. and C.C. Culvenor. 1987. Tunicamycin and corynetoxin poisoning in sheep. Aust. Vet. J. 64:232-235.

Jasieniuk, M., R. Ahmad, A.M. Sherwood, J.L. Firestone, A. Perez-Jones, W.T. Lanini, C. Mallory-Smith, and Z. Stednick. 2008. Glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) in California: distribution, response to glyphosate, and molecular evidence for an altered target enzyme. Weed Science 56:496-502.

Johnston, M.S., S.S. Sutherland, C.C. Constantine and D.J. Hampson. 1996. Genetic analysis of *Clavibacter toxicus*, the agent of annual ryegrass toxicity. Epidemiol. Infec. 117:393-400.

Kessell, D. 2010. Annual ryegrass toxicity – current situation. Farmnote #417, Department of Agriculture and Food, Western Australia. February 2010.

Kowalski, M.C., D. Cahill, T.J. Doran and S.M. Colegate. 2007. Development and application of polymerase chain reaction-based assays for *Rathayibacter toxicus* and a bacteriophage associated with annual ryegrass (*Lolium rigidum*) toxicity. Aust. J. Exp. Agr. 47:177–183.

Masters, A.M., A.R. Gregory, R.J. Evans, J.E. Speijers and S.S. Sutherland. 2006. An enzyme-linked immunosorbent assay for the detection of *Rathayibacter toxicus*, the bacterium

involved in annual ryegrass toxicity, in hay. Aust. J. Agr. Res. 57:731-742.

Masters, A.M., B. Samarasinghe, M.J. Kalkhoven, G.L. den Hollander, and D.G. Palmer. 2011 Improvements to the immunoassay for detection of *Rathayibacter toxicus* in hay. Crop and Pasture Science 62: 523-530.

Masters, A.M., B. Samarasinghe, M. Kalkhoven, L. den Hollander, and D.G. Palmer. 2014. A semi-quantitative enzyme-linked immunosorbent assay for *Rathayibacter toxicus*, the bacterium involved in annual ryegrass toxicity. Crop and Pasture Science 65 (12): In Press (doi.org/10.1071/CP14052).

May, C. and P.L. Stewart. 1998. Development of a toxin-binding agent as a treatment for tunicaminyluracil toxicity: protection against tunicamycin poisoning of sheep. Aust. Vet. J. 76:752-756.

McKay, A.C. and K.M. Ophel. 1993. Toxigenic *Clavibacter*/*Anguina* associations infecting grass seedheads. Ann. Rev. Phytopath 31:151-167.

McKay, A.C. and I.T. Riley. 1993. Sampling ryegrass to assess the risk of annual ryegrass toxicity. Aust. Vet. J. 70:241-243.

McKay, A.C., K.M. Ophel, T. Reardorn and J. Gooden. 1993. Livestock death associated with *Clavibacter toxicus*/*Anguina* sp. infection in seedheads of *Agrostis avenacea* and *Polypogon monspeliensis*. Plant Dis. 77:635-641.

McWilliam, A.S. and P. Vogel. 1988. Immunological studies with liposome- bound corynetoxins. Vet. Immunol. Immunopathol. 19:21-30.

Murray, T.D. 1986. Isolation of *Corynebacterium agropyri* from 30- to 40-year- old herbarium specimens of *Agropyron* species. Plant Dis. 70:378-380.

Nickle, W.R. 1991. Manual of agricultural nematology. Marcel Dekker Inc., NY.

Ophel, K.M., A.F. Bird and A. Kerr. 1993. Association of bacteriophage particles with toxin production by *Clavibacter toxicus*, the causal agent of annual ryegrass toxicity. Phytopathology 83:676-681.

Penno M.A.S., A. Bacic, S.M. Colegate, P. Hoffmann and W.P. Michalski. 2009. Identifying candidate serum biomarkers of exposure to tunicamycins in rats using two-dimensional electrophoresis. J Proteome Res 8:2812-2826.

