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Vulpia myuros and the annual ryegrass toxicity organisms, *Anguina funesta* and *Clavibacter toxicus*

Ian T. RILEY

Department of Agriculture, Baron-Hay Court, South Perth, Western Australia 6151, Australia.

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Summary – *Vulpia myuros* was found to be a host for both *Anguina funesta* and *Clavibacter toxicus* (the organisms responsible for annual ryegrass toxicity) in an infested *Lolium rigidum* pasture in Western Australia. The reproduction of *A. funesta* in *V. myuros* was inefficient both in the field and in container-grown plants. The number of galls produced was low, many of the eggs laid did not hatch and many second-stage juveniles did not reach the survival stage. The multiplication rates in container-grown plants were insufficient to maintain the nematode population in *V. myuros* alone. *V. myuros* populations are unlikely to be important in the management of pasture infested with *A. funesta*.

Résumé – Vulpia myuros *et les organismes responsables de la toxicité annuelle du ray-grass*, Anguina funesta *et* Clavibacter toxicus – *Vulpia myuros* a été observé comme hôte d'*Anguina funesta* et de *Clavibacter toxicus* – agents de la toxicité annuelle du ray-grass – dans un pâturage de l'ouest australien. La multiplication d'*A. funesta* sur *V. myuros* est insignifiante, aussi bien au champ qu'en pots. Peu de galles sont produites, une grande proportion des œufs pondus n'éclosent pas et bon nombre de juvéniles n'atteignent pas le stade de survie (dauerlarva). Les taux de multiplication obtenus en pots, de l'ordre de 0,8 à 0,1, ne permettent donc pas aux populations du nématode de se maintenir sur *V. myuros*. En conséquence, il n'est probablement pas nécessaire de tenir compte des populations de nématodes présentes sur *V. myuros* dans la gestion des pâturages infestés par *A. funesta*.

Key-words : Nematodes, *Lolium rigidum, Vulpia myuros, Anguina funesta, Clavibacter toxicus,* annual ryegrass toxicity.

Annual ryegrass (*Lolium rigidum* Gaudin) infected with the bacterium, *Clavibacter toxicus* Riley & Ophel, 1993, and its vector nematode, *Anguina funesta* Price, Fischer & Kerr, 1979 are responsible for the poisoning of livestock known as annual ryegrass toxicity (ARGT). The host range of *A. funesta* is known to include *Lolium* spp. (ryegrasses), *Festuca* spp. (fescues) and *Vulpia myuros* (L.) C. C. Gmelin (rat's tail fescue) under experimental conditions (Price, 1973; Riley & McKay, 1991; Chatel; 1992), however only *L. rigidum* has been found infested in the field. In contrast, *C. toxicus* has been found on a range of grasses (including *Avena* spp., *Danthonia caespitosa* Gaudich., *Phalaris* spp.) growing in ryegrass pastures heavily infested with *A. funesta* (Chatel *et al.*, 1979; Chatel, 1992). None of these grasses have been confirmed as hosts of the nematode; rather it is likely that in ryegrass pasture with high populations of *A. funesta* the nematode carries *C. toxicus* into grasses in which it cannot reproduce but in which the bacterium can grow (Riley & McKay 1991).

Although, *C. toxicus* is dependant on a nematode vector it is not dependant solely on *A. funesta*. *Clavibacter toxicus* has been found in association with another *Anguina* sp. in *Agrostis avenacea* J. F. Gmelin and *Polypogon monspeliensis* (L.) Desf. in New South Wales and South Australia, respectively (McKay *et al.*, 1993). This association is responsible for livestock poisoning known as flood plain staggers (Bryden *et al.*, 1991). Also, *Anguina tritici* has been demonstrated to carry *C. toxicus* into wheat under experimental conditions (Riley, 1992).

