

# Axenizing and Culturing Endomigratory Plant-Parasitic Nematodes using Pluronic F127, Including its Effects on Population Dynamics of *Pratylenchus penetrans*<sup>1</sup>

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**Abstract:** A non-chemical technique for surface sterilizing plant-parasitic nematodes for aseptic cultures is described. The method is most applicable to nematodes with active migratory infective stages and requires only a few starting specimens. Rate of achieving a primary aseptic culture with the technique ranged from 60%–100% depending on the conditions of the specimens collected for culturing. Aseptic cultures of species of *Meloidogyne*, *Rotylenchulus*, *Pratylenchus*, and *Radopholus* initiated with the method remained contamination-free after 12 months of maintenance in tomato root explant or alfalfa callus cultures. Further studies of Pluronic F127, a polyol gel medium employed in the technique to confine the spread of contaminating bacteria or fungi associated with the nematodes, showed that the polyol gel was a suitable support medium for culturing corn root explant, alfalfa callus tissues, and consequently *Pratylenchus* species including *P. agilis*, *P. brachyurus*, *P. scribneri*, and *P. penetrans*. During the course of 10 months, *P. penetrans* reared in polyol-base medium followed a standard biological growth curve, multiplied to a higher population density, maintained a similar female-to-male ratio, and possessed a similar tendency to reside inside or outside host tissues as did *P. penetrans* reared in agar-base medium. The percentages of *P. penetrans* juveniles in the sub-populations residing outside or inside the host tissues reared in polyol-base medium also were similar to and fluctuated temporally in like manner as those reared in agar-base medium. Members of these sub-populations from the polyol- or agar-base were equally infective and reproductive after 9 months of culturing.

**Key words:** aseptic culture, axenizing technique, *Meloidogyne*, nematode, Pluronic F127, *Pratylenchus agilis*, *Pratylenchus brachyurus*, *Pratylenchus penetrans*, *Pratylenchus scribneri*, population dynamics, *Radopholus*, *Rotylenchulus*, sterilization.

Initiation of an aseptic culture of a plant-parasitic nematode species requires surface sterilization of the nematode. Chemical sterilization has been so far the most common and effective means of achieving this goal (6). However, the chemicals used may cause high nematode mortality, thereby requiring a large number of starting specimens. Also, a chemical suitable to axenize one species of nematodes may not be applicable to another species, as sensitivities to chemicals for nematode species are different (6). Therefore, it is desirable to have a means of axenizing a small number of nematodes without the potential deleterious effects of

chemicals and, at the same time, applicable equally to most, if not all, the nematode species. Pluronic F127 (BASF, Parsippany, NJ), a block co-polymer gel of propylene oxide and ethylene oxide (polyglycol), was found to inhibit the growth of many bacteria, fungi, or actinomyces without affecting the growth of selected nematode species at 20% (w/v) (11). Encapsulation of the nematodes to be axenized in the polyglycol would ensue retardation of growth of associated microbial contaminants without affecting nematode movement. The resultant capsule, when placed in agar- or gelrite-base substrate, should allow the nematodes inside to move out freely through the substrate, migrating ahead of or liberating themselves from any growing microbial contaminants, such as root tips of plants genetically transformed by *Agrobacterium rhizogenes* rid themselves of the bacterial incitant as the roots grow through thick agar (13,20).

A few plant-parasitic nematodes have been reared successfully in the Pluronic F127-base medium (11). The Pluronic

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F127 gel system has the potential advantages that nematodes, plant tissues, their excretory-secretory products, or other labile biologically active factors associated with the culture could be extracted easily and nondestructively by simply shifting to liquefying temperatures. Pluronic F127 has been found useful as agar substitute in culture media to detect and measure microbial enzyme production (8). Therefore, the polyol-base medium may similarly be used to examine the various aspects of nematode biology as well as host-parasite relationships. However, the effects of Pluronic F127 on plant tissue growth and on nematode population dynamics during culture have not been reported in detail and must be investigated before any such use. The objectives of this research were to i) test if passage of nematodes encapsulated in Pluronic F127 was an effective means of freeing the nematodes from contaminating bacteria or fungi in agar or gelrite medium; ii) determine the suitability of Pluronic F127, via comparing to agar, as a support base for culturing plant tissues and *Pratylenchus* species; and iii) examine and compare the population dynamics of *P. penetrans* cultured in the polyol- and agar-base medium.

