Quantitative Assessment of *Anguina* sp. and *Rathayibacter rathayi* in *Dactylis glomerata* Seed Production Fields in Oregon and Estimates of Yield Loss

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ABSTRACT

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Anguina sp. is a nematode that infests the inflorescence of orchardgrass and forms galls that replace the seed. *Anguina* sp. is also a vector of the bacterial pathogen *Rathayibacter rathayi*, which causes galls or gummosis in orchardgrass (*Dactylis glomerata*) panicles. The percentage of orchardgrass panicles infected or percentage of seed loss from *Anguina* sp. or *R. rathayi* in five commercial orchardgrass seed-production fields in Oregon during 2003 and 2004 was determined. The percentage of panicles with *Anguina* sp. ranged from 9 to 24%, although the percentage of seed replaced by *Anguina* sp. was less than 0.2%. The number of galls per panicle ranged from 1 to 29. However, more than 50% of *Anguina*-infested panicles contained only a single *Anguina* gall and few panicles had eight or more galls. The percentage of panicles with *R. rathayi* ranged from 3 to 27%. Percentage of seed loss from *R. rathayi* ranged from 0.1 to 7.3%. Seed loss in orchardgrass seed-production fields assessed for both *Anguina* sp. and *R. rathayi* was found to be as great as 8%. The number of *Anguina* galls remaining in fields following harvest ranged from 0 to 40 per square meter.

Additional keywords: bacterial head blight, Rathay's disease, seed gall, yellow berry

Orchardgrass (*Dactylis glomerata* L.) is an important forage grass grown for seed in the Willamette Valley, OR. The nematode *Anguina* sp. and the bacterium *Rathayibacter rathayi* are important pathogens that impact the orchardgrass seed industry directly through seed loss or indirectly through prevention of export due to their presence in seed shipments.

The *Anguina* sp. that infests orchardgrass is undescribed, but it is known to be distinct from *Anguina agrostis*, a common seed gall nematode of *Agrostis* spp. in Oregon (8). The nematodes invade the immature ovary, replacing the seed with dark purple galls that can contain as many as 2,000 nematode juveniles when mature, which coincides with maturity of orchardgrass seed (1,12). However, unlike the common bentgrass nematode, *A. agrostis*, which causes characteristic elongation of glumes, lemma, and palea (4), the orchardgrass nematode causes no morphological change to the lemma, palea, or

glumes, and there is no obvious change in appearance of infected plants (1). Thus, detection of *Anguina* sp. in orchardgrass is difficult. Development of a scarifier to remove lemma and palea from seed provided a means to quantify *Anguina* sp. galls in seed samples (1). In postharvest samples of orchardgrass seed in 2000, *Anguina* sp. and *R. rathayi* were found in 40 and 48% of samples, respectively (1). However, the preharvest population density of *Anguina* sp. within seed-production fields and proportion of galls returned to the field during harvest are unknown. In addition, it is not clear what impact *Anguina* sp. has on seed yield in orchardgrass.

The genus *Anguina* is a well-known vector of *Rathayibacter* spp. (3,9,10,14). *R. rathayi* is an important panicle-infecting bacterium of orchardgrass in Oregon (1,7). Cells of *Rathayibacter* spp. in soil that contact *Anguina* spp. during their migration from galls to plants adhere to the nematode cuticle and are carried into the plant with the nematode. Bacteria that proliferate before gall initiation form a yellow gummosis and those that proliferate after that stage kill the nematode and result in yellow, bacterially colonized galls (1,11). In orchardgrass, the bacterial growth is often extensive, resulting in a yellow gummosis of the panicle that envelops a portion of or the entire panicle. There are no seed produced in florets cov-

ered by the viscous exudate. The gummosis is most pronounced at head emergence through flowering. The disease is commonly referred to as Rathay's disease, yellow berry, or bacterial head blight. However, quantitative estimates of yield reduction in orchardgrass infected with *R. rathayi* and its vector *Anguina* sp. are lacking.

The objectives of this study were to (i) assess the preharvest level of *Anguina* sp. within commercial orchardgrass fields, (ii) estimate the number of galls remaining in the field after harvest, and (iii) estimate yield loss in orchardgrass from *Anguina* sp. and *R. rathayi* infection.

