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Summary

Nematode levels were assessed for woodchips taken from sirex-infested trees in South Australia during July 1988. These trees were sampled to determine the percentage of sirex infected with nematodes that emerged during January to April 1989. A logistic equation was fitted to the data from 20 trees to quantify the relationship between the nematode level for woodchips and the percentage of sirex infected with nematodes. Knowledge of this relationship will allow an assessment of the nematode level in a plantation immediately before the inoculation period. Managers can prescribe nematode inoculations based on these results. An operational procedure for woodchip sampling is suggested. However, further testing is needed to verify the relationship in other regions and to determine a reliable period for sampling.

Introduction

The parasitic nematode *Deladenus siricidicola* Bedding is considered the key biological control agent of *Sirex noctilio* F. in Australia (Bedding and Akhurst 197 4, Taylor 1981, Neumann and Morey 1984). Bedding (1967, 1972) first described the biology of *D. siricidicola* and its dimorphic life cycle. Nematodes in the infective stage can enter a sirex larva and ultimately sterilize the adult female sirex. Nematodes are transported to other trees as the female sirex disperses and attempts to oviposit. Nematodes in the fungus-feeding stage multiply and disperse within a sirex-infested tree. Knowledge of the fungus-feeding cycle enables this nematode to be mass-reared in laboratory cultures for artificial inoculation into sirex-infested trees (Bedding and Akhurst 1974).

The level of nematode infection in a sirex population should be assessed to determine the need for artificial inoculation (Haugen *et al.* 1990). Previous assessment methods involved caging logs and dissecting the emerging sirex (e.g., Neumann and Morey 1984). This method is labour-intensive, and plantations must be sampled six months prior to the inoculation period. Another limitation is that nematode levels assessed from the caged samples are not for the same generation of sirex which the inoculations are targeted. A method for estimating the nematode infection level in a sirex generation just prior to the inoculation period would provide managers with current data to make more informed decisions.

R. A. Bedding and R. J. Akhurst (pers. comm.) sampled woodchips to detect the presence of *D. siricidicola* in sirex-infested trees. They collected woodchips along the bole of a tree, soaked them in water for 24 hrs, then examined the water under a microscope to detect nematodes. This technique was used to monitor the geographic spread of the nematode from an inoculation site and to determine the percentage of trees naturally inoculated with nematodes by sirex.

Objectives of our study were to quantify the relationship between the nematode level in woodchips and the percentage of sirex infected with nematodes, and to propose an operational sampling procedure, if this relationship gave a reliable prediction.

Materials and methods

Seven compartments were selected to provide a wide range of nematode infection levels, based on results from the 1988 sirex emergence period. These compartments were located in *Pinus radiata* D. Don plantations throughout southeastern South Australia and had been planted during 1974-1976.

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Cumulative sirex-associated tree mortality ranged from 3-42% in these compartments at the end of 1988.

Three sirex-infested trees were felled in each compartment during 25-29 July 1988. Woodchip samples were collected every metre along the bole of each tree from the base to a small-end diameter of 7 cm. Prior to collecting a woodchip, the bark and cambium were removed from a 10 x 10 cm area on the bole to expose the sapwood. Then a woodchip, approximately 6 x 6 x 1.5 cm, was cut out of the sapwood with an axe. Each woodchip was immediately placed into a separate 400 ml plastic container with approximately 150 ml of water (enough to cover the woodchip). Woodchips were soaked for about 24 hrs. The water was carefully decanted until approximately 20 ml remained, then the remaining water was swirled and poured into a petri plate (9 cm in diameter). The number of nematodes in the plate was estimated by counting nematodes in a proportion of the plate using a stereoscopic microscope at 40x, and a rating was recorded (Table 1).

The same 21 trees were assessed for the percentage of sirex infected with nematodes. Each tree was cut into five equal strata (butt, lower, mid, upper, top) during December 1988. The log from each stratum was placed into a separate cage (200 L drum with fly-wire screen secured over the top). Male and female sirex that emerged from January to April 1989 were dissected to determine the percentage infected with nematodes for each of the 105 cages.

Table 1. Nematode ratings for woodchip samples.

