Livestock Deaths Associated with *Clavibacter toxicus*/*Anguina* sp. Infection in Seedheads of *Agrostis avenacea* and *Polypogon monspeliensis*

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ABSTRACT

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Flood plain staggers, a recently discovered poisoning of livestock, has been linked to *Clavibacter toxicus* infection in the seedheads of blown grass, *Agrostis avenacea*, in northern New South Wales and annual beardgrass, *Polypogon monspeliensis*, in the southeast of South Australia. The same bacterium on annual ryegrass, *Lolium rigidum*, causes the poisoning of livestock known as annual ryegrass toxicity. Strains of *C. toxicus* from *A. avenacea* and *P. monspeliensis* were indistinguishable from strains from *L. rigidum* based on colony morphology, serological reactions, and bacteriophage specificity. Bacteriophages isolated from *C. toxicus* on the three hosts were indistinguishable from each other based on DNA restriction patterns. In allozyme studies, considerable variation was observed between the *C. toxicus* strains from the three hosts, but the variation was within the range exhibited by a single species. *C. toxicus* is carried into *L. rigidum* by a seed gall-forming nematode, *Anguina funesta*. *Anguina* nematodes are also associated with *C. toxicus* infection of *A. avenacea* and *P. monspeliensis*. Allozyme studies indicate that the same *Anguina* species probably infects both grasses, and that it is not *Anguina funesta*, *Anguina agrostis*, *Anguina tritici*, or the species found on velvetgrass (*Holcus lanatus*). This is the first recording of a nematode other than *Anguina funesta* as a vector for *C. toxicus*. The new vector broadens the range of grasses that the bacterium can infect.

Additional keywords: livestock poisoning

In September 1990, a new livestock problem caused the deaths of cattle grazing senesced pasture along flood plains of the Bogan River in the northwest of New South Wales, Australia. In the following 4 mo, this condition was observed on 31 properties along the flood plains of a number of rivers in the Darling catchment area in the Bourke and adjoining Moree districts. Mortalities totaled 1,722 cattle, 2,466 sheep, and 11 horses; and the problem became known locally as flood plain staggers (5).

The localities where stock were poisoned were flooded or received heavy rain during winter and were dominated by blown grass, *Agrostis avenacea* C.C. Gmelin. Feeding trials were conducted to identify the source of the toxins. Cattle fed samples of blown grass exhibited clinical symptoms similar to those of animals poisoned in the field (2). The symptoms were identical to those of corynetoxin poisoning, the cause of annual ryegrass toxicity (6).

An orange, crystalline material resembling dried bacterial slime was observed on distorted seedheads of *A. avenacea* at several locations where stock had been poisoned (5). This material resembled *Clavibacter toxicus* Riley & Ophel (21) infection on the seedheads of the annual ryegrass *Lolium rigidum* Gaudin, which is associated with annual ryegrass toxicity (6). Close examination of the seedheads of the *A. avenacea* used in the feeding trial revealed the presence of seed galls containing second-stage juveniles of an *Anguina* species of nematode. These galls were much larger than uninfected seed and resembled those produced by *Anguina agrostis* (Steinbuch) Filipjev on *Agrostis capillaris* L. (31).

Annual ryegrass toxicity occurs in the cropping regions of South Australia and Western Australia (8,12), where the nematode *Anguina funesta* Price, Fisher and Kerr (14) carries the bacterium into *L. rigidum*. It was first recorded in 1956 in the midnorth of South Australia, and in 1980 it was recorded in South Africa (26).

The discovery of a bacterium and nematode complex on *A. avenacea* associated with stock losses prompted renewed interest in a livestock problem known as Stewarts Range syndrome 1,000 km away in the southeast of South Australia. Stewarts Range syndrome has caused livestock losses for more than 20 yr (7). In 1990, 40 producers reported outbreaks, and stock losses included 2,000 sheep and 40 cattle (Colin Trengove, *personal communication*). Losses occur during the summer months in pastures prone to flooding during winter and dominated by annual beardgrass, *Polypogon monspeliensis* (L.) Desf. A range of distorted *P. monspeliensis* seedheads was collected from a paddock in which cattle had recently died from Stewarts Range syndrome. Examination of the seedheads revealed the presence of dried bacterial slime and galls containing second-stage juveniles of an *Anguina* species.

This paper reports the results of studies to identify the bacteria and nematodes on the *A. avenacea* and *P. monspeliensis* that were associated with the livestock deaths.

