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THE EFFECTS OF STORAGE CONDITIONS ON SUBSEQUENT HATCHING OF *GLOBODERA ROSTOCHIENSIS*

by
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Summary. Three-year old cysts of *Globodera rostochiensis* were stored under dry, wet and humid conditions at 5, 10, 15, 20 and 25 °C for periods ranging from two to twelve months. At two, four, six, eight, ten and twelve months intervals, eggs were hatched at 20 °C (for those cysts stored at 5, 10, 15 and 25 °C) and 25 °C (for cysts stored at 20 °C). The highest emergence occurred in cysts stored wet at 15 °C and above, while the least emergence occurred in cysts stored dry at 10 ad 15 °C. It is suggested that emergence is influenced by storage humidity regimes and to a lesser extent by storage temperatures and time periods. These findings corroborate the suggestion that *G. rostochiensis* shows preference for wet conditions with medium temperatures, which are near to conditions occuring in nature during spring and early summer in the United kingdom.

Diapause has only recently been recognised in *Globodera rostochiensis* (Hominick, *et al.*, 1985; Muhammad, 1990) and *G. pallida* (Muhammad, 1990). The process of both induction and termination of diapause in potato cyst nematodes has been subject of detailed studies. Lewis and Mai (1957; 1960) reported that storage of cysts at up to 24 °C or alternating between 0 °C and 24 °C in dry soil had no effect on emergence. However, storage in moist soil under the same temperature regimes resulted in increased emergence. Shepherd and Cox (1967) reported that cysts stored moist hatched better than those stored dry, and cysts exposed to 30 °C for 6 weeks had their diapause broken. Bishop (1955) and Oostenbrink (1967), however, showed that alternation of temperatures resulted in higher emergence compared to constant temperature storage. Attempts to understand conditions necessary for maximum hatching were not limited to potato cyst nematodes, other plant parasitic nematodes were also studied. Banyer and Fisher (1971; 1971a) suggested that dormancy in *Heterodera avenae* is broken by the onset of low temperature and enhanced by the duration of the cold temperature. Also Antoniou (1983) reported that in *Meloidogyne naasi*, chilling interrupted by short periods of warmth stimulated hatching in diapausing eggs. However, de Guiran (1979a; 1980) reported the presence of diapause in *M. incognita* but found that neither temperature nor humidity had an influence in ending diapause.

From the foregoing reasonings it appears there is little known about the combined effects of different moisture conditions and storage temperatures over a long storage period on the hatching behaviour of potato cyst nematodes. Therefore, investigations were undertaken on the pre-conditioning effects of storage temperatures on juvenile emergence from batches of cysts stored under dry, wet and humid conditions for various periods. It was anticipated that experiments would show (a) whether cysts stored at temperatures other than constant 20 °C vary in their hatching response; (b) whether different humidity regimes during various storage periods could modify hatching behaviour and (c) whether combinations of different temperature and humidity regimes could influence hatching behaviour in three-year old post harvest cysts of *G. rostochiensis.*

Diapause had been shown to occur only in new cysts and storage of such cysts for a period of one year was shown to overcome the diapause (Hominick, *et al.*, 1985; Muhammad, 1990). However, no work had been done to investigate the hatching behaviour of cysts older than one year and not exposed to hatching conditions.

Materials and methods

Globodera rostochiensis (Woll.) Mulvey *et* Stone cysts were obtained from the Scottish Crop Research Institute (SCRI) and at the time of experiments cysts were three years old after harvest. From the time of harvest at SCRI to their arrival at Silwood Park, Ascot (SP), cysts were stored