Rating	Estimated No. of nematodes/plate	Observation
0	0	no nematodes found in entire plate
1	1– 400	nematodes found after searching part of the plate
2	400–1000	1–2 nematodes in first field-of-view (16 mm²)
3	1000–8000	3–20 nematodes in first field-of-view
4	>8000	>20 nematodes in first field-of-view

Table 2. Rating of nematode level in woodchip samples from each metre along the bole of 21 sample trees.

Cpt.	Tree No.	Strata: Butt	Lower	Mid	Upper	Top	Tree[1] Mean
A	1	1 2 3	3 3 3	3 3 3 3	3 3 4	3 3 3	3.1
A	2	0 3 3	3 3 3	2 3 3	3 3 4	4 4 3	3.2
A	3	0 4 3	1 4 4	4 2 4	4 3 0	1 0 0	2.3
B	4	0 0 0	3 3 4	4 4 4	4 3 3	3 3	3.5
B	5	0 3	3 4 4	4 4 4	4 4 4	4 4	3.9
B	6	0 0 0	0 0 0	3 2 3	3 3 3	2 0 1	1.7
C	7	0 1 2	2 1 2	1 4 3	2 1 2	4 3	2.3
C	8	4 3 4	3 4 4	4 4 4	4 4 1	4 1	3.4
C	9	0 0	0 0	0 0 0	0 0	2 1	0.3
D	10	1 0	0 0 0	0 0 0	0 0 0	0 0	0.0
D	11	0 1	1 0 1	0 0	0 0 2	4 4	1.2
D	12	0 1 1	1 1 1	1 2 1	1 2 2	2 0	1.3
E	13	1 1	0 1	0 1 0	1 1	0 1	0.6
E	14	0 0	0 0	0 0	0 3	3 0	0.8
E	15	0 0	0 0	0 0	3 3	0 0	0.8
F	16	0 3 4	4 3 3	3 3 4	4 3 4	3 3 4	3.4
F	17	0 0	0 0	2 4 4	3 4	3 4	2.7
F	18	0 0 0	1 4 4	4 4 4	4 4 4	4 4	3.7
G	19	0 0	0 0 0	0 0 3	3 0 0	0 0	0.5
G	20	0 0	1 3 3	3 1 0	0 0 0	0 0	1.0
G	21	1 0 4	3 3 3 3	3 3 3	3 3 3 3	3 3 3	3.0

[1]Mean rating for a tree calculated from lower, mid, upper, and top strata.

Table 3. Number of *Sirex noctilio* dissected from each stratum within a tree.

Cpt.	Tree No.	Strata					Tree Total
		Butt	Lower	Mid	Upper	Top	
A	1	158	138	192	79	154	721
A	2	99	35	156	31	133	454
A	3	26	13	65	48	68	220
B	4	20	101	13	14	15	163
B	5	1	6	48	19	80	154
B	6	22	1	4	9	69	105
C	7	37	81	45	55	107	325
C	8	1	1	55	12	110	179
C	9	0	21	122	72	48	263
D	10	21	101	152	64	3	341
D	11	14	137	135	80	192	558
D	12	122	96	104	75	65	462
E	13	86	165	204	80	117	652
E	14	6	101	228	187	167	689
E	15	3	77	64	126	96	366
F	16	39	99	194	48	91	471
F	17	0	1	24	9	26	60
F	18	58	58	25	25	67	233
G	19	38	265	154	79	94	630
G	20	84	334	357	139	246	1160
G	21	11	264	225	39	285	824

Table 4. Percentage of *Sirex noctilio* infected with nematodes[1] for each stratum within a tree and for the entire tree.

