Killing of Bulb Eelworm by Hot-water Treatment

RECENTLY there has been concern about the efficiency of hot-water treatment to eradicate the stem and bulb eelworm (*Ditylenchus dipsaci* (Kühn) Filipjev) from *Narcissus* stocks. Following Staniland's work[1], it was the standard practice in Britain to treat bulbs for 3 h at 110° F (43·3° C) rather than the 4 h which both Ramsbottom's[2] and Van Slogteren's[3] work suggested. More recently[4] 4 h at 110° F (43·3° C) or 3 h at 111° F (43·9° C) has been recommended to improve control. These treatments were based on *in vitro* treatments of the nematodes in water, and while *in vivo* tests with bulbs did not contradict the *in vitro*, the results have been extended to practical treatments of eelworms in bulbs assuming that longer (approximately 60 min) would be required to allow the centre of the bulbs to reach treatment temperature.

By using New Blue *R* stain[5] to differentiate between dead and live nematodes, it has been possible to compare the effects of treatment *in vitro* and *in vivo*, although staining may underestimate mortality. The treatments were applied to badly infested bulbs at the end of August when many of the nematodes in the bulbs showed chains of oil droplets typical of the wool-forming pre-adult stage. Nematodes were either treated in the bulb tissue and afterwards washed from the central fleshy scales, care being taken that dead and live forms were washed out proportionately, or were treated in distilled water in thin-walled glass capillary tubes after extraction, by Seinhorst mistifier[6], from the central scales.

The capillary tubes, bulbs or bulbs with capillary tubes inserted to the centre, were submerged in a laboratory controlled-temperature water bath for 3 h at 44·4° C ± 0·03° C. Treatment started immediately the containers were submerged, for the drop in water temperature (*c.* 0·3° C) was regained within a few seconds. Results are shown in Table 1.

Table 1. THERMAL MORTALITY OF *D. dipsaci*
(Estimates are based on means of counts of 4th and small 5th stages from eight replicates)

Medium	Treated in bulbs	Cooled in bulbs	Mean proportion killed (angular transformation)	Percentage killed (de-transformed)	Standard deviation of mean angle
1. Bulb tissue	yes	yes	72	90	1·8
2. Water	yes	yes	40	41	2·9
3. Water	no	no	41	43	2·8
4. Water	yes	no	38	38	1·8
5. Water	no	yes	37	36	2·0

Note: Abbott's correction (ref. 7) for control mortality was used.

The difference between the first two treatments was striking, and yet the only difference between them was that the second was *in vitro* and the first *in vivo*. The third treatment subjected the nematodes to immediate heating, rising to bath temperature in a few seconds, and a similar immediate cooling to room temperature: yet there was little difference between this and the second, *in vitro*, treatment in which the nematodes were subjected to heating and cooling slowly as the bulb tissue changed temperature. The results of the last two treatments suggest that slow change of temperature has little effect on the time for the killing of the nematode even when the slow heating causes a shorter treatment as in the fourth or slow cooling extends the treatment as in the fifth.

Hastings[8] quoted correspondence from Weiss showing the difficulty of killing nematodes that escaped from bulbs into the water of the treatment tank and recommended the use of a nematicide in the bath. Formalin was suggested as it also acted as a fungicide, and later Chitwood and Blanton[9] showed that formalin was an effective nematicide at high temperatures although not at room temperature. They recommended that 0·5 per cent formalin should be used in the bath, that the solution should not be used for more than two treatments and that the bath should be topped up with the same solution between treatments. Later, Staniland[10] suggested the use of 0·25 per cent chlorophenol in hot water baths to kill wool-forming pre-adults. The difficulty of killing this stage has been noted by several workers, for example, Hastings and Newton[11]. This may be because such worms are on the outside of bulbs, and are treated in water rather than in bulb tissue, and the results in Table 1 support this explanation. All stages, other than the eggs, seem to be equally susceptible when treated in bulb tissue (Table 2).

Table 2. KILLING OF *D. dipsaci* BY HEAT TREATMENT OF BULBS
(Estimates are based on means of counts from six replicates)

Stage	Mean proportion killed (angular transformation)	Percentage killed (de-transformed)	Standard deviation of mean angle
Eggs	47	54	8·9
2nd	81	97	4·7
3rd	81	97	2·6
4th	78	96	3·3
5th	78	96	3·4

Note: Abbott's correction for control mortality was used.

The experiment from which these results were obtained was similar to the previous one, but treatments in capillary tubes were omitted. Due to the difficulty in defining the stages while counting large numbers, each stage noted in the table may have included a few of the stage above.

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An External Effect of Inorganic Nitrogen in Root Nodulation

THE inhibition of nodulation by combined nitrogen was first reported almost a century ago. The results of most studies lend support to a theory proposing that the effect of combined nitrogen is wrought within the plant, the end-result being determined by the amount of carbohydrate available for root and nodule growth. This theory, however, does not explain the different effects obtained when different nitrogen compounds were used at the same nitrogen-level[1,2]. Also some experiments[3–6] have suggested that there was a local (external) effect of combined nitrogen in inhibiting nodulation.

Rhizobia have the ability to reduce nitrate to nitrite and to convert tryptophan to indolyl-3-acetic acid (IAA). It also has been reported that nitrite catalytically destroys IAA[7]. Theoretically, it could be presumed that, when both nitrate and tryptophan are added to a rhizobial media, nitrite and IAA production would occur and that there would be a concomitant destruction of IAA by the nitrite produced.

A mixture of *R. japonicum* (strain 117, a high nitrite-producing strain) and *R. meliloti* (strain *Su* 388, a high IAA-producing strain) was added to the following reaction mixtures: (1) tryptophan, (2) tryptophan + nitrate