MATERIALS AND METHODS

All samples were collected in 2003 and 2004 from each of five orchardgrass fields (cv. Potomac) with a history of *Anguina* sp. or *R. rathayi*. Fields were located between Albany and Tangent, OR. All fields were in production longer than 3 years and the same fields were sampled in 2003 and 2004.

Preharvest estimates of *Anguina* sp. and *R. rathayi*. Previous studies (1) determined that the best time to assess orchardgrass for *Anguina* sp. infestation was during flowering, when the *Anguina* galls were turning red. At this stage of development, florets could be easily spread out under a dissecting microscope with dark-field illumination to observe the reddish-colored galls under the lemma or palea. Fifty panicles, collected arbitrarily along each of two diagonal transects in each field, were assessed for *Anguina* sp. and *R. rathayi*. To avoid bias in selection of panicles, reproductive tillers were selected at random from the base of plants without examining the attached panicles. Galls of *Anguina* sp. or *R. rathayi* were counted on each panicle. The palea was removed from each suspect flower to verify presence of a gall. Galls of *R. rathayi* were characteristic yellow. *R. rathayi* that formed a gummosis was quantified on each panicle by visually estimating the proportion of the panicle affected. Flowers that were colonized by *R. rathayi*, beyond a discrete gall but not affecting more than one to several florets, were counted separately. In a previous study (7), the bacterial galls and yellow gummosis on orchardgrass in Oregon were found to be associated with *R. rathayi*.

In 2003 and 2004, a separate assessment of galls of *R. rathayi* and *Anguina* sp. was

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conducted. One hundred panicles were collected arbitrarily while walking along each of four transects in a W pattern in each field within 1 week of cutting. Tillers were selected at random from the base of plants without examining the attached panicles. Tillers without panicles were excluded from counts of total panicles. Panicles were air dried for 2 weeks, then hand threshed. The lemma and palea were removed using a scarifier, as previously described (1), and the number of galls of *Anguina* sp. and *R. rathayi* were visually counted under a dissecting microscope. Seed number per panicle was estimated from each transect by dividing the total seed (caryopses, without lemma or palea) by the total seed heads collected for that transect.

Yield loss estimates. Each gall of *Anguina* sp. represents a single seed. Thus, yield loss from *Anguina* sp. equals the percentage of seed replaced by galls. A similar relationship applies to galls of *R. rathayi*. To estimate seed loss from galls or individual flowers infested with *R. rathayi*, counts of galls or infested flowers for each panicle at flowering were divided by the mean seed (caryopses) per panicle, determined from mature panicles collected just prior to harvest (as described above), and then multiplied by 100 to obtain the percentage. Because seed does not develop in the presence of the extensive bacterial growth, proportion of seed loss corresponds to the proportion of panicle engulfed in gummosis. All panicles with gummosis were examined under a dissecting microscope to determine if any seed were present in bacterially colonized flowers. The percentage of seed loss from gummosis for each panicle was determined by multiplying the percentage of each panicle with gummosis (visually estimated) by mean seed per panicle. Mean seed per panicle was determined from mature panicles collected within 1 week of cutting (as described above). Yield loss from *R. rathayi* was estimated from the

sum of loss from gummosis, galls, and infested flowers.

Postharvest in-field estimates of *Anguina* sp. Following harvest, eight sites were chosen at random throughout each field. At each site, residues from a 0.25-m² quadrant were collected by hand and placed in paper bags. A vacuum was used to collect seed, galls, and other fine debris from the soil surface. Seed and galls were separated from residues through a series of seed-cleaning operations, including hand screens, air columns, and indent cylinders. These procedures were relatively gentle and not sufficient to fracture galls.

Statistical analysis. A *t* test was used to compare sample means, based on meaningfully paired observations, as described by Steel and Torrie (13), and included mean values from each of the five fields. The test was used to determine whether percentage of panicles with galls, galls per panicle, or percentage of seed replaced by *Anguina* sp. or *R. rathayi* differed significantly between 2003 and 2004. In addition, the *t* test was used to determine if *Anguina* sp. or *R. rathayi* galls per panicle, or percentage seed replaced by *Anguina* sp. or *R. rathayi*, differed significantly between flowering and maturity in 2003 or 2004. All paired combinations were analyzed individually.