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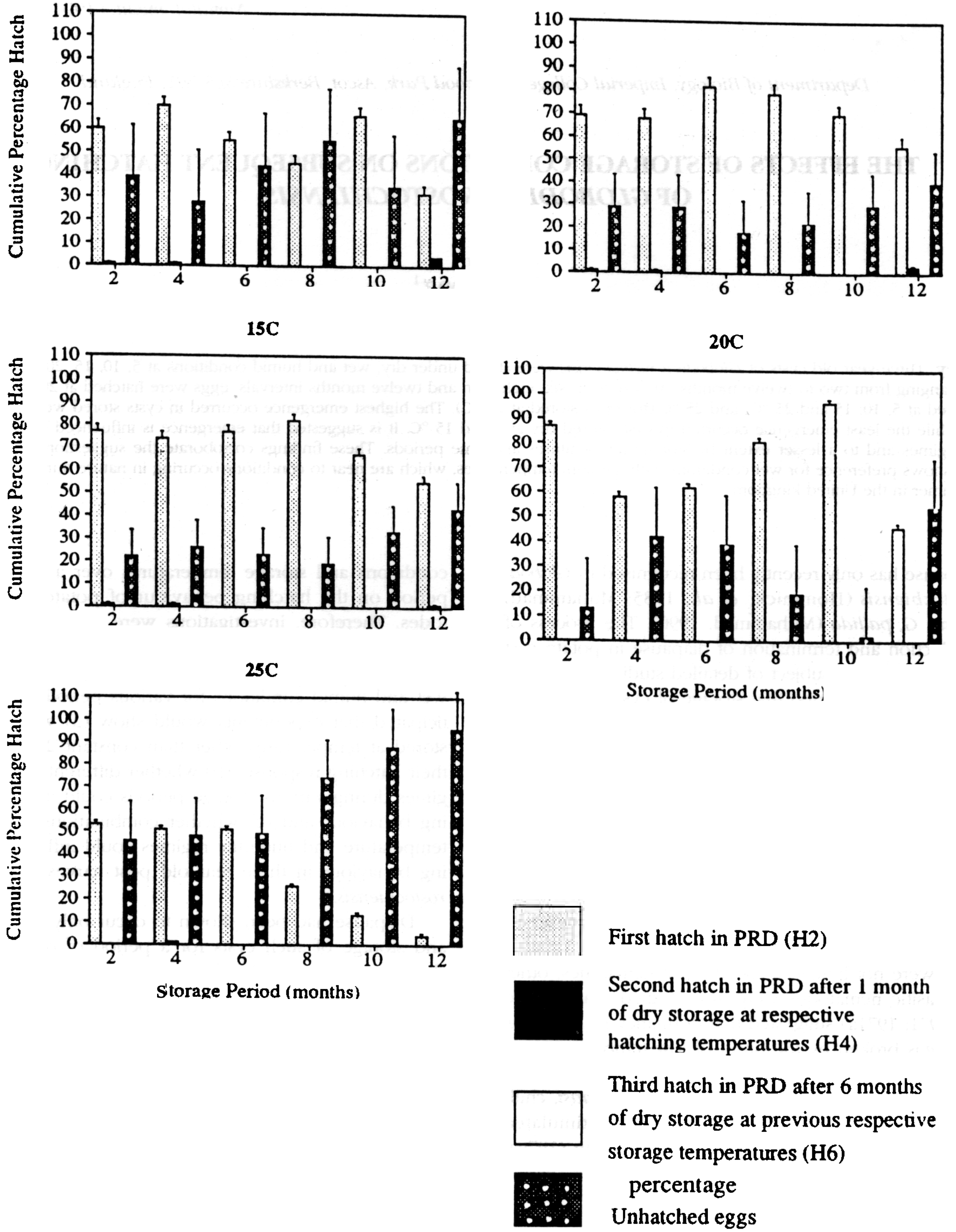

Fig. 1 - Cumulative percentage hatch (juveniles) and percentage unhatch (eggs) of *Globodera rostochiensis* cysts stored dry over a period of two, four, six, eight, ten and twelve months at 5, 10, 15, 20 and 25 °C. Cysts hatched in potato root diffusate at 20 and 25 °C (cysts stored at 5, 10, 15 and 25 °C were hatched at 20 °C and those stored at 20 °C were hatched at 25 °C). Lines above bars indicate mean standard errors.

at room temperature in the dark. At SP they were stored at constant 20 °C in the dark until required for experimentation.