Penno M.A.S., S.M. Colegate, W.P. Michalski and P. Hoffmann. 2012. Detection and measurement of carbohydrate deficient transferrin in serum using immune-capture mass spectrometry: Diagnostic applications for annual ryegrass toxicity and corynetoxin exposure. Res Vet Sci 93:611-617.

Postnikova, E., I. Agarkova, S. Altundag, F. Eskandari, A. Sechler, A. Karahan, A.K. Vidaver, W. Schneider, M. Ozakman and N.W. Schaad. 2009. *Rathayibacter iranicus* isolated from asymptomatic wheat seeds in Turkey. Plant Path. 58:796.

Postnikova, E., I. Agarkova, I. Riley, S. Alderman, C. Ocamb, A. Vidaver and N. Schaad. 2004. Identification of *Rathayibacter rathayi* in Oregon and Maryland. APS Annual Meeting, July 31-August 4, 2004. Anaheim, CA, Phytopathology 94: S85.

Retallick, M.A., S.M. Colegate and W.P. Michalski. 2006. A toxicoproteomic approach to

understanding annual ryegrass toxicity. Poisonous Plants: Global Research Solutions. K. E. Panter, T. L. Wierenga and J. A. Pfister (eds). Cambridge, MA, CABI: 230-234

Riley, I.T. 1992a. *Anguina tritici* is a potential vector of *Clavibacter toxicus*. Aust. Plant Path. 21:147-148.

Riley, I.T. 1992b. Paddock sampling for management of annual ryegrass toxicity. J Agr. West. Aust. 33:51–56.

Riley, I.T. 1994. *Dilophospora alopecuri* and decline in annual ryegrass toxicity in Western Australia. Aust. J. Agric. Res. 45:841-850.

Riley, I.T. 1995. *Vulpia myuros* and the annual ryegrass toxicity organisms, *Anguina funesta*, and *Clavibacter toxicus*. Fund. Appl. Nemat. 18:595-598.

Riley, I.T. 1996. *Dilophospora alopecuri* on *Lolium rigidum* and *Holus lanatus* in southeastern Australia. Aust. Plant Path. 25:255-259.

Riley, I.T. and M.J. Barbetti. 2008. Australian anguinids: their agricultural impact and control. Aust. Plant Path. 37:289-297.

Riley, I.T. and G.S. Gill. 1994. Relationship between herbicide resistance in *Lolium rigidum* and populations of *Anguina funesta*, the nematode vector in annual ryegrass toxicity. Aust. J. Exp. Agric. 34:633-635.

Riley, I.T. and A.C. McKay. 1991. Inoculation of *Lolium rigidum* with *Clavibacter* sp., the bacterium responsible for toxicity of annual ryegrass. J. Appl. Bact. 71:302-306.

Riley, I.T., and K.M. Ophel. 1992. *Clavibacter toxicus* sp. nov., the bacterium responsible for annual ryegrass toxicity in Australia. Int. J. Syst. Bact. 42:64-68.

Riley, I.T., A.R. Gregory, J.G. Allen and J.A. Edgar. 2003. Poisoning of livestock in Oregon in the 1940s to 1960s attributed to corynetoxins produced by *Rathayibacter* in nematode galls in chewing fescue (*Festuca nigrescens*). Vet. Hum. Toxicol. 45:160-162.

Riley, I.T., A.R. Gregory, J.G. Allen and J.A. Edgar. 2004. Bacteria and corynetoxin-like toxins in nematode seed-galls in *Festuca nigrescens* from North America and New Zealand. in: Poisonous Plants and Related Toxins. T. Acamovic, C.S. Stewart and T.W. Pennycott (eds). Wallingford, UK, CABI Publishing: 50- 55.

Riley, I.T., A. Schmitz and P. de Silva. 2001. *Anguina australis*, a vector for *Rathayibacter toxicus* in *Ehrharta longiflora*. Aust. Plant Path. 30:171-175.

