SOME BIOLOGICAL EFFECTS ON PLANT NEMATODES AND THEIR HOSTS OF THE ORGANO-PHOSPHORUS COMPOUND THIONAZIN.

by

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ABSTRACT

The organophosphorus compound Thionazin appears to have systemic properties in that control of Heterodera rostochiensis was achieved in pot experiments where tomatoes were drenched with solutions of the compound two weeks after planting. The presence of Thionazin in root diffusate at concentrations as low as 0.125 p.p.m. had a marked inhibitory effect on hatch of H. rostochiensis larvae. Plant parasitic nematodes were only moderately affected when exposed "in vitro" to the compound but Turbatrix aceti was markedly affected at low concentrations suggesting that the compound had to be ingested for it to be highly nematicidal. When Thionazin was incorporated into agar and Fusarium oxysporum and Agaricus campestris were cultured on this substrate, Ditylenchus myceliophagus and Aphelenchus avenae placed on the cultures were severely affected at concentrations of Thionazin as low as 1 p.p.m. These nematodes exposed to the compound "in vitro" were far less affected. The fungi appeared to take up the compound in very small quantities.

Using labelled Thionazin, absorption by dormant narcissus and tulip bulbs when immersed in solutions of the compound, and uptake and translocation by the roots and foliage were demonstrated. Uptake by dormant bulbs was passive and the relationship between log uptake and the log of the exposure time was linear. Spectrophotometric analysis indicated that the compound was rapidly broken down in both narcissus and tulip bulbs.

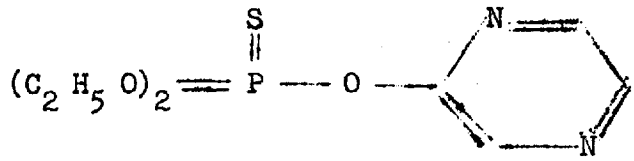
Field experiments showed that by dipping narcissus and tulip bulbs in solutions of Thionazin up to 99% control of Ditylenchus dipsaci living

in the bulbs could be achieved. Very good control of D. dipsaci was obtained over a period of three years when narcissus plants were drenched with Thionazin, but poor control was obtained with similar treatments of tulips. The compound was more phytotoxic to tulip than narcissus but generally the phytotoxicity, at concentrations of Thionazin that gave good nematode control, was low.

TABLE OF CONTENTS.

GENERAL INTRODUCTION.

The organophosphorus compound Thionazin (formerly known as Zinophos and "Experimental Nematicide" E.N. 18133) was discovered by Cyanamid's Stamford Laboratories in 1956. The structural formula of the compound is as follows:



It is a liquid and has a solubility in water of 0.1%. It has a high mammalian toxicity with an oral $L.D._{50}$ value of 4.

The reasons for the toxicity of some organophosphorus compounds to nematodes is by no means clearly understood although the demonstration of the presence in nematodes of acetylcholine and cholinisterase (Melanby, 1955; Krotov, 1957; Rohde, 1960) suggests that the effect may well be due to the phosphorylation and resultant inactivation of enzyme systems. This is one biological effect of the compound and others would include its lethality, its selectivity and its systemic properties, and it is these latter considerations that have been studied in this thesis. The term toxicity - the capacity to produce injury - has been loosely used throughout and in this context means lethality. Thus, the toxicity of the compound has been examined by the measurement of its effect on the development of nematode populations, and to a lesser extent its effect on plants as measured by plant growth; its selectivity as measured by its effect on a few different nematode

species and genera; and its systemic properties as measured by the uptake of the compound by some plants and the effect on nematodes living on these plants.

This type of study is of particular importance in the field of nematology, as although the discovery of wide ranges of compounds, many having high selective toxicities, has opened up new possibilities in the control of noxious organisms, the biologist involved in the control of nematodes has relatively limited chemical resources at his disposal. The bulk of the nematicides used today are compounds which are biologically effective because of fumigant action - Berck (1964) defines a fumigant as a chemical which at a required temperature and pressure can exist in a gaseous state in sufficient concentration to be lethal to a given organism - which implies that this type of compound acts in the vapour phase. Although it is possible under favourable conditions for some organophosphorus compounds like D.D.V.P. and Parathion, and no doubt Thionazin too, to exert a so-called fumigant action by yielding small amounts of toxicants in the vapour phase, organophosphorus compounds, where they are nematicidal, certainly act in a very different way than do the widely used nematicidal halogenated hydrocarbon soil fumigants like D.D., E.D.B., D.B.C.P. and C.B.P. Evidence for this is given by the vapour pressures of the compounds, that for Thionazin is 3×10^{-3} mm. Hg at 25°C., while the value for E.D.B. is 11 mm. Hg. Thus, among this group of compounds with different physical, chemical and biological properties, may well be found some by which nematodes can be controlled not only more efficiently than is achieved by accepted

conventional methods, but also in situations where for reasons of phyto-toxicity or low nematode toxicity, the conventional methods have failed. As the effects of nematode infestations become to a greater extent the limiting factor in plant growth, so the necessity for research in this field becomes more imperative.

In reading this thesis it will become obvious that the work is limited to a large extent to the investigation of the control of nematodes in bulbs by chemical methods, and this has indeed been the main objective in this study. In this process a number of the biological effects of Thionazin have been elucidated, and a number of additional experiments have been carried out to obtain further information on the properties of the compound. However, the author has attempted throughout not to go outside the limits of what Zuckerman (1961) has termed "objective basic research", and each experiment has been planned so as to give information that would either in itself be useful or would assist in explaining the results obtained elsewhere.

PART I. POT AND LABORATORY TESTS ON THE NEMATICIDAL PROPERTIES OF THIONAZIN.

Section I. The Effect of Thionazin on Heterodera rostochiensis.

A. Pot Experiments to establish the Effect of Time of Application on H. rostochiensis.

Many workers have tested the effects of the application of chemicals to pots infested with Heterodera spp. and assayed the results by various means. Peters (1952a) gives an account of the conduct of pot experiments and the limitations inherent in this type of experimentation while Hague and Omidvar (1962) discuss the merits of different means of assay. Thionazin has been quite widely reported on- Cooper and Sasser (1962) give an account of its field application for the control of nematodes in peanuts; Motsinger and Morgan (1960) report control of root knot on tobacco and Motsinger (1961) again reports on further evaluations.

An aspect which has received relatively little attention is the effect of time of application on the nematode populations. Schindler and Henneberry (1962) tried two times of application in their work on roses and found no difference, in fact obtained no control of Xiphinema americanum and Hoplolaimus spp. However, Den Ouden and Kaai (1963) reporting on the use of the chemical in microplots to control H. rostochiensis found that the application of the chemical two weeks after planting gave better control than treatments at five and eight weeks after planting and that repeated applications did not result in better control.

The purpose of the experiments here was to obtain more information about these possibilities.

Materials and Methods.

Soil containing approximately one cyst of Heterodera rostochiensis per 2 gm. of soil was collected and the soil thoroughly mixed by coning and quartering and sieved to get rid of the coarser particles and to give a good tilth. The soil was then added to 8" glazed cylindrical clay pots at the rate of 2 litres of soil per pot. The treatments consisted of four concentrations of the chemical, 0, 12.5 p.p.m., 25 p.p.m. and 50 p.p.m. with three treatment occasions, viz. 3 weeks before planting, 2 weeks after planting and 4 weeks after planting. The experiment was replicated three times. The pots were placed in the greenhouse, treatments randomised as regards position on the greenhouse bench, and three weeks after the first treatments the pots were planted to well developed Eurocross A tomato seedlings. Treatment was carried out by drenching the pots with 500 c.c. of water containing the chemical at the various concentrations. Though Thionazin is soluble in water up to 1000 p.p.m. the chemical was formulated with volumes of acetone and Triton X 100 equal to that of Thionazin, and this done because there was some settling out when the slightly impure commercial technical grade was added to water. The control treatments received a quantity of acetone and Triton X 100 equal to that in the highest treatment of the chemical.

After five months the plants were removed from the soil and the cysts washed out according to the method of Fenwick (1940) and counted, and their content determined according to Bijloo (1954).

Results.

Very high kills are obtained from all treatments so the criteria here are more "difference in effects" than "degree of effect". Results are expressed as percentage mortalities and analysis of variance was carried out on the angular transformation of these data.

Table 1. Mean percentage mortalities of larvae per 200 gm. soil (mean of 3 replicates).

Treatment Time	Conc. of Chemical p.p.m.			Mean
	12.5	25.0	50.0	
3 weeks before planting	90	85	91	89
2 weeks after "	91	95	98	95
4 " " "	83	90	91	88
Mean:	88	90	93	

Table 2. Mean percentage killed based on the counts of cysts per 200 gm. soil (means of 3 replicates).

Treatment Time	Conc. of Chemical p.p.m.			Mean
	12.5	25.0	50.0	
3 weeks before planting	67	72	75	71
2 " after "	83	86	92	87
4 " " "	66	76	80	74
Mean:	72	78	82	

Analysis of the data (see Tables 57A and 57B in the Appendix) indicated that no matter what criterion of evaluation was used, i.e. cysts per gramme or larvae per gramme, the overall effect of time of application of the treatments led to a significantly high variance at the 0.1% level and that of concentration at the 1% level. Analysis of the concentration factor at each individual time of treatment showed that these were significantly different in the post-plant treatments but not the pre-plant treatment (see Table 58 in Appendix). Finally analysis of the percentage decrease or reduction of cyst content (see Table 57C in Appendix) showed that both time and concentration factors were non-significant.

B. Tests to establish the Effect of Thionazin on Hatch of H. rostochiensis larvae.

Ever since Baunacke (1922) reported that larvae of Heterodera rostochiensis were stimulated to hatch by a diffusate from the roots of growing potato plants, this phenomenon has been carefully studied. Wallace (1963) reports in detail on some of the physical factors involved, and much work has been done on the stimulation of hatch using artificial substances (Clarke and Shepherd, 1964; Wallace, 1956). The hatching test has been widely used to establish the effects of nematicides; treated cysts being placed in root diffusate, the hatch estimated and the effect of treatment so measured (Hague, 1959; Peachey, Rao and Chapman, 1963). What does not seem to have been examined very carefully is the hatch of Heterodera spp. in the presence of both nematicide and root diffusate. There are examples

of this type of study, but the compounds involved are not generally regarded as being nematicidal, though Smedley (1939) examined the effects of sub-lethal dilutions of isothiocyanates. Morgan (1925) observed that the diffusate from mustard had an apparently inhibitory effect on the hatch of potato root eelworm. Carroll and McMahon (1937) report that the presence of iron oxide in water used for the collection of root diffusate also inhibited hatch in Heterodera spp. Johnson and Townsend (1949) found that ammonium carbonate inhibited emergence when the level of free ammonia reached 100 p.p.m. and that inhibition was thus correlated with a high pH value. Hague (1958) reports that the removal of water from hatching factor inhibits hatch, this fact being due to the increased concentration of the hatching factor or an increase in the salts or other substances present, and finally Schreiber and Sembner (reported by Shepherd, 1962) claimed that certain solanaceous plants produce a diffusate containing factors inhibiting to H. rostochiensis.

It would seem logical in examining the effects of a nematicide on cyst hatch to measure this effect in the presence of both the chemical and the root diffusate - this presumably is the condition which prevails in the soil when the nematicide is applied around a growing plant, and this observation is what has been attempted here.

Materials and Methods.

The hatching tests were carried out according to the method described by Fenwick and Widdowson (1958). Preliminary experiments indicated

that the presence of Thionazin in root diffusate at concentrations of 0.125 p.p.m. inhibited hatch slightly while a concentration of 0.5 p.p.m. had a distinct effect (see Appendix Tables 59 and 60). For this reason the treatments consisted of concentrations of Thionazin of 0.125 p.p.m., 0.5 p.p.m. and 2.0 p.p.m. in root diffusate, the experiment being replicated six times initially and running over a period of 16 weeks in total. After four weeks the replicates were split into two programmes, one receiving only root diffusate, the other continuing to receive root diffusate plus Thionazin. After eight weeks all treatments consisted of root diffusate only.

The concentrations of the chemical were obtained by making up concentrations of Thionazin of 4, 1 and 0.25 p.p.m. and adding 1 c.c. of these solutions to 1 c.c. of root diffusate; in the control treatment 1 c.c. of distilled water was added to the diffusate to eliminate any dilution effect in the other treatments. Hatch was estimated weekly, the diffusate and chemical therefore being renewed once per week.

Results.

The results obtained are illustrated in Fig. 1, where the cumulative mean hatch is plotted on a log scale against time. The cumulative mean hatch values after different periods are given below.

Explanation of Fig. 1.

a = Root diffusate only.

b = Exposure of cysts to a concentration of 0.125 p.p.m. of Thionazin in root diffusate for 4 weeks followed by root diffusate only.

b_1 = Exposure of cysts to a concentration of 0.125 p.p.m. of Thionazin in root diffusate for 8 weeks followed by root diffusate only.

c = As for b, but using a concentration of 0.5 p.p.m. of Thionazin.

c_1 = As for b_1, but using a concentration of 0.5 p.p.m. of Thionazin.

d = As for b, but using a concentration of 2.0 p.p.m. of Thionazin.

d_1 = As for b_1, but using a concentration of 2.0 p.p.m. of Thionazin.

FIG. 1.

Hatching of Heterodera rostochiensis cysts in response to low concentrations of Thionazin in the presence of root diffusate

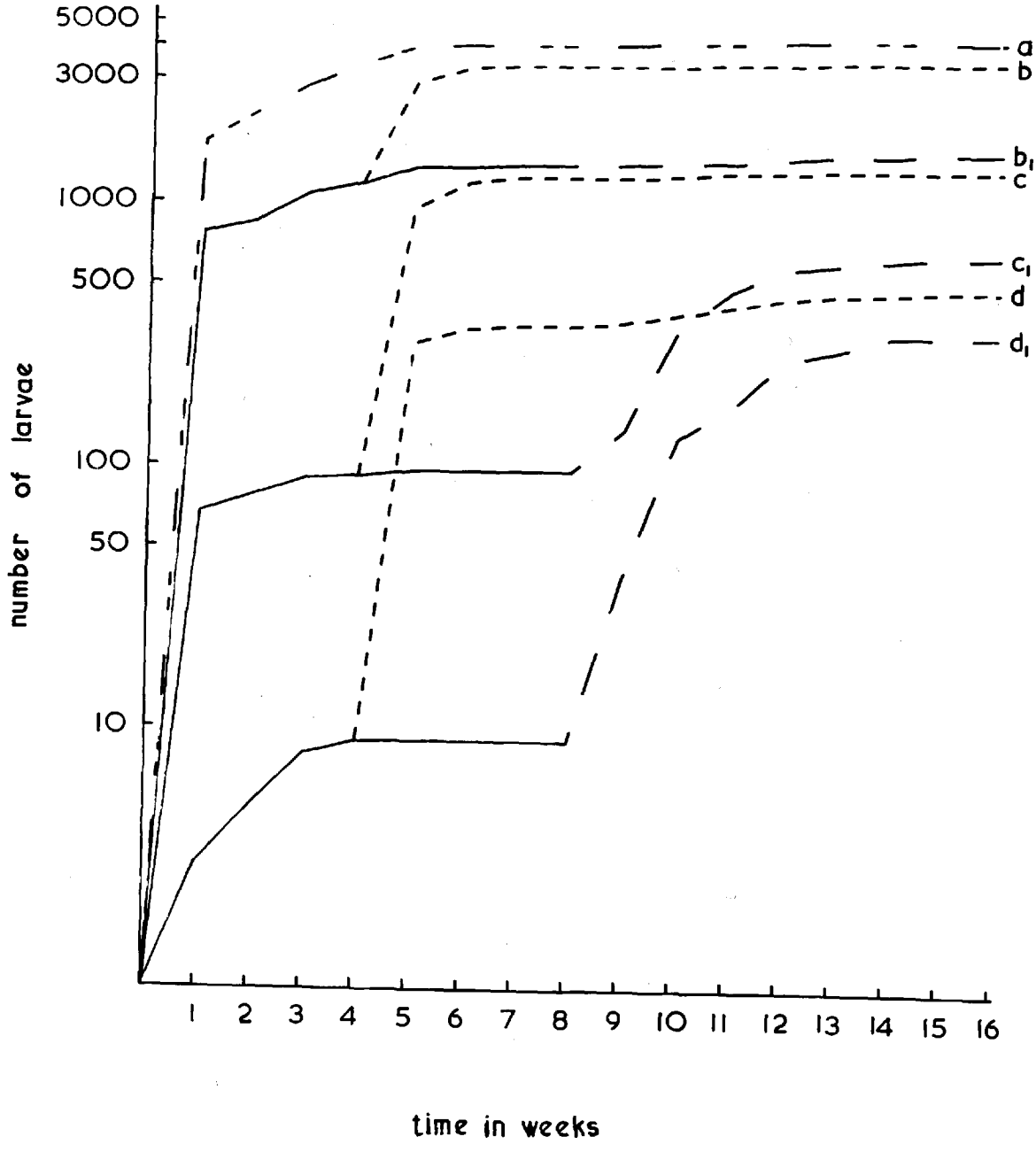


Table 3. Cumulative mean hatch after 4 weeks from H. rostochiensis cysts exposed to root diffusate containing Thionazin in solution (means of 6 replicates).

Conc. of chemical p.p.m.	0	0.125	0.5	2.0
No. of larvae	3381	1177	103	8

The effect of concentration here is highly significant (P = 0.1) (see analysis in Appendix Table 61).

Table 4. Cumulative mean hatch after eight weeks under two programmes of H. rostochiensis cysts exposed to root diffusate containing Thionazin in solution (means of 3 replicates).

Conc. of chemical p.p.m.		0	0.125	0.5	2.0	Mean
No. of larvae:	Programme 1	3473	2976	1296	336	2020
	Programme 2	4534	1706	56	9	1576
Mean		4003	2341	676	173	1798

Table 62 in the Appendix shows the analysis of these data. The concentration factor is significantly variable at the 0.1% level as is the programme factor, with their interaction significant at the 1% level.

Table 5. Cumulative mean hatch after sixteen weeks under two programmes of H. rostochiensis cysts exposed to root diffusate containing Thionazin in solution (means of 3 replicates).

Conc. of chemical in p.p.m.		0	0.125	0.5	2.0	Mean
No. of larvae:	Programme 1	3615	3296	1715	238	2275
	Programme 2	4790	1872	495	183	1881
Mean		4203	2584	1105	421	2078

After sixteen weeks the concentration factor is again significantly variable at the 0.1% level while programmes just fail to achieve significance at the 5% level (see table 63 in the Appendix).

Section II. The Effect of Thionazin on Ditylenchus dipsaci.

A. Effect of "in vitro" Exposure of D. dipsaci to Thionazin.

It would seem that the first way of testing the nematicidal or other properties relating to nematodes, of any chemical would be to put the eelworms in a solution of the chemical and in some way to measure the effect, if any, on the nematode. The obvious difficulty here is to measure what effects there are and to interpret these. The so-called "movement assay" is widely used and is a convenient criterion for measuring "mortality" although its shortcomings are obvious. Motionless individuals are not necessarily dead, nor will they necessarily die soon. Conversely, those which exhibit motion are not necessarily capable of attacking a host. Staining methods for distinguishing dead nematodes are moderately plentiful - Shepherd (1962a) reports on the use of New Blue R, Fenner (1962) on Phloxine B and Doliwa (1956) on Chrysoidin. It is the writer's opinion, having used these stains, that they require a very practised eye and were thus unsatisfactory in this study as being too subjective.

The work in this section was an attempt to obtain some information on the chemical Thionazin by exposing Ditylenchus dipsaci "in vitro" to various concentrations of the chemical for different periods of time at different concentrations, and to measure the effects by "movement".

Materials and methods.

In all cases in the following description only 4th stage larvae and adults were counted (these being easily distinguishable from the other

larval forms though not easily from each other). The nematodes used were obtained from infested tulip bulbs by means of the Seinhorst mistifier.

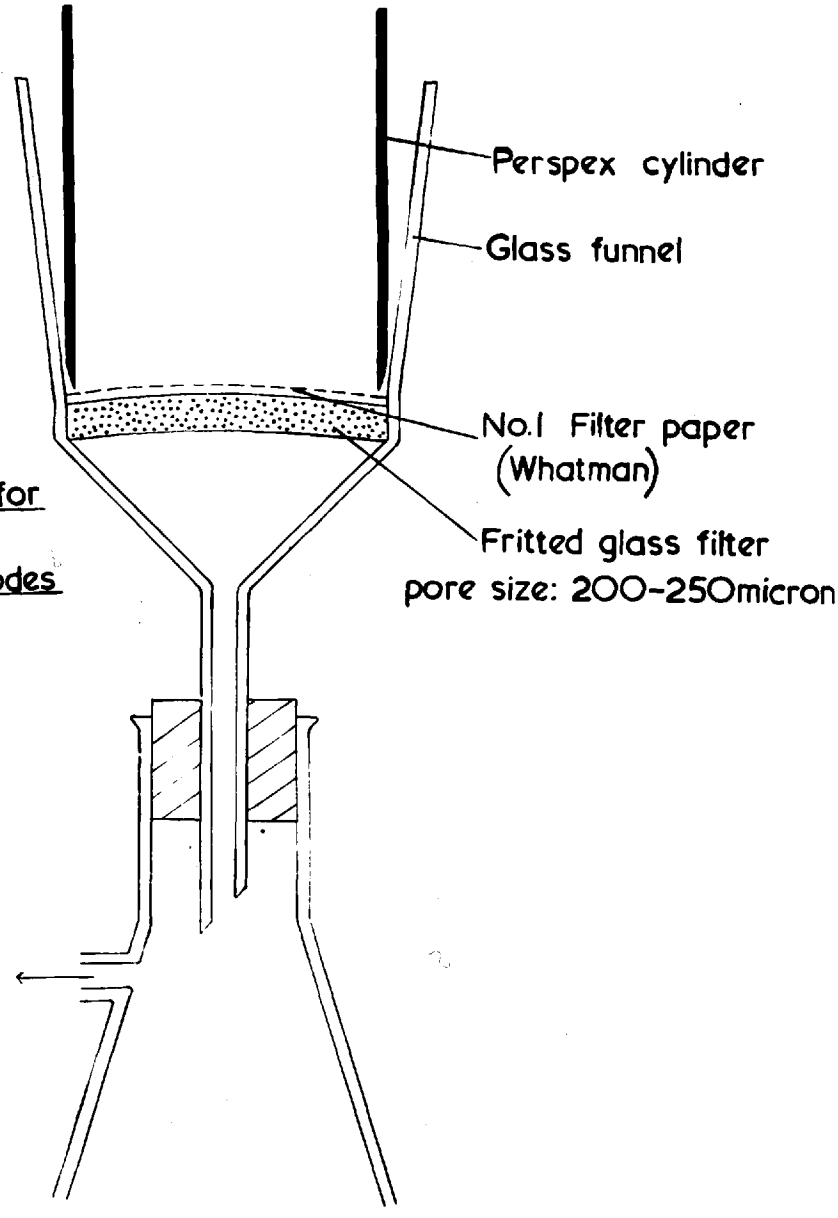
Treatments were carried out in the bath described by Purnell (1963) except for the fact that the apparatus was altered to take two extra containers. Solubilised concentrations of the chemical were prepared to give concentrations of the nematicide of 1800, 600 and 200 p.p.m. when the suspension of nematodes was added, treatments were carried out at 80°F., 90°F. and 100°F. for 2, 6 and 18 hours and assays carried out at 1, 5 and 10 days. The control treatments contained concentrations of acetone and Triton X 100 equal to that used to solubilise the highest concentration of the chemical, solubilisation being achieved by using equal quantities of chemical, solvent and emulsifier. Air was bubbled through the containers holding the treated nematodes where the assay was delayed for 5 or 10 days. Each treatment was replicated twice.

The method of assay described by Purnell (1963) was to wash the nematodes over three 300 mesh sieves to remove all traces of the nematicide. This worker reports a loss of 40% of the nematodes in this process.

The method used here was slightly different. The nematicide-nematode suspension was poured on a filter paper in the apparatus illustrated in Fig. 2 and filtered under pressure, washed by pouring a further two litres of water over it, continuing the pressure filtration. The filter paper was then removed and inverted over two Coldstream cotton wool filter discs lying on a brass sieve over a petri dish of water (see Fig. 3).

The effectiveness of this method for carrying out movement assays

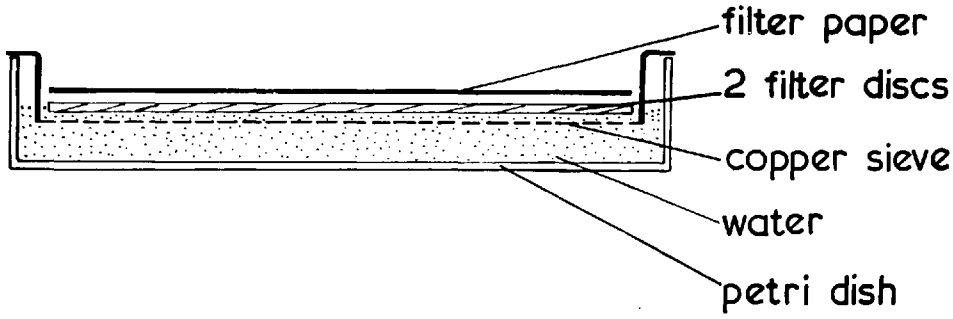
Fig. 2.



Filtration apparatus for separation of nematodes from nematicide.

Fig. 3.

Equipment for nematode movement assay



was measured as follows. Individual samples of nematodes were counted and the nematodes killed using 0.1% Lugols solution. When all the nematodes were observed to be inactivated the suspension was filtered as described above, and in one experiment inverted over one Coldstream filter disc, in another over two discs. In a third case the nematodes were killed by heating to 65°C., filtered as above and inverted over two discs. Results are given below.

Table 6. Number of inactivated nematodes passing through Coldstream cotton wool filter discs after 18 hours.

A. Nematodes, inactivated with 0.1% Lugols solution, on one Coldstream disc.

Sample No.	Original Nematode Count	Number passing through	% passing through
1	148	16	10.8
2	265	8	3.0
3	161	10	6.2
4	215	8	3.7

B. Nematodes, inactivated with 0.1% Lugols solution, passing through two Coldstream discs.

Sample No.	Original Nematode Count	Number passing through	% passing through
5	211	1	0.5
6	193	2	1.0
7	194	2	1.0
8	172	2	1.2

C. Nematodes, inactivated by heat, passing through two Coldstream discs.

Sample No.	Original Nematode Count	Number passing through	% passing through
9	169	2	1.2
10	188	2	1.1
11	125	2	1.6
12	191	2	1.1

In all cases the nematodes that had passed through the filters appeared to be inactive.

The method was suitable for withholding inactivated nematodes - the final criteria would be its suitability for allowing active nematodes to pass through.

A similar experiment to that described above was carried out except that the nematodes were not inactivated. After periods of time of ½, 1, 4, 6 and 12 hours the copper sieves containing the filter papers and filter discs were taken off the petri dishes and replaced on fresh water and the nematodes that had passed through counted. The results are given in Table 7.

This method effectively retains inactivated nematodes and allows active eelworms to pass through. The counts in Tables 6 and 7 represent actual numbers of nematodes and are not estimates. In all cases the final suspension obtained from the petri dishes was filtered down on a 1 - 1.5 μ pore size fritted glass funnel to a suitable volume for counting. The suspension was then washed off the filter into the counting dish.

Table 7. Active nematodes passing through two Coldstream filter discs after different periods of time.

Sample	Original Count	½ hour		1 hour		4 hours		6 hours		12 hours	
		Count	Total %	Count	Total %	Count	Total %	Count	Total %	Count	Total %
1	162	128	79.0	11	85.8	13	93.8	2	95.0	3	96.9
2	161	128	79.5	7	83.8	14	92.5	0	92.5	0	93.7
3	174	156	89.6	10	95.4	0	95.4	0	95.4	0	95.4
4	215	180	83.7	10	88.3	7	91.6	1	92.0	3	93.4
5	126									118	93.7
6	84									82	97.6
									Mean % Recovery after 12 hours		95.1

Results.

The overall results expressed as angular transforms of the percentage mortality are given in the Appendix in Table 64. The analysis of variance of the data is given in Table 65. The overall treatment effects are given in Table 8.

The marginal means are not always the exact values from the data in the table nor is the general mean therefore always 35, this being due to the retransformation of the data from angles and rounding of the decimals.

Table 8. Overall effect on movement of D. dipsaci of various treatments. Effects expressed as mean % mortalities (retransformed from angles).