MATERIALS AND METHODS

Bacterial samples. All bacterial strains used in this study and their sources are listed in Table 1. Unless otherwise indicated, bacterial strains were grown at 26 C on modified 523 agar (523M) as described by Riley and Ophel (21). *C. toxicus*-infected nematode galls used in toxin assays were collected from *L. rigidum* near Geranium, South Australia.

Nematode samples. At the time of this investigation, only dauer second-stage juveniles of the nematodes were available on *A. avenacea* and *P. monspeliensis*. Two populations of the *Anguina* species on *A. avenacea* were sampled from neighboring properties on the Bogan River 10 km upstream from Gongolgon, New South Wales; and two populations of the nematode on *P. monspeliensis* were sampled from one farm near Lucindale and another near Conmurra in South Australia. These samples were compared by allozyme electrophoresis with the following nematode populations used in earlier studies to determine the status of *Anguina funesta* (23): *Anguina funesta* from *L. rigidum* collected near Geranium, South Australia; *Anguina agrostis*, population 16 (23), from Annat, New Zealand; *Anguina* species from velvetgrass, *Holcus lanatus* L., in the Belair National Park, South Australia; and a recent collection of *Anguina tritici* (Steinbuch) Chitwood from near Three Springs, Western Australia.

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Toxic activity of samples. Yellow, crystalline material from infected seedheads was embedded in 523M agar (22). Bacterial galls from *L. rigidum* known to contain annual ryegrass toxicity toxins were used as a positive control in all experiments. Plates with embedded galls were exposed to chloroform for 30 min and left at 4 C overnight before being overlaid with 3 ml of soft buffered agar (0.2 M phosphate buffer, pH 7.3, 0.7% purified agar) containing 500 µl of a turbid suspension of *Clavibacter tritici* (Hutchinson) Davis et al strain CS4. Toxicity was assessed by the diameter of the clear zone.

Isolation of bacteria. Crystalline material from distorted seedheads was surface-sterilized for 5 min each in a 1:5 dilution of Milton antibacterial solution (1% available chlorine as sodium hypochlorite, 16.5% sodium chloride), followed by 70% ethanol. Material was then rinsed three times in sterile distilled water before maceration in 500 µl 523M broth. Samples that had undergone some decomposition were surface-sterilized by exposure to chloroform for 30 min before rinsing and maceration. All samples were spread on 523M agar and incubated for 7–14 days at 26 C. Small, raised, bright yellow, circular colonies were picked and purified further on 523M agar.

Bacterial identification. Serological relationships between the strains of *Clavibacter* species from *A. avenacea* and *P. monspeliensis* were examined by immunodiffusion and enzyme-linked immunosorbent assay (ELISA). Polyclonal antisera used in immunodiffusion were raised to *C. toxicus* (CS14), *C. iranicus* (Carlson & Vidaver) Davis et al (CS13), *C. tritici* (CS21), and *C. rathayi* (Smith) Davis et al (CS26) (16). ELISA was performed with antiserum raised to *C. toxicus* CS14 with a modification of the method described by Riley and McKay (20). Bacterial strains tested were suspended in sterile buffered saline and adjusted to the same optical density at 600 nm.

To isolate the bacteriophage, portions of bacteria-infected seedheads were surface-sterilized as described, macerated, and inoculated into 10 ml of sterile 523M broth amended with 0.1 µg/ml of Mitomycin C (Sigma Chemical Co.). Cultures were incubated by shaking at 25 C for 6 days. Aliquots (10 µl) were tested on bacterial lawns of *C. toxicus* strains CS14 and CS28. Plates were examined for lysis after 24 hr. Plaques were suspended in 1 ml of SM buffer

Table 1. Bacterial strains

Strain	Species	Host	Location	Source[y]
CS4	*Clavibacter tritici*	*Triticum aestivum*		NCPPB 255
CS12	*C. tritici*	*T. aestivum*	Iran 1966	ICMP 2628
CS21	*C. tritici*	*T. aestivum*		NCPPB 1857[z]
CS13	*C. iranicus*	*T. aestivum*		NCPPB 2253[z]
CS26	*C. rathayi*	*Dactylis glomerata*		NCPPB 2980[z]
CS7	*C. rathayi*	*D. glomerata*	New Zealand 1968	ICMP 2573
CS14	*C. toxicus*	*Lolium rigidum*	Murray Bridge, South Australia 1983	NCPPB 3552[z]
CS28	*C. toxicus*	*L. rigidum*		WSM188
CS35	"*Corynebacterium agropyri*"	*Agropyron smithii*	Montpelier, ID 1945	CA-1
CS36	...	*Agrostis avenacea*	Gongolgon, New South Wales 1990	
CS37	...	*A. avenacea*	Gongolgon, New South Wales 1990	
CS38	...	*Polypogon monspeliensis*	Lucindale, South Australia 1990	
CS39	...	*P. monspeliensis*	Lucindale, South Australia 1990	

