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Anhydrobiosis of *Pratylenchus coffeae*

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ABSTRACT

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P. coffeae responded to slow desiccation by bending their bodies into tight coils, loose coils, and C-shape. Coiling of nematodes is an indication of anhydrobiosis. The percent coiling was higher on the lids of the Petri dishes in the desiccation treatment than in the desiccated medium but the survival rate was higher in the medium. The percent coiling in the desiccated medium increased with time, but the highest percentage of survival did not coincide with the highest percentage of coiling. The bending of nematode bodies to form coils may have been interfered by the particles around the nematodes in the medium as well as in the soil, which may affect the relationship between survival and coiling. The lesion nematodes survived longer in the desiccated medium than in the moist medium. Based on the present findings, one can deduct that moist fallow would be more effective than dry fallow for the control of *P. coffeae* by preventing the nematodes from entering the state of anhydrobiosis. The nematodes could be kept for 9 months with 36.1% survival which was sufficient for the purpose of maintaining the inoculum source for sub-culturing the nematodes with minimum cost of resources.

Key words: anhydrobiosis, coiling, desiccation, *Pratylenchus*, preservation, survival

INTRODUCTION

Desiccation is an environmental stress organisms frequently encounter. Some organisms, including plants [15], and animals [3], are capable of surviving desiccation by entering a state of quiescence called anhydrobiosis [4]. Anhydrobiosis of nematodes has been reviewed by researchers from time to time [4, 5, 8. 30,]. Anhydrobiotic ability exists in nematodes of different groups: animal-parasitic [18, 21], entomopathogenic [11, 12, 14], saprophytic [1, 24], and plant-parasitic [26, 28, 31]. Among the plant-parasitic nematodes, aerial plant parasites encounter more rapid changes in environment than the root parasites and hence evolved stronger ability to survive desiccation. Although the root parasites are not in obvious need for enduring desiccation because of the protection of soil, some of them do posses anhydrobiotic ability. Soil is a relatively stable environment in terms of moisture changes comparing to the air, however, there may be niches in the soil where moisture is below the level necessary for maintaining normal biological activities of the nematodes.

Among the root parasites, anhydrobiosis has been reported for the reniform nematode *Rotylenchulus reniformis* [26], the root-knot nematode *Meloidogyne javanica* [23], the citrus nematode *Tylenchulus semipenetrans* [27, 28], and the lesion nematode *Pratylenchus penetrans* [22]. There has been no report on the anhydrobiosis of *Pratylenchus coffeae* (Zimmermann) Filipjev & Schuurmans Stekhoven. In the routine culture of *P. coffeae* with Gamborg's B5 medium [29] in my laboratory, coiled nematodes were observed from time to time to appear on the lids of the Petri dishes. Coiling is an indication of anhydrobiosis of nematodes [6, 23, 24, 26]. *P. coffeae* is one of the important plant-parasitic nematodes in Taiwan. Their ability to enter the state of anhydrobiosis

will affect the control strategy for this nematode since anhydrobiosis has been reported to enhance the survival of nematodes [12, 26] and their tolerance to adverse conditions [7, 28]. This paper reports the anhydrobiotic survival of *P. coffeae* under different incubation time. Successful induction of anhydrobiosis is highly dependent on the rate of desiccation [17]. Since the preliminary test indicated that the microenvironment in the Parafilm sealed Petri dishes with Gamborg's B5 medium provided the suitable rate of desiccation conducive to anhydrobiosis of *P. coffeae*, the present research was conducted under the same condition.

MATERIALS AND METHODS

Nematodes

The lesion nematode *P. coffeae* was originally collected from the experimental station of the National Taiwan University and cultured on excised tomato roots in Gamborg's B5 medium (GIBCOBRL) following surface sterilization with H_2O_2 (3%) for 2 min.

Preparation of medium and culturing of excised tomato roots

Seeds of cherry tomato (Known-You Seed Co., LTD, Kaohsiung, Taiwan) were surface sterilized with 70% ethanol for 2 min and 0.6 % sodium hypochlorite for 10 min and sown on 0.6% water agar plates. Root tips, about 1 cm in length, were cut four days after sowing and transferred to Gamborg's B5 medium (GB5), five pieces per plate. GB5 was prepared according to the manufacturer's instruction (Life Technologies, Inc., New York, U.S.A.) with the addition of 0.8% agar. The aliquot of 20 ml of the autoclaved medium was dispensed into each Petri dish (9 cm diam.). After transferring the root tips, the Petri dishes were sealed with two strips of Parafilm M (PM-996, Pechiney Plastic Packaging, Menasha, WI, U.S.A.) and incubated at 25℃ for 5 days before inoculation of nematodes.