#### MATERIALS AND METHODS

Unless otherwise stated, all operations were performed aseptically at room temperature (22–23 C). All nematode and plant tissue cultures were grown in 9-cm-d petri plates and incubated at 25 C in the dark. In experiments for objective 1, agar- and gelrite-base media were minced in a blender to facilitate nematode recovery; in experiments for objectives 2 and 3, the agar- and polyol-base media were equilibrated at 15 C for at least 30 minutes before the nematode extractions. In studies involving Pluronic polyol as a support base, Bacto-agar was used as a control.

**Root explant and callus cultures:** Root explants of tomato (*Lycopersicon esculentum* Miller cv. Rutgers) were cultured on Murashige and Skoog medium (MS) (16) supplemented with 2% sucrose prepared in ei-

ther 1.5% (w/v) agar (Difco Laboratories, Detroit, MI) or 0.25% gelrite (Kelco, Merck & Co., San Diego, CA). Two surface-sterilized tomato seeds (11) were placed near the edge of the petri plates containing MS and allowed to germinate in the dark at 25 C for 3–4 days, when the radicles were 2–3 cm long. The epicotyls were removed with a sterile scalpel; roots were allowed to grow for another 3–5 days. These plates (Fig. 1D) were selected for immediate use as bait plates to surface sterilize nematodes; plates not used immediately were placed at 4 C until use. Alfalfa

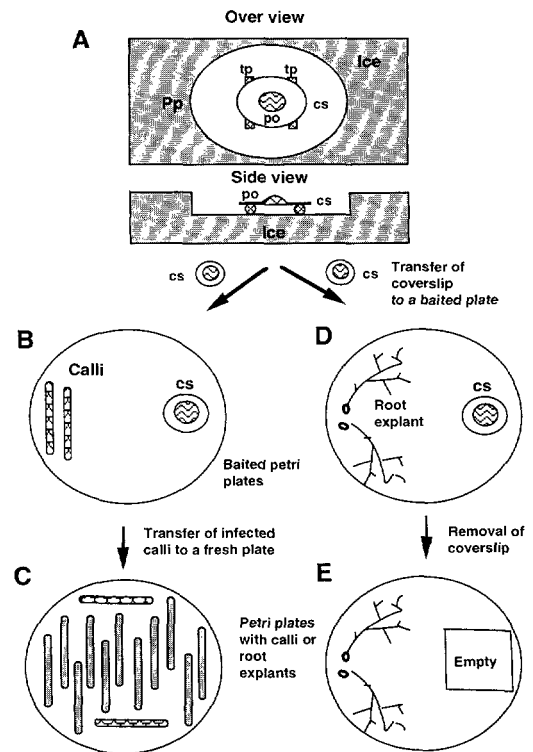


FIG. 1. Flow diagram of the nematode surface-sterilization process, including a schematic of the setup. A) A drop of Pluronic polyol (po) on a coverslip (cs) ready to receive nematode specimens. The coverslip is supported by two pieces of wooden toothpick (tp) (1–2 cm) on a small petri plate (Pp) (5-cm-d) sitting on crushed ice (ice). B) A coverslip with nematode specimens being transferred to a petri plate baited with alfalfa callus tissues at a distal end. C) Infected calli from B being transferred to a fresh callus plate. D) A coverslip with nematode specimens being transferred to a petri plate baited with root explants at a distal end. E) The coverslip in D was carefully removed along with a block of agar or gelrite, leaving only the infected root explants.