[y] ATCC = American Type Culture Collection, Rockville, Maryland; NCPPB = National Collection of Plant Pathogenic Bacteria, Harpenden, England; ICMP = International Collection of Microorganisms from Plants, (formerly Plant Disease Division Culture Collection), DSIR, Mt. Albert Rd., Private Bag, Auckland, New Zealand; WSM = Western Australian Department of Agriculture, Baron-Hay Ct., South Perth, WA 6151, Australia. CA = strain-identification code used by T. D. Murray, Washington State University, Pullman 99164-6430.
[z] Type strain for the species.

Fig. 1. *Clavibacter toxicus* infection preventing seedhead emergence in *Agrostis avenacea.*

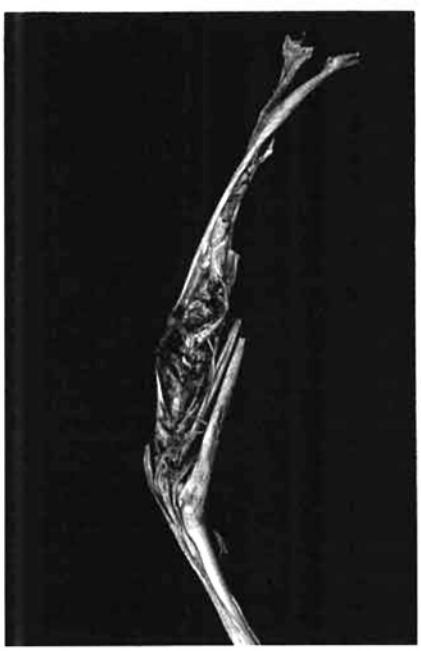

Fig. 2. *Clavibacter toxicus* infection preventing seedhead emergence in *Polypogon monspeliensis.*

Fig. 3. Enlarged spikelet on *Agrostis avenacea* (center) infected by *Anguina* sp. nematode.

Fig. 4. Left to right, healthy *Agrostis avenacea* seed separated from glumes, *Anguina* sp. infected spikelet, seed gall produced by the nematode, and seed gall infected with *Clavibacter toxicus*.

Fig. 5. Left to right, healthy *Polypogon monspeliensis* seed, *Anguina* sp. infected spikelet, seed gall produced by the nematode, *Clavibacter toxicus*-infected nematode gall, and nematode gall initiated in branch primordia on the rachilla.

(25) amended with 5 μl of chloroform and incubated at room temperature for 3–4 hr. Bacteriophages were stored in SM buffer plus chloroform at 4 C. The titre of phage stock cultures was determined by sequentially diluting the phage in SM buffer. A 100-μl aliquot of each dilution was mixed with 100 μl of a fresh CS14 broth culture, and the mixture was overlaid in 3 ml of soft buffered agar onto a 523M plate. The number of PFU at each dilution was determined after 2 days of incubation at 26 C. The phages were designated A1 from *A. avenacea*, P1 from *P. monspeliensis*, and L1 (NCPPB 3778) from *L. rigidum*.

The host ranges of the bacteriophages were examined with fresh cultures of type strains of *C. iranicus* CS13, *C. rathayi* CS26, *C. tritici* CS21, *C. toxicus* CS14, and strains isolated from *A. avenacea* CS36 and CS37, and *P. monspeliensis* CS38 and CS39. Bacteria were inoculated into soft buffered agar overlays and incubated to form a uniform lawn. Bacteriophages isolated from *C. toxicus* on *L. rigidum*, *A. avenacea*, and *P. monspeliensis* were added in 10-μl drops, and plates were examined for lysis after 24 hr of incubation at 26 C (17).

To prepare the bacteriophages for DNA analysis, 500 μl of a 10^8 PFU/ml solution of each bacteriophage was added to 500-ml log phase cultures of CS14. Cultures were grown for 7 days, after which lysis was observed. Bacteriophages were purified by a modification of the method described for lambda phage by Sambrook et al (25). Phage preparations were purified on a single cesium chloride gradient for 18 hr at 40,000 rpm. DNA extraction from the purified bacteriophage was done as described by Sambrook et al (25). Restriction enzyme analysis was performed with *Sal*I, *Eco*RI, and *Hin*dIII (Boehringer Mannheim Australia) under the conditions recommended by the manufacturer.

