1982

Investigations of ryegrass toxicity.

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1982 Summary of Results of Field Experiments

Investigations of Ryegrass Toxicity

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CONTROL OF SPRAYTOPPING

Experiment: 82KA28

Location: D. Holmes, Gnowangerup

Aim: Earlier trials at Katanning (79KA14, 80KA26) showed that a single spray of paraquat in pasture just after ear emergence will reduce the number of galls produced. A proportional reduction in toxicity of the pasture is expected. Subsequent trials (81KA48, 81NA45, 81MO45) showed that the timing of the spray in relation to plant development was the same in different areas of the state and that as well as reducing the numbers of galls produced, spraying also reduced the viability of nematodes within the galls that did survive.

The trial reported here was done to compare two new chemicals, "glean" and "roundup", with paraquat, the herbicide currently recommended for spraytopping.

Treatments: The experiment was done in a toxic paddock leased from Holmes. As in previous years the area was inoculated with galls to give a uniform and heavy level of infection. The experimental design comprised four randomized blocks each including three chemical treatments and an untreated control. Each treatment was applied to one plot per block at intervals of one week between September 15 and November 17. Paraquat was applied at a rate of 550 ml per ha, glean at 20 g per ha and roundup at 300 ml per ha. A wetting agent was incorporated with each.

Methods: At maturity, plant tops were harvested from 1.0 m^2 quadrats per plot and weighed to give an estimate of pasture production. The samples have subsequently been threshed to separate the galls and further measurements are currently being done. Only the effects of the chemicals on pasture production are available for this report.

Results: The following table gives the average weight of pasture harvested from the experimental plots treated with herbicides. Based on the growth stage of the ryegrass, the optimum time to spray with paraquat to control toxicity and reduce gall numbers was between September 22 and October 6. There was no significant difference in the amount of pasture produced on plots sprayed with any of the three herbicides during this period. With later sprays, significantly less pasture survived sprays with glean and roundup than that sprayed with paraquat.

Comments: These results suggest there is no advantage in using either glean or roundup in preference to paraquat to control annual ryegrass toxicity on the basis of pasture production. The only advantage of using these chemicals may be from superior control of the organisms. This aspect has yet to be evaluated.

The most serious disadvantage of using spraytopping as a strategy for controlling ARGT is the considerable loss in pasture production and the growing threat of predisposing to erosion. Farmers should be made aware of these factors when spraytopping as a strategy is considered.

Pasture production (kg/ha) on experimental plots spraytopped with the herbicides paraquat, glean and roundup

Date of spray	Herbicide		
	paraquat	glean	roundup
September 15	267	540	778
September 22	541	514	382
September 29	458	549	507
October 6	378	605	546
October 13	576	467	570
October 20	847	647	549
October 27	715	538	615
November 5	973	533	677
November 10	782	555	626

The untreated controls averaged 751 kg/ha

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EMERGENCE OF NEMATODE LARVAE FROM GALLS

Experiments: 82KA29, 82NA33, 82MO19

Location:
D. Holmes, Gnowangerup
R. Dawson, Bulyee
J. Follett, Wongan Hills

Aim: To study the emergence of larvae of *Auguina agrostis* from galls at three different locations in the state and to evaluate any differences in the patterns of emergence in relation to any variations in climate. To determine whether all the larvae emerge from the galls in a single season, and if not whether further emergence occurs during a second season.