Desiccation

Approximately 1.5 cm^2 of medium with roots and nematodes from a one-month-old culture of *P. coffeae* on GB5 was transferred to the 5-day-old excised root culture. For slow desiccation of the nematodes, the Petri dishes were sealed with two strips of Parafilm after inoculation and incubated at 25 ℃ for 1 month, 3 months, 6 months, and 9 months. The sealed GB5 plates were weighed at the beginning and each sampling time to keep track of the water loss. Desiccation of nematodes was prevented in another set of GB5 plates by adding 1 ml of sterile distilled water to each plate after inoculation and before sealing the Petri dishes. These Petri dishes were sealed with four strips of Parafilm and then kept in air-tight zip-lock plastic bags. They were kept at 25℃ for the same period of time as described in the above and weighed at the beginning and each sampling time. All the procedures were performed under sterile condition. There were four replicates for each treatment. The second experiment was carried out two weeks after the first batch of experiment.

Coiling and survival of nematodes

At each sampling date, coiled nematodes were counted and then re-hydrated. Parallel lines, approximately 0.5 cm apart, were drawn with a fine blue marking pen at the bottom of Petri dishes (divided into 12 sections) to facilitate the counting of nematodes under dissecting microscope. The nematodes were counted in four categories: tight coils, loose coils, curved in C-shape, and active nematodes. Nematodes crawled to the lids of Petri dishes were counted in the same way. After counting the nematodes out side of the roots in each plate, sterile distilled water was added to the lids of Petri dishes and the agar plate for re-hydration and then roots were carefully removed to minimize error from nematodes emerging from inside the roots. The nematodes in the medium were then extracted with modified Baermann funnels [10] and collected after 72 h, then counted under dissecting microscope. Active nematodes on the lids of Petri dishes after re-hydration were counted under dissecting microscope. Percentage of survival in the medium was calculated as number of nematodes recovered from Baermann funnels divided by number of nematodes counted per plate. Percentage of survival of nematodes on the lids of Petri dishes was calculated as number of active nematodes after re-hydration divided by total number of nematodes on the lids.

Arcsine square root transformation was performed for percentages of all the above experiments before statistical analysis. One-way analysis of variance was carried out

with SAS 9.1 software (SAS Institute, Cary, NC, USA) and treatment means were compared with Duncan's multiple range test at the 95% level of confidence.

RESULTS

Desiccation of medium

The inoculated GB5 plates sealed with two strips of Parafilm desiccated slowly with time while those sealed with four strips of Parafilm and kept in zip-lock plastic bags remained moist (Fig. 1). There was 52.0 % water loss in the desiccated plates but only 3.2 % water loss in the moist plates by the end of nine months.

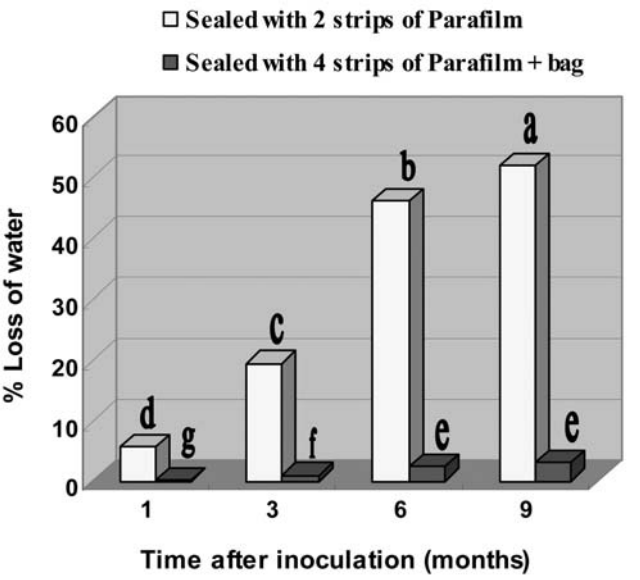

Fig. 1. Percentage of water loss in Gamborg's B5 medium in Petri dishes at 25℃. Bars with different letters are significantly different at $P=0.05$ according to Duncan's multiple range test after arcsine square root transformation.