Cpt.	Tree No.	Strata					Tree Total
		Butt	Lower	Mid	Upper	Top	
A	1	98	97	93	95	97	96
A	2	89	100	99	92	100	95
A	3	100	69	95	97	71	90
B	4	100	100	100	100	64	97
B	5	*	*	88	98	100	94
B	6	73	*	*	*	100	93
C	7	100	99	91	89	73	90
C	8	*	*	82	97	100	92
C	9	*	62	76	63	90	76
D	10	5	1	0	0	*	1
D	11	100	72	84	54	88	72
D	12	94	73	76	55	35	71
E	13	14	24	4	7	23	13
E	14	*	12	18	41	46	30
E	15	*	95	92	68	20	61
F	16	100	98	98	100	90	98
F	17	*	*	100	*	100	100
F	18	98	98	100	100	100	99
G	19	45	3	48	24	5	20
G	20	94	95	63	6	47	61
G	21	91	99	100	100	100	99

[1] "*" = less than 10 sirex dissected; percentage not given.

Results

By Stratum

Nematode ratings from the woodchip samples were recorded for each tree by their position along the bole, and then partitioned into corresponding strata (Table 2). Each tree had at least two woodchip samples from each stratum. Generally, nematode ratings of adjacent woodchips were the same or differed by a single rating increment. Also, ratings were usually consistent within a stratum of a tree.

The number of sirex dissected was recorded for each cage (Table 3). Fourteen cages had less than 10 sirex dissected, and these cages were not included in the stratum analysis. The percentage of sirex infected with nematodes was recorded for each cage (Table 4).

Nematode ratings from woodchips (Table 2) and infection percentages from the caged logs (Table 4) were well correlated (r = 0.63) for the stratum data (n = 91). Separate correlations for each stratum showed data from the butt stratum (n = 15) were poorly correlated (r = 0.25), while data from each of the other stratum were well correlated (r = 0.66 to 0.84). Data from the butt stratum were excluded from the set, and the degree of correlation improved (r = 0.75). A plot of these data (n = 76) revealed that the infection level of sirex was consistently >50% when the nematode rating of woodchips was >2.0 (Figure 1). Infection levels were more variable when nematode ratings were less than 1.0. Seven of the observations gave "false negatives' (i.e., woodchips did not detect nematodes, but >40% of the sirex were infected).

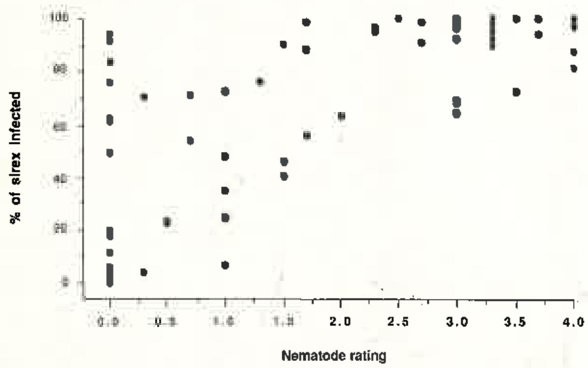

Figure 1: Plot of mean nematode rating from woodchips and percentage of sirex infected with nematodes for observations from the lower, mid, upper, and top strata with more than 10 sirex dissected (n = 76).

By Tree

Nematode ratings from the lower, mid, upper, and top strata were averaged for each of the 21 trees (Table 2). The mean ratings for the trees ranged from 0.0 to 3.9 on a scale of 0 to 4. The percentage of sirex infected with nematodes ranged from 1-100% for these 21 trees (Table 4). Twelve trees had sirex with infection levels greater than 90%; while only four trees had sirex with infection levels less than 50%.

Nematode ratings and infection percentages were well correlated (r = 0.80) for the whole-tree data (n = 21). A plot of these data revealed a distinct curve (Figure 2), except for one tree (Tree 9). In "Tree 9", nematodes were not found in the woodchips from the lower, mid, and upper strata, but 62-76% of the sirex emerging from these strata were infected. To generate a more conservative model, this tree was deleted from the data set as an outlier.

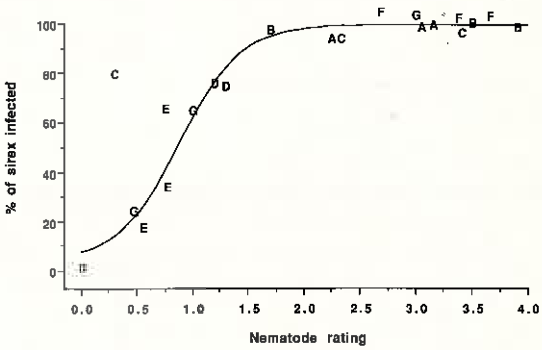

Figure 2: Plot of mean nematode rating from woodchips and percentage of sirex infected with nematodes for tree observations (n = 21). Letters denote the compartments. A logistic curve was fitted to these data (except Tree 9 from Cpt C).