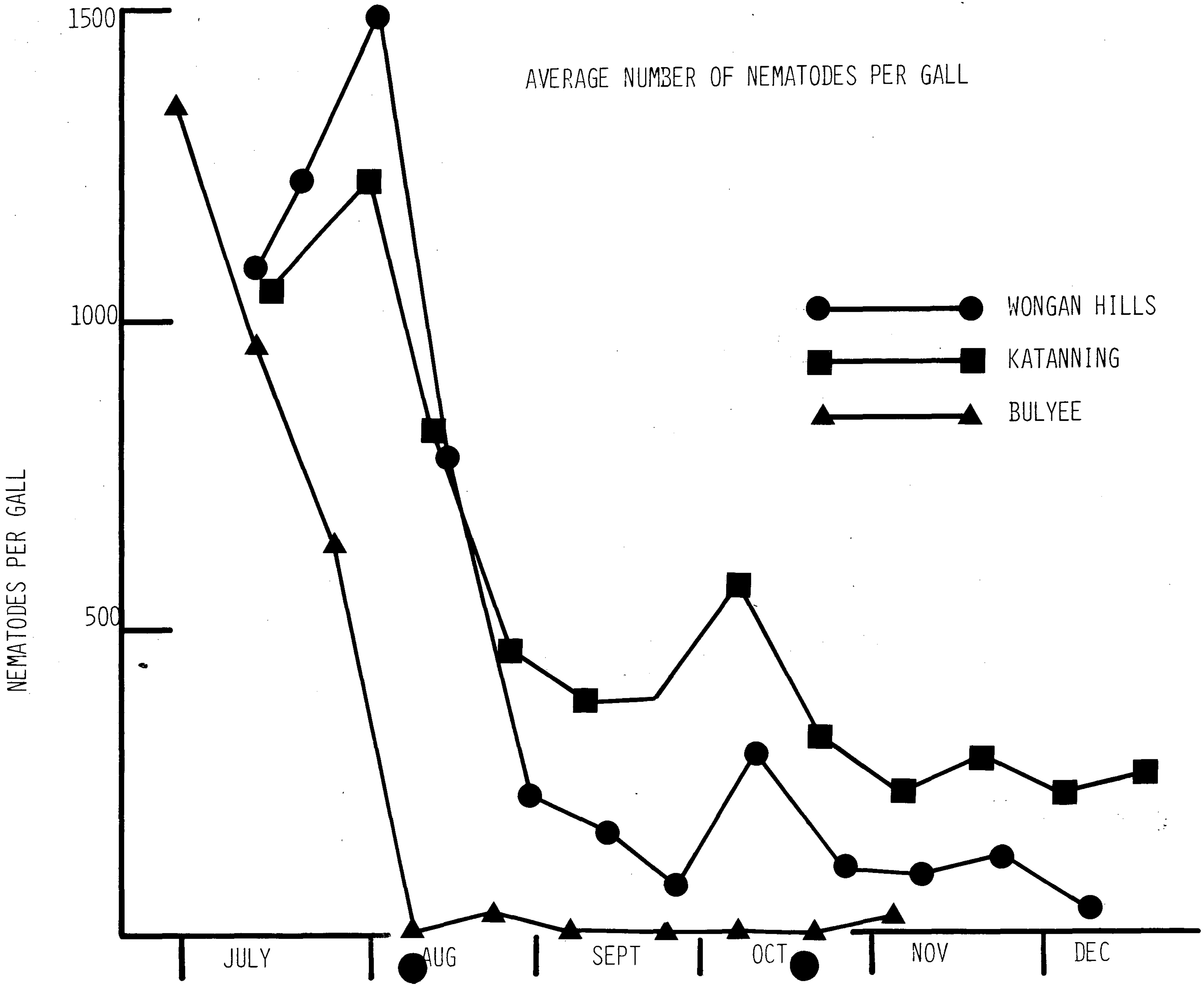
Methods: Two nylon mesh bags (approximately 30 mm x 30 mm) each containing 10 nematode galls were placed in 144 sampling trays. Forty eight of these sampling trays, each comprising a 20 cm length of 8 cm PVC pipe closed off at one end with nylon mesh (1 mm aperture), were randomized between 4 blocks and lightly covered with soil at each of the 3 sites. At each site one gall bag from a tray in each block was sampled at fortnightly intervals for 12 sampling times commencing four weeks after opening rain. The sampling is to be repeated, taking the remaining gall bag from each tray in 1983. The number of larvae in the galls collected in 1982 were counted and records were kept of temperature and rainfall throughout the season.

Results: The following figure shows the average number of larvae remaining in the galls from each sampling and the table shows the average weekly climatic data recorded at the three sites for the periods from when opening rains occurred to when larvae began to emerge from galls.

Opening rains occurred four weeks earlier at Corrigin and Wongan Hills than they did at Gnowangerup while larval emergence from the galls commenced 10, 8 and 13 weeks after opening rain at Gnowangerup, Corrigin and Wongan Hills, respectively.