Coiling of nematodes

There were coiled nematodes on the lids of Petri dishes (Fig. 2 A) and in the desiccated medium (Fig. 2 B) but not in the moist medium at 3, 6, and 9 months after inoculation. There were variations in coils, some very tight (Fig. 2 A and 2B) and some loose (Fig. 2 C). Some nematodes bent into C-shape (Fig. 2 D). All the coiled and C-shaped nematodes were motionless while a small percentage of nematodes were moving in the desiccated medium at 3 and 6, and even 9 months after inoculation (Fig 3). There were some nematodes with motion posture (wavy) but were motionless (Fig. 4). The percentage of coiled nematodes increased with time (Fig.3). There were 100 % and 78.7 % coiled nematodes, including tight coils and loose coils, on the lids of Petri dishes and in the desiccated medium, respectively, at 9 months after inoculation (Fig 4). Comparison of the bending of nematode bodies between the lids of Petri dishes and the desiccated medium showed that there were more tight coils on the lids and more loose coils and C-shaped nematodes in the medium (Fig. 4).

Survival of nematodes

There were more nematodes survived in the desiccated medium than on the lids of Petri dishes at 6 and 9 months after inoculation (Fig. 5). The nematodes survived longer in the desiccated medium than in the moist medium. Survival of nematodes decreased with time both on the lids of Petri dishes and in the medium. There were 24.7 % of nematodes survived on the lids of Petri dishes and 36.1 % survived in the desiccated medium at 9 months after inoculation. The intestines of *P. coffeae* in the desiccated medium were dark (Fig. 2B and 2C) while those in the moist medium were transparent (Fig. 2F) indicating starvation.

Infectivity of the survived nematodes

The survived nematodes from both the lids of Petri dishes and the desiccated medium were capable of infecting tomato roots grown in GB5 medium (Fig. 6) and turned the roots brown as the fresh nematodes did in the regular culture approximately 21 days after inoculation.

DISCUSSION

It has been reported that survival of nematodes increases with rising level of humidity to which they were exposed [6, 20, 23, 26, 27]. Higher humidity implicated slower rate of desiccation. Kikawada, *et al.* [17] reported that with the evaporation rate of 0.22 ml day^{-1}, all of the larvae of the African chironomid *Polypedilum vanderplanki* recovered after rehydration while those dehydrated quickly at 0.75 ml day^{-1} did not revived after rehydration. In the present studies, although sealed with Parafilm, water evaporated from the medium in the Petri dishes and gradually passed