Allozyme electrophoresis was used to compare *Clavibacter* strains from both *A. avenacea* and *P. monspeliensis* with *C. toxicus* strains CS14 and CS28, *C. tritici* strain CS12, *C. rathayi* strain CS7, and "*Corynebacterium agropyri*" strain CS35. Sources for the strains, and the procedure for their preparation for electrophoresis, are given in Riley (16) and Riley et al (24). Allozyme electrophoresis was conducted on Cellogel (Chemetron, Milan) according to the procedures given in Richardson et al (15).

The following enzymes for 22 presumptive loci were scored: adenylate kinase (AK, EC 2.7.4.3), alkaline phosphatase (AP, EC 3.1.3.1), esterase (EST, EC 3.1.1.?), guanine deaminase (GDA, EC 3.5.4.3), aspartate aminotransferase (GOT, EC 2.6.1.1), glutathione reductase (GSR, EC 1.6.4.2), hexokinase (HK, EC 2.7.1.1), isocitrate dehydrogenase (IDH, EC 1.1.1.42), cytosol aminopeptidase (LAP, EC 3.4.11.1), mannose-6-phosphate isomerase (MPI, EC 5.3.1.8), purine-nucleoside phosphorylase (NP, EC 2.4.2.1), dipeptidase (PEP-A, EC 3.4.13.?), tripeptide aminopeptidase (PEP-B, EC 3.4.11.?), proline dipeptidase (PEP-D, EC 3.4.13.?), phosphoglycerate mutase (PGAM, EC 5.4.2.1), phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), phosphoglycerate kinase (PGK, EC 2.7.2.3), phosphoglucomutase (PGM, EC 5.4.2.2), pyruvate kinase (PK, EC 2.7.1.40), triose-phosphate isomerase (TPI, EC 5.3.1.1), UTP-glucose-1-phosphate uridylyltransferase (UGPP, EC 2.7.7.9), and nucleoside-phosphate kinase (UMPK, EC 2.7.4.4).

Nematode identification. Infected samples of *A. avenacea* and *P. monspeliensis* and gall contents were examined with a dissecting microscope. Allozyme electrophoresis was used to compare juveniles of the *Anguina* species from these samples with *Anguina* species from *L. rigidum*, *A. capillaris*, *H. lanatus*, and *Triticum aestivum* L. Nematodes were prepared for electrophoresis as described in Riley et al (23). Allozyme electrophoresis was conducted on Cellogel according to standard procedures (15).

The following enzymes for 22 presumptive loci were scored: aminoacylase

Fig. 6. Nematode seed galls on *Polypogon monspeliensis* inflorescences. Seed plus glumes have fallen from the top half of the inflorescence on the left, and all uninfected seed has fallen from the inflorescence on the right.

Fig. 7. Nematode gall colonized by the bacterium and initiated on the rachilla of *Polypogon monspeliensis*.

(ACYC, EC 3.5.1.14), fructose-bisphosphate aldolase (ALD, EC 4.1.2.13), enolase (ENOL, EC 4.2.1.11), esterase (EST, EC 3.1.1.?), fumarate hydratase (FUM, EC 4.2.1.2), glyceraldehyde-3-phosphate dehydrogenase (GAPD, EC 1.2.1.12), aspartate aminotransferase (GOT, EC 2.6.1.1), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), glycerol-3-phosphate dehyrogenase (GPD, EC 1.1.1.8), glucose-6-phosphate isomerase (GPI, EC 5.3.1.9), isocitrate dehydrogenase (IDH, EC 1.1.1.42), malate dehydrogenase (MDH, EC 1.1.1.37), mannose-6-phosphate isomerase (MPI, EC 5.3.1.8), dipeptidase (PEP-A, EC 3.4.13.?), tripeptide aminopeptidase (PEP-B, EC 3.4.11.?), proline dipeptidase (PEP-D, EC 3.4.13.?), phosphoglycerate mutase (PGAM, EC 5.4.2.1), phosphoglycerate kinase (PGK, EC 2.7.2.3), pyruvate kinase (PK, EC 2.7.1.40), and triose-phosphate isomerase (TPI, EC 5.3.1.1).