A. Effect of Temperature.

Temperature	% Mortality
80°F.	12
90°F.	32
100°F.	66
MEAN	35

B. Effect of Exposure Time.

Exposure (hrs.)	% Mortality
2	23
6	32
18	51
MEAN	35

C. Effect of Concentration of Thionazin.

Concentration p.p.m.	% Mortality
200	26
600	39
1800	41
MEAN	35

D. Effect over Examination Time.

Examination Time (Days)	% Mortality
1	36
5	30
10	39
MEAN	35

E. Effect over Temperature x Time Exposed Interaction.

Temp. °F. / Time (hrs.)	100	90	80	Mean
2	39	23	10	23
6	61	28	13	32
18	91	47	13	51
Mean	66	32	12	35

As can be seen from the analysis the main effects are significant, with temperature differences very marked. The reasons for the significant two-factor interactions are not obvious and the author cannot give an explanation for them; with the exception of the large FT interaction they should probably be ignored. The reason for the highly significant Temperature x Exposure Time interaction is that the lethal effect of increasing time of exposure operates markedly at 100°F. but scarcely at all at 80°F., as Table 8E shows.

Probably the most notable factor is that although the effects of Thionazin concentration are significant, the compound has no very marked contact effect on the movement of Ditylenchus dipsaci adults or fourth stage larvae, even at 1800 p.p.m.

B. "In vitro" Toxicity to D. dipsaci of Possible Breakdown Products of Thionazin.

Results in Section II A (page 19) indicate that Thionazin has only limited effect on movement of D. dipsaci when these nematodes are exposed to the chemical "in vitro". Allen (1960) maintains that there is little evidence to support the idea of the necessity of penetration of a nematicide through the cuticle. He continues that it is easily observed that when nematodes are exposed to water solutions of vital stains, the penetration is most rapid through the oral opening, phasmids, vulva and anus. He concludes that there is no reason to assume that most nematicidal chemicals do not enter the body of the nematode through these avenues as well as through the cuticle. If this theory is correct then one of many possible explanations of the results obtained in Section IIA is that in plants or in soil Thionazin is broken down to some other compound that is nematicidal, and that this process does not take place in the "in vitro" studies. Dr. F. Call treated Thionazin in various ways conducive to breakdown and the products of these treatments and other compounds were tested "in vitro" against D. dipsaci.

Materials and Methods.

The method described and used in Section IIA is a satisfactory one but it does require quite a large volume of treating liquid to maintain adequate stirring. This equipment was used because it was available and because it included facilities for temperature variations.

The method used in this examination was similar to that described by Simard (1964) with minor modifications (see Fig. 4). In this study 2 cc. of Thionazin were delivered into stoppered reaction tubes (Quickfit tubes No. 1314/23) and to this was added 2 cc. of the nematode suspension. (Where this technique is referred to later in this text, the volume of liquid used is not always as stated here, but the general procedure is the same).

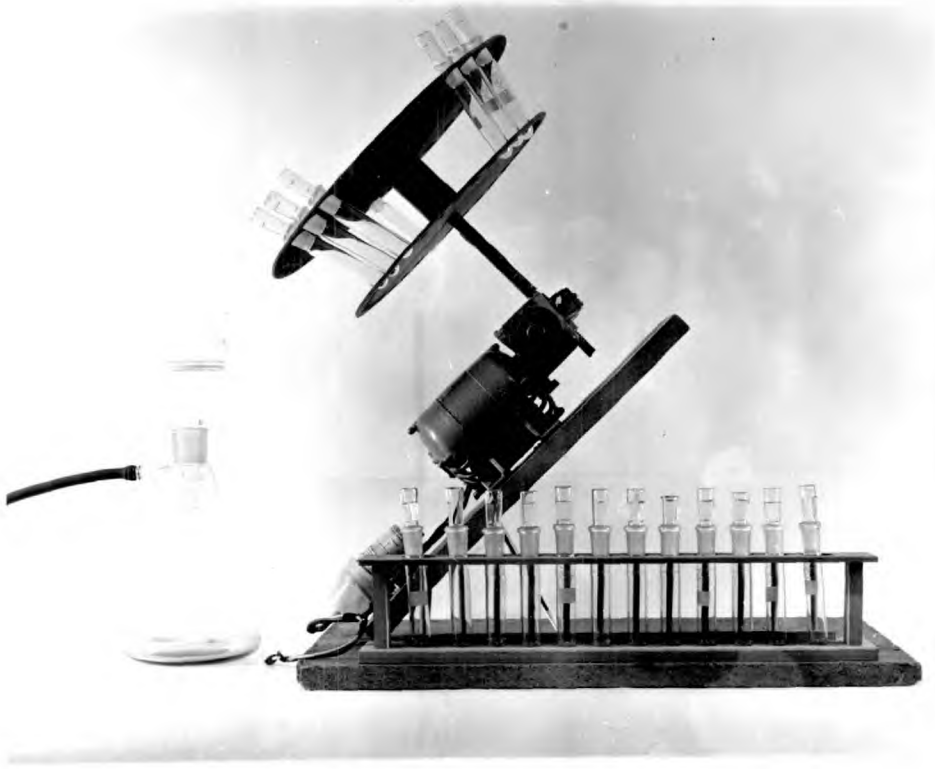
The tubes were placed on a turntable (see Fig. 4) which provided gentle agitation, and kept in a constant temperature room maintained at 25°C. After treatment the tubes were removed and the contents poured and washed on to 7 cm. Whatman No. 1 filter paper folded on glass funnels. The suspension was allowed to filter and the nematodes washed by allowing fresh water to filter through on four occasions. After washing, the filter papers were removed from the funnels and inverted on the movement-assay equipment illustrated in Fig. 3. The nematodes were allowed to migrate overnight and the number of nematodes that had passed through estimated.

The compounds tested were:

A) The product of acid hydrolysis of Thionazin.

B) " " " alkaline " " "

Fig. 4. Turntable, reaction tubes and fritted glass funnel for *in vitro* toxicity tests.



C) The product obtained when Thionazin was incubated for 24 hours with dormant narcissus bulb material.

D) The product obtained when Thionazin was incubated for 24 hours with growing narcissus bulb material.

E) The oxygen analogue of Thionazin.

F) Sodium pyrazinolate.

G) Water control.

The oxygen analogue of Thionazin and sodium pyrazinolate were provided by the manufacturers of Thionazin. The compounds were tested at about 500 p.p.m. for 18 hours at 25°C., each treatment being duplicated.

Results.

The results in the table below are expressed as percentage inactivated or possibly killed, being calculated in each case on the number of nematodes used initially.

Table 9. Effect of "in vitro" exposure to various possible forms of Thionazin on movement of D. dipsaci (mean of two replicates).

Compound	% inactivated
A	3.5
B	15.0
C	8.2
D	10.7
E	17.4
F	7.2
G	5.5

The above results are obviously negative though there has been some inhibition of movement. It is a failing in this experiment not to have included unmodified Thionazin but what was being looked for was a high kill, and this was not obtained. Possibly the exposure time should have been longer, or the concentration higher but it would seem that none of the compounds tested were highly biologically active.

Section III. The Effect of Thionazin on Ditylenchus myceliophagus.

A. Effect on Ditylenchus myceliophagus, living on Agaricus campestris, of exposure of the Fungus to Thionazin.

The effect of the addition of chemicals to the substrate of fungi on which nematodes are living has to the author's knowledge never been studied. This field might well lend itself to critical study, for to quote Townshend (1964) "mycophagous nematodes lend themselves to critical examinations of factors affecting nematode populations, as fungi and their substrates can be manipulated with considerable facility". Presumably treatment could be carried out in two obvious ways. Firstly the fungus could be irrigated with the chemical, or secondly the chemical could be incorporated in the substrate and the fungus cultured on this.

This type of study should give additional information on the biological properties of the chemical, even if this information is limited to the fact that the chemical has no fungicidal effects when the fungus is subjected to it in this way.

Materials and methods.

Irrigation treatments were carried out as follows. Well developed cultures of Agaricus campestris on potato-dextrose agar (P.D.A.) were bathed for 24 hours with 10 cc. volumes of Thionazin at 0, 10, 100 and 1000 p.p.m. formulated with alrodyne and propylene glycol and one treatment of 10 p.p.m. prepared from an analytical grade Thionazin in water. After irrigation the excess fluid was poured off after which the plates were left to dry for 24 hours, and then inoculated with 1 cc. suspensions of Ditylenchus mycelio-

phagus containing about 130 nematodes. After one week the nematodes were extracted (see later) and their numbers estimated. The experiment was replicated three times.

The second system, the impregnation of the substrate with Thionazin utilised a suggestion by Dr. B.E.J. Wheeler (personal communication) which approximates the method used by Eckert (1962). A known weight of Thionazin was dissolved in a known volume of analar acetone to give concentrations twenty times as great as that required for the treatments. Twenty cc. of distilled water was added to the requisite number of P.D.A. tablets in McCartney tubes and autoclaved in the usual way at 15 p.s.i. pressure for 15 minutes and thereafter cooled to and held at approximately 60°C. A volume of 1 cc. of the relevant acetone/Thionazin solution was added to each tube and vigorously shaken. This would give a concentration of Thionazin in the agar of approximately 1/20 of that in the acetone/Thionazin solution. The control treatments received 1 cc. of acetone only. Acetone boils at 56°C. so it is probably fair to assume that most of it evaporated on contact with the agar. The agar was then poured into sterile petri dishes and allowed to stand open until the agar had solidified. When the plates had cooled they were inoculated with Agaricus campestris and incubated for two weeks at 25°C. Each plate was then inoculated with a 1 cc. suspension of D. myceliophagus and again incubated at 25°C. for two weeks.

Nematodes were extracted from the cultures using the Oostenbrink direct cotton wool filter extraction technique as described by Townshend (1964), the only difference to Townshend's description being that no

sterilising solution was added. The fungal culture was roughly chopped up with a spatula, placed on a facial tissue and left for 24 hours after which the sieve was removed and the number of nematodes that had passed through estimated.

Results.

The results of the irrigation experiment are given below. The nematode inoculum per plate was about 130 nematodes.

Table 10. Number of Ditylenchus myceliophagus nematodes obtained from cultures of Agaricus campestris previously irrigated with Thionazin (mean of 3 replicates).

Concentration of chemical p.p.m.	No. of Nematodes	Population Growth Factor
0	1660	12.8
10 (analytical grade)	178	1.4
10 (technical ")	95	0.7
100 (" ")	104	0.8
1000 (" ")	57	0.4

An obvious difference exists between the control and the other treatments indicating that whereas the population on the control plates has multiplied 12 fold, none of the plates bathed with Thionazin have supported reproduction except perhaps the treatment using the analytical grade of the chemical. However, analysis of the log transformation of

the data indicates that the treatments were not significant.

An unsuccessful attempt was made to subculture the plates of fungus on fresh potato dextrose agar.

In the agar impregnation experiment the chemical was incorporated as described and approximately 200 nematodes added in one c.c. of water when the fungus was well developed. The number of nematodes extracted after two weeks is given below.

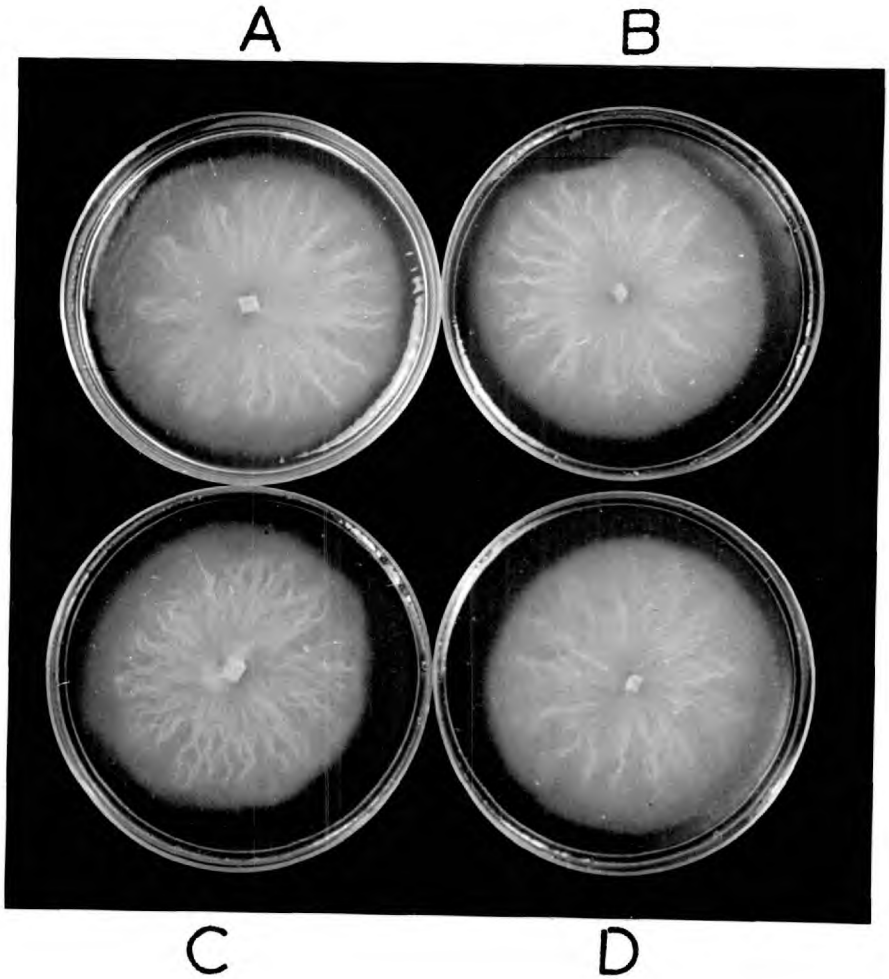
Table 11. Number of D. myceliophagus obtained from plates of Agaricus campestris when the agar was impregnated with Thionazin (mean of 3 replicates).

Concentration of chemical p.p.m.	No. of Nematodes	Population Growth Factor	% Mortality
0	9583	47.9	-
0.25	2400	12.0	75.0
1.0	380	1.9	96.1
4.0	5	0.02	99.9

There was no obvious effect on the mycelial growth of the fungus. Fig. 5 shows the effect on mycelial growth of Agaricus campestris where concentrations of the chemical of 0, 10, 50 and 250 p.p.m. were incorporated in the agar.

Obviously the incorporation of Thionazin into the agar has a very marked effect on the nematodes living on the fungus and that at very

Fig. 5. The effect on the mycelial growth of Agaricus campestris subsequent to the incorporation of different concentrations of Thionazin in the medium.



A = Thionazin at 0 p.p.m.
B = Thionazin at 10 p.p.m.
C = Thionazin at 50 p.p.m.
D = Thionazin at 250 p.p.m.

low concentrations, indicating the presence of a highly toxic compound.

B. The Effect of "in vitro" Exposure of Ditylenchus myceliophagus to Thionazin on Reproduction of the Nematodes.

The possibility exists that by exposing nematodes to Thionazin "in vitro", their reproductive potential is reduced, and an experiment was conducted to establish this. D. myceliophagus nematodes were extracted from mushroom cultures and the nematodes exposed to concentrations of Thionazin of 0, 200, 600 and 1800 p.p.m. of the chemical - formulated with propylene glycol and alrodyne - for 18 hours at 80°F., the experiment being conducted as described in Section IIA. After treatment the nematodes were allowed to pass through cotton wool filters. There was a small degree of inactivation. The nematode suspensions were concentrated to give approximately equal concentrations of the nematodes and 1 c.c. of each suspension was added to well developed mushroom cultures on potato-dextrose agar. Approximately 260 nematodes were put on each plate, the experiment was replicated four times, and the nematodes extracted after three weeks.

Results.

Table 12. Number of D. myceliophagus obtained from an initial inoculum of the nematode exposed to Thionazin "in vitro" and cultured on A. campestris (mean of 4 replicates).

Conc. of chemical p.p.m.	No. of Nematodes	Population Growth Factor
0	5947	22.9
200	7718	29.7
600	7797	30.0
1800	6013	23.1

The analysis of variance of the log transformation of the data indicates that treatments did not fluctuate significantly, reproduction was therefore equal in all cases and exposure to the chemical in this way had no effect on reproduction.

Section IV. The Effect of Thionazin on Aphelenchus avenae.

A. Effect on Aphelenchus avenae, living on Fusarium oxysporum, of Exposure of the Fungus to Thionazin.

The results in Section IIIA indicate that Thionazin incorporated into the substrate on which Agaricus campestris is growing has a marked effect on Ditylenchus myceliophagus living on the fungus. It would be of value to test this effect on another fungus and another nematode.

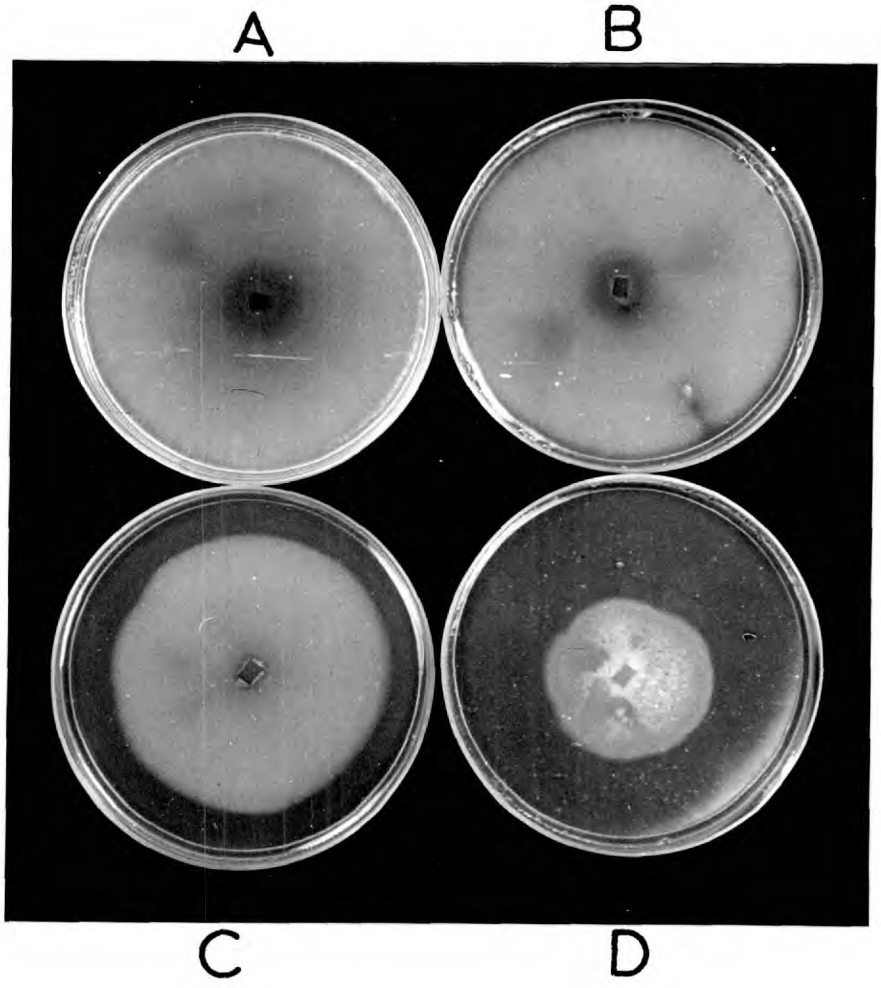
Materials and methods.

Cultures of Fusarium oxysporum and of Aphelenchus avenae were obtained and Thionazin was incorporated into the potato dextrose agar as described in Section IIIA (page 32) and the plates inoculated with the fungus. When these were well grown the plates were inoculated with the nematode. Preliminary experiments indicated that complete control was not achieved even at concentrations of Thionazin of 25 p.p.m. but that concentrations as low as 1 p.p.m. gave good control. Concentrations of 0, 0.04, 0.2 and 1 p.p.m. were used, the experiment being replicated four times. Each plate received about 100 nematodes.

Results.

Within three days of inoculating the plates with the nematodes, there was an obvious effect on the movement of the nematodes. Those on the control plates moved smoothly and were active, whereas those on the high treatment plates exhibited greatly reduced activity and tended to move in a jerky manner. Many of the nematodes adopted a "rolled up" posture

Fig. 6. The effect on the mycelial growth of Fusarium oxysporum subsequent to the incorporation of different concentrations of Thionazin in the medium.



A = Thionazin at 0 p.p.m.
B = Thionazin at 5 p.p.m.
C = Thionazin at 50 p.p.m.
D = Thionazin at 500 p.p.m.

though they continued to move for a long period of time.

Again, as in the case of Agaricus campestris, there was no obvious effect on the growth of the fungus. Fig. 6 shows the effect on development of the fungus of far higher concentrations of the chemical. At a 5 p.p.m. Thionazin concentration, the fungus grew as well as it did in the untreated controls.

Table 13. Number of Aphelenchus avenae obtained from plates of Fusarium oxysporum when the agar was impregnated with Thionazin (mean of 4 replicates).

Conc. of Chemical p.p.m.	No. of Nematodes	Population Growth Factor	% Mortality
0.0	3560.0	35.6	-
0.04	3873.7	38.7	-
0.2	1368.7	13.7	61.6
1.0	63.5	0.6	98.2

Again, as was the case in Section IIIA, there is a very marked effect on the nematodes, again at very low concentrations, indicating that Thionazin, or a breakdown product of Thionazin, is very highly nematicidal when applied in this way.

B. The Effect of "in vitro" Exposure of Aphelenchus avenae to Thionazin on Reproduction of the Nematode.

As in Section IIIB, the effects recorded in Table 13 could be due to the nematodes failing to reproduce, or move through the facial tissue, because of being in contact with the nematicide for long periods. The following experiment was carried out to determine if this was the case.

Materials and methods.

Aphelenchus avenae were exposed to concentrations of Thionazin of 0, 4, 40 and 400 p.p.m. in water for two weeks at 25°C. The experiment was replicated three times (about 200 nematodes being used per treatment) and carried out in stoppered quick-fit tubes, the conduct of this experiment being exactly as described in Section IIB except that every three days the tubes were opened to allow air exchange. After two weeks the nematodes were allowed to migrate through facial tissues and counted. Twenty nematodes from the bulked samples of each treatment were randomly selected and placed on well developed colonies of Fusarium oxysporum and incubated at 25°C. for three weeks. After this period the nematodes were extracted and counted.

Results.

Exposure to Thionazin for two weeks at concentrations up to at least 40 p.p.m. does not appear to have any effect on the ability of nematodes capable of movement to reproduce and to produce viable offspring.

Table 14. Effect on movement of Aphelenchus avenae exposed to Thionazin concentrations for 2 weeks (mean of 3 replicates).

Conc. of chemical p.p.m.	No. of Nematodes	% Mortality
400	16	89
40	94	34
4	91	36
0	142	-

Table 15. Number of Aphelenchus avenae obtained from an initial inoculum of nematodes exposed to concentrations of Thionazin for two weeks and cultured on F. oxysporum.

Conc. of chemical p.p.m.	No. of Nematodes	Population Growth Factor
0	9030	451.5
4	11585	579.2
40	12300	615.0
400	0	0.0

Section V. The Effect on Turbatrix aceti of "in vitro" Exposure to Thionazin

As shown in Sections II, III and IV Thionazin has a limited effect on both movement and reproduction on plant parasitic nematodes when exposed to the chemical "in vitro". The use of free-living bacterial-feeding nematodes for similar treatments could provide additional information on the properties of the chemical because of the apparently more active movement, different feeding habits and structural differences of these nematodes. A number of experiments were carried out in an attempt to detect what, if any, effects Thionazin had on Turbatrix aceti.

Materials, Methods and Results.

The nematodes were extracted from well developed cultures by the method described by Peters (1952), exposed to the chemical by the method described in Section IIB, and initially an assay conducted by movement observation. Turbatrix aceti exhibits negative geotaxis and Peters (1952) reports that the nematodes do not move readily through cotton wool filters. However, observations indicated that the population available would move through two Coldstream cotton wool filter discs as shown in the table below.

Table 16. Movement of Turbatrix aceti through cotton wool filters after 6 hours.

No. of Nematodes used	Recovery	% Recovery	Mean % Recovery
336	243	72.3	
376	316	84.0	
344	274	79.9	78.7

Freshly extracted T. aceti were exposed to concentrations of Thionazin of 0, 4, 40 and 400 p.p.m. in water for 27 hours at 25°C., washed free of the nematicide and allowed to migrate overnight (as in Section IIB). The nematodes that had passed through were killed using 0.1% Lugols solution and their number estimated. The results are given below.

Table 17. Effect, on movement of Turbatrix aceti, of exposure to Thionazin for 18 hours at 25°C. (mean of 3 replicates).

Conc. of Thionazin p.p.m.	No. of Nematodes
0	824
4	645
40	854
400	671

There would appear to be no effect on movement of the eelworms but observation of treated nematodes showed that whereas the nematodes in the controls moved very rapidly and exhibited geotaxis, a very distinct effect of exposure to the chemical was the fact that after a short period of treatment the nematodes tended to lose the ability to congregate at the highest point in the solution, became less active, and were found at the base of the tube even though movement still took place - this movement was distinctly jerky, very uneven and quite different to that of normal untreated T. aceti. Nematodes exposed to a concentration of the chemical of 400 p.p.m.

had dropped down to the base of their container in 90 minutes but were still moving very actively - relative to the movement of Ditylenchus dipsaci for example - after 72 hours though the movements were very jerky in appearance.

The movement assay has obvious limitations in this case as it gives no indication of the above effects.

T. aceti lends itself well to reproduction studies because it reproduces rapidly and easily under laboratory conditions. The following experiment was carried out to measure what effects, if any, Thionazin had on T. aceti exposed to the chemical.

Freshly extracted T. aceti were exposed "in vitro" to concentrations of Thionazin of 25 p.p.m. and 5 p.p.m. for various periods. After treatment the nematodes were thoroughly washed on a 1 - 1.5 μ pore size fritted glass funnel and the nematodes poured into 3" x 1" specimen tubes. To these was added a few c.c. of vinegar which had previously been inoculated with the filtrate from a well developed culture of T. aceti. Peters (1952) points out that 0.5% of T. aceti passed through a Number 1 Whatman filter paper and thus the following precaution was taken in obtaining the inoculum. The culture was poured through two Whatman No. 50 filter papers and the filtrate examined microscopically to ensure that no larvae had passed through. This ensured, the inoculum was added to the vinegar.

The number of nematodes used for each of the three replicates was approximately 250. After three weeks the total number of nematodes was counted, the results are given in Table 18.

Table 18. Effect of "in vitro" exposure to 25 p.p.m. Thionazin on T. aceti (mean of 3 replicates).

Exposure Time (hrs.)	No. of Nematodes	Population Increase Factor
½	770	3.1
1	775	3.1
2	587	2.3
4	600	2.4
8	465	1.8
Control	1380	5.5

Table 19. Effect of "in vitro" exposure to 5 p.p.m. of Thionazin on T. aceti (mean of 3 replicates).

Exposure Time (hrs.)	No. of Nematodes	Population Increase Factor
2/3	1567	6.3
2	1150	4.6
6	1097	4.4
18	547	2.2
Control	1337	5.3

T. aceti is a bacterial-feeding nematode and presumably takes in bacteria all the time and the possibility exists that it also takes in Thionazin which is in solution in its surroundings. Goodey (1951) states

that nematodes can be anaesthetised with dichloro-diethyl ether and as presumably nematodes would not feed while anaesthetised, the following experiment was carried out.

A volume of 1 c.c. of freshly extracted T. aceti containing about 200 eelworms was exposed to an equal volume of water in which some dichloro-diethyl ether had been dissolved, as suggested by Goodey (1951) and this exposure continued for ½ hour in sealed tubes after which time all activity had either ceased or had been drastically reduced. A volume of 2 c.c. Thionazin solution was then added to the tubes to give a concentration of 25 p.p.m., the controls or other treatments receiving equal volumes of water, and the tubes again sealed. After exposure for 2 hours the nematodes were washed free of the chemicals and placed in specimen tubes containing vinegar and bacterial inoculum as described previously. After 3 weeks the total number of nematodes were counted, the results are given below.

Table 20. The Effect of "in vitro" exposure for 2 hours to Thionazin in the presence and absence of dichloro-diethyl ether on reproduction of T. aceti (means of 2 replicates).

A. Number of nematodes.

Conc. of chemical p.p.m.	No Ether	Plus Ether
0	1545	707
25	662	900

B. Percentage Mortality.

Conc. of chemical p.p.m.	No Ether	Plus Ether
0	0	54
25	57	42

C. Population Growth Factor.

Conc. of chemical p.p.m.	No Ether	Plus Ether
0	7.7	3.5
25	3.3	4.5

The results in this section are considered in the discussion. What is obvious though is that the exposure of Turbatrix aceti to Thionazin "in vitro" has a marked effect on the nematode, and this effect is apparent even at very low concentrations and short exposure times.

Section VI. Discussion.