RESULTS

Pre- and postharvest estimates of *Anguina* sp. and *R. rathayi*. Mean seed per panicle ranged from 339 to 429 in 2003 and 405 to 479 in 2004 (Table 1). Percentage of panicles with *Anguina* sp. ranged from 9 to 24% (Table 1). Mean number of galls per panicle, based on panicles collected at flowering, ranged from 0.16 to 1.3. Percentage of seed replaced by *Anguina* sp. (seed loss) per panicle, based on panicles collected at flowering, ranged from 0.03 to 0.38 (Table 1). Mean *Anguina* sp. galls per panicle collected near harvest ranged from 0.13 to 0.49. Percentage of seed replaced by *Anguina* sp. (seed loss), based on panicles collected near harvest,

ranged from 0.03 to 0.13 (Table 1). The number of galls per single panicle ranged from 0 to 29. However, more than twice as many panicles had a single gall than two, and a relatively low frequency of panicles had eight or more galls (Fig. 1).

Percentage of panicles with *R. rathayi*, based on panicles collected at flowering, ranged from 3 to 27% (Table 2). *R. rathayi* galls per panicle, based on panicles collected at flowering, ranged from 0 to 0.32 (Table 2). The mean number of flowers per panicle with *R. rathayi* ranged from 0.03 to 1.36. Percentage of seed loss from *R. rathayi* ranged from 0.1 to 7.3 (Table 2). Mean number of *R. rathayi* galls per panicle at panicle maturity ranged from 0.02 to 0.45 (Table 2). There was no indication that bacterial infestation on one part of the panicle affected seed development on unaffected parts of the panicle.

Panicles were found to contain *Anguina* sp. or *R. rathayi* alone as well as in combi-

Fig. 1. Frequency of number of galls of *Anguina* sp. per orchardgrass panicle among five infested fields in 2003 and 2004.

Table 1. Orchard grass seed per panicle, percentage of panicles with galls of *Anguina* sp., *Anguina* sp. galls per panicle, and percentage of seed replaced by *Anguina* sp., assessed at flowering; and galls per panicle and percentage seed replaced by *Anguina* sp., assessed at orchardgrass maturity; in orchardgrass seed-production fields in Oregon in 2003 and 2004

Field	Seed per panicle ^a		Panicles with galls at flowering (%) ^b		Galls per panicle at flowering ^b		Seed replaced at flowering (%) ^c		Galls per panicle at maturity ^d		Seed replaced at maturity (%) ^e	
	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004
1	339 ± 70	416 ± 39	22 ± 2.1	24 ± 5.7	1.30 ± 1.04	0.55 ± 0.24	0.38 ± 0.31	0.13 ± 0.06	0.24 ± 0.13	0.49 ± 0.08	0.07 ± 0.03	0.11 ± 0.04
2	355 ± 90	434 ± 29	18 ± 5.7	9 ± 4.2	0.96 ± 0.52	0.38 ± 0.28	0.27 ± 0.15	0.09 ± 0.07	0.48 ± 0.35	0.17 ± 0.09	0.13 ± 0.08	0.04 ± 0.04
3	429 ± 39	479 ± 59	23 ± 6.4	10 ± 2.8	0.74 ± 0.06	0.16 ± 0.00	0.17 ± 0.01	0.03 ± 0.00	0.27 ± 0.08	0.13 ± 0.06	0.06 ± 0.04	0.03 ± 0.02
4	403 ± 19	405 ± 40	23 ± 9.9	9 ± 1.4	0.39 ± 0.13	0.42 ± 0.37	0.10 ± 0.03	0.10 ± 0.09	0.15 ± 0.05	0.20 ± 0.07	0.04 ± 0.01	0.05 ± 0.01
5	373 ± 59	460 ± 69	17 ± 4.2	13 ± 4.2	0.36 ± 0.03	0.71 ± 0.66	0.10 ± 0.01	0.15 ± 0.14	0.19 ± 0.07	0.16 ± 0.04	0.05 ± 0.03	0.04 ± 0.01

^a Mean ± standard deviation (SD) based on seed threshed from 100 panicles collected within 1 week of harvest from each of four transects from each field.