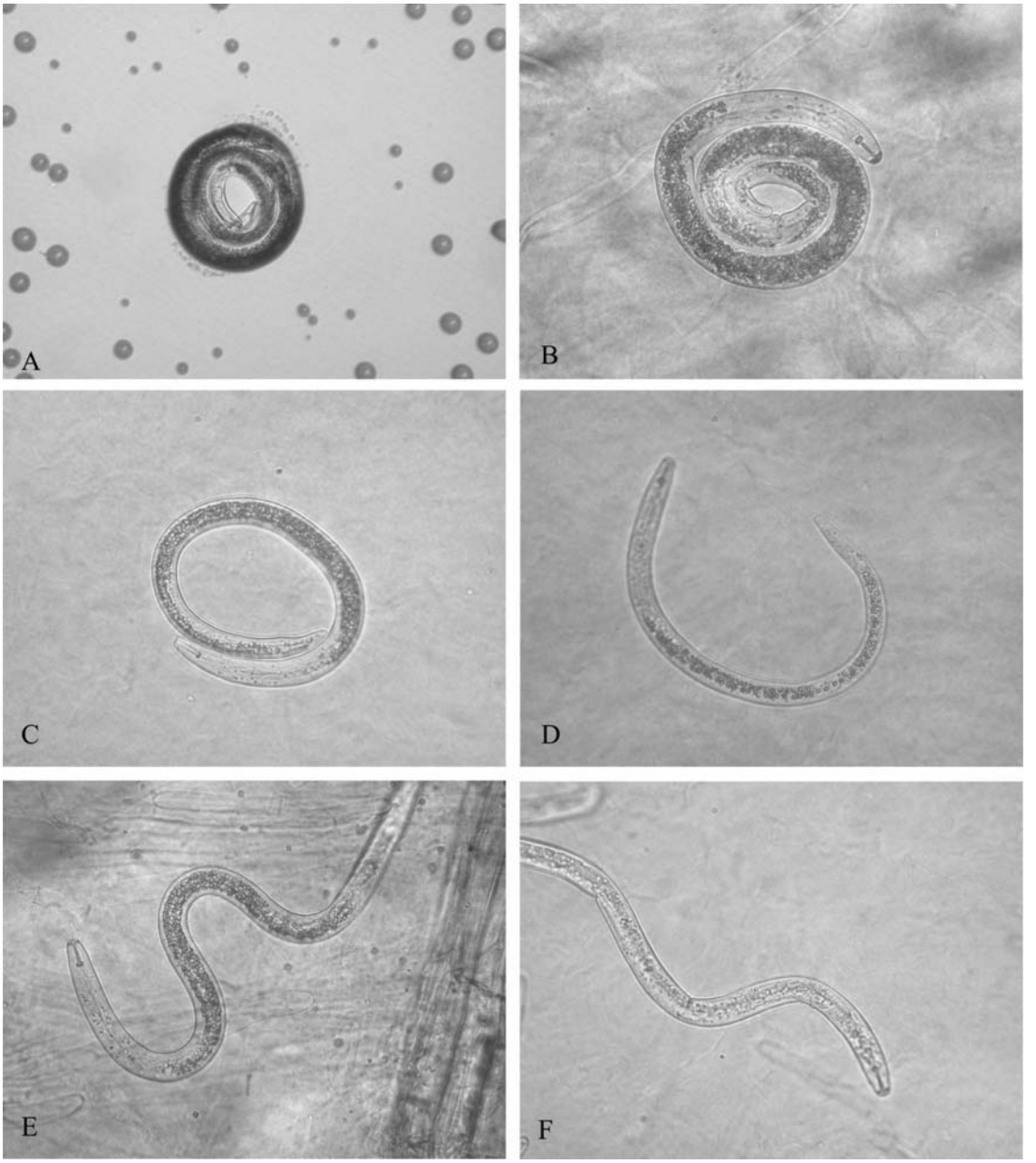

Fig. 2. Shapes of *Pratylenchus coffeae* in desiccated or moist treatments. (A) Tight coil on lid of Petri dish in desiccation treatment; (B) Tight coil in desiccated GB5 medium; (C) Loose coil in desiccated GB5 medium; (D) C-shaped nematode in desiccated GB5 medium; (E) Shape of active nematode in fresh culture; (F) Transparent intestine of nematode in moist medium, contrasted with the dark intestines of the coiled nematodes.

through Parafilm and hence the medium desiccated with time (Fig. 1). By the end of 9 months, 52.0 % of water had evaporated from the medium in the Petri dishes with two layers of Parafilm seal, which is equivalent to the average of 0.19 % of water loss per day. The slow rate of desiccation was favorable for the induction of anhydrobiosis of *P. coffeae* as indicated by the coiling of nematodes (Fig. 2A-C).

The percent coiling was higher on the lid than in the medium in the desiccation treatment (Fig. 4). Although in the same sealed Petri dish, nematodes on the lid could be expected to experience higher degree of desiccation than those in the medium and consequently higher percentage of coiling. The percent coiling in the desiccated medium increased with time while the medium became drier. These results are similar to the reports on the citrus nematode *T.*

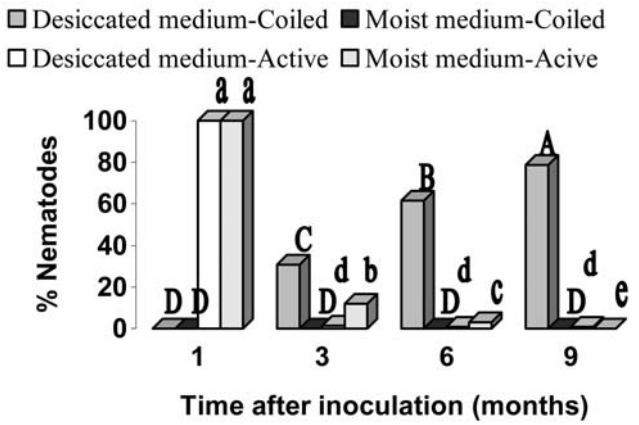

Fig. 3. Percentages of coiled and active nematodes in desiccated and moist Gamborg's B5 medium. Bars with different letters are significantly different at $P=0.05$ according to Duncan's multiple range test after arcsine square root transformation.