Comments: Conditions appeared to be more favourable for larval emergence at Corrigin in 1982 than at the other two sites, with larvae commencing to emerge 8 weeks after opening rain and all the larvae emerging within a further six weeks. Emergence was delayed at the other two sites and by the end of the season between six and 20 per cent of the original larval content still remained in the galls. Sampling in the second season will determine whether these larvae are still viable.

These observations suggest that after the nematodes become hydrated by the opening rain they may have a low temperature incubation requirement before they can become active and emerge from galls. Such mechanisms have been reported for other species of nematodes where they prevent premature activity in false seasonal breaks.


AVERAGE NUMBER OF NEMATODES PER GALL
NEMATODES PER GALL
1500
1000
500
JULY
AUG
SEPT
OCT
NOV
DEC
WONGAN HILLS
KATANNING
BULYEE

A suggested optimum temperature for this incubation period would be around 15^{o} maxiomum and 5^{o} minimum as occurred for approximately six weeks prior to larval emergence at both Corrigin and Wongan Hills. The lower temperatures at Gnowangerup could have prolonged the incubation period at this site and higher temperatures at Wongan Hills for approximately 6 weeks after opening rains commenced could have delayed the nematodes from becoming active earlier.

Controlled environment laboratory studies are being done to investigate further the process of emergences.

Climatic data for experimental sites at Gnowangerup, Corrigin and Wongan Hills for the periods from when opening rains occurred to when nematode larvae began to emerge from galls

Date	Gnowangerup Max. temp.	Gnowangerup Min. temp.	Gnowangerup Rain	Corrigin Max. temp.	Corrigin Min. temp.	Corrigin Rain	Wongan Hills Max. temp.	Wongan Hills Min temp.	Wongan Hills Rain
May 2- 8				21	7	14	22	11	11
9-15				22	7	1	23	11	4
16-22				17	6	4	20	9	0
23-29	16	7	9	18	10	9	19	11	4
June 30- 5	15	3	1	19	4	0	20	9	1
6-12	14	3	8	18	6	20	19	8	32
13-19	11	2	10	15	6	24	17	7	47
20-26	11	1	3	14	3	3	15	3	0
July 27- 3	10	4	5				15	5	3
4-10	12	3	6				17	7	5
11-17	12	4	3				18	7	16
18-24	12	3	6				14	6	10
25-31	13	3	3				17	5	1

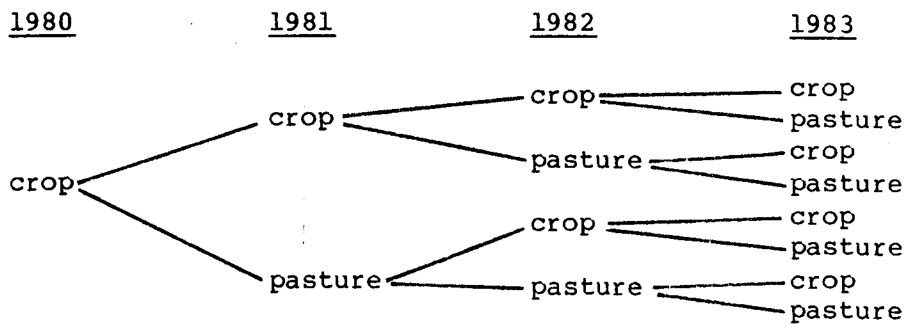
EFFECTS OF LONG TERM CROPPING

Experiment: 81KA57

Location: D. Holmes, Gnowangerup

Aim: To determine the long term effect of grass free cropping programmes on the persistence of annual ryegrass toxicity, and the feasability of resowing these areas with ryegrass and maintaining a minimal disease pasture.

Treatments: An experimental area selected in the paddock leased from Holmes was cropped in 1980 without grass control to establish a high initial level of infection. The experiment, replicated in four blocks, comprised the following split-plot design:



From 1981 onwards each crop was to be kept free of ryegrass using herbicides and the subsequent pasture plot was to be resown with ryegrass.