Batches of 50 cysts were placed on a 7 mm diameter 45 µm nylon hatching sieves (Muhammad, 1990, which were then placed in a well of a 24-well Linbro culture plate (Flow Laboratories, UK) and stored dry at 5, 10, 15, 20 and 25 °C respectively. Each plate with its contents was stored at its respective storage temperatures in the dark for periods ranging from two to twelve months. At two month intervals, four replicates of 50 cysts were soaked in 1 ml of sterilized tap water for two weeks at their respective hatching temperatures (cysts stored at 5, 10, 15 and 25 °C were hatched at 20 °C, while cysts stored at 20 °C were hatched at 25 °C). At the end of the soaking period cysts were set to hatch at their respective hatching temperatures (20 or 25 °C) in 1 ml of potato root diffusate (hatching medium) and emerging juveniles were counted weekly while changing the hatching medium.

When emergence was less than 100 juveniles/replicate/week, further hatching was stopped and cysts were stored dry on their hatching sieves at their respective hatching temperatures (20 or 25 °C) for one month. At the end of the one month storage period, cysts were again soaked in 1 ml of sterilized tap water at their respective hatching temperature (20 or 25 °C) for one week and then hatched in 1 ml of potato root diffusate. Emerging juveniles were counted weekly when changing the hatching medium, and hatching was continued until emergence was less than 50 juveniles/replicate/week. The cysts were then stored dry on the hatching sieves at the initial storage temperatures (5, 10, 15, 20 or 25 °C) for six months. At the end of this storage period, cysts were again soaked in 1 ml of sterilized tap water for one week and then hatched in 1 ml of potato root diffusate with emergence counted weekly when changing the hatching medium. Hatching was continued until emergence was less than 10 juveniles/replicate/week, when the cysts were broken open and the number of viable eggs counted and percentage hatch determined.

Similar protocol as described for dry storage was followed for wet stored cysts, except that cysts were stored wet in 2 ml of sterilized tap water during the first and second storage. Also, during wet storage, cysts were closely monitored to ensure that there was sufficient sterilized tap water at all times to keep them wet. Fungal growth that appeared during storage was removed with a soft brush when necessary.

Similar protocol described for dry storage was also followed for humid stored cysts, except that cysts were stored humid during first and second storage. Humid chambers were created by lining the inside of plastic sandwich boxes with tight fitting lids with rolls of tissue paper wetted with sterilized tap water. Culture plates containing batches of cysts were placed into the humid box and closed tightly with the lid. Humid chambers were closely monitored to ensure that the tissue rolls remained wet and occasionally the lids were opened to allow circulation of air. Fungal growth was checked and controlled as in wet storage.

The statistical package "Statistix" (NH Analytical Software, St. Paul MN 55117, USA) was used to perform a two way analysis of variance (ANOVA) on angularly transformed percentages.

Results

The results of the hatching of dry-stored cysts are shown in Fig. 1. Cysts hatched at all storage temperatures and periods during the first hatching period. Emergence from cysts stored at 5, 10 and 15 °C was not significantly different between various storage periods; while emergence in cysts stored at 20 and 25 °C were significantly different between the various storge periods (P<0.05). Cysts stored at 5 °C had no pattern in their emergence; however, at 10 and 15 °C storage, emergence increased with period of storage up to the eighth month before declining in the subsequent months. At 25 °C storage, emergence was about 50% in the first six months followed by a sharp decline in the subsequent months. At 25 °C storage, emergence was about 50% in the first six months followed by a sharp decline in the subsequent months. The highest emergence occurred in cysts stored at 20 °C, with about 90% in the first two months, about 60% in the fourth and sixth months and peak of about 95% in the tenth month. A common feature in all the storage temperatures was decline in emergence in the twelfth month of storage.

Second and third hatchings had little effect on emergence even though there was a large percentage of unhatched eggs (Fig. 1). The percentage of unhatched eggs was not significantly different between the different storage periods in cysts stored at 5, 10 and 15 °C. However, in cysts stored at 20 and 25 °C, the percentage unhatched eggs was significantly different (P<0.05) between the different storage periods. At 25 °C the percentage unhatched eggs remained the same in the first six months of storage with about 45%, thereafter it increased with an increase in storage period reaching a peak of about 95% in the twelfth month. However, at 20 °C there was no clear pattern except that a peak of about 50% was attained in the twelfth month of storage.

