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The publisher's version can be accessed at:

- https://dx.doi.org/10.1163/187529291X00204

The output can be accessed at: https://repository.rothamsted.ac.uk/item/8688w/lethal-temperatures-for-eggs-of-globodera-rostochiensis-determined-by-staining-with-new-blue-r.

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Nematologica 37 (1991): 225-240, E. J. Brill, Leiden

SHORT COMMUNICATIONS

K. Evans*): *Lethal temperatures for eggs of* Globodera rostochiensis, *determined by staining with New Blue R.*

Heat treatment of soil to kill potato cyst nematodes (*Globodera pallida* (Stone) and *G. rostochiensis* (Woll.)) has been used for the protection of tomato plants grown in glasshouses in the U.K. (Southey, 1978). The soil was heated by steaming and reached a temperature of 100°C. However, alternative methods of culture and sources of supply of tomatoes mean that this control strategy is no longer relevant to UK agriculture. Furthermore, the procedure was expensive, mainly because of the large volume of soil to be treated. However, there are instances in which it would be desirable to kill potato cyst nematodes in a small volume of soil. Triffitt & Hurst (1935) described experiments in which they showed that substantial numbers of cysts can adhere to seed potato tubers grown on infested land. They referred to work which showed that the stem and bulb nematode *Ditylenchus dipsaci* (Kühn) could be killed in bulbs without harming the plant tissue by immersion in hot water, a treatment which has since been refined for different applications, most of which use a treatment temperature of about 45°C for a period of up to 3 h (Southey, 1978). Triffitt & Hurst (1935) speculated that infested seed tubers could be de-contaminated by similar hot water treatments, so investigated the thermal death point for juveniles contained within cysts. Batches of cysts were treated at temperatures from 43.3 to 54.4°C for periods of up to 60 min. Following these treatments the cysts were exposed to potato root diffusate and the ability of juveniles to hatch was observed. Exposure for 30 min or more to 46.1°C caused a delay in hatching of about a month and exposure for a similar time to 48.9°C appeared to kill the entire cyst contents, or at least to prevent any hatch under the test conditions. Unfortunately, the exposure times and temperatures required to kill the nematodes are likely to damage seed potato tubers.

A situation in which relatively small amounts of soil may need to be decontaminated is that where biological material contaminated by soil is passed to an area or country through quarantine procedures. In this context, waste soil must be decontaminated before it can be disposed of safely. Most plant-parasitic organisms are killed by exposure to 60°C for 30 min (Baker, 1962).

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The description of the use of New Blue R as a stain which differentiates between living and dead nematodes by Shepherd (1962) provided a rapid and simple assay for assessing lethal effects of control treatments, and was used as an assay in the work described in this paper to determine the lethal temperature-time combinations for *Globodera rostochiensis*, using a nematode population from Prattsburgh, New York.

In order to control time of exposure accurately, by minimising the warm up time, the experiments were carried out with free eggs of potato cyst nematodes. These were obtained by soaking batches of about 1000 clean cysts in water overnight and crushing them on an aluminium channel as described by Reid (1955). The crushed cysts plus contents were washed through three nested sieves of 140, 200 and 400 meshes to the inch. Cyst fragments remained on the 140 mesh, many juveniles were retained on the 200 mesh and all the eggs plus some juveniles were recovered from the 400 mesh. Passing this mixture through the 200 and 400 mesh sieves twice more removed virtually all the juveniles and left just eggs, with a yield of about 160,000 eggs per batch of 1000 cysts. Aliquots of several thousand eggs were placed in 5 mm diameter test tubes in 0.5 ml of water. All heat treatments were in an aluminium block Thermolyne Dri-bath, at temperatures ranging from 40 to 70°C. In the first experiment eggs were treated at 40, 45, 50, 55 or 60°C for ½, 1, 2, 4 or 7 h, stained in 0.05% New Blue R for 24 h and then counted as either stained (dead) or unstained (viable). In the second experiment eggs were treated for ½ h at 5°C intervals from 40 to 70°C but at 1°C intervals for those temperatures between 55 and 65°C before staining and counting. In both experiments, eggs were cooled rapidly after treatment by the addition of a large excess of New Blue R solution.

Fig. 1 shows that treatment for up to 7 h at 40 or 45°C did not cause juveniles in eggs to take up stain but that 4 h or more at 50°C resulted in large numbers of juveniles taking up the stain. For batch processing of soil in a decontamination facility, a short treatment time would be advantageous. Fig. 1 also shows that a ½ h exposure had little effect on staining of juveniles at 55°C but a large effect at 60°C. This is why 1°C intervals were used between 55 and 65°C in the second experiment, when treatment time was for a standard ½ h period. Fig. 2 shows that a marked transition temperature can be identified at which treatment for 30 min makes juveniles in eggs of *G. rostochiensis* stainable by New Blue R. This temperature was between 58 and 59°C.

Bird & McClure (1976) treated *Meloidogyne javanica* (Treub) eggs at various temperatures for 30 min and estimated the proportion in which juveniles could be stained by osmic acid. They found a transition temperature of abut 62°C (but not so marked as that in Fig. 2) which they attributed to changes in the properties of lipoprotein membranes in the lipid layer of the eggshell. These changes increased the permeability of the eggshell to osmic acid. In the same

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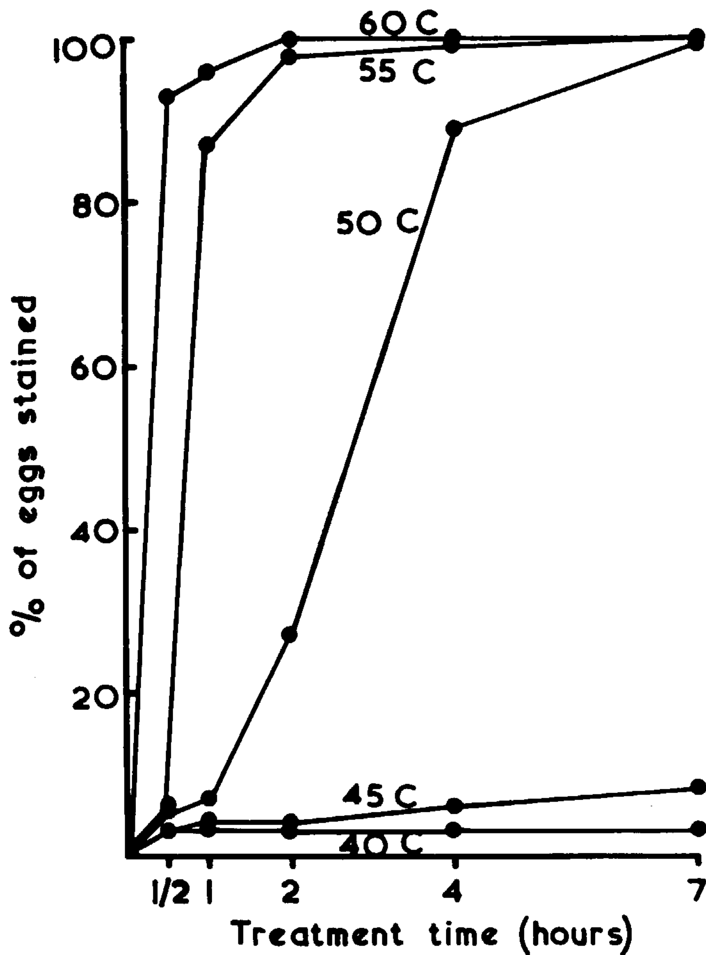

Fig. 1. Percentages of *G. rostochiensis* juveniles inside eggs stained by New Blue R after treatment at 40, 45, 50, 55 or 60°C for periods of ½ to 7 hours.

way, exposure to temperatures of 59°C or greater for 30 min may have increased the permeability of *G. rostochiensis* eggshells to New Blue R. Once the stain was able to pass through the eggshell it was able to stain the juvenile inside the egg. The lethal time-temperature combination for the juvenile may have been the same as that required to effect permeability changes in the eggshell. However, it is likely that juveniles were killed by exposure for 30 min to temperatures lower than 59°C because lightly stained juveniles were noted after treatment at temperatures as low as 50°C. This would accord much more closely with the results found by Triffitt & Hurst (1935) and any apparent disparity between results may be a reflection of the fact that New Blue R used as a vital stain assay on whole eggs actually measures eggshell permeability

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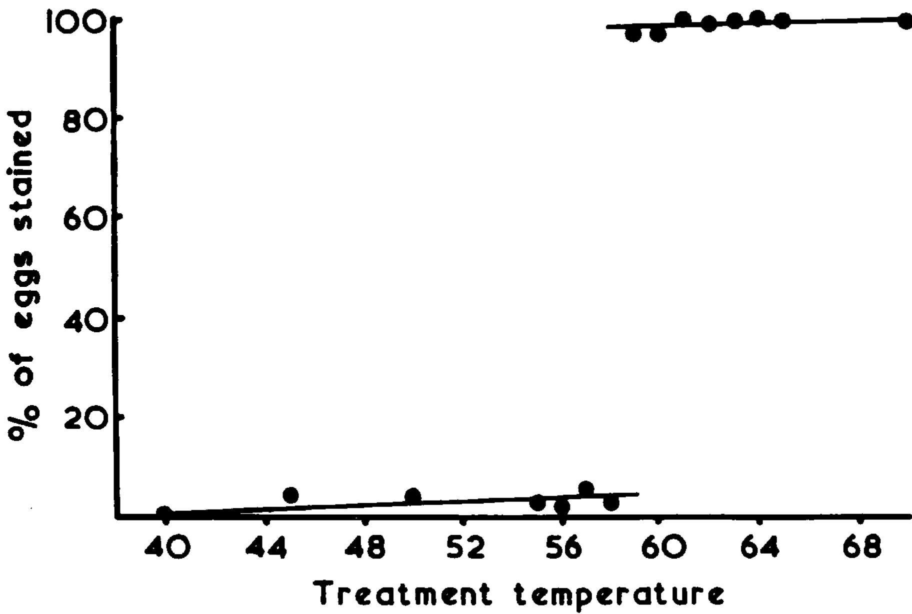

Fig. 2. Percentages of *G. rostochiensis* juveniles inside eggs stained by New Blue R after treatment at temperatures from 40 to 70°C for ½ hour.

rather than the vitality of the juvenile within. Normally, the eggshell would be permeable to the stain if the juvenile were dead but in the special circumstances of these experiments it is possible that juveniles were killed at a temperature lower than that required to make the eggshell permeable to New Blue R. Thus, it would appear to be erring on the safe side to suggest that exposure to 59°C for 30 min would kill any *G. rostochiensis* contaminating soil removed from quarantine samples. However, it should be stressed that the thermal sensitivity of *G. rostochiensis* juveniles inside cysts is greatly dependent on the moisture level (LaMondia & Brodie, 1990), with juveniles in dry cysts less sensitive than those in water soaked cysts; subsequent hatching from cysts of *G. rostochiensis* and *G. pallida* exposed in dry conditions to a range of temperatures was not affected at temperatures less than 90°C (Stone & Webley, 1975). It may be possible to exploit the relative sensitivity of juveniles in moist conditions in the field by covering the soil with clear plastic sheeting in order to raise the soil temperature and kill the nematodes by 'solarization'. LaMondia and Brodie (1983) describe experiments in which up to 98.6% of potato cyst nematodes were killed to a depth of 10 cm in soil by such treatment. Kill was always enhanced when drip irrigation was applied to maintain soil moisture levels. Such tactics could perhaps be added to the range of management strategies available for control.

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WALTER SUDHAUS[1]): *Check list of species of* Rhabditis *sensu lato (Nematoda: Rhabditidae) discovered between 1976 and 1986.*

> *"The prevailing tendency to raise repeatedly the rank of comparatively small groups, without taking a sufficiently broad view of the classification of the nematodes as a whole, seems to have brought about too wide a separation of groups which, when considered together, are obviously closely related to each other"* (Baylis & Daubney, 1926, p. 193-194).

There is a continuing debate among taxonomists about "splitting" and "lumping". "Excessive splitting at the generic level is bad, relationships being thereby lost; excessive lumping hides relationships" (Keast, 1977, p. 265). Systematics should reflect the order existing in nature. This is best realized by cladistic analysis. The monophyly of a group must be verified by apomorphies. This has not been the case in most groups of nematodes; several are paraphyletic or even polyphyletic. Systematics, however, has the practical task of mastering the variety that is present in nature and of facilitating exchange of information on subjects of investigations. In this respect, excessive splitting is helpful neither to workers dealing with relatively wide groups nor to those who are interested in general questions.

The monophyly of the genus *Rhabditis* Dujardin, 1845 is proven by the glottoid apparatus of the stoma as an apomorphous character. This group was split

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