(*Medicago sativa* L. cv Drummer) calli were initiated (11) and cultured on modified White's (MW) medium (19) in 1.5% agar or 0.25% gelrite. Bait plates with calli (Fig. 1B) for axenizing nematodes were prepared by transferring 2–3 pieces of 10-day-old callus tissues to blank MW plates.

*Surface sterilization of nematodes (Experiment 1)*: Individuals from nine nematode populations (four *Meloidogyne*, two *Pratylenchus*, two *Radopholus*, and one *Rotylenchulus* species) were used to evaluate the effectiveness of surface sterilization. Approximately 50 nematode individuals consisting of migratory infective stages (with equal numbers of males and females for amphimictic species) from roots or soil (2) were individually picked and placed in a drop (25–50  $\mu$ l) of 20% (w/v) Pluronic F127 polyol (11) on an ice-cold circular sterile coverslip (12 mm, 0.13–0.17 mm thick) (Fig. 1A). The coverslips containing the nematodes were equilibrated at 25 or 30 C for 15 minutes for the drops to gel. Each coverslip was then inverted and transferred to a point most distal in a petri plate baited with 2–4 pieces of sterile tomato root explants (Fig. 1D) or alfalfa callus tissues (Fig. 1B), with the drop containing the nematodes in contact with the agar or gelrite surface. The inoculated plates were then incubated at 25 C. As soon as nematode tracks appeared around the baits, or when bacteria or fungal growth occurred (1–2 days) on the polyol drop, the contaminated gelrite or agar portion ( $\frac{1}{5}$ – $\frac{1}{4}$  of the petri dish) was carefully removed (Fig. 1E). These plates were further incubated, allowing the nematodes to multiply. Alternatively, the nematode-infected callus tissues from the bait plates (Fig. 1B) could be transferred to a new plate containing fresh callus tissues (Fig. 1C).

Five plates of each nematode population prepared in this manner were observed monthly for contamination. At 4 months, nematodes (adults, juveniles, or eggs) were extracted from the callus tissues, root explants, or the support base (gelrite or agar) by the Baermann funnel method (2). Eggs of *Meloidogyne* and *Rotylenchulus* spp. were

extracted from egg masses on roots with 0.05% NaOCl (2). A culture was considered established if egg-laying females appeared and nematode population density increased in the absence of contamination after 120 days. Some original and second-generation plates continued to be incubated at 25 C; samples of nematodes and host tissues from these plates were tested for sterility by passage through nutrient, glucose peptone, and potato dextrose agar (PDA) (4) at the end of 4 and 12 months.

*Evaluation of Pluronic F127 as support base for short-term culturing of corn root explants and Pratylenchus species (Experiment 2)*: Corn root explants (*Zea mays* L. cv. Sweet Sue) maintained in MS medium in petri plates using 1.5% (w/v) agar or 20% (w/v) polyol as a support base were inoculated with ca. 250 mixed life-stages of each *Pratylenchus* spp. when the explants were 5 days old. The maintenance of the stock *Pratylenchus* cultures and preparation of inocula for the experiment were conducted according to methods described by Huettel (7). After 6 weeks of incubation at 25 C in the dark, nematodes in the roots, callus tissues, or support base were estimated in the following manner (hereafter noted as the SS procedure): Root explants or calli were equilibrated at 15 C, separated from the support base (polyol or agar), and then transferred separately to 250-ml Erlenmeyer flasks containing 100 ml of sterile water. The flasks were placed in a wrist-action shaker (21) and agitated at 60 strokes per minute for 3 days. The contents of the flasks were then passed through a 700- $\mu$ m pore sieve nested over a 37- $\mu$ m pore sieve. The nematodes collected on the bottom sieve were counted, while the roots on the upper sieve were dried at 60 C for 48 hours and then weighed.