A negative exponential curve and a logistic curve were fitted to these data (n = 20), and the logistic equation (1) gave a better fit.

INFECT = 95.46 / [1 + EXP(3.26 — 3.68 * CHIP)] (1)

where INFECT is the percentage of sirex infected with nematodes for a tree, EXP is the base of natural logarithms (approximately 2.71826) raised to the power specified, and CHIP is the mean nematode rating of woodchips from the lower, mid, upper, and top strata of a tree. This model accounted for 99.3% of the variation in the sums of squares.

Ratings from woodchip samples were accurate in distinguishing differences in infection levels of sirex from trees within a compartment. For example, 20, 61, and 99% of the sirex emerging from the three trees in "Cpt G" were infected with nematodes, and the woodchip samples accurately discriminated these differences with ratings of 0.5, 1.0, and 3.0, respectively.

Discussion

Based on these results, the level of nematodes in woodchips should be useful for predicting the percentage of sirex infected with nematodes. The section of the curve that is critical for making inoculation decisions is when infection levels are between 0-30% (woodchip ratings of 0.0-1.0). Unfortunately, this section of the curve was constructed using data from only four trees. Additional data are needed to confirm the relationship at this end of the curve.

This relationship between woodchip samples and infection levels (Figure 2) was determined under the conditions prevailing in southeastern South Australia during 1988 and for the stated parameters (e.g., woodchips sampled during late July, plantations aged 12-14 years, and 3-42% sirex-associated tree mortality). Further investigations are needed to test the precision and versatility of this model under other conditions. Initially, this model should be validated with data from other regions. Also, the stability of the nematode ratings needs to be determined from May-August, so a reliable period for sampling can be specified.

The rating system used for this study (Table 1) was simplistic and unrefined. Now that the critical section of the curve has been determined, further investigations of the relationship within this section should be conducted to improve and verify the model. The number of nematodes per plate should be used to quantify the relationship, instead of a rating system. A more definitive rating system can be devised for operational use after this relationship has been quantified.

Suggested operational sampling procedure

The knowledge of the relationship between nematode ratings from woodchips and percentage of sirex infected with nematodes can be applied now, even though this model has not been fully tested or validated. Woodchip sampling should be used in plantations where the nematode level is unknown and a significant increase in sirex-associated tree mortality has occurred. Results from the woodchip sampling will provide the information to make inoculation decisions. The following procedure is suggested for operational use.

The basic unit for sampling is a locality (i.e., a group of adjacent compartments in the same age class and with the same silvicultural history; assume 100-200 ha per locality for this discussion). At least 20 sirex-infested trees should be selected for woodchip sampling throughout a locality with a bias away from previous inoculation sites. Eight woodchips should be collected from each tree (two woodchips from each stratum, excluding the butt). Each woodchip should be assessed individually. The number of nematodes in a sample should be estimated by counting nematodes in sections of the plate representing 5-10% of the entire plate, and the rating recorded according to Table 1. Then, a mean nematode rating for a tree can be calculated from the eight values, and the infection level estimated from the curve in Figure 2.

Inoculations are not warranted if most of the sampled trees in a locality are estimated to have greater than 30% of the sirex infected with nematodes. At these levels, nematodes are considered to be well established, and further inoculations will not substantially increase the infection level in the sirex population. If the mean infection level is estimated to be less than 20%, further inoculations are probably warranted. However, critical interpretation of the data is the responsibility of the manager, and factors such as the range in the nematode ratings, geographic pattern of the ratings within a locality, and density of sirex-associated tree deaths should be considered in the decision process. Using this procedure, woodchips can be collected at a rate of 10-20 trees per worker-day, and the samples can be examined at a rate of 30-40 trees per worker-day. An assessment of a locality may take two or three worker-days, but it may save hundreds of worker-days if the results show inoculations are not needed.

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