^b Mean ± SD based on 50 panicles collected at flowering from each of two transects from each field.

^c Mean ± SD based on 50 panicles collected at flowering from each of two transects from each field. Calculated by dividing galls per panicle by mean seed per panicle from each of two transects.

^d Mean ± SD based on four transects per field. Calculated by dividing total galls collected by total panicles per transect, based on 100 panicles collected within 1 week of harvest from each of four transects from each field.

^e Mean ± SD based on 100 panicles collected from each of four transects within 1 week of cutting. Calculated by dividing number of galls by number of galls + total seed and then multiplying by 100 to obtain percentage for each of four transects per field (100 panicles collected per transect within 1 week of cutting).

nation. The proportion of panicles with *Anguina* sp. and *R. rathayi* in combination ranged from 10 to 40%, depending on field and year (Fig. 2).

Mean values of percentage of panicles with *Anguina* sp. or *R. rathayi* at flowering, galls of *Anguina* sp. or *R. rathayi* per panicle, percentage of seed replaced by *Anguina* sp. or *R. rathayi* at flowering, *Anguina* sp. or *R. rathayi* galls per panicle at flowering, or seed per panicle were similar in 2003 and 2004. Significantly ($P = 0.05$) more *Anguina* sp. galls per panicle were found at flowering than at maturity in 2003 but not in 2004; similar results were found for percentage of panicles with *Anguina* sp. *R. rathayi* gall numbers did not differ significantly ($P = 0.05$) between flowering and maturity in either 2003 or 2004, although there was a suggestion of greater gall numbers at maturity in 2003 with a significance at $P = 0.10$.

Mean density of *Anguina* sp. galls remaining within fields following harvest ranged from 2.4 to 18.4 per square meter (Table 3). Up to 40 galls per square meter were recovered.

DISCUSSION

The number of *Anguina* sp. galls per panicle ranged from 0 to 29, with more than half the infested panicles containing only a single gall. A similar range of 1 to 58 *Anguina* sp. galls per infested panicle was observed in *Polypogon monspeliensis* in grazing lands in South Australia (2). A low level of *Anguina* sp. galls of about 0.1% by weight was reported among orchardgrass seed in the United Kingdom (12), consistent with previous reports from the United States (1). The low level of *Anguina* sp. found among seed heads corresponded to a low level of galls returned to the field during harvest. The population of galls remaining in the field following harvest ranged from 0 to 40 per square meter. Given a mean number of 1,200 nematode juveniles per gall (1), total population of nematodes would range from 0 to 48,000 per square meter. The level of *Anguina* sp. infestation in orchardgrass differs significantly from the occurrence of *A. agrostis* in Oregon on bentgrass, in which

seed loss from some fields reached 50 to 70% annually (5).

The genus *Anguina* is a known vector of *Rathayibacter* spp. However, the means of spread of *R. rathayi* within orchardgrass seed heads is not well known. Unlike other *Anguina*–*Rathayibacter* spp. associations, in which discrete galls are produced, gummosis from *R. rathayi* can engulf a single flower to an entire panicle. In the field, discrete heads typically are observed, with no indication of disease spread from infected to adjacent heads. Seed loss from *R. rathayi* is due primarily to bacterial gummosis. Much of the bacterial gummosis covering the seed heads is returned to the field with straw residue during harvest. No attempts were made to quantify the level of *R. rathayi*-contaminated residues returned to the field during harvest.

Although significant yield reductions (30 to 70%) from *Anguina*–*Rathayibacter* spp. can occur in wheat (15), seed loss in orchardgrass in Oregon from *Anguina* sp. and *R. rathayi* did not exceed 8%. In the cereal grains, grain quality is reduced by

Anguina and *Rathayibacter* spp. (6). In orchardgrass, partially infected seed were not observed and seed appeared either healthy or were replaced or destroyed by *Anguina* sp. or *R. rathayi*.