Schneider, D.J. 1981. First report of annual ryegrass toxicity in the Republic of South Africa. Onderstepoort J. Vet. Res. 48:251-255.

Shaw, J.N. and O.H. Muth. 1949. Some types of forage poisoning in Oregon cattle and sheep. J. Am. Vet. Med. Assoc. 114:315-317.

Smith, E.F. 1914. Rathay's disease of orchardgrass. Pages 155-160 *in*: Bacteria in Relation to Plant Diseases. Vol. 3, Vascular Diseases. Carnegie Institution of Washington, Publication No. 27, Vol. Three. Gibson Brothers, Washington, DC.

Stirling, G.R., J.M. Stanton and J.W. Marshall. 1992. The importance of plant-parasitic nematodes to Australian and New Zealand agriculture. Aust. Plant Path. 21:104-115.

Stuart, S.J., B.J. Leury and J.A. Edgar. 1992. Plasma clearance of tritiated- tunicamycin in sheep. Proc. Nutr. Soc. Aust. 17:137.

Stynes, B.A. and A.F. Bird1982. Development of galls induced in *Lolium rigidum* by *Anguina agrostis*. Phytopathology 82:336-346.

Stynes, B.A. and A.F. Bird. 1983. Development of annual ryegrass toxicity. Aust. J. Agric. Res. 34:653-660.

Subbotin, S.A. and I.T. Riley. 2012. Stem and gall forming nematodes. Pages 521-577, In: Practical Plant Nematology (Eds Manzanilla-Lopez R, Marban-Mendoza M). (Biblioteca Básica de Agricultura, Grupo Mundi-Prensa, Mexico).

Takatsuki, A., K. Arima and G. Tamura. 1971. Tunicamycin, a new antibiotic. I. Isolation and characterization of tunicamycin. J. Antibiot (Tokyo) 24:215-223.

Takatsuki, A. and G. Tamura. 1971a. Tunicamycin, a new antibiotic. II. Some biological properties of the antiviral activity of tunicamycin. J. Antibiot. (Tokyo). 24:224-231.

Takatsuki, A. and G. Tamura. 1971b. Tunicamycin, a new antibiotic. III. Reversal of the antiviral activity of tunicamycin by aminosugars and their derivatives. J. Antibiot. (Tokyo) 24:232-238.

Than, K.A., Y. Cao, A. Michalewicz and J.A. Edgar. 1998. Development of a vaccine against annual ryegrass toxicity. In: Toxic Plants and Other Natural Toxicants. T. Garland and A.C. Barr (eds). Wallingford, UK, CAB International, pages 165-168.

Than, K., S. Colegate and J. Edgar. 2002. Development of a quantitative ELISA for screening fodder for corynetoxins. A report for the Rural Industries Research and Development Corporation. RIRDC Publication No 02/118. RIRDC Project No. CSA-3A: 31 p.

USDA-Economic Research Service. 2009. U.S. Beef and Cattle Industry: Background Statistics and Information. http://www.ers.usda.gov/news/BSECoverage.htm.

Vogel, P., D.S. Petterson, P.H. Berry, J.L. Frahn, N. Anderton, P.A. Cockrum, J.A. Edgar, M.V. Jago, G.W. Lanigan, A.L. Payne and C.C. Culvenor. 1981. Isolation of a group of glycolipid toxins from seedheads of annual ryegrass (*Lolium rigidum* Gaud.) infected by *Corynebacterium rathayi*. Aust. J. Exp. Biol. Med. Sci. 59:455-467.

Yan, G. and I.T. Riley. 2003. Fermentation period, moisture content, and substrate effects on inoculum effectiveness of *Dilophospora alopecuri*: A biocontrol agent of annual ryegrass toxicity. Biol Control. 26:174-179.