The high kills obtained in the pot tests described in Section I are of interest but give little specific information on the chemical. Peters (1952) points out that higher kills are to be expected in experiments carried out in pots than under field conditions. In fact the high kills could possibly disguise some effects due to concentration and time of treatment. Of particular interest is the effect of time of treatment, the treatment 2 weeks after planting being more effective than 3 weeks before planting or 4 weeks after planting. The post-plant treatment effects are in agreement with the findings of Den Ouden and Kaai (1963) and also some unpublished findings of the author, using Meloidogyne sp. Soil drenching of tomato plants with Thionazin more effectively prevented egg mass formation when applied within 9 days of the nematodes entering the plant than after 9 days - this under optimum conditions when egg mass formation took place after 21 days. The explanation of Den Ouden and Kaai (1963) for their results is that the nematodes are probably killed by the chemical just before or while penetrating the plant, whereas once they are in the plant they are no longer affected. The literature yields very little information on the rate of hatch and plant penetration under conditions similar to this experiment where well developed tomato plants were placed in pots containing the cysts. It is conceivable that under these conditions hatching factor would be "available" immediately, that hatching would commence very quickly and presumably penetration of the plants would take place. Indeed Mr. G.L.

James (personal communication) has found considerable numbers of nematodes in plants within 4 days of planting under similar conditions. Fenwick and Reid (1953), Peters (1953) report inter alia on penetration but use potato chits where presumably production of hatching factor would not be as soon.

It would seem that two possibilities exist - firstly that within two weeks many larvae have hatched and have not yet entered the plant, and are prevented from doing so by contact with the chemical, whereas after 4 weeks they have entered the plant and are no longer affected, as suggested by Den Ouden and Kaai (1963). The other possibility is that they are killed within the plant and that the larvae are more susceptible at an early stage than later on, perhaps at an initial actively feeding stage. After four weeks a larger number of the larvae have passed this susceptible stage. The effect of concentration is significant at two weeks, and only just so after 4 weeks - this latter explanation could account for the phenomenon.

The fact that the effect of concentration is not significant in the pre-plant treatment might be explained by the findings in the hatching experiment described in Section II. Here long exposure of cysts for 8 weeks in a solution of root diffusate containing 0.5 p.p.m. of Thionazin resulted in an apparently high mortality (see later) and possibly the lowest treatment of 12.5 p.p.m. was sufficiently high to result in this effect. Assay methods too could be too insensitive to measure small differences.

The hatching tests in Section II give two interesting considerations, one of apparent mortality the other of inhibition. The inhibition of hatching is by no means a new consideration and examples are given in the foreword to Section II. The inhibitory effects of the organo-phosphorus insecticide Systox on the hatch of H. rostochiensis cysts are reported by Sasser, Feldmesser and Fassuliotis (1951), and on Meloidogyne spp. egg masses again by Sasser (1952). Concentrations were higher, being of the order of 20,000 p.p.m. for exposure periods of the order of 24 hours. Concentrations here are far lower and the obvious inhibitory effect can be clearly seen in Fig. 1 where as soon as the Thionazin is removed, the hatch increases. The apparent inhibitory effects shown in Table 4 are expressed as percentages below.

Table 21. The percentage inhibition of hatch of cysts of H. rostochiensis exposed to concentrations of Thionazin in root diffusate for 8 weeks.

Conc. of Thionazin p.p.m.	% Inhibition
0.125	72.4
0.5	98.8
2.0	99.9

The other effect, that of apparent mortality can be measured by the differences in the concentration totals in Table 5. As can be seen from Fig. 1, the lines on the graph remain horizontal after about 13 weeks

indicating that, presumably, all hatch has ceased. The final values as percentage mortalities are given below.

Table 22. Percentage mortality of total hatch after 16 weeks from *H. rostochiensis* cysts exposed to Thionazin in the presence of root diffusate for 8 weeks.

Conc. of Thionazin p.p.m.	% Mortality
0.125	38.6
0.5	73.8
2.0	90.0

"Programme" effects are not significant after 16 weeks, this no doubt partly due to "dilution" by control counts, but it is therefore justifiable to calculate the above on the "concentration" totals for 16 weeks. It would seem that exposure of the cysts to Thionazin for 8 weeks does not significantly decrease the total hatch after 16 weeks as compared with exposure for 4 weeks. This effect is just not significant at the 5% level after 16 weeks and with the wide fluctuations in hatch, the experimental evaluation could be insensitive.

The results of the contact tests measured by movement are probably open to criticism. If it is assumed that the movement assay is a suitable measure then this compound has distinct limitations as a nematicide if Allen's (1960) idea of entry by many avenues is accepted. If passive entry through the body openings is sufficient to kill nematodes then either this

compound is a poor nematicide, or it is broken down in the soil or by the plant to a more active contact agent, or this is an exception to the rule. The possibility exists that the concentrations are not sufficiently high or that the exposure time is not long enough. An exposure time of two weeks was used in the case of Aphelenchus avenae (Table 14) and this gave a 36% mortality at a concentration of 4 p.p.m. However, the fact that a ten fold increase in concentration, i.e. 40 p.p.m. gave no greater kill leads to the possibility that within this range of concentrations the absence of a source of food may be more important. Certainly exposure in this way does not seem to have any marked effect on reproduction of either Aphelenchus avenae or Ditylenchus myceliophagus (see Table 15 and 12) though the shorter period of exposure in the latter case is a criticism of this experiment.

That a very active breakdown product is formed remains a possibility, and the organophosphorus compound Systox provides a good example of such a phenomenon. This will be more fully discussed in Part V, page 144. The fact that none of the compounds tested in Section IIB were toxic as tested by movement assay, is no reason to reject this theory.

What is of particular note however is the fact that considerable kills are obtained when the chemical is incorporated into the fungal substrate and nematodes placed on the fungus. To the writer's knowledge this is a new approach to the evaluation of a nematicide, and though its wide applicability is doubtful, in this study it has given useful information. The concentrations used were very low, indicating a very active compound. There are a number of possibilities, the more obvious being firstly that

the fungus takes up the chemical and by feeding upon the fungus the nematode ingests the Thionazin or a breakdown product and is so killed, or rendered sterile. Secondly in the presence of the fungus, the compound might be converted to a highly active form and kill by contact. With the low concentrations used, this seems least likely. Thirdly, by the very presence of the chemical in the fungal substrate, the mycelium growing through the substrate is enveloped by the chemical and by this rather sophisticated means the chemical enters the body of the nematode.

This trend of thought, the killing of nematode populations by active ingestion of the chemical is to some extent confirmed by the results in Section V using Turbatrix aceti. The greater effect on these nematodes and the effect at low concentrations naturally led to speculation that the different feeding habits of these nematodes may play a part. The movement assay results which appear in Table 17 are of interest from the academic standpoint and as later results indicate, this method of assay obviously has big failings - indeed with the reduced movement of these nematodes and the fact that treated nematodes do not exhibit negative geotaxis, it is conceivable that treated nematodes will move through the cotton wool filters more successfully than would untreated ones.

In the reproduction studies in Tables 18 and 19 the controls have reproduced to a level five times that of the inoculum - this is approximately in agreement with Peters (1952) who reports that T. aceti populations tend to increase in geometric progression and under favourable conditions a population will double itself in a week. Though rigid conclusions cannot

be drawn from a small experiment of this nature, there does appear to be something approaching a concentration x time relationship in some cases. For example, 18 hours exposure at 5 p.p.m. gives nearly the same reproduction rate as 25 p.p.m. for 4 hours. In other cases, however, this does not seem to apply.

Finally, there are the results of the anaesthetisation experiment, which appear in Table 20. Though this was nothing more than a preliminary investigation and a crude one at that, it is probably fair to assume that the effect on the nematode in the presence of ether and Thionazin should approximate the sum of these individual effects. At least the combined effect should be larger than any single effect. This is not the case and a valid conclusion is therefore that the nematodes when deactivated by the ether are less susceptible to the chemical. Presumably in this state the nematodes are not feeding.

PART II. STUDIES ON THE UPTAKE OF THIONAZIN BY SOME PLANTS

INTRODUCTORY COMMENT TO PART II.

In preliminary investigations, Purnell (1963) showed that by dipping infested narcissus bulbs in Thionazin, *D. dipsaci* could be controlled and it would therefore seem of value to establish whether Thionazin was taken up by the bulbs when applied in different ways, and the extent of this uptake. Further, because of the results obtained with fungi in Part I, Sections IV and V, experiments were conducted to establish whether this nematicidal compound could be taken up by the fungi in question.

Section I. Studies on the Uptake of Thionazin by Narcissus and Tulip.

The degree of uptake of organophosphorus compounds by plants, and the residues of these compounds remaining in plants after applications have been very thoroughly examined. Quite naturally the bulk of this work has been carried out on compounds that are in accepted use, and thus, as might be expected, very little has been published in this field on Thionazin. This compound was discovered relatively recently and its commercial use has been very limited. The only information available to the author is (i) the work of Bowery (unpublished) on the detection of the alkaline hydrolysis product of Thionazin in green peanut foliage by spectrophotometric methods, (ii) that of Kiigemagi and Terriere (1963) on the detection of Thionazin in various crops and in the soil by spectrophotofluorometric means and (iii) Call's unpublished data on the uptake of the compound by the dried roots of narcissus bulbs.

The methods used generally for the detection of organophosphorus compounds in vegetable material utilise gas chromatography equipment or spectrophotometers, neither of which were available at the beginning of this study, though by the end a spectrophotometer was available to the author. Another possible technique is that described by Laws and Webley (1961) but this is very time-consuming. As Dr. F. Call had been successful in labelling Thionazin with tritium (^{3}H) by a modification of the method suggested by Garnett (1961), and as a small amount of this labelled material

(and later a small amount of ^{14}C labelled Thionazin) was available, it was decided to carry out uptake studies initially using this labelled material and finally by means of spectrophotometric methods.

A. Autoradiographic methods.

The utilisation of the photographic plate in this type of study is extremely useful because of its simplicity. This type of detector is unfortunately far less sensitive than any counter (Taylor, 1963) but where a qualitative result is required to locate or study the distribution of a substance within an organ or tissue,this type of passive element is very convenient.

Autoradiographs were obtained by cutting the treated bulbs into halves with a very sharp knife and firmly pressing the cut surfaces without sliding on to Kodak Industrial G X-ray film in a darkroom fitted with a safelight. This operation was carried out to get the bulb juices from the cut surface on to the X-ray film. The casettes containing the X-ray film were stored for two weeks in the case of the tritiated Thionazin and four days for the ^{14}C-labelled material. The X-ray film was fully developed using Kodak D19B high-contrast developer and fixed in the usual way.

Exposure of the bulbs to the labelled material was simply by applying the compound to the foliage by means of a camel hair brush in the case of the foliar uptake study, by placing the roots of bulbs into a water solution of the compound in the case of the root uptake study, and by immersing the dormant bulb in an aqueous solution of Thionazin to establish uptake under these conditions.

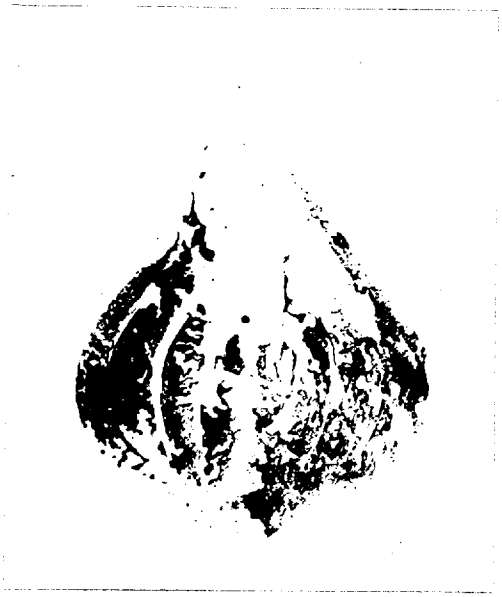


Fig. 7.

Autoradiograph demonstrating the uptake of tritiated Thionazin by a dormant tulip bulb immersed in a solution of the chemical.

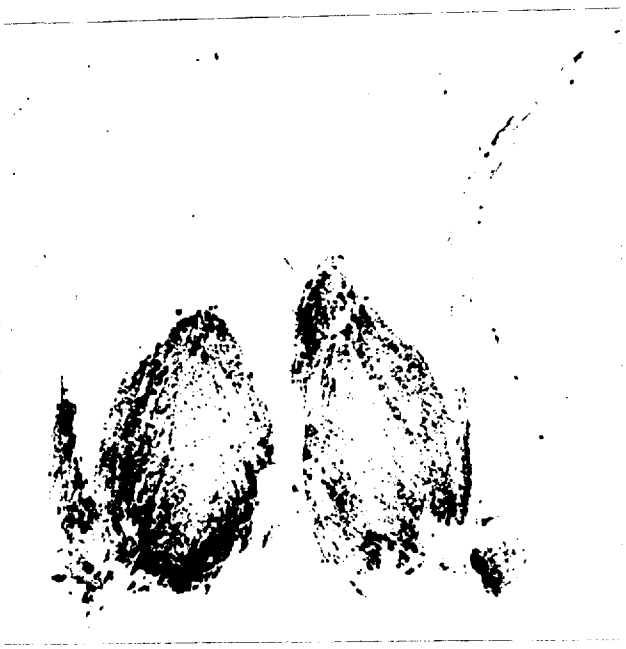


Fig. 8. Autoradiograph demonstrating foliar uptake of ^{14}C-labelled Thionazin by a tulip plant.

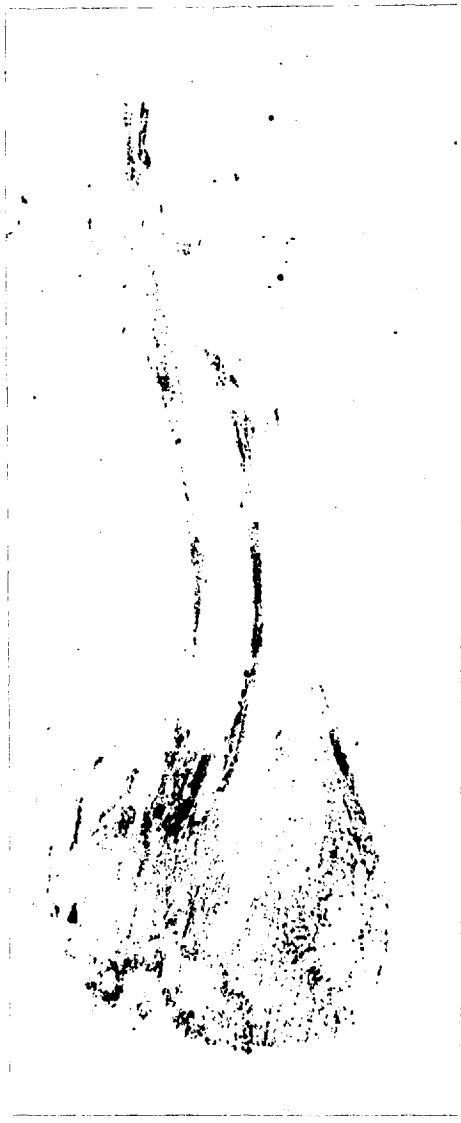


Fig. 9.

Autoradiograph demonstrating root uptake of ^{14}C-labelled Thionazin by a tulip plant.

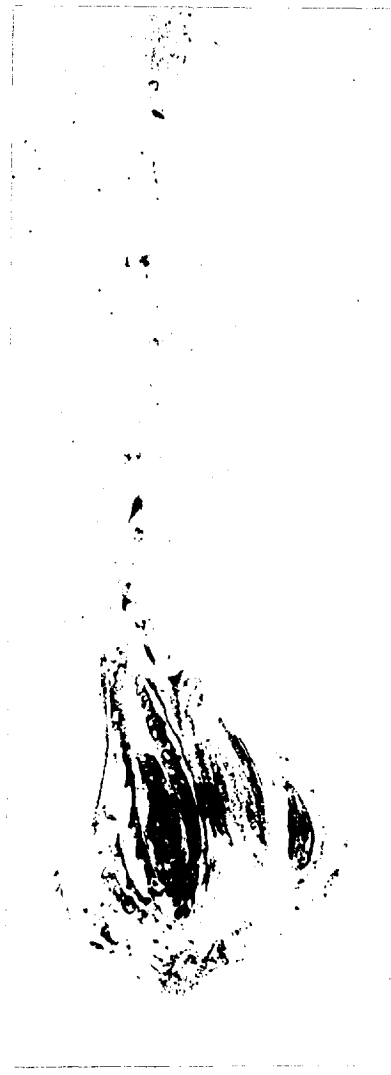


Fig. 10.

Autoradiograph demonstrating foliar uptake of tritiated Thionazin by a tulip plant.

Fig. 11.

Autoradiograph demonstrating the uptake of tritiated Thionazin by a dormant narcissus bulb in a solution of the chemical.

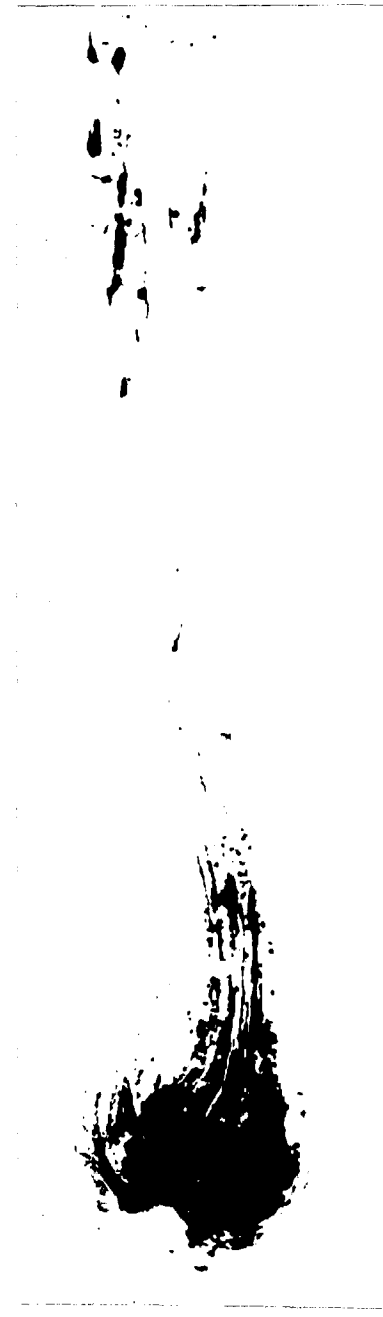


Fig. 12.

Autoradiograph demonstrating foliar uptake of tritiated Thionazin by a narcissus plant.

Fig. 13.

Autoradiograph demonstrating root uptake of ^{14}C-labelled Thionazin by a narcissus plant.

Fig. 14.

Autoradiograph demonstrating foliar uptake of ^{14}C-labelled Thionazin by a narcissus plant.

Results.

The autoradiographs are reproduced in Figures 7 - 14. It is clear that Thionazin moves into the bulb regardless of the method of application, and regardless too of the isotope, the result being the same for both tritiated and Carbon 14 labelled material. From the autoradiographs it would seem that the compound is mainly concentrated in the centre of the bulb, particularly so in the case of narcissus, and though this is what might be expected if the point of entry is via the neck of the bulb, another explanation for this result is that as the autoradiograph is dependent upon the bulb juices getting on to the film, there could possibly be less fluid further from the centre away from the central growing point of the bulb.

The compound was rapidly translocated within the bulb, this applying equally to foliar and root uptake. Within one hour of applying labelled Thionazin to the tip of a 9" leaf of narcissus, traces of the compound could be detected in the bulb itself by autoradiography. The same situation existed for root uptake and it could be that uptake and translocation is in fact faster, the one hour interval being the shortest tested.

B. Scintillation Counter Methods.

Since autoradiography is only crudely quantitative (although valuable for studying spatial distribution), in order to determine the amount of material taken up by the bulb, one or other of the available

counting methods must be used in which the actual number of atomic disintegrations per unit time is measured. Since a highly sensitive and efficient liquid scintillation counter was available, this was used for the quantitative work.

In this method the substance to be assayed is added to a solution containing a complex organic "scintillator" in a special vial with a fused silica window. This window can be brought into intimate optical contact with the window of a photomultiplier cell which is connected to a suitable scaler for counting the resulting electrical pulses. Each atom disintegrating by β decay emits an electron which excites the scintillator chemical. This, on reverting to its original unexcited state emits a flash of light converted by the photomultiplier to an electrical pulse which is counted by the scaler. There is an unavoidable background count due to stray radiation. The scintillation head was a Nuclear Enterprises N.E. 5503 unit feeding pulses to an Isotope Developments Type 1700 scaler.

One difficulty is that certain compounds normally present in plant tissues may absorb the energy of the electron ejected by the radioisotope and prevent its conversion to light energy, the phenomenon being known as quenching. Such interfering substances must therefore be removed by a "clean-up" process before assay.

Extraction and "clean-up" methods.

The extraction method used initially approximated that of Kiigemagi and Terriere (1963) and Call (unpublished data). The bulb was

weighed and initially macerated in a Kenwood food mixer in the presence of anhydrous sodium sulphate (to absorb water) at the rate of 1.5 gramme of sodium sulphate per gramme of bulb material. This gave a granular distribution of finely divided bulb material and sodium sulphate and to this was added hexane at the rate of 3 c.c. per gramme of bulb material and the resultant preparation was homogenised for 5 minutes. The solvent was then filtered off from the bulb material under pressure and the homogenised sodium sulphate - bulb material mixture further washed with 25 c.c. of hexane. The hexane solution was then quantitatively transferred to a Kuderna - Danish evaporative concentrator, a chip of porcelain added to prevent bumping and the volume reduced to approximately 5 c.c. The remaining hexane was taken off under an airstream.

Unfortunately the extract obtained in this way consisted of a heavy green wax-like substance which quenched in the scintillation counter to the extent of 90%. The obvious way to remove this would be to use an adsorption chromatography column and though this succeeded in removing the pigment there appeared to be tritium exchange in the columns as, though Thionazin came through and could be detected by spotting the filtrate on filter paper and spraying with Cook's (1954) dibromosuccinimide - fluoroscein reagents, very reduced radioactivity was detected when the sample was assayed in the scintillation counter. The same situation existed using celite and carbon columns. This difficulty could be overcome by eluting the column with acetone when complete recovery of the tritiated Thionazin could be achieved, but the pigment moved through the column at exactly the same speed as the Thionazin under these conditions. A large number of

different solvents were used but in all cases the pigment was removed. The ^{3}H-labelling of the compound also appeared to be rather labile and it was found that the activity was greatly reduced by heating in the presence of water. Extraction with dimethyl sulphoxide reduced the amount of pigment very considerably but this compound quenched heavily when added to the scintillator and the Thionazin could not be extracted from it.

The procedure which gave the best results, though recovery was only in the region of 50%, was the following. The pigmented wax-like extract containing the Thionazin was taken to dryness under an airstream and the resultant preparation taken up in 3 c.c. of acetone. The tube was then thoroughly shaken and when the contents had dissolved an equal volume of cold water was added. The wax-like substances immediately appeared to flocculate and the contents of the tube were filtered through a Whatman No. 31 filter paper previously dampened with a 50% acetone water solution into a separating funnel containing 25 c.c. of hexane and the flocculent further washed with 50% aqueous acetone. The separating funnel was thoroughly shaken and the layers allowed to separate. The hexane layer was removed and quantitatively transferred again to a Kuderna-Danish concentrator, and the volume reduced to about 5 c.c. This volume was finally reduced under an airstream to about 2 c.c., the volume accurately measured and 0.5 c.c. of the solution placed in a vial with N.E. 213 scintillator fluid and assayed in the single sided scintillation counter available. E.H.T. voltage was 1000 volts, discrimenator bias voltage 6 volts, and the gate 6 - 50 volts, conditions found to give an efficiency for tritium of $\sim$ 5% with a background of $\sim$ 1 c.p.s. (Call, unpublished). The solution so

obtained did not quench more than 5%. The mean percentage recovery over six determinations was 49.6% for narcissus.

The bulbs were immersed in a solution of Thionazin of 200 p.p.m. for 1/3, 1, 3, 9 and 27 hours and in the case of the tulips the bulbs were weighed immediately before treatment and again immediately after treatment, the increase in weight during treatment so being established. The activity of the test solution was established to be 0.989 counts per second per µg. of Thionazin and the extraction process described previously was carried out immediately after treatment, the bulbs first being washed with acetone to remove any surface contamination. The narcissus experiment was replicated five times and the tulip experiment four times. A small amount of Triton X 100 was added to the treating liquid to reduce the surface tension of the solution and enhance penetration into the bulb.

A further study to establish whether there was any decrease in the concentration of Thionazin in the treating solution was carried out. Duplicated 1 c.c. samples of the solution were taken after each treatment period and the Thionazin extracted with 10 c.c. of hexane, the volume reduced and the activity measured in the scintillation counter. The treating liquid took up a certain amount of pigment and some of this was extracted by the hexane.

Results.

The results are given in Table 23, these figures being corrected for the percentage recovery of the compound in the extraction process.

Table 23. Uptake in µg. per g. of bulb material of tritiated Thionazin by dormant tulip and narcissus bulbs immersed in a solution of 200 p.p.m. of the compound (means of 5 replicates for narcissus and 4 replicates for tulip).

Exposure time (hours)	1/3	1	3	9	27
Tulip	4.1	6.9	8.6	19.7	32.6
Narcissus	3.7	5.3	7.9	11.4	16.5

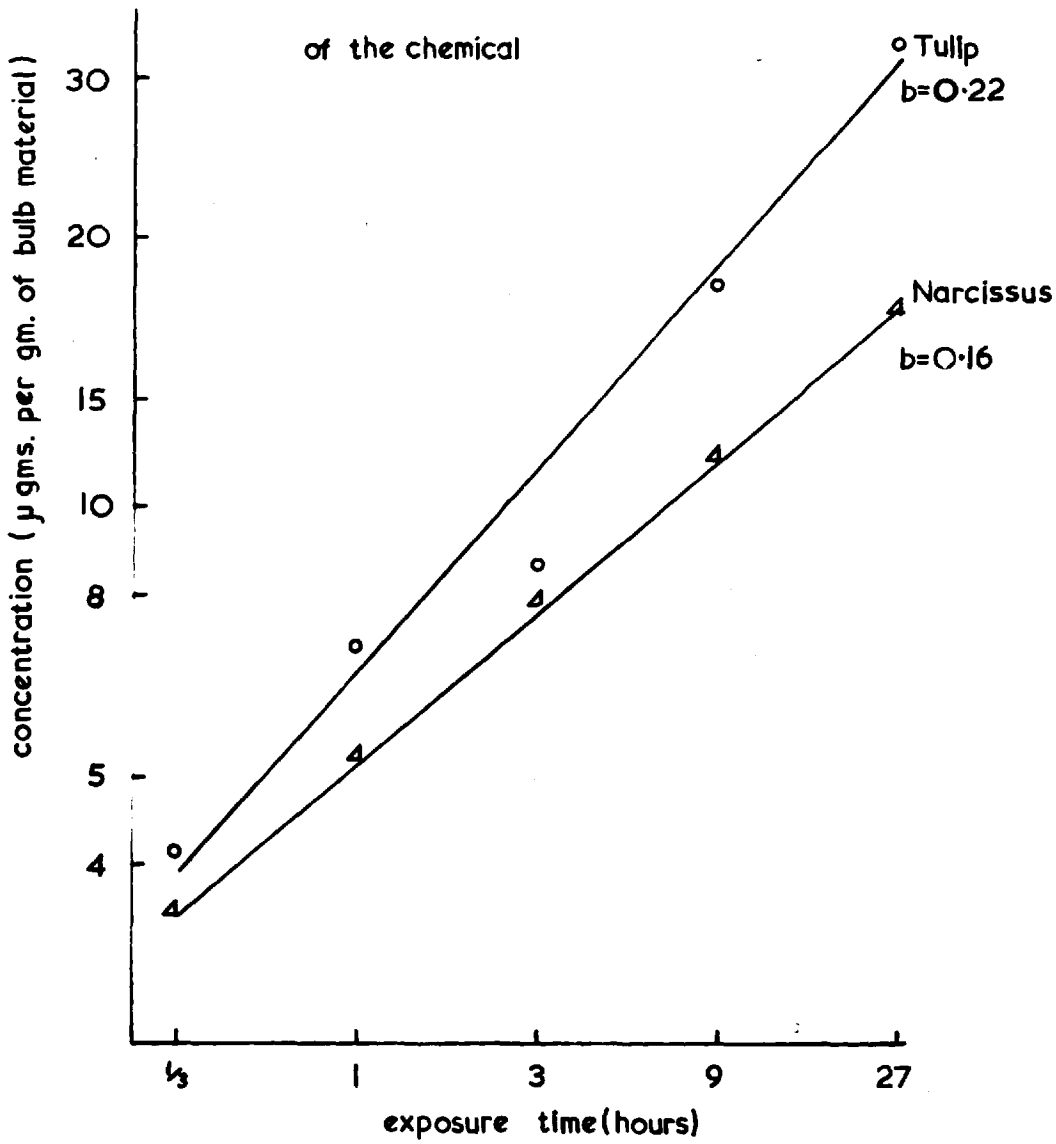
The analysis of variance of these data (transformed to 1000log x values) appears in the appendix Tables 66 and 67. For both narcissus and tulip the linear term removes a very large proportion of the variability of y leaving a non-significant residual. Fig.15 illustrates the results.