The results of the hatching of wet stored cysts are shown in Fig. 2. Cysts hatched at all storage temperatures and periods during the first hatching period. There was no significant difference in emergence between cysts stored for various periods at 15, 20 and 25 °C. However, emergence in cysts stored at 5 and 10 °C was significantly different (P<0.05) between different storage periods. At 5 °C

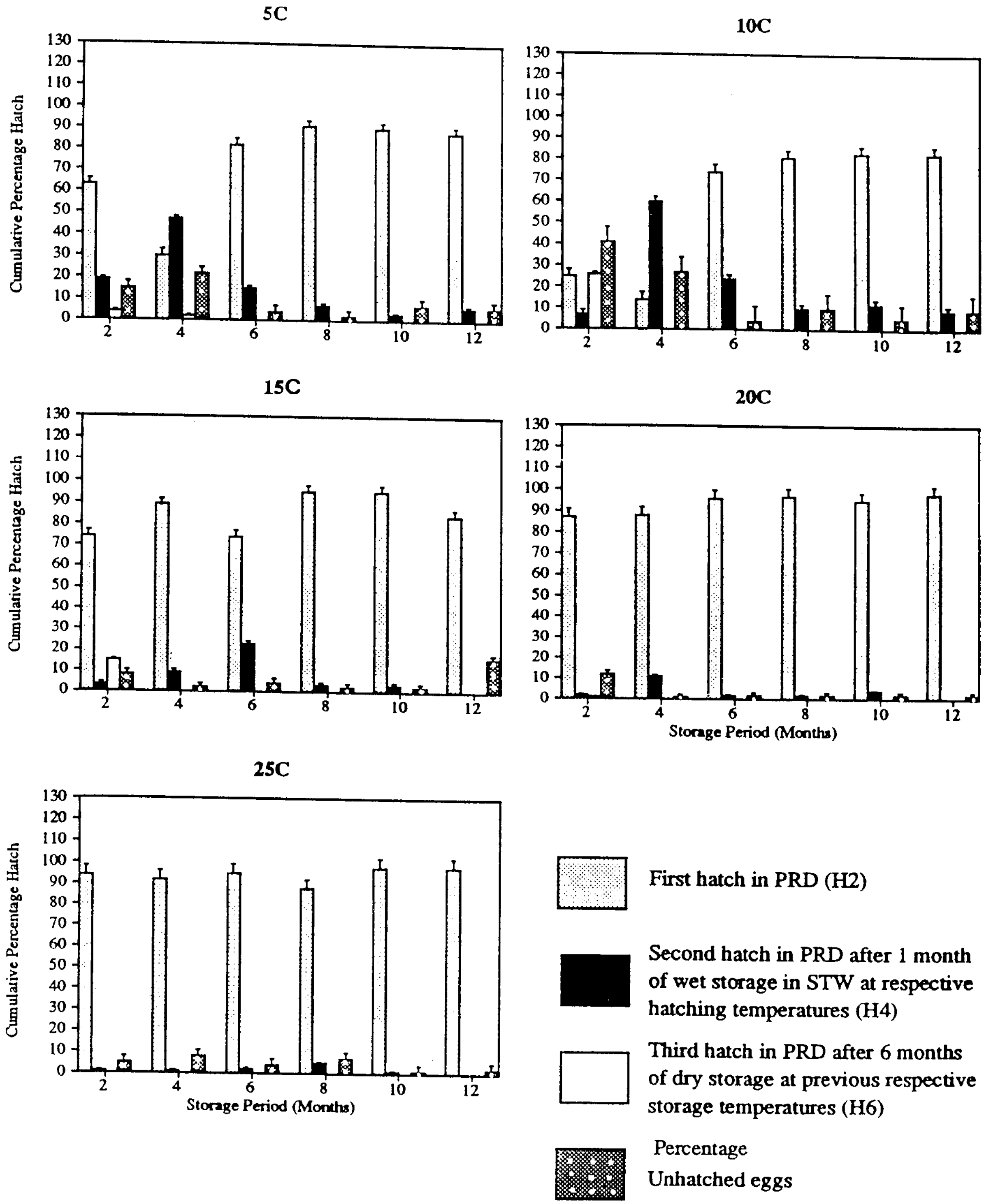

Fig. 2 - Cumulative percentage hatch (juveniles) and percentage unhatch (eggs) of *G. rostochiensis* cysts stored wet over a period of two, four, six, eight, ten and twelve months at 5, 10, 15, 20 and 25 °C. Cysts hatched in potato root diffusate at 20 and 25 °C (cysts stored at 5, 10, 15 and 25 °C were hatched at 20 °C and those stored at 20 °C were hatched at 25 °C). Lines above bars indicate mean standard errors.

storage, emergence started at about 60% then declined to about 30% in the fourth month; thereafter emergence increased with storage periods reaching peaks of about 90% in the last six months. At 10 °C storage, emergence started slowly in the first four months of storage, then it increased with storage period for the last eight months with peaks of about 90% in the last six months.

Emergence during second hatching (Fig. 2) was significantly different (P<0.05) between various storage periods at all storage temperatures. At 5 and 10 °C storage, emergence reached peaks of about 45% and 65% respectively in the fourth month of storage before declining in the subsequent months. At other storage temperatures, peak emergence were about 25% in the sixth month at 15 °C, 10% in the fourth month at 20 °C and about 5% in the eighth m:onth at 25 °C. Third hatching (Fig. 2) had no effect on emergence at 20 and 25 °C storage. However, emergence occurred at 5, 10 and 15 °C storage during the first two months of storage only.

Percentage unhatched eggs (Fig. 2) was not significantly different between various storage periods at 15, 20 and 25 °C. However, at 5 and 10 °C storage percentage unhatched eggs was significantly different (P<0.05) between the various storage periods. At 5 °C storage, percentage unhatched eggs increased from 15% in the first two months of storage to about 20% in the fourth month; while at 10 °C percentage unhatched eggs declined from 40% in the first two months to about 25% in the fourth month. A common feature of this two storage temperature was the continuous decline of unhatched eggs after the fourth months of storage to less than 10% in the remaining storage periods.

