Plant Pathology Bulletin 17: 65-68, 2008

利用資源回收紙類培養根瘤線蟲

蔡碧雲

臺北市 國立台灣大學植物病理與微生物學系
聯絡作者：電子郵件：bieyntm@ntu.edu.tw　傳眞：02-23636490
接受日期：97 年 2 月 19 日

摘　要

蔡碧雲. 2008. 利用資源回收紙類培養根瘤線蟲 植病會刊17 ：65-68.

本研究利用資源回收紙類作爲支撐介質，製作培養袋以種植綠豆苗來培養 *Meloidogyne incognita*。資源回收紙類大部分皆對綠豆苗有不良影響，但泡水一天除去有害化學物質後，可明顯改善綠豆苗之生長。除牛皮紙信封袋外，供試回收紙類，包括麥當勞、肯德基、及星巴克等速食店之紙袋，超市紙袋，及用過之白色影印紙等，皆可用來作爲支撐介質種植綠豆苗培養線蟲。試驗結果顯示，每株苗接種 500 隻二齡幼蟲，將培養袋平放一天再掛起於生長箱內，可成功培養出大量之根瘤線蟲。以最普遍之用過的影印紙生產線蟲量來計算，一個內部容積爲 73 ×49×135 cm^3 之生長箱，可生產之 *M. incognita* 可達一千四百多萬隻。若只採用孵化後三天內之線蟲，以符合某些實驗對接種原新鮮度之嚴格要求，一個生長箱每三天累積之產量最高爲七百二十多萬隻，最低尙可達二百四十萬隻左右。利用資源回收紙類製作之培養袋不僅可培養線蟲，亦可用於各種研究，尤其適合根部病徵之發展的連續性觀察。本培養法對溫室空間不足者當有助益。

關鍵詞：環保、南方根瘤線蟲、*Meloidogyne incognita*、線蟲培養、接種原

線蟲之培養是線蟲學研究之基礎。一般線蟲之培養主要考量在於所耗費之人力、物力、空間等之成本。植物寄生性線蟲除極少數可用眞菌培養者[6] 外皆爲絕對寄生，其培養需寄主植物或植物組織。苜蓿癒合組織曾被用來培養 *Radopholus similis*[1]，利用組織培養基培養之切根 (excised roots) 也可用來培養線蟲[5]。這些方法優點在節省空間，缺點爲需無菌操作。根據本研究室之經驗，有時因不愼污染而損失整批線蟲，甚至造成絕種的窘境。以盆缽培養線蟲無上述缺點，但是耗費較多勞力，且佔用很多空間。Tsai[5] 用進口生長袋 (Seed-Pack Growth Pouch, Mega International of Minneapolis) 種植綠豆苗培養 *Meloidogyne javanica*。此法比盆缽培養操作輕便，節省勞力，且節省空間。若與以植物組織培養線蟲之法比較，此法不需無菌操作，且不需高溫高壓滅菌，較節約能源。

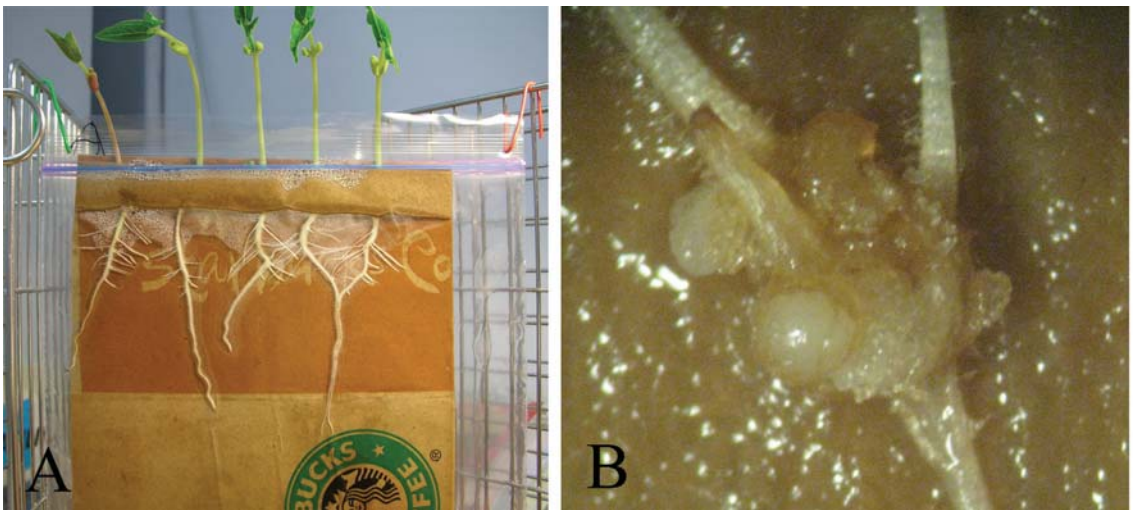
進口之生長袋主要靠塑膠袋內之特製紙張來支撐種子並吸收水分。若能利用資源回收紙類作爲支撐介質置於塑膠袋內來種植寄主植物培養線蟲，不僅可降低成本，且可爲環保盡一份心力。本研究之目的在於測試常見之資源回收紙類，是否有適合作爲支撐介質來培養線蟲者，期能發展出環保又簡易之線蟲培養法。