The results of the study to establish whether there was any decrease in the concentration of the treating liquid appear in Table 24. Analysis of these data indicated that "intervals" and "samples" were not significant showing that Thionazin was passively taken up with the treating solution.

Table 24. Concentration in counts per second per c.c. of treating solution after narcissus bulbs had been immersed in it for various periods of time.

Intervals (hrs.)	1	3	9	27	Mean
1st Sample	272.5	254.0	277.0	232.0	258.9
2nd Sample	285.8	282.2	280.4	270.0	279.6
Mean	279.1	268.1	278.7	251.0	269.2

Fig. 15. Uptake of tritiated Thionazin by bulbs immersed in a solution containing 200 p.p.m. of the chemical



Finally the mean uptake of Thionazin by tulip bulbs (variety Merry Widow) was compared to the mean increase in weight of the bulbs over the treatment periods and the data are given in Table 26.

Table 26. Comparison of the increase in bulb weight (mg./g. of bulb material) and uptake of tritiated Thionazin (µg./g. of bulb material) by tulip bulbs immersed in a solution of the compound (means of 4 replicates).

Exposure Time (hours)	Wt. Increase (mg./g.)	Thionazin Uptake (µg./g.)
1/3	18.8	4.1
1	36.4	6.9
3	44.2	8.6
9	82.2	19.7
27	149.9	32.6

The correlation coefficient for these data was calculated ($r = 0.995$) and showed that the uptake of the treating solution in mg./g. and uptake of Thionazin in µg./g. was very highly correlated. This would indicate again that the uptake of the chemical was purely passive and that knowing the concentration of the treating solution and the increase in weight of the bulb over the period of treatment, the concentration of Thionazin in the bulb could be calculated.

This study indicated that the uptake of Thionazin by the bulb is

dependent on the uptake of the treating liquid and that the former, in logarithmic transformation is in turn dependent on the logarithm of the exposure time. Finally, it would seem that the tulip variety used in this study took up a larger quantity of Thionazin than did narcissus (variety Fortune) under the same conditions.

C. Spectrophotometric Methods.

Many chemical compounds have a characteristic absorption spectrum - in this context, a curve showing the amount of radiation absorbed at each wavelength. This characteristic can be described in terms of transmission which is the ratio of the radiant energy transmitted by the sample to the energy incident upon the sample. The relationship between these two measurements is given by the following equation:

$$\text{ABSORBANCE} = \log \frac{1}{\text{Transmission (T)}}$$

(T being expressed as a decimal fraction).

Quantitative spectrophotometry is based upon the fact that the absorbance of an absorbing material is dependent upon its concentration (Beckman, in Zweig, 1963).

The particular advantage of the spectrophotometer over the isotope assay is its specificity, so that measurements are made of the actual compound and not of its breakdown products as might be the case using the labelled compound.

It seemed of value to establish that it was in fact Thionazin

that was measured in the previous experiments and to obtain some information on the persistence of Thionazin in the narcissus and tulip bulbs.

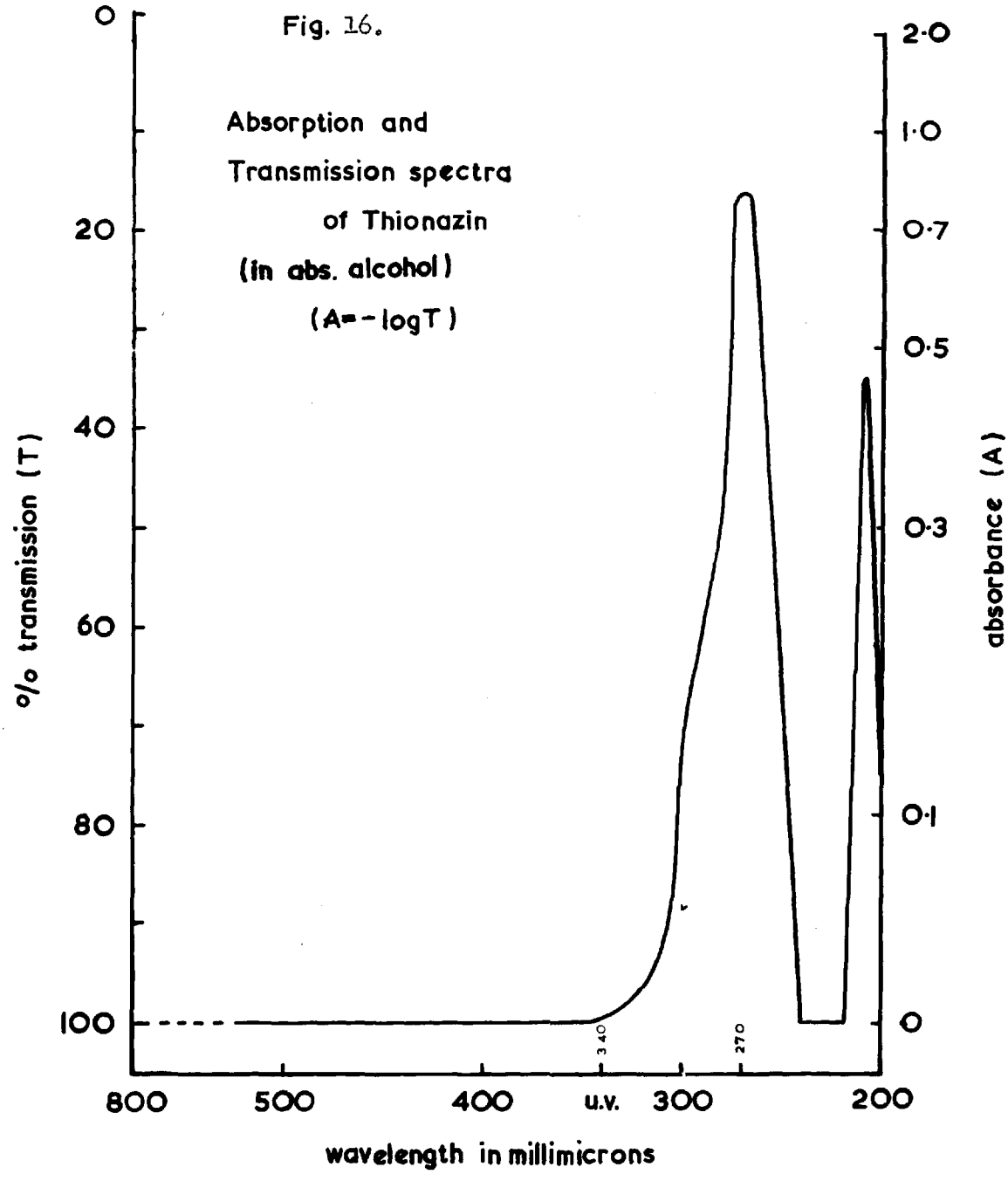
Materials and methods.

The spectrophotometer used was a Beckman DB in which a sample is directly compared with a reference solution over the wavelength range 200 - 800 mμ. Transmission and Absorbance can be read from a scale while Transmission can be scanned at either 10 or 40 mμ per minute and recorded by a chart recorder to give a spectral transmission curve.

Thionazin was found to give an absorbance peak in the ultra violet at approximately 270 mμ wavelengths and another smaller peak at about 220 mμ - see Fig. 16 - this confirming the findings of the Cyanamid Co. Absorbance at 270 mμ was plotted against concentration and this gave a slightly curved line. To calibrate the apparatus the "least squares" straight line was fitted.

Extraction of the Thionazin from the plant material was carried out as described previously (Section IB), the only difference initially being that all hexane was removed by evaporation under vacuum and the residue taken up in a known volume of absolute alcohol for the spectrophotometric assay. However, it was found that contaminants gave peaks in the same region as Thionazin, and this difficulty was partially overcome by passing the extract through an alumina chromatography column and eluting with hexane. The hexane was again removed under vacuum and the residue taken up in absolute alcohol. The cleanup was not yet adequate but by

Fig. 16.


Absorption and
Transmission spectra
of Thionazin
(in abs. alcohol)
(A=-logT)
0
20
40
60
80
100
% transmission (T)
2·0
1·0
0·7
0·5
0·3
0·1
0
absorbance (A)
340
270
800
500
400
u.v.
300
200
wavelength in millimicrons

putting the extract from untreated bulbs in the reference cell of the spectrophotometer, the Thionazin peaks could be clearly seen. The contaminants still interfered to some extent, this varying from sample to sample but rough preliminary studies could be carried out.

The aim in using the spectrophotometer was twofold: firstly to check that it was in fact Thionazin that was being measured in the previous study and secondly, to measure the extent of breakdown of Thionazin within the bulb.

For this latter experiment, narcissus and tulip bulbs were weighed and treated by immersion for 3 hours in a solution of Thionazin of 200 p.p.m. prepared from the analytical grade of the compound. After treatment the bulbs were dried, re-weighed and placed in sealed plastic bags containing reservoirs of water to maintain a high humidity at 25°C. This was done to simulate the conditions under which rapid growth would take place, and in fact over the period of the experiment the shoots of the bulbs did make considerable growth. The bulbs were dipped periodically in aretan to prevent fungal growth. At each interval of 1, 3, 9 and 27 days the Thionazin of each of four bulbs was extracted and the concentration of Thionazin present in the sample measured using the Beckman DB spectrophotometer available. The expected amounts of Thionazin could be calculated by the increases in bulb weights and then could be compared to those obtained, correcting for efficiency of extraction.

Results.

To establish whether in using the scintillation counter actual Thionazin was being measured, extracts containing tritiated Thionazin were assayed by both the spectrophotometer and the scintillation counter. The results are given below.

Table 27. Comparison of the amount of tritiated Thionazin in µg of Thionazin detected in tulip after 9 hours in a solution containing 200 p.p.m. of the compound.

Scintillation counter assay	Spectrophotometer assay
58.1	63.5
46.4	57.7
55.5	64.0
51.6	67.2

The samples were assayed by means of the scintillation counter first, the sample put through an alumina column, eluted with a large volume of hexane, the hexane removed, the sample taken up in absolute alcohol and the spectrophotometer assay carried out using the extract from an untreated bulb in the reference cell. The necessary correction for the sample used in the scintillation counter was made for the spectrophotometer assay. The determinations are remarkably similar if it is considered how many errors could be present. The results might indicate that the recovery of Thionazin was in fact higher than estimated. This

could be due to the apparent instability of the labelling.

The results of the study of the possible breakdown of Thionazin in the bulb are given in Table 28. The data have not been analysed as the results are clear-cut. The values for each of the replicates are given to show the degree of variability. The expected concentration of Thionazin was calculated on the increase in weight of the bulb, knowing the concentration of the treating solution.

Table 28. Amount of Thionazin in µg/g. of bulb material extracted from tulip and narcissus bulbs after different periods of time after immersion in Thionazin solution of 200 p.p.m. for 3 hours.

Time Interval (Days)	Sample Number	Narcissus		Tulip	
		Obtained	Expected	Obtained	Expected
1	1	5.5	5.1	4.7	5.1
	2	3.3	3.9	3.1	6.1
	3	6.5	6.6	3.4	3.1
	4	4.7	5.7	3.3	5.3
	Total	20.0	21.3	14.5	19.6
	Mean	5.0	5.3	3.6	4.9
3	1	0	4.6	0	7.5
	2	4.1	4.6	1.6	5.2
	3	4.2	5.6	2.9	8.8
	4	0	4.1	1.8	6.4
	Total	8.3	18.9	6.3	27.9
	Mean	2.1	4.7	1.6	7.0
9	1	0	4.7	1.3	5.8
	2	0	6.0	0.6	5.8
	3	0	4.6	0.7	6.7
	4	0	3.8	0	4.9
	Total	0	19.1	2.6	23.2
	Mean	0	4.8	0.7	5.8
27	1	0	5.4	0	4.5
	2	0	4.0	0	6.6
	3	0	4.4	0	6.7
	4	0	5.5	-	-
	Total	0	19.3	0	17.8
	Mean	0	4.8	0	5.9

Because of the contaminants present, the evaluations, particularly at 27 days after exposure, were difficult to carry out and probably therefore too much reliance should not be placed on these data. However, what does seem to be certain is that there is a decrease in the concentration of Thionazin in both tulip and narcissus bulbs over time, and this can only be accounted for by the breakdown of the compound to other forms by the plants.

Section II. Studies on the Uptake of Thionazin by Fusarium oxysporum and Agaricus campestris.

The uptake of chemical compounds by fungi has received relatively little attention though in recent years a fair amount of work has been carried out in this field.

Schütte (1956) discusses the phenomenon of translocation in fungi, and points out that agarics translocate readily, this being demonstrated by the use of dyes. Abbot and Grove (1959) report on the uptake and translocation of triphenyl tetrazolium chloride by Phycomyces blakesleeanus and the same authors (1959a) using griseofulvin found that significant amounts of this compound were not taken up by P. blakesleeanus though the compound disappeared slowly from the media on which other fungi were grown. These authors demonstrated the breakdown of triphenyl tetrazolium chloride by the fungi using microspectrophotometric techniques. Grossbard and Stranks (1959) were unsuccessful in demonstrating the translocation of cobalt-60 or caesium-137 in various fungi but did not include either Fusarium oxysporum or Agaricus campestris. Lucas (1960) demonstrated that the mycelium of Phycomyces nitens and Chaetonium spp. were only able to transport phosphorus-32 under certain limited conditions. These latter workers also showed that there was no activity beyond the edge of the colony indicating that the phosphorus was so absorbed into the mycelium as not to diffuse out into the medium. Thrower and Thrower (1961) demonstrated the movement of ^{14}C-labelled glucose by various fungi and Monson and Sudia

(1963) showed that the ions ^{65}Zn, ^{35}S, ^{89}Sr and ^{32}P were readily absorbed by the hyphae of Rhizoctonia solani and were readily translocated throughout the mycelium, also that the age of the mycelium had an effect on the translocation of ^{32}P. Finally, Littlefield, Willcoxson and Sudia (1965) also demonstrated the translocation of ^{32}P in the hyphae of Rhizoctomia solani.

The results obtained in Part I, Sections IV and V, indicate that Thionazin may be taken up by Agaricus campestris and Fusarium oxysporum and it would be of value to establish whether this was indeed so. The work mentioned above illustrated translocation, and though a positive result in this type of study would indicate uptake, a compound taken up by the fungi would not necessarily be translocated. Abbot and Grove (1959) made the point that compounds taken up by the mycelium in direct contact with the nutrient medium may be absorbed and immobilised or may be translocated to the growing tips of the hyphae either unchanged or in a chemically modified form. The work described in this section was an attempt to discover whether Thionazin was taken up by Fusarium oxysporum and Agaricus campestris.

Materials, methods and results.

The simplest way to show that a fungus takes up a particular compound might be to grow the fungus on a medium containing the compound and to determine after a period of time the amount of the compound present in the fungus. The difficulty would be to separate the fungus from the medium and to prevent or remove, any surface contamination by the compound.

Initial work was done using a modification of the system proposed by Tribe (1957). Tritiated Thionazin was incorporated into P.D.A. as in Part I, Section IV, and a sterilised cellophane sheet placed over the medium and the plate inoculated with Fusarium oxysporum above the cellophane. When the fungus was well developed it was peeled off the cellophane, homogenised in the presence of hexane and the activity of the suspension measured in a scintillation counter. A blank treatment using unlabelled material was also included. The result indicated the presence of Thionazin, the fungus on the labelled material giving a mean count of 3.7 counts per second, the fungus on the unlabelled material a count of 0.7 counts per second. The obvious weakness of this method was the fact that the lower surface of the fungal colony could be contaminated with the labelled material. In addition, there was no direct link between the substrate and the fungus. In an attempt to overcome this an idea suggested by Dr. B.E.J. Wheeler (personal communication) was put into practice. "Millipore" filter discs (Millipore Filter Corp., Massachusetts) of various pore diameters were placed on agar impregnated with ^{14}C-labelled Thionazin and the fungus inoculated on the plate. In this case the fungal hyphae grew through the pores and the colony developed both above and below the filter disc. When the fungus was well developed the discs were pulled off and inverted on X-ray film to obtain autoradiographs. Because of heavy contamination the background showed up very darkly and even repeated washings of the filter discs did not remove it. Because of the limited growth of the fungus above the filter disc, not sufficient of the mycelium could be obtained

to do a scintillation count. It could not therefore be established whether the mycelium contained any of the labelled material.

Finally a system was developed using possible translocations to measure uptake utilising a hyphal bridge and satisfying the primary condition that the isotope could not move away from the source in any way other than by means of the hyphal strands of the mycelium. ^{14}C-labelled Thionazin was incorporated into P.D.A. (as described previously) which was poured into petri dishes. In this agar was placed a small watch glass and into this was put potato dextrose agar free from Thionazin. Care was taken to ensure that at no stage was there any contact between the labelled and unlabelled material. The plates were then inoculated on the labelled P.D.A. and the fungus allowed to grow up over the edge and on to the agar in the watch glass, this being achieved by a hyphal bridge (see Fig. 17).

An attempt was then made to determine whether any of the labelled Thionazin was present in the fungal colony or agar in the watch glass.

Firstly, the agar discs from the watch glasses containing the fungus were boiled in water, cooled to 60°C. and this liquid added to heated hexane in separating funnels and thoroughly shaken. The hexane layer was removed, the volume reduced by distillation and presence of the isotope established by means of the scintillation counter. To ascertain whether the fungus secreted the compound into the medium, the agar in the watch glass was separated according to the presence or absence of the fungus and the above extraction carried out on the different sections. The results are given in Table 29.

Table 29. Radioactivity of fungal cultures grown from potato dextrose agar containing ^{14}C-labelled Thionazin (values being difference between background and sample counts in counts/second).

Fungus	Fungal Area	Agar beyond Fungal Area	Total
Fusarium oxysporum	1.9	3.4	4.3
	6.2	3.1	9.3
	6.4	2.9	9.3
	5.4	2.2	7.6
Mean	4.9	2.9	7.7
Agaricus campestris	2.2	4.0	6.2
	17.6	3.8	21.4
	2.8	4.5	7.3
	3.2	5.1	8.3
Mean	6.5	4.4	10.9

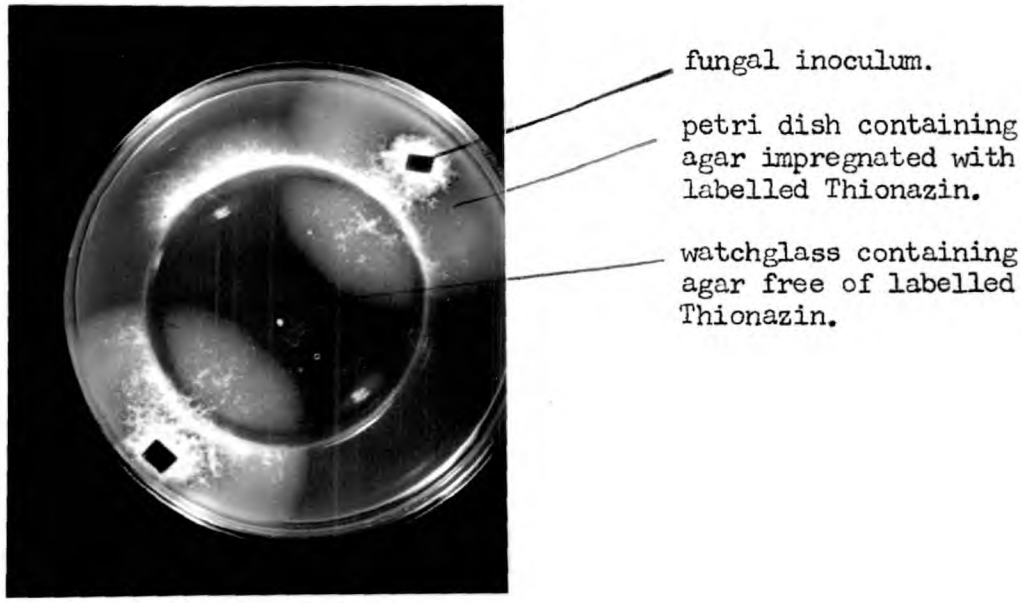
What is obvious is that the isotope is present - the above values mean nothing more than this. No measure was made of the recovery as indeed there was no way of knowing how much of the chemical was taken up by the fungus. It would seem though that the amount was relatively low.

In the final step, an attempt was made to obtain autoradiographs of the fungal cultures in the watch glasses. The cultures were grown as previously and when the colonies were well developed the agar from the watch glasses was wrapped in a very fine terylene film and placed on X-ray film in a deep freeze. After one, two and three weeks films were developed. The reason for carrying out the process in a deep freeze was to prevent the fungus from continuing to grow on the film. Also, when

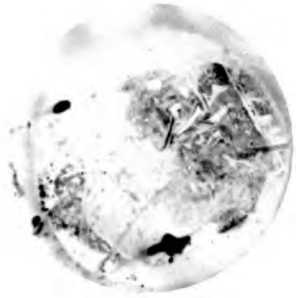
the agar was frozen it could be pushed on to the film without being flattened out.

All the autoradiographs showed the presence of the isotope, but generally they were poor, giving no information on the spatial distribution of the compound. The folds in the terylene film below the agar discs appear to have had an effect on the pattern obtained. Fig. 18 illustrates the autoradiograph obtained from a culture of Agaricus campestris. Though these are disappointing, they do confirm that the compound is taken up by the fungi.

Fig. 17. Arrangement for detecting uptake and translocation of ^{14}C-labelled Thionazin by fungi.



Autoradiograph demonstrating uptake and translocation of ^{14}C-labelled Thionazin by Agaricus campestris.



Section III. Discussion.

In retrospect the results obtained in the dormant bulb uptake study are perhaps obvious. It could be expected that some of the water in which the bulbs were immersed would enter at the neck and move down into the bulb between the scales and that a chemical in solution or dispersion would be carried down too - the concentration of the chemical within the bulb being related to the amount of solution entering the bulb, which in turn is manifested by the increase in weight of the bulb. Presumably therefore this uptake of water or treating solution, and therefore uptake of the chemical, is directly related to the moisture content of the bulb and to the variety of bulb if for example differences exist in the spacing between the bulb scales. This latter point might explain for example why the tulip bulb takes up more of the chemical - calculated on a per gramme of bulb weight basis - than did the narcissus bulbs in this experiment. A tulip bulb in cross-section shows very marked and distinct gaps between the "scales".

Probably an important factor from the biological point of view which has not been studied is the distribution of the chemical within the bulb. It would seem a fair assumption that the greatest concentration would be in the centre of the bulb where the link with the neck - the apparent point of entry - is most direct, and this is to some extent confirmed by the autoradiographs. However, as pointed out, the apparent distribution in the autoradiographs could be misleading. It is interesting

to note that Call (unpublished data) in examining the distribution of Thionazin in the bulb when taken up via the roots, found the greatest concentration of the compound in the outer scales and a considerably reduced concentration in the centre of the bulb.

In the breakdown over time study, an interesting situation arises. It would seem that this compound is rapidly broken down, although, as stated previously, the cleanup of the bulb extract was by no means as good as it could be, and this would make detection rather difficult. However there is no doubt that this general trend exists. The manufacturers of the compound, in a personal communication, report the presence of Sodium pyrazinolate in tulips within a week of treatment and Thionazin in bulbs boiled immediately after treatment - boiling would presumably destroy the enzyme complexes present and prevent breakdown of the compound, if indeed enzymatic breakdown takes place. These latter results must be regarded with some reserve, as the concentration of the compound found in the bulbs was tremendously high and obviously far exceeded what could have been taken up by the bulb if uptake were a purely passive process.

Because of the lack of time, no attempt was made to establish whether any breakdown products were present in the bulbs. However this should not be very difficult and could give useful information - what would be of particular interest would be to establish the persistence in the bulbs of these breakdown products. The recovery of the labelled material too was very low and this could no doubt be considerably improved. The use of the tritiated compound has drawbacks, particularly as the labelling

appeared to be particularly labile under certain conditions. However, Kiigemagi and Terriere (1963) using far more time-consuming and elaborate extraction methods achieved recoveries as low as 34% from peppermint oil, and were unable to extract any Thionazin from oranges. In the present work the rate of extraction from tulip varied between 37.2% and 62.2% over 20 extractions as calculated against the projected uptake, and though this fluctuation was not significant it does represent a wider range than was the case for narcissus and this is difficult to explain. Certainly these rates of recovery could be greatly improved.

A further interesting biological property of Thionazin is the fact that it moves into the bulb both from the foliage and from the roots. This two-way movement of organo-phosphorus compounds is unusual but what is probably vital here is the fact that these plant forms are very specific in that the bulb is the focus of leaves and roots. It was encouraging to establish that this movement could be traced using both tritiated and ^{14}C-labelled Thionazin.

Although the results of the autoradiographs obtained in the fungal uptake study are disappointing, there appears to be sufficient evidence to suggest that the compound is taken up and translocated by the fungi in question, though possibly in very small amounts.

Thionazin has therefore very marked systemic properties being taken up and translocated by plants as widely different as fungi and flower bulbs.

PART III. FIELD EXPERIMENTS USING THIONAZIN FOR THE CONTROL OF DITYLENCHUS DIPSACI IN NARCISSUS.

A. The Application of the Chemical to Dormant Bulbs.

Introduction.

The history of nematode control in bulbs is well reported, Purnell (1963) giving a summary of the main events. In most cases control has consisted of exposing the bulbs to hot water to which has been added a certain concentration of formaldehyde. Newton et al. (1933) treated narcissus bulbs with a silver nitrate - potassium cyanide solution under vacuum and postulated that a chemical treatment would be unsuitable because not sufficient chemical could enter the bulb to control the nematodes. Generally the approach has been to add chemicals to hot water thus increasing the effect of hot water treatment. It appears that Purnell (1963) was the first to show that the addition of chemicals to cold water dips held promise for the control of nematodes in bulbs and the work outlined here has been carried out to further investigate this possibility. The experiments described have been conducted over a period of two years.

Materials and Methods.

The two tanks used for these experiments were those described by Purnell (1963) being similar in design to that suggested by Weaving (1960). The only difference to Purnell's set-up was that a 220/240 volt Stuart Turner pump delivering approximately 1000 gallons per hour was coupled to the second tank thus allowing for circulation of the contents of both tanks. Each tank was equipped to take three wire mesh baskets stacked one on top of the other and in the 1963 experiments these were used to

contain the bulbs. Each basket had a mesh division down the centre allowing two lots of bulbs to be treated in each basket and ensuring that there would be no mixing of these samples. In 1964,12" × 24" nylon reticules were used, these being weighted and suspended in the treating liquid. In this way the number of treatments being carried out at any one time could be greatly increased thus considerably shortening the time required to complete any set of experiments. Also this greatly reduced the volume of chemical solution used as the bags could be suspended in a relatively small volume of liquid.

The narcissus bulbs used in the 1963 experiments were "Helios" variety from a stock grown at Ashurst Lodge and originally from Cornwall. One stock was heavily infested with Ditylenchus dipsaci, the other lightly so. The stocks were lifted and sorted into various grades; i.e. first year bulbs or chips, second year bulbs or "rounds" and older bulbs.

The "rounds" were further sorted to give bulbs of approximately equal size and these were then coned and quartered three times and sixty randomly selected bulbs were used for each treatment. This process was carried out for both the infested and un-infested stocks,thus giving sixty bulbs of each stock for each treatment. The process for the 1964 experiments was much the same except that only infested material was used, the variety being Golden Harvest from a stock in Cornwall. In this case two lots of 30 bulbs were randomly selected and each treatment was thus duplicated.

The 1963 trial consisted of a fully factorial experiment using

concentrations of Thionazin of 0, 200, 600 and 1800 p.p.m. with exposure periods of 2, 6 and 18 hours at temperatures of 80°, 90° and 100°F. The Thionazin was dissolved in equal volumes of acetone and Triton X 100 and added to the requisite volume of water in the treating bath. At the higher concentrations solubilisation did not take place but with the agitation provided by the circulating pumps the emulsion at no stage broke. A volume of acetone and Triton X 100 equivalent to the highest treatment concentration was added to the controls.