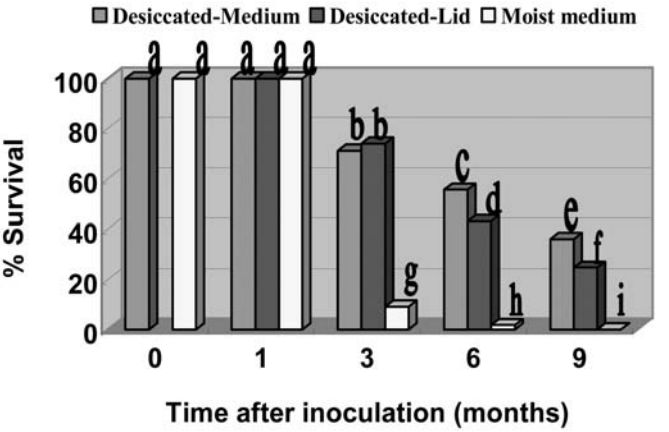

Fig. 5. Survival of nematodes in desiccated Gamborg's B5 medium and on the lids of Petri dishes and in moist medium. Bars with different letters are significantly different at $P=0.05$ according to Duncan's multiple range test after arcsine square root transformation.

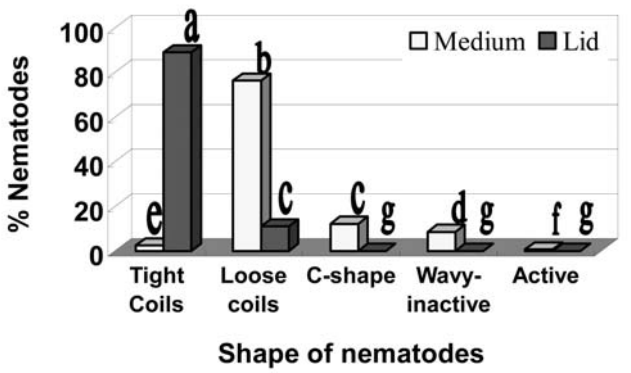

Fig. 4. Shapes of nematodes in desiccated Gamborg's B5 medium and on the lids of Petri dishes at 9 months after inoculation. Bars with different letters are significantly different at $P=0.05$ according to Duncan's multiple range test after arcsine square root transformation.

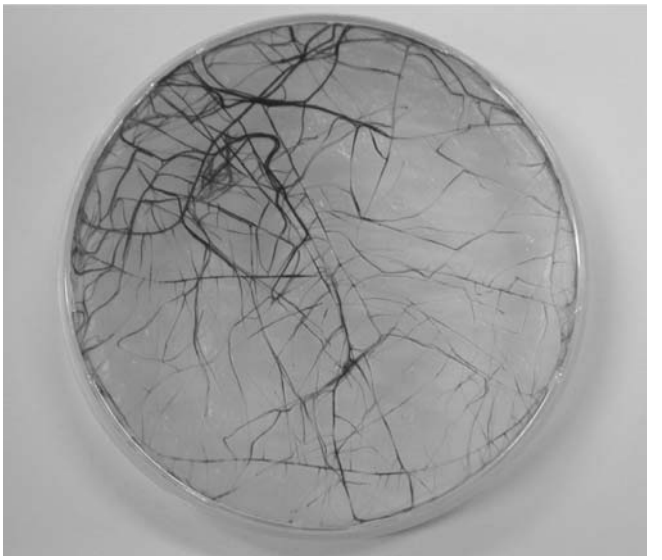

Fig. 6. Brown tomato roots resulted from infection by survived anhydrobiotic *Pratylenchus coffeae*.

semipenetrans [28] and nematodes from the McMurdo Dry Valleys of Antarctica [24] where coiling was related to degree of desiccation. Another possible reason for higher percentage of coiling on the lid than in the medium was the interference to the bending of nematode bodies. Although *P. coffeae* could move freely through the agar in GB5 medium, they were surrounded by particles of agar and might not bend into coils as freely as on the lids of Petri dishes and hence reduced the percentage of coiling in the medium.