With humid stored cysts emergence occurred at all storage temperatures and periods during the first hatching period (Fig. 3). Emergence in cysts stored at 5, 10, 15 and 20 °C was significantly different (P<0.05) between the different storage periods, while in cysts stored at 25 °C there was no significant difference. Cysts stored at 25 °C had the highest emergence, with a peak of about 80%. However, emergence from cysts stored at 5, 10, 15 and 20 °C had no specific pattern. Those stored at 5 and 10 °C had their peak emergence of about 90% in the eighth month of storage; at 15 °C storage peak emergence was also about 90% but in the tenth month of storge; while at 20 °C storage there were two peaks of about 90% in the fourth and eighth months of storage.

Emergence was significantly different (P<0.05) between the different storage periods at all storage temperatures during the second hatching period (Fig. 3). In cysts stored at 5, 10 and 15 °C emergence occurred at all storage periods except the tenth month; with peak emergence in the fourth month of storage with about 30% at 5 °C, 50% at 10 °C and 60% at 15 °C. At 20 °C storage, emergence occurred during all storage period with a peak of about 35% in the twelfth month of storage. The least emergence occurred in cysts stored at 25 °C, where peak emergence was about 10% in the first four months of storage. There was no emergence during third hatching periods at all storage temperatures and periods, even though there were large number of unhatched eggs.

Percentage unhatched eggs (Fig. 3) was significantly different (P<0.05) between the different storage periods at 5 and 10 °C storage. The highest percentage of unhatched eggs was about 25% at 5 °C and 35% at 10 °C. However, at 15, 20 and 25 °C storage percentage unhatched eggs was not significantly different between the different storage periods, with unhatched eggs ranging from 20-30%.