*Evaluation of long-term effects of Pluronic polyol on growth of alfalfa calli and reproduction of P. penetrans (Experiment 3)*: Alfalfa callus tissues (2 pieces per tube) were established in 2.5  $\times$  15 cm culture tubes containing 20 ml MW per tube, using 1.5% (w/v) agar or 20% (w/v) polyol (11) as sup-

port base. Each culture tube was inoculated with a 0.1-ml sterile suspension of 500 mixed life-stages of *P. penetrans* when its calli were 10 days old. Maintenance and preparation of *P. penetrans* inoculum were conducted according to Riedel and Foster (19) and Kable and Mai (9), respectively. At monthly intervals for 10 months, samples of five tubes from each support base treatment were harvested. The nematodes were extracted separately from callus tissues and support base in each tube culture as described above. A random sample of 100 nematode individuals was selected and the sex ratio and proportion of juveniles and adults within the sample determined. In some cases, the callus tissues collected on the 700- $\mu$ m sieve were rinsed with deionized water, dried for 48 hours at 60 C, and then weighed. Nematode numbers in the calli and in the support base were summed to estimate the population yielded per tube culture. An additional 20 tubes of the callus tissues was grown in either support base in the absence of nematodes to assess the effects of the polyol itself on tissue growth. Dry tissue weights of these controls, along with those of inoculated treatments, were determined at 0, 3, 6, and 10 months after the start of the experiment.

*Infectivity and reproduction of P. penetrans reared in agar- or polyol-base medium (Experiment 4):* Inocula of *P. penetrans*, each consisting of 2,000 mixed life-stages extracted separately from the callus tissues and either support base (agar or polyol) of 9 month-old cultures, were inoculated onto ten 6-day-old pea seedlings (*Pisum sativum* L. cv. Wando) growing in a 10-cm-d clay pot containing loamy sand. Roots of five plants from each treatment were harvested after 1 week, stained with acid fuchsin (3), and the number of nematodes inside counted to determine infectivity. Roots of another five plants from each treatment were again harvested after 6 weeks, weighed, and then cut into 2–3-cm segments. Nematodes were extracted from 1-g sample of root segments by the SS procedure, and from 100 cm<sup>3</sup> samples of the

accompanying soil by the Baermann method (2). Numbers of nematodes in both roots and soil were counted subsequently and totaled to estimate the degree of nematode reproduction.

*Experimental design and statistical analysis:* In experiments 1 and 4, each of the treatments (nematode population or inoculum source, respectively) was replicated five times and arranged in a randomized complete block design (RCB). In experiment 2, the treatments (support base) were also each replicated five times and arranged in RCB within a *Pratylenchus* species or control. In experiment 3, the two-factorial treatments (support base and month) were each replicated 10 times and arranged in a split-plot design, with month being the main plots and support base the sub-plots. Treatment means were compared using SAS Student's *t*-tests (Experiment 2) or were subjected to analysis of variance using the SAS GLM procedures (SAS version 6.0, SAS Institute Inc., Cary, NC) (experiments 1,3,4) for treatment effects. Where applicable, means were also separated by Fisher's least significant differences if a significant treatment effect was detected. Except for experiment 1, all experiments were repeated once. Only one set of data was reported here since results for the repeat tests were similar.

## RESULTS AND DISCUSSION

The new surface-sterilization technique made use of the previous observation (M. Ko, data not shown) that the selected nematodes migrated faster than the growth of most fungi or bacteria. Indeed, more than 60% of the nematodes were devoid of detectable microbial contaminants after they migrated some distance (about 5 cm) in the agar- or gelrite-substrate contained in the petri plates (Table 1). Contaminants were not observed when some of the 'clean' nematodes or nematode-infected tissues were plated onto rich media consisting of nutrient agar, glucose peptone, or PDA during these periods (4 and 12 months). Apparently, the Pluronic

TABLE 1. Nematodes (mixed life-stages) and egg numbers from 120-day-old cultures of alfalfa calli or tomato root explants. Cultures were initiated with about 50 infective stages of nematodes surface sterilized by passage through 0.25% gelrite.