The percent survival of *P. coffeae* was higher in the medium than on the lids (Fig. 5), yet the later had higher percentage of coiling. In the desiccated medium, the best survival rate (Fig. 5) did not coincide with the highest percentage of coiling (Fig. 3) either. Crowe and Madin [6] and Tsai [26] also reported similar phenomena on *Aphelenchus avenae* and *R. reniformis*. It is agreed among researchers that coiling is an indication of anhydrobiosis [6, 9, 23, 24, 26], yet highest percentage of survival did not coincide with highest percentage of coiling. It would seem that some factor other than desiccation was also affecting the relationship between survival and coiling. One possibility is that the bending of nematode bodies to form coils may

be interfered by the particles around the nematodes as indicated by the difference between the lids of Petri dishes and the agar plates in the present studies. For example, some nematodes bent into C-shape in the desiccated medium (Fig. 2D) which could probably be the result of interference from the agar particles, for there were no C-shaped nematodes on the lids of Petri dishes where nematodes could bend freely without interference. In the soil, nematodes move freely through the pores between soil particles, however, bending to form coils would take wider space then when they were moving and thus some soil particles may get in the way of bending.

There were 0.7% nematodes remained active in the desiccated medium at 9 months after inoculation (Fig. 3), although moving slower than those in the fresh culture. The result showed that certain individuals in a population are more tolerant to desiccation than the others.

It is interesting to note that in the process of photographing under microscope, the coiled nematodes started to regain activity without the addition of water. The pictures of coiled nematodes (Fig. 2B and 2C) were taken directly from the sealed Petri dishes. It is unlikely that moisture in the air could penetrate the Parafilm seal and sip through the medium during photographing. The obvious changes in the microenvironment in the sealed Petri dish were the intense light and the heat generated by the light during photographing. There has been no report on the activation of anhydrobiotic nematodes by light or heat. It might be possible that the heat caused the agar to release some remaining moisture which was not available to the nematodes in the desiccated medium before photographing. However, the possibility of activation of anhydrobiotic nematodes by light or heat *per se* can not be ruled out without further studies.

The lesion nematodes survived longer in the desiccated medium than in the moist medium (Fig. 5). This was probably because the nematodes remained active in the moist medium even after their food source was exhausted and thus depleted their energy reserves. The desiccated *P. coffeae* preserved their energy by entering anhydrobiosis. Anhydrobiotic nematodes are in a state of quiescence in which the metabolic activities decreased to non-detectable level [30, 4] and the energy of the organisms conserved. The intestines of *P. coffeae* in the desiccated medium were dark while those in the moist medium were transparent (Fig. 2B, 2C, and 2F) after the same duration of time after inoculation, indicating the differential rate of consumption of energy reserves between the desiccated and non-desiccated nematodes. These findings are in conformity with those reported for the reniform nematode, *R. reniformis* [26]. Tsai studied the survival of the reniform nematode at various soil moisture levels and found that the nematodes survived better in dry soil than in moist soil and the lowest survival was at -0.15 bar which was near the field capacity where the nematodes remained active and then died due to starvation. Understanding of the relationship between moisture and nematode survival can help in developing an effective strategy for nematode control. Since *P. coffeae* is an obligate parasite, absence of the host plant will lead to eventual death of the nematodes. Based on the present findings, one can deduct that moist fallow would be more effective than dry fallow for the control of *P. coffeae* by preventing the nematodes from entering the state of anhydrobiosis. Soil moisture is not even in the field, there may be niches in the field where soil moisture was conducible for anhydrobiosis of the nematodes. Anhydrobiosis should be considered when formulating control strategies for nematodes, such as solarization, because the anhydrobiotic nematodes are more resistant to unfavorable conditions [6, 7, 19, 28]. Solarization controls nematodes by raising soil temperature with solar energy. However, desiccation has been reported to increase the tolerance of nematodes to temperature extremes [13]. Therefore, soil should be kept moist to prevent the effect of anhydrobiosis of nematodes in solarization.

Anhydrobiosis presented a convenient way of nematode preservation. There are usually more than one nematode species cultured in a laboratory and not all of them are actively in use all the time, and keep sub-culturing the nematodes which are not actively in use is a waste of man power and materials. Therefore, researchers have studied measures for nematode preservation. Triantaphyllou and McCabe [25] preserved root-knot and cyst nematodes in liquid nitrogen. Irdani, *et al.* [16] reported the preservation of *Bursaphelenchus* spp. in a mechanical freezer at -140 ℃ for six months using 25% ethylene glycol solution as the cryoprotectant and a rapid-cooling protocol involving immersion in liquid nitrogen at -196 ℃ before storage. There has been no report on the