Methods: At the end of each season 20 x 0.5 m^2 quadrats wee harvested from each plot established with pasture that season. The samples were bulked from each plot, the plant material weighed and the galls and seeds recovered by threshing. The number of galls containing nematodes, those colonized by bacteria and the weight of ryegrass seed present was recorded for each plot.

900.

Results: No measurements were made on the plots in 1981 but the following measurements are given for an adjacent experimental area that received identical treatment:

Treatment - crop 1980, pasture 1981 measurements (per 10 m^2)

- pasture weight 530
- nematode galls 1,180
- bacterial galls 2,740
- total galls 3,920

the measurements for 1982 are averaged per 10 m^2 as follows:

Measurement	Treatment		Level of significance
	Pasture 1981 Pasture 1982	Crop 1981 Pasture 1982	
Pasture weight	476	773	n.s.
Seed weight	29	37	n.s.
Nematode galls	306	288	n.s.
Bacterial galls	237	58	*
Total galls	543	346	n.s.

Comments: The level of infection was high in 1981 as expected in pasture following a crop where there was no ryegrass control. By 1982 in the second year of pasture, the infection level had dropped naturally by almost 84 per cent. This agrees with field observations that show infected paddocks are most toxic to graze within 12 months of cropping and the risk of toxicity declines in subsequent seasons.

The level of infection in the alternative treatment, that was cropped successively in 1980 and 1981, was comparable to the area in its second year of pasture. In this treatment, the good ryegrass control achieved in the 1981 crop using hoegrass prevented the buildup of infection normally observed after a crop. This suggests that good ryegrass control in a crop will minimize the subsequent risk of toxicity, but possibly more than one season of grass free cropping is necessary to ensure a complete break in the nematode life cycle. Clearly, sufficient nematodes persisted either on the odd ryegrass plant that survived in the 1981 crop or in galls still present from 1980 to maintain a low level of infection. Nevertheless, further work may show that there is sufficient reduction from this single treatment to prevent subsequent toxicity.

A further encouraging aspect of this treatment showed that resowing with ryegrass gave a pasture stand at least comparable with the alternative treatment in a second year of pasture without any increase in the level of infection. Furthermore, in this instance, there were significantly fewer galls colonized by bacteria which means the pasture was less toxic. This results suggests that sowing with ryegrass to re-establish pasture after grass free cropping would be practicable where the infection level has been substantially reduced.

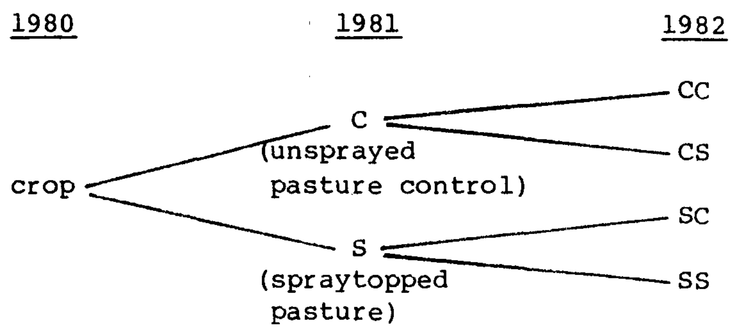
LONG TERM EFFECTS OF SPRAYTOPPING

Experiment: 81KA58

Location: D. Holmes, Gnowangerup

Aim: To evaluate current spraytopping recommendations to control annual ryegrass toxicity and to monitor the level of infection in pasture one and more years after spraytopping treatment.

Treatments: An experimental area selected in the paddock leased from Holmes was cropped in 1980 without grass control to establish a high initial level of infection. The experiment, comprising the following split-plot design, was replicated in eight blocks:



Treatment C represents unsprayed pasture control areas and S represents areas sprayed with paraquat one week after first head emergence according to the recommended spraytop control measure (550 ml/ha). Treatments CC, CS, SC and SS represent treatments applied in successive years 1981 and 1982.

Methods: At the end of the season 10 x 0.5 m^2 quadrats were harvested from each plot. The samples were bulked from each plot, the plant material weighed and the galls and seeds were recovered by threshing. The number of galls containing nematodes, those colonized by bacteria and the weight of ryegrass seed present was recorded for each plot.