Species	Host/location	Nematodes or eggs/plates	Contamination (%) <sup>c</sup>
<i>Meloidogyne incognita</i>	Ginger-Hilo, Hawaii	5,000 <sup>a</sup>	20
<i>Meloidogyne arenaria</i>	Taro-Wainiha, Kauai	8,000 <sup>a</sup>	40
<i>Meloidogyne konaensis</i>	Coffee-Kona, Hawaii	7,500 <sup>a</sup>	20
<i>Meloidogyne javanica</i>	Pineapple-Haliimaili, Maui	8,000 <sup>a</sup>	40
<i>Pratylenchus brachyurus</i>	Pineapple-Kunia, Oahu	22,000 <sup>b</sup>	20
<i>Pratylenchus</i> sp.	Coffee-Poamoho, Oahu	20,000 <sup>b</sup>	0
<i>Rotylenchulus reniformis</i>	Pineapple-Kunia, Oahu	10,000 <sup>a</sup>	40
<i>Radopholus</i> sp.	Banana-Kona, Hawaii	30,000 <sup>b</sup>	20
<i>Radopholus</i> sp.	Calathea-Hilo, Hawaii	35,000 <sup>b</sup>	0

<sup>a</sup> Average number of eggs from the roots rounded to the nearest 1,000.

<sup>b</sup> Average number of vermiform stages from both roots and support base rounded to the nearest 1,000.

<sup>c</sup> Percentage of plates contaminated during the 120-day period.

polyol drop in the coverslip, though unable to completely suppress bacterial or fungal growth, concentrated the nematode specimens into a small area and slowed the growth of these contaminants to allow nematodes to migrate ahead of the contaminant growth. The polyol may also act as a cleansing agent as the nematode specimens migrate out from the drop. If needed, antibiotics or fungicides may be incorporated into the polyol drop to further deter bacterial or fungal growth.

Aseptic cultures were achieved in at least 60% of the plates for all nematode populations tested (Table 1). Some plates became contaminated within a few days after inoculation, but most remained free of contamination during the 12 months of this experiment. In general, the more active nematodes (e.g., *Radopholus* sp.) were the least contaminated.

Population densities increased in all the uncontaminated plates from the initial population (Table 1). *Pratylenchus* and *Radopholus* species increased 400–700 fold, which is at the lower range of multiplication achieved in other culturing systems during the same period (3 months) (5,18, 22,23). The low nematode multiplication rates might be attributed to the lower initial population density (Pi) and different culture medium and substrate used in our experiments. Permanent aseptic cultures of these nematodes were accomplished by transfers of nematode-infected tissue or

egg masses (for root-knot or reniform nematodes) from the primary aseptic cultures onto daughter plates with fresh callus tissues as was done with *Radopholus* spp. or *Pratylenchus* spp. (7).

A major advantage of the technique is that only a few nematode specimens are needed to start an aseptic population. The technique may be applicable to other nematodes such as *Ditylenchus*, *Aphelenchoides*, and *Heterodera* or *Globodera*-species that have active migratory juvenile infective stages. A single egg mass of *Meloidogyne* or *Rotylenchulus* species with hatching juveniles may be used to replace the polyol drop, and no coverslip would be needed (M. Ko, data not shown). Furthermore, the technique may also be used to initiate an aseptic culture of obligate nematode endoparasites such as *Pasteuria* species, since the technique results in little nematode mortality. Some disadvantages of the technique seem to be its cumbersome nature and a requirement for timely preparation of the tissue plates before the experiment. However, most procedures involved (e.g., initiation, growth, and maintenance of callus tissues or root explants) are also required of routines associated with other means of surface sterilization.