The 1964 experiments consisted of duplication of dips of $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 4 and 8 hours at concentrations of Thionazin of 0, 500, 1000, 2000, 4000 and 8000 p.p.m. In this case the formulation was the commercially available 25% Thionazin/propylene glycol/alrodyne formulation. In the controls equivalent amounts of propylene glycol and Triton X 100 were added - alrodyne, the emulsifier used in the commercial preparation was not available and for this reason Triton X 100 was used. A treatment of 1000 p.p.m. for 1, 2, 4 and 8 hours using the same formulation as in the 1963 experiments was included to observe any formulation effect. All these treatments were carried out at ambient temperature which varied between 60° and 70°F.

A standard hot water treatment of 112°F. for 3 hours in 0.5% formaldehyde plus Triton X 100 as a wetter was also carried out for purposes of comparison. Precautions were taken to avoid contamination between the different batches after treatment had taken place.

In the 1963 experiments the dipped bulbs were planted out in

rows 2'6" apart with approximately one bulb diameter distance between bulbs. In the 1964 experiments the dipped bulbs were planted in rows 5 ft. apart and bulbs about 6" apart in the rows which considerably eased cultural practices and simplified phytotoxicity determinations.

Nematodes were extracted from the chopped bulb material over a period of eighteen hours using a Seinhorst mistifier as adapted by Webster (1962). Sturrock (1961) showed that this method of extracting nematodes could be used for quantitative measurements.

In the case of the 1968 experiments the bulbs were lifted and twenty bulbs randomly selected from each treatment. The bulbs were finely chopped using a Kenwood Food mixer, the chopped material mixed and a fixed volume of approximately 130 c.c. taken from this bulk. An equivalent volume of chopped leaf material was taken, again from a mixed sample, added to the bulb material and the nematodes extracted. The process in 1964 was slightly different. Here the first twenty bulbs in the row were lifted - this done to eliminate any operator bias in selection of the bulbs which could have occurred in the system used in 1963. Each bulb was then cut to give a transverse section approximately $\frac{1}{2}$" thick, sections being taken at random over the "length" of the bulb, i.e. between the neck and the root or base plate. The sections were chopped, this time using a Skyline Food Chopper, the bulk pooled and a volume equal to that used in the 1963 evaluations taken. This system was regarded as superior to the practice of cutting each bulb into four quarters and selecting one of these. This latter system could lead to bias in that as the symptoms of

nematode infestation are clearly seen by the "brown ring" of infestation, the operator could introduce an element of bias in selection. The reason for using this approach of sampling individual bulbs and not chopping the whole bulb as in 1963 was because of the enormous amount of work involved in this process. The reason for taking a volume of bulb material rather than a weight - which would have ensured equal sized samples - was again for speed. It is conceivable that under ideal conditions the nematode infestations of bulbs could change drastically over a short period of time and this was to be avoided if comparisons were to be valid. The reason for using a 130 c.c. volume of bulb material was because easily cleaned, non-breakable containers of this size happened to be available. After each treatment sample had been processed, all the equipment used was placed in boiling water to prevent contamination.

The water containing the nematodes plus heavy deposits of starch obtained from the mistifier was immediately passed through a 10 cm. diameter 350 mesh (45 micron) sieve, the liquid being kept to one side of the sieve. The sieve was washed with a gentle flow of water and then inverted into a funnel over a tall 150 c.c. beaker. The material remaining on the sieve was washed into the beaker, this being achieved finally by washing the sieve from both ends with water under pressure. By holding the sieve up to the light, any nematodes sticking on the mesh could be clearly seen and any "carry-over" from one sample to the next avoided.

The advantage of this process was that the volume of water containing the nematodes was rapidly reduced, and also the amount of starch

in the sample considerably reduced. Loss of fourth stage larvae and adults of Ditylenchus dipsaci did not exceed 10% and averaged 6% over 6 determinations. The nematodes in the 150 c.c. beakers were allowed to stand for a minimum of 6 hours and then the top 5/6 of the liquid siphoned off.

The above process was carried out in all the 1965 evaluations. In the previous work the containers from the mistifiers were allowed to stand at 4°C. for 6 hours, four fifths of the volume was siphoned off and the remainder poured into tall 250 ml. beakers and again allowed to stand for a minimum of 6 hours before siphoning off approximately 5/6 of the liquid. This latter system was that used by Purnell (1963). The disadvantage of this system was firstly the amount of time required to process any single sample and the considerable amount of carrying about of the samples. Secondly the presence of starch in the sample made counting and identification particularly difficult. Attempts in 1964 to get rid of the starch by means of enzymes proved unsuccessful. The starch could be quite easily flocculated with aluminium but large numbers of nematodes were trapped in this process.

In all cases only fourth stage larvae and adult D. dipsaci were counted, this being done in open counting dishes. Counts were, where possible, of the order of 100 and the sample size was varied, where necessary, to achieve this, as suggested by Jones (1955).

Purnell (1963) showed that counts of healthy flowers was a satisfactory measure of phytotoxicity and this scheme was adopted. In the

1963 experiments flower counts were carried out on the uninfested treated bulbs and in the 1964 experiments on the infested bulbs treatments.

Finally, the use of "spikkel" counts - these are aberrations caused on the leaves by the nematodes - was adopted in the 1963 evaluations, these being scored on a presence or absence basis, no measure being made of the degree of spikkeling.

Results.

1) Effect on Nematode Populations.

The geometric means of the treatment effects in the 1963 dip experiments are given in Table 30. Only 4th stage larvae and adult Ditylenchus dipsaci were counted.

Table 30. Number of D. dipsaci extracted from narcissus bulbs dipped in solutions of Thionazin for different times at different concentrations.

Treatment Means (geometric means transformed from log values)

Temperature		Time	
80°F.	2,729	2 hours	3,404
90°F.	5,117	6 hours	1,824
100°F.	593	18 hours	1,340

Concentration	
0 p.p.m.	23,550
200 p.p.m.	3,733
600 p.p.m.	1,590
1800 p.p.m.	119

As a result of the high phytotoxicity in the 18 hour/100°F./1800 p.p.m. treatment in this experiment, no bulbs grew and no nematode count could thus be obtained. For the purposes of the analysis of variance the missing plot technique suggested by Snedecor (1962) was made use of.

The actual nematode counts appear in the Appendix Table 68A and the Analysis of Variance of the (100 log x) transformation of the data in Table 68B. "Concentration" and "Temperature" factors are significant at the 0.1% level while that for "Time" and all the interactions are non-significant. Because the possible dilution effect of the zero concentration treatments might influence the analysis of the "Time" factor, the analysis was carried out omitting these data (see Table 68C in the Appendix). The picture is unchanged. The critical differences were calculated and the results appear in the Appendix Table 68D. In the case of the temperature effects, there were no significant differences between the 80°F. and 90°F. treatments but these were significantly different at the 0.1% level to the 100°F. treatment. In the case of the concentration effects, all treatments are significantly different.

Another evaluation carried out in the 1963 dip experiment was to count the number of plants showing spikkel lesions, and these were expressed as a percentage of the number of plants present in each plot. The results of these observations are given in Table 31.

Table 31. Percentage of narcissus plants showing spikkel lesions where the bulbs had been dipped in Thionazin solutions.

Treatment means

Temperature		Exposure Time	
80°F.	56.9	2 hours	46.6
90°F.	52.0	6 hours	52.0
100°F.	35.7	18 hours	45.9

Concentration

0 p.p.m.	68.5
200 p.p.m.	50.9
600 p.p.m.	38.1
1800 p.p.m.	35.1

Analysis of variance of the angular transformation of these data is given in the Appendix Table 69. The "Concentration" factor is significant at the 0.1% level and that for "Temperature" at the 1% level. The critical differences were calculated for these factors and these show (Table 69) that while the differences between the 80° and 90° treatments were not significant, that between 90°F. and 100°F. just not was/significant at the 1% level. In the case of the concentration factor, the difference between zero concentration and 200 p.p.m. is significant at 1% with the 1800 p.p.m. treatment significantly better than 200 p.p.m. at the 5% level. As in the case of the nematode counts these data were re-analysed excluding the values for the zero concentrations and the analysis appears in Table 70 in the Appendix. "Concentration" and "Temperature" factors are significant

at the 5% level. With the possible diluting effect of the zero concentration treatments excluded, the difference between the 200 p.p.m. and 600 p.p.m. concentration now becomes just significant at the 5% level.

The above spikkel determinations were carried out on a strictly present or absent basis and no attempt was made to measure the degree of spikkelling. This method would therefore not distinguish clearly between heavy and light infestations. Table 32 gives a comparison of spikkel count evaluations and nematode counts.

Table 32. Comparison of spikkel counts and nematode populations of narcissus bulbs exposed to Thionazin for two hours at 100°F.

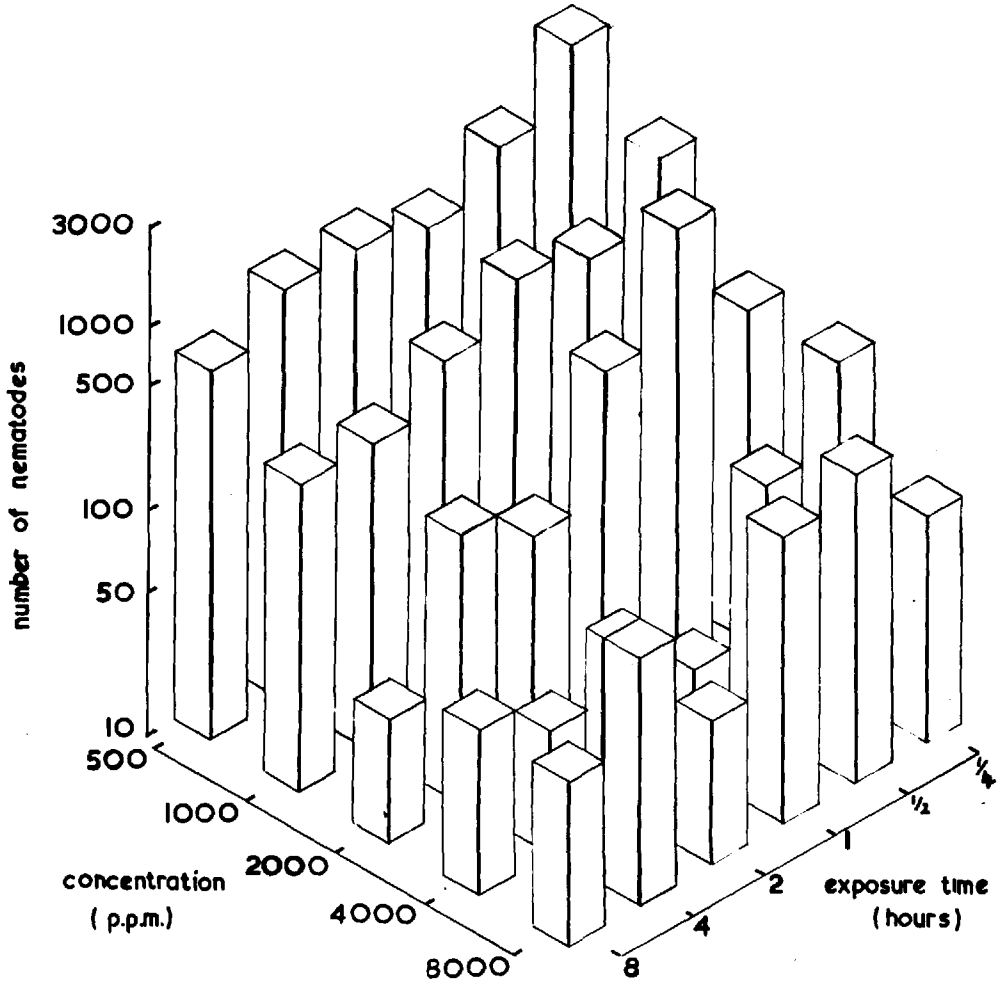
	Concentration of Thionazin (p.p.m.)	
	0	1800
% of plants showing spikkels	43.5	25.0
Nematode count	19400	80

Obviously the relationship between the above two treatments over the two methods of evaluation is quite different, and it would seem that the nematode counts give a far clearer picture of infestation than do spikkel determinations, and for this reason this latter method was not used in the 1964 evaluations.

What is interesting from Table 32 is the fact that treatment of the bulbs seems to generally reduce the number of nematodes per bulb and not so much the number of bulbs infested.

FIG . 19.

Stereograph showing effect of concentration and exposure time on the number of nematodes in Narcissus bulbs dipped in Thionazin solution



The results of the 1964 dip experiment are illustrated in Fig. 19 and the actual nematode counts given below.

Table 33. Numbers of D. dipsaci extracted from infested bulbs immersed in solutions of Thionazin. (Means of 2 replicates).

Exposure Time (hours)	Concentrations of Thionazin (p.p.m.)						Mean	Mean excluding 0 p.p.m.
	0	500	1,000	2,000	4,000	8,000		
¼	39,237	2,592	1,263	366	444	117	7,336	956
½	12,125	1,183	607	1,491	171	354	2,655	761
1	6,287	732	812	381	32	249	1,415	441
2	3,937	936	516	138	69	54	941	343
4	48,312	1,047	285	201	39	159	8,340	346
8	8,012	664	351	42	63	66	1,533	237
Mean	19,651	1,192	639	436	136	166	3,703	~515

Standard hot water treatment 249.

The analysis of variance of the logarithmic transformation of the data appears in the Appendix Table 71A. The data were re-analysed making allowance for the zero concentration treatments as suggested by Professor B.G. Peters (personal communication) (see Table 71B) and finally the data were analysed leaving out all the zero concentration values (see Table 71C). In all cases the "Exposure Time" factor was significant at the 1% level and that for "Concentrations" at the 0.1% level with the interaction being non-significant. The critical differences were calculated (see Table 71D) and though all concentrations of Thionazin are significantly different to the zero concentration treatment, the only other significant concentration difference lies between 2000 p.p.m. and higher concentrations and 1000

p.p.m. and lower concentrations. The differences between exposure times are not as clear but generally it would seem that an exposure time of 1 hour or longer is significantly different to an exposure period of half an hour or shorter, with no other differences being significant.

As the interaction between the "concentration" and "exposure time" factors was not significant, these two factors can be examined separately. Using orthogonal polynomials the linnearity of these two factors was examined (see Table 72 in the Appendix) and in each case the sum of squares for the linnear term removed over 80% of the total variability and was significant at the 1% level. In each case the data were plotted against the number of nematodes and the "least squares" line fitted (see Fig. 20). The slope for the "concentration" parameter is greater than for "exposure time" indicating that a given multiple of the concentration is more effective than the same multiple of the "exposure time". The two lines cross between the quarter and half hour exposure periods indicating that after this point the effect of concentration - on the nematode population inside the bulbs - is greater than that of exposure time, this at least for the concentrations used in this experiment.

As the positive diagonals in Table 33 (omitting zero concentration) have the same concentration-time product (C.T.P.) this situation can easily be examined. It would seem that no simple C.T.P. relationship exists and indeed in this type of study it would not be expected. Table 34 shows the relationship between the C.T.P. and nematode control.

FIG. 20.

Overall effect of Thionazin concentration and exposure time on the number of nematodes in Narcissus bulbs

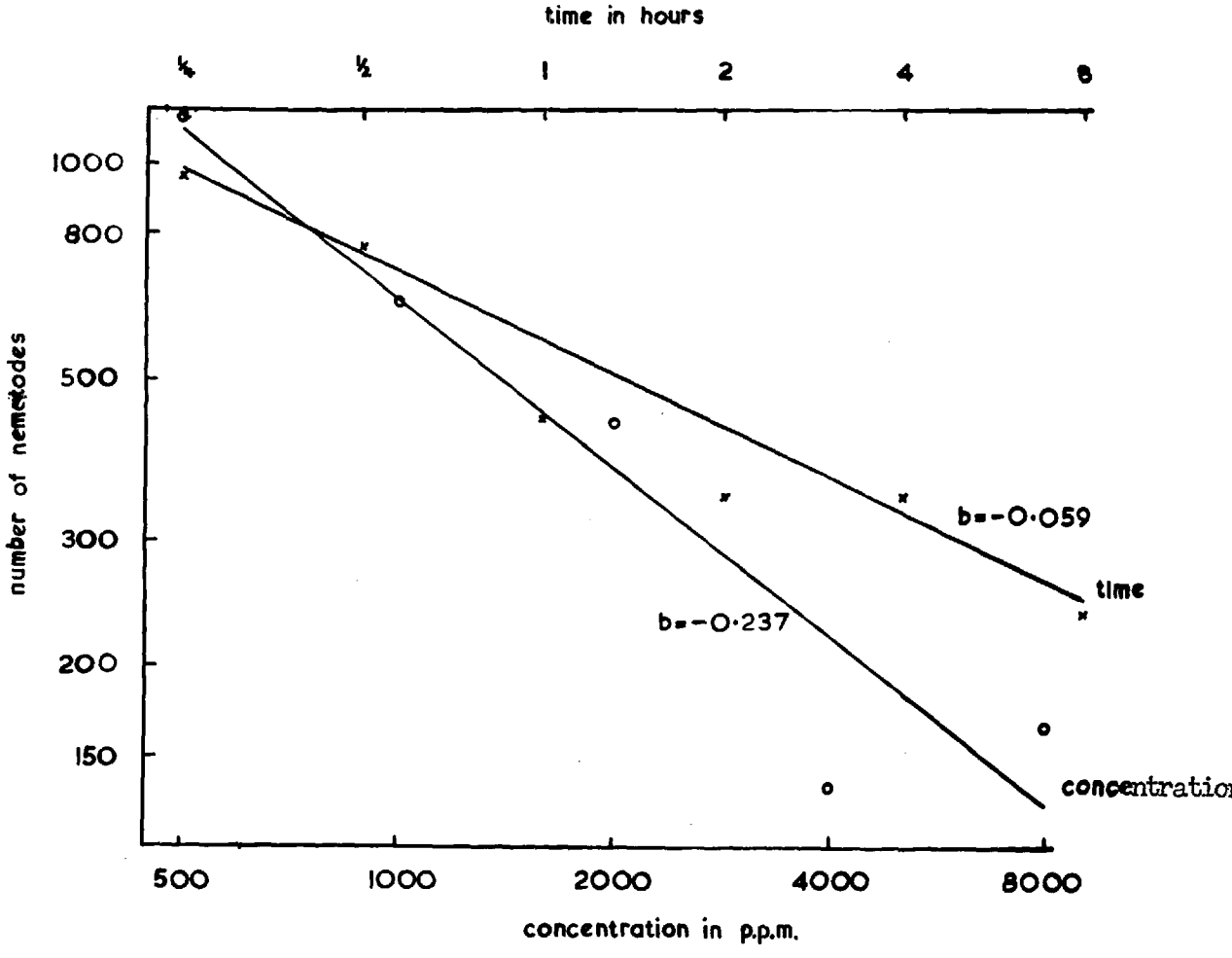


Table 34. Relationship between C.T.P. and nematode control in narcissus bulbs exposed to Thionazin.

C.T.P.	Mean Number Nematodes	Weight	% of 1/8 C.T.P.
1/8	2,592	1	100
1/4	1,224	2	47
1/2	568	3	22
1	921	4	36
2	446	5	17
4	295	5	11
8	218	4	8.4
16	45	3	1.7
32	111	2	4.3
64	66	1	2.5

N.B. The column headed "Weight" shows the number of cells in Table 33 sharing the same C.T.P.

Finally, as an acetone-Triton X 100 - Thionazin formulation was used in the 1963 experiments, and the Thionazin was formulated with propylene glycol and alrodyne in 1964, a treatment using the former formulation was included in the 1964 experiments. The results are given in Table 35.

Table 35. The number of D. dipsaci extracted from infested narcissus bulbs immersed in 2000 p.p.m. of Thionazin formulated in two ways. (Means of 2 replicates).

Exposure Time (hours)	Propylene glycol, alrodyne formulation	Acetone/Triton X 100 Formulation
1	381	1,223
2	138	239
4	201	510
8	42	762

The above data were analysed and the formulation factor was significant at the 5% level. A possible explanation for this difference could be the fact that the commercially prepared formulation was more stable and that there was some settling out in the Acetone - Triton formulation.

2) Phytotoxicity evaluations.

The mean number of flowers from the uninfested bulbs treated with Thionazin in the 1963 experiments are given in Table 36. The bulbs in these plots were weighed before planting with the intention of lifting and weighing them after two years to get a long-term measure of phytotoxicity. Unfortunately in the second year the zero concentration treatments showed signs of nematode damage and this approach was abandoned because of the possible confounding effect of the presence of nematodes.

Table 36. Mean number of healthy flowers produced by narcissus bulbs immersed in solutions of Thionazin at different temperatures for different times.

Treatment Means

Temperature		Exposure Time	
80°F.	32.1	2 hours	29.2
90°F.	27.2	6 hours	26.2
100°F.	15.2	18 hours	19.1
Mean	24.8	Mean	24.8

Concentrations

0 p.p.m.	30.9
200 p.p.m.	24.0
600 p.p.m.	26.2
1800 p.p.m.	18.2
Mean	24.8

The actual flower counts appear in the Appendix Table 73A and the Analysis of Variance of the data in Table 73B. The "Temperature" factor is significant at the 1% level while the "concentration" and "exposure time" factors are significant at the 5% level. The critical differences were calculated and appear in the Appendix Table 73C. In the case of "temperature", the 100°F. treatment is significantly more phytotoxic than the 90°F. treatment at the 1% level, with no significant differences existing between 80°F. and 90°F. In the case of exposure time the 18 hour treatment is significantly different to the 2 hour treatment at the 1% level and to the 6 hour treatment at the 5% level. The 1800 p.p.m. concentration is significantly more phytotoxic than 0 p.p.m. at the 1% level but none of the other comparisons are significantly different.

The flower counts for the 1965 determinations are given in Table 37. Generally there appeared to be small visual differences between the treatments.

Table 37. Total number of healthy flowers produced by narcissus bulbs immersed in solutions of Thionazin - 1964 experiment. (Means of 2 replicates).

Exposure Time (hours)	Concentration of Thionazin (p.p.m.)						Mean
	0	500	1,000	2,000	4,000	8,000	
1/4	15	21	24	19	19	21	19.8
1/2	16	15	24	18	22	22	19.5
1	16	20	39	25	22	18	23.3
2	18	16	26	18	20	24	20.3
4	21	14	26	17	14	18	18.3
8	14	22	22	20	18	29	20.8
Mean	16.7	18.0	26.8	19.5	19.2	22.0	20.4

Normal Hot Water Treatment 12.

The data were analysed and the analysis of variance appears in the Appendix Table 74A. The "Concentration" factor is significant at 1% level with the other factors being non-significant. The critical differences were calculated and these appear in the Appendix Table 74B. Some treatments have produced more flowers than the control and the reason for this is probably because the nematode populations in the controls have reduced flowering. This is therefore a very unsatisfactory measure of phytotoxicity, but it is probably fair to say that these treatments were not particularly phytotoxic as all the plants grew vigorously. All treatments are patently different to normal hot water treatment but this comparison is not a fair one as the hot water treatment was not carried out at the stage of growth optimum for this type of treatment, and phytotoxicity is thus higher than it would normally be.

B. The Application of the Chemical to Growing Bulbs.

Introduction.

The use of a chemical with systemic properties for the control of nematodes is obviously a very desirable state of affairs, and some success has been achieved in this field, though this generally on a small scale. Peacock (1960) reports on the use of systemic chemicals for the control of root-knot and also (Peacock, 1963) on the inhibition of root knot by foliar application of sodium fluoroacetate and maleic hydrazide. Sasser (1952) reports on the foliar application of Systox for the control of Meloidogyne spp. The work of Bergenson (1955) stimulated the work described here. This latter worker found that the foliar application of a number of organo-phosphorus compounds effectively controlled D. dipsaci in daffodils. The aim of the work described here was to establish whether Bergenson's results could be repeated on a field scale, to see whether time of application of the chemical was relevant and to gain information on the effect of concentration of the chemical and the volume of water in which it is applied.

Materials and methods.

Two field experiments were put down, one treated in 1963, the other in 1964.

In the 1963 experiment narcissus bulbs (variety Helios) known to be infested with D. dipsaci were planted out in square plots 4 sq. metres in area at a density of 100 bulbs per plot. The chemical was applied at

two stages of bulb growth, i.e. early application when about 70% of the plants were budding and a late application when the flowers of about 70% of the plants had died back. Thionazin was applied in volumes equivalent to 450, 900 and 1800 gallons per acre at concentrations of 4000 and 8000 p.p.m. An additional treatment of 12000 p.p.m. at 900 gallons per acre was included. Each treatment was replicated four times. Application was by means of a standard watering can with a fine rose. After four months when the foliage had died down, five randomly selected bulbs were lifted from each plot, weighed and the nematodes extracted as described in Part III, A. The means of the five individual counts were taken to give the estimate of the nematode population of the plot.

The following year the number of flowers produced were counted to give a measure of phytotoxicity. Ten bulbs randomly selected from each plot were dug up, chopped in a Kenwood Food Mixer, the material bulked and a 130 c.c. volume of both bulb and foliar material taken and the nematodes extracted. In year 3 the method used was that described in Part III, A (page 89) where the bulbs were sectioned, the sections bulked and the nematodes extracted from a sample of bulb and foliar material.

In the second experiment, i.e. the 1964 experiment, again using "Helios" variety narcissus bulbs, bulbs were planted out into 16 square feet plots at the rate of 50 bulbs per plot. Each plot had a sufficiently large guard area around it to ease application of the treatments and the design of the experiment was a thrice replicated randomised block arrangement. Application of the chemical solution was again by means of a

watering can to give in each case a volume of water on the plot equivalent to 900 gallons per acre with concentrations of 5000 and 10,000 p.p.m. Two separate experiments were carried out here, one on bulbs known to be heavily infested with D. dipsaci and the other on a so-called uninfested stock which was, as it turned out, slightly infested. The treatments were carried out at the following times:

A) When the roots of the bulbs were well developed - this being ascertained by digging up a number of bulbs planted at the same time but not included in the experiment;

B) When the bulb shoots were just above ground level;

C) When the shoots were about 4" long;

D) When the plants showed signs of bud development; and

E) Untreated control.

The above experiment was repeated with infested and uninfested bulbs in an attempt to measure (a) the efficacy of the treatment in controlling nematodes, in the infested bulbs and (b) phytotoxicity, unaffected by nematodes, in the uninfested bulbs. Unfortunately, the infested bulbs were so heavily infested with nematodes that they made very poor growth and this experiment had therefore to be abandoned. However, the "uninfested" bulbs made very good growth in 1964 and all except the control treatments again in 1965. Flower counts to estimate phytotoxicity could therefore be carried out.

Results.

1) Effect on Nematode Populations.

The design of the 1963 drench experiment proved to be highly unsatisfactory. With the different treatment combinations it could be expected that interactions might occur, and these could not be examined in this design. The approach therefore was to analyse the experimental results according to the design and then, omitting the control and 1200 p.p.m. concentration treatments, re-analyse the data. The mean counts for the 1963 evaluations of the experiment appear in Table 38.

Table 38. Geometric means of the numbers of D. dipsaci extracted from narcissus plants drenched with Thionazin in 1963. (1963 counts, means of 4 replicates).

Treatment / Time	Volume / galls/acre	Concentrations p.p.m. 4,000	8,000	12,000	Mean
Early	450	263	186	71	225
	900	331	426		276
	1800	63	151		107
	Mean	219	254	71	
Late	450	3,981	589	363	2,285
	900	155	75		198
	1800	851	151		501
	Mean	1,662	272	363	
	General mean	1,159	263	217	

Control Treatments Mean = 10,470

The nematode counts were analysed and the analysis of variance of the (100 log x) transformation appears in the Appendix Table 75A. Critical differences were calculated and these show that all treatments were significantly different to the 4000 p.p.m./450 gallons per acre treatment and the control at the 1% level.

The geometric means of the nematode counts excluding the 1200 p.p.m./900 gallons per acre and zero concentration treatments are given in Table 39. The analysis of variance of these data transformed to (100 log x) values is given in the Appendix Table 75B.

Table 39. The numbers of D. dipsaci extracted from narcissus bulbs when the plants had been treated with Thionazin. (1963 counts).

Treatment means (geometric values transformed from logs).

Concentrations		Times	
4,000 p.p.m.	3,758	Early	2,004
8,000 p.p.m.	2,075	Late	3,890

Volumes	
450 gallons/acre	5,794
900 " "	2,009
1,800 " "	1,866

The "Volume" factor is significant at the 5% level with the other main factors non-significant. The "t" test shows that there is a significant difference at the 5% level between the 450 gallon and 900 gallon per acre treatments with the 1800 gallon per acre treatment not

being significantly different to the 900 gallon treatment. The Time x Concentration interaction is significant at the 5% level and that for Time x Volume at the 1% level. This is discussed later.

The plots were sampled again in 1964. The mean counts for all the treatments are given in Table 40.

Table 40. Geometric means of numbers of D. dipsaci extracted from narcissus plants drenched with Thionazin in 1963.
(1964 counts - means of 4 replicates).