RESULTS

Plant symptoms. Bacterially infected inflorescences of *A. avenacea* and *P. monspeliensis* were distorted and sometimes did not emerge from the boot (Figs. 1 and 2). On *A. avenacea,* the dried bacterium was visible on the inflorescence. On *P. monspeliensis,* many of the bacterially infected inflorescences were blackened by microbial growth. Close examination revealed the orange, brittle bacterial exudate, especially under the leaf sheath covering the partly emerged inflorescences.

On *A. avenacea,* galled spikelets were often much larger than normal (Fig. 3). Galled spikelets varied up to 17 mm in length and resembled those produced on *A. capillaris* by *Anguina agrostis* (31). Growth of the glumes was often stimulated; the lemma varied from absent to as long as the glumes; and palea were absent. The fine hairs and awn on healthy lemma were absent on infected spikelets. Galls varied in size up to about 6 mm long. The brown galls contained second-stage juvenile nematodes, and the orange galls were filled with bacteria (Fig. 4). The number of nematodes in the brown galls averaged 1,342 each in six galls and ranged from 433 to 2,427 per gall. Galled spikelets often remained on the inflorescence after the seed had fallen. If the galls separated from the inflorescence, they detached below the glumes; seed detached above the glumes, leaving them attached to the inflorescence.

On *P. monspeliensis,* seed and stem galls were observed (Fig. 5). Galled spikelets were larger than normal and varied up to 19 mm in length. Growth of the glumes and lemma was stimulated, with the glumes sometimes shorter than the lemma. The hairs and awns on healthy glumes and lemmas were absent on infected spikelets. Stem galls appeared to have developed in branch primordia rather than floral primordia (Fig. 5). Galls containing bacteria were orange, and those containing second-stage juvenile nematodes were brown (Fig. 5). Numbers of second-stage juveniles averaged 893 each in six seed galls and ranged from 420 to 1,376; they averaged 1,587 each in six stem galls and ranged from 648 to 2,871. Galled spikelets often remained attached to the inflorescence after the seed had fallen (Fig. 6). Galled and uninfected spikelets detached below the glumes. Stem galls did not appear to detach from the rachilla (Fig. 7).

Toxic activity and bacterial isolation from infected material. Toxins from the orange crystalline material on infected seedheads of *A. avenacea* and *P. monspeliensis* inhibited the growth of *C. tritici* in the plate assay (22). The zones were similar in clarity and size to those produced by the corynetoxins from bacteria-infected galls from *L. rigidum.*

Bacterial identification. Small, yellow colonies consistent in appearance with *C. toxicus* were present after 7–10 days of incubation, in samples from all areas.

Immunodiffusion showed a strong reaction between the strains from *A. avenacea* and *P. monspeliensis* and antiserum raised to CS14, but not to any of the other antisera tested. Gall macerates and bacterial strains gave ELISA reactions that were quantitatively similar to CS14.

The bacteriophage associated with *C. toxicus* on *L. rigidum* lysed all *Clavibacter* strains from *A. avenacea* and *P. monspeliensis.* Bacteriophages were isolated from bacterial exudates from *A. avenacea* and *P. monspeliensis.* These lysed cultured bacteria from *A. avenacea* and *P. monspeliensis,* and three *C. toxicus* strains from *L. rigidum.* DNA restriction patterns for all three bacteriophages were identical (Fig. 8).

Allozyme electrophoresis. The nine strains under examination sorted into eight electrotypes. The allelic profiles of these are given in Table 2. The two strains from *A. avenacea* had identical allelic profiles. A matrix of percent difference between electrotypes over all loci was prepared, and a phenogram derived from unweighted pair group method analysis (UPGMA) was constructed (30).

The phenogram (Fig. 9) shows four major "lineages" that differ, on average, more than 70%. These lineages correspond to the four named species, with the strains from *A. avenacea* and *P. monspeliensis* clustering with *C. toxicus* at an average difference of 17% or less. Within this cluster, the two strains from