Pluronic F127 polyol was a suitable support base for short-term culturing of corn root explant and several *Pratylenchus* species (Table 2). The dry weights of root explants infected with *Pratylenchus* spp. at the

TABLE 2. Growth of corn root explants and reproduction of *Pratylenchus* spp. in agar- or polyol-base medium.<sup>a</sup>

<i>Pratylenchus</i> species	Root dry weight (g)		Number of nematodes <sup>b</sup>	
	Agar (1.5% w/v)	Polyol (20% w/v)	Agar (1.5% w/v)	Polyol (20% w/v)
<i>P. agilis</i>	0.092 a	0.136 b	2,040 a	5,730 b
<i>P. brachyurus</i>	0.098 a	0.118 a	1,192 a	4,344 b
<i>P. scribneri</i>	0.096 a	0.164 b	330 a	2,157 b
Check (None)	0.104 a	0.155 b	0 a	0 a

<sup>a</sup> Means of five replicates. Means with same letter in same row within the same variable are not significantly different ( $P = 0.05$ ) according to Student's  $t$  test.

<sup>b</sup> Measured at 6 weeks after inoculation ( $P_i = 250$ ).

end of 6 weeks in the polyol-base were higher than those in the agar-base and did not differ from the control ( $P = 0.05$ ) (Table 2). *P. agilis*, *P. brachyurus*, and *P. scribneri* were about 3, 4, and 6 times more abundant in the polyol than in agar, respectively (Table 2). The higher counts of *Pratylenchus* species in the polyol-base were not due to difference in the nematode extraction efficiencies, which were determined in a preliminary experiment by methods described by MacGuidwin (12). The efficiencies of extracting nematodes from agar- (69.3%) or polyol- (68.3%) base did not differ ( $P = 0.05$ ); neither were the efficiencies of extraction from the callus tissues reared in agar (57.3%) or in polyol (60.4%) ( $P = 0.05$ ).

The Pluronic polyol was a suitable support base for culturing alfalfa callus tissues (Fig. 2). Dry weight of nematode-free callus tissues in agar- or polyol-base increased 2-fold during the first 3 months but remained essentially the same ( $P = 0.05$ ) for the next 7 months. Alfalfa calli inoculated with *P. penetrans* in both support bases appeared progressively darker and smaller than the uninfected callus tissues. However, dry weights of the infected calli in the polyol decreased to 70% of their initial weights, whereas those in the agar increased to 135% of their initial weights over 6 months (Fig. 2). This may be caused in part by the much higher number of *P. penetrans* in the polyol feeding on the callus tissues (Fig. 3). Thus, the propagation of nematodes did not necessarily correlate positively with growth of callus tissues.

Similar observations have also been reported with other *Pratylenchus* species on alfalfa calli (15).

Over the 10-month period, *P. penetrans* population densities in agar-base medium fluctuated in a bimodal manner (with peaks at months 3 and 8, and a trough at month 6), whereas the population growth curve in polyol-base medium had distinct lag (1 month), exponential (3 months), stationary (4 months), and degenerating (2 months) phases (Fig. 3A). The polyol-base was more suitable than agar-base in terms of supporting nematode reproduction. As much as 35% more *P. penetrans* developed per tube in the polyol- as in agar-base medium (Fig. 3A). Three times as many nematodes per gram of tissue were supported in the polyol as in the agar (Fig. 3B). The higher nematode numbers in the

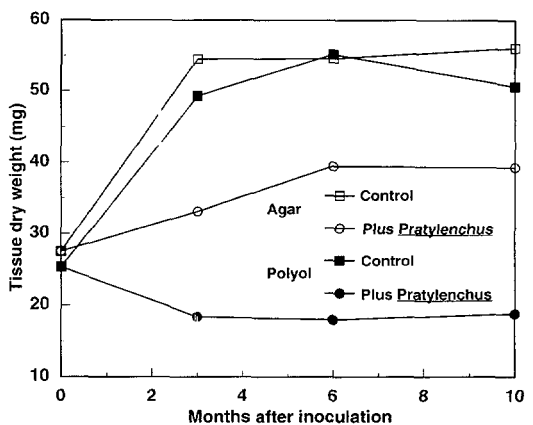


FIG. 2. Effects of *Pratylenchus penetrans* on growth of alfalfa callus tissues in agar- or polyol-base MW medium. MW = modified White's medium.