Treatment / Time	Volume / galls/acre	Concentrations p.p.m. 4,000	8,000	12,000	Mean
Early	450	33.0	591.7	344.6	312.3
	900	91.0	467.8		301.1
	1800	188.0	128.0		158.0
	Mean	104.0	395.8	344.6	
Late	450	504.8	413.0	19.4	458.9
	900	107.0	98.8		75.1
	1800	59.0	29.4		44.2
	Mean	223.6	180.4	19.4	
	General mean	163.8	288.1	182.0	

Control Treatments Mean = 26,150.

Again, because of the inadequacy of the design it was impossible to learn which of the factors (and/or interactions) were significant, and for this reason the data were re-examined ignoring the 1200 p.p.m. and zero concentrations. The results are given in Table 41.

Table 41. Number of D. dipsaci extracted from narcissus plants where the plants had been treated with Thionazin in 1963. (1964 counts). Treatment means (geometric means transformed from logs).

Times		Concentrations	
Early	76.4	4,000 p.p.m.	106.1
Late	120.4	8,000 p.p.m.	86.7

Volumes	
450 gallons/acre	252.7
900 " "	81.9
1800 " "	42.6

The analysis of variance of these data transformed to log (x + 1) values appears in the Appendix Table 75C. None of the factors nor the interactions is significant.

The experimental plots were again sampled in 1965 and the numbers of D. dipsaci estimated. The control plots now had no bulbs growing on them at all and the 4,000 p.p.m. concentration, 450 gallons per acre volume treatment plots for both early and late applications had very few bulbs on them, and these were obviously very heavily infested with D. dipsaci and were not sampled. The other treatments were not significant and there was no large build-up in nematode numbers as compared with the 1964 estimations.

Table 75D in the Appendix shows all the values over the three years expressed as percentages mortality.

FIG 21.

Effect of Thionazin on yield and nematode population when applied as a soil drench to narcissus bulbs.

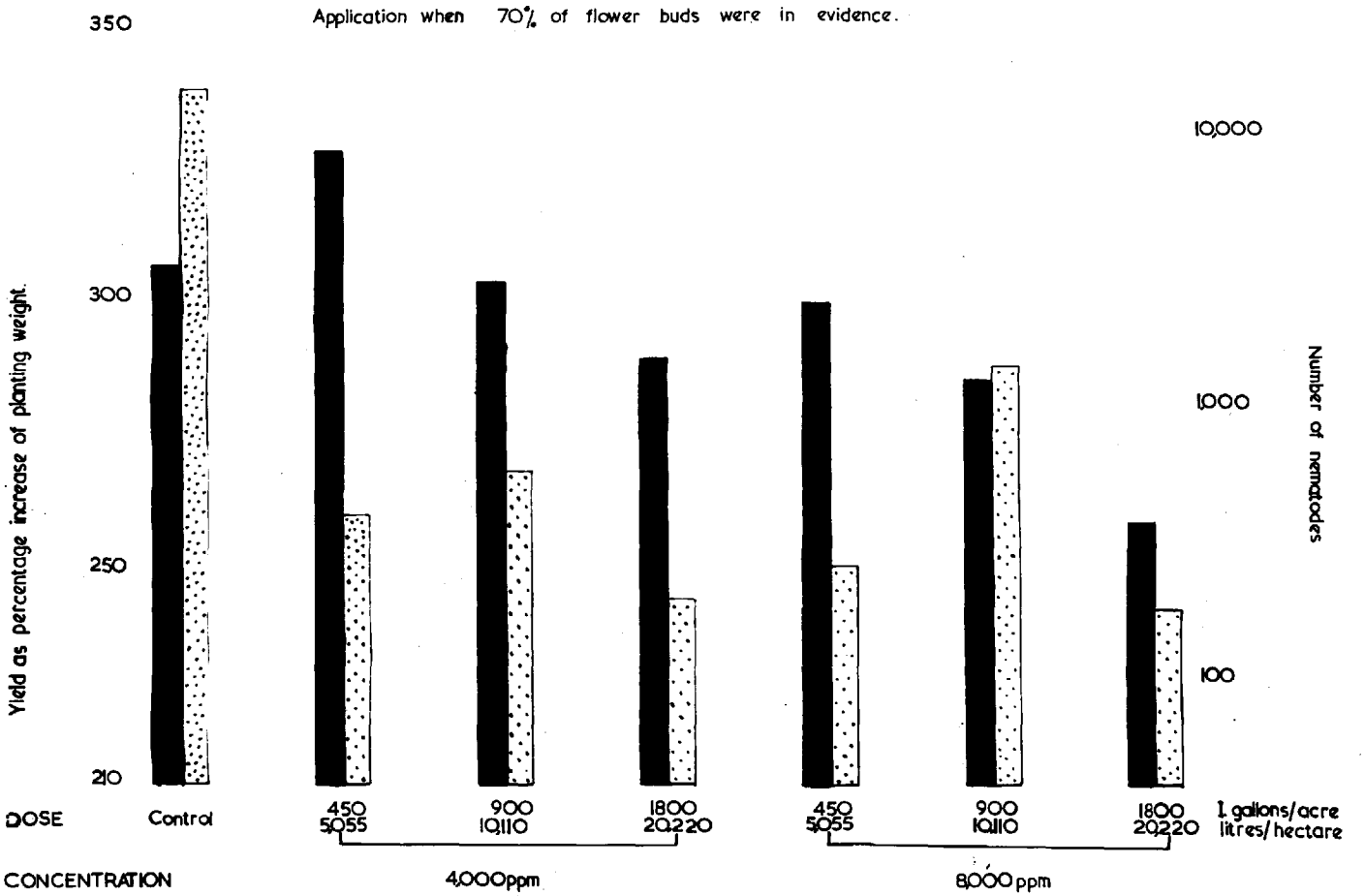
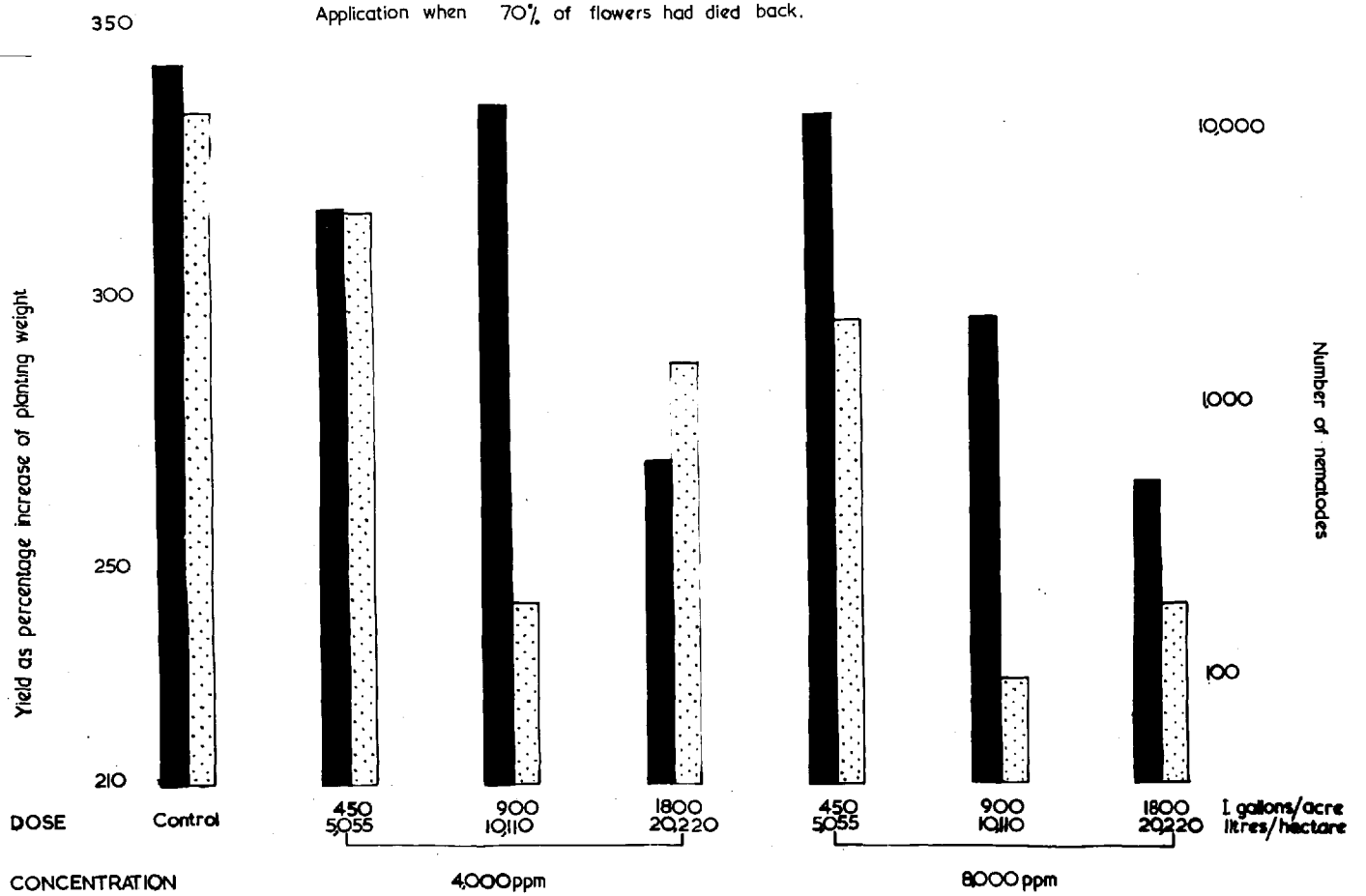


FIG 22.

Effect of Thionazin on yield and nematode population when applied as a soil drench to narcissus bulbs.



2) Phytotoxicity Evaluations.

Evaluations for the first (1963) drench experiment were carried out after the plants had died down in 1963 and measurements were based upon the percentage increase in weight of the bulbs over the planting weights. These measurements were based upon the weights of ten randomly selected bulbs from each plot and the calculation based upon one tenth of the original planting weight. The data was analysed and the treatments were significant at the 1% level. The critical differences appear in Table 76A. As was the case in the nematode population counts, the design was not suitable for measuring the effects of the individual treatment factors or their interactions and the data were therefore re-analysed leaving out the 1200 p.p.m. and zero concentration treatments. The analysis of these data appear in the Appendix Table 76B, and the treatment means in Table 43.

Table 43. Percentage increase in bulb weight in year of treatment of narcissus plants drenched with Thionazin.

Treatment means.

Concentrations		Times	
4,000 p.p.m.	305.5	Early	292.4
8,000 p.p.m.	288.5	Late	301.5

Volumes

450 gallons/acre	317.7
900 " "	303.5
1800 " "	269.6

The "Volume" factor is significant and this is due to the fact that the 1800 gallon per acre treatment is significantly more phytotoxic than the other two volume treatments, while no significant difference exists between these.

The percentages increase over planting weight in these treatments are illustrated in Figures 21 and 22.

In the following year the measure of phytotoxicity was based upon flower counts, and the results appear in Table 76C in the Appendix. The effects of the different treatment factors appear in Table 44.

Table 44. Number of healthy flowers produced by narcissus bulbs one year after the plants had been drenched with Thionazin.

Treatment means.

Concentrations		Times	
4,000 p.p.m.	63.5	Early	60.5
8,000 p.p.m.	61.5	Late	64.5
Mean	62.5	Mean	62.5

Volumes

450 gallons/acre	58.2
900 " "	67.6
1800 " "	61.8
Mean	62.5

The only factor which is significant is that of "Volume" and the 900 gallon per acre treatment has yielded significantly more flowers than the 450 gallon treatment (see Table 76D in Appendix).

No evaluations were made in 1965 because obviously the effect

of nematode population on flower yield would confound that of phytotoxicity.

The number of flowers produced by the uninfested bulb treatments in 1964 and 1965 are given in Table 46.

Table 46. Number of healthy flowers produced by narcissus bulbs where the plants had been drenched with Thionazin at different stages of growth at 900 gallons per acre (means of 3 replicates).

Treatment means.

Concentrations		Year	
5,000 p.p.m.	31.8	1964	23.2
10,000 p.p.m.	28.1	1965	36.7
Mean	29.9	Mean	29.9

Time of Treatment

A	33.1
B	32.0
C	27.4
D	34.6
E	22.4
Mean	29.9

The actual number of flowers produced appear in Table 76E in the Appendix and the analysis of variance of these data in Tables 76F and 76G.

None of the factors were significant in 1964 and though significant differences existed in the 1965 counts, the control treatments yielded the lowest number of flowers indicating that the measurements were no longer confined to phytotoxic effects but that nematode populations in the bulbs were playing a part.

C. Discussion.

Probably the most significant consideration coming out of this work is the fact that very considerable nematode control - at least as good as standard hot water treatment - can be achieved by simply dipping the bulbs in solutions of Thionazin and it is the author's opinion that this will revolutionise the well-established practices for the control of nematodes in narcissus bulbs.

The fact that differences between exposure times as measured by nematode counts in the 1963 experiment were not significant was rather hard to accept, particularly in the light of the fact that concentration effects were significant. Apart from anything else, it is traditional to accept that where a compound is biologically active, the longer the exposure to the compound, the more manifest will be the effect. However, the fact that the effects of exposure time are different in the 1964 trial, and here very short exposure times were included leads to the belief that an optimum amount of treating liquid enters the bulb in a period of less than two hours. This being the case, the more marked differences between concentration effects are quite understandable. This fact has become obvious as a result of the uptake studies and will be discussed more fully later.

The effects of temperature are obvious (Table 68) this being no doubt the physical effect of heat. It is hard to understand why the interaction between exposure time and temperature is not significant. It seems reasonable to expect that there would be a rapid increase in kill at

100°F. over the exposure period, relative to the 80°F. treatments.

As shown in the text, "spikkel" evaluations are possibly insensitive which is unfortunate because this method of evaluation is far simpler and less time-consuming than are nematode extractions and counts. However there was quite good overall agreement between spikkel and nematode count evaluations.

As regards phytotoxicity in the dipping experiment, the differences between temperature effects are to be expected, the young developing flower within the bulb obviously being sensitive to heat. Again it is strange that the exposure time and temperature interaction is not significant.

As regards practical use of the compound, the results of the 1964 study are reassuring. Here the only differences which exist are between the zero and the other treatments. Possibly the reason for this is the fact that the nematode populations within the controls in some way influenced flower development - certainly though, there were no obvious visual differences in the vigour of the plants. Any phytotoxicity as a result of the treatments was light. The reason why concentration effects were significant in the 1963 experiment and not in the 1964 experiment could be that the varieties used in the two years were different.

The drench experiment presents a very interesting picture. In the year of treatment significant differences existed between the treatments as regards nematode control. There were no significant differences between treatment effects in the second year and obvious differences were present

again in year three, very few plants growing in the lowest treatment. These results are not easily explained - the expected outcome of this type of treatment (assuming that it was effective in controlling nematodes) would be to get the sort of results obtained in year one and then to have a steady increase in the numbers of nematodes in the treatments with conceivably a lower rate of increase in the higher treatments in the second year, and the same general trend in the third year with a lesser difference between the higher and lower treatments if this trend existed in year two. This has not been the case. In year one the higher treatments gave, generally, better control, in year two there were no differences and in year three the higher treatments again maintained a higher control, though over all the years D. dipsaci were present in the bulbs. A possible explanation of the results is as follows. If it is postulated that a certain optimum concentration of the chemical is necessary to give good nematode control then it is possible that in the first year not sufficient of the chemical had been taken up by the plant - evidence for this is the fact that generally there was poorer control in the later treatments - these being where many of the flowers had died back. It is reasonable to assume that the plants were perhaps growing less actively at this stage and that uptake by both the roots and the foliage was reduced or perhaps that the effects of the chemical were not yet manifest. In year two there were no differences and it could be that sufficient of the chemical persisted in the soil in all treatments to give an optimum concentration in the bulbs, thus inhibiting the rapid build-up of nematodes.

Sampling in year two was carried out in May. In year three, again in May, thus giving a full year between evaluations, the lower treatments, both early and late applications, had practically died away, the higher treatments still giving good growth. An explanation here is that possibly sufficient of the chemical still persisted in the soil, was taken up by the roots of the bulbs, and controlled the nematodes in the higher treatments but in the lower treatments the concentration of the compound in the soil had fallen to a point too low for the optimum concentration to be maintained in the bulb. Indeed, a colleague, N.E. Scopes (personal communication), detected the presence of a biologically active compound in the soil by means of a bioassay using aphids placed on the leaves of wheat seedlings grown in soil from the high treatments, though the author (unpublished data) was unable to detect the presence of Thionazin by spectrophotometric means. This could have been due to inadequate cleanup of the soil extract or more possibly that Thionazin had broken down in the soil into some other form.

A possible explanation for the significant Time x Volume interaction in the 1963 counts might be that the foliage of the plants during the early treatment was highly active and took up the compound readily, while in the later treatment this was not the case and a particular volume of water was necessary to carry the compound to the root area for uptake to occur. The same explanation could account for the significant Time x Concentration interaction.

The results of the 1964 drench are quite clear, phytotoxicity as

measured by flower counts does not occur, the difference between the control and other treatments in the 1965 counts being due probably to nematode effects in the controls. It was obviously a failure in these drench experiments not to use lower rates and volumes, but at the time the experiments were planned, the manufacturers of Thionazin were stressing the fact that if indeed the bulbs did take the material up, it would be via the roots.

Finally it may be said that this compound is apparently highly active against D. dipsaci applied by the means described and very good nematode control in narcissus can be obtained by its use.

PART IV. FIELD EXPERIMENTS USING THIONAZIN FOR THE CONTROL OF DITYLENCHUS DIPSACI IN TULIP.

A. Application of the Chemical to Dormant Bulbs.

Introduction.

Tulips, like narcissus, are susceptible to D. dipsaci, and as a result of their sensitivity to hot water treatment, nematode control is very difficult. For this reason tulip bulbs were exposed to Thionazin dips to establish whether this chemical was biologically active against these nematodes when applied in this manner and what effect the chemical had on the nematode's host.

Materials and Methods.

The equipment used and the treating procedure adopted were exactly the same as for the narcissus dip experiments described in Part III (page 86). The sampling, chopping and extraction techniques were also exactly the same as for narcissus, the techniques applied in different years also being the same.

The bulbs used in the 1963 experiments were of a so-called "mixed variety" from a stock in Lincolnshire and those for the 1964 experiment were of the variety Rose Copeland. The bulbs had been graded previously and all that was necessary was to remove the damaged bulbs, cone and quarter the stock and randomly select the bulbs for each treatment.

The 1963 experiments consisted of a factorial experiment using concentrations of Thionazin at 0, 200, 600 and 1800 p.p.m., exposure times of 2, 6 and 18 hours at ambient temperature (which here fluctuated between 55°F. and 65°F.) 80°, 90° and 100°F.

The 1964 trials consisted of duplicated treatments using exposure periods of 4, 8, 16 and 32 hours at concentrations of 0, 500, 1000, 2000, 4000 and 8000 p.p.m. of Thionazin.

Nematodes were extracted as described previously, and in 1963 "lesion" counts were carried out. Lesions in this context are what the name implies - in the presence of heavy infestations of D. dipsaci lesions appear on the stems of tulip plants, and the basis of evaluation here was on a presence or absence criterion.

Phytotoxicity evaluations were carried out on the basis of healthy wellformed flowers as was the case in the narcissus experiments. In the 1963 experiments preplanting weights of the bulbs were recorded and the weights after lifting again recorded. Differences in weights were calculated as percentage increase in weight over planting weight. Shortly after flowering these bulbs had however suffered a heavy attack of Botrytis spp. infestation and it was very likely that this could have affected the weights of the bulbs.

Flower counts in tulips presented very considerable difficulties as most bulbs produced healthy flowers, the length of the stem and the size of the flower being apparently more closely related to phytotoxicity. Total flower counts would thus not give a valid measure and the operator thus had to decide whether a bloom was up to standard or not. The criterion used was whether the operator regarded the particular bloom as "saleable" or not.

Results.

1) Effect on nematode populations.

The actual counts for the 1963 dip experiments are given in the Appendix Table 79A. These counts reflect estimations of 4th stage larvae and adult D. dipsaci only. Because of the very high phytotoxicity in the 100°F. range, counts could not be obtained for all these treatments.

Table 47. Number of D. dipsaci extracted from tulip bulbs dipped in solutions of Thionazin for different times at different temperatures.

Treatment means (geometric means transformed from log values).

Temperature		Time	
Ambient	4463	2 hours	2922
80°F.	2163	6 hours	2429
90°F.	1126	18 hours	1531

Concentrations	
0 p.p.m.	9198
200 p.p.m.	2647
600 p.p.m.	1692
1800 p.p.m.	583

The analysis of variance of the data in Table 47 transformed to (1000 log x) values and excluding all treatments at 100°F. appears in the Appendix Table 79B. Both the "Temperature" and "Concentration" factors are significant at the 0.1% level while that for "Time" was not significant. The critical differences were calculated and these showed that all the temperature treatments were significantly different at the 5% level and that the

90°F. treatment was significantly different to the ambient temperature treatment at the 0.1% level. In the case of the various concentrations, all were significantly different to the zero concentration at least the 1% level while the 1800 p.p.m. treatment was better than the 600 p.p.m. concentration at the 1% level and the 200 p.p.m. treatment at the 0.1% level (see Table 79B in the Appendix).

The possibility exists that the zero concentration treatments over "Time" are "diluting" this factor, and for this reason the data were re-analysed excluding all zero concentration effects (see Table 79C in the Appendix). The "Time" factor now becomes significant at the 5% level and as can be seen from the critical differences calculated in Table 79C, this difference exists between the 18 hour and other exposure periods. The other factors are now significant at the 1% level and for the sake of interest relevant critical differences have been calculated here too - see Appendix Table 79C. The geometric means of these treatments are given in Table 47B.

Table 47B. Number of D. dipsaci extracted from tulip bulbs dipped in solutions of Thionazin for different times at different temperatures.

Geometric treatment means transformed from log values and excluding all zero concentration effects.

Temperature		Exposure Time	
Ambient	2270	2 hours	1954
80°F.	1684	6 hours	1659
90°F.	684	18 hours	807

Concentrations	
200 p.p.m.	2647
600 p.p.m.	1692
1800 p.p.m.	584

Another evaluation carried out in the 1963 experiments was lesion counts. The tulip plants were examined individually and the presence or absence of the lesions recorded, and as in the case of the narcissus "spikkel" counts, no measure of the extent of lesion formation was made. The percentage of plants showing lesions based upon the total number of flower stems in each plot were calculated and these percentages transformed to angular values.

Table 48. Treatment means expressed as the mean angular values of the percentage of tulip plants showing lesions after being dipped in solutions of Thionazin.

Temperature		Exposure Time	
Ambient	40.2	2 hours	36.4
80°F.	35.5	6 hours	36.7
90°F.	31.5	18 hours	34.1

Concentration	
0 p.p.m.	43.3
200 p.p.m.	35.2
600 p.p.m.	32.7
1800 p.p.m.	31.7

The analysis of these data appears in the Appendix Table 80. The "Temperature" and "Concentration" factors are significant at the 1% level while that for "Exposure Time" is non-significant. The "t test" shows that the 90°F. treatment is significantly different to the Ambient treatment at the 1% level and very nearly so at the 0.1% level while all the Thionazin concentrations are significantly different to the zero concentration

but not significantly different from each other. This method of evaluation has therefore distinct limitations being unsuitable for detecting differences unless they are large. As this method could not detect differences between the concentrations, and as it was the effects of the chemical which were of particular interest, the method of evaluation was not made use of in the 1964 experiments.

The nematode counts for the 1964 experiments were carried out in early May 1965 and it would seem that this was too early as large numbers of nematodes were not extracted. The counts for these experiments are given in Table 49.

Table 49. Nematode counts per unit volume of tulip bulb material from bulbs dipped in solutions of Thionazin (means of 2 replicates).

Exposure Time (hours)	Conc. of Thionazin (p.p.m.)						Mean excluding 8000 p.p.m. treatment	Mean excluding 8000 p.p.m. and 0 p.p.m. treatments
	0	500	1000	2000	4000	8000		
4	4046	248	74	125	35	195	906	121
8	2025	1002	72	53	179	45	666	338
16	1775	200	15	19	44		411	70
32	2154	133	72	109	49		503	91
Mean	2500	395	58	77	77		618	153

Because of very high phytotoxicity in the 8000 p.p.m. concentration range, no bulbs grew in the longer exposure time treatments over this concentration and no nematode population estimates could be obtained. The analysis of variance of the (1000 log x) transformation of the data appears in the Appendix Table 81. The "Concentration" factor is significant at the

0.1% level while the "Exposure Time" factor and the interactions are non-significant. The "t" test shows all Thionazin concentrations to be significantly different to the zero concentration treatment at the 0.1% level with the 1000 p.p.m. and 2000 p.p.m. concentrations significantly better than 500 p.p.m. at the 1% level and the 4000 p.p.m. treatment just not significantly better than the 500 p.p.m. treatment at the 0.1% level.

2) Phytotoxicity evaluations.

In both the 1963 and 1964 experiments the treatments were examined and counts made of the number of so-called "saleable" flowers. However, in each case the determinations were carried out on infested bulbs, and if nematode infestations have any effect on flower production - and it is probably fair to assume that this is the case - then obviously these recordings are not a true reflection of the effect of the treatment on flower production. Presumably, assuming that the treatments control nematodes, flower counts would be low at the lower treatments as a result of nematode damage and low again at the higher treatments because of phytotoxicity. If these considerations are kept in mind then some value can be attached to the evaluations in this section. The actual counts and the analysis of variance of the data (excluding effects at 100°F.) appear in the Appendix Tables 82A and 82B.

Table 50. The number of healthy flowers produced by tulip bulbs after immersion in solutions of Thionazin (1963 experiments).

Treatment means.

Exposure Times		Temperatures	
2 hours	22.3	Ambient	18.1
6 hours	16.4	80°F.	14.2
18 hours	7.3	90°F.	13.8

Concentrations

0 p.p.m.	13.1
200 p.p.m.	16.0
600 p.p.m.	19.1
1800 p.p.m.	13.0

All the factors are significant when tested against the triple interaction, but if first-order interactions are used as a measure of error the only significant factor is time of exposure. It is quite possible that the triple interaction estimate is abnormally low by chance, in which case the other factors should be ignored as of doubtful significance. The "t" test shows that in the case of the "Exposure Time" treatments, the 2 hour treatment is significantly less phytotoxic at the 1% level than the 6 hour treatment and this at the 0.1% level when compared with the results of 18 hour exposure. The difference between the 6 hour and 18 hour treatments is also significant at the 1% level.

The Temperature x Concentration interaction is significant at the 1% level and this is displayed in Table 82C in the Appendix. This significance appears to come from the zero concentration over temperature counts, and is a reflection of the fact that the effect of temperature is dependent

on concentration, and vice versa. It is notable that the greatest number of healthy flowers came from the 600 p.p.m. treatments at ambient temperature, and the least from 0 p.p.m., also at ambient.

In the bulb yield study the bulbs were lifted and weighed and these weights expressed as percentage increase in weight over the planting weights. The results are given in Table 51 and the analysis of variance appears in the Appendix, Table 83.

Table 51. The percentage increase in tulip bulb weight over planting weight after immersion in solutions of Thionazin (1963 experiment).

Treatment Means.

Temperature		Exposure Time	
Ambient	175.5	2 hours	209.5
80°F.	183.8	6 hours	192.3
90°F.	179.4	18 hours	136.9

Concentrations

0 p.p.m.	187.2
200 p.p.m.	176.3
600 p.p.m.	187.5
1800 p.p.m.	167.2

The "Time" factor is significant at the 5% level with none of the other factors nor the interactions significant. These "Time" treatment differences lie between the 18 hour and other exposure periods. No doubt had the 100°F. treatments been included, the "Temperature" factor would also have been significant. As mentioned earlier, the fungal attack might have influenced things but in the light of the above results it would seem

that flower counts give a far more sensitive measure of the effect of Thionazin on the plant. However if the aim of treatment is to produce bulbs rather than flowers then it would seem quite suitable to make use of longer exposure treatments of the compound should this, for some reason, be desirable.

The flower count determinations for the 1964 experiment are open to the same criticisms as those for 1963. However these bulbs were not as heavily infected with nematodes as were the bulbs in the 1963 experiments and the nematode effects, if these exist, would not be as obvious.

Table 52. Number of healthy flowers produced by tulip bulbs after immersion in solutions of Thionazin (1964 experiments - means of 2 replicates).

Exposure Time (hours) \ Concentration p.p.m.	0	500	1000	2000	4000	8000	Mean
4	25	23	18	8	10	4	15
8	26	21	15	8	4	1	13
16	25	17	4	0	0	0	8
32	22	4	0	0	0	0	4
Mean	25	16	9	4	4	1	10

Obviously phytotoxicity is very marked over all treatments, though in the lower exposure periods it does not appear to be too severe.