Of the hosts for *A. funesta*, other than *L. rigidum*, the only species that occurs commonly in the self-regenerating pastures associated with cereal cropping in Western Australia and South Australia where ARGT occurs is *V. myuros* (Rossiter, 1966). It may be possible that *V. myuros* supports the reproduction of *A. funesta* in the field. Also, given the broad host range of *C. toxicus*, *V. myuros* may be colonized by *C. toxicus* in the field. However to date, *V. myuros* has only been reported to be a host for *A. funesta* under experimental conditions using a high inoculation rate which resulted in a maximum of two galls per plant (Riley & McKay, 1991).

Reproduction of *A. funesta* and *C. toxicus* in *V. myuros* may have important implications for the management of ARGT. It is unlikely that *V. myuros* itself would represent a toxicity problem, as it is rapidly-maturing, not highly productive (Smith *et al.*, 1972) and not palatable to livestock when mature. However, because *V. myuros* is early-maturing, it may allow *A. funesta* to escape herbicide treatments used to control the nematode in *L. rigidum* (McKay *et al.*, 1992). The treatment of the pasture with herbicides to control seed-set in ryegrass tends to increase the proportion of *V. myuros* in the pasture by reducing competition from other grasses

(Dowling *et al.*, 1992, 1993; Leys *et al.*, 1993). *V. myuros* may act as a reservoir of the ARGT organisms, and may need to be considered in the management of *A. funesta* infested pastures.

The aim of this study was to evaluate *V. myuros* as a host for *A. funesta* and *C. toxicus* under field conditions in Western Australia and the significance of *V. myuros* for the management of ARGT.

Materials and methods

Examination of field infection

Mature *V. myuros* and *L. rigidum* seedheads were collected in November 1992 from a site 4 km East of Ballidu, Western Australia (30° 36′ S, 116° 46′ E). At this site, *L. rigidum* was heavily infested with *A. funesta* and *C. toxicus*, and *D. caespitosa* was found with *C. toxicus* infection. The seedheads were examined under a dissecting microscope before being threshed by hand using a ribbed rubber mat. The nematode galls in *V. myuros* are difficult to detect reliably by the methods used for *L. rigidum* (McKay & Riley, 1993). To improve detection, the threshed *V. myuros* was soaked overnight and small quantities examined in a water-filled Petri-dish on which a 10 mm grid had been drawn to assist in systematic examination of the sample. The soaking increased the transparency of the palea and lemma and by adjusting the balance of transmitted and incident light normal and bacterially-colonized *Anguina funesta* galls could be detected with a dissecting microscope. Gall numbers in both *V. myuros* and *L. rigidum* samples were determined and the number of nematode eggs and juveniles determined in about 20 galls from each host. *C. toxicus* was identified following isolation by cultural characteristics (Riley & Ophel, 1992) and immunodiffusion (Riley, 1987).

Culture of *A. funesta* in container-grown *V. myuros*

To determine the efficiency of *A. funesta* reproduction in *V. myuros*, the grass was grown in pots in the open at South Perth, Western Australia (31° 57′ S, 115° 51′ E) in 1993. Nematode inoculum consisted of varying levels of *A. funesta* galls from *L. rigidum* or *V. myuros*. The inoculation treatments were 32 and 128 galls from *L. rigidum* per pot and 10 g per pot of threshed *V. myuros* seedheads containing galls, giving approximatively 32 000, 128 000 and 2 500 viable juveniles respectively. The galls from *L. rigidum* were collected from a site about 12 km north-west of Ballidu in the November 1992. The *V. myuros* seedheads used as inoculum were collected from Ballidu as described above and treated with trifluralin to reduce seed germination. The trifluralin treatment was 100 µg/ml trifluralin for 24 h at 5 °C followed by six changes of water at 1 h intervals. Preliminary tests had shown that this treatment had no apparent effect on the viability of *A. funesta* juveniles in galls from *L. rigidum*.