preservation of *Pratylenchus* species. Although cryopreservation has its advantages, it requires special equipments and complicated procedures. Utilizing the anhydrobiotic property of the nematodes discovered in the present studies, preservation of *P. coffeae* in desiccated GB5 medium is easy, convenient, and economical. The nematodes could be kept for 9 months with 36.1 % survival. The survival rate was not as high as *Bursaphelenchus* spp. in the cryopreservation, nevertheless, it was sufficient for the purpose of maintaining the inoculum source for sub-culturing the nematodes with minimum cost of resources. The anhydrobiotic nematodes have been used successfully as inoculum source for the subculture of *P. coffeae* after rehydration with sterile water (Fig. 6). Although there is no previous report on the utilization of desiccation for preservation of plant-parasitic nematodes, it has been documented for mice sperm preservation [(2)].

LITERATURE CITED

1. Aroian, R.V., Carta, L., Kaloshian, I., and Sternberg, P.W. 1993. A free-living *Panagrolaimus* sp. from Armenia can survive in anhydrobiosis for 8.7 years. J. Nematol. 25: 500-502.
2. Bhowmick, S., Zhu, L., McGinnis, L., Lawitts, J., Nath, B. D., Toner, M., and Biggers, J. 2003. Desiccation tolerance of spermatozoa dried at ambient temperature: production of fetal mice. Bio. Repro. 68: 1779-1786.
3. Clegg, J. S. 2005. Desiccation tolerance in encysted embryos of the animal extremophile, *Artemia*. Integr. Comp. Biol. 45:715-724
4. Cooper, A. F., Jr. and Van Gundy, S. D. 1971. Senescence, quiescence, and cryptobiosis. Pages 297-318 *in* Plant parasitic nematodes, vol. II. B. M. Zuckerman, W. F. Mai, and R. A. Rohde eds. Academic Press, New York, 347pp.
5. Crowe, J. H., Hoekstra, F. A., and Crowe, L. M. 1992. Anhydrobiosis. Ann. Rev. Physiol. 54:579-99.
6. Crowe, J. H., and Madin, K. A. C. 1975. Anhydrobiosis in nematodes: evaporative water loss and survival. J. Exp. Zool. 193: 323-334.
7. Demeure, Y. 1980. Biology of the plant-parasitic nematode *Scutellonema* cavenessi Sher, 1964: anhydrobiosis. Revue Nématol. 3: 283-290.
8. Demeure, Y., and Freckman, D. W. 1981. Recent advances in the study of anhyrobiotic nematodes. Pages 205-226 *in*: Plant parasitic nematodes, vol. III. B. M. Zuckerman, W. F. Mai, and R. A. Rohde eds. Academic Press, New York, 508 pp
9. Freckman, D. W., Kaplan, D. T., and Van Gundy, S. D. 1977. A comparison of techniques for extraction and study of anhydrobiotic nematodes from dry soils. J. Nematol. 9: 176-81.
10. Goodey, J. B. 1957. Laboratory methods for work with plant and soil nematodes. Her Majesty's Stationery Office, London. 47 pp.
11. Grant, J. A., and Villani, M. G. 2003. Soil moisture effects on entomopathogenic nematodes. Environ. Entomol. 32: 80-87.
12. Grewal, P.S. 2000. Enhanced ambient storage stability of an entomorphogenic ematode through anhydrobiosis. Pest Management Sci. 56: 401-406.
13. Grewal, S.K., and Grewal, P. S. 2003. Effect of osmotic desiccation on longevity and temperature tolerance of *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae) J. Parasitol. 89: 434-438.
14. Grewal,P. S., and Jagdale, G. B. 2002. Enhanced trehalose accumulation and desiccation survival of entomopathogenic nematodes through cold preacclimation. Biocontrol Sci. and Tech. 12: 533-545.
15. Hoekstra, F. A. 2005. Differential longevities in desiccated anhydrobiotic plant systems. Integr. Comp. Biol. 45:725-733.
16. Irdani, T., Carletti, B., Ambrogioni, L., and Roversi, P. F. 2006. Rapid-cooling and storage of plant nematodes at -140℃. Cryobiology 52: 319-322.
17. Kikawada, T., Minakawa, N., Watanabe, M., and Okuda, T. 2005. Factors inducing successful anhydrobiosis in the African Chironomid *Polypedilum vanderplanki*: Significance of the larval tubular nest. Integr. Comp. Biol. 45: 710-714.
18. Lettini, S. E., and Sukhdeo, M. V. K. 2006. Anhydrobiosis increases survival of Trichostrongyle nematodes. J. Parasitol. 92: 1002-1009.
19. O'Dell, S. J. and Crowe, J. H. 1979. Freezing in nematodes: the effects of variable water contents. Cryobiology 16: 534-541.
20. Perry, R. N. 1977. Desiccation survival of larvae and adult stages of the plant parasitic nematodes, *Ditylenchus dipsaci* and *D. myceliophagus*. Parasitology 74: 139-148.
21. Pit, D. S. S., Blotkamp, J., and Polderman, A. M. 2000. The capacity of the third-stage larvae of *Oesophagostomum bifurcum* to survive adverse conditions. Ann. of Tropical Med. & Parasitology 94: 165-171.
22. Townshend, J.L. 1984. Anhydrobiosis in *Pratylenchus penetrans*. J. Nematol. 16: 282-289.
23. Towson, A. J., and Apt, W. J. 1983. Effect of soil