供試回收紙類包括麥當勞、肯德基、及星巴克等速食店之紙袋，超市紙袋 (Jasons Market Place，以下簡稱 JMP 超市)，牛皮紙信封袋，及用過之白色影印紙 (Double A, Advance Paper Co., Ltd.)。因進口生長袋原已在本研究室成功培養根瘤線蟲，故將其作爲對照組。先將紙袋剪開除去有黏膠處。將紙對摺成兩層，剪成 14×19 cm^2 之長方形後噴以 70% 酒精表面消毒。晾乾後將紙之上方 3 cm 向下摺，再向上摺疊成 1.5 cm 深之凹槽以放置種子。沿凹槽之摺線用鑽子每隔約 0.3 cm 打一個洞。將紙塞進 16.5×14.9 cm 之夾鍊塑膠袋 (小三明治袋)。然後用 迴紋針製作掛塑膠袋之掛勾。另一組先將裁好之紙泡水一天，其間換水三次，晾乾後其餘步驟同上。綠豆 (*Vigna radiata* (L.) Wikzek)由超級市場購得。將種子以 70% 乙醇消毒四分鐘，沖洗後泡

在水中備用。將四粒種子排放在紙之凹槽，蓋上棉花。加水後掛在28℃ 生長箱內預先綁好之兩條平行繩子。大約二至三天澆一次水。播種後三天疏苗成一株。在播種後四天測量主根及莖之長度，並計算側根數。各處理四重複。本試驗重複兩次。試驗結果發現回收紙類皆對綠豆苗有不良影響，但經泡水一天除去有害化學物質後，明顯改善綠豆苗主根長度及側根數。除牛皮紙信封袋外，供試資源回收紙類皆可用來種植綠豆苗培養線蟲(圖一A)。

M. incognita 由國立台灣大學農場取得，培養於進口生長袋中之綠豆苗。卵塊成熟後取下於孵卵裝置[5]孵化。每天收集幼蟲保存於15℃ 備用。線蟲產量之測試如下。用各回收紙類製作之培養袋及進口生長袋種植綠豆。播種後五天每株苗接種2 ml 懸浮液內含約500 隻線蟲。培養袋於生長箱平放一天後掛起。接種後31 天計算根瘤及卵塊數，並將卵塊於28℃ 孵化。每天收集，直到收集不到線蟲爲止。最後計算線蟲總數。另一批實驗用肯德基、JMP 紙袋及用過之影印紙製作之培養袋及進口生長袋種植綠豆，步驟同上，但收集孵化之幼蟲時僅採用1-3 天、4-6 天，及7-9 天所收集者。各處理四重複。以上各試驗重複兩次。

試驗結果顯示，利用資源回收紙類可成功培養出大量之根瘤線蟲(圖一B)。各紙類中，根瘤數以用過之影印紙最高，而線蟲產量以麥當勞、星巴克紙袋爲最高(表一)。肯德基紙袋之卵塊數並不比影印紙及JMP超市紙袋者低，但線蟲產量卻較少。然而其產量仍相



圖一、用資源回收紙類種植綠豆苗培養線蟲。A. 星巴克紙袋種植綠豆苗，B. 回收紙培養袋所生產之根瘤線蟲卵塊。

Fig. 1. Recycled papers as the supporting substrates for growing mungbean seedlings for culturing nematodes. A. Growth of mungbean seedlings supported by Starbucks Coffee brown bag, B. Egg masses of *Meloidogyne incognita* produced in culture pouch with recycled paper as the supporting substrate.

表一、資源回收紙類對*Meloidogyne incognita* 在綠豆苗之根瘤數及線蟲產量之影響

Table1. Effects of recycled papers on galling and yields of *Meloidogyne incognita* on mungbean

Variety of recycled papers	Number of galls per root system[1]	Number of egg masses per root system[1]	Number of J_2 yielded per root system[1]
Brown bags – McDonald's	59.5 bc	119.3 a	75636 ab
Brown bags – Kentucky Fried Chicken	50.8 d	86.0 bc	51984 d
Brown bags – Starbucks Coffee	54.3 cd	97.8 b	79851 a
Grocery bags – Jasons Market Place	54.8 cd	80.3 c	72513 b
Used Xerox paper	64.3 ab	83.8 c	61387 c
Control (Imported growth pouch)	67.5 a	91.8 bc	76530 ab

[1] Means within each column with different letters are significantly different at P=0.05 according to the Duncan's multiple range test.