Thirty-two *V. myuros* seedlings (5 day-old) were transplanted into replicate pots (280 mm diameter) for each treatment at two times (4 June and 30 July). Galls from *L. rigidum* were spread on the soil surface at planting and the *V. myuros* seedheads were likewise applied one week later when the transplants had established. Avon River loam (5.5 % clay, 3.0 % silt and 41.0 % fine sand and 50.5 % coarse sand) from Northam, WA was used as potting medium and 17 g of slow release fertilizer (Osmocote, 2-3 months) applied. The plants were harvested at physiological maturity to minimize loss of galls through shattering, oven dried (60 °C) and examined for bacterial and nematode colonisation as described above. The recovery of galls was estimated by examining sub-samples of the stover for galls not removed by threshing.

Results

Bacterial gummosis was found on a small proportion of *V. myuros* seedheads from Ballidu. Nematode galls, both normal and bacterially-colonized galls (Fig. 1) were found in the threshed seedheads. Gall concentration was 104 galls/10 g with 12 % being bacterially-colonized, compared with 360 galls/10 g and 66 % bacterially-colonized for *L. rigidum* at the same site. Counts of nematode eggs and juveniles within the galls from *V. myuros* and *L. rigidum* are presented in Table 1. Although the total number of eggs laid per gall (the sum of eggs and juveniles at maturity) in *V. myuros* was about one third of that in galls from *L. rigidum*, the number that hatched was only one tenth of that in *L. rigidum*. Not only was the hatch lower in *V. myuros*, the proportion of second stage juveniles that had developed to the survival (dauer) stage was considerably lower. Viable juveniles were seen in only six of 21 galls from *V. myuros* and these represented only 29 % of the juveniles in the six galls. In contrast, 82 % of the juveniles in *L. rigidum* were viable and only one gall did not contain any viable juveniles.

The results of the pot experiment are presented in Table 2. No galls were produced with the *V. myuros* inoculum. Galls were produced in pots inoculated with galls from *L. rigidum*, but the low number of juveniles in the galls represented effective multiplication rates of less than one. The multiplication rates for juveniles were approximately 0.8 and 0.4 for 32 and 128 galls applied in June and 0.3 and 0.1 in July. The number of viable juveniles could not be determined for these galls as oven drying of the samples had killed the juveniles. Since about 60 % of the eggs had hatched in the galls before drying, compared to only about 20 % at Ballidu in the previous season, it is likely that the proportion of viable juveniles would have been greater than the 29 % in the Ballidu galls. Multiplication rates were clearly insuffi-

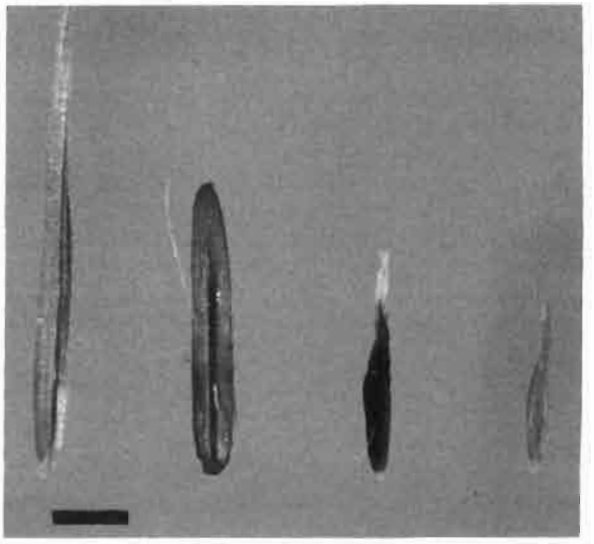

Fig. 1. *Seed and galls in* Vulpia myuros; *from left to right, caryopsis within the palea and caryopsis, seed-gall formed by* Anguina funesta *and seed-gall colonized by* Clavibacter toxicus. (Scale bar = 1 mm).