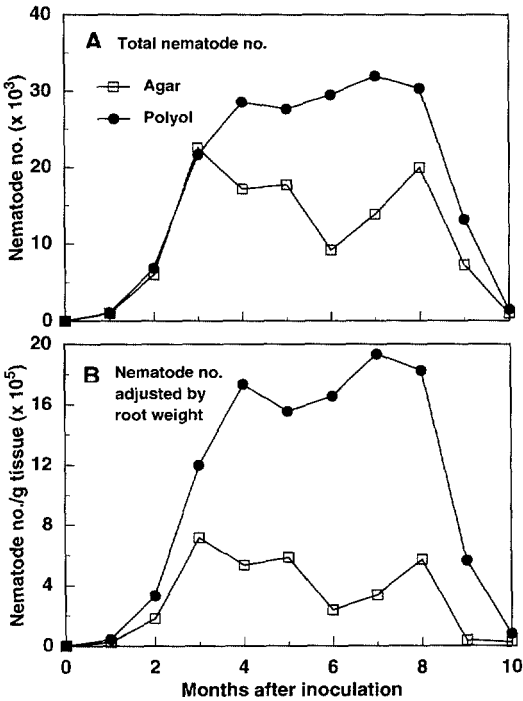


FIG. 3. Temporal change in the total population densities of *Pratylenchus penetrans* in alfalfa callus tissues reared in agar- or polyol-base MW medium. A) Number of nematodes per culture tube. B) Number of nematodes per g dry tissue. MW = modified White's medium.

polyol might be attributed to more efficient penetration of the roots by the nematodes in the substrate, effectively providing a higher Pi. Inability to penetrate roots was reported as the limiting factor for failure of *Ditylenchus destructor* to reproduce on excised clover and tomato roots (5) or *P. zeae* on excised maize roots (14) in agar. Alternatively, callus cells growing in the polyol might have provided a more suitable substrate or growing conditions for the nematodes.

Differences in the percentage of the nematode population appearing outside the callus tissues in the agar- (39.4%) or polyol- (37.5%) base were not statistically significant ( $P = 0.05$ ) (Fig. 4). However, percentage of the population appearing outside the calli in either support base fluctuated temporally and tended to correspond to the phases of the growth curves, especially for the nematodes reared in

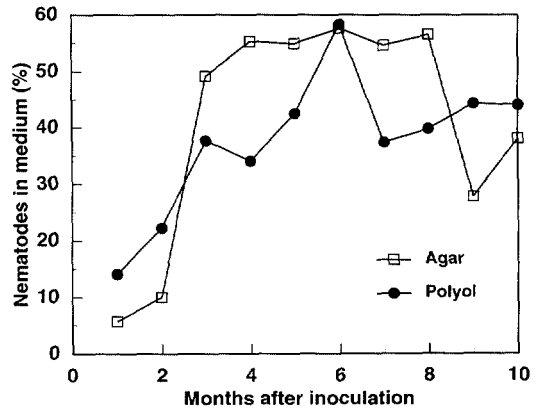


FIG. 4. Temporal changes in percentage of *Pratylenchus penetrans* residing outside the alfalfa callus tissues reared in agar- or polyol-base MW medium. MW = modified White's medium.

agar-base medium (Fig. 4). The percentage of population outside the calli in either support base was lowest in the lag, considerable at the exponential, and highest in the middle of the stationary phases of growth (Fig. 4). These data provide further evidence that a portion of *Pratylenchus* species resides outside the host tissues, regardless of whether the medium is soil (12), agar (17), or, in this case, the polyol. Nematode numbers outside the host tissue generally increase as the culture ages, when rate of host tissue senescence is high (12). However, in our experiment, their early exit from host tissues, even at the exponential phase of growth (when the rate of tissue senescence was supposedly low), further indicates that migration of nematodes from host tissues is not necessarily a response to host senescence or depleted food source.