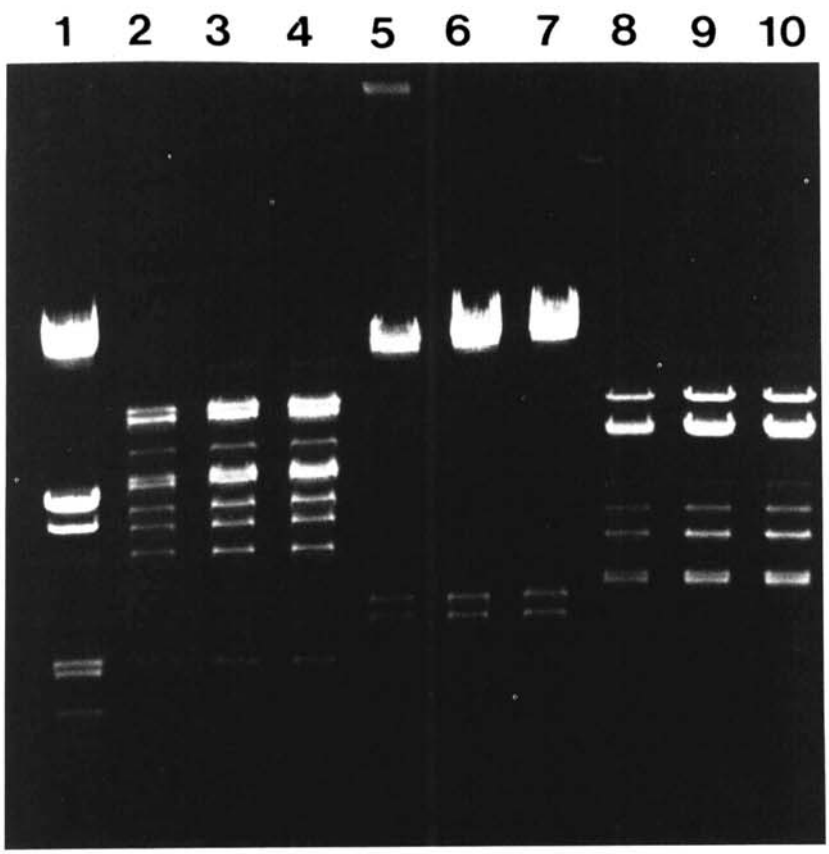

Fig. 8. DNA restriction patterns of a bacteriophage isolated from *Lolium rigidum* galls (L1), *Agrostis avenacea* galls (A1), and *Polypogon monspeliensis* galls (P1). Lane 1 = *Hin*dIII/*Eco*R1 digest of lambda bacteriophage DNA for molecular-size standard (21.226, 5.148, 4.277, 3.53, 2.027, 1.904, 1.584, 1.33 base pairs); lanes 2–4 = *Sal*I digests; lanes 5–7 = *Eco*R1 digests; and lanes 8–10 = *Hin*dIII digests of P1, A1, and L1, respectively.

Table 2. Allelic profiles for *Clavibacter* strains

	Clavibacter spp./strain							
Locus	*C. toxicus* CS14	*C. toxicus* CS28	*"Corynebacterium agropyri"* CS35	*C. tritici* CS12	*C. rathayi* CS7	From *Agrostis avenacea* CS36, CS37	From *Polypogon monspeliensis* CS38	From *Polypogon monspeliensis* CS39
Ak	a	a	c	b	c	a	a	a
Ap	a	b	c	d	c	b	b	b
Est	a	a	c	c	b	a	a	a
Gda	e	d	c	b	a	f	e	e
Got	a	a	b	d	c	a	a	a
Gsr	a	a	a	b	a	a	a	a
Hk	a	a	c	b	b	a	a	a
Idh	a	a	b	d	c	a	a	a
Lap	b	b	c	d	a	b	a	b
Mpi	b	b	e	d	c	e	a	a
Np	a	a	a	b	a	a	a	a
PepA	a	a	b	b	...	a	a	a
PepB	a	a	c	c	b	a	a	a
PepD	b	b	c	d	d	b	b	a
Pgam	a	a	b	c	d	a	a	a
6Pgd	a	a	b	b	c	a	a	a
Pgk	b	b	a	c	a	b	b	b
Pgm	a	a	b	d	c	a	a	a
Pk	c	c	c	c	a	c	b	c
Tpi	a	a	d	c	b	a	a	a
Ugpp	d	d	c	b	a	d	d	d
Umpk	a	a	d	c	b	a	a	a

the genus *Polypogon* differ from each other at 18% of loci and from *A. avenacea* and *Lolium* strains at an average of 15.5%.