B. Application of the Chemical to Growing Bulbs.

Introduction.

As in the case of the narcissus drench experiments, this work was initiated as a result of work carried out by Bergenson (1955) on Easter Lilies and narcissus. The aim of the experiments described here was to measure what effect, if any, the application of Thionazin to tulips would have on the nematodes within the tulips and on the tulip plants themselves.

Materials and Methods.

Two field experiments were carried out. In the first, tulip bulbs from a *D. dipsaci*-infested stock of mixed varieties were planted out into 16 square feet plots at the rate of 50 bulbs per plot. Each plot had a sufficiently large guard area around it to ease the application of the treatments. The design was a randomised block arrangement with three replicates. Application of the chemical solution was by means of a watering can to give in each case a volume of water on the plot equivalent to 900 gallons per acre, with concentrations of 5000 and 10,000 p.p.m.

Treatment times were as follows:

A) When the roots of the bulbs were well developed - this being ascertained by digging up a number of bulbs planted at the same time but outside the experiment.

B) When the tulip shoots were just above the ground.

C) When the shoots were about 4" long.

D) When the plants showed signs of bud development.

E) A combination of A and D.

F) Untreated controls.

When the plants were well developed they were examined for lesions (as in Part IV, A), the number of well developed ("saleable") flowers recorded and finally the bulbs dug up and nematode extractions carried out.

The limitations of this experiment seemed to be that:

1) It would only give information on soil drenches.

2) It would not show whether foliar application of the chemical was effective, a technique far simpler and more acceptable than drenches.

3) It would give information only on high application rates of the chemical.

With the above considerations in mind the following experiment was carried out. An area in a field of tulips (variety Rose Copeland) which had just been topped and were showing signs of nematode damage was selected and a fully randomised duplicated experiment with all combinations of the following treatments put down.

Concentration of Thionazin: 0, 2.5, 5, 10, 20 lbs a.m./acre.

Volume of carrier: 100, 200, 400 gallons/acre.

Application was by means of a pressurised sprayer and every attempt was made to get the spray evenly distributed over the plot. When the foliage had died down the plots were dug up and the nematodes extracted from a sample of twenty bulbs randomly selected from each plot. The plots were further sampled and forty randomly selected bulbs from each plot were planted out to establish whether the effect of the treatment was perhaps delayed and could be detected a year later.

Results.

1) Effect on nematode populations.

The nematode counts for the first drench experiment - this being the experiment on the "mixed" varieties - are given in Table 53. The analysis of the logarithmic transformation of these data appears in the Appendix Table 84.

Table 53. Number of D. dipsaci extracted from tulip bulbs drenched with Thionazin at different stages of growth at 900 gallons per acre (means of 3 replicates).

Time of Treatment	Number of Nematodes		
	Concentration of Thionazin p.p.m.		Mean
	5,000	10,000	
A) At root development.	6,683	21,030	13,857
B) Shoots just above ground level.	4,587	1,420	3,004
C) Shoots ~4" long.	4,725	22,225	13,475
D) At budding.	6,175	9,883	8,029
E) Combination A & D.	8,558	9,088	8,823
F) Control.	6,315	10,260	8,288
Mean	6,173	12,317	9,245

Analysis of these data showed that none of the factors was significant.

The nematode population estimations from the second experiment, this being the trial on tulip variety Rose Copeland, are given in Table 54.

Table 54. Number of D. dipsaci extracted from tulip bulbs where the plants were treated with Thionazin just after "topping" (means of 2 replicates).

Concentration of Thionazin lbs a.m./acre	Volume of water (gallons/acre)			Mean
	100	200	400	
0	1,515	1,795	587	1,299
2.5	181	286	1,212	559
5	1,420	1,056	136	870
10	892	2,367	448	1,235
20	2,978	160	178	1,105
Mean	1,397	1,132	512	1,014

The analysis of variance of the (1000 log x) transformation of these data appears in the Appendix Table 85A. None of the factors is significant. The plots from which the above data were obtained were sampled, the bulbs planted out and nematode extractions carried out a year later - this being done to establish whether any long term effect existed which could not be detected by the earlier evaluations. The results appear in Table 55.

The analysis of variance of the logarithmic transformation of the data in Table 55 appears in the Appendix Table 85B, and shows that none of the factors is significant, indicating that no nematode control is achieved.

The nematode counts in Table 55 cannot be directly compared to those in Table 54 as the amount of plant material used for the evaluations in 1965 was half that used for the evaluations in Table 54.

Table 55. The number of D. dipsaci extracted from tulip bulbs a year after the plants had been treated with Thionazin (means of 2 replicates).

Concentration of Thionazin lbs a.m./acre	Volume of water (gallons/acre)			Mean
	100	200	400	
0	1,787	11,024	147	4,319
2.5	316	361	194	290
5	342	1,102	2,555	1,333
10	246	216	477	313
20	823	213	131	389
Mean	703	2,583	701	1,329

2) Phytotoxicity evaluations.

The results of the first experiment in 1964 are given in Table 56.

Table 56. Number of healthy flowers obtained from tulip bulbs drenched with Thionazin at different stages of growth at the rate of 900 gallons per acre (means of 3 replicates).

Time of Application	Conc. of Thionazin p.p.m.		Mean
	5,000	10,000	
A) At root development.	31	28	29.5
B) Shoots just above ground level.	27	23	25.0
C) Shoots 4" long.	26	21	23.0
D) At budding.	22	19	20.5
E) Combination A & D	26	19	22.5
F) Control (no treatment).	26	28	27.0
Mean	26.3	23.0	24.6

The above data were analysed (see Table 86 in the Appendix) and none of the factors was significant indicating that these treatments were not phytotoxic.

No recordings were made of phytotoxicity in the second foliar and drench experiment on the Rose Copeland variety. However there were no obvious visual effects except for brown discoloured markings which appeared on the leaves of the treated plants within a week of application of the chemical. These did not appear to affect the plants in any obvious way and there was no wilting of the leaves.

The plots were sampled and bulbs planted out again at the end of the season. Bulbs from all the treatments grew well and there were no obvious visual differences between treatments in the following year.

C. Discussion.

The generally greater degree of phytotoxicity in tulips as compared to narcissus is obvious and well illustrated by the effects on plant growth of exposure to temperatures of 100°F. (see Table 82A). This sensitivity to temperature and the obvious effects of concentration of Thionazin and exposure time (see Table 82A in the Appendix) make nematode control very difficult, and obviously reduce the value of Thionazin for this purpose.

However, quite considerable nematode control was achieved in the 1963 experiments on the mixed varieties of tulips, and what is interesting is the fact that when the possible diluting effects of the zero treatments was excluded, the differences between exposure times - as measured by nematode counts - were significant. This is different to what was achieved with narcissus but is perhaps in line with what might be expected from the uptake study in Part II. The effects of Temperature and Concentration are similar to the findings for narcissus, and again the reason for the Temperature and Exposure Time interaction not being significant is not clear. The 1964 trial does show that nematodes can be controlled quite effectively but phytotoxicity was so high generally that these estimations are not worth very much. The rate of nematode development is probably related in some way to the vigour of the host plant, and certainly the tulips exposed to the higher treatments were not vigorous. The experiment in 1964 was badly conceived - it would have been far more

fruitful to use far shorter exposure times and possibly have included a higher concentration. The reason for doing the experiment as it was done was firstly because nematode control in the 1963 experiment was not as good as for narcissus and the fact that exposure time differences were significant where the zero treatments were not included in the analysis.

Again, as regards evaluation of these experiments, the value of lesion counts appears to be limited, this method being unsuitable for detecting anything but very large differences. This is particularly unfortunate because this is a very simple and easy system, the lesions showing up very clearly on the flower stems. Possibly if some method of measuring the extent of lesion formation could be perfected this method of evaluation could be adopted.

The phytotoxicity observations of the dipped bulbs indicate that, as mentioned before, tulip bulbs are easily damaged by this type of treatment. The analysis of the 1963 experiment (Appendix Table 82B) shows that the Exposure Time factor is significant at the 0.1% level - no doubt had the data for the 100°F. set of treatments been included, "Temperature" too would have been very highly significant. Why the Concentration by Temperature interaction should be particularly significant is hard to decide. The Temperature by Time interaction would be expected to be very high, this probably would have been the case had there been estimations for higher temperature effects. It is interesting to note though that even at a relatively low temperature of 90°F., the flower producing potential of the bulbs was affected.

Little can be said about the foliar application of Thionazin. There appears to have been no control of nematodes by this type of treatment and it is difficult to think of any further experiments which could be carried out along these lines. A drawback of the experiments was the obviously big fluctuations in the populations of nematodes in the bulbs and this would make the analysis insensitive - however what is clear is that there certainly has not been any large degree of control as was the case in the narcissus experiments. Phytotoxicity at the levels and rates of Thionazin used does not seem to create a problem.

The next step along the lines of the experiments in this section would be to do further dipping experiments using far shorter exposure periods at ambient temperatures. This approach could well lead to the fixing of optimum concentrations and exposure times and create a situation where good control could be achieved in tulips which would constitute a major breakthrough in the field of nematode control.

PART V. GENERAL CONCLUSIONS.

This thesis is divided into four parts, each being to some extent complete in itself. There are the greenhouse and laboratory studies, the uptake observations and the studies of the effect of treating narcissus and tulip bulbs with Thionazin. As these each form an independent study, each is followed by a discussion of the main points arising out of the particular experiments carried out and the aim under this heading of "General Conclusions" is to discuss in general terms the points which arise out of the work as a whole. In this context probably the most relevant observation is the fact that though the Thionazin appears to have a limited effect on plant parasitic nematodes when they are exposed to it "in vitro" it appears to be very highly nematicidal when the nematodes are exposed "in vivo". Kreutzer (1963) makes the point that the degree of toxicity of a chemical to a soil microorganism (and to this the author feels could be added plant microorganisms) in pure culture is markedly different from that shown against the same organism in the natural habitat. The first involves only a chemical x organism interaction, the second a chemicalxorganismxsoil interaction. This worker has given this explanation of the greater toxicity of compounds, in general, when the organism is exposed "in vitro" as compared to "in vivo" exposure. In this study the position is reversed - the compound has very limited obvious effects when the nematode is exposed to it "in vitro", but a very marked effect, and that at a very low concentration, when the plant parasitic nematodes studied were exposed "in vivo", but Kreutzer's (1963)

principle still applies. This situation is not unknown in nematology. Tarjan (1950) working on Sodium selenate has shown that this compound has no obvious effect "in vitro" on Pratylenchus spp. but a substantial effect "in vivo", controlling these nematodes in boxwood. This latter worker has postulated that this control might be due to ingestion of the chemical in ionised form; the selenium may combine with another substance in the plant tissue to form a toxic principle or that another substance toxic to the nematodes may be activated by the presence of the selenium.

Similar explanations could account for the results obtained by the author in this study. The fact that Thionazin has had a distinct effect on Turbatrix aceti "in vitro" tends to the belief that ingestion of the compound is necessary before it is nematicidal. Further evidence for this is given by the fact that when narcissus and tulip bulbs are immersed in a solution of Thionazin the nematodes are controlled, and the presence of the compound in the bulbs has been demonstrated. The same situation exists for Ditylenchus myceliophagus and Aphelenchus avenae living on their fungal hosts.

However, the disappearance of Thionazin, in its original form, at a relatively rapid rate from bulbs has been demonstrated in this study, and the author has been unable to extract Thionazin from the soil in the narcissus drench experiment, even though an obvious effect on the nematodes is evident. Slootweg (1963) reported that uninfested tulip bulbs dipped in Thionazin and planted in soil infested with Ditylenchus dipsaci were significantly less infested than untreated bulbs, growing in the same soil, at the end of the

season. This would indicate that the bulbs are in a sense resistant to the nematodes, and an obvious explanation for this is the fact that Thionazin, or a biologically active derivative, has existed in the plant for a considerable period. As the author has been unable to detect Thionazin in the bulbs after a relatively short period after exposure to the compound, it would seem that a biologically active breakdown product is effective here. The breakdown of organophosphorus compounds to biologically active derivatives is known, and Hartley (1952) reports on the breakdown of "Systox" in plants. This compound contains two isomers of the active ingredient, so called Demeton O and Demeton S, the insecticidal properties of the compound are thought to be due mainly to the presence of Demeton S, and Demeton O isomerises to form this compound. Demeton S in turn further degrades to two toxic derivatives, and it is these which are regarded as making the compound very insecticidal. Another example is given by Thomas and Bennett (1954) who inter alia report that Schradan may be converted within the plant tissues to give a very active metabolite. Obviously this compound x plant interaction is highly complicated.

However, in spite of (or as a result of) the foregoing, nematodes in bulbs have been controlled and this at very low concentrations. In the 1963 study where the bulbs were dipped for two hours in a solution of 200 p.p.m. of Thionazin, the concentration of the compound in the bulb would probably have been in the region of 6 p.p.m. - this calculated on the basis of the findings in the uptake study in Part II of this work. This would certainly indicate that the compound is highly active biologically, and this

is confirmed in the fungal feeding nematode study. Newton et al. (1934) in postulating that chemical control of nematodes in bulbs was not possible because not sufficient of the chemical could enter the bulb was obviously wrong, though presumably with the compounds he had available to him this was true.

A point which can well be made here is one regarding the tremendous variability in the nematode populations of tulip and narcissus bulbs. Purnell (1963) extracted the nematodes from twelve randomly selected narcissus bulbs - these from the same stock used in the early work in this thesis - and found that the numbers of D. dipsaci ranged from zero to 5380 nematodes per bulb over these twelve extractions. Shah (unpublished data) extracted the nematodes from thirty individual tulip bulbs - these from the stock used in the latter part of the work on tulips in this thesis - and found that the numbers of D. dipsaci ranged between 12 and 10,300 nematodes per bulb. The results of this type of problem are obvious - treatment effects have to be very marked to be detected and in this way smaller, but possibly valuable effects go undetected.

The autoradiographs in Part II of this study indicate that the compound moves readily into the bulb, and as nematode control is achieved, this compound can be termed a chemotherapeutant. Crowdy (1952) uses the term chemotherapy to describe the treatment of plant diseases with chemicals which are, to a greater or lesser extent, mobile within the host. The term here is used in contrast to protection where generally the action takes place outside the plant. Horsfall and Dimond (1951) define chemotherapy as the

treatment of a host with a compound so that the action of the compound occurs inside the host when the host is diseased. The compound may (a) kill the pathogen as it enters the host plant, (b) free the host of the established pathogen, (c) mitigate the disease. As Slootweg (1963) has shown, it would seem that treatment with Thionazin provides some form of protection as well and the requirements of Horsfall and Dimond (1951) are therefore fulfilled. Foliar and root uptake of the compound is presumably truly systemic, the chemical being transported, as it were, by the plant. In the case of the bulb dip, the chemical is applied at the neck of the bulb and has an effect elsewhere though movement here is due to the fact that the compound is carried by some outside vehicle, in this case its solvent, water. However, having reached a particular site, the chemical would presumably have to be taken up by the plant for it to be nematicidal. As Crowdy (1952) points out, the effect of a systemically distributed chemical on plant disease is a very complicated biological system to study and is generally limited to the application of the chemical at one end of the system, and measurement of the effect at the other. In terms of nematode control, the results obtained in this study are possibly an example of what Oostenbrink (1964) terms harmonious control of nematode infestation, or perhaps even more applicable, specific nematode control. It is probably safe to assume that, accepting that this compound is nematicidally effective only when it is ingested, only those nematodes feeding upon plant material are affected.

As regards nematode control, the 1964 narcissus dip experiment results are in line with what might be postulated from the uptake study in

Part II. The overall effect of concentration of Thionazin is more marked than the effect of length of dip or immersion in the compound, and as the uptake study shows, the uptake of Thionazin is very rapid at first and then tails off, this fact being more marked in the case of narcissus than in the tulip study. It seems that just so much liquid is taken up by the bulb and the concentration within the bulb is therefore related to its concentration in the liquid absorbed. The reason why the effects of dipping time in the 1963 experiment on narcissus were not significant could be that within the first two hours of treatment a very considerable portion of the total possible uptake has already taken place. According to this, a very short exposure period to a high concentration should in principle be adequate to obtain the optimum concentration of the compound in the bulb. In practice a longer exposure at a lower concentration may be more biologically effective in that this might result in a more uniform distribution of the compound within the bulb. From the practical point of view of nematode control in bulbs, such treatments as presoaking would probably be undesirable, in fact it may be preferable to slightly desiccate bulbs before treatment to increase the uptake. It is interesting to note that in the 1963 tulip dip experiments where the effects of the zero treatments are ignored, the effects of exposure times are significantly different. An explanation of this is given by the uptake study in Part II - the effect of exposure time on uptake of the compound is greater in tulip than it is in narcissus.

The drench experiment results are difficult to explain. In narcissus there has been a marked effect, and though the uptake of the

compound by tulips has been demonstrated, no differences in nematode control could be detected. Obviously in these experiments there was a tremendous variation in the degree of infestation of individual bulbs, and this could have reduced the sensitivity of the assay.

The results of the "in vitro" toxicity tests on D. dipsaci in Part I and the results of the similar treatments "in vivo" in Parts III and IV cannot be compared. What is interesting in these studies is the fact that though the temperature-time interaction is significant in the "in vitro" study, this is not the case in the "in vivo" observations. It could be that at the higher temperature treatments the temperature within the bulb did not reach that of the treating liquid though with an exposure period of 18 hours this is difficult to accept. A more likely explanation is the fact that the bulb acted as an insulator to some extent, and that the temperature within the bulb went up more slowly and that the nematode was not therefore subjected to the shock of sudden exposure to a higher temperature, and was slowly acclimatised. Green (1964) makes the point that so-called acclimatization to heat may be an important factor in nematode resistance to temperature effects.

Nothing has been said about the effect of Thionazin on the hosts of the nematodes and there is little that can be said. In the case of tulip, phytotoxicity is more marked than in the case of narcissus, but it would seem that generally the effect of the compound on the host, at concentrations which have a marked effect on the nematode, is very limited. This applies too to the studies on the fungal hosts, Fusarium oxysporum and

Agaricus campestris.

The nematicidal effect on Heterodera rostochiensis is interesting, and the author has postulated earlier that part of this effect may be due to ingestion by these nematodes of the compound. Another very distinct property is the inhibitory effect of very low concentrations of the compound on hatch of larvae from cysts.

Finally, the following generalisations made by some workers are of interest in the light of the findings in this study. Christie and Perry (1951) postulate that in field plot experiments any soil nematodes probably reflect the comparative nematicidal properties of a compound just as accurately as do root knot nematodes or any other plant parasitic nematodes. Allen (1960) in discussing the properties of a successful nematicide lists, in addition to many other properties, the need for a nematicide to have very special properties as regards vapour pressure and diffusion, and in so doing equates nematicides with soil fumigants. These generalisations are obviously incorrect in the light of what has been elucidated in the course of this study on the biological properties of Thionazin.

As a result of the work detailed in this thesis, the utilisation of Thionazin has been accepted for the very specific end use of nematode control in flower bulbs, and a large quantity of the compound will be used for this purpose in the coming season. The field of nematode control is expanding and this study has to some extent contributed to this expansion.

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APPENDIX.

The following are the abbreviated symbols used in the appendix.

x = Significant at the 5% level of probability.

xx = Significant at the 1% level of probability.

xxx = Significant at the 0.1% level of probability.

n.s. = Not significant.

D.F. = Degrees of Freedom.

S.S. = Sums of Squares.

M.S. = Mean Square.

F. = Variance ratio.

C.D. = Critical difference.

Table 57. Analysis of variance of data obtained from pots treated with Thionazin at different concentrations and different times.

A. Analysis of angular transformation of the percentage mortality of larvae/200 gm. soil.

Source	D.F.	S.S.	M.S.	F.	P.
Treatment Times	2	38,313	19,156	20.2	xxx
Concentrations	2	16,003	8,001	8.4	xx
C × T	4	10,805	2,701	2.8	n.s.
Error	18	17,012	945		
Total	26	82,133			

B. Analysis of angular transformation of percentage mortality based on cyst counts per 200 gm. soil.

Source	D.F.	S.S.	M.S.	F.	P.
Treatment Times	2	69,124	39,562	22.3	xxx
Concentrations	2	26,465	13,232	7.5	xx
C × T	4	2,587	646	<1	n.s.
Error	18	31,957	1,775		
Total	26	130,133			

C. Analysis of angular transformation of percentage reduction in cyst content.

Source	D.F.	S.S.	M.S.	F.	P.
Treatment Times	2	10,511	5,255	2.3	n.s.
Concentrations	2	5,053	2,527	1.1	n.s.
C × T	4	9,218	2,305		
Total	8	24,782			

Table 58. Analysis of the angular transformation values of the percentage mortality of larvae of H. rostochiensis per 200 gm. of soil treated at different times and concentrations.

A. Analysis of variance of effects of treatment two weeks before planting.

Source	D.F.	S.S.	M.S.	F.	P.
Concentrations	2	4,467	2,233	2.8	n.s.
Error	6	4,808	801		
Total	8	9,275			

B. Analysis of variance of effects of treatment two weeks after planting.

Source	D.F.	S.S.	M.S.	F.	P.
Concentrations	2	13,733	6,866	5.6	x 5%
Error	6	7,294	1,215		
Total	8	21,027			

C. Analysis of variance of effects of treatment four weeks after planting.

Source	D.F.	S.S.	M.S.	F.	P.
Concentrations	2	8,598	4,299	5.2	x 5%
Error	6	4,911	818	(F.05 = 5.14)	
Total	8	13,509			

Table 59. Hatch of H. rostochiensis cysts exposed to concentrations of Thionazin plus root diffusate for one week and then root diffusate only for two weeks.

A. Hatch after one week (mean of 3 replicates).

Conc. of chemical (p.p.m.)	Mean Hatch
0	993
0.5	2
2.0	2
10.0	3

B. Cumulative hatch after two weeks after one week exposure to Thionazin.

Conc. of chemical (p.p.m.)	Mean Hatch
0	1507
0.5	210
2.0	82
10.0	10

C. Cumulative hatch after four weeks after one week exposure to Thionazin.

Conc. of chemical (p.p.m.)	Mean Hatch	% Hatch
0	1791	-
0.5	329	18.4
2.0	149	8.3
10.0	49	2.7

Table 60. Hatch of H. rostochiensis cysts exposed to Thionazin plus root diffusate for one week (means of 4 replicates).

Conc. of chemical (p.p.m.)	Mean Hatch
0	1574
0.125	1042
0.062	1600

Table 61. Analysis of variance of cumulative hatch of H. rostochiensis cysts exposed to Thionazin plus root diffusate for 4 weeks (log transformation).

Source	D.F.	S.S.	M.S.	F.	P.
Concentrations	3	24,851	8,284	124.9	xxx 0.1%
Error	20	1,327	66.35		
Total	23	26,178			

Table 62. Analysis of variance of cumulative hatch after eight weeks of H. rostochiensis cysts exposed to Thionazin plus root diffusate under two programmes (log transformation of data).

Source	D.F.	S.S.	M.S.	F.	P.
Concentrations C	3	13,362	4,454	35.8	xxx 0.1%
Programmes P	1	2,709	2,709	21.8	xxx 0.1%
P × C	3	2,545	845	6.8	xx 1%
Error	16	1,987	124		
Total	23	20,603			

Table 63. Analysis of variance of cumulative hatch after 16 weeks of H. rostochiensis cysts exposed to Thionazin plus root diffusate under different programmes (log transformation of data).

Source	D.F.	S.S.	M.S.	F.	P.
Concentrations C	3	41,151	13,717	37.16	xxx 0.1%
Programmes P	1	1,559	1,559	4.22	n.s.
C × P	3	1,868	623	1.68	n.s.
Error	16	5,906	369		
Total	23	50,484			

Table 64. Angular Transformations of Percentage Mortalities of D. dipsaci exposed to Thionazin in vitro.

Temperature		100°F.				90°F.				80°F.				Totals
Concentration p.p.m.		200	600	1800		200	600	1800		200	600	1800		
2 Hour Exposure	E	34.45	42.13	33.21		35.06	36.27	32.58		23.58	18.43	21.97		
	1	27.97	33.83	35.06		27.27	24.35	30.00		19.37	25.84	28.66		
	DAY	62.42	75.96	68.27	206.65	62.33	60.62	62.58	185.53	42.95	44.27	50.63	137.85	530.03
	E	42.71	43.85	40.98		19.37	28.66	38.65		14.18	18.43	8.13		
	5	30.66	50.18	46.15		15.34	25.10	27.97		9.97	12.92	23.58		
	DAY	73.37	94.03	87.13	254.53	34.71	53.76	66.62	155.09	24.15	31.35	31.71	87.21	496.83
	E	33.83	35.67	40.40		33.21	25.10	36.27		8.13	23.58	24.35		
	10	34.45	45.00	47.87		33.21	21.13	30.66		21.97	20.27	8.13		
	DAY	68.28	80.67	88.27	237.22	66.42	46.23	66.93	179.58	30.10	43.85	32.48	106.43	523.23
TOTAL		204.07	250.66	243.67	698.40	163.46	160.61	196.13	520.20	97.20	119.47	114.82	331.49	1550.09
6 Hour Exposure	E	55.55	67.21	67.21		9.97	28.66	28.66		8.13	25.84	20.27		
	1	44.43	56.17	54.94		17.46	33.21	33.21		8.13	22.79	25.84		
	DAY	99.98	123.38	122.15	345.51	27.43	61.87	61.87	151.17	16.26	48.63	46.11	111.00	607.68
	E	38.65	58.69	44.43		9.97	27.97	27.27		24.35	9.97	17.46		
	5	51.94	30.00	62.73		21.97	21.97	37.46		18.43	15.34	9.97		
	DAY	90.59	88.69	107.16	286.44	31.94	49.94	64.73	146.61	42.78	25.31	27.43	95.52	528.57
	E	39.82	60.00	50.77		34.45	50.77	57.42		22.79	27.27	33.21		
	10	53.13	38.67	50.18		14.18	62.03	57.42		28.66	25.84	31.31		
	DAY	92.95	98.67	100.95	292.57	48.63	112.80	114.84	276.27	51.45	53.11	64.52	169.08	737.92
TOTAL		283.52	310.74	330.26	924.52	108.00	224.61	241.44	574.05	110.49	127.05	138.06	375.60	1874.17
18 Hour Exposure	E	64.90	77.08	75.82		42.13	40.98	42.13		24.35	23.58	38.06		
	1	73.57	74.66	74.66		49.60	49.02	47.87		19.37	23.58	22.79		
	DAY	138.47	151.74	150.48	440.69	91.73	90.00	90.00	271.73	43.72	47.16	60.85	151.73	864.15
	E	57.87	77.08	77.08		18.43	54.94	43.28		8.13	25.84	18.43		
	5	56.79	78.46	81.87		14.18	53.13	56.79		12.92	8.13	24.35		
	DAY	114.66	155.54	158.95	429.15	32.61	108.07	100.07	240.75	21.05	33.97	42.78	97.80	767.70
	E	53.73	80.03	80.03		39.82	51.94	49.60		21.97	24.35	25.84		
	10	74.66	78.46	71.57		39.23	48.45	39.23		8.13	8.13	36.27		
	DAY	128.39	158.49	151.60	438.48	79.05	100.39	88.83	268.27	30.10	32.48	62.11	124.69	831.44
TOTAL		381.52	465.77	461.03	1308.32	203.39	298.46	278.90	780.75	94.87	113.61	165.74	374.22	2463.29
GRAND TOTAL		869.11	1027.17	1034.96	2931.24	474.85	683.68	716.47	1875.00	302.56	360.13	418.62	1081.31	5887.55

Table 65. Analysis of variance of angular transformation of percentage mortalities of *D. dipsaci* exposed to Thionazin "in vitro".

Source	D.F.	S.S.	M.S.	F.	P.
Concentration C	2	2865.3	1433	30.9	xxx 0.1%
Temperature F	2	31900.2	15955	343.8	xxx 0.1%
Examination Time E	2	873.5	436	9.4	xxx 0.1%
Time exposed T	2	7938.0	3969	85.5	xxx 0.1%
C x F	4	390.6	97	2.1	n.s.
C x E	4	127.6	32	<1	n.s.
C x T	4	354.2	88	1.9	n.s.
F x E	4	619.0	154	3.3	x 5%
F x T	4	4797.1	1199	25.8	xxx 0.1%
E x T	4	669.7	167	3.6	x 5%
C x F x E	8	492.9	61	1.3	n.s.
C x F x T	8	821.0	102	2.2	x 5%
F x E x T	8	1346.8	168	3.6	x 5%
E x T x C	8	1109.8	138	3.0	x 5%
C x F x T x E	16	689.2	43	<1	n.s.
Error	81	3756.2	46		
Total	161	58751.5			

Table 66. Analysis of uptake data of Thionazin (1000 log x transformation) by dormant narcissus bulbs immersed in a solution of the chemical. (Orthogonal Polynomial Table).