Results: The measurements given in the following table are averaged per 10 m^2 so they can be compared with results of the adjacent experiment (81KA57) which was designed to look at the effects of long term cropping.

Comments: The results show that spraytopping significantly reduced the level of infection in all treated areas by comparison with the untreated control (CC). The greatest reductions were seen in the areas sprayed in 1982 (CS and SS) but there was also good residual control in the areas sprayed only in 1981 (SC). Most significantly, the greatest reductions in all treatments were in the numbers of galls that became colonized by bacteria. This suggests there is a more marked reduction in toxicity than there is in the actual level of infection. A similar pattern was also seen in the long term cropping experiment (81KA57).

Unfortunately, the benefit of reducing infection must be balanced against a considerable loss in pasture production, at least in the year of spraying. As observed in other experiments, the production of pasture in the second year following cropping was poorer than in the first year. A further reduction of about 40% occurred when spraytopping was done that year (CS and SS versus CC and SC). However, the effect of spraytopping in the first year did not persist through to the second year (CC versus SC and CS versus SS) when pasture production was comparable to an unsprayed area.

The effect of spraytopping on pasture production
and on the development of galls

Measurements[A]	Treatments[B]				Analyses of variance comparisons[C]:		
	CC	SC	CS	SS	Main treatments	Sub treatments	Main x sub treatment interactions
Pasture wt (g)	314	326	208	180	n.s.	***	n.s.
Seed wt (g)	29	30	16	16	n.s.	***	n.s.
Nematode galls	84	60	36	40	n.s.	*	n.s.
Bacterial galls	60	22	2	4	n.s.	*	n.s.
Total galls	144	82	38	44	n.s.	*	n.s.

[A] Measurements averaged per 10 m^2

[B] Treatment CC was unsprayed pasture in 1981 and 1982, SC was spraytopped in 1981 but not sprayed in 1982, etc.

[C] Treatments applied in 1981 were the main treatments and those applied in 1982 the sub treatments

DEVELOPMENT OF TOXICITY

Experiment: 81KA47, 81NA47, 81MO47

Location: C. Butterworth, Katanning
D. Holmes, Katanning
A. Price, Corrigin
J. Follett, Wongan Hills

Aim: To monitor the amount of toxin present in galls from the time of first head emergence until maturation of the ryegrass pastures. To relate the onset and development of toxicity to plant growth stages and climatic conditions in the different regions affected by annual ryegrass toxicity. To relate the level of toxicity to pasture density and to the number of galls present in the pasture.

Methods: At each experimental site, an area known to be affected with annual ryegrass toxicity was subdivided into four blocks each comprising 10 plots measuring 1 x 2 m. The sites were innoculated with 175 galls per sq. m. to help achieve a uniform heavy infection. Approximately 14 per cent of the galls were colonised by bacteria, the remainder contained nematodes.

At each site four plots, one from each block, were harvested at intervals of one week from head emergence until all plots were sampled.