Percentage of juveniles in the nematode sub-population residing outside (Fig. 5A) or inside (Fig. 5B) the host tissues in agar or polyol fluctuated widely and similarly over the 10-month period. In all cases, percentage of juveniles in the total nematode population was the highest in the early part of the stationary phase of growth (Figs. 3,5). Percentages of juveniles among the sub-populations inside (32.6%) and outside (32.9%) the host tissues in agar were not different, neither were the per-

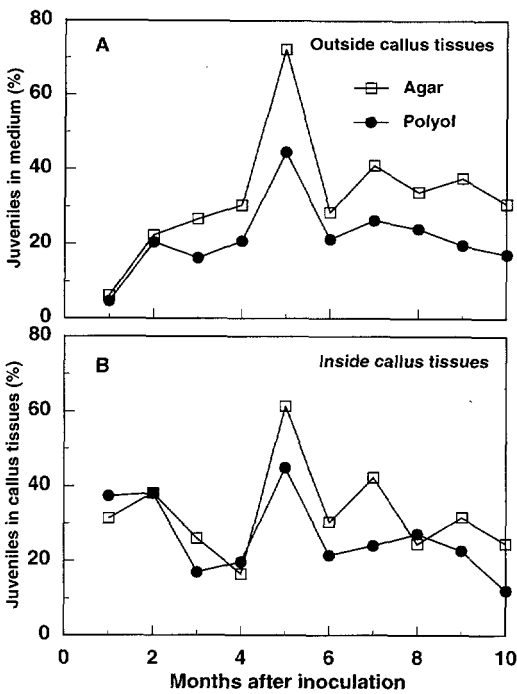


FIG. 5. Temporal changes in proportion (as percentage of total number of all life-stages) of *Pratylenchus penetrans* juveniles from alfalfa callus tissues reared in agar- or polyol-base MW medium. A) Percentage of juveniles outside calli. B) Percentage of juveniles inside calli. MW = modified White's medium.

centages inside (26.4%) and outside (21.5%) the host tissues in polyol ( $P = 0.05$ ), indicating that migration of *P. penetrans* from the host tissues onto the surrounding substrate was not specific to a particular life-stage and the proportions were similar regardless of the support base used.

The female-to-male ratios of the nematode populations from either support base also fluctuated widely over the period. The sex ratios among the sub-populations residing inside the callus tissues in agar (1.14) and in polyol (1.24), and among the sub-populations residing outside the callus tissue in agar (1.28) and in polyol (1.05), were not different ( $P = 0.05$ ).

At the end of week 1, the percentage of inoculant *P. penetrans* (number of nematodes found in roots divided by 2,000, the Pi) from 9-month-old polyol- or agar-base culture found inside roots of test pea

plants was 38.8% and 36.1%, respectively. These numbers were not different ( $P = 0.05$ ), indicating that *P. penetrans* reared in polyol- or agar-base were equally infective. Nematode numbers recovered from pea plants at the end of 6 weeks averaged 208/100 cm<sup>3</sup> soil and 250/g root using nematodes extracted from the polyol as inoculum, and 178 per 100 cm<sup>3</sup> soil and 203/g root using nematodes extracted from agar as inoculum. These numbers, representing a 3-4-fold increase in population after conversion to total numbers per pot, are not different ( $P = 0.05$ ). That is, nematodes reproduced similarly, irrespective of whether they originated from the agar-, polyol-base, or from the callus tissues.

No egg data were obtained in our population dynamics studies due to limitation of the extraction techniques used. Enzymatic digestion of plant tissues (1,10) would have been the preferred technique if rapid egg recovery were to be anticipated. Despite such deficiency, *P. penetrans* reared in polyol-base medium clearly shows similar ectoparasitic behavior as those reported in literature (12,17) and similar population dynamics as those reared here in agar. Populations from both types of culture declined abruptly after 8 months and had similar sex-ratio, percentage composition of juveniles, and likelihood to reside outside host tissues. The polyol cultivation system thus has no adverse effect on *P. penetrans* and may be employed specifically to study the role and fate of those individuals outside the host tissues, the factors causing their exodus from host tissues, and also those leading to their abrupt population decline. Such information is necessary to manage this nematode.

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