Total y	$y\xi^1_1$	$y\xi^1_2$	$y\xi^1_3$	$y\xi^1_4$	Σ
2813	- 5626	+ 5626	- 2813	+ 2813	
3534	- 3534	- 3534	+ 7068	-14136	
4486	0	- 8972	0	+26916	
5199	+ 5199	- 5199	-10398	-20796	
6074	+12148	+12148	+ 6074	+ 6074	
Σ 22106	+ 8187	+ 69	- 69	+ 971	
Σz^2	67026969	4761	4761	942841	
$\Sigma n^1(\xi^1)^2$	50	70	50	350	
Σ^2	1342539.3	68.0	95.4	2693.8	1343396.5
$\frac{\Sigma^2}{\Sigma n^1(\xi^1)^2}$					

Table 66 cont. Analysis of Variance.

Source	S.S.	D.F.	M.S.	F.	P.
Linnear	1342539.3	1	1342539.3	152.3	xxx 0.1%
Quadratic	68.0	1			
Cubic	95.4	1	952.7	n.s.	
Quartic	2693.8	1			
Treatments	1343396.5	4	335849.1	38.1	xxx 0.1%
Error	176334.5	20	8816.7		
Total	1519731.0	24			

Table 67. Uptake of tritiated Thionazin in µg/g. of bulb material by tulip bulbs immersed in a solution of the chemical (1000 log x transformation).

Analysis of Variance.

Source	D.F.	S.S.	M.S.	F.	P.
Linnear	1	223776	223776	95.4	xxx 0.1%
Other	3	5685	1862	<1	
Treatments	4	229451	57364	24.5	xxx 0.1%
Replicates	3	4501	1500	<1	
Error	12	28149	2346		
Total	19	262101			

Table 68. Number of nematodes extracted from narcissus bulbs dipped in solutions of Thionazin for different times at different temperatures (1963).

A.

Temperature	Conc.p.p.m.	Exposure Time (hours)		
		2	6	18
80°F.	0	45,600	28,800	30,000
	200	16,160	12,000	6,300
	600	600	480	8,000
	1800	260	100	60
90°F.	0	56,400	70,000	58,800
	200	27,400	4,800	4,080
	600	6,000	8,160	4,520
	1800	960	200	60
100°F.	0	19,400	9,600	1,300
	200	1,240	400	440
	600	1,240	320	320
	1800	80	120	-

Table 68 Cont.

B. Analysis of Variance (log x 100 transformation).

Source	D.F.	S.S.	M.S.	F.	P.
Temperatures T	2	55602	27801	22.76	xxx 0.1%
Concentrations C	3	245185	81728	66.93	xxx 0.1%
Times D	2	10216	5108	4.18	n.s.
D x C	6	8441	1407	1.15	n.s.
D x T	4	5212	1303	1.07	n.s.
C x T	6	11241	1874	1.53	n.s.
D x T x C	11	13426	1221		
Missing plot	1	-			
Total	34	349323			

C. Analysis of Variance (log x 100 transformation) of above data excluding all zero treatments.

Source	D.F.	S.S.	M.S.	F.	P.
Concentrations	2	108865	54432	31.4	xxx 0.1%
Temperatures	2	39665	19832	11.5	xxx 0.1%
Times	2	7747	3873	2.24	n.s.
Error	19	32874	1730		
Missing plot	1				
Total	26	189151			

D. Critical Differences.

Treatment		Mean	Difference
Temperature	80°F.	343.6	
			27.3
	90°F.	370.9	
			93.6
	100°F.	277.3	

Critical Difference $t_{.05}$ = 31.4
$t_{.01}$ = 44.3
$t_{.001}$ = 63.2

Treatment		Mean	Difference
Concentrations	0 p.p.m.	437.2	
			80.0
	200 "	357.2	
			37.0
	600 "	320.2	
			112.4
	1800 "	207.8	

Critical Difference $t_{.05}$ = 36.3
$t_{.01}$ = 51.2
$t_{.001}$ = 73.0

Table 69. Number of narcissus plants showing spikkels after the bulbs had been dipped in solutions of Thionazin for different times at different temperatures.

Analysis of variance of the angular transformation of the percentage of plants showing spikkels.

Source	D.F.	S.S.	M.S.	F.	P.
Time	2	179.75	89.8	1.2	n.s.
Concentration	3	2770.54	923.5	11.8	xxx 0.1%
Temperature	2	1269.87	634.9	8.1	xx 1%
Error	28	2180.42	77.9		
Missing plot	1	-			
Total	35	6400.58			

Critical Differences.

	Treatment	Mean	Difference	C.D.
Temperature	80°F.	50.7		
			4.8	$t_{.05}$ = 7.3
	90°F.	45.9		
			9.5	$t_{.01}$ = 9.9
	100°F.	36.4		
Concentration	0 p.p.m.	58.2		
			12.6	$t_{.05}$ = 8.5
	200 "	45.6		
			7.7	$t_{.01}$ = 11.5
	600 "	37.9		
			2.1	
	1800 "	35.8		

Table 70. Number of plants showing spikkel lesions after being dipped in Thionazin solutions for different times at different temperatures

Analysis of variance of the angular transformation of the percentage of plants showing spikkels and excluding all zero concentration effects.

Source	D.F.	S.S.	M.S.	F.	P.
Time	2	29.25	14.62	<1	n.s.
Concentration	2	483.13	241.56	3.9	x 5%
Temperature	2	516.79	258.39	4.2	x 5%
Error	19	1172.60	61.7		
Missing plot	1				
Total	26	2201.77			

Table 70 cont.

Critical Differences.

Treatment		Mean	Difference	
Temperature	80°F.	42.9		
			0.1	
	90°F.	42.8		
			9.2	
	100°F.	33.6		C.D.
				$t_{.05}$ = 7.7
				$t_{.01}$ = 10.5
Concentration	200 p.p.m.	45.6		
			7.7	
	600 "	37.9		
			2.1	
	1800 "	35.8		

Table 71. Number of nematodes extracted from infested narcissus bulbs immersed in solutions of Thionazin for different times (1964 experiment).

A. Analysis of variance (log transformation) of all data.

Source	D.F.	S.S.	M.S.	F.	P.
Times T	5	39057	7811.4	4.07	xx 1%
Concentrations C	5	374587	74917.4	39.05	xxx 0.1%
T x C	25	49425	1977.0	1.03	
Error	36	69065	1918.5		
Total	71	532134			

B. Analysis of variance (log transformation) making allowance for dummy treatments.

Source	D.F.	S.S.	M.S.	F.	P.
Times T	5	39057	7811.4	3.88	xx 1%
Concentrations C	5	374587	74917.4	37.28	xxx 0.1%
T x C	20	36115	1805.7		
Error	41	82375	2009.1		
Total	71	532134			

Table 71 cont.

C. Analysis of variance (log transformation) omitting all zero treatment effects.

Source	D.F.	S.S.	M.S.	F.	P.
Times T	5	38152	7630.4	4.1	xx 1%
Concentrations C	4	105950	26487.5	14.4	xxx 0.1%
T x C	20	37021	1851.1	1.0	n.s.
Error	30	55267	1842.2		
Total	59	236390			

D. Critical Differences.

Treatment		Mean	Difference	Critical Differences
Concentration	0 p.p.m.	399		
			100	$t_{.05}$ = 36
	500 "	299		
			32	$t_{.01}$ = 48
	1000 "	267		
			44	$t_{.001}$ = 62
	2000 "	223		
			28	
	4000 "	195		
			4	
	8000 "	191		

Exposure Time (ignoring zero concentration effects).

½ hour	273		
		8	
¼ "	265		
		38	$t_{.05}$ = 39
1 "	227		
		2	$t_{.01}$ = 53
4 "	225		
		5	
2 "	220		
		19	
8 "	201		

Table 72. Number of nematodes extracted from infested narcissus bulbs immersed in solutions of Thionazin for different times (1964 experiments).

Analysis of variance using orthogonal polynomials and ignoring zero treatment effects.

Source	D.F.	S.S.	M.S.	F.	P.
Linnear	1	31085	31085	16.8	xxx 0.1%
Other	4	7067	1767	<1	n.s.
Times T	5	38152	7630	4.1	xx 1%
Linnear	1	96879	96879	52.4	xxx 0.1%
Other	3	9071	3027	1.6	n.s.
Concentrations C	4	105950	26487	14.3	xxx 0.1%
T x C	20	37021	1851	1.0	n.s.
Error	30	55267	1842		
Total	59	236390			

Table 73A. Total number of healthy flowers produced by narcissus bulbs immersed in solutions containing Thionazin (1963 experiment).

Temperature	Concentration p.p.m.	Time (hours)		
		2	6	18
80°F.	0	31	25	31
	200	38	32	27
	600	32	34	43
	1800	37	41	14
90°F.	0	33	28	36
	200	23	36	19
	600	30	33	30
	1800	36	22	1
100°F.	0	32	34	28
	200	28	13	0
	600	17	17	0
	1800	13	0	0

Table 73 B. Number of healthy flowers produced from narcissus bulbs immersed in solutions containing Thionazin (1963 experiment).

Analysis of Variance.

Source	D.F.	S.S.	M.S.	F.	P.
Concentrations C	3	747	249	4.01	x 5%
Temperatures T	2	1822	911	14.69	xx 1%
Time D	2	646	323	5.21	x 5%
D x C	6	614	102	1.65	n.s.
C x T	6	826	121	1.95	n.s.
T x D	4	109	27	0.43	n.s.
D x T x C	12	743	62		
Total	35	5507			

Table 73 C. Critical differences.

Treatment		Mean	Difference
Temperature	80°F.	32.1	
			4.9
	90°F.	27.2	
			12.0
	100°F.	15.2	
	Critical Difference		$t_{.05}$ = 6.9
			$t_{.01}$ = 9.7
			$t_{.001}$ = 13.6
Exposure Time	2 hours	29.2	
			3.0
	6 hours	26.2	
			7.1
	18 hours	19.1	
	Critical Difference		$t_{.05}$ = 6.9
			$t_{.01}$ = 9.7
Concentration	0 p.p.m.	30.9	
			4.7
	600 "	26.2	
			2.2
	200 "	24.0	
			5.8
	1800 "	18.2	
	Critical Difference		$t_{.05}$ = 8.1
			$t_{.01}$ = 11.3

Table 74. Total number of healthy flowers produced by narcissus bulbs immersed in solutions containing Thionazin (1964 experiment).

A. Analysis of variance of all data.

Source	D.F.	S.S.	M.S.	F.	P.
Concentrations C	5	499	99.8	3.71	xx 1%
Times T	5	53	10.6	<1	n.s.
C x T	25	480	19.2	<1	n.s.
Error	36	970	26.9		
Total	71	2002			

B. Critical Differences.

	Treatment	Mean	Difference	Critical Difference
Concentrations	0 p.p.m.	16.2		
			2.2	
	500 "	18.4		$t_{.05} = 4.3$
			0.5	
	4000 "	18.9		$t_{.01} = 5.7$
			0.4	
	2000 "	19.3		
			2.6	
	8000 "	21.9		
			2.5	
	1000 "	24.4		

Table 75 A. The number of D. dipsaci extracted from narcissus bulbs drenched with Thionazin in 1963.

Analysis of variance of 1963 counts (100 log x transformation).

Source	D.F.	S.S.	M.S.	F.	P.
Blocks	3	2378	793	<1	n.s.
Treatments	15	301381	20092	6.8	xxx 0.1%
Error	45	131954	2932		
Total	63	435713			

Table 75 A cont.

Critical Differences.

Treatment			Mean	Difference	C.D.
Concentration p.p.m.	Volume gallons/acre	Time			
4000	1800	Early	180	5	$t_{.05}$ = 78
12000	900	Early	185	3	$t_{.01}$ = 103
8000	900	Late	188	30	$t_{.001}$ = 135
8000	1800	Early	218	0	
8000	1800	Late	218	1	
4000	900	Late	219	8	
8000	450	Early	227	15	
4000	450	Early	242	10	
4000	900	Early	252	4	
1200	900	Late	256	7	
8000	900	Early	263	14	
8000	450	Late	277	16	
4000	1800	Late	293	67	
4000	450	Late	360	42	
CONTROL			402		

Table 75 B. Number of D. dipsaci extracted from narcissus bulbs drenched with Thionazin in 1963 (1963 counts).

Analysis of variance of (log x 100) transformation and excluding zero and 1200 p.p.m. concentrations.

Source	D.F.	S.S.	M.S.	F.	P.
Time T	1	9660	9660	3.4	n.s.
Concentration C	1	7747	7747	2.7	n.s.
Volume V	2	23956	11978	4.2	x 5%
T x C	1	16901	16901	5.9	x 5%
T x V	2	42885	21443	7.5	xx 1%
C x V	2	3760	1880	<1	
Error	38	108595	2858		
Total		213504			

Critical Differences.

Treatment	Mean	Difference	
Volume gallons/acre			
450	276	46	$t_{.05}$ = 38
900	230	2	$t_{.01}$ = 51
1800	228		

Table 75 C. Numbers of D. dipsaci extracted from narcissus bulbs in 1964 when the plants had been drenched with Thionazin in 1963.

Analysis of variance of log (x + 1) transformation of data and excluding zero and 1200 p.p.m. concentration treatments.

Source	D.F.	S.S.	M.S.	F.	P.
Times T	1	0.51	0.51	<1	n.s.
Volumes V	2	4.93	2.47	2.6	n.s.
Concentrations C	1	0.14	0.14	<1	n.s.
T x V	2	0.71	0.36	<1	n.s.
T x C	1	0.02	0.02	<1	n.s.
V x C	2	3.24	1.62	1.7	n.s.
Error	38	36.54	0.96		
Total	47	46.09			

Table 75 D. Percentage mortalities over three years of nematodes in narcissus bulbs drenched with Thionazin.

Time of Application		Volume (gallons/acre)	Concentration p.p.m.		
			4000	8000	12000
EARLY	1963	450 900 1800	97 96 99	98 91 99	99
	1964	450 900 1800	99 99 98	95 99 99	95
	1965	450 900 1800	- 95 97	92 98 99	99
LATE	1963	450 900 1800	57 84 88	83 92 98	94
	1964	450 900 1800	84 98 99	95 97 99	99
	1965	450 900 1800	- 97 98	97 99 99	99

Table 76 A. Percentage increase in weight in year of treatment over planting weight of narcissus bulbs drenched with Thionazin.

Critical Differences.

Treatment			Mean	Difference
Concentration p.p.m.	Volume gallons/acre	Time		
8000	1800	Early	257	8
8000	1800	Late	265	4
4000	1800	Late	269	9
12000	900	Late	278	6
8000	900	Early	284	7
12000	900	Early	291	4
8000	900	Late	295	3
8000	450	Early	298	4
4000	900	Early	302	13
4000	450	Late	315	8
CONTROL MEAN			323	3
4000	450	Early	326	6
8000	450	Late	332	2
4000	900	Late	334	

$t_{.05} = 44$

$t_{.01} = 59$

Table 76B. Percentage increase in weight in year of treatment over planting weight of narcissus bulbs drenched with Thionazin.

Analysis of variance of data excluding 1200 p.p.m. and zero concentration treatments.

Source	D.F.	S.S.	M.S.	F.	P.
Volumes V	2	19555	9778	7.2	xx 1%
Concentrations C	1	3468	3468	2.6	n.s.
Times T	1	1008	1008	<1	n.s.
V x C	2	1081	541	<1	n.s.
V x T	2	1491	745	<1	n.s.
C x T	1	850	850	<1	n.s.
Error	38	51228	1348		
Total	47	78681			

Critical Differences.

Treatment	Mean	Difference
Volumes (gallons/acre)		
450	317.7	
		14.2
900	303.5	
		33.9
1800	269.6	

$t_{.05} = 26.3$
$t_{.01} = 35.1$
$t_{.001} = 46.2$

Table 76 C. Number of healthy flowers produced by narcissus bulbs one year after the plants had been drenched with Thionazin.

Analysis of Variance.

Source	D.F.	S.S.	M.S.	F.	P.
Blocks	3	424	141	1.66	n.s.
Treatments	15	14143	943	11.09	xxx 0.1%
Error	45	3827	85		
Total	63	18394			

Table 76 C cont.

Critical Differences.

Treatment			Mean	Difference	C.D.
Concentration p.p.m.	Volume gallons/acre	Time			
8000	900	Late	71		
				1	
8000	1800	Late	70		
				0	
4000	1800	Late	70		$t_{.05}$ = 13
				1	
4000	900	Early	69		$t_{.01}$ = 18
				3	
12000	900	Late	66		$t_{.001}$ = 23
				1	
8000	900	Early	65		
				0	
4000	900	Late	65		
				1	
4000	1800	Early	64		
				1	
8000	450	Early	63		
				3	
1200	900	Early	60		
				1	
4000	450	Early	59		
				1	
8000	450	Late	58		
				5	
4000	450	Late	53		
				10	
8000	1800	Early	43		
				20	
CONTROL MEAN			23		

Table 76 D. Number of healthy flowers produced by narcissus bulbs one year after the plants had been drenched with Thionazin.

Analysis of variance excluding the highest and zero concentration treatments.

Source	D.F.	S.S.	M.S.	F.	P.
Volumes V	2	744	372	4.4	x 5%
Concentrations C	1	48	48	<1	n.s.
Times T	1	184	184	2.2	n.s.
V x C	2	492	246	2.9	n.s.
V x T	2	1032	516	6.1	xx 1%
C x T	1	363	363	4.3	x 5%
Blocks	3	229	76	<1	n.s.
Error	35	2968	85		
Total	47	6060			

Critical Differences.

Treatments Volumes (gallons/acre)	Mean	Difference
450	58.2	
		3.6
1800	61.8	
		5.8
900	67.6	

$t_{.05} = 6.5$

$t_{.01} = 8.7$

Table 76 E. Number of healthy flowers produced by narcissus bulbs drenched with Thionazin at different stages of growth at 900 gallons/acre (means of 3 replicates).

Time of Treatment	1964 Concentration p.p.m.		1965 Concentration p.p.m.	
	5000	10000	5000	10000
A	22.3	27.7	33.3	49.0
B	25.3	20.0	45.7	37.7
C	25.0	14.7	42.0	27.7
D	26.0	25.3	52.7	34.3
E	23.3	22.7	22.7	22.7

Table 76 F. Analysis of variance of 1964 counts.

Source	D.F.	S.S.	M.S.	F.	P.
Time T	4	126	31.5	1.2	n.s.
Concentration C	1	41	41.0	1.6	n.s.
T x C	4	206	51.5	1.9	n.s.
Error	20	530	26.5		
Total	29	903			

Table 76 G. Analysis of Variance of 1965 counts.

Source	D.F.	S.S.	M.S.	F.	P.
Time T	4	1747	436.7	11.6	xxx 0.1%
Concentration C	1	187	187.0	4.9	x 5%
T x C	4	1090	272.5	7.2	xxx 0.1%
Error	20	751	37.6		
Total	29	3775			

Critical Difference.

Treatment		Mean	Difference	
Time of Treatment	D	43.5		$t_{.05}$ = 7.3
			1.8	
	B	41.7		$t_{.01}$ = 10.0
			0.6	
	A	41.1		$t_{.001}$ = 13.6
			6.3	
	C	34.8		
			12.1	
	E	22.7		

Table 79A. Nematode counts per unit volume of tulip bulb material from bulbs dipped in solutions of Thionazin.

Temperature	Concentration p.p.m.	Time (hours)		
		2	6	18
Ambient	0	35520	18400	59800
	200	2380	8800	4680
	600	4700	1740	4100
	1800	1480	1640	200
80°F.	0	4060	3380	7000
	200	7600	8960	1480
	600	1720	2740	1480
	1800	1060	1040	140
90°F.	0	6500	7100	2720
	200	1900	1000	340
	600	1440	370	910
	1800	660	400	400
100°F.	0	6400	-	-
	200	3500	-	-
	600	770	-	-
	1800	-	-	-

Table 79 B. Number of nematodes extracted from tulip bulbs dipped in solutions of Thionazin for different times at different temperatures.

Analysis of variance (log x 1000 transformation).

Source	D.F.	S.S.	M.S.	F.	P.
Temperatures T	2	21482	10741	13.68	0.1% xxx
Concentrations C	3	66354	22118	28.17	0.1% xxx
Times D	2	5019	2509	3.19	n.s.
D x C	6	8535	1422	1.8	n.s.
D x T	4	2166	541	<1	n.s.
C x T	6	10194	1699	2.16	n.s.
D x T x C	12	9415	785		
Total	35	123165			

Table 79 B cont.

Critical Differences.

Treatment		Mean	Difference	Critical Difference
Temperature	Ambient	3649.6		
			314.7	$t_{.05}$ = 248.5
	80°F.	3334.9		
			283.4	$t_{.01}$ = 347.7
	90°F.	3051.5		
				$t_{.001}$ = 491.3
Concentration	0 p.p.m.	3963.7		
			540.9	
	200 p.p.m.	3422.8		$t_{.05}$ = 287.7
			194.4	
	600 p.p.m.	3228.4		$t_{.01}$ = 402.6
			462.0	
	1800 p.p.m.	2766.4		$t_{.001}$ = 568.9

Table 79 C. Analysis of variance excluding all zero treatments data.

Source	D.F.	S.S.	M.S.	F.	P.
Temperature T	2	13209	6604	10.14	xx 1%
Concentration C	2	20465	10232	15.71	xx 1%
Time exposure D	2	7509	3754	5.76	x 5%
D x C	4	5729	1432	2.19	n.s.
D x T	4	3773	943	1.44	n.s.
C x T	4	3984	996	1.52	n.s.
D x T x C	8	5209	651		
Total	26	59878			

Table 79 C cont.

Critical Difference.

Treatment		Mean	Difference	C.D.
Temperature	Ambient	3356.0		
			129.8	
	80°F.	3226.2		
			390.7	
	90°F.	2835.5		
Time	2 hours	3290.8		$t_{.05}$ = 277.8
			71.0	
	6 "	3219.8		$t_{.01}$ = 402.6
			312.8	
	18 "	2907.0		$t_{.001}$ = 605.8
Concentration	200 p.p.m.	3422.8		
			194.4	
	600 "	3228.4		
			462.0	
	1800 "	2766.4		

Table 80. Percentage of tulip flower stems showing lesions after being dipped in Thionazin solutions for different times at different temperatures.

Analysis of variance (angular transformation of data).

Source	D.F.	S.S.	M.S.	F.	P.
Times	2	49.98	24.99	<1	n.s.
Concentrations	3	733.02	244.34	6.94	xx 1%
Temperatures	2	464.58	232.29	6.59	xx 1%
Error	28	985.70	35.20		
Total	35	2233.28			

Table 80 cont.

Critical Differences.

Treatment		Mean	Difference	C.D.
Temperature	Ambient	40.2	4.7	$t_{.05}$ = 4.9
	80°F.	35.5	4.0	$t_{.01}$ = 6.6
	90°F.	31.5		$t_{.001}$ = 8.8
Concentration	0 p.p.m.	43.3	8.1	$t_{.05}$ = 5.5
	200 "	35.2	2.5	$t_{.01}$ = 7.4
	600 "	32.7	1.0	$t_{.001}$ = 9.9
	1800 "	31.7		

Table 81. Number of nematodes extracted from tulip bulbs immersed in solutions of Thionazin for different times.

Analysis of variance (log x 1000 transformation).

Source	D.F.	S.S.	M.S.	F.	P.
Concentrations C	4	184743	46185	6.5	0.1% xxx
Times T	3	13618	4539	2.6	n.s.
C x T	12	15522	1293	<1	
Error	20	34771	1739		
Total	39	248654			

Critical Differences.

Treatment		Mean	Difference	
Concentration	0 p.p.m.	3367.5	1013.3	
	500 "	2354.2	643.8	$t_{.05}$ = 433.4
	2000 "	1710.4	69.1	$t_{.01}$ = 591.8
	1000 "	1641.3	70.6	$t_{.001}$ = 791.9
	4000 "	1570.7		

Table 82 A. Number of healthy flowers produced by tulip bulbs after immersion in solutions of Thionazin (1963).

Temperature	Concentration p.p.m.	Exposure Time		
		2	6	18
Ambient	0	6	6	10
	200	26	16	16
	600	32	31	25
	1800	22	15	12
80°F.	0	26	17	9
	200	22	13	3
	600	20	21	6
	1800	22	7	4
90°F.	0	24	20	0
	200	26	20	2
	600	12	26	0
	1800	30	5	0
100°F.	0	3	0	0
	200	1	0	0
	600	0	0	0
	1800	0	0	0

Table 82 B. Number of healthy flowers produced by tulip bulbs after immersion in solutions of Thionazin for different times at different temperatures.

Analysis of variance (excluding results at 100°F.)

Source	D.F.	S.S.	M.S.	F.	P.
Times T	2	1386	693.0	39.4	xxx 0.1%
Concentrations C	3	234	77.4	4.4	x 5%
Temperatures F	2	137	68.5	3.9	x 5%
T x C	6	338	56.3	3.2	x 5%
C x F	6	614	102.3	5.8	xx 1%
F x T	4	374	93.5	5.3	x 5%
T x C x F	12	211	17.6		
Total	35	3294			

Table 82 B cont.

Critical Differences.

Treatments		Mean	Difference	C.D.
Exposure Times	2 hours	22.3	5.9	$t_{.05}$ = 3.7
	6 "	16.4	9.1	$t_{.01}$ = 5.2
	18 "	7.3		$t_{.001}$ = 7.3
Temperatures	Ambient	18.1	3.9	$t_{.05}$ = 3.7
	80°F.	14.2	0.4	$t_{.01}$ = 5.2
	90°F.	13.8		$t_{.001}$ = 7.3
Concentrations	0 p.p.m.	13.1	2.9	$t_{.05}$ = 5.3
	200 p.p.m.	16.0	3.0	$t_{.01}$ = 6.1
	600 p.p.m.	19.0	6.0	$t_{.001}$ = 8.4
	1800 p.p.m.	13.0		

Table 82 C. Interaction.

2-way table showing relationship between concentration and temperature treatments. (Mean No. of healthy flowers).

Concentration p.p.m.	Temperature			
	Ambient	80°F.	90°F.	Mean
0	22	52	44	39
200	58	38	48	48
600	88	47	38	58
1800	49	33	35	39
Mean	54	43	41	46

Table 83. The percentage increase in tulip bulb weight over planting weight after immersion in solutions of Thionazin for different times at different temperatures.

Analysis of Variance.

Source	D.F.	S.S.	M.S.	F.	P.
Concentrations C	3	2567	856	<1	n.s.
Time T	2	34536	17268	9.8	x 5%
Temperature F	3	417	209	<1	n.s.
C x T	6	11794	1965	1.1	n.s.
C x F	9	7764	862	<1	n.s.
T x F	6	7992	1332	<1	n.s.
C x T x F	6	10539	1756		
Total	35	75609			

Critical Difference.

Treatment		Mean	Difference	
Exposure Time	2 hours	209.5	17.2	$t_{.05}$ = 37.7
	6 "	192.3	55.4	$t_{.01}$ = 58.1
	18 "	136.9		$t_{.001}$ = 92.6

Table 84. Number of nematodes extracted from tulip bulbs (mixed varieties) drenched with Thionazin at different stages of growth.

Analysis of variance (log transformation of data).

Source	D.F.	S.S.	M.S.	F.	P.
Blocks	2	2.78	1.39	2.76	n.s.
Treatments	11	4.15	0.38	<1	n.s.
Error	22	11.09	0.504		
Total	35	18.02			

Table 85. Number of D. dipsaci extracted from tulip bulbs (variety Rose Copeland) receiving foliar and drench applications of Thionazin in 1964.

A. Analysis of variance of 1964 counts (log x 1000 transformation).

Source	D.F.	S.S.	M.S.	F.	P.
Concentrations C	4	44913	11288	<1	n.s.
Volumes V	2	25575	12787	<1	n.s.
C x V	8	147029	18378	<1	n.s.
Error	15	383143	25542		
Total	29	600660			

B. Analysis of variance of 1965 counts (log transformation).

Source	D.F.	S.S.	M.S.	F.	P.
Concentrations	4	0.97	0.24	<1	n.s.
Volumes	2	0.01	0.005	<1	n.s.
Error	23	10.33	0.45		
Total	29	11.31			

Table 86. Number of healthy flowers produced by tulip bulbs (mixed varieties) drenched with Thionazin at different stages of growth.

Analysis of Variance.

Source	D.F.	S.S.	M.S.	F.	P.
Blocks	2	85	42.5	1.65	n.s.
Treatments	11	476	43.3	1.68	n.s.
Error	22	569	25.7		
Total	35	1130			