Web Resources

Information on Rathayibacter poisoning can be obtained from internet searches using ARGT, Rathayibacter toxicus, or Ryegrass staggers as a key word and the websites listed below:

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 Environment and Primary Industries Victoria
http://www.depi.vic.gov.au/home

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/

The Merck Veterinary Manual
http://www.merckmanuals.com/vet/toxicology/ryegrass_toxicity/annual_ryegrass_staggers.html

Appendices

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

Referring to	Common names for galls, disease, or pathogen
Anguina tritici and/or *Rathayibacter tritici* in wheat	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
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
Anguina agrostis in bentgrass (*Agrostis* spp.)	bentgrass nematode

Structure of Corynetoxins (CTs) Produced by *Rathayibacter toxicus*.

Corynetoxins (CTs) produced by *R. toxicus* are structurally and functionally similar to tunicamycins (TMs) produced by the bacterium *Streptomyces iysosuperficus* (Takatsuki et al., 1971; Takatsuki and Tamura, 1971a, 1971b). Structurally, CTs produced by *R. toxicus* contain a N-acetylglucosamine tunicaminyluracil core structure, but vary in the length, terminal branching formation and hydroxylation states of their fatty acid chains (Eckardt, 1983) (Figs. A1, A2, and Table A2). The structural and functional similarity between the CTs produced by *R. toxicus* and TMs by *S. iysosuperficus* allows the use of *S. iysosuperficus* TMs as a substitute in evaluating functions of *R. toxicus* CTs *in vitro* and *in vivo*. Indeed, many effects of *R. toxicus* CTs on cells and animals were obtained using *S. iysosuperficus* TMs in cell and animal models, and numerous reports confirmed that the effects of the two on cells and animals are indistinguishable (Allen, 2004; Allen et al., 2006; Finnie, 2006). The half-life for *R. toxicus* CTs should be short, based on studies of *S. iysosuperficus* 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 *R. toxicus* CTs as glycolipids has never been reported in affected animal species. *R. toxicus* CTs may be extracted from the bacterial gall-rich concentrate purified with HPLC (Than et al., 2002; Vogel et al., 1981).

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

S. iysosuperficus		***R. toxicus***	
TM	**Fatty acid residues (R)**	**CT**	**Fatty acid residues (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-$(CH_2)_{10}$-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-
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-
		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).

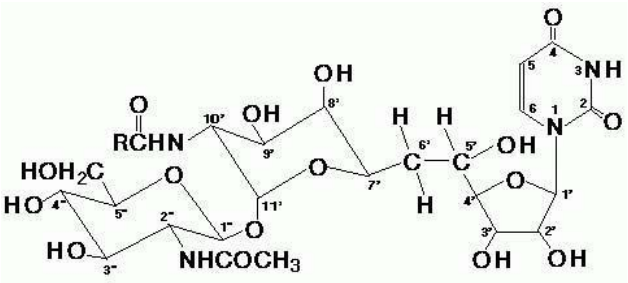
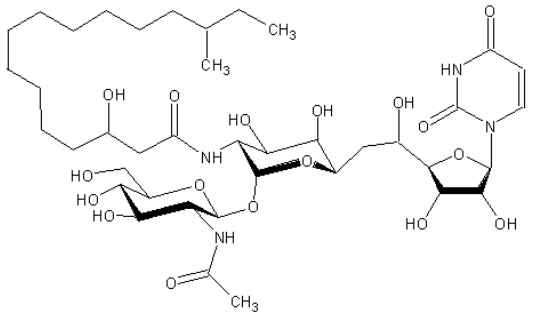


Figure A1. The core structure tunicaminyluracil is identical in *Rathayibacter toxicus* corynetoxins and *Streptomyces iysosuperficus* tunicamycins (Eckardt, 1983). R represents the fatty acid residues of various lengths, terminal branching and hydroxylation states (see Appendix Table A2).



Corynetoxin H 17a

Figure A2. Corynetoxin H17a, one of the major components of the *Rathayibacter toxicus* corynetoxins (Eckardt, 1983).