Panicles of orchardgrass can be infected with *Anguina* sp. or *R. rathayi* alone or in combination. *Anguina* sp. is assumed to carry *R. rathayi* and occurrence of *R. rathayi* alone is likely when the nematodes are killed by bacterial proliferation. Significantly more galls of *Anguina* sp. per panicle occurred at flowering than at maturity in 2003. Considering significance at $P = 0.10$, more *R. rathayi* galls were observed at maturity than at flowering in 2003. This suggests that *R. rathayi* colonization of *Anguina* sp. galls may continue beyond flowering.

Although some variation in the level of *R. rathayi* may occur, there is no indication from this or previous studies (1) that the level of *Anguina* sp. and *R. rathayi* is increasing in Oregon orchardgrass seed-production fields. The population of *Anguina* sp. within seed-production fields is likely to be limited by removal of galls during harvest and death of *Anguina* sp. from bacterial colonization. However, the population dynamics and increase of *Anguina* sp. in commercial seed-production fields in the absence of *R. rathayi* has not been established.

Additional information is needed on the population dynamics of *Anguina* sp. and *R. rathayi* and the factors that regulate interaction of the two pathogens. Little is

Fig. 2. Proportion of panicles with *Rathayibacter rathayi* only, both *Anguina* sp. and *R. rathayi*, or *Anguina* sp. only, based on total panicles from five orchardgrass fields in 2003 and the same fields in 2004.

Table 2. Percentage of panicles with galls of *Rathayibacter rathayi*, galls per panicle, percentage of seed replaced by *R. rathayi* at flowering, and *R. rathayi* galls per panicle at maturity in orchardgrass seed-production fields in Oregon in 2003 and 2004

Field	Panicles with <i>R. rathayi</i> at flowering (%) ^a		<i>R. rathayi</i> galls per panicle at flowering ^a		Seed replaced by <i>R. rathayi</i> at flowering (%) ^b		<i>R. rathayi</i> galls per panicle at maturity ^c	
	2003	2004	2003	2004	2003	2004	2003	2004
1	19 ± 1.4	13 ± 9.9	0.17 ± 0.11	0.11 ± 0.07	2.8 ± 0.5	0.6 ± 0.5	0.27 ± 0.14	0.19 ± 0.06
2	23 ± 7.1	18 ± 8.5	0.20 ± 0.05	0.03 ± 0.04	2.8 ± 0.2	3.2 ± 1.3	0.45 ± 0.26	0.04 ± 0.03
3	27 ± 12.7	3 ± 1.4	0.32 ± 0.10	0.00 ± 0.00	7.3 ± 3.8	0.1 ± 0.1	0.38 ± 0.23	0.02 ± 0.02
4	19 ± 4.2	18 ± 8.5	0.06 ± 0.02	0.11 ± 0.16	1.8 ± 0.8	1.0 ± 1.3	0.12 ± 0.04	0.07 ± 0.4
5	16 ± 1.8	3 ± 2.8	0.07 ± 0.01	0.15 ± 0.10	3.3 ± 1.0	5.1 ± 3.8	0.11 ± 0.02	0.02 ± 0.01

^a Mean ± standard deviation (SD) based on 50 panicles collected at flowering from each of two transects from each field.

^b Mean ± SD based on 50 panicles collected at flowering from each of two transects from each field. Percentage of seed loss includes loss from gummosis, galls, and infested flowers.

^c Mean ± SD galls per panicle based on 100 panicles from each of four transects. Total galls from threshed seed were divided by 100 panicles to obtain galls per panicle for each transect.

Table 3. Estimates of number of galls of *Anguina* sp. in orchardgrass fields following harvest in 2004

Field	<i>Anguina</i> sp. galls/m ²	
	Mean ± SD ^a	Range
1	8.4 ± 6.4	0–20
2	9.2 ± 9.2	0–28
3	2.4 ± 4.4	0–12
4	18.4 ± 19.6	0–40
5	4.0 ± 7.2	0–20

^a Mean and standard deviation (SD) based on examination of residues collected from 0.25-m² areas at each of eight random sites from each field.

known of the longevity of *R. rathayi* in soils or of the movement of *R. rathayi* in contaminated, wind-blown residues. There currently are no management strategies in place for control of *R. rathayi* in orchardgrass. Presumably, elimination of *Anguina* sp. from a field would result in no *R. rathayi* infection, although this hypothesis remains untested.

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