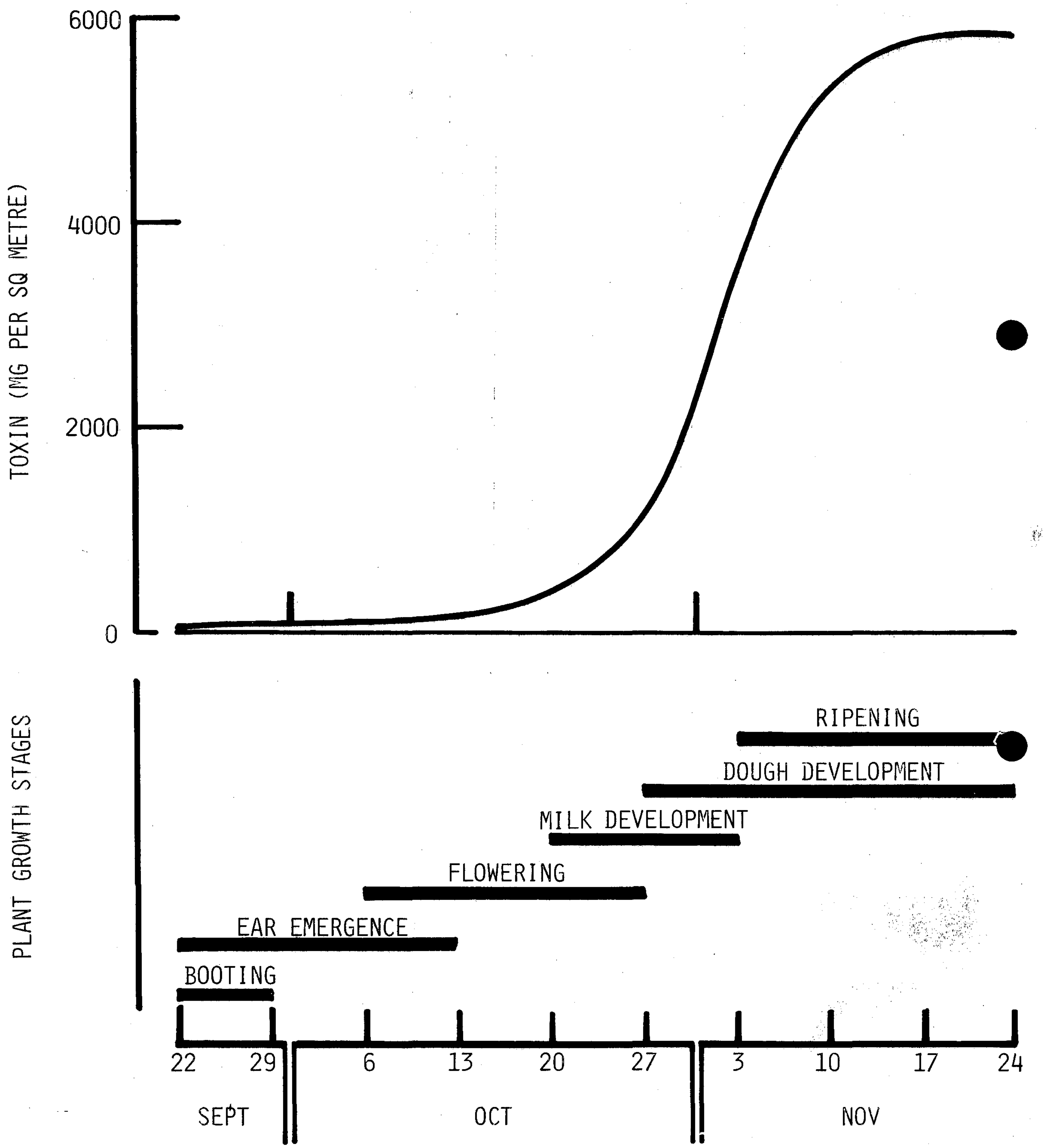
The total number of ryegrass heads, their weights, and the number of galls containing either nematodes or bacteria were estimated for each sample, and the growth stage of the ryegrass was recorded.

Toxicity tests were done on chemical extracts from the infected material collected at each sampling. A sensitive microassay based on the ability of the toxin to inhibit the growth of bacteria *in vitro* was used for these tests.

Results: Preliminary results concerning the density of pasture and the numbers of galls present in pasture at each site during the season were reported in the 1981 experimental summary. Results of the toxicity tests subsequently completed are shown in the following figure. As the results were similar for all sites, only those for Katanning are included in this summary.

Comments: As suggested from field observations, there was a considerable delay between the time when galls became colonized by bacteria and when the galls actually became toxic. Even though galls were fully developed by early October, there was no significant accumulation of toxin until November. Levels of toxin up to 459 µg per m^2 in pasture prior to and during anthesis increased rapidly during the final four harvests (corresponding to ripening) and reached a maximum of 6,292 µg per m^2.

ACCUMULATION OF TOXIN IN PASTURE HARVESTED AT WEEKLY INTERVALS AND GROWTH STAGES OF RYEGRASS AT CORRESPONDING TIMES



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This seasonal pattern is observed each year in field outbreaks of annual ryegrass toxicity. The first outbreaks are typically recorded around Katanning during the last week of October or the first week of November each year. This suggests a close relationship between the physiological maturation of the plant and the accumulation of toxin.