water potential on survival of *Meloidogyne javanica* in fallow soil. J. Nematol. 15: 110-15.
24. Treonis, A. M., and Wall, D. H. 2005. Soil nematodes and desiccation survival in the extreme arid environment of the Antarctic Dry Valleys. Integr. Comp. Boil. 45:741-750.
25. Triantaphyllou, A.C., and McCabe E. 1989. Efficient preservation of root-knot and cyst nematodes in liquid nitrogen, J. Nematol. 21: 423-426.
26. Tsai, B. Y. 1978. Anhydrobiosis of the reniform nematode: survival and coiling. M.S. Thesis. University of Hawaii. 63 pp.
27. Tsai, B. Y., and Van Gundy, S. D. 1988. Comparison of anhydrobiotic ability of the citrus nematode with other plant parasitic nematodes. Pages 983-992 *in*: Proceedings of the sixth International Citrus Congress. R. Goren and K. Mendel eds. Margraf Scientific Books, Weikersheim.
28. Tsai, B. Y., and Van Gundy, S. D. 1989. Tolerance of proto-anhydrobiotic citrus nematodes to adverse conditions. Revue Nématol. 12: 107-112.
29. Tsai, B. Y. 2001. Effect of nematode age and plant species on the penetration speed of *Melodogyne javanica* and *Pratylenchus coffeae*. Plant Pathol. Bull. 10: 65-70. (in Chinese).
30. Van Gundy, S. D. 1965. Factors in survival of nematodes. Ann. Rev. Phytopathol. 3: 43-68.
31. Wharton, D. A., Rolfe, R. N., and Perry, R. N. 2000. Electrophysiological activity during recovery from anhydrobiosis in fourth stage juveniles of *Ditylenchus dipsaci*. Nematol. 2: 881-886.

摘 要

蔡碧雲. 2008. *Pratylenchus coffeae* 之脫水殘存. 植病會刊17：17-24. (台北市 國立台灣大學植物病理與微生物學系；電子郵件：bieyntm@ntu.edu.tw；傳眞：02-23636490)

P. coffeae 在緩慢脫水後身體彎曲成緊密之圈狀，寬鬆圈狀，及 C-形。線蟲彎曲成圈狀是脫水殘存 (anhydrobiosis) 之特徵。緩慢脫水處理之培養基其培養皿蓋子上之線蟲彎曲成圈狀之比例高於培養基中者，但是培養基中之線蟲存活率卻高於培養皿蓋子上者。培養基中線蟲彎曲成圈狀之比例隨著時間而增加，然而最高之存活率並不與最高之圈狀線蟲之比例相對應。在土壤中及培養基中線蟲之彎曲皆可能受到身體周圍物體之阻礙因而影響線蟲存活率與圈狀線蟲之比例間的關係。根腐線蟲在脫水之培養基中存活率比在濕潤之培養基中者高。脫水殘存之線蟲對不良環境較具抵抗性，因此本研究結果有助於根腐線蟲田間防治策略之研擬，如改變土中溼度以降低線蟲存活率，並提供了一個以乾燥方式來保存根腐線蟲之方法。保存九個月後尚有36.1% 之線蟲存活，足以供應繼代培養所需之接種原。乾燥保存法可大幅減少維持線蟲繼代培養所消耗之人力與物力資源。

關鍵詞：脫水殘存、anhydrobiosis、*Pratylenchus*、培養