Table 1. *Eggs and juveniles within* Anguina funesta *seed-galls collected from* Vulpia myuros *and* Lolium rigidum *growing in the field near Ballidu, Western Australia in 1992.*

		Vulpia myuros	*Lolium rigidum*
Galls examined		21	22
Eggs	Range	2-541	0-860
	Mean ± S. E.	257 ± 34	234 ± 55
Juveniles	Range	0-368	0-2424
	Mean ± S. E.	75 ± 21	915 ± 132
Total	Range	2-887	0-2428
	Mean ± S. E.	333 ± 50	1149 ± 125

cient to maintain a population of *A. funesta* on *V. myuros*.

No bacterially-colonized galls were found in the container-grown *V. myuros* however one head with gummosis was found in a pot sown in June with 128 galls applied. Bacterial inoculum was not specifically applied in this experiment, although there was sufficient bacterial contamination of the nematode galls for moderate levels of bacterial colonisation of galls produced in *L. rigidum* in concurrent experiments.

Examination of stover indicated that the recovery of galls from the *V. myuros* seedheads was about 80 %; the data above have not been adjusted for this recovery rate. Adjustment would increase the multiplication rates, but even so the rates would be insufficient to maintain a population of *A. funesta*.

Table 2. *Reproduction of* Anguina funesta *in container-grown* Vulpia myuros *inoculated with either* A. funesta *galls from* Lolium rigidum *or threshed* V. myuros *seedheads containing galls at two sowing times at South Perth in 1993*

Treatment	Galls/pot (n = 2)	Eggs/gall ± S.E. (n = 16)	Juveniles/ gall ± S.E. (n = 16)
Junes			
32 *A. funesta* galls	92		
128 *A. funesta* galls	216	137 ± 28	240 ± 52
V. myuros inoculum	0		
July			
32 *A. funesta* galls	74		
128 *A. funesta* galls	100	65 ± 15	96 ± 37
V. myuros inoculum	0		

Discussion

V. myuros was found to be a host, under natural conditions, for both the nematode and bacterium responsible for ARGT. This is a new host record for *C. toxicus* and further confirms that its host range within the grasses appears to be limited only by the availability of a suitable vector. *Clavibacter toxicus* colonizes the spaces around the developing seedhead but does not invade or degrade the tissue. Damage to the plant is caused by mechanical constriction imposed by the gummy nature of the bacterium. The bacterium is an opportunistic colonizer of grass seedheads and *Anguina* seed-galls rather than a plant-pathogen in a strict sense. A broad host-range is consistent with these observations.

Vulpia myuros was found to be a host of *A. funesta* under field conditions. The number of galls per plant was greater at Ballidu than produced under experimental conditions in Adelaide (34° 56′ S, 138° 36′ E), South Australia (Riley & McKay, 1991), but the viability of the juveniles was lower. Although the total egg-lay was similar, less than 10 % of eggs developed to survival-stage juveniles in Ballidu compared to about 50 % in Adelaide. Ballidu has a warmer climate and shorter growing season than Adelaide, so *V. myuros* develops faster allowing less time for the *A. funesta* eggs to hatch and juveniles to mature. The maturity of galls cultured in Perth was intermediate, with 60 % of eggs hatching compared to 80 % in Adelaide and 20 % in Ballidu.

The rapid development of *V. myuros* greatly limits its potential as a host for *A. funesta*. In Perth, *A. funesta* was unable to maintain its population density on *V. myuros*. Given that the areas in Western Australia where ARGT continues to be a problem are in districts with shorter growing season than Perth, it is unlikely that *V. myuros* holds any significance for the management of ARGT. If *L. rigidum* were eradicated from a pasture and *V. myuros*

became dominant, *A. funesta* populations are likely to decline and eventually die out. Eradication of *L. rigidum* is unlikely to be achieved in fields used for cropping in W. A., so the reproduction of *A. funesta* in *L. rigidum* will always be more significant than in *V. myuros*. Although *V. myuros* is an unwanted component of wheatbelt pastures, it may have one beneficial side-effect in that it traps *A. funesta* juveniles that may otherwise reproduce more effectively in *L. rigidum*.

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