表二、資源回收紙類三天內所產之*Meloidogyne incognita* 二齡幼蟲之產量

Table2. Three-days yields of *Meloidogyne incognita* on mungbean with selected recycled papers as the supporting substrates in culture pouches

Variety of recycled papers	Number of J_2 yielded[1]		
	1~3 DAH[2]	4~6 DAH	7~9 DAH
Brown bags – Kentucky Fried Chicken	13302 d	20561 b	11300 c
Grocery bags – Jasons Market Place	24221 c	27596 a	12156 b
Used Xerox paper	30218 a	22158 b	10724 c
Control (Imported growth pouch)	26167 b	27259 a	15741 a

1 Means within each column with different letters are significantly different at P=0.05 according to the Duncan's multiple range test.
[2] DAH= Days after hatch commenced.

當可觀，一株綠豆苗可生產五萬多隻線蟲，相當於繁殖了 100 倍以上。二齡幼蟲每三天之產量如表二。第一批產量以用過之影印紙爲最高，達三萬隻以上。第二批以 JMP 超市紙袋爲最高，達二萬七千隻以上。雖然各個實驗所需線蟲接種原之量不同，但根據文獻所載，一般實驗室或溫室試驗所需線蟲量，大約在一百多萬隻以內[3]。一個內部容積爲 73 ×49 ×135 cm^3 之普通生長箱，可容納約 240 個環保培養袋。以一般研究室最普遍之用過的影印紙來計算，一個生長箱可生產之根瘤線蟲可達一千四百多萬隻，足以供應一般研究用。某些實驗對接種原之新鮮度要求較嚴格，只採用孵化後三天內之線蟲[2, 4]。即使如此，用過之影印紙所製作之環保培養袋也足以供應此等接種原需求（表二)。本培養法對溫室空間不足者當有助益。本線蟲培養系統不僅可用來培養線蟲，亦可用於各種研究，例如篩選抗病品種或寄主與線蟲之交互關係之研究，尤其適合根部病徵之連續性觀察。除綠豆外，理論上只要是寄主植物之種子本身具備足夠養分供苗生長以完成線蟲生活史者皆可用於本培養系統，以免添加肥料後有其他微生物在培養袋中生長。

引用文獻 (LITERATURE CITED)

1. Elsen, A., Lens, K., Nguyet, D.T.M., Broos, S., Stoffelen, R., and Waele, D. de. 2001. Aseptic culture systems of *Radopholus similis* for *in vitro* assays on *Musa* spp. and *Arabidopsis thaliana*. J. Nematology 33: 147-151.
2. Jin, R. D., Suh, J. W., Park, R. D., Kim, Y. W., Krishnan, H. B., and Kim, K. Y. 2005. Effect of chitin compost and broth on biological control of *Meloidogyne incognita* on tomato (*Lycopersicon esculentum* Mill.) Nematology 7: 125-132.
3. Melakeberhan, H., Brooke, R. C., and Webster, J. M. 1986. Relationship between physiological response of French beans of different age to *Meloidogyne incognita* and subsequent yield loss. Plant Pathol. 35: 203-213.
4. Oka,Y. 2001. Nematicidal activity of essential oil components against the root-knot nematode *Meloidogyne javanica*. Nematology 3: 159-164.
5. Tsai, B. Y. 2001. Effect of nematode age and plant species on the penetration speed of *Meloidogyne javanica* and *Pratylenchus coffeae*. Plant Pathol. Bull. 10:65-70. (in Chinese with English abstract)
6. Tzeng, C. Y., and Lin, Y. Y. 2005. The intraspecific variation of *Aphelenchoides besseyi* populations in Taiwan. Plant Pathol. Bull. 14: 67-75. (in Chinese with English abstract).

ABSTRACT

Tsai, B. Y. 2008. Recycled papers as supporting substrates for root-knot nematode culture. Plant Pathol. Bull.17: 65-68. (Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan；E-mail: bieyntm@ntu.edu.tw, Fax: 02-23636490)

Recycled brown bags from fast food restaurants, including McDonald's, Kentucky Fried Chicken, and Starbucks Coffee, grocery bags from a supermarket, and Xeroxed paper were used as the supporting substrates in zip lock plastic bags to grow mungbean seedlings for culturing *Meloidogyne incognita*. The phytotoxicity of recycled papers to the mungbean seedlings was eliminated by soaking the papers in water for one day prior to use. Each seedling was inoculated with 500 second-stage juveniles of *M. incognita* and then placed horizontally for 24 h prior to hanging up the pouches in the growth chamber. In a growth chamber, with the interior size of 73×49×135 cm^3, the nematode yield could reach 14 millions of nematodes with recycled Xerox paper as the supporting substrate. The yield of three-day old or younger second-stage juveniles which were preferred as the nematode inoculum by certain researchers was 2.4-7.2 millions nematodes per growth chamber. This nematode culture system with recycled papers can provide more than sufficient nematodes for researches and it is space-saving, environment-friendly, energy-saving, and easy to handle. It is also a good tool for other researches and is especially good for continuous observations on the symptom development of the root systems.

Key words: environment-friendly, root-knot nematode, *Meloidogyne incognita*, nematode culture, inoculum