Nematode identification. Allozyme gels gave clear single bands for each locus for each sample except for Mdh2 in *A. avenacea,* Pgam in *H. lanatus,* and Tpi in *P. monspeliensis,* where the bands exhibited were consistent with those expected for pooled diploid individuals with two alleles for each locus. The six nematode samples gave six unique electrotypes, the allelic profiles for which are shown in Table 3. Because each sample came from pooled individuals, it was not possible to estimate gene frequencies for the three samples where two alleles were present at a locus. We therefore used percent fixed difference as a measure of genetic divergence between electrotypes (15). A matrix of the percentage of loci not sharing any alleles between pairs of samples was prepared, from which a phenogram (Fig. 10) derived by UPGMA was constructed (30). The phenogram (Fig. 10) shows that there are five major "lineages" that differ, on average, more than 55%. The test samples from *A. avenacea* and *P. monspeliensis* cluster at 14% fixed difference and together form one of the lineages that differs more than 65% from the remaining species under test.

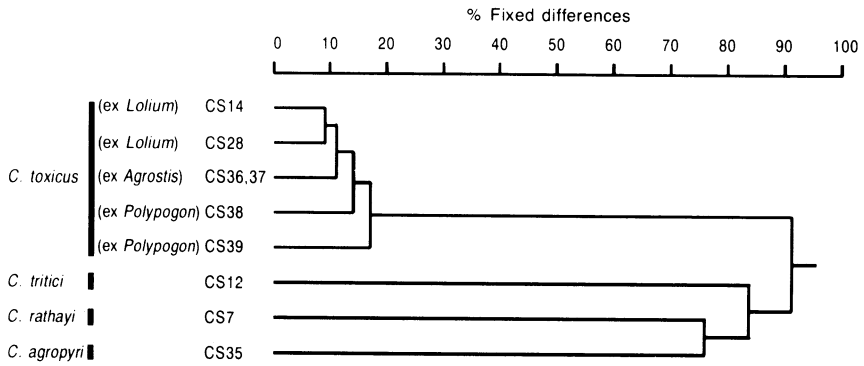

Fig. 9. Phenogram showing relationship among *Clavibacter* electrotypes from allozyme data based on unweighted pair group method analysis.

DISCUSSION

In colony morphology, serological reactions, and bacteriophage specificity, the bacteria isolated from the inflorescences of *A. avenacea* and *P. monspeliensis* were indistinguishable from strains of *C. toxicus* isolated from *L. rigidum* associated with annual ryegrass toxicity. Also, the bacteriophage isolated from galls on *A. avenacea* and *P. monspeliensis* was identical to that previously isolated from *L. rigidum* galls (17), as were the toxins (J. Edgar, *unpublished*); and the pathology of animals affected by flood plain staggers and Stewarts Range syndrome was the same as that for annual ryegrass toxicity (2,7).

Based on allozyme data, the bacterial strains from *A. avenacea* and *P. monspeliensis* clustered with known strains of *C. toxicus* at an average difference of 17%. This is within the range exhibited by strains of a single species of other bacteria (3,27). The data obtained from the few strains tested show that significant variation exists within *C. toxicus.*

In the allozyme studies of dauer second-stage juveniles, the *Anguina* nematodes from *A. avenacea* and *P. monspeliensis* differed from *Anguina funesta* at 68% and 59% of loci, respectively, and from the other species at more than 65%. Genetic differences at this level are strong evidence that the *Anguina* from *A. avenacea* and *P. monspeliensis* are specifically distinct from the other species tested. However, the genetic difference of 14% between the nematodes from *A. avenacea* and *P. monspeliensis* is within the variation expected for allopatric populations of a single species (15). Nonetheless, these differences are significant, and further studies will be required to resolve the taxonomic status of this group.

To avoid a proliferation of names for describing stock deaths caused by *C. toxicus,* it is proposed that flood plain staggers replace Stewarts Range syndrome and be used as the name to describe livestock poisonings associated with *A. avenacea* and *P. monspeliensis.* In cropping areas of South Australia and Western Australia, annual ryegrass toxicity is established as the name for livestock poisonings associated with *L. rigidum.* Collectively, annual ryegrass toxicity and flood plain staggers are corynetoxin poisonings (6).

Table 3. Allelic profiles of *Anguina* species including those associated with annual ryegrass toxicity, flood plain staggers, and Stewarts Range syndrome[z]

Locus	*Anguina* juveniles collected from hosts					
	Polypogon monspeliensis	*Agrostis avenacea*	*Lolium rigidum*	*Holcus lanatus*	*Triticum aestivum*	*Agrostis tenuis*
Acyc	b	b	b	b	c	a
Ald1	d	b	c	b	d	a
Ald2	a	a	a	a	a	a
Enol	b	b	b	a	b	b
Est	b	b	e	d	a	c
Fum	b	b	c	c	a	c
Gapd	a	a	a	a	b	a
Got	c	c	b	b	c	a
Gpd	e	e	d	a	c	b
G6pd	c	c	c	a	a	b
Gpi	b	b	a	a	a	a
Idh	a	a	b	b	b	b
Mdh1	c	c	c	c	b	a
Mdh2	a	a,b	d	c	c	c
Mpi	b	b	a	c	b	d
PepA	a	a	b	b	c	c
PepB	b	d	b	a	a	c
PepD	b	b	b	c	b	a
Pgam	d	d	a	b,c	c	b
Pgk	e	c	b	a	c	d
Pk	c	c	b	a	c	d
Tpi	a,c	a	c	a	b	b

[z] Alleles are designated alphabetically in order of increasing mobility, with 'a' being the most cathodal.

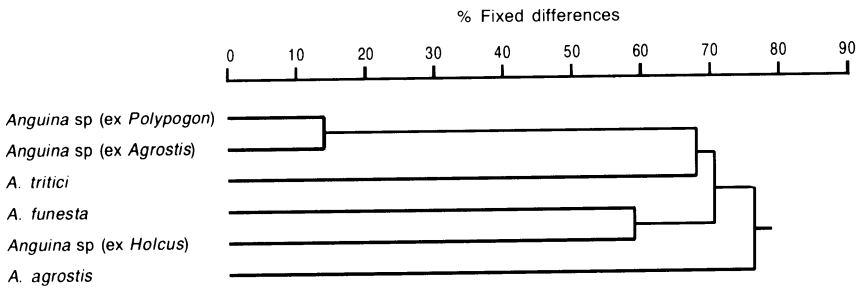

Fig. 10. Phenogram showing relationship of *Anguina* populations from allozyme data based on unweighted pair group method analysis.

The discovery of a new vector for *C. toxicus* is disturbing, because it increases the known range of grasses that can be infected. *A. avenacea* and *P. monspeliensis* are in the tribe Avenacea, subtribe Agrostidinae (29). The host grasses of *Anguina funesta* are in the tribe Poeae and include *Lolium* species, *Festuca* species, and *Vulpia myuros* (L.) C.C. Gmelin, although the last is a poor host (13,19).

With the assistance of a suitable vector, *C. toxicus* may infect a wide range of grasses. It has been observed on the inflorescences of *Avena fatua* L., *Danthonia caespitosa* Gaudich., *Phalaris minor* Retz., and *Phalaris paradoxa* L. (4); but these were all in fields of heavily infested *L. rigidum*. *Anguina funesta* was probably the vector, since some second-stage juveniles of this nematode will congregate around the meristems of nonhost grasses (19). However, *C. toxicus* will adhere to other *Anguina* species, including *Anguina agrostis, Anguina tritici,* and a species found on *H. lanatus,* although the proportion that it attaches to varies among populations (18,19). More work is needed to determine whether these nematodes will carry the bacterium into their respective hosts.

While stock losses caused by *C. toxicus* have only been reported in Australia and South Africa, it may be causing losses elsewhere, possibly in association with different *Anguina* species. Annual ryegrass toxicity closely resembles the livestock poisoning associated with Chewings fescue in Oregon between 1939 and 1960 (9,28); and in Russia, rabbits died after being fed *Anguina agrostis* galls from *Agrostis stolonifera* L. and *Agrostis tenuis* Sibth. (10).

C. toxicus appears to survive in a wide range of environments. In the cropping areas where annual ryegrass toxicity has been recorded, the rainfall varies from 300 mm to 600 mm per annum (12). In northern New South Wales, flood plain staggers was discovered following several successive seasons of winter rainfall pattern and flooding. The rainfall in this area is unreliable. In the east it tends to be summer dominant and >650 mm; it grades to <350 mm in the west and is distributed more uniformly throughout the year. In the southeast of South Australia, affected pastures are usually flooded for between 1 and 5 mo during the growing season.

Infected inflorescences of hosts of *Anguina funesta* usually appear normal, but the inflorescences of *A. avenacea* and *P. monspeliensis* infested with the nematode associated with flood plain staggers show distinctive symptoms. Despite this distinctive appearance, infected *P. monspeliensis* was not found for more than 20 yr. Also, the clinical symptoms and gross pathology in affected animals are not sufficiently specific to confirm a diagnosis of corynetoxin poisoning (1,2,11,26). Therefore, workers investigating the cause of stock poisonings where affected animals show a convulsive syndrome, often with high mortality rates, and where the grass has senesced, should consider *C. toxicus* as the possible source of the toxin.

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