Discussion

Emergence was greater among cysts stored under wet conditon, regardless of storage temperatures, than in cysts stored either humid or dry. Storage period had no influence on wet-stored cysts as emergence at 15 °C and above was maximal irrespective of storage period. These differences in emergence are not uncommon in *G. rostochiensis* as the cysts are continuously exposed to different moisture levels and fluctuating temperatures in British agricultural soils (Jones, 1975; 1975a). Irrespective of storage conditions, eggs of *G. rostochiensis* still exhibited diapause but with different duration. The extended diapause was observed in cysts stored dry while the least in cysts stored wet.

Differences in emergence between wet and humid stored cysts may be attributed to lack of free water in humid stored cysts to enhance the physiological activities of the juveniles. This can be deduced from Robinson *et al.* (1987) who reported that hatched juveniles of *G. rostochiensis* stored at various moisture levels used their lipid reserves rapidly at soil moisture levels corresponding to the point of inflection of the soil moisture curve.

The effects of storage temperature on wet stored cysts during second hatching was greatest at 5 and 10 °C, which resulted in further emergence (Fig. 2). In humid stored cysts, there was a further emergence at all storage temperatures, though with variable percentages depending on storage periods (Fig. 3). This contrasts with absence of emergence during third hatching period except in wet stored cysts where there was emergence in the second month of storage at 5, 10 and 15 °C. Emergence in wet and humid stored cysts when compared to complete cessation of emergence in dry stored cysts during the same hatching period and temperature indicates that *G. rostochiensis* prefers those conditions to which it is usually exposed to in nature. This corresponds to spring when rain is abundant and hosts are available for infection.

In contrast to wet and humid stored cysts, emergence in dry stored cysts occurred only during the first hatching

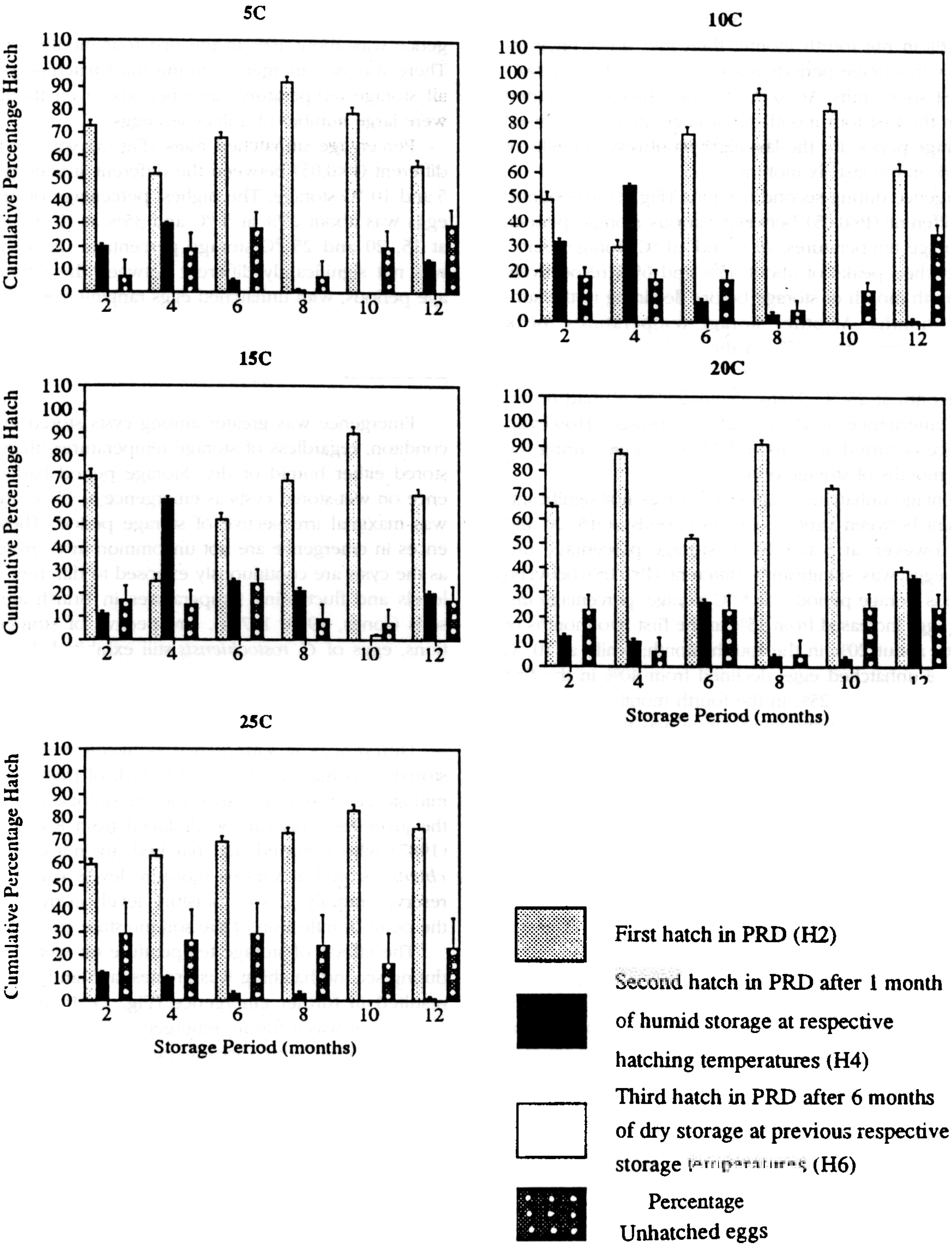

Fig. 3 - Cumulative percentage hatch (juveniles) and percentage unhatch (eggs) of *G. rostochiensis* cysts stored humid over a period of two, four, six, eight, ten and twelve months at 5, 10, 15, 20 and 25 °C. Cysts hatched in potato root diffusate at 20 and 25 °C (cysts stored at 5, 10, 15 and 25 °C were hatched at 20 °C and those stored at 20 °C were hatched at 25 °C). Lines above bars indicate mean standard errors.

period (Fig. 1) with the highest emergence occurring in cysts stored at 10, 15 and 25 °C. Second and third hatching had no influence on emergence of juveniles at all storage temperatures and periods. Lack of moisture may have inhibited the physiological activities of the juveniles during the first and second dry storage periods of the cyst. Robinson *et al.* (1987) showed that *G. rostochiensis* juveniles utilize their lipid reserves commensurate with moisture levels which suggests that level of activity of the nematode is correlated with degrees of moisture available to the juveniles. However, cysts of *G. rostochiensis* are not exposed to dry condition in nature for long periods, which is reflected in the emergence pattern in dry stored cysts.

In general, these results agree with those of Shepherd and Cox (1967) who showed that when cysts are stored dry or moist at various temperatures for various periods, the moist stored cysts showed higher emergence than dry stored cysts. However, in *M. incognita* de Guiran (1979) reported that the hatching of eggs was delayed and decreased proportionally to the time of their previous stay in water-saturated soil. These differences in response to levels of moisture by *G. rostochiensis* and *M. incognita* point to the adaptive capacity of the two species. *G. rostochiensis* being an inhabitant of wet regions, is not inhibited by excessive moisture levels in its hatching ability whereas *M. incognita*, being an inhabitant of dry region is deterred by excessive moisture levels in its hatching ability.

Oostenbrink (1967) reported that storage of cysts of *G. rostochiensis* under wet conditions is noxious to the hatchability of the cyst and hatching of such cysts at 23 °C after incubation at 5 °C results in low emergence. The results presented in this work do not support these observations. Oostenbrink (1967) attributed his observations to microbial activities and fungal disintegration of the cyst wall. In natural soil these conditions are an integral part of the soil system to which the cysts are continuously exposed. There was no evidence in the work reported in this paper to show disintegration of the cyst wall as a result of fungal activities.

Potato cyst nematodes normally begin to infect their hosts in spring, which is also a period of rainy season; while from late summer to autumn and winter they remain dormant in the field. In *G. rostochiensis* population reported in this work, emergence occurred at all storage temperatures and humidity regimes, with the highest emergence in wet stored cysts at 15 °C and above. This suggest the preference of *G. rostochiensis* for wet conditions with medium temperature, which is near conditions available in nature during spring and early summer in the UK. Generally, emergence in *G. rostochiensis* cysts is strongly influenced by storage humidity regimes and to a lesser